THE MEASUREMENT AND SIGNIFICANCE
OF ATP POOLS
IN ACTIVATED SLUDGE TREATING
COKE OVEN LIQUORS.

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Submitted by ALAN ROBERT CLARKE to the University of Aston
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SUMMARY

Coke oven liquor is a toxic wastewater produced in large quantities by the Iron and Steel, and Coking Industries, and gives rise to major effluent treatment problems in those industries.

Conscious of the potentially serious environmental impact of the discharge of such wastes, pollution control agencies in many countries have made progressively more stringent quality requirements for the discharge of the treated waste.

The most common means of treating the waste is the activated sludge process. Problems with achieving consistently satisfactory treatment by this process have been experienced in the past. The need to improve the quality of the discharge of the treated waste prompted attempts by TOMLINS to model the process using Adenosine Triphosophate (ATP) as a measure of biomass, but these were unsuccessful.

This thesis describes work that was carried out to determine the significance of ATP in the activated sludge treatment of the waste. The use of ATP measurements in wastewater treatment were reviewed. Investigations were conducted into the ATP behaviour of the batch activated sludge treatment of two major components of the waste, phenol, and thiocyanate, and the continuous activated sludge treatment of the liquor itself, using laboratory scale apparatus.

On the basis of these results equations were formulated to describe the significance of ATP as a measured activity and biomass in the treatment system. These were used as the basis for proposals to use ATP as a control parameter in the activated sludge treatment of coke oven liquor, and wastewaters in general. These had relevance both to the treatment of the waste in the reactor and to the settlement of the sludge produced in the secondary settlement stage of the treatment process.

KEYWORDS:

ADENOSINE TRIPHOSPHATE   ACTIVATED SLUDGE   COKE OVEN LIQUOR

DEDICATION

This work is dedicated to my wife who tended and nourished the Wild Seed that brought forth the Crop. Like the Farmer and his Harvest, not one grain bears her name, but no one doubts the Love and the Light that produced it.

……

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Excuse me if my Intoxication with the Joys of Soul seems to distract me. What you call Achievement is but a shooting star, the similitude of a million brighter stars on the Journey. Time enough to bathe in the Ecstasy of their Glow at the Journey's End.

A.R.C.

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CHAPTER 1

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INTRODUCTION

This thesis reports the results of a project sponsored by the British Steel Corporation and the Science Research Council, and carried out in collaboration with Aston University. The aim of that project was to investigate the measurement and significance of Adenosine Triphosphate (ATP) pools in the Activated Sludge Treatment of Coke Oven Liquors, and to use this parameter to develop a practical mathematical model for the control of the treatment system. In so doing it was hoped that this would enable the British Steel Corporation to improve the design and operation of plant treating this liquid waste, and so make a valuable contribution to the overall control of effluent disposal at Steel Works.

As the title of the thesis would suggest, the research involved the application of several scientific disciplines, including microbiology, biochemistry, analytical chemistry, civil engineering, mathematics and computer science. The interdisciplinary nature of the work was possible due to the interest and facilities offered by the British Steel Corporations laboratories on Teesside and at Battersea, and Aston University's Interdisciplinary Higher Degrees (IHD) Scheme, Biological Sciences and Civil Engineering Departments, and in particular due to the interest shown by those members of these departments acknowledged earlier.

The results obtained were of direct practical importance to the British Steel Corporation, and offer considerable potential in enabling them to realise the aims outlined above. In addition the results are of significance outside the Steel Industry, particularly to those involved in the effluent problems of the ever important and expanding coal and coke industries of the world, as well as to those involved in the biological treatment of wastewaters of domestic and other industrial origins.

Very large volumes of water are required to operate an integrated iron and steel works, and one of the long term objectives of the Steel Industry in any country must be to improve water and effluent management. This chapter describes the essential aspects of water-management in the Iron and Steel Industry, the significance of coke oven effluent production, and the need for that management to be efficient. Most of the problems described are common to any Iron and Steel Industry, and although many of the examples described concentrate on the problems of the British Steel Industry, and the British Steel Corporation (BSC) in particular, other examples are given to illustrate the similar problems encountered in other countries, and to give the subject world perspective.

As well as describing the reason for investigating the treatment of the coke oven effluent component of the Industry's wastewater production, this chapter also outlines the reasons for concentrating on the activated sludge treatment of the waste.

1. WATER AND EFFLUENT MANAGEMENT IN THE IRON AND STEEL INDUSTRY

The Iron and Steel Industry is a major user of water, and it will be shown here that there are sound financial, environmental and legal reasons for ensuring the efficient use and disposal of water in the industry. First however it is necessary to consider the

Industry's use of water in some detail.

1.1 Water Usage in the Iron and Steel Industry

The Central Water Planning Unit reported in January 1979 (1) that there were about 150 steelworks in England and Wales, and that although there was an approximately equal number owned by BSC and private industry, BSC was responsible for 85-90% of the crude steel output, and for 94% of the annual water demand of the industry at that time. That annual water demand was considerable and amounted to 485 x $10^6 m^3$.

This demand can be seen in perspective if it is compared with other demands on water in England and Wales. For example, the average daily water consumption for purely domestic purposes in England and Wales is generally estimated to be about 140 litres per head per day. On this basis the Steel Industry's demand reported above was equivalent to the annual domestic water consumption of 9-10 million people. (Actually, only a small proportion of the Industry's water demand is met by the public potable water supply required by domestic consumers, but the comparison does serve to illustrate the magnitude of the water demand).

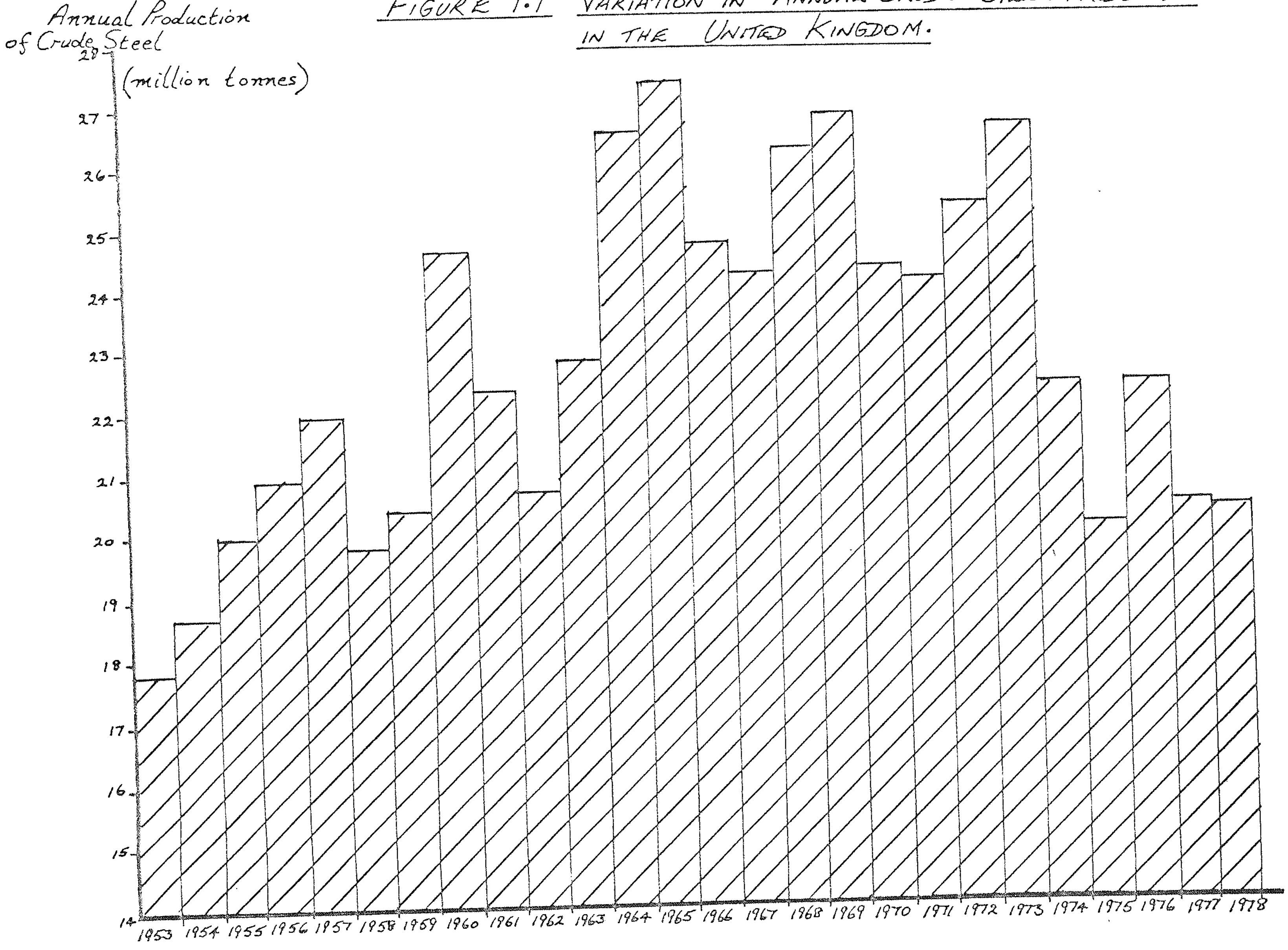
The Steel Industry's water demand can perhaps be seen in better perspective when compared with the total national water demand. Of the estimated average precipitation of 400 x $10^6 m^3$ per day (2) in England and Wales, only about 200 x $10^6 m^3$ per day is available to replenish watercourses and recharge underground water-bearing strata, the balance being lost by evaporation and plant transpiration. There is an unequal distribution of this water however, both geographically and seasonally so that only a proportion of it is practically available for our domestic, industrial, agricultural, and other uses, and that which is available needs to be carefully managed by the Water Authorities (WA's) charged with ensuring supply and distribution. The total water demand in England and Wales has been estimated to be 23 x $10^6 m^3$ per day(3) (excluding the mainly estuarine water used by the CEGB power stations for cooling purposes) about half of which is used by industry.

The Steel Industry's reported annual demand of 485 x $10^6 m^3$ therefore represented of the order of 6% of the total demand, or 12% of the industrial water demand in England and Wales.

Although the annual water demand by the Iron and Steel Industry in England and Wales in considerable, the demand in BSC has in fact shown a marked downward trend for several years and this trend is expected to continue. The annual demand was calculated to be 836 x $10^6 m^3$ in 1968 and this had reduced to 570 x $10^6 m^3$ p.a. in 1976.

There are several factors which have contributed to this reduction in demand. One is that although water demand is not directly proportional to steel output, it has some bearing. Figure 1-1 summarises the United Kingdom (UK) steel output

FIGURE 1.1 VARIATION IN ANNUAL CRUDE STEEL PRODUCTION IN THE UNITED KINGDOM.



since 1953.(4) It shows that steel production increased and reached a maximum in the 1960's but has shown a consistent decline since then. To put the UK steel production in context with world production Table 1-1 shows the relative position(5) of the UK in the world as a steel producer in 1977. It shows that although the UK is a large producer of steel, it is not a major world producer, or even the largest producer in Europe. It is also interesting to note that although there has been a decline in UK crude steel production during the last decade, world crude steel production over the last eighty years has increased dramatically (Figure 1-2) and this is related to the increases in population, rising living standards, and Third World Development.

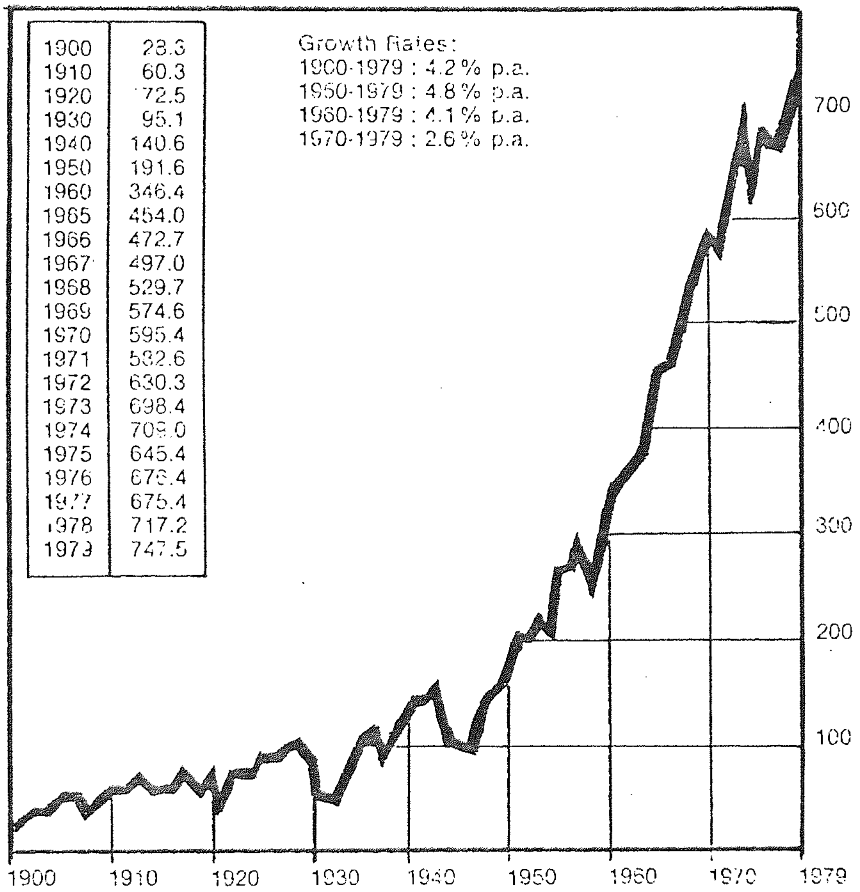
The actual water demand of steel plants is influenced by a great many factors and varies considerably. The demand on an integrated steel works can vary from as high as 170 m^3/tonne of finished steel produced in a works not practising water conservation, to as low as $3m^3$/tonne where water re-use and recycling is efficiently employed. Water demand also tends to be related to the age of the works being considered. Older works tend to be far less efficient in their use of water than modern ones which reflects modern concern with water conservation and water pollution control and the legal and financial sanctions associated with them, of which more will be said later.

Older works were built at a time when there was less concern

TABLE 1-1 LEAGUE TABLE OF COUNTRIES WITH LARGEST CRUDE STEEL PRODUCTION AND CONSUMPTION, AND THEIR IMPORT AND EXPORT OF FINISHED STEE PRODUCTS IN 1977 (ALL VALUES IN MILLION TONNES)

COUNTRY	PRODUCTION	CONSUMPTION	EXPORTS	IMPORTS
U.S.S.R.	146.7	147.0	7.5	6.6
U.S.A.	113.2	133.9	1.9	17.2
JAPAN	102.4	63.2	33.4	0.2
FED. REP. GERMANY	39.0	33.0	15.4	11.1
CHINA	23.4	29.9	5.0	
ITALY	23.3	20.7	6.7	5.2
FRANCE	22.1	19.6	10.1	8.2
U.K.	20.5	19.7	4.4	3.8
POLAND	17.8	18.7	1.8	2.4
BELGIUM/ LUXEMBORG	15.6	4.0	12.2	3.1
CZECHOSLOVAKIA	15.1	11.2	3.5	0.5
CANADA	13.6	12.7	2.1	1.4
ROMANIA	11.5	11.9	1.5	1.9
BRAZIL	11.3	12.0	0.4	0.9
SPAIN	11.2	9.1	2.7	1.1
INDIA	10.0	8.7	1.5	0.5
SOUTH AFRICA	7.3	4.7	2.1	0.1
AUSTRALIA	7.3	6.1	2.5	1.5
GERMAN DEM. REP.	6.9	10.2	1.7	4.2
MEXICO	5.6	5.9	0.3	0.5

(SOURCE: INTERNATIONAL IRON AND STEEL INSTITUTE).



1900	28.3
1910	60.3
1920	72.5
1930	95.1
1940	140.6
1950	191.6
1960	346.4
1965	454.0
1966	472.7
1967	497.0
1968	529.7
1969	574.6
1970	595.4
1971	582.6
1972	630.3
1973	698.4
1974	709.0
1975	645.4
1976	678.4
1977	675.4
1978	717.2
1979	747.5

Growth Rates:
1900-1979 : 4.2 % p.a.
1950-1979 : 4.8 % p.a.
1960-1979 : 4.1 % p.a.
1970-1979 : 2.6 % p.a.

Fig. 1·2 World crude steel production

FIGURE I.2 World Crude Steel Production[5] with such matters, when water costs had a negligible affect on the cost of the finished product,(6) and consequently had a low priority in the design of works.

Efficient water management however is not only related to the age of the works but also to the relative abundance of water supplied. The least efficient works tend to be those where water is abundant and there are several raw water sources available, and the most efficient those totally dependant upon public water supply.

There are various sources of water available to the industry, these being largely dependant upon the geographical location of the works. Public water supply however supplies only a small fraction of the total demand for industrial usage, this being 6.5% of the total annual demand for BSC in England and Wales in 1968/69. In the case of the private sector steel industry, public water supply is estimated to contribute about 40% of total requirements, the balance for both sectors of industry being met by direct abstraction. However, as the private sector water demand forms only about 6% of the total for the Iron and Steel Industry it is estimated that less than 8% of the total annual demand of the industry is met by the public supply. The demand for water by BSC and the private sector in England and Wales from various sources is summarised in Table 1-2.

The water demand of the steel industry is therefore large, although it varies considerably from plant to plant depending

TABLE 1-2 DEMAND FOR WATER BY BRITISH STEEL CORPORATION (BSC) AND THE PRIVATE SECTOR STEEL INDUSTRY FROM VARIOUS SOURCES

DIRECT ABSTRACTION	BSC 1968/69[1] ANNUAL WATER DEMAND $10^6 m^3$		BSC 1976 5 ANNUAL WATER DEMAND $10^6 m^3$		ESTIMATED PRIVATE SECTOR ANNUAL WATER DEMAND $10^6 m^3$
INLAND SURFACE WATERS	227	244 (28.6%)	244.9	253.7 (44.5%)	
GROUND WATER	17		8.8		
LAND DRAINAGE SEWAGE EFFLUENT & OTHERS		19 (2.2%)		35.4 (6.2%)	17.5 (60.3%)
TIDAL SOURCES		526 (61.5%)		223.6 (39.2%)	
PUBLIC WATER SUPPLY					
INDUSTRIAL USE	56	66 (7.7%)		57.7 (10.1%)	11.5 (39.7%)
DOMESTIC USE	10				
TOTAL		855 (100%)		570.4 (100%)	29 (100%)

on a great many factors including age, abundance of water supply, volume of steel production, and the operation and design of works.

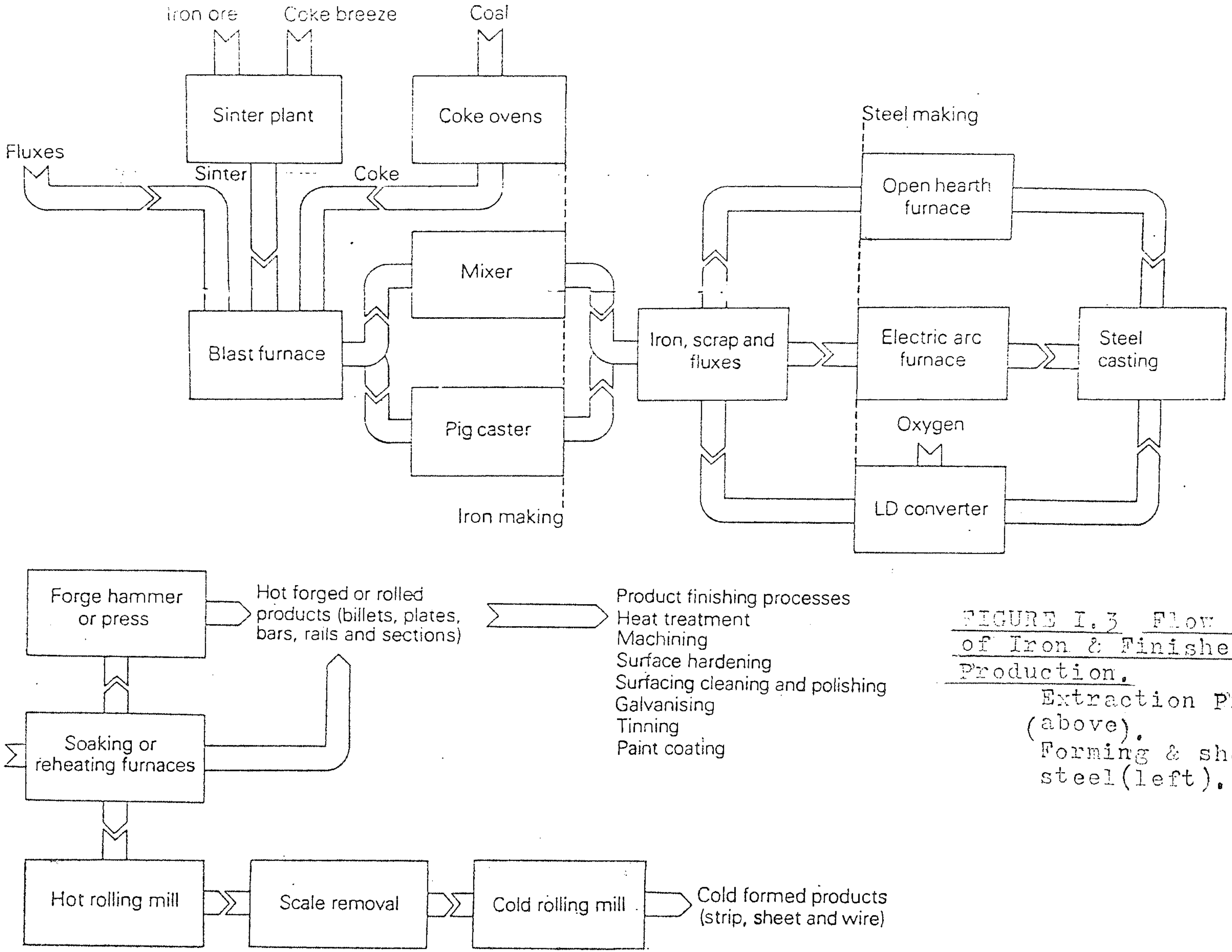
Before considering the more specific uses of water at plant level, and the sources of effluent, it will be useful to briefly summarise the Iron and Steel Production Process.

1.2 The Iron and Steel Production Process

The operations of an integrated iron and steel works can be divided into three parts, iron making, steel making, and the forming and shaping of the steel along with other finishing processes to produce the finished product. These are illustrated diagramatically in Figure 1-3, and briefly described as follows:

Iron Making

This includes two other processes, coke making and sintering. In the coke making process coal is carbonised in coke ovens to produce metallurgical coke. Coke breeze is mixed with fine or crushed iron ore and ignited on a continuous grate in the sintering process. The resultant product (sinter) is a porous mechanically strong iron-rich material. This sinter, along with coke and fluxes is loaded into a blast furnace into which pre-heated air is blown and the iron ore is reduced to molten iron, and tapped from the base of the furnace, as is the slag



<u>FIGURE I.3 Flow Diagram of Iron & Finished Steel Production.</u>
Extraction Processes (above).
Forming & shaping of steel (left).

that is also produced.

Steel Making

There are 3 alternative steel-making processes. In two of them the basic oxygen (BOS) and the open hearth process, the molten iron from the blast furnace along with steel scrap and fluxes is used to charge the process. In the BOS process, which is the method used in most modern steel plants, a pure oxygen jet is directed at the surface of the hot metal to refine it into steel. The third method, the electric arc process, begins with cold steel scrap. The molten steel from these processes then passes to the next stage.

Steel Forming, Shaping and Finishing Processes

The molten steel produced is then cast and may pass through a variety of forming, shaping and other finishing processes as described in Figure 1-3 to produce the finished product.

1.3 Use of Water in an Integrated Iron and Steel Works

The main uses of water in an iron and steel works may be listed as follows:

1. For cooling plant, equipment and various products of the process.
2. Dust and fume control.
3. Waste product transport.

4. Other uses e.g. steam raising, product rinsing.

By far the largest volumes of water are used in cooling where it is necessary for prevention of damage to plant, much of which operates at high temperatures. Blast furnaces used in iron making for examples are used at temperatures as high as 1500^{o}C.

Water cooling systems are of two types:

1. <u>Direct</u> - This performs a combined cooling and cleaning function, accounts for about 30% of the water circulating in a typical modern integrated plant, and is subject to varying degrees of contamination. It is used for blast furnace gas cleaning and BOS fume cleaning for example.

2. <u>Indirect</u> - These are used purely for cooling and are used for example in condensers, furnace elements, and compressors. This sytem accounts for about 70% of the water circulating in a typical integrated plant and does not become contaminated, other than by heat.

Water demand for an integrated steel plant can vary, as described earlier, from 3m^3 to 170m^3/tonne of finished steel produced. Less than 3m^3 of water/tonne is actually consumed in the process, most of this being lost to the atmosphere, so that the size of the demand is largely a reflection of the degree to which recirculation and re-use is practised. The maximum demands are found in plants with once-through water systems. The low demands are typically found in modern plants where water conservation measures are rigorously applied. Table 1-3 summarises the circulation flows for a modern BSC integrated works producing 4M tonnes of steel per year.(7)

The water demands of the Steel Industry can therefore be considerably reduced by recirculation and re-use, but the choice of whether to employ such measures is made at each individual plant mainly on the basis of the relative costs of conservation measures, water availability, and Water Authority constraints on effluents. Generally however, these last two factors are the major influence and the overall strategy is towards conservation. The overriding influence on reducing the water-demand of the UK Steel Industry at present however relates more to the current world wide slump in steel demand, and also to the over production abroad by more efficient (and often heavily subsidised) steel plants aggressively competing in the world steel market. This, along with the decision in the UK to concentrate steel production in a few large modern steelworks in line with the policy advocated in the Beswick Report of 1973, and the financial and industrial relations problems related to the international slump, has meant that in the next few years we shall see an acceleration in the closure of the smaller, older and less efficient plants.

1.4 Effluents Produced in the Iron and Steel Industry

Effluents arise from various stages in the iron and steel production process and the origin of these was indicated in

TABLE 1.3 WATER USE AND CIRCULATION FLOW IN A BSC INTEGRATED STEELWORKS PRODUCING 4M TONNES PER ANNUM

PLANT	INDIRECT COOLING WATER SYSTEM QUANTITY			DIRECT COOLING WATER SYSTEM		
	MAIN USE	M^3/min	% TOTAL	MAIN USE	M^3/min	% TOTAL
COKE OVENS	GAS PRIMARY COOLERS	75	7.9	GAS COOLING AND PURIFICATION	15	1.6
BLAST FURNACES	FURNACE AND FURNACE BLOWER CONDENSER COOLING	350	36.7	GAS COOLING AND CLEANING	75	7.9
STEEL FURNACES	FURNACE AND EQUIPMENT COOLING	70	7.3	FUME COOLING AND CLEANING	35	3.7
CONTINUOUS CASTING	MOULD AND MACHINE COOLING	35	3.7	QUENCHING HOT STEEL	10	1.0
ROLLING MILLS	COOLING ELECTRICS AND HYDRAULICS	30	3.1	ROLL COOLING, PRODUCT QUENCHING, SCALE WASHDOWN	130	13.6
POWER GENERATION	CONDENSER COOLING	85	8.9			
OTHERS, INCLUDING SINTER PLANT		30	3.1			
TOTAL		675	70.7		265	27.8

OTHER WATER USES INCLUDING PROCESS WATER, BOILER FEED, AND DOMESTIC USES ACCOUNT FOR REMAINING 1.5%.

Figure 1-3, and the major pollutants are summarised in Table 1-4. Of the various effluents produced those arising from the coke ovens are the strongest, the most complex, and consequently the most difficult to treat. It was for this reason that the project chose to concentrate on the treatment of this waste, and its chemical nature and treatment are considered here in some detail.

In the coke-making process water is required for direct and indirect cooling of the coke oven gas produced in the carbonisation process, and in some of the distillation processes at the by-product plant and for coke quenching. The major part of the effluent arises from the moisture in the coal itself. The waste liquor produced in the gas cleaning process is termed "ammoniacal liquor". This is a strong liquor and contains a complex mixture of organic and inorganic chemicals, including free and fixed ammonia, phenols, thiocycanate, thiosulphate, sulphides, and cyanides. Much of the free and fixed ammonia, and "Devil Gases" containing hydrogen sulphide, carbon dioxide, hydrogen cyanide and phenol, are removed from the liquor by further processing. The "spent liquor" that remains forms the major effluent from the coking process.

Another liquor, termed "Devil Liquor", is produced and represents the condensate from the distillation of benzole solution arising from treatment of the ammoniacal liquor, and is generally added to the "spent liquor".

TABLE 1.4 MAIN DIRECT COOLING RECIRCULATION SYSTEMS AND THE MAJOR POLLUTANTS THAT CONTAMINATE THE WATER IN AN INTEGRATED IRON AND STEEL WORKS

PLANT	PROJECTS	MAJOR POLLUTANTS
Rolling Mills	Roll cooling Product Quenching Scale Washdown	Scale Particles, lubricating oils, hydraulic fluids
Continuous Casting	Spray cooling of hot steel	Scale Particles, lubricating oils, hydraulic fluids
Blast Furnaces	Gas cooling and cleaning	Dust particles, cyanide, fluoride, lead, zinc
BOS Furnaces	Fume cooling and cleaning	Iron oxide particles, fluoride
Coke Ovens	Gas cooling and purification	Tar-oils, ammonia, phenols, thiocyanate, cyanide, thiosulphate

Water used to quench hot coke in the cokemaking process, containing a high suspended solids content, is also added to the spent liquor, and the resultant effluent from the coking process is variously termed coke oven liquor, coke oven effluent, or carbonisation effluent. The composition of coke oven liquor is complex and highly variable, and is dependant upon the type of coal carbonised, the carbonisation temperature, and the method and operation of plant used to recover ammonia and by-products from the process. Table 1-5 includes a list of the main components of coke oven liquor for a typical carbonisation plant and illustrates the wide variation in composition that may occur.

1.5 Treatment of Coke Oven Liquor

About 10 million tonnes of coal were carbonised in the UK in 1978, and the Iron and Steel Industry accounted for about 70% of this. As between 0.13 and 0.23m^3 of ammoniacal liquor is produced per tonne of coal carbonised, it is evident that a considerable volume of waste liquor requires disposal each year. The coke oven liquor produced from the coking process represents only a small fraction of the water in use at an integrated iron and steel works (Table 1-3). However, as its composition would indicate, coke oven liquor is a very polluting waste, exerting a large oxygen demand when discharged to a watercourse or sewage treatment system, as well as being toxic in low concentrations to the flora and fauna found in both, and it gives rise to the major effluent treatment problem of the

Table 1·5 Showing a) Typical Composition Of A Coke Oven Liquor
b) Typical Consent Conditions For Discharge Of Treated Liquor To River
c) Toxicity Of Some Individual Components To Freshwater Fish

Component	Liquor Content		Consent Conditions.	Fish Toxicity
	Average	Range		
	mg/l	mg/l	mg/l	mg/l
PV	1900	1400-2700	100	
Mono-Phenols	650	450-800	5	1-20
Total Phenols	900	750-1200	20	
Thiocyanate	120	90-170	5	
Ammonia	100	80-200	100	
Free Cyanide	13	5-20	0.5	0.04-0.1

Iron and Steel Industry.

The toxic nature of coke oven liquor is reflected in the stringent Consent Conditions set by Water Authorities for discharge of the treated effluent to a river, the actual quality requirements being dependant mainly upon the relative flows of the effluent and river, the present river quality, and the WA's objectives for the river's future use and quality. A typical example is shown in Table 1-5, along with some limited toxicity data for some of the components of the liquor.

Some of the components of the liquor are very toxic to river life, although it is difficult to quote exact toxic levels as these are dependant upon very many factors including photolytic action, microbial degradation, pH, water hardness and temperature. In addition the toxicity of a mixture of components may be greater than the sum of the toxicities of the individual components of the mixture, and sub-lethal concentrations may still be of significance to the well-being of the river.

Toxicity of phenolics has been studied on a variety of micro-organisms,(8) (yeast, protozoa, and bacteria), and also on algae,(9) duckweed, and various invertebrates(10)(11) and vertebrates.(12) Quoted values for acute toxicity to phenol vary from less than 0.5 to 1,840 mg/l. Higher phenols and phenol derivatives, also present in liquor tend to be more toxic. Chlorinated phenols are particularly toxic to some species, toxicity levels being measured in μg/l. Chlorinated phenols although not normally found in steel works effluent, are produced when water containing phenol is abstracted and chlorinated for public water supply. Such water has an objectionable medicinal taste for phenol levels measured in parts per billion (ppb).

In one sense there is no problem in treating carbonisation effluents because it is technically possible to treat them to whatever standard is required. The problem lies however, as with any wastewater treatment process, in being able to achieve the required treatment practically and on a full-scale within reasonable capital and operational costs. As with any commercial enterprise therefore, it is important to the Iron and Steel Industry that it achieves the desired treatment objectives with the minimum of cost and this generally dictates the treatment method(s) chosen. The methods chosen may vary from plant to plant due to a number of factors including the effluent quality required (dictated mainly by geographical considerations, the quality of the receiving waters, and the quality objectives of the WA for those waters), the iron and steel making capacity and the operation of the plant and therefore the volume and strength of the liquor to be treated, and the financial and technical resources of the plant concerned.

The costs involved therefore are very important and need to be considered. Before doing so however, the treatment methods available will be briefly summarised.

The methods available(13) can be divided into three major categories, concentration processes, chemical oxidation processes, and biological oxidation processes.

1.5.1 Methods for Coke Oven Liquor Treatment

1.5.1.1 Concentration Processes

Adsorption and Ion Exchange

This involves concentration of the pollutants onto an adsorbtive medium. Adsorbants can vary and activated carbon, ion-exchange media,(14) coal, fly-ash, colliery spoil heaps, and other mineral media have all been used.

The cost of activated carbon and ion-exchange media is such that they need to be regenerated, and this leaves a concentrated solution of the pollutants which requires further treatment or disposal. The other cheaper absorbants do not generally require regeneration, and the contaminated material may be carbonised or dumped. The latter alternative is now less attractive in England and Wales than it used to be since the implementation of Part 1 of the Control of Pollution Act 1974. Because of the toxic nature of the adsorbed pollutants there are very few tipping sites which would be allowed to accept the contaminated material, and the transportation costs and charges for dumping at those sites that can accept them are generally high and continue to rise.

The use of activated carbon, ion exchange resins, and other adsorbative materials is likely to remain restricted because of the high costs involved.

Distillation

This is generally accepted as a necessary stage of ammoniacal liquor treatment.(15)(16) Free ammonia is first distilled off into dilute sulphuric acid, followed by liberation of fixed ammonia by the addition of lime, and a further similar distillation stage. Such treatment reduced the ammonia concentration from about 2000 mg/l to 100-200 mg/l.

Distillation in steam has also been successfully used for dephenolation in the Moppers process to achieve up to 95% recovery of monohydric phenols. No information regarding the costs of the process is available.

Foam Fractionation

This process(17),(18),(19) uses surface active agents which combine with organic molecules and gas bubbles, and allow the concentration of the organic molecules in a foam which can be separated from the main solution. Although several workers have investigated its use on laboratory scale plant, there are no reports known of its use on full-scale plants and its potential lies in the polishing of final effluents for phenol removal from biologically treated wastes, for which the costs would appear to be low.

Hyperfiltration (Reverse Osmosis) and Ultrafiltration

Concentration of pollutants can be achieved by membrane (20)(21)(22) filtration, with ultrafiltration involving the removal of colloidal particles and hyperfiltration the removal of low molecular weight dissolved materials, although the boundary between the two is not always discernible. Both have been investigated for removal of phenolic compounds from coal gasification wastewaters, but there is no information regarding its practical use for full-scale treatment of coke oven liquors or of the likely cost.

Chemical Precipitation and Coagulation

Chemical precipitation and coagulation (23)(24) has been used for suspended solids and tar removal prior to further treatment. Precipitation of organic compounds reducing phenol and thiocyanate concentrations has been reported where the process was used before or between stages in a two-stage biological system. Removal of pollutant in this manner may facilitate dumping of the waste.

Solvent Extraction

Various solvents (25)(26) have been successfully used with various extractor apparatus to achieve dephenolisation of coke oven liquors. Inorganic and some organic pollutants are not removed and further treatment of some other form is required. The market value of the extracted phenol generally dictates whether it is economic to use the process.(25)(26) Synthetically produced phenol tends to be cheaper so that solvent extraction is rarely used for treatment of the wastewater.

1.5.1.2 Chemical Oxidation Processes

Ozonisation

Ozone has been used on a small scale for oxidation of coke oven liquors,(27)(28) with some oxidation of thiocyanate and cyanide to cyanate, and with phenol removal approaching 100% being achieved. (The oxidation of thiocyanate to cyanide has the disadvantage however of making the effluent more toxic). Ozone does not appear to have been used on full-scale plant for treating the waste due to the capital cost of the ozone generator and to projected high operating costs. Costs however appear acceptable for its use as a polishing process for removal of phenol after biological treatment.

Electrolytic Oxidation

Phenols, thiocyanates and cyanides can be oxidised electrolytically but the process is only economic at low pollutant concentrations and laboratory-scale experiments suggest that its economic application would be restricted to polishing biologically treated effluents.(29)(30)

Incineration and Catalytic Combustion

Various incineration and catalytic combustion processes (31) have been proposed for coke oven effluent treatment and although it would seem possible to operate this for a full-scale plant, all the evidence indicates that this would be an expensive process.

Other Oxidation Methods

A large number of methods have been proposed including the use of nitric acid, chlorine, chlorine dioxide, and hydrogen peroxide.(32) No evidence of use of these methods on full-scale plant is known and operational difficulties and high costs are likely to inhibit their commercial application on this scale. Some methods, for example metal-catalysed hydrogen peroxide, have potential for use as a polishing process for removal of phenols from biologically treated effluents.

1.5.1.3 Biological Oxidation Processes

Biological oxidation processes are most commonly used for treatment of coke oven liquors.(33) This is because they are generally less costly than non-biologial methods, and that most of the pollutants can be treated in one process. Table1.6 summarises various methods used for treatment of coke oven liquor, and their relative value for removal of

TABLE 1.6.

Summary of Various Methods used for Treatment of Coke Oven Liquors.

	Major Components that can be removed.			
	ØOH	CNS	NH_3	Comments
CONCENTRATION PROCESSES				
Adsorption and Ion Exchange	+	+	+	Adsorbed material requires further treatment or disposal
Distillation	+ (In Steam)	-	+	NH_3 reduced to 100-200 mg/l
Chemical Precipitation	+	+	-	-
Solvent Extraction	+	-	-	-
CHEMICAL OXIDATION PROCESSES				
Ozonisation	+	-	-	Some oxidation of thiocyanate to cyanide
Electrolytic Oxidation	+	+	-	Only economic at low concentrations. Main application likely to be polishing biologically treated effluents
Incineration and Catalytic Combustion	+	+	+	An expensive process
BIOLOGICAL TREATMENT ACTIVATED SLUDGE PROCESS	+	+	+	Removal of all major components possible

\+ indicates full or partial removal of component possible the various components of coke oven liquor. This confirms that most of the components can potentially be removed by biological oxidation. The higher cost and difficulty in practically applying them to full-scale treatment works generally means that chemical oxidation and adsorption processes, which might otherwise be viable alternatives, are rarely chosen in preference to a biological process for large scale application.

Four basic types of biological processes have been used, the percolating filter or packed-tower process, the rotating disc process, the fluidised bed process, and the activated sludge process.

Packed-Tower Process

Work has been carried out using packed towers filled with a suitable plastic media for biological treatment.(34) Results indicate that the packed tower may have application at plants where large volumes of a weak liquor are to be treated. It would also seem to have good potential for partial treatment of liquors, for example prior to discharge to a public sewer or further biological treatment. However, the process has a number of disadvantages which make it unsuitable for many full-scale plants where an effluent suitable for direct discharge to most rivers is required. The towers are not recommended for treatment of limed liquors due to the precipitation of calcium salts on the packing, or for when complete thiocyanate removal is required.

However, when used as a pre-treatment step, or for treatment of weak liquors, or when the effluent quality requirements for discharge to the watercourse are not stringent, then its requirements for a smaller ground area and simpler foundations may occasionally make it more economic than the activated sludge process.

Rotating Disc Process

Laboratory and pilot-scale rotating discs have been used to successfully treat coke oven liquors,(35) and it has been shown to have a number of advantages over the activated sludge process.(36) The process requires no sludge recycling, foaming is eliminated, low sludge wastage can be practised, and it appears to be more resilient to some disturbances. The British Carbonisation Research Association reached the conclusion that the process is not economically viable for the commercial treatment of coke oven liquors. However, there is still active interest in the process in Europe, particularly in Belgium and Italy, and this includes its application to ammonia removal.

Fluidised Bed Process

In this process sand or activated carbon is used as the attachment medium for biological growth. The wastewater is introduced into the base of a column containing the medium at a rate which maintains the medium and attached growth in suspension. The large surface area that results allows a large surface area for biomass/waste contact and produces high reaction rates. The process has been successfully pilot tested, and is a recent development in biological treatment that is receiving much interest. Advantages claimed for the process are that it achieves complete treatment, at faster rates than other suspended growth systems and requires a smaller area for the plant. No information is available as to the practicallities of applying the process to full-scale treatment of coke oven liquors, and evidence suggests that it is not likely to be an economically viable alternative to activated sludge treatment of the waste.(37)

Activated Sludge Process

This is the most widely used process for treatment of (38) liquors. Single, two or three stage plants have been used, and satisfactory removal of all the main liquor components can be achieved, and effluents suitable for direct discharged to a watercourse.

However, despite the wide usage of the activated sludge process for coke oven liquor treatment, plants treating the waste often fail to perform satisfactorily, and coke oven liquors still represent a major effluent treatment problem to the Iron and Steel Industry. A large amount of research has already been conducted so that a greater understanding of the process can be gained. The continuing failure to operate full-scale plants satisfactorily however suggests that many of the basic principles governing the design, operation and control of the process have yet to be elucidated and the main aim of the present project is to progress research in this area so that more efficient design of plants treating carbonisation effluents can be achieved.

1.6 Water Costs for the Iron and Steel Industry

It has been established that the Iron and Steel Industry is a major user of water, that the industry can if desired considerably reduce this usage, and that for a number of reasons the water demand, although still very large, has already fallen significantly in recent years. It has also been demonstrated that the industry produces large volumes of toxic effluents, of which the most important are those arising from the coking process, and that the technology exists to reduce the hazards that they represent to the environment to an acceptable level.

The industry's behaviour with respect to water conservation and pollution control has important environmental consequences, but as for any commercial enterprise any measures taken towards improvements in these areas must to some extent be justified by financial and legal considerations. The legal aspects concerned will be considered later along with the role of WA's charged with interpreting and enforcing the relevant legislature.

The financial constraints and inducements to the industry to promote efficient management of water use and disposal are briefly considered here.

There are considerable problems in accurately estimating the Iron and Steel Industry's costs for water services. Water is, after all, only one of the raw materials that are intimately involved in the many and varied steps of iron and steel production, and isolating its costs from the many others is obviously difficult. Estimates of the overall costs quoted in the literature therefore can best be regarded as giving an order of magnitude guide to costs relative to the overall costs for steel production.

It has been estimated that about 6% of the capital costs of a new BSC steel works can be attributed to the equipment required for water supply, use and efficient disposal.

Actual costs for water services of course will vary considerably from works to works due to the wide variations in water management practice, the reasons for which were outlined earlier. However, the charges made by WA's to BSC for the financial year 1977-78 for external water services, that is for the supply of potable water, charges for abstractions and other services charges, are summarised in Table 1-7, and total approximately £11m. It can be seen that although potable water supply only accounts for about 8-10% of the industry's water demand, it accounts for about 50% of the external water costs. It is of course less costly to use water from sources other than the public potable water supply, and the latter is only used where there is a specific requirement for such high quality water, that is, for domestic and a small number of other purposes, or where other sources of water are absent or inadequate. There is therefore an obvious financial incentive to the industry to avoid use of public potable water supply whenever possible. Water of lower quality than the public supply is generally adequate for the vast majority of uses (see Table 1-8) and is of course used where available. These other sources of water however are not without cost, and it is often a source of some surprise within the steel industry that they should be charged by WA's for abstracting water for which often there is negligible WA involvement.

The unit charges (pence m^{-3}) for direct abstractions are of course considerably less than for potable water from the public supply. However, a small proportion of this abstracted water may require further treatment and need to pass through a softening plant, and some may additionally need to be demineralised, and this further treatment increases water costs. Table 1-9 shows the water costs from abstraction to effluent discharge for a BSC integrated works producing 2.8M tonnes of

TABLE 1-7

External water service costs, BSC 1977-8

	£M
Charges for potable water from public supply system	5.5
Charges for direct abstractions	1.5(estimate)
Miscellaneous service charges (via rates)	4.0(estimate)
Total	11.0

TABLE 1-8

Qualities of water required in an integrated steelworks

Type	Important quality parameters	Main use
Potable	Physically, chemically and biologically wholesome	Drinking, amenity, air conditioning
High-grade industrial	Low total disolved solids, low organic matter	Steam raising
General	Low suspended solids, non corrosive, non scale-forming	Cooling condensers, furnaces, rolling mills, gases
Low-grade industrial	Absence of gross pollution	Coke and slag quenching

TABLE/9

Water costs from abstraction to effluent discharge for an integrated works producing 2,8M tonnes pa (1977)

	Quantity 10^6 m^3/pa	pence m^{-3}	Cost pence tonne^{-1} steel
Direct abstraction	10.08	0.303	1.1
Softening	7.57	2.793	7.6
Demineralisation	0.68	28.55	6.9
Distribution	10.08	3.157	11.4
Recirculation/effluent treatment -			
indirect cooling	350.00	0.378	47.3
direct cooling	150.00	1.539	82.5
Potable water	0.59	15.224	3.2
Total			160.0

steel p.a., and which practises extensive water recirculation and re-use. Costs quoted are inclusive of those associated with the operation and maintenance of the water system, and of capital depreciation calculated linearily over a 15 year period. The 1977 estimate of £1.60 per tonne however did not include charges for miscellaneous services made through the rates, and with this included total cost per tonne of steel produced was estimated to be £2.20. In the example shown in Table 1-9 water recirculation and incircuit treatment accounted for 81% of the total costs. Although this would appear to be a powerful disincentive to practise water conservation, it can be assumed that the water management strategy chosen in this case was that which reflected the optimum solution to all the factors affecting water and operational costs for the plant, plus a number of non-cost factors, primarily those ultimately associated with the role and powers invested in the WA's.

To put the costs in some overall perspective, although the £11m p.a. raw material cost that water represented to BSC in 1977-78 was large it nevertheless formed less than 1% of the £1300m spent by the corporation for all its raw materials in 1976-77. Similarly the estimated total water cost of £2.20 per tonne of steel (1977) only represented about 1% of the selling price of £200-£220 per tonne of general steel product at that time.

This section has briefly outlined the recent costs of water to the industry in Britain. These costs, particularly those due to charges from WA's have risen significantly in recent years. This is simply illustrated by inspection of the charges to BSC's Scunthorpe works (1972/78) potable water and also their total freshwater charges, shown in Table 1-10. Present trends for industry as a whole indicate that the rate of increase in WA charges for water supply and effluent treatment are likely to accelerate in the forseeable future in line with government policy that WA's should be self-financing, and with the WA's aspirations for water conservation and pollution control.

The role of legislation and the WA's in promoting more efficient water management in industry is now considered in greater detail.

1.7 Water Conservation and Pollution Control, and the Role of the Water Authorities in England and Wales

Despite the moderately high average precipitation values for England and Wales quoted earlier, geographical and seasonal variations, and the increasing demand for water (expected to reach 45 x $10^6 m^3$ per day in the year 2000) mean that it has become increasingly important that the nation efficiently manages its water usage, and in particular encourages water conservation in industry. This need for water conservation, along with increasing awareness of the existing and potential environmental impact of many wastewaters, particularly those from industry, has meant that statutory protection of the environment has progressively increased over a number of decades.

TABLE 1.10 POTABLE WATER AND TOTAL FRESHWATER CHARGES MADE BY THE ANGLIAN WATER AUTHORITY TO BSC's SCUNTHORPE WORKS

YEAR ENDING 31st MARCH	POTABLE WATER pence/m^3	TOTAL FRESHWATER pence/m^3
1972	-	1.22
1973	3.51	1.15
1974	3.51	1.41
1975	4.26	1.75
1976	7.33	2.07
1977	10.40	4.23
1978	13.20	

Because the Iron and Steel Industry is such a major user of water and produces large volumes of very hazardous wastes which have potentially very serious consequences for the environment, the changes in legislature and the increasing role of the WA's affect them more than most industries.

With the implementation of the 1973 Water Act on 1st April 1974, responsibility for water resource management and development in England and Wales passed to 10 Regional Water Authorities. Now known just as Water Authorities, they took over the duties previously shared between some 200 water undertakings, 1,400 local authorities and 29 river authorities, each WA being responsible for the whole hydrological cycle in the entire catchment area of one or more major rivers. The 10 WA's were linked to a central authority, the National Water Council (NWC) which was composed of representatives from the WA's and it is here that decisions are made on a national water policy, influenced where appropriate by the policy of the European Economic Community (EEC).

The most comprehensive piece of legislature to date relating to pollution control has been the 1974 Control of Pollution Act, although many important sections of this have yet to be implemented, and regulation is still largely governed by previous legislation, the most important of which has been summarised as follows:

<u>The Public Health (Drainage of Trade Premises) Act 1937</u> gives

the right to discharge trade effluents to public sewers. The consent of the sewerage authority, now the WA, is required however, and they can impose quality and quantity conditions on the discharge to ensure protection of the sewerage system and the ultimate receiving watercourse, and also charge for the conveyance and treatment of the waste.

The Rivers (Prevention of Pollution) Act 1951 means that industrial discharges to non-tidal watercourses from new or altered outlets require consent from the WA's. Consent can be refused or subject to quality and quantity conditions.

The Clean Rivers (Estuaries and Tidal Waters) Act 1960 extended the provisions of the former act to certain specified estuaries.

The Rivers (Prevention of Pollution) Act 1961 further extended control to pre-1951 discharges, although not those to tidal waters and estuaries. (Control could be extended to discharges to specified tidal waters and sea but only by specific orders from the Secretary of State under the 1951 Act).

The Water Resources Act 1963 controls discharges to underground waters and these require the consent of WA's.

As well as establishing the WA's, another important consequence of the 1973 Water Act mentioned previously was that it obliged WA's to meet their costs entirely from charges levied. This has meant that WA's continue to increase their charges for supply of water and for treatment of effluents discharged to public sewers. Only discharges controlled by the above 1937 Act are at present subject to charges, these being based on the strength and volume of the effluent, and the cost to the WA of providing the necessary treatment facilities. The 1974 Act however makes provision for enacting sections of the 1973 Water Act which allows WA's to levy charges on direct discharges to watercourses, and this would be in accord with EEC policy of making the polluter pay the cost of remedial action against pollution.

Implementation of Part II of the Control of Pollution Act will have other potentially important consequences for all dischargers and will strengthen existing legislation to produce a most comprehensive control of water pollution. One important consequence is that it will mean that virtually all discharges to inland and coastal water, including those to land as these may ultimately reach a watercourse or underground waters, will be controlled, and this will include many discharges that were previously exempt.

The 1974 Act also has very important provisions which give the general public more access to information regarding discharges, anc more opportunity for them to object to new discharges, and to pursue prosecutions for unsatisfactory discharges.

Applications for consent for new discharges will need to be advertised in the London Gazette and a local newspaper by the

WA concerned, unless the discharge is not considered likely by the WA to have a significant effect upon the receiving waters. The WA must then consider any complaints made during a 6 week period following the initial advertisement, and where complaints are not upheld, appeal can be made to the Secretary of State who can order a public enquiry.

Where there is a failure to agree conditions or if consent is refused within 3 months of the application, the consent is deemed refused under the Act. This is in direct contrast to the 1951 Rivers (Prevention of Pollution) Act where the consent was deemed to have been given after the three months following application if there was no contrary notification by the WA. Under this earlier legislation many consents were granted by default because WA's were unable to investigate all of the applications, particularly those relating to the often very large number of small discharges, with the result that some discharges which were unsatisfactory in relation to their effect upon the receiving watercourse, were consented.

The 1974 Act will require that WA's keep Registers detailing applications for consents, consents granted, and the results of analyses of effluent samples and the action taken on the basis of those results, for discharges other than those to the public sewers. These Registers must be made available for inspection by the public.

Although registers of consents are kept in accordance with

Section 7(7) of the Rivers (Prevention of Pollution) Act 1951, only those people who in the opinion of the WA have an interest in the outlet, land, or premises from which the discharge flows, are allowed to inspect the relevant records, and this considerably limits access. Furthermore, Section 12 of the 1961 Act expressly forbids WA's from giving any information relating to an application for a consent concerning an industrial discharge to any person or body without the permission of the applicant himself. Existing legislation therefore inhibits public access to information, makes objections to intended discharges unlikely until after they have been started, and this combined with the absence of any formal procedures for objecting to the granting of a consent, makes public involvement in this area highly unlikely.

Implementation of the 1974 Act, as well as making far more information available to the public, will also remove certain restrictions on the rights and difficulties of members of the general public to bring prosecutions for water pollution offences. The previous requirement for the permission of Attorney-General has been removed.

The new Act is therefore in theory a daunting prospect for industry, requiring them to seek consent for discharges for which they were previously effectively exempt, as well as exposing them to the possibility of prosecution from any member of the public for any unsatisfactory discharge. Prosecutions are at present initiated at the discretion of the WA's and these tend in practice to be small in number, and generally only pursued when gross negligence or highly uncooperative behaviour on the part of the polluter is involved.

The fears of industry with respect to prosecutions by the public under the new Act are however likely to be largely unfounded, and the new freedoms with regard to information access and pursuance of prosecutions by individuals is likely to be more apparent than real.

For example, it is the intention of the Department of the Environment that information available in the new Registers, including the analytical data, will be purely factual. The lack of any interpretation therein will mean that very few members of the public will understand the significance of the information provided and therefore be able to act upon it.

The fear of prosecution extends to the WA's themselves, who will be liable to such action when unsatisfactory discharges occur from their own sewage treatment works. WA's are in fact the largest causers of water pollution and a very large proportion of their discharges fail to meet consent conditions issued under earlier legislation by themselves or their predecessors. In theory there is the prospect of WA's whose duty it is to prevent water pollution, being subject to mass prosecutions initiated by private individuals for its many unsatisfactory discharges. In practice WA's will be taking action to prevent this happening. The most likely course of action will be to change the basis on which its treatment works are assessed for consent conditions.

One possible method will be to base consent conditions on the effect of the discharge on the receiving watercourse, and additionally to set these on a statistical basis so that the limits will be those expected of the discharge for 90 or 95% of the time, or at least for 90 or 95% of the samples taken. Another possibility is that consent conditions will be issued on the statistical basis described, but based on the past performance of the treatment works concerned, with some additional margin to ensure that the quality of the discharge will effectively never exceed the consent conditions.

There are sound scientific reasons for a statistical approach to consent conditions and on basing them on the likely effect on the receiving stream. Adoption of such practices however would make the information available in the new Registers even more incomprehensible to the general public. This, along with any decision to modify consent conditions in the way last described, may be regarded as expedient by the WA's, but would be viewed by many others as unethical, and whilst it might fall within the letter of the new law it would certainly not follow the spirit that generated it.

Whatever the action taken by WA's with respect to their own discharges, and such action will require the approval of the

Secretary of State, industry can take heart because "ethics" will probably dictate that the same approach be made to their discharges.

The immediate impact of implementation of Part II of the 1974 Act on industry, and particularly the Iron and Steel Industry, is likely therefore to be less than imagined. However, the above comments should not detract from the long term general trend for effluent discharges which is to increase charges for supply and treatment of water. These demands by WA's for improvements in effluent quality are certain to continue, but are likely as before to be ultimately decided on the basis of what the industry concerned can reasonably be expected to afford.

1.8 Water Pollution Control Legislation in the USA

It is useful to briefly contrast British legislation with that in the USA to demonstrate that concern with water pollution is not confined to Britain, and to illustrate that the legislative pressures on the British Steel Industry are modest when compared with their American counterparts.

In the USA the 1972 Federal Water Pollution Control Act Amendments(39) empowered the US Environmental Protection Agency (EPA) to establish standards for industrial discharges to navigable waterways. Under Section 301 of the act industrial discharges were required to comply with effluent standards by the

1st July, 1977 by application of the "best practicable control technology economically achievable" (BPCTCA). More important however is the requirement that the discharges comply with more stringent effluent standards by 1st July 1983 by application of the "best available technology economically achievable" (BATEA).

Federal regulations lay down effluent limitation guidelines (ELG's) according to the use of Best Available Technology for different sectors of industry, including the Iron and Steel Industry. The ELG's for an industry represent the effluent loadings attainable by the highest degree of water treatment and recycling deemed achievable in the whole of that particular industry in the USA using existing economical technology. The ELG's are given as values which should not be exceeded by the average load over 30 consecutive days, with the additional requirement that the maximum daily effluent load per unit of production should never exceed the ELG by more than a factor of 3.

ELG's are given on the basis of the weight of pollutant produced per unit weight of product produced, and there are no specific limits on the discharge flow rate or concentration which are so characteristic of the consent conditions system in Britain. They are however generally based on specified values for discharge flow per unit of production and these assume a high degree of water recycling. In fact the ultimate goal of the USEPA appears to be to achieve total recycle of water in the

Iron and Steel Industry.(40)(41)

The standards required by the USEPA are far higher than the equivalent standards required by WA's in Britain. For example, based on the current ELG values quoted for coke and blast furnace iron production for the Iron and Steel Industry, and the discharge volumes used to calculate the ELG values, the equivalent phenol concentrations of the effluent discharges will need to be less than 0.5 parts per billion, a value incidently too small to measure by current laboratory analytical methods. This compares with a typical consent condition for a British Steel works for phenol of 5-10 parts per million.

Many, not least some Americans, would question the need for such stringent control of water use and effluent discharges from the Iron and Steel Industry. The fact remains however that some form of strict control on the quality of effluents from the industry is common to most countries of the world. This, and the increasing international awareness of the need for controlling water and other pollutions mean that the interest in and the significance of the subject matter of this project extends beyond the British Steel Industry to those internationally concerned with improving the performance of biological treatment plants as part of the strategy to reduce world water pollution.

1.9 The Coke Oven Liquor Problem - A Wider Perspective

Coke oven liquors are of course not only produced in the Iron and Steel Industry but are produced wherever coal is carbonised, that is, in the Coking Industry in general. The oven coke consumption of the steel industry is of course very large, as can be seen for the values for the industry in the EEC, shown in Table 1-11A for the years 1977-79. When the overall coke consumption figures for the Community are considered, as in Table 1-11B, the results confirm that the Iron and Steel Industry is indeed the major user, at least in Europe, being responsible for about 8% of the coke consumed.

However, a better perspective of the size and distribution of the coke oven liquor problem can be gained by considering the overall coal and coke data for the world. The reported quantities of coal carbonised in various parts of the world are recorded in Table 1-12, and it can be seen that these quantitites are considerable.

A better comprehension of the size and distribution of world coke production, and the associated problems with coke oven liquor production can be gained from Table 1-13 which shows figures for 1975. In 1975 world supplies of coking coal were about 625 million tonnes, of which about 80% was produced in six countries, USSR, USA, West Germany, Poland, Australia, and China, and were about 23% greater than in 1960. About 80% of of these supplies were actually used for coke production.

TABLE 1-11A OVEN COKE CONSUMPTION BY THE IRON AND STEEL INDUSTRY MEETING IN THE EUROPEAN COMMUNITY

	1977	1978	1979
Belgium	5.438	5.815	5.920
Denmark	0.060	0.061	0.060
W. Germany	16.241	17.480	17.750
France	10.242	10.240	10.595
Ireland	0.010	0.010	0.010
Italy	6.316	6.010	6.205
Luxembourg	2.006	2.050	2.200
Netherlands	1.937	2.230	2.235
United Kingdom	8.725	8.265	8.835
TOTAL	50.975	52.161	53.810

TABLE 1-11B OVEN-COKE CONSUMPTION BY SECTOR IN THE EUROPEAN COMMUNITY

	1977	% TOTAL	1978	% TOTAL	1979	% TOTAL
Iron and Steel Industry	51.0	81.2	52.2	83.0	53.8	83.4
Other Industries	4.9	7.8	4.6	7.3	4.7	7.3
Domestic Heating	5.6	8.9	5.1	8.1	4.9	7.6
Others	1.3	2.1	1.0	1.6	1.1	1.7
TOTAL	62.8	100.0	62.9	100.0	64.5	100.0

TABLE 1-12.

COAL CARBONISED IN THE COKING INDUSTRY.

COUNTRY	1975	1976	1977	1978
		MILLION TONNES		
U.K	-	19.01	14.91	-
USA	83.60	84.70	77.74	71.39
SOUTH AFRICA	-	-	6.92	-
FRANCE	-	-	6.20	-

- indicates value not available.

TABLE 1-13 WORLD COKE PRODUCTION

	MILLION TONNES	% TOTAL
EEC	78.9	21.8
W. EUROPE	87.7	24.2
ASIA	58.0	16.0
AFRICA	3.8	1.1
AMERICA	62.3	17.2
AUSTRALIA	5.2	1.5
WESTERN WORLD	217.2	60.0
EASTERN BLOC	144.9	40.0
WORLD	362.1	100.0

	%		%
UK	4.4	AUSTRALIA	1.5
W. GERMANY	9.6	USSR	23.1
OTHER EUROPE	10.2	CHINA	6.9
INDIA	2.3	POLAND	5.0
JAPAN	13.3	CZECHOSLOVAKIA	3.0
OTHER ASIA	0.5	N. KOREA	0.6
AFRICA	1.1	OTHER EASTERN BLOC	1.3
USA	14.3		
OTHER AMERICA	2.9		

Between 1963 and 1974 annual coal consumption for coke-making increased from 380 to 470 million tonnes, and this represented a rate of increase of 2.0 per cent per annum. The use of coking coal for other purposes increased from 94 to 150 million tonnes per annum, representing a rate of increase of 4.3 per cent per annum. It is interesting to note that in the EEC countries during the same time period, coking coal production decreased from 218 to 96 million tonnes per annum, and consumption for coke-making fell from 150 to 91 million tonnes per annum.

However world coke production increased by 28 per cent (2.1 per cent per annum) between 1963 and 1975 showing a continuing world demand for coke and the likely increase in interest and problems associated with the waste liquor produced from the coking process. The very large world coke reserves (Table 1.14) along with the increasing world population, rising living standards, and industrialisation of the Third World, including its increasing demand for steel , and the search for viable energy sources alternative to the world's oil reserves, means that the rate of demand for coal and coke is likely to increase, as are the associated pollution problems.

1.10 Choice of Project

The preceding sections have demonstrated that the Iron and Steel Industry is a major user of water, and that there are indeed sound financial, environmental, and legal reasons for

TABLE 1-14 WORLD COAL RESERVES

COAL RESERVES Mtce	GEOLOGICAL RESOURCES		RESOURCES RECOVERABLE UNDER PRESENT TECHNICAL AND ECONOMIC CONDITIONS	
	HC	S & BC	HC	S & BC
AMERICA	1,308,541	1,408,838	126,839	71,081
EUROPE	535,664	55,241	94,210	33,762
AFRICA	172,714	190	34,033	90
AUSTRALASIA	213,890	49,034	18,164	9,333
ASIA	5,494,025	887,127	219,226	29,626
TOTAL WORLD	7,724,834	2,400,430	492,472	143,892

Source: World Energy Conference, World Energy Resources 1985-2020, I.P.C. Science & Technology Press 1978.

HC = Hard Coal

S & BS = subbituminous and brown coal

1tce = 29.30 GJ approx. ≡ 1t at average calorific value.

ensuring the efficient use and disposal of water in the industry. Water use in the industry can be considerably reduced by recycling and re-use, and there are few technical problems involved for most of the waters in use at an integrated iron and steel works. (The degree to which water conservation and re-use is actually practised is influenced by the many factors that have already been discussed.). A small proportion of the water in use however does present problems because it becomes seriously contaminated and requires extensive treatment before it can be safely re-used or discharged to a watercourse.

One major contribution to this problem is of course that water involved in the direct cooling and purification of gases from coke ovens, giving rise to coke oven liquor, is one of the most complex and difficult wastewaters to treat in the industry. As has already been described, coke oven liquors also represent one of the most potentially serious hazards to the environment from the Steel Industry, and is the subject of stringent controls by the appropriate government agencies most everywhere in the world.

This concern with the problems that coke oven liquor represents in the water management of the Iron and Steel Industry, and of course the most important justification for investigating the treatment of this wastewater in this project. Research into coke oven liquor treatment is, as will as be evident from work reported earlier in the chapter, not new, and indeed the large amount of research into the subject may be taken as evidence of the importance attached to achieving more efficient and economical treatment of the waste.

The decision to concentrate research in the project on the activated sludge treatment of the waste is based on the fact that such biological treatment represents the most economical method of large scale treatment, and that such treatment is still the choice of most Steel Works and the Coking Industry. Although it is technically possible to chemically purify coke oven liquors consistently to any standard required, the choice of purely chemical considerations means that the cost advantages of biological treatment are outweighted by the consequences of failure to consistently produce the effluent standards required.

Not unexpectedly therefore the British Steel Corporation, along with the other large steel companies of the world, have been involved with a number of projects researching into the problems of coke oven liquor treatment. The project reported here followed an earlier project sponsored by BSC which sought to develop a dynamic model for the activated sludge treatment of the waste. That project, the subject of research by TOMLINS, was unsuccessful in producing a model which represented the behaviour of plant treating the waste. One part of the project, however, involved the investigation of the use of Adenosine Triphosphate (ATP) as a measure of the active biomass in an activated sludge plant. Although TOMLINS was unsuccessful in using this parameter to develop a working model of the treatment process, and in adequately explaining the ATP results reported, the results stimulated further interest in the subject.

It was the failure of many workers, including TOMLINS, to adequately and successfully explore the potential of using ATP measurements to achieve a greater understanding of the activated sludge process that led to the decision to explore that potential for the treatment of coke oven liquor waste in this project. A details survey of the research involving ATP measurements in the field of wastewater treatment forms the main subject of the following chapter.

CHAPTER 2

THE USE OF ATP MEASUREMENTS

A LITERATURE REVIEW

Introduction

2.1 ATP - Its Importance in the Cell and the Enzymic Basis of Assay

2.2 ATP Studies in Pure Cultures

2.3 Application to Wastewater Treatment

2.4 Adenylate Energy Charge Measurements

2.5 Discussion

THE USE OF ATP MEASUREMENTS

A LITERATURE REVIEW

Introduction

The decision to investigate the measurement and significance of ATP pools in the activated sludge treatment of coke oven liquor was, as described in the previous chapter, partly influenced by the work of TOMLINS. His research, also sponsored by the British Steel Corporation, was directed towards the development of a dynamic model for the activated sludge treatment of the wastewater. TOMLINS'(42) work, although unsuccessful in developing a model that would adequately describe the behaviour of plant treating the waste, did report some interesting and unpredicted results involving the measurement of ATP, and these will be considered in more detail later.

Tomlins, and other workers who have used ATP in the field of wastewater treatment, have failed, in the author's opinion, to adequately explain the observed ATP behaviour or to realise the potential of ATP both as a tool for better understanding the biological behaviour of treatment systems, and as a parameter to be used in their control.

This chapter reviews the use of ATP measurements generally, and more particularly their use in wastewater treatment research.

2.1 ATP - Its Importance in the Cell and the Enzymic Basis of Assay

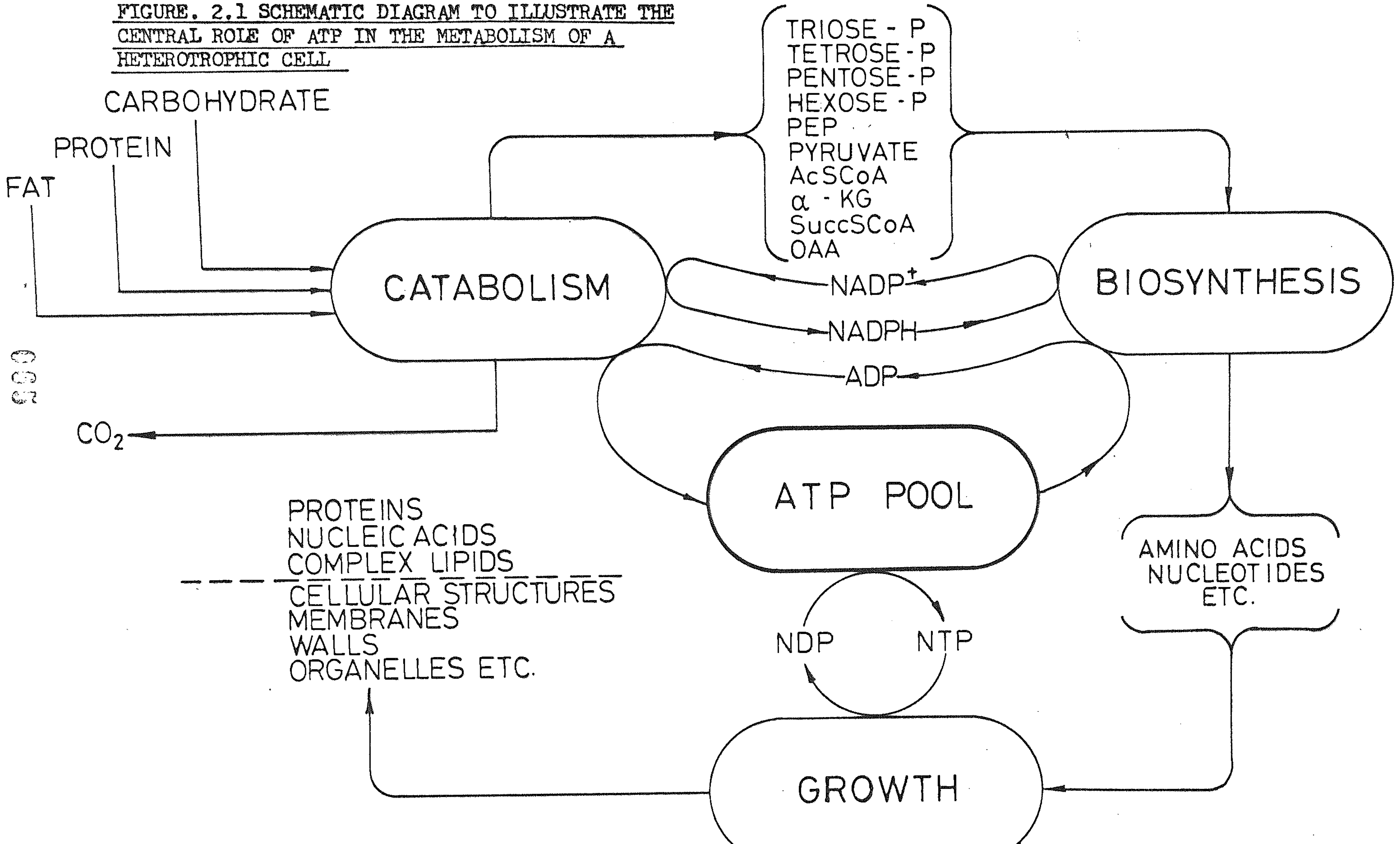
ATP is a high energy compound and primary energy donor in all living cells.(43) Commonly also described as the unit of energy currency in the cell it is intimately involved, directly or indirectly, with all aspects of cellular metabolism. Figure 2.1 for example illustrates the involvement of ATP in the various aspects of the metabolism of an heterotrophic cell.

Outside the cell, ATP is very unstable and hydrolyses rapidly, and in natural conditions it can therefore be regarded as a compound found exclusively in living cells. Because of the unstable nature of ATP special precautions need to be adopted when extracting and preserving biological samples which require ATP analysis.

ATP occurs in very low concentrations in living organisms and therefore a sensitive method is required for its analysis. Such a method has been developed on the basis of McELROY's(44) work who showed in 1947 that the luminescence of firefly extracts could be restored by the addition of ATP, and that there was an absolute requirement for ATP for this bioluminescence to occur. Subsequent work by McELROY, SELIGER and WHITE(45) led to the discovery of the mechanism by which the enzymes, luciferase, present in the extracts, produced light. The mechanism, depicted in Figure 2.2 involved ATP as cosubstrate, and the light produced was shown to be proportional to the quantity of ATP present.

McELROY and STREHLER(46) reported in 1949 that following the addition of ATP to firefly extracts there was an initial burst of light, followed by a rapid decline to a low and uniform level of emission. STREHLER and TOTTER(47) in 1952 demonstrated that the addition of arsenate buffer to the assay system resulted in an intermediate level of emission, with the luminescene decaying steadily and exponentially with time, and this modification is now generally employed in the assay procedure.

FIGURE. 2.1 SCHEMATIC DIAGRAM TO ILLUSTRATE THE CENTRAL ROLE OF ATP IN THE METABOLISM OF A HETEROTROPHIC CELL



$$\underset{\text{Luciferase}}{E} + \underset{\text{Luciferin}}{LH_2} + ATP \xrightleftharpoons{Mg^{++}} \underset{\substack{\text{Luciferyl adenylate}\\ \text{complex}}}{E \cdot LH_2 \cdot AMP} + \underset{\text{Pyrophosphate}}{P.P}$$

$$E \cdot LH_2 \cdot AMP + O_2 \longrightarrow E + \text{Product} + CO_2 + AMP + \text{Light}$$

Figure **2·2** Reaction of ATP with firefly enzymes.

employed in the assay procedure.

Although ATP is the primary energy donor and high energy compound in the cell, other nucleoside triphosphates (NTP's), which are also high energy compounds, are also found in the cell. MCELROY and GREEN(48) have shown that ATP is the only such compound that will produce light with purified firefly extracts. HOLM-HANSEN and BOOTH (49) however have shown that two other NTP's, cytidine - 5 - triphosophate (CTP) and inosine - 5 - triphosphate (ITP), found in cells, produce luminescence equivalent to that of ATP with the crude firefly extracts available commercially. This has been shown to be due to the presence of transphosphorylase enzymes in the crude extracts.

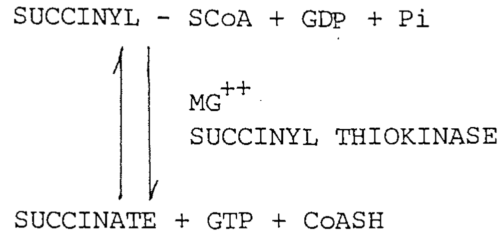
The apparent reduced specificity of crude extracts, however, is not regarded as serious as the NTP's have similar energy values within the cell and are produced directly from ATP by the action of nucleoside diphosphokinase on the nucleoside diphosophates;

FIGURE 2.3

$$\begin{tikzcd} \text{NUCLEOSIDE TRIPHOSPHATE} + \text{ADP} \arrow[d, shift left, "\text{Mg}^{++}\ \text{NUCLEOSIDE DIPHOSPHOKINASE}"] \\ \text{NUCLEOSIDE DIPHOSPHATE} + \text{ATP} \arrow[u, shift left] \end{tikzcd}$$

Guanosine triphosphate (GTP) has generally been described as an exception to this in that it is mainly produced in the tricarboxylic acid cycle by the action of the enzyme succinyl thiokinase on succinyl CoA;

FIGURE 2.4



$$\text{SUCCINYL - SCoA + GDP + Pi} \underset{\text{SUCCINYL THIOKINASE}}{\overset{\text{MG}^{++}}{\rightleftharpoons}} \text{SUCCINATE + GTP + CoASH}$$

and not necessarily directly from ATP as for the other NTP's, in reaction (Figure 2.3). In fact, in animal systems both GDP/GTP, and IDP/ITP can function as cosubstrates in reaction (Figure 2.4), whilst in Escherichia coli and higher plants ADP/ATP functions as the cosubstrate.(50)

Whatever the precise metabolic pathways involved in the production of NTP's, all NTP's can be regarded as equivalent in energy value to ATP, and can enter the metabolic pool of high energy currency by the nucleoside diphosphokinase reaction.

Most ATP assay methods employ the luciferase enzyme system of firefly extracts, and are based on the principle that the light produced by the reaction is proportional to the ATP present. Instruments have been developed by a number of workers (including FORREST(51), HOLM-HANSEN and BOOTH(49), and LYMAN and DE VINCENZO(52)), and others are available commercially, which are based on the detection of the luminescent response to the enzyme by a photo-multiplier tube in a light-protected box, and amplification of the signal produced so that it may be registered on a recorder. Other workers (including ADDANKI et al.(53), and COLE et al.(54), have used liquid scintillation counters to measure the luminescence produced by the enzyme. A non-enzymic method using high pressure liquid chromatography(55) (HPLC) has been used in medical research for the analysis of ATP, but the assay time for each sample of up to one hour compares with a time measured in seconds using the luciferase system, and it clearly has limited application where large numbers of samples require analysis.

2.2 ATP Studies in Pure Cultures

A large proportion of the ATP studies that have been carried out have involved pure cultures of microorganisms rather than the mixed cultures that are typically found in natural ecosystems, and of course in wastewater treatment systems.

D'EUSTACHIO and LEVIN(56) investigated the cellular ATP content of 3 species of bacteria, Escherichia coli, Pseudomonas fluorescens, and Bacillus subtilis, and reported in 1967 that this remained relatively constant throughout lag, exponential, and stationary growth phases. In later work D'EUSTACHIO and JOHNSON(57) reported the endogenous cellular ATP content of 13 aerobic bacterial species which they chose as representative inhabitants of contaminated systems. These showed a range from 0.22-1.03 fg ATP/cell with a mean value of 0.5 fg ATP/cell. A linear correlation between the endogenous cellular ATP content and plate counts of bacteria was also found.

ALLEN(58) showed that the endogenous level of ATP per unit biomass, remained relatively constant when compared to the cellular organic content, throughout all phases of growth for several species of bacteria. HOLM-HANSEN (59), (60) also demonstrated this for several species of algae and zooplankton. HAMILTON and HOLM-HANSEN, (61) and DAWES and LARGE (62) showed that the ATP content per viable cell also remained relatively constant during periods of starvation.

ATKINSON, (63) and CHAPMAN et al. (64) have also shown that a wide range of cells can maintain optimal ATP concentrations during rapid loss or gain of energy sources.

A large number of studies have therefore indicated that the cellular ATP content of microorganisms in pure cultures remains relatively constant. These studies suggest therefore that ATP may form a useful and valid measure of living biomass, and the value of ATP as an indicator of living cells has been confirmed by the findings of HOLM-HANSEN and BOOTH, (59), (65) who showed that ATP is rapidly broken down in extracellular solution or in dead cells. Other workers have, however, reported variations in cellular ATP content in pure cultures.

STRANGE, WADE and DARK (66) found that the ATP content of Aerobacter aerogenes varied with oxygen tension and substrate concentration, and that the ATP contents were markedly reduced when the cells were subjected to anaerobic conditions or high substrate concentrations. FORREST and WALKER (67), (68) found that the cellular ATP content in starved cultures of Streptococcus faecolis remained constant under endogenous conditions, for a period which was related to the substrate concentration prior to the starvation condition, but then declined rapidly.

FORREST(69) also studied the cellular ATP content of Streptococcus faecalis during growth, and reported that it was constant during the log phase, and stablised again in the stationery phase.

These variations in cellular ATP content are in fact important and their significance will be considered in more detail in the next section where the results of ATP studies in wastewater treatment are considered. For now, however, it may be concluded that the bulk of pure culture studies have indicated that the cellular ATP content of microorganisms remains relatively constant. As such, this has formed the basis for its use and proposed use by several workers as a measure of living biomass. PAERL and WILLIAMS,(70) among others, for example have indicated its value as an accurate measure of total living microbial biomass in a variety of aquatic ecosystems. It also formed the basis for TOMLINS(42) choice of ATP as a measure of living biomass in the activated sludge treatment of coke oven liquors, of which more will be said in the following section.

2.3 Application to Wastewater Treatment

The application of ATP determinations to wastewater treatment has been a more recent development.

PATTERSON, BREZONICK, and PUTMAN(71) investigated the measurement and significance of ATP in laboratory model activated sludge systems.

They reported that the ATP levels in the activated sludge were relatively constant under endogenous conditions. They also found that the ATP pool reflected the toxicity of pH and heavy metals, and they thus indicated the potential of ATP as a measure of sludge activity and viable biomass, and its use as a tool for operational control of activated sludge treatment systems. WEDDLE and JENKINS(72) investigated the use of ATP using laboratory and field activated sludge systems and were also able to indicate the potential value of ATP for this purpose.

Later work by BREZONIK and PATTERSON(73) with laboratory batch and continuous activated sludge units confirmed the use of ATP as a specific measure of sludge activity, and of viable biomass under constant environmental conditions. They also investigated the ATP response to temperature, dissolved oxygen, substrate loading, and nickel, cyanide, mercury, and copper toxicants. They found that there was no marked change in the ATP pool in response to temperature variations falling within the normal operating range of most activated sludge plants. The ATP pool was responsive to changes to and from anoxic conditions, and to heavy metal toxicants, and was affected by substrate loading. KAO, CHIU, FAN and ERICKSON(74) investigated ATP levels in pure and mixed cultures of E. coli and Pseudomonas aeruginosa, and found significantly lower biomass and ATP yields in mixed culture than in pure cultures, indicating that interaction between bacterial species may have a marked influence on the system parameters. The same workers(75) investigated ATP levels in continuous and batch activated sludge systems, and found that dilution rate significantly affected sludge activity and cellular

ATP content. In the continuous systems they found that at high dilution rates the sludge had a high metabolic activity and low ATP pool, whilst the converse was true at low dilution rates. They concluded that changes in ATP concentration reflected not only changes in cell viability, but also population shifts and altered growth phases in the cultures, and that there needed to be better understanding of the relationship between ATP concentration and activated sludge performance before its full potential as an operational control tool could be realised.

CHU, ERICKSON, and FAN(74) investigated the dynamic behaviour of laboratory activated sludge systems by subjecting the system to step changes in influent waste concentrations, recycle flow rate, and sludge wastage rate, and measuring ATP, chemical oxygen demand (COD), and cell dry weight in the process. They showed that the dissolved ATP concentration was affected by the dissolved COD level, and that following a step increase in influent COD, variation in ATP/MLSS appeared to be directly related to variations in dissolved COD, for a considerable time afterwards. They found that the ATP response to a step increase and a step decrease of the same input variable were not symmetrical. The ATP/MLSS ratio was found to depend on the food to organism ratio in the aeration tank. They also found that the response of ATP and MLSS to transient conditions were different.

LEVIN, SCHROT, and HESS(76) investigated the applicability of ATP monitoring to the control of a 20 mgd Activated Sludge Sewage Treatment Plant. Over a period of one month the mixed liquor biomass was controlled by regulation of the return sludge rate, using the mixed liquor ATP content as the control parameter. The investigation demonstrated the close relationship between sludge ATP content and metabolic activity on a full scale plant and good plant performance was maintained throughout the test using the ATP measurements.

STATHAM and LANGTON(77) also investigated the use of ATP measurements for a full-scale Activated Sludge Sewage Treatment Plant. They showed that the sludge ATP content was related to the sludge volume index (SVI) except for very high SVI values, and that the ATP content was also related to the degree of aeration provided. They advocated the use of the Solids Retention Method (SRT) method for controlling the plant, and indicated that ATP monitoring could play a valuable role in this.

UPADHYAYA and ECKENFELDER(78) also measured ATP in an activated sludge plant. Investigating the use of biodegradable fraction as an activity parameter for a continuous laboratory scale activated sludge plant, they found that the cellular ATP content of the sludge remained constant at different organic loadings.

The research reported by TOMLINS,(42) in which he attempted to use ATP as a measure of living biomass for the determination of various kinetic parameters for the development of a dynamic model of the activated sludge treatment of coke oven liquors, has already been briefly refered to. He monitored ATP levels in laboratory scale batch reactors, using sludge from a full-scale plant treating liquor which had been acclimatised to treat either thiocyanate or phenol, in a basal medium of treated coke oven liquor.

ATP was measured because MLSS and mixed liquor volatile suspended solids (MLVSS) were found to be inadequate measures of the active biomass in activated sludges treating thiocyanate solutions. The decision to use ATP measurements was made on the assumption that the cellular ATP content of organisms was constant and could therefore be used to directly measure the active cell content of sludge. TOMLINS(42) was unsuccessful in using this approach to develop a model which would describe the behaviour of full-scale plant treating coke oven liquor. The ATP behaviour of the sludges observed in his experiments was not that expected if ATP had simply been a measure of living biomass, and TOMLINS, CHIDLEY, COLES, and HAWKES(79), put forward the hypothesis of a substrate storage model to explain preliminary results obtained with batch sludges treating phenol solutions. The significance of TOMLINS(42) work, and that of other researchers, to the project is discussed later.

The key role played by ATP as the unit of energy currency in the cell has already been outlined. Some researchers have considered the energy state of cells in terms of Adenylate Energy Charge (E.C), which includes a consideration of both the level of ATP, and its lower energy precursors, ADP and AMP. The postulated significance of E.C to cellular energy metabolism is not considered here, but a detailed description can be found by ATKINSON.(43) The definition of E.C and a brief summary of the work relevant to the project that has been carried out, is considered in the following section.

2.4 Adenylate Energy Charge Measurements

Adenylate energy charge was defined by ATKINSON and WALTON (80) as follows:

$$\text{ADENYLATE ENERGY CHARGE} = \frac{(\text{ATP}) + \tfrac{1}{2}(\text{ADP})}{(\text{ATP}) + (\text{ADP}) + (\text{AMP})}$$

ATKINSON and various coworkers have in fact been involved in much of the research published on E.C measurements.

CHAPMAN, FALL, and ATKINSON (81) investigated the ATP content of starved cultures of E. coli and found that ATP was conserved in non-viable cells, and that the adenylate energy charge was an indicator of viability.

CHAPMAN, FALL, and ATKINSON (81) found that actively growing E.coli cells maintained an adenylate energy charge of about 0.9, but that this was reduced to about 0.5 in starved cells, and below this value cells become increasingly non-viable but still contained some ATP.

BALL and ATKINSON (82) found that Saccharomyces cerevisiae cells grown aerobically maintained an E.C value of 0.8 to 0.9 during prolonged starvation. However cells grown anaerobically showed a rapid decrease in E.C value when subjected to starvation conditions. The yeast cells remained viable at E.C values below 0.1.

SWEDES, SEDO and ATKINSON (83) investigated the relation of growth and protein synthesis to the Adenylate Energy charge in an Adenine-requiring mutant of E.coli. Their work suggested that the rate of protein synthesis and the capacity for growth were more sensitive to changes in E.C value than to ATP concentration, and that whilst growth still occured at ATP levels of one third the normal level, it was not observed when the E.C value had fallen by only 10%.

The most significant work to date involving E.C measurements has been that completed by SIMPSON, STATHAM, HUDSON, and HUGHES.(84) They investigated the use of ATP and E.C measurements in a study of the model activated sludge treatment of waste from the manufacture of ion exchange resins. The authors maintained that ATP was a reliable measure of total biomass, but that it was not necessarily a measure of viability, and that E.C was a better measure of the latter.

2.5 Discussion

The most important conclusion to be derived from the ATP studies involving mixed culture systems is that ATP represents some measure of the metabolic activity of the microorganisms involved. However, results of some of the research reported appears to contradict these findings. For example, one of the findings of UPADHYAYA and ECKENFELDER(78) reported earlier who used a continuous laboratory-scale activated sludge plant, was that the cellular ATP content of the sludge remained constant at different organic loading rates. It might be expected, that the metabolic activity of a sludge would vary with organic loading rates, and that therefore the ATP level, if it was a measure of metabolic activity, would also vary.

Similarly, the concept of ATP as a measure of activity is not consistent with the findings of much of the pure culture research described, which suggested that cellular ATP remains constant in varying growth conditions in which cellular activity would be expected to change.

The evaluation here of the research reported is that ATP appears to represent both a measure of living biomass and metabolic activity in a system involving pure or mixed cultures of organisms. It is suggested that the cause of the apparent conflict in reports of constant cellular ATP levels, and levels varying with activity, lies in the interpretation of experimental results.

Research in which constant cellular ATP levels have been reported in varying growth conditions, have generally specified that the levels remained "relatively" constant. Re-examination of the data generally shows that variations in ATP levels have occured, and these variations have presumably not been considered significant, perhaps because they have been attributed to experimental error, by the researchers involved. The variations in ATP levels that can be observed by re-examining the data may well be significant, and indeed reflect the changes in cellular activity that would be expected with the changing environment of the cells. (A more detailed examination of the ATP data reported by other researchers, may prove useful, later in the project, in investigating the possible significance of ATP in coke oven liquor treatment).

This division of thinking on the role of ATP into the concept of it just as a measure of living biomass, assuming a constant cell content, and the concept of it as a measure of activity, is an important one. It does however represent an issue which, it is suggested, can be resolved fairly simply at this stage of the project by further such examination of existing data. Of most direct importance to the present project are the results reported by TOMLINS in his investigations. TOMLINS decision to use ATP measurements with his activated sludge systems was based on the assumption that the cellular ATP content was constant and that ATP therefore represented a direct measure of living biomass. Indeed he used much of his ATP data as a direct measure of cell concentration for the determination of kinetic constants for use in mathematical models of the activated sludge treatment process. It is not intended to examine TOMLINS or other workers, ATP data in detail here. However, the increases in ATP concentrations for example in TOMLINS work, following the addition of substrate to a starved sludge, were of a magnitude and velocity that could not reasonably be accounted for by cellular reproduction. In fact, along with the activated sludge studies by many other workers, TOMLINS results would seem best explained by postulating some role for ATP as a measure of activity.

Examination of the research to date therefore suggested that ATP has great potential as a measure of both living biomass and activity. The use of ATP seems to be particularly attractive for activated sludge systems where only a small proportion of the solids comprising the sludge are living cells responsible for metabolising the waste.

Measures such as MLSS which determine the total solids of the sludge, or of protein, DNA and RNA for example, which determine components of biological origin but fail to distinguish between living and dead cells, have been used in controlling such treatment systems. The ability to measure that proportion of the sludge actually degrading the waste would be an important advantages in controlling such systems. Various measures such as dehydrogenase activity and plate count methods have been used with varying degrees of success, but no method appears to be as potentially simple, direct, and useful as ATP.

The results upon which these conclusions are based have been available for sometime, and a few of the workers described earlier could also be credited with successful identification of these. However, there seems to have been little progress in realising this potential, and it is suggested that the main reasons for this lie in:

1. A failure to consider and understand the ATP behaviour of sludges in terms of the sub-cellular biochemistry of the sludge organisms i.e. to understand the ATP behaviour of the cellular components of the sludge.

2. A failure to define the "activity" that ATP is supposed to represent in a manner that can be quantitatively useful in the control of activated sludge processes in general.

The failure to quantitatively define "activity" in terms of relating ATP to the other factors important in controlling a treatment plant is seen as having its origin in 1.

The investigation of the significance of ATP in this project is seen as best approached by exploring the two weaknesses in present research described above. In particular, it is felt particularly important that efforts should be made to quantitatively define the significance of ATP to the activated sludge process, rather than continue the generally qualitative approach made in the activated sludge work described earlier. Although the project is concerned with activated sludge treatment of coke oven liquor, any progress in achieving the aims outlined is likely to have application to activated sludge processes in general.

CHAPTER 3

MODELLING OF THE ACTIVATED SLUDGE PROCESS

Introduction

3.1.1. The Activated Sludge Process
3.1.2 The Complete-Mixing Activated Sludge Process
3.2.1 The Kinetics of Biological Growth
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MODELLING OF THE ACTIVATED SLUDGE PROCESS

Introduction

In the previous chapter it was suggested that ATP had potential both as a measure of active biomass and activity. The investigation of this potential in terms of the activated sludge treatment of coke oven liquor of course forms the main subject of the present project. Whatever the results of those investigations however, ATP is unlikely to have any real practical importance in the operation of a plant treating the waste unless its significance can be quantitatively defined. An obvious way in which its use might be quantified would be to incorporate it into a kinetic model of the treatment process, since such a model requires a measure of biomass. It will be recalled that TOMLINS has attempted to use ATP as a measure of active biomass in his models of the process. A project whose main aim is to investigate the significance of ATP in the treatment of coke oven liquor might therefore logically be expected to consider the significance of that parameter to the mathematical modelling of the process.

As an introduction to the modelling work undertaken later in the project, this chapter outlines the main types of models that have been developed to describe the activated sludge treatment of wastes in general.

It is perhaps worth noting that despite the large amount of research conducted into modelling of activated sludge processes in general, attempts to produce a practically useful model which adequately describes the treatment of coke oven liquor have so far been unsuccessful. Any progress made towards developing such a model for the treatment of this waste is likely therefore to be welcomed by the Iron and Steel, and Coking industries.

3.1.1 The Activated Sludge Process

The activated sludge process, one of the two major processes used for the biological treatment of wastewaters, (the other being percolating filters), is actually less than 70 years old. Its discovery is attributed to ARDEN and LOCKETT of Manchester Corporation, England, who published a series of three papers (85) (86) (87) in 1914-1915 entitled "The oxidation of sewage without the aid of filters." Since their early experiments, which were carried out on a fill and draw basis, full-scale activated sludge plants have found wide use throughout the world. Various types of plant have been developed and the process continues to be the subject of much research so that the process is now generally regarded as an effective and economical form of biological treatment for most wastewaters.

The principles of the process, illustrated diagramatically in Figure 3.1, are simple. The wastewater, screened and settled to remove most non-colloidal solids, is supplied to micro-organisms, mainly bacteria in a reactor which metabolise the waste producing simple metabolic products, mainly carbon dioxide and water, and an increased mass of microorganisms produced by the growth on the waste. Sufficient turbulence is maintained in the reactor to ensure adequate aeration for

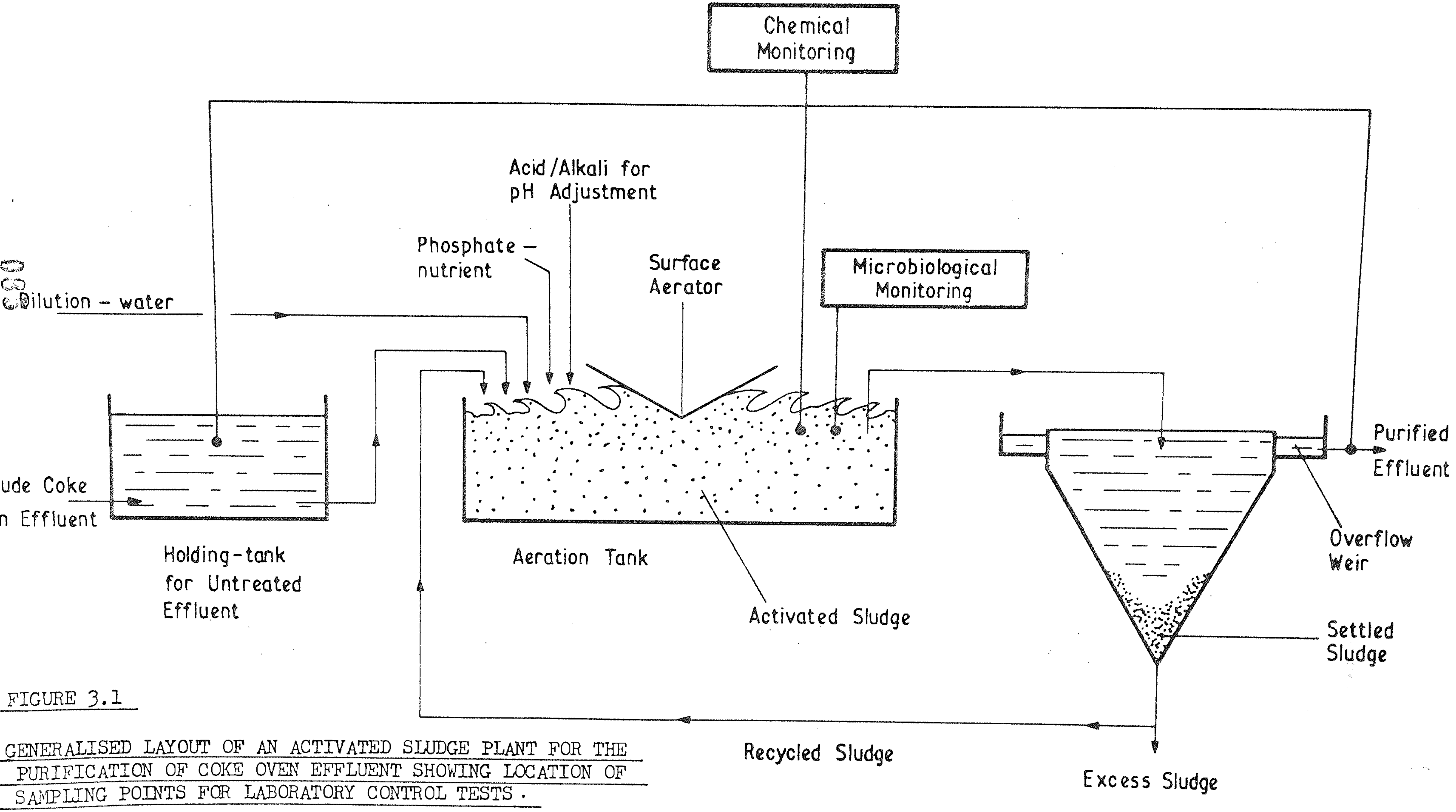


FIGURE 3.1

GENERALISED LAYOUT OF AN ACTIVATED SLUDGE PLANT FOR THE PURIFICATION OF COKE OVEN EFFLUENT SHOWING LOCATION OF SAMPLING POINTS FOR LABORATORY CONTROL TESTS.

metabolism of the waste. As part of the continuous process, illustrated in Figure 3.1, the biological solids produced are settled in a settling tank, a small proportion of which are removed from the system, and the remainder fed back to the reactor to maintain an adequate level of "sludge" microorganisms for effective metabolism of the waste. The net result of the process is the conversion of the waste into simple and generally harmless metabolic products which can be discharged to a watercourse, and the production of microorganisms which can be removed from the treated wastewater by settlement for further treatment or disposal. An important characteristic of this treatment process is the ability of the collection of microorganisms involved in the treatment of the waste to form flocs, an organic matrix in which the treatment organisms are embedded. Such floc formation facilitates the simple gravity settlement of microorganisms, and their subsequent recycling or removal. Without this property, control of the biomass, in the form of free-floating organisms, would be impractical for most treatment purposes.

3.1.2 The Complete-Mixing Activated Sludge Process

Figure 3.1 illustrates the principle of the activated sludge process, and also the conventional form in which the treatment process is generally used. A wide variety of methods are now available for ensuring an adequate supply of oxygen to the organisms responsible for oxidising the waste in the process and for ensuring appropriate mixing and therefore contact of the organisms with the waste.

In addition, since its invention, a wide variety of more fundamental modifications to the process have arisen, generally to overcome the problems associated with the treatment of particular wastes and/or operating conditions. The important modifications include taperedaeration, incremental loading (or step aeration), contact stabilisation, extended aeration, high rate and complete-mixing processes.

Of these, the complete-mixing system of continuous treatment forms the main continuous system considered in this chapter and the project generallly. A complete-mixing system, as the name implies, is one in which aeration and mixing conditions in the reactor are such that the environmental conditions of the treatment organisms can be regarded as uniform throughout the reactor. In such conditions treatment of the waste and growth of the microorganisms can similarly be regarded as uniformly distributed throughout the reactor. Descriptions of the various aeration and mixing systems, and of the other treatment modifications listed, are available in any good textbook describing wastewater treatment and are not described here.

For the purposes of the project, there are practical advantages in considering the complete-mixing system because it is a simpler system to mathematically model, and the continuous system which can most easily be simulated with laboratory-scale apparatus. However, these advantages are merely fortuitous, and there are sounder reasons for justifying the considerations of the complete-mixing system in the project. The most important is that a large number of activated sludge systems treating coke oven liquor are, or aspire to be, complete-mixing systems, and the results of the project are therefore likely to have more direct significance to existing plants treating the waste.

The process also has a number of advantages over other modifications. EIDSNESS(88) for example pointed out that high quality effluents were possible with low retention periods in the system. McKINNEY (89) noted that the system was conducive to the maintenance of a high concentration of microorganisms acclimatised to the treatment of the waste. This was an advantage over the conventional activated sludge process which operates on a plug-flow basis in which the environment of the microorganisms is continually changing as they pass through the reactor.

FURNESS(90) notes that an important advantage of the complete-mixing system was that it showed more resistance to shock loading and toxic effects than other systems. This is a particularly important characteristic of the process when it is used to treat wastes such as coke oven liquor whose strength and toxicity can vary considerably during its production at a given plant at any time. The ability of the system to achieve very rapid and complete-mixing of the influent with the reactor contents minimises and buffers any potential adverse effects on the microorganisms which in other systems would be localised and more pronounced.

Some workers (91), (92) have shown that a complete-mixing system can treat higher organic loads than the conventional system.

The various activated sludge processes developed each have their own characteristics of course, and the decision upon which to use is made by balancing the relative merits and disadvantages of each. The complete-mixing system is not without its disadvantages. TOMLINSON (93) has suggested, for example, that sludge bulking may be more likely in the complete-mixing process than in the conventional process. It is also important to note that much of the research work that has been published has been concerned primarily with domestic sewage, and some care is needed in extrapolating conclusions from such work to the treatment of a complex industrial waste such as coke oven liquor. Whilst it is accepted that other types of activated sludge process may be successfully applied to the treatment of coke oven liquor, the evidence suggests that the advantages offered by the complete-mixing system, or a system containing a significant complete-mixing component, particularly in buffering shock load and toxic effects, appear to commend its use more than any other for treatment of the waste.

3.2.1 The Kinetics of Biological Growth

A number of equations have been formulated by various workers to represent the behaviour of the activated sludge process in terms of substrate utilisation and biological solids production. Most of these are based on the equations developed by MONOD, (94) who was probably the first worker to mathematically model the growth of bacteria on a continuous basis. He formulated the following equation relating bacterial growth and substrate utilisation producing the hyperbolic relationship depicted in Figure 3.2;

EQUATION 3.1

$$\mu = \mu_{max.} \frac{S}{K_S + S}$$

where μ = the specific growth rate of the microorganisms (time^{-1})

$\mu_{max.}$ = the maximum specific growth rate (time^{-1})

S = substrate concentration (mass/vol)

K_S = substrate concentration at half maximum growth rate (mass/vol)

MONOD formulated the following equation to described the rate of substrate utilisation;

EQUATION 3.2

$$\frac{ds}{dt} = \frac{X}{Y} \cdot \mu_{max.} \frac{S}{K_s + S}$$

where X = cell concentration (mass/vol)

Y, the yield coefficient was defined by the following relationship between the growth of the bacteria and substrate utilisation;

EQUATION 3.3

$$- \frac{dX}{ds} = \frac{\text{weight of bacteria formed}}{\text{weight of substrate used}} = Y$$

Y was assumed to be a constant.

He expressed the growth rate of the microorganisms thus;

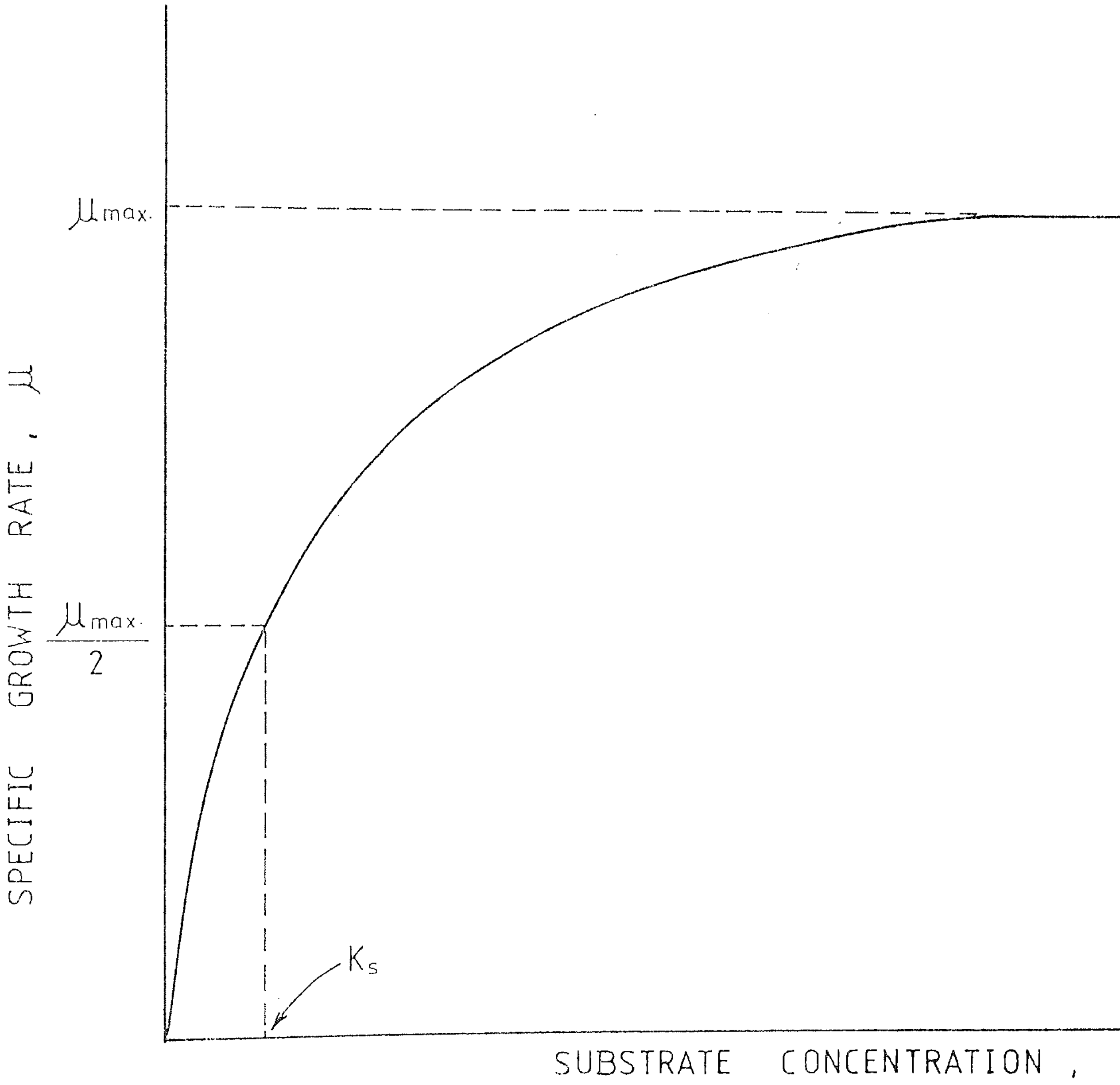
EQUATION 3.4

$$\frac{dX}{dt} = \mu X$$

where $\frac{dX}{dt}$ = the rate of microbial growth

Figure 3·2 Dependence of specific growth rate on substr
concentration, as predicted by Monod kinetics

$$\mu = \mu_{max.} \frac{S}{K_s + S}$$



Many workers have used MONOD's equations as the basis for mathematically modelling the activated sludge process, with varying degrees of success. McKINNEY,(95) HERBERT,(96) KESHEVAN et al,(97) REYNOLDS and YANG,(98) and RANANATHAN and GAUDY (99) for example have each developed models for various activated sludge processes based on the MONOD equations, but each was used to describe the behaviour of a single species systems operating in steady state conditions. TENCH and MORTON,(100) however, assuming an homogenous sludge, and using Biochemical Oxygen Demand (BOD) as a measure of substrate, applied the MONOD equations to an activated sludge system treating multiple substrates, and found that they did not describe the observed treatment behaviour.

TUCEK et al.(101) have questioned the application of MONOD equations to multispecies, multisubstrate systems, and in fact several workers have discussed the justification of applying the model to complete-mixing activated sludge plants, including JONES,(102) McKINNEY,(103) and LEE ,(104) and have indicated that there is little justification for doing so. MONOD's model was applied to a specific set of conditions, the growth of pure bacterial cultures in aerobic culture conditions without recycle, that were very different from the complex operating, mixed culture, and multisubstrate conditions typically found in activated sludge plants.

The most successful application of the MONOD equations has been by DOWNING et al.(105) in their nitrification studies. JONES(106) has suggested however that this success was due to the fact that the growth conditions of the organism studied, Nitrosomonas sp., an autotroph, on the substrate, ammonia, were effectively very similar to the pure culture, single substrate conditions used by MONOD. As such, the MONOD model had been successfully applied to a rather special case which was very different from that which applied to the typical multisubstrate utilisation of the hererotophic organisms found in the activated sludge process.

TISCHLER and ECKENFELDER (107) however have concluded that the MONOD equations could be successfully applied to a mixed culture system, by applying them to each of those being removed individually and then summing them. Each individual substrate removal was found to be zero order.

MONOD's equations have been modified by several workers so that they may better describe treatment by the activated sludge system. JONES (108) for example expanded the model to take account of the activity of those cells in an activated sludge which were non-viable but were nevertheless active in substrate utilisation;

EQUATION 3.5

$$-\frac{ds}{dt} = \frac{X^v}{Y} \cdot \mu\,\max \cdot \frac{S}{K_s + S} + X^a \cdot \frac{R}{K_m + S}$$

where X^v = the concentration of viable cells growing on the substrate (mass/vol)

X^a = the concentration of cells metabolising the substrate without consequent growth (mass/vol)

R = the maximum specific reaction rate

K_m = the Michaelis constant, the concentration, S, of the substrate at half the maximum reaction rate (mass/vol)

LEE et al.'s (104) development of MONOD's model included allowance for the kinetics of dispersed-phase and flocculent bacteria. JACQUART et al.'s (109) development allowed for the "storage" of substrate by cells as well as its metabolism.

Most workers include a decay or maintenance coefficient in the MONOD equations to allow for the net biomass loss due to endogenous respiration. The change in active biomass due to endogenous respitation is proportional to the active mass present, and can be represented as;

EQUATION 3.6

$$\frac{dX}{dt} = K_d X$$

where K_d = the decay or maintenance coefficient.

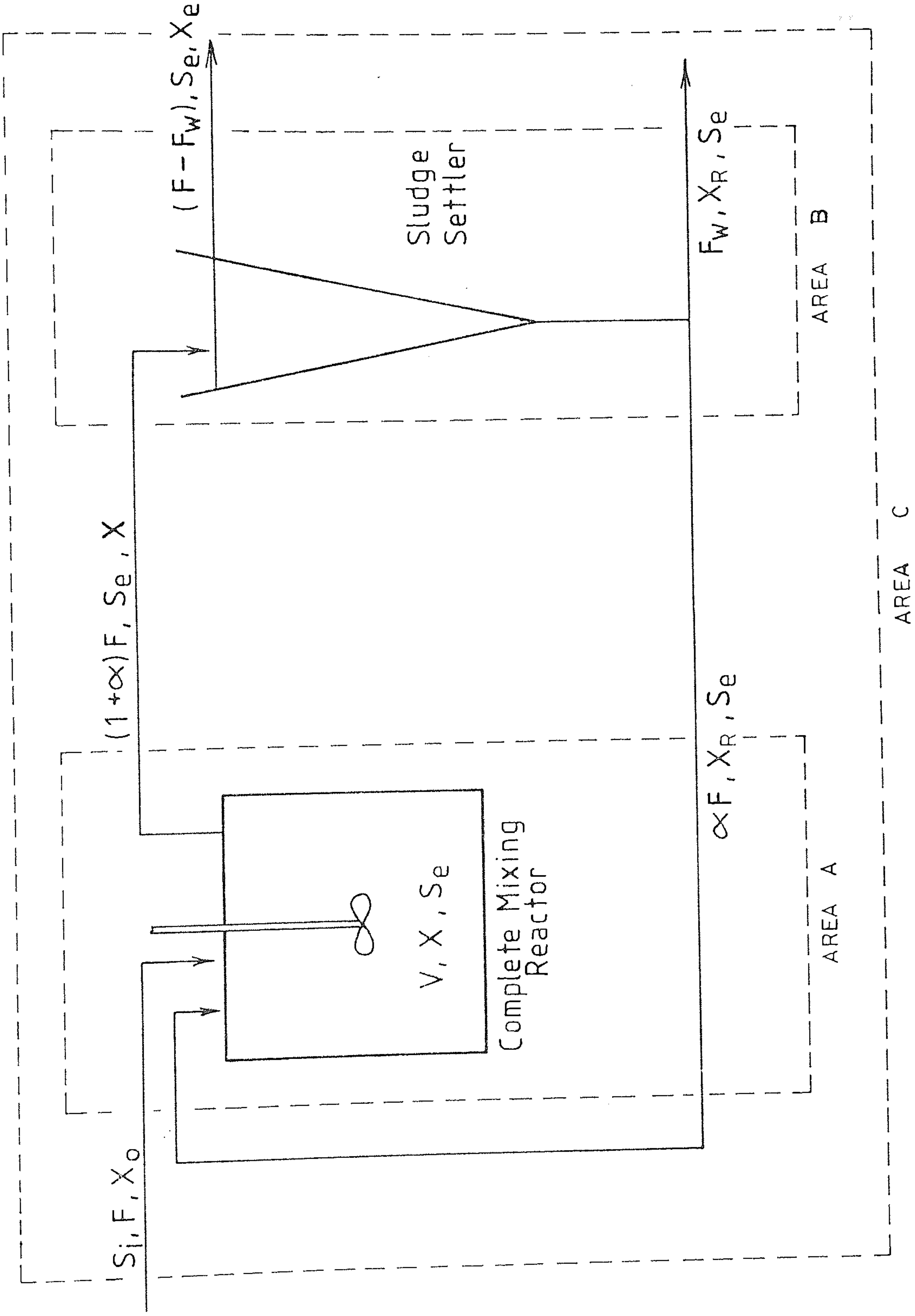
The MONOD growth equations have been used with varying degrees of success in modelling the activated sludge process, and although they have been modified by several workers, they still form the basis of most models published. Other, non-MONOD type, growth equations have been successfully applied, although these are not reviewed here.

The more specific application of MONOD growth equations to the modelling of the activated sludge process is now considered.

3.2.2 Application of Growth Kinetics to the Modelling of the Complete-Mixing Activated Sludge Process

The application of growth kinetics to the modelling of the complete-mixing activated sludge process is, at its most elementary, a simple process which requires a consideration of the material balance of substrate and biomass in the treatment system. This section is intended as no more than a brief introduction to the principles involved, with the object of forming the basis for more detailed consideration later in the project.

Figure 3.3 shows a flow diagram of a single stage complete-mixing activated sludge treatment system. It includes a reactor of volume V, where the waste is metabolised by the organisms comprising the sludge and which receives a continuous flow F of influent, with a substrate concentration of S_i, which is reduced to concentration S in the reactor. The contents of the reactor overflow to a settler where sludge is recycled to the reactor, and some wasted at a flow rate of F_w. The


S_i, F, X_o
Complete Mixing Reactor
V, X, S_e
$(1+\alpha)F, S_e, X$
Sludge Settler
$(F-F_w), S_e, X_e$
F_w, X_R, S_e
$\alpha F, X_R, S_e$
AREA A
AREA B
AREA C

concentration of biomass, X, in the reactor is determined by the growth of organisms produced by metabolising the waste plus the concentration of the recycled sludge X_R. The flow rate of recycled sludge is αF, where α is the recycle ratio. The biomass contents of influent and effluent are X_o and X_e respectively.

To simplify the model here the following assumptions are made;

1. That no biomass enters the system in the influent, or leaves in the final effluent i.e. $X_o = X_e = 0$

2. That changes due to growth and endogenous respiration outside the reactor vessel V, that is in the settler and recycle system, are negligible.

With these assumptions the mass balance equations constructed about the reactor, or the system as a whole, will be the same.

The material balance of biomass in the system gives;

NET ACCUMULATION OF BIOMASS	=	INCREASE DUE TO RECYCLE FLOW	+	INCREASE DUE TO GROWTH	-	DECREASE DUE TO ENDOGENOUS RESPIRATION	-	DECREASE DUE TO FLOW FROM REACTOR

EQUATION 3.7

which in terms of the symbols used in Figure 3.3 may be represented thus;

EQUATION 3.8

$$\frac{VdX}{dt} = \alpha FX_R + V\mu X - K_d.XV - F(1+\alpha)X$$

Similarly the material balance equation for substrate gives;

NET DECREASE OF SUBSTRATE	=	INCREASE DUE TO INFLOW TO REACTOR	-	DECREASE TO OUTFLOW FROM REACTOR	-	INCREASE DUE TO CELL GROWTH

EQUATION 3.9

which in terms of symbols may be represented thus;

$$\frac{Vds}{dt} = FS_i + \alpha FS - F(1+\alpha)S - V\mu\frac{X}{Y}$$

EQUATION 3.10

Using $D = \frac{F}{V}$ where D = the dilution rate

EQUATIONS 3.8 and 3.10 can be simplified to give the following;

For the change of biomass;

$$\frac{dX}{dt} = D\alpha X_R + (\mu - K_d)X - D(1+\alpha)X$$

EQUATION 3.11

For the change in substrate;

EQUATION 3.12

$$\frac{dS}{dt} = D(S_i + \alpha S) - D(1+\alpha)S - \mu\frac{X}{Y}$$

EQUATIONS 3.11 and 3.12 now constitute a simple model of the complete-mixing activated sludge process which has been constructed from the material balance equations for the treatment system, and on the metabolic and microbiological principles that form the basis of MONOD's equations.

3.2.3 Types of Model

Mathematical models can be classified in various ways, but the usual types found described in wastewater treatment studies are dynamic or steady-state models, and deterministic or stochastic models.

EQUATIONS 3.11 and 3.12 are an example of a dynamic model of the activated sludge process and describe a continuous and changing system. By contrast a steady-state model assumes that the system under consideration is in equilibrium, that is, the system variables are independant of time. EQUATIONS 3.11 and 3.12 would become a steady-state model of the treatment process if they were set so that $\frac{dS}{dt}$ and $\frac{dX}{dt}$ were equal to zero.

Steady-state models are used in the design and operational modelling of activated sludge plants and are generally simpler and easier to construct than their dynamic counterparts. An important factor in determining the success of any model, in terms of describing the system that it is intended to represent, is the degree to which the numerical values of the various components of the model can be determined. In the simple model developed earlier, for example, this would mean the determination of the values of the various kinetic coefficients. It is generally easier practically to determine the values of these in a steady-state wastewater treatment system than in a non-steady-state system.

The value of a model will depend upon the use to which it is to be put. A steady-state model may be of limited value in describing the day-to-day or hour-to-hour changes in a system that is inherently dynamic. Activated sludge plants, and particularly those treating coke oven liquor, are generally operated in conditions which fluctuate widely and continuously, and are therefore far from steady-state. The value of a steady-state model of such a process may be limited to describing the limits of operation of the plant between which adequate treatment may be expected overall, or it may have greater or no value at all. Steady-state models of dynamic processes may be regarded as a step towards the development of a more complex and dynamic model, and may be useful for example in the determination of the value of kinetic constants required in the dynamic system. Because of the dynamic nature of the treatment of coke oven liquor, dynamic modelling of the process is likely to be the most potentially rewarding direction in which to pursue modelling studies.

3.3.1 Microbiology of the Activated Sludge Treatment of Coke Oven Liquor, and its Application to the Modelling of the Process

The chemical composition of coke oven liquor is, as was indicated in Chapter 1, both variable and highly complex. For the liquor to be rendered safe for discharge to a watercourse the activated sludge used to treat the waste must contain all of the organisms, in appropriate concentrations and growth conditions, necessary to oxidise the harmful components to harmless products. It will become evident from this brief review that the successful microbial degradation of all the harmful components of the waste involves, potentially at least, a rather complex system in which the removal of any one component is influenced, often adversely, by the presence of the other components, and involves often very different types of organisms with different and often conflicting optimum conditions for growth.

Quantitatively, there are three main components of coke oven liquor, monohydric phenol, thiocyanate, and ammonia. This section, and the project generally, is mainly concerned with the removal of two of these, phenol and thiocyanate. This should not however be taken as diminishing the importance of ammonia removal, or of the other components of the system such as polyhydric phenols and cyanide for example, and the reasons for concentrating on phenol and thiocyanate removal will be outlined later.

It has been demonstrated that the activated sludge process is capable of reducing all of the important components to an adequate level for discharge to a watercourse. The problem has been however that it is frequently difficult to do this consistently. This applies particularly to the removal of the thiocyanate component, and frequent and inexplicable breakdowns in the performance of plants treating the waste, in terms of thiocyanate removal, are common. An insight into the possible causes of this and other types of unsatisfactory treatment performance can be gained by a consideration of the microbiology of the process.

There have been several studies concerned with the identification of these organisms responsible for the degradation of the components of coke oven liquor. JONES,(106) for example, used a laboratory activated sludge plant fed with a synthetic solution of phenol and thiocyanate, and inoculated with a mixed population of bacteria from garden soil. After a period of acclimatisation, and once oxidation of the components had been achieved, he undertook to identify the bacterial species present. He found three different genera of phenol tolerant bacteria, Vibrio, Actinomycetes, and Pseudomonas, and five different species of thiocyanate-tolerant bacteria belonging to the genus Thiobacillus.

The British Carbonisation Research Association (BCRA)(110) in a preliminary investigation of the microorganisms occurring in the activated sludge treatment of carbonisation effluents in bench-scale and pilot plant apparatus, isolated 61 strains of bacteria and fungi, which could be characterised into 14 different groups, from the system. The bacteria isolated included members of the following genera, *Pseudomonas*, *Flavobacterium*, *Aeromonas*, *Micrococcus*, *Bacillus*, and *Thiobacillus*. Table 3.1 gives a summary of the bacteria isolated in that study. Two groups of ciliated protozoa, the Holotricha (or free-swimming ciliates) and Peritricha (stalked ciliates), and rotifers were also found. They concluded that the activity of these protozoa helped to produce a good quality effluent and that their presence was an indicator of good plant performance.

JONES and CARRINGTON(108) isolated organisms from a laboratory activated sludge plant treating carbonisation liquor. They isolated 8 strains, two of which grew on phenol, and one on thiocyanate, as the sole energy course. Their studies concentrated on these last three strains. The thiocyanate oxidising organism was found to be similar to *Thiobacillus thioparus*, and the phenol oxidising organisms were *Comamonas sp.*, a member of the Moraxella/Acinetobacter group. The latter organisms grew on a more restricted range of phenols than the former, would not grow on xylenols or cresols (also components of liquor), and had properties similar to *Achromobacter sp. NCIB8250*.

TABLE 3.1 BACTERIA ISOLATED, IN A STUDY[110] BY THE BRITISH CARBONISATION RESEARCH ASSOCIATION, FROM PILOT ACTIVATED SLUDGE PLANTS TREATING COKE OVEN EFFLUENTS

ORGANISM		NUMBER OF DIFFERENT SPECIES ISOLATED
FUNGI		3 (2 YEASTS)
ENTEROBACTERIA GROUP	1 sp.	7
	2 sp.	1
PSEUDOMONAS GROUP	1 sp.	2
	2 sp.	10
	3 sp.	2
	4 sp.	2
FLAVOBACTERIUM	sp.	5
AEROMONAS	sp.	7
MICROCOCCUS	sp.	2
BACILLUS	sp.	11
THIOBACILLUS	sp.	6
UNIDENTIFIED		3
	TOTAL	61

The behaviour of the 3 strains studied in pure and mixed culture, and in batch and continuous reactors, was very interesting and served to indicate the complexity of the ecological relationship between the organisms involved in the activated sludge treatment of coke oven liquor. In pure culture the growth of the thiocyanate - utilising organism was unaffected by the presence of 100mg/l of phenol, but was completely inhibited in mixed culture with another organism degrading phenol. Studies on the two phenol-degrading bacteria in batch and continuous systems demonstrated stimulation and inhibitions of growth dependant upon the relative concentrations of the two organisms. Also their work with pure cultures of Comamonas sp., and the work by JONES, JANSEN and McKAY(111) using Achromobacter NCIB8250, has indicated that growth of these organisms on phenol exibits substrate inhibition which can be described by HALDANE's(112) substrate inhibition equation;

EQUATION 3.13

$$\upsilon = \frac{V}{1 + K_s/S + S/K_i}$$

where

υ = the observed reaction rate

V = the maximum reaction rate in the absence of inhibition

K_s = the saturation constant

K_i = the inhibition constant

S = limiting substrate concentration

The oxidation of thiocyanate in the treatment of coke oven liquor was at the time considered to be solely due to the action of autotrophic bacteria, such as the Thiobacillus described by HAPPOLD, JOHNSTONE, ROBERTS and YOUATT,(113) and as already noted Thiobacillus spp. have been isolated in several studies on the liquor. HIAM(114) for example isolated autotrophic thiocyanate-degrading bacteria Thiobacillus thioparus and Thiobacillus denitrificans from a sludge treating liquor. STAFFORD and CALLELY(115) have however isolated a heterotophic bacterium, a pseudomonad similar to Pseudomonas stutzeri, which can utilise phenol and thiocyanate simulataneously. HIAM has also isolated an organism from sludge treating coke oven liquor which oxidised phenol and thiocyanate simultaneously, and this organism resembled Pseudomonas putida. Thiocyanate removal was inhibited by low ammonia levels in the former however and therefore would probably be inactive in removing thiocyanate in the conditions normally found in plant treating the waste. With HIAM's isolate however, growth was suppressed at high ammonia concentrations (300mg/l), and this coupled with the fact that it was isolated from a system treating liquor (unlike STAFFORD and CALLELY) suggests it may have significance in plant treating the waste. HIAM also found that his strain was not inhibited by aniline, pyridine, or catechol at the highest levels normally found in treatment plant.

The ability of heterotophs to remove thiocyanate (using it as a nitrogen source) from plant treating the waste has great potential for improving treatment perfomance. The growth and metabolic characteristics of autotrophs and heterotrophs are very different. The latter generally have higher growth rates, and the presence of a thiocyanate-utilising heterotroph would tend to increase the stability of a plant in terms of thiocyanate removal since it would be less prone to "wash-out" from hydraulic overloading. (Heterotrophs also tend to be less sensitive to inhibition by toxic compounds, another factor which might be expected to improve treatment stability). However, the isolation of such a heterotroph from a plant treating liquor is not proof that that organism has any significance in thiocyanate removal in the system. The frequent failure of plants to remove thiocyanate efficiently may be taken as presumptive evidence that it has little or no significance. Alternatively, the fact that in some plants thiocyanate treatment appears more stable than in others may be a reflection of the degree to which heterotrophic or autotrophic thiocyanate removal predominates.

WOODWARD,STAFFORD, and CALLELY(116) used respirometry to establish whether the behaviour of a sludge towards the oxidation of thiocyanate and thiosulphate was that expected of a Thiobacillus or heterotrophic type of culture. The method used was to establish whether the stoichiometric oxidation of the substrate, thiosulphate for example, was consistent with that found (by HAPPOLD et al(113) by a model Thiobacillus, Thiobacillus thiocyanoxidans as represented by the following

$$S_2O_3^{2-} + H_2O + 2O_2 \longrightarrow SO_4^{2-} + H_2SO_4$$

Their sludge was found to oxidise thiocyanate with a stoichiometry similar to that reported by HAPPOLD,(113) and YOUATT(117) for <u>T. thiocyanooxidans</u>, and oxidation was therefore presumed to be predominately autotrophic.

In their experiments they used the organism isolated by STAFFORD and CALLELY, <u>Ps. stutzeri</u>, as the model heterotroph able to utilise thiocyanate. Their discovery that <u>T. thiocyanooxidans</u> was inhibited by thiocyanate concentrations > 20 mM served as an additional test to distinguish between autotrophic and heterotrophic removel of thiocyanate. Other workers have also reported inhibition in thiobacilli able to rapidly oxidise thiocyanate at thiocyanate concentrations >20mg/l.

Later work by HIAM,(118) however, has thrown doubt upon the value of testing the sensitivity of the sludge to high thiocyanate concentrations to distinguish between autotrophic and heterotrophic removal. HIAM, using samples of sludge from a full-scale plant treating coke oven liquor, found that thiocyanate removal was inhibited by high thiocyanate concentrations. but that when the period of exposure was increased beyond 4 hours, recovery from inhibition occured. Later samples failed to show the initial inhibition due to high thiocyanate concentrations, whilst still showing other features characteristic of thiobacilli, such as maintenance of thiocyanate oxidation in the absence of organic compounds.

HIAM's findings in this respect deserve further investigation because the ability of autotrophs that degrade thiocyanate to tolerate high thiocyanate concentrations would have important implications for the stability of the treatment process with respect to thiocyanate removal. If thiocyanate oxidation by autotrophs was inhibited at concentrations greater than 20 mg/l in treatment plants then this could form the basis for much of the treatment failure with respect to thiocyanate removal observed in full-scale plants, and great care would need to be taken at plants to avoid any over-loading or shock-loading of the system that would elevate concentrations beyond the critical level. However, if the inhibitied thiobacilli were able to rapidly adapt to higher thiocyanate concentrations, or if there were a rapid shift in population to thiobacilli able to oxidise thiocyanate at higher concentrations, both possible explanations of HIAM's findings, then loading conditions that raised reactor thiocyanate levels would not be expected to have more than limited adverse effects on treatment efficiency.

Another possibility that should be considered of course is that the test procedures used by HIAM had failed to properly distinguish between autotrophic and heterotrophic thiocyanate removal, and that the removal in the presence of high thiocyanate concentrations apparently due to autotrophs was in fact due to heterotrophs.

This comparison between HIAM's work and the work of STAFFORD and CALLELY, does not appear to have been made before, and the suggestions put forward in the discussion above may warrant further consideration later in the project.

The phenomenum of substrate inhibition by bacteria metabolising phenol has already been mentioned and has been described by several workers, including JONES and CARRINGTON,(108) JONES, JANSEN and McKAY,(111) and PAWLOWSKY.(119) PAWLOWSKY determined the kinetics of 2 different populations of organisms, one predominantly filamentous, and the other non-filamentous, in batch cultures using simple mineral media containing phenol as substrate. He also found that higher phenol concentrations were inhibitory to the organisms concerned, and that the effect could be explained by HALDANE's equation. However, the concentration of phenol at which inhibition became significant varied appreciably. In work carried out by JONES et al.(108) phenol-oxidising bacteria were 50% inhibited by 95mg/l of phenol. HIAM(118) reported only 20% inhibition at the same concentration using samples of sludge taken from a reactor treating liquor. The inhibition reported by PAWLOWSKY(119) for this non-filamentous growth was of the same magnitude as that reported by JONES et al, but inhibition by the filamentous growth was negligible at the 95mg/l level.

Phenol, as well as inhibiting its own metabolism, also inhibits the degradation of thiocyanate. JONES and CARRINGTON's(108) results reported earlier, in which the thiocyanate-degrading organism grew in pure culture unaffected by a phenol concentration of 100mg/l, but completely inhibited in a mixed culture containing an organism degrading phenol, indicates that the inhibition is not caused due to the presence of phenol itself. They suggested that the inhibition was associated with the relatively high growth rate of the phenol-oxidising organism. They also noted that phenol and thiocyanate could be removed at the same time in a completely-mixed activated sludge plant where there was a uniformly low growth rate, whereas in a plug flow system, removal was sequential. PANKHURST,(120) investigating the oxidation of spent gas liquor reported a similar phenomenon in batch culture, where inhibition was due to the growth of another organism on phenol, rather than due to phenol itself.

Other workers have indicated that thiobacilli are sensitive to the presence of phenol, and other organic substances present in coke oven liquor have also been found to be inhibitory. HUTCHINSON and WHITE,(121) for example, found that thiobacilli were absent in many activated sludge plants treating carbonisation effluents, but present in bacteria beds. The sensitivity of the thiobacilli to phenol and oxidised catechol was demonstrated and the suggestion was made that film accumulation on filter beds offered protection for the thiobacilli.

The levels of phenol reported to be inhibitory to thiocyanate oxidiation have varied widely. HAPPOLD et al,(113) for example, reported that phenol levels of 1000mg/l were very inhibitory to thiocyanate oxidation by an organism resembling *Thiobacillus thiocyanoxidans*. Reporting on the treatment of coke oven liquor, HUTCHINSON(121) found that phenol level of 100mg/l inhibited autotrophic thiocyanate oxidation, whilst LYNN(122) reported that 50mg/l was totally inhibitory. McLEE and MOORE (123) reported that for thiocyanate removal to proceed satisfactorily in coke oven liquor treatment total phenol levels should be reduced to 10-20mg/l.

STAFFORD,(124) and DOWNING (125) have indicated that phenol and cresols also inhibit nitrification. STAFFORD demonstrated inhibition at phenol and cresol levels greater than 3mg/l and that inhibition was 100% at 10mg/l. Nitrification rarely occurs in a single stage activated sludge plant treating coke oven liquor, and a second stage reactor is generally added to the system where nitrification is a treatment objective. In such cases therefore it is desirable that the first stage is operated to produce very low phenol levels. Other components of the liquor have also been shown to be inhibitory to nitrification including various pyridine derivatives. These tend to be more resistent to degradation than phenol, but are more likely to be oxidised in plant conditions that produce low phenol effluent concentrations.

Reports on the performance of activated sludge plants treating coke oven liquor, for example the National Coal Board's Fishburn Plant, have indicated that the occurrence of nitrification is associated with poor thiocyanate removal. Nitrification is generally associated with a lowering of pH in the treatment system to a level below that conducive to efficient thiocyanate, and also phenol, oxidation. Thiocyanate and phenol removal activity however can be regained simply by restoring the optimum pH.

The bacteriostatic action of phenol on cultures of bacteria is well known, and various workers have investigated the basis for this action. DAGLEY, DAWES, and MORRISON (126) suggested that it was due to the inhibition of the generation of essential metabolic intermediates. They found that low concentrations of glutamate, methionine, and succinate countered the lag in growth of Escherichia coli caused by phenol. ASHMORE et al. (127) studied the phenolic inhibition of thiocyanate removal in the treatment of coke oven liquor and suggested that it was partially oxidised polymeric phenols, such as oxidised catechol, that were responsible for inhibition. BAIRD et al. (128) also suggested that inhibitive effects were due to the production of toxic oxidation intermediates rather than to phenol itself.

The presence of phenol therefore directly or indirectly inhibits the metabolism of other components on coke oven liquor. Adding to the complexity of the system, the removal of phenol itself has been shown to be influenced by the presence of other liquor components. Phenol removal, as well as being subject to substrate inhibition, has also been shown to be influenced by the ammonia concentration; KOSTENBADER and FLECKSTEINER (129) have indicated that high ammonia concentrations reduce phenol removal. More recent work by NEUFELD and VALIKNAS (130) has indicated that phenol degradation is inhibited by thiocyanate and that this becomes significant to the design and operation of a plant treating liquor when low effluent phenol concentrations are required.

A further illustration of the complex biochemistry of the treatment process is that some organic compounds at low concentrations (between 2-5mg/l) have been shown to enhance treatment efficiency, and these have been the subject of patents. Work in this area has been centered at the BCRA, and chemicals, or growth factors, shown to improve treatment of liquor include p-aminobenzoic acid (PABA), p-hydroxybenzoic acid,(131) nicotinic acid, isonicotinic acid, pyruvic acid, and certain amino acids.(132) CATCHPOLE and COOPER (133) investigated the effect of PABA addition to activated sludge treating ammonium thiocyanate and found that the retention time for satisfactory removal could be reduced to half that for the system without the growth factor. It was suggested that PABA was not itself the growth factor, but was metabolised to a compound that was able to relieve enzymes suppressed by inhibitors. Pyruvic acid has been suggested as the compound effective intracellularly in producing this effect, and the suggestion has been supported by the effectiveness of pyruvic acid addition itself to sludges and also by later experiments.(134) Glucose has also been shown to enhance treatment efficiency, and its effectiveness may be due to its conversion to pyruvic acid by glycolysis in the cell.

Further work at BCRA,(135) particularly by COOPER and CATCHPOLE (136) has indicated that once the addition of growth factor to sludge has established the appropriate modification in metabolism, further addition may not be required. Also their investigation into the effect of growth factors on nitrification enabled a complete nitrified effluent to be produced from a coke oven waste containing 200mg/l ammonia, without the need for an additional treatment stage.

It is worth noting that this growth factor phenomenon is not confined to the treatment of coke oven liquor. Several other examples are known, and the action of glutamate, methionine, and succinate in restoring normal growth to E. coli cells inhibited by phenol, reported earlier, is one such example. The precise action of the intracellular level of the various compounds shown to enhance treatment efficiency is not known. All of the compounds listed and shown to have this effect, however, are important compounds in the metabolism of the cell, and there are sound reasons for presuming that their action is to influence the activity of certain key enzymes in the cell which control the direction of metabolism.

It is also worth noting that the spectacular improvements in treatment efficiency reported resulting from the use of growth factors in coke oven liquor treatment often have not been confirmed by plant operatives using the chemical to relieve poor plant performance. This may however be due in part to a failure of plant operatives to fully understand the cause of the poor treatment performance, and growth factors may have been used in inappropriate conditions, where, for example, inhibition was not the cause, or was of a type or degree where they would be ineffective.

3.3.2 Applications to the Treatment Process

It will be evident that the treatment of coke oven liquor in the activated sludge process involves a very complex ecosystem, with a large number of different organisms interacting in the complex and highly variable growth environment of the waste-water in the reactor.

Ideally perhaps, a model of the biological treatment system would need to include a consideration of all the individual species of organisms present, and their behaviour towards each other and to the many chemical components of the liquor. However, in reality this would neither be practical or desirable. It would not be practical because we do not have, and could not easily obtain the biological information required. It would not be desirable since such a model may well be unmanageable, and would also be unnecessary. The main requirement of the model envisaged in this type of project would be that it represented the treatment process to the extent that practical design and operation of the treatment plant could be achieved. Such a model should be as simple as possible; the more complex the model the more parameters that require determination for application of the model, and the practical usefulness of a model of this type is generally inversely proportional to the information needed to apply it.

The simple kinetic model of the activated sludge process developed earlier assumed that the treatment system behaved as though it contained a single homogenous population of bacteria. Such an assumption may represent a valid simplification of the microbiology of the system when the metabolic behaviour of all the component microbes is similar. The review of the microbiology of coke oven liquor treatment however revealed a system containing organisms with very different metabolic characteristics. The kinetics of autotrophs and heterotrophs are typically very different, and a model of the process should therefore make allowance for this.

An elementary simplification of the system for coke oven liquor treatment would be to consider only the biological removal of the waste's three main components, phenol, thiocyanate, and ammonia. At its simplest therefore the system could be considered as containing three distinct populations of organisms, each responsible for removal of one of the components, a heterotrophic population being responsible for phenol removal, and different autotrophic populations responsible for thiocyanate removal and nitrification. Consideration of heterotrophic thiocyanate removal, plus the influence of the other populations and chemical components on the component removal by any one population, would of course complicate the model further.

An early decision in the pesent project was that research should be concentrated, initially at least, on the removal of phenol and thiocyanate components from the liquor, and the assumption was made that a distinct population of organisms was responsible for the removal of each of these components. Low priority was given to consideration of ammonia removal because of the limited time and resources available to the project, and because nitrification is not a treatment objective at many full-scale plants treating liquor. The priority given here to phenol and thiocyanate removal reflects that generally applied by plant operators responsible for treatment of the waste.

The approach, outlined in this chapter, to the modelling of the activated sludge treatment of coke oven liquor, forms the main basis for testing the significance of the ATP measurements to be made in the project and, if possible, for the development of a practically useful model of the process.

CHAPTER 4

INVESTIGATION OF THE ATP BEHAVIOUR OF LABORATORY-SCALE BATCH ACTIVATED SLUDGE REACTORS TREATING THIOCYANATE SOLUTIONS

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INVESTIGATION OF THE ATP BEHAVIOUR OF LABORATORY-SCALE BATCH ACTIVATED SLUDGE REACTORS TREATING THIOCYANATE SOLUTIONS

Introduction

It was decided at an early stage in the project to consider the biological treatment of coke oven liquor in terms of the removal of its three main components, phenol, thiocyanate, and ammonia, and to give priority to consideration of the former two. It was also decided to assume, initially at least, that there existed three distinct populations of organisms in activated sludge treating the waste, each being responsible for the degradation of just one of the components. The reasons for this simplification of the system were outlined in the previous chapter and were to form the basis for the investigation into the ATP behaviour of activated sludge treating coke oven liquor waste, and for exploring the potential of ATP measurements for the development of a mathematical model describing the overall treatment process.

The project concentrated initially on the biological removal of the thiocyanate component, and the investigation was carried out using laboratory-scale batch activated sludge reactors. The decision to use laboratory-scale batch reactors followed the precedent set by the project sponsors in using this method and apparatus in their earlier investigations, but also reflected the limited practical facilities available which meant that larger scale and continuous reactor studies were not possible at the start of the project.

It was evident from the previous chapter that the microbiology and biochemistry of the activated sludge treatment of liquor is complex.

Particularly important is the fact that the many chemical components of the liquor appear to influence the removal of any one component. It was felt important therefore that, given the simplication of the system regarding the three main components, and that a batch rather than continuous mode of operation was to be used, the general growth environment of the sludge organisms in the laboratory experiments, should, as far as possible, resemble those found in full-scale plants treating the waste. The decision was made therefore, that experiments should be conducted using a basal medium for the sludge of treated coke oven liquor from a laboratory continuous reactor treating the waste. In this way the growth medium would contain a large proportion of those chemical components of the liquor which, although only present in small concentrations, had been demonstrated to be important because of their inhibitiory effect on sludge organisms and their resistance to biodegradation. Although it was not practical in this project to carry out complex chemical analysis of the basal medium so that the growth conditions could be better defined, a large degree of consistency in the quality of the treated liquor used as the basal medium was assumed. This approach was favoured rather than an alternative of using the simple chemically defined mineral media used by some researchers which might be expected to produce growth conditions, and therefore results, which would be less relevant and more difficult to extrapolate to full-scale systems.

The initial experiments carried out to investigate the ATP behaviour of batch activated sludges treating liquor were therefore conducted on the basis of the approach outlined here, and these are now described in detail.

4.1. Establishment of a Thiocyanate Batch Activated Sludge

The batch reactor studies were carried out using reactors of the type shown in Figure 4.1. Four litre quantities of activated sludge from a full-scale treatment plant treating coke oven liquor at the British Steel Works, Orgreave, Sheffield, were added to each of two such reactors. These were used to develop sludges acclimatised to treating thiocyanate as the sole substrate in the following manner. They were fed on a batch basis, generally once a day, with aliquots of a stock solution of potassium thiocyanate solution (60,000mg/l), and with small quantities of a phosphate solution, and a solution containing trace elements. The composition of these solutions was recorded later.

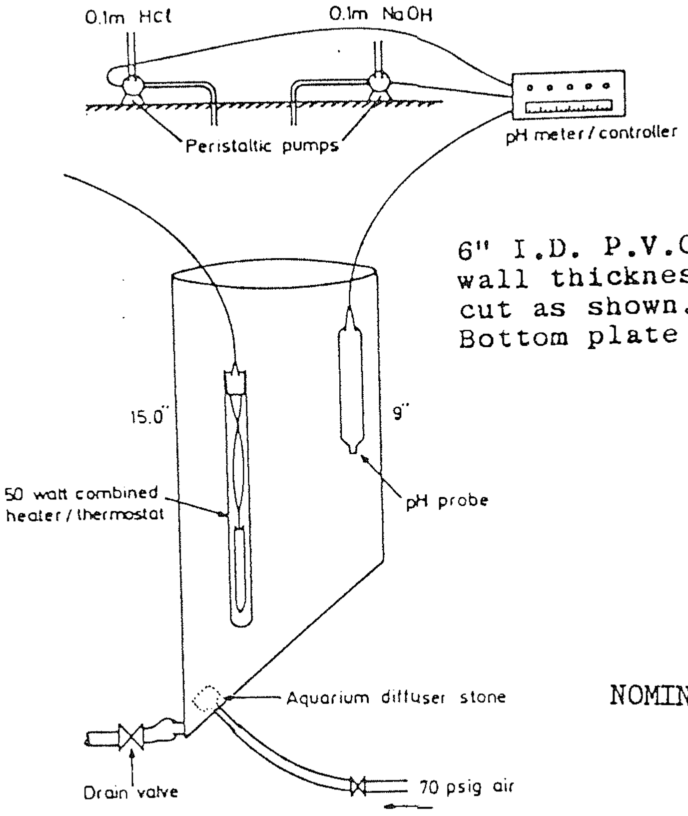
The temperature of each reactor was maintained constant ($\pm 0.5^{o}$C) by means of a 50 watt combined heater/thermostat at a chosen temperature which generally varied between 20^{o}C and 30^{o}C. Initially the pH of each reactor was maintained at about 7.0 by means of an industrial pH controller system, the reactors being fed with 0.1M hydrochloric acid or 0.1M sodium hydroxide as appropriate, via small peristaltic pumps whenever the pH deviated from the set range. Later, the controller system was removed, and the pH was adjusted, generally once a day, to the range pH 7.0-8.0 by the appropriate manual addition of the acid or alkali described.

Periodically, small quantities of the sludge were wasted from the reactors, and less frequently the contents of each reactor were allowed to settle, the supernatent discarded ,and replaced by effluent from small laboratory continuous reactors treating coke oven liquor. The reactors were also cleaned regularly to remove mineral deposits which formed on the walls and the diffuser stones replaced as necessary to ensure that the aeration rate was adequate to maintain complete mixing in the reactor.

The thiocyanate concentration, pH, temperature, and sludge volume (SV_{10}) were monitored daily, and additions to the reactors and sludge wastage were made on the basis of the results of these tests.

The reactors were maintained for a period of several months as described so that the sludges developed were able to treat high concentrations of thiocyanate on a batch basis in a basal medium of treated coke oven liquor. The maximum concentration of thiocyanate fed to the reactors was increased from a low level at the start of the acclimatisation period, when the sludge had first been obtained from the full-scale reactor, to a higher level over a period of weeks. After this acclimatisation period, the maximum daily concentration upon batch addition of stock thiocyanate solution was generally about 500mg/l, but substrate additions giving concentrations exceeding 1000mg/l were occasionally made.

The treatment of the batch reactors described was carried out on a daily basis during the normal working week, but less frequently or not at all, outside that period. The larger additions of thiocyanate were made immediately preceding these periods, when daily attention to the reactors was not possible, to avoid long periods of substrate deprivation in the reactors.



6" I.D. P.V.C. piping
wall thickness 0.25"
cut as shown.
Bottom plate welded.

NOMINAL CAPACITY: 5.5 l

FIGURE 4.1. REACTOR & ASSOCIATED APPARATUS USED IN BATCH REACTOR STUDIES

Stock Thiocyanate Solution 60,000mg/l SCN^-

100.4g potassium thiocyanate were made up to 1 litre with distilled water.

Stock Phosphate Solution approx. 3,000mg/l as P.

10g of sodium hexametaphosphate $(NaPO_3)_6$ was made up to 1 litre with distilled water.

Trace Metals Solution

(Added to reactor in the proportions 10mls solution per 1 litre of basal medium).

Composition

EDTA	50g
$ZnSO_4.7H_2O$	22g
$CaCl_2$	5.54g
$MnCl_2.4H_2O$	5.06g
$FeSO_4.7H_2O$	4.99g
$(NH_4)_6Mo_7O_{24}.4H_2O$	1.1g
$CuSO_4.5H_2O$	1.57g

Distilled water to 1 litre

Preparation

The EDTA was dissolved in about 300ml of distilled water.

The pH of the solution was then increased to a little greater than 7.0 by the addition of sodium hydroxide solution. Each of the remaining components were then dissolved in the solution and distilled water added to make the volume up to 1 litre.

4.2 Preliminary Investigations

A number of experiments were performed to establish the ATP behaviour of batch reactors treating thiocyanate. The principle design of these experiments was that one of the batch reactors (labelled B1 and B2) described, was starved of substrate for a period prior to the experiment, and then a number of parameters were monitored at intervals over varying time periods, following the additions of a single dose of thiocyanate solution. (The period of starvation in these and all subsequent experiments was defined as having commenced when the Lovibond test for thiocyanate (see Method 4.3.4.1), using an appropriate volume of supernatent from settled sludge from the reactor, indicated a thiocyanate concentration $<$ 5mg/l). The parameters monitored in these initial experiments were thiocyanate, ATP, and mixed liquor suspended solids (MLSS), and these were determined by taking samples of the mixed liquor and treating or storing them in an appropriate manner for later analysis.

The precise methods employed in the experiments varied, generally as the result of the experience gained from earlier experiments. A summary of the parameters monitored and the variation in experimental methods employed in this first series of experimental runs follows, and this precedes a detailed account of the methods and materials used in the experiments.

4.2.1 Experimental Runs TH1 and TH2

The activated sludge was starved for a period of at least 12 hours prior to each run, and batch reactor B1 was used. Initial temperature and pH conditions were recorded and the mixed liquor suspended solids, and thiocyanate concentrations were determined using Methods 4.3.3 and 4.3.4.2 respectively. The butanol/octanol method (Method 4.3.5.1.1.) was used to extract ATP from the sludge solids filtered from samples of the reactor sludge prior to the ATP analysis using Method 4.3.5.2. The ATP concentrations of the filtrates produced following the filtration of reactor sludge samples were also determined using Method 4.3.5.2.

ATP concentrations were not determined in Run TH2.

In each run thiocyanate concentrations of the sludge filtrate were determined immediately after all of the sludge samples had been taken, and this was the practive adopted in other runs unless otherwise stated.

4.2.2 Experimental Runs TH3-8

The activated sludge was starved for a period of at least 12 hours for Run TH3, at least 24 hours for Run TH4, and 1-2 hours for Run TH5. The starvation period prior to the addition of thiocyanate was not known for Runs TH6-8. Reactor B1 was used for all runs except Run TH8 in which reactor B2 was used. Initial temperature and pH conditions were recorded for each run, and pH was also periodically determined during the course of Run TH6. MLSS determinations were only made for Runs TH4 and TH5.

Samples taken from the reactor for ATP analysis were frozen immediately in a dry ice/acetone mixture as described later, prior to being stored in a refrigerator at -18°C, and then thawed and ATP extracted at a later date using the DMSO extraction method prior to ATP analysis. This procedure replaced the immediate analysis of filtered sludge solids following extraction by the butanol/octanol method used for Run TH1. The filtrates produced during MLSS determinations were used for the determination of filtrate ATP concentrations in Runs TH3 and TH4. As in Run TH1 filtrate ATP concentrations were determined immediately using Method 4.3.5.2.

Table 4.1 summarises the experimental conditions and parameters monitored in Runs TH1-8.

TABLE 4.1. : SUMMARY OF EXPERIMENTAL CONDITIONS AND PARAMETERS MONITORED IN RUNS THI-8

Exptl runs	Stock scn added	Reactor B1	Reactor B2	Starvation period hrs	Ph		ATP Sludge	ATP Filtrate	ATP Total	MLSS	CNS	CN	NH_3	SO_4	NO_2	NO_3	Redox Potential
TH1	20	+	-	12	-	-	+	+	-	+	-	-	-	-	-	-	-
TH2	20	+	-	12	-	-	-	-	-	+							
TH3	20	+	-	12	-	-	+	+	-	+	-	-	-	-	-	-	-
TH4	20	+	-	24	-	-	-	+	+	+	-	-	-	-	-	-	-
TH5	30	+	-	1-2	-	-	-	-	+	+	-	-	-	-	-	-	-
TH6	20	+	-	NR	-	+	-	-	+	-	-	-	-	-	-	-	-
TH7	10	+	-	NR	-	-	-	-	+	-	-	-	-	-	-	-	-
TH8	10	-	+	NR	-	-	-	-	+	-	-	-	-	-	-	-	-

+ = Parameter monitored - = Parameter not monitored NR = Not Recorded

4.3 Methods and Materials

4.3.1 Temperature

A-10 to 50°C or -10 to 100°C range mercury-in-glass thermometer was used to measure all the temperatures.

4.3.2 pH

pH was determined using a combined pH/reference electrode and either an Orion Model 701 Digital pH/mV Meter (Orion Research Inc., Mass., USA), or an EIL Model 7050 Laboratory pH Meter (Electronic Instruments Ltd., Chertsey, England).

The system was calibrated with freshly prepared pH 4.0 and 9.0 buffers as recommended by the manufacturers before each experiment. Both types of pH meter used incorporated automatic temperature compension.

4.3.3 Mixed Liquor Suspended Solids

Millipore membrane filters (4.7cm Type HA 0.45μm) were dried at 105°C, and cooled in a desiccator containing silica gel, and then weighed. Suspended solids were determined by filtering samples of known volume through the membranes using Millipore Filter Apparatus and the membranes then dried, cooled, and weighed as before. The MLSS concentration was determined using the following equation;

$$\text{MLSS Concentration} = \frac{(A - B)}{V} \times 10^{6} \text{ mg/l.}$$

where B = the weight of the dried membrane in grms.

A = " " " " " " + dried solids in grms.

V = the volume of sample taken in mls.

Samples of activated sludge were taken from the reactor using a 10ml graduated pipette which had a wide orifice permitting the passage of large floc particles, and facilitating rapid filling and emptying which enabled well-mixed and representative samples to be taken. A "blow-out" type pipette was used and each sample constituted the entire contents of the pipette which were rapidly expelled into the filtration apparatus. The volume of the pipette used was determined by accurately weighing the same volume of distilled water expelled by the clean pipette, using the known density of water. This volume was found to be 10.9mls.

4.3.4 Thiocyanate

4.3.4.1 Modified Lovibond Comparator Test

A simplified version of the standard Lovibond test was used to enable rapid approximations of the thiocyanate concentration of the supernatent from settled samples of activated sludge from reactors B1 and B2. Ferric chloride reagent used in the test was prepared as follows:-

Reagent

100grms of hydrated ferric chloride ($FeCl_3.6H_2O$) were dissolved in water containing 50mls concentrated hydrochloric acid, and diluted to a volume of 500mls.

Test

A volume of the sample was added to the test cell and diluted with water to the 10ml mark. 1.0ml of ferric chloride reagent was then added to the cell and mixed. This cell was then placed in the Comparator, along with a reference cell which had been similarly treated but contained no sample. The colour of the treated sample was matched as near as possible with that of one of the glass colour standards in the Comparator Disc 3/63, covering the range 5-70mg/l of thiocyanate ion. The test was repeated with appropriate dilution of the sample where the concentration in the test cell exceeded the range of the disc.

4.3.4.2 Ferric Nitrate Method

Accurate determinations of thiocyanate concentration in samples were made using this method.

As with the above method, the principle of the method is that a red colour is developed upon addition of a solution of a ferric salt, in this case ferric nitrate, to a solution containing thiocyanate, and the intensity is determined colorimetrically.

Reagents

As for all other analytical operations carried out in the project, analytical grade, generally ANALAR, chemicals, and laboratory distilled water, were used.

N/10 Silver Nitrate

8.495g of dried silver nitrate were dissolved in water and diluted to 500ml in an amber volumetric flask.

Nitric Acid 40% V/V

Ferric Ammonium Sulphate Solution

30g of ferric ammonium sulphate $Fe_2(SO_4)_3(NH_4)_2SO_4.23H_2O$ were dissolved in 100ml water.

Nitrobenzene

Stock Thiocyanate Solution Approx. 5g/l CNS^-

4.5g of potassium thiocyanate were dissolved in water and diluted to 500ml in a volumetric flask. The solution was standardised as follows:-

Standardisation Procedure

20.0mls of N/10 silver nitrate solution were pipetted into a 250ml conical flask, and 5.0mls of dilute nitric acid, 1.0ml ferric ammonium sulphate solution, and 1ml nitrobenzene added and the flask well shaken. This was titrated with the stock thiocyanate solution until faint brown colour was produced, which did not disappear on shaking.

The thiocyanate concentration of the stock solution was calculated using:

$$\text{Thiocyanate as } CNS^{-} = \frac{116200\text{mg/l}}{V}$$

where V = volume of stock thiocyanate solution used in the titration (mls)

Thiocyanate Solution 100mg/l CNS

20.0ml of stock thiocyanate solution was diluted to 1 litre in a volumetric flask.

Ferric Nitrate Solution

100g of ferric nitrate $Fe(NO_3)_3.9H_2O$ was dissolved in water containing 25mls nitric acid SG1.42 and made up to a volume of 500ml with water.

Mercuric Chloride - saturated solution.

Preparation of Calibration Graph

Aliquots of the 100mg/l thiocyanate solution were pipetted into a series of 100ml volumetric flasks to cover the range up to 2.5mg CNS^-, and diluted with water to give a volume of about 80ml. A flask containing no thiocyanate was used as a blank. The solutions were then treated as described under "Analytical Procedure". The optical densities of the solutions, minus that of the blank solution, was used to prepare a calibration graph relating optical density to mg. thiocyanate. This was a linear relationship within the range specified.

Analytical Procedure

An aliquot of each sample was pipetted into a 100ml volumetric flask, and 1.0ml of dilute nitric acid, and 10.0ml ferric nitrate solution added. The solutions were made up to a volume of 100ml with water and mixed well. The flasks were allowed to stand at room temperature for 10 minutes, and then the optical density of each solution determined in 10mm cells at 515nm using a Unicam SP30 UV Spectrophotometer. The optical density of test solutions was made with reference to water in the reference cell. The optical density of the blank solution was subtracted from that of the test solutions, and the thiocyanate content of the samples using the calibration graph.

In those experiments where activated sludge samples were filtered for MLSS determination, the filtrates were used for thiocyanate determinations. Where MLSS concentrations were not determined, one drop of saturated mercuric chloride solution was added to each sample which was allowed to settle. The supernatent from the settled samples was used for thiocyanate analysis. (Mercuric salts suppress the formation of the red colour in the assay, but at these concentrations used to inhibit the metabolism of the sludge samples, the effect was not found to be significant). When filtered samples were not used an additional blank solution was prepared for each sample, containing the same volume of sample as in the test solution, diluted with water to 100mls, but no reagents. The optical density of this solution was additionally subtracted from that of the test solution to correct for absorbance by fine suspended material.

4.3.5 ATP Analysis

4.3.5.1 Extraction Techniques

1.3.5.1.1. The Butanol/Octanol Method

Samples of activated sludge were taken from the reactor using a 10ml graduated pipette and were filtered through a pre-weighed Millipore membrane filter (4.7cm Type HA 0.45μm) using the Millipore Filter Apparatus in exactly the same manner described for MLSS determinations. The vacuum was released and the filtrate run from the apparatus and stored for further analysis. Where the

ATP concentration of the filtrate was determined this was carried out immediately using the method described later.

5.0mls of butanol was then added to the membrane and filtered solids, and after 30 seconds the vacuum was re-applied to the apparatus and the butanol extract collected in the extraction funnel. The membrane was then washed with 2 x 5.0ml aliquots of MOPS buffer, and filtered through the membrane and collected in the funnel with the butanol extract. 10.0mls of octanol was then added directly to the funnel containing the butanol/MOPS buffer extract and the mixture shaken. The layers were separated by centrifuging the mixture at about 3,000rpm for 3 minutes in an MSE bench centrifuge. The upper butanol/octanol layer was removed by pipetting, and the volume of the bottom aqueous layer containing ATP was determined to a tenth of a millilitre, and then kept in and ice bucket and assayed in the manner described later.

4.3.5.1.2 The DMSO Method

Introduction

The Butanol/Octanol extraction method outlined above presented a number of practical problems when it came to analysing the large number of activated sludge samples taken at short time intervals in the experimental runs described. The most important of these were:

1. Limited supplies of the apparatus shown in Figure 4.1. meant that it was not possible to use clean ATP-free apparatus for each sample extracted. Repeated use of the same apparatus meant that cross contamination between samples was inevitable.

2. Due to the size of the apparatus involved and the number of operations involved in the procedure, it was difficult to maintain apparatus and extracts at the cool temperatures that were consistent with the maintenance of the stability of the ATP extracted from the samples.

3. The extraction method was found to be a very time consuming procedure which meant that it was not practical to take samples at the short time intervals required in the experiments.

The large amount of time required for the butanol/octanol extraction procedure proved to be the most serious disadvantage of the method.

Simple Preservation

The analysis of sludge samples for ATP during the experiments described earlier severely limited the sampling frequency and the number of parameters that could be monitored, because of the time requirements for the extraction and analysis procedures. It was realised that this problem could be overcome if samples for ATP analysis could be stored in some manner so that they could be analysed at a convenient time when the experimental runs had been completed. This principle and the following simple procedure represents a new approach to this problem and an important development that appears to be unique to this project.

1-2ml samples of sludge were taken from the experimental reactor with a disposable Pasteur pipette and expelled into small glass test-tubes, which were immediately frozen in a dry ice/acetone mixture. Duplicate tubes were sealed, labelled and stored at -18°C for later analysis.

These frozen samples were later thawed and extracted using the DMSO method and analysed for ATP. The ATP concentrations of samples which were extracted and analysed immediately were not found to be significantly different from duplicate samples which were frozen in the manner described for periods up to 6 months and then subsequently thawed and analysed.

(All glassware used in this procedure, and the subsequent extraction and assay procedure, was chemically clean, and either sterilised in an autoclave, or heated in an oven for several hours at 105°C and then allowed to cool in clean or aseptic conditions first before its use was required in the experiment).

DMSO Extraction Procedure

The extraction procedure used was a modification of a method recommended in the Instruction Manual for the instrument used for the ATP analysis of samples, the Du Pont 760 Luminescence Biometer. The reagents used were as follows:

Reagents

Low Response (LR) Water

Laboratory distilled water was acidified with one drop of concentrated hydrochloric acid per litre and boiled for five minutes. This was neutralised (pH meter) with dilute sodium hydroxide and sterilised by autoclaving in McCartney or 100ml capped bottles. These were stored in a refrigerator (2-5°C).

MOPS Buffer

0.01M MOPS buffer was prepared as follows: 2.09g of morpholinopropane sulphonic acid (MOPS) (Hopkins and Williams molecular weight 209.3) per litre was dissolved in LR water. This was adjusted to pH 7.4 (pH meter) with sodium hydroxide solution and sterilised by autoclaving in McCartney or 100ml capped bottles. These were also stored in a refrigerator (2-5°C).

90% DMSO

The extractant was prepared daily by diluting 9 parts dimethyl sulphoxide (DMSO) (B.D.H. Chemicals, Analar) with 1 part MOPS buffer.

Preparation of Glassware

Normal chemically clean glassware was rinsed three times with 0.2N hydrochloric acid, then three times with laboratory distilled water, then three times with MOPS buffer (to neutralise any remaining acid) and finally rinsed with several washes of LR water.

Alternatively, the chemically clean glassware was sterilised by autoclaving, or heated in an oven at 105°C for several hours and then allowed to cool in a clean or aseptic environment just before it was required.

Extraction

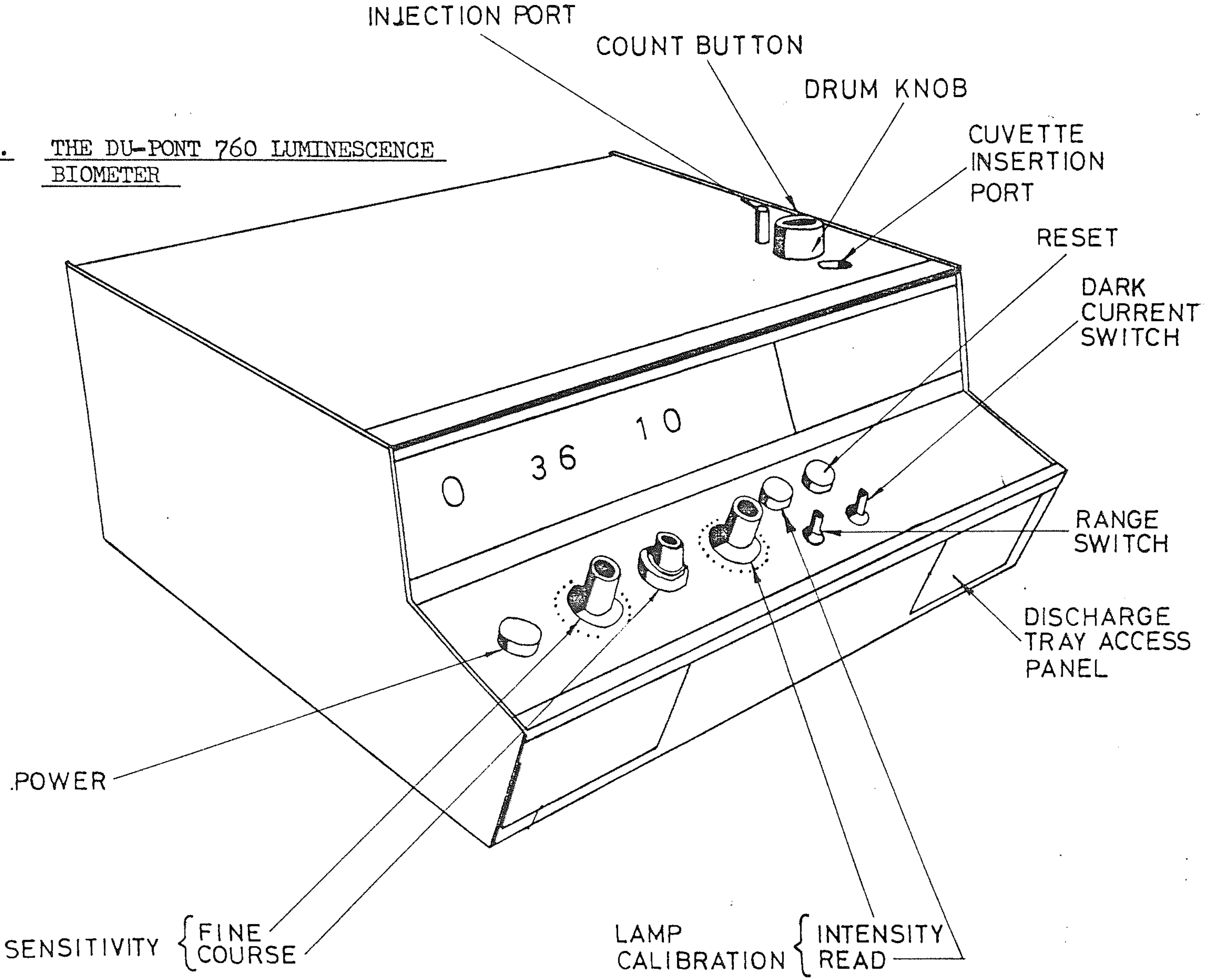
Small test tubes containing 1.0ml of 90% DMSO were prepared shortly before they were required and stored in a refrigerator (2-5°C), and removed as needed. A sample tube stored at -18°C, was thawed rapidly by holding the base of the tube under a running cold water tap. As soon as the sample had completely thawed, it was shaken to ensure a homogenous suspension and a 0.1ml or 0.2ml sample taken immediately using an automatic pipette (Beckton and Dickenson Pipettor 259615 and disposable tips 259616) and added to one of the tubes containing 1.0ml of 90% DMSO. The latter tube was then mixed for about 10 seconds by hand and then allowed to stand in an ice bath for 2 minutes. 5.0mls of sterile MOPS buffer was then added to the tube and the contents mixed. The ATP content was then determined by injecting 10μl aliquots into the Biometer.

4.3.5.2 Determination of the ATP Content of Samples

The Du Pont 760 Luminescence Biometer shown in Figure 4.2 was used throughout the project. Unlike the sample preservation and extraction procedures described earlier, which were either developments original to the project, or modifications of methods employed by other researchers, the ATP assay procedure used was exactly that recommended by the instrument's manufacturers, and this has been described in Appendix 1.

10ml aliquots of extracts were used for each determination. These were injected into the instrument using a 50μl Hamilton precision syringe (Style 705N, point style no.3) mounted in a Shandon Repro-jector, as shown in Figure 4.3. Use of this injection system rather than manual injection using the syringe only, was found to be essential to obtain reproducible results for each sample.

FIGURE 4.2. THE DU-PONT 760 LUMINESCENCE BIOMETER



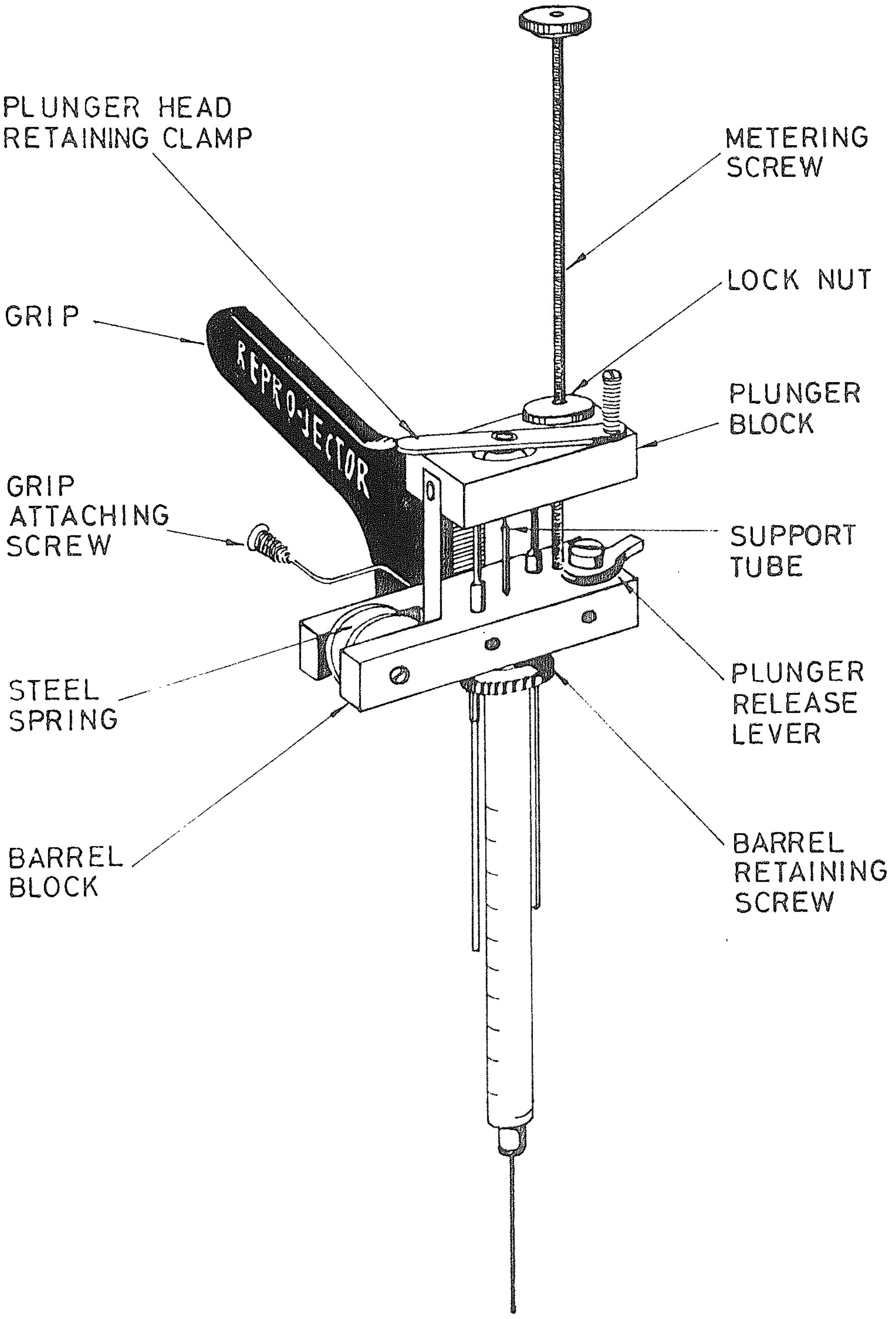


Fig 4-3 Repro-Jector and Precision Syringe Assembly

4.4 Results

The results from experimental runs TH1-8 are recorded in Tables 4.2 to 4.9 and plotted in the graphs shown in Figures 4.4 to 4.11.

4.5 Further Investigations

The results from the preliminary investigations of the ATP behaviour of the batch reactors treating thiocyanate solutions are discussed in detail later. On the basis of these results it was decided to continue the investigation, and the experiments described earlier were modified appropriately, particularly in respect of extending the range of parameters monitored during each run.

The parameters monitored during some or all of the experimental runs during this second series of runs were, pH and temperature, MLSS, ATP, thiocyanate, cyanide, ammonia, sulphate, nitrite and sulphide concentrations, and redox potential. The methods used for the determination of some of these parameters also required some investigations before they could be usefully applied to the batch experiments, and the details of these experiments are described in Chapter 5.

The principle design of this second series of batch experiments was essentially the same as that described in the first series. A summary of the parameters monitored, and of any variations in experimental methods employed in this second series of experimental

TABLE 4-2 RESULTS OF EXPERIMENT TH1

TIME HRS-MINS	CNS mg/l	A.T.P.		M.L.S.S. g/l
		FILTERED SLUDGE X10^6fg/ml	FILTRATE X10^7fg/ml	
0-00	5.5	0.512	2.825	6.70
1-05	301.4	2.335	3.547	5.22
1-20	293.2	0.434	4.110	5.08
1-45	276.7	0.470	3.845	5.33
2-00	260.3	0.461	-	-
2-15	274.0	0.886	-	6.34

TABLE 4-3 RESULTS OF EXPERIMENT TH2

TIME HRS-MINS	CNS mg/l	MLSS g/l
0-00	3.94	5.91
0-05	0	5.80
0-10	0	6.03
0.15	0	6.00
0.20	203	5.77
0-25	207	5.99
0-30	283	5.84
0-35	168	5.74
0-40	217	5.87
0-45	146	5.87
0-50	305	5.81
0-55	323	5.89
0.58	322	-
1.00	319	6.02
1-03	322	-
1-05	323	5.83
1-08	321	-
1-10	319	5.82
1-13	314	-
1-15	320	6.02
1-18	314	-
1-20	312	5.89
1-23	312	-
1-25	309	5.88
1-28	307	-
1-30	307	5.77
1-33	307	-
1-35	307	5.82
1-38	304	-
1-40	302	5.72

TABLE 4-4 - RESULTS OF DUPLICATE THIOCYANATE DETERMINATIONS (EXPERIMENT TH2)

TIME HRS-MINS	E^{1cm}_{515nm} TEST SOLUTION (DUPLICATE SAMPLES)	E^{1cm}_{515nm} MEAN VALUE	MEAN (CNS) mg/l
2-00	0.690 0.686	0.688	306
2-05	0.621 0.681	0.651	289
2-10	0.626 0.684	0.655	291
2-35	0.593 0.612	0.603	267
2-40	0.632 0.656	0.644	286
2-45	0.581 0.636	0.609	270
2-50	0.630 0.618	0.624	277
2-55	0.560 0.616	0.588	260

TABLE 4-5 RESULTS OF EXPERIMENT TH3

TIME HRS-MINS	CNS mg/l	A.T.P. FILTERED SLUDGE X10^7fg/ml	A.T.P. FILTRATE X10^6fg/ml	M.L.S.S. g/l
0-00	5.5	1.45	0.30	6.90
0-10	293.2	1.28	4.00	5.25
0-20	298.7	1.99	5.09	5.56
0-30	295.9	-	3.50	5.60
0-40	284.9	2.60	5.17	5.59
0-50	284.9	7.00	3.49	5.03
1-00	282.2	3.81	7.13	5.23
1-10	268.5	5.84	5.20	5.44
1-20	265.8	6.07	7.18	5.19
1-35	263.0	4.41	5.02	5.02
1-45	263.0	4.04	4.67	5.14
1-55	260.3	-	2.44	-
2-00	290.4	-	2.37	4.81
2-10	249.3	5.79	3.50	-
2-15	282.2	7.95	2.18	5.11
2-25	249.3	-	5.41	-
2-30	337.0	9.58	2.05	5.59
3-00	227.4	-	4.23	-

TABLE 4-6 RESULTS OF EXPERIMENT TH4

TIME HRS-MINS	CNS mg/l	ATP		MLSS g/l
		SLUDGE FILTRATE x10^5fg/ml	TOTAL SLUDGE x10^8fg/ml	
0-00	0	10.97*	-	-
0-03	-	-	2.04	-
0-05	0	3.053*	-	19.09
0-09	-	-	2.12	-
0-11	0	6.15*	-	15.01
0-13½	-	-	2.13	-
0-18	0	1.26*	-	18.90
0-20	-	-	1.75	-
0-23	0	0.96*	-	19.10
0-26	-	-	2.68	-
0-29	231	0.65*	-	18.66
0-31	-	-	3.51	-
0-33	282	0.46*	-	18.88
0-36	-	-	3.16	-
0-39	247	0.43*	-	18.38
0-42	-	-	3.84	-
0-44	251	1.493*	-	19.33
0-47	-	-	4.12	-
0-48	270	0.57	-	18.66
0-52	-	-	4.75	-
0-54	233	0.70	-	19.02
0-58	-	-	4.20	-
1-00	255	0.22	-	17.70
1-04	-	-	4.87	-
1-07	-	-	4.52	-
1-08	-	-	4.03	-
1-09	-	-	1.06	-
1-10	-	-	4.25	-
1-11	363	1.54*	-	18.13

* MEAN RESULT OF 2 SAMPLES.

TABLE 4-7 RESULTS OF EXPERIMENT TH5

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^7fg/ml	MLSS g/l
0-00	17	-	8.77
0-03	-	1.24	-
0-05	11	-	8.65
0-08	-	1.18	-
0-18	-	0.903	-
0-20	14	-	8.79
0-23	-	1.37	-
0-25	382	-	8.68
0-28	-	2.105	-
0-30	402.5	-	8.76
0-33	-	2.765	-
0-35	425.5	-	8.66
0-38	-	3.243	-
0-40	397	-	8.61
0-43	-	3.425	-
0-48	-	2.735	-
0-50	310.5	-	8.77
0-53	-	3.530	-
0-55	387	-	8.74
0-58	-	2.460	-
1-00	364	-	8.58
1-03	-	3.180	-
1-05	347.5	-	8.77
1-08	-	2.645	-
1-10	373	-	8.73
1-13	-	2.980	-
1-15	350	-	8.63
1-18	-	2.995	-
1-20	353	-	8.75

TABLE 4-7 continued

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^7$fg/ml	MLSS g/l
1-23		2.075	-
1-25		-	8.64
1-28		1.555	-
1-30		-	8.85
1-33		2.360	-
1-35		-	8.77
1-38		3.275	-
1-40		-	8.73
1-43		3.455	-
1-45		-	8.77
1-48		3.035	-
1-50		-	8.84
1-55		-	8.70
1-58		3.175	-
2-00		-	8.76
2-03		3.255	-
2-05		-	8.67
2-08		3.605	-
2-10		-	8.80
2-13		3.005	-
2-15		-	8.82
2-18		3.485	-
2-20		-	8.70
2-23		2.995	-
2-25		-	8.62
2-28		3.230	-
2-30		-	8.76
2-33		3.325	-
2-35		-	8.71

TABLE 4-7 continued

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^7$fg/ml	MLSS g/l
2-38		3.235	-
2-40		-	8.82
2-43		3.765	-
2-45		-	8.76
2-48		3.205	-
2-50		-	8.76
2-53		3.210	-
2-55		-	8.72
2-58		2.645	-
3-00		-	8.73
3-03		3.030	-
3.05		-	8.77
3-08		2.860	-
3-10		-	8.75
3-13		3.250	-
3-15		-	8.72
3-18		2.980	-
3-20		3.015	-
3.23		-	8.94
3-26		-	9.06
3-28		2.830	-
3-30		-	8.82
3-33		3.170	-
3-35		-	8.86
3-38		3.040	-
3-40		-	8.96
4-43		2.990	-
3-45		-	8.86
3-48		2.900	-

TABLE 4-7 continued

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^7$fg/ml	MLSS g/l
3-50		-	8.78
3-53		2.355	-
3-55		-	8.85
3-58		1.950	-
4-00		-	8.77
4-03		2.770	-
4-05		-	8.77
4-08		1.860	-
4-10		-	8.78
4-15		-	8.76
4-18		2.560	-
4-20		-	8.72
4-23		2.090	-
4-25		-	8.95
4-28		0.948	-
4-30		-	8.88
4-33		1.990	-
4.35		-	8.79
4-38		2.080	-
4-40		-	9.07
4-43		1.850	-
4-45		-	8.79
4-48		1.680	-
4-50		-	9.01
4-53		1.400	-
4-56		-	8.89
4-58		2.610	-
5-00		-	8.91
5-03		2.260	-
5-05		-	9.02

TABLE 4-7 continued

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^7fg/ml	MLSS g/l
5-08		2.870	-
5-10		-	8.89
5-13		2.740	-
5-15		-	8.89
5-18		3.230	-
5-20		-	8.98
5-23		2.960	-
5-25		-	8.94
5-28		2.865	-
5-30		-	8.85
5-33		3.110	-
5-35		-	8.99
5-39		3.0870	-
5-40		-	8.94
5-43		2.405	-
5-45		-	8.88
5-48		2.244	-
5-50		-	8.82
5-53		2.820	-
5-55		-	8.80
5-58		3.055	-
6-00		-	8.87
6-03		2.620	-
6-05		-	8.90
6-10		-	9.25
6-13		2.130	-
6-28		1.395	-
6-30		-	9.07
6-33		2.500	-

TABLE 4-7 continued

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^{7}fg/ml	MLSS g/l
6-35		-	9.02
6-38		3.050	-
6-40		-	8.97
6-43		1.080	-
6-45		-	9.14
6-48		1.420	-
6-50		-	9.10
6-53		1.280	-
6-55		-	9.28
6-58		1.200	-
7-00		-	9.32
7-03		1.290	-
7-07		-	9.24
7-12		-	9.17

TABLE 4-8 RESULTS OF EXPERIMENT TH6

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$fg/ml	pH
0-00	12	4.99	6.93
0-05	4	-	-
0-07	4	5.39	-
0-11	3	-	-
0-13	3	4.37	-
0-20	2	4.81	-
0-24	323	5.70	-
0-27	293	-	-
0-30	294	7.13	-
0-32	292	-	-
0-35	291	-	-
0-40	288	6.20	-
0-45	288	-	-
0-50	281	6.94	-
0-55	282	6.76	-
0-57	-	-	6.85
0-58	-	-	6.84
1-00	279	7.01	-
1-06	274	6.26	-
1-09	-	-	6.84
1-11	276	5.55	-
1-15	275	6.48	-
1-18	-	-	6.83
1-20	272	6.79	-
1-25	271	6.54	-
1-30	270	6.79	-
1-35	268	-	-
1-40	264	-	-
1-44	-	-	6.83
1-45	267	6.32	-

TABLE 4-8 continued

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$fg/ml	pH
1-50	262	-	6.83
1-56	265	-	-
2-00	260	6.05	-
2-05	262	6.39	-
2-10	257	-	6.82
2-15	250	-	-
2-20	253	6.60	5.82
2-30	251	6.26	6.82
2-35	248	-	-
2-40	248	-	-
2-46	248	6.42	-
2-50	247	6.32	-
2-53	-	-	6.82
2-55	243	-	-
3-00	242	-	-
3-05	242	-	-
3-11	236	6.85	6.81
3-15	237	-	-
3-29	-	-	6.81
3-30	231	-	-
3-35	228	6.32	6.81
3-40	-	7.10	-
3-45	216	6.54	-
3-48	-	-	6.81
3-53	-	-	6.80
3-56	222	6.26	-
4-00	223	6.42	-
4-05	216	7.29	-
4-10	220	-	-
4-15	220	6.67	-

TABLE 4-8 continued

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$fg/ml	pH
4-20	221	-	-
4-25	222	7.22	-
4-30	216	-	6.80
4.35	216	5.77	-
4.37	-	-	6.79
4-40	2.13	-	-
4-53	218	8.06	-
5-04	203	-	-
5-15	203	7.78	-
5-20	201	6.42	6.79
5-30	200	-	-
5-41	197	-	-
5-43	-	-	6.78
5-48	194	6.42	-
6-00	-	-	6.78
6-15	-	-	6.77
15-50	44	2.76	6.72
14-00	40	-	-
14-12	37	-	-
14-32	30	3.93	-
14-42	27	5.21	-
14-52	31	5.24	-
15.02	21	4.77	-
15-15	6	4.34	-
15-22	10	4.99	-
15-29	6	-	-
15-36	6	3.26	-
15-56	5	4.50	-
16-03	3	4.96	
16-11	3	-	-
16-15	4	-	-

TABLE 4-9 RESULTS OF EXPERIMENT TH7

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$fg/ml
0-00	23	12.35
0-10	9	11.01
0-20	10	10.77
0-30	125	14.91
0-40	118	13.80
0-52	116	15.22
1-00	110	14.45
1-10	100	15.66
1-20	97	15.47
1-39	88	15.63
1-46	79	15.93
1-55	72	15.59
2-04	66	14.28
2-08	59	16.18
2-14	60	12.43
2-19	42	12.06
2-31	27	-
2-51	22	10.68
2-55	19	-
2-56	11	-
3-10	6	-

TABLE 4-10 RESULTS OF EXPERIMENT TH8

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^8fg/ml
0-05	21	4.43
0-15	3	3.94
0-25	8	4.03
0-35	126	4.93
0-45	138	4.71
0-55	132	15.48
1-05	125	6.42
1-15	106	-
1-25	114	5.64
1-30	81	13.05
1-40	123	5.33
1-50	114	5.95
2-00	109	6.54
2-06	105	5.61
2-10	104	6.37
2-16	101	4.42
2-24	90	5.05
2-33	-	4.26
2-52	63	-
2-58	54.8	-
3-13	54.8	-
3-20	49.3	-

FIGURE 4.4 RESULTS OF EXPERIMENT TH1

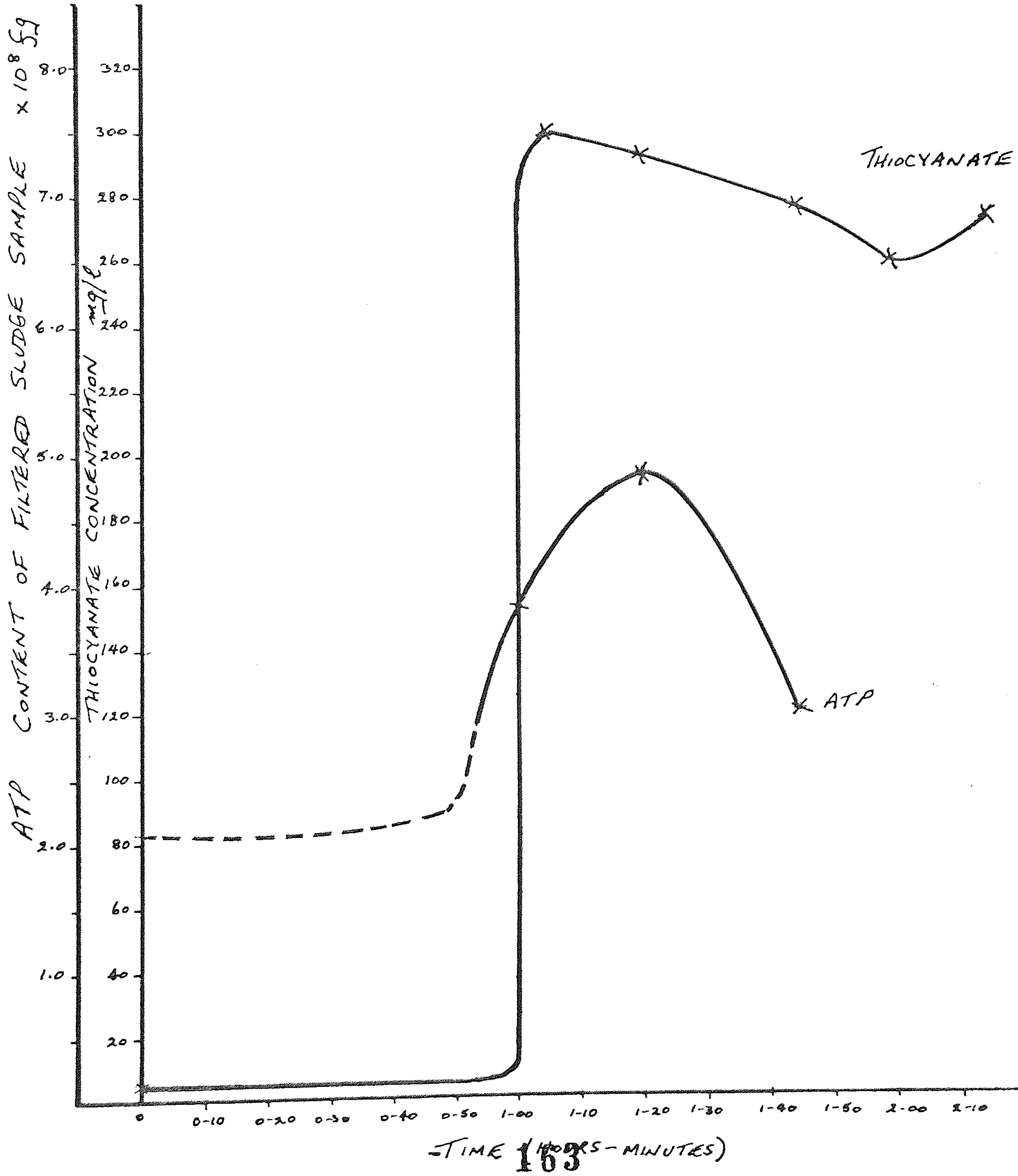


FIGURE 4.5 RESULTS OF EXPERIMENT TH2

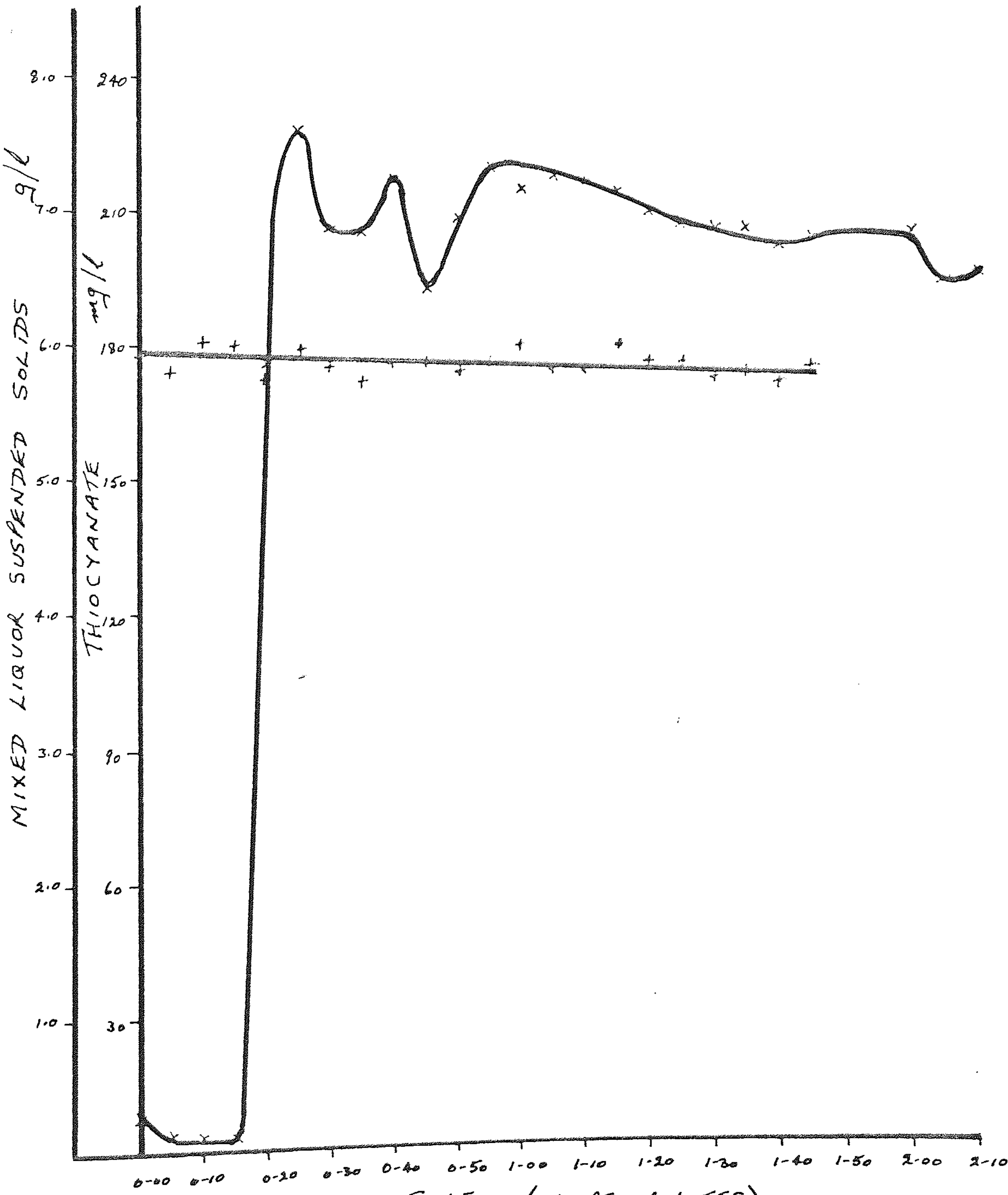


FIGURE 4.6 RESULTS OF EXPERIMENT TH3

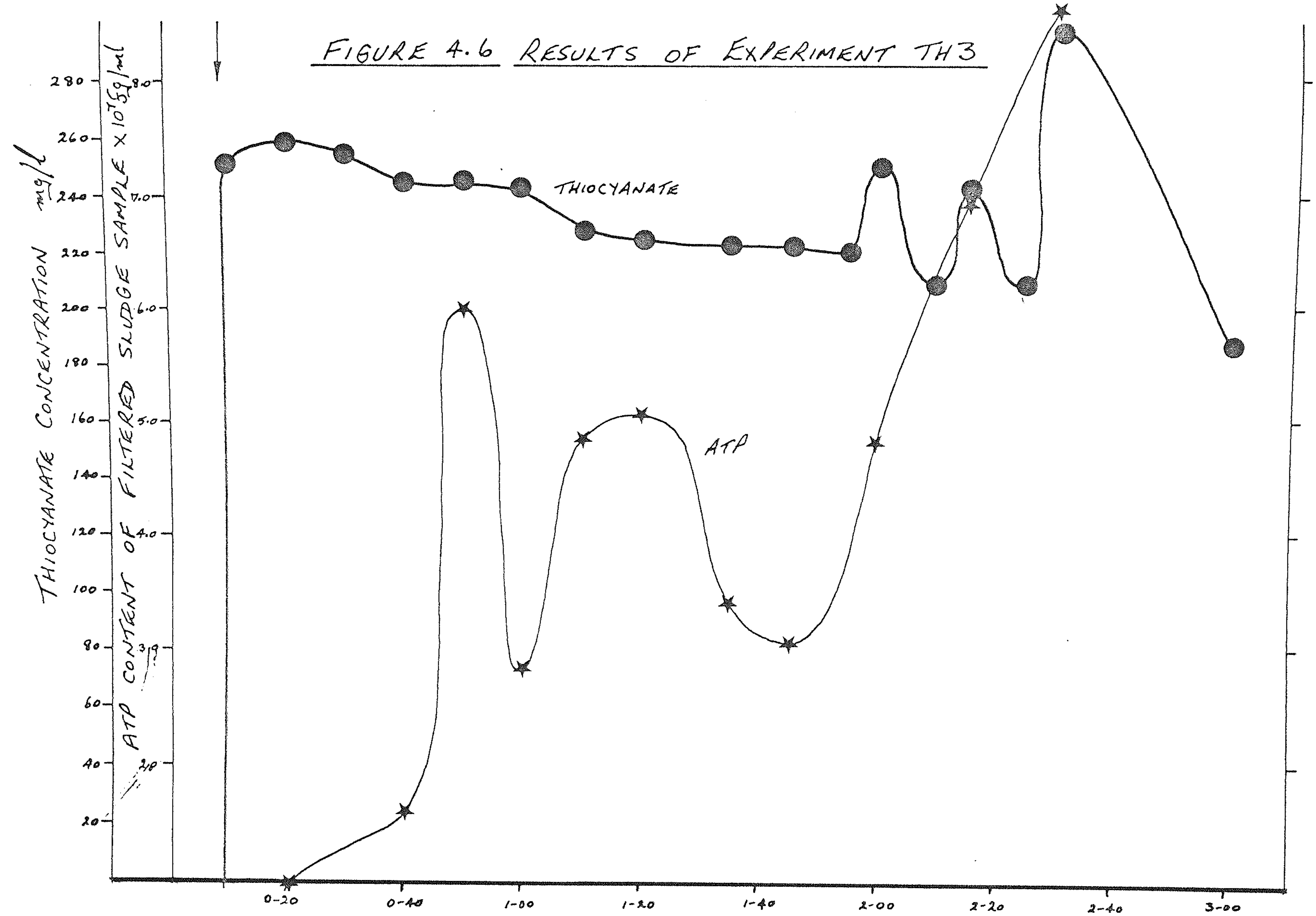
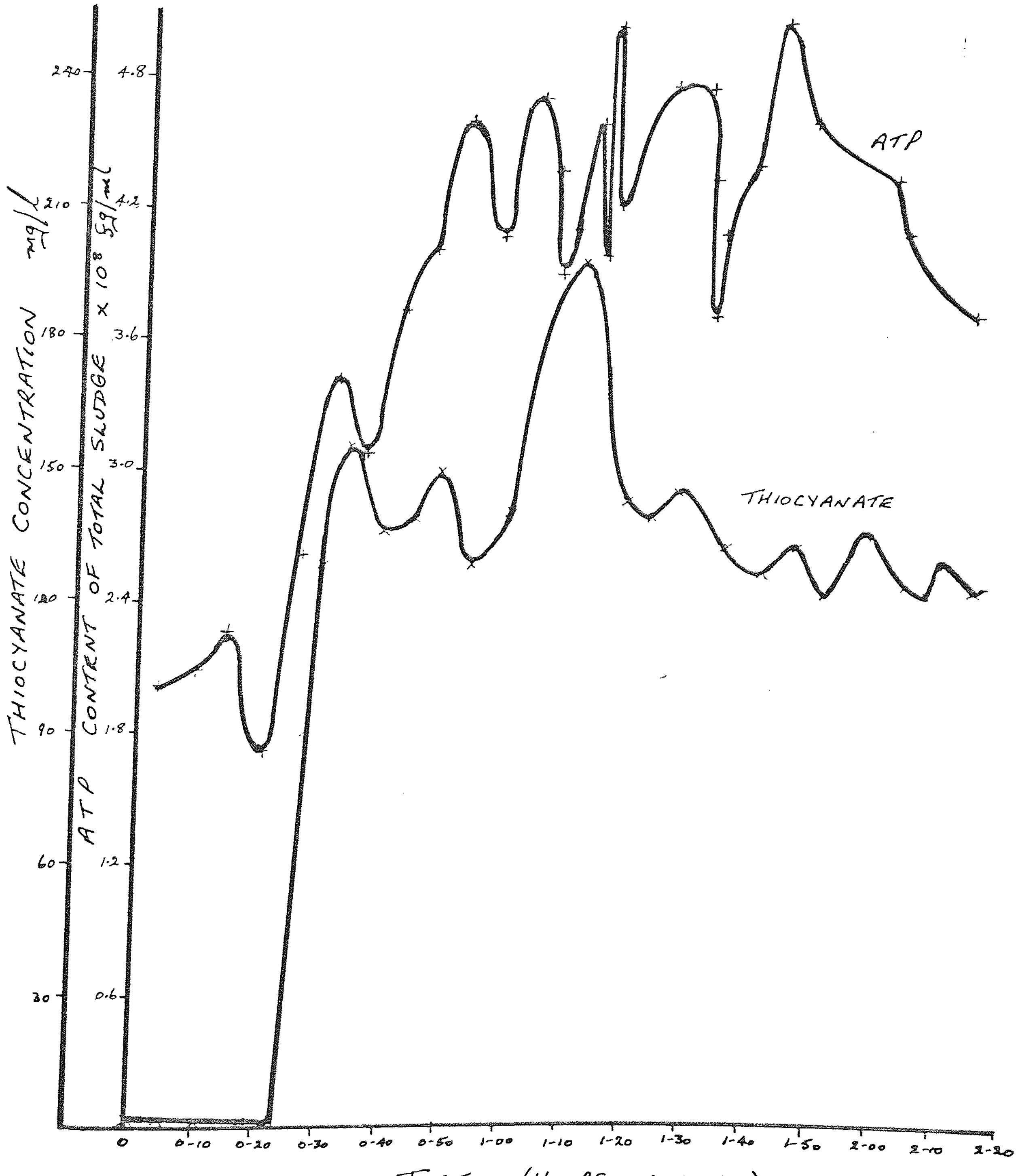


FIGURE 4.7 RESULTS OF EXPERIMENT TH4



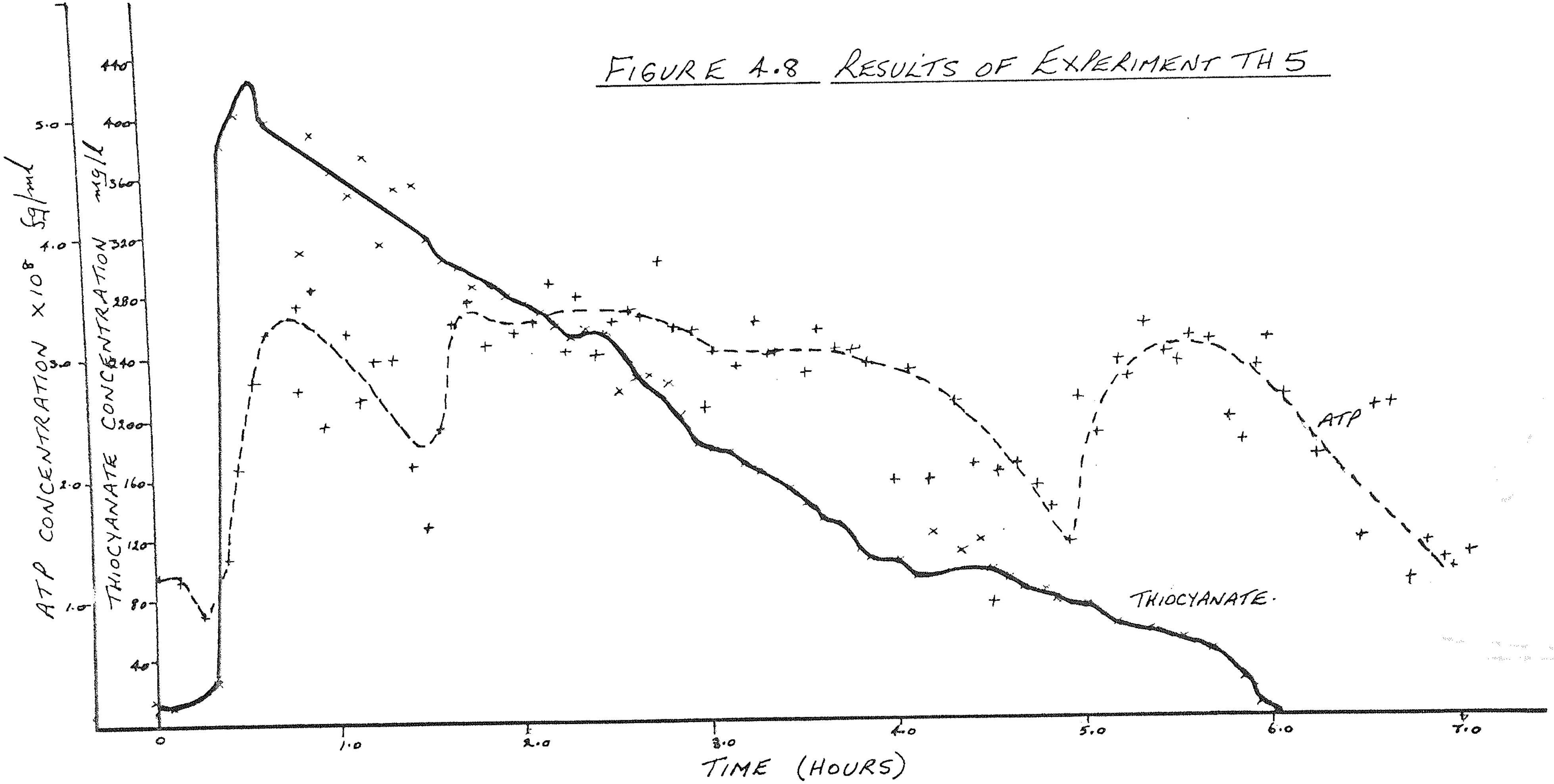


FIGURE A.8 RESULTS OF EXPERIMENT TH 5

FIGURE 4.9 RESULTS OF EXPERIMENT TH6

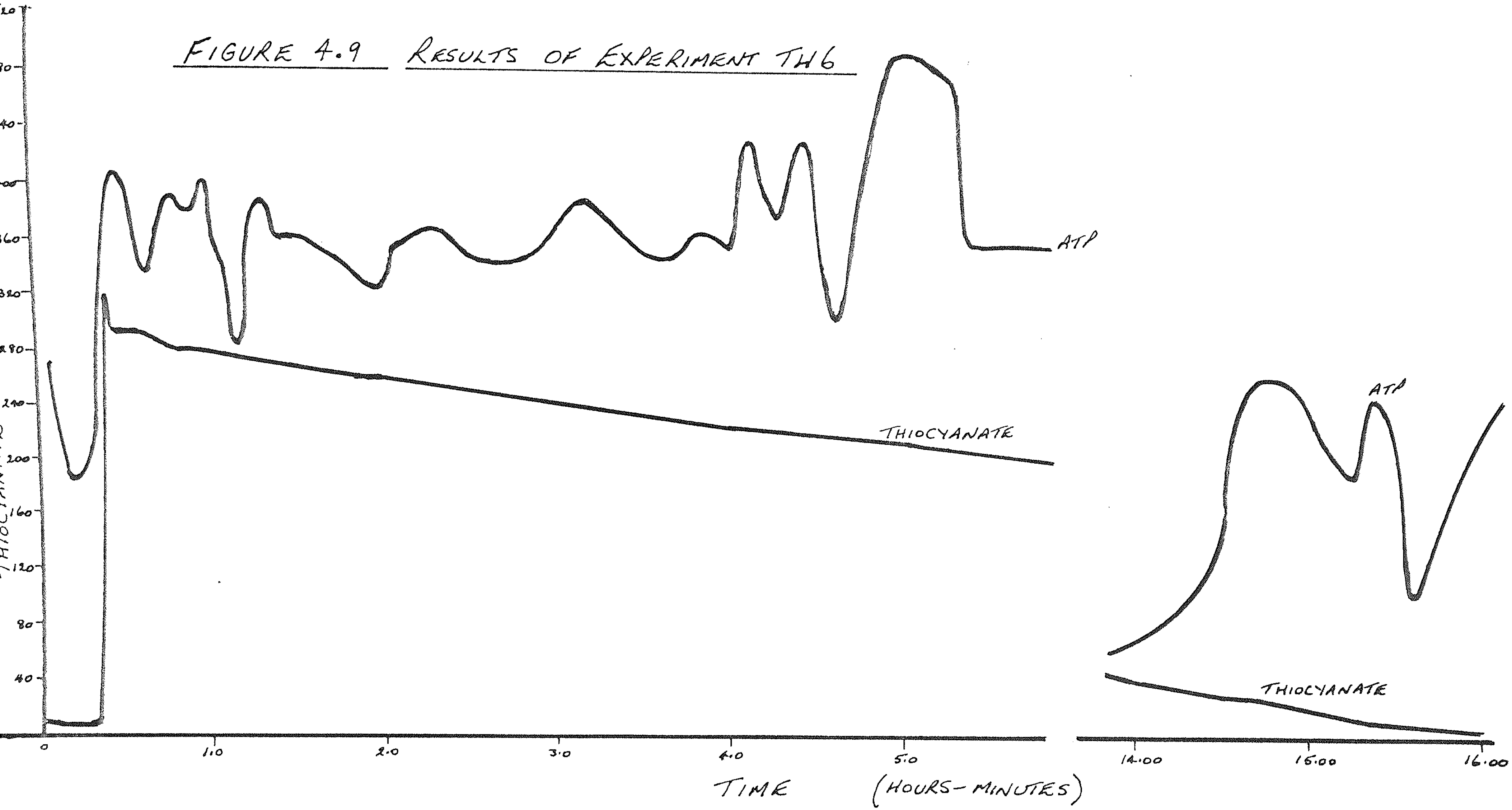
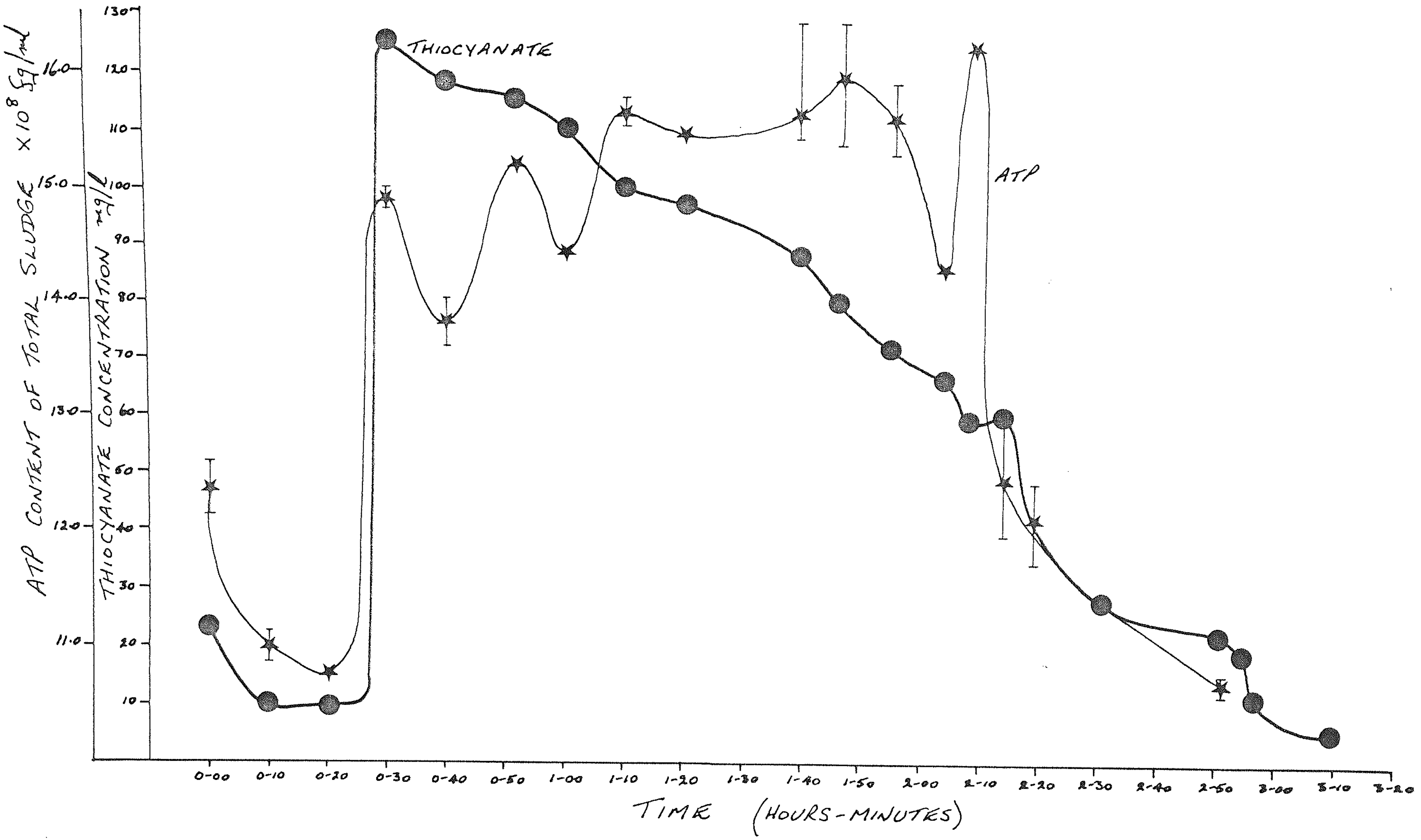


FIGURE 4.10 RESULTS OF EXPERIMENT TH7



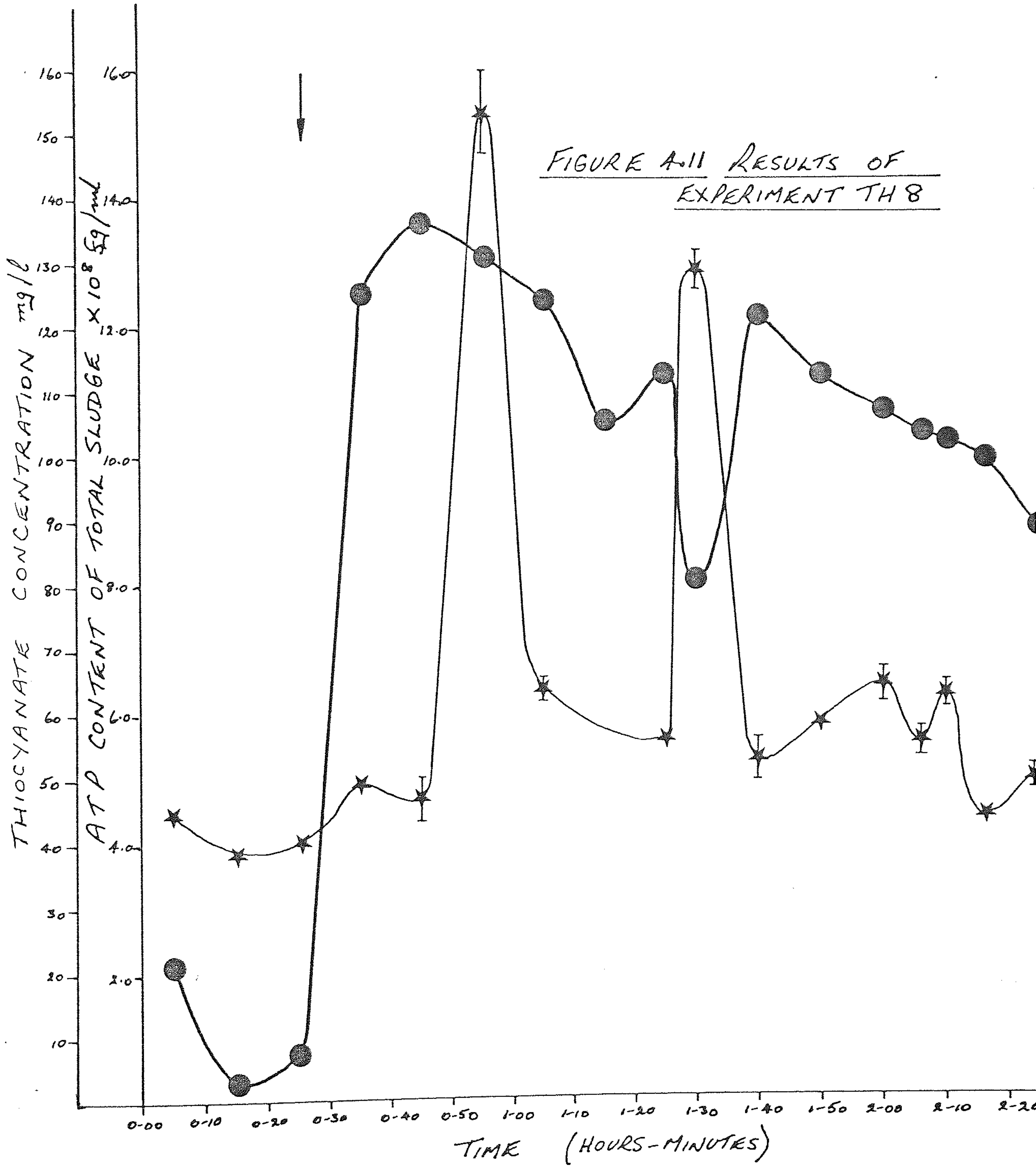


FIGURE A.11 RESULTS OF EXPERIMENT TH8

runs follows, and precedes a detailed account of those methods and materials not previously described.

4.5.1 Experimental Runs TH9-13

The actual period of starvation prior to the start of the experiment and addition of substrate in all of this second series of runs was not known. MLSS, pH, temperature, ATP and thiocyanate determinations were made using the methods previously described. Cyanide determinations were performed for runs TH9 and 10 using the cyanide electrode method described later. Ammonia concentrations were determined using the phenol-hypochlorite method described in Chapter 5.

Batch reactor B1 was used for runs TH9, 11, and 13, and reactor B2 for runs TH10 and 12.

4.5.2 Experimental Runs TH14-17

The parameters monitored for runs TH9-11 were also monitored in runs TH14-17, with the exception that no cyanide or MLSS determinations were made. In addition, redox potential was monitored in runs TH16 and 17 using the method described later.

Reactor B1 was used for runs TH14 and 15, and reactor B2 for runs TH16 and 17.

Unlike the experimental runs described earlier, in which consecutive runs for a given reactor, were carried out separated by a period varying from a day to several weeks, runs TH14 and 15 for reactor B1, and TH16 and 17 for reactor B2, followed each other immediately, another dose of substrate being added as soon as tests indicated that the previous addition had been oxidised.

4.5.3 Experimental Runs TH18-21

The parameters monitored for runs TH14-17 were also monitored in runs TH14-17, with the exception that redox potential measurements were only made for runs TH20 and 21. In addition, sulphate and nitrite determinations were made using the methods described later.

Batch reactor B1 was used for runs TH18 and 19, and reactor B2 was used for runs TH20 and 21. Runs TH18 and 19 for reactor B1, and runs TH20 and 21 for reactor B2, followed each other immediately.

4.5.4 Experimental Runs TH22 and 23

Runs TH22 and 23 used batch reactors B1 and B2 respectively. pH and temperature, and ATP, thiocyanate, and ammonia concentrations were monitored in both runs. Sulphate, nitrate, and nitrite concentrations were also monitored in run TH23 using the methods described later.

Table 4.32 summarises the experimental conditions and parameters monitored in runs TH9-23.

4.6 Methods and Materials

4.6.1 Cyanide

This was determined using the method described in Appendix 2.

4.6.2 Ammonia and Sulphate

These were determined using the methods described in Chapter 5.

4.6.3 Redox Potential

This was determined as outlined in Chapter 6.

4.6.4 Phenol

4-Aminophenazone Method

Accurate determinations of phenol in samples were made using this method, which was based on a method recommended by the Institution of Gas Engineers.(137) The principle of the method is that a red colour is produced as the result of the reaction of monohydric phenols with 4-aminophenazone, and the intensity is determined colorimetrically.

Reagents

Hydrochloric Acid Solution

50ml of concentrated hydrochloric acid were diluted to 100ml with water.

Ammonium Hydroxide Solution

10 ml of ammonium hydroxide solution (S.G. 0.88) were diluted to 100ml of water.

4-Aminophenazone Solution

2g of 4-aminophenazone were dissolved in 100mls of water. This was kept in an amber bottle and stored in a refrigerator at 2-5°C when not in use. This was discarded and fresh reagent prepared every 10-14 days,

Potassium Ferricyanide Solution

8g of potassium ferricyanide, $K_2Fe(CN)_6$, were dissolved in water and made up to 100mls. The reagent was stored in a refrigerator at 2-5°C when not in use. It was discarded and fresh reagent prepared every 10-14 days.

Stock Phenol Solution 1000mg/l

1. 00g of phenol was dissolved in water and made up to 1 litre in a volumetric flask.

Standard Phenol Solution 50mg/l

5.0ml of stock phenol solution was diluted to 100mls in a volumetric flask. This solution was freshly prepared as required.

Preparation of Calibration Graph

Aliquots of standard phenol solution were pipetted into 50ml volumetric flasks. Water was added to each flask to bring the volume to about 35mls. Two drops of hydrochloric acid solution and 5.0mls of ammonium hydroxide solution were then added to each and mixed. The pH of each solution was then adjusted to 9.6±0.2, using test papers, by the appropriate addition of acid or alkali solutions. 2.0ml of 4-aminophenazone solution were then added to each flask, mixed, made up to the mark with water, and mixed again. 1.0ml of potassium ferricyanide solution was then pipetted into each flask, the contents well mixed, and then allowed to stand for 10 minutes. A blank solution was prepared using the same procedure but omitting the addition of standard phenol solution. The optical density of each solution was determined in 10mm cells at 410nm, against a reference cell containing water, using a Unicam SP30 UV Spectrophotometer. The optical density of the blank solution was subtracted from that of the standard solutions.

A calibration graph was plotted relating optical density to mgs phenol in the 50ml flask.

Analysis of Samples

Samples were diluted, if necessary to produce solutions containing 10 to 20mg/l of phenol. Filtered samples were used wherever possible. Where settled or centrifuged samples were used, an additional solution was prepared for each sample, containing the same volume of sample as in the test solution, diluted with water to 50mls, but no reagents. The optical density of this solution was additionally subtracted from that of the test solution to correct for absorbance by fine suspended material .

Aliquots of the samples were pipetted into 50ml volumetric flasks and were analysed using the procedure described for the standard solutions above, and the phenol concentrations of the samples determined from the calibration graph.

4.6.5 Nitrite

The method used was that recommended by the Institute of Gas Engineers.(137)

Principle

The method depends on the formation of a pink azo-dye when the diazonium compound formed by the action of nitrous acid on sulphanilic acid is coupled with N- (1-naphthyl) ethylenediamine.

Reagents

Sulphanilic Acid Solution

30.0g of potassium hydrogen sulphate and 3.5g of sulphanilic acid were dissolved in water and diluted to 1 litre.

N- (1-Naphthyl) Ethylenediamine dihydrochloride Solution 0.04%

0.08g of the solid was dissolved in 200mls of water and stored in the dark.

Stock Nitrite Solution

0.370g of sodium nitrite, which had been previously dried at 105°C and stored in a desiccator over phosphorus pentoxide, was dissolved in freshly boiled and cooled water and diluted to 1 litre. The solution was kept in an amber flask and stored in the dark.

Standard Nitrite Solution 1.5mg/l Nitrite Nitrogen

20.0mls of the stock nitrite solution were diluted to 1 litre in a volumetric flask. This solution was freshly prepared before each batch of determinations.

Procedure

1, 2, 3, 4, and 5ml volumes respectively of standard nitrite solution were pipetted into five 50ml volumetric flasks. An appropriate volume of each sample, which had been previously filtered through a Millipore filter (Type HA 0.45) was pipetted into a 50ml volumetric flask. An additional flask containing water rather than standard or sample was prepared and was subsequently used as the reagent blank.

2.5ml of sulphanilic acid was pipetted into each flask and mixed, and after an interval of 10 minutes, 2.5ml of N-(1-naphthyl) ethylenediamine dihydrochloride solution added and mixed. The solutions were allowed to stand for 20 minutes, and then diluted to the 50ml mark with distilled water and mixed well.

The optical density of the contents of each flask was then measured with reference to the reagent blank in 1cm cells at 550nm using the Unicam SP30 UV Spectraphotometer.

A graph was plotted relating the concentration of nitrite-nitrogen against optical density readings, and this was used to determined the concentraion in samples. (Where samples were highly coloured an additional blank solution was prepared for each sample containing an equivalent volume of sample, with the N- (1-naphthyl) ethylenediamine dihydrochloride solution omitted).

4.6.6 Nitrate

The method used was that recommended by Pye Unicam for use with its SP series of spectrophotometers for the determination of nitrate in water.

Principle

Nitrate is determined directly spectrophotometrically in aqueous solutions on the basis of its strong absorbance at about 200nm. A correction has to be applied to this when the samples concerned contain organic constituents as these also absorb at this wavelength. The correction is generally applied by relating the absorbance at 200nm with that at the correction wavelength of 275nm.

Reagent

Twice Distilled Water

This was prepared by re-distilling deionised or distilled water from an all-Pyrex still. This was used for the preparation of all standard solutions, dilutions, and blanks.

Stock Nitrate Solution

0.7221g of Analar potassium nitrate was dissolved and made up to 1 litre with twice distilled water in a volumetric flask. (1ml solution ≡ 0.100mg nitrogen ≡ 0.443mg nitrate).

Standard Nitrate Solution

100ml of stock nitrate solution was diluted to 1 litre in a volumetric flask using twice distilled water. (1ml solution ≡ 10µg nitrogen ≡ 44.3µg nitrate).

Hydrochloric Acid IN

1 part concentrated hydrochloric acid was diluted with 11 parts of twice distilled water.

Calibration

0-50ml volumes of standard nitrate solution were pipetted into 50ml volumetric flasks and made up to the mark with twice distilled water to prepare nitrate standards in the range 0.10mg/l.

Procedure

Samples were filtered through Millipore filters (Type HA 0.45) after prior treatment with a small concentration of powdered activated carbon. The absorbance of standards and samples was read at 210nm and 275 nm using 1cm cells, using the Unicam SP30 UV spectrophotometer. The absorbance of the test solutions was determined with reference to twice distilled water in the reference cell. A calibration graph was drawn relating absorbance at 210nm with concentration of nitrate nitrogen in mg/l in the standards. Correction was made for the organic content of the samples by subtracting four times the 275nm value from the 210nm absorbance value;

$$A = A_{210} - 4\,A_{275}$$

4.7 Results

A summary of the Tables and Figures showing the results from individual experiments was recorded in Table 4.33.

23 batch experiments were conducted in the present investigation, the ATP behaviour being investigated in 20 of those. In those 20 experiments, the degradation of thiocyanate was monitored to completion or nearly so, in 13 of them (TH5-7, 9, 10, 12-18, and 21). It was possible in the majority of the 13 cases to establish a general trend in the ATP behaviour of the sludges. This was summarised in a general form in Figure 4.27, the main features of which could be summarised as follows;

1. The ATP level or pool in the sludge showed a large and almost immediate rise following the addition of thiocyanate, to a level that was, or approached, maximal for the experiment.

2. The ATP pool was maintained at a high and reasonably constant level for a period following substrate addition, and then declined graduatlly as thiocyanate degradation continued, reaching a minimum value at a thiocyanate concentration of about 80mg/l.

TABLE 4-11. RESULTS OF EXPERIMENT TH9.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$ fg/ml	MLSS g/l	CYANIDE mg/l
0-02	16	-	-	0.5-0.6
0-43	14	-	2.38	0.8
1-06	-	1.5435	-	-
1-10	12	-	2.63	-
1-15	10	-	2.59	-
1-23	-	-	-	-
1-26	-	2.4395	-	-
1-31	292	-	2.37	0.9
1-40	-	-	-	-
1-46	310	-	2.50	-
1-53	-	5.705	-	-
2-01	303	-	2.37	0.6
2-10	-	6.090	-	-
2-17	-	5.915	-	-
2-18	284	-	2.56	-
2-29	-	5.39	-	-
2-35	271	-	2.40	-
2-41	-	6.16	-	-
2-44	262	-	2.50	-
2.50	-	5.635	-	-
2-55	261	-	2.30	-
3-00	-	5.845	-	-
3-03	252	6.685	2.33	-
3-14	227	-	2.20	-

TABLE 4-11. RESULTS OF EXPERIMENT . CONTINUED.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^8 fg/ml	MLSS g/l	CYANIDE mg/l
3-22	-	6.335	-	-
3-32	-	6.230	-	-
3-35	213	-	2.39	-
3-42	-	6.195	-	-
3-47	218	-	2.36	-
3-54	6.230	-	-	-
4-00	199	-	2.22	-
4-13	-	5.985	-	-
4-20	178	-	2.19	-
4-28	-	-	-	-
4-46	157	-	2.21	-
4-57	-	5.670	-	-
5-01	138	-	2.16	-
5-12	121	-	2.03	-
5-17	-	6.125	-	-
5-31	-	5.040	-	-
5-42	98	-	2.15	-
5-52	-	3.990	-	-
5-57	100	-	2.22	-
6-01	-	3.220	-	-
6-02	94	-	2.20	-
6-06	-	3.430	-	-
6-07	83	-	2.17	-
6-12	-	3.465	-	-

TABLE 4-11. CONTINUED.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$fg/ml	MLSS g/l	CYANIDE mg/l
6-13	84	-	2.26	-
6-21	-	4.025	-	-
6-22	82	-	2.16	-
6-29	75	-	2.06	-
6-53	-	3.430	-	-
6-55	52	-	2.17	-
7-14	-	4.095	-	-
7-15	26	-	2.13	-
7-24	-	3.112	-	-
7-25	18	-	2.21	-
7-38	-	1.5435	-	-
7-39	16	-	2.22	-
7-45	17	-	1.98	-

TABLE 4-12. RESULTS OF EXPERIMENT TH10.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^8 fg/ml	MLSS g/l	CYANIDE mg/l
0-07	22	-	-	0
0-48	15	-	-	0.4
1-08	-	5.145	-	-
1-13	16	-	2.64	-
1-18	24	-	2.64	-
1-24	-	4.515	-	-
1-28	-	4.620	-	-
1-36	314	-	2.59	0.6
1-42	-	7.700	-	-
1-50	345	-	2.63	-
1-55	-	8.575	-	-
2-04	295	-	2.56	1.1
2-11	-	8.540	-	-
2-22	-	8.540	-	-
2-26	276	-	2.65	-
2-30	-	8.750	-	-
2-38	261	-	2.63	-
2-43	-	12.705	-	-
2-46	255	-	2.63	-
2-52	-	12.670	-	-
2-57	231	-	2.64	-
3-02	-	12.390	-	-
3-06	283	-	2.71	-

TABLE 4-12. RESULTS OF EXPERIMENT. CONTINUED.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$ fg/ml	MLSS g/l	CYANIDE mg/l
3-10	-	12.495	-	-
3-17	222	-	2.67	-
3-24	-	11.760	-	-
3-27	188	-	2.70	-
3-33	-	11.795	-	-
3-43	-	10.395	-	-
3-50	160	-	2.66	-
3-55	-	12.005	-	-
4-06	123	-	2.30	-
4-14	-	9.625	-	-
4-23	104	-	2.59	-
4-29	-	7.735	-	-
4-49	61	-	2.53	-
4-58	-	9.450	-	-
5-07	34	-	2.48	-
5-18	-	9.730	-	-
5-25	18	-	2.31	-
5-32	-	-	-	-
5-35	-	5.005	-	-
5-40	-	3.395	-	-
6-25	20	-	2.44	-
6-28	-	6.300	-	-

TABLE 4-13. RESULTS OF EXPERIMENT TH11.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^8 fg/ml	MLSS g/l	pH
0-02	9	-	-	-
0-05	-	-	-	7.00
0-26	11	-	1.18	-
0-46	264	-	1.63	-
1-07	-	3.3005	-	-
1-10	285	-	1.53	-
1-18	-	3.108	-	-
1-22	-	2.674	-	-
1-25	288	-	1.50	-
1-35	-	3.0835	-	-
1-39	-	3.297	-	-
1-57	-	3.269	-	-
2-00	291	-	1.56	-
2-05	-	3.2305	-	-
2-14	-	3.381	-	-
2-20	293	-	1.50	-
2-28	-	3.0135	-	-
2-34	299	-	1.57	-
2-45	-	2.9995	-	-
2-56	-	3.36	-	-
2-58	-	-	-	5.40
ADJUSTED TO pH 7.40 WITH 5% NaOH				
3-01	-	-	-	7.40
3-02	-	1.9075	-	7.40

TABLE 4-13. CONTINUED.

TIME HRS-MINS	CNS mg/1	ATP TOTAL SLUDGE X10^{8} fg/ml	MLSS g/1	pH
3-08	279	-	1.66	-
3-12	-	-	-	7.49
3-25	-	-	-	7.23
3-34	-	-	-	7.20
3-37	274	-	1.61	-
3-40	-	2.5515	-	-
3-43	-	3.29	-	-
3-49	-	3.0275	-	-
3.50	-	-	-	7.19
3-52	268	-	1.67	-
4-03	-	3.045	-	-
4-24	-	-	-	7.11
4-34	-	3.01	-	7.10
4-40	258	-	1.68	-
5-05	272	-	1.90	6.89
5-09	-	3.535	-	-
5-14	-	-	-	7.05
5-26	-	-	-	7.00
5-33	-	3.3565	-	-
5-35	-	-	-	7.00
5-39	248	-	-	-
6-09	-	-	-	6.99
6-12	242	-	1.66	-
6.15	-	3.57	-	-
7-07	203	-	1.58	-
7-11	-	3.381	-	6.90

TABLE 4-13. CONTINUED.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^8 fg/ml	MLSS g/l	pH
7-57	157	-	-	-
8-02	-	3.1815	-	-
10-39	-	-	-	7.15

TABLE 4-14. RESULTS OF EXPERIMENT TH12.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^{8} fg/ml	MLSS g/l	pH
0-05	-	-	-	7.0
0-08	7	-	-	-
0-32	4	-	1.12	-
0-49	239	-	1.17	-
0-56	-	4.305	-	-
1-08	-	4.655	-	-
1-14	248	-	0.94	-
1-19	-	4.655	-	-
1-23	-	5.285	-	-
1-29	2.39	-	1.09	-
1-36	-	4.69	-	-
1-40	-	4.445	-	-
1-58	-	4.305	-	-
2-06	209	-	1.13	-
2-09	-	4.62	-	-
2-15	-	4.62	-	-
2-25	182	-	1.06	-
2-32	-	4.55	-	-
2-48	-	4.62	-	-
2-50	145	-	1.08	-
3-03	-	-	-	6.8
3-05	-	4.48	-	-
3-20	109	-	1.85	-
3-42	-	3.57	-	-

TABLE 4-14. CONTINUED.

TIME HRS-MINS	CNS mg/1	ATP TOTAL SLUDGE X10^8 fg/ml	MLSS g/1	pH
3-45	59	-	-	-
3-48	-	3.29	-	-
4-04	-	3.395	-	-
4-24	8	-	0.93	-
4-30	76	-	0.94	-
4-35	-	3.115	-	-
5-29	5	-	-	-
8-04	-	2.0475	-	-
8-07	-	3.3635	-	-
8-16	-	3.024	-	-
8-50	-	3.2865	-	-

TABLE 4-15. RESULTS OF EXPERIMENT TH13.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^8 fg/ml	MLSS g/l	pH
-0-02	-	-	-	7.44
-0-01	-	3.406	-	-
0-02	0	-	8.46	-
0-04	-	-	-	7.43
0-05	-	2.951	-	-
0-06	0	-	-	-
0-10	-	-	-	7.42
0-11	-	5.285	-	-
0-12	318	-	-	7.40
0-14	-	-	-	7.36
0-16	-	4.830	-	7.33
0-17	351	-	-	-
0-21	-	7.035	-	-
0-22	321	-	-	-
0-27	-	6.790	-	7.25
0-28	287	-	-	-
0-30	-	-	-	7.23
0-34	-	-	-	7.20
0-35	-	8.435	-	7.21
0-36	248	-	-	-
0-41	-	6.020	-	7.20
0-43	212	-	8.74	-
0-45	-	-	-	7.19
0-46	-	11.410	-	-
0-51	161	-	8.55	-
0-53	-	-	-	7.19

TABLE 4-15. CONTINUED.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$ fg/ml	MLSS g/l	pH
0-54	-	13.475	-	-
0-59	-	-	-	7.18
1-00	-	13.825	-	-
1-02	105	-	8.83	-
1-07	-	7.105	-	-
1-08	119	-	8.93	7.18
1-10	-	-	-	7.19
1-12	-	13.720	-	-
1-13	-	-	-	7.18
1-14	36	-	8.91	-
1-17	-	10.500	-	7.17
1-23	0	-	9.01	-
1-27	-	-	-	7.18
1-29	-	-	-	7.23
1-29½	-	-	-	7.24
1-31	-	-	-	7.25
1-32	-	7.595	-	-
1-33	0	-	8.88	7.25
1-34	-	-	-	7.24
1-36	-	-	-	7.26
1-37	0	-	8.63	-
1-40	-	6.685	-	7.25
1-43	-	6.475	-	7.25

TABLE 4-15. CONTINUED.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^8 fg/ml	MLSS g/l	pH
1-44	0	-	-	-
1-46	-	-	-	7.24
1-49	-	4.515	-	7.24
1-50	0	-	-	-
2-00	-	-	-	7.22
2-00½	-	-	-	7.23
2-01	-	3.395	-	7.24
2-02	0	-	8.66	-
2-04	-	-	-	7.23
2-05	-	3.140	-	-
2-08	-	-	-	7.24
2-11	-	-	-	7.24
2-12	0	-	8.80	-
2-14	-	-	-	7.23
2-15	-	3.413	-	-
2-16	0	-	8.99	-
2-17	-	-	-	7.22
2-20	-	3.955	-	-
2-22	0	-	8.76	-
2-24	-	-	-	7.24
2-26	-	2.779	-	7.22

TABLE 4-15. CONTINUED.

TIME HRS-MINS	CNS mg/1	ATP TOTAL SLUDGE $X10^8$ fg/ml	MLSS g/1	pH
2-29	0	-	8.98	-
2-31	-	-	-	7.23
2-32	-	3.147	-	-

TABLE 4-16. RESULTS OF EXPERIMENT TH14.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$ fg/ml	AMMONIA mg/l	pH
-0-03	-	3.437	-	-
0-00	-	3.238	-	7.25
0-02	0	-	-	-
0-05	0	-	1783	-
0-07	-	3.465	-	-
0-31	-	3.535	-	-
0-33	0	-	2146	7.25
0-35	-	3.990	-	-
0-39	0	-	1730	-
0-40	-	3.535	-	-
0-45	5.0 mls STOCK CNS ADDED			
0-47	-	8.470	-	-
0-48	35	-	1741	-
0-51	-	3.710	-	-
0-52	28	-	1825	7.20
0-55	-	2.818	-	-
0-56	15	-	1813	7.20
1-02	21	-	1884	-
1-03	-	8.505	-	-
1-06	1	-	1825	-
1-07	-	7.280	-	7.20
1-10	9	-	1866	-
1-11	-	7.560	-	-
1-15	-	5.915	-	-
1-16	0	-	1783	-

TABLE 4-16. CONTINUED.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^3$ fg/ml	AMMONIA mg/l	pH
1-19	-	5.005	-	-
1-20	0	-	1902	-
1-23	-	-	-	7.20
1-29	0	-	1819	-
1-30	-	4.340	-	-
1-38	-	-	-	7.19
1-39	-	4.130	-	-
1-40	0	-	1842	-
1-42	-	-	-	7.19

TABLE 4-17. RESULTS OF EXPERIMENT TH15.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$ fg/ml	AMMONIA mg/l	pH
3-08	-	-	-	7.20
3-09	0	-	1771	-
3-10	-	3.99	-	-
3-13	-	2.905	-	-
3-15	0	-	1819	-
3-22	-	4.76	-	7.19
3-23	76	-	1866	-
3-29	-	5.28	-	-
3-30	66	-	1819	-
3-34	-	4.90	-	-
3-43	33	-	1777	-
3-47	-	7.17	-	-
3-53	-	6.16	-	-
3-54	-	-	-	7.29
3-55	5	-	1836	-
3-58	-	-	-	7.30
4-01	-	5.075	-	-
4-02	0	-	1878	-
4-10	-	5.11	-	7.30
4-11	0	-	1831	-
4-21	0	-	1824	-
4-25	-	5.285	-	-
4-27	0	-	1831	-
4-36	-	-	-	7.15
5-30	-	-	-	7.11

TABLE 4-18. RESULTS OF EXPERIMENT TH16.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^8 fg/ml	AMMONIA mg/l	pH	REDOX mV
-0-01	-	10.465	-	7.20	-
0-01	-	13.055	-	-	-
0-03	0	-	981	-	-
0-06	0	-	1022	-	-
0-08	-	10.920	-	-	-
0-32	-	12.110	-	-	89
0-34	0	-	1010	-	90
0-36	-	11.200	-	-	-
0-41	0	-	1016	-	-
0-42	-	12.600	-	-	-
0-45	-	-	-	-	89
0-48	-	-	-	-	52
0-49	-	26.705	-	-	52
0-50	-	-	1028	-	-
0-52	-	-	-	-	49
0-53	-	19.215	-	-	-
0-54	16	-	1034	-	-
0-55	-	-	-	-	49
0-57	-	19.285	-	-	-
0-58	13	-	1284	-	-
0-59	-	-	-	-	47
1-00	9	-	1022	-	-
1-01	-	27.930	-	-	-
1-02	-	-	-	-	48
1-04	-	25.305	-	-	-

TABLE 4-18. CONTINUED.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^{8}fg/ml	AMMONIA mg/l	pH	REDOX mV
1-05	10	-	1010	-	49
1-06	-	-	-	-	49
1-07	-	-	-	-	49
1-08	-	20.755	-	-	-
1-09	6	-	993	-	-
1-10	-	-	-	-	50
1-11	-	-	-	-	51
1-13	4	-	1004	-	-
1-14	-	19.355	-	-	-
1-15½	-	-	-	-	57
1-17	-	20.685	-	-	-
1-18	1	-	1088	-	-
1-19	-	-	-	-	61
1-21	-	27.300	-	-	-
1-23	0	-	1016	-	68
1-29	-	-	-	-	76
1-31	-	-	-	-	77
1-32	0	-	1082	-	-
1-34	0	-	1177	-	-
1-35	-	9.345	-	-	78
1-37	-	9.730	-	-	-
1-40	-	-	-	-	80
1-42	-	-	-	7.20	78

TABLE 4-19. RESULTS OF EXPERIMENT TH17.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^8 fg/ml	AMMONIA mg/l	pH	REDOX mV
3-09	-	-	-	7.20	-
3-10	-	-	-	-	-
3-11	0	-	1189	-	81
3-12	-	10.115	-	-	-
3-13	-	-	-	-	-
3-14	-	11.55	-	-	-
3-15	-	-	-	-	-
3-16	0	-	1094	-	-
3-19	-	-	-	-	82
3-20	STOCK CNS ADDED				
3-21	-	-	-	-	36
3-22	-	-	-	-	32
3-23	-	-	-	-	29
3-24	-	16.625	-	-	-
3-25	49	-	1010	-	-
3-26	-	-	-	-	24
3-29	-	-	-	-	-
3-30	-	-	-	-	20
3-31	-	21.91	-	-	-
3-34	-	-	-	-	21
3-42	29	-	1022	-	-
3-43	-	-	-	-	-

TABLE 4-19. CONTINUED.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$fg/ml	AMMONIA mg/l	pH	REDOX mV
3-44	-	22.05	-	-	-
3-47	-	-	-	-	-
3-52	-	21.805	-	-	-
3-53	-	-	-	-	25
3-55	-	-	-	-	-
3-56	15	-	1028	-	26
3-58	-	-	-	-	28
4-00	-	20.615	-	-	29
4-01	-	-	-	-	30
4-02	-	-	-	-	-
4-03	7	-	1022	-	-
4-07	-	22.26	-	-	35
4-08	7	-	1046	-	37
4-10	-	-	-	-	-
4-11	-	-	-	-	41
4-12	-	-	-	-	42
4-13	-	-	-	-	45
4-17	-	18.62	-	-	-
4-18	1	-	1117	-	59
4-20	-	-	-	-	61
4-21	-	-	-	-	-
4-25	-	-	-	-	-

TABLE 4-19. CONTINUED.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^{8}fg/ml	AMMONIA mg/l	pH	REDO mV
4-26	-	-	-	-	65
4-27	-	-	-	-	-
4-28	0	-	1076	-	-
4-29	-	11.515	-	-	-
4-36	-	-	-	-	69
4-37	-	-	-	7.10	-
5-30	-	-	-	-	74

TABLE 4-20. RESULTS OF EXPERIMENT TH18.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$ fg/ml	AMMONIA mg/l (MEAN)	p^H	NITRITE mg/l	SUBSTR mg/l
-0-01	-	-	-	6.90	-	-
	PH ADJUSTED WITH 5% NaOH					
0-00	-	-	-	7.50	-	-
0-01	12	-	1725	-	0	5.06
0-02	-	2.870	-	7.49	-	-
0-11	2	-	2141	-	0	6.4
0-20	-	3.332	-	-	-	-
0-28	-	3.091	-	-	-	-
0-30	-	-	-	7.40	-	-
0-34	20.0 mls STOCK CNS ADDED					
0-42	238	-	1684	-	0	6.2
0-44	-	6.475	-	-	-	-
0-52	-	-	-	7.20	-	-
0-53	-	6.720	-	-	-	-
0-54	248	-	2239	-	0	6.2
1-01	-	-	-	7.15	-	-
1-06	173	-	1796	-	0	4.7
1-09	-	6.580	-	-	-	-
1-11	188	-	2013	-	0	5.2
1-13	-	6.790	-	-	-	-
1-19	-	-	-	7.10	-	-
1-27	-	3.045	-	-	-	-
1-29	103	-	1745	-	0	4.8
1-31	-	3.64	-	-	-	-

TABLE 4-20. CONTINUED.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$ fg/ml	AMMONIA mg/l (MEAN)	pH	NITRITE mg/l	SULPHA mg/l
1-33	115	-	-	7.09	0	8.10
1-40	-	-	-	7.08	-	-
1-47	-	5.215	-	7.08	-	-
1-48	90	-	1931	-	0.40	6.5
1-53	-	5.425	-	-	-	-
2-07	-	4.55	-	-	-	-
2-08	1	-	1850	7.04	0	5.36
2-10	-	4.41	-	-	-	-
2-11	2	-	2077	-	0	7.39
2-20	-	-	-	7.11	-	-

TABLE 4-21. RESULTS OF AMMONIA DETERMINATIONS IN EXPERIMENT TH18.

TIME HRS-MINS	AMMONIA mg/l	MEAN VALUE mg/l
0-01	1752 1697	1725
0-11	2175 2107	2141
0-42	1710 1657	1684
0-54	2275 2203	2239
1-06	1824 1767	1796
1-11	2045 1980	2013
1-29	1773 1717	1745
1-48	1962 1900	1931
2-08	1879 1820	1850
2-11	2110 2043	2077

TABLE 4-22. RESULTS OF EXPERIMENT TH19

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $X10^8$ fg/ml	AMMONIA mg/l (MEAN)	pH	NITRITE mg/l	SULPHAT mg/l
3-36	-	-	-	7.19	-	-
3-43	-	4.025	-	-	-	-
3-44	21	-	2165	-	2.20	6.90
3-51	-	3.325	-	-	-	-
3-53	-	-	-	-	-	-
3-53	-	-	-	7.19	-	-
3-54	-	7.525	-	-	-	-
3-55	534	-	1941	-	0.39	5.46
3-58	-	3.378	-	-	-	-
3-59	613	-	1979	-	3.22	6.44
4-01	-	3.78	-	7.02	-	-
4-02	615	-	2053	7.05	0	6.05
4-04	-	-	-	7.00	-	-
4-05	674	-	2046	-	0.16	5.83
4-07	-	8.05	-	6.99	-	-
4-40	556	-	1708	-	0.57	5.53
4-42	-	7.035	-	6.85	-	-
4-45	-	4.445	-	-	-	-
4-46	512	-	2056	-	0	6.44
4-55	-	-	-	6.85	-	-
5-05	-	3.50	-	-	-	-
5-15	-	4.55	-	-	-	-
5-23	-	10.605	-	-	-	-

TABLE 4-22. RESULTS OF EXPERIMENT TH19, CONTINUED.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^2 fg/ml	AMMONIA mg/l (MEAN)	pH	NITRITE mg/l	SULPHA mg/l
5-25	-	-	-	6.79	-	-
5-31	336	-	1847	-	1.04	6.01
5-33	-	8.82	-	-	-	-
5-34	-	-	-	6.75	-	-
5-36	356	-	2152	-	0.09	6.21
5-57	-	3.059	-	-	-	-
6-02	250	-	1918	-	0.21	5.2
6-03	-	-	-	6.69	-	-
6-24	-	8.61	-	-	-	-
6-26	181	-	2206	-	0.47	7.8
6-31	-	-	-	6.60	-	-
6-38	-	8.575	-	-	-	-
6-40	177	-	2107	-	6.81	7.5
7-05	-	4.41	-	-	-	-
7-10	74	-	1826	-	-	-
7-26	-	-	-	6.52	-	-
7-28	-	2.230	-	-	-	-
7-30	50	-	2168	-	0.33	5.7
7-36	-	3.367	-	6.60	-	-
8-19	-	-	-	6.70	-	-

TABLE 4-23. RESULTS OF AMMONIA DETERMINATIONS IN EXPERIMENT TH19.

TIME HRS-MINS	AMMONIA mg/l	MEAN VALUE mg/l
3-44	2199 2130	2165
3-55	1972 1910	1941
3-59	2010 1947	1979
4-02	2086 2020	2053
4-05	2079 2013	2046
4.40	1735 1680	1708
4-46	2089 2023	2056
5-31	1876 1817	1847
5-36	2186 2117	2152
6-02	1948 1887	1918
6-26	2241 2170	2206
6-40	2141 2073	2107
7-10	1797 1855	1826
7-30	2203 2133	2168

TABLE 4-24. RESULTS OF EXPERIMENT TH20.

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE $\times 10^8$ fg/ml	AMMONIA mg/l (MEAN)	pH
0-00	-	-	-	7.19
0-02	-	-	-	-
0-03	-	15.47	-	-
0-04	37	-	1034	-
0-07	32	-	1262	-
0-08	-	-	-	-
0-22	38	-	1189	-
0-30	-	-	-	-
0-31	-	12.915	-	-
0-35½	20.0 mls STOCK CNS ADDED			
0-38	235	-	1386	-
0-39	-	32.025	-	-
0-52	-	24.36	-	-
0-53	-	-	-	-
0-58	242	-	1341	-
1-00	-	32.55	-	-
1-01	192	-	1311	-
1-02	-	-	-	-
1-05	-	33.005	-	-
1-11	-	-	-	-
1-17	152	-	1092	-
1-18	-	22.925	-	-
1-31	-	-	-	-

TABLE 4-24. CONTINUED.

TIME HRS-MINS	CNS mg/1	ATP TOTAL SLUDGE X10^{8} fg/ml	AMMONIA mg/1 (MEAN)	p^{H}
1-33	-	-	-	-
1-36	-	24.745	-	-
1-38	84	-	1164	-
1-40	-	-	-	-
1-42	-	24.29	-	-
1-43	78	-	1519	-
1-47	-	-	-	-
1-52	-	24.92	-	-
1-54	73	-	1563	-
1-59	47	-	1596	-
2-01	-	20.93	-	-
2-13	-	11.655	-	-
2-21	31	-	1266	-
2-24	-	-	-	-
2-25	-	-	-	7.10
2-35	-	-	-	-
2-36	-	-	-	-
3-45	-	-	-	7.10

TABLE 4-24. CONTINUED.

TIME HRS-MINS	REDOX mV	NITRITE mg/l	SULPHATE mg/l
0-00	80	-	-
0-02	80	-	-
0-03	-	-	-
0-04	-	0.91	4.38
0-07	-	0.73	6.76
0-08	-	-	-
0-22	83	0.55	6.21
0-30	83	-	-
0-31	-	-	-
0-35½	20 mls STOCK CNS ADDED		
0-38	19	0.58	5.95
0-39	15	-	-
0-52	3	-	-
0-53	2	-	-
0-58	4	0.36	6.35
1-00	-	-	-
1-01	-	3.46	5.83
1-02	7	-	-
1-05	-	-	-
1-11	7	-	-
1-17	10	0.01	5.23
1-18	11	-	-
1-31	18	-	-
1-33	19	-	-

TABLE 4-24. CONTINUED.

TIME HRS-MINS	REDOX mV	NITRITE mg/l	SULPHATE mg/l
1-36	-	-	-
1-38	22	1.38	4.12
1-40	23	-	-
1-42	-	-	-
1-43	26	3.68	7.15
1-47	30	-	-
1-52	-	-	-
1-54	38	2.16	6.76
1-59	-	1.30	7.74
2-01	-	-	-
2-13	-	-	-
2-21	-	3.51	5.89
2-24	ca 60	-	-
2-25	-	-	-
2-35	ca 64	-	-
2-36	76	-	-
3-45	-	-	-

TABLE 4-25. RESULTS OF AMMONIA DETERMINATIONS IN EXPERIMENT TH20

TIME HRS-MINS	AMMONIA mg/l	MEAN VALUE mg/l
0-04	1042 1046 1013	1034
0-07	1286 1270 1230	1262
0-22	1198 1160 1210	1189
0-38	1408 1363	1386
0-58	1386 1339 1297	1341
1-01	1325 1283 1324	1311
1-17	1108 1073 1096	1092
1-38	1170 1133 1190	1164
1-43	1525 1477 1556	1519
1-54	1592 1573 1523	1563
1-59	1611 1560 1618	1596
2-21	1256 1217 1324	1266

TABLE 4-26. RESULTS OF EXPERIMENT TH21

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^8 fg/ml	AMMONIA mg/l (MEAN)	pH
4-51	20	-	1211	-
4-54	-	14.665	-	-
5-16	-	20.02	-	-
5-17	37	-	1366	-
5-19	40 mls STOCK CNS ADDED			
5-20	-	29.68	-	-
5-21	499	-	1432	-
5-25	-	-	-	-
5-28	612.5	-	1462	-
5-30	-	24.605	-	-
5-33	-	-	-	-
5-58	-	27.51	-	-
6-00	315	-	1207	-
6-03	-	-	-	-
6-25	-	28.105	-	-
6-29	339	-	1195	-
6-32	-	-	-	7.08
6-39	-	31.045	-	-
6-43	312	-	1314	-
7-06	-	25.865	-	-
7-07	259	-	1551	-
7-26	-	-	-	-
7-29	-	24.85	-	-
7-33	144	-	1327	-

TABLE 4-26. CONTINUED

TIME HRS-MINS	CNS mg/l	ATP TOTAL SLUDGE X10^8 fg/ml	AMMONIA mg/l (MEAN)	pH
7-39	132	-	1630	-
8-13	36	-	1626	-
8-16	-	12.705	-	-
8-17	20	-	1750	-
8-20	-	12.565	-	-
8-22	-	-	-	7.20
8-50	-	-	-	-

TABLE 4-26. RESULTS OF EXPERIMENT TH21

TIME HRS-MINS	REDOX mV	NITRITE mg/l	SULPHATE mg/l
4-51	80	2.04	4.98
4-54	-	-	-
5-16	-	-	-
5-17	81	0.44	5.85
5-19	40 mls STOCK CNS ADDED		
5-20	8	-	-
5-21	3	0.97	7.25
5-25	-7	-	-
5-28	-12	1.22	7.27
5-30	-	-	-
5-33	-13	-	-
5-58	-12	-	-
6-00	-	0.22	5.66
6-03	-11	-	-
6-25	-7	-	-
6-29	-6	0.04	4.97
6-32	-	-	-
6-39	-	-	-
6-43	-3	0.49	6.13
7-06	2	-	-
7-07	3	0.82	6.03
7-26	9	-	-
7-29	-	-	-

TABLE 4-26. CONTINUED.

TIME HRS-MINS	REDOX mV	NITRITE mg/l	SULPHATE mg/l
7-33	13	1.96	5.05
7-39	16	0.01	6.90
8-13	63	1.38	5.91
8-16	-	-	-
8-17	-	0.51	7.69
8-20	-	-	-
8-22	66	-	-
8-50	29	-	-

TABLE 4-27. RESULTS OF AMMONIA DETERMINATIONS, IN EXPERIMENT TH21.

TIME HRS-MINS	AMMONIA mg/l	MEAN VALUE mg/l
4-51	1242 1215 1177	1211
5-17	1388 1377 1333	1366
5-21	1442 1397 1458	1432
5-28	1477 1430 1480	1462
6-00	1218 1180 1222	1207
6-29	1212 1205 1167	1195
6-43	1348 1318 1277	1314
7-07	1550 1576 1527	1551
7-33	1360 1332 1290	1327
7-39	1638 1587 1666	1630
8-13	1635 1583 1660	1626
8-17	1752 1697 1800	1750

TABLE 4-28.RESULTS OF EXPERIMENT TH22

TIME HRS-MINS	CNS mg/l	AMMONIA mg/l	pH
0.00	1	1142	6.51
0-12	1	1276	6.53
0-18	1	1251	6.51
0-22	5	1026	-
0-28	PH ADJUSTED TO 7.32		
0-30	2	1320	7.38
0-40	1	1137	7.37
0-44	20 mls STOCK CNS ADDED		
0-45	220	1036	7.35
0-50	255	1156	-
1-00	234	1185	7.18
1-05	230	1226	7.13
1-18	191	1215	-
1-23	176	1244	-
1-29	157	1040	7.04
1-34	-	1124	7.02

TABLE 4-28 CONTINUED

TIME HRS-MINS	CNS mg/l	AMMONIA mg/l	pH
1-38	-	971	7.03
1-43	-	1137	-
1-47	-	1171	7.02
1-56	-	1150	7.01
2.00	-	1170	7.02
2-07	-	1017	7.02
2.11	-	1257	7.02
2.15	-	1286	7.02
2.20	-	1095	-
2-33	-	1161	7.11
2-40	-	1049	7.14

TABLE 4-29. RESULTS OF AMMONIA DETERMINATIONS IN EXPERIMENT TH22.

TIME HRS-MINS	AMMONIA mg/l	MEAN VALUE mg/l
0.00	1044 1283 1100	1142
0-12	1228 1361 1238	1276
0-18	1139 1371 1242	1251
0.22	965 1064 1050	1026
0-30	1288 1346 1327	1320
0-40	1014 1107 1290	1137
0-45	1005 1072 1030	1036
0-50	1112 1153 1203	1156
1-00	1158 1192 1206	1185

TABLE 4-29. CONTINUED

TIME HRS/MINS	AMMONIA mg/l	MEAN VALUE mg/l
1-05	1185 1239 1255	1226
1-18	1135 1295	1215
1-23	1182 1260 1290	1244
1-29	971 1086 1063	1040
1-34	1024 1143 1205	1124
1-38	907 988 1019	971
1-43	1094 1165 1153	1137
1-47	1130 1211 1173	1171
1-56	1080 1115 1254	1150
2-00	1054 1210 1247	1170
2-07	967 995 1090	1017
2-11	1222 1255 1295	1257

TABLE 4-29. CONTINUED.

TIME HRS-MINS	AMMONIA mg/1	MEAN VALUE mg/1
2.15	1238 1312 1309	1286
2-20	1055 1091 1139	1095
2-33	1088 1183 1212	1161
2-40	1042 1055 1050	1049

TABLE 4-30. RESULTS OF EXPERIMENT TH 23.

TIME HRS-MINS	CNS mg/l	AMMONIA mg/l (MEAN)	pH	NITRATE mg/l	SULPHATE mg/l
0-15	8	414	7.19	7.14	-
0-27	6	2264	7.17	0	-
0-31	5	2265	-	6.22	-
0-36	4	2315	7.16	1.57	-
0-43	15 mls STOCK CNS ADDED				
0-45	191	2284	-	5.62	6.72
0-48	211	2291	-	6.54	7.32
0-52	192	2270	7.12	5.88	7.01
0-55	183	2279	7.11	-	7.29
1-03	159	2290	7.11	0	7.37
1-08	137	2251	-	0	7.46
1-11	125	2330	-	0	7.44
1-21	83	2294	7.07	4.18	7.06
1-24	69	2334	-	0	7.25
1-29	47	2307	7.05	1.31	7.17
1-32	31	2293	7.06	-	6.76
1-36	15	2399	7.06	0	6.70
1-47	5	2323	7.07	3.53	6.87
1-54	2	2368	7.05	3.40	7.15
2-00	2	2378	-	1.44	7.22
2-08	3	2334	-	-	6.99
2-12	5	2372	7.06	0	6.90
2-20	4	2371	7.05	-	6.90

TABLE 4-31. RESULTS OF AMMONIA DETERMINATIONS IN EXPERIMENT TH23

TIME HRS-MINS	AMMONIA mg/l	MEAN VALUE mg/l
0-15	414	414
0-27	2252 2275	2264
0-31	2268 2261	2265
0-36	2306 2324	2315
0-45	2309 2259	2284
0-48	2294 2288	2291
0-52	2270	2270
0-55	2276 2281	2279
1-03	2290	2290
1-08	2188 2313	2251
1-11	2326 2333	2330
1-21	2294	2294
1-24	2319 2349	2334
1-29	2298 2316	2307
1-32	2293	2293
1-36	2399	2399
1-47	2297 2348	2323
1-54	2397 2338	2368
2-00	2400 2356	2378

TABLE 4-31. CONTINUED.

TIME HRS-MINS	AMMONIA mg/l	MEAN VALUE mg/l
2-08	2323 2345	2334
2-12	2402 2341	2372
2-20	2371	2371

TABLE 4-32 SUMMARY OF EXPERIMENTAL CONDITIONS AND PARAMETERS MONITORED IN RUNS TH9-23

Exptl runs	Stock cns added	Reactor		Starvation period hrs	Ph	ATP			MLSS	CNS	CN	NH_3	SO_4	NO_2	NO_3	Redox Potentia
		B1	B2			Sludge	Filtrate	Total								
TH9	20	+	-	NR	-	-	-	+	+	+	+	+	-	-	-	-
TH10	20	-	+	NR	-	-	-	+	+	+	+	+	-	-	-	-
TH11	20	+	-	NR	+	-	-	+	+	+	-	+	-	-	-	-
TH12	20	-	+	NR	-	-	-	+	+	+	-	+	-	-	-	-
TH13	20	+	-	NR	+	-	-	+	+	+	-	+	-	-	-	-
TH14	5	+	-	NR	+	-	-	+	-	+?	-	+	-	-	-	-
TH15	10	+	-	NR	+	-	-	+	-	+?	-	+	-	-	-	-
TH16	5	-	+	NR	+	-	-	+	-	+?	-	+	-	-	-	+
TH17	10	-	+	NR	+	-	-	+	-	+?	-	+	-	-	-	+
TH18	20	+	-	NR	+	-	-	+	-	+	-	+	+	+	-	-
TH19	40	-	+	NR	+	-	-	+	-	+	-	+	+	+	-	-
TH20	20	-	+	NR	+	-	-	+	-	+	-	+	+	+	-	+
TH21	40	-	+	NR	+	-	-	+	-	+	-	+	+	+	-	+
TH22	20	+	-	NR	+	-	-	+	+	+?	-	+?	-	-	-	-
TH23	15	-	+	NR	+	-	-	+	+	+	-	+	+	+	+	-

TABLE 4.33

SUMMARY OF TABLES AND FIGURES SHOWING RESULTS FROM INDIVIDUAL EXPERIMENTS

EXPT.	TABLES	FIGURES
TH1	4.2	4.4
TH2	4.3, 4.4	4.5
TH3	4.5	4.6
TH4	4.6	4.7
TH5	4.7	4.8
TH6	4.8	4.9
TH7	4.9	4.10
TH8	4.10	4.11
TH9	4.11	4.12
TH10	4.10	4.13
TH11	4.13	4.14
TH12	4.14	4.15
TH13	4.15	4.16
TH14	4.16	4.17
TH15	4.17	4.18
TH16	4.18	4.19
TH17	4.19	4.20
TH18	4.20, 4.21	4.21
TH19	4.22, 4.23	4.22
TH20	4.24, 4.25	4.23
TH21	4.26, 4.27	4.24
TH22	4.28, 4.29	4.25
TH23	4.30, 4.31	4.26

FIGURE 4.12 RESULTS OF EXPERIMENT TH 9

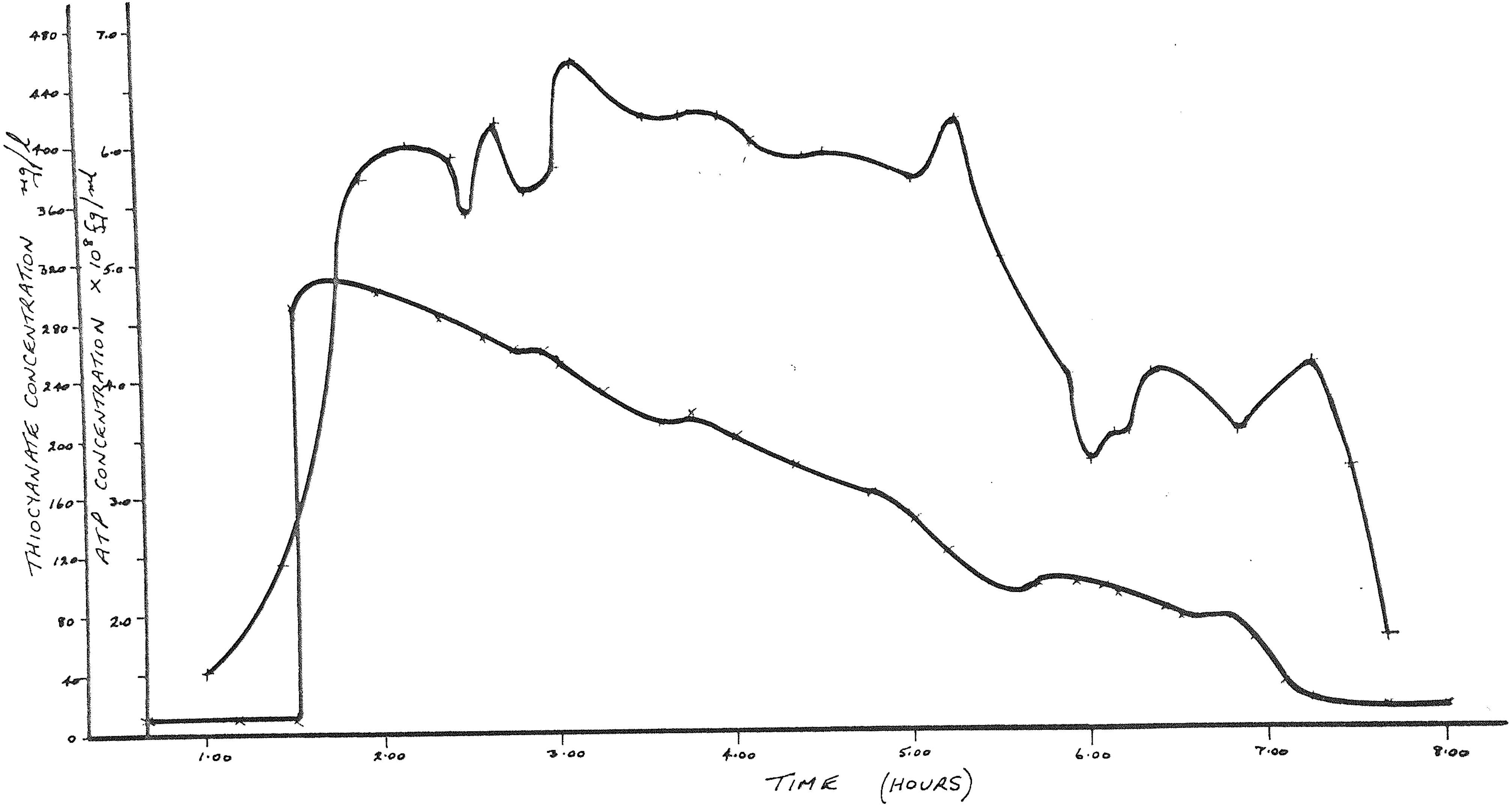


FIGURE 4.13 RESULTS OF EXPERIMENT TH10

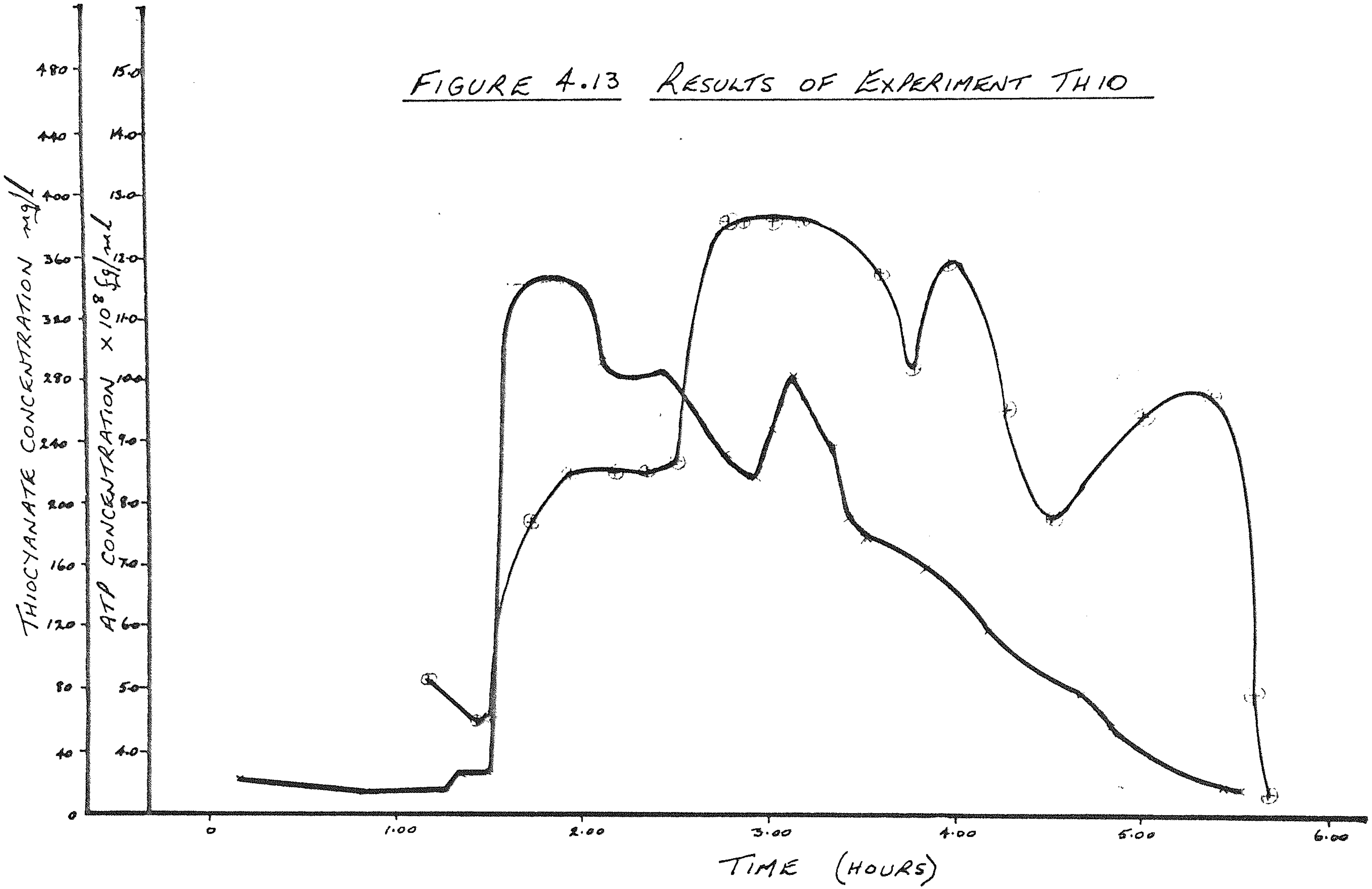
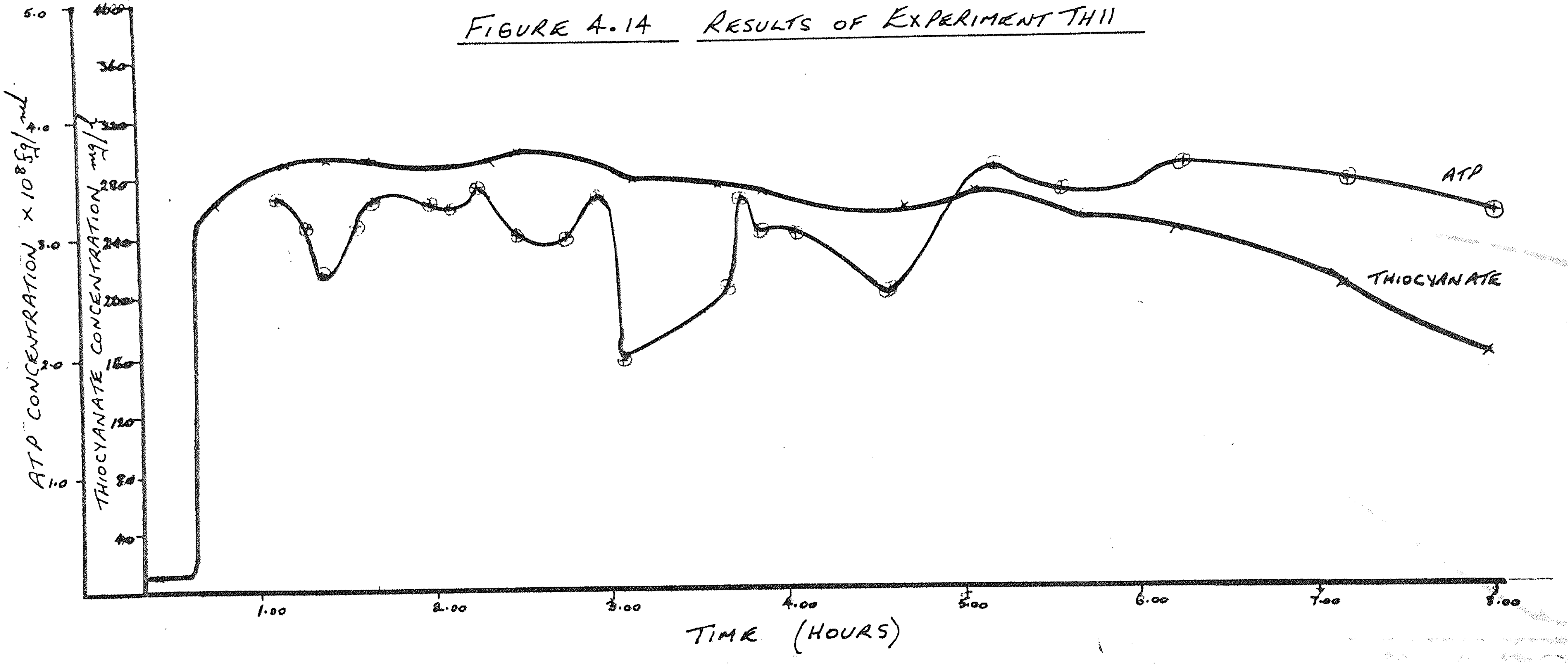


FIGURE A.14 RESULTS OF EXPERIMENT TH11



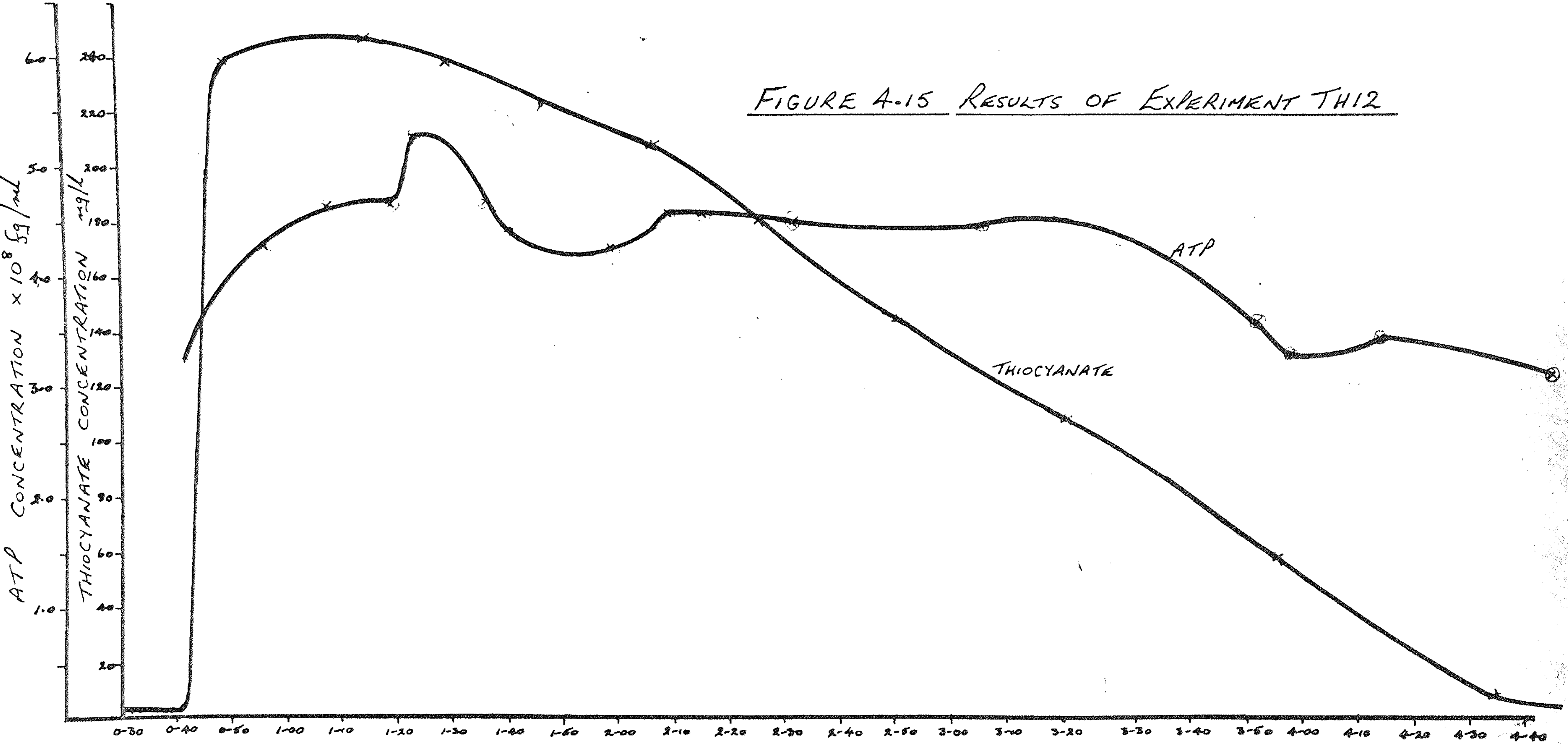


FIGURE 4.15 RESULTS OF EXPERIMENT THI2

FIGURE A.16 RESULTS OF EXPERIMENT TH13

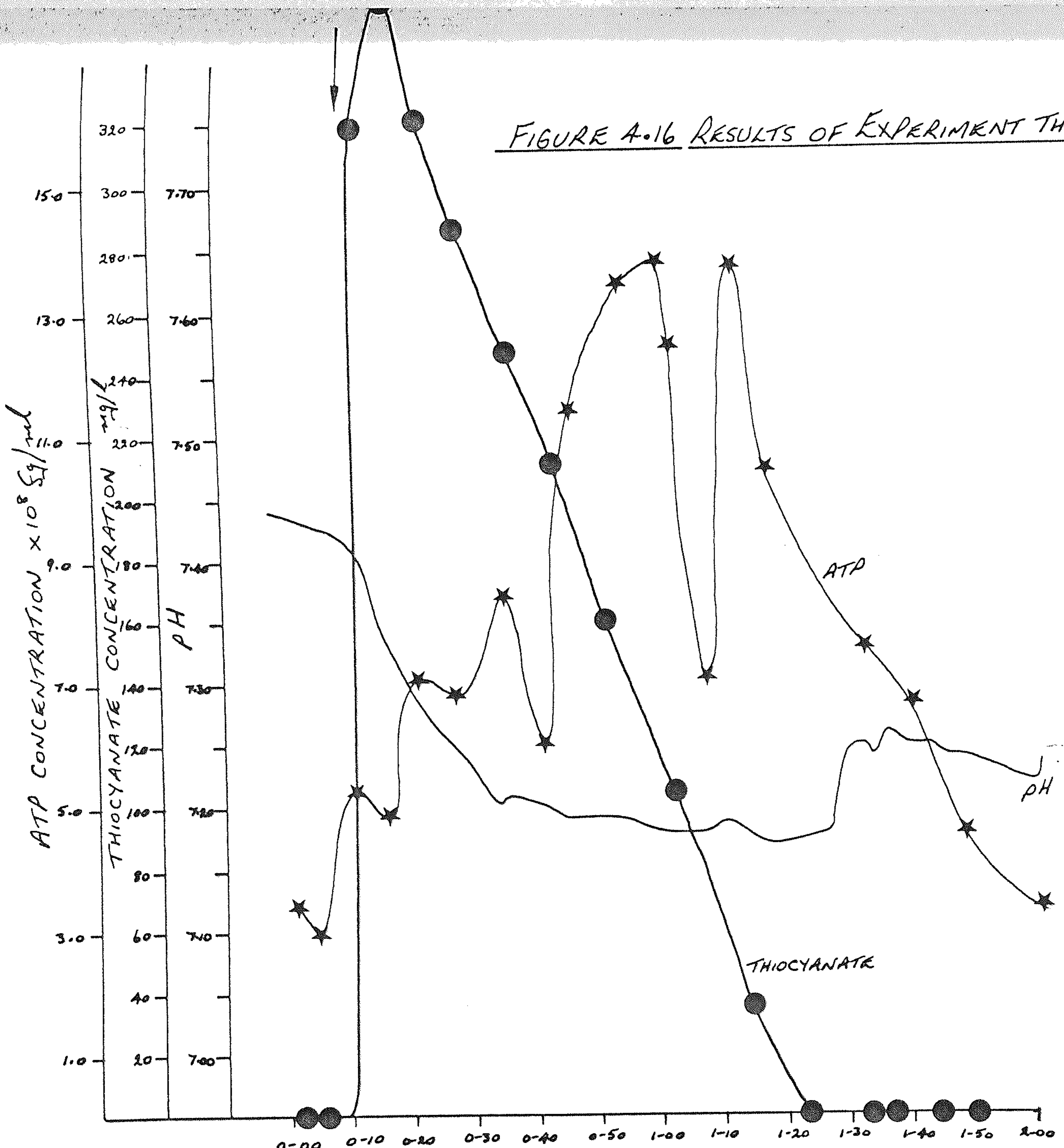


FIGURE 4.17 RESULTS OF EXPERIMENT TH14

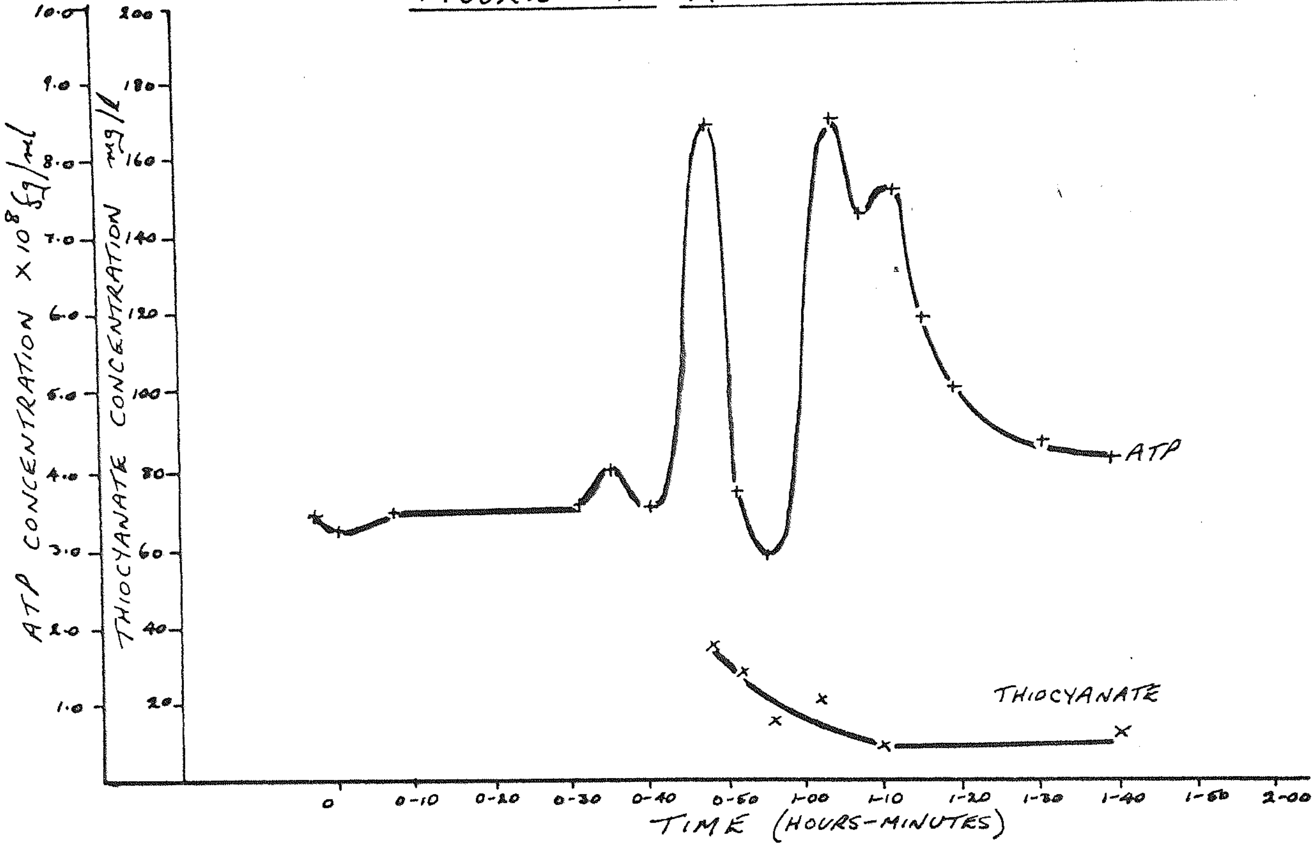


FIGURE 4.18 RESULTS OF EXPERIMENT TH15

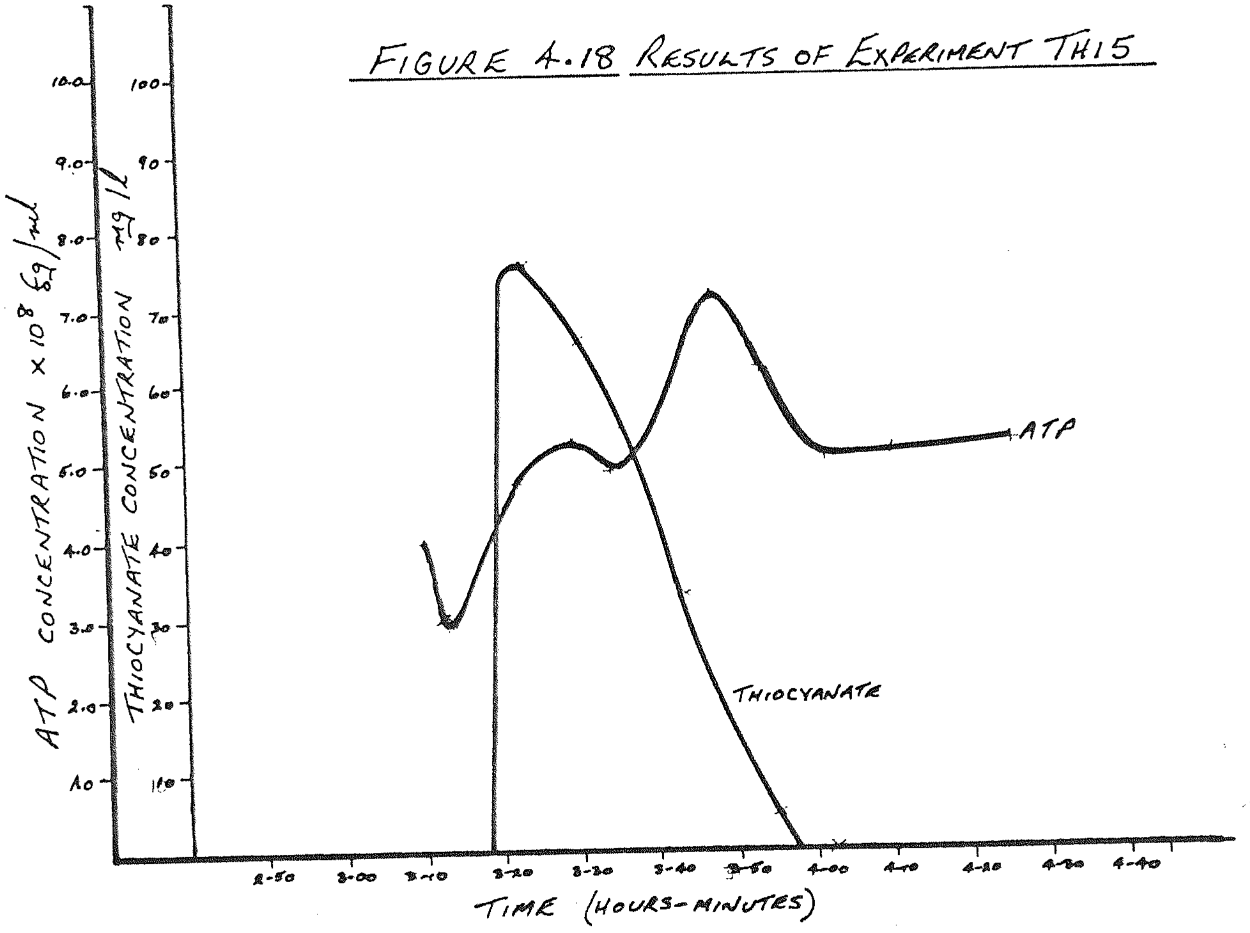


FIGURE A.19 RESULTS OF EXPERIMENT TH16

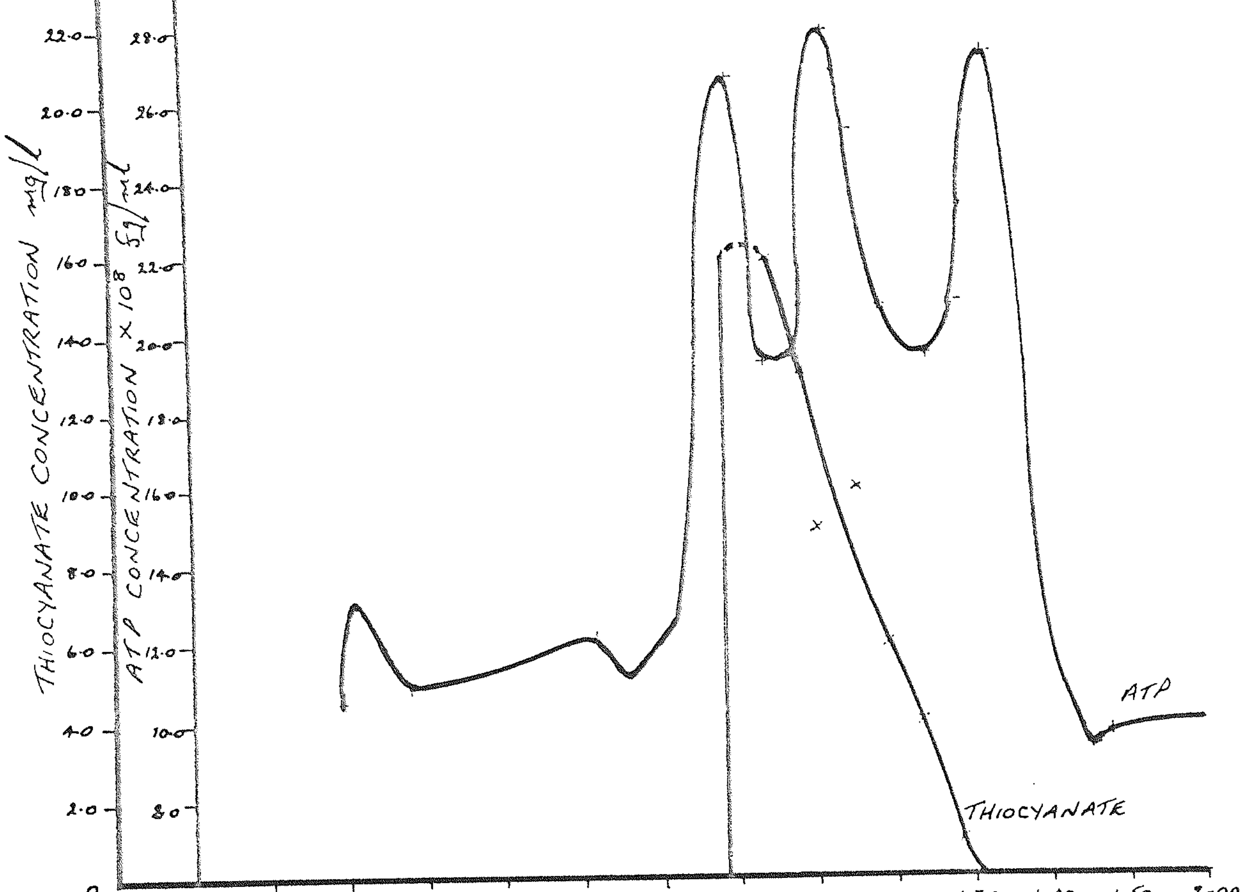


FIGURE A.20 RESULTS OF EXPERIMENT TH 17

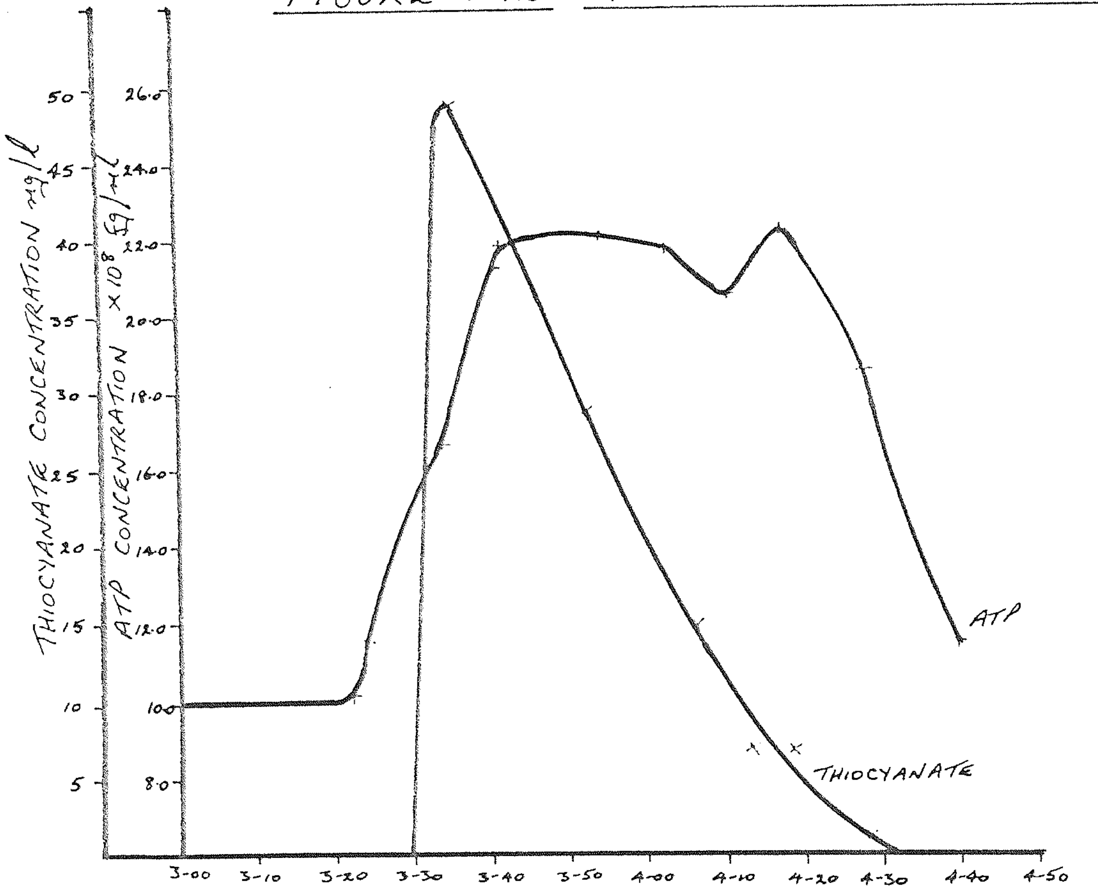
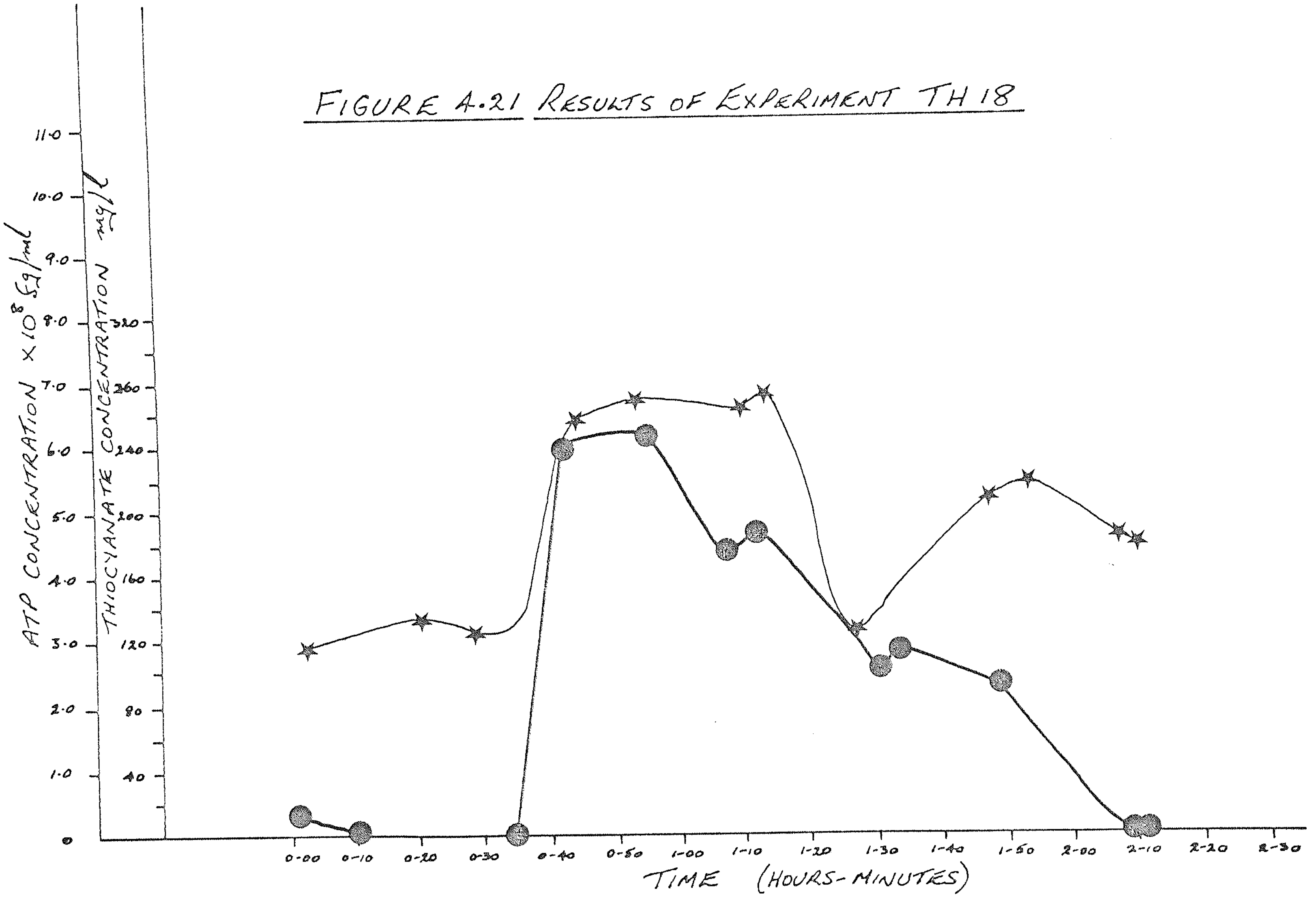


FIGURE A.21 RESULTS OF EXPERIMENT TH 18



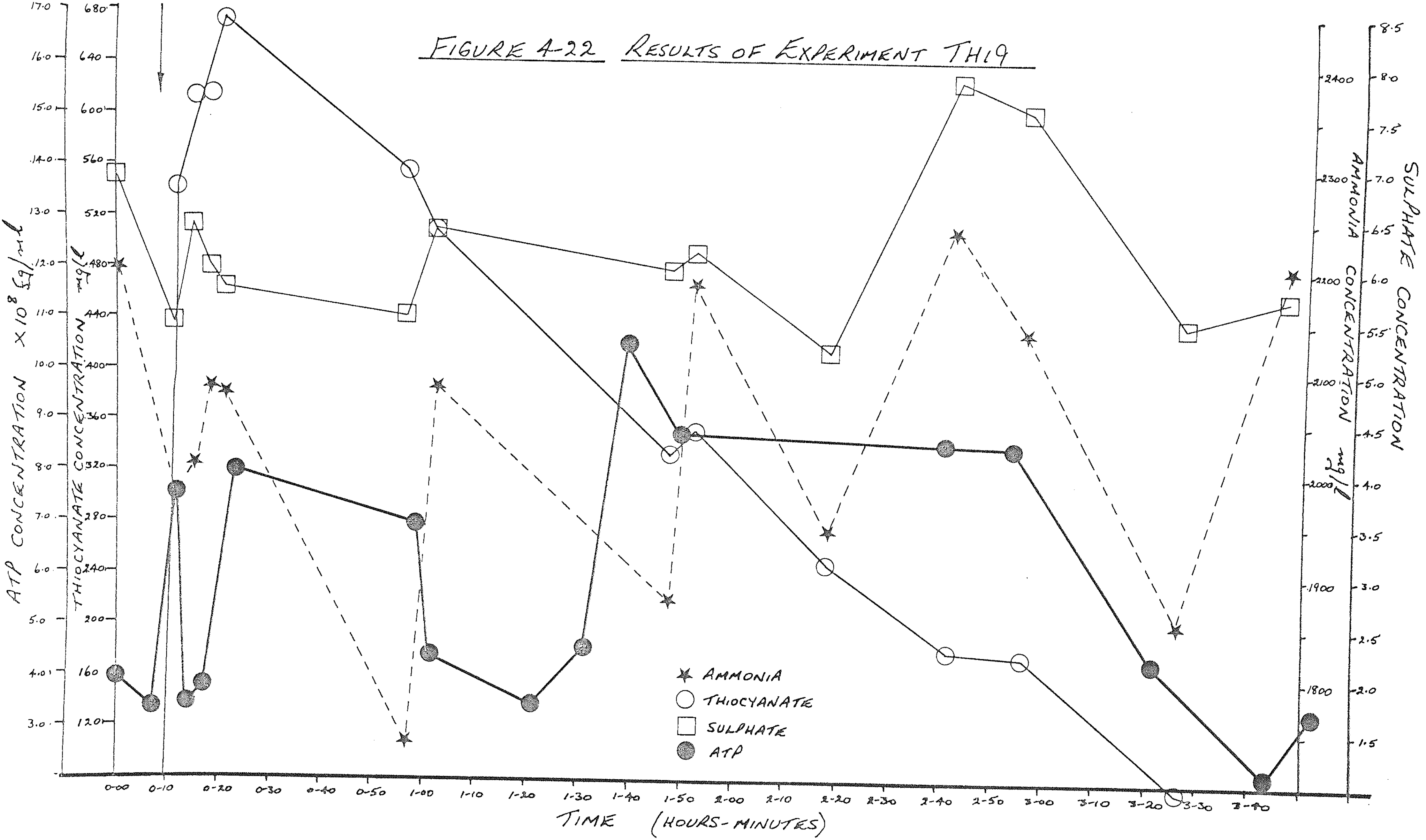


FIGURE 4-22 RESULTS OF EXPERIMENT TH19

FIGURE 4.23 RESULTS OF EXPERIMENT TH20

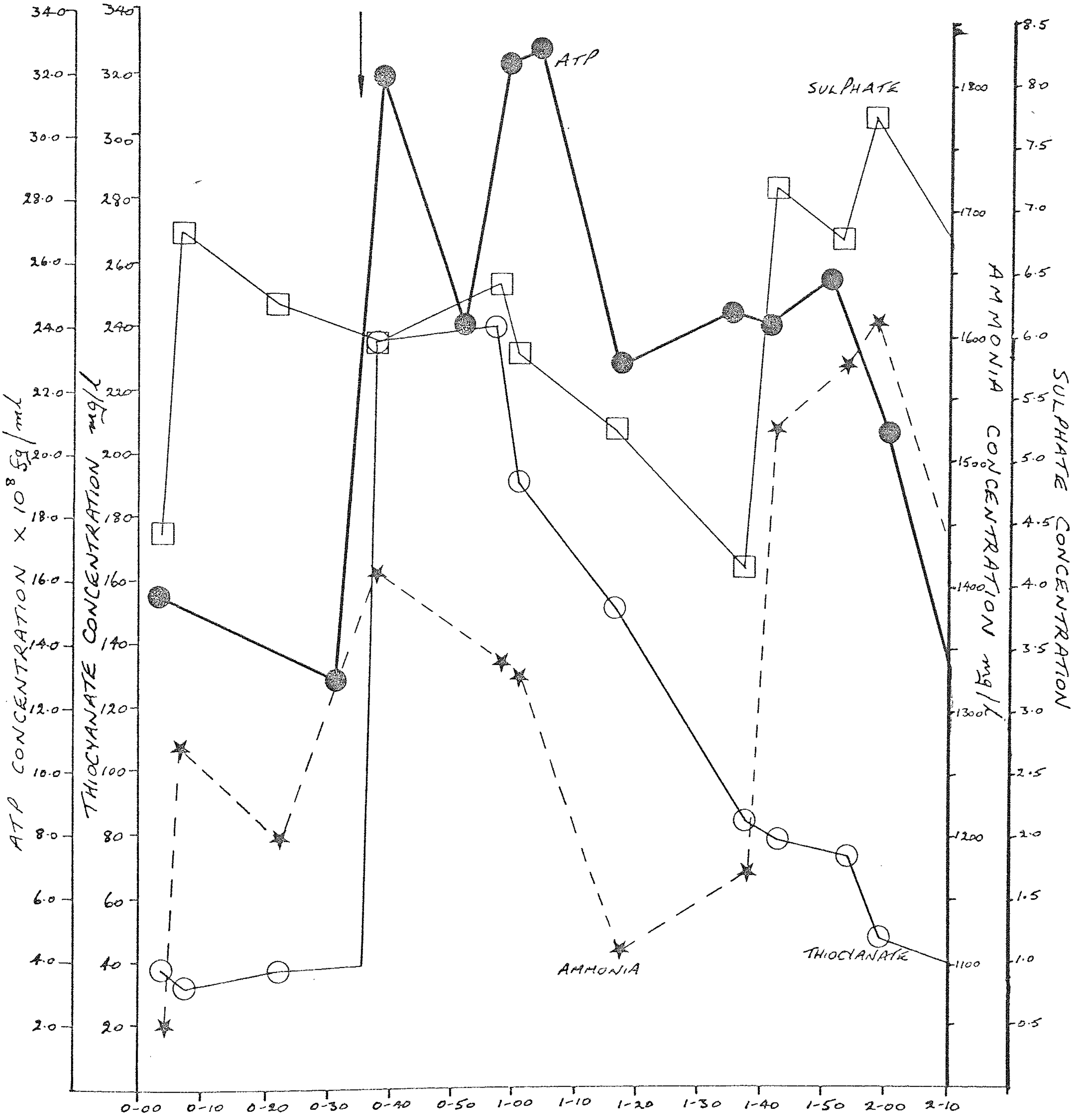
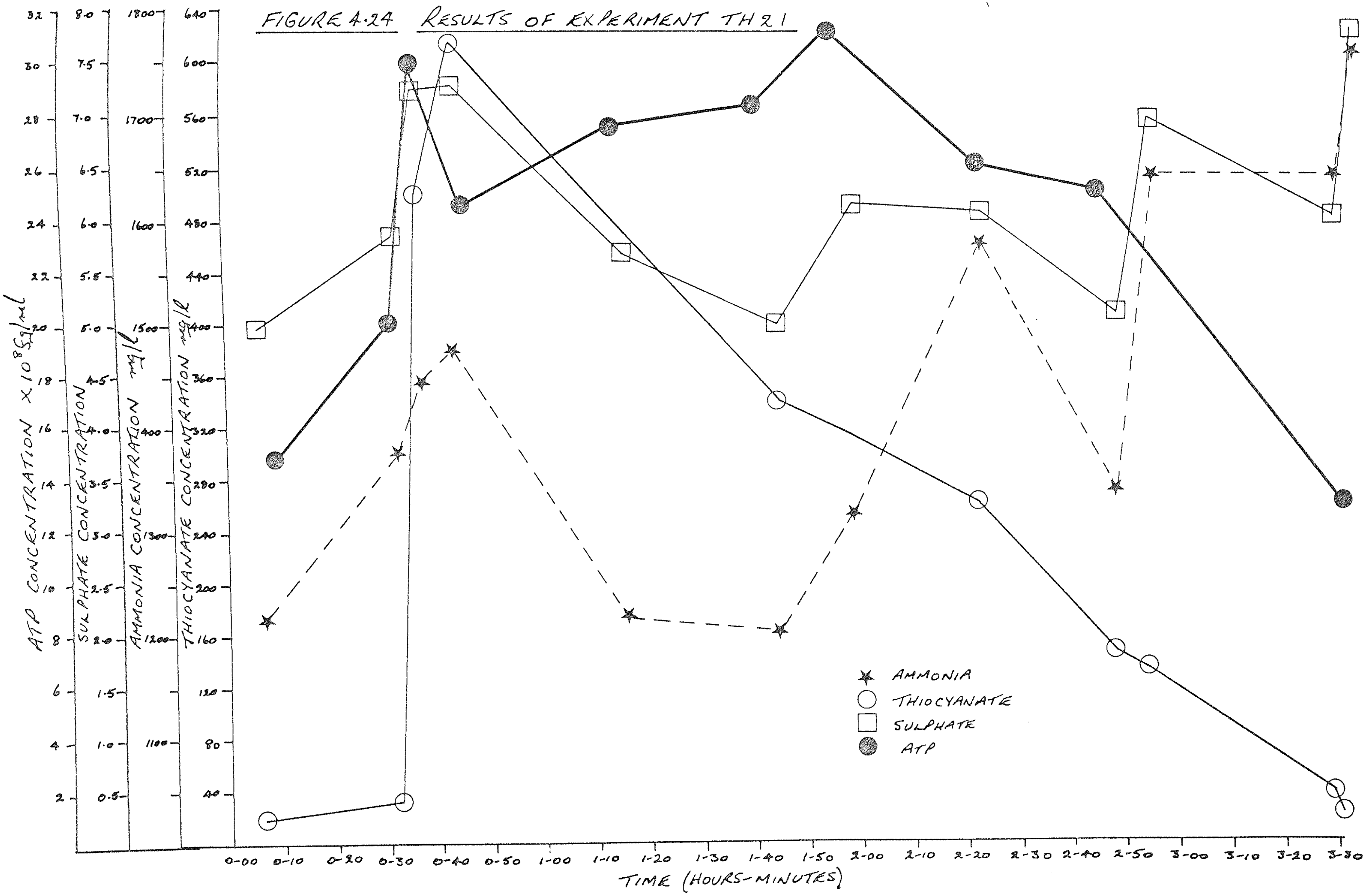


FIGURE 4·24 RESULTS OF EXPERIMENT TH21



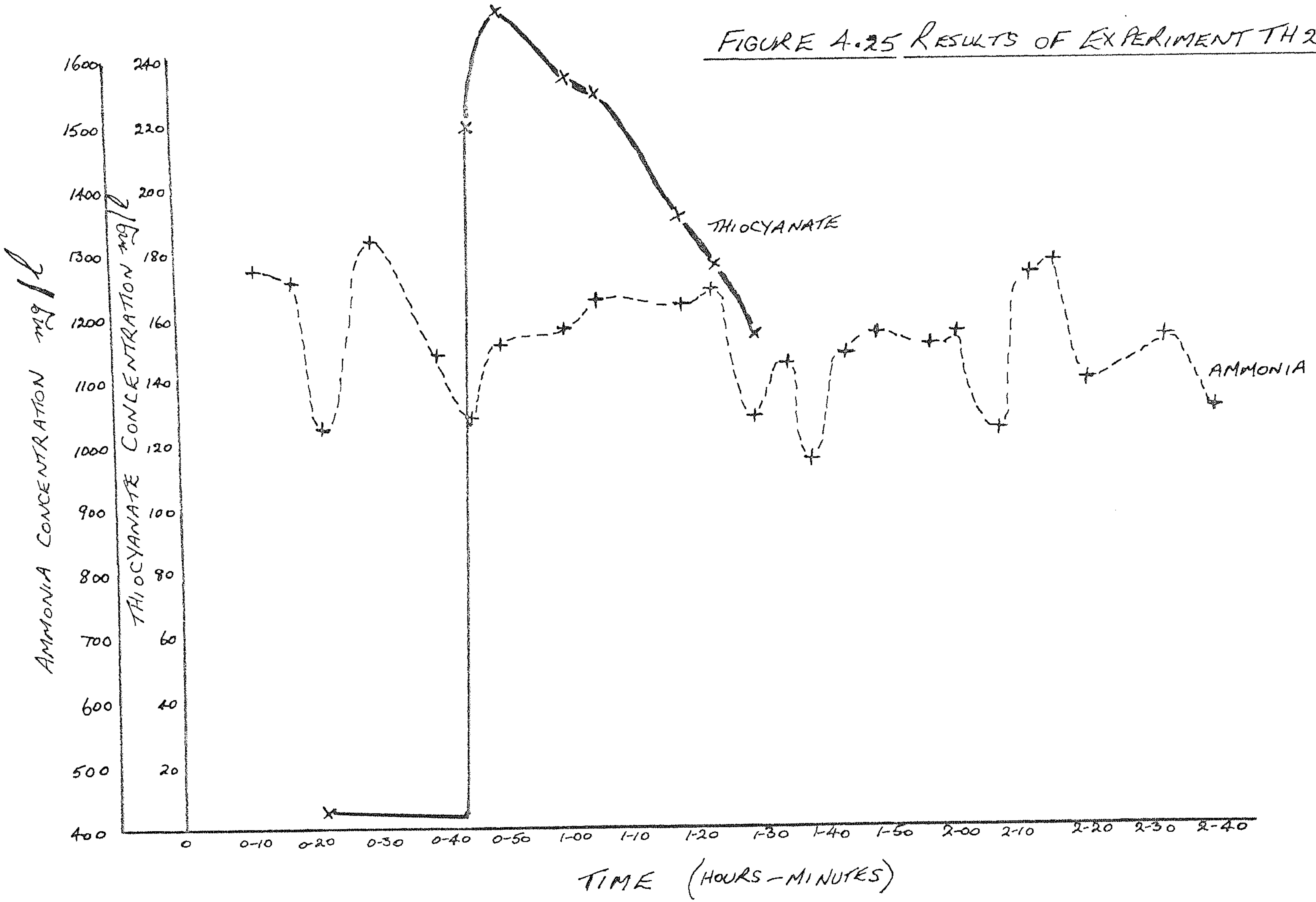
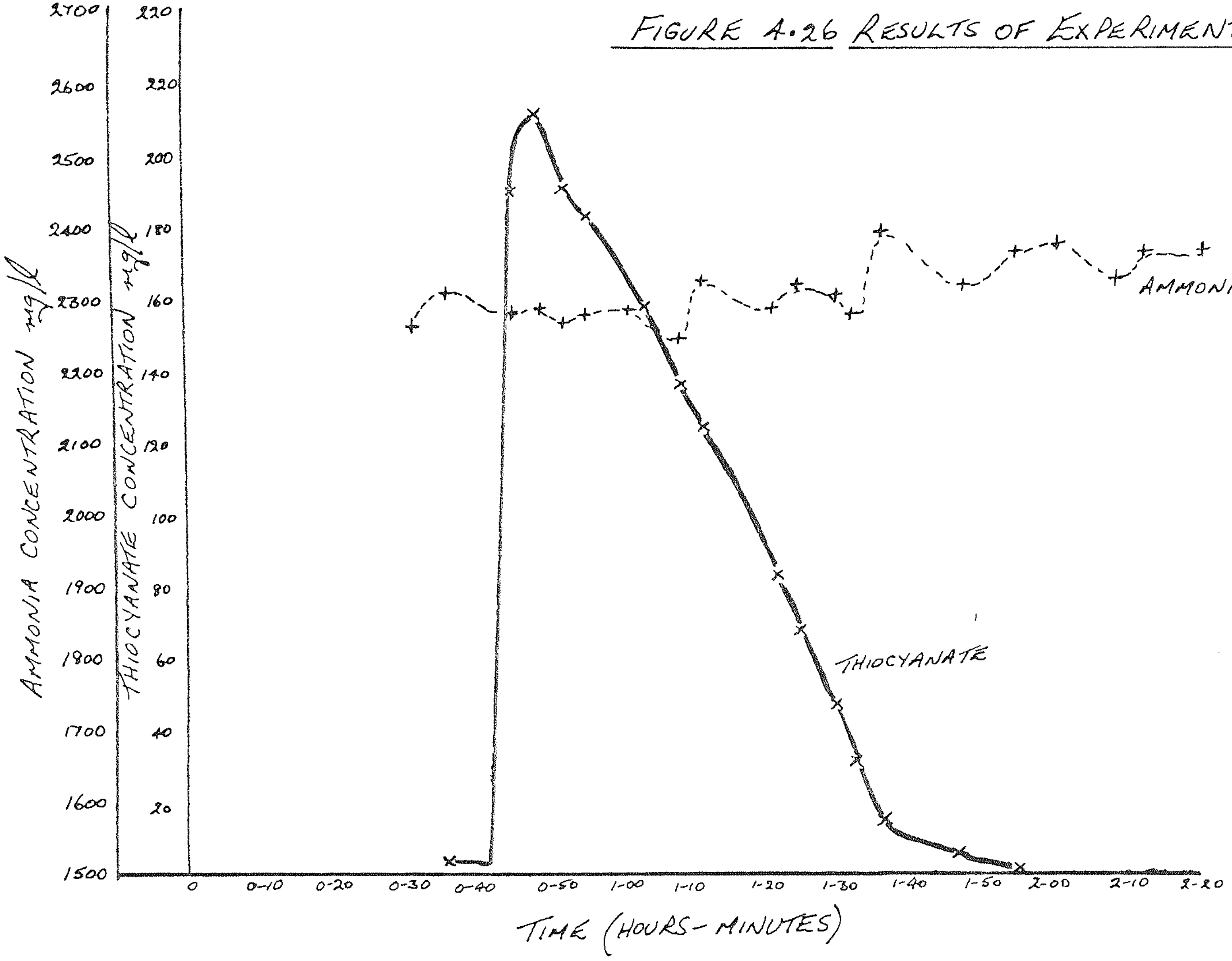


FIGURE A.25 RESULTS OF EXPERIMENT TH22

FIGURE A.26 RESULTS OF EXPERIMENT TH23



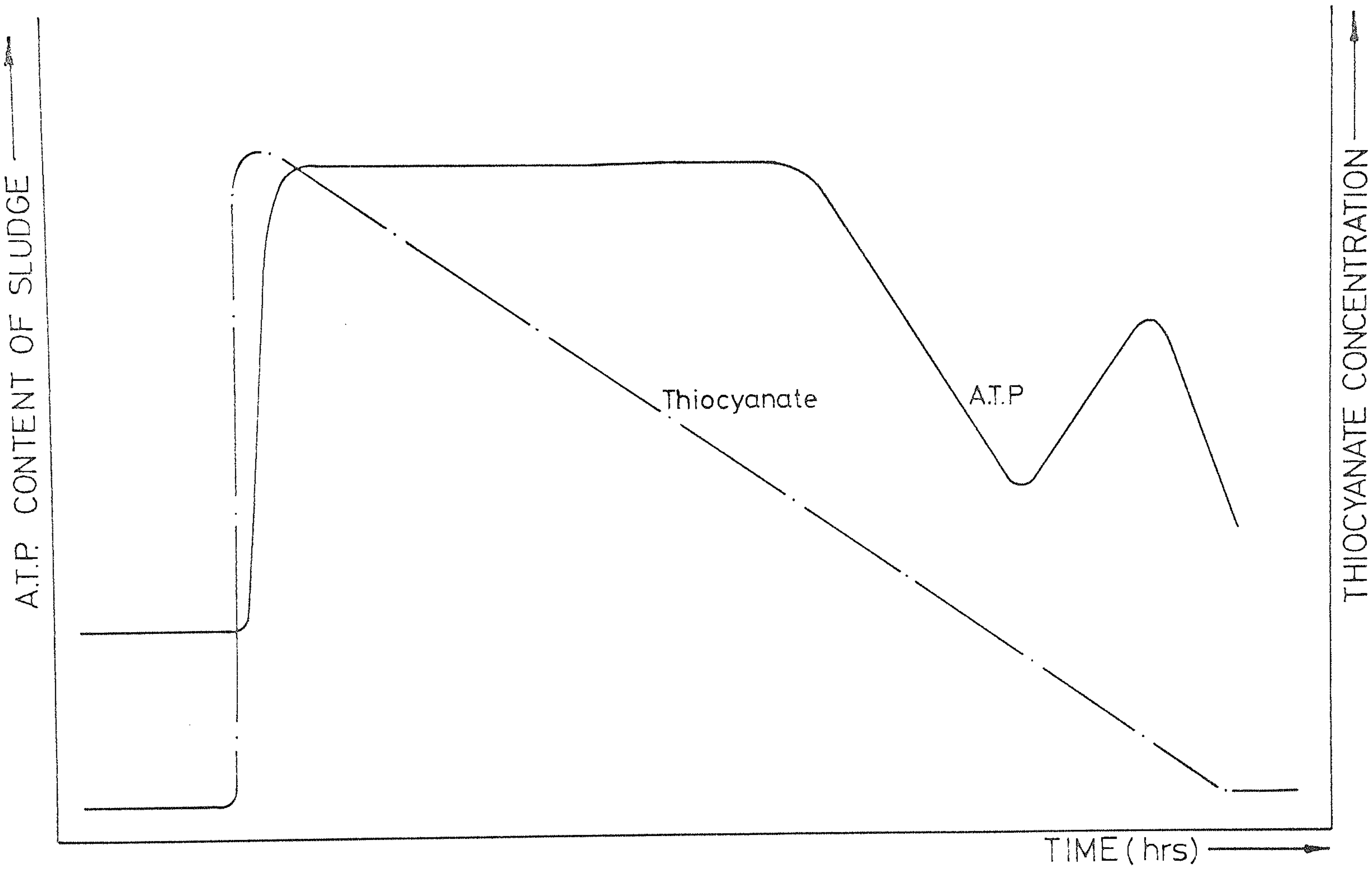


FIGURE 4.27 TYPICAL FLUCTUATIONS IN ATP POOL IN LABORATORY BATCH ACTIVATED SLUDGE REACTOR TREATING THIOCYANATE SOLUTIONS

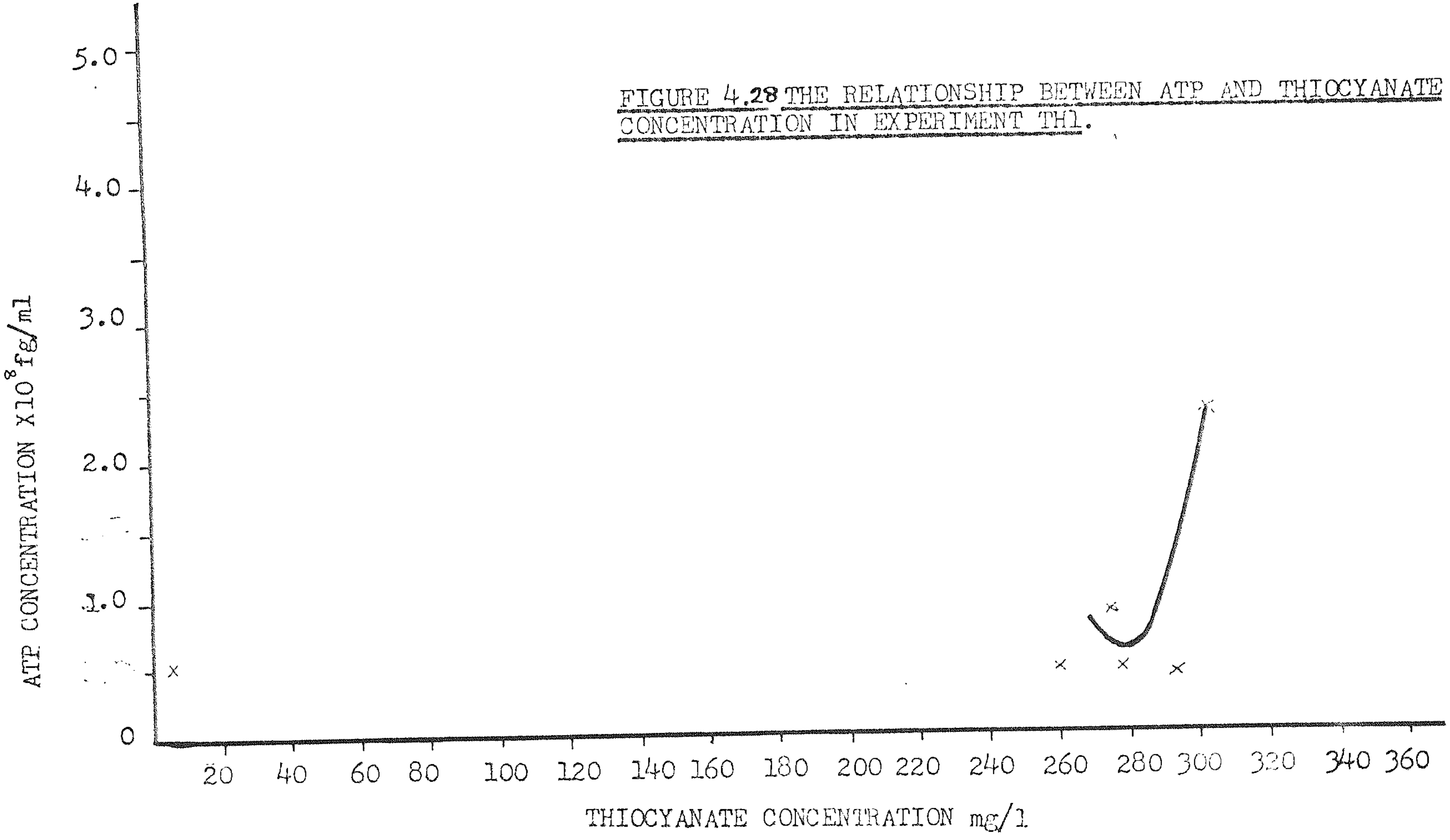


FIGURE 4.28 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH1.

FIGURE 4.29 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH3.

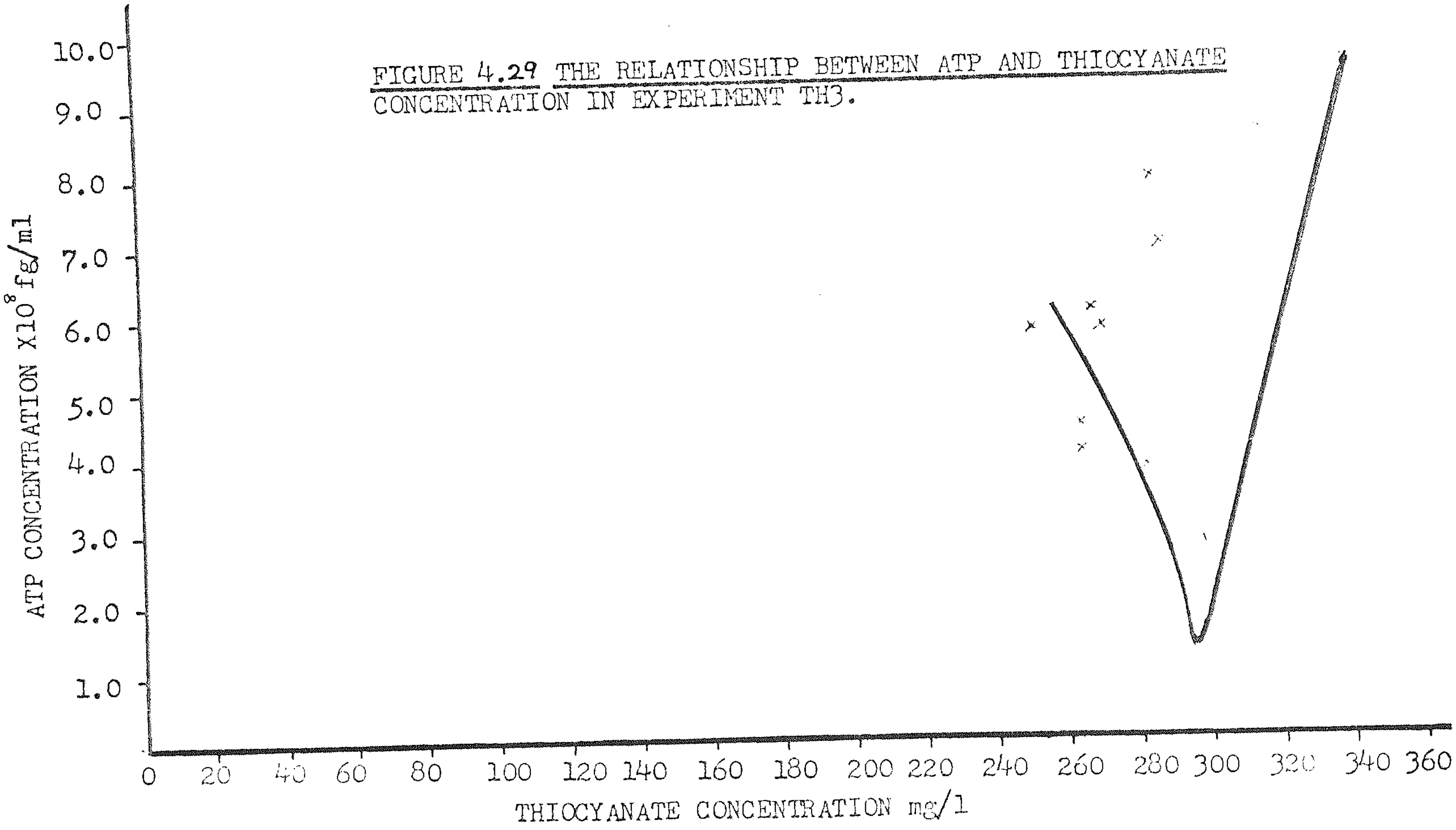


FIGURE 4.30 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH4

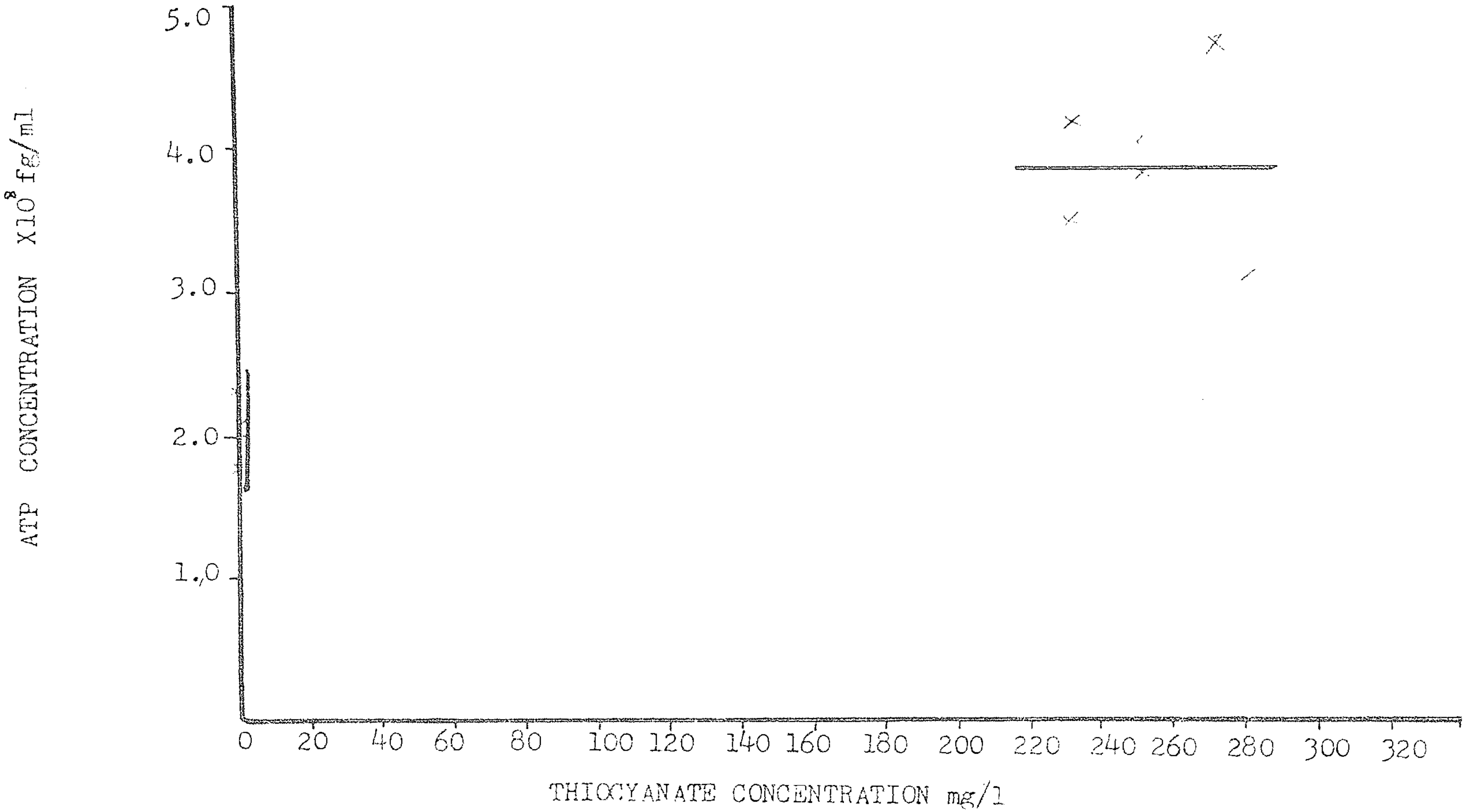


FIGURE 4.31 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH5.

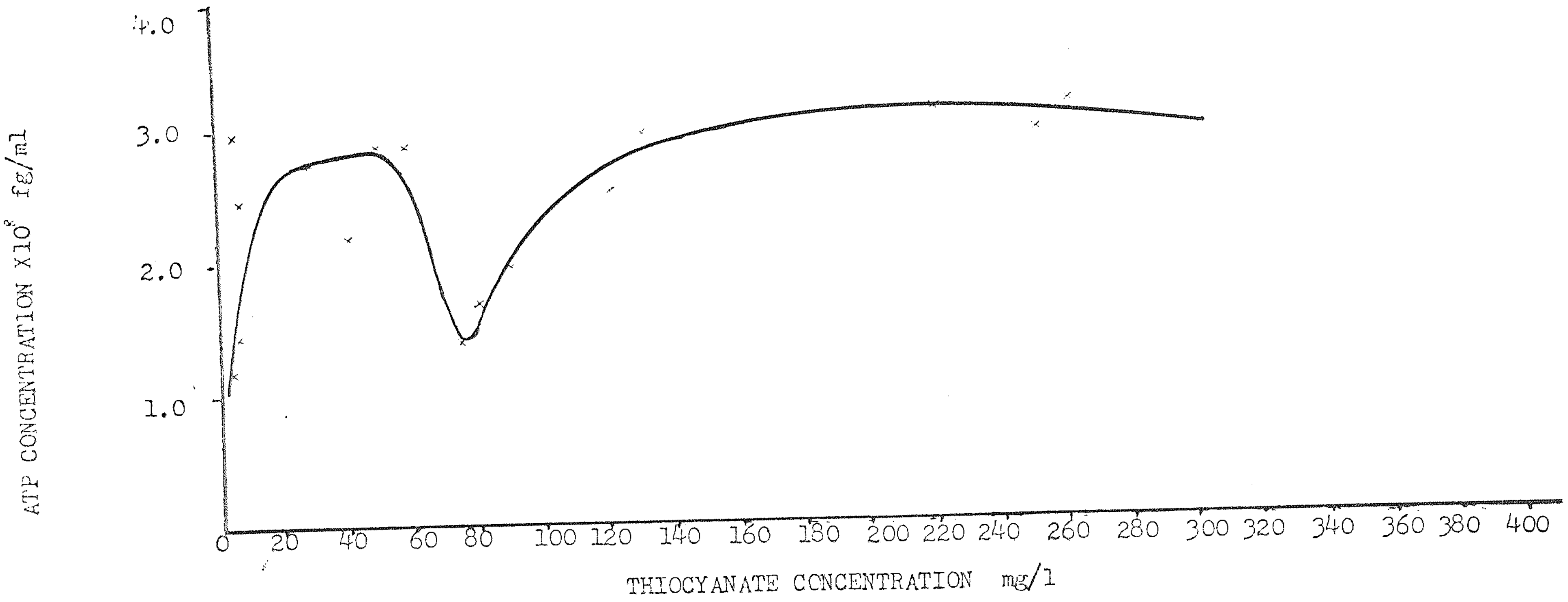


FIGURE 4.32 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH6.

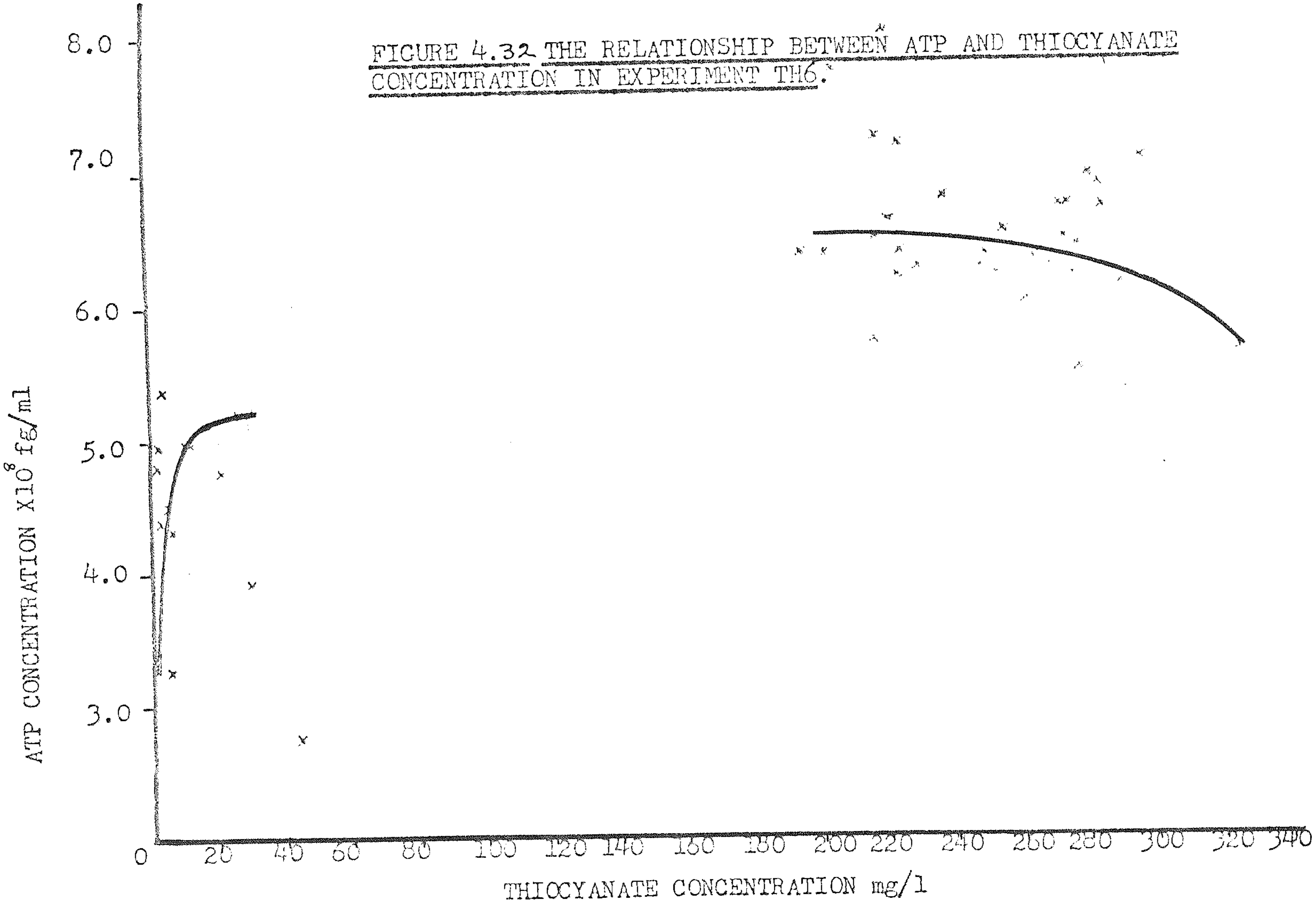


FIGURE 4.33 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH7.

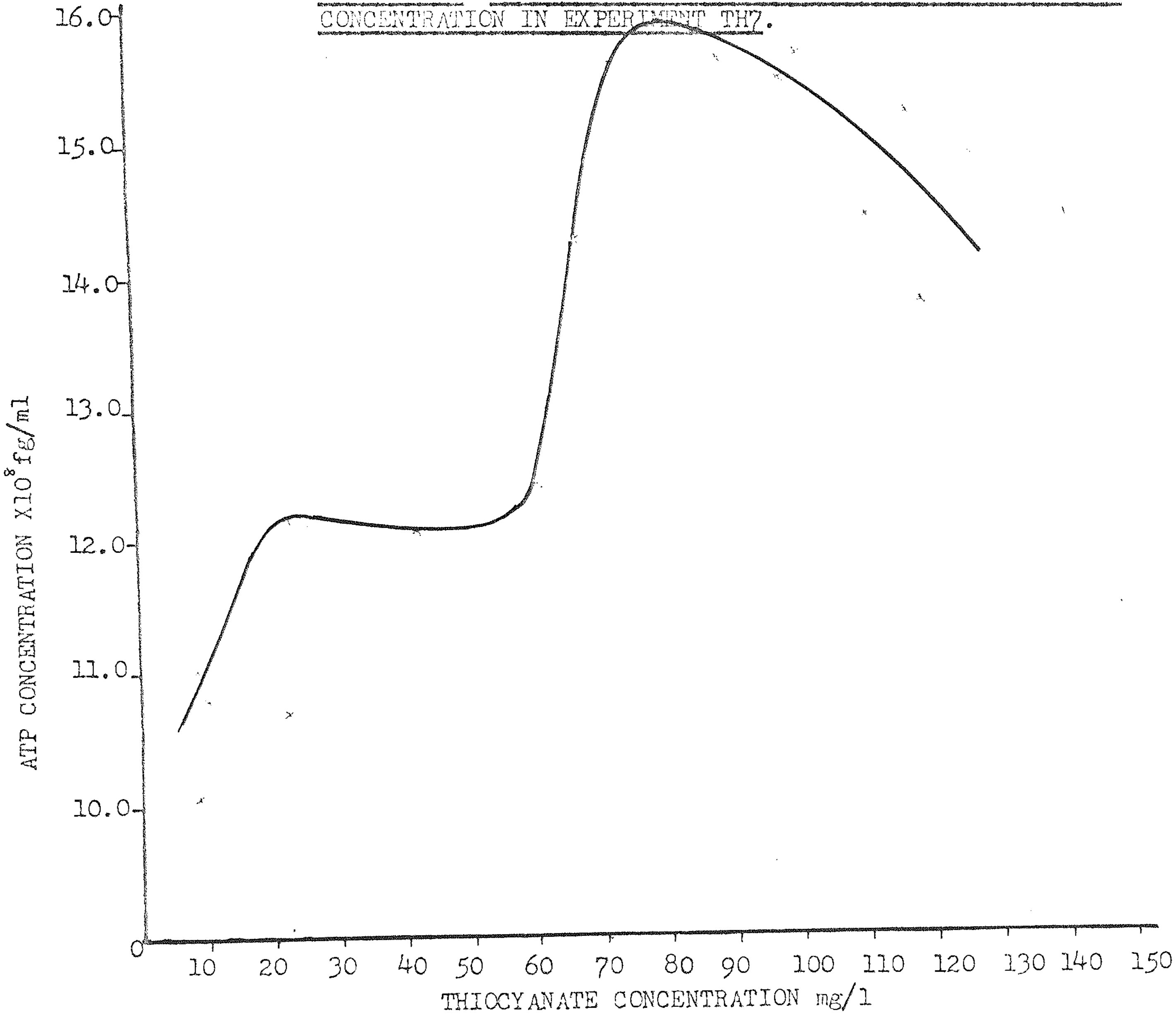
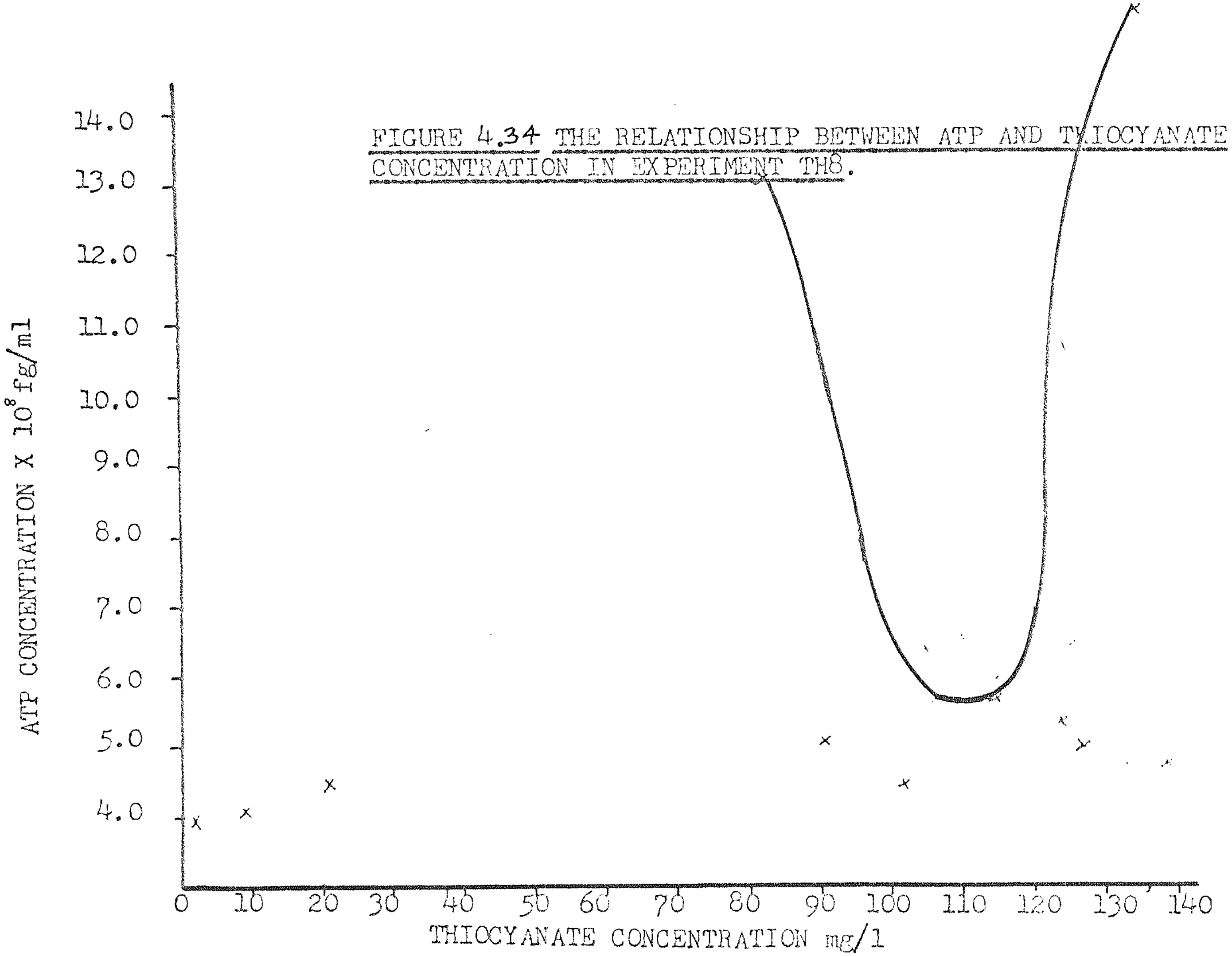


FIGURE 4.34 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH8.



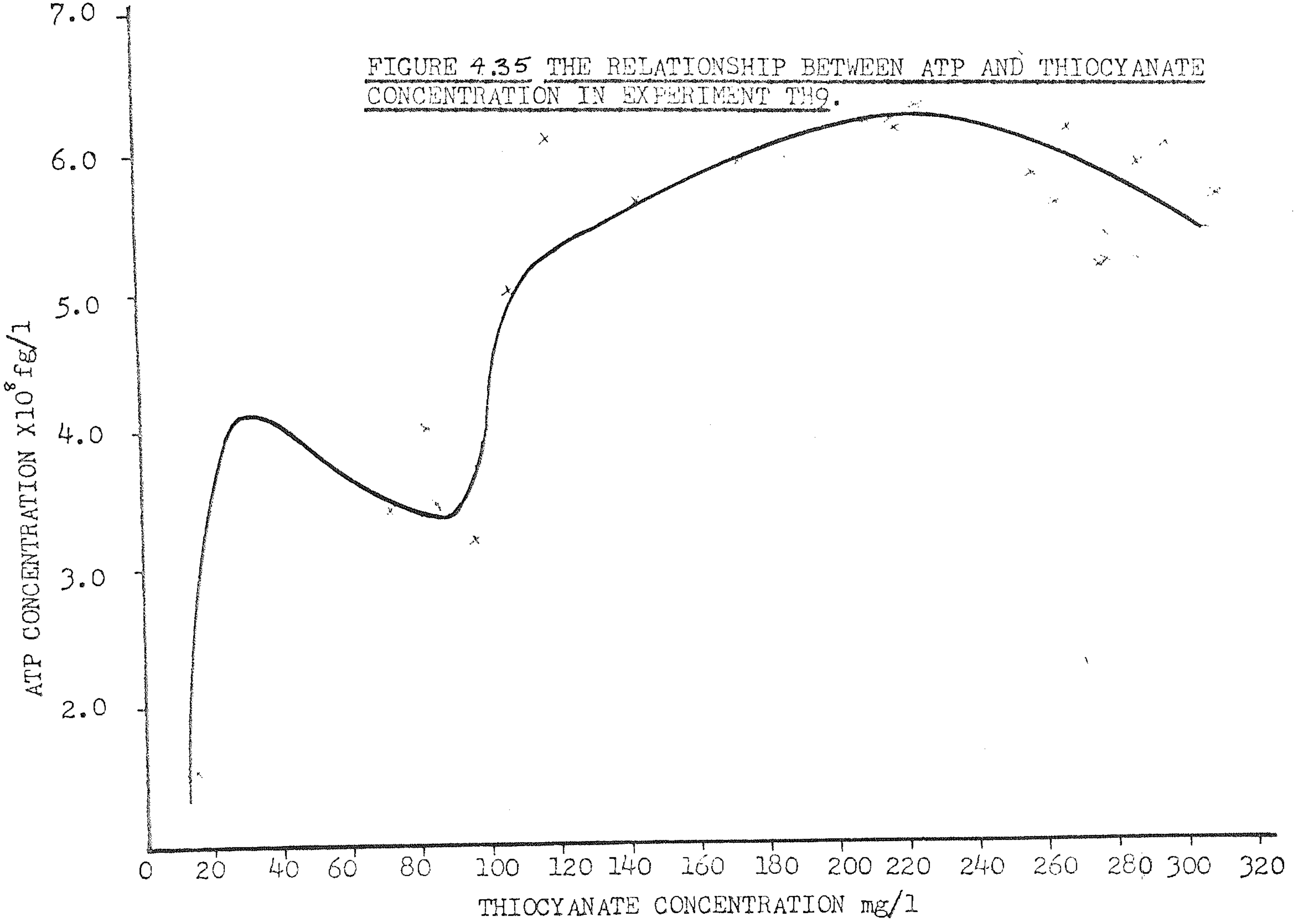


FIGURE 4.35 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH9.

FIGURE 4.36 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH10.

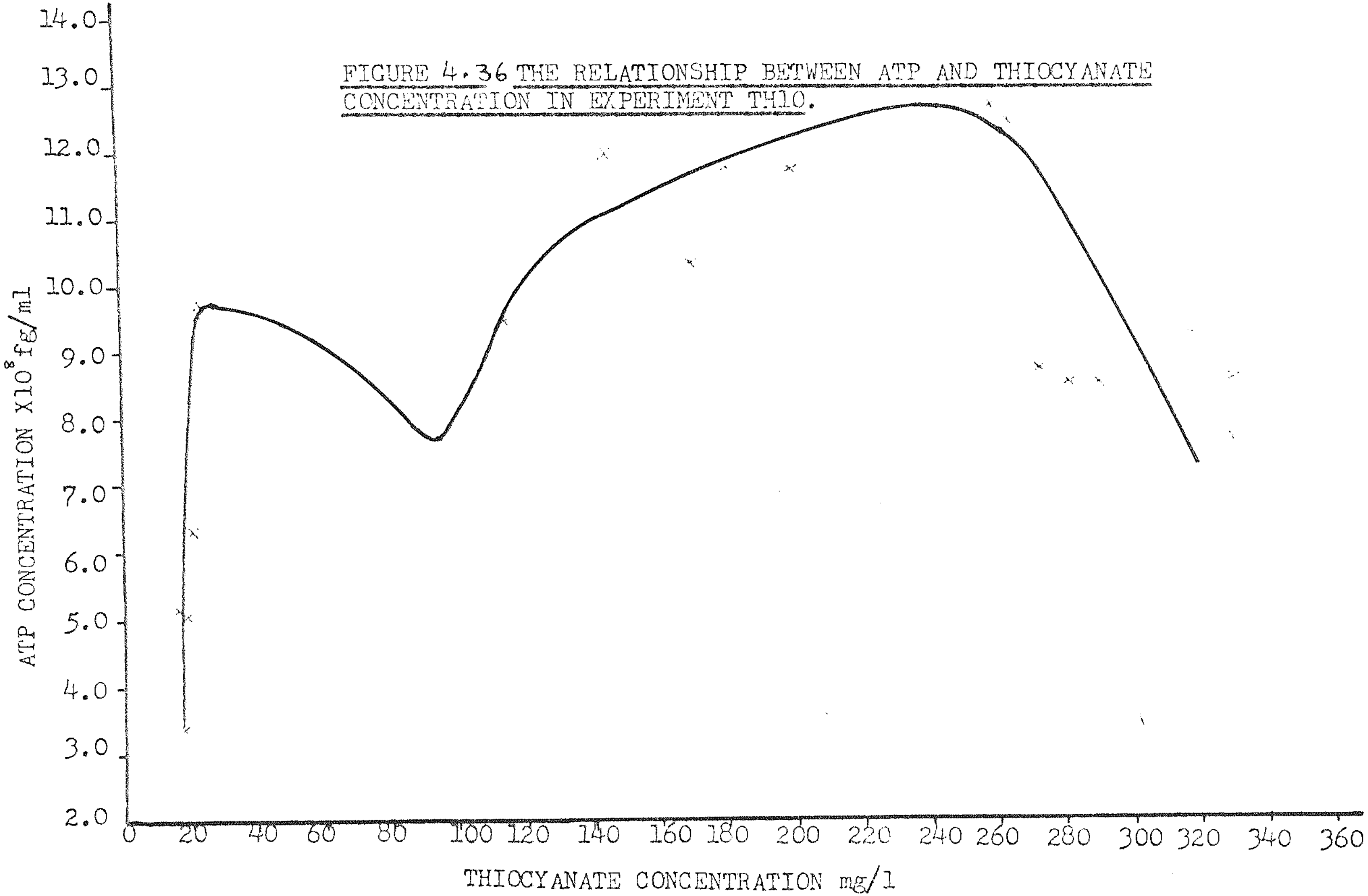


FIGURE 4.37 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT, TH11.

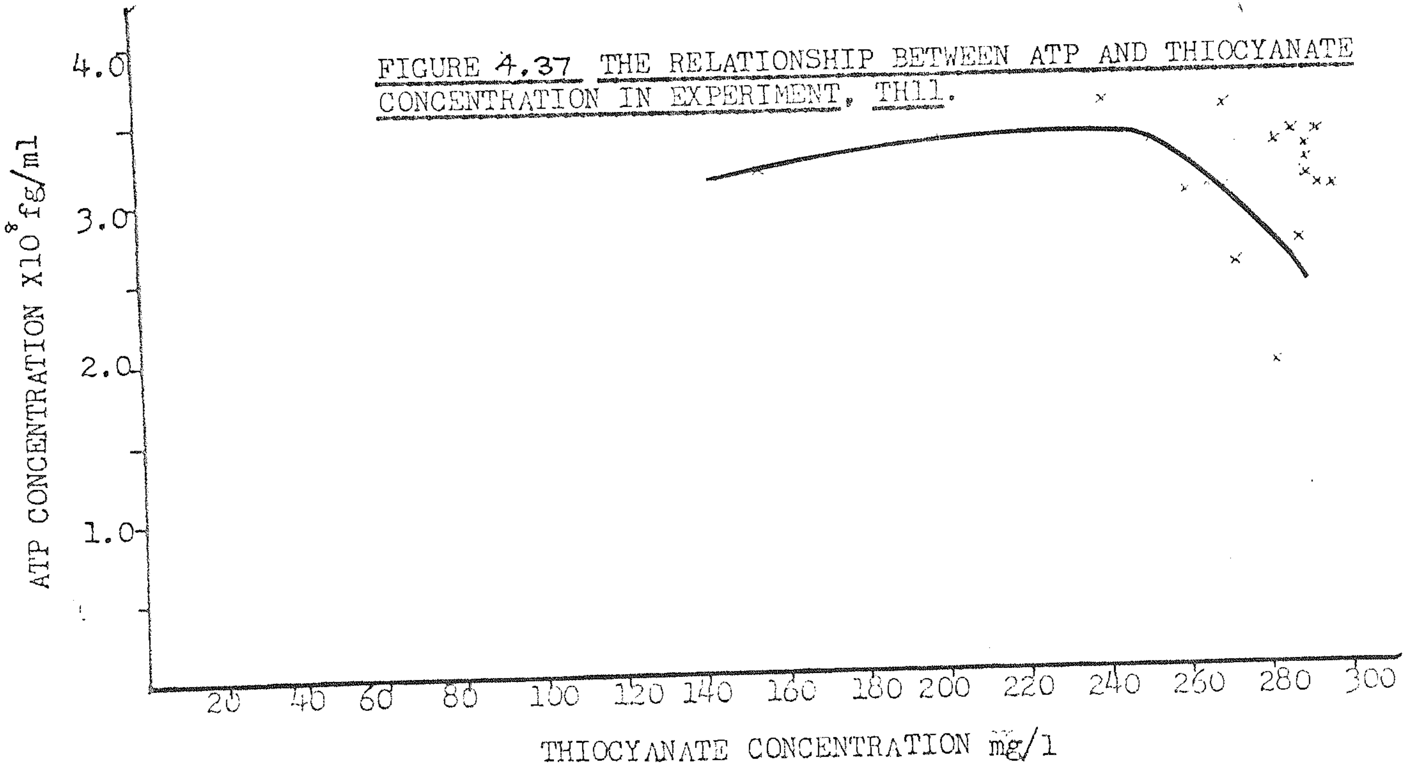
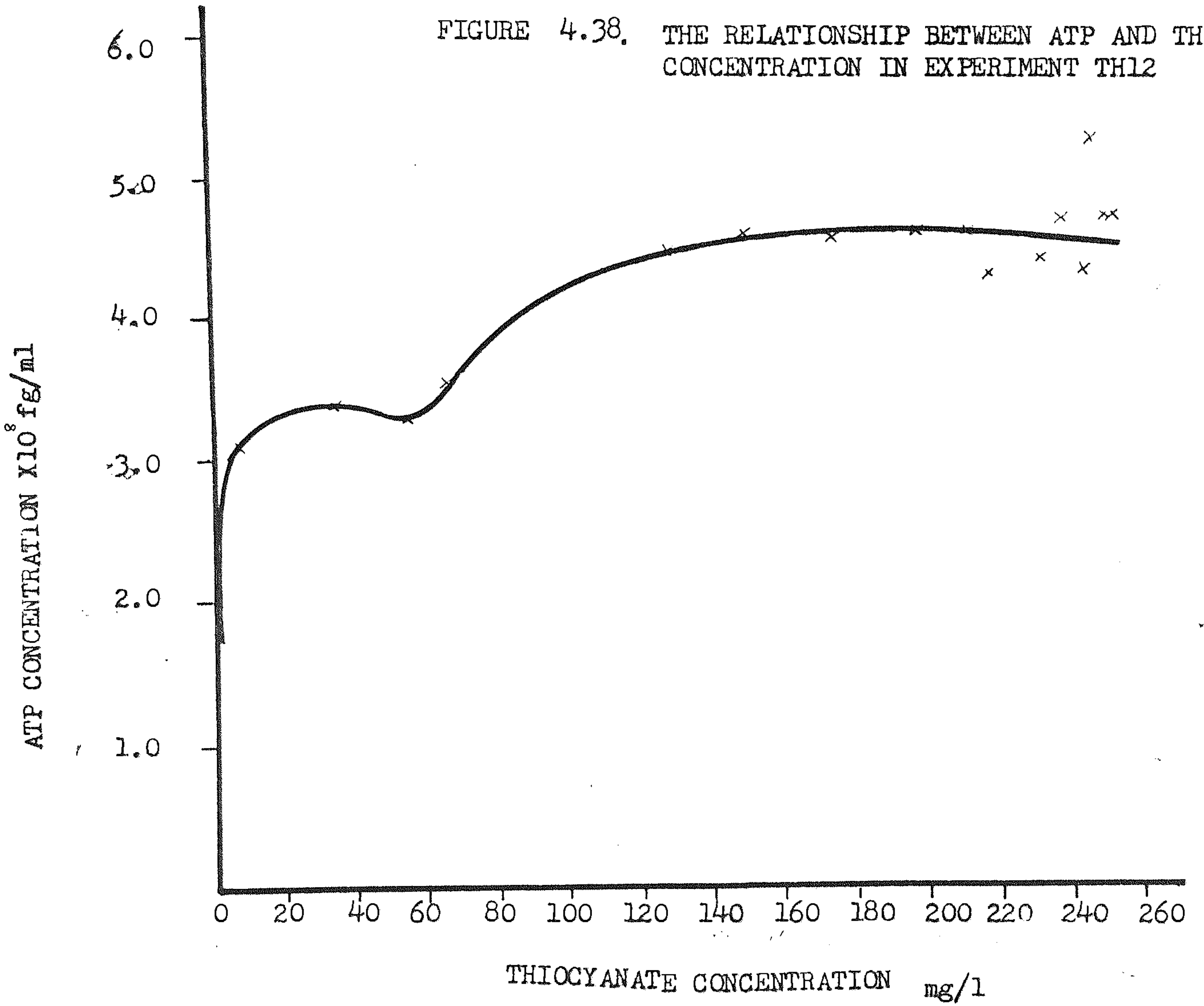


FIGURE 4.38. THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH12



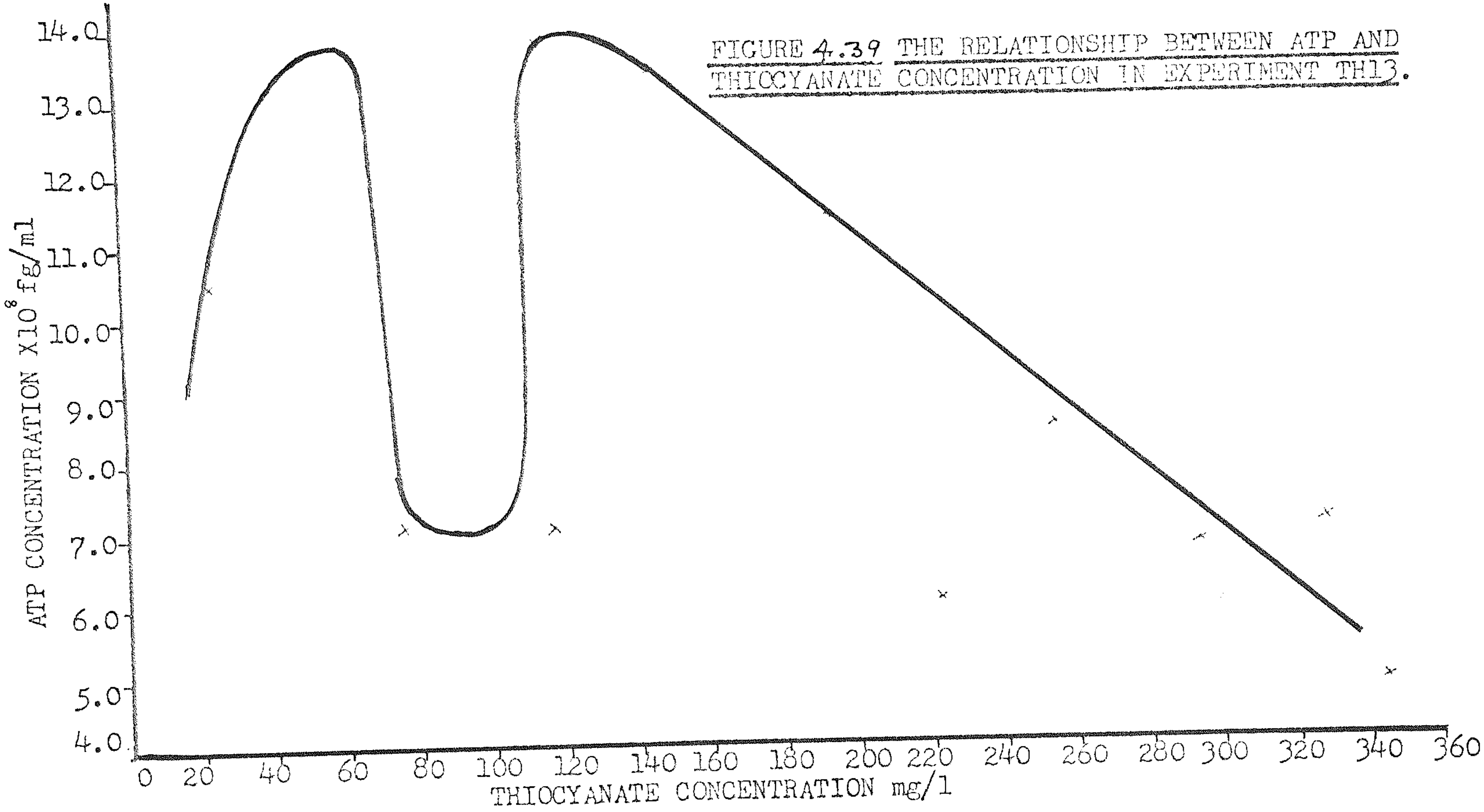


FIGURE 4.39 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH13.

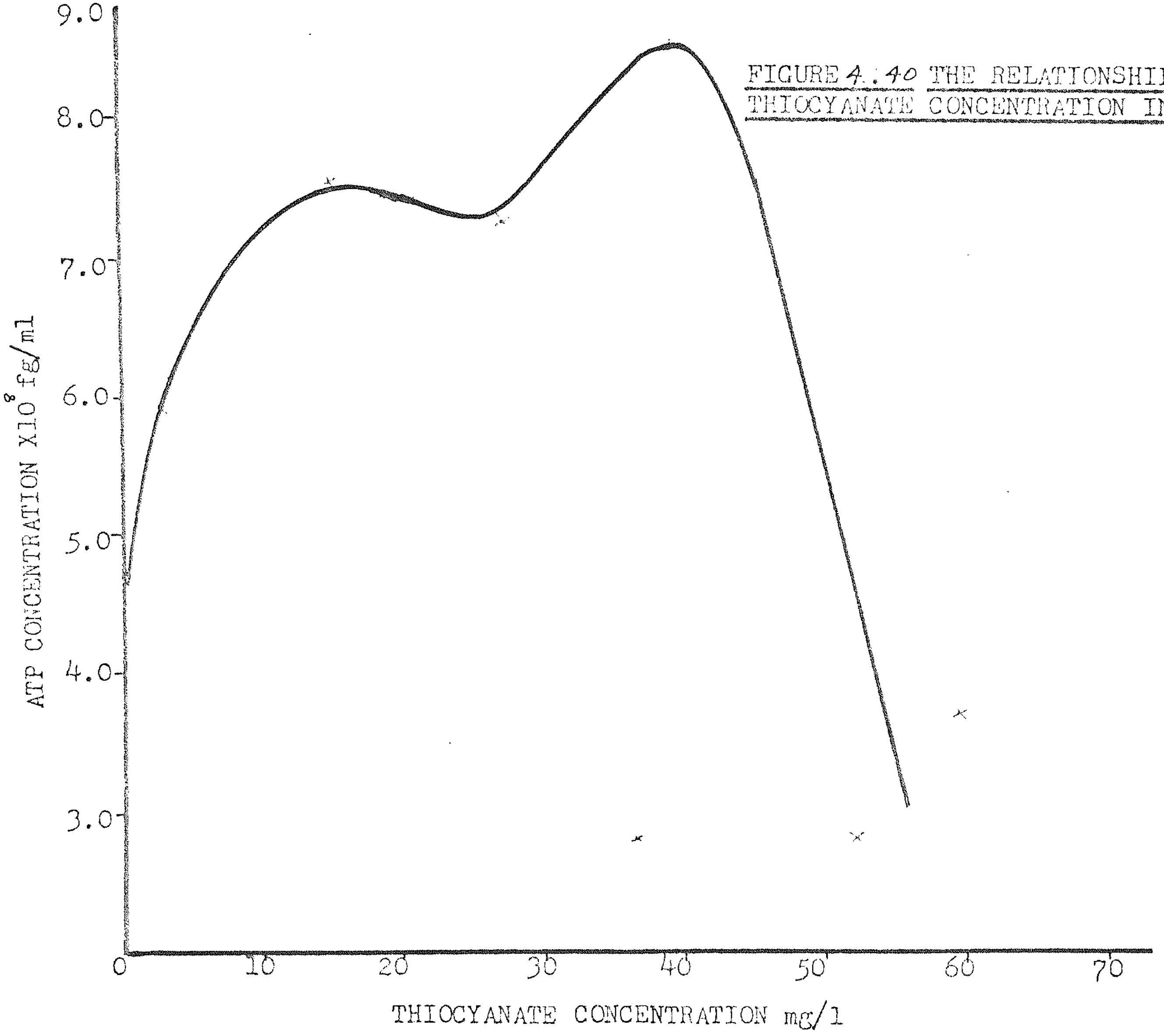
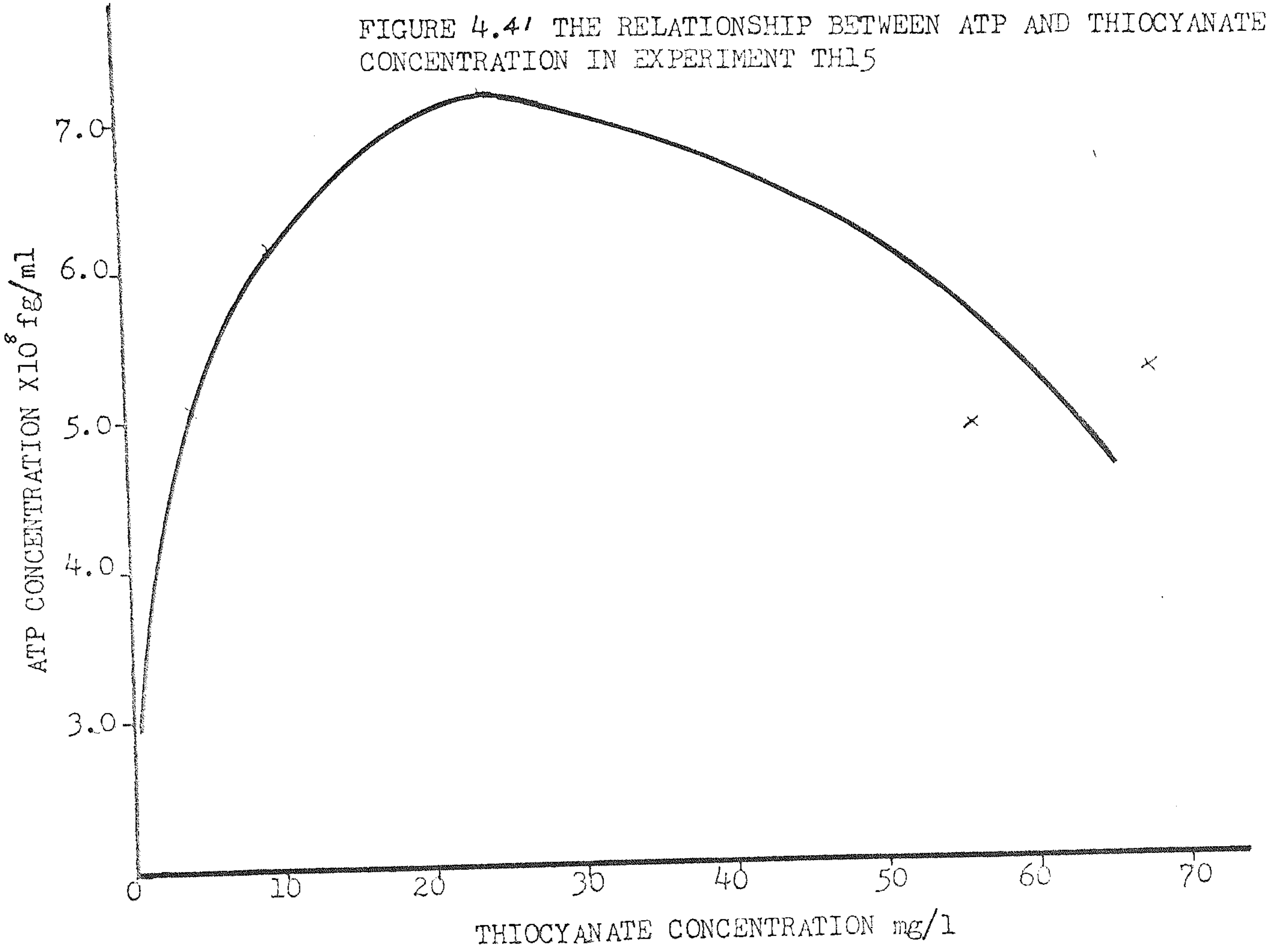


FIGURE 4.40 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH14.

FIGURE 4.41 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH15



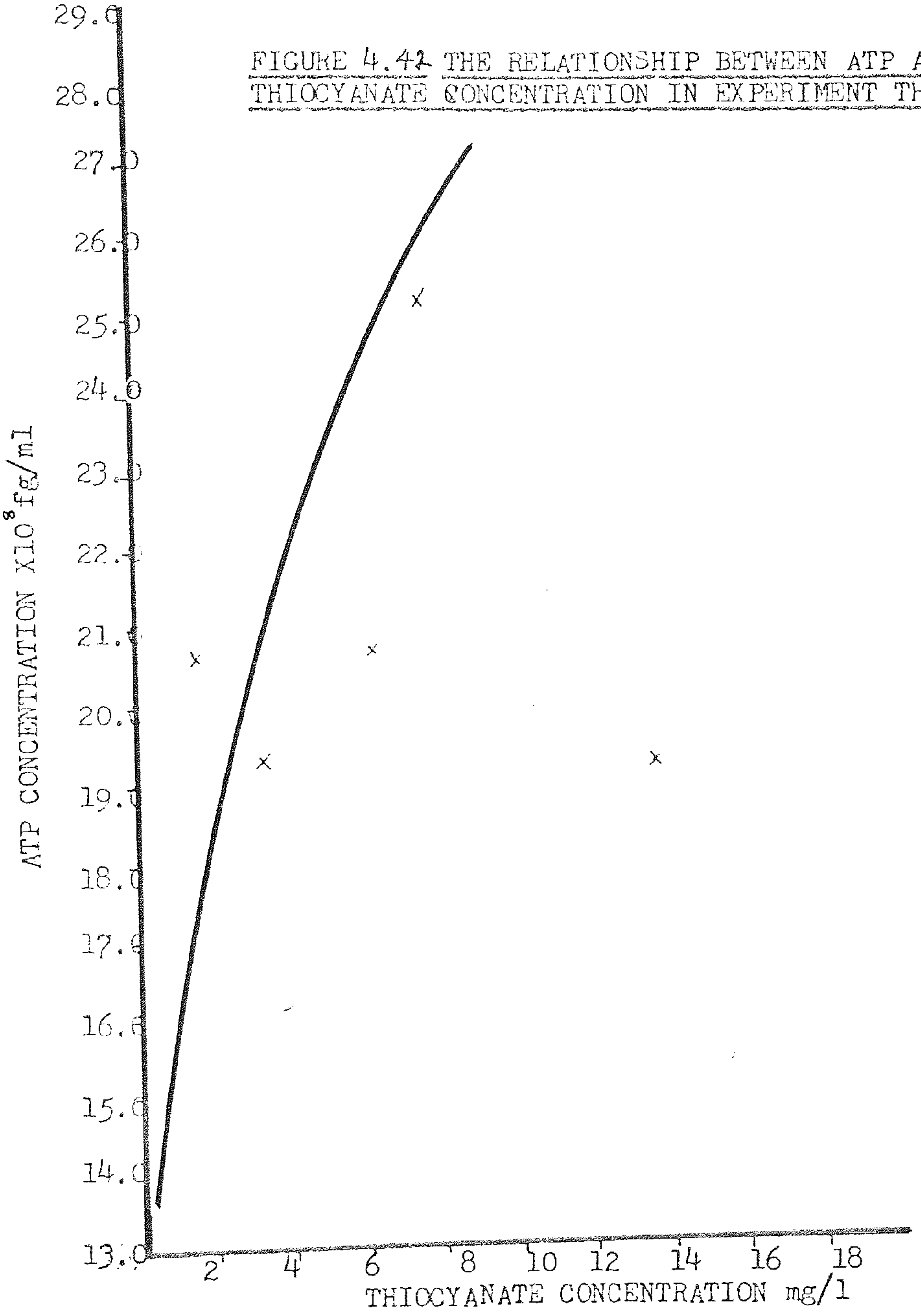


FIGURE 4.42 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH16.

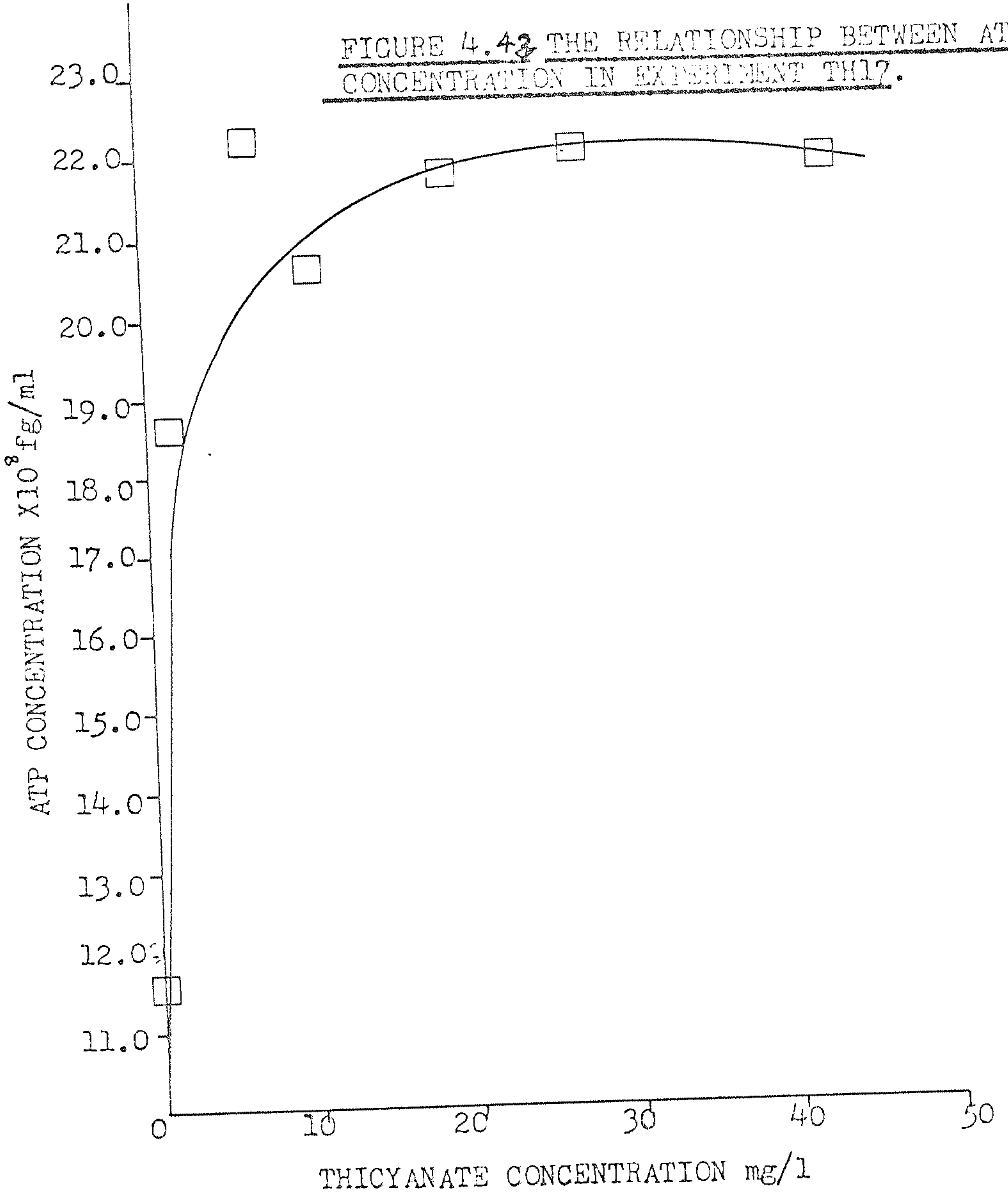


FIGURE 4.42 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH17.

FIGURE 4.44 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH18.

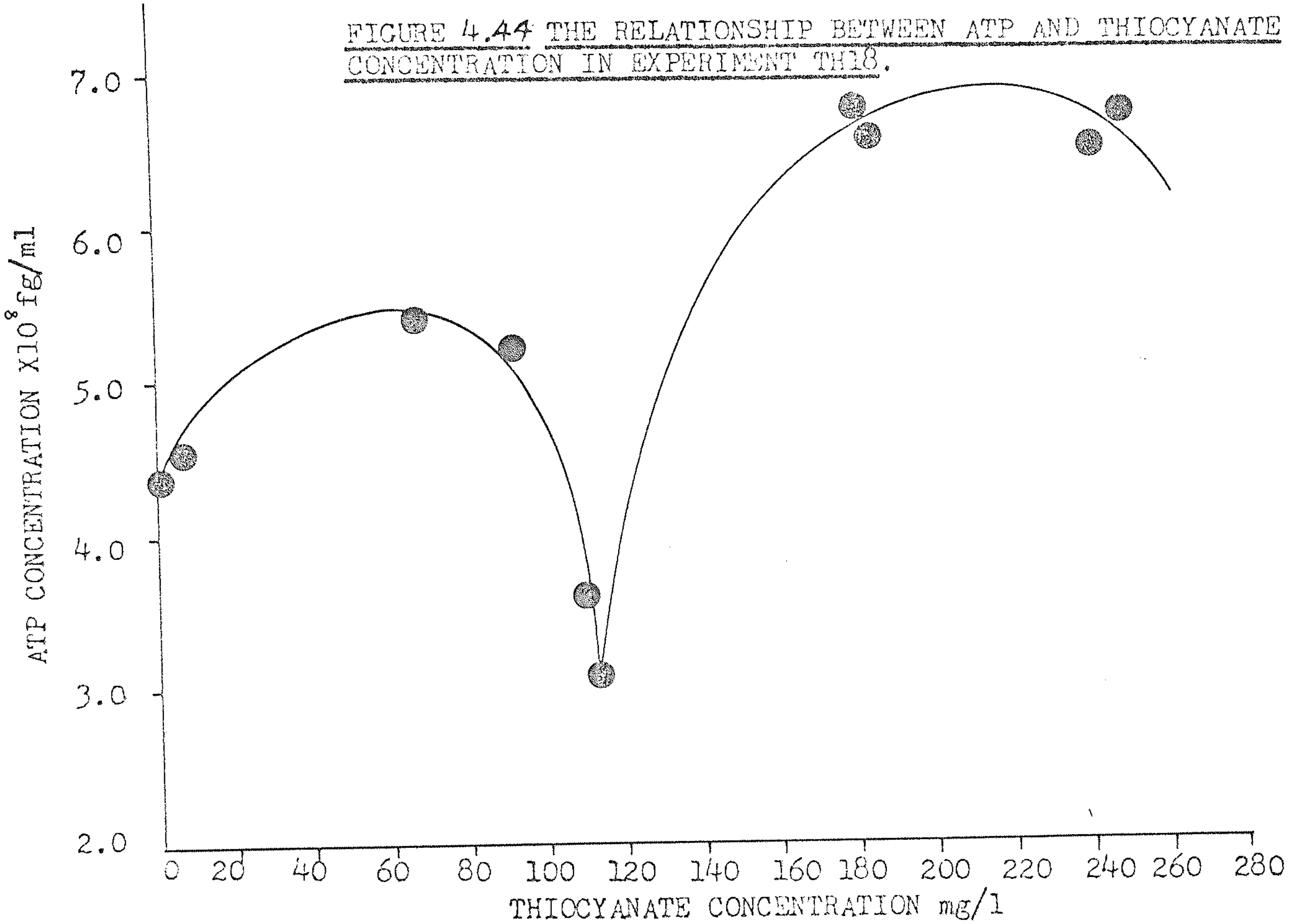
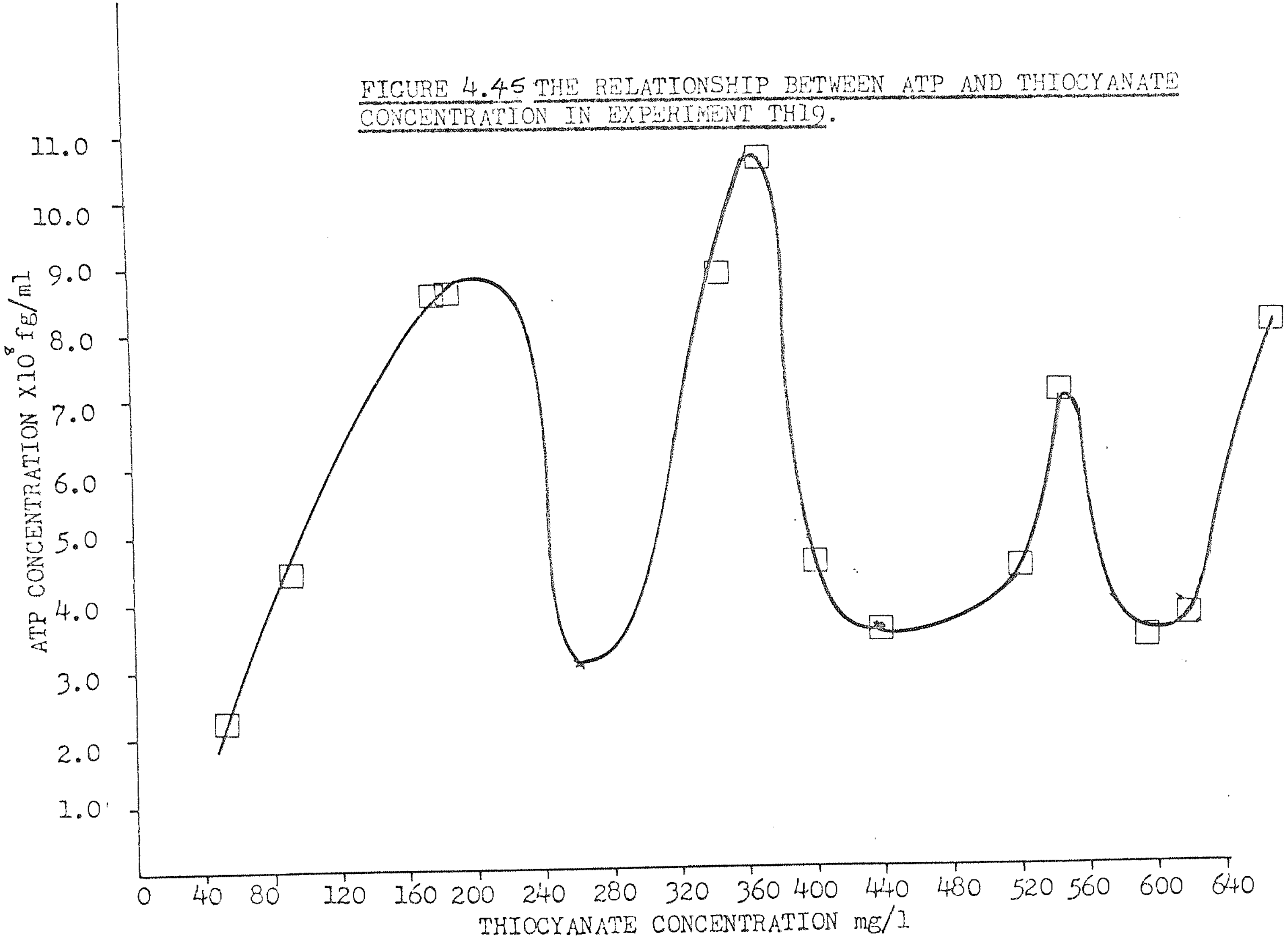


FIGURE 4.45 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH19.



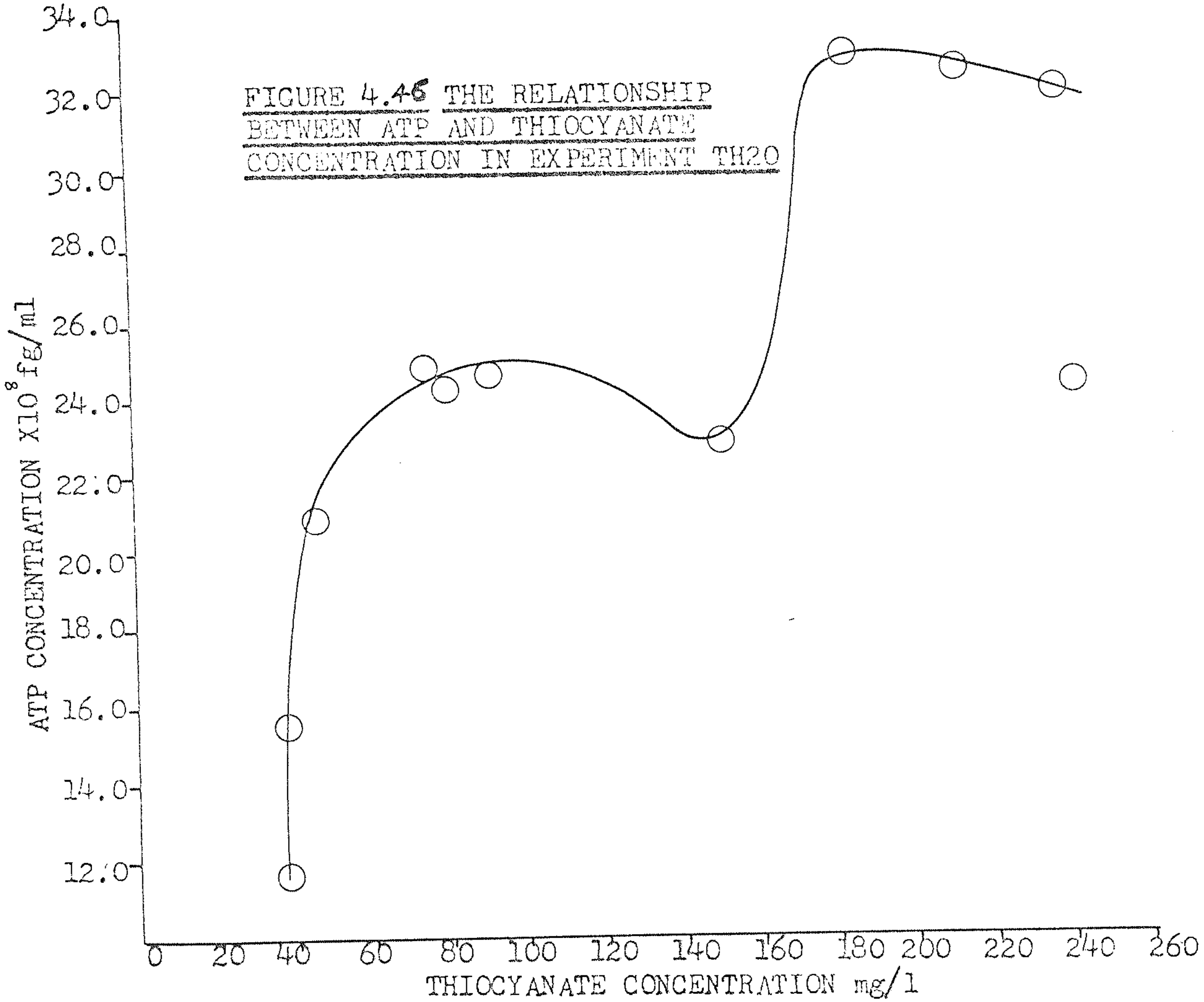


FIGURE 4.46 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH20

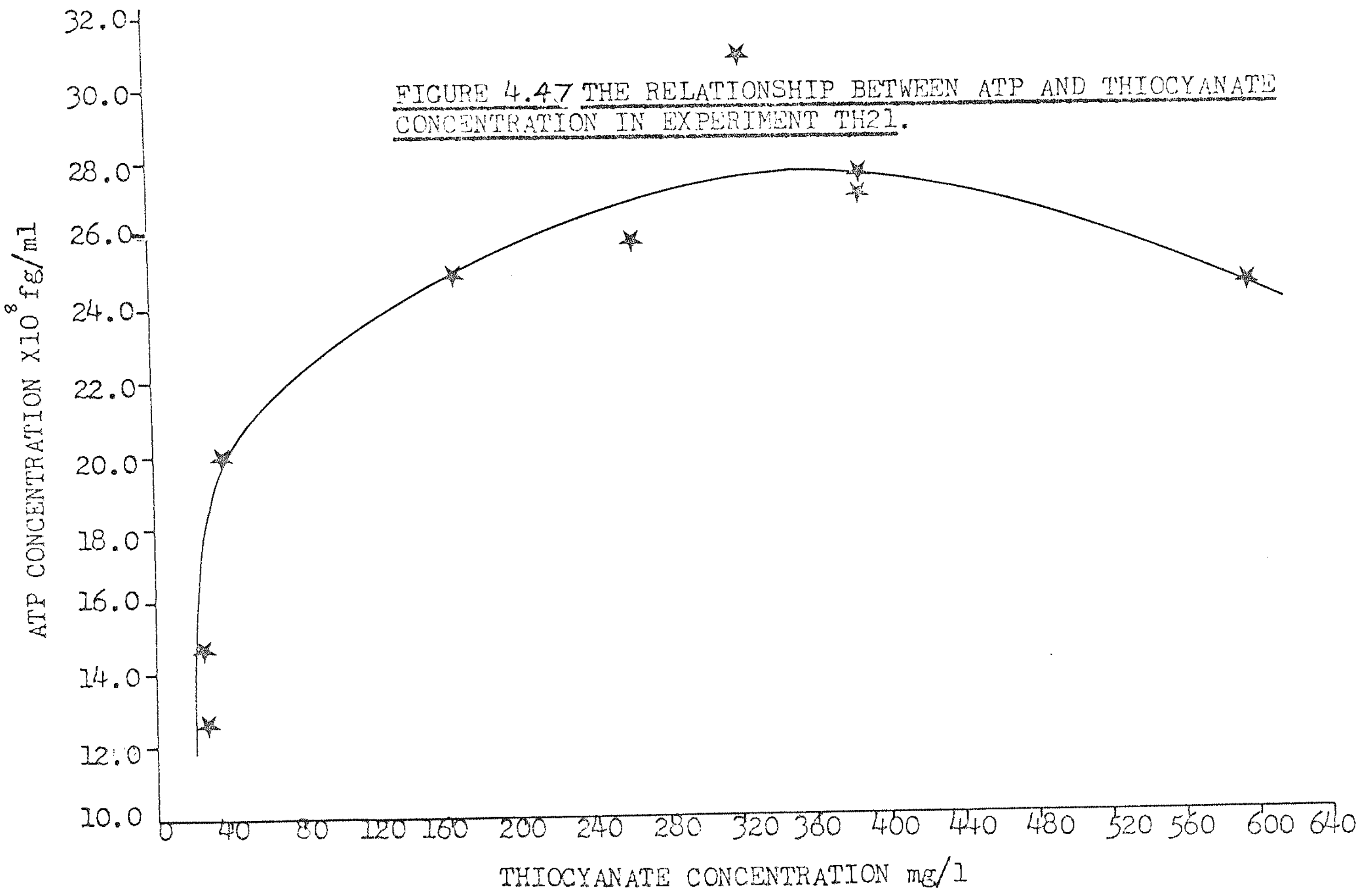
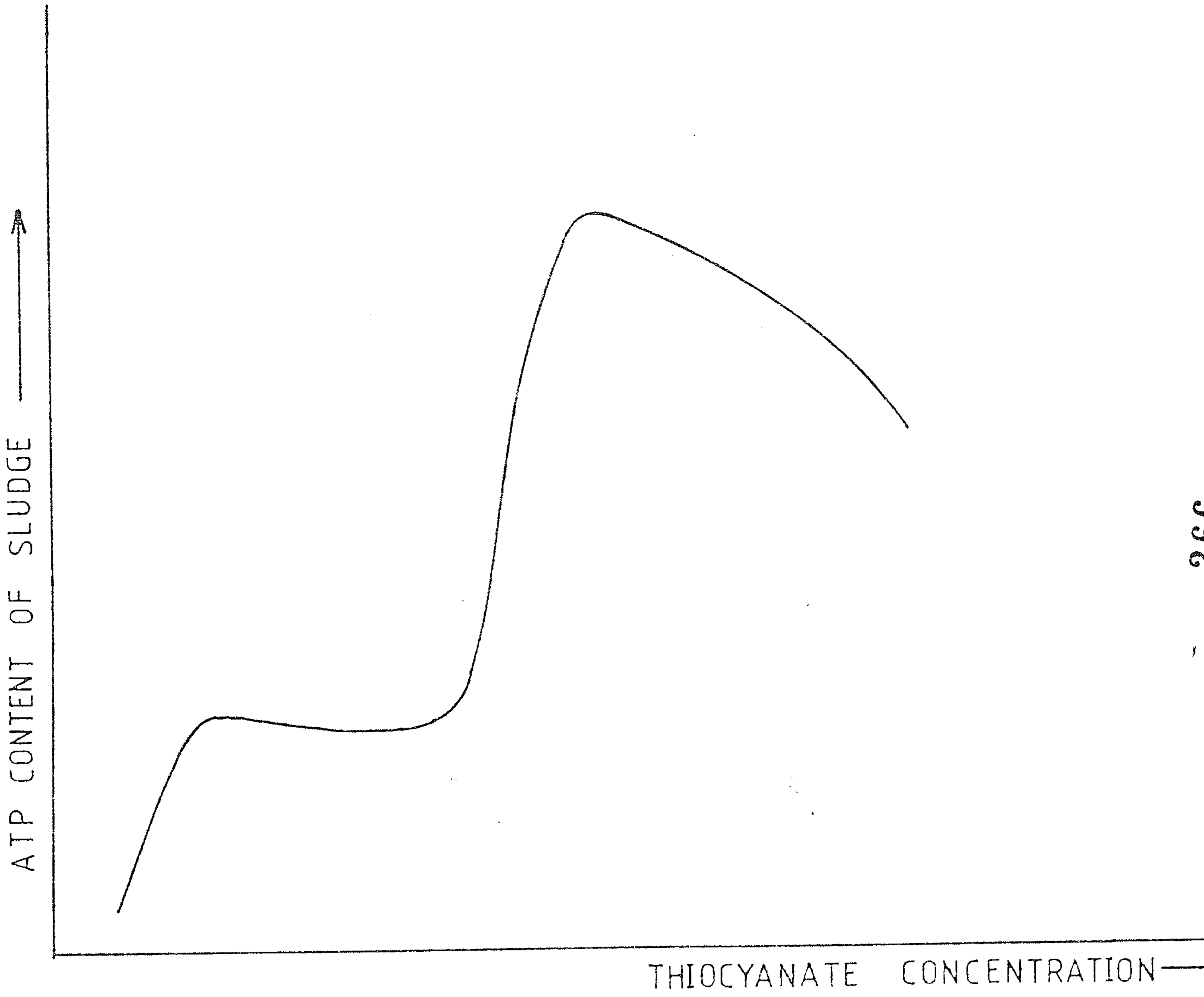


FIGURE 4.47 THE RELATIONSHIP BETWEEN ATP AND THIOCYANATE CONCENTRATION IN EXPERIMENT TH21.

Figure 4·48 Typical relationship between sludge ATP content and substrate concentration in batch reactors treating thiocyanate solutions.



3. Following this there was a rise in the ATP level to a new peak value in the concentration range 20-60mg/l, followed by a decline to an ATP value similar to that at the start of the experiment before substrate addition.

The occurrence of the second and smaller peak in the ATP pool towards the end of each batch experiment, within a reasonably constant thiocyanate concentration range, indicated that this behaviour may indeed have been related to the thiocyanate concentration in the sludge. The observed behaviour occured despite wide variations both in the initial thiocyanate concentration following dosing, the rate of thiocyanate degradation, and the total time taken for degradation to occur.

In order to clarify this possible relationshop between the ATP pool and sludge thiocyanate concentration graphs were plotted relating these in Figures 4.28 - 4.47 . With the exception of experiments TH1, 3, 4, 6, 7, and 8, ATP determinations were performed on samples taken at different times to those taken for thiocyanate analysis. In these cases the thiocyanate concentration corresponding to the times at which ATP determinations were made, were determined by extrapolation using the results of thiocyanate determinations performed immediately before and after each ATP result, assuming a linear rate of change over each period considered. The results obtained using this procedure were used to plot the Figures.4.28-4.48 In most cases the sampling frequency for substrate determinations was such as to make the errors involved in this procedure small.

From these graphs a general relationship between sludge ATP content and the substrate concentration in the sludges was found which could be generalised in the form shown in Figure 4.48. This representation of the data made this apparent relationship more obvious in that 2 distinct peaks in ATP levels are evident, the smaller in the 0.80mg/l range, and the larger above 80mg/l.

This relationship was clearly evident in Figures 4.33, 4.35, 4.36 4.44, 4.46 representing data from experiments TH7, 9, 10, 18 and 20. It was not evident in Figures for experiments TH1, 3, 4, 8, 11, and 17 respectively, but in these experiments the data available did not cover the relevant range of substrate concentrations. Of these however, experiments TH14-17 clearly demonstrated the existence of the first (lower) peak in the thiocyanate range 0.80mg/l, and these results were consistent with those typified by Figure 4.48 within the lower concentration range. The results for experiments TH6, 12 and 13 also indicated the existence of 2 peaks, although there was insufficient data to demonstrate this as clearly as in other experiments. In experiments TH19 and 21 the sampling frequency was too low for sufficient data to be gathered to define the graph adequately in the thiocyanate concentration ranges concerned.

It may be concluded that the relationship shown in Figure 4.48 was evident in a large number of the experiments, and where it was not the results obtained were at least consistent with this relationship within the limits of the concentration ranges used, or a low sampling frequency had prevented adequate definition of the relationship.

4.8 Discussion

4.8.1 Establishment of a General Trend in ATP Behaviour

The establishment of a general trend in the ATP behaviour of batch sludges treating thiocyanate, as shown in Figure 4.27 represented an important development in the project. Early work in the project not presented here, and analysis of the results presented by TOMLINS for batch sludges treating thiocyanate, indicated that there was a difficulty in establishing consistent ATP behaviour in the batch experiments. A discussion and re-analysis of the results obtained by TOMLINS was reported later in the project and was not considered here. However, it would seem that the reason for the success in establishing the general pattern of behaviour reported here was in part a reflection of the research conducted early in the project into improving the facilities and techniques for ATP determinations at the sponsor's research laboratories.

Serious errors in ATP determinations can arise due to its labile nature if not properly stored before analysis, and from contamination of samples by extraneous microbial-bearing material. The attention paid to improving facilities and techniques for cleaning apparatus, sterilising glassware and solutions, and for storage of these and samples was found to be an important contributory factor in obtaining reproducible results. In particular the simple technique described earlier for freezing and storing sludge samples proved invaluable in this connection as it enabled larger numbers of sludge samples to be taken in an experiment. Perhaps the most important consequence of this was that ATP analysis no longer needed to be carried out immediately but could be postponed until a convenient later date, so making more time available for ensuring good sampling and analytical techniques.

4.8.2 Interpretation of ATP Data

The decision to use ATP as a measure of biomass at the start of the project described by TOMLINS was based on the assumption that the ATP content of a cell remained relatively constant. It was obvious however from the work reported here and by TOMLINS that the immediate and large change in the ATP concentration of the batch sludges following the addition of substrate was far greater than could be expected by an increase in biomass due to growth. The response of the sludge to substrate addition clearly indicated that ATP represented some measure of sludge activity as well as the active biomass content of the sludge. Other workers of course have made similar suggestions for other activated sludge systems. In view of the intimate and intricate involvement of ATP in the metabolic activity and regulation of the cell, it would have been surprising in fact if the ATP content of the cells in the sludge, in the changing environmental conditions of the experiments described had remained constant.

However, observation that the ATP level in the sludge changed with environmental conditions and was therefore indicative of a change in sludge activity, although interesting, was of itself of little practical value to the project. The relationship between the ATP level and sludge activity clearly needed to be defined in a more precise manner if it was to be useful in gaining a greater insight into the behaviour of sludges treating thiocyanate and coke oven liquor, and in the mathematical modelling of the treatment system.

4.9 An Hypothesis to Explain the Observed ATP Behaviour

On the basis of the results described it was possible to postulate the hypothesis that the sludge ATP level, above a basal level, represented a measure of the specific growth rate.

The basal level was considered to be that level of ATP found in the sludge in the absence of substrate, and represented the ATP concentration corresponding to endogenous metabolic conditions. The relationship between specific growth rate and substrate concentration as predicted by the Monod growth kinetics discussed in Chapter 3, could be represented graphically as shown in Figure 3.2. Inspection of the graphical results presented earlier for the relationship between ATP and thiocyanate concentration revealed that there were obvious differences from those shown for the relationship between specific growth rate and substrate concentration in

Figure 3.2. To explain this is was proposed that the results for the sludges reflected the activity of 2 groups or populations of organisms, each of the 2 peaks in graphs of the type shown in Figure 4.48 predominantly representing the activity of one of the 2 populations. The precise relationship between ATP level and thiocyanate concentration in the results presented earlier was not always clear because of the limited data, but the results did indicate a decline in ATP activity for each of the proposed populations once the maximum level had been reached.

To account for this it was proposed that each of the populations exhibited substrate inhibition of the type described by HALDANE and considered in Chapter 3.

It was proposed therefore that the ATP behaviour of the sludges reflected the activity of two populations of organisms;

a) One population active at only low levels of substrate, and exhibiting substrate inhibition at thiocyanate concentrations greater than about 60mg/l. Their existence would explain the sudden rise in ATP level towards the end of each of the batch experiments described in this chapter.

b) One population of organisms predominantly active at higher levels of substrate, and also exhibiting substrate inhibiition.

These proposals therefore represented an initial approach to accounting for the role of ATP as a measure of activity. It was evident from these proposals, and the results presented, that the ATP content of sludge organisms was not constant but varied with the activity of the organisms. However, for a given species of organism it might be expected that the ATP content of a cell would be constant for given conditions of activity, and with all other conditions being constant (e.g. pH, temperature, etc.), for a given substrate concentration. This observation would have little practical value in most circumstances since the activity of the organisms would be expected to change continually in the presence of substrate as a result of the metabolism and removal of the substrate. However, no such difficulty would be expected in the absence of substrate, and it was proposed therefore that the cellular ATP level could be regarded as a constant in endogenous conditions, and as such represent a potentially useful measure of active biomass at this basal level of activity. In this way ATP could be potentially useful both as a measure of the active biomass and activity in activated sludge systems.

The ATP behaviour with respect to substrate concentration could be regarded as an "activity profile" of the sludge under consideration. The hypothesis that 2 distinct populations or organisms were involved obviously required further investigation and explanation before it could be fully described how the behaviour of each could be defined to produce the net result observed. There could also be other explanations to explain the ATP behaviour observed, but clearly whatever the explanation, the relationship between the ATP level and sludge activity would need to be defined in a more precise manner before it could have a really useful function in the modelling and control of the treatment system.

However, before further considering these points it was felt important to establish that the results observed were a real reflection of the response of the sludge to different substrate concentrations.

A number of investigations were therefore conducted to explore this relationship between ATP and substrate, and the role of ATP as a measure of activity. These were designed to measure the response of sludges to different substrate concentrations, whilst maintaining as many of the other factors likely to affect activity constant, and in particular to exclude the changes in sludge conditions in terms of accumulation of product and metabolic intermediates that may occur in lengthy batch experiments. This was done by investigating the initial ATP response of starved sludges to thiocyanate dosing and this work was described later. Also, to exclude the possibility that the ATP behaviour observed merely represented interferance in the ATP assay by thiocyanate, the effect of thiocyanate on the assay was also investigated, and this work was described in Chapter 5.

4.10 Other Investigations Conducted in the Project

The work described so far has referred only to the activated sludge treatment of the thiocyanate component of coke oven liquor. Similar work was carried out to investigate the ATP behaviour of batch sludges treating phenol, later in the project. The ATP behaviour of sludges treating phenol was well-established by TOMLINS, so only limited investigations of the type described here in Chapter 4, were carried out. These experiments were modified to include consideration of the effects of multiple phenol additions and were described in Chapter 7.

The batch experiments described in Chapter 7 were likely to be subject to the same limitations as those described here in Chapter 4. Experiments were therefore conducted to investigate the initial ATP response of sludges fed phenol analagous to the experiments described in Chapter 6 for thiocyanate-treating sludges. This work was described in Chapter 8.

The work described so far has only considered the batch treatment of the two main components of coke oven liquor, phenol and thiocyanate. In order to relate the ATP behaviour of these systems to the behaviour of continuous systems treating liquor itself, experiments were conducted which examined the ATP behaviour of a laboratory-scale reactor treating coke oven liquor. This experiment was described in Chapter 9.

The work described in Chapter 10 investigated the use of another measure of sludge activity which has been used by other researchers, oxygen uptake rate, or OUR. These investigations were carried out in conjunction with the continuous reactor study of Chapter 9, with the aim of comparing the use of the two measures of activity OUR and ATP later in the project.

Chapter 11 describes the results of design and operational modelling of the activated sludge treatment of coke oven liquor for phenol removal. This work involved analysis of performance data for pilot and full-scale plants treating the waste. It was not possible to investigate the ATP behaviour of full-scale plants treating coke oven liquor, and this work was conducted with the aim of comparing the knowledge gained concerning the growth kinetics of the system with that obtained using ATP and laboratory scale experiments, later in the project.

The experiments described in Chapter 5 to 11 were conducted in parallel and largely independently of each other. The significance of the results of these separate investigations to the overall aims of the project, were discussed along with the results of the investigations described in this chapter, in Chapter 12.

CHAPTER 5

INVESTIGATION OF ANALYTICAL TECHNIQUES

AN ASSESSMENT OF ANALYTICAL METHODS FOR SULPHATE AND AMMONIA DETERMINATIONS, AND OF THE EFFECT OF THIOCYANATE ON ATP DETERMINATIONS

Introduction

5.1 The colorimetric Determination of Ammonia in Coke Oven Effluent (COE). An Assessment of the application to COE analysis of the method originally proposed by HARWOOD and KÜHN for analysis of natural waters.

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5.3 Investigation of the Effect of Thiocyanate on ATP Determinations

5.3.1 Introduction
5.3.2 Reagents
5.3.3 Method
5.3.4 Results
5.3.5 Discussion and Conclusions

INVESTIGATION OF ANALYTICAL TECHNIQUES

AN ASSESSMENT OF ANALYTICAL METHODS FOR SULPHATE AND AMMONIA DETERMINATIONS, AND OF THE EFFECT OF THIOCYANATE ON ATP DETERMINATIONS

Introduction

Two of the analytical methods used in the project, the determination of ammonia using the phenol-hypochlorite method, and the turbidimetric determination of sulphate, required some preliminary investigation before they could be applied in the activated sludge experiments.
Also the results from the batch experiments described in the previous chapter required that the effect of thiocyanate on the ATP assay technique be investigated. This chapter describes the results of those investigations.

5.1 The Colorimetric Determination of Ammonia in Coke Oven Effluent (COE)

An assessment of the application to COE analysis of the method originally proposed by HARWOOD and KÜHN(138) for analysis of natural waters

5.1.1. Introduction

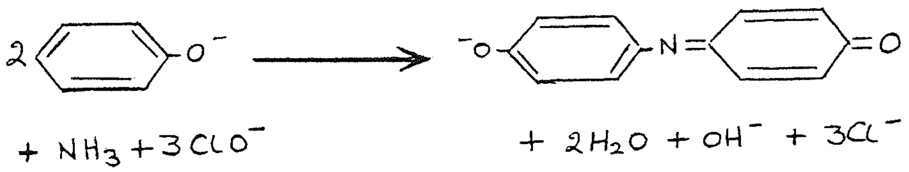
Ammonia is typically found in high concentration in COE. A knowledge of its concentration in the activated sludge treating coke oven waste and in the final effluent is important because ammonia is toxic to, and exerts an oxygen demand on, a watercourse receiving such waste, and inhibitory to many of the organisms found in treatment plants purifying coke oven liquors.

Analysis of activated sludges and effluents for ammonia from laboratory-scale plants treating coke oven wastes is routinely carried out at British Steel's Battersea Research Laboratory, which is the location at which much of the present project's work is centred, and at many other of the sponsor's laboratories. The recommended method for ammonia analysis involves distillation of the sample followed by absorption of the released ammonia in dilute acid and titration. However, SUTCLIFFE and JONES(139) have indicated that the hydrolysis of nitrogenous material may occur in the distillation method. This possibility, particularly the hydrolysis of thiocyanate (which may be present in high concentrations) to ammonia, has meant that the method is generally considered unsatisfactory. Also, the distillation method has been found to be very time consuming, and where large numbers of samples regularly require to be analysed, as is frequently the case at British Steel's laboratories, the method has proved to be impractical.

In the past the simple colorimetric Nessler method of ammonia determination has been used. However, a number of workers, including TETLOW and WILSON(140), have indicated this method to be sensitive to many variables, and SUTCLIFFE and JONES have indicated that this includes the age of the reagent.

Experience at the Battersea Research Laboratory has indicated that the method is particularly subject to interference from the variable constituents of coke oven effluent.

More recently, ammonia determinations at the Battersea Laboratory have been based on the reaction of ammonia with phenol and hypochlorite and spectrophotometric determination (at 630nm) of the blue-coloured indophenol compound produced. The overall reaction leading to the production of this compound is generally accepted to be as follows:



The method of HARWOOD and KÜHN (138) in particular is used, in which sodium nitroprusside is used to catalyse the above reaction. Recent experience of this latter method at the Battersea Laboratory has suggested the possible interference by thiocyanate for accurate determinations in samples containing high concentrations of ammonia ($>$200 mg/lNH_3).

The aim of the present experiment was to investigate these two factors, and so assess the value of the method for accurate determination of ammonia in coke oven effluents containing high ammonia and/or thiocyanate concentrations.

EXPERIMENTAL

5.1.2 Reagents

Analytical grade (ANALAR) chemicals were used wherever possible for reagent preparation. Laboratory distilled water which was then passed through a deioniser, was used.

Phosphate Buffer

5% W/V Na_3PO_4 solution.

Phenol Stock

500g phenol were dissolved in methanol and diluted to 800ml with methanol. The reagent was stored in a refrigerator at 2-5°C.

Sodium Hydroxide Solution 27% W/V

270g NaOH pellets were dissolved in water, cooled and diluted to 1 litre.

Phenate Reagent A

15ml of the phenol stock and 0.02g sodium nitroprusside were diluted to 100ml with water.

Sodium Hypochlorite Solution 3.5% Cl

20mls of sodium hypochlorite solution (Fisons Technical Grade 8% min. available chlorine) were diluted to 45mls with water.

Reagent B

Equal volumes of sodium hypochlorite solution (3.5% Cl) and 27% sodium hydroxide solution were mixed, and diluted to 50ml.

Reagents A and B were stored in amber bottles in a refrigerator (2-5^{o}C), but allowed to reach room temperature before use.

Stock Ammonium Chloride Solution

3.141g dried NH_4Cl was dissolved in water and diluted to 1 litre in a volumetric flask.

Standard Ammonium Chloride Solution 10µg/ml NH_3

10.0mls of stock ammonium chloride solution was diluted to 100mls of the latter well-mixed solution was diluted to 100mls in a second volumetric flask and mixed to produce a final solution containing 10µg/ml NH_3.

Thiocyanate Solution 100mg/1 CNS^-

This was prepared as previously described in the Ferric Nitrate

Method of thiocyanate determination.

5.1.3 Method

5.1.3.1 Preparation of Calibration Graph

Aliquots of standard ammonium chloride solution (containing 10 to 100µg NH_3) were pipetted into 50ml volumetric flasks and 4.0ml buffer added. All the flasks were then diluted to about 25mls with water, and 10.0mls Reagent A, and 5.0mls Reagent B added to each flask, and the volume of each flask diluted to 50mls with water. Each flask was mixed thoroughly. A blank solution was prepared in exactly the same manner but did not contain ammonium chloride solution. The optical density of each solution was read in 10mm cells, against a reference cell containing water, at 630nm using a Unicam SP30UV Spectrophotometer. The reading of the blank solution was subtracted from that for the other solutions, and a calibration graph drawn relating optical density to the ammonia content of each flask. (All standard solutions used in the preparation of the calibration graph were prepared in duplicate).

5.1.3.2 Investigation of the Effects of Thiocyanate

A series of 50ml volumetric flasks were prepared containing 3.0ml of standard ammonium chloride solution.

Aliquots of standard thiocyanate solution were added to the flasks, and the ammonia determination carried out as described above. The determination was also performed on flasks containing standard ammonium chloride solution but no thiocyanate. All determinations were performed in duplicate. The effects of the addition of thiocyanate solution on the ammonia determination were represented graphically.

5.1.3.3. Accuracy of Determinations for Samples Containing High Ammonia Concentrations

The supernatents from centrifuged samples of activated sludge treating coke oven liquor were used as samples. 1.0ml of each sample was pipetted into 100ml volumetric flasks and diluted to volume with water. This was performed in duplicate for each sample. 10.0ml aliquots of each of these solutions were pipetted into 50ml volumetric flasks and the ammonia determinations carried out as described above. The results were used to determine the ammonia concentration of the samples, and comparison of the duplicate results enabled an assessment of the accuracy of the procedure to be made.

5.1.4 Results

The results obtained from the calibration procedure were recorded in Tables 5.1 and the calibration graph produced

Table 5.1 Results of Ammonia Analyses (Using the HARWOOD and KÜHN Method) Used to Prepare Calibration Graph.

μg NH_3 in 50ml	E^{630nm}_{1cm} VS H_2O	E^{630nm}_{1cm} - Blank	Mean	S.D.	Coeff. of Variation %
Blank	0.066	-	-	-	-
10	0.252	0.186	0.193	0.009	4.7
10	0.265	0.199			
30	0.580	0.514	0.504	0.015	3.0
30	0.559	0.493			
50	0.926	0.860	0.826	0.049	5.9
50	0.857	0.791			
100	1.712	1.646	1.661	0.020	1.2
100	1.741	1.675			
				Mean	3.7

from this data shown in Figure 5.1. The regression line shown had a correlation coefficient of r = 0.9992, and Beer's Law was obeyed over the concentration range, 0 - 100µg NH_3 in 50ml, investigated. The method was reproducible, giving a mean coefficient of variation of 3.7% over this concentration range.

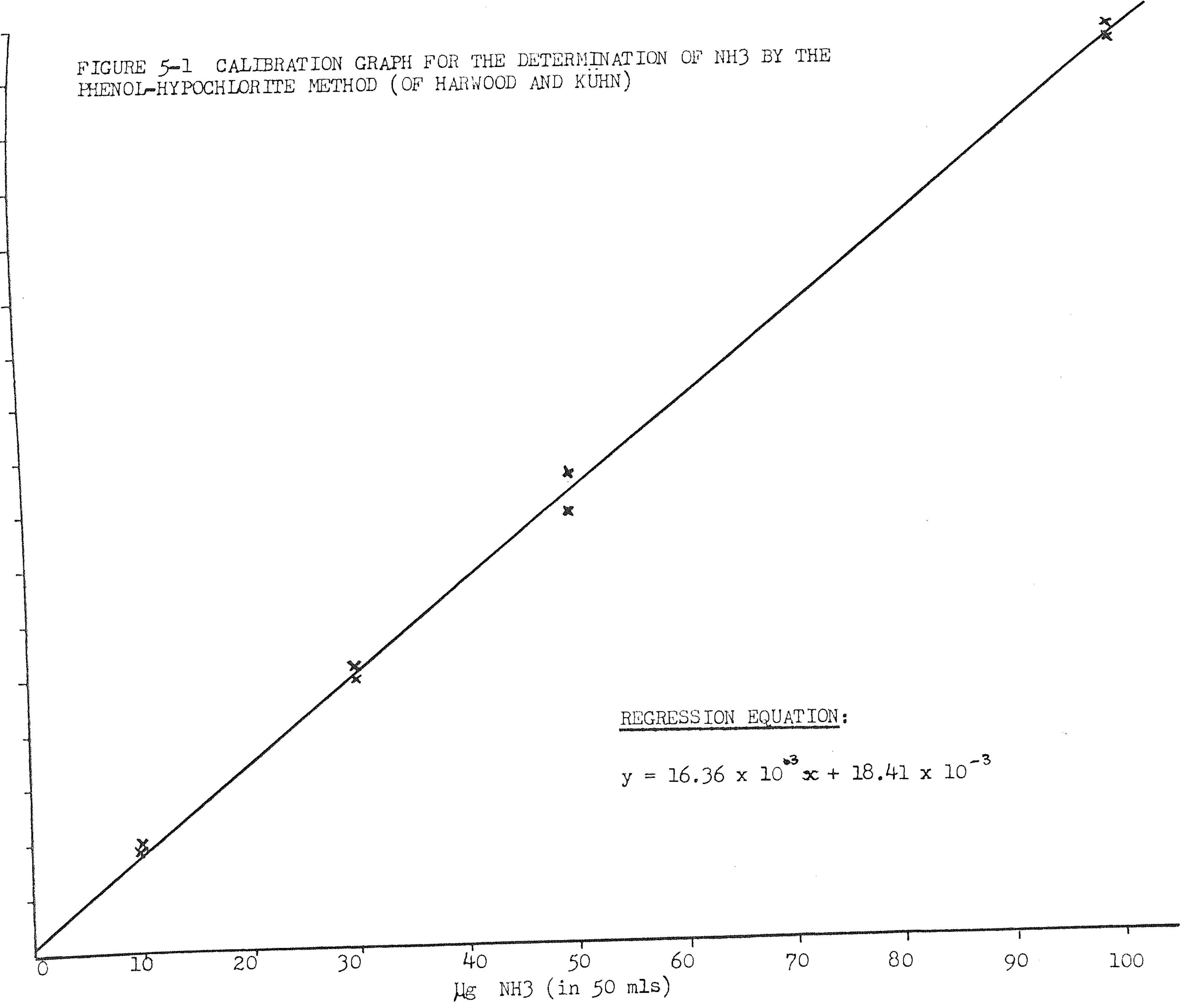
The results obtained for the analysis of samples of supernatants obtained from the centrifugation of various samples of activated sludge treating coke oven liquor were recorded in Table 5.2 A mean coefficient of variation of 7.5% was found for the samples analysed.

The results obtained from the investigation of the effects of thiocyanate on the determination were recorded in Table 5.3. The mean coefficient of variation for the solutions analysed was 2.2%. The results were plotted in the graph shown in Figure 5.2, which shows the effect of the presence of various levels of thiocyanate on the readings recorded for the ammonia concentration in the solutions tested. The ammonia concentration recorded was reduced as the thiocyanate content of the solution under test was increased. A linear relationship was indicated for the thiocyanate concentration used, with a correlation coefficient of 0.988.

5.1.5 Discussion

The results presented in Table 5.2 indicated that HARWOOD and

FIGURE 5-1 CALIBRATION GRAPH FOR THE DETERMINATION OF NH3 BY THE PHENOL-HYPOCHLORITE METHOD (OF HARWOOD AND KÜHN)



REGRESSION EQUATION:

$y = 16.36 \times 10^{-3} x + 18.41 \times 10^{-3}$

KÜHN's method of ammonia analysis gave sufficiently reproducible results to justify its use for the analysis of coke oven effluents.

The results presented in Figure 5.2 clearly indicated that high thiocycanate concentrations reduced the colour intensity in the ammonia assay, producing significant errors in the assay results in the conditions employed.

The ammonia content of coke oven effluent varies considerably and will of course be highest in the untreated liquor. If 360mg/l is taken as a mean value for each liquor, then from Figure 5.2 it can be deduced that the concentration of thiocyanate in the sample necessary to lower the ammonia concentration determined to a value 10% below the real value, the thiocyanate concentration would need to be 2000mg/l. The latter is 5 to 10 times higher than the typical thiocyanate concentration found in the influent.

The effect of thiocyanate on the ammonia determination would appear to be dependant upon the relative concentration of the two components. The results indicated that in reality a significant deviation ($\geqslant$ 10%) in the value determined for the ammonia concentration from the true value, was only likely to occur when there was a combination of a very low ammonia concentration and a relatively high thiocyanate concentration. For example, a 10% reduction in the determined ammonia concentration at the 36mg/l level would still only occur with

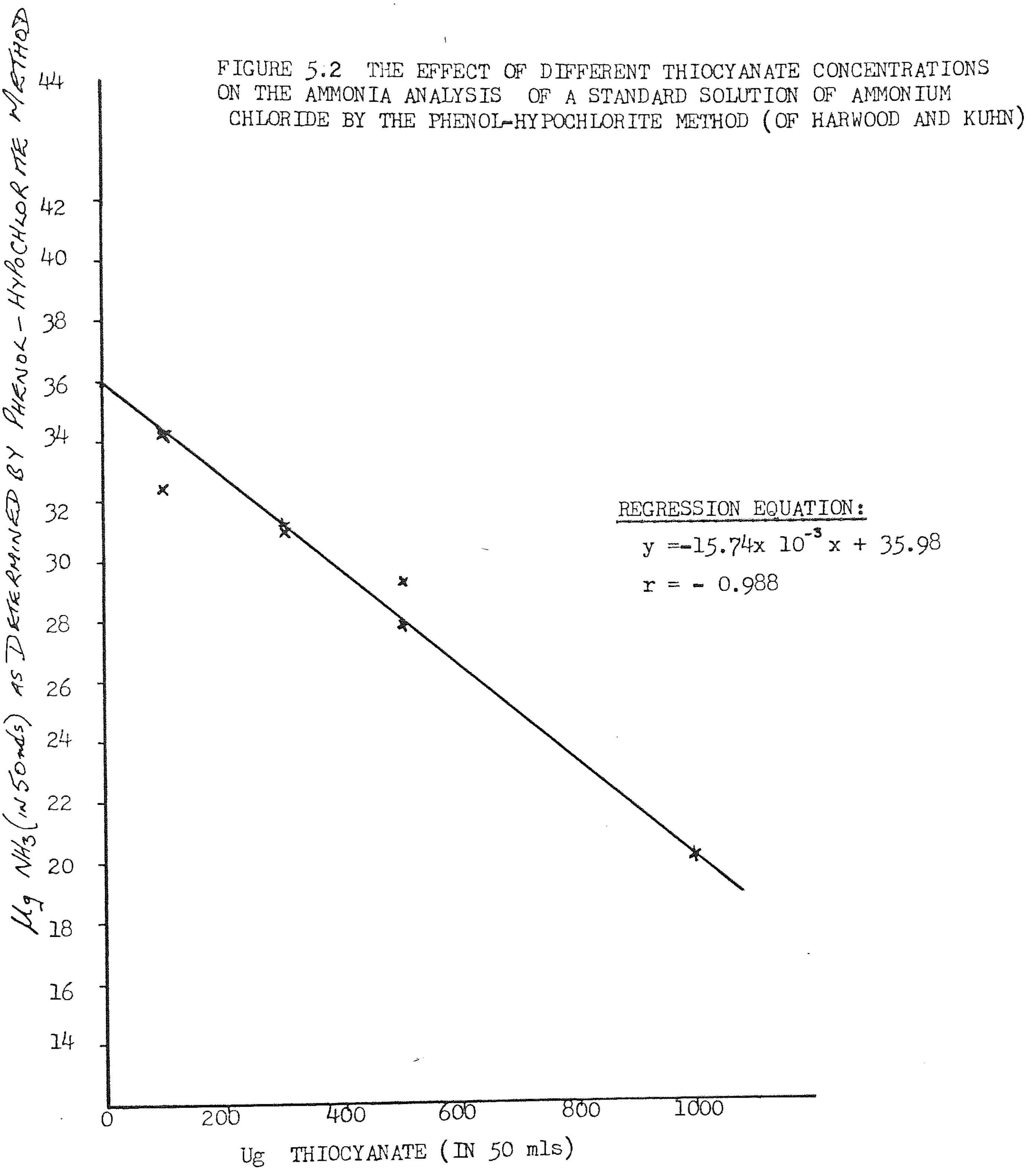
Table 5.2 Ammonia Analyses of Samples of Supernatants Obtained By Centrifugation of Various Activated Sludges Treating Coke Oven Liquors.

Sample	E_{1cm}^{630nm} VS H_2O	E_{1cm}^{630nm} - Blank	NH_3 In Sample mg/l	Mean NH_3 mg/l	S.D.	Coeff. of Variation %
Blank	0.071	-	-	-	-	-
A	0.163	0.092	45.0	42.2	4.0	9.5
A	0.152	0.081	39.4			
R8	0.764	0.693	412	395.5	23.3	5.9
R8	0.709	0.638	379			
R7	0.732	0.661	393	428.5	50.2	11.7
R7	0.849	0.778	464			
G2	0.796	0.725	432	464.0	45.3	9.8
G2	0.901	0.830	496			
B	1.112	1.041	625	627.0	2.6	0.4
B	1.118	1.047	629			
					Mean	7.5

Table 5.3 The Effect of Different Thiocyanate Concentrations on The Ammonia Analysis of a Standard Solution of Ammonium Chloride.

Sample	Thiocyanate µg in 50ml	Ammonia Reading µg in 50ml	Mean NH_3 Reading µg in 50ml	S.D.	Coeff.of Variation %	% change in NH_3 reading due to presence of thiocyanate
1	0	37.51	36.78	1.04	2.8	-
2	0	36.04				
3	100	34.27	33.35	1.30	3.9	-9.3
4	100	32.43				
5	300	31.01	31.14	0.18	0.6	-15.3
6	300	31.26				
7	500	29.32	28.59	1.04	3.6	-22.3
8	500	27.85				
9	1000	20.15	20.15	0	0	-45.2
10	1000	20.15				
				Mean	2.2	

FIGURE 5.2 THE EFFECT OF DIFFERENT THIOCYANATE CONCENTRATIONS ON THE AMMONIA ANALYSIS OF A STANDARD SOLUTION OF AMMONIUM CHLORIDE BY THE PHENOL-HYPOCHLORITE METHOD (OF HARWOOD AND KUHN)



a thiocyanate concentration of 200mg/l.

It will have been evident from earlier descriptions in the project that the ammonia concentration in untreated coke oven liquor is typically greater than that of the thiocyanate concentration. Similarly, in treated liquor thiocyanate is invariably metabolised before ammonia so that the ammonia concentration typically remains greater than the thiocyanate concentration.

The mechanism by which thiocyanate interferes in the ammonia assay was not obvious from the results obtained. However, the details of the mechanism of interference, as with the details of the mechanism involved in indophenol formation itself, are not required to make practical use of the results obtained.

5.1.6 Conclusions

It may be concluded that although the investigation demonstrated that thiocyanate interfered in the determination of ammonia by the phenol-hypochlorite method, this interference was not likely to be significant for most samples of liquor and sludge taken from a plant treating coke oven liquor.

5.2 The Turbidimetric Determination of Sulphate in Coke Oven Effluent

An assessment of a simple and rapid method developed for the determination of sulphate in large numbers of samples using the Analmatic Automatic Analyser

5.2.1 Introduction

The method described was developed by the author by simple modifications of standard methods of sulphate determination. Modification of existing methods was necessary to enable rapid analysis of large numbers of coke oven effluent samples. The recommended gravimetric method was not found to be practical for this purpose.

5.2.2. Principles

The principle of the method used was that soluble sulphate was precipitated in the presence of barium chloride solution to form a precipitate of barium sulphate under controlled conditions. The amount of precipitate formed is directly proportional to the soluble sulphate content of the test solution, providing that there is an excess of barium ions present. Determination of the sulphate content of the sample was dependant upon quantitative determination of the precipitate formed. Insoluble material was removed from the sample prior to the addition of the barium chloride solution to avoid interferance in the determination.

In the method to be described the precipitate formed was maintained in colloidal suspension by incorporating a stabilising reagent in the assay mixture, and the suspension was estimated turbidimetrically. A detailed account of the theory and measurement of scattered radiation can be found elsewhere and is not described here.

The turbidance of suspensions may be measured by conventional use of a spectrophotometer in the visible light region, and involved measurement of the power of the transmitted beam P_t relative to the power of the incident or illuminating beam P_o. The turbidance, corresponding to absorbance, of the solution can be obtained by the relation;

$$S = \log \frac{P_o}{P_t} = k\ b\ c \qquad \text{(EQUATION 5.1)}$$

where S = turbidance

k = the turbidity coefficient

b = the path length

c = the concentration in grams per litre

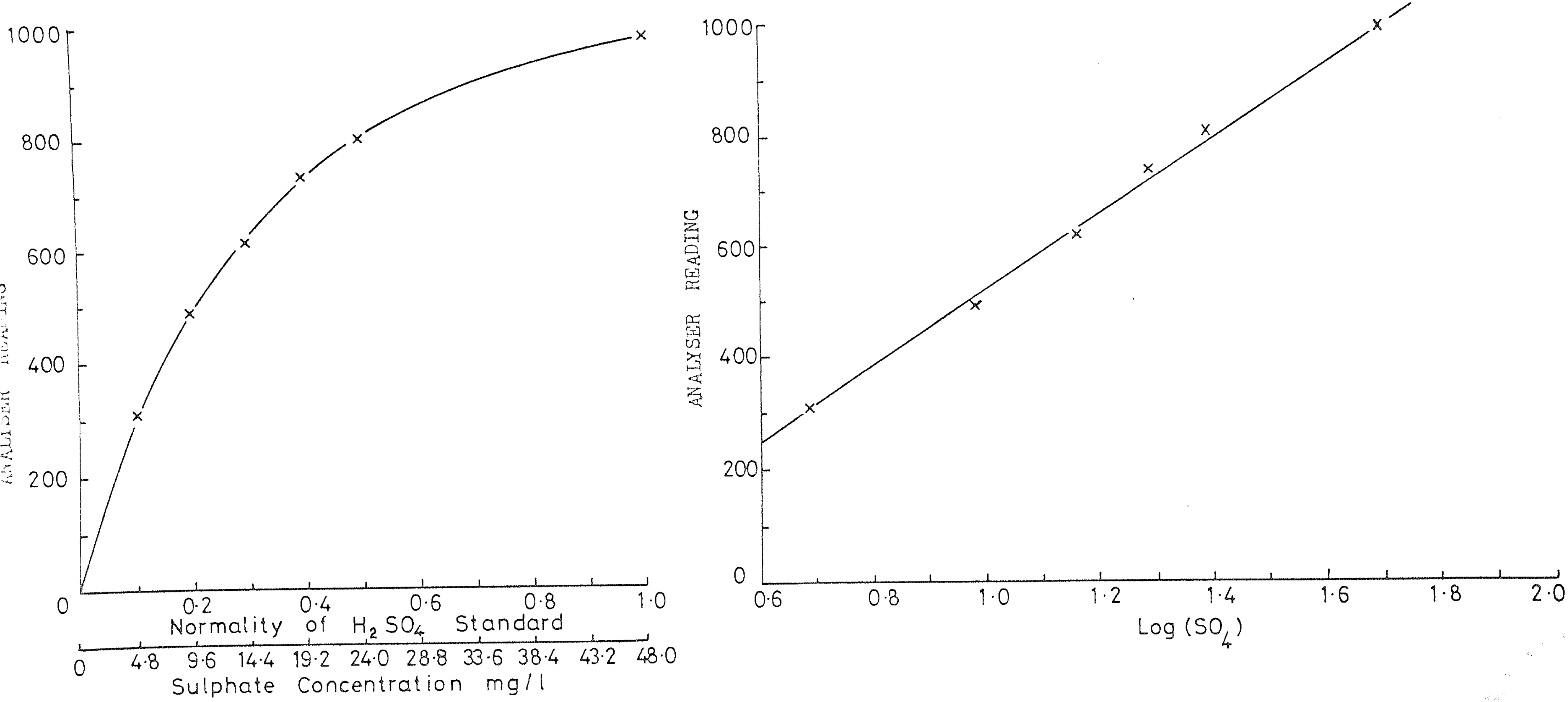
This expression is generally only valid for dilute suspensions because at higher concentrations a large proportion of the light that would have been deflected from the line of the incident/ transmitted beam actually becomes deflected back along its line by multiple scattering.

(Methods are available for measurement of the amount of light scattered rather than the light that passes through the sample without deflection (transmitted light), and the general method involved is called nephelometry. The above equation is not applicable in the latter method).

In a spectrophotometer of the type incorporated in the Analmatic Automatic Analyser used in this method, the power of the transmitted beam passing through a sample cell is measured with reference to the power of the incident beam which passes through a reference cell. P_o and P_t are therefore measured, and the instrument gives a range of transmittance readings which in the case of this instrument can be calibrated using standard solutions. On the basis of Equation 5.1 it can be seen that the transmittance reading on the instrument should be linearly related to the log. of the sulphate concentration. It should therefore be possible to derive the sulphate concentration of samples by determination of their transmittance on the calibrated spectrophotometer and use a of a graphical plot of the type shown later in Figure 5.3.

The investigation described assesses a simple and rapid method, developed on the basis of the above principles, for the determination of sulphate in large numbers of coke oven effluent samples using the Analmatic Automatic Analyser.

FIGURE 5.3 CALIBRATION OF THE ANALMATIC AUTOANALYSER FOR SULPHATE DETERMINATION



EXPERIMENTAL

5.2.3 Reagents

Stabilising Reagent

135g of calcium chloride $CaCl_2 \cdot 2H_2O$ were dissolved in 200ml of water and 50ml of concentrated hydrochloric acid, with swirling and cooling. 500ml of diethylene glycol was added, followed by the addition of water to give a final volume of 1 litre, and shaking to produce an homogenous solution.

Barium Chloride Solution 5% W/V

5g of barium chloride $BaCl_2 . 2H_2O$ were dissolbed in 100ml of water. (This was the same solution recommended by the Institute of Gas Engineers(137) for the gravimetric determination of sulphate).

Standard Sulphate Solution 48.03g/l SO_4

1.000N sulphuric acid, H_2SO_4, was used. (Analar anhydrous Na_2SO_4 was a prefered standard but was not available at the time of this investigation).

5.2.4 Method

The Analmatic Automatic Analyser (Baird and Tatlock) was used in the investigation. The instrument was operated exactly as recommended by the manufacturers, the details of which were described in the operating manual. Sample and standard dilution, reagent addition, and optical density determinations and calibration procedures were summarised as follows:

Stage 1 - Sample and standards were diluted with distilled water (0.1ml sample, blank, or standard + 5.0ml water) using syringes 5 and 6.

Stage 2 - 1.0ml of stabilising reagent was added to the diluted test solutions using syringe No. 3.

Stage 3 - 3.0mls of barium chloride solution was added to each test solution using syringe No. 4.

Stage 4 - The optical density of each solution was determined immediately at 420mm, with distilled water being used in the reference cell.

1.0N, 0.5N, 0.4N, 0.3N, and 0.1N sulphuric acid solutions were used as standards for calibration of the instrument of the calibration curve.

The spectrophotometer was set to give full deflection (i.e. reading 1.00) and the printer to give a reading of 999 for the 1.0N standard. Distilled water, which had been treated with reagents in exactly the same manner as the standards and samples, was used as the blank solution with which to check and set zero readings on the spectrophotometer and printer.

Typically 10 blank solutions and 10 standard solutions were used to calibrate the instrument, and 50-60 samples were analysed in each batch of determinations. Instrument calibration was checked by including a full series of blanks and standards with each set of samples analysed.

5.2.5 Results

The results obtained from the calibration procedure were recorded in Table 5.4 and were plotted in Figure 5.3. The plot of instrument reading in relation to the logarithm of the sulphate concentration was linear, with a regression line of correlation coefficient equal to 0.998. The results shown in this plot were typical of those produced on each occasion that the calibration procedure and analysis of samples was carried out.

The results obtained agreed with those predicted earlier from theoretical considerations, and the method developed and described proved entirely satisfactory for sulphate analysis of coke oven effluent samples.

The concentration of sulphate in samples was determined using the linear plot shown in Figure 5.3 or the regression equation in Table 5.3.

TABLE 5.4 RESULTS OF CALIBRATION PROCEDURE FOR SULPHATE DETERMINATION, AND REGRESSION ANALYSIS OF THE LINEAR PLOT SHOWN IN FIGURE 5.3

H_2SO_4 STANDARD	(SO_4) mg/l	$\log(SO_4)$	E_{420nm} READING
1.0N	48	1.6812	897
1.0N	48	1.6812	989
0.5N	24	1.3802	801
0.4N	19.2	1.2833	732
0.3N	14.4	1.1584	614
0.2N	9.6	0.9823	490
0.1N	4.8	0.6812	307

REGRESSION EQUATION

$y = mx + c$

where $x = \log(SO_4)$

$y = E_{420nm}$ READING

m = slope

c = y intercept

Using the data above

$c = 174.64$

$m = 694.24$

correlation coefficient = $r = 0.998$

5.3 Investigation of the Effect of Thiocyanate on ATP Determinations

5.3.1 Introduction

The experiments investigating the ATP behaviour of batch activated sludges treating thiocyanate, described in Chapter 4, indicated that the ATP response of the sludge was related to the thiocyanate concentration of the system in a manner typified by Figure 4.51. The significance of this behaviour was discussed earlier. It was noted in that discussion that it was important to establish that the ATP results observed, particularly those at the 20-80mg/l level, represented the actual response of the sludge and were not an artifact of the ATP assay system. The possibility therefore that the results observed may merely have represented the response of the luciferase enzyme system to thiocycante had to be checked.

The following experiment investigated the possible interference of thiocycante in the ATP assay by examining the ATP content of frozen samples of a standard ATP solution containing different concentrations of thiocyanate. The concentrations used in these standard solutions covered the range of thiocyanate concentrations found in the extracted sludge samples assayed earlier in the project.

EXPERIMENTAL

5.3.2 Reagents

Clean ATP-free apparatus, prepared as described in Chapter 4, was used for the preparation of reagents, and throughout the rest of the investigation.

ATP Diluent

This was prepared as described in Appendix 1.

Stock Thiocyanate Solution 5g/1 CNS^-

4.5g of potassium thiocyanate were dissolved in ATP diluent and diluted to 500ml in a volumetric flask with the diluent.

Thiocyanate Solution 100mg/1 CNS

20.0ml of stock thiocyanate solution was diluted to 1 litre with ATP diluent in a volumetric flask.

5.3.3. Method

119.3mg of crystalline adenosine - 5′ - triphosphate disodium salt trihydrate (Sigma Chemicals), equivelant to 100.0mg ATP, was weighed accurately and dissolved in 100ml of ATP diluent in a 100ml volumetric flask. From this soltution three

10:100 serial dilutions were made with ATP diluent using volumetric pipettes and flasks to prepare a solution with a final concentration of 10mg ATP/ml (1×10^9 fg/ml).

10.0ml aliquots of this solution were pipetted into each of nine 100ml volumetric flask. 1, 2, 3, 4, 5, 6, 8 and 9ml aliquots of thiocyanate solution were pipetted into eight of these flasks, which when diluted to volume with ATP diluent gave solutions containing 1, 2, 3, 4, 5, 6, 8, and 9mg/l respectively of thiocyanate and 1×10^8 fg/ml ATP. The remaining flask was also diluted to volume to give a blank solution containing no thiocyanate and the same concentration of ATP. All nine flasks were immediately placed in an ice-bath to keep the ATP stable.

Using 10ml glass syringes, 2ml aliquots of the final solutions were dispensed into small clean test-tubes without delay. These were immediately frozen by gently immersing the bottom of the tubes in a dry ice/acetone mixture. The tubes were capped and stored in a large sealed jar containing silica gel desiccant at -18°C.

The luminescence Biometer was calibrated as described in Appendix 1. Each frozen solution was then defrosted and the ATP concentration determined by injection of 10mls of the thawed solution in the same manner employed for ATP determination of the sludge extracts described in Chapter 4. The ATP concentration was determined for the frozen samples corresponding to each of the thiocyanate concentrations used, and also for the blank solution.

5.3.4 Results

The results obtained were recorded in Table 5.5 and a graph plotted relating the ATP concentration reading of the samples to their thiocyanate content in Figure 5.4 . Linear regression analysis was performed to determine the "best fit" straight line through the data points.

5.3.5 Discussion and Conclusions

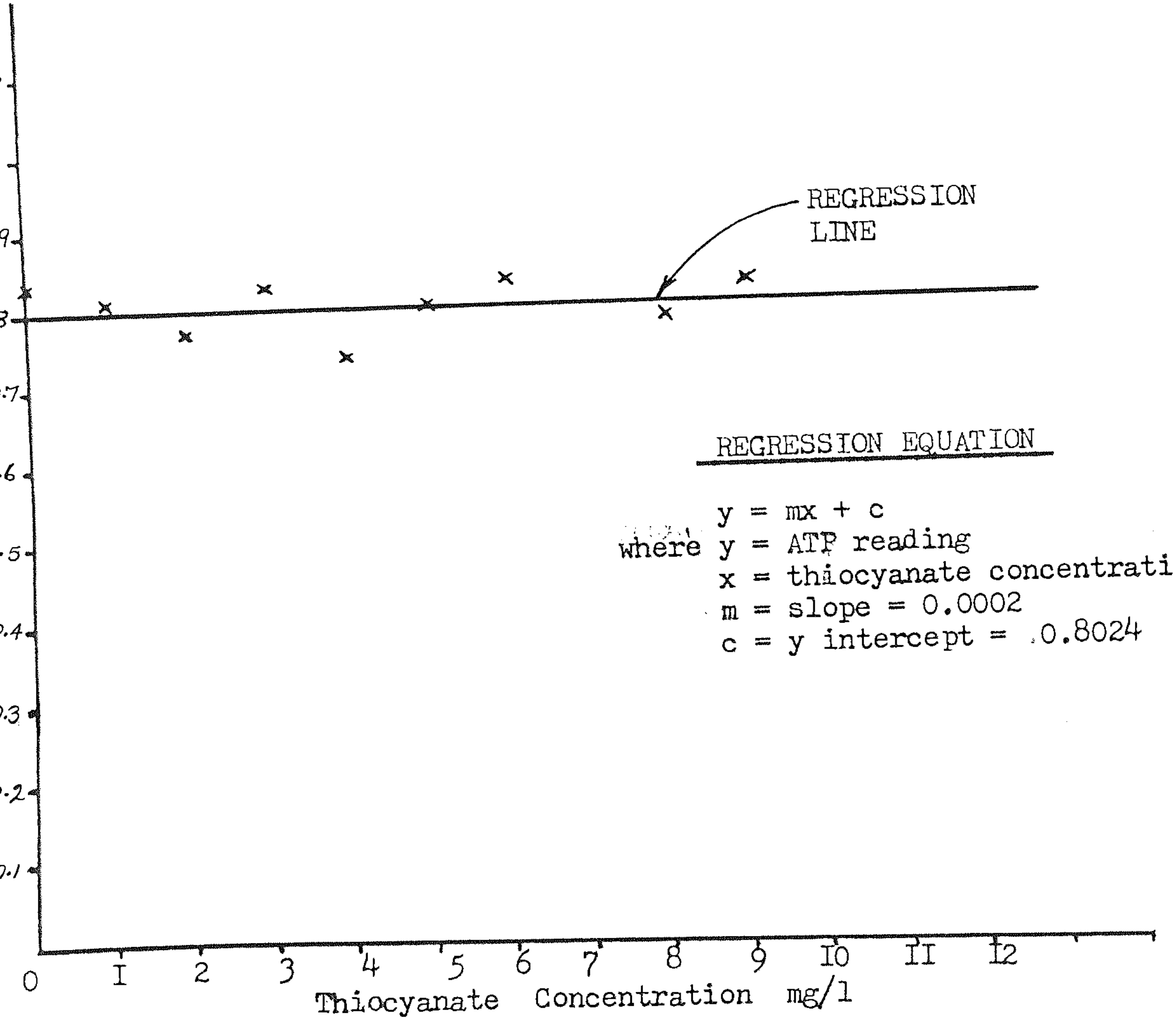
The ATP readings of all the samples was somewhat lower than would have been predicted on the basis of the standard ATP solution prepared. A value of 1×10^8 fg/ml would have been expected. The lower values actually found were attributed to the instability of ATP in dilute solution and the fact that the diluted ATP solutions prepared in the investigation were left to stand at room temperature for a longer period than typically used in preparation of standard ATP solutions (as described in Appendix 1), due to the need for quantitative additions of thiocyanate.

The deviation in readings from the regression line was also somewhat greater than typically found following the instrument calibration procedure described in Appendix 1, where successive ATP determinations on ATP standards typically produced deviation

TABLE 5.5 RESULTS OF THE ATP ANALYSIS OF SAMPLES OF STANDARD ATP SOLUTION CONTAINING VARIOUS CONCENTRATIONS OF THIOCYANATE

(CNS) IN SAMPLE mg/l	ATP READING x 10^8 fg/ml
0	0.831
1.0	0.813
2.0	0.775
3.0	0.833
4.0	0.745
5.0	0.811
6.0	0.845
8.0	0.800
9.0	0.843

FIGURE 5.4 INVESTIGATION OF THE EFFECT OF THIOCYANATE ON ATP DETERMINATIONS .



REGRESSION EQUATION

$y = mx + c$

where y = ATP reading

x = thiocyanate concentration

m = slope = 0.0002

c = y intercept = 0.8024

in readings not greater than ± 0.02×10^8 fg/ml. Experience in the project however demonstrated the importance of immediately cooling dilute ATP solutions and freezing the tubes of standard solutions prepared from them without delay. The greater deviation found during the investigation was believed to reflect not only the extended time taken, but also the variations in times taken, for the preparation of the different thiocyanate/ATP standards prepared.

The results did not indicate any significant trend in the variation of ATP readings with the thiocyanate content of the samples in the concentration ranges employed.

The ATP analysis of sludge samples taken in the investigation described in Chapter 4 and subsequently in the project, involved a prior extraction procedure such that the initial sludge sample was diluted by a factor of 35 before ATP analysis. Therefore the thiocyanate concentrations employed in the standard solutions used in this investigation should be multiplied by the same factor to give the range of concentrations that these would have represented in sludge samples. This range was 0-315mg/l. The results indicated that the apparent variation in sludge ATP content with thiocyanate content found in the batch experiments described in Chapter 4 were not due to interferance by thiocyanate in the ATP assay.

CHAPTER 6

INVESTIGATION OF THE INITIAL ATP RESPONSE OF BATCH ACTIVATED SLUDGES TREATING THIOCYANATE IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR

Introduction

6.1 Experimental Details

    6.1.1 Experiment F

    6.1.2 Experiment J and K

6.2 Results

6.3 Discussion

INVESTIGATION OF THE INITIAL ATP RESPONSE OF BATCH ACTIVATED SLUDGES TREATING THIOCYANATE IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR

Introduction

The hypothesis, described in Chapter 4, that ATP represented a measure of specific growth rate as well as a measure of living biomass was largely based on results showing the type of relationship shown in Figure 4.27. These results represented the ATP content of the sludge at different substrate concentrations during the course of an individual experiment in which the treatment of a thiocyanate solution in a batch reactor was investigated. The results of the type shown in Figure 4.27 were obtained for the majority of the experiments described earlier, and although each graph of that type was only derived from a single batch experiment, the reproducibility of the relationship shown for a large number of experiments with different conditions tended to support the hypothesis described.

Nevertheless, during the course of the degradation of a slug of thiocyanate solution in a batch experiment there would, predictably be major changes in the environment in the sludge, including the formation of large quantities of the products of its metabolism. The ATP behaviour observed during those experiments may well therefore have reflected factors other than substrate concentration. It was felt important therefore to investigate the relationship between the ATP pool and substrate concentration in such a manner that the potential interferences from the various other chemical changes occuring during such experiments were minimised.

The approach adopted was to monitor the initial ATP response of batch sludges immediately before and after the addition of various concentrations of substrate before there were any other significant changes in the chemical environment of the sludge organisms. The aim of doing so was to check the relationship between substrate and ATP previously found and thereby to some degree assess the validity of the hypothesis described earlier.

6.1 Experimental Details

Three experiments, F, J and K, were performed to investigate the initial ATP response to batch activated sludges treating thiocyanate in a basal medium of treated coke oven liquor. The batch reactors B1 and B2 used in the batch experiments described earlier in the project were used as the source of sludge.

The general design of the experiments was that aliquots of one of the batch reactors were used in a series of test runs, in each of which a quantity of concentrated thiocyanate solution was added. The substrate and ATP concentrations were monitored for a short period before and after the addition of the Stock Thiocyanate Solution, along with various other parameters. Different quantities of the stock solution were added in each test so that the ATP response to a range of different substrate concentrations was obtained. Every effort was made to ensure that all other conditions remained constant in each test.

6.1.1 Experiment F

The contents of batch reactor B1, maintained as described in Chapter 4, were allowed to settle and the supernatant decanted. The settled sludge remaining was then diluted to about 700mls with a 1:1 solution of treated coke oven liquor and water and aerated for several hours. Aeration was then stopped, the sludge allowed to settle, and the supernatant decanted. The settled sludge was then diluted to a volume of 12 litres with treated coke oven liquor which had been obtained from laboratory continuous reactors, and which had also been the source of the basal medium used in earlier batch experiments. The sludge was well-aerated to ensure aerobic conditions and complete mixing for a period of several hours. Air was delivered via diffuser stores suspended in the sludge and fed by a compressed air supply. The temperature of the sludge was maintained at 22°C by means of a heater/thermostat suspended in the sludge.

An 800ml aliquot of the well-mixed activated sludge was used for each of 7 test runs, the sludge from each completed test being stored separately from the initial 12 litre reservoir of sludge. The pH and temperature of each aliquot of sludge taken was determined immediately prior to its use in a test run, using the methods described in Chapter 4. Each test run was conducted using the apparatus shown in Figure 6.1.

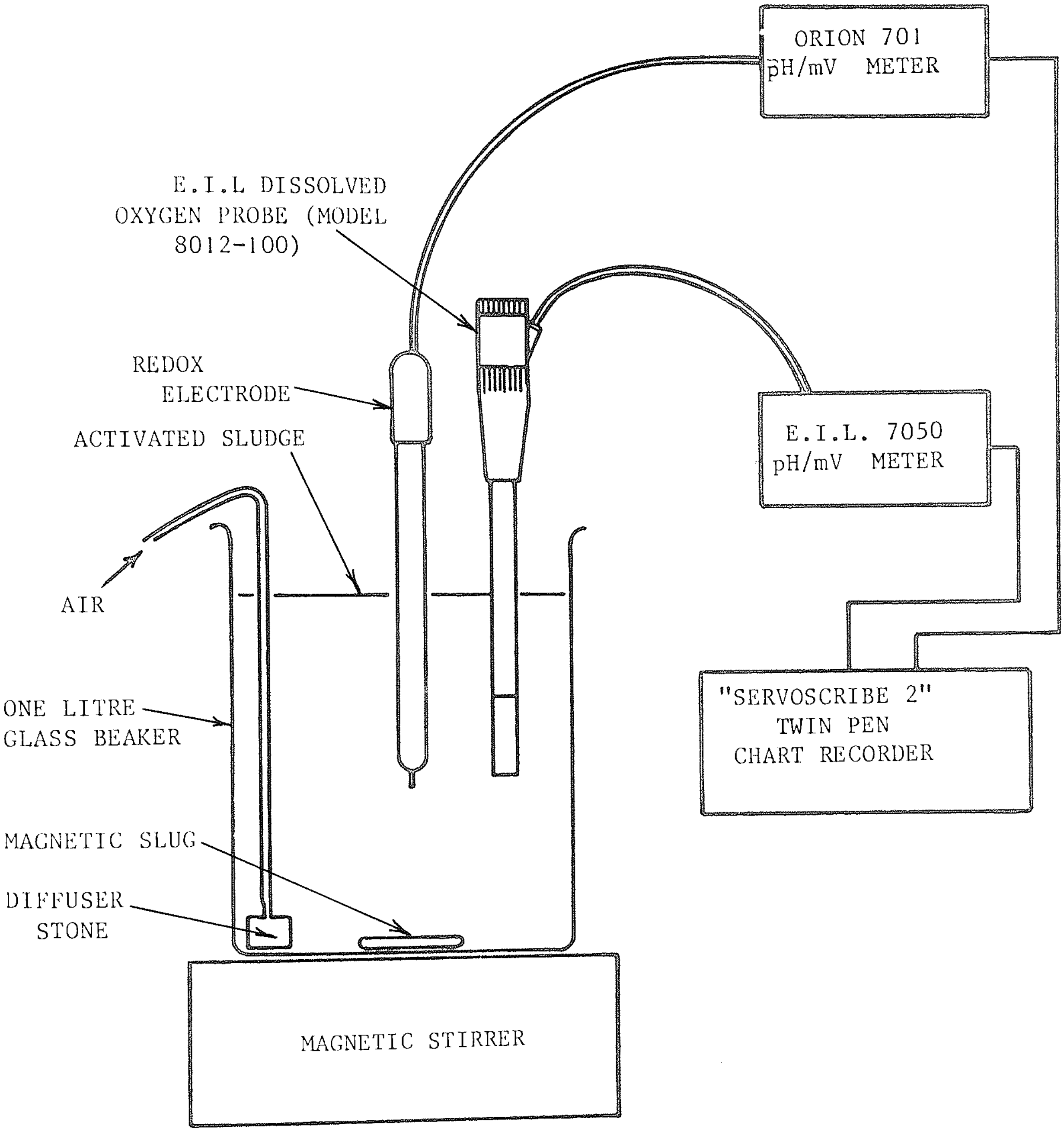


FIGURE 6.1 DIAGRAM TO ILLUSTRATE THE APPRARTUS USED IN EXPERIMENT F AND SUBSEQUENT SIMILAR EXPERIMENTS

The aliquot of sludge was added to the one litre beaker and the magnetic stirrer started. The stirring rate and air supply rate to the test reactor were maintained constant throughout the test, and the same for all test runs in the experiment.

Samples for ATP analysis were taken periodically using a Pasteur pipette, about 2mls being pipetted into a glass test tube and immediately frozen in a dry ice/acetone mixture for each sample. Samples were stored in a refrigerator at -18°C and analysed at a later date using the method described in Chapter 4.

Samples of thiocyanate analysis were taken periodically using a 10ml pipette and filtered through a pre-weighed membrane filter. The filtrate was used to determine the thiocyanate concentration of the sample, and the membrane and filtered solids used to determine the suspended solids concentration. The sampling methods and analytical procedures described in Chapter 4 were used.

The redox potential and dissolved oxygen concentration were monitored continuously throughout each test run. Redox measurements were made using the redox electrode which was used in conjunction with a reference electrode and an Orion 701 pH/mV meter. Dissolved oxygen was measured using an oxygen electrode (E.I.L D.O Probe Model 8012-100) in conjunction with an

EIL 7050 pH/mV meter which was calibrated in the usual manner using the procedure recommended by the manufacturers. Both electrodes were connected to a calibrated Servoscribe twin-pen chart recorder which was used to make a continuous record of these parameters in each test run.

A quantity of stock thiocyanate solution (60,000 mg/l) was added to the sludge in the beaker 10-15 minutes after the start of each test run. Samples for thiocyanate and ATP analysis were taken during the period before, and over a period of about 20 minutes after, the addition of substrate.

At the end of each run the sludge was poured into a storage reservoir and the beaker and electrodes cleaned to remove sludge contamination before the addition of a further aliquot of sludge from the 12 litre reservoir and the start of the next test run.

Seven test runs were carried out sequentially as described, a different volume of stock thiocyanate solution being added in each of six of the runs. In the last test run approximately the same volume of stock solution was added as in the first run.

At the end of the experiment, the aliquots of sludge used in the test runs were combined with that remaining in the 12 litre reservoir and the sludge allowed to settle. The supernatant was decanted and the settled sludge returned to reactor B1 where treated coke oven liquor was added to nearly fill the reactor, and the sludge then maintained on a daily basis as described in Chapter 4.

6.1.2 Experiments J and K

These experiments were conducted on the same basis as Experiment F, with a few differences.

Batch reactor B2, maintained as described in Chapter 4, was used in these experiments. The contents of the reactor were emptied into a large 12 gallon plastic container and 5 litres of tap water added. 5mls of trace metal concentrate and 20mls of phosphate solution, whose compositions were detailed earlier in the project, were also added and the pH adjusted to 7.4. The resultant sludge was well aerated and mixed for a period of several hours to produce endogenous conditions and this reservoir of sludge was then used in Experiment J.

Experiment J was conducted in a similar manner to Experiment F using the apparatus shown in Figure 6.1 with a few exceptions. Dissolved oxygen measurements were not made. Redox measurements were made as previously described, and in addition the response of sulphide and thiocyanate electrodes monitored in each test run. An Orion sulphide electrode, and a thiocyanate electrode were used. The sulphide electrode was connected to the EIL 7050 pH/mV meter and the output recorded on the twin-pen Servoscribe chart recorder along with redox measurements. The thiocycanate electrode was connected to another Orion 701 pH/mV meter and the output recorded on a single pen Servoscribe chart recorder.

Nine test runs were carried out in series, a different volume of stock thiocyanate solution being added in each of eight of the runs. Approximately the same volume of stock solution was added to the penultimate test runs as in the first, and this, as in Experiment F and subsequent similar experiments served as a control test to check the consistency in the behaviour of the sludge. Small quantities of a stock sulphide solution were also added in test runs 1 and 2.

The results obtained from Experiment J were recorded in Table 6.3 and plotted in Figures 6.9 to 6.17.

At the end of the experiment the aliquots of sludge used in the test runs and that remaining in the reservoir were combined and allowed to settle. The supernatent was decanted and the settled sludge returned to reactor B2. This was diluted with treated coke oven liquor (which formed the basal medium) to fill the reactor and the sludge was fed and maintained as described in Chapter 4.

Experiment K was performed the following day, again using reactor B2 and the same procedures described for Experiment J, with a few exceptions. Cyanide analysis of filtrate samples used for thiocyanate determinations was also carried out using the method described later. Six test runs were performed, with stock thiocyanate solution additions giving a higher range of thiocyanate concentrations than used in Experiment J. A control test run was not carried out. The sludge used in the test runs and that remaining in the reservoir were combined, and returned to reactor B2 as in previous experiments.

A summary of the conditions and parameters monitored in Experiments F, J, and K was recorded in Table 6.1.

6.2 Results

The results of Experiments F, J, and K were recorded in Tables 6.2, 6.3, and 6.4 respectively, and in Figures 6.2 to 6.8, 6.9 to 6.17, and 6.18 to 6.23 respectively.

The mean ATP level before and after the addition of substrate was determined for each test run in each of the three experiments. These values were used to determine the mean ATP concentrations per mg of sludge before and after thiocyanate addition in each of the test runs using the following equation;

$$\begin{array}{l}\text{MEAN ATP CONTENT} \\ \text{PER mg DRIED SLUDGE}\end{array} = \frac{\text{MEAN ATP CONTENT PER ML. SLUDGE} \times 10.9}{\text{(MEAN) SUSPENDED SOLIDS PER SAMPLE}}$$

TABLE 6.1 SUMMARY OF THE PARAMETERS MONITORED IN EXPERIMENTS F, J AND K

EXPT.	ADDITIONS STOCK CONS	INITIALLY STARVED ?	T°C	pH	S.S	CNS	CN	NH_3	ATP	ELECTRODES			
										REDOX	S	CNS	D.O
	mls												
F	0.5 - 5.0	NO	+	+	+	+	-	-	+	+	-	-	+
J	0.1 - 1.0	YES	+	+	+	+	-	-	+	+	+	+	-
K	1.0 - 2.2	YES	+	+	+	+	+	-	+	+	+	+	-

TABLE 6.2 THE RESULTS OF EXPERIMENT F

	TIME HRS-MINS.		REDOX mV	SS mg/ 10.9ml sple	(CNS) mg/l	ATP fg/ml	D.O. mg/l
TEST 1	EXPT.	RUN					
	0-26	0-00	+10.0	--	----	----	9.1
	0-27	0-01	----	31.4	82.3	----	---
	0-32	0-06	----	---	----	4.060×10^7	---
	0-33	0-07	+39.0	----	----	----	9.1
	0-36	0-10	+45.2	32.3	82.3	----	9.2
	0-37	0-11	----	----	----	4.095×10^7	---
	0-39	0-13	----	----	----	3.640×10^7	---
	0-41	0-15	0.5ml STOCK CNS ADDED				
	0-42	0-16	----	----	----	3.675×10^7	---
	0-44	0-18	+55.6	----	115.6	----	---
	0-46	0-20	+59.7	----	----	3.185×10^7	---
	0-49	0-23	+62.3	----	----	3.675×10^7	9.1
	0-50	0-24	+63.3	33.0	120.0	----	---
	0-55	0-29	+64.2	----	----	3.395×10^7	9.1
	0-56	0-30	+66.3	----	----	----	9.1
	0-59	0-33	+68.5	32.4	122.7	----	9.1
	0-60	0-34	----	----	----	3.500×10^7	---
TEST 2							
	1-06		----	----	----	----	---
	1-11	0-00	----	----	----	2.958×10^7	---
	1-12	0-01	----	26.9	87.8	----	---
	1-13	0-02	+12.1	----	----	----	8.9
	1-14	0-03	----	----	----	3.091×10^7	---
	1-18	0-07	+20.0	27.2	81.2	----	8.9
	1-20	0-09	+26.3	----	----	3.430×10^7	8.8-8.9
	1-25	0-14	1ml STOCK CNS ADDED				
	1-27	0-16	+42.4	----	----	3.028×10^7	8.8
	1-28	0-17	+44.3	26.9	92.7	----	8.8
	1-32	0-21	+51.0	----	----	----	8.8
	1-34	0-23	+54.0	----	----	2.870×10^7	8.8
	1-35	0-24	+55.3	26.6	160.4	----	8.8
	1-37	0-26	----	----	----	2.940×10^7	---
	1-40	0-29	+60.6	----	----	3.269×10^7	8.8

TABLE 6.2 CONTINUED

	TIME HRS-MINS		REDOX	SS	(CNS)	ATP	D.O.
TEST 3	EXPT.	RUN					
	1-45		----	---	----	---	---
	1-48	0-00	+40.0	20.7	94.3	---	8.5
	1-50	0-02	+46.1	---	----	$2.681x10^7$	8.5
	1-53	0-05	+54.0	---	----	$2.912x10^7$	8.5
	1-55	0-07	+56.5	---	----	$2.688x10^7$	8.5-8.6
	1-58	0-10	+60.6	Ca. 2MLS	STOCK CNS	ADDED	8.5-8.6
	2-00	0-12	+62.7	---	----	$3.360x10^7$	8.6
	2-01	0-13	+63.7	20.6	218.6	---	---
	2-03	0-15	+65.3	---	----	$3.815x10^7$	---
	2-07	0-19	+69.0	---	----	$3.255x10^7$	8.6
	2-12	0-24	+72.8	---	----	$3.955x10^7$	8.6-8.7
	2-20	0-32	+77.6	---	----	$3.815x10^7$	8.6-8.7
	2-21	0-33	+78.2	20.4	238.0	---	8.7-8.8
TEST 4							
	2-32	0-00	+27.6	---	----	---	8.6-8.7
	2-33	0-01	+31.6	22.5	103.1	---	8.6-8.7
	2-34	0-02	+34.9	---	----	$3.815x10^7$	8.6-8.7
	2-36	0-04	+42.4	---	----	$3.850x10^7$	8.6-8.7
	2.39	0-07	+52.1	---	----	$3.745x10^7$	8.6-8.7
	2-40	0-08	Ca.	3MLS	STOCK CNS	ADDED	
	2-41	0-09	+55.3	---	----	$3.920x10^7$	8.7-8.8
	2-43	0-11	+58.9	22.2	274.6	---	8.7
	2-44	0-12	+60.5	---	----	$3.290x10^7$	8.8
	2-49	0-17	+67.3	---	----	$3.465x10^7$	8.7-8.8
	2-52	0-20	+70.4	---	----	---	---
	2-57	0-25	+74.8	---	----	$3.500x10^7$	---
	2-59	0-27	+76.0	22.4	306	---	8.8-8.9
TEST 5	3-08	0-00	+31.2	34.7	117.8	---	8.5-8.6
	3-10	0-02	---	----	----	$3.990x10^7$	---
	3-14	0-06	+42.8	----	----	$4.235x10^7$	8.6-8.7
	3-15	0-07	+45.1	----	----	$5.740x10^7$	8.6
	3-16	0-08	Ca. 4mls	CNS ADDED			
	3-18	0-10	+47.8	----	----	$4.830x10^7$	8.7
	3-19	0-11	+49.3	35.2	356.6	---	8.7
	3-21	0-13	+52.1	----	----	$5.355x10^7$	8.7-8.8
	3-24	0-16	+55.9	----	----	---	8.7
	3-31	0-23	+64.3	----	----	$5.600x10^7$	8.8
	3-34	0-26	+66.1	34.0	403.0	---	8.8-8.9
			+67.9	----	----	$5.425x10^7$	8.8-8.9

TABLE 6.2 CONTINUED

TEST 6	3-56	0-00	+48.6	36.1	128.2	----	8.3-8.4
	3-58	0-02	+53.6	----	----	5.460x10^{7}	8.4
	4-02	0-06	+61.9	----	----	4.830x10^{7}	---
	4-03	0-07	+62.4	----	----	6.020x10^{7}	8.5
	4-05	0-09	Ca. 5mls STOCK CNS ADDED				
	4-07	0-11	+64.7	----	----	5.390x10^{7}	8.5-8.6
	4-08	0-12	+65.7	37.8	385.0	----	8.5-8.6
	4-10	0-14	+67.5	----	----	6.265x10^{7}	8.5-8.6
	4-14	0-20	+71.0	----	----	6.125x10^{7}	8.5-8.6
	4-20	0-24	+75.1	----	----	6.860x10^{7}	8.6
	4-25	0-29	+78.0	----	----	6.755x10^{7}	8.6
	4-27	0-31	+78.9	37.1	446.7	----	8.6
TEST 7	4-37	0-00	+18.3	34.5	131.5	----	8.5
	4-39	0-02	+22.9	----	----	5.950x10^{7}	8.5-8.6
	4-43	0-06	----	----	----	6.335x10^{7}	---
	4-46	0-09	+50.4	----	----	5.355x10^{7}	8.6
	4-48	0-11	Ca. 0.5ml STOCK CNS ADDED				
	4-50	0-13	+57.2	----	----	4.200x10^{7}	8.6-8.7
	4-53	0-16	+62.4	35.5	115.6	----	---
	4-58	0-21	+68.9	----	----	5.005x10^{7}	8.7-8.8
	5-05	0-28	+75.4	----	----	4.165x10^{7}	8.7-8.8
	5-07	0-30	----	----	----	4.480x10^{7}	---
	5-10	0-33	+78.5	----	----	5.110x10^{7}	8.8-8.9
	5-12	0-35	+79.7	----	118.4	----	8.8

pH OF SLUDGE THROUGHOUT EXPT. = 7.5

STOCK CNS = 60,000mg/l THIOCYANATE SOLUTION

TABLE 6.3 THE RESULTS OF EXPERIMENT J

EXPERIMENT J

TEST 1

Time (hrs/ mins)	pH	Temp °C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings Redox	Electrode Readings Sulphide	Electrode Readings Thiocyanate
0-00	7.4	--	-	-	-	+100	+99	-2.6
0-04	-	-	-	-	-	+98	+97	-3.9
0-06	-	-	19.8	0	-	-	-	-
0-09	-	-	-	-	-	+100	+95	-4.5
0-10	-	-	-	-	3.71	-	-	-
0-11	-	-	-	0	-	-	-	-
0-14	-	-	-	-	3.71	-	+94	-5.1
0-15	-	-	-	-	3.64	+104	-	-
0-19	0.1ml stock thiocyanate solution added					+108	+92	-5.5?
0-20	-	-	-	-	4.445	-	-	-6.4
0-21	-	-	-	-	-	+112	+91	-6.8
0-22	-	-	-	6.6	-	-	-	-
0-23	-	-	-	-	-	+110	+91	-6.9
0-26	-	-	-	-	4.305	-	-	-
0-27	-	-	-	-	-	+110	+91	-7.4
0-28	-	-	-	-	4.165	-	-	-
0-29	-	-	-	6.6	-	-	+91	-
0-30	-	-	-	-	-	+109	+90	-7.8
0-32	-	-	-	-	4.06	-	+90	-
0-34	0.1ml stock sulphide solution added							
0-36	-	-	-	-	3.675	-	+40	-
0-38	-	-	-	-	5.88	+102	+65	-9.0
0-41	0.1ml stock sulphide solution added					+106	+76	-9.1
0-41½	-	-	-	-	-	+5 wentLO	-25	-9.5
0-42	-	-	-	-	-	*26	*3	-9.5
0-43	-	-	-	-	-	+76	+37	-9.4
0-44	-	-	-	-	-	+92	+52	-9.4
0-45	-	-	-	5.2	-	-	-	-
0-46	-	-	-	-	-	+103	+67	-9.5

TABLE 6.3 CONTINUED

EXPERIMENT J

TEST 2

Time (hrs/ mins)	pH	Temp °C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings Redox	Electrode Readings Sulphide	Electrode Readings Thiocyanate
0-56	-	-	-	-	-	+106	+84	-8.9
1-00	-	-	-	0	-	+108	+88	-9.6
1-01	-	-	-	-	4.935	+111	+88	-9.7
1-03	-	-	23.3	0	-	-	-	-
1-04	-	-	-	-	3.01	+112	+90	-9.7
1-07	-	-	-	-	-	+114	+90	-9.7
1-08	0.2mls stock thiocyanate solution added							
1-09	-	-	-	-	-	+114	+90	-10.5
1-10	-	-	-	-	5.005	-	+89	-10.6
1-11	-	-	-	11.4	-	+114	+89	-10.6
1-14	-	-	-	-	3.15	-	-	-10.5
1-15	-	-	-	-	-	+114	+90	-10.5
1-16	-	-	-	13.4	-	-	-	-
1-17	-	-	-	-	3.01	+116	+90	-10.4
1-21	-	-	-	-	3.2305	-	-	-
1-23	0.1ml stock sulphide solution added					+116 before addition	+90	-10.1
1-23½	-	-	-	-	-	+4	-5	-10.3
1-24	-	-	-	-	-	+28	+1	-10.3
1-24½	-	-	-	-	-	+60	+25	-
1-25	-	-	-	-	3.255	-	+38	-
1-26	-	-	-	-	-	+97	+54	-10.2

TABLE 6.3 CONTINUED

EXPERIMENT J

TEST 3

Time (hrs/ mins)	pH	Temp °C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings		
						Redox	Sulphide	Thiocyanate
1-32	-	-	-	-	-	+104	+77	-8.3
1-38	-	-	-	-	-	+112	+87	-8.6
1-42	-	-	-	-	-	+114	+90	-8.5
1-48	-	-	23.9	O	-	+116	+92	-8.6
1-49	-	-	-	-	3.85	-	+92	-8.7
1-51	-	-	-	-	3.675	+118	+92	-
1-52	-	-	-	-	3.255	-	-	- Before addition
1-54	0.3mls stock thiocyanate solution added							
1-55	-	-	-	-	4.725	+119	+91	-10.5
1-56	-	-	-	17.5	-	+118	+91	-11.4
1-59	-	-	-	-	5.53	+118	+90	-11.5
2-00	-	-	-	-	-	+118	+90	-11.4
2-03	-	-	-	-	4.62	+118	+90	-11.5
2-04	-	-	-	-	-	+118	+90	-11.5
2-05	-	-	-	13.4	-	-	-	-
2-08	-	-	-	-	-	+120	+91	-11.5
2-11	-	-	-	-	4.48	+120	+91	-11.5

TABLE 6.3 CONTINUED

EXPERIMENT J

TEST 4

Time (hrs/ mins)	pH	Temp °C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings		
						Redox	Sulphide	Thiocyanate
2-16	-	-	-	-	-	+114	+92	-9.0
2-20	-	-	22.7	3.2	-	+118	+92	-10.0
2-21	-	-	-	-	3.605	-	-	-10.0
2-23	-	-	-	-	4.55	-	+92	-9.8
2-24	-	-	-	-	-	+111	+92	-9.7
2-25	-	-	-	-	-	+114	+92	-9.7
2-27	-	-	-	-	4.165	+119	+92	-9.6
2-28	0.4mls stock thiocyanate solution added							
2-29	-	-	-	-	3.43	+114	+91	-10.9
2-30		(15.7 - bulk)		-	-	+114	+90	-11.3
2-34	-	-	-	28.4	-	+116	+89	-11.4
2-35	-	-	-	-	5.355	+116	+89	-11.4
2-37	-	-	-	-	-	+114	+89	-11.3
2-38	-	-	-	-	-	+116	+89	-11.3
2-44	-	-	-	-	5.215	+116	+89	-11.2
2-50	-	-	-	-	4.76	+117	+89	-11.1
2-55	-	-	-	-	-	+118	+89	-10.9

TABLE 6.3 CONTINUED

EXPERIMENT J

TEST 5

Time (hrs/ mins)	pH	Temp °C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings Redox	Electrode Readings Sulphide	Electrode Readings Thiocyanate
3-16	-	-	-	-	-	+122	+88	-12.1
3-21	-	-	-	-	-	+124	+88	-11.9
3-22	-	-	24.0	0	-	+124	+88	-11.9
3-23	-	-	-	-	4.62	-	-	-
3-24	-	-	-	-	-	+124	+88	-11.9
3-25	-	-	-	-	4.095	-	+88	-11.9
3-28	-	-	-	-	4.2	+125	+88	-11.8
3-29	0.5ml stock thiocyanate solution added							
3-30	-	-	-	-	6.965	+124	+88	-13.5
3-31	-	-	-	-	-	+123	+87	-13.9
3-33	-	-	-	33.9	-	-	-	-
3-36	-	-	-	-	6.475	-	+86	-14.2
3-39	-	-	-	-	-	+120	+86	-15.0
3-40	-	-	-	-	4.865	+119	+86	-15.1
3-42	-	-	-	-	-	+118	+86	-15.2
3-44	-	-	-	-	4.655	+118	+86	-15.2
3-46	-	-	-	-	5.285	-	-	-
3-47	-	-	-	-	-	+118	+86	-15.2
3-48	-	-	-	-	-	+119	+86	-15.1
3-49	-	-	-	-	-	+119	+86	-15.1
3-50	-	-	-	-	6.125	+119	+86	-15.1

TABLE 6.3 CONTINUED

EXPERIMENT J

TEST 6

Time (hrs/ mins)	pH	Temp °C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings		
						Redox	Sulphide	Thiocyanate
4-07	-	-	-	-	-	+122	+87	-12.7
4-13	-	-	23.7	4.5	-	+128	+88	-12.0
4-16	-	-	-	-	-	-	-	-
4-21	-	-	-	-	4.655	+128	+89	-11.3
4.23	-	-	-	-	4.34	-	-	-
4-26	0.7mls stock thiocyanate solution added							
4-27	-	-	-	-	5.635	+124	+87	-13.1
4-31	-	-	-	-	4.48	+116	+85	-14.4
4-34	-	-	-	55.1	-	-	+84	-
4-35	-	-	-	-	5.775	+116	+84	-14.5
4-38	-	-	-	-	-	+118	+84	-14.4
4-40	-	-	-	-	5.18	-	+84	-14.3
4-43	-	-	-	-	-	+118	+85	-14.2
4-44	-	-	-	-	-	+118	+85	-14.2
4-45	-	-	-	-	5.46	+119	+85	-14.2
4-54	-	-	-	-	-	+117	+85	-13.8

TABLE 6.3. CONTINUED

EXPERIMENT J

TEST 7

Time (hrs/ mins)	pH	Temp ^{o}C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings Redox	 Sulphide	 Thiocyanate
5-08	-	-	-	-	-	+122	+86	-13-5
5-09	-	-	24.0	5.9	-	-	-	-
5-10	-	-	-	-	4.515	+125	+85	-13.2
5-13	-	-	-	-	3.15	+128	+86	-12.8
5-15	-	-	-	-	3.535	+128	+86	-12.5
5-16	0.85mls stock thiocyanate solution added							
5-17	-	-	-	-	5.53	+127	+84	-15.5
5-18	-	-	-	-	-	+126	+84	-16.1
5-19	-	-	-	-	-	+123	+83	-16.5
5-20	-	-	-	-	4.725	-	+83	-
5-21	-	-	-	-	-	+115	+82	-16.7
5-22	-	-	-	-	-	+120	+82	-16.8
5-24	-	-	-	68.7	-	+121	+82	-16.8
5-25	-	-	-	-	-	+120	+82	-16.8
5-26	-	-	-	-	4.62	-	-	-
5-29	-	-	-	-	5.145	-	-	-
5-31	-	-	-	-	6.02	+122	+82	-16.5

TABLE 6.3 CONTINUED

EXPERIMENT J

TEST 8

Time (hrs/ mins)	pH	Temp °C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings Redox	 Sulphide	 Thiocyanate
5-41	-	-	-	-	-	+116	+84	-12.0
5-45	-	-	26.0	0	-	-	+85	-
5-46	-	-	-	-	3.57	+119	+85	-12.7
5-54	-	-	-	-	3.185	+118	+85	-11.7
5-55	-	-	-	-	3.115	+120	+85	-11.6
5-56	0.1ml stock thiocyanate solution added					+120	+85	-11.5
5-57	-	-	-	-	2.905	+115	+85	-11.8
5-59	-	-	-	-	-	+115	+84	-11.8
6-00	-	-	-	-	4.025	+114	+83	-11.7
6-02	-	-	-	8.6	-	-	+83	-
6-07	-	-	-	-	3.43	-	+83	-11.4
6-08	-	-	-	-	-	+123	+83	-11.3
6-10	-	-	-	-	3.465	+122	+83	-11.2

TABLE 6.3 CONTINUED

EXPERIMENT J

TEST 9

Time (hrs/ mins)	pH	Temp °C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings Redox	 Sulphide	 Thiocyanate
6-22	-	-	-	-	-	+128	+85	-15.0
6-23	-	-	-	-	3.36	-	+86	-14.9
6-25	-	-	26.0	0	-	-	+86	-
6-26	-	-	-	-	3.605	+130	-	-
6-30	-	-	-	-	3.227	+128	+86	-13.6
						before addition		
6-32	1.0ml stock thiocyanate solution added					+130	+87	-13.3
6-33	-	-	-	-	4.235	+126	+85	-16.2
6-35	-	-	-	-	3.815	+124	+84	-17.3
6-36	-	-	-	-	-	+124	+84	-17.6
6-38	-	-	-	74.9	-	+123	+83	-17.8
6-39	-	-	-	-	-	+124	+83	-17.9
6-40	-	-	-	-	4.025	-	-	-
6-43	-	-	-	-	3.92	+122	+82	-17.8
6-46	-	-	-	-	3.185	+122	+82	-17.7
6-48	-	-	-	-	-	+122	*83	-17.6

J71- 4.865×10^7

TABLE 6.4 THE RESULTS OF EXPERIMENT K

EXPERIMENT - K

TEST 1

Time (hrs/ mins)	pH	Temp °C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings		
						Redox	Sulphide	Thiocyanate
0.00	7.6	16.8 (bulk)	-	-	-	+128	+87	-13.3
0.01	-	-	24.8	0		-	-	-
0.02	-	-	-	-	2.6495	-	-	-
0.03	-	-	-	-	2.1665	+129	+87	-13.2
0.04	-	-	-	-	3.0275	-	-	-13.1
0.05	1.0ML OF STOCK THIOCYANATE SOLUTION ADDED							
0.06	-	-	-	-	4.375	+128	+85	-15.5
0.09	-	-	-	-	-	+124	+83	-16.6
0.10	-	-	-	-	2.8875	-	+83	-16.9
0.11	-	-	-	-	-	+122	+82	-17.0
0.13	-	-	-	75.8	-	-	+81	-17.0
0.14	-	-	-	-	3.3075	+104	+81	-16.9
0.15	-	-	-	-	-	+106	+81	-17.0
0.17	-	-	-	-	3.122	+106	+80	-16.9
0.35				-	2.828	+58	+82	-16.6

TABLE 6.4 CONTINUED

EXPERIMENT K

TEST 2 (STIRRING RATE INCREASED)

Time (hrs/ mins)	pH	Temp °C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings		
						Redox	Sulphide	Thiocyanate
0.55	-	-	-	-	-	+122	+87	-14.6
0.56	-	-	16.5	2.5	-	-	-	-
0.57	-	-	-	-	1.9215	+118	+87	-14.6
0.59						+118	+87	-14.5
1.02	-	-	-	-	-	+120	+87	-14.8
1.03	-	-	-	-	2.1665	-	-	-
1.04	-	-	-	-	-	+125	+87	-15.1
1.05	-	-	-	-	1.82	+126	+87	-15.3
1.06	1.2 MLS STOCK THIOCYANATE SOLUTION ADDED							
1.07	-	-	-	-	3.283	+126	+85	-18.5
1.08	-	-	-	-	-	+123	+84	-19.5
1.10	-	-	-	-	3.199	-	-	-20.5
1.14						+104	+82	-21.1
1.16	-	-	-	57.2	-	+101	+81-82	-21.3
1.17	-	-	-	-	-	+101	+81	-21.3
1.18	-	-	-	-	3.64*	-	+80-81	-
1.22	-	-	-	-	-	+98	+83	-21.4
1.26					-	-	-	-
1.28	-	-	-	-	3.129	-	-	-

TABLE 6.4 CONTINUED

EXPERIMENT K

TEST 3

Time (hrs/ mins)	pH	Temp °C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings Redox	Electrode Readings Sulphide	Electrode Readings Thiocyanate
1.35	-	-	-	-	-	+101	+89	-15.1
1.40	-	-	-	-	-	+114	+88	-18.1
1.41	-	-	-	-	2.4045	-	-	-18.6
1.44	-	-	-	-	-	+125	+88	-18.7
1.47	-	-	24.0	0	-	-	-	-
1.48	-	-	-	-	2.8945	+122	+88	-18.8
1.49	-	-	-	-	-	+121	+88	-18.8
1.50	-	-	-	-	2.6705	+122	+88	-18.8
1.51	1.6MLS STOCK THIOCYANATE SOLUTION ADDED							
1.52	-	-	-	-	3.255	-	+85	-22.4
1.53	-	-	-	-	-	-	-	-23.8
1.54	-	-	-	-	-	+113	+84	-23.3
1.55	-	-	-	-	2.87	-	-	-24.6
1.56	-	-	-	-	-	+114	+83	-24.9
1.59	-	-	-	60.5	-	+116	+83	-25.5
2.00	-	-	-	-	3.178	+116	+82	-25.7
2.01	-	-	-	-	-	+116	+82	-25.7
2.02	-	-	-	-	3.045	+116	+82	-25.7

TABLE 6.4 CONTINUED

EXPERIMENT K

TEST 4

Time (hrs/ mins)	pH	Temp °C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings Redox	Electrode Readings Sulphide	Electrode Readings Thiocyanate
2.10	-	-	-	-	2.905	+112	+86	-20.1
2.14	-	-	25.7	2.5	-	+112	+86	-19.7
2.15	-	-	-	-	2.786	+116	+86-87	-19.5
2.16	-	-	-	-		+121	+87	-19.4
2.17	1.8 MLS STOCK THIOCYANATE SOLUTION ADDED							
2.18	-	-	-	-	3.3985	-	+83-84	-23.4
2.19	-	-	-	-	-	+110	+83	-24.7
2,21	-	-	-	-	-	+112	+83	-25.7
2.24	-	-	-	-	4.725	+112	+82	-26.2
2.25	-	-	-	-	-	+117	+82	-26.4
2.26	-	-	-	-	3.339	+110	+82	-
2.27	-	-	-	141.3	-	-	-	-
2.28	-	-	-	-	3.3355	+119	+82	-26.7

TEST 5

2.40	-	-	24.8	3.1	-	+130	+87	-20.6
2.41	-	-	-	-	1.981	-	-	-
2.44	-	-	-	-	3.311	+136	+88	-20.3
2.45	-	-	-	-	-	+136	+88	-20.3
2.46	-	-	-	-	1.5925	+138	+88	-20.1 to 20.2
2.47	2.0ML STOCK THIOCYANATE SOLUTION ADDED							
2.48	-	-	-	-	4.34	+129	+84	-24.4
2.50	-	-	-	-	-	+ 123 to 124	+84	-26.4
2.51	-	-	-	-	-	+123 to 124	+84	-26.8
2.53	-	-	-	-	2.73	+123 to 124	+83	-27.2
2.55	-	-	-	142.9	-	-	-	-
2.56	-	-	-	-	3.297	+122 to 123	+83	-27.6
2.57	-	-	-	-	-	+123 to 124	+83	-27.6 to 27.7
2.58	-	-	-	-	-	+123	+83	-27.7
3.01	-	-	-	-	3.535	+126	+83	-27.6

TABLE 6.4 CONTINUED

EXPERIMENT K

TEST 6

Time (hrs/ mins)	pH	Temp °C	Suspended solids	Thiocyanate	ATP x107	Electrode Readings Redox	Electrode Readings Sulphide	Electrode Readings Thiocyanate
3.09	-	-	-	-	2.2365	+119	+87	-21.7
3.11	-	-	27.2	4.2	-	+119	+87	-22.1
3.12	-	-	-	-	1.785	-	-	-
2.13	-	-	-	-	-	+126	+88	-22.3
3.14	-	-	-	-	-	+108	+88	-22.3
3.15	2.2 MLS STOCK THIOCYANATE SOLUTION ADDED							
3.16	-	-	-	-	2.499	+112	+84	-27.0
3.19	-	-	-	-	-	+117	+83	-30.0
3.20	-	-	-	-	2.8035	+117	+83	-30.2
3.24	-	-	-	146.2	-	-	-	-
3.25	-	-	-	-	2.639	+119	+82	-30.8
3.28	-	-	-	-	2.3695	+114	+82	-31.0

FIGURE 6.2 RESULTS OF EXPERIMENT F. TEST F1

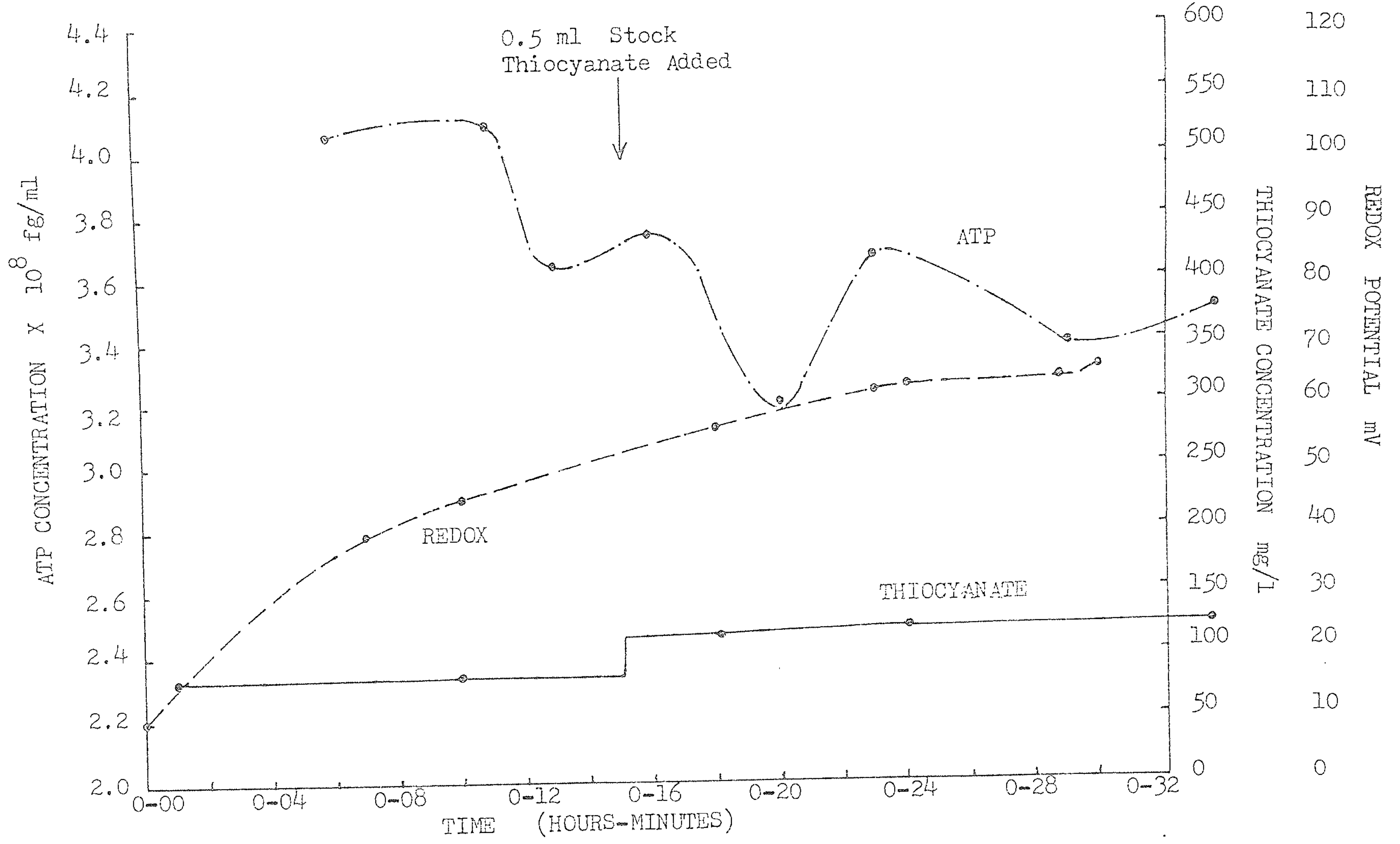


FIGURE 6.3 RESULTS OF EXPERIMENT F. TEST F2.

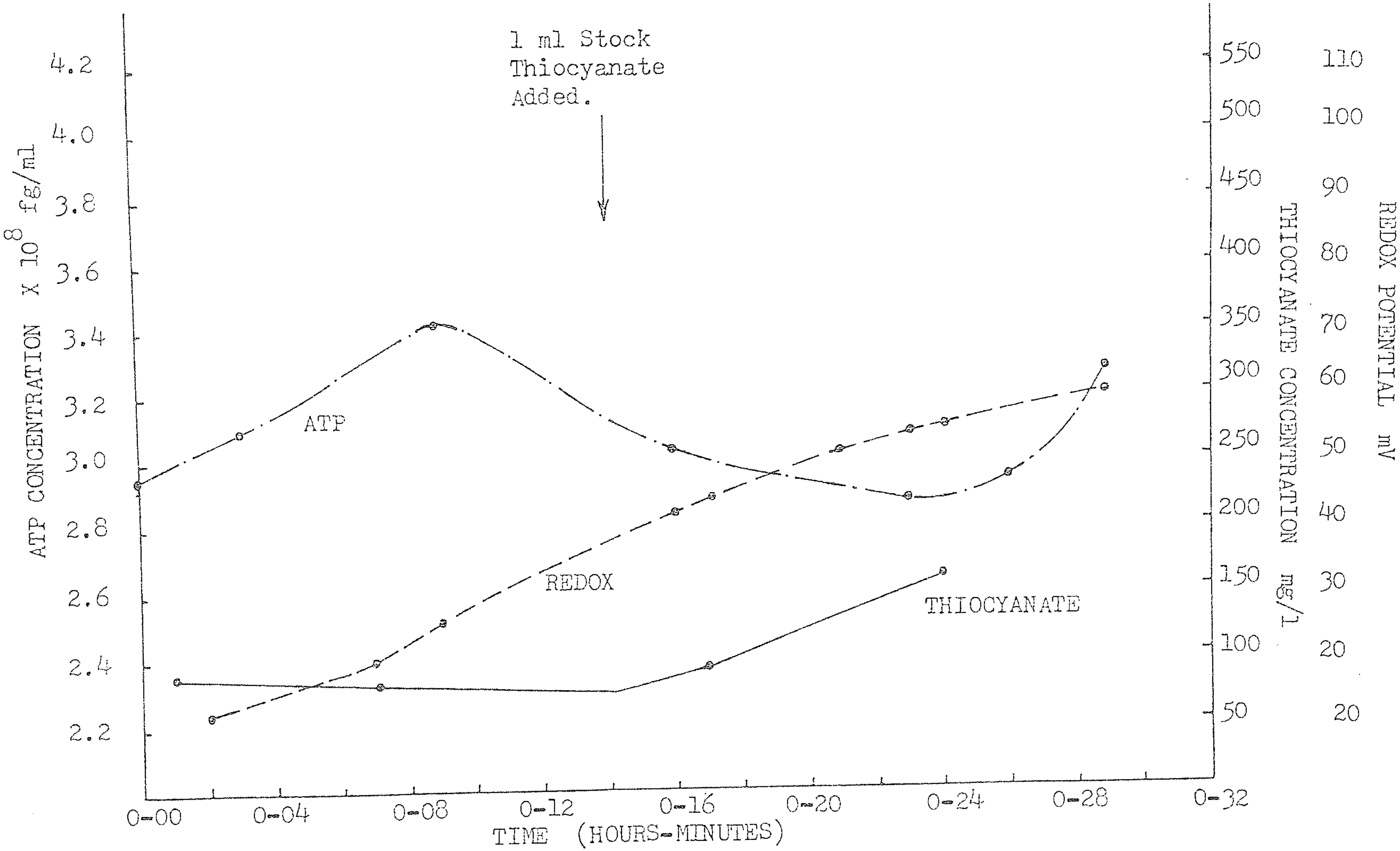


FIGURE 6.4 RESULTS OF EXPERIMENT F. TEST F3.

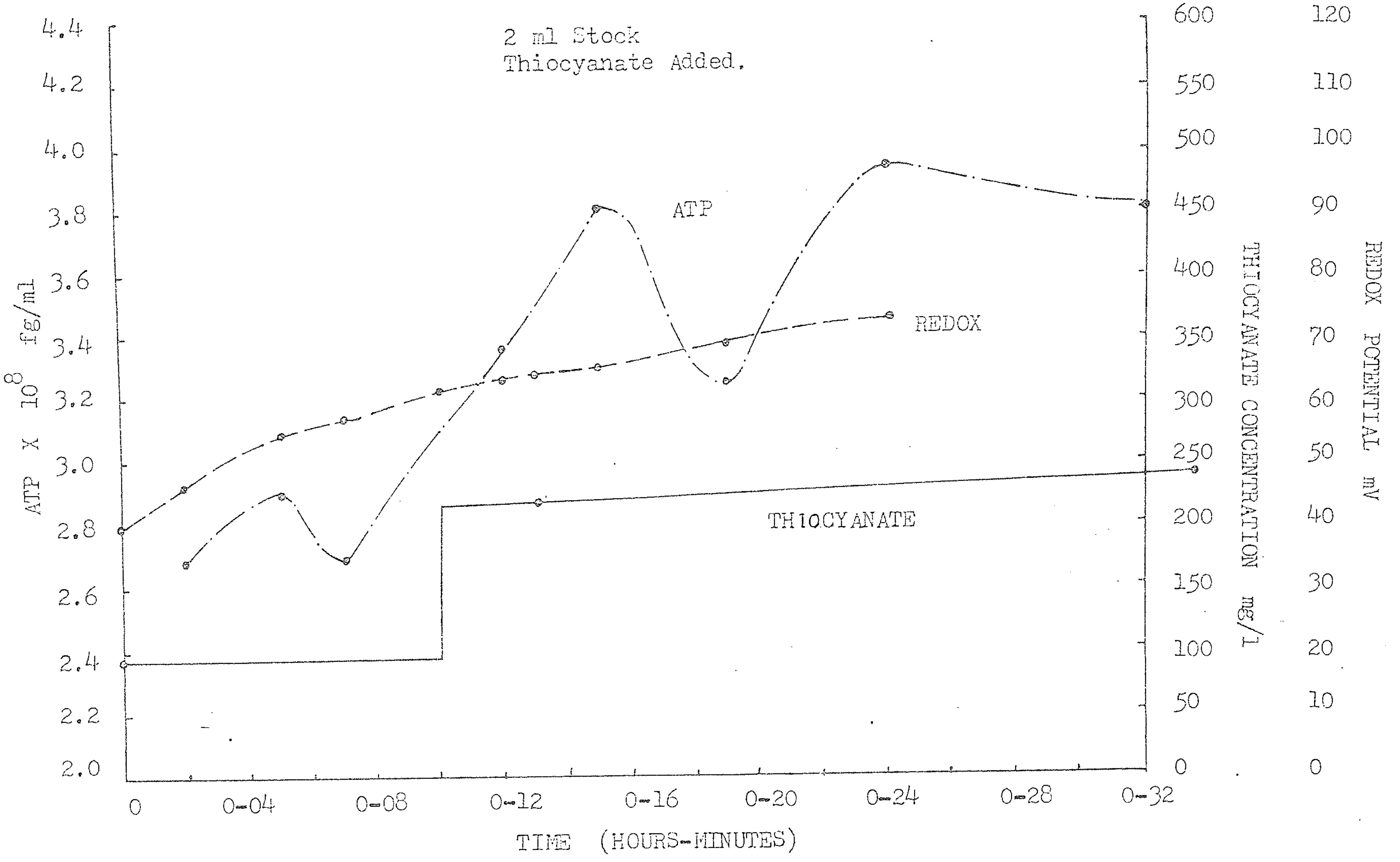


FIGURE 6.5 RESULTS OF EXPERIMENT F. TEST F4.

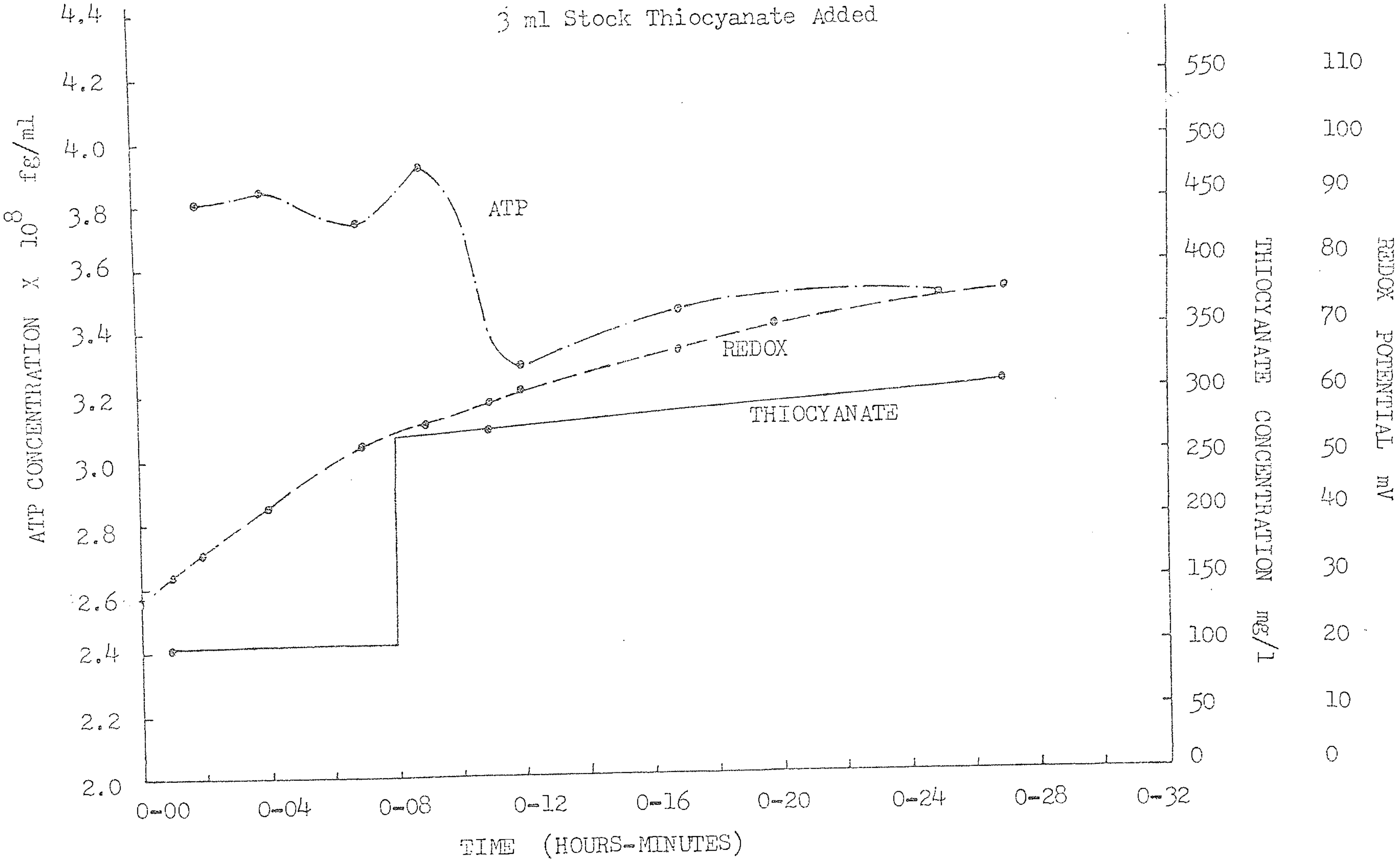


FIGURE 6.6 RESULTS OF EXPERIMENT F. TEST F5.

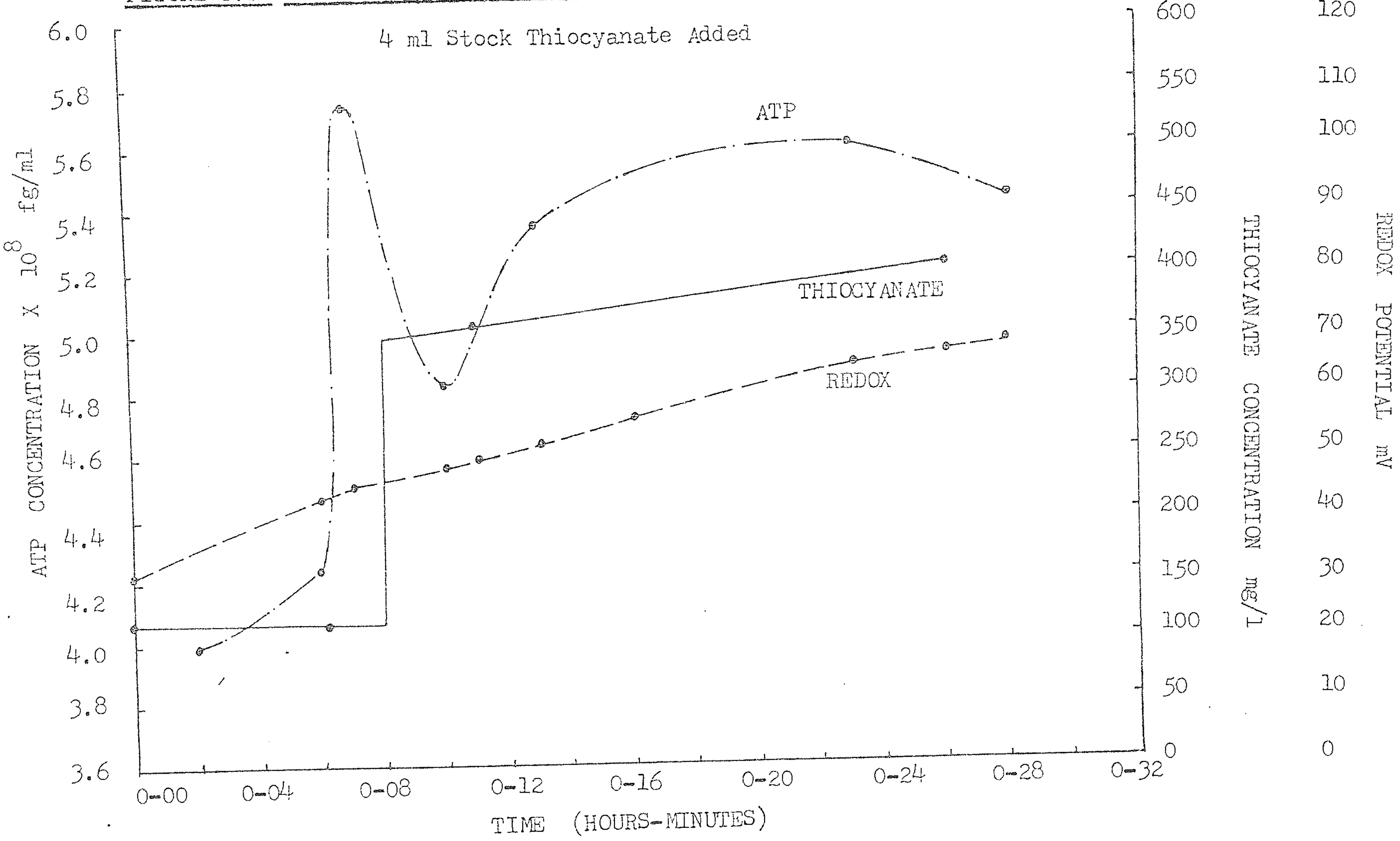


FIGURE 6.7 RESULTS OF EXPERIMENT F . TEST F6.

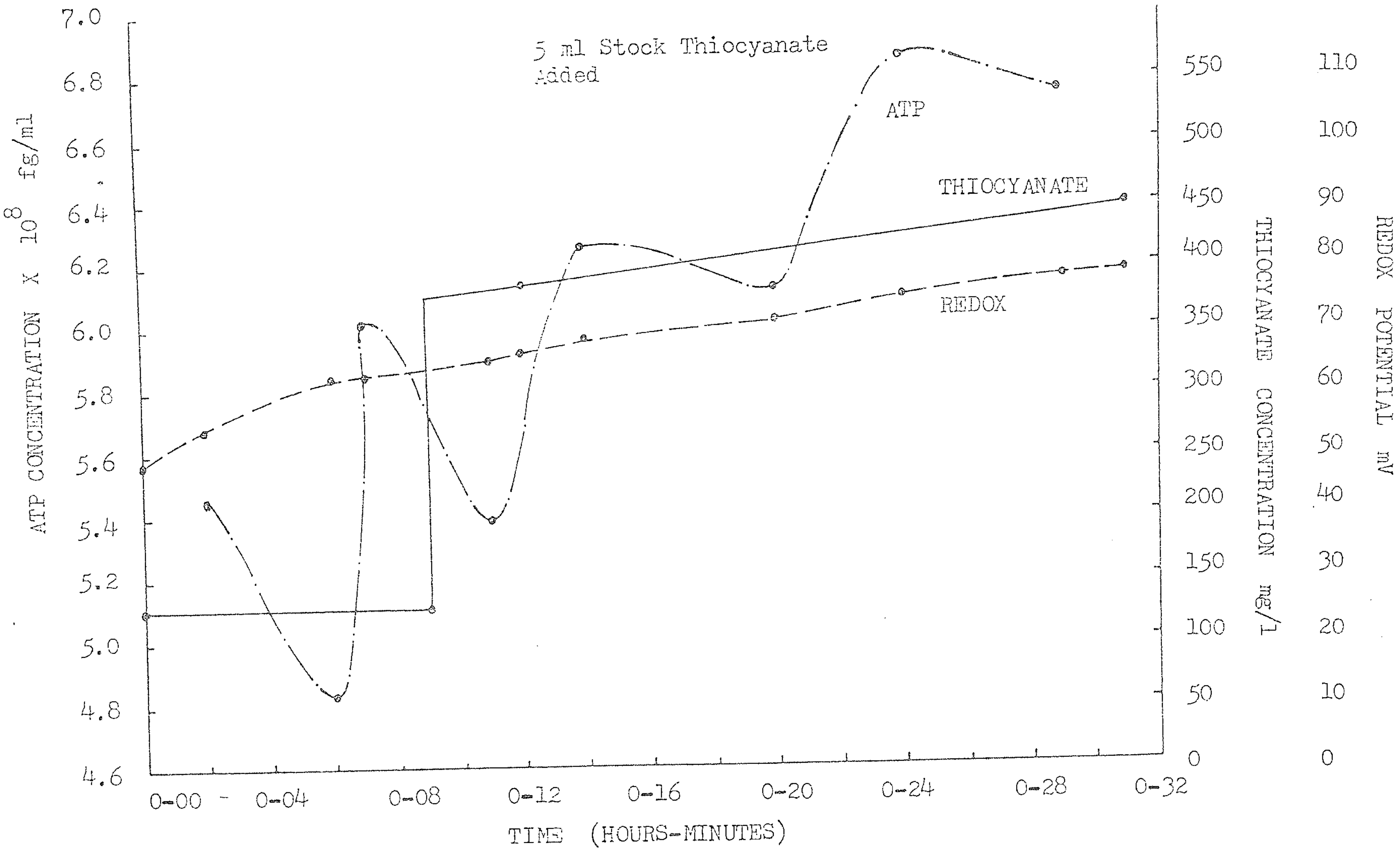


FIGURE 6.8 RESULTS OF EXPERIMENT F. TEST F7.

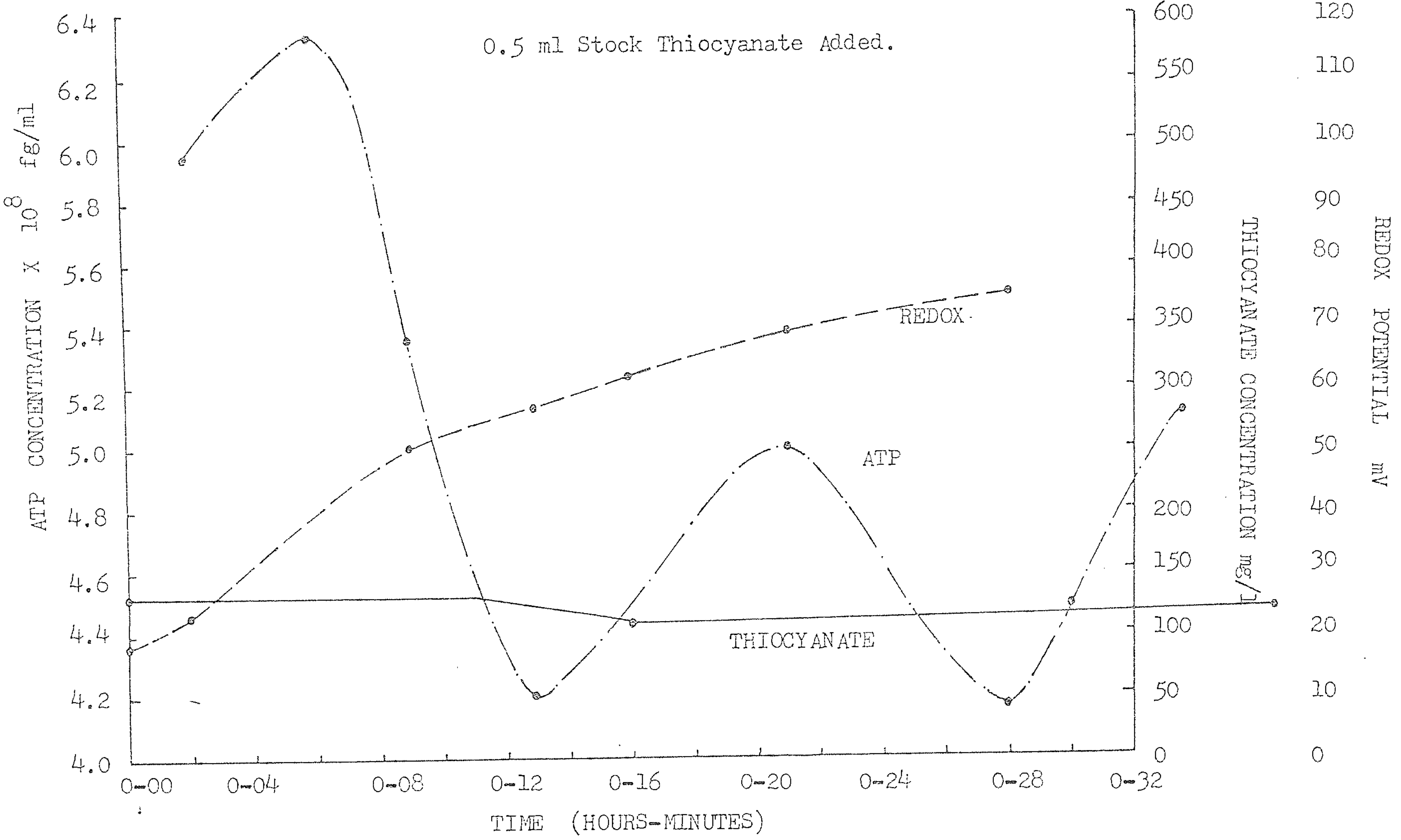


FIGURE 6.9 RESULTS OF EXPERIMENT J. TEST J1.

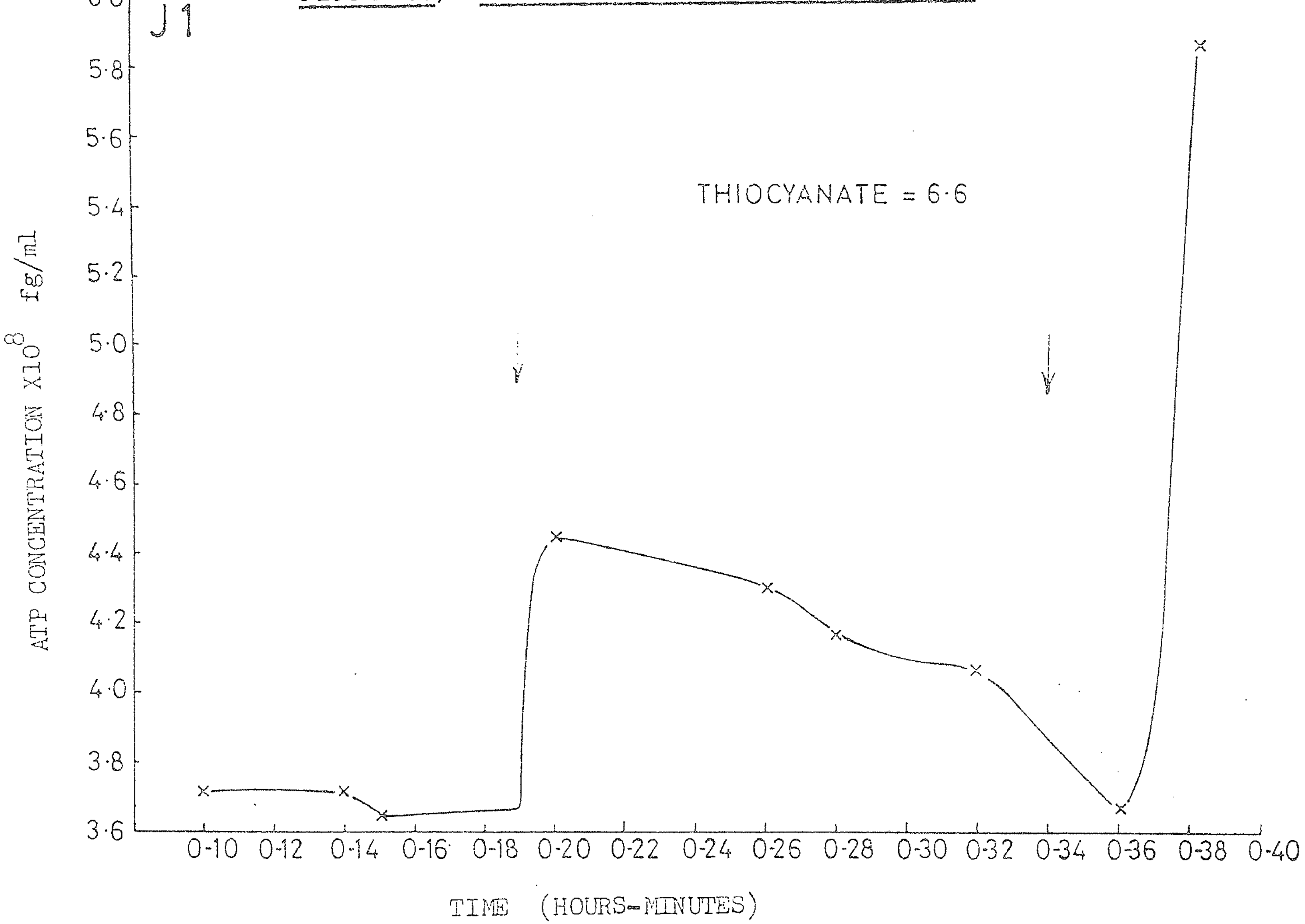


FIGURE 6.10 RESULTS OF EXPERIMENT J. TEST J2.

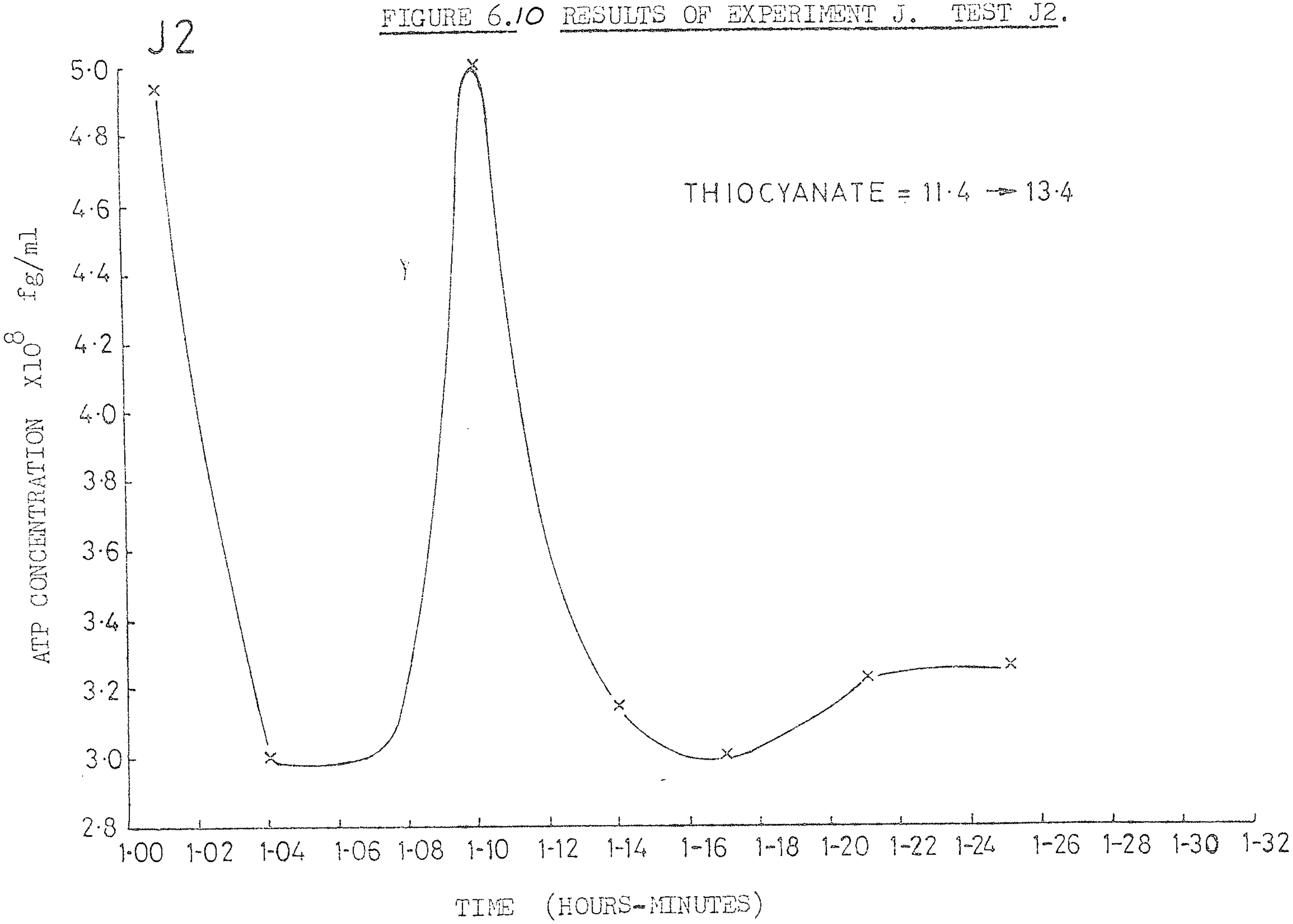


FIGURE 6.11 RESULTS OF EXPERIMENT J. TEST J3.

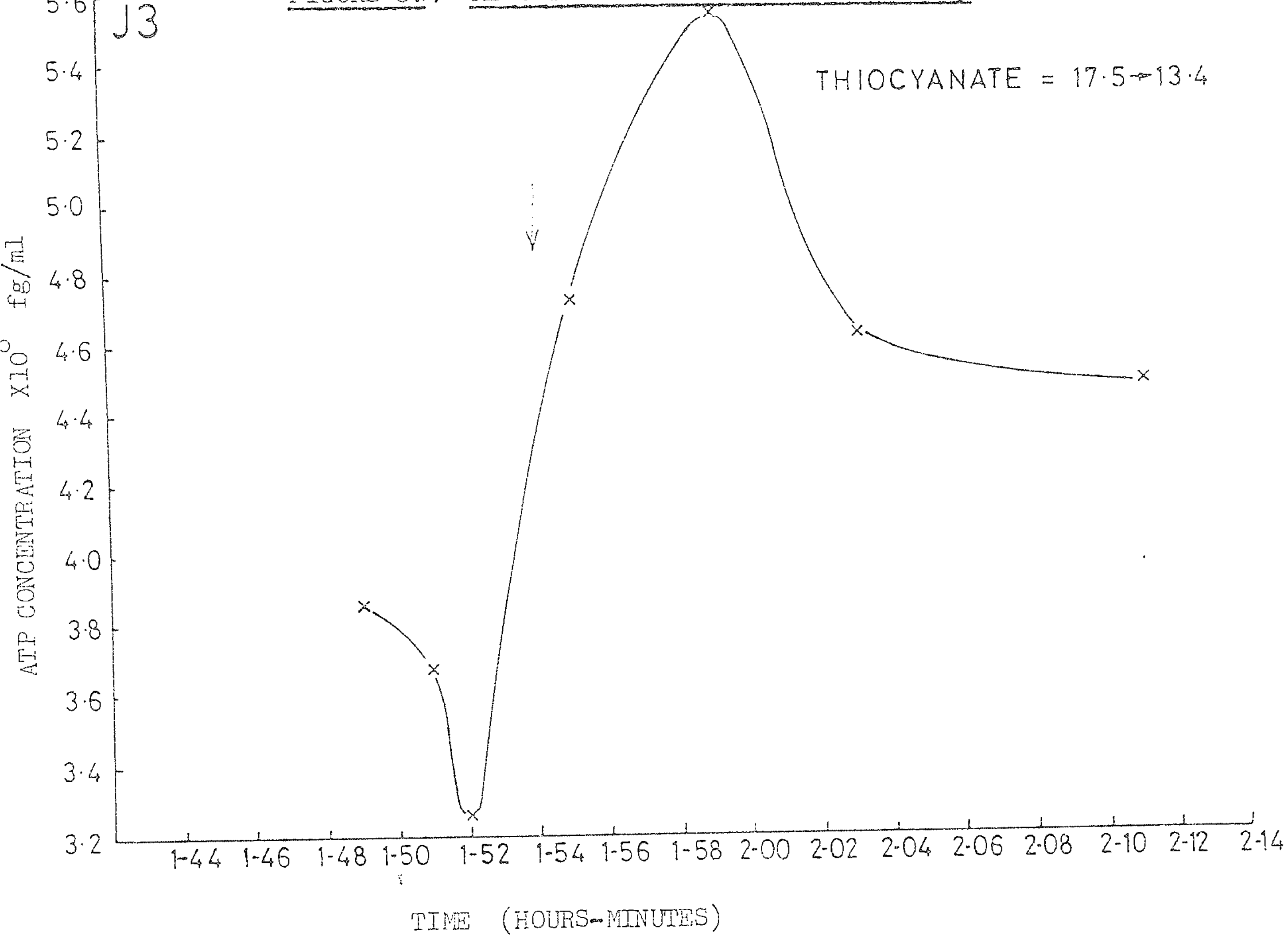


FIGURE 6.12 RESULTS OF EXPERIMENT J. TEST J4.

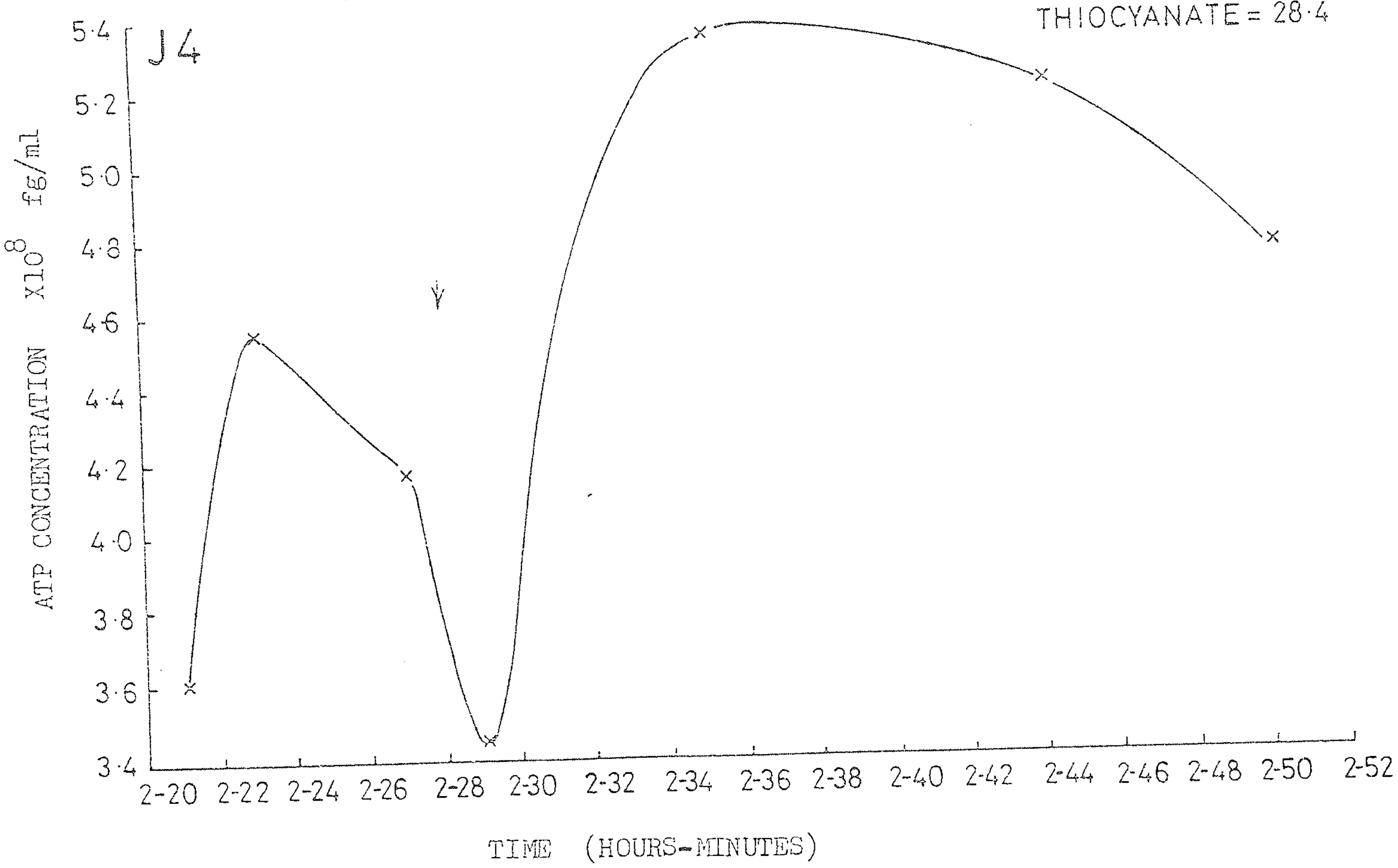


FIGURE 6./3 RESULTS OF EXPERIMENT J. TEST J5.

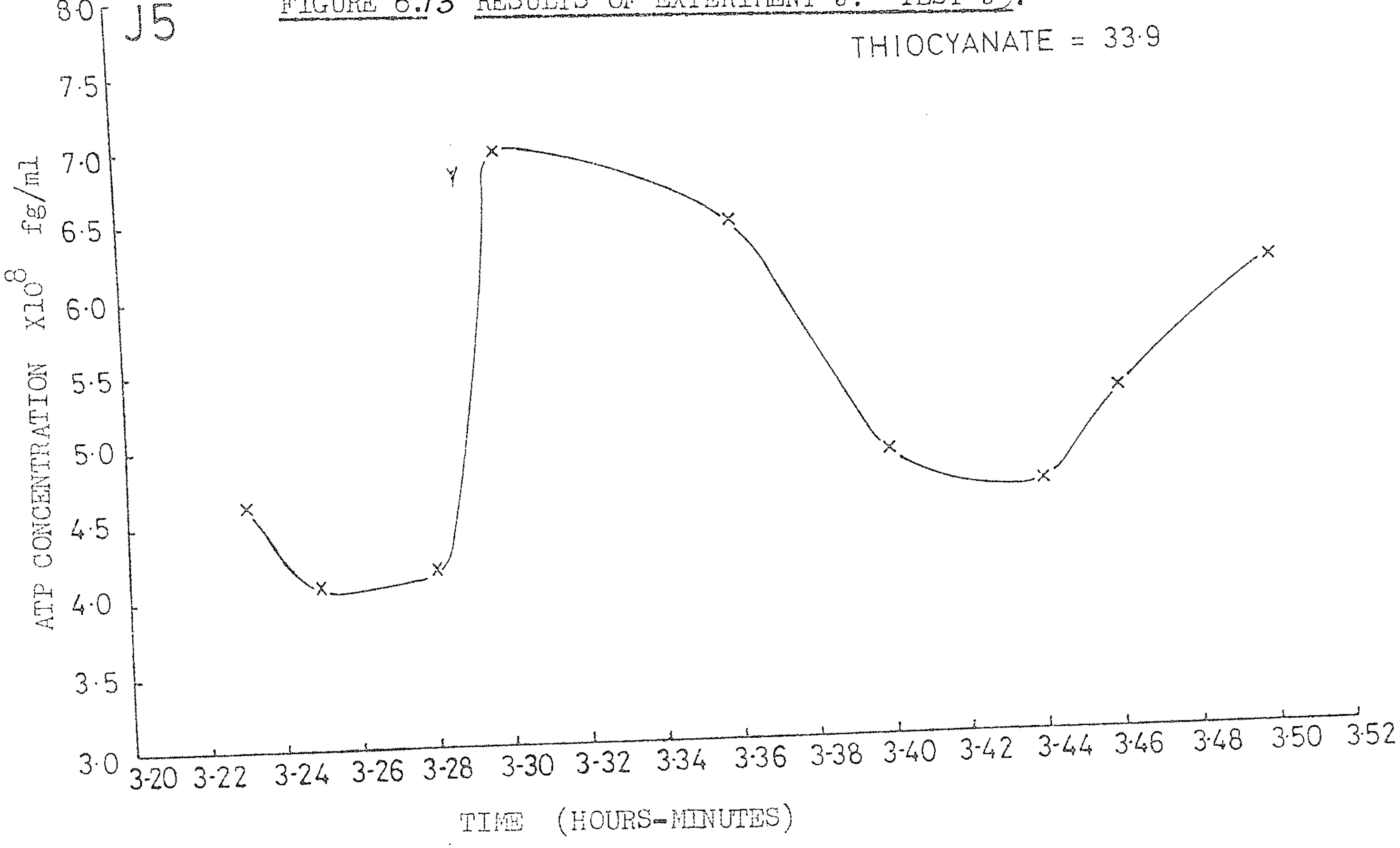
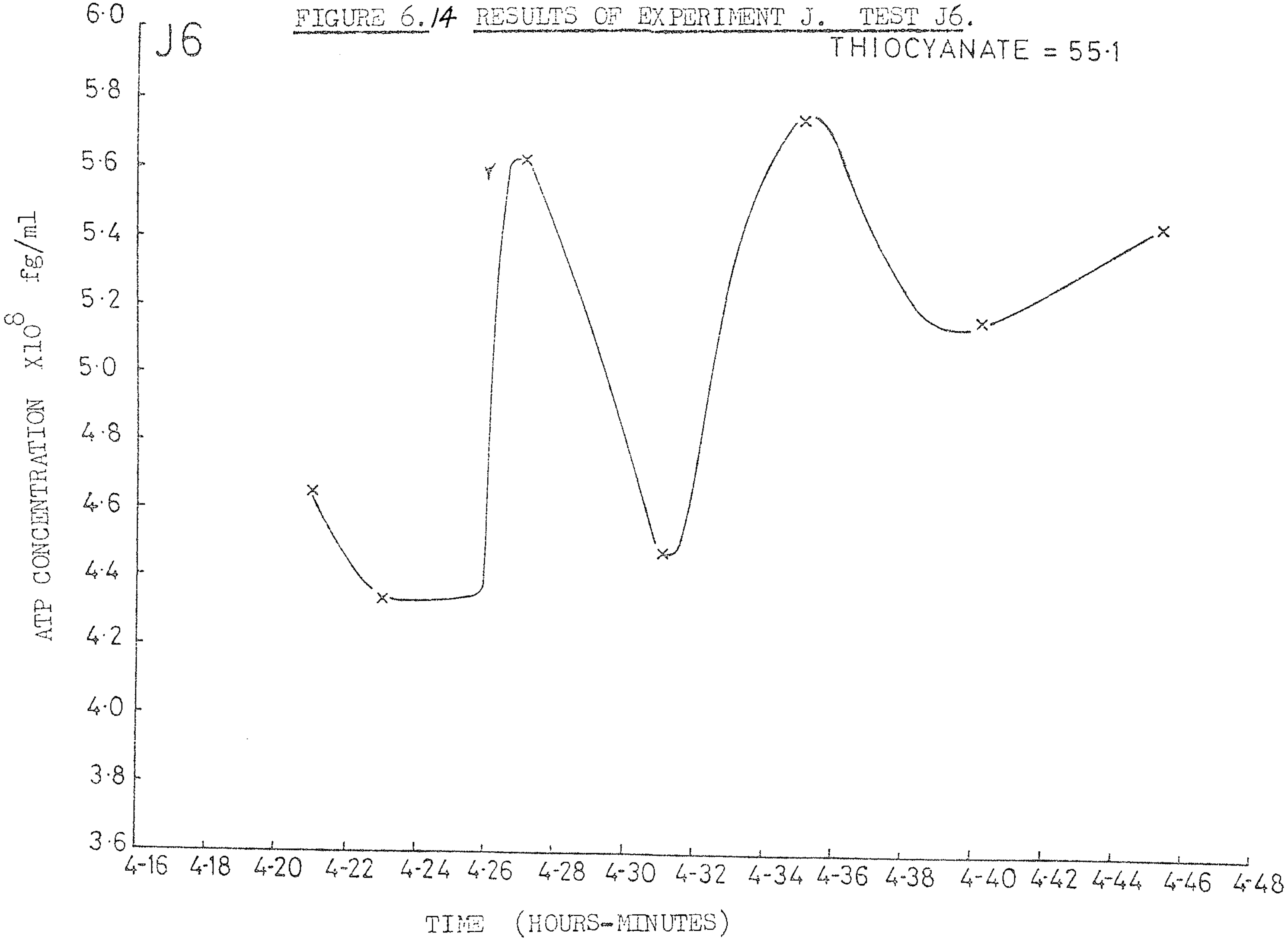


FIGURE 6.14 RESULTS OF EXPERIMENT J. TEST J6.



THIOCYANATE = 55·1

FIGURE 6.15 RESULTS OF EXPERIMENT J. TEST J7.

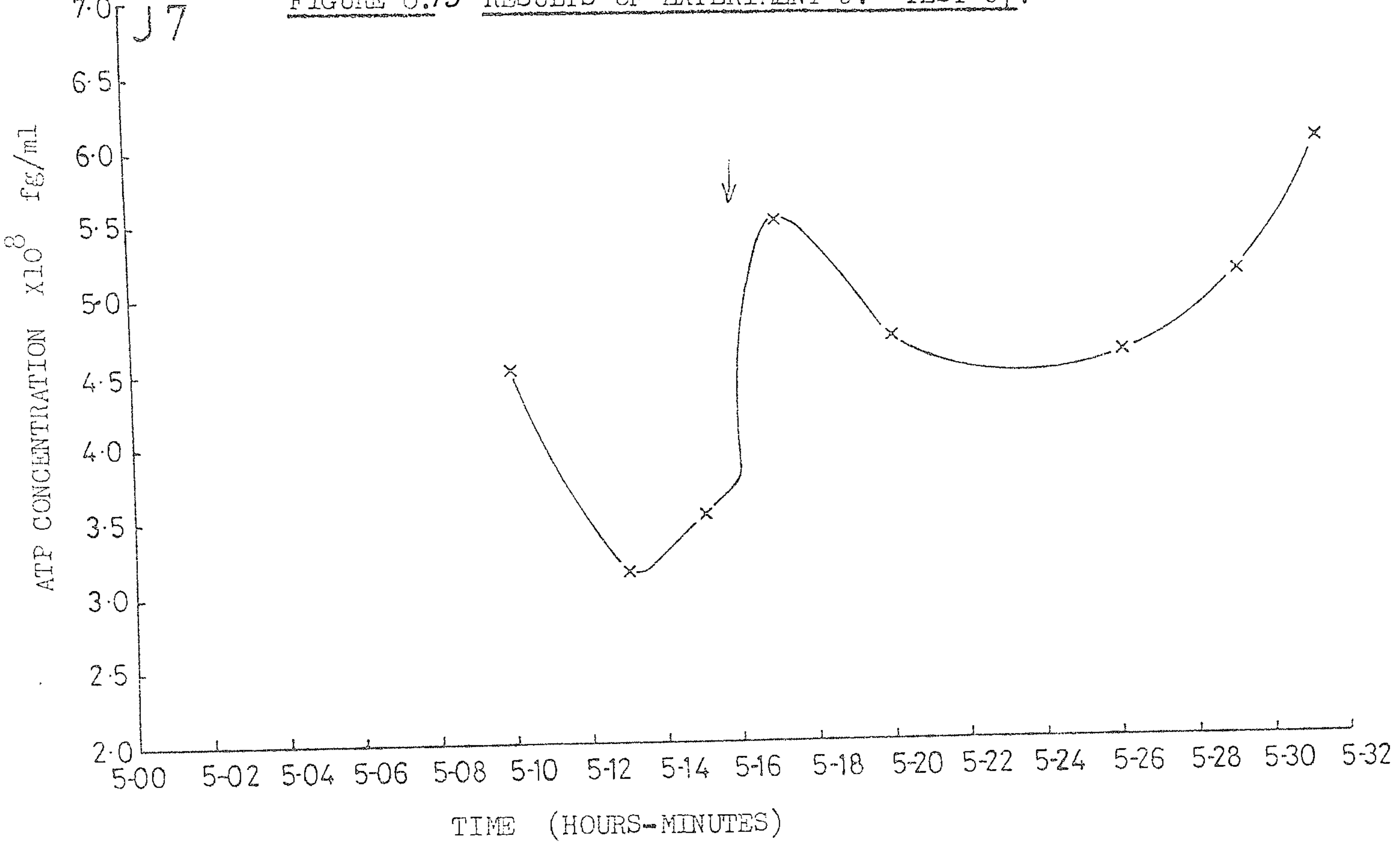


FIGURE 6.16 RESULTS OF EXPERIMENT J. TEST J8.

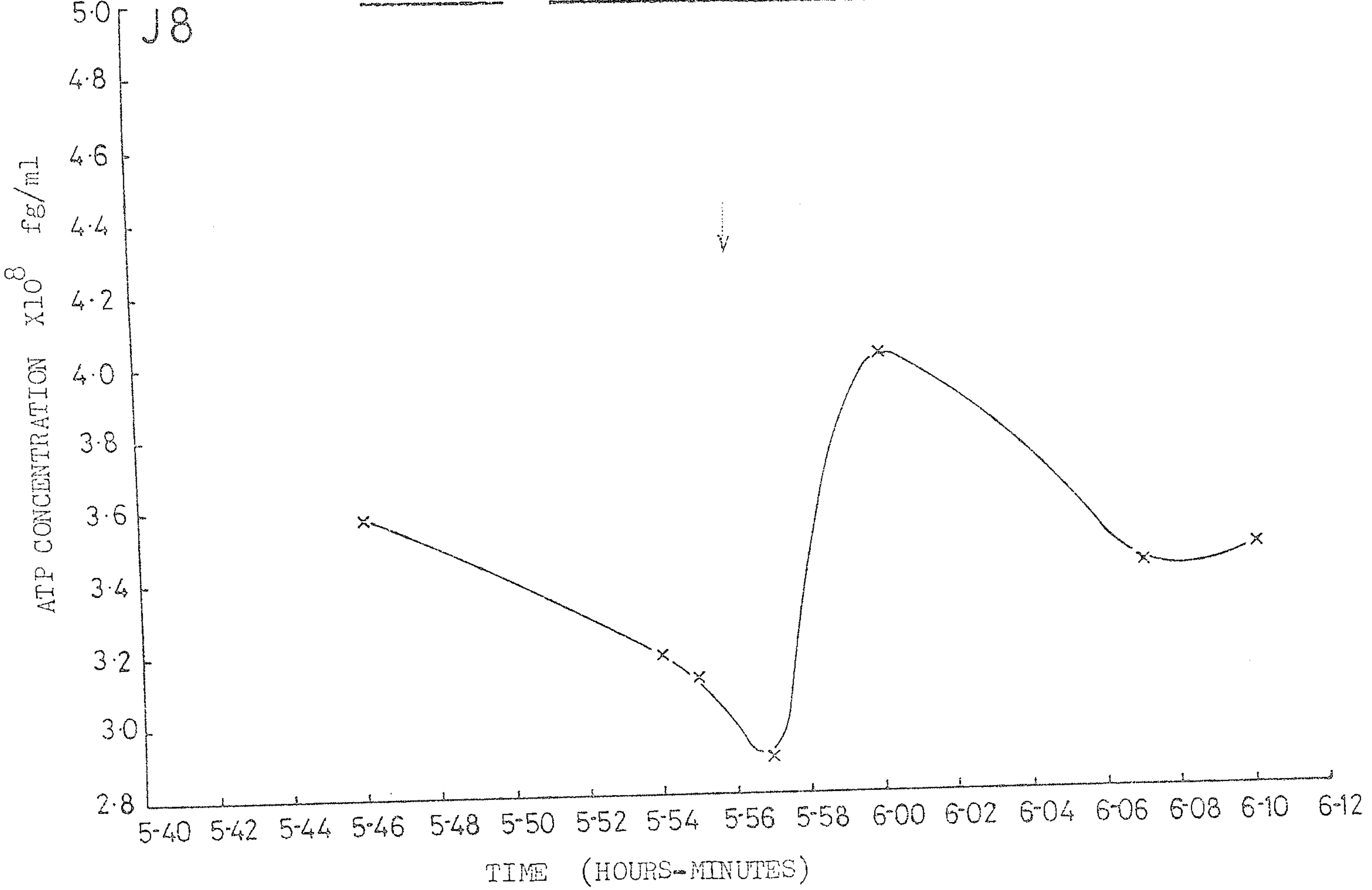


FIGURE 6./7 RESULTS OF EXPERIMENT J. TEST J9.

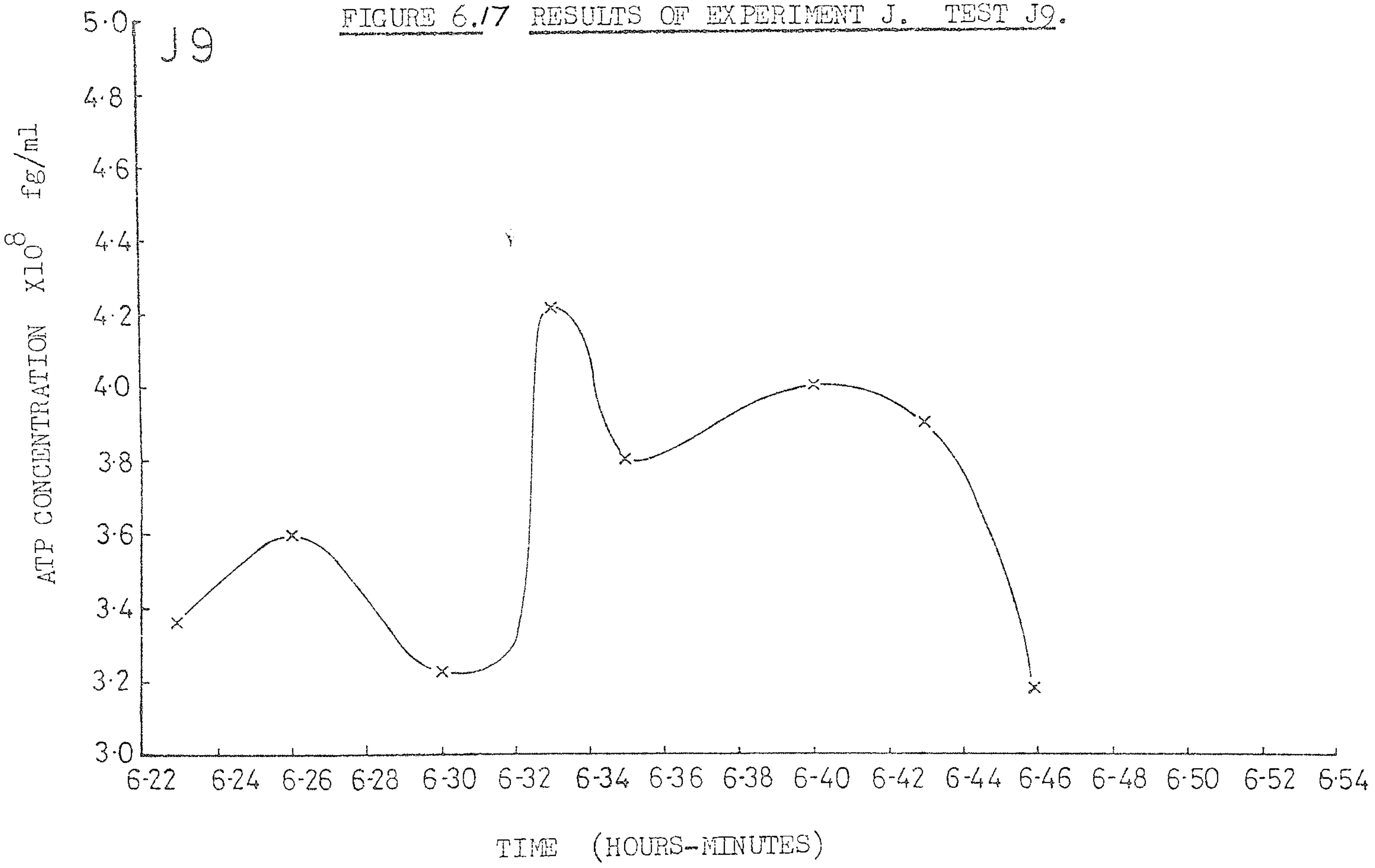


FIGURE. 6.18 RESULTS OF EXPERIMENT K. TEST K1.

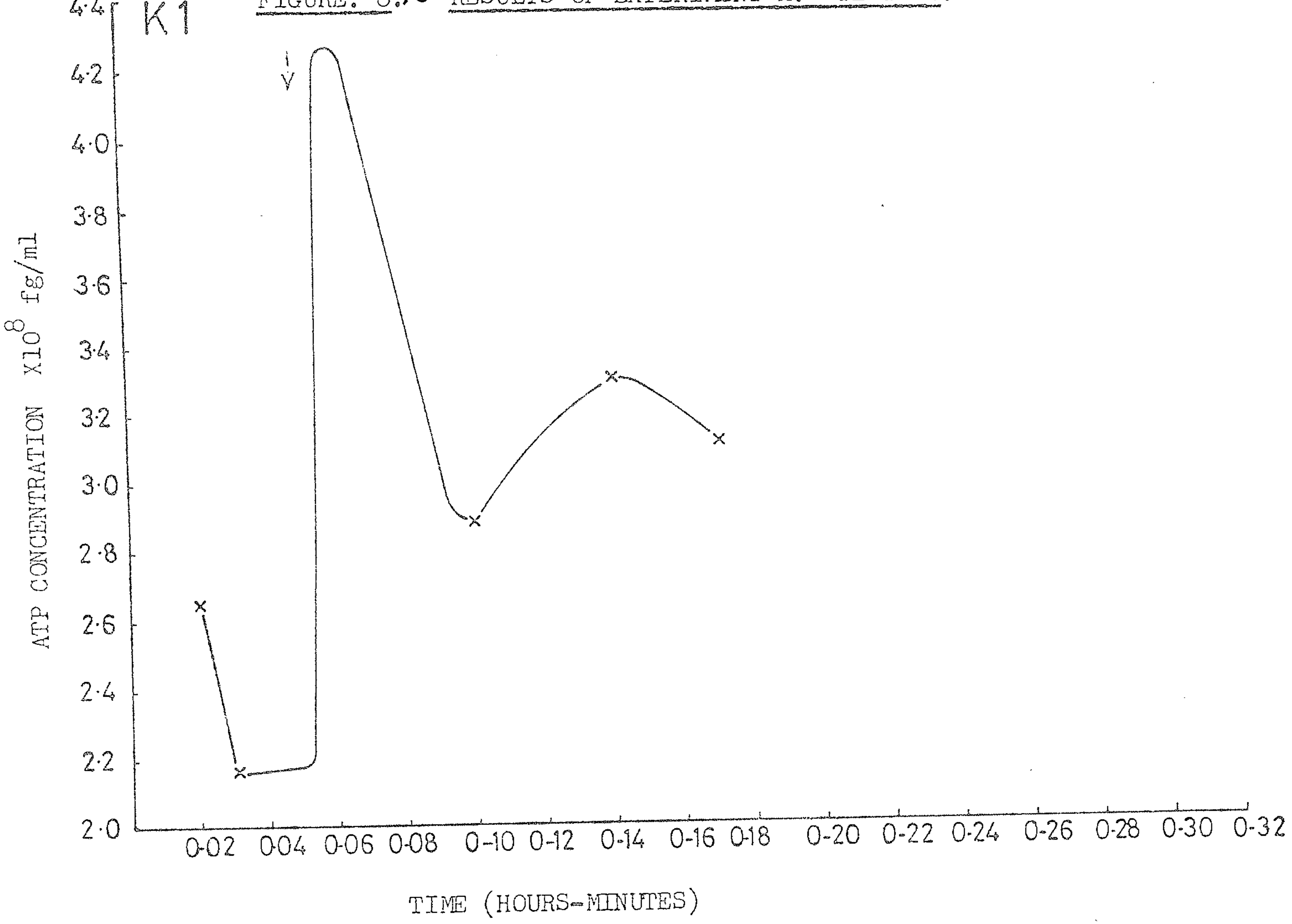


FIGURE 6.19 RESULTS OF EXPERIMENT K. TEST K2.

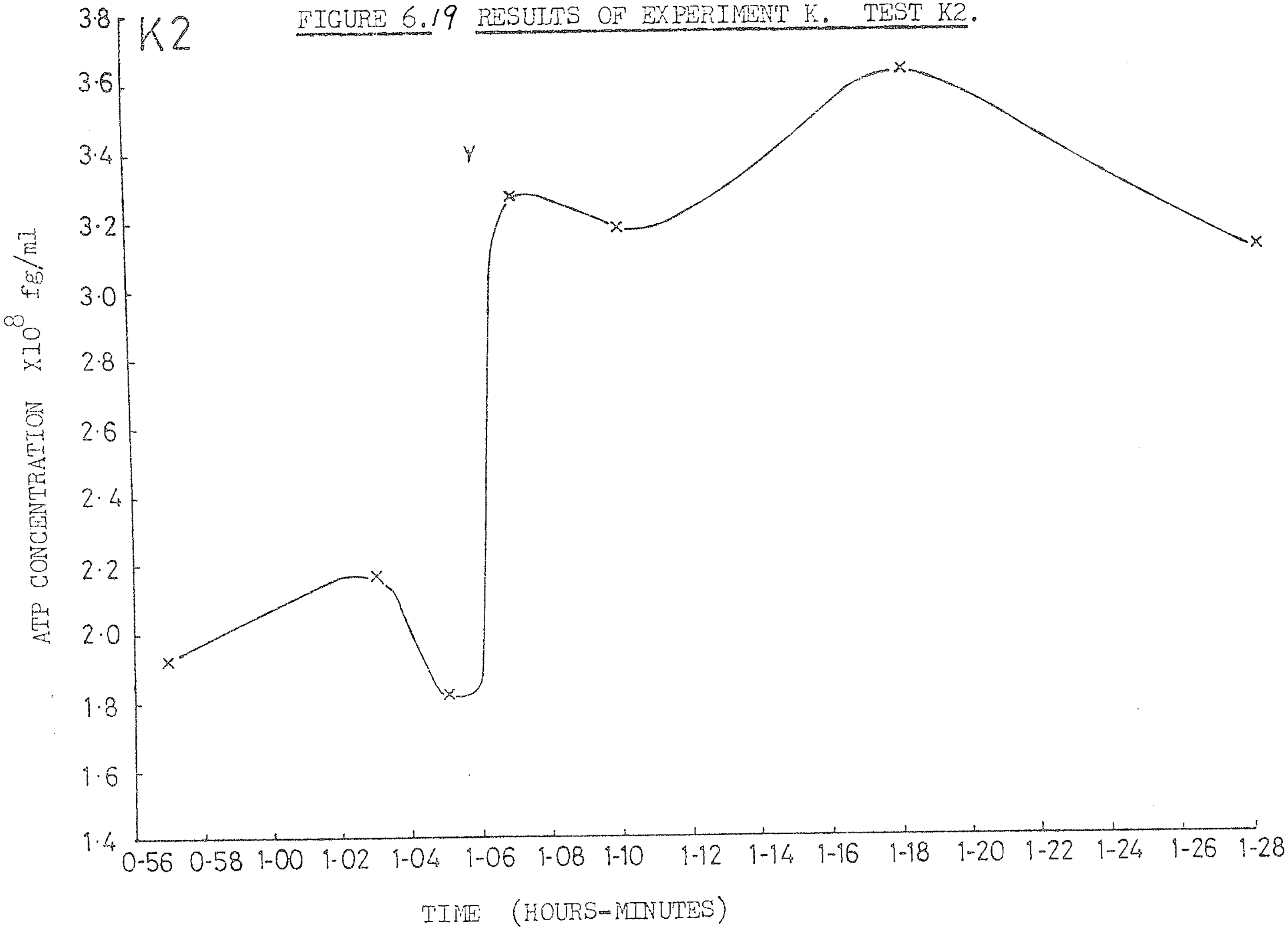


FIGURE 6.20 RESULTS OF EXPERIMENT K. TEST K3.

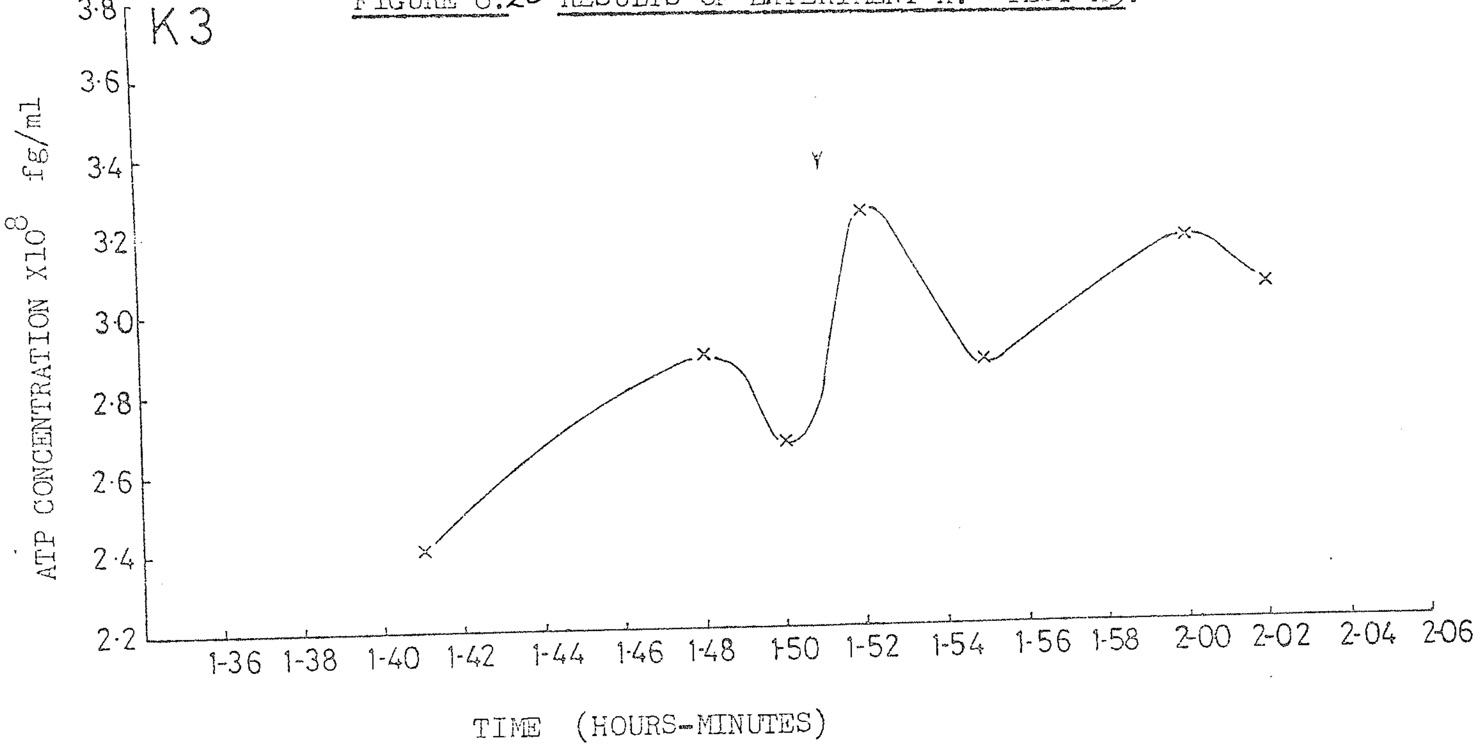


FIGURE 6.21 RESULTS OF EXPERIMENT K. TEST K4.

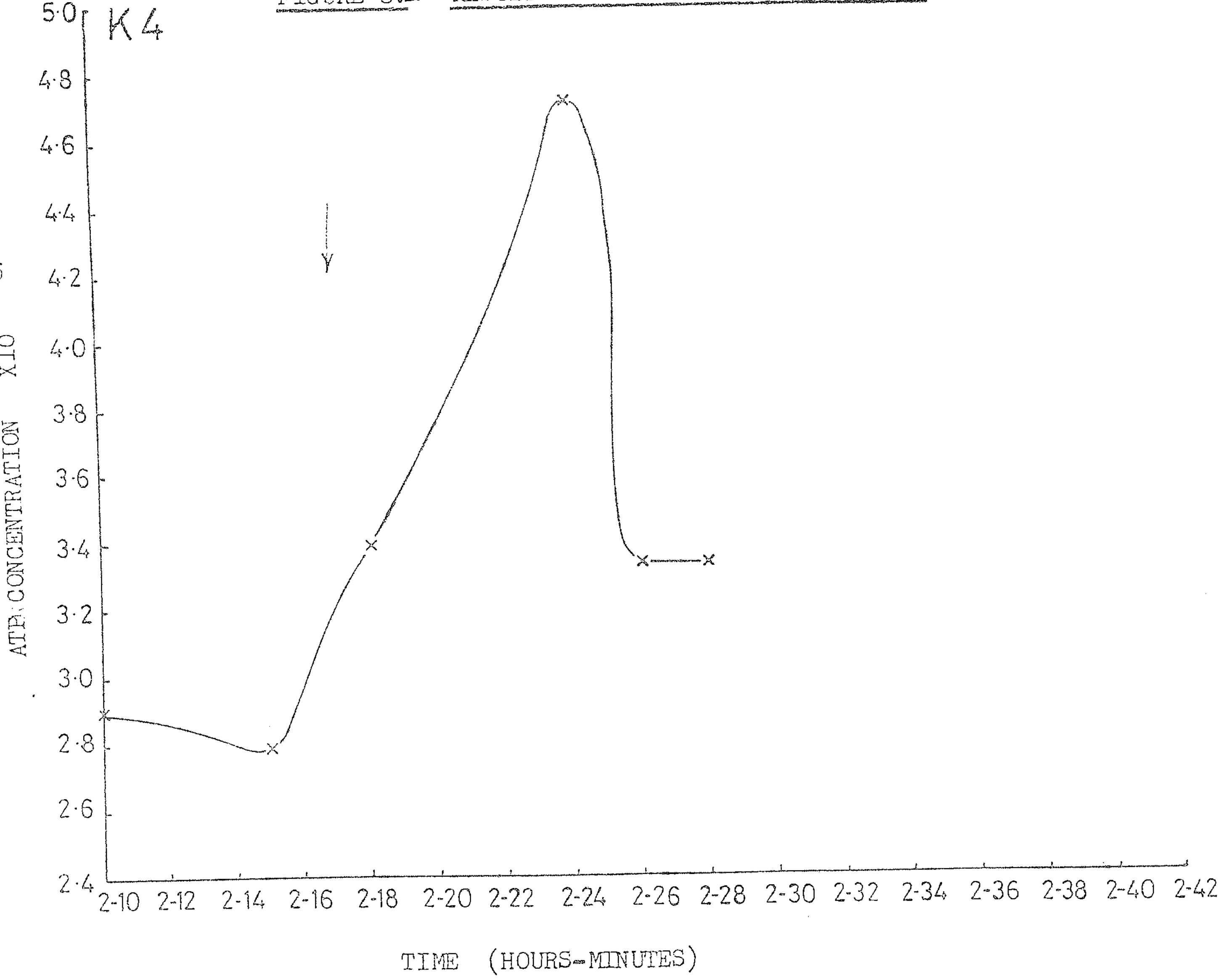


FIGURE 6.22 RESULTS OF EXPERIMENT K. TEST K5.

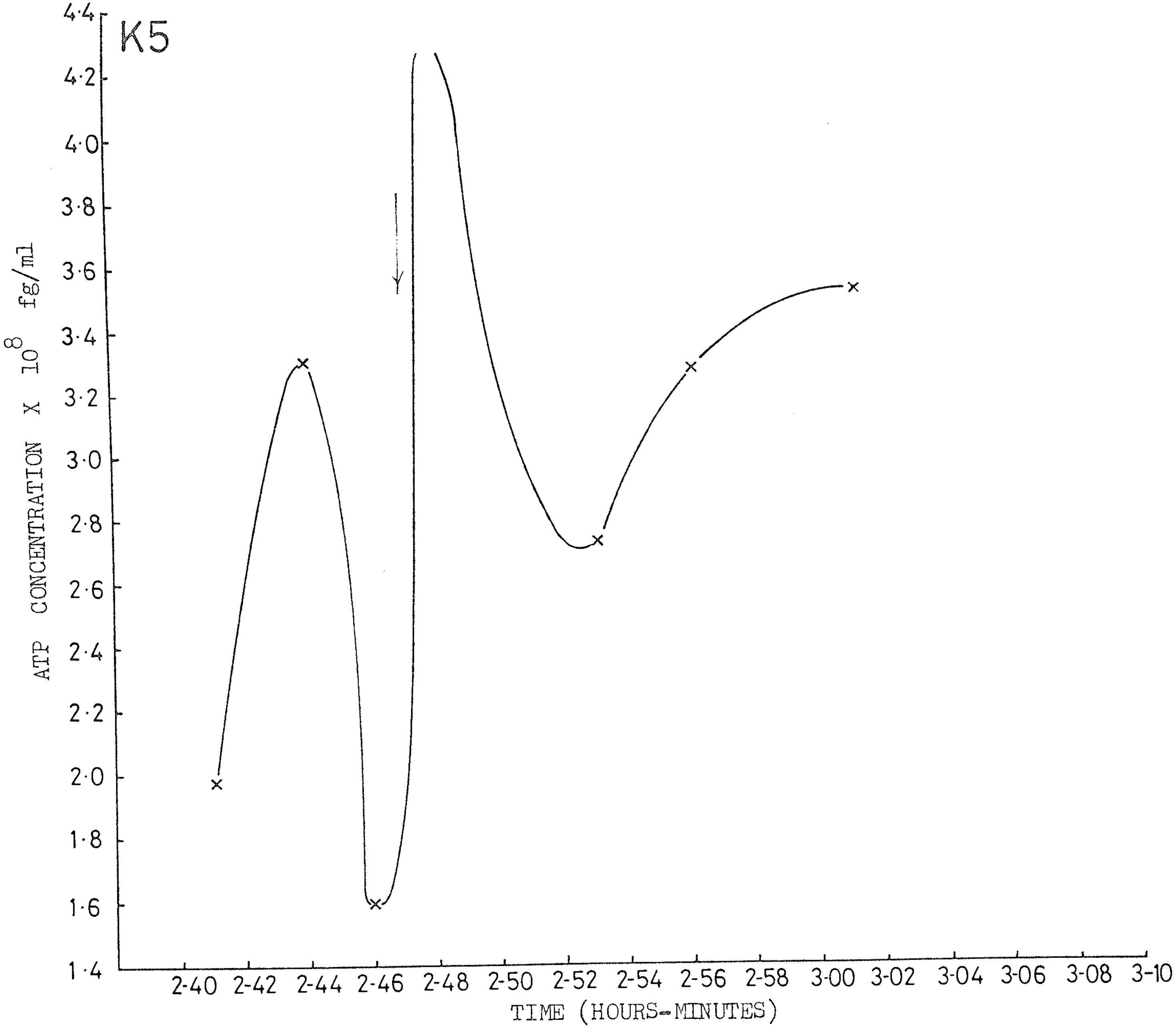


FIGURE 6.23 RESULTS OF EXPERIMENT K. TEST K6.

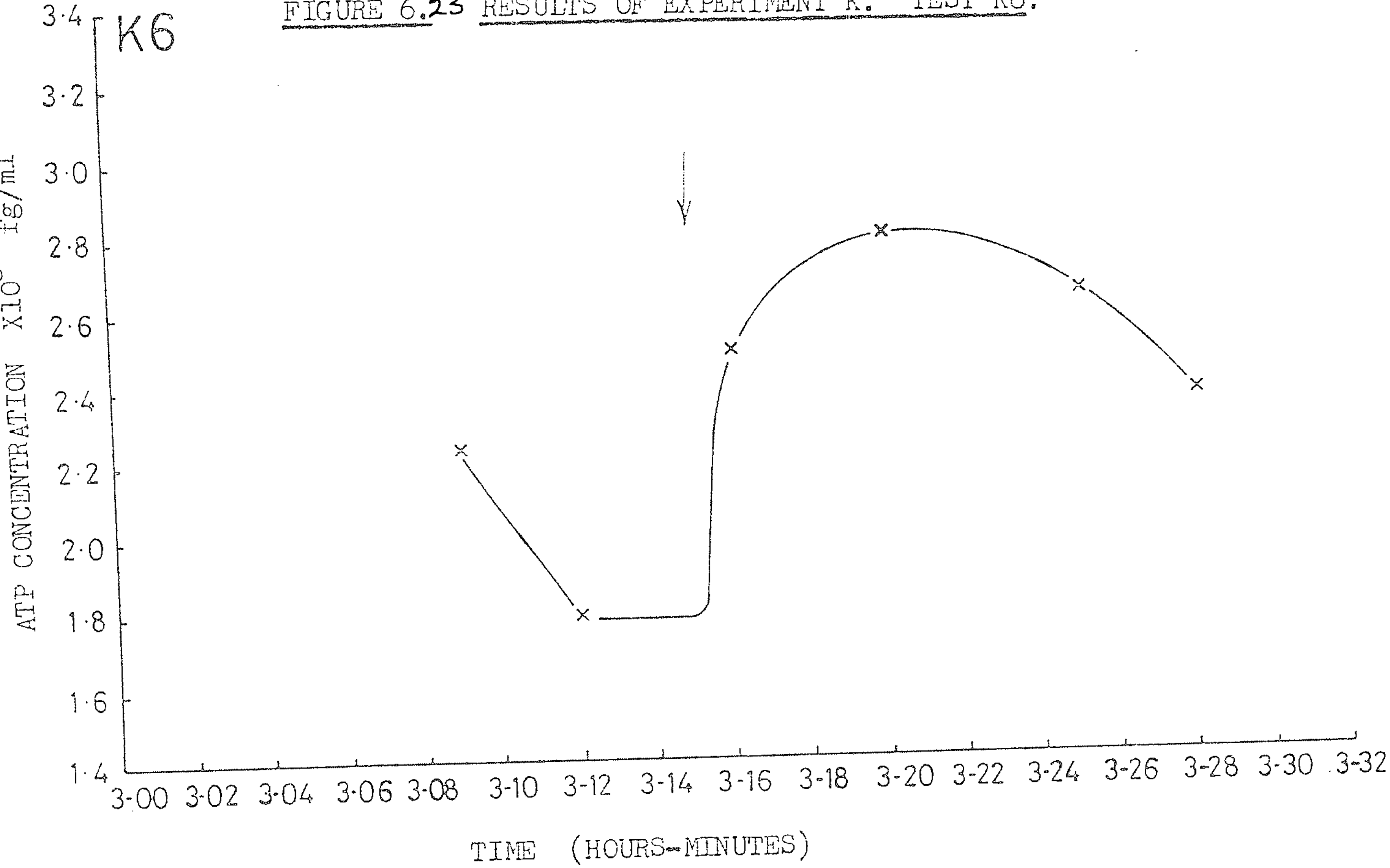
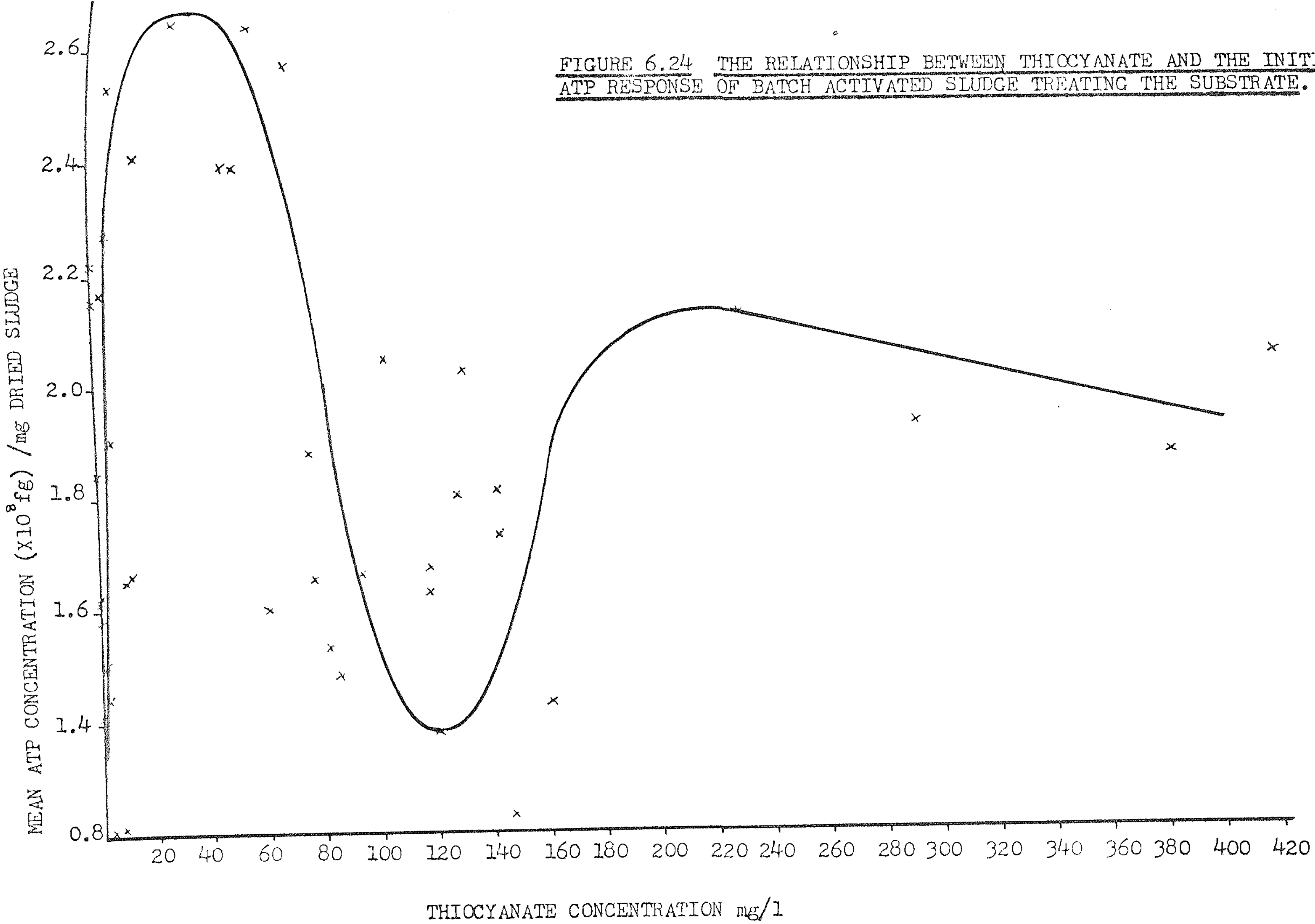


FIGURE 6.24 THE RELATIONSHIP BETWEEN THIOCYANATE AND THE INITIAL ATP RESPONSE OF BATCH ACTIVATED SLUDGE TREATING THE SUBSTRATE.



The mean values of the suspended solids results in each test were used in calculating these values where more than one suspended solids determination had been carried out. The factor of 10.9 in the above equation arose from the fact that a pipette delivering 10.9ml of sludge was used to obtain the sample for suspended solids analysis.

These results were recorded in Table 6.5, along with the thiocyanate concentration in each test run before and after substrate addition. As with the suspended solids values, mean values were recorded where more than on determination had been carried out. A graph was plotted in Figure 6.24 relating the ATP concentration of the sludges in all three tests to thiocyanate concentration.

6.3 Discussion

The results of some individual test runs indicated some large fluctuations in ATP behaviour, and these were discussed later in the project, Over all, however, from examination of Figure 6.24, produced from plotting some 44 data points, the results indicated that the ATP/thiocyanate behaviour showed a similar trend to that reported for the batch experiments. (The results showed a peak in the ATP pool in the 20-70mg/l range, followed by a sharp decline in ATP level above this range, followed by the second peak at higher substrate levels).

The significance of these findings was discussed later in the project, along with a more detailed consideration of the other results obtained in the investigation.

TABLE 6.5. ANALYSIS OF EXPERIMENTAL RESULTS.

EXPT	CNS(MEAN) BEFORE mg/l	AFTER mg/l	ATP (MEAN) $\times 10^{8}$ fg/ml	SUSPENDED SOLIDS(MEAN) mg/10.9ml sple	ATP(MEAN)/ mg SLUDGE
F1	82.3	-	3.9317	32.275	1.3278
	-	119.4	3.486		1.1773
F2	84.5	-	3.597	26.9	1.2264
	-	160.4	3.0268		1.2264
F3	94.3	-	2.7603	20.567	1.4629
	-	228.3	3.64		1.9291
F4	103.1	-	3.8033	22.367	1.8535
	-	290.3	3.5438		1.7270
F5	117.8	-	4.655	34.633	1.4650
	-	379.8	5.3025		1.6688
F6	128.2	-	5.4367	37	1.6016
	-	415.9	6.279		1.8500
F7	131.5	-	5.88	35	1.8312
	-	117	4.592		1.4301
J1	0	-	3.6867	19.8	2.0295
	-	6.6	4.2438		2.3362
J2	0	-	3.01	23.3	1.4081
	-	12.4	3.1302		1.4643
J3	0	-	3.5933	23.9	1.6388
	-	15.45	4.8388		2.2068
J4	3.2	-	4.1067	22.7	1.9719
	-	28.4	5.11		2.4537
J5	0	-	4.305	24.0	1.9552
	-	33.9	5.7283		2.6016
J6	4.5	-	4.4975	23.7	2.0685
	-	55.1	5.306		2.4403
J7	5.9	-	3.7333	24.0	1.6955
	-	68.7	5.208		2.3653
J8	0	-	3.29	26.0	1.3793
	-	8.6	3.4563		1.4490
J9	0	-	3.3973	26.0	1.4243
	-	74.9	3.9988		1.6764
K1	0	-	2.6145	24.8	1.1491
	-	75.8	3.304		1.4522
K2	2.5	-	1.9693	16.5	1.3009
	-	57.2	3.3128		2.1885
K3	0	-	2.6565	24.0	1.2065
	-	60.5	3.087		1.4020
K4	2.5	-	2.8455	25.1	1.2357
	-	141.3	3.6995		1.6066
K5	3.1	-	2.2948	24.8	1.0086
	-	142.9	3.4755		1.5275
K6	4.2	-	2.0108	27.2	0.8058
	-	146.9	2.5778		1.0330

CHAPTER 7

INVESTIGATION OF THE ATP RESPONSE OF BATCH ACTIVATED SLUDGES TREATING PHENOL IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR TO MULTIPLE ADDITIONS OF PHENOL

Introduction

7.1 Experimental Details
    7.1.1 Experiment E-I
    7.1.2 Experiment E-II
7.2 Results

INVESTIGATION OF THE ATP RESPONSE OF BATCH ACTIVATED SLUDGES TREATING PHENOL IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR TO MULTIPLE ADDITIONS OF PHENOL

Introduction

The ATP behaviour of batch activated sludges acclimatised to treating phenol in a basal medium of treated coke oven liquor was well-established by TOMLINS. Unlike the results of his batch experiments with thiocyanate treating sludges, there was a large degree of consistency in the results of his batch experiments with phenol treating sludges.

In view of this it was not felt necessary to investigate the ATP response of batch sludges treating phenol to the same degree as employed for sludges treating thiocyanate. Some experiments were necessary, however, in order to check TOMLINS' findings for such sludges. Two such experiments were performed which were similar to the batch experiments described for the thiocyanate sludges in Chapter 4. The experiments were modified, however, to consider the ATP response of the sludges to multiple additions of phenol. This chapter describes the conduct and results of those experiments.

7.1 Experimental Details

Two batch activated sludges (in reactors labelled B3 and B4) acclimatised to treating phenol in a basal medium of treated coke oven liquor were prepared earlier in the project. These were prepared in a similar manner to that employed for reactors B1 and

B2 treating thiocyanate. Reactor B4 was used as the source of sludge in the following two experiments.

The principle of the experiments was that a batch activated sludge acclimatised to treating phenol in a basal medium of coke oven liquor was starved for several hours to produce endogenous conditions and then fed periodically with doses of concentrated phenol solution. Substrate, ATP and other parameters were monitored periodically or continuously as practicable before and after the addition of substrate.

7.1.1 Experiment E-I

The experiment was carried out using the entire contents of reactor B4 which was contained in a beaker of the type shown in Figure 4.1. Aeration was achieved by supplying compressed air to a diffuser stone at the base of the reactor, and was sufficiently vigorous to maintain complete mixing. The sludge was starved of substrate for a period of several hours before the start of the experiment to produce endogenous conditions.

Redox potential and dissolved oxygen concentration were monitored continuously throughout the experiments, using the electrodes and meters described for measurement of these parameters in Chapter 6. The electrodes were positioned at a depth of approximately 8cm in the sludge. ATP and suspended solids concentrations in the sludge were determined periodically using the sampling and analytical procedures described in earlier experiments. The filtrates produced for suspended solids determinations were analysed for phenol using the analytical method described earlier in the project.

4.4ml of trace metal concentrate and 5.4ml of stock phosphate solution were added to the reactor 24.5 and 26 minutes respectively after the start of the experiment. Approximately 5ml aliquots of stock phenol solution were added to the reactor 39 and 119 minutes after the start of the experiment. The parameters indicated were monitored over a period of 148 minutes and then over a period of 35 minutes, 13 hours after the start of the experiment.

The results obtained were recorded in Table 7.1 and presented graphically in Figure 7.1.

7.1.2 Experiment E-II

This experiment was carried out approximately 24 hours after the start of Experiment E-I using the same sludge and apparatus described above, and was conducted in a similar manner. 5.0mls of stock phosphate solution were added just before, and additions of stock phenol solution made 24 minutes, 2hrs 33mins, 2hrs 55mins, 3hrs 13mins, 3hrs 37mins and 3hrs 59mins after, the start of the experiment. The parameters described in the previous experiment were monitored. In addition, sludge filtrate samples were also analysed for Total Organic Carbon (TOC) using a Tocsin Mark II analyser. The results obtained were recorded in Table 7.2 and presented graphically in Figure 7.2.

7.2 Results

The results of experiments EI and EII were recorded in Tables 7.1 and 7.2 respectively and in Figures 7.1 and 7.2 respectively.

These results were similar to, and consistent with the results reported by TOMLINS for batch sludges treating phenol in as much that a sharp decline in the ATP pool resulted on every occasion from the addition of phenol followed by a gradual rise in the level.

These results were compared with TOMLINS'S findings and discussed in detail later in the project.

TABLE 7.1 THE RESULTS OF EXPERIMENT E-I

TIME	REDOX	SS	PHENOL	D.O	A.T.P.
HRS-MINS.	mV	mg/ 10.9 ml sple	mg/l	mg/l	X 10^8 fg/ml
STARTING pH = 7.53					
0-01	--	27.0	4.0	---	---
0-05	--	28.0	---	---	---
0-12	--	---	---	---	0.6335
0-14	--	28.3	5.0	---	---
0-15	--	---	---		0.7665
0-17	--	27.3	---	---	----
0-19	--	--	---	---	0.8225
0-22	---	25.8	---	---	----
0-24	---	---	---	---	1.096
0-24½	4.4 MLS TRACE METAL CONCENTRATE				
0-26	5.4 MLS PHOSPHATE SOLUTION (3000ppm)				
0-28	---	---	---	---	1.694
0-29	-16.0	---	---	---	---
0-32	-18.0	---	---	9.3	---
0.38	-17.0	---	---	9.3	---
0-39	5MLS STOCK PHENOL SOLUTION ADDED				
0-40	---	28.3	67	---	---
0-42	---	---	---	---	1.145
0-45	-18.0	27.4	---	8.8	---
0-52	---	---	---	---	1.040
0-53	-16.0	---	---	8.8	---
0-55	---	26.5	71	---	---
0-59	-16.0	---	---	8.8	---
1-01	-15.0	---	---	8.9	---
1-02	---	---	---	---	1.064
1-04	---	27.3	---	---	---
1-09	---	27.3	64	---	---
1-11	-14.0	---	---	8.85	1.460
1-24	-12.0	---	---	8.8	---
1-27	---	29.0	---	---	---
1-29	-12.0	---	---	8.8	1.085
1-33	---	26.8	50	---	---
1-35	-12.0	---	---	8.9	1.537

TABLE 7.1 CONTINUED

EXPERIMENT E-I

TIME	REDOX	SS	PHENOL	D.O.	A.T.P.
1-37	---	27.8	---	---	---
1-41	---	26.4	45.5	---	---
1-43	---	---	---	---	1.379
1-48	-11.0	26.7	---	8.8	---
1-56	-11.0	---	---	8.7	---
1-58	-11.0	---	---	8.8	2.261
1-59	5MLS STOCK PHENOL SOLUTION				
2-00	-11.0	---	---	8.8	2.548
2-01	---	26.9	97	---	---
2-06	-10.0	28.6	---	9.1	---
2-07	---	---	---	---	1.477
2-13	---	27.4	105	---	---
2-20	---	23.1	---	---	1.502
2-27	-11.0	27.1	100	9.0	---
2-28	---	---	---	---	1.411
13-11	---	---	---	---	---
13-13	-8.0	---	---	9.4	---
13-26	---	---	---	---	1.449
13-28	---	26.5	4.5	---	---
13-33	---	24.7	---	---	---
13-35	---	---	---	---	1.152

FIGURE 7.1 RESULTS OF EXPERIMENT E-I.

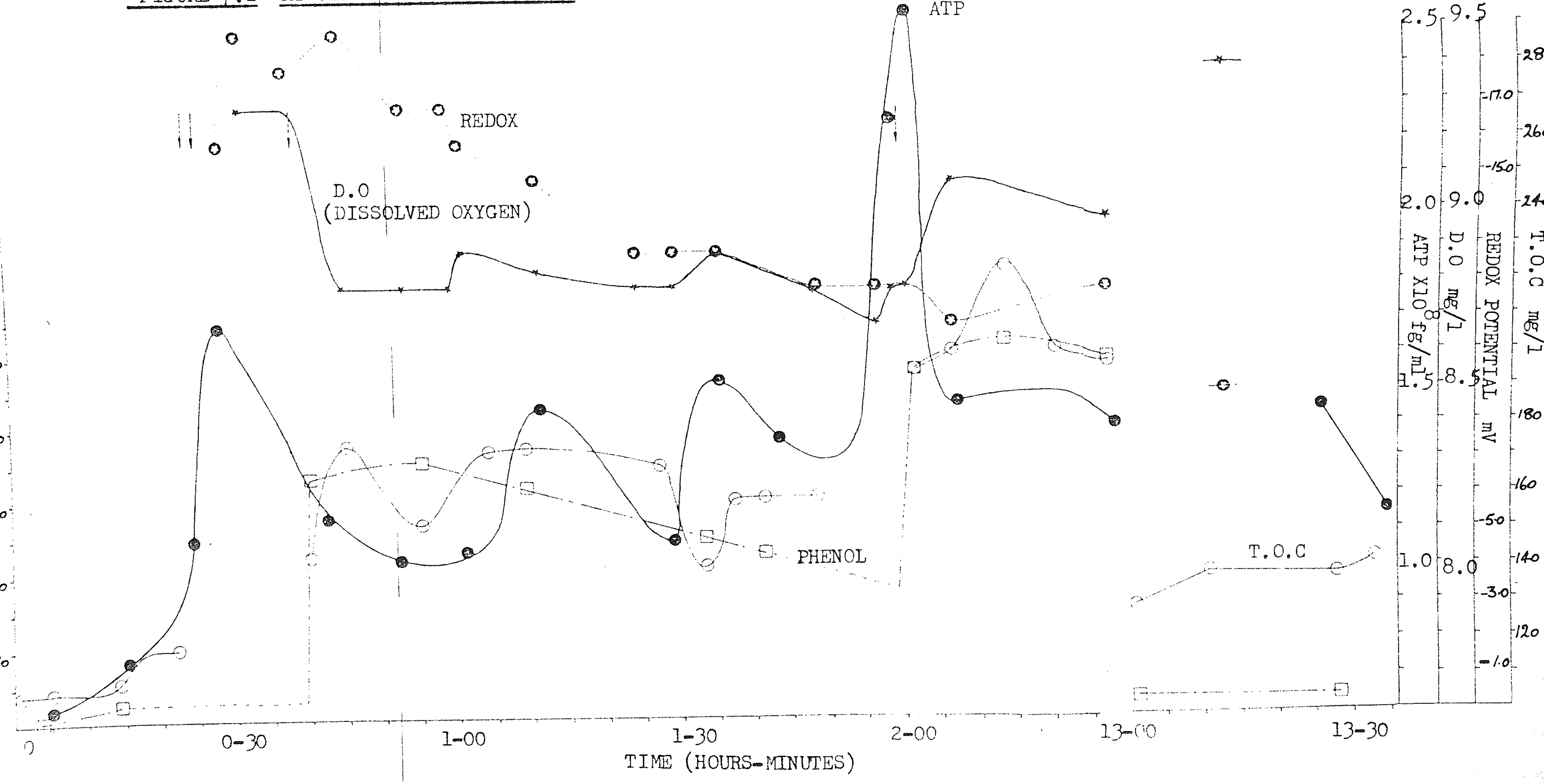


TABLE 7.2 THE RESULTS OF EXPERIMENT E-II

TIME HRS-MINS	REDOX mV	SS mg/10·9ml/sple.	PHENOL mg/l	D.O. mg/l	A.T.P. $\times 10^{?}$ fg/ml
-0-03	5.0MLS PHOSPHATE SOLUTION				
-0-01	-1.0	---	---	---	---
0-01	---	30.1	3) 2) 2.5	---	---
0-05	---	28.4	---	---	---
0-09	-1.0	---	---	9.3	---
0-10	---	---	---	---	1.981
0-12	---	27.7	8) 3) 5.5	---	---
0-13	-0.6	---	---	9.4	---
0-14	---	---	---	---	
0-22	---	25.8	---	---	
0-24	Ca. 3MLS STOCK PHENOL SOLUTION				
0-26	---	---	---	---	
0-27	---	25.4	29) 33) 31	---	---
0-28	-0.7	---	---	9.05	---
0-32	---	26.3	---	---	---
0-39	-1.0	---	---	8.7-8.8	
0-41	---	26.8	29) 33) 31	---	---
0-45	---	---	---	---	
0-49	-0.4	25.2	---	8.75	---
0-55	---	25.9	28) 28) 28	---	---
1-00	0.0	---	---	8.90	---
1-02	---	---	---	---	
1-10	0.0	---	---	8.80	---
1-12	---	---	---	---	1.442
1-13	---	24.5	---	---	---
1-17	---	27.0	17) 15) 16	---	---
1-21	---	---	---	---	1.596
1-22	-0.9	---	---	8.80	---
1-24	---	25.8	---	---	---
1-41	-0.5	---	---	8.95	1.792
1-43	-0.5	25.6	4) 3) 3.5	9.3	---
1-45	---	---	---	---	1.701
1-48	---	---	---	---	1.855
1-49	---	29.0	---	---	---
1-55	---	---	---	---	2.030
1-56	-0.9	---	---	9.4	---
1-5	---	26.5	1) 1) 1	---	---

TABLE 7.2 CONTINUED

2-00	-1.2	---	---	9.4	---
2-02	---	---	---	---	1.796
2-03	---	25.7	---	---	---
2-04	-1.5	---	---	9.4	---
2-08	-1.6	---	---	9.4	1.694
2-25	---	26.7	---	---	---
2-27	---	---	---	---	3.084
2-30	-1.8	---	---	9.45	---
2-33	Ca 5MLS STOCK PHENOL SOLUTION			---	---
2-35	---	---	---	---	2.720
2-36	-2.2	---	49) 57) 53	9.20	---
2-38	---	---	---	---	2.314
2-41	-2.7	25.3	---	8.9	---
2-46	---	---	---	---	2.979
2-48	-2.0	---	64) 65) 64.5	8.85	---
2-54	-1.3	---	---	8.80	---
2-55	Ca 5MLS STOCK PHENOL SOLUTION				
2-56	-1.0	---	---	9.00	---
2-59	---	25.8	113) 118) 115.	---	---
3.00	---	---	---	---	1.810
3-05	---	---	---	---	1.589
3-06	-0.3	---	---	8.90	---
3-10	-0.1	---	---	9.0	1.733
3-13	Ca 5MLS STOCK PHENOL SOLUTION				
3-15	---	---	---	---	2.664
3-16	---	26.8	171) 183) 177	---	---
3-17	0.0	---	---	9.0	---
3-21	-0.1	---	---	9.1	2.500
3-22	---	---	---	---	---
3-35	---	29.7	190) 191) 190.5	---	---
3-36	-0.3	---	---	9.0	1.491
3-37	Ca 5MLS STOCK PHENOL SOLUTION				
3-40	-0.2	---	---	9.1	---
3-41	---	25.5	244) 252) 248	---	---
3-41	---	---	---	---	1.600
3-50	-0.4	---	---	9.05	1.726
3-52	-1.0	---	---	9.05	---
3-57	---	---	---	---	2.097
3-59	---	---	---	---	---

FIGURE 7.2 RESULTS OF EXPERIMENT E-II

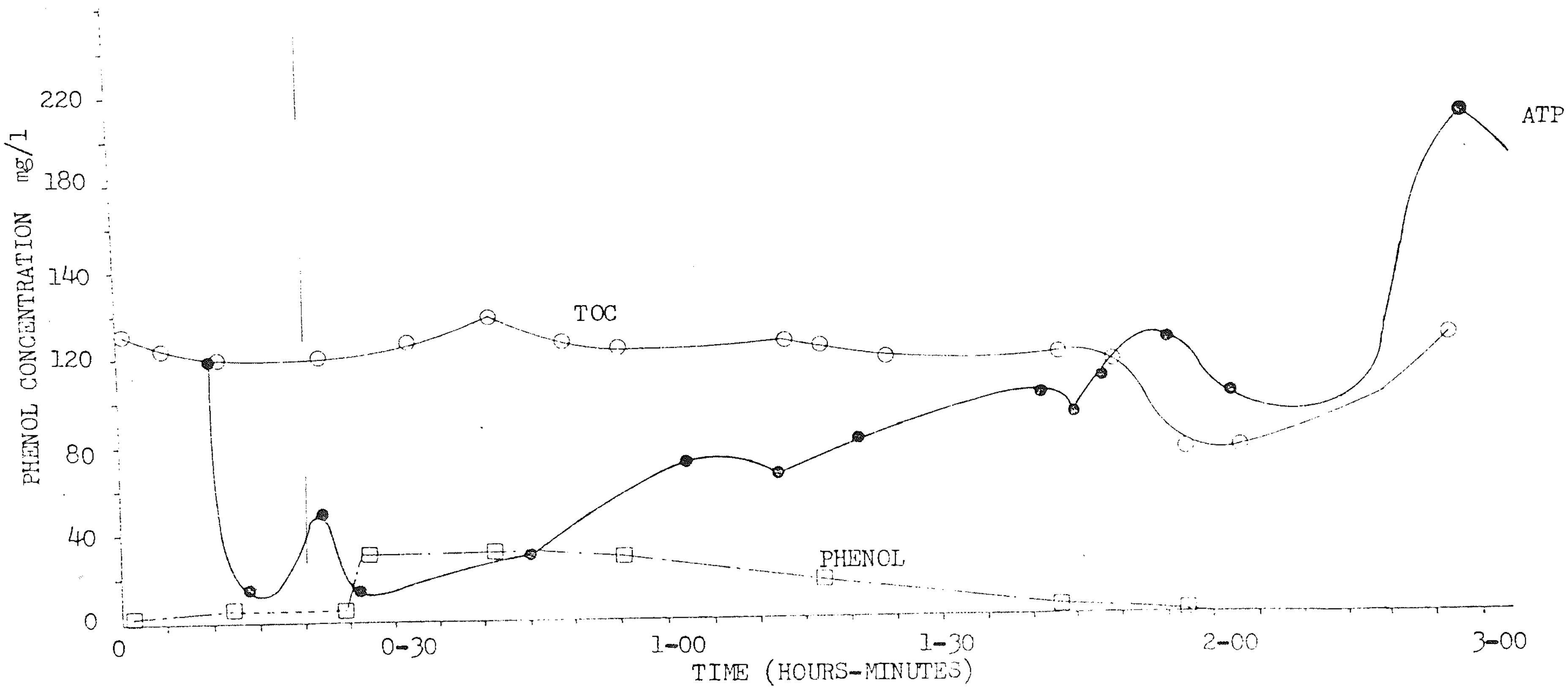
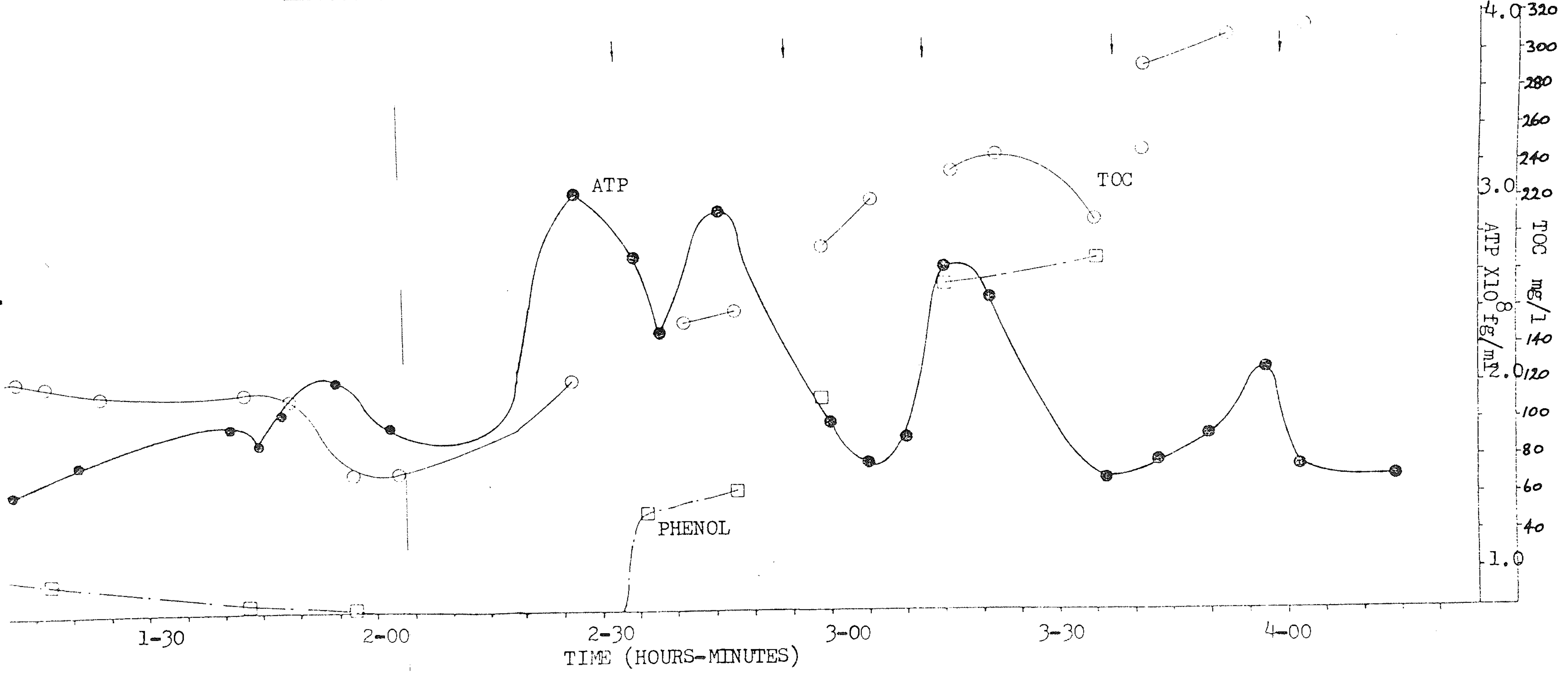


FIGURE 7.2 RESULTS OF EXPERIMENT E-II continued.



CHAPTER 8

INVESTIGATION OF THE INITIAL ATP RESPONSE OF BATCH ACTIVATED SLUDGES TREATING PHENOL IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR

Introduction

8.1 Experimental Details
  8.1.1 Experiment G
  8.1.2 Experiment P
  8.1.3 Experiment R
8.2 Results
8.3 Discussion

INVESTIGATION OF THE INITIAL ATP RESPONSE OF BATCH ACTIVATED SLUDGES TREATING PHENOL IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR

Introduction

The previous chapter described two experiments which investigated the ATP response of batch activated sludges treating phenol in a basal medium of treated coke oven liquor, to multiple substrate additions. A natural extension of these experiments and those described by TOMLINS, was to consider the initial ATP response of such sludges to different quantitative additions of substrate. These experiments were similar to those described for the thiocyanate-treating sludges described in Chapter 6 and were performed for essentially the same reasons. This chapter describes the conduct and results of those experiments.

8.1 Experimental Details

Three experiments, G, P, and R were performed to investigate the initial ATP response of batch activated sludges treating phenol in a basal medium of treated coke oven liquor. The batch reactor B3 referred to earlier was used as the source of sludge.

The general design of the experiments were the same as those described for the experiments described in Chapter 6. Aliquots of batch reactor B3 were used in a series of tests, in each of which a quantity of concentrated phenol solution was added. Phenol and ATP concentrations were monitored for a short period before and after the addition of the stock phenol solution along with various other parameters. Different quantities of stock substrate solution were added in each test in order that the ATP response to a range of different substrate concentrations was obtained. As in the previous series of experiments with thiocyanate, every effort was made to ensure that all other conditions were identical in each test.

8.1.1 Experiment G

This experiment was similar in most respects to that described for experiments F, J and K of the thiocyanate-treating sludge, and used the same apparatus shown in Figure 6.1.

In this experiment, the four litres of sludge in batch reactor B3 was used without dilution. This sludge was aerated to achieve complete mixing for a period of $>$ 24 hours without substrate addition to ensure endogenous conditions. 600ml aliquots of the starved sludge were taken for each test run.

Samples for ATP and substrate analysis were taken as described earlier and redox potential and dissolved oxygen monitored as before. Phenol determinations on the filtrates were made, using the method described earlier.Four test runs were carried out, a different volume of stock phenol solution (60,000mg/l) being added in each run.

At the end of the experiment all of the sludge was returned to reactor B3 and this was maintained in a similar manner to that

TABLE 8.1. SUMMARY OF THE PARAMETERS MONITORED IN EXPERIMENTS G, P AND R.

EXPT	ADDITIONS STOCK PHENOL	INITIALLY STARVED?	T°C	pH	S.S	PHENOL	CN	NH_3	ATP	REDOX	S	CNS	D.O
										ELECTRODES			
	mls												
G	0.5-5.0	YES	-	-	-	+	-	-	+	+	-	-	+
P	0.1-0.9	YES	+	+	+	+	-	-	+	+	-	-	-
R	1.0-2.2	YES	+	+	+	+	-	-	+	+	-	-	-

described for reactors B1 and B2 earlier.

The results obtained from the experiment were recorded in Table 8.2 and plotted in Figures 8.1 to 8.4.

8.1.2 Experiment P

These experiments were conducted in a similar manner to that indicated for experiment G. Reactor B1 was used as the source of sludge for the experiment. In this experiment, however, the four litres of sludge in reactor B3 were put into a large plastic container and diluted with 5 litres of treated coke oven liquor. This reservoir of sludge was well aerated to ensure an adequate supply of oxygen to the constituent micro-organisms and to ensure complete mixing. Aeration was carried out for a number of hours before the start of the experiment to ensure endogenous conditions. 800ml aliquots of the sludge were used for each of the test runs. The pH and temperature of each aliquot was measured as previously described, immediately prior to its use in each test run.

Samples for ATP, phenol and suspended solids analysis were taken as described earlier, and the redox potential monitored as in Experiment G.

Eight test runs were conducted, a different volume of stock phenol solution being added to each of seven of the runs. In the last test run, approximately the same volume of stock solution was added as in the first to produce a control run.

At the end of the experiment the aliquots of sludge used in the test runs were combined with that remaining in the reservoir and the sludge allowed to settle. The supernatant was decanted and the settled sludge returned to reactor B3. Treated coke oven liquor was added to nearly fill the reactor, and the sludge then maintained in a similar manner to that employed for reactors B1 and B2, the reactor being fed with stock phenol solution.

The results obtained from the experiment were recorded in Tables 8.3 and plotted in Figures 8.5 to 8.12.

8.1.3 Experiment R

This experiment was conducted approximately 24 hours after Experiment P using the contents of the reactor B3. The parameters monitored in each of the test runs and the general conduct of the experiment were exactly as described for the previous experiment.

Only six test runs were conducted in this experiment, however, with larger additions of stock phenol solution being made in each test run than in Experiment P. A control run was not carried out.

At the end of the experiment, the sludge used in the test runs was combined with that remaining in the reservoir, and treated as described at the end of the previous experiment.

The results obtained from the experiment were recorded in Table 8.4 and plotted in Figures 8.13 to 8.18.

8.2 Results

The results from experiments G, P and R were recorded in Tables 8.2, 8.3, and 8.4 respectively, and in Figures 8.1 to 8.4, 8.5 to 8.12, and 8.13 to 8.18 respectively.

In nearly all of the test runs performed the sludge showed a rise in ATP level following the addition of phenol followed by a decline in the ATP level.

The mean ATP level following phenol addition was calculated for each test run (Table 8.5), and this was used to calculate the mean ATP value/mg of dried sludge following substrate addition (Table 8.6) using equation 6.1. A graph was plotted relating the mean ATP concentration/mg dried sludge to the phenol concentration following substrate addition in Figure 8.19. A similar graph was plotted in Figure 8.20 relating the maximum ATP concentration found/mg dried sludge to phenol concentration following substrate addition.

Both of these graphs are very similar in appearance, and a graph relating the maximum ATP concentration following phenol addition

TABLE 8.2 RESULTS OF EXPERIMENT G.

TEST G1.

TIME HRS-MINS	REDOX mV	D.O mg/ml	ATP X10^9 fg/ml	PHENOL mg/ml
0-00	-	-	-	-
0-01	-1.8	9.8-10.0	1.124	-
0-03	-	-	-	5.0
0-05	-4.5	9.8-9.9	1.950	-
0-06	-	-	1.071	-
0-09	ca. 0.5ml PHENOL ADDED			
0-12	-8.2	9.6-9.7	1.166	-
0-13	-9.2	9.6	-	36.5
0-14	-10.2	-	1.285	-
0-16	-11.2	9.6	1.684	-
0-18	-12.0	9.5	1.610	-
0-20	-12.6	9.5	1.264	-

TEST G2.

TIME HRS-MINS	REDOX mV	D.O mg/ml	ATP X10^9 fg/ml	PHENOL mg/ml
0-29	-	-	-	-
0-33	-	-	-	9.5
0-34	-8.6	9.6	1.589	-
0-38	-9.0	-	9.975	-
0-46	ca. 1 ml PHENOL ADDED			
0-48	-11.4	9.4-9.5	·8 40	-
0-49	-12.7	9.4-9.5	-	70.0
0-51	-13.2	9.3-9.4	1.057	-
0-53	-	-	1.365	-
0-57	-	-	1.439	-
1-01	-15.9	9.2-9.3	2.408	-

TABLE 8.2 CONTINUED.

TEST G3.

TIME HRS-MINS	REDOX mV	D.O mg/ml	ATP $X10^8$fg/ml	PHENOL mg/ml
1-14	-9.5	9.8	$1.579x10^8$	-
1-15	-9.9	-	$1.435x10^8$	-
1-16	2ml PHENOL ADDED			
1-18	-	-	$9.415x10^7$	-
1-20	-13.7	9.7	-	185
1-22	-	-	$1.442x10^8$	-
1-24	-	-	1.099	-
1-29	-	-	1.043	-
1-46	-16.5	9.6	-	-
TEST G4.				
1-56	-5.1	10.1	9.345	-
2-02	ca. 5ml PHENOL ADDED			
2-04	-14.2	-	1.285	-
2-07	-16.4	10.0	-	865
2-08	-16.6	-	1.348	-
2-10	-16.8	10.0	1.34	-
2-14	-17.2	9.9-10.0	1.036	-

pH THROUGHOUT EXPT = 7.6.

STOCK PHENOL = 60,000mg/l PHENOL SOLUTION.

TABLE 8.3 SUMMARY OF RESULTS FOR EXPERIMENT P. TEST P1.

TIME HRS-MINS	PHENOL	ATP $X10^8$ fg/l	REDOX mV
0-00	-	-	0.00
0-01	3.0-3.5	-	0
0-02	-	1.61	0
0-03	-	1.8375	0
0-15	-	1.5785	-8
0-17	ca. 0.1 ml STOCK PHENOL SOLUTION ADDED		
0.18	-	1.855	-
0-19	-	-	-11
0-20	-	-	-12
0-24	-	1.8375	-13
0-26	-	1.7955	-
0-28	-	1.631	-14
0-33	3.5	-	-16
0-34	-	2.0685	-17
0-36	-	-	-17
0-37	-	2.037	-18
0-51	-	-	-21
0-52	-	1.575	-22
			TEST P2.
1-00	-	-	-28
1-06	-	1.8795	-26
1-10	3.5	-	-27
1-11	-	1.82	-
1-17	-	1.827	-28
1-18	ca. 0.2 ml STOCK PHENOL SOLUTION ADDED		
1-19	-	2.0405	-29
1-23	-	2.128	-31

TABLE 8.3 CONTINUED.

TIME HRS-MINS	PHENOL	ATP X10^8 fg/l	REDOX mV
1-28	-	2.3205	-30
1-29	4.5	-	-
1-35	-	1.5715	-30
1-38	-	-	-31
1-42	-	-	-31
1-43	-	1.6765	-31
1-46	-	-	-31
			TEST P3.
1-52	-	-	-
1-53	-	1.61	-39
1-55	-	-	-35
2-06	-	1.9355	-32
2-09	4.0	-	-31
2-11	-	1.9285	-31
2-12	ca. 0.3 mls STOCK PHENOL SOLUTION ADDED		
2-13	-	2.1945	-32
2-14	-	-	-33
2-15	-	2.3205	-33
2-17	-	-	-33
2-18	-	2.4535	-32
2-20	-	-	-32
2-23	-	-	-31
2-24	-	2.3275	-30
2-29	-	2.366	-28
2-31	4.0	-	-

TABLE 8.3 CONTINUED.

TIME HRS-MINS	PHENOL	ATP X10^8 fg/l	REDOX mV
2-32	-	2.334	-
2-34	-	-	-27
2-36	-	-	-27
			TEST P4.
2-55	3.5-4.5	-	-32
2-56	-	1.8795	-
3-01	-	1.694	-32
3-04	-	-	-32
3-05	-	1.596	-32
3-07	ca. 0.4 mls STOCK PHENOL SOLUTION		
3-09	-	1.729	-34
3-11	-	1.5645	-34
3.16	-	1.7675	-33
3-20	5.5	-	-33
3-21	-	1.6135	-33
3-24	-	-	-33
3-26	-	1.4875	-
3-32	-	1.435	-33
			TEST P5.
3-48	-	-	-38
3-53	3.5	-	-33
3-54	-	1.26	-33
3-55	-	1.9005	-33
3-57	-	-	-32
3-58	-	1.8655	-32
3-59	ca. 0.5 mls STOCK PHENOL SOLUTION ADDED		

TABLE 8.3 CONTINUED.

TIME HRS-MINS	PHENOL	ATP X10^8 fg/l	REDOX mV
4-00	-	2.128	-32
4-06	22.0	-	-32
4-07	-	2.2715	-32
4-12	-	2.4045	-31
4-13	-	-	-31
4-15	-	-	-31
4-16	-	2.464	-31
4-22	-	2.3065	-31

TEST P6.

TIME HRS-MINS	PHENOL	ATP X10^8 fg/l	REDOX mV
4-38	3.0	-	-31
4-39	-	1.9845	-30
4-42	-	1.645	-30
4-45	-	1.778	-30
4-46	ca. 0.7 mls STOCK PHENOL SOLUTION ADDED		
4-47	-	2.6705	-30
4-48	-	-	-31
4-57	30	-	-30
4-58	-	1.911	-30
5-01	-	2.597	-29
5-06	-	2.576	-29
5-10	-	2.576	-29
5-16	-	3.059	-30

TEST P7.

RESEVOIR SLUDGE TEMP. 84 F. TEST REACTOR COOLED TO 74 F BEFORE STARTING TEST 7.

TIME HRS-MINS	PHENOL	ATP X10^8 fg/l	REDOX mV
6-13	4.0	-	-31
6-15	-	2.7055	-22

TABLE 8.3 CONTINUED.

TIME HRS-MINS	PHENOL	ATP $X10^8$ fg/l	REDOX mV
6-16	-	2.7755	-20
6-19	-	2.8805	-20
6-20	ca. 0.9 mls STOCK PHENOL SOLUTION ADDED		
6-21	-	3.031	-20
6-26	-	3.2445	-21
6-30	-	3.3705	-20
6-32	40.5	-	-20
6-35	-	3.269	-20
6-38	-	-	-20
6-40	-	2.7545	-20
			TEST P8.
6-54	-	-	-
7-02	-	-	-25
7-03	-	-	-25
7-08	4.0	-	-24
7-09	-	2.5235	-24
7-10	-	-	-24
7.11	-	2.1875	-
7-15	-	2.3695	-
7-17	-	-	-23
7-18	ca. 0.1 ml STOCK PHENOL SOLUTION ADDED		
7-19	-	-	-23
7-22	-	-	-24
7-23	-	2.464	-24
7-27	3.0	-	-
7-28	-	2.4045	-24
7-33	-	2.0755	-24
7-38	-	-	-24

TABLE 8.4 SUMMARY OF RESULTS FOR EXPERIMENT R.

TEST R1.

TIME HRS-MINS	PHENOL	ATP X10^8 fg/l	REDOX mV
0-00	-	-	-8
0-02	-	2.8175	-6
0-03	5.0	-	-
0-04	-	3.3565	-6
0-09	-	3.0625	-7
0-11	1.0 ml STOCK PHENOL SOLUTION ADDED		
0-12	-	3.311	-9
0-15	-	4.34	-10
0-17	40.0	-	-10
0-21	-	3.2165	-10
0-22	-	-	-10
0-26	-	3.346	-11
0-28	-	-	-11
0-31	-	3.297	-12
0-35	-	3.311	-14

TEST R2.

TIME HRS-MINS	PHENOL	ATP X10^8 fg/l	REDOX mV
0-44	-	-	-23
1-11	-	-	-23
1-12	-	3.3915	-23
1-15	3.0	-	-24
1-16	-	3.255	-24
1-20	-	3.1605	-24
1-22	1.2 mls STOCK PHENOL SOLUTION ADDED		
1-25	-	2.835	-
1-26	-	-	-25
1-29	-	3.01	-

TABLE 8.4 SUMMARY OF RESULTS FOR EXPERIMENT R.

TIME HRS-MINS	PHENOL	ATP $X10^8$ fg/l	REDOX mV
1-32	-	-	-24
1-33	51.0	-	-24
1-34	-	3.1465	-24
1-35	-	-	-24
1-36	-	-	-24
1-37	-	2.94	-24
1-39	-	-	-24
1-41	-	-	-24
1-42	-	2.765	-24
1-44	-	-	-25
1-48	-	2.66	-25
TEST R3.			
2-02	-	-	-29
2-03	-	3.276	-29
2-11	-	2.8035	-27
2-13	2.0	-	-27
2-14	-	2.9155	-27
2-15	1.4 mls STOCK PHENOL SOLUTION ADDED		
2-16	-	2.695	-28
2-18	-	3.136	-28
2-22	-	2.555	-27
2-26	-	-	-26
2-27	44.0	-	-26
2-29	-	2.765	-26
2-32	-	3.15	-26
2-35	-	2.877	-26
2-40	-	-	-26

TABLE 8.4 CONTINUED.

	TIME HRS-MINS	PHENOL	ATP $\times 10^8$ fg/l	REDOX mV
	2-41	-	3.416	-
	2-45	-	3.4125	-27
TEST R4.				
	3-05	-	-	-28
	3-06	4.0	-	-
	3-07	-	2.8665	-28
	3-10	-	2.856	-28
	3-13	-	2.814	-28
	3-14	1.6 mls STOCK PHENOL SOLUTION ADDED		
	3-15	-	2.695	-29
	3-16	-	-	-29
	3-19	-	3.22	-29
	3-24	97.0	-	-28
	3-25	-	3.0765	-28
	3-26	-	-	-28
	3-27	-	3.08	-27
	3-31	-	2.7895	-27
	3-37	-	3.01	-27
	3-41	-	-	-27
	3-43	-	-	-27
	3-45	-	-	-28
	3-46	-	3.15	-28
TEST R5.				
	3-54	-	3.416	-
	3-56	6.5	-	-32
	3-57	-	3.388	-31
	4-00	-	3.115	-31

TABLE 8. CONTINUED.

TIME HRS-MINS	PHENOL	ATP X10^{8}fg/l	REDOX mV
4-01	1.9 mls STOCK PHENOL SOLUTION ADDED		
4-02	-	3.605	-31
4-05	-	3.535	-31
4-12	126.0	-	-28
4-13	-	3.71	-28
4-15	-	-	-28
4-19	-	2.94	-28
4-22	-	3.85	-28
4-39	-	2.6845	-29
4-43	3.5	-	-28
4-44	-	2.702	-28
4-50	-	2.6145	-28
4-51	2.2 mls STOCK PHENOL SOLUTION		
4-52	-	3.339	-28
4-57	-	3.0555	-27
4-59	108.0	-	-27
5-00	-	3.1395	-26
5-05	-	2.051	-26
5-09	-	-	-26
5-10	-	2.366	-
5-15	-	3.0345	-26

pH = 7.4 Temperature = 76°F

FIGURE 8.1 RESULTS OF EXPERIMENT G. TEST G1

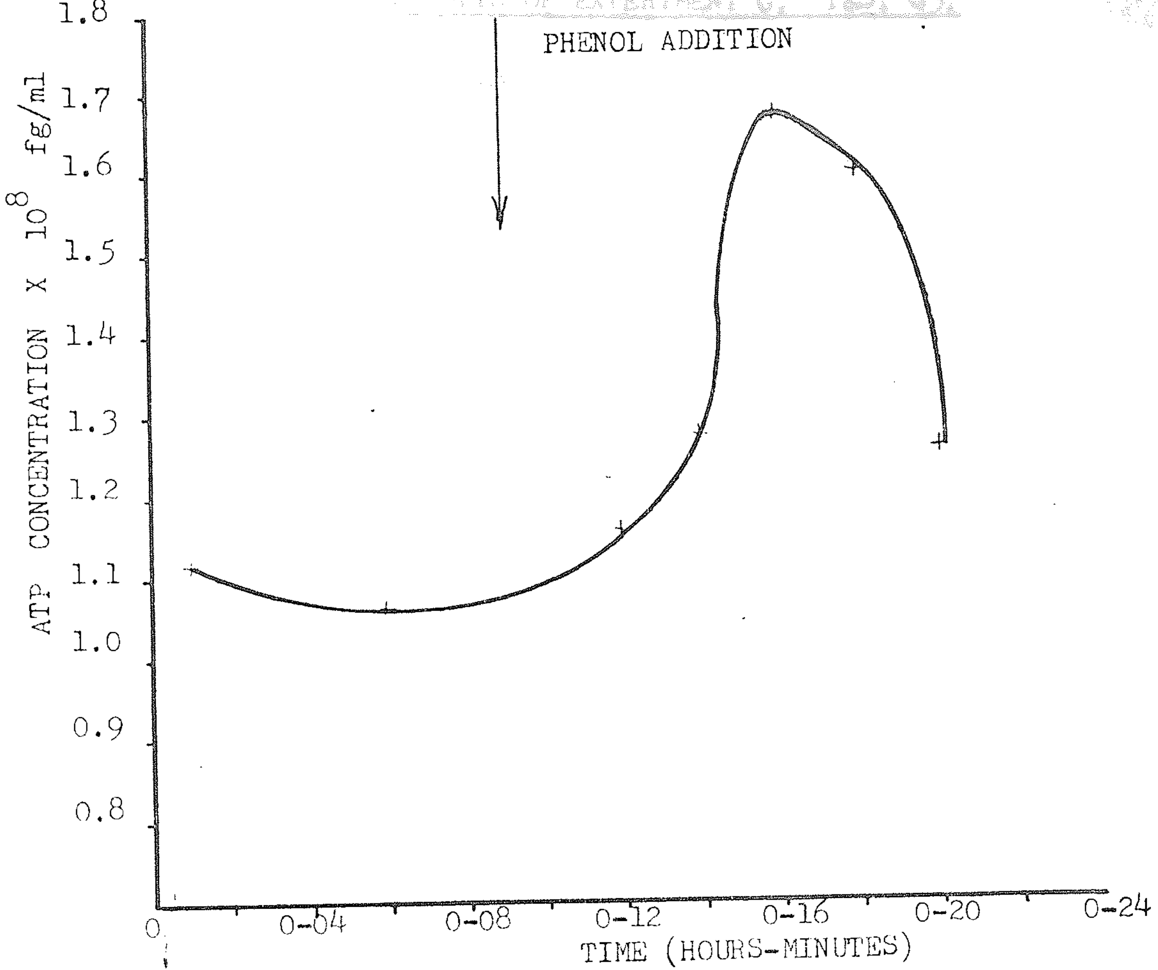


FIGURE 8.2 RESULTS OF EXPERIMENT G. TEST G2

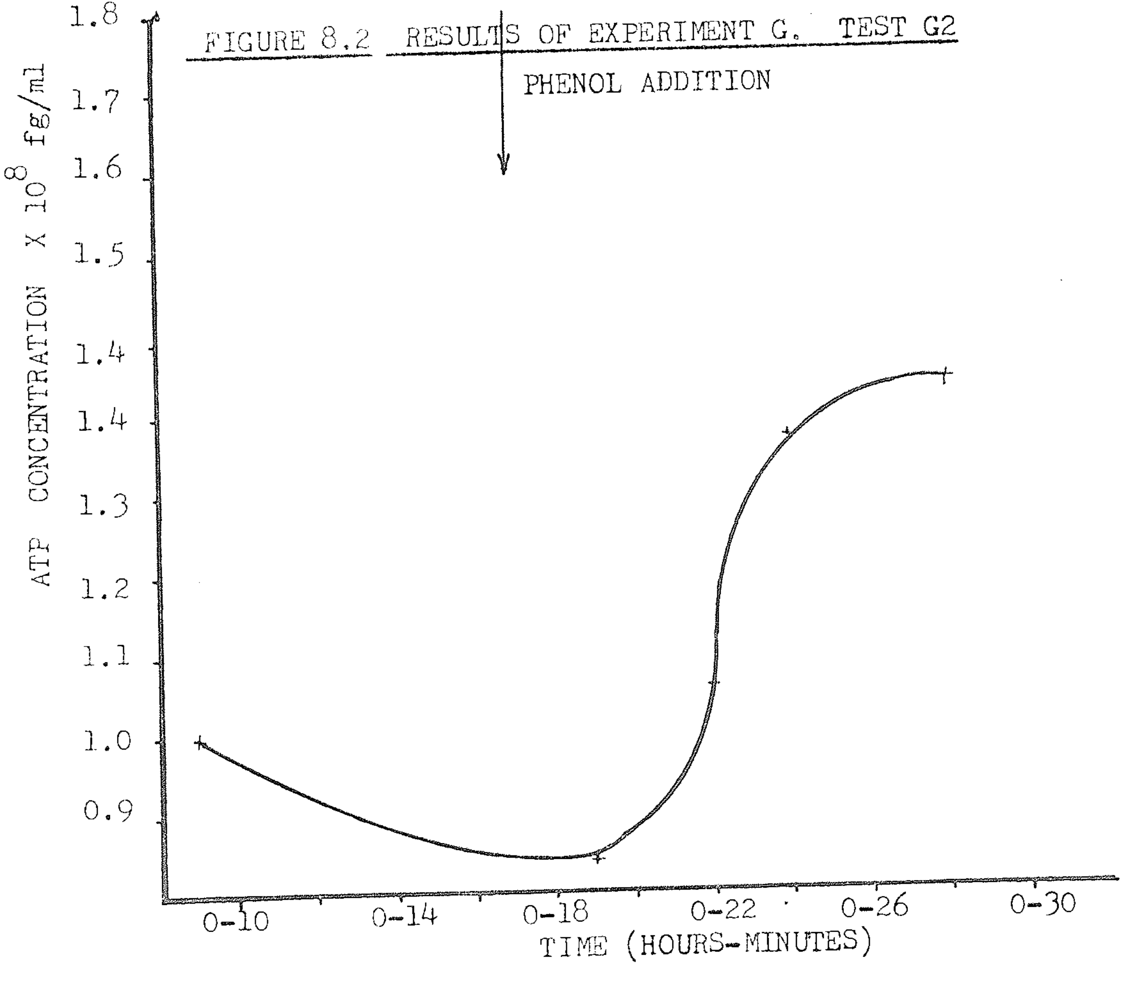


FIGURE 8.3 RESULTS OF EXPERIMENT G. TEST G3.

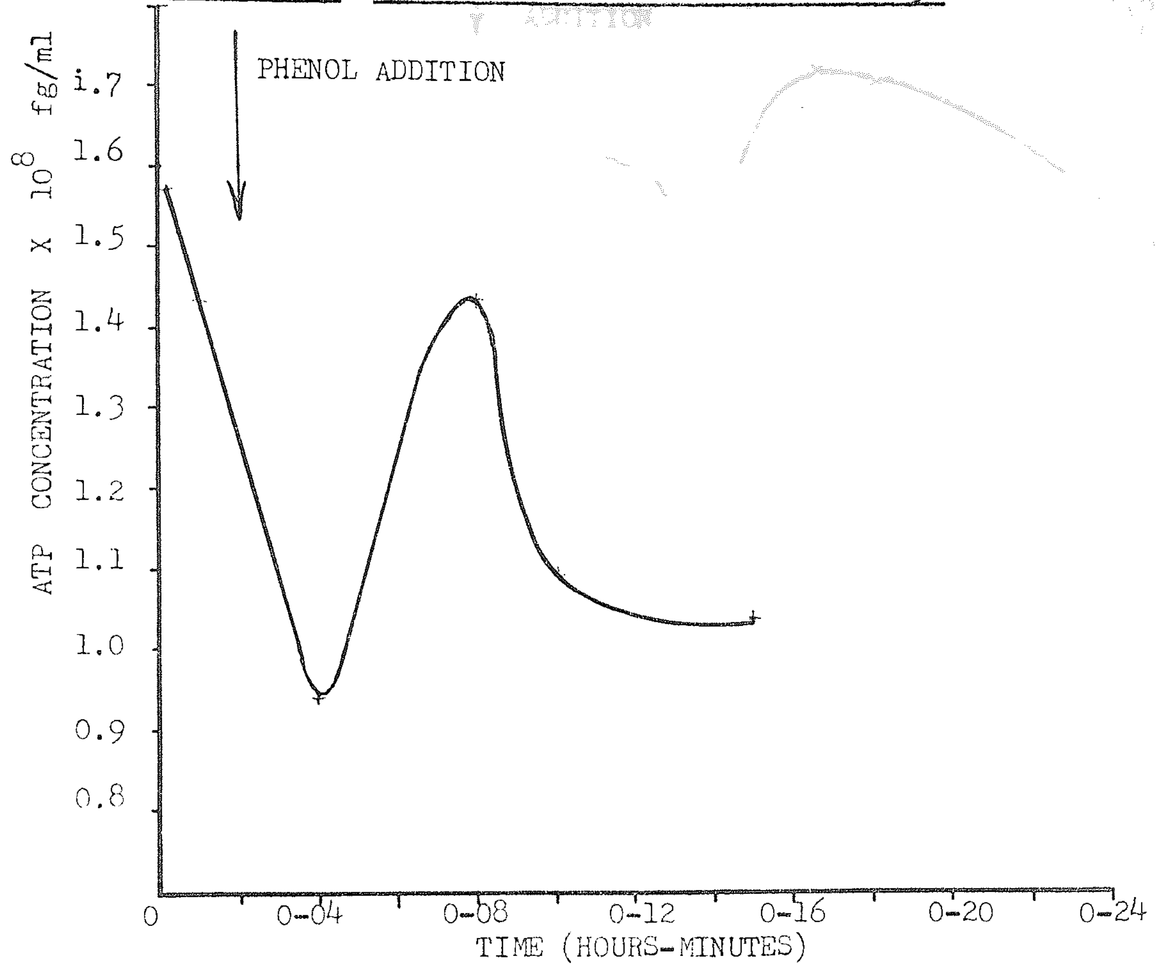


FIGURE 8.4 RESULTS OF EXPERIMENT G. TEST G4.

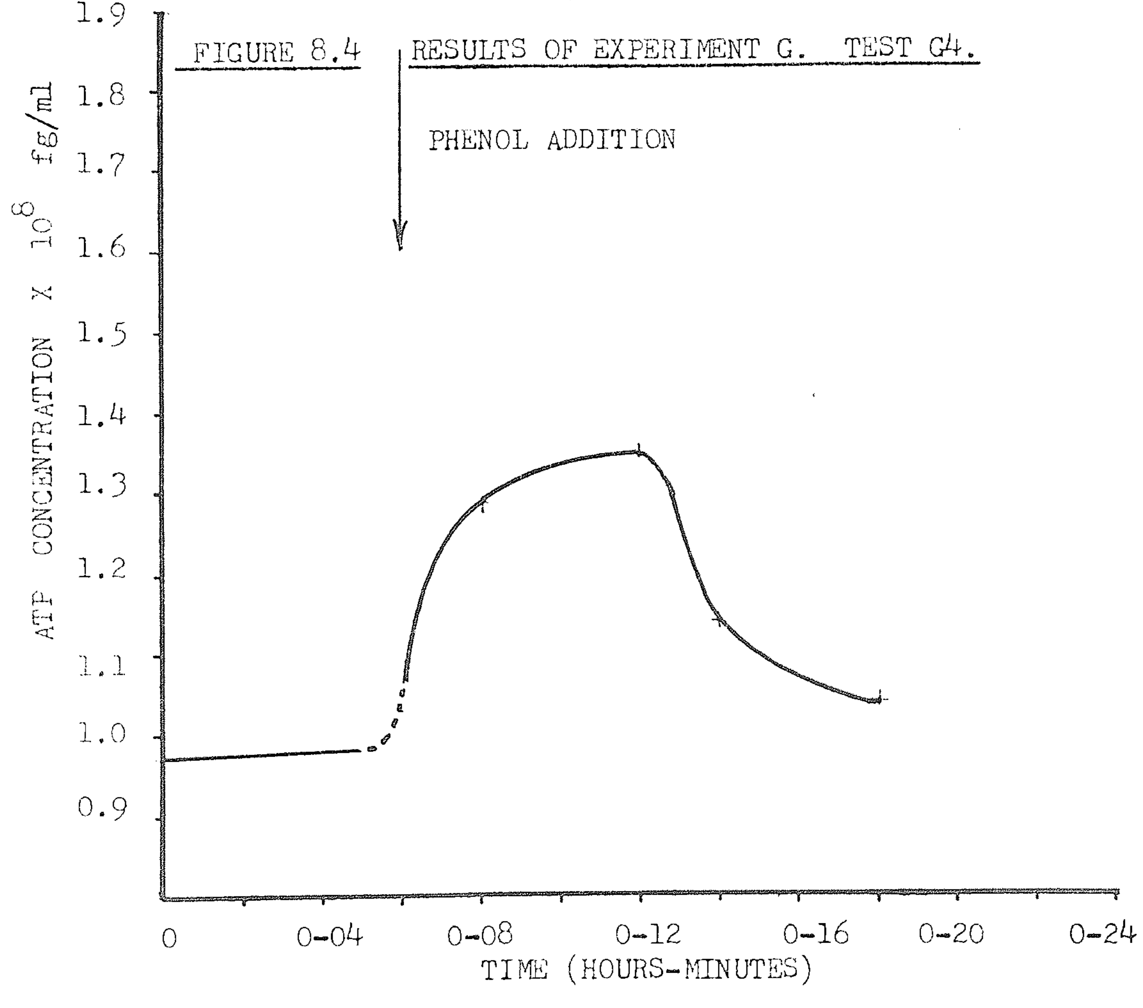


FIGURE 8.5 RESULTS OF EXPERIMENT P. TEST P1.

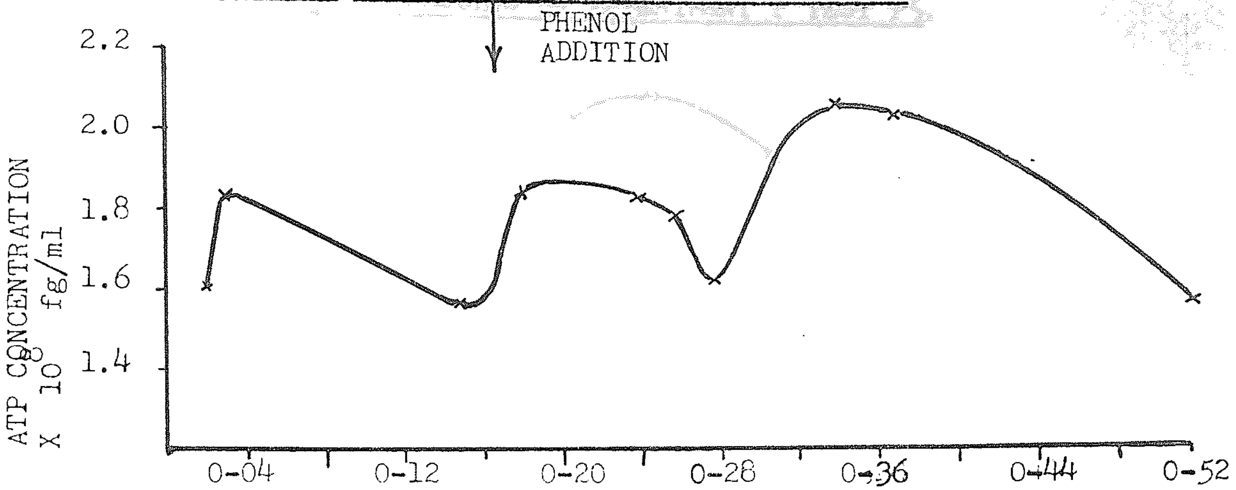


FIGURE 8.6 RESULTS OF EXPERIMENT P .TEST P2.

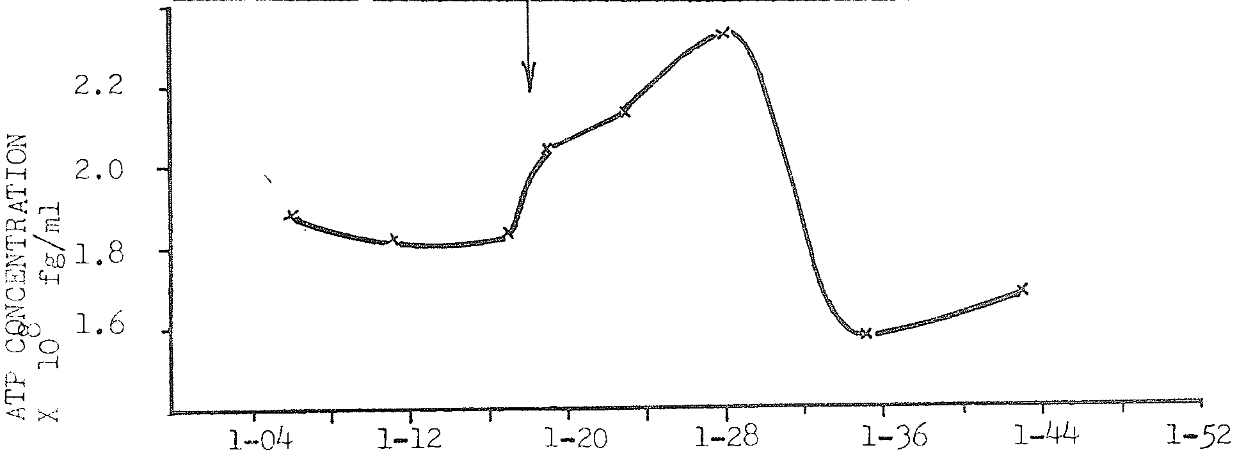


FIGURE 8.7 RESULTS OF EXPERIMENT P. TEST P3.

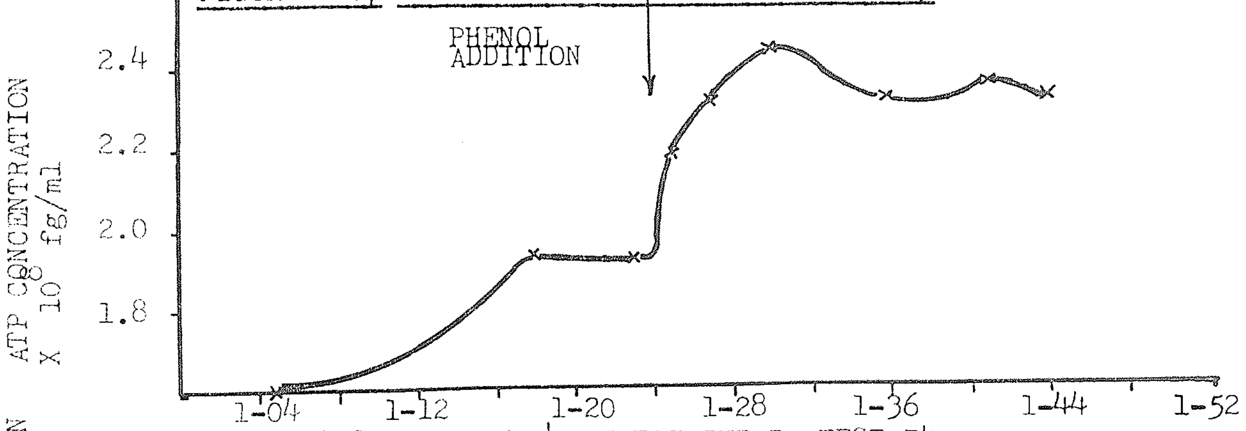


FIGURE 8.8 RESULTS OF EXPERIMENT P. TEST P4.

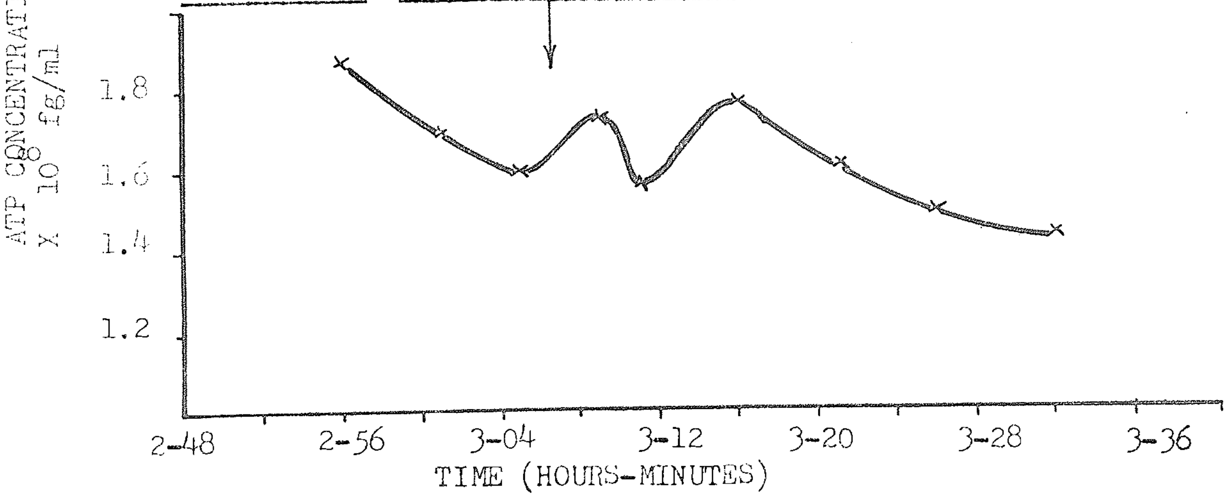


FIGURE 8.9 RESULTS OF EXPERIMENT P TEST P5.

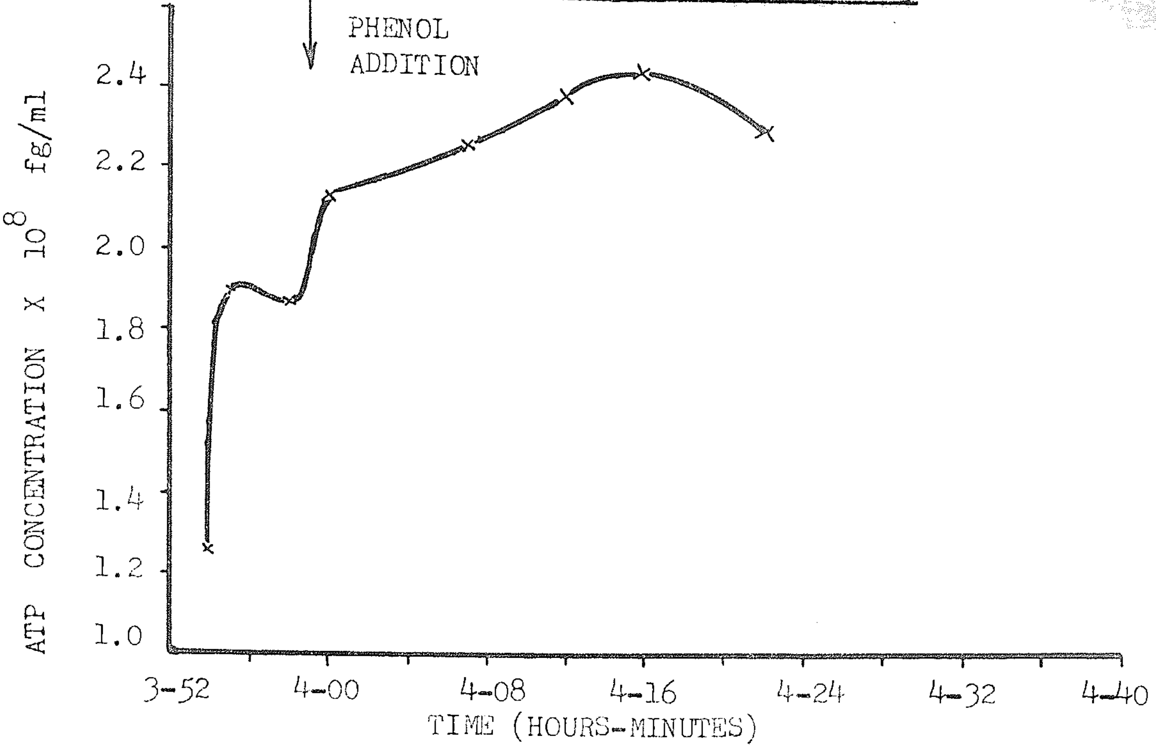


FIGURE 8.10 RESULTS OF EXPERIMENT P . TEST P6.

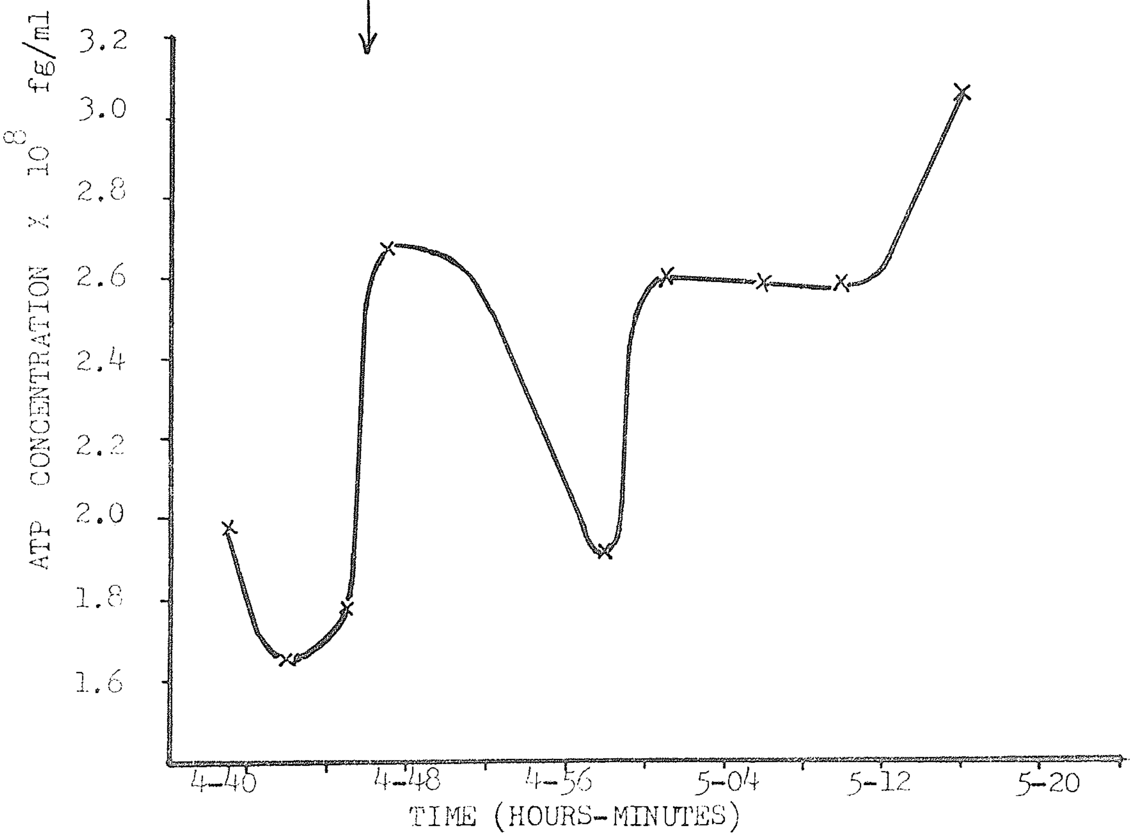


FIGURE 8.13 RESULTS OF EXPE[illegible]

TEST [illegible]

FIGURE 8.11 RESULTS OF EXPERIMENT P. TEST P7.

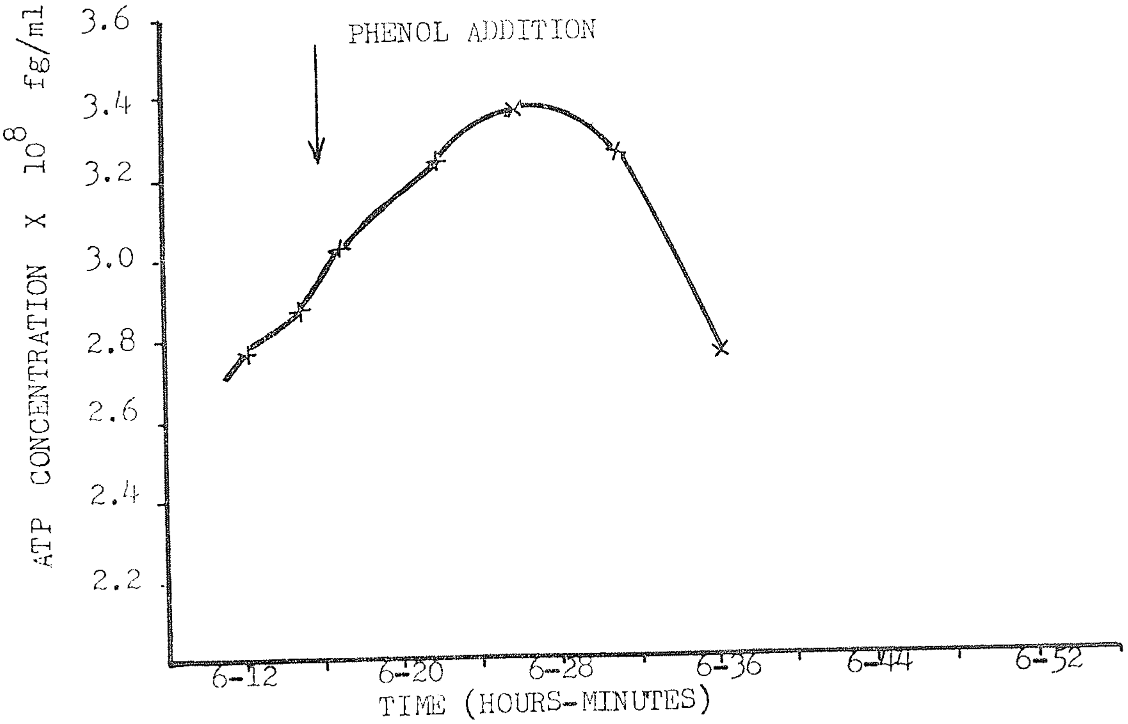
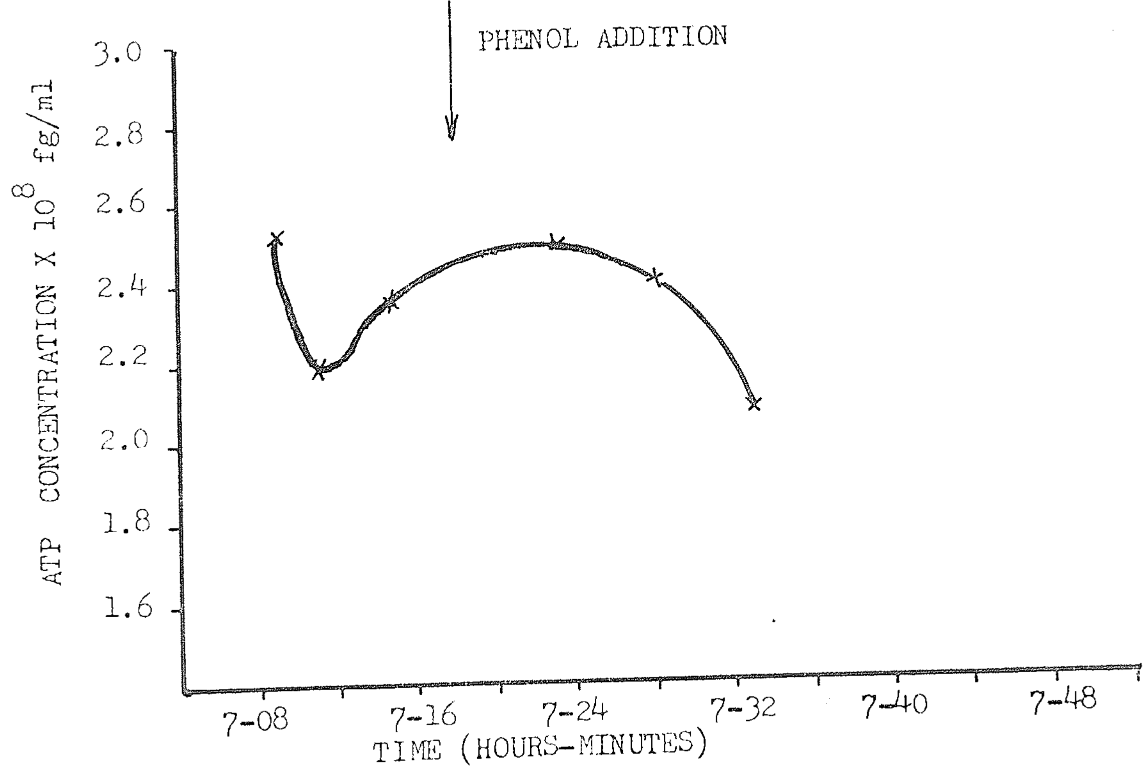


FIGURE 8.12 RESULTS OF EXPERIMENT P. TEST P8.



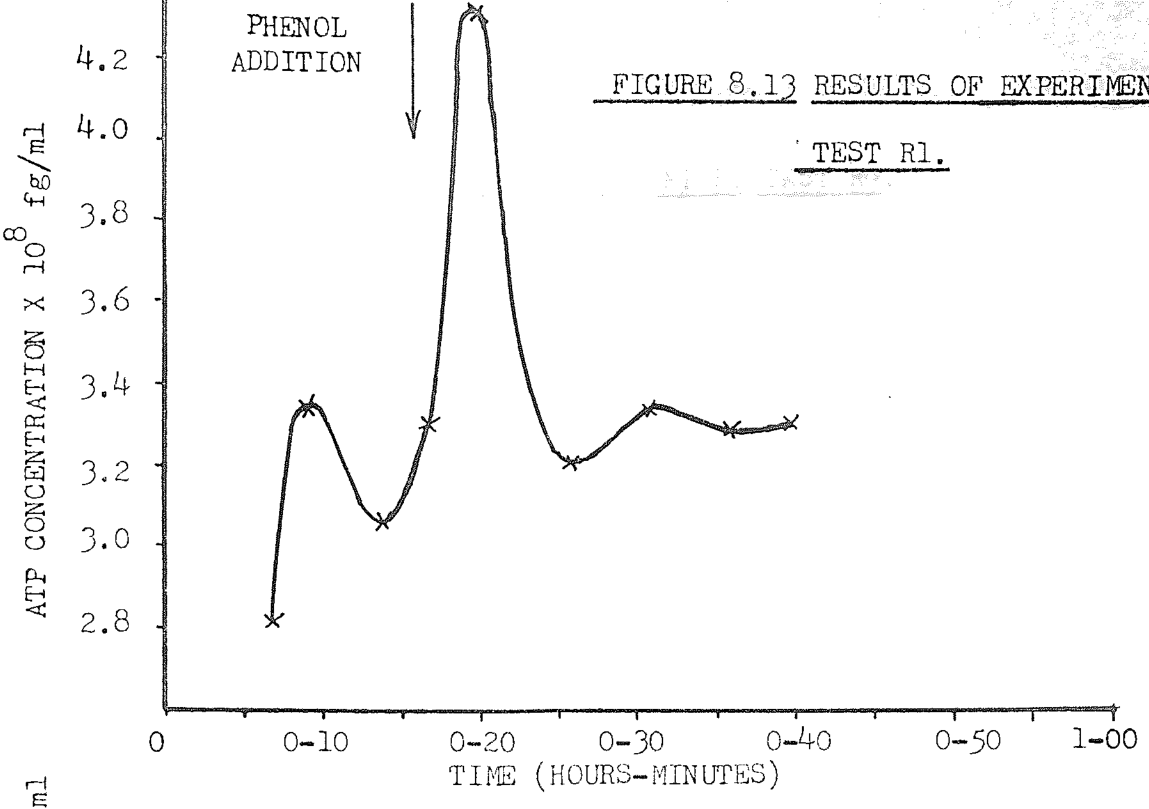


FIGURE 8.13 RESULTS OF EXPERIMENT R.

TEST R1.

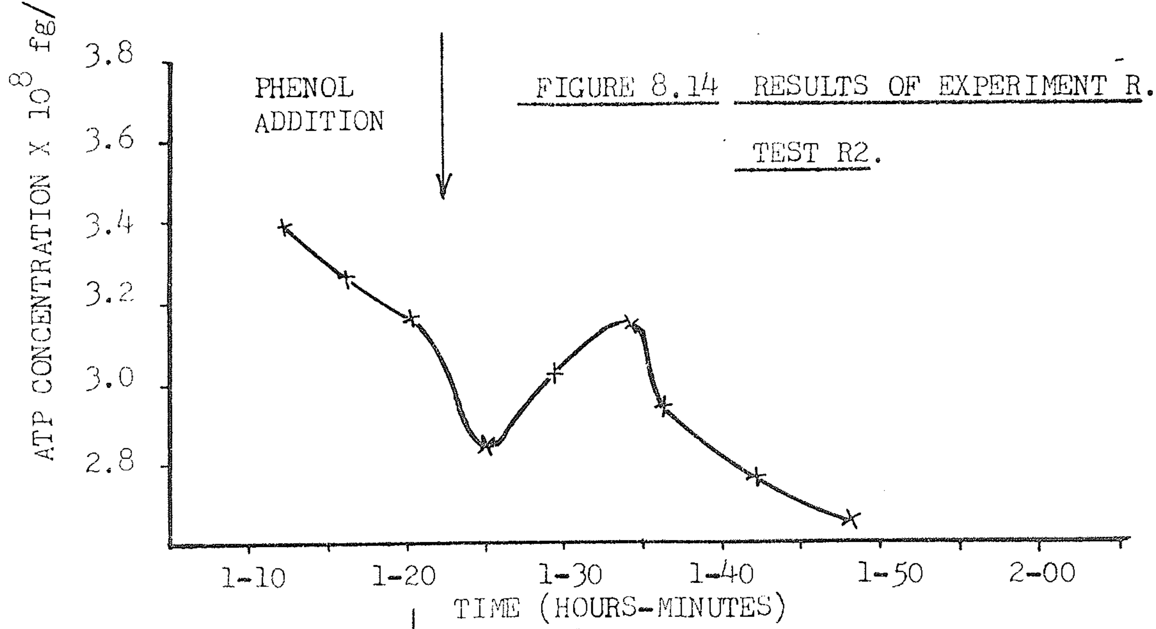


FIGURE 8.14 RESULTS OF EXPERIMENT R.

TEST R2.

FIGURE 8.15 RESULTS OF EXPERIMENT R.

TEST R3.

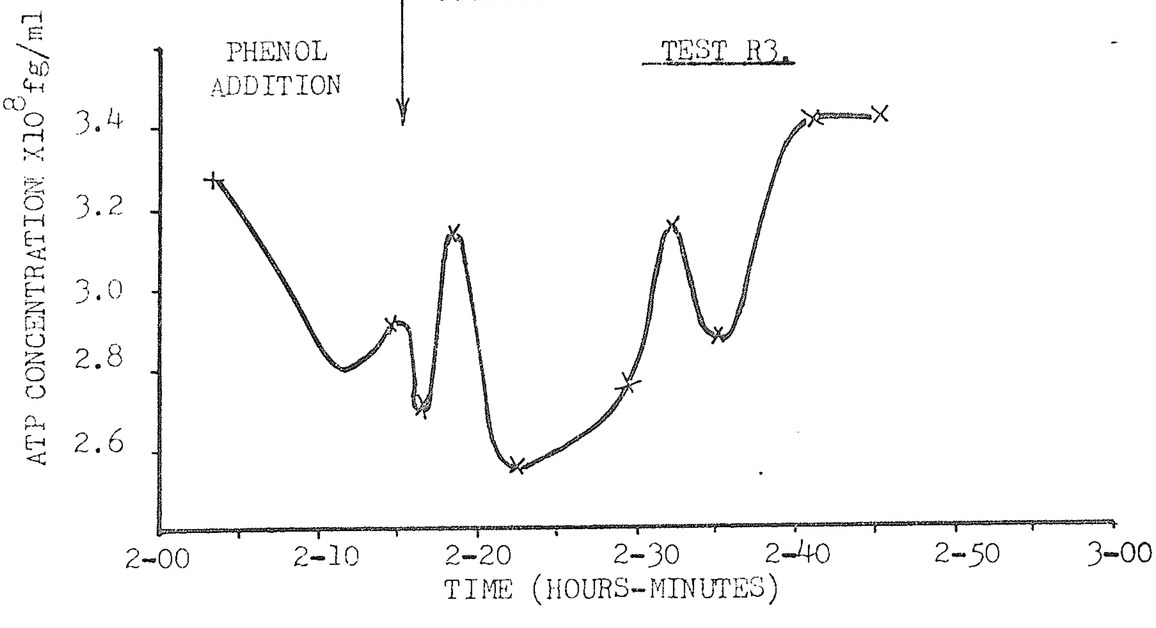


FIGURE 8.16 RESULTS OF EXPERIMENT R. TEST R4.

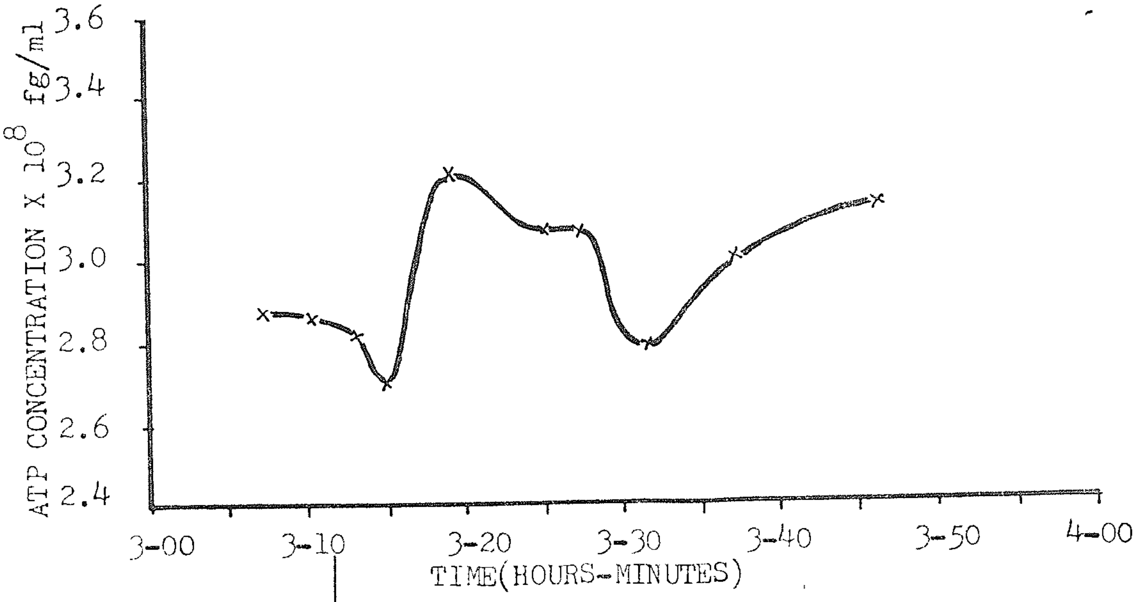


FIGURE 8.17 RESULTS OF EXPERIMENT R. TEST R5.

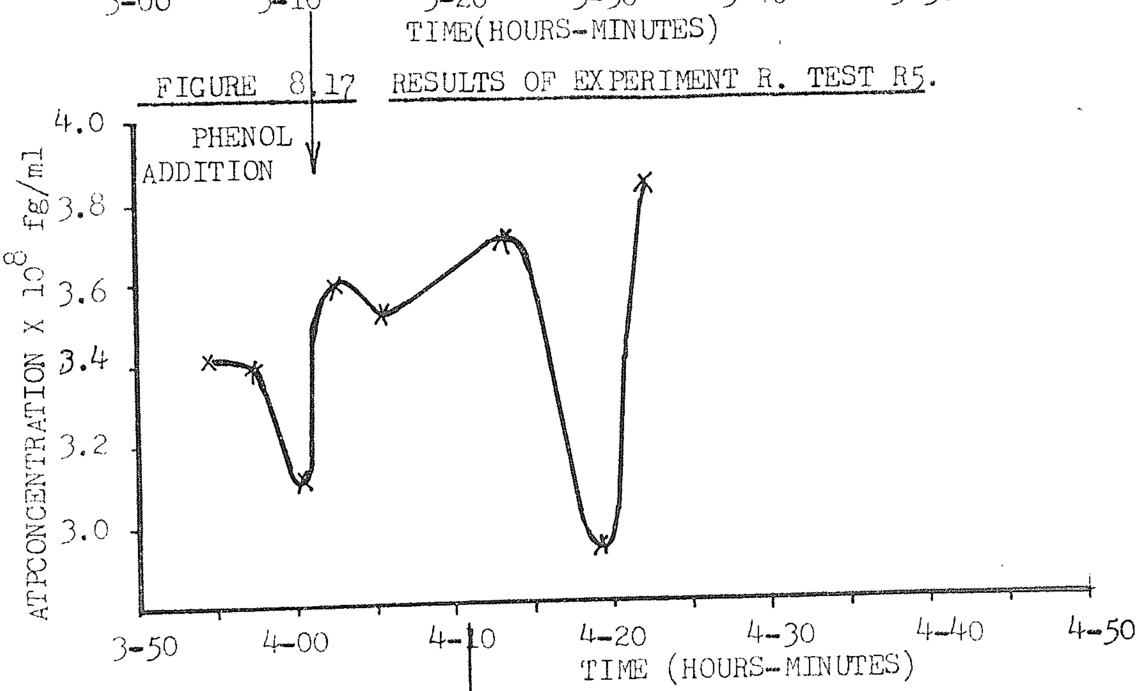
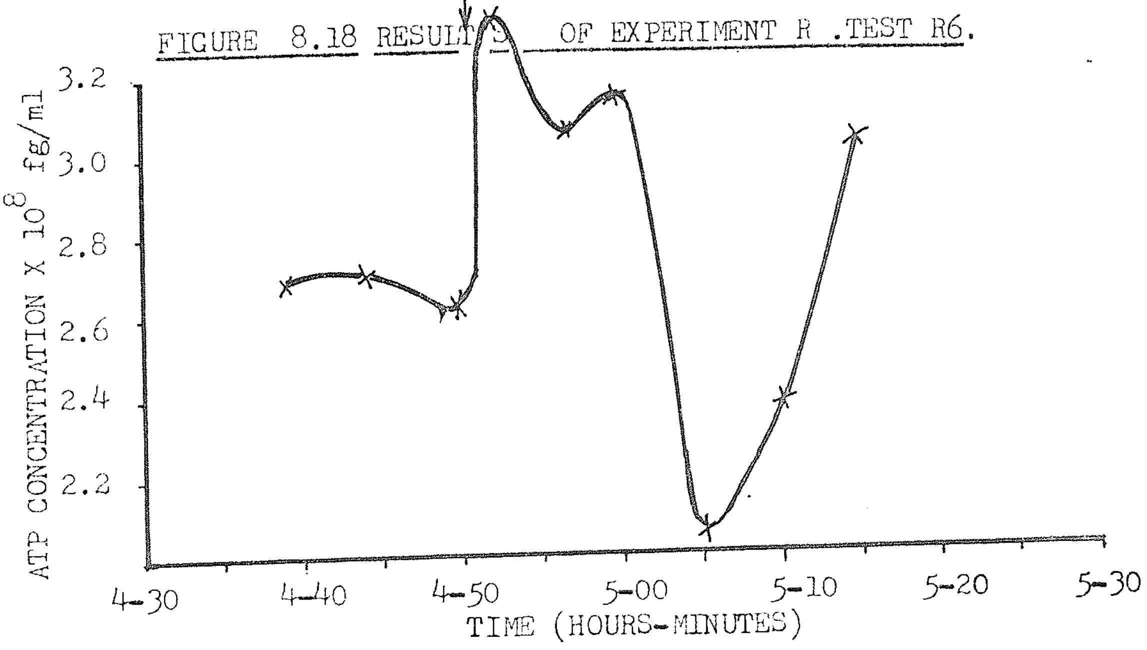


FIGURE 8.18 RESULTS OF EXPERIMENT R .TEST R6.



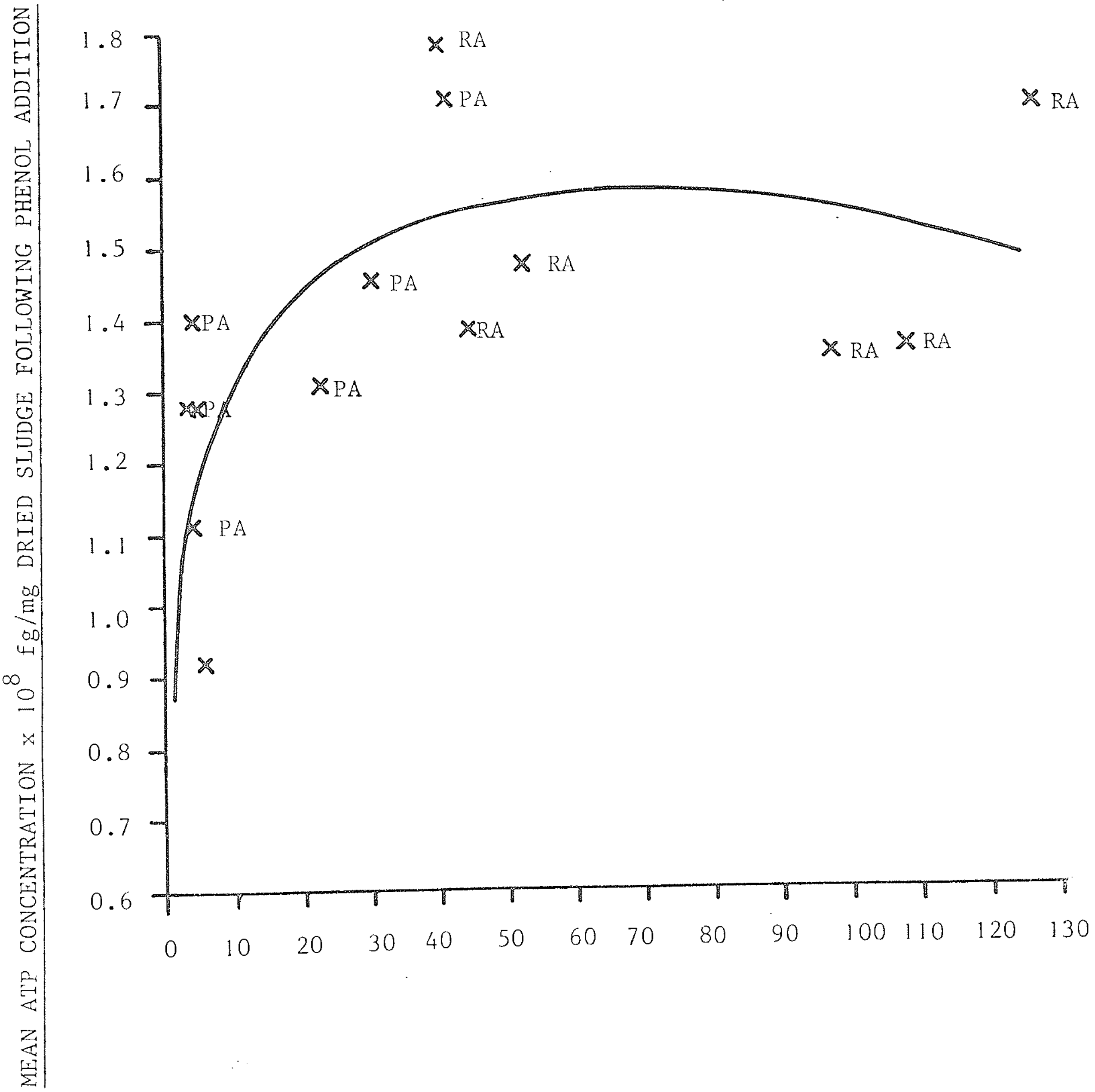
TEST RUN	[PHENOL]		mean [ATP]		Max [ATP] AFTER	SUSPENDED SOLIDS (SS)	
	BEFORE	AFTER	BEFORE (B)	AFTER (A)		BEFORE	AFTER
	mg/l		$\times 10^8$ fg/ml		$\times 10^8$ fg/ml	mg	
G1	5.0	36.5	1.3817	1.4018	1.684	-	-
G2	9.5	70.0	1.2933	1.4218	2.408	-	-
G3	-	185	1.507	1.1314	1.442	-	-
G4	-	865	0.973	1.2008	1.348	-	-
P1	3.0-3.5	3.5	1.6753	1.8285	2.0685	17.7	17.9
P2	3.5	4.5	1.8422	1.9474	2.3205	18.1	16.6
P3	4.0	4.0	1.8247	2.3327	2.4535	16.5	18.2
P4	3.5-4.5	5.5	1.7232	1.5995	1.7675	18.9	18.9
P5	3.5	22.0	1.6753	2.3149	2.464	-	19.3
P6	3.0	30.0	1.8025	2.5649	3.059	-	19.2
P7	4.0	40.5	2.7872	3.1339	3.3705	-	20.0
P8	4.0	3.0	2.3602	2.3147	2.464	-	19.7
R1	5.0	40.0	3.0788	3.4703	4.34	-	21.1
R2	3.0	51.0	3.269	2.8928	3.1465	-	21.3
R3	2.0	44.0	2.9983	3.0008	3.416	-	23.5
R4	4.0	97.0	2.8455	3.003	3.22	-	24.1
R5	6.5	126.0	3.3268	3.528	3.85	-	22.5
R6	3.5	108.0	2.667	2.8309	3.339	-	22.5

TABLE 8.5

TEST RUN	max [ATP] after/mg SS	mean [ATP] after/mg SS	$\frac{[ATP]\ (A) - (B)}{SS}$	$\frac{[ATP]\ (A) - (B)}{ATP\ (B)}$
	← $\times 10^8$ fg/mg		→	
G1	-	-	-	0.0145
G2	-	-	-	0.0994
G3	-	-	-	-0.2492
G4	-	-	-	0.2341
P1	1.2596	1.1134	0.0938	0.0914
P2	1.5237	1.2787	0.0661	0.0571
P3	1.4694	1.3971	0.3191	0.2784
P4	1.0194	0.9225	-0.0713	-0.0718
P5	1.3916	1.3074	0.3612	0.3818
P6	1.7366	1.4561	0.4328	0.4230
P7	1.8369	1.7080	0.1890	0.1244
P8	1.3633	1.2807	-0.0252	-0.0193
R1	2.2420	1.7927	0.2022	0.1272
R2	1.6102	1.4804	-0.1925	-0.1151
R3	1.5844	1.3919	0.0012	0.0008
R4	1.4563	1.3582	0.0712	0.0554
R5	1.8651	1.7091	0.0975	0.0605
R6	1.6176	1.3714	0.0794	0.0615

TABLE 8.6

FIGURE 8.19 THE RELATIONSHIP BETWEEN THE MEAN ATP CONCENTRATION PER mg DRIED SLUDGE AND PHENOL CONCENTRATION FOLLOWING PHENOL ADDITION



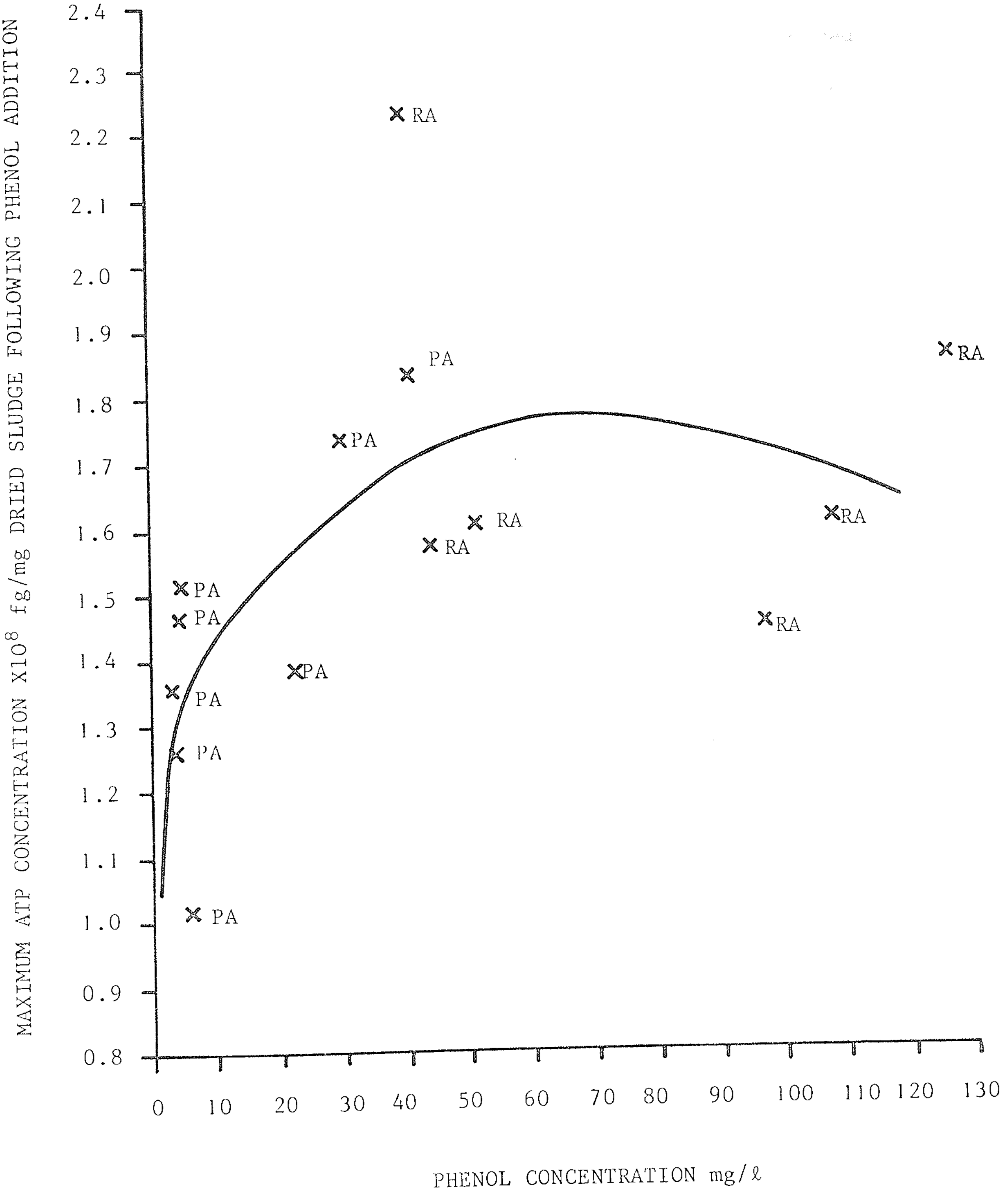


FIGURE 8.20 THE RELATIONSHIP BETWEEN THE MAXIMUM ATP CONCENTRATION PER mg DRIED SLUDGE AND PHENOL CONCENTRATION FOLLOWING PHENOL ADDITION.

to the corresponding mean value, shown in Figure 8.21, showed a linear relationship with a correlation coefficient of 0.96.

8.3 Discussion

The lines drawn through the data points in Figures 8.19 and 8.20 represented only a preliminary interpretation of the data, and these results were discussed in more detail later in the project. Some further limited examination of the data was however performed at this stage in the project.

Examination of the data in these two graphs indicated a scatter of the points about the lines drawn which it was considered may have been significant. There was a variation in the mean ATP level in the sludges and the suspended solids concentrations between test runs in the experiments, reflecting it was believed variations in the sludge concentrations used in the runs. To correct for these variations, and to give a better representation of the relationship between the ATP pool and substrate concentration, the change in the mean ATP concentration following substrate addition per mg of dried sludge was calculated in Table 8.6. This was done by subtracting the mean ATP value following phenol addition from the mean ATP value before addition for each test run and the change per mg dried sludge calculated in a similar manner to that described earlier. A graph was plotted relating the change in ATP concentration/mg dried sludge to phenol concentration in Figure 8.22.

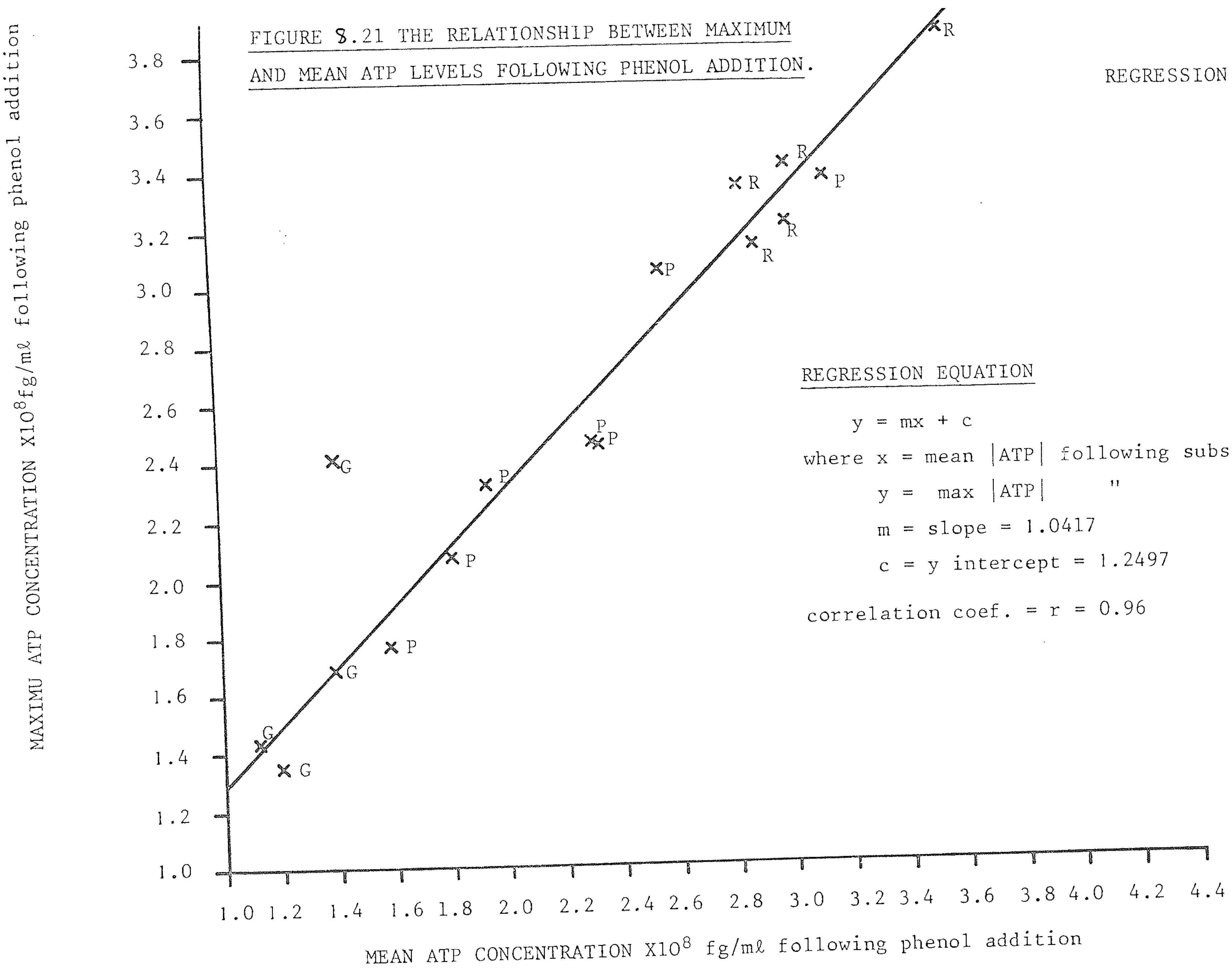


FIGURE 8.21 THE RELATIONSHIP BETWEEN MAXIMUM AND MEAN ATP LEVELS FOLLOWING PHENOL ADDITION.

REGRESSION EQUATION

$y = mx + c$

where x = mean $|ATP|$ following substrate addition

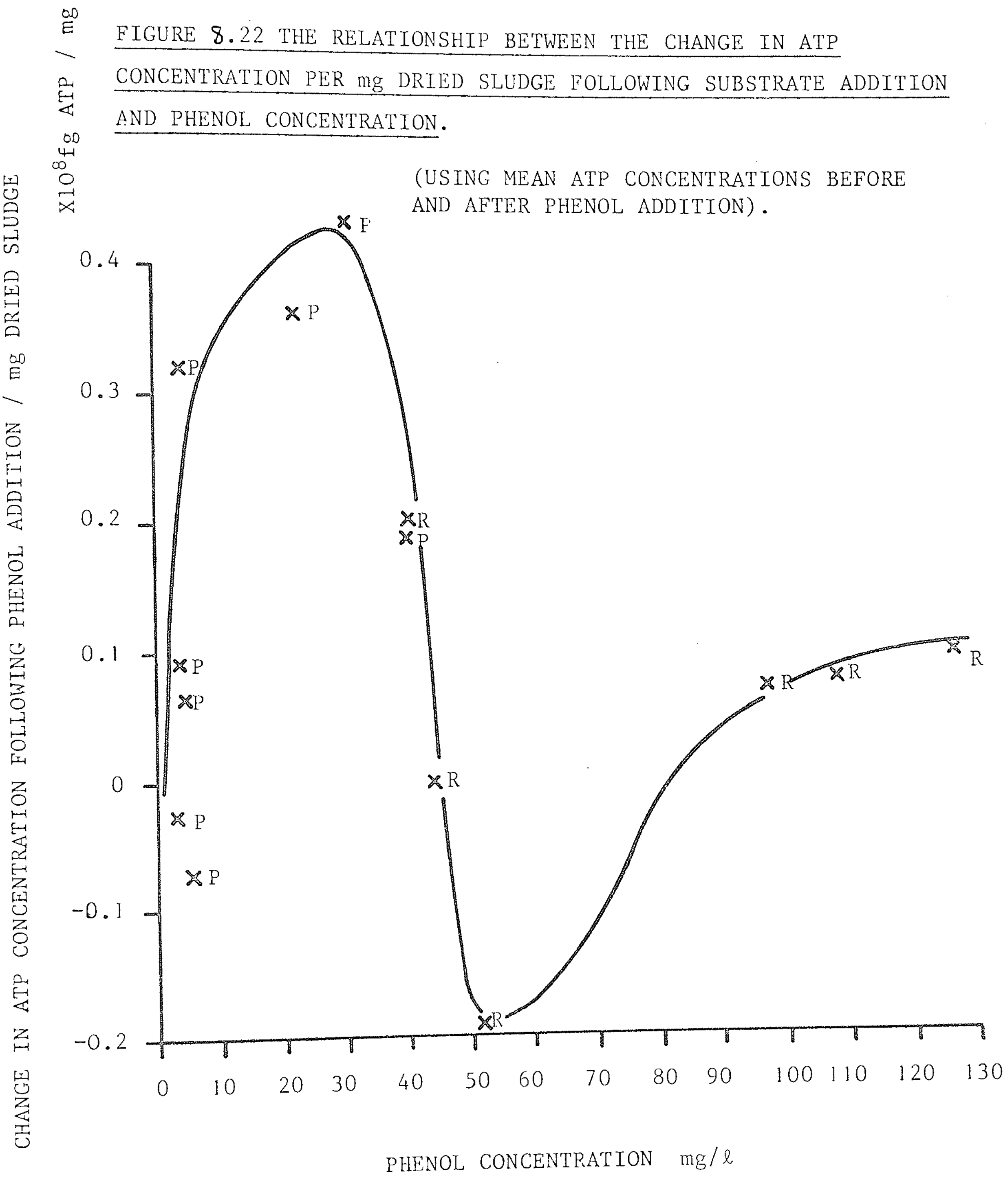
y = max $|ATP|$ " " "

m = slope = 1.0417

c = y intercept = 1.2497

correlation coef. = r = 0.96

FIGURE 8.22 THE RELATIONSHIP BETWEEN THE CHANGE IN ATP CONCENTRATION PER mg DRIED SLUDGE FOLLOWING SUBSTRATE ADDITION AND PHENOL CONCENTRATION.



(USING MEAN ATP CONCENTRATIONS BEFORE AND AFTER PHENOL ADDITION).

Suspended solids determinations were not performed in Experiment G. In order that this data may also be included in analysis of this type it was decided to relate the change in ATP concentration, calculated as described earlier, to the mean ATP concentration before phenol addition. It was suggested in Chapter 4 that in constant conditions of substrate concentration, (of which conditions in which endogenous metabolism prevailed represented a particular case where the extracellular substrate concentration was zero), the ATP level of the sludge could be used as a measure of active biomass.

As the conditions at the start of each test run were considered constant regarding substrate concentrations, on the basis of the hypothesis outlined in Chapter 4, the mean ATP levels before substrate addition could be considered to be linearly related to biomass. The change in ATP level following phenol addition per unit ATP concentration before addition, could therefore be considered a valid means of correcting for variations in sludge concentrations between test runs.

For each test run therefore the mean ATP concentration following phenol addition was subtracted from the mean ATP value before addition, as before, and this value divided by the mean ATP value before addition. These values, recorded in Table 8.5, were related to sludge phenol concentration following phenol addition in Figure 8.23.

A graph relating the ATP content of the sludge before phenol addition in each test run for Experiments P and R to the suspended solids

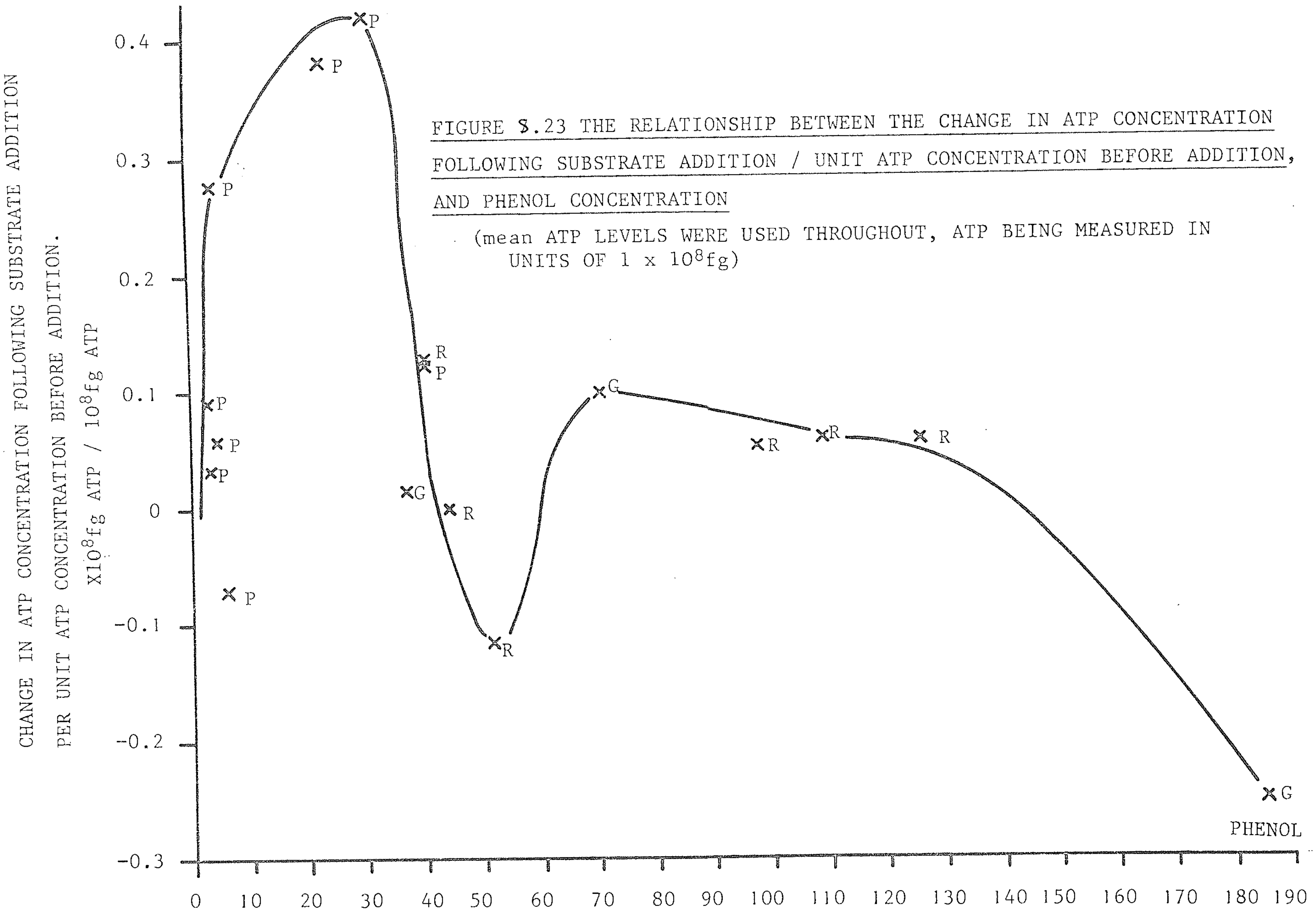


FIGURE 5.23 THE RELATIONSHIP BETWEEN THE CHANGE IN ATP CONCENTRATION FOLLOWING SUBSTRATE ADDITION / UNIT ATP CONCENTRATION BEFORE ADDITION, AND PHENOL CONCENTRATION

(mean ATP LEVELS WERE USED THROUGHOUT, ATP BEING MEASURED IN UNITS OF 1 x 10^8fg) content, recorded in Figure 8.24, produced a linear relationship with a correlation coefficient of 0.80. This was taken as evidence supporting the assumptions made on the basis of the hypothesis described in Chapter 4, and for justifying the consideration of the relationship shown in Figure 8.23. Examination of Figure 8.24 showed that the regression line would not pass through the origin. This could be explained by assuming that the sludge samples taken contained a quantity of non-active biomass. This assumption was consistent with reports by other workers that activated sludges in general typically contain a large proportion of non-active material.

From the relationship shown in Figure 8.24, the fact that very similar trends were shown in Figures 8.22 and 8.23, was to be expected. Figure 8.23 included data from Experiment G, which was useful in further clarifying the relationship shown in Figure 8.22 which did not.

The results of the graphs shown in Figures 8.22 and 8.23 appeared to show two peaks in the ATP behaviour of the sludges with respect to substrate, which seemed similar in some respects to the type of behaviour found earlier for thiocyanate-treating sludges.

The significance of these findings and of other results in this investigation were considered later in the project.

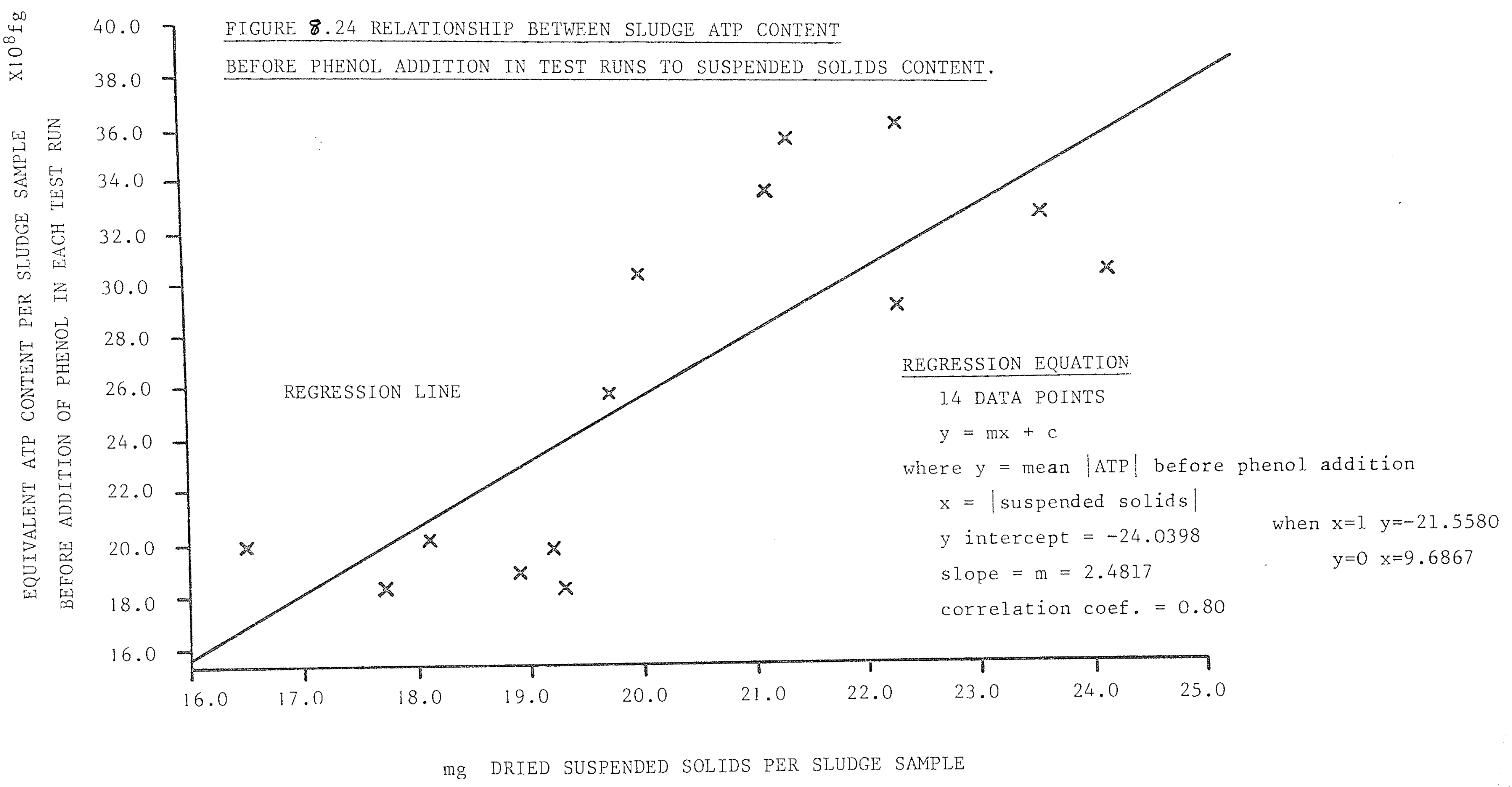


FIGURE 8.24 RELATIONSHIP BETWEEN SLUDGE ATP CONTENT BEFORE PHENOL ADDITION IN TEST RUNS TO SUSPENDED SOLIDS CONTENT.

REGRESSION EQUATION

14 DATA POINTS

y = mx + c

where y = mean |ATP| before phenol addition

x = |suspended solids|

y intercept = -24.0398

slope = m = 2.4817

correlation coef. = 0.80

when x=1 y=-21.5580

y=0 x=9.6867

CHAPTER 9

INVESTIGATION OF THE ATP BEHAVIOUR OF LABORATORY-SCALE CONTINUOUS COMPLETE-MIX ACTIVATED SLUDGE REACTORS TREATING COKE OVEN LIQUOR

Introduction

9.1 Continuous Reactor Study

9.2 Methods and Materials

- 9.2.1 Source of Sludge and Waste Liquor
- 9.2.2 Reactor
- 9.2.3 Parameters monitored
- 9.2.4 Flow Rate
- 9.2.5 Reactor Maintenance and Sampling Procedure

9.3 Results

9.4 Analysis and Discussion of Results

- 9.4.1 Thiocyanate Removal
- 9.4.2 ATP and Reactor Phenol Concentration
- 9.4.3 ATP and Growth Rate
- 9.4.4 ATP and OUR

INVESTIGATION OF THE ATP BEHAVIOUR OF LABORATORY-SCALE CONTINUOUS COMPLETE-MIX ACTIVATED SLUDGE REACTORS TREATING COKE OVEN LIQUOR

Introduction

Previous investigations of the ATP behaviour of sludges in the project were confined to laboratory batch reactors treating only a single component of coke oven liquor at any one time in a basal medium of treated liquor. Initial experiments involved using concentration ranges for these components, and growth conditions, which were very different from those typically found in full-scale plants treating the waste. The second series of experiments, which investigated the initial ATP response of starved batch sludges to the addition of various substrates, used lower substrate concentration ranges, but as in the first series of experiments sludges acclimatised to the treatment of a single liquor component were used. The simplification of the treatment system represented by these two approaches was it was believed important at the start of an investigation into the ATP behaviour of the treatment of such a complex waste.

It was evident however at the start of the project that there maybe some difficulty in extrapolating findings from the batch system employed, treating a single liquor component, to full-scale plant treating coke oven liquor. It was unfortunately not possible to conduct any ATP investigations on full-scale plant treating liquor as was desired. Plant performance data for pilot and full-scale plants treating the waste were made available however, and this was used in Chapter11to determine the value of kinetic parameters, and in the design and operational modelling of the process. This enabled comparisons to be made later in the project between the results of that work and the conclusions made on the basis of the batch experiments.

Given the limitations regarding ATP investigations with full-scale systems in the project, investigation of the ATP behaviour of a laboratory-scale continuous reactor treating coke oven liquor, rather than of individual liquor components, was therefore a natural development in the project.

This chapter described such an experiment and the results obtained were compared later in the project with those from the batch experiments described earlier, and were used to further develop theories regarding the significance of ATP.

9.1 Continuous Reactor Study

An important aspect of this work was that a fresh sample of activated sludge collected from a full-scale plant treating coke oven liquor, and a supply of the liquor being treated at the plant, were used at the start of the experiment. Conditions with respect to type of activated sludge and waste liquor used therefore simulated as far as possible those found in full-scale plants.

In order to facilitate the determination of kinetic parameters for modelling the treatment system, attempts were made to run the reactor at a series of different steady-state conditions. (The principles involved in this approach were discussed in Chapter 11).

The reactor was therefore operated as far as practical in constant conditions, particularly with regard to feed strength and influent flow rate, for periods of 5-10 days. A new set of conditions was applied at the end of each period, commencing the start of the next, by altering the influent flow rate by adjustment of the metering pump setting, or by altering the strength of the influent. Alteration in feed strength was achieved by additions of a concentrated phenol solution to the feed reservoir.

The changes in operating conditions marking the start of each new steady-state condition were evident from the tables of results presented later.

A summary of the conditions and conduct of the continuous reactor study follows.

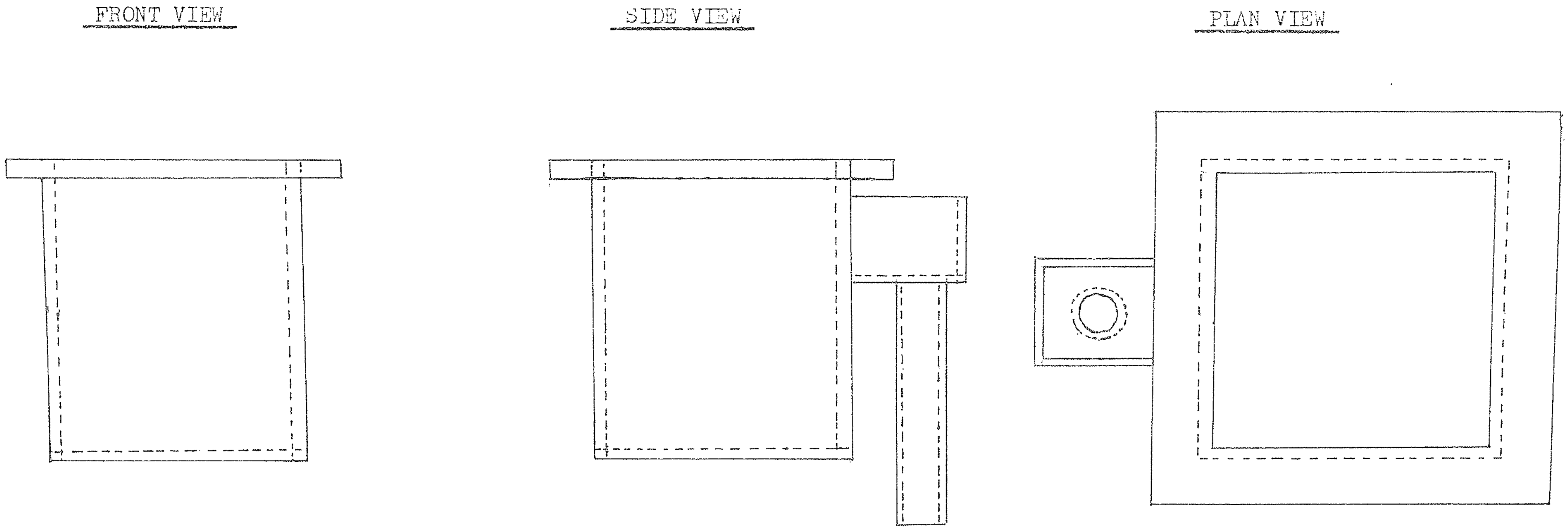
9.2 Methods and Materials

9.2.1 Source of Sludge and Waste Liquor

Liquor and activated sludge collected from the BSC treatment works at Scunthorpe was used for the study.

9.2.2 Reactor

This was constructed from perspex sheet and had the dimensions shown in Figure 9.1.



MATERIAL: PERSPEX SHEET AND TUBING

APPROX. 1/4 SCALE.

FIGURE 9.1 DIAGRAM OF REACTOR USED IN THE CONTINUOUS REACTOR STUDY.

A diagramatic representation of the apparatus used in the experiment was recorded in Figure 9.2.

9.2.3 Parameters monitored

Samples of reactor and recycle sludge were generally taken once daily and used for suspended solids determinations using the procedure described in Chapter 4. The filtrates were used for phenol, thiocyanate, ammonia, nitrate and nitrite determinations using the methods described earlier in the project. Phosphate was determined using a Technicon auto-analyser and the standard method recommended for that instrument.

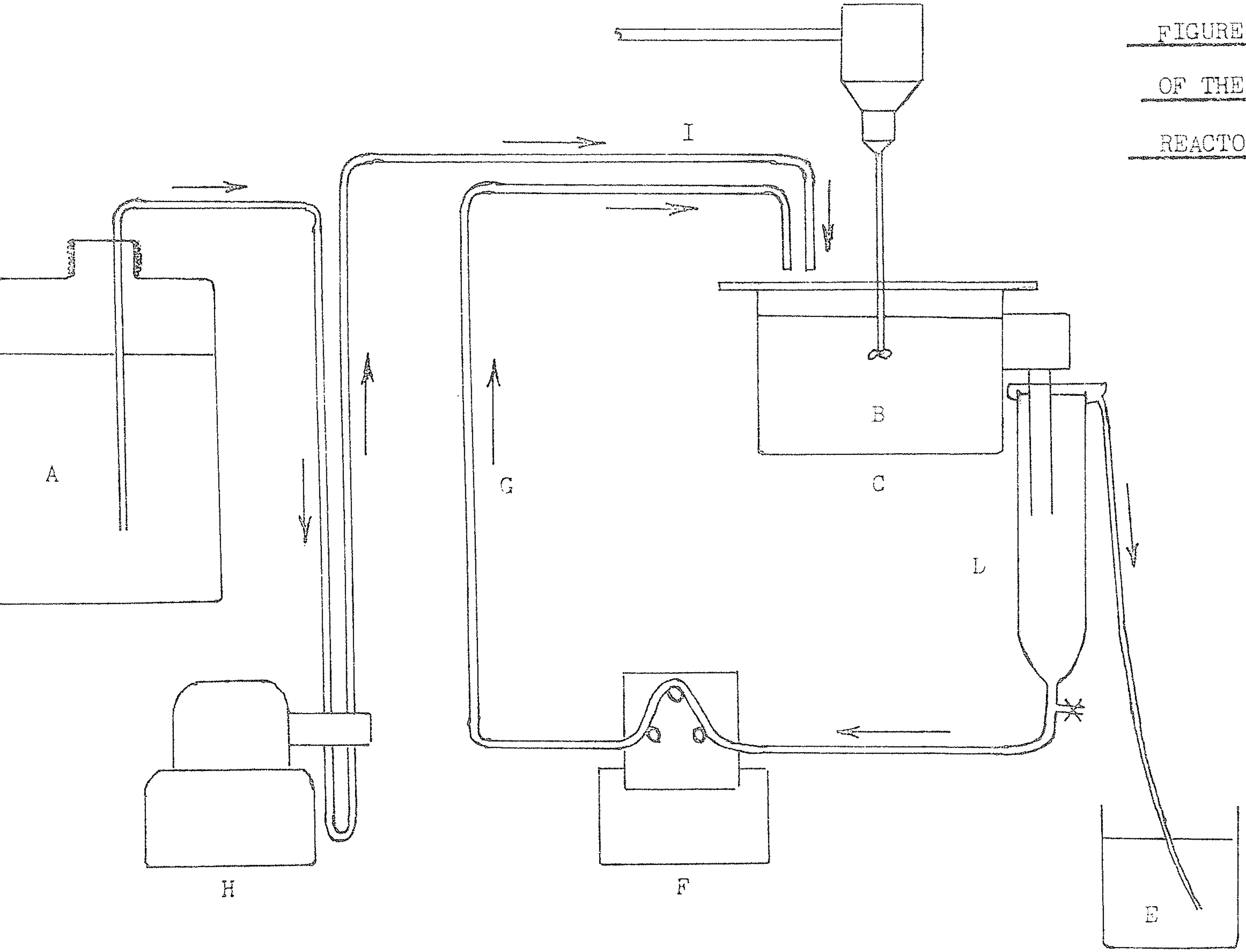
OUR was determined using 5ml samples of reactor sludge as described in Chapter 10. Duplicate samples of reactor sludge were taken and analysed for ATP using the procedures described for the batch experiments.

9.2.4 Flow Rates

Influent and recycle flows were measured by collecting the flow from each flow line in a 100ml measuring cylinder, and using a stopwatch to record the time taken for collection of a known volume. The contents of the measuring cylinders were returned to the reactor after determination of the flow rates.

Effluent flow rate was determined by collecting the effluent from the reactor in a plastic container over a measured period of several hours on each day. The volume of the collected

FIGURE 9.2 DIAGRAMMATIC REPRESENTATION OF THE APPARATUS USED IN THE CONTINUOUS REACTOR STUDY.



A - INFLUENT RESEVOIR
B - ACTIVATED SLUDGE
C - REACTOR
D - SETTLER
E - COLLECTED EFFLUENT
F - PERISTALTIC PUMP
G - RECYCLE FLOW
H - METERING PUMP
I - INFLUENT FLOW

effluent was determined using a 1 litre measuring cylinder. 50ml samples of the well-mixed effluent were taken for suspended solids determination and other analysis as outlined above.

9.2.5 Reactor Maintenance and Sampling Procedure

The stirrer used to maintain complete-mixing conditions in the reactor was operated at a constant speed and was lubricated regularly.

Influent, recycle, and effluent tubing was inspected daily and given appropriate attention to avoid blockages. Tubing used in conjunction with the peristaltic pump on the influent and recycle lines was replaced regularly to avoid leakages due to tubing fatigue.

The internal walls of the reactor were cleaned by scraping twice daily, once one hour before sampling was begun, to minimise wall growth.

Sampling was carried out at approximately the same time on each day and a set schedule used for monitoring the reactors. In particular, "ATP" samples of reactor sludge were taken before any other. Flow rates were determined after ATP, reactor and recycle sludge samples had been taken to ensure as near as possible that the sludge samples were representative of the behaviour of the reactor on that day.

Any unusual sludge conditions or losses of sludge observed during the experiment were recorded.

9.3 Results

The results were recorded in Table 9.1. and Figure 9.3.

9.4 Analysis and Discussion of Results

9.4.1 Thiocyanate Removal

There were two periods during the experiment when efficient thiocyanate removal was achieved, corresponding to days 1 to 7, and 20 to 38. A rapid deterioration in removal was observed from days 8 to 18, and from day 39 onwards.

Attempts were made to correlate these observed periods of poor removal with other parameters monitored during the experiment. There appeared no obvious relation between the poor removal and reactor phenol, ammonia, nitrite, nitrate, and phosphate concentrations, or with influent flow rate or solids retention time. The only factor common to both periods of unsatisfactory removal was that new batches of feed stock had been started 24-48 hours prior to the start of deterioting performance. The most likely explanation for treatment deterioration was therefor assumed to be that some of the containers of liquor used in the experiment contained liquor components toxic to thiocyanate removal.

TABLE 9.1

RESULTS OBTAINED FROM CONTINUOUS REACTOR STUDY

	INFLUENT					REACTOR										EFFLUENT
DAY	FLOW RATE	PHENOL	CNS	NH_3	PO_4	T^oC	pH	SV_{10} /100	MLSS	PHENOL	CNS	NH_3	PO_4	ATP	VOL WASTED	S.S
1	2.2632	192.7	257.5	808	74	20.0	6-6.5	56	7133	3.23	0	400	27	7.236	35.5	150
2	2.2176	247.7	257.5	816	75	20.0	6-6.5	57.5	6810	1.18	0	592	49	7.326	35.0	150
3	2.3760	243.2	254.6	832	83	20.0	6-6.5	54	8221	1.59	0	688	54	9.846	41.0	150
4	2.2856	246.1	256.7	832	77	20.0	6-6.5	64	6142	1.79	0.6	752	67	9.504	36.5	135
5	2.0880	196.2	248.7	808	79	20.0	6	70	6469	0.71	0.6	872	78	10.044	36.2	143
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	2.1864	249.4	271.5	1220	5	20.0	6	84.5	7022	0.50	0	992	53	4.896	36.0	217
8	2.3664	249.4	269.3	1250	4	20.0	6.5-7	62	6984	1.53	60.8	1160	42	7.272	36.0	229
9	1.8984	249.4	272.9	1220	5	20.0	6.5-7	58	6793	1.53	110.7	1220	32	6.948	37.3	205
10	1.9032	232.9	264.1	1300	6	20.0	6.5-7	58	6693	1.74	141.5	1340	26	6.84	36.2	217
11	2.0400	245.3	267.8	1290	5	20.0	6.6-7	62	5672	1.74	173.8	1460	22	7.02	38.8	217
12	2.0808	722.8	273.7	1110	14	20.0	6.517	57	5006	1.94	190.0	1400	18	10.098	38.5	215
13	2.0808	702.2	297.2	1220	13	20.0	6.5-7	60	4206	1.53	206.1	1540	18.4	9.828	38.0	235
14	2.0496	672.8	284.0	1220	13.2	20.0	7	60	3488	1.53	210.5	1490	18.8	8.676	37.8	225

BLE 9.1 CONTINUED

	INFLUENT					REACTOR										EFFL.
Y	FLOW RATE	PHENOL	CNS	NH_3	PO_4	T^oC	pH	SV_{10} /100	MLSS	PHENOL	CNS	NH_3	PO_4	ATP	VOL WASTED	S.S
	2.1192	699.3	305.2	1230	12.4	20.0	7	30	1734	1.53	199.5	1400	38.0	7.02	39.0	225
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2.2224	437.9	287.6	980	348	20.0	7	25	1683	4.82	165.8	1290	363	10.08	38.7	473
3	2.1768	417.9	278.1	1020	343	20.0	-	24	2943	2.76	132.0	1320	520	11.16	39.0	473
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0	2.1192	409.7	292.0	1020	363	20.0	7	43	2956	2.35	5.8	1370	615	-	38.0	458
1	2.0736	420.0	292.0	950	343	20.0	7	23	1989	20.85	5.8	1320	605	7.164	40.0	488
2	2.148	421.4	-	-	-	20.0	-	-	3026	2.76	9.0	1400	705	9.144	38.8	473
3	2.4624	393.3	311.8	1110	595	20.0	7	36	2833	1.12	0.6	1460	775	7.788	45.0	140
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	2,412	615.2	375.7	1320	69	20.0	6.5-7	26	2674	3.58	5.0	1820	340	9.864	39.0	183
28	2.4168	631.7	386.0	-	-	20.0	6.5-7	-	2893	4.82	0.6	-	-	11.88	39.5	183

E 9.1 CONTINUED

Y	INFLUENT					REACTOR										EFFL.
	FLOW RATE	PEHNOL	CNS	NH_3	PO_4	T^oC	pH	SV_{10}	MLSS	PHENOL	CNS	NH_3	PO_4	ATP	VOL WASTED	S.S
	2.424	631.7	385.2	1380	64	20.0	6.5-7	59	3075	3.58	5.8	1700	215	12.06	42.8	180
0	2.4264	639.9	395.5	1420	70	20.0	6.5-7	58	3043	3.58	2.8	1740	161	11.16	27.5	180
1	2.4048	634.4	-	-	-	20.0	6.5-7	64	3149	11.81	0	1740	146	9.36	27.5	180
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	2.496	-	350.0	675	27	20.0	6.5-7	63	2897	1.53	5.0	1305	88	10.188	40.0	180
4	2.5248	-	350.7	675	23	20.0	6.5-7	73	2697	1.53	2.8	1220	58	11.484	39.6	180
5	2.388	-	352.9	675	24	20.0	6.5-7	65	2604	0.71	4.3	1110	30	12.6	39.9	180
6	2.316	-	359.6	700	36	20.0	6.5-7	59	2348	1.1	3.6	1030	50	16.2	40.5	180
7	-	-	-	-	-	20.0	-	-	2286	1.1	11.6	945	45	-	41.8	180
38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	-	-	-	-	-	20.0	-	-	2167	1.5	72.6	950	47	-	40.0	180
40	2.4312	-	441.8	980	75	20.0	6.5-7	25	2272	1.51	136.2	1035	59	14.94	36.0	180
41	2.4312	-	412.4	1050	71	20.0	6.5-7	27	2237	2.3	190.7	1090	60	16.272	36.5	180
42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

All of the feed liquor used was collected on a single occasion in large plastic containers from a pipe feeding the inlet reservoir at the BSC Scunthorpe treatment works. However, because a large number of containers were filled the time taken to complete liquor collection was such that the quality of the flow to the works, and therefore the liquor collected, may have varied significantly during that period. This may provide an explanation why, if toxic inhibition was the cause of poor treatment, that only some containers of feed liquor caused a deterioration in thiocyanate removal.

9.4.2 ATP and Reactor Phenol Concentration

Efficient phenol removal was achieved throughout the 41 day period following the start of the experiment, so that on all but two days the reactor phenol level was less than 5mg/l, levels of 20.85mg/l and 11.81mg/l being found on days 21 and 31 respectively.

Graphs relating the reactor ATP concentration per ml of sludge, and ATP concentration per mg of suspended solids to reactor phenol concentration were plotted in Figures 9.4 and 9.5 respectively. These indicated that a maximum ATP level was achieved at about 2 mg/l phenol, with the levels declining at greater phenol concentrations. The existence of just two data points indicating this decline was not in itself conclusive evidence that this trend was real. However, when examined in the context of similar trends exhibited by the batch and respirometric studies reported elsewhere in the project, it may be

FIGURE 9.3 GRAPHICAL REPRESENTATION OF RESULTS OF THE CONTINUOUS REACTOR STUDY.

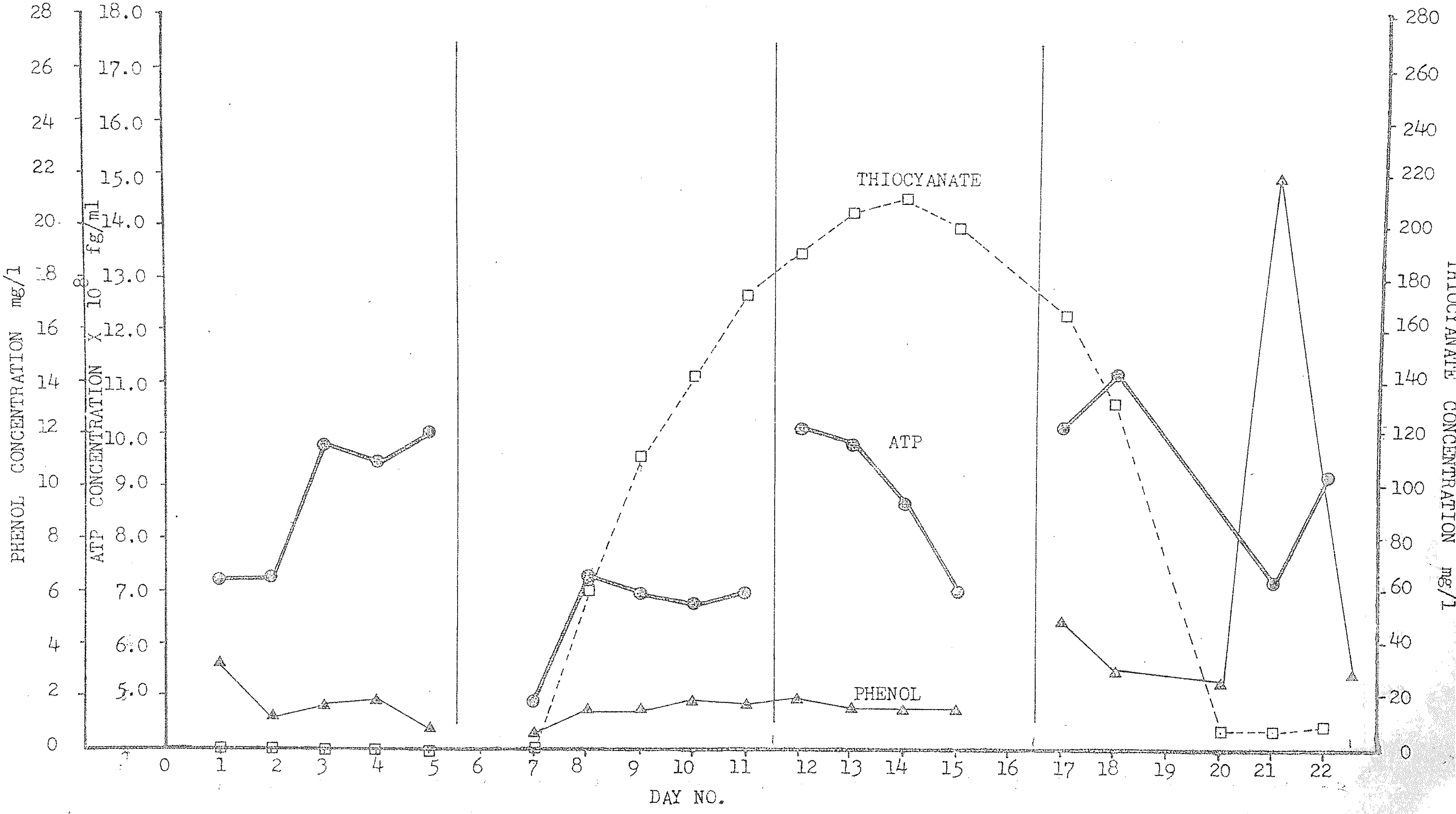


FIGURE 9.3 CONTINUED.

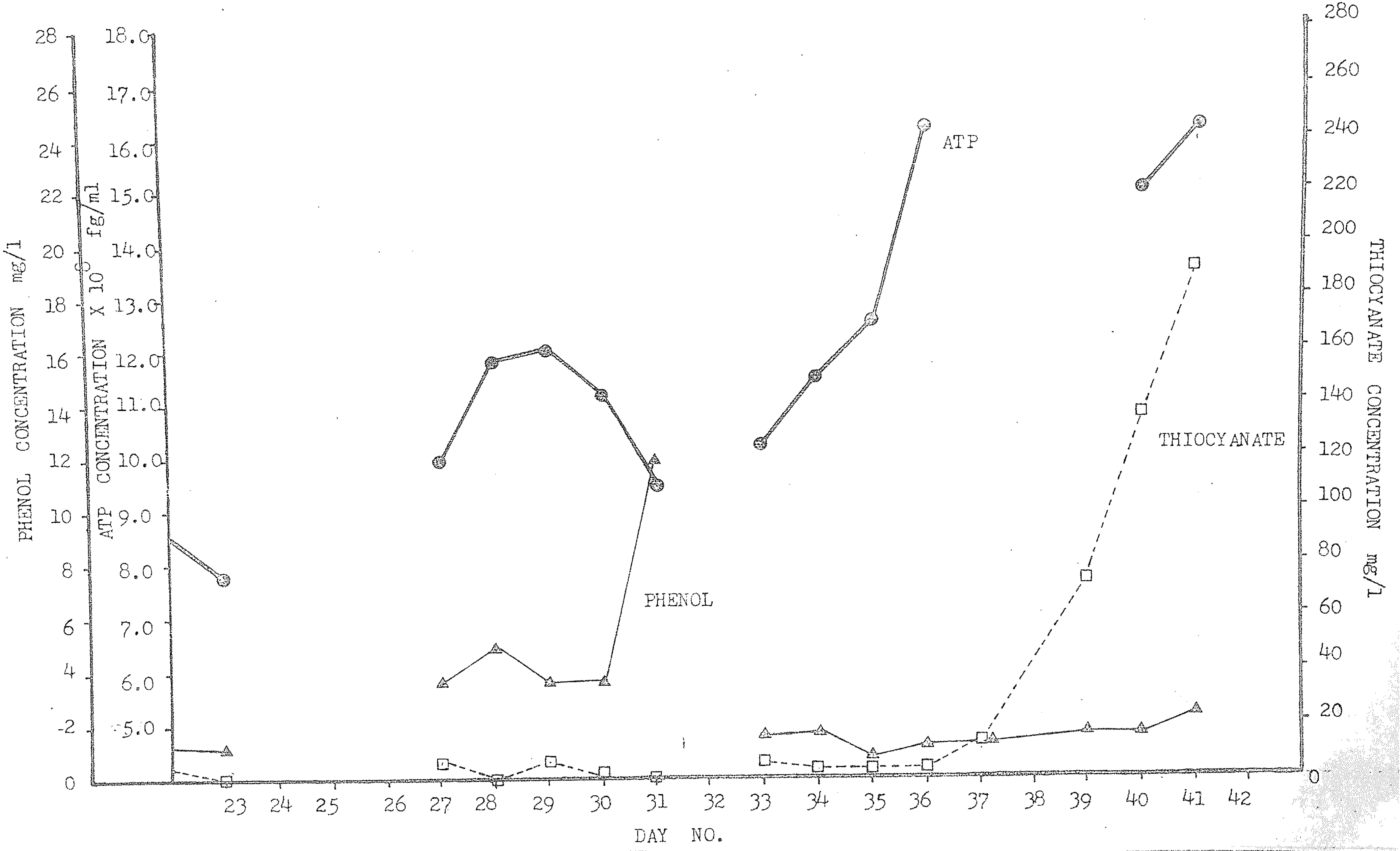


FIGURE 9.4. THE RELATIONSHIP BETWEEN ATP CONCENTRATION /ml SLUDGE AND REACTOR PHENOL CONCENTRATION

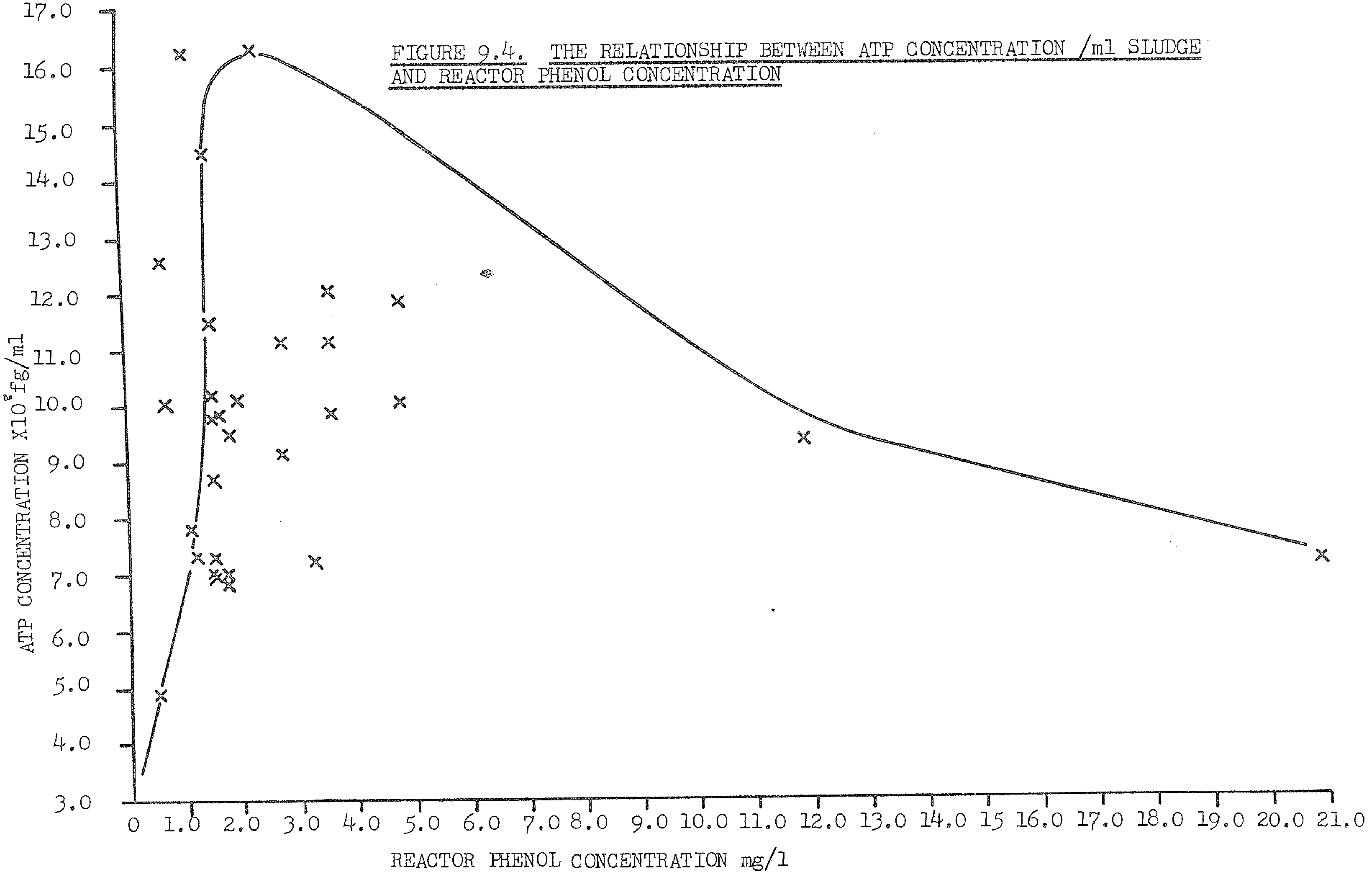
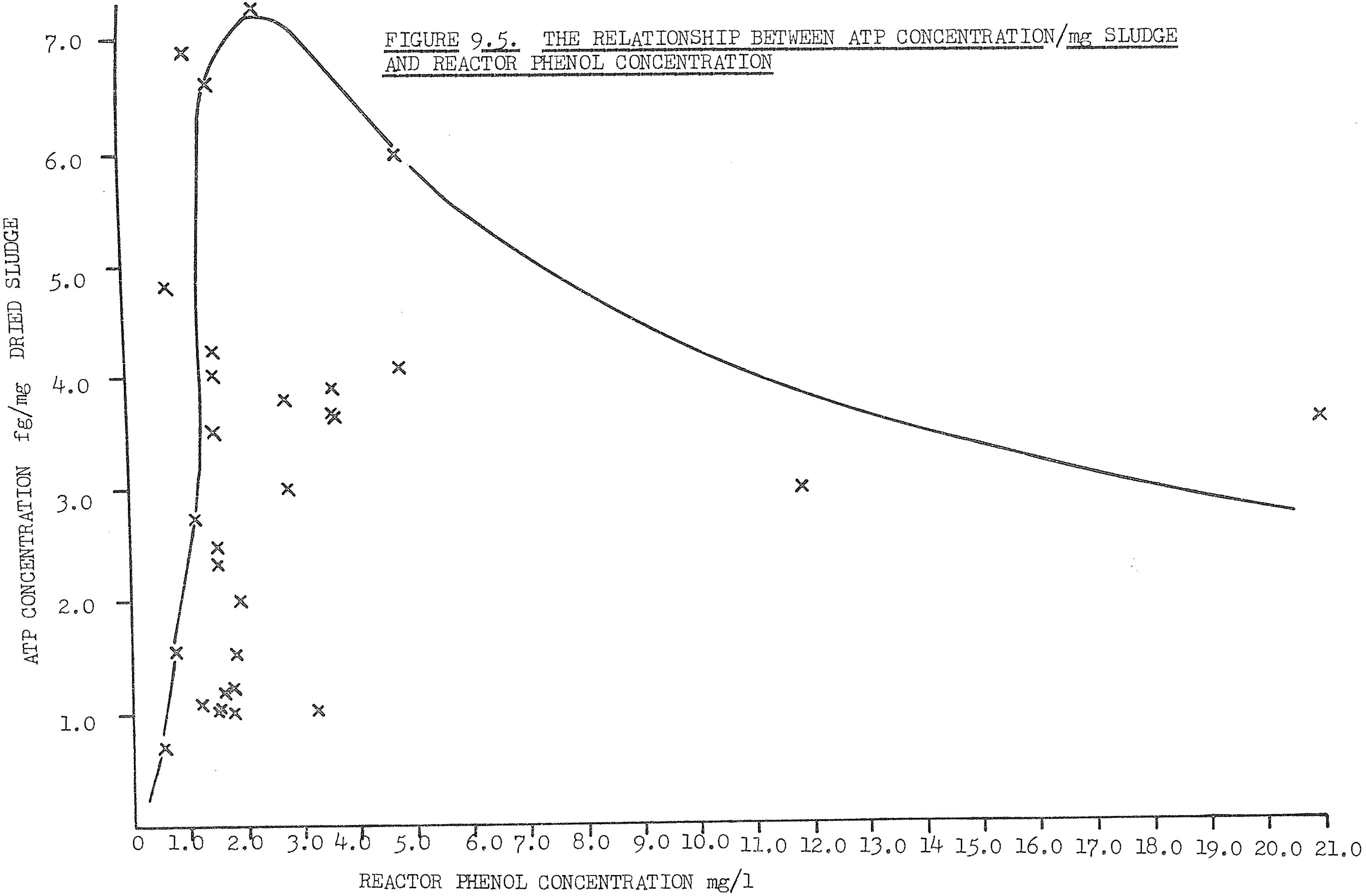


FIGURE 9.5. THE RELATIONSHIP BETWEEN ATP CONCENTRATION/mg SLUDGE AND REACTOR PHENOL CONCENTRATION



seen as further evidence of substrate inhibition in the metabolism of phenol by sludges treating coke oven liquor.

9.4.3 ATP and Growth Rate

The hypothesis was postulated in Chapter 4 that ATP represented a measure of growth rate. The data from this experiment was used to test that hypothesis.

On the basis of the steady-state models described in Chapter the net growth rate of the sludge on each day was determined using the following equation;

EQUATION 9.1

$$\mu_n = \frac{F_w X_w + (F - F_w) X_e}{VX}$$

where μ_n = net growth rate

F_w = wasted sludge flow rate

X_w = biomass concentration in wasted sludge

F = influent flow rate

X_e = effluent biomass concentration

X = reactor biomass concentration

V = volume of reactor

The net growth rate is related to specific growth rate by;

EQUATION 9.2

$$\mu_n = \mu - Kd$$

where μ = specific growth rate

Kd = a constant, the decay coefficient.

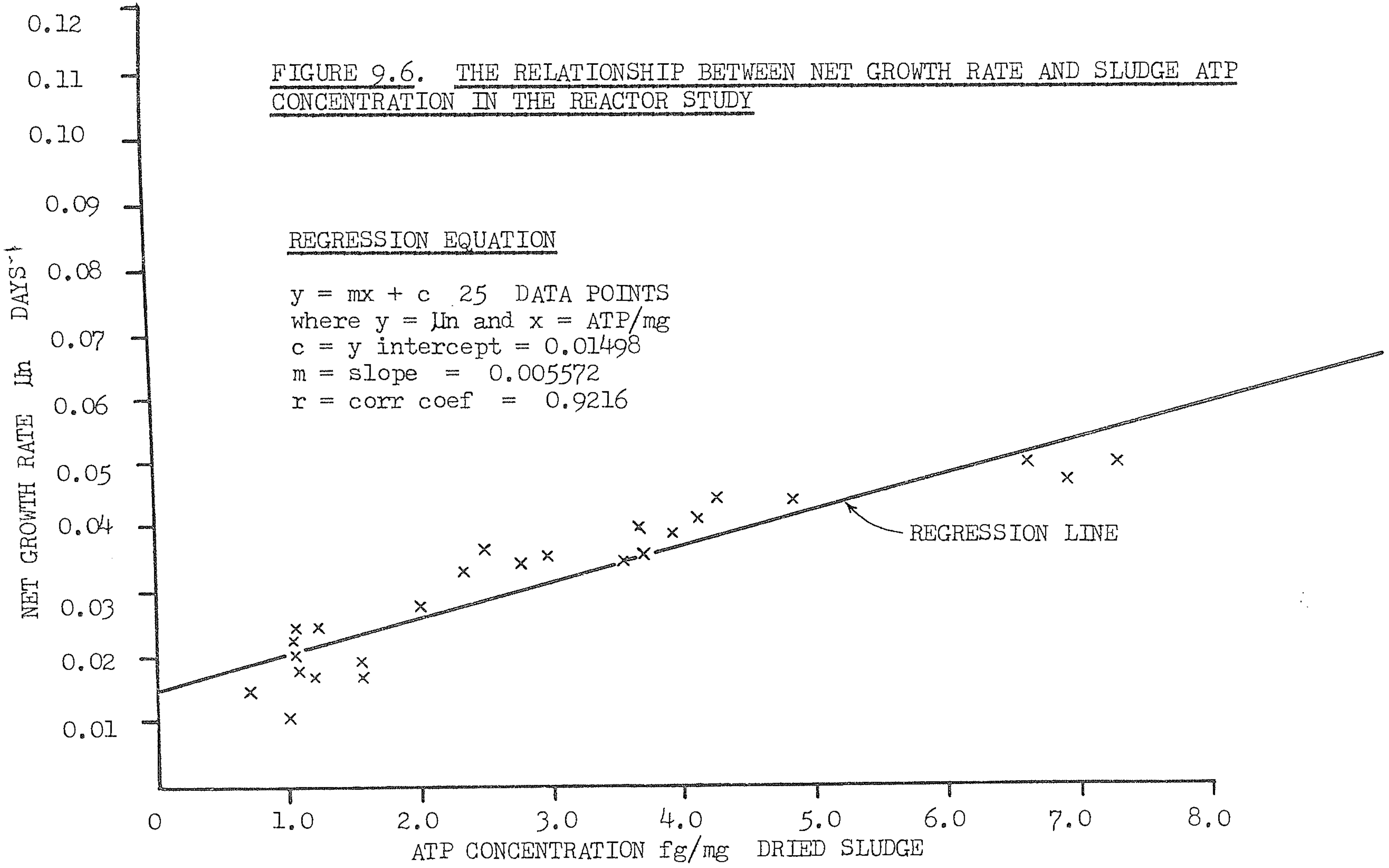
In determining the net growth rate from the experimental data the assumption was made that conditions approximated to steady-state on each of the days considered. On days 15-21 and 27, significant losses of sludge occurred from the apparatus due to the collection of thickened sludge at the top of the settler, much of which overflowed and was lost down the outside walls of the apparatus. The conditions on these days were therefore less likely to approach steady-state, and in addition it was not possible to accurately determine the total loss of solids from the system on these occasions. Plant performance data relating to these days was not considered therefore in the net growth rate determinations.

When the plant was performing satifactorily the only loss of solids from the system was in the effluent, and that due to sampling. The value used for F_w in the above equation was taken as the total volume of reactor sludge samples taken on the day, and the value for X_w as the reactor suspended solids concentration.

A graph was plotted in Figure 9.6 relating the net growth rate to ATP concent per mg dried sludge, and this produced a linear relationship with a correlation coefficient of 0.92. This therefore appeared to confirm the hypothesis described earlier in the project that ATP represented a measure of growth rate.

A graph relating sludge ATP concentration per unit volume to the MLSS concentration, shown in Figure 9.7 , showed a poor

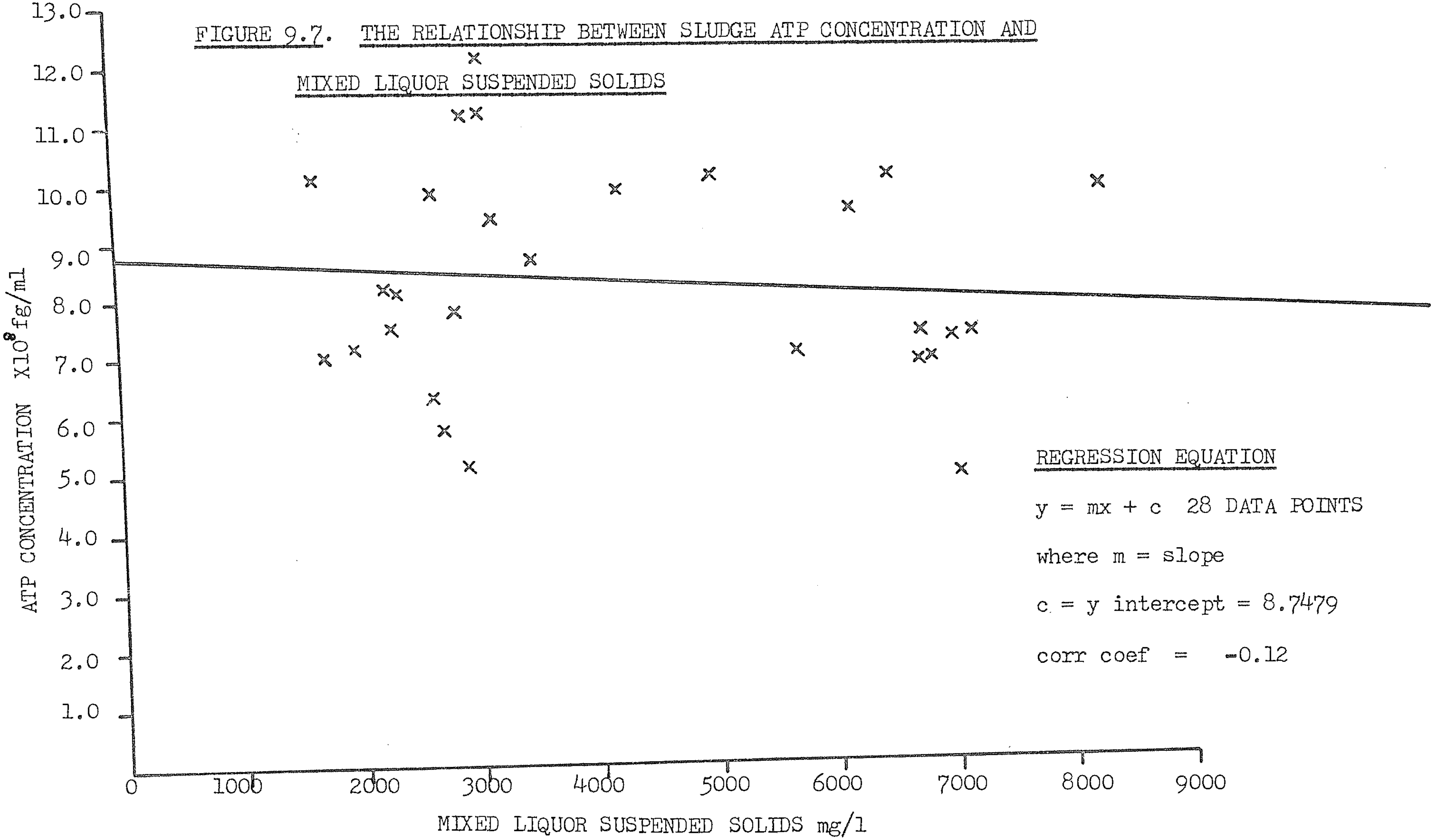
FIGURE 9.6. THE RELATIONSHIP BETWEEN NET GROWTH RATE AND SLUDGE ATP CONCENTRATION IN THE REACTOR STUDY



REGRESSION EQUATION

$y = mx + c$ 25 DATA POINTS
where $y = \mu n$ and $x = \mathrm{ATP/mg}$
$c = y$ intercept $= 0.01498$
$m =$ slope $= 0.005572$
$r =$ corr coef $= 0.9216$

FIGURE 9.7. THE RELATIONSHIP BETWEEN SLUDGE ATP CONCENTRATION AND MIXED LIQUOR SUSPENDED SOLIDS



REGRESSION EQUATION

$y = mx + c$ 28 DATA POINTS

where m = slope

c = y intercept = 8.7479

corr coef = -0.12

correlation. This was taken as evidence confirming that ATP represented more than just a measure of biomass.

9.4.4 ATP and OUR

OUR values were determined on 10 occasions during the experiment using the method described in Chapter 10. The results of these determinations, and the corresponding ATP values, were recorded in Table 9.2 . On some of the days several OUR determinations were carried out, generally when respirometric tests of the type described in Chapter 10 were performed, and the mean of these determinations were used in subsequent graphical analysis. On all occasions samples were taken for ATP analysis immediately before OUR determinations were started.

A graph was plotted in Figure 9.8 , relating ATP to OUR and a linear relationship with a correlation coefficient of 0.97 found.

The results of this experiment were discussed further in the project, along with the results of other ATP experiments.

TABLE 9.2. OUR AND ATP RESULTS FROM CONTINUOUS REACTOR STUDY

DAY	OUR			ATP
	No. OF DETERMINATIONS	STD DEVIATION	MEAN VALUE * mgO_2/min	MEAN VALUE $X10^8$ fg/ml
7	1	-	0.120	4.896
8	1	-	0.179	7.272
9	19	0.038	0.168	6.948
22	4	0.005	0.239	9.144
23	19	0.020	0.153	7.788
24	12	0.020	0.234	12.132
30	14	0.026	0.229	11.160
44	1	-	0.050	0.336
46	1	-	0.028	0.335
47	1	-	0.019	0.224

* value for 5ml of sludge

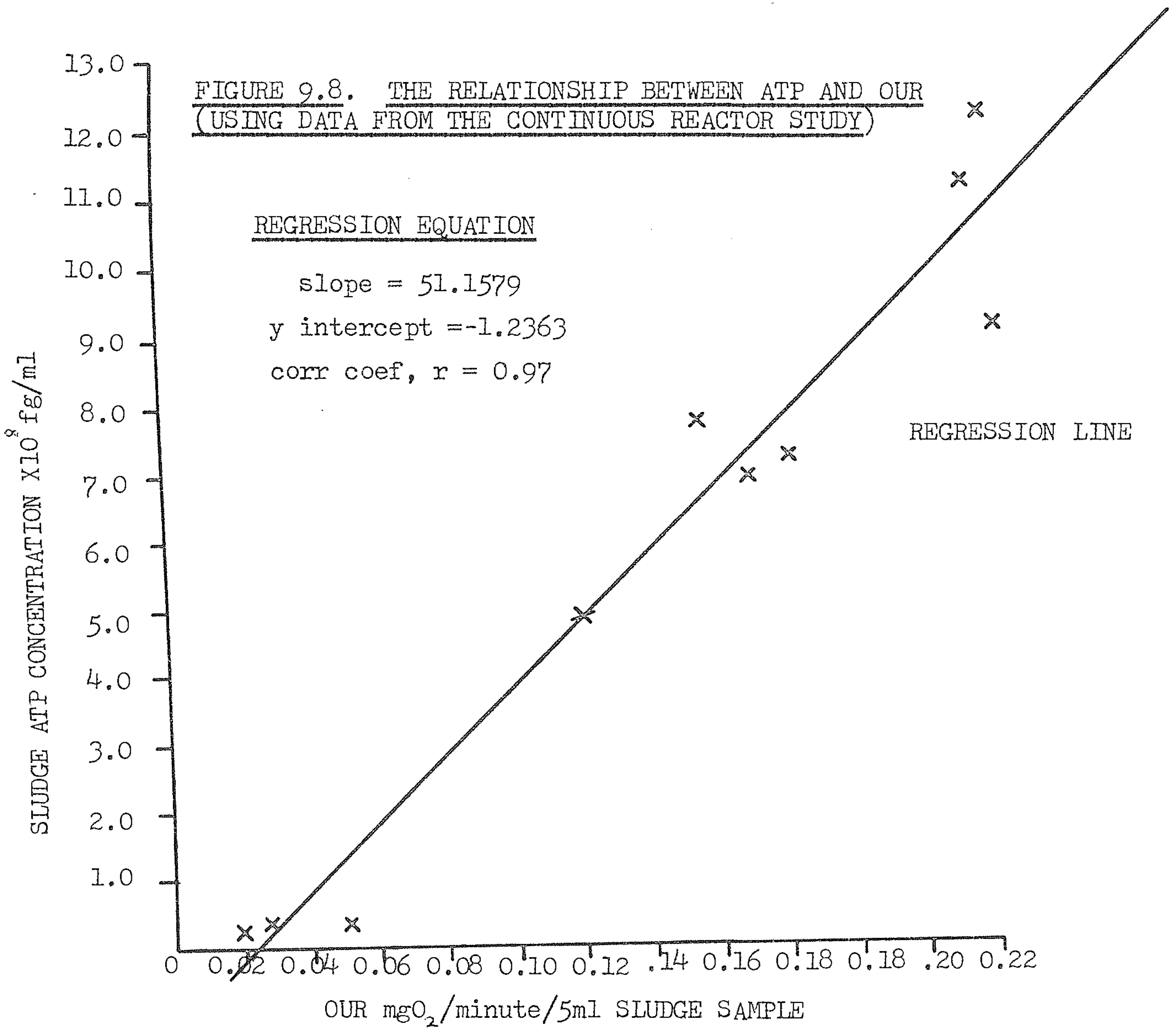


FIGURE 9.8. THE RELATIONSHIP BETWEEN ATP AND OUR (USING DATA FROM THE CONTINUOUS REACTOR STUDY)

REGRESSION EQUATION

slope = 51.1579

y intercept =-1.2363

corr coef, r = 0.97

CHAPTER 10

RESPIROMETRIC TESTING OF A LABORATORY-SCALE CONTINUOUS REACTOR TREATING COKE OVEN LIQUOR

Introduction

10.1 Principle
10.2 Operation
10.3 Calibration
10.4 Respirometric Testing of Activated Sludge
10.5 Investigations
10.6 Results
10.7 Results Analysis and Discussion
    10.7.1 Tests Conducted on Day 9 (Table 10·1)
    10.7.2 Tests Conducted on Day 30 (Table 10·2)
    10.7.2.1 Phenol
    10.7.2.2 Thiocyanate
    10.7.3 Tests Conducted on Day 23 (Table 10·3)
    10.7.3.1 Phenol
    10.7.3.2 Ammonia
    10.7.3.3 Effect of Ammonia on Phenol Oxidation
    10.7.3.4 Glucose
    10.7.3.5 Effect of Thiocyanate on Phenol Oxidation

RESPIROMETRIC TESTING OF A LABORATORY-SCALE CONTINUOUS REACTOR TREATING COKE OVEN LIQUOR

Introduction

Earlier work in the project indicated the role of ATP both as a measure of living biomass and activity. The potential for using ATP as a measure of these was indicated at the start of the project from a knowledge of the intimate involvement of ATP in all aspects of the metabolism of the cell, as typified by Figure 2.2 . It was obvious from that figure that the generation of ATP in the cell was also intimately related to the respiration of the cell, and the relation between these was discussed elsewhere in the project.

The respiration of sludges, measured in terms of the oxygen uptake rate (OUR) has already been investigated by a number of workers for various activated sludges, but the author is not aware of any published data relating to the treatment of coke oven liquor. The following brief research investigated the respiration activity of sludge taken on a number of days from a laboratory continuous reactor treating coke oven liquor, with the aim of gaining some additional insight into the kinetics of the treatment of the wastewater. In addition, because of the known link between the respiratory activity of cells and their cellular ATP production, it was hoped that comparison between both measures of activity might provide some additional insight into the significance of ATP in sludges treating coke oven liquor.

Respirometric Tests

These were carried out using the Rank oxygen electrode system (Rank Bros; Bottisham, Cambridge). Figure 10.1 shows a diagram of the electrode, and Figure 10.2 a schematic representation of the electrode and associated apparatus required for the tests.

10.1 Principle

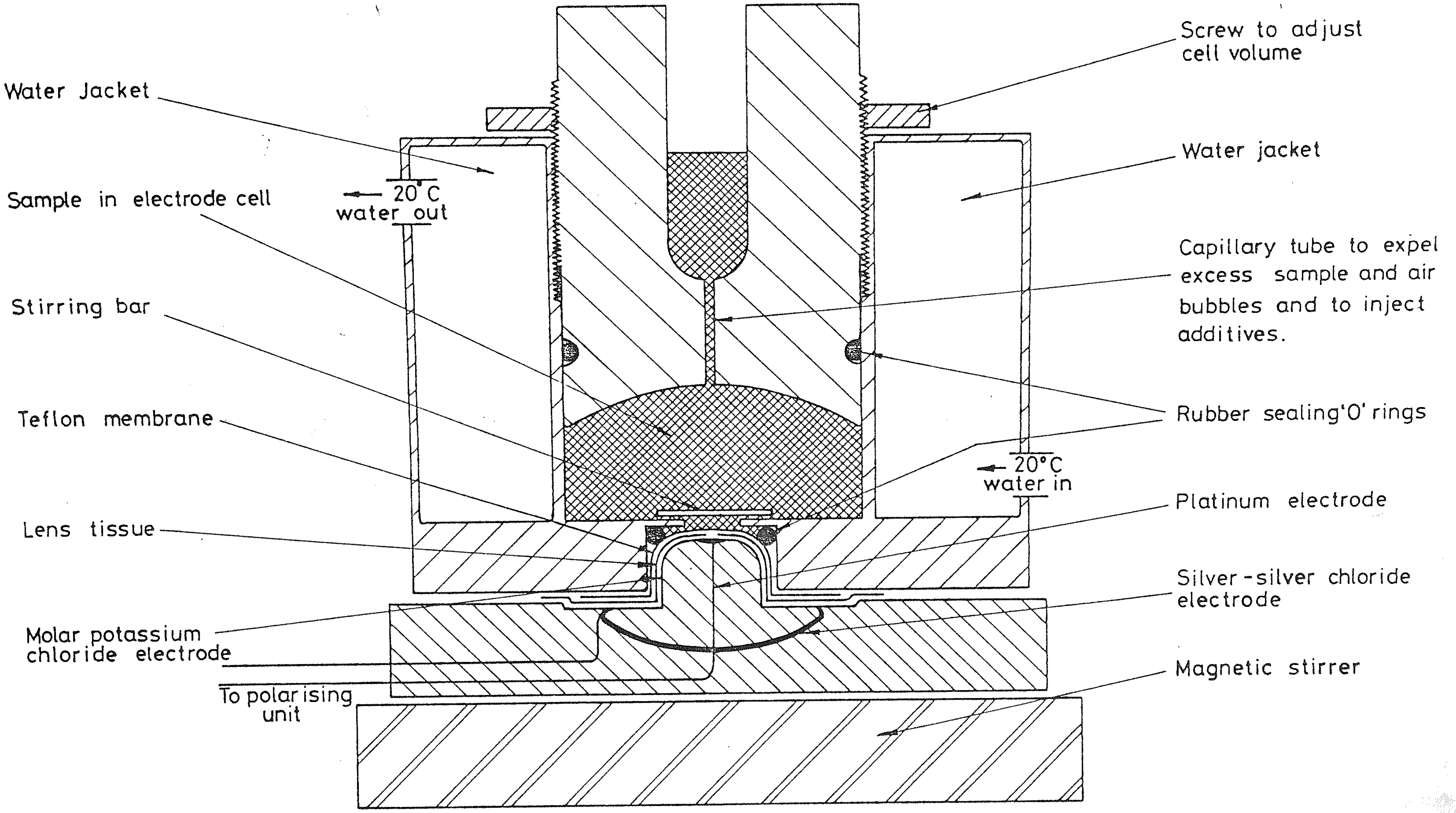
The Rank oxygen electrode operates on the principle of the Clark electrode. The electrode is composed of two half cells, using a platinum cathode in conjunction with a silver-silver chloride anode. Both electrodes are in contact with a saturated solution of potassium chloride and are separated from the test solution by a thin membrane, generally made of Teflon.

The principle of the electrode is that 4 electrons are generated at the anode which are used to reduce each molecule of oxygen diffusing through the membrane from the test solution.

ANODE	$4\,Ag + 4\,Cl^-$	$4\,AgCl + 4\,e^-$
CATHODE	$4\,H^+ + 4\,e^- + O_2$	$2\,H_2O$
	$4\,H^+ + 4\,Ag + 4\,Cl^- + O_2$	$4\,AgCl + H_2O$

If an appropriate voltage is applied across the electrodes (0.5 - 0.8V) the current measured is proportional to the oxygen concentration in the test solution.

FIGURE 10.1 DIAGRAMMATIC REPRESENTATION OF A VERTICAL SECTION THROUGH THE RANK OXYGEN ELECTRODE.



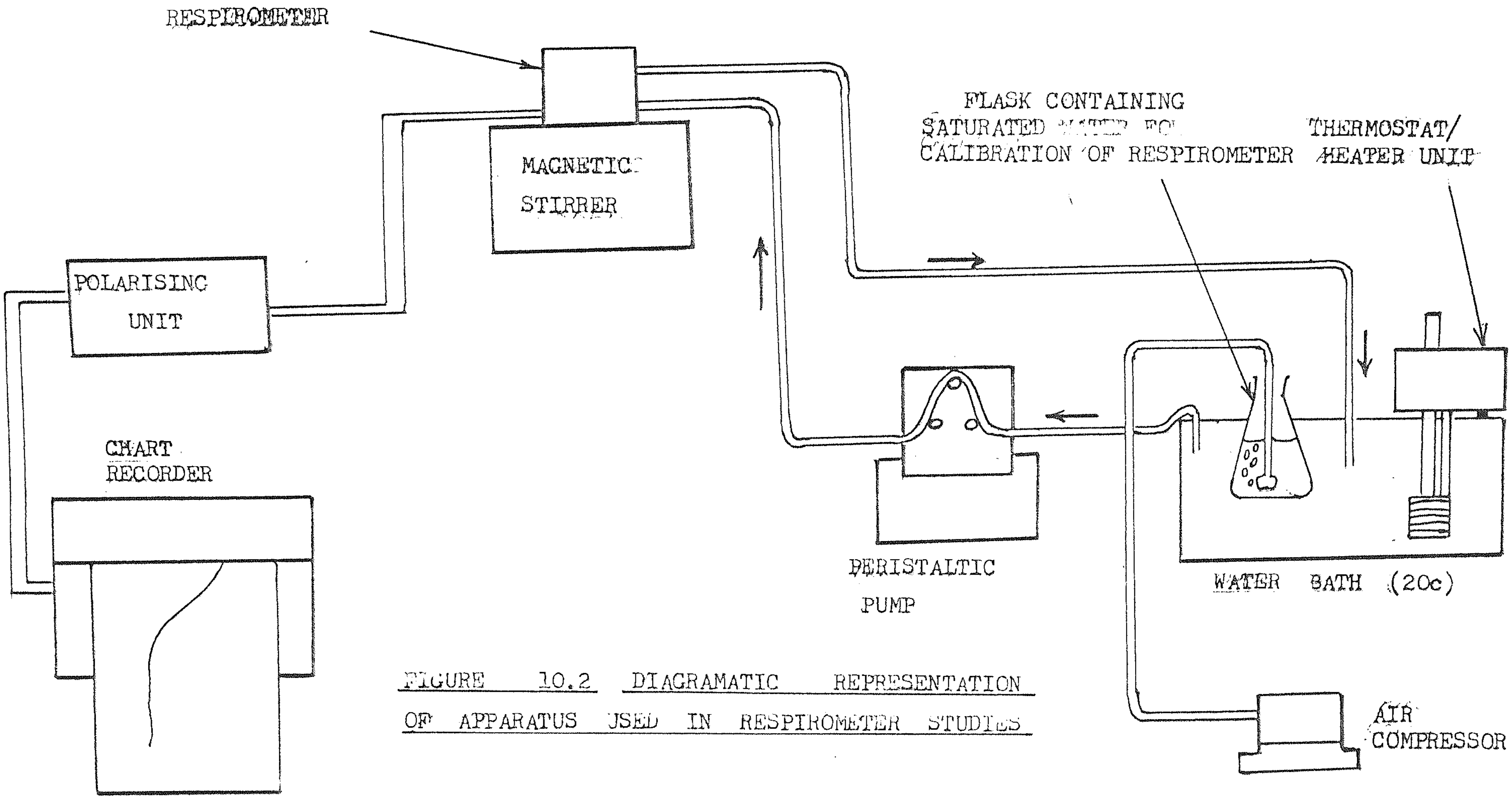


FIGURE 10.2 DIAGRAMATIC REPRESENTATION OF APPARATUS USED IN RESPIROMETER STUDIES

10.2 Operation

The respirometer was placed on a magnetic stirrer which was adjusted so that complete mixing was achieved when the cell contained 5mls of activated sludge. Water was continuously pumped to the water jacket, by means of a peristaltic pump, from a water bath maintained at 20°C. The electrode was connected to a polarising unit and recorder (Single-pen Servoscribe) as recommended by the manufacturers. Water circulation in, and electrical connection to, the apparatus was made at least 30 minutes before the respirometer was calibrated to ensure temperature and electrical equilibration.

10.3 Calibration

The electrode was calibrated on each day immediately before respirometric testing was required. A conical flask containing distilled water was placed in the water bath at 20°C and aerated for 10-15 minutes using a diffuser stone and a small compressed air pump to obtain a 100% saturated solution equivalent to an oxygen concentration of 9.2mg/l. After rinsing the electrode three times with the saturated solution, this solution was used to set the upper point of the calibration and the recorder was adjusted to give full deflection.

The lower point of the calibration was set, again after rinsing three times with the standard to be measured, using a freshly prepared 5% solution of sodium sulphite, equivalent to a zero oxygen concentration. The recorder was adjusted to give zero deflection.

10.4 Respirometric Testing of Activated Sludge

5.0ml samples of activated sludge were used in each test. Before and after each test the cell was rinsed well by successively filling and emptying it with distilled water to avoid cross-contamination of samples. (The cell was left filled with distilled water when not is use). Immediately before the addition of sample the inner walls of the cell were carefully dried with tissue to remove the majority of residual water. In all operations care was taken to avoid damage to the membrane.

The 5.0ml aliquots of activated sludge were pipetted without delay from the reactor, using a 10ml graduated pipette with a wide outlet orifice, into the electrode cell from which the plunger had been removed. The latter was then inserted and lowered by means of the screw to expell any air bubbles present, and until sludge had risen just past the top of the capillary tube. Dissolved oxygen concentrations were monitored continuously on the calibrated recorder. The various additions made to the sludge during tests were made by injection through capillary tube in the plunger using Hamilton 50μl microsyringes.

10.5 Investigations

These were of two types, and all were carried out during the continuous reactor study described earlier in Chapter 9;

a) The tests were used on a number of days to determine the oxygen uptake rate (OUR), of the sludge in the reactor by calculating the oxygen consumption relative to time using the chart recorder readings. As samples of sludge from the reactor were tested without delay, the tests also served to measure the dissolved oxygen concentration in the reactor. In both types of investigation the samples of sludge used in the tests were returned to the reactor following completion of testing. The results of these investigations were recorded in Table 9.2 along with the other daily monitoring data from that investigation.

b) On days 9, 23, and 30, during the continuous reactor studies, tests were carried out to investigate the effect of the addition of various substrates, or mixtures of substrates, on the OUR of the sludge. These additions, of 5-50μl quantities, were made to the sludge sample in the respirator by means of micro-syringes, once the initial OUR of the sludge had been determined. The following standard solutions, or mixtures of them were used.

SOLUTION

A	POTASSIUM THIOCYANATE	10g CNS/l
B	POTASSIUM THIOCYANATE	1.0g CNS/l
C	PHENOL	10.0g/l
D	PHENOL	1.0g/l
E	AMMONIUM CHLORIDE	20.0g NH_3/l
F	GLUCOSE	10.0g/l

TABLE 10-1 RESULTS OF RESPIROMETRIC TESTS ON SAMPLES TAKEN FROM CONTINUOUS REACTOR ON DAY 9

(all concentrations in mg/l)

TEST	INITIAL D.O.	INITIAL OUR	ADDITIONS mls STC		SUBSTRATE & CONCENTRATION		OUR	FOLLOWING OUR
1	7.68	0.218	50	D	PHENOL	10.0	0.455	-
2	7.82	0.200	10	D	"	2.0	1.680	0.308
			10	D	"	2.0	1.890	0.330
3	7.70	0.210	20	D	"	4.0	1.605	0.330
4	7.52	0.227	-		-		-	-
5	7.78	0.223	30	D	"	6.0	1.600	-
6	7.80	0.140	30	D	"	6.0	1.560	0.263
7	7.65	0.175	40	D	"	8.0	1.483	-
8	7.73	0.154	50	D	"	10.0	1.433	-
9	7.82	0.187	5	D	"	1.0	1.620	0.300
			5	D	"	1.0	1.380	0.420
			5	D	"	1.0	1.800	0.360
			5	D	"	1.0	1.83	0.360
			5	D	"	1.0	2.04	0.390
10	7.65	0.198	15	D	"	3.0	1.380	0.345
			15	D	"	3.0	1.690	0.225
11	7.52	0.090	25	D	"	5.0	1.470	0.353
12	7.53	0.138	45	D	"	9.0	1.410	-
13	7.62	0.153	10	C	"	20.0	1.300	-
14	7.48	0.135	20	C	"	40.0	1.210	-
15	7.30	0.150	30	C	"	60.0	1.127	-
16	7.50	0.160	40	C	"	80.0	1.054	-
17	7.50	0.161	50	C	"	100.0	0.936	-
18	7.40	0.129	5	C	"	10.0	1.397	-
19	7.44	0.135	25	C	"	50.0	1.126	-

TABLE 10-2 RESULTS OF RESPIROMETRIC TESTS ON SAMPLES TAKEN FROM CONTINUOUS REACTOR ON DAY 30

(all concentrations in mg/l)

TEST	INITIAL D.O.	INITIAL OUR	ADDITIONS mls STD		SUBSTRATE & CONCENTRATION		OUR	FOLLOWING OUR
A	4.08	0.248	30	D	PHENOL	6.0	5.700	-
B	4.30	0.234	40	D	"	8.0	5.400	-
C	4.20	0.233	30	D	"	6.0	5.33	-
D	4.20	0.214	50	D	"	10.0	5.700	-
E	4.10	0.300	20	D	"	4.0	5.400	-
F	3.80	0.205	10	D	"	2.0	5.265	0.429
			5	D	"	1.0	4.320	0.375
G	3.90	0.200	10	B	CNS	2.0	0.318	-
			50	B	"	10.0	0.360	-
			50	B	"	10.0	0.360	-
			50	B	"	10.0	0.366	-
H	3.90	0.255	10	A	"	20.0	0.366	-
			20	A	"	40.0	0.386	-
			50	A	"	100.0	0.340	-

TABLE 10-3 RESULTS OF RESPIROMETRIC TESTS ON SAMPLES TAKEN FROM CONTINUOUS REACTOR ON DAY 23

(all concentrations in mg/l)

TEST	INITIAL D.O.	INITIAL OUR	ADDITIONS mls STD	SUBSTRATE & CONCENTRATION	OUR	FOLLOWING OUR
1A	5.60	0.180	10 D	PHENOL 2.0	4.275	0.385
			10 D	" 2.0	4.028	-
2A	5.50	0.180	30 D	" 6.0	3.938	-
3A	5.70	0.162	50 D	" 10.0	3.255	-
4A	5.20	0.162	10 D	" 2.0	3.080	0.279
			10 D	" 2.0	3.225	0.338
5A	5.10	0.175	20 D	" 4.0	3.330	0.333
6A	5.10	0.171	30 D	" 6.0	3.135	-
7A	5.30	0.132	40 D	" 8.0	3.225	-
8A	5.30	0.145	10 (D/A 1:1)	PHENOL 1.0 CNS 10.0	3.200	0.317
			20 "	PHENOL 2.0 CNS 20.0	3.890	0.375
9A	5.30	0.138	10 (D/A/ H_2O 1:0.8: 0.2)	PHENOL 1.0 CNS 8.0	2.595	0.344
			20 "	PHENOL 2.0 CNS 16.0	3.240	0.678
10A	5.30	0.163	10 (D/A/ H_2O 1:0:6: 0.4)	PHENOL 1.0 CNS 6.0	3.514	0.296
			20 "	PHENOL 2.0 CNS 12.0	3.353	0.585
			30 "	PHENOL 3.0 CNS 18.0	3.66	-
11A	5.30	0.136	10 (D/A/ H_2O 1:0:4:.6)	PHENOL 1.0 CNS 4.0	4.950	0.296
			20 "	PHENOL 2.0 CNS 8.0	3.609	0.474
			30 "	PHENOL 3.0 CNS 12.0	3.675	-
12A	5.50	0.163	10 (D/A/ H_2O 1:0:2:0:8)	PHENOL 1.0 CNS 2.0	2.730	0.163
			20 "	PHENOL 2.0 CNS 4.0	3.863	0.590
			30 "	PHENOL 3.0 CNS 6.0	3.675	-

The new OUR following the addition of substrate was also measured. Where changes in oxygen consumption indicated that oxidation of the added substrate was complete, the new OUR (FOLLOWING OUR) was measured. In some cases further additions of substrate were made to the same sample and the procedure repeated.

The initial OUR, substrates added, and subsequent OURs, were recorded for each test. The results corresponding to days 9, 23, and 30 of the continuous reactor study were recorded in Tables 10.1, 10.3, and 10.2 respectively. The tests conducted on each of those days investigated the effect on the OUR of a range of substrate concentrations, and the substrates and ranges used were summarised later.

10.6 Results

a) The results of the daily monitoring of D.O concentration and OUR in the continuous reactor study were recorded and discussed in Chapter 9.

b) The results of the investigation into the effect of the addition of various substrates on the OUR in respirometric tests on the sludge from the continuous reactor studies already described were recorded in Table 10.1, 10.2, and 10.3. An analysis and discussion of these results follows.

10.7 Results Analysis and Discussion

10.7.1 Tests Conducted on Day 9 (Tables 10.1)

Phenol

A graph was plotted relating OUR to phenol concentration in each respirometric test in Figure 10.3. Only data relating to the OUR following the first addition of substrate was used in plotting the graph. The results of this graph indicated that phenol oxidation in the sludge was subject to substrate inhibition of the type described by HALDANE in enzyme kinetic studies and represented by an equation of the form;

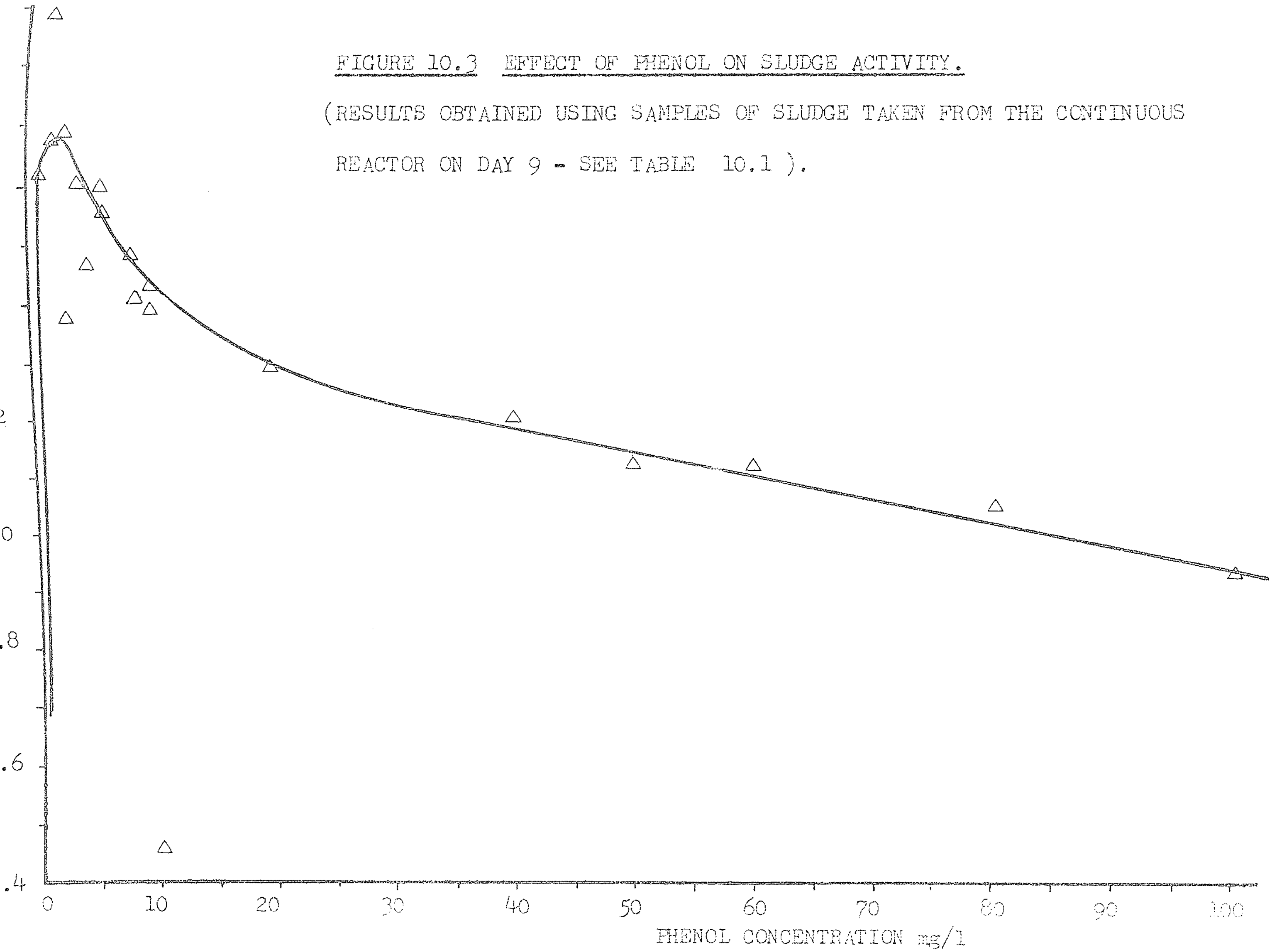
$$v = \frac{Vmax.}{1 + K_s/S + S/K_i}$$

which was discussed earlier in the project.

The results of the routine monitoring of the reactor in the continuous study described in Chapter 9 indicated a reactor phenol concentration of 1.53mg/l, and thiocyanate and ammonia concentrations of 11.7mg/l and 1220mg/l respectively, on day 9. The respirometric tests on this day indicated that the sludge was active with respect to phenol oxidation, and with such a low reactor phenol level a zero concentration was assumed in the sludge in each test before the injection of the standard

FIGURE 10.3 EFFECT OF PHENOL ON SLUDGE ACTIVITY.

(RESULTS OBTAINED USING SAMPLES OF SLUDGE TAKEN FROM THE CONTINUOUS REACTOR ON DAY 9 - SEE TABLE 10.1).



substrate solution. With this assumption, and that the substrate inhibition observed in Figure 10.3 could be approximated to that described by HALDANE the values for the two constants K_s and K_i in the above equation were estimated using graphical analysis analogous to that employed in enzyme kinetic studies. Using V = OUR to S = the phenol concentration, a plot of 1/V vs 1/S was used in Figure 10.4 to estimate the value for K_s. Similarly a plot of 1/V vs S was used to estimate the value of K_i in Figure 10.5. This produced values of K_s and K_i of 0.41mg/l and 181mg/l respectively. These two values could be used to estimate the substrate concentration at which the OUR was optimal S_{opt}, using

$$S_{opt} = \sqrt{K_s K_i}$$

and this was found to be 8.6mg/l. This was a more accurate estimate of the value for S_{opt} than that apparent in the free-hand graph drawn to fit the data in Figure 10.3.

10.7.2 Tests Conducted on Day 30 (Table 10.2)

10.7.2.1 Phenol

As for Table 10.1 data, a graph was plotted relating OUR to phenol concentration, and this was recorded in Figure 10.6. Also, as in the previous section, a zero phenol concentration

FIGURE 10.4 EFFECT OF PHENOL ON SLUDGE ACTIVITY.

ESTIMATION OF SATURATION CONSTANT K_S FOR SLUDGE EXHIBITING SUBSTRATE INHIBITION OF THE HALDANE TYPE. (DATA DERIVED FROM TABLE 10.1 DAY 9).

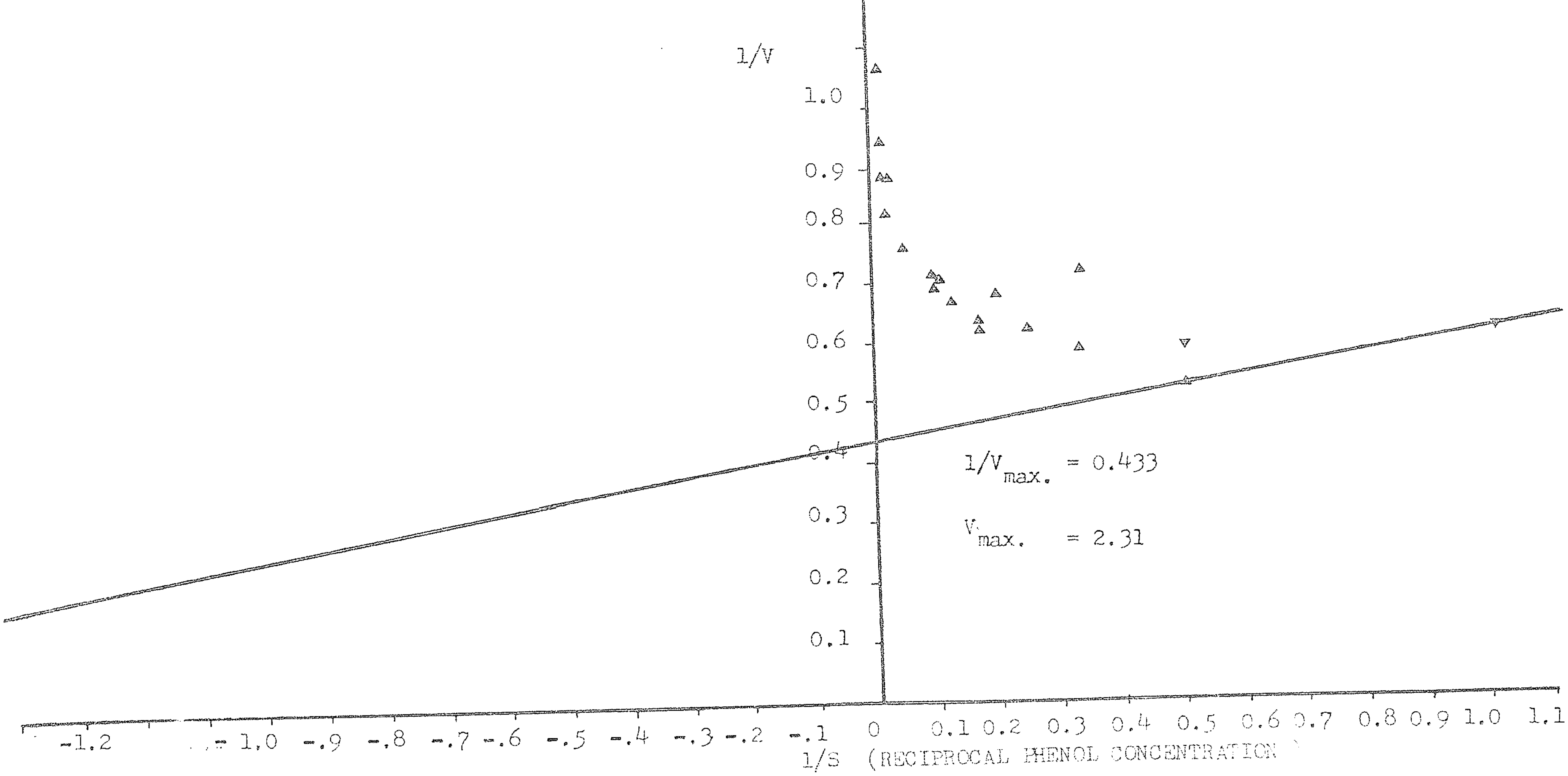
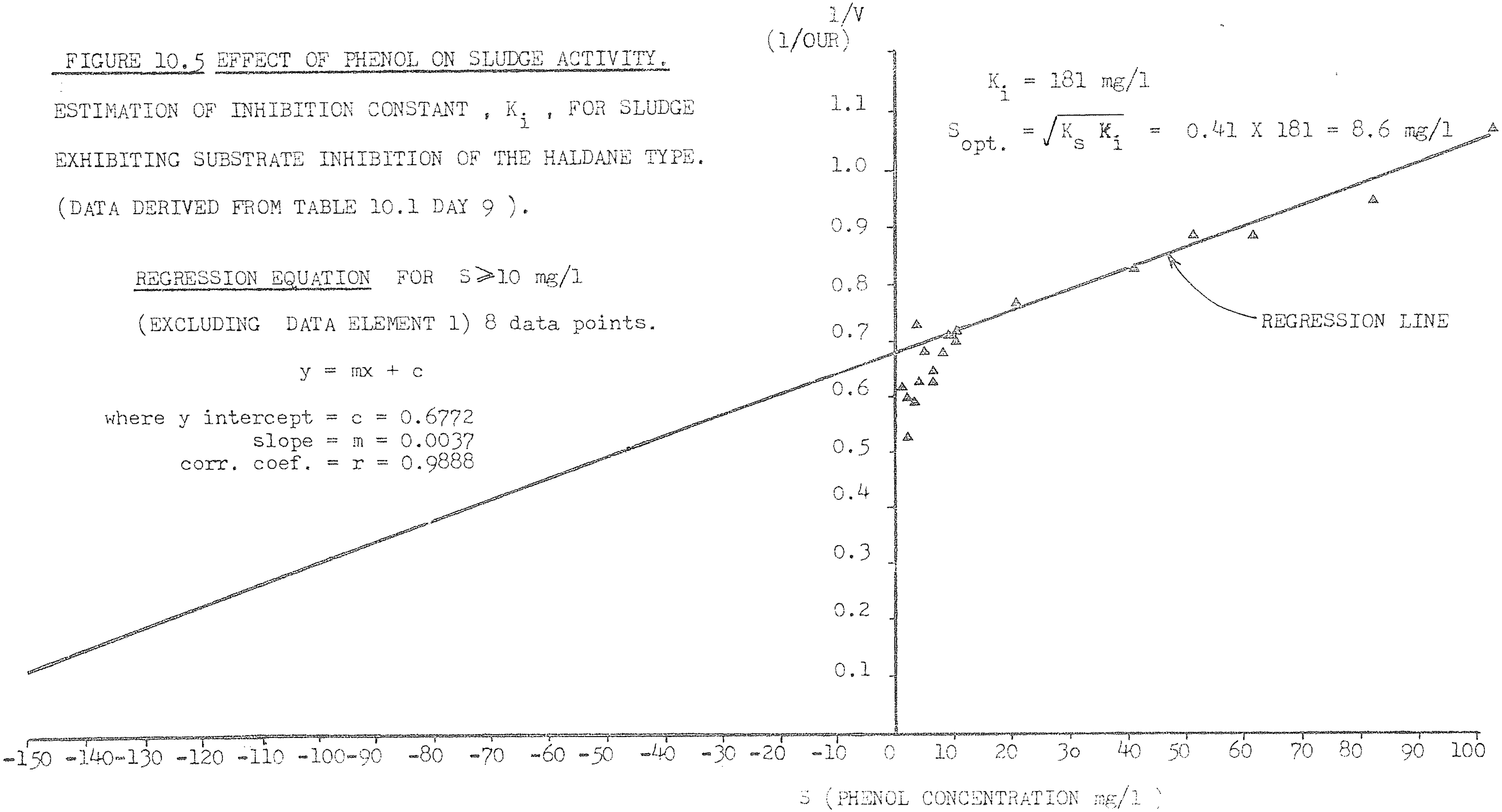


FIGURE 10.5 EFFECT OF PHENOL ON SLUDGE ACTIVITY.

ESTIMATION OF INHIBITION CONSTANT , K_i , FOR SLUDGE EXHIBITING SUBSTRATE INHIBITION OF THE HALDANE TYPE.

(DATA DERIVED FROM TABLE 10.1 DAY 9).



REGRESSION EQUATION FOR $S \geqslant 10$ mg/l

(EXCLUDING DATA ELEMENT 1) 8 data points.

$$y = mx + c$$

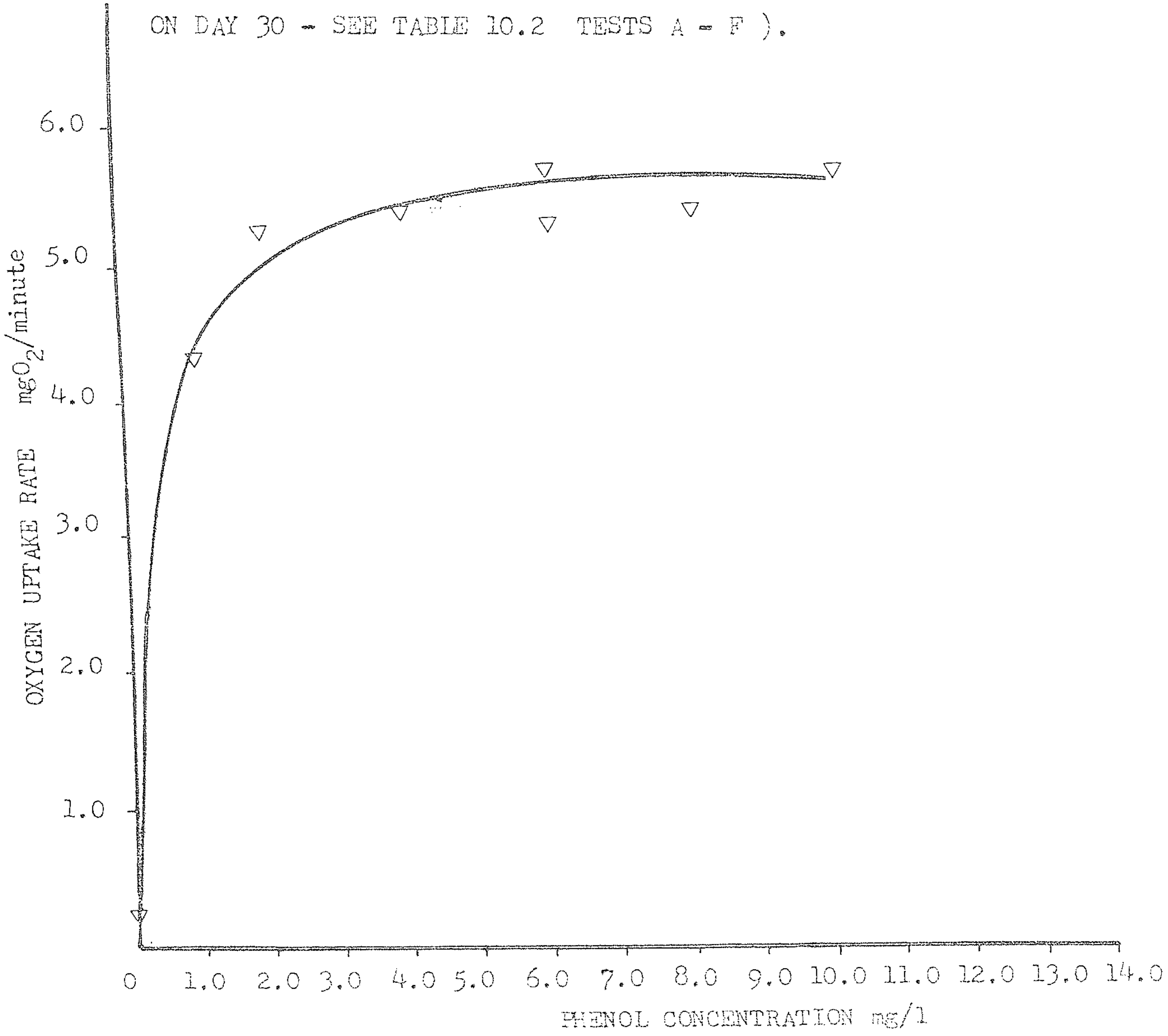
where y intercept = c = 0.6772
slope = m = 0.0037
corr. coef. = r = 0.9888

K_i = 181 mg/l

$S_{opt.} = \sqrt{K_s K_i} = 0.41 \times 181 = 8.6$ mg/l

FIGURE 10.6 EFFECT OF PHENOL ON SLUDGE ACTIVITY.

(RESULTS OBTAINED USING SAMPLES OF SLUDGE TAKEN FROM THE CONTINUOUS REACTOR ON DAY 30 - SEE TABLE 10.2 TESTS A - F).



was assumed in each test sample of sludge just before the injection of the standard solution. This graph failed to indicate evidence of substrate inhibition. This was consistent however with the results of the analysis of the data in Table 10.1 in which inhibition only became obvious at higher concentrations than employed here. The phenol concentration at which OUR was maximal was also consistent with the value for S_{opt} found on day 9.

The value for K_s was determined by using the double reciprocal plot of 1/V vs 1/S as earlier, and linear regression analysis. The result obtained for K_s in Figure 10.7 was 0.33mg phenol/litre and was very similar to the value of 0.41mg/l obtained for Table 10.1 data.

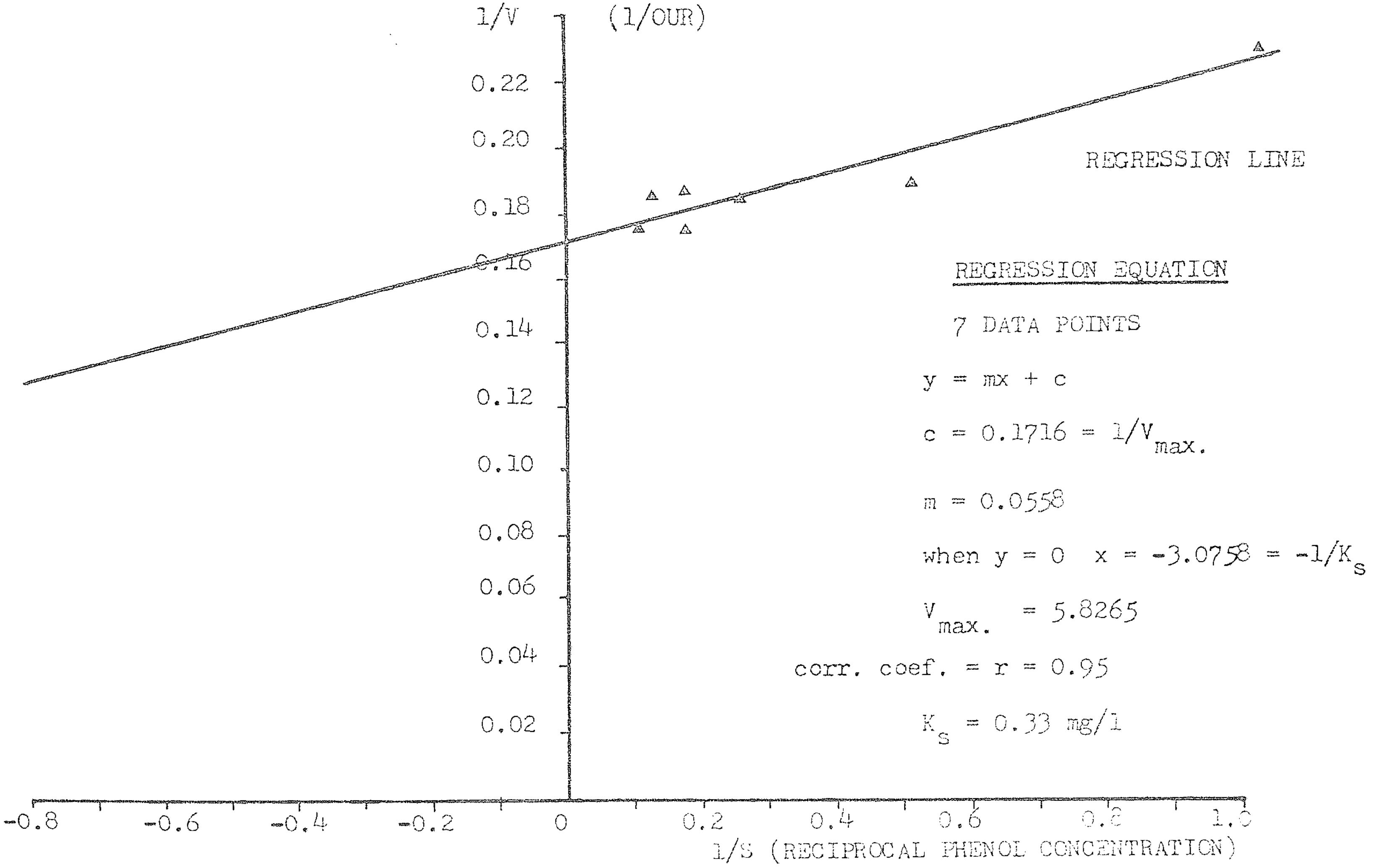
10.7.2.2 Thiocyanate

A graph was plotted in Figure 10.8 relating OUR to the thiocyanate using the data from tests G and H. The concentration of thiocyanate in the respirometer cell following each successive injection of thiocyanate in each test was assumed to approximate to the cumulative total of thiocyanate injected in the test for the purposes of plotting the graph. This assumption was made on the basis that;

1. The initial thiocyanate concentration in the sludge taken from the reactor for the test was nearly zero and could be assumed to be negligible at the start of each test.

FIGURE 10.7 EFFECT OF PHENOL ON SLUDGE ACTIVITY.

ESTIMATION OF SATURATION CONSTANT K_s . (TABLE 10.2 TESTS A-F, DAY 30).



REGRESSION EQUATION

7 DATA POINTS

$y = mx + c$

$c = 0.1716 = 1/V_{max.}$

$m = 0.0558$

when $y = 0 \quad x = -3.0758 = -1/K_s$

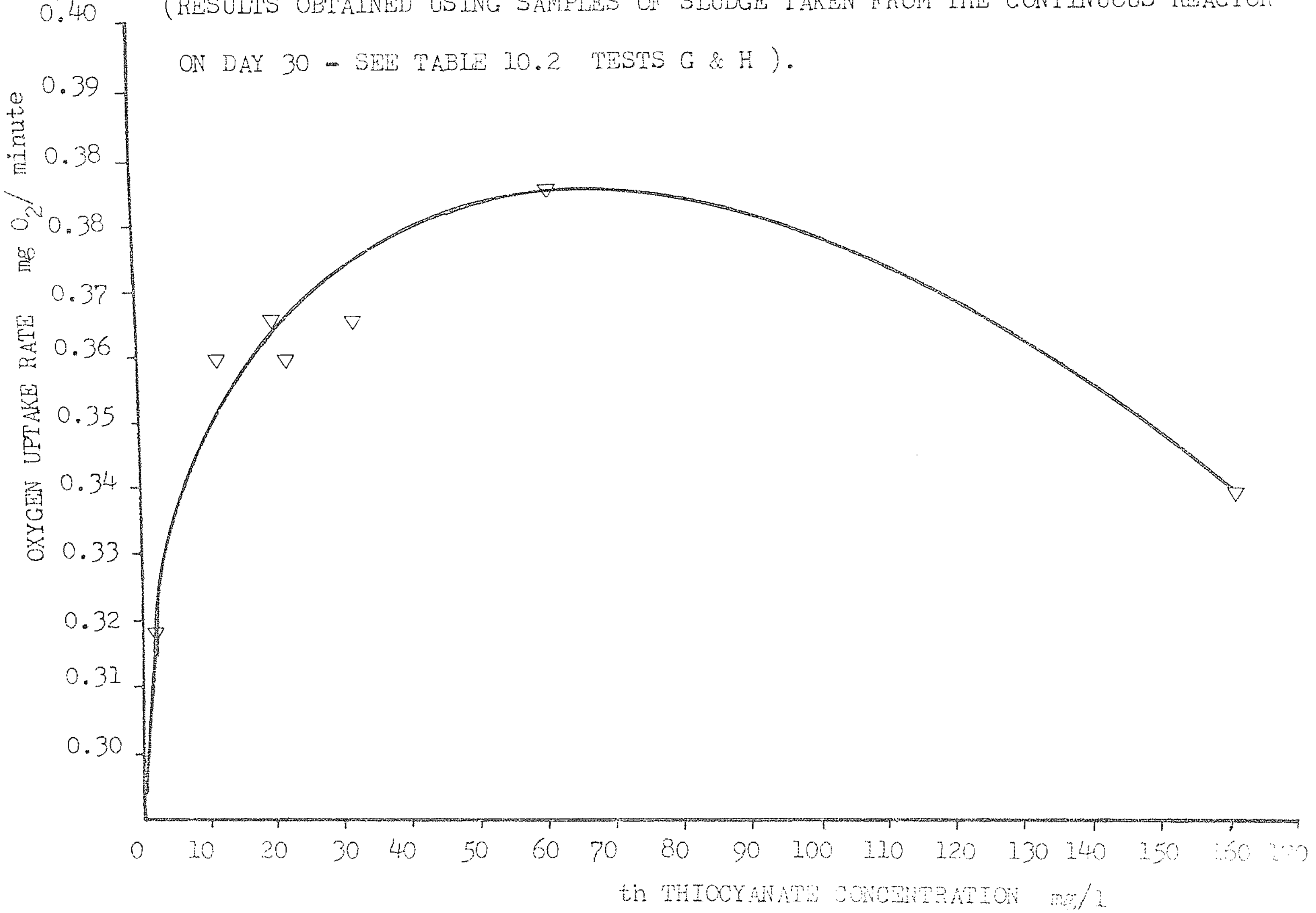
$V_{max.} = 5.8265$

corr. coef. = $r = 0.95$

$K_s = 0.33$ mg/l

FIGURE 10.8 EFFECT OF THIOCYANATE ON SLUDGE ACTIVITY.

(RESULTS OBTAINED USING SAMPLES OF SLUDGE TAKEN FROM THE CONTINUOUS REACTOR ON DAY 30 - SEE TABLE 10.2 TESTS G & H).



2. Only a short period separated successive injections of substrate in both tests.

3. The rate of thiocyanate oxidation by the sludge was apparently small.

The sludge phenol concentration was assumed to be negligible at the start of each test because very low levels were recorded in reactor sludge samples taken later on that date.

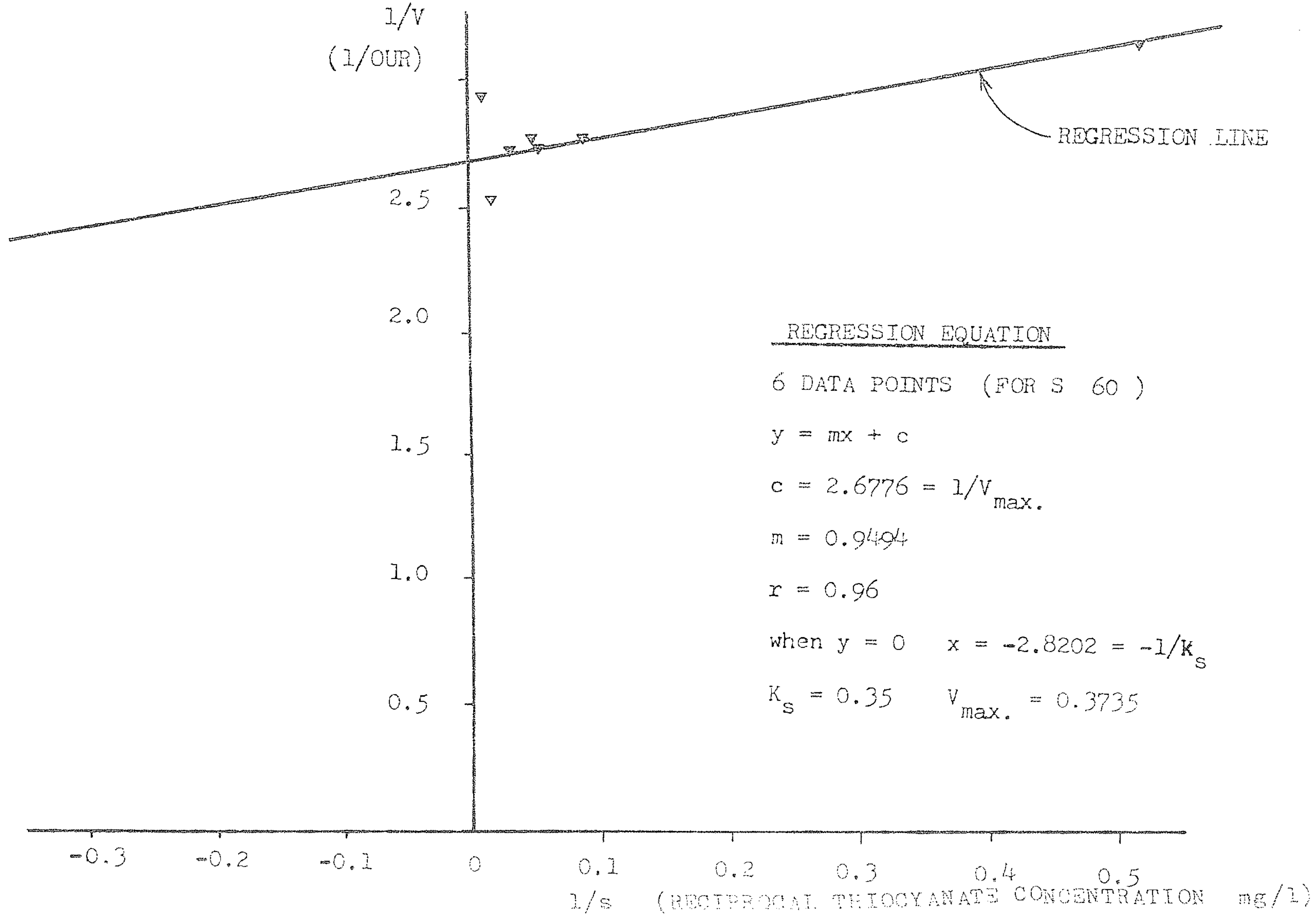
Examination of Figure 10.8 appeared to indicate that thiocyanate oxidation in the sludge was subject to substrate inhibition. Such a conclusion however could only be made on the basis of the single observation of the OUR at the thiocyanate concentration of 160mg/l in test H, and was clearly unwise on the basis of such limited data.

The value of K_s was determined by using the double reciprocal plot of 1/V vs 1/S as earlier, and linear regression analysis. The result obtained for K_s in Figure 10.9 was 0.35mg/l.

(If the sludge was assumed to exhibit substrate inhibition with respect to thiocyanate oxidation Figure 10.10 shows that the value for K_i determined as described earlier, would be of the order of 690mg/l).

FIGURE 10.9 EFFECT OF THIOCYANATE ON SLUDGE ACTIVITY.

ESTIMATION OF SATURATION CONSTANT K_s (TABLE 10.2 TESTS G & H , DAY 30).



REGRESSION EQUATION

6 DATA POINTS (FOR S 60)

$y = mx + c$

$c = 2.6776 = 1/V_{max.}$

$m = 0.9494$

$r = 0.96$

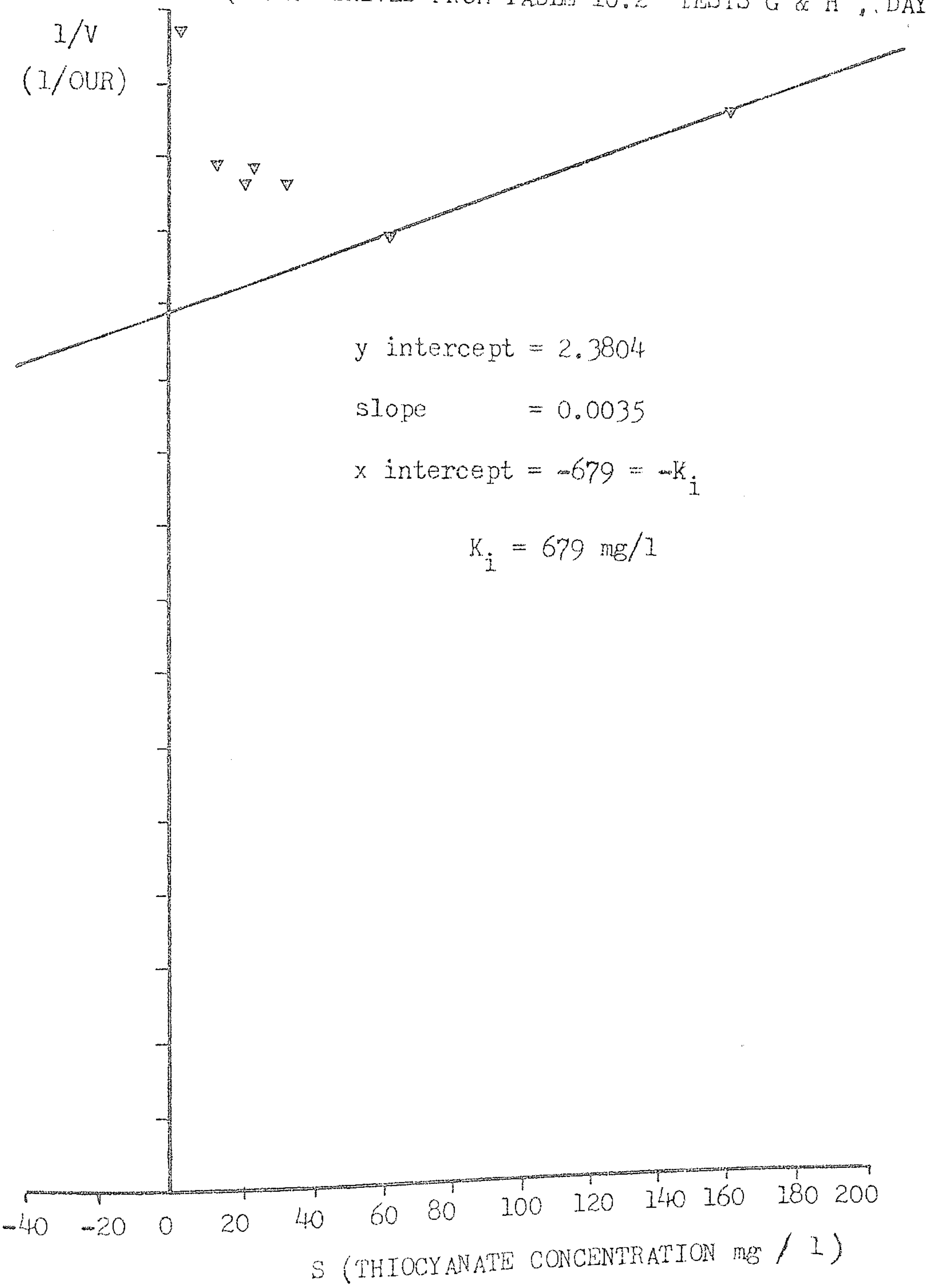
when $y = 0$ $x = -2.8202 = -1/K_s$

$K_s = 0.35$ $V_{max.} = 0.3735$

FIGURE 10.10 EFFECT OF THIOCYANATE ON SLUDGE ACTIVITY.

ESTIMATION OF INHIBITION CONSTANT K_i ASSUMING SLUDGE EXHIBITS SUBSTRATE INHIBITION OF THE HALDANE TYPE.

(DATA DERIVED FROM TABLE 10.2 TESTS G & H , DAY 30).



y intercept = 2.3804

slope = 0.0035

x intercept = -679 = $-K_i$

K_i = 679 mg/l

10.7.3 Tests Conducted on Day 23 (Table 10.3)

10.7.3.1 Phenol

A graph was plotted in Figure 10.11 relating OUR to phenol concentration as earlier, using the results of tests 1A-7A. The graph produced was similar to those plotted in Figures 10.3 and 10.6 in as much that OUR became maximal in the phenol concentration range 2-10mg/l. There was some indication of substrate inhibition in Figure 10.11 but there were insufficient data to confirm this conclusively or to accurately determine the value of K_s. Visual inspection of the graph however indicated a value for K_s similar to that found in the tests analysed earlier.

10.7.3.2 Ammonia

Tests 22A to 31A inclusive were conducted to investigate;

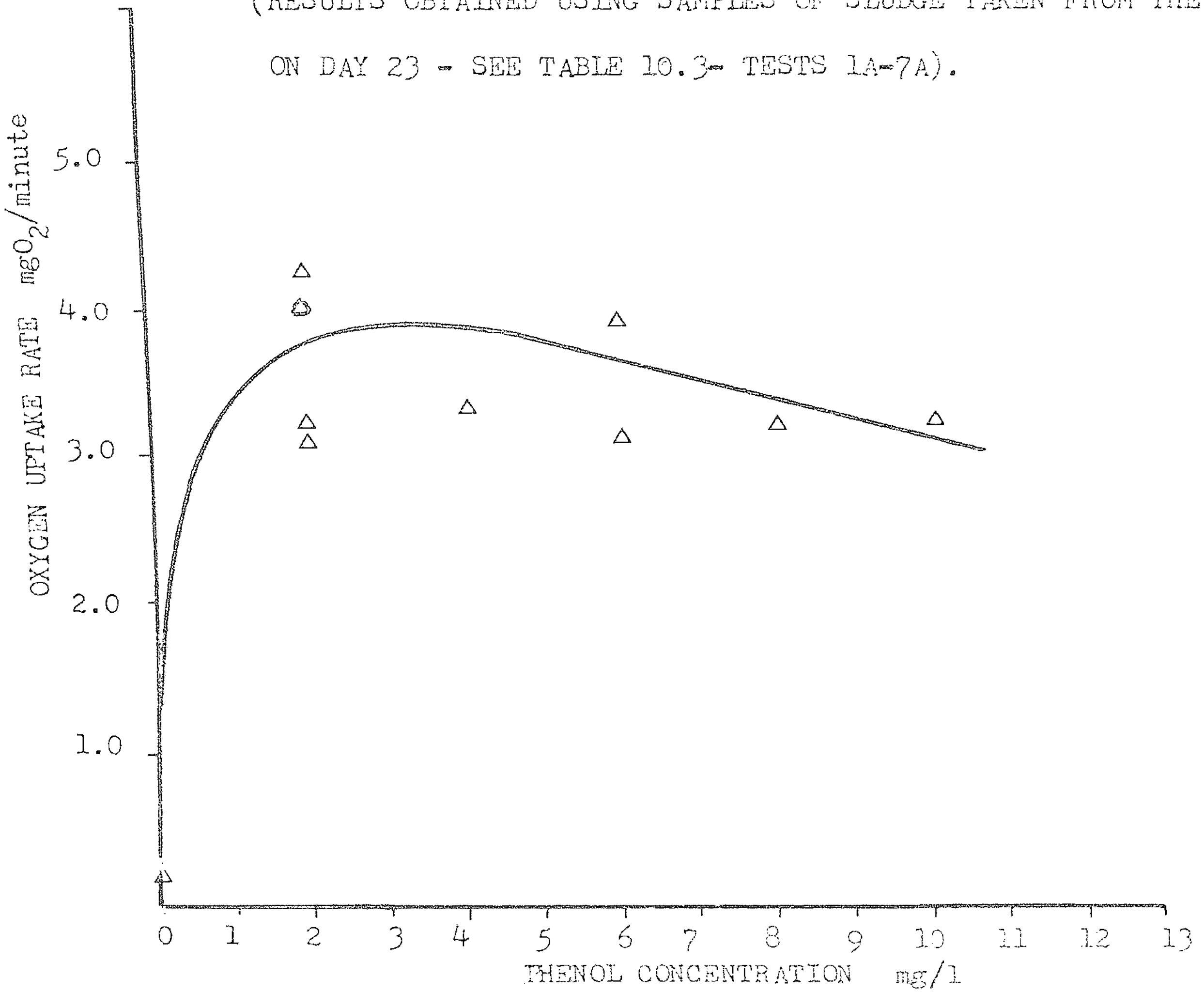
1. The occurance and kinetics of nitrification in the sludge.

2. The effect of ammonia on phenol oxidation.

In each test run an aliquot of the stock ammonia solution was injected after the initial OUR had been recorded and before the injection of the phenol solution. OURs were measured after the injection of each substrate. Phenol, thiocyanate, and

FIGURE 10.11 EFFECT OF PHENOL ON SLUDGE ACTIVITY.

(RESULTS OBTAINED USING SAMPLES OF SLUDGE TAKEN FROM THE CONTINUOUS REACTOR ON DAY 23 - SEE TABLE 10.3- TESTS 1A-7A).



ammonia analysis of the reactor sludge on the day of these tests gave results of 1.12mg/l, 0.6mg/l and 1460mg/l respectively, and so phenol and thiocyanate concentrations were assumed negligible at the start of each test.

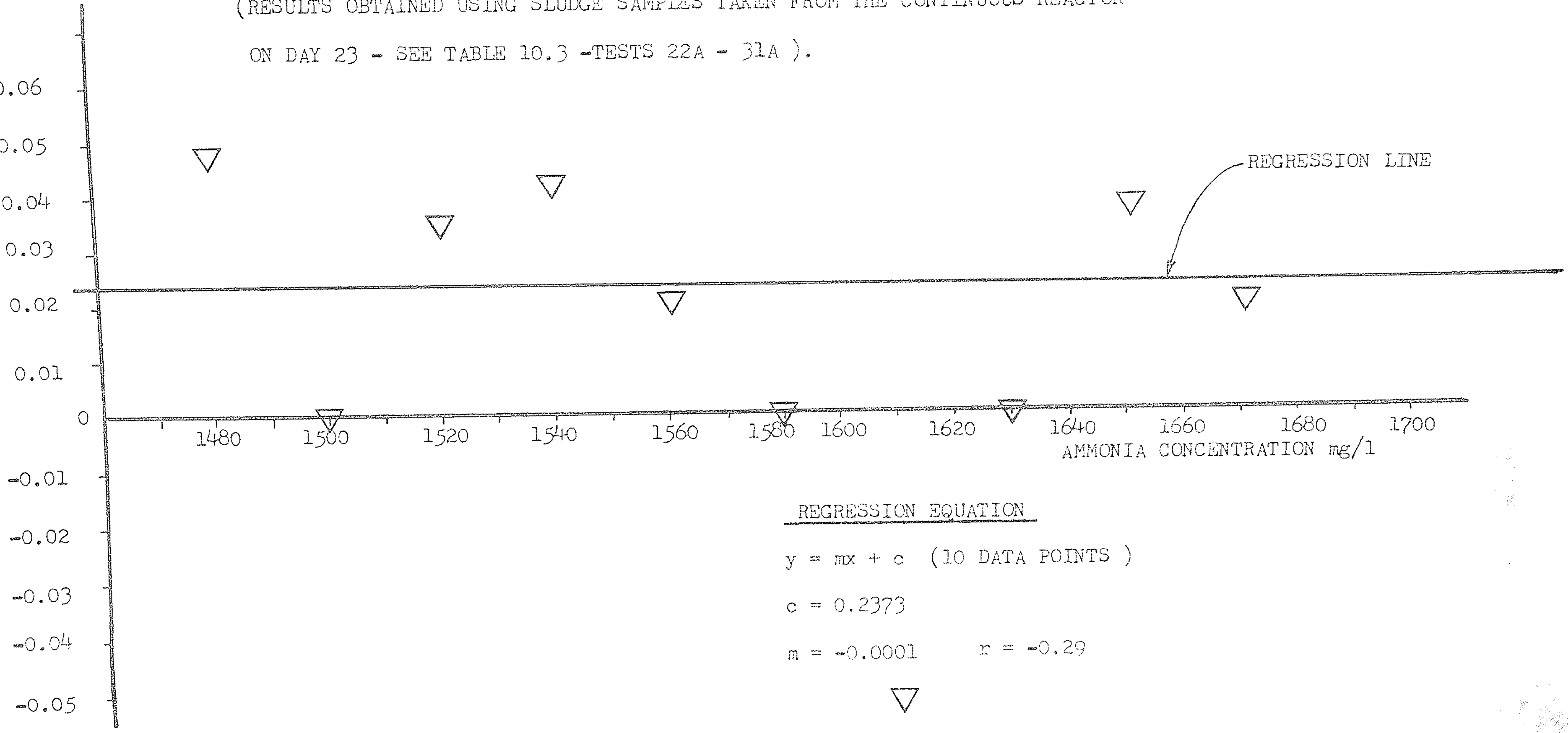
The change in the OUR in each test run following the addition of the stock ammonia solution was very small and may merely have reflected the errors in measuring the gradient of the dissolved oxygen curves on the chart records. A graph was plotted in Figure 10.12 relating the change in OUR following the injection of ammonia to the resultant ammonia concentration in the sludge sample under test. The resultant ammonia concentration was calculated by summing the initial ammonia concentration (1460mg/l) and that due to the addition of the stock ammonia solution. The "best-fit" line was drawn following linear regression analysis. The equation for this line had a negligible slope, and a correlation coefficient which indicated that the changes in OUR following the ammonia additions were not significant. These results and the low initial OUR's observed in these test runs, which were consistent with endogenous respiration, were concluded to be indicative of a non-nitrifying sludge.

10.7.3.3 Effect of Ammonia on Phenol Oxidation

A graph was plotted in Figure 10.13 relating OUR following injection of phenol to the ammonia concentration, using the results of test runs 21A-31A inclusive. A regression line was drawn which had a negative slope and indicated that phenol

FIGURE 10.12 EFFECT OF AMMONIA ON SLUDGE ACTIVITY.

(RESULTS OBTAINED USING SLUDGE SAMPLES TAKEN FROM THE CONTINUOUS REACTOR ON DAY 23 - SEE TABLE 10.3 -TESTS 22A - 31A).



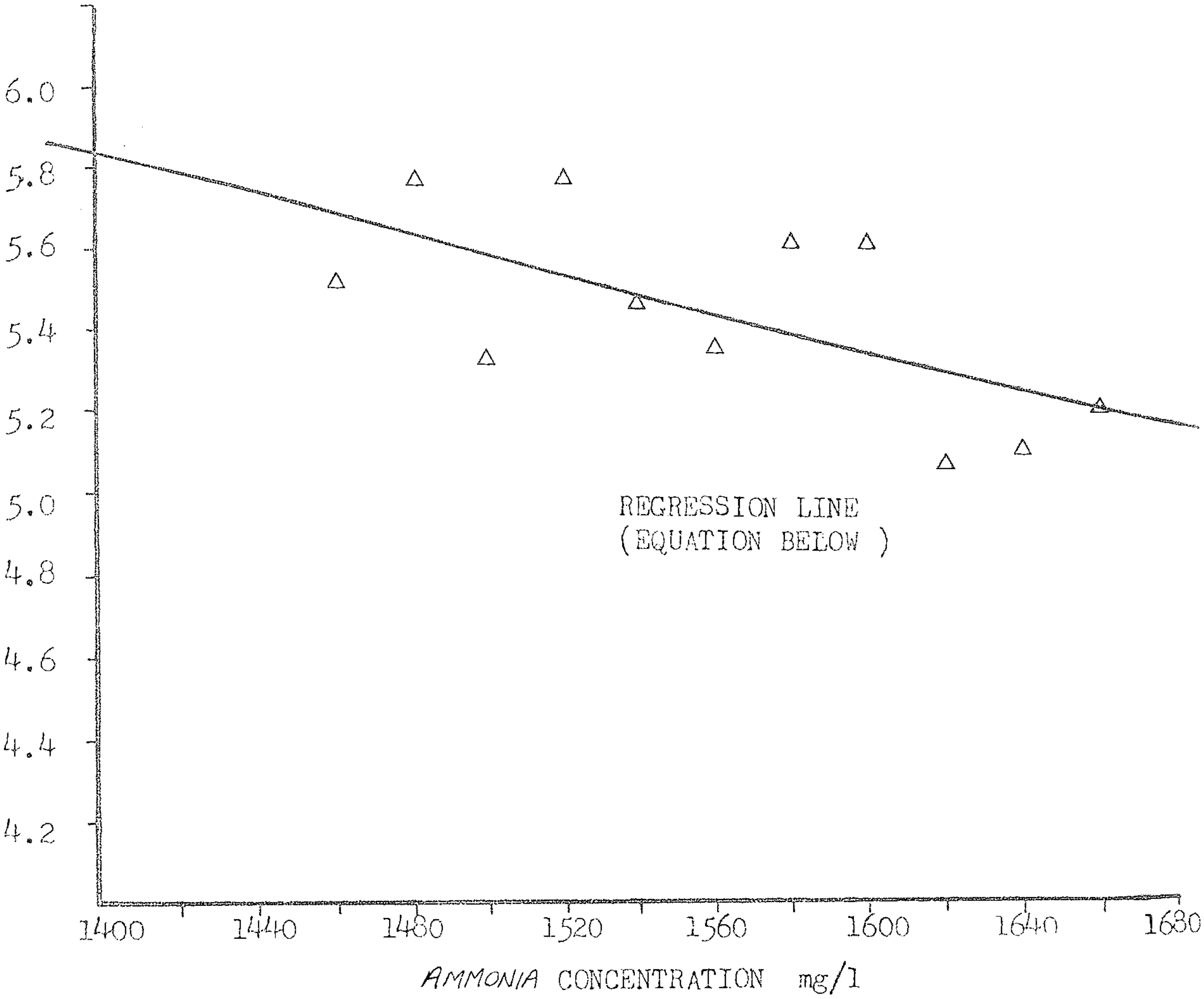
REGRESSION EQUATION

$y = mx + c$ (10 DATA POINTS)

$c = 0.2373$

$m = -0.0001$ $r = -0.29$

FIGURE 10.13 EFFECT OF AMMONIA ON PHENOL OXIDATION.
(DAY 23 TABLE 10.3)



LINEAR REGRESSION ANALYSIS

TEST RUN	AMMONIA CONCENTRATION x	OUR (PHENOL) y
21A	1460	5.535
22A	1500	5.355
23A	1540	5.500
24A	1580	5.648
25A	1620	5.100
26A	1660	5.240
27A	1560	5.385
28A	1600	5.655
29A	1520	5.800
30A	1640	5.138
31A	1480	5.790

REGRESSION EQUATION

11 DATA POINTS
$y = mx + c$

y intercept = $c = 9.006$

slope = $m = -0.0023$

corr. coef. = $r = 0.61$

With Degrees Of Freedom $\phi = 9$ and a calculated r value = 0.61 the correlation between AMMONIA CONCENTRATION (x) and OUR due to PHENOL OXIDATION is significant at the 95% confidence level.

($P = 0.95$).

oxidation was inhibited by ammonia. The correlation between OUR and ammonia concentration was shown to be significant at the 95% confidence level.

10.7.3.4 Glucose

There was little or no significant increase in the OUR of the sludge in response to the addition of glucose and there appeared to be no significant effect on phenol oxidation when phenol was subsequently added to the sludge in test 21A. This test, and other similar tests which were not recorded here, were intended as a preliminary study to indicate whether glucose could act as a major substrate for a sludge treating coke oven liquor. It will be recalled from Chapter 3 that research at the BCRA had indicated that glucose, and other compounds, could be used to improve treatment stability and performance in activated sludge plants treating coke oven liquor. The possible reasons for these improvements were discussed in that Chapter.

Although these experiments did not investigate the long term effects of glucose on sludge activity, it was clear that in the short term that glucose was not utilised as a major substrate in the way that phenol was.

10.7.3.5 Effect of Thiocyanate on Phenol Oxidation

Tests 8A-19A inclusive were conducted to investige the effect of thiocyanate on phenol oxidation. Four different phenol concentrations, 1, 2, 3, and 4mg/l were chosen, and the effect of a range of thiocyanate concentrations on each assessed. These various mixtures of phenol and thiocyanate concentrations were achieved by preparing and then injecting into the respirometer cell appropriate mixture and dilutions of stock solutions, as outlined in Table 10.3.

A graph was plotted in Figure 10.14 relating the OUR on the thiocyanate concentration for each of the four phenol concentrations. From this representation of the results it appeared that the OUR was independent of the thiocyanate concentrations at phenol concentrations of 3 and 4mg/l. The mean differences in OUR observed at these two phenol concentrations approximated to those predicted by the relationships shown earlier for phenol oxidation in the absence of thiocyanate.

In Figure 10.16 the graph plotted related the sludge OUR to the phenol concentration for each thiocyanate concentration employed. This representation of the results showed that OUR became maximal at a phenol concentration of 2-4mg/l, and this was consistent with the general trend shown in the phenol vs OUR graphs plotted earlier. Visual inspection of the graph at phenol levels of 2 and 3mg/l indicated that the maximal values of the OUR were inversely related to the thiocyanate level,

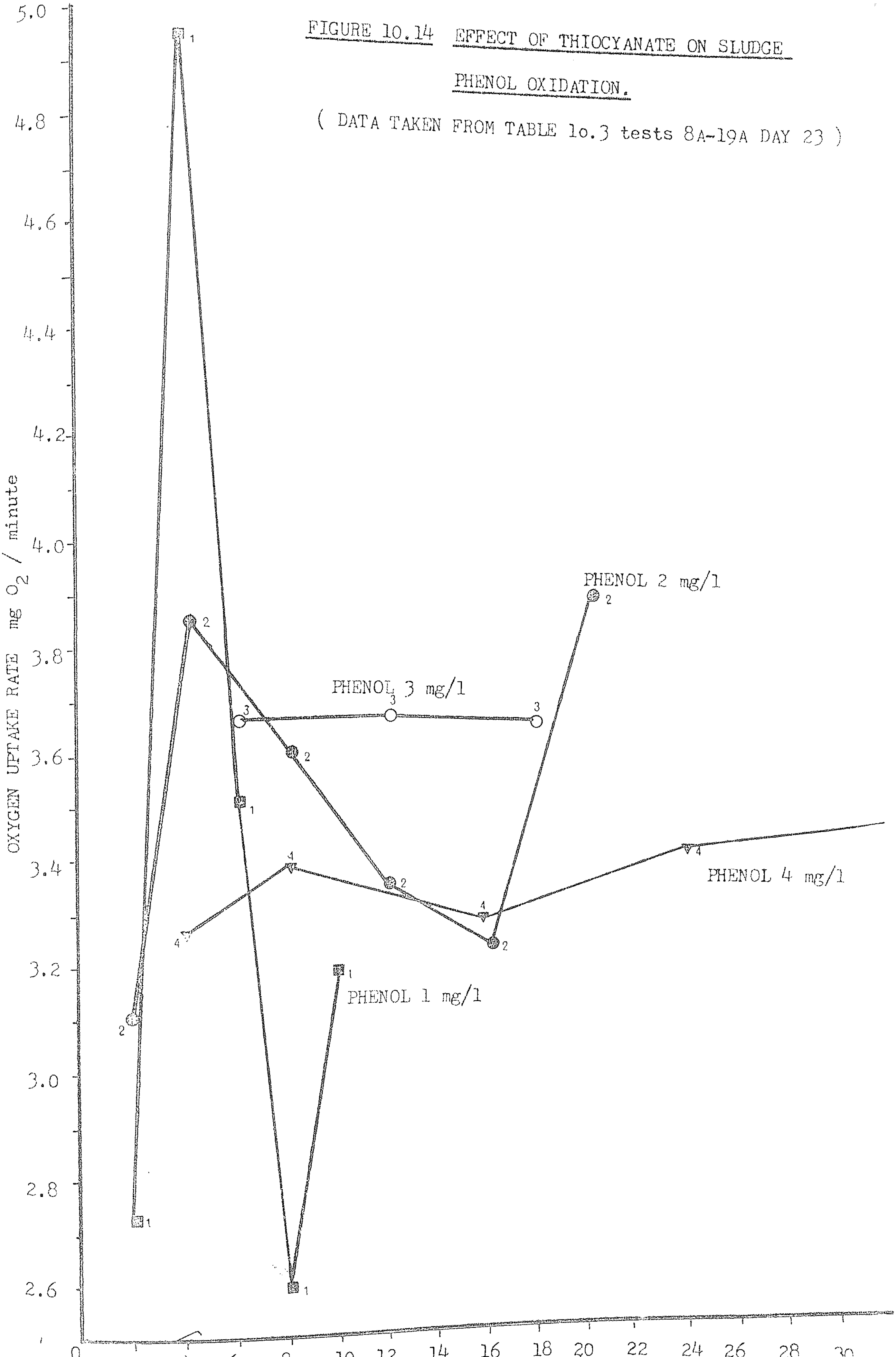


FIGURE 10.14 EFFECT OF THIOCYANATE ON SLUDGE PHENOL OXIDATION.

(DATA TAKEN FROM TABLE 10.3 tests 8A-19A DAY 23) with the exception of the value corresponding to a thiocyanate concentration of 2mg/l. The differences in these maximal values are small, but may have indicated thiocyanate inhibition of phenol oxidation at these phenol concentrations. These differences were no longer apparent however at the 4mg/l phenol level. The possibility of thiocyanate inhibition of phenol oxidation was investigated by further graphical analysis of the data for tests 8A-19A.

Inhibitions caused by a chemical on an enzyme or microbial system are generally categorised into 3 types, competitive, non-competitive, and uncompetitive. These type of inhibition can be identified by graphical analysis of the kinetic data, and by comparing the results of the analysis with the patterns of behaviour characteristic of the various types of inhibition. Figure 10.15 shows 2 examples of graphical methods used to identify the various types of inhibition. (More complex types of inhibition, and other graphical methods of analysis are known, substrate inhibition, described earlier, being one such example).

The graphical method of plotting 1/V vs i shown in Figure 10.15 was applied to the analysis of the data in this section, and Figure 10.16 shows the results of a plot of the reciprocal of OUR vs thiocyanate concentration.

The results of linear regression analysis of the data from tests 8A-19A were also summarised in Figure 10.16 and regression lines corresponding to each phenol concentration drawn.

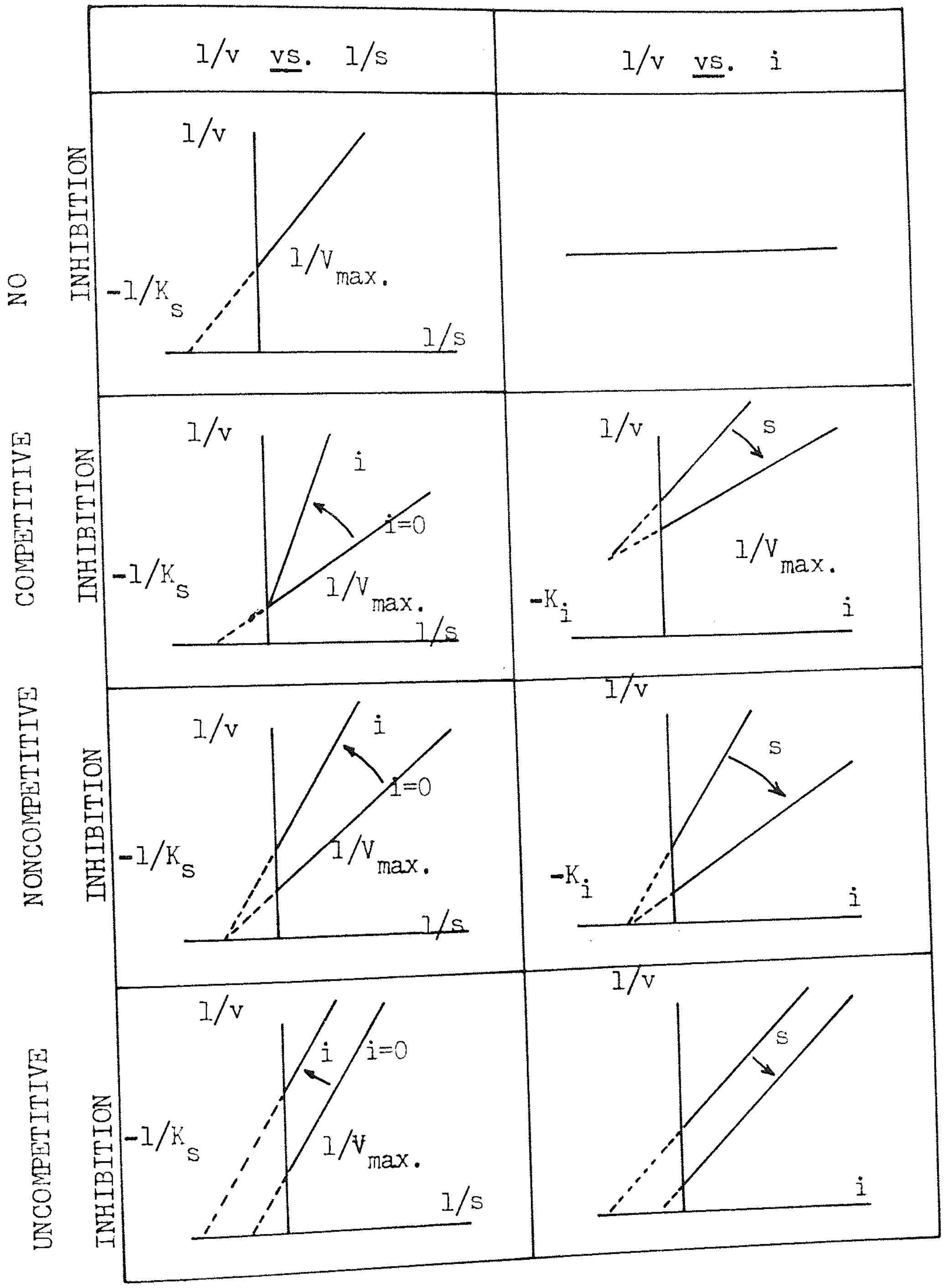
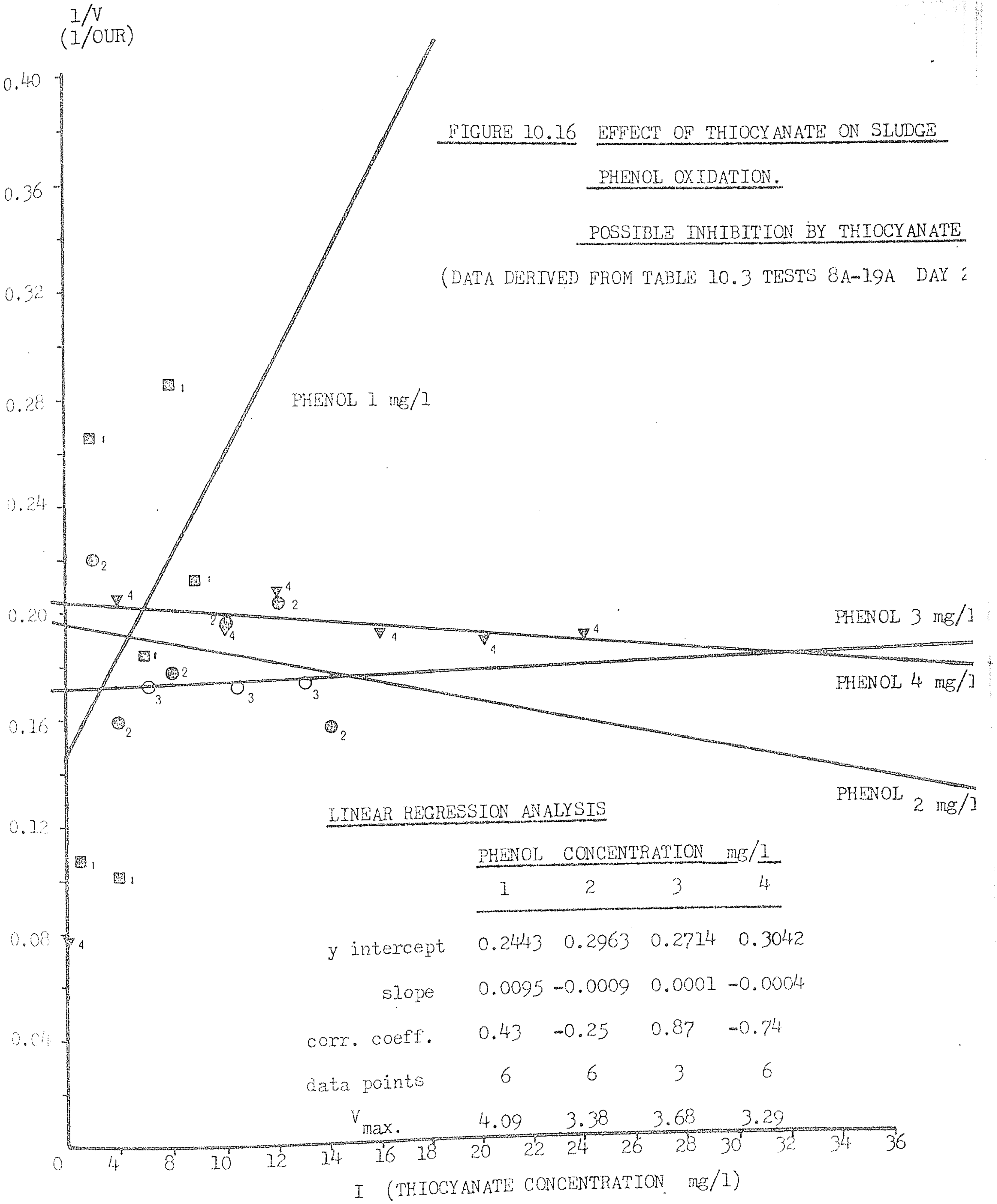


FIGURE 10.15 GRAPHICAL METHODS FOR THE ANALYSIS OF SOME KINETIC SCHEMES.

FIGURE 10.16 EFFECT OF THIOCYANATE ON SLUDGE PHENOL OXIDATION.

POSSIBLE INHIBITION BY THIOCYANATE

(DATA DERIVED FROM TABLE 10.3 TESTS 8A-19A DAY



LINEAR REGRESSION ANALYSIS

	PHENOL CONCENTRATION mg/l			
	1	2	3	4
y intercept	0.2443	0.2963	0.2714	0.3042
slope	0.0095	-0.0009	0.0001	-0.0004
corr. coeff.	0.43	-0.25	0.87	-0.74
data points	6	6	3	6
$V_{max.}$	4.09	3.38	3.68	3.29

The graphs drawn for phenol concentrations of 2, 3, and 4mg/l were almost horizontal indicating the absence of thiocyanate inhibition. Extrapolation of these lines to zero thiocyanate concentration produced values for Vmax (1/y intercept value) of 3.38, 3.68, and 3.29mg O_2/minute for phenol concentrations of 2, 3, and 4mg/l. The mean of these values, 3.45mg O_2/ minute, was very similar to the mean of the maximal OUR value of 3.5) mg O_2/min calculated from Figure 10.11 for phenol concentrations of 2.10mg/l.

The graph drawn corresponding to a phenol concentration of 1mg/l in Figure 10.16 was very different from those produced for higher concentrations, and indicated some form of inhibition at this level. As with earlier analysis of the data in this section, the results produced corresponding to the phenol concentration of 1mg/l were not consistent with the trends shown at higher phenol concentrations. No obvious reasons were apparent, or explanations offered here for this behaviour.

The significance of the results reported in this chapter were discussed later in this report.

CHAPTER 11

MODELLING OF THE DESIGN AND OPERATION OF THE COMPLETELY-MIXED ACTIVATED SLUDGE TREATMENT OF COKE OVEN LIQUOR FOR PHENOL REMOVAL, USING PILOT AND FULL-SCALE PLANT PERFORMANCE DATA

Introduction

11.1 Design and Operational Models for Continuous Activated Sludge Treatment

11.2 Experimental Procedures

  11.2.1 Pilot Plant Studies

  11.2.2 Full-Scale Plant Studies

11.3 Results and Discussion

  11.3.1 Determination of Kinetic Constants for Phenol Oxidation from Pilot Plant Studies

  11.3.2 Determination of the Kinetic Constants for Phenol Oxidation from Full-Scale Plant Data

  11.3.3 Design and Operation of Plant for Phenol Removal

  11.3.4 Comparison with Kinetic Coefficients for Phenol Oxidation in Simple Mineral Media

  11.3.5 Significance of Substrate Inhibition

  11.3.6 Thiocyanate and Ammonia Removal

11.4 Summary

MODELLING OF THE DESIGN AND OPERATION OF THE COMPLETELY-MIXED ACTIVATED SLUDGE TREATMENT OF COKE OVEN LIQUOR FOR PHENOL REMOVAL, USING PILOT AND FULL-SCALE PLANT PERFORMANCE DATA

Introduction

There are a variety of different methods of modelling the activated sludge process and for determining the value of the various kinetic constants required, and these were briefly discussed in Chapter 3. A number of workers, including TOMLINS for example, have used laboratory-scale batch reactor studies to estimate the value of the kinetic constants. However, it was evident from TOMLINS' studies on the treatment of coke oven liquor and from studies on domestic wastewater treatment, that difficulties are often encountered in relating kinetic data or models derived from laboratory or even pilot-scale plants to those treating the waste in full-scale plants. It would be useful therefore to be able to derive the value of the kinetic constants from data collected from pilot and full-scale plants treating coke oven liquor and to compare these with those values derived from laboratory studies. Design and operational modelling techniques applied to domestic wastewater treatment studies, do not appear to have been applied to the study of the treatment of coke oven liquors, but would appear to have this potential value for determination of kinetic constants.

Models describing the treatment of liquor at laboratory scale level will only have practical value if they can be shown to have relevance to full-scale treatment of liquor. A first approach in testing such models is to compare the value of kinetic parameters derived from laboratory and full-scale plants. Close correlation between these values will indicate a good basis for further extrapolating findings from laboratory scale work to full-scale plant, providing of course that the same fundamental modelling approach has been used in analysing both types of plant.

The examination of plant performance data from full-scale plants, and comparisons with laboratory work does of course form a sound basis for testing and developing the model described earlier. The development of a design and operational model of the treatment of coke oven liquor would have considerable practical usefulness in the operation of existing and future plants in defining not only the dimensional basis for the construction of the treatment works, but also the limits in the operation of the system within which satisfactory treatment may be expected. As described in Chapter 3, such a model would be likely to be less useful than a dynamic model of the process, but may be viewed as a preliminary stage in the development of the latter.

This chapter considers the application of design and operational modelling of the treatment process, and continues the approach made earlier of considering the treatment of the major components of the waste individually, with only phenol removal being considered in detail here.

11.1 Design and Operational Models for Continuous Activated Sludge Treatment

Mathematical models developed by McKINNEY,(141) ECKENFELDER,(142) and LAWRENCE(143) and McCARTY, and more recently GAUDY,(144), (145), (146) are commonly used by design engineers to predict reactor biomass and effluent substrate concentrations in steady state conditions in activated sludge systems with and without organism recycle. The use of these models in design have largely replaced the older "rules of thumb" techniques which are used to design many of the older plants. (These models do not seem to have been applied to plants treating coke oven liquor, the majority of which appear to have been designed on the same basis as plants treating domestic sewage, a mistake compounded by the use of outdated design techniques).

The design models have been based on the construction of material balance equations for the treatment systems, and on the basic metabolic and microbiological principles used to develop the equations described in Chapter 3. Figure 11.1 shown overpage shows a flow diagram of such a treatment system, a single stage complete mixing activated sludge system, whose operation was described in Chapter 3.

The material balance equations for substrate and biomass used by MCKINNEY, ECKENFELDER, LAWRENCE and MCCARTY, and GAUDY, are shown in Table 11.1. From these it can be seen that GAUDY'S model is significantly different from the others, in that it includes X_R and α as control factors in the model. This has arisen because GAUDY considered the material balances for X and S around the reactor, and around the settler, (areas enclosed by A and B in Figure 11.1) separately. In contrast, the other investigators applied the equations to the entire system, the area enclosed by C in Figure 11.1.

FIGURE II.I FLOW DIAGRAM OF A SINGLE-STAGE COMPLETE-MIXING ACTIVATED SLUDGE PLANT.

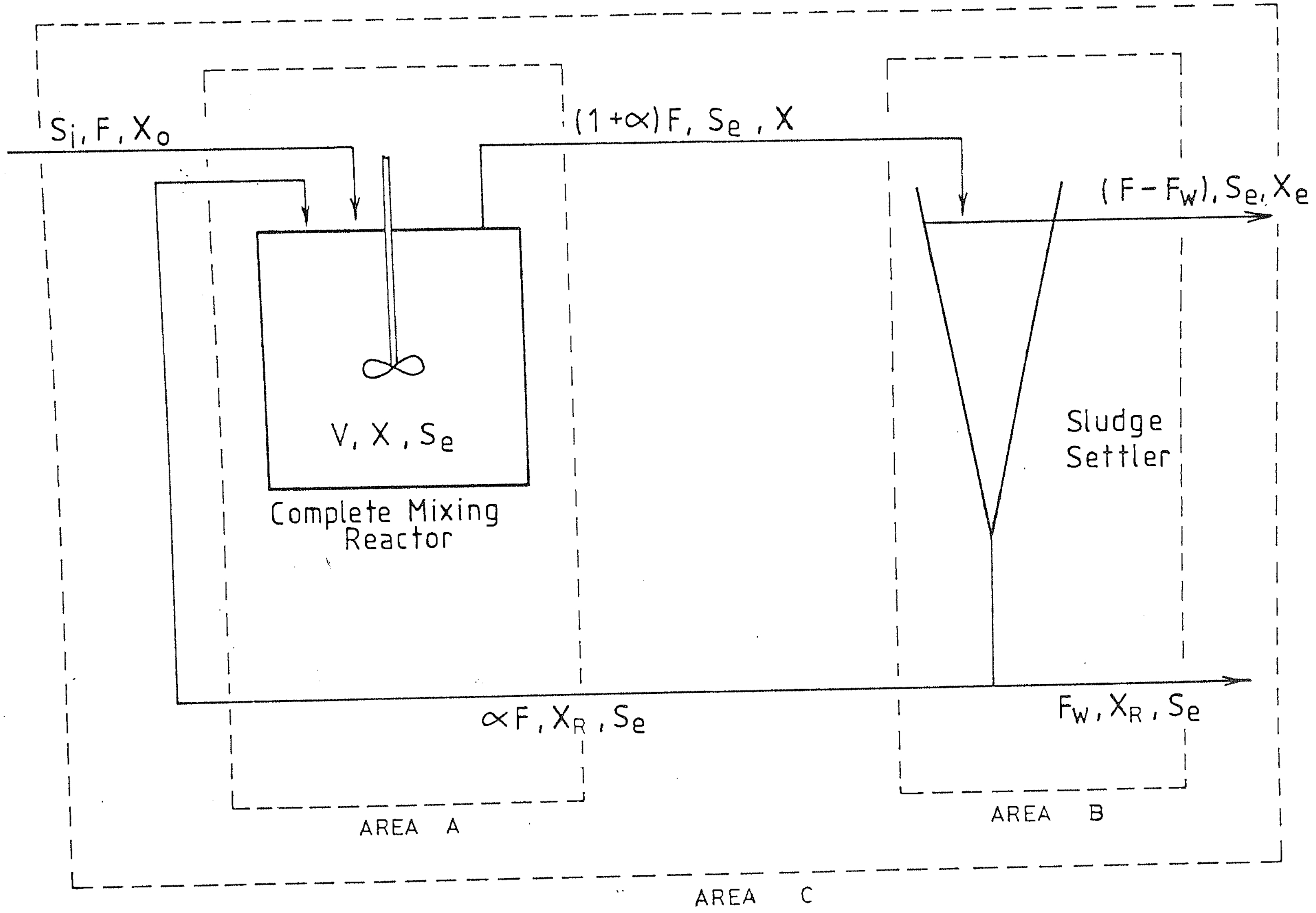


TABLE II.I THE MATERIAL BALANCE EQUATIONS USED BY VARIOUS RESEARCHERS.

MODEL	MATERIALS BALANCE FOR BIOMASS					MATERIALS BALANCE FOR SUBSTRATE			
	NET ACCUMULATION OF CELLS	INCREASE DUE TO INFLOW TO REACTOR	INCREASE DUE TO GROWTH	DECREASE DUE TO AUTODIGES-TION	DECREASE DUE TO OUTFLOW FROM REACTOR	NET DECREASE OF SUBSTRATE	INCREASE DUE TO INFLOW TO REACTOR	DECREASE DUE TO OUTFLOW FROM REACTOR	DECREAS DUE TO CELL GROWTH
CKENFELDER	$\frac{dX.V}{dt}$	$= F X_o$	$+ Y_t(Si-Se)F$	$- K_d X V$	$- (F-F_w)X_e - F_w X_R$	$\frac{dS.V}{dt}$	$= F S_i$	$- F S_e$	$- K_e X S_e V$
GAUDY	$\frac{dX.V}{dt}$	$= FX_o + \alpha F X_R$	$+ \frac{\mu_{max} X \cdot Se.V}{Ks + Se}$	$- K_d X V$	$- F(1+\alpha) X$	$\frac{dS.V}{dt}$	$= FS_i + \alpha F S_e$	$- F(1+\alpha) S_e$	$- \mu_{max}\left(\frac{X}{Y_t}\right)\left(\frac{Se}{Ks+Se}\right)$
LAWRENCE AND McCARTY	$\frac{dX.V}{dt}$	$= FX_o$	$+ \frac{Y_t \cdot K \cdot Se.V}{Ks + Se}$	$- K_d X V$	$- (F-F_w)X_e - F_w X_R$	$\frac{dS.V}{dt}$	$= FS_i$	$- FS_e$	$- KXV \frac{Se}{Ks + S}$
McKINNEY	$\frac{dX.V}{dt}$	$= F X_o$	$+ Y_t.K_m.S.V$	$- K_d X V$	$-(F-F_w)X_e - F_w X_R$	$\frac{dS.V}{dt}$	$= F S_i$	$- F S_e$	$- K_m S_e V$

GAUDY'S model appears to have distinct advantages over the others because it enables the designer and treatment works operator to observe the effects of all three controllable variables, dilution rate D, α, and X_R that control the treatment system. This can be done by applying the equations for prediction of reactor biomass X, effluent substrate, S_e, and sludge production X_W, shown in Table 11.2 to the treatment system.

The kinetic constants K_s, μ_{max}, Y_t, and K_d can be determined, for example, from laboratory scale or pilot plant treating the waste under consideration. The plant is operated under a variety of growth conditions by varying D, α , and X_R, and for each set of conditions, substrate and biomass concentrations in the system are measured when steady-state conditions are achieved.

From this data, the net growth Rate μ_n, the observed yield coefficient Y_o, and specific utilisation U can be determined for GAUDY'S model using his equations;

$$\mu_n = \frac{F_w X_w + (F - F_w)\ X_e}{VX} \qquad \underline{\text{EQUATION 11.1}}$$

$$Y_o = \frac{F_w X + (F - F_w)\ X_e}{F(S_i - S_e)} \qquad \underline{\text{EQUATION 11.2}}$$

$$U = \frac{S_i - S_e}{X} \cdot \frac{F}{V} \qquad \underline{\text{EQUATION 11.3}}$$

Table II.2 EQUATIONS USED BY GAUDY FOR PREDICTION OF X , S_e , AND X_w .

$$X = \frac{Y_t\,[S_i - (1 + \alpha)S_e] + \alpha X_R}{(1 + \alpha) + \frac{K_d}{D}} \qquad (\quad)$$

$$S_e = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \qquad (\quad)$$

where

$$a = \mu_{max.} - (1 + \alpha)D - K_d$$

$$b = D[\,S_i - (1 + \alpha)K_s] - \frac{\mu_{max.}}{1 + \alpha}\left(S_i + \frac{\alpha X_R}{Y}\right) + K_d\left(\frac{S_i}{1 + \alpha} - K_s\right)$$

$$c = K_s D S_i + \frac{K_d}{1 + \alpha} K_s S_i$$

$$X_w = VX\left[D\left(1 + \alpha - \alpha\frac{X_R}{X}\right)\right] \qquad (\quad)$$

and Y_t, and K_d determined graphically using the equations;

$$\frac{1}{Y_o} = \frac{1}{Y_t} + \frac{K_d}{Y_t} \cdot \frac{1}{\mu_n} \qquad \text{EQUATION 11.4}$$

and

$$\mu_n = Y_t U - K_d \qquad \text{EQUATION 11.5}$$

The net growth rate

$$\mu_n = \mu - K_d \qquad \text{EQUATION 11.6}$$

and also

$$\mu_n = \frac{1}{\theta_c} \qquad \text{EQUATION 11.7}$$

where θ_c = the solids retention time.

The values of μ_{max} and K_s can be determined graphically using the Monod equation (3.1)

LAWRENCE and McCARTY (143) defined 4 biological solids retention time, terms in their design model;

θc = biological solids retention time
(reciprocal equation (11.7))

θ_c^d = value of θc to be used in design

θ_c^m = minimum biological solids retention time
at which
$S_e = S_i$

and

$\left.\theta_c^m\right]$ lim = limiting value of θ_c^m which occurs

when

$$S_i \gg K_s$$

θ_c^m represents the lower value of θc at which complete failure of the biological process occurs. Below θ_c^m the organisms are removed from the system at a rate greater than their growth rate, so that eventually no organisms remain in the system. Obviously it is desirable that θ_c^d be significantly greater than θ_c^m, and LAWRENCE and McCARTY used the ratio θ_c^d/θ_c^m to give a safety factor for the system.

Inclusion of the θ_c^m or $\left.\theta_c^m\right]$lim value in a design and operational model is very useful, and LAWRENCE and MCCARTY'S definitive equations can be redefined to produce the following equations consistent with GAUDY'S model.

$$\theta_c^m = \frac{\mu\text{max}.\ S_i}{K_s + S_i} - K_d \qquad \underline{\text{EQUATION 11.8}}$$

and

$$\left.\theta_c^m\right]\text{lim} = \frac{1}{\mu\text{max}. - K_d} \qquad \underline{\text{EQUATION 11.9}}$$

11.2 Experimental Procedures

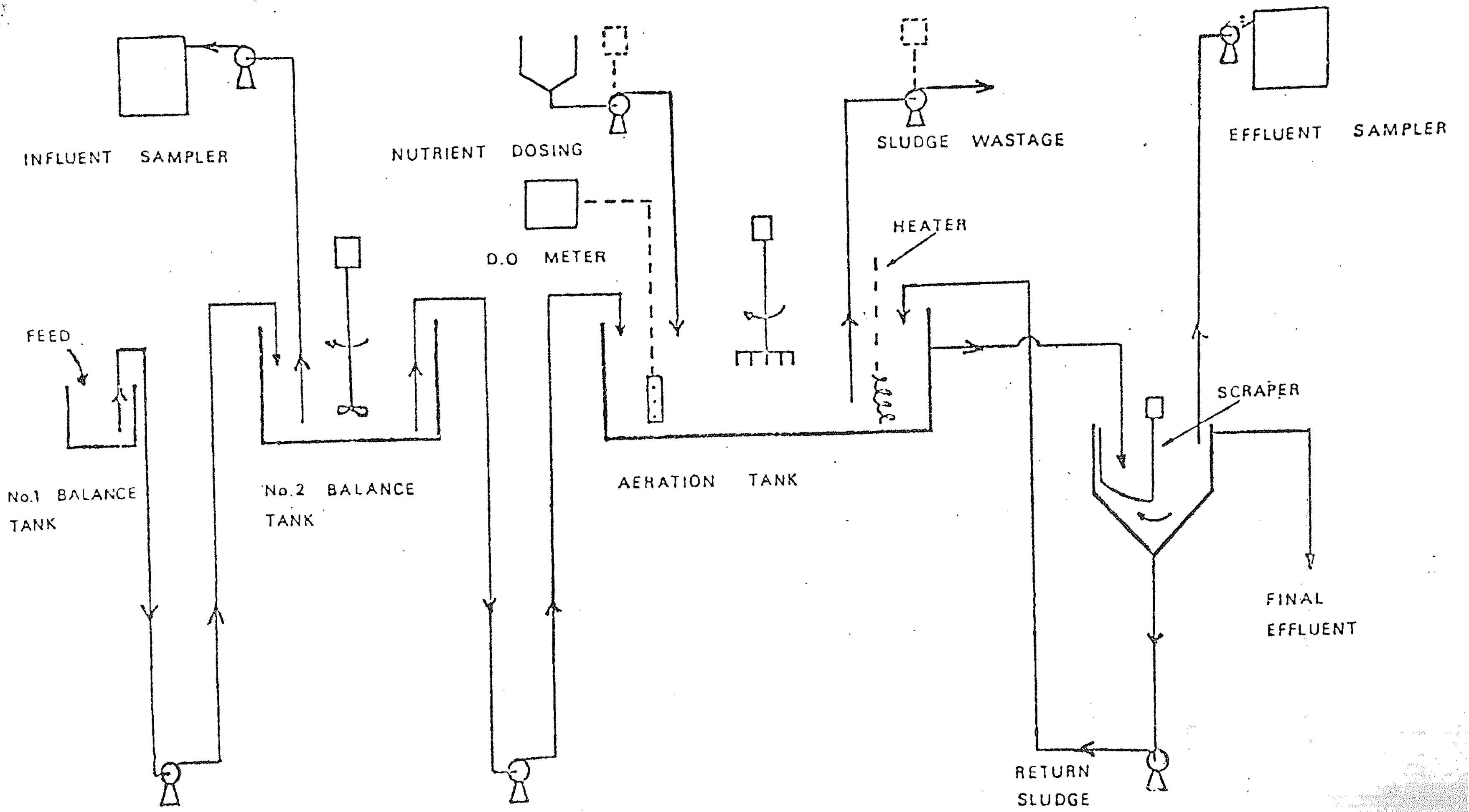
11.2.1 Pilot Plant Studies

The sludge and coke oven liquor used in this study were derived from the full-scale plant described later.

The pilot plant was of the complete mixing type with recycle, with a reactor of 50 litres capacity. Adiagramatic representation of the plant is shown in Figure 11.2 The data used for the present kinetic study was that collected over a 9 week period from May to July. The plant was operated at ambient temperature (mean daytime temperature 19°C). Three different sets of flow conditions were used during the period considered, and these were consecutively;

Condition	Flow Rate Of Influent	Flow Rate of Waste Sludge
	1/d	1/d
1	33.12	1.5650
2	59.04	2.9520
3	59.04	4.1328

The following measurements, relevant to this study, were made daily; influent flow rate, waste sludge flow rate, temperature, dissolved oxygen saturation, reactor pH, reactor suspended solids, and the monophenol concentration of influent and effluent.


INFLUENT SAMPLER
NUTRIENT DOSING
SLUDGE WASTAGE
EFFLUENT SAMPLER
D.O METER
HEATER
FEED
SCRAPER
No.1 BALANCE TANK
No.2 BALANCE TANK
AERATION TANK
FINAL EFFLUENT
RETURN SLUDGE

Samples for phenol analysis were taken automatically at least every 2 hours, and analysed using a colorimetric method using an Analmatic Automatic Analyser (Baird and Tatlock). The mean of the daily results were used in this study. Suspended solids analysis was made using dry weight determination and was performed once daily. To conform with Safety Regulations the parameters listed were only monitored on weekdays.

The pilot plant results were summarised in Table 11.3. A period of at least 5 days was allowed to pass after each change of flow conditions to allow for the plant to acclimatise to the new conditions, and the data collected during that period not used in this study. All measurements made after that time were assumed to approximate to steady-state conditions, and the data used along with EQUATIONS 11.1, 11.2, and 11.3 to determine the various values, shown in Table 11.4, necessary for the determination of Y_t, K_d, $\mu_{max.}$, and K_s. The values of K_d and Y_t were determined graphically using EQUATIONS 11.4 and 11.5, and the results shown in Figures 11.3 and 11.4 respectively.

11.2.2 Full-Scale Plant Studies

Data were collected for a full-scale complete-mixing activated sludge plant treating coke oven liquor. The plant concerned had formed the source of the sludge used in the pilot plant described earlier, and also the source of the limed liquor. A flow diagram of the plant is shown in Figure 11.5.

Table 11.3 Summary of Results from Pilot Plant Studies.

Si	Se	X	Fw	F
mg/l	mg/l	mg/l	1/d	1/d
215	2	1400	1.656	33.12
199	2	1380	1.656	33.12
377	7.6	2900	2.952	59.04
390	2	3000	2.952	"
477	3.9	4700	4.1328	"
480	4.7	5960	"	"
488	3.75	7220	"	"
545	3.5	8600	"	"
678	2.9	11,000	"	"
696	4.8	12,600	"	"
423	5.5	10,400	"	"
394	3.3	12,300	"	"
384	3.5	12,700	"	"
315	3	9000	"	"
258	3.2	9200	"	"
268	3	9200	"	"
337	3.3	8700	"	"
295	4	7400	"	"
280	3.7	8400	"	"
334	3.5	8400	"	"
359	3.2	7500	"	"
398	3.25	8000	"	"
575	3.4	8700	"	"
501	3.8	8000	"	"
617	6.6	7200	"	"
674	13.75	6700	"	"
408.3	5.67	2800	"	"
715.8	7.25	5300	"	"

Table II-1 Summary of Values for the Determination of Yt and Kd for Pilot Plant Studies.

Yo	1/Yo	U	μn	θc
0.3732	2.6792	0.1008	0.0376	26.5852
0.3985	2.5096	0.0946	0.0377	26.5393
0.4182	2.3909	0.1504	0.0629	15.8962
0.4111	2.4326	0.1527	0.0628	15.9288
0.7151	1.3985	0.1139	0.0850	11.7657
0.8973	1.1144	0.0942	0.0845	11.8345
1.0629	0.9408	0.0792	0.0842	11.8797
1.1289	0.8858	0.0743	0.0839	11.9143
1.1543	0.8663	0.0725	0.0837	11.9540
1.2895	0.7755	0.0648	0.0835	11.9721
1.7660	0.5663	0.0471	0.0837	11.9457
2.2275	0.4489	0.0375	0.0835	11.9630
2.3608	0.4236	0.0354	0.0835	11.9731
2.0490	0.4880	0.0409	0.0839	11.9223
2.5640	0.3900	0.0327	0.0838	11.9261
2.4653	0.4056	0.0340	0.0838	11.9261
1.8529	0.5397	0.0453	0.0839	11.9164
1.8120	0.5519	0.0464	0.0841	11.8850
2.1618	0.4626	0.0388	0.0840	11.9100
1.8073	0.5533	0.0465	0.0840	11.9100
1.5017	0.6659	0.0560	0.0841	11.8878
1.4422	0.6934	0.0583	0.0840	11.9007
1.0817	0.9245	0.0776	0.0839	11.9164
1.1450	0.8734	0.0734	0.0840	11.9007
0.8409	1.1892	0.1001	0.0842	11.8791
0.7244	1.3804	0.1164	0.0843	11.8631
0.5099	1.9612	0.1698	0.0866	11.5503
0.5337	1.8737	0.1588	0.0847	11.8025

FIGURE II.3 DETERMINATION OF Kd AND Yt FOR PHENOL OXIDATION FROM PILOT PLANT STUDIES.

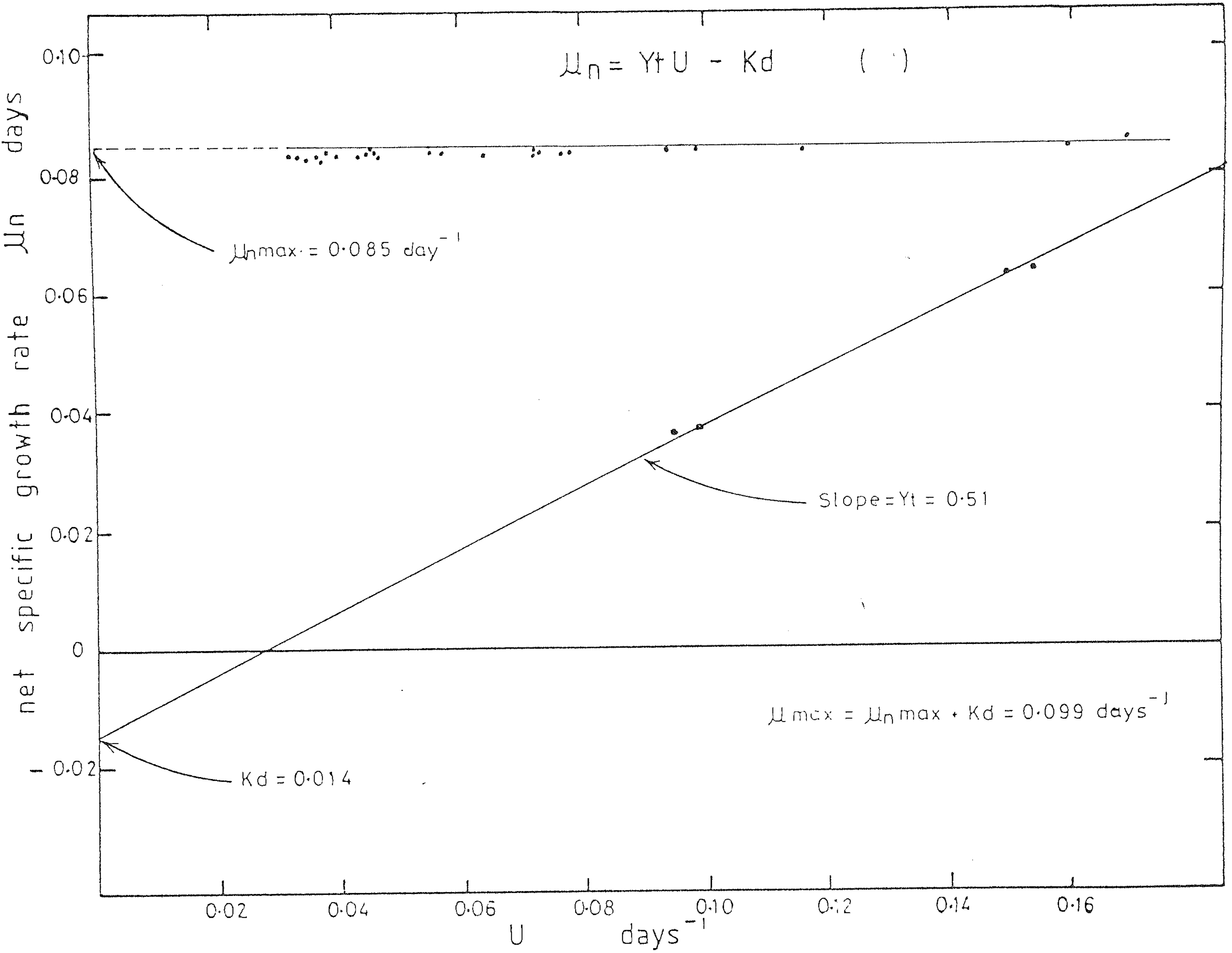
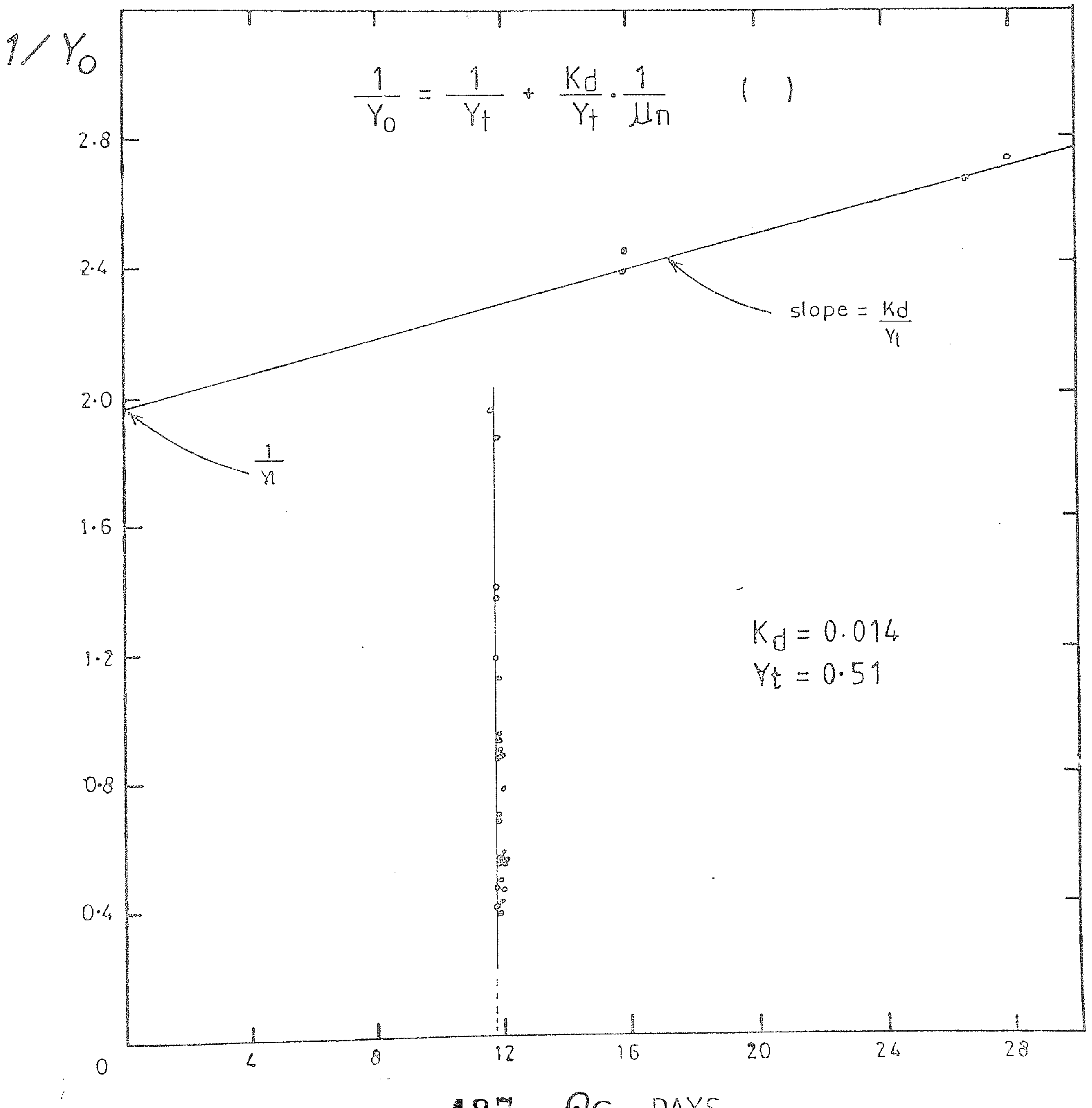


Figure 11·4 Determination of K_d and Y_t for Phenol Oxidation from Pilot Plant Studies·



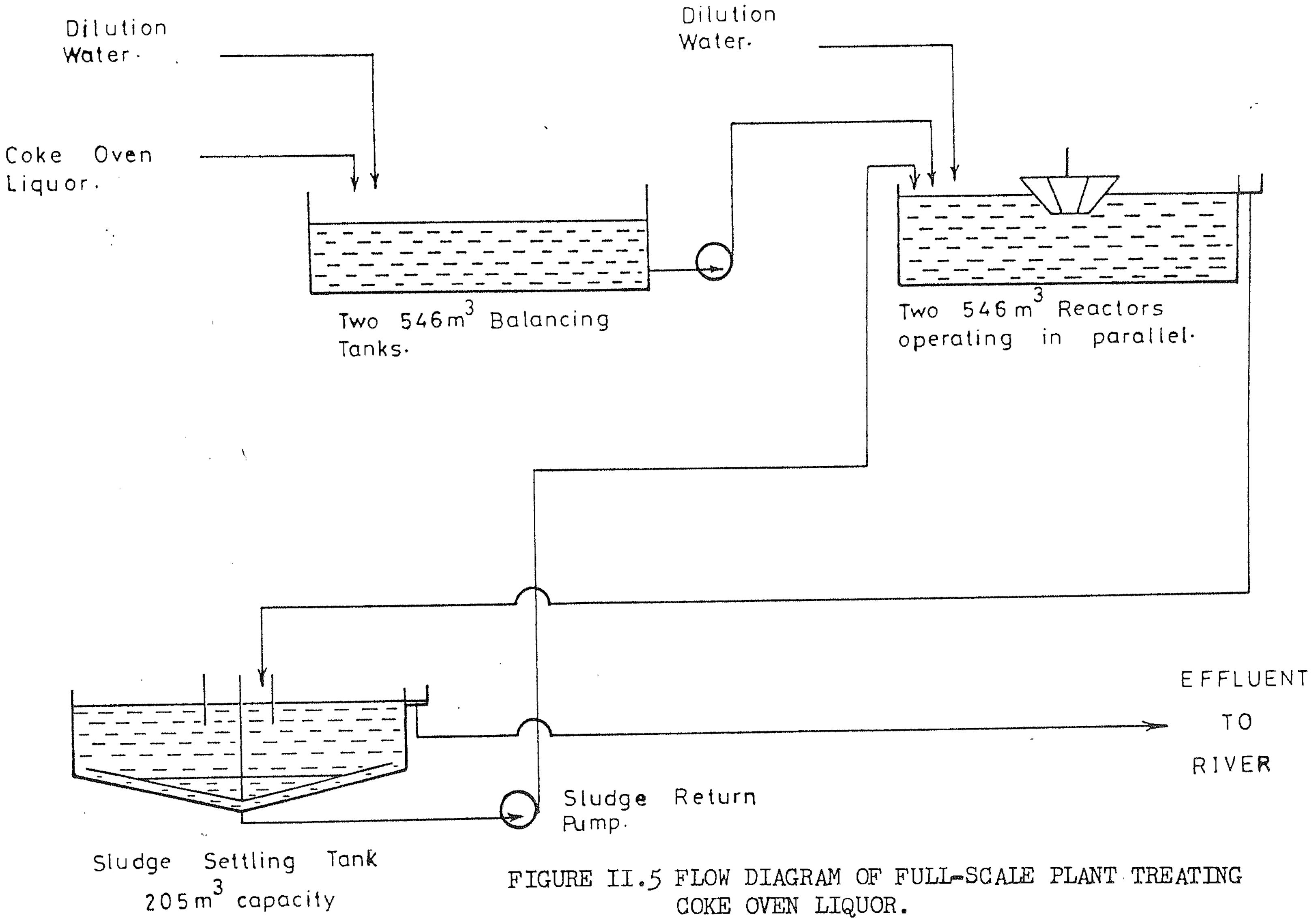


FIGURE II.5 FLOW DIAGRAM OF FULL-SCALE PLANT TREATING COKE OVEN LIQUOR.

Waste liquor from the coke oven works was fed to one of two 546m^3 balancing tanks, the second balancing tank acting as an overflow. Liquor from either of these tanks was pumped at equal rates to the 2 identical reactors operating in parallel. Effluent from the reactors passed to a single 205m^3 settling tank, and effluent from this passed to the river. The works was run at a constant sludge recycle rate, 545·4m^3/day, and without sludge wastage during the period covered by the data.

The daily plant results for various concentrations of reactor suspended solids taken over a period of a week of the plants operation in February this year, were recorded in Table 11.5. The results for each of the reactors operating in parallel were averaged. It was assumed that the behaviour of the plant approximated to steady-state conditions during the period considered. As for the pilot plant data, the values necessary for the determination of Y_t, K_d, $\mu_{max.}$, K_s were calculated using EQUATIONS 11.1, 11.2, and 11.3, and recorded in Table 11.6. The values of K_d and Y_t were determined graphically and shown in Figures 11.6 and 11.7.

11.3 Results and Discussion

11.3.1 Determination of Kinetic Constants for Phenol Oxidation from Pilot Plant Studies

The pilot plant results were summarised in Table 11.3. The values necessary for the determination of K_d and Y_t from

Table 11.5 Summary of Results from Full-Scale Plant Studies.

S_i	S_e	X	X_e	F
mg/l	mg/l	mg/l	mg/l	m^3/day
671	1.2	7094	86	272.7
671	3.7	7259	86	"
885	1.8	7280	185	"
885	1.8	6082	185	"
F_w = 0		V = 545.4m^3		

Table 11.6 Values for the Determination of Y_t and K_d for Full Scale Plant Studies.

Y_o	$1/Y_o$	U	Jln	θc
0.1284	7.7884	0.0472	0.00606	164.9767
0.1289	7.7583	0.0460	0.00592	168.8140
0.2095	4.7741	0.06066	0.01271	78.7027
0.2095	4.7741	0.07261	0.01521	65.7514

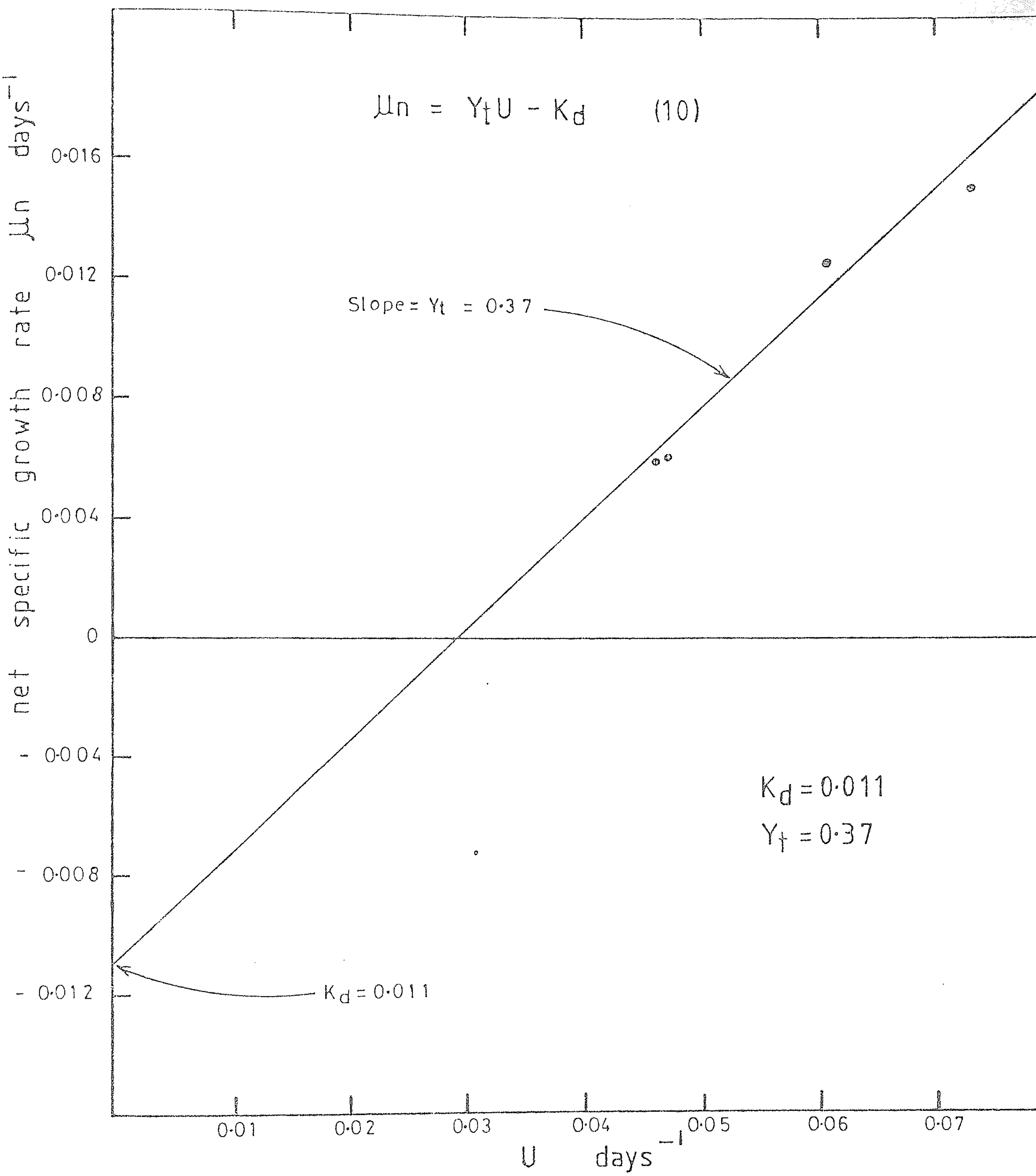
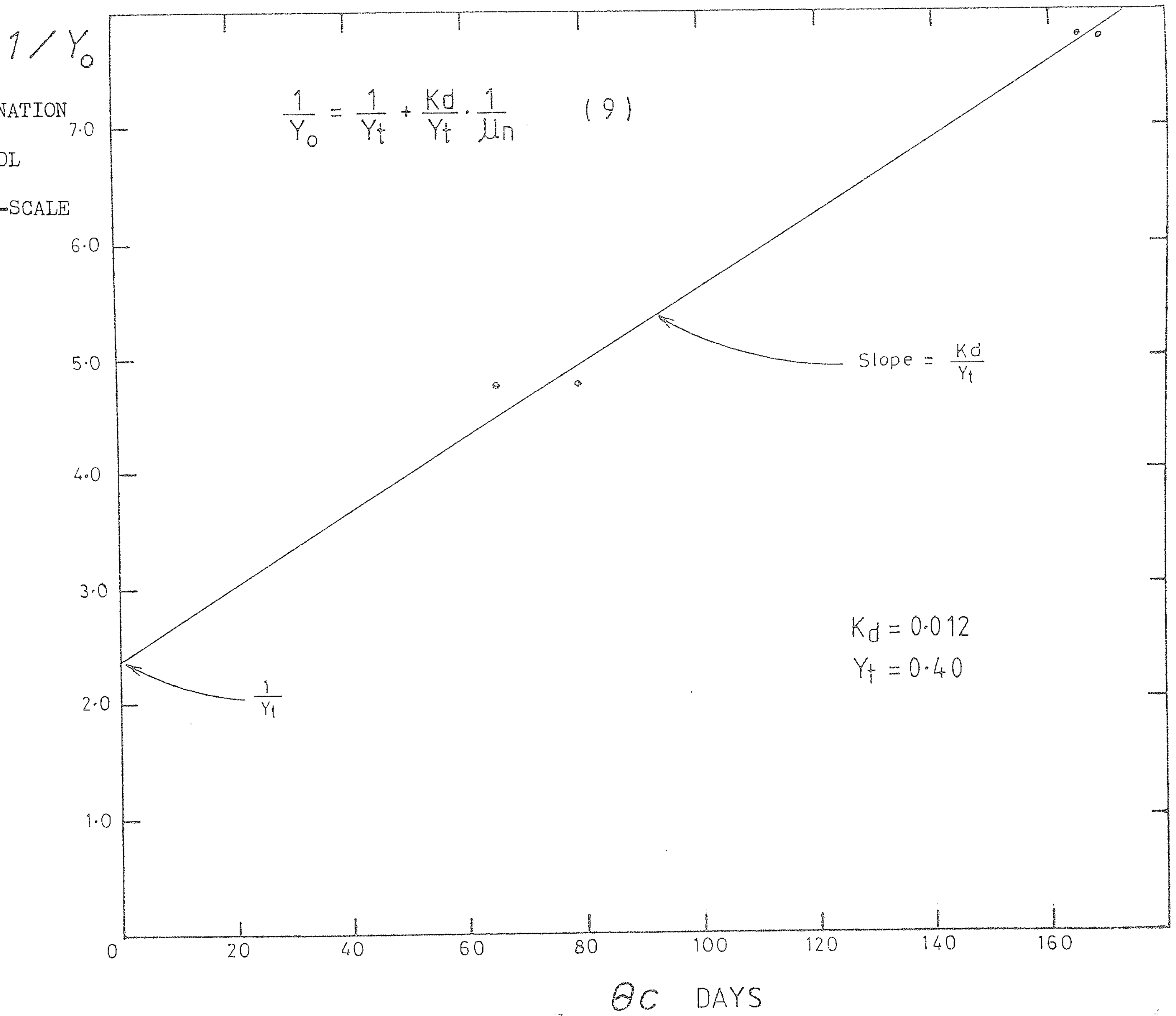


Figure II·6 Determination of K_d and Y_t for Phenol Oxidation from Full-Scale Plant Studies

IGURE 11.7 DETERMINATION F K_d & Y_t FOR PHENOL XIDATION FROM FULL-SCALE LANT STUDIES.



EQUATIONS 11.4 and 11.5 were determined using EQUATIONS 11.1, 11.2 and 11.3 and the results shown in Table 11.4, and plotted in Figures 11.3 and 11.4.

Each of the figures showed 2 straight lines through the data points. The horizontal and vertical lines in Figures 11.3 and 11.4 respectively were not predicted by the design models outlines earlier. However, the results could be explained if it was assumed that $S \gg K_s$. The values for K_d and Y_t were determined in the figures using the sloped lines as shown.

Inspection of the data in Table 11.4, and extrapolation of the horizontal line in Figure 11.3 indicated a maximum net specific growth rate of 0,0850 day^{-1}. $\mu_{max.}$ was calculated from the relationship

$$\mu_{n}\text{max.} = \mu\text{max.} - K_d \qquad \underline{\text{EQUATION 11.10}}$$

and found to be 0·0988 day^{-1}.

If the assumption was made that $S \gg K_s$ then the equation;

$$\frac{ds}{dt} = \frac{X}{Y}\mu\text{max.}\frac{S}{K_s + S} \qquad \underline{\text{EQUATION 11.11}}$$

used to develop the design models could be approximated to;

$$\frac{ds}{dt} = KX \qquad \underline{\text{EQUATION 11.12}}$$

where K = a constant, the maximum rate of substrate utilisation per unit mass of organisms, and equal to $\mu_{max.}/Y_t$.

EQUATION 11.12 is a zero order reaction with respect to substrate concentration.

If the assumption that reactor substrate levels were much greater than the K_s value was correct, then from EQUATION 11.12 a graph of the relationship between substrate utilisation and reactor biomass should be linear with a slope equal to $\mu_{max.}/Y_t$, for the data where the net specific growth rate, μ_n was maximal.

A graph of substrate utilisation rate, U, vs reactor biological solids, X, for the data from Tables 11.4 and 11.5 where μ_n equalled or very nearly equalled 0.085 day produced a linear relationshop, thus supporting the assumption made. This graph was shown in Figure 11.8. Line A in the figure showed the "best fit" through all of the data points, whilst Line B showed the "best fit" for data points where $X < 11{,}000$mg/l. The latter showed the best correlation.

Using the value for Y_t determined earlier, $\mu_{max.}$ calculated from the slope of Line B ($\mu_{max.}/Y_t$) was found to be 0.083 day^{-1}, showing good agreement with the earlier determined value.

The value of K_s was computed by determining the value that

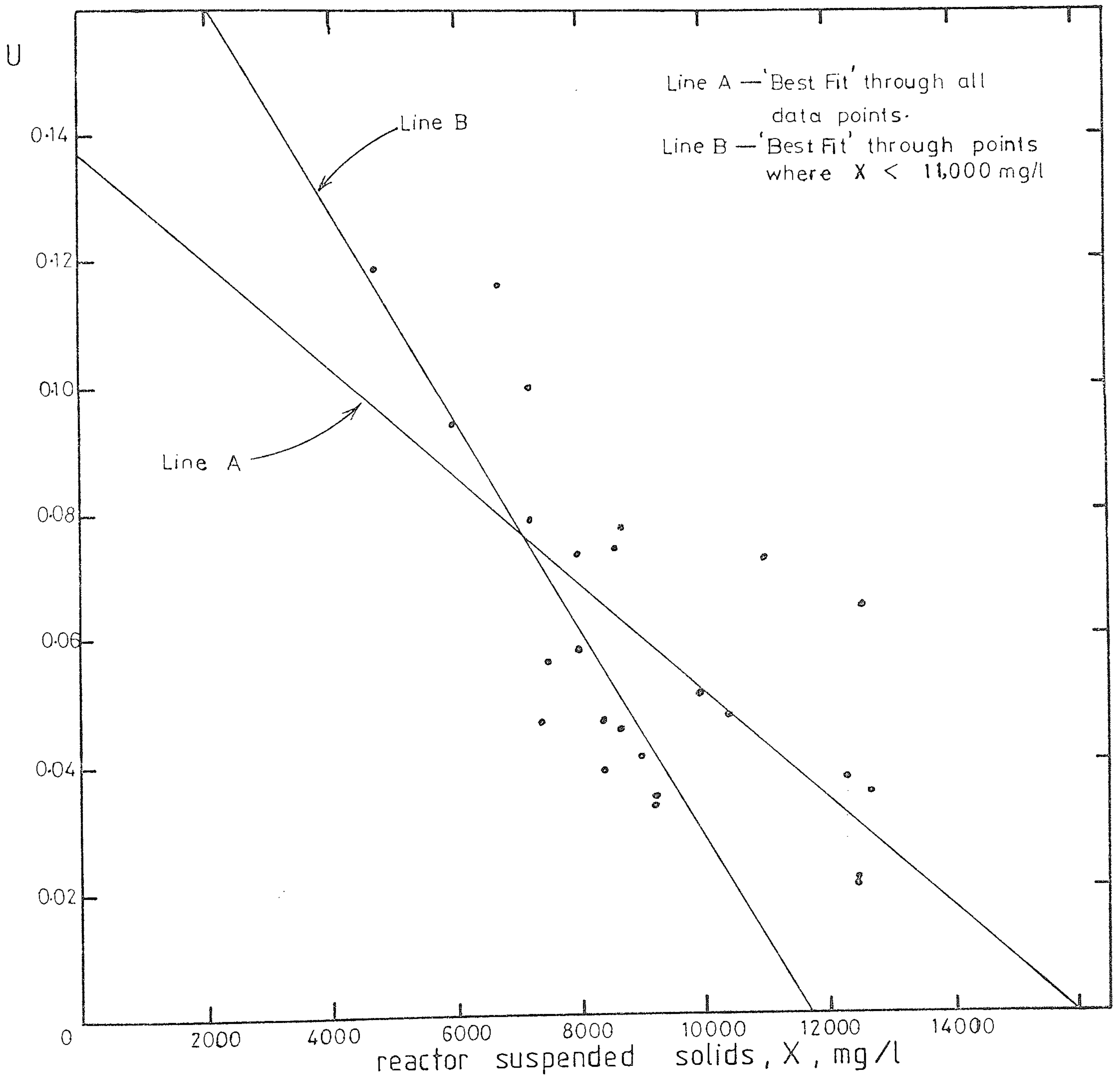


Figure 11·8 Plot of Phenol utilisation rate, U, vs reactor suspended solids for Pilot Plant data where $\mu_n \approx \mu_n$ max.

would best fit all of the calculated specific growth rate values for the plant data. The value for K_s was estimated to be 0.1.

11.3.2 Determination of the Kinetic Constants for Phenol Oxidation from Full-Scale Plant Data

The very limited data available was recorded in Table 11.5, and the values necessary to calculate Y_t and K_d determined from EQUATIONS 11.1, 11.2, and 11.3, and recorded in Table 11.5. The values of Y_t and K_d were determined from EQUATIONS 11.4 and 11.5 in Figures 11.6 and 11.7. The calculated values compared favourably with those determined from the pilot plant studies.

Because of the limited data available, the absence of the clear indication of the value for $\mu_{max.}$ found in the pilot plant studies, and because of the likely errors in the effluent phenol values quoted, it was not practical to estimate $\mu_{max.}$ and K_s values for the full-scale plant data.

Experience has shown throughout the project that accurate determination of very low levels of mono-phenol in effluent from plants treating coke oven liquor is difficult, and the analytical methods used are subject to interference by other components of the effluent.

In the batch activated sludges treating phenol described earlier in the project, interference only became significant in filtered sludge samples containing very low levels of phenol, and values of up to 5mg/l were recorded for samples where the growth conditions of the experiment indicated that zero values were to be expected. Such interferences could in part be removed by modifications to the analytical method used.

11.3.3 Design and Operation of Plant for Phenol Removal

The affect of the kinetic constants determined from the pilot plant studies, and the 3 controllable design factors $\bar{t}$ (or 1/D), X_R, α, and S_i on reactor biological solids, X, and effluent substrate concentration S_e, can be determined using equations described by GAUDY. Using the values $_{max.} = 0.099\ \text{day}^{-1}$, $K_s = 0.1$, $Y_t = 0.51$, and $K_d = 0.014\ \text{day}^{-1}$, the effect of various values of the control factors and S_i on effluent phenol concentrations were illustrated in Figures 11.8a and 11.8b. The values chosen for the control factors cover the range found by the authors for full-scale plants treating coke oven liquors.

Graphs of this type can be used to select the appropriate design and operational characteristics of a plant to produce the desired effluent quality. It can be seen from Figures 11.9a and 11.9b that the values of S_i and $\bar{t}$ play an important part in determining the quality of the final effluent. Most full-scale plants treating coke oven waste experience large variations in both the phenol content and flow rate of the coke oven liquor arriving at the treatment plant. These variations could be reduced by the provision of suitable balancing and pumping facilities before the reactor stage is reached. To ensure a consistent quality effluent the plant could be designed to treat the highest influent phenol levels and flow rates expected, and it can be seen that as S_i increases and $\bar{t}$ decreases serious constraints are imposed on the options available for combining α, X_R, and $\bar{t}$ to produce the desired effluent quality.

The choice of whether to provide extra balancing facilities to minimise influent variations, or to design the reactor to cope with larger variations in conditions, is one that must be made considering the overall cost optimisation of the treatment systems. Clearly, however, the information provided by the model and Figures 11.9a and 11.9b will play a small but significant part in deciding the options available.

Figure 11.10 shows that the biomass concentration is not significantly affected by the mean hydraulic retention time. Although X remains relatively constant EQUATION 11. shows that θc increase as $\bar{t}$ increases. In practice this means that for a given $\bar{t}$ value for a plant θc or μ_n can be varied by changing reactor biological solids X by manipulation of α or X_R. The results from the pilot studies indicate that where μ_n is approximately maximal, the substrate utilisation rate is directly proportional to X, and zero order with respect to S.

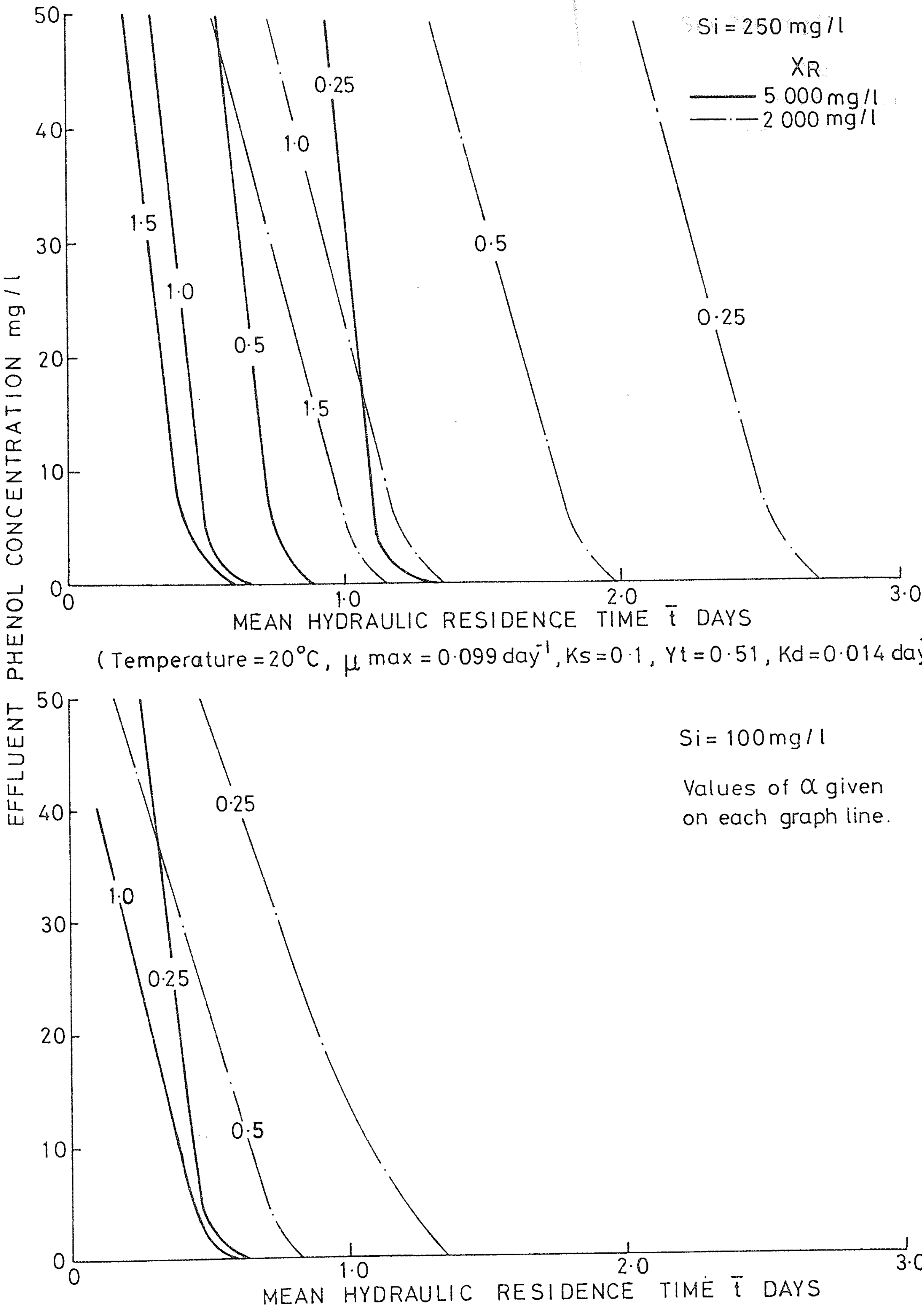
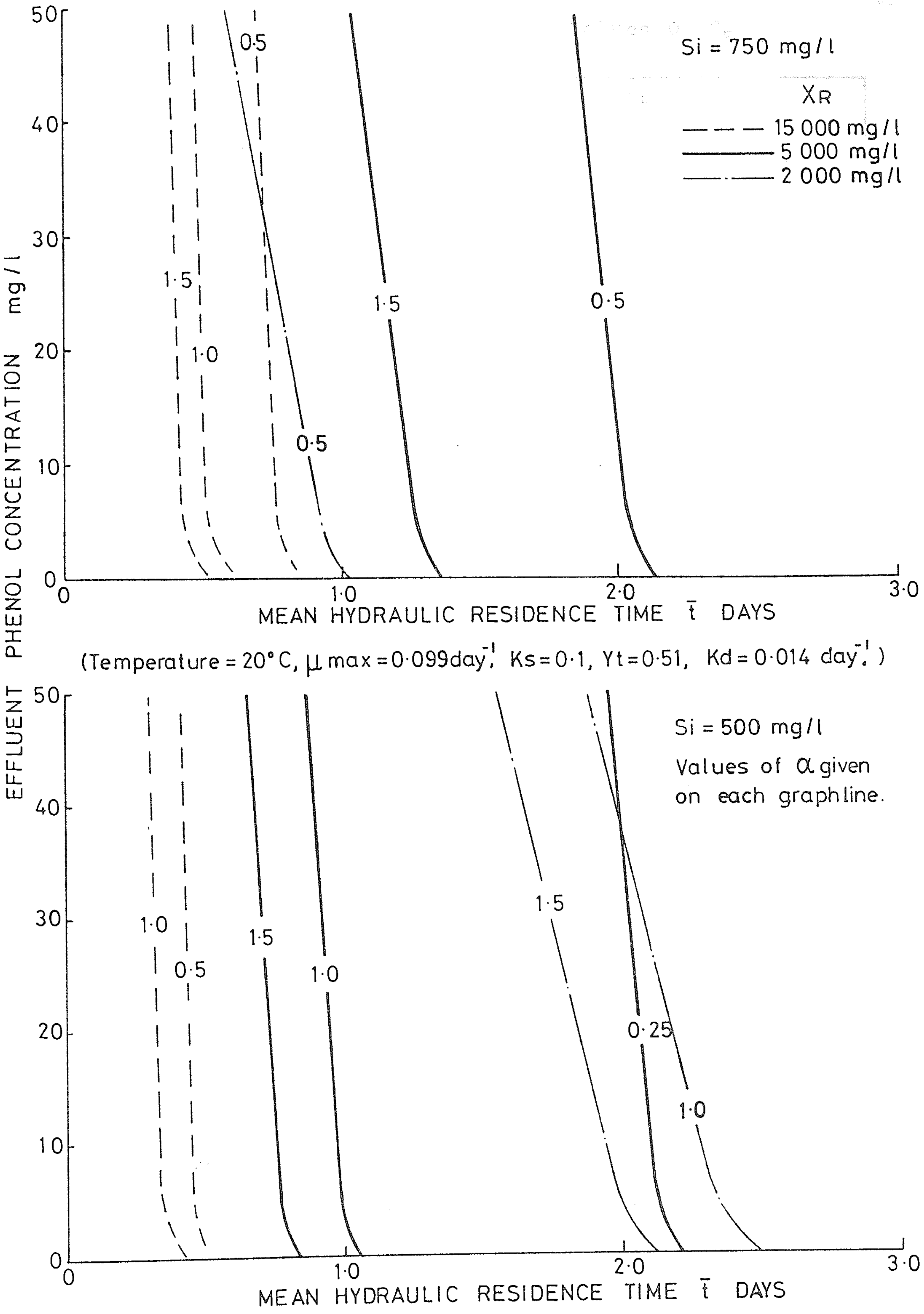


Fig 11·9a Illustrating the Effects of $\bar{t}$, α, X_R and Si on Effluent Phenol Concentration Using Gaudy's Model.



(Temperature = 20°C, μ max = 0.099 day^{-1}, Ks = 0.1, Yt = 0.51, Kd = 0.014 day^{-1})

Fig. 11.9b Illustrating the Effects of $\bar{t}$, α, X_R and Si on Effluent Phenol Concentration Using Gaudy's Model.

Table 11.7 Effect Of Y_t On Predicted Values Of S_e And X For Phenol Oxidation

S_i	Y	$X_R = 15000$mg/l		$X_R = 5000$mg/l	
		S_e	X	S_e	X
		mg/l	mg/l	mg/l	mg/l
250	0.1	0	7460	0.01	2495
	0.2	0.01	7473	0.03	2507
	0.3	0.01	7485	0.04	2520
	0.4	0.02	7498	0.07	2532
	0.5	0.02	7510	0.10	2545
	0.6	0.03	7522	0.15	2557
	0.7	0.03	7535	0.22	2569
	0.8	0.04	7547	0.36	2582
	0.9	0.04	7550	0.68	2594
	1.0	0.05	7572	2.05	2604

$\mu_{max} = 0.099$ day^{-1} $K_d = 0.014$ day^{-1} $K_s = 0.1$
$D = 1$ $\alpha = 1$

Table 11.8 Effect Of K_s On Predicted Values Of S_e And X For Phenol Oxidation

S_i	K_s	$X_R = 15000$mg/l		$X_R = 5000$mg/l	
		S_e	X	S_e	X
		mg/l	mg/l	mg/l	mg/l
250	0.1	0.02	7511	0.10	2546
	0.5	0.10	7511	0.51	2546
	1.0	0.20	7511	1.01	2545
	2.0	0.41	7510	1.98	2545
	5.0	1.02	7510	4.75	2544
	10.0	2.03	7510	8.89	2541

$\mu_{max} = 0.099$ day^{-1} $Y_t = 0.51$ $K_d = 0.014$ day^{-1}
$D = 1$ $\alpha = 1$

The minimum biological solids retention time is given by EQUATION 11.9 , as S_i and $S_e \gg K_s$; and equals 11.76 days and represents the value at which there is complete treatment failure, i.e. where $S_e = S_i$. In practice "treatment failure" can be used to describe periods when the effluent fails to meet the required standards, and the conditions where this will occur can be predicted from graphs such as Figures 11.9a and 11.9b. It is important however that the plant be designed to avoid conditions where θ_c approaches $\left.\theta_c^m\right]$ lim.

It was indicated earlier that errors in determining low effluent phenol concentrations made estimation of K_s difficult, and Table 11.8 shows the affect of varying the value of K_s on S_e and X over the range 0.1 to 10.0 using the values of Y_t, K_d, and μ_{max}. calculated earlier. The results indicated that changing the value of K_s has little effect on S_e and X, particularly at the lower end of the range where kinetic studies indicated the actual value lies.

Table 11.7 shows the affect of varying the value of Y_t on S_e and X using values for the other kinetic constants calculated earlier, and the values for control parameters indicated. The results indicated that the effect on S_e and X was also very small using a range of values for Y_t which exceed the limits of error for the values calculated from pilot and full-scale studies.

11.3.4 Comparison with Kinetic Coefficients for Phenol Oxidation in Simple Mineral Media

PAWLOWSKY,(147) and JONES(148) have described the kinetics of phenol oxidation in simple mineral media. PAWLOWSKY determined the kinetics of 2 different population of organisms, one predominately filamentous, the other nonfilamentous, in batch cultures. The organisms were obtained from laboratory continuous cultures. JONES examined the growth kinetics of Bacterium NCIB 8250 in a two stage continuous culture.

Both investigators found that higher phenol concentrations were inhibitory to the organisms concerned, and that the effect on the organisms could be explained by HALDANE'S(112) model for substrate inhibition. The value of the kinetic constants determined from these studies were summarised in Table 11. 9 . PAWLOWSKY considered a number of substrate inhibition models, and found no significant difference between them in terms of fitting the experimental data; only the results for the HALDANE model were recorded in the Table 11. 9 .

The results obtained in, this study ,for Y_t fell within the range found by both investigators, and the value for K_s compared well with that reported by JONES. The value obtained for μ_{max}. was however considerably lower than that found by JONES or PAWLOWSKY, and it is suggested that this reflects the more hostile and inhibitory environment experienced by the organisms growing in coke oven liquor.

Table 11.9

Kinetic Constants For Phenol Oxidation In Simple Mineral Media Reported By Pawlowsky, And Jones

Investigator	μ_{max}	K_s	Ki	Y_t	Comments
	hr	mg/l	mg/l		
Pawlowsky	0.260	25.4	173	0.545 0.070	Non-Filamentous Growth
"	0.223	5.86	934.5	0.616 0.070	Filamentous Growth
Jones	0.29	1	110	0.32-0.7	Bacterium NC1B8250

Although the results of the studies presented here did not indicate substrate inhibition, the possibility of substrate inhibition of phenol oxidation in coke oven liquor treatment should still be considered.

In fact, it was evident from the ATP and respirometric work with sludges treating phenol and coke oven liquor described earlier in the project that substrate inhibition does occur, and HIAM has also indicated such inhibition from respirometric studies of sludges treating the waste.

11.3.5 Significance of Substrate Inhibition

HALDANE'S model for substrate inhibition takes the form;

$$\mu = \frac{\mu_{max.}}{1 + K_s/S + S/K_i} \qquad \text{EQUATION 11.13}$$

where K_i = the inhibition constant

In the absence of data from the studies presented here, for determination of a value for K_i, the values found by the above investigators will be used to assess the possible significance of substrate inhibition. Using these values, the effect of substrate inhibition on growth rate can be seen from Figure 11.11. ANDREWS,(149) and YANO and KOGA(150), considered the effects of substrate inhibition of the type shown in EQUATION 11.13 on the stability of treatment systems. YANO and KOGA described three steady-states of practical

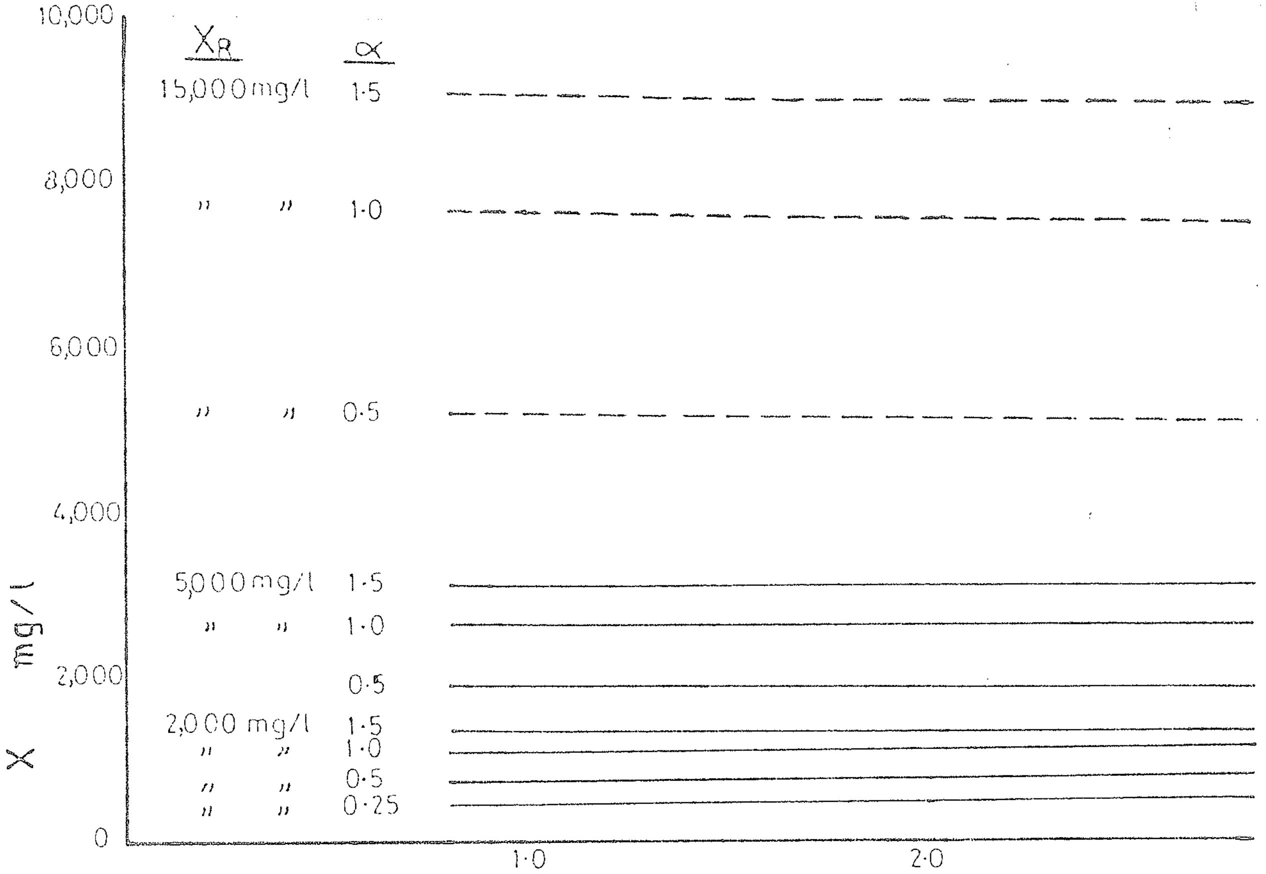


Figure 11·10 ILLUSTRATING THE EFFECTS OF $\bar{t}$, α, AND X_R ON X.

[Si = 250 mg/l, Yt = 0·51, Kd = 0·014 day^{-1}]

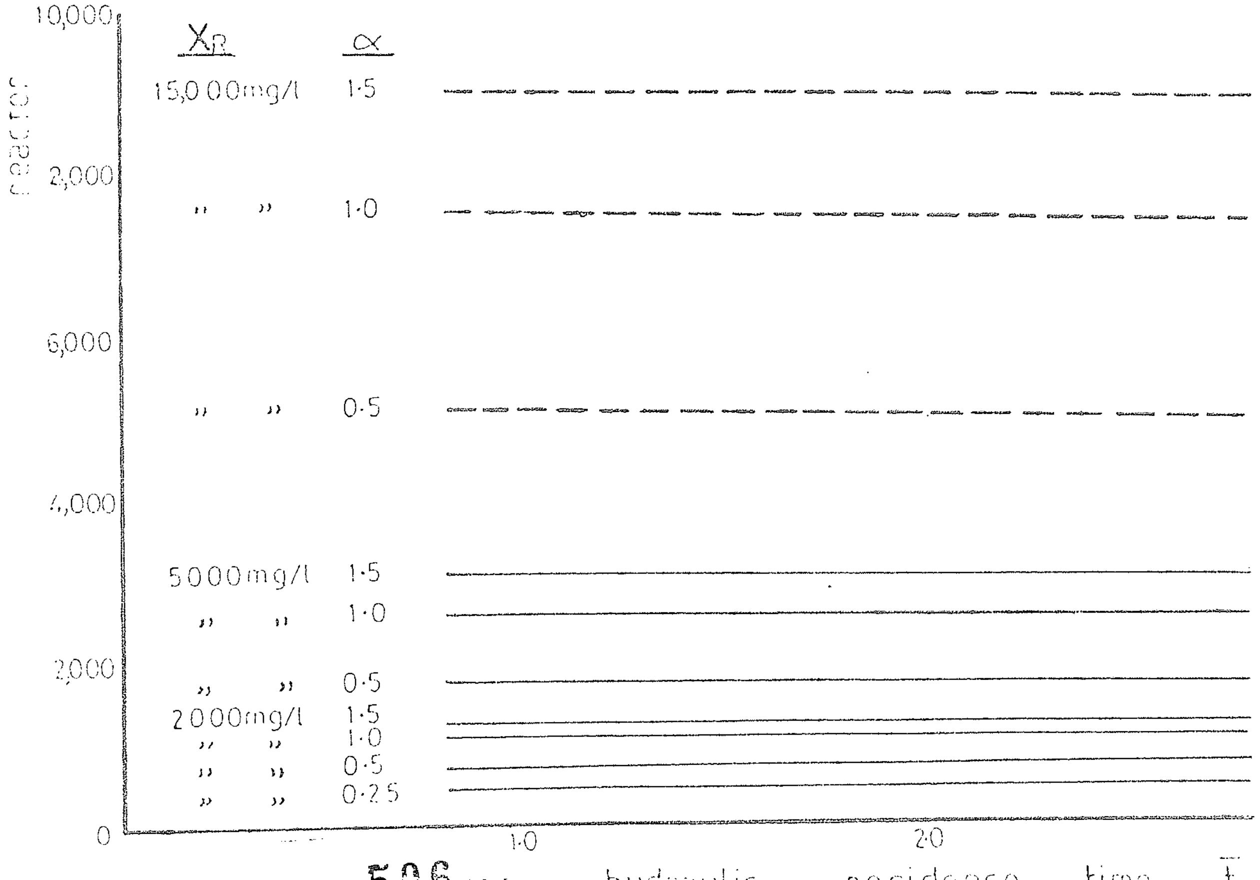
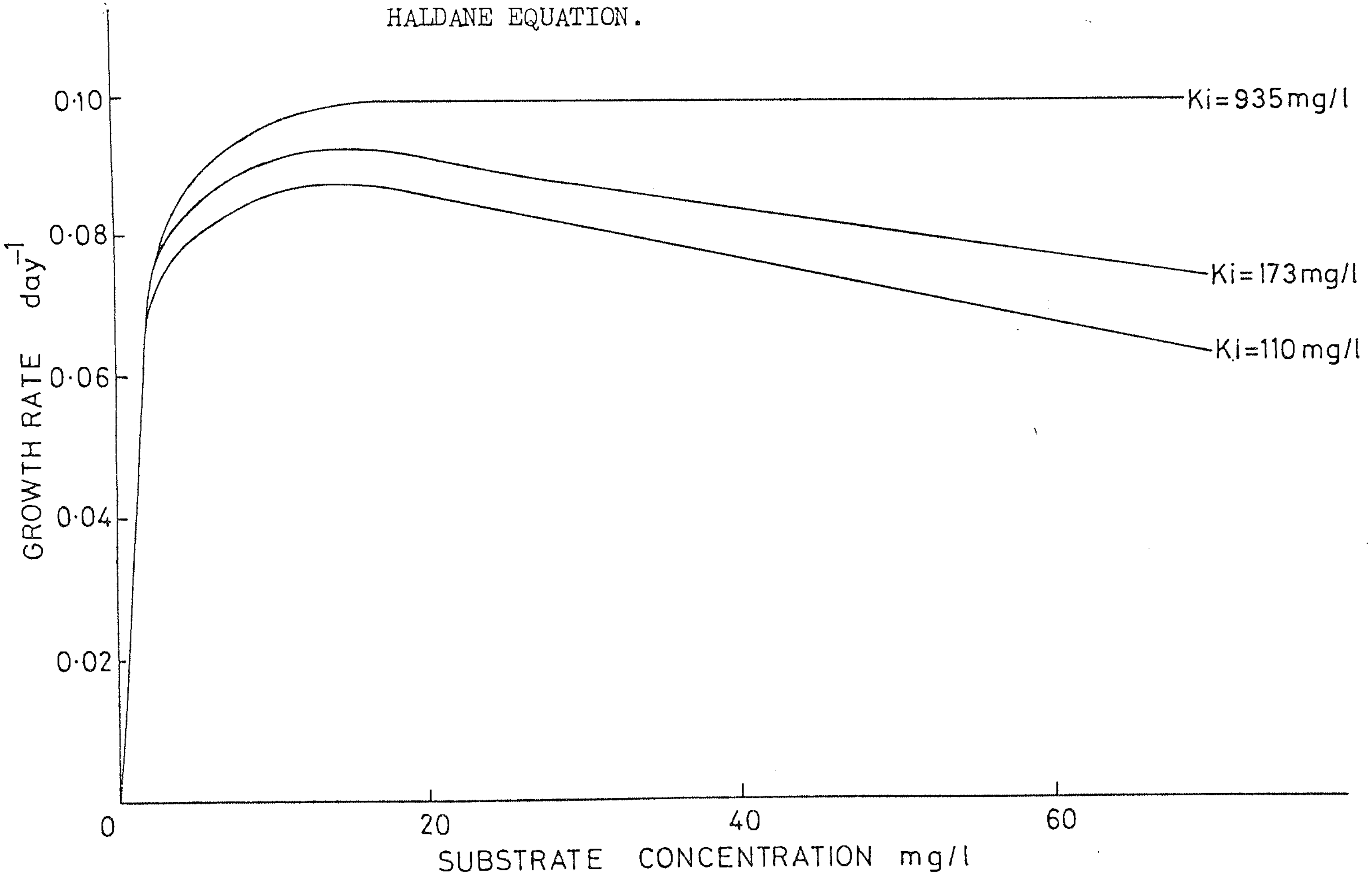


FIGURE II.II ILLUSTRATING THE EFFECT OF SUBSTRATE INHIBITION ON GROWTH RATE FOR DIFFERENT Ki VALUES USING THE HALDANE EQUATION.



importance. Two of these were stable states existing at the high biomass and low substrate concentrations typically found in treatment plants. The third state was unstable and corresponds to wash-out conditions.

Instability and wash-out begin to occur when $S > \sqrt{K_i K_s}$, and therefore treatment plant should operate below the value where inhibition occurs, that is when $S < \sqrt{K_i K_s}$. At non-inhibitory levels of phenol in the reactor, the model and design and operational graphs described earlier are entirely applicable, and they can be used to ensure that non-inhibitory levels of substrate are obtained. The value of S at which inhibition occurs for phenol oxidation, using the various values for K_s and K_i in Table 11. 9 , are 66.3mg/l and 74mg/l for non-filamentous and filamentous growth respectively (PAWLOWSKY), and 10.5mg/l and 3.3mg/l for $K_s = 1$ and $K_s = 0.1$ respectively (JONES). This critical value of S is very dependent upon the value of K_s, and the problems of accurately determining low K_s values have already been indicated.

The initial results from batch studies of phenol oxidation in a basal medium of coke oven effluent, using ATP as a measure of activity, suggested that inhibition occurred at approximately 20 mg/l, and HIAM'S results could be interpreted to give a similar value.

11.3.6 Thiocyanate and Ammonia Removal

STAFFORD,(124) and DOWNING,(105) have indicated that phenol and cresols inhibit nitrification. STAFFORD demonstrated inhibition at phenol and cresol levels > 3mg/l and that inhibition was 100% at 10mg/l.

Nitrification rarely occurs in a single stage activated sludge plant treating coke oven liquor, and a second stage reactor is generally added to the system where nitrification is a treatment objective. In such cases therefore it is desirable that the first stage is operated to produce very low phenol levels.

Other components of the liquor have been shown to be inhibitory to nitrification including various pyridine derivatives. These tend to be more resistant to degradation than phenol, but are more likely to be oxidised in plant conditions that produce low phenol effluent concentrations.

TOMLINS(42) suggested that thiocyanate removal may be inhibited by phenol. However, it is suggested that the most common cause of failure to remove thiocyanate from full-scale plants, is failure to operate the plant at sufficiently high solids retention times. Thiocyanate removal is achieved by autotrophic bacteria, which like the autotrophs responsible for nitrification, require long θ_c values for stable growth in continuous culture.

STAFFORD,(115) and HIAM(114) have demonstrated the existence of heterotrophic bacteria in sludge treating coke oven liquor which are able to degrade thiocyanate. However, studies carried out by HUTCHINSON,(121) and STAFFORD have indicated that if thiocyanate oxidation was predominantly carried out by heterotrophs rather than autotrophs, that thiocyanate removal in the treatment system would be more stable than it is generally observed to be.

STAFFORD(151) has reported the minimum retention time ($\bar{t}$) for a variety of coke oven liquors of varying strengths, limed and unlimed, necessary for efficient thiocyanate removal. However, although the use of minimum $\bar{t}$ values is a useful "rule of thumb" for general design of plants, or valuable when applied to a particular plant in set operating conditions, θc is the most useful design parameter to use. θc is related to $\bar{t}$ by

$$\frac{1}{\theta c} = \frac{1}{\bar{t}} \left(1 + \alpha - \frac{X_R}{X}\right)$$

EQUATION 11.14

and although θc increases as $\bar{t}$ increases, the value of θc can be changed for a given $\bar{t}$ by changing α or X_R.

In the absence of data concerning α and X_R for the reported minimum values of $\bar{t}$ for thiocyanate removal it is not possible to test the hypothesis that the minimum values for solids retention time are higher than those for phenol removal.

Efficient phenol removal therefore appears important where ammonia removal is required, and may also be of significance in thiocyanate removal. However, design and operation of plant to produce adequate solid retention times are likely to be the most important factors to be considered.

11.4 Summary

The growth kinetics for phenol removal during the complete mixing activated sludge treatment of coke oven liquor were examined using pilot and full-scale plants. Current design and operational models were briefly reviewed, and the significance of the determined kinetic constants in determining the specific design and operation of plant discussed.

The growth kinetics of phenol removal in coke oven liquor were compared with those for phenol removal in simple mineral media reported by other investigators. The magnitude of $\mu_{max.}$ was considerably less than that reported for phenol removal in simple mineral media, and this was attributed to the more inhibitory nature of coke oven liquor.

The results reported failed to indicate the existence of substrate inhibition reported for phenol removal in simple mineral media, probably because non-inhibitory levels of phenol were used in the experiments. Results of other investigations, have indicated the existence of substrate inhibition in the treatment system, and the significance of such inhibition to the design and operation of plant treating coke oven liquor was discussed.

The significance of efficient phenol removal to the removal of thiocyanate and ammonia components of coke oven liquor was also discussed.

CHAPTER 12

THE SIGNIFICANCE OF ATP MEASUREMENTS IN THE ACTIVATED SLUDGE TREATMENT OF COKE OVEN LIQUOR - A DISCUSSION

Introduction

12.1 The Use of Activated Sludges

12.2 The ATP Behaviour of Batch Sludges Treating Thiocyanate

    12.2.1 Establishment of a General Trend in ATP Behaviour

    12.2.2 Comparison with the Trend in ATP Behaviour Reported by TOMLINS

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12.3 The ATP Behaviour of Batch Sludges Treating Phenol

    12.3.1 A Comparison with the Results Reported by TOMLINS

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    12.3.3 The Significance of the Initial ATP Response of Batch Sludges to Different Phenol Loadings (CHAPTER 8)

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    12.3.5 Respirometric Testing of Phenol Removal
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    12.4.1 Qualitative Explanations of the Significance of ATP
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THE SIGNIFICANCE OF ATP MEASUREMENTS IN THE ACTIVATED SLUDGE TREATMENT OF COKE OVEN LIQUOR - A DISCUSSION

Introduction

The analysis and discussion of the results of experiments investigating the ATP behaviour of batch sludges treating thiocyanate, described in Chapter 4, formed the basis of the investigations described in Chapters 5-11. These investigations were conducted largely independently of each other. This chapter discusses all of the experimental results obtained in the project with the object of determining the significance of ATP measurements in the activated sludge treatment of coke oven liquor.

12.1 The Use of Batch Activated Sludges

It was evident from the work described in Chapters 1-3 that coke oven liquor is a wastewater that is chemically both complex and highly variable in composition, and that this complexity is reflected in the microbiology and biochemistry of the activated sludge treatment of the liquor.

In view of this complexity, the decision to consider the biological treatment of the liquor in terms of the removal of its three main components, phenol, thiocyanate, and ammonia, and to give priority to the former two, therefore represented a practical simplification of the system upon which to base the ATP studies. Included in this simplification was the assumption that there existed, three distinct populations of organisms in activated sludge treating the waste, each being responsible for the degradation of just one of those three components.

The decision to investigate the activated sludge treatment of each of those three components using batch reactors was a less obvious first approach. Ideally of course the sludges and conditions employed in the investigations should have been identical with, or resembled as near as possible, those found in full-scale plants treating the waste. Laboratory-scale batch reactors were used however because limited practical facilities prevented use of large scale and continuous reactors at the start of the project. Later in the project of course it became possible to carry out continuous reactor studies on a laboratory scale, and this enabled the work described in Chapter 9 to be performed. The use of sludge derived from full-scale plant treating coke oven liquor, and the use of biologically treated coke oven liquor as the basal medium in the sludges acclimatised to treating individual liquor components, did however ensure some realistic representation of the sludge and component organisms found in full-scale continuous systems treating liquor. This use of batch systems also had the obvious advantage that it enabled comparison between the projects results and those obtained by TOMLINS(42) in similar experiments.

12.2 The ATP Behaviour of Batch Sludges Treating Thiocyanate

12.2.1 Establishment of a General Trend in ATP Behaviour

The establishment of a general trend in the ATP behaviour of batch sludges treating thiocyanate, shown in Figure 4.27 was an important development in the project. A large and almost immediate rise in the ATP pool following thiocyanate addition was a notable characteristic of that general trend. This behaviour confirmed that ATP represented a measure of activity rather than just biomass, as the velocity and magnitude of the increase was not consistent with an increase in biomass due to bacterial reproduction.

The significance of the two peaks in ATP concentration evident in Figure 4.27 was discussed in Chapter 4, and included the proposal that each represented the activity of a distinct population of organisms. It was noted earlier that the occurrence of these peaks in each batch experiment appeared to be related to the prevailing thiocyanate concentration in the reactor. The same general trend occur despite variations between batch experiments in the experimental conditions employed. Graphical plots of the relationship between ATP and substrate concentration in each of the batch experiments served to clarify this apparent relationship. Graphs of the type shown in Figure 4.51 were produced. This relationship between ATP and substrate may however have been more apparent than real, and may have reflected the response to factors other than substrate. The reproducibility of this relationship for a large number of batch experiments despite variations in experimental conditions did however provide evidence indicating that the relationship was real. Further experiments were conducted to check this relationship, but before considering those it would be useful to compare the results of the batch experiments with those obtained by TOMLINS.

12.2.2 Comparison with the Trend in ATP Behaviour Reported by TOMLINS

The batch experiments conducted by TOMLINS involving activated sludge acclimatised to treating thiocyanate in basal medium of treated coke oven liquor, were very similar to those described in Chapter 4. He performed a total of 13 experimental runs. In 5 of these, which he called runs ATP-T1 to ATP-T5, the sludge ATP level was monitored in each run during the batch degradation of a single dose of thiocyanate. In run ATP-T6 ATP was monitored over a period of 24 hours in the absence of thiocyanate.

The remaining 8 runs were conducted in 4 pairs with the aim of investigating the effect of phenol addition. For each pair this involved sub-dividing a batch of sludge into 2 to form identical batch reactors of sludge, and adding a similar volume of stock thiocyanate solution to each. A volume of phenol solution was added to one member of each pair, the other member acting as control. These four pairs of experiments were labelled Runs ATP-1 (a) and (b), ATP-12 (a) and (b), ATP-13 (a) and (b), and ATP-14 (a) and (b).

Graphical representations of the results reported by TOMLINS were recorded in Figures 12.1 to 12.6.

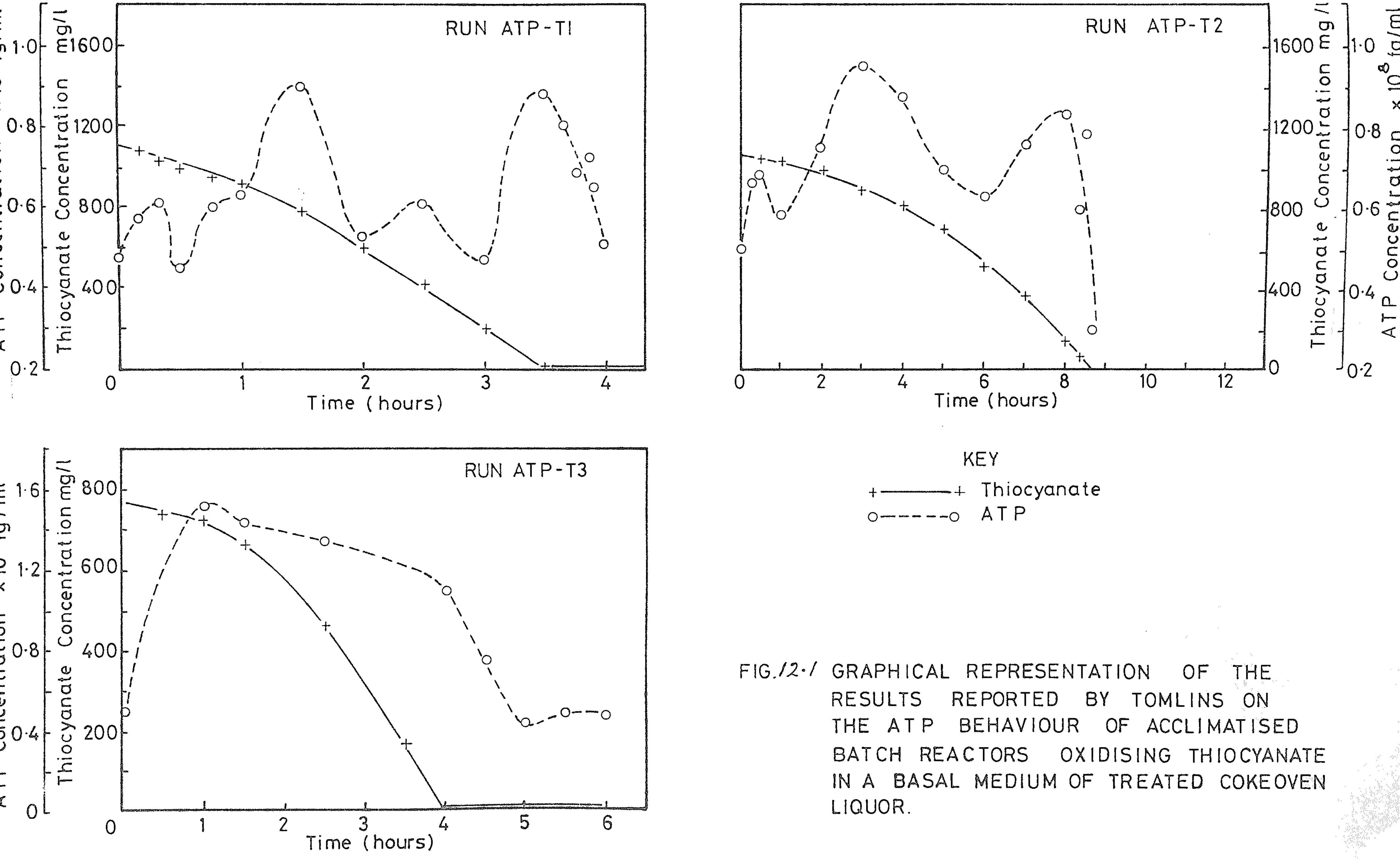


FIG. 12·1 GRAPHICAL REPRESENTATION OF THE RESULTS REPORTED BY TOMLINS ON THE ATP BEHAVIOUR OF ACCLIMATISED BATCH REACTORS OXIDISING THIOCYANATE IN A BASAL MEDIUM OF TREATED COKEOVEN LIQUOR.

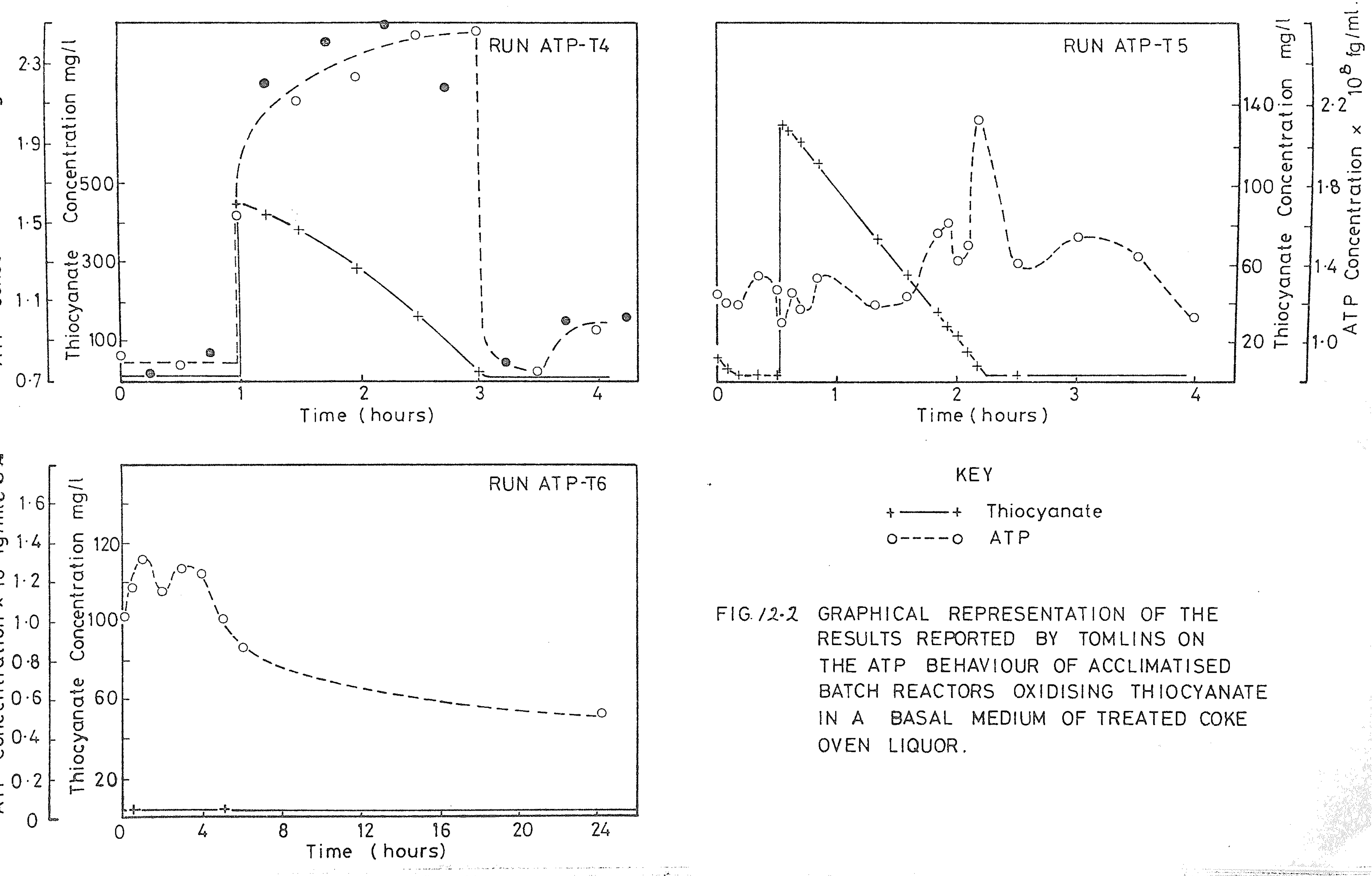


FIG. /2·2 GRAPHICAL REPRESENTATION OF THE RESULTS REPORTED BY TOMLINS ON THE ATP BEHAVIOUR OF ACCLIMATISED BATCH REACTORS OXIDISING THIOCYANATE IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR.

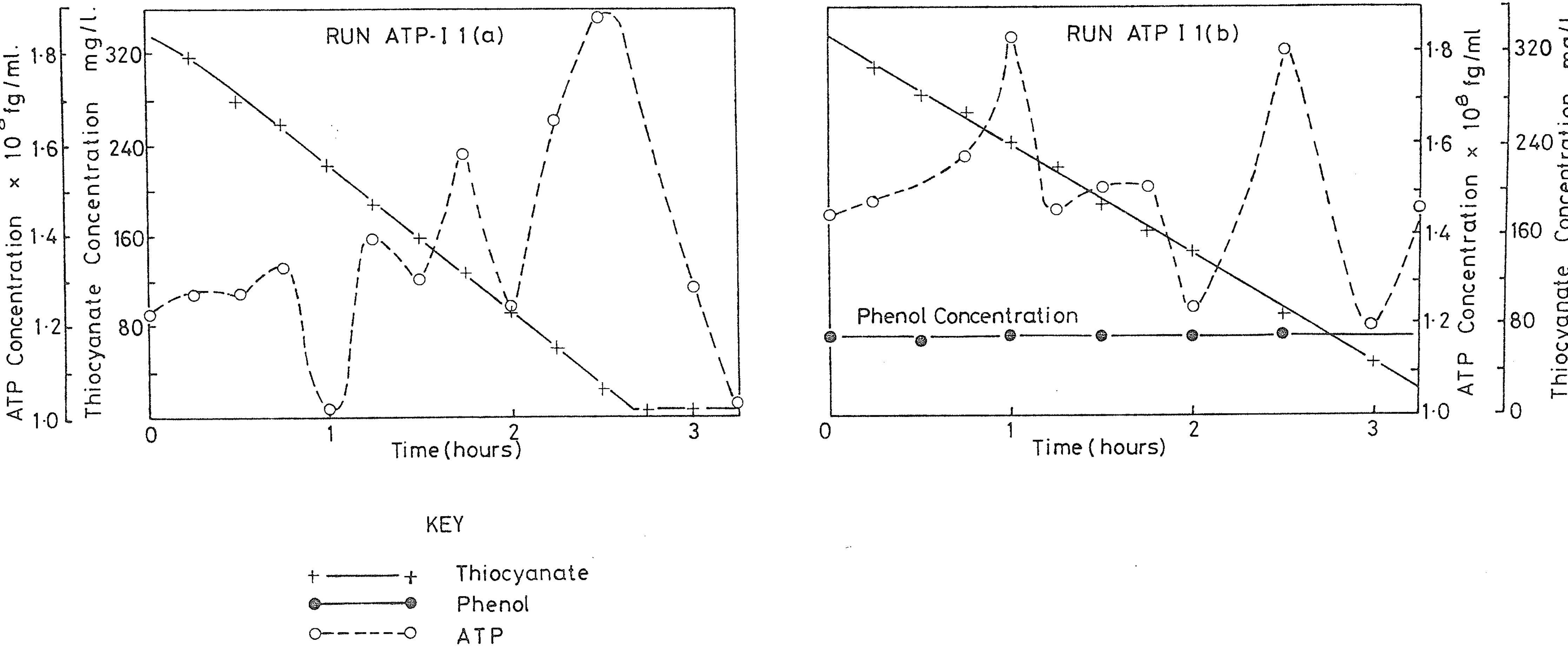


FIG./2·3 GRAPHICAL REPRESENTATION OF THE RESULTS REPORTED BY TOMLINS INVESTIGATING THE EFFECT OF PHENOL ADDITION ON THE ATP BEHAVIOR OF ACCLIMATISED BATCH REACTORS OXIDISING THIOCYANATE IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR.

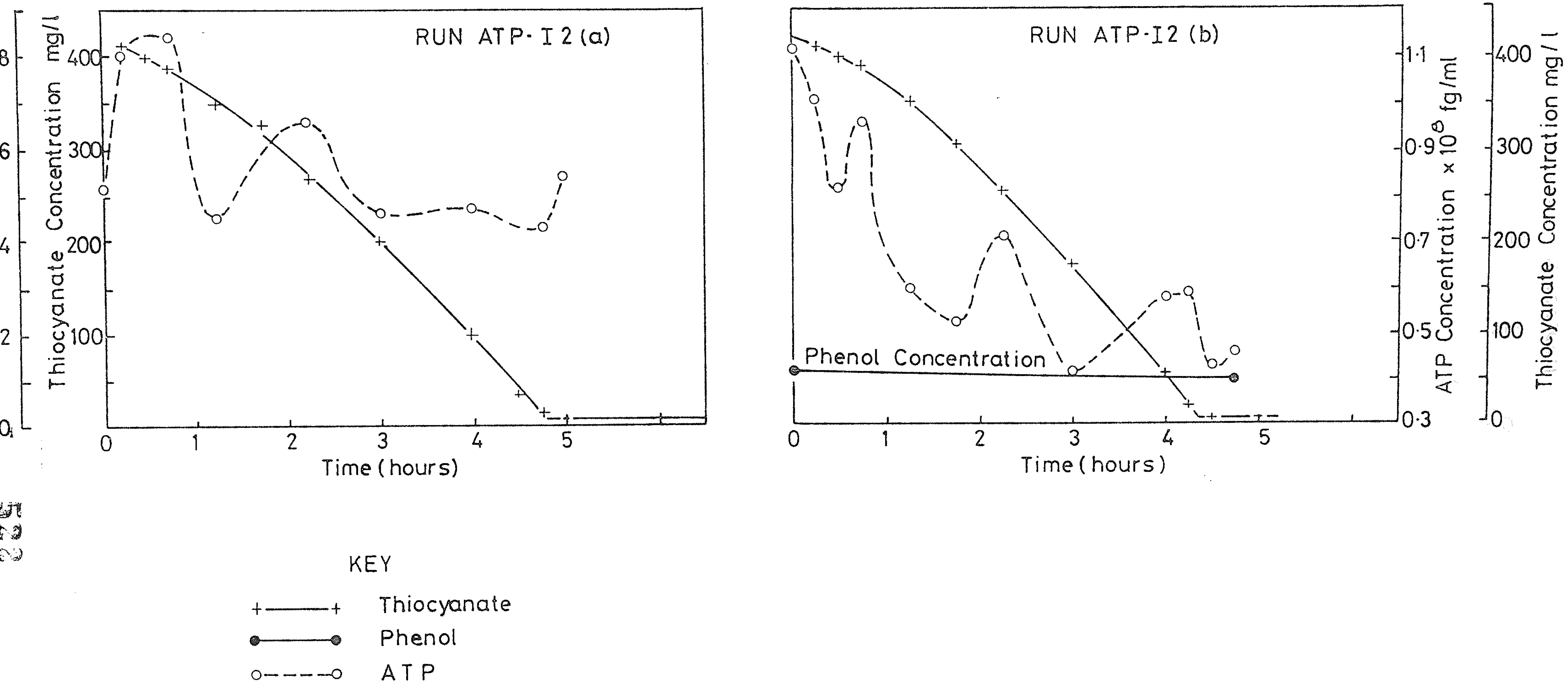
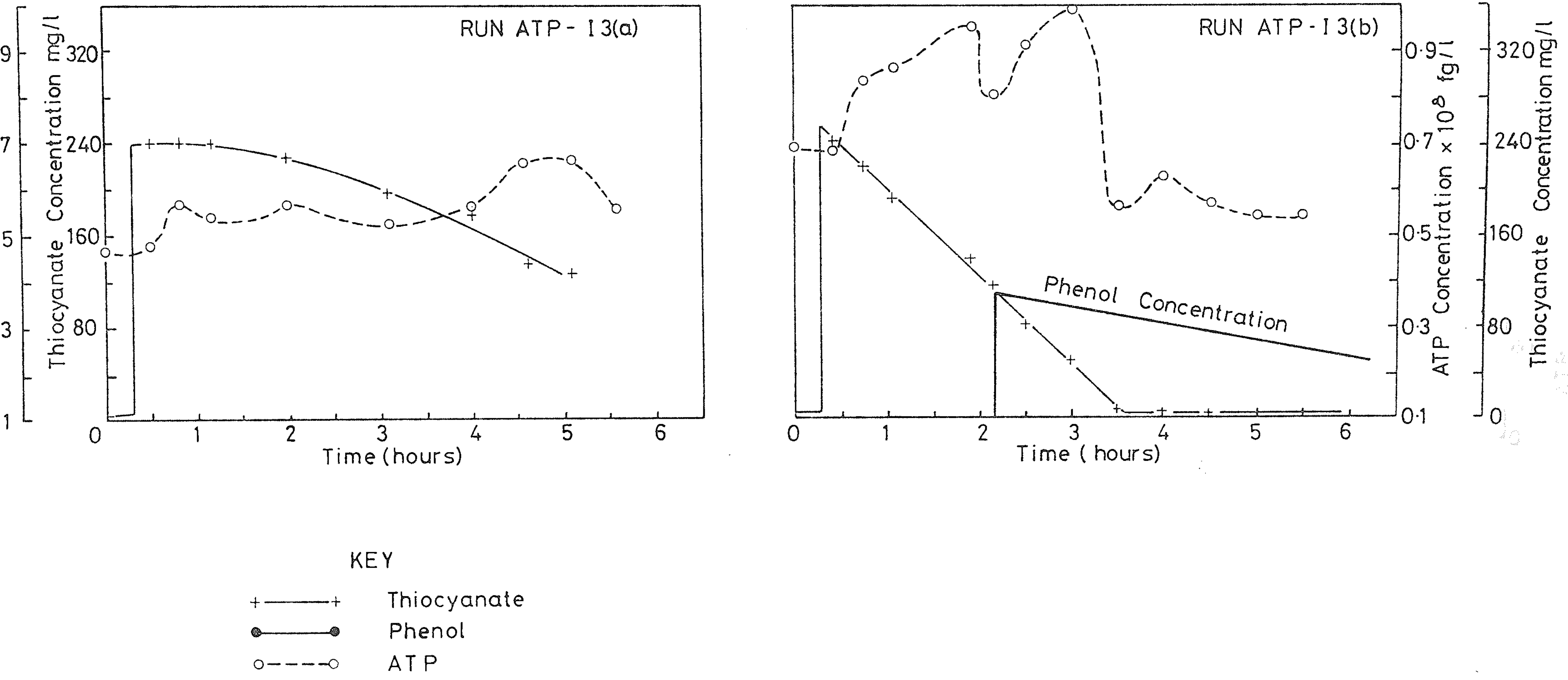


FIG. 12·4 GRAPHICAL REPRESENTATION OF THE RESULTS REPORTED BY TOMLINS INVESTIGATING THE EFFECT OF PHENOL ADDITION ON THE ATP BEHAVIOUR OF ACCLIMATISED BATCH REACTORS OXIDISING THIOCYANATE IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR.



FIG/2·5 GRAPHICAL REPRESENTATION OF THE RESULTS REPORTED BY TOMLINS INVESTIGATING THE EFFECT OF PHENOL ADDITION ON THE ATP BEHAVIOUR OF ACCLIMATISED BATCH REACTORS OXIDISING THIOCYANATE IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR.

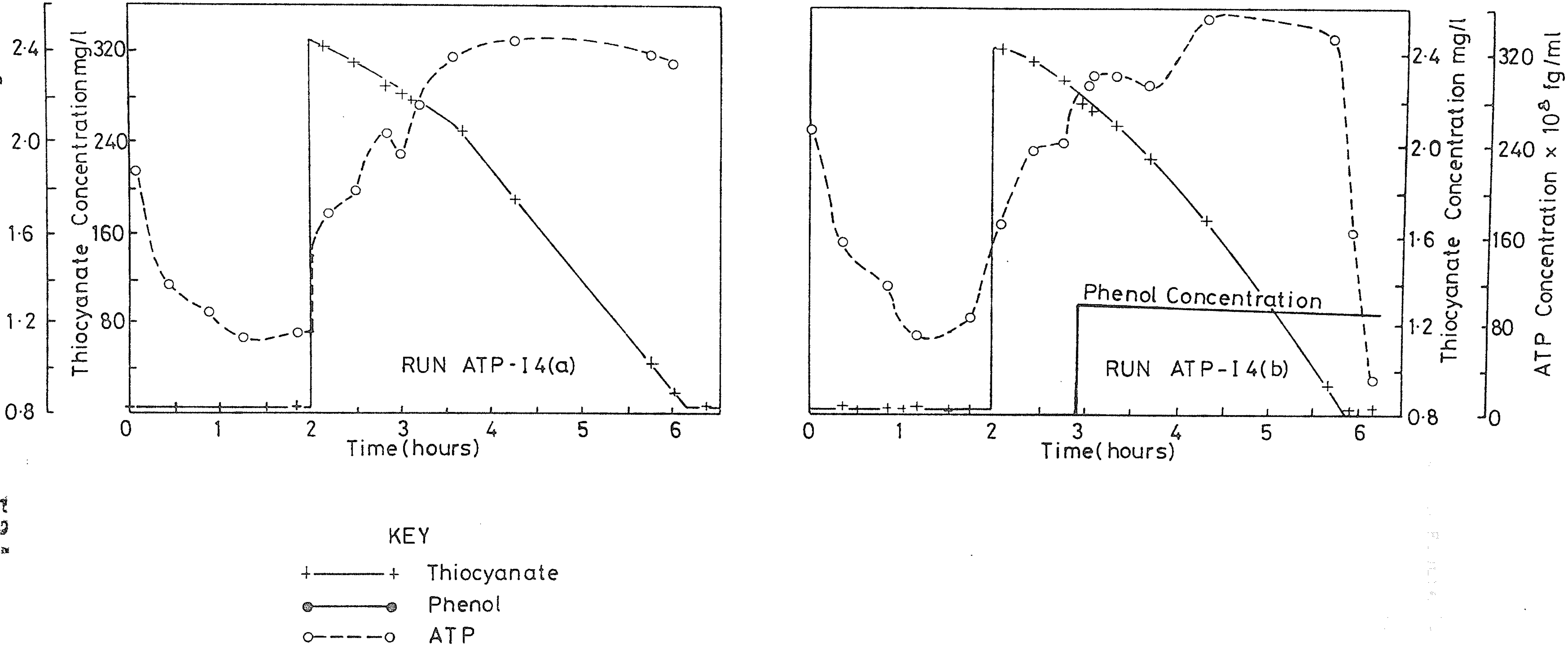


FIG 12·6 GRAPHICAL REPRESENTATION OF THE RESULTS REPORTED BY TOMLINS INVESTIGATING THE EFFECT OF PHENOL ADDITION ON THE ATP BEHAVIOUR OF ACCLIMATISED BATCH REACTORS OXIDISING THIOCYANATE IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR.

TOMLINS reported a general trend (shown in Figure 12.7 in the ATP beahviour of the batch sludges. This trend differed quite markedly from that found earlier in the project. Closer examination of TOMLINS results (rather than the general trend reported by him), however reveals some important similarities with the trend observed in this project. 7 of the 12 runs in which thiocyanate was added showed a clear increase in the sludge ATP pool towards the end of degradation of the added thiocyanate. The occurrence of these peaks in each of these runs, relative to the prevailing reactor thiocyanate concentration was recorded in Table 12.1.

The reported thiocyanate concentration, or range of concentrations at which the peak in activity occurred, generally corresponded to that reported in Chapter 4 for the occurrence of the lower peak, that is, in the concentration range 20-60 mg/l. It is suggested that errors in determining thiocyanate at low concentrations in runs where very high initial concentrations were used, may account for some variation in the location of this peak in activity in TOMLINS results. The low frequency of ATP analysis contributed to the difficulty in precisely locating this peak and may also explain the failure to identify this peak at all in other runs. ATP was not monitored at all below a thiocyanate level of 127mg/l in run ATP-13 (a) and identification of this peak was not therefore possible in this case.

At higher thiocyanate concentrations the general trend reported

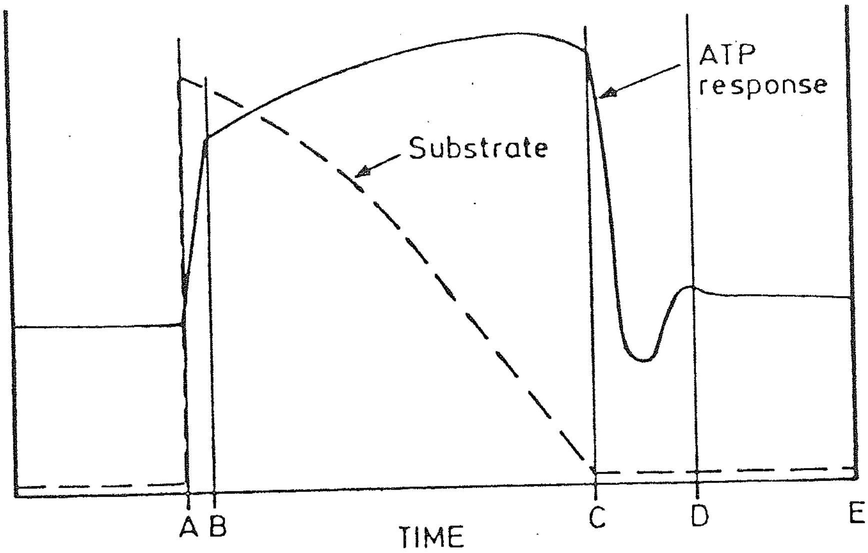


FIGURE I2.7 THE GENERAL TREND REPORTED BY TOMLINS FOR THE ATP BEHAVIOUR OF BATCH SLUDGES TREATING THIOCYANATE.

TABLE 12.1 The Occurrence Of Peaks in ATP levels relative to Thiocyanate Concentration in the range 0-80 mg/l observable by examination of TOMLINS results for Batch Sludges degrading Thiocyanate.

EXPERIMENTAL RUN	THIOCYANATE CONCENTRATION AT WHICH PEAKS (OR MAX.) ATP LEVELS WERE OBSERVED IN THE GIVEN RANGE.
	mg/l
ATP-T1	4.9
ATP-T2	31
ATP-T3	--
ATP-T4	22 *
ATP-T5	28,8
ATP-I1a	23
ATP-I1b	16 *
ATP-I2a	5.5*
ATP-I2b	17
ATP-I3a	--
ATP-I3b	49
ATP-I4a	40*
ATP-I4b	23*

* denotes thiocyanate concentration at which the maximum ATP level was recorded in the range 0-80 mg/l & where limited ATP data prevented identification of the point as a peak within the given range.

by TOMLINS was broadly similar to that found in this project, though in both cases wide fluctuations in ATP were sometimes observed, and these are discussed later. The large and almost immediate rise in ATP level following thiocyanate addition was of course the feature most evident in both of the reported trends.

It may be concluded therefore that there is good evidence to suggest that TOMLINS results show similar trends in ATP behaviour to that observed in the larger number of experiments reported in Chapter 4, although this trend is less obvious in TOMLINS results, and would not itself have been obvious by examination of his results alone.

There were obvious dangers in concluding that the ATP behaviour of the batch sludges in Chapter 4 was necessarily related to thiocyanate concentration on the basis of those batch experiments alone, for the reasons already outlined. The fact that the apparent relationship between ATP and substrate was observed in a large number of the batch experiments, despite variations in experimental conditions, and that similar evidence for the relationship was provided by TOMLINS work, did suggest that the relationship was real.

The experiments described in Chapter 6 were conducted to determine the initial ATP response of batch sludges to the thiocyanate addition, with the aim of reducing variation in, and standardising where possible, the other factors that might have been responsible for fluctuations in ATP level.

A graph was plotted in Figure 6.24 relating the mean ATP concentration per unit mass of sludge to thiocyanate concentration. This graph confirmed the type of relationship reported for the batch experiments in Chapter 4. It showed a peak in activity in the 20-70mg/l thiocyanate range, followed by a sharp decline at concentrations greater than 80mg/l, and a further peak at still higher concentrations. As in the batch experiments of Chapter 4, there was some scatter of the data points in the region of this decline in activity. The data points in this region are however derived from all three experiments described in Chapter 6, and despite the scatter of points, the trend shown was clear. The results from Chapter 6 did therefore confirm the relationship between the ATP pool and thiocyanate concentration proposed in Chapter 4.

12.2.3 Thiocyanate and its Possible Interferance in the ATP Assay Procedure

The possibility that the ATP behaviour observed, and particularly the decline observed at thiocyanate concentrations greater than about 80mg/l, merely reflected the response of the luciferase enzyme system used in the ATP assay procedure to thiocyanate, rather than the sludges response to the substrate, was investigated in Chapter 5. Some small variations in the ATP levels were recorded in the graph plotted in Figure 5.4 which related the ATP concentration found for samples containing a standard ATP solution and different thiocyanate concentrations. (The thiocyanate concentrations employed were in the range found in extracted sludge samples assayed in the project). These variations were small however and could be attributed to the practical problems in conducting an experiment of that type. The source of the possible errors that gave rise to those variations were discussed in Chapter 5.

The variations observed appeared to bear no relation to thiocyanate concentration and were not of a magnitude which would explain the variations in ATP charactistic of the general trend reported for the batch sludges in Chapter 4 or the behaviour shown in Figure 6.24.

The results confirmed that the variations in ATP behaviour observed for sludges in the project were not an artifact of the ATP assay procedure.

12.2.4 Respirometric Testing of Thiocyanate removal

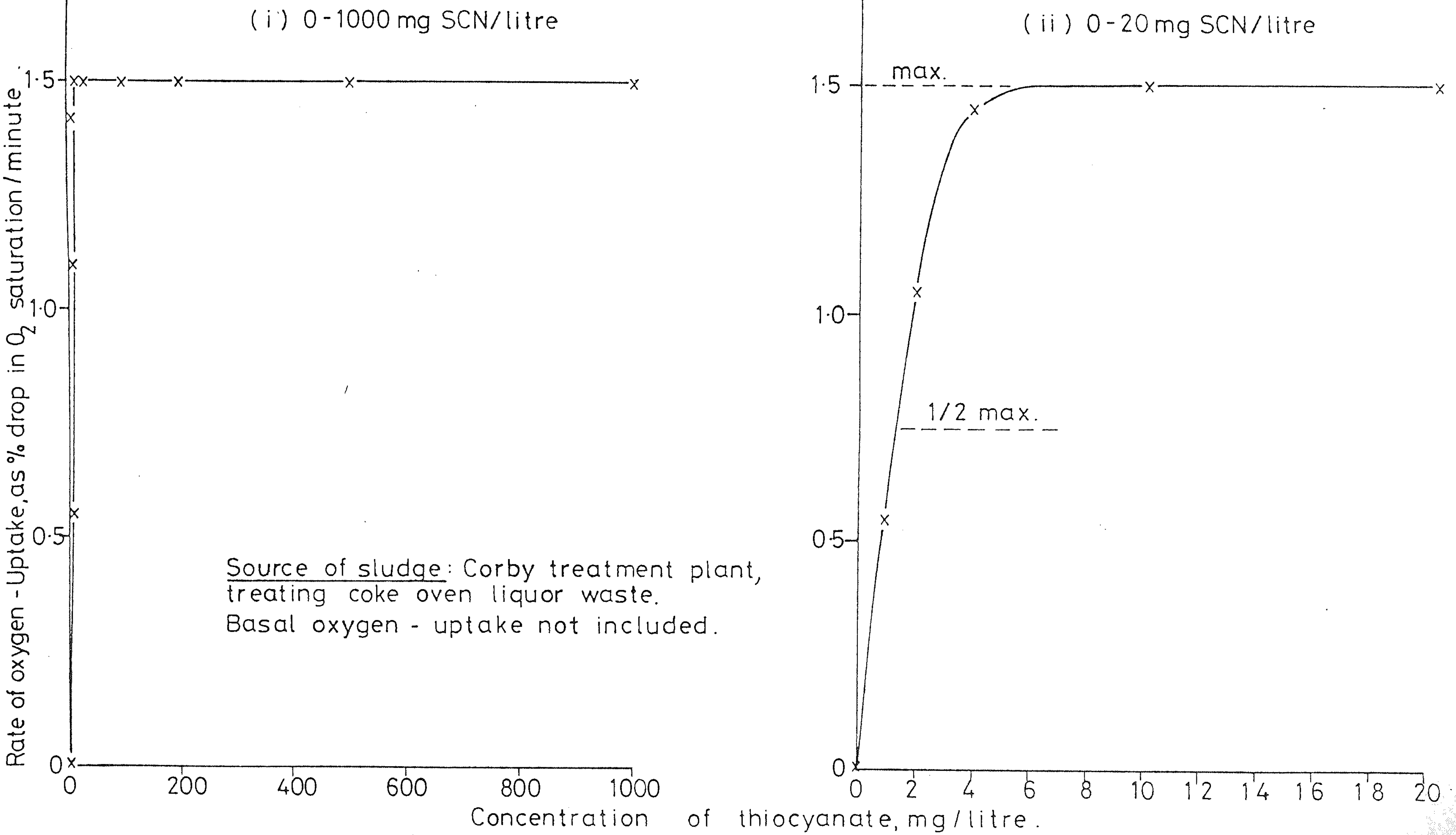
Respirometric tests were conducted on samples of sludge taken from a laboratory-scale continuous reactor treating coke oven liquor. These tests were described in Chapter 10. The decision to investigate the respirometric behaviour of sludges in terms of OUR was based on the knowledge that it had been employed by other researchers as a measure of activity for sludges treating domestic wastewater, and that the generation of ATP in the cell was known to be intimately related to the respiration of the cell.

There was no known published data concerning the application of these tests to sludge treating coke oven liquor, although work had been conducted by HIAM (152)(and reported in an BSC Internal Research Report) on samples of sludge taken from a full-scale plant treating the liquor.

Tests were conducted in Chapter 10 which measured the OUR of samples of sludge from the continuous reactor in response to the addition of different concentrations of thiocyanate. A graph was plotted in Figure 10.18 relating OUR to thiocyanate concentration. Only limited data was available to investigate this relationship. The graph produced showed a single peak and a form which indicated that oxygen uptake was inhibited by concentrations greater than 60mg/l. The limited data available meant that it was not possible to define this relationship as precisely as would have been desirable, and in particular to determine whether there were 2 peaks in respiratory activity analogous to the 2 peaks observed for ATP activity.

As noted in Chapter 3, a number of researchers have reported the phenomenon of substrate inhibition in thiobacilli metabolising thiocyanate, and the decline in both ATP and OUR activity at higher thiocyanate levels confirmed this phenomenon in sludges metabolising thiocyanate, The results of the respirometric tests conducted by HIAM referred to above, and shown in Figure 12.8 , did not however provide any evidence of substrate inhibition. The difference in these results may relate to whether heterotrophic or autotrophic removal of

FIGURE I2.8 RESULTS OF RESPIROMETRIC STUDIES (FROM BSC INTERNAL RESEARCH REPORT)
EFFECT OF THIOCYANATE ON SLUDGE ACTIVITY.



(i) 0-1000 mg SCN/litre

(ii) 0-20 mg SCN/litre

Source of sludge: Corby treatment plant, treating coke oven liquor waste.
Basal oxygen - uptake not included.

thiocyanate was occurring. Substrate inhibition of thiocyanate removal is a characteristic of autotrophic removal by thiobacilli, and has not been reported for heterotrophic removal where thiocyanate serves as a nitrogen rather than a primary energy source.

STAFFORD and CALLELY(115) reported for example that T. Thiocyanooxidans was inhibited by thiocyanate concentrations greater than 20mM, that is greater than about 60mg/l. The inhibition evident from the respirometric tests, reported in Chapter 10 was therefore consistent with their findings for that organism which has been isolated from the sludge treating coke oven liquor.

The results of the batch experiments described in Chapter 4 and 6 showed 2 peaks in ATP activity relative to thiocyanate concentration. The characteristics of the peak in the lower concentration range resembled those of the peak found in the respirometric studies and may therefore have reflected the behaviour of a population of thiobacilli resembling T. Thiocyanooxidans.

Before considering the significance of ATP in activated sludge treatment further, it may be useful to consider the results obtained for phenol removal first.

12.3 The ATP Behaviour of Batch Sludges Treating Phenol

12.3.1 A Comparison with the Results Reported by TOMLINS

The ATP behaviour of batch sludges treating thiocyanate, already discussed, showed a consistent relationship between ATP and thiocyanate concentrations. The appearance of 2 distinct peaks in that relationship was the basis for the proposal that the observed behaviour could be attributed to 2 distinct populations of organisms.

It would be useful therefore to analyse the results obtained with phenol-treating sludges to investigate whether a similar relationship between ATP and substrate concentration existed in this case also.

As noted in Chapter 7, the ATP behaviour of batch sludges acclimatised to treating phenol in a basal medium of treated coke oven liquor was well-established by TOMLINS. The experiments described in that chapter were conducted in order to check that TOMLINS findings applied to the phenol-treating sludges being maintained at the time of the project. The experiments described in this project differed from those described by TOMLINS in that they considered the ATP response of the sludges to multiple additions of phenol.

The results of experiments E1 and E11 described in Chapter 7 appeared consistent with the findings of TOMLINS in as much that a sharp decline in the ATP level of the sludge followed phenol addition.

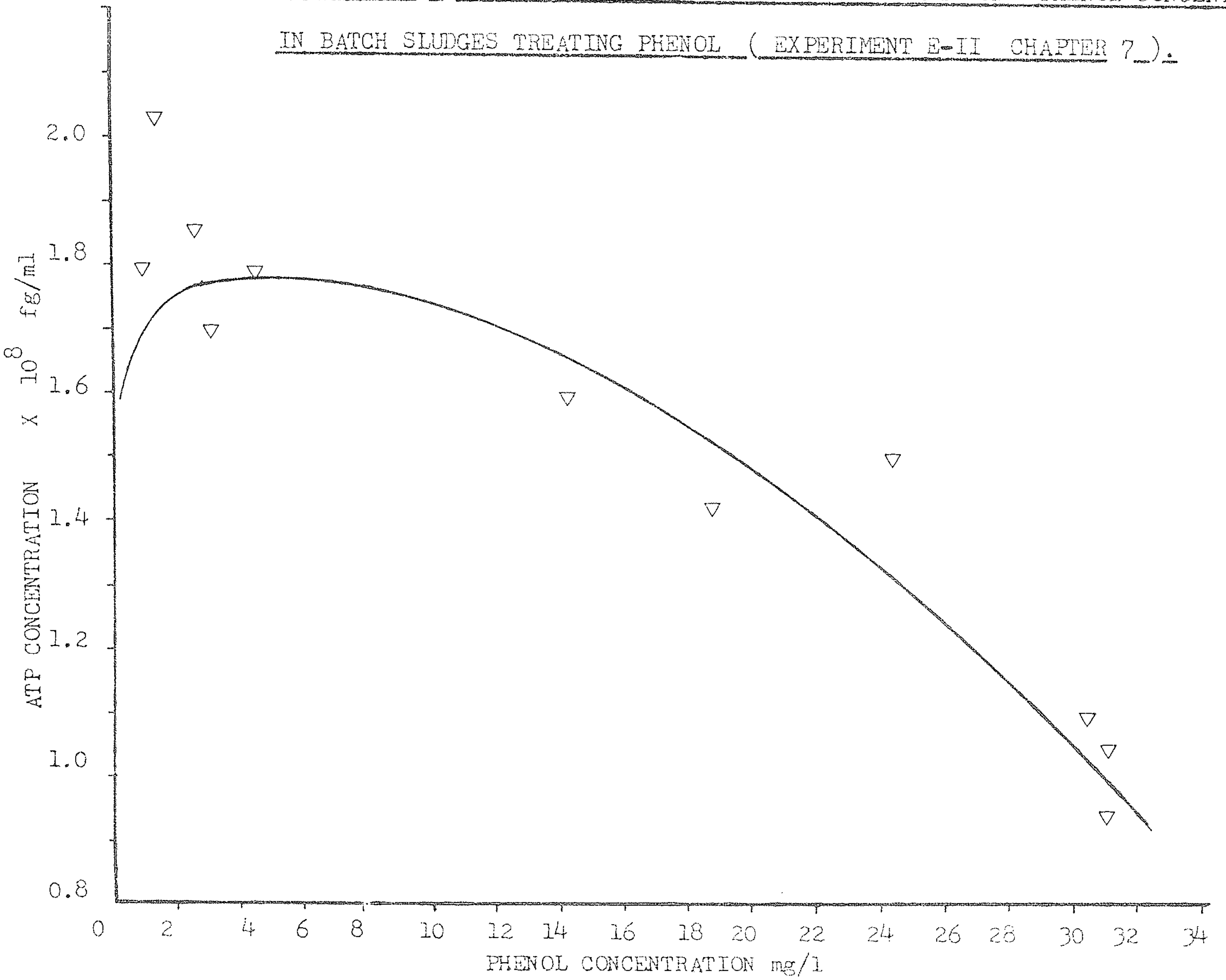
In both experiments E1 and E11 the ATP behaviour of the sludge was maintained for sometime after the first addition of phenol before any further additions were made. Large fluctuations in the ATP level were observed during this period in experiment E1, but these were largely absent for the corresponding period in experiment E11.

The procedure applied earlier for the thiocyanate treating sludges, of relating ATP and substrate concentrations, was applied to the results of experiment E11 for this period following the first addition of phenol. The graph relating these two parameters was plotted in Figure 12.9 . This graph showed a maximum ATP level at about 2mg/l, and declined at higher phenol concentrations. As with the thiocyanate-treating sludges, this could be interpreted as evidence of substrate inhibition, which has already been well-established by other researchers as a phenomenon characteristic of phenol-metabolising organisms.

In contrast to experiment E11, the degradation of the first addition of phenol was not allowed to continue to completion in experiment E1 before a further addition was made, and it was therefore not possible to test for this relationship in this case.

In experiment E11 the level of ATP following the metabolism

FIGURE 12.9 THE RELATIONSHIP BETWEEN REACTOR ATP AND PHENOL CONCENTRATIONS IN BATCH SLUDGES TREATING PHENOL (EXPERIMENT E-II CHAPTER 7).



of the first addition of phenol was noticeably higher than at the start of the experiment. This could be attributed to the increase in the mass of organisms resulting from the metabolism of the substrate.

It will be recalled that it was proposed in Chapter 4 that ATP represented a measure both of active biomass and activity. In the batch experiments involving degradation of thiocyanate, the increase in ATP concentration following the complete degradation of the substrate over the level in the starved condition before the start of the experiment was very small or negligible.

This difference between the two types of experiment may be explained by assuming that the thiocyanate-treating system contained predominantly autotrophs, whilst the phenol-treating system of course contained heterotrophs. The growth characteristics of autotrophs are such that the increase in biomass expected would be small and possibly undetectable, whilst the opposite would be expected in the heterotrophic system.

(These growth characteristics of autotrophs would also explain why TOMLINS was unable to use suspended solids measurements effectively for the determination of the growth rate and yield coefficient of his thiocyanate-degrading sludges. The very small increase in suspended solids expected from the autotrophic metabolism of a single dose of thiocyanate, as well as the apparently large errors involved in the procedure he employed for suspended solids determinations meant, as he discovered, that such measurements were unsuitable for kinetic parameter determination in that sludge system).

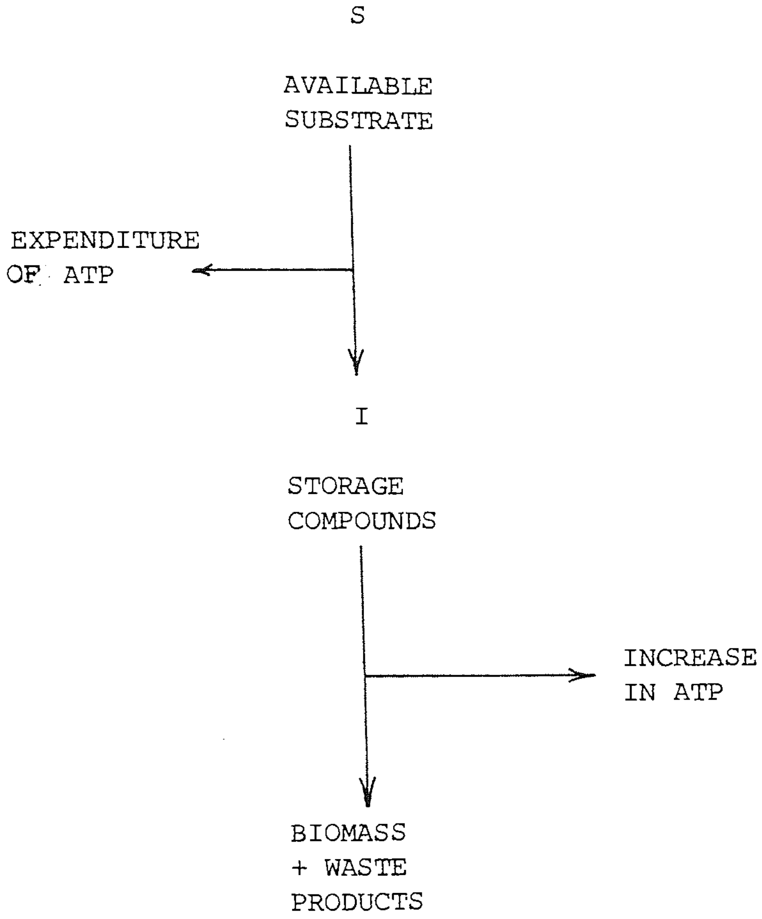
This difference in the growth characteristics of autotrophs and heterotrophs also meant that the ATP/substrate concentration relationships depicted graphically were less accurate for the phenol systems than for the thiocyanate systems. This was because the ATP component due to biomass was significant in the former and not in the latter. The interference in this relationship by changes in biomass, and other factors that may affect ATP levels, was reduced by use of experiments of the type described in Chapter 6.

Continuing the examination of experiment E11, it was not practical to explore the ATP/substrate concentration relationship throughout the remaining period in that experiment, when several further additions of phenol were made, due to the wide variations in ATP level observed. During this period of the experiment each addition of phenol was followed by a decline in ATP level follwed by a recovery to a level lower than that before the addition. It is suggested that the fact that recovery was to a lower level than before substrate addition reflected the effect of further inhibition in activity due to substrate inhibition. The moderately rapid decline and recovery pattern observed at these higher phenol concentrations was not observed following the first addition in experiment E11, but was for the first addition in experiment E1. The concentration of phenol resulting from the first addition of phenol in experiment E1 was higher than that in experiment E11, and it may be that this phenomenum is confined to these higher phenol levels.

This behaviour may perhaps be explained by a concept similar to TOMLINS storage model. It would be useful therefore to consider TOMLINS results for phenol-treating sludges and his storage model before proceeding further with analysis of this projects results.

12.3.2 TOMLINS Storage Model as an Explanation of the ATP Behaviour of Sludges Treating Phenol

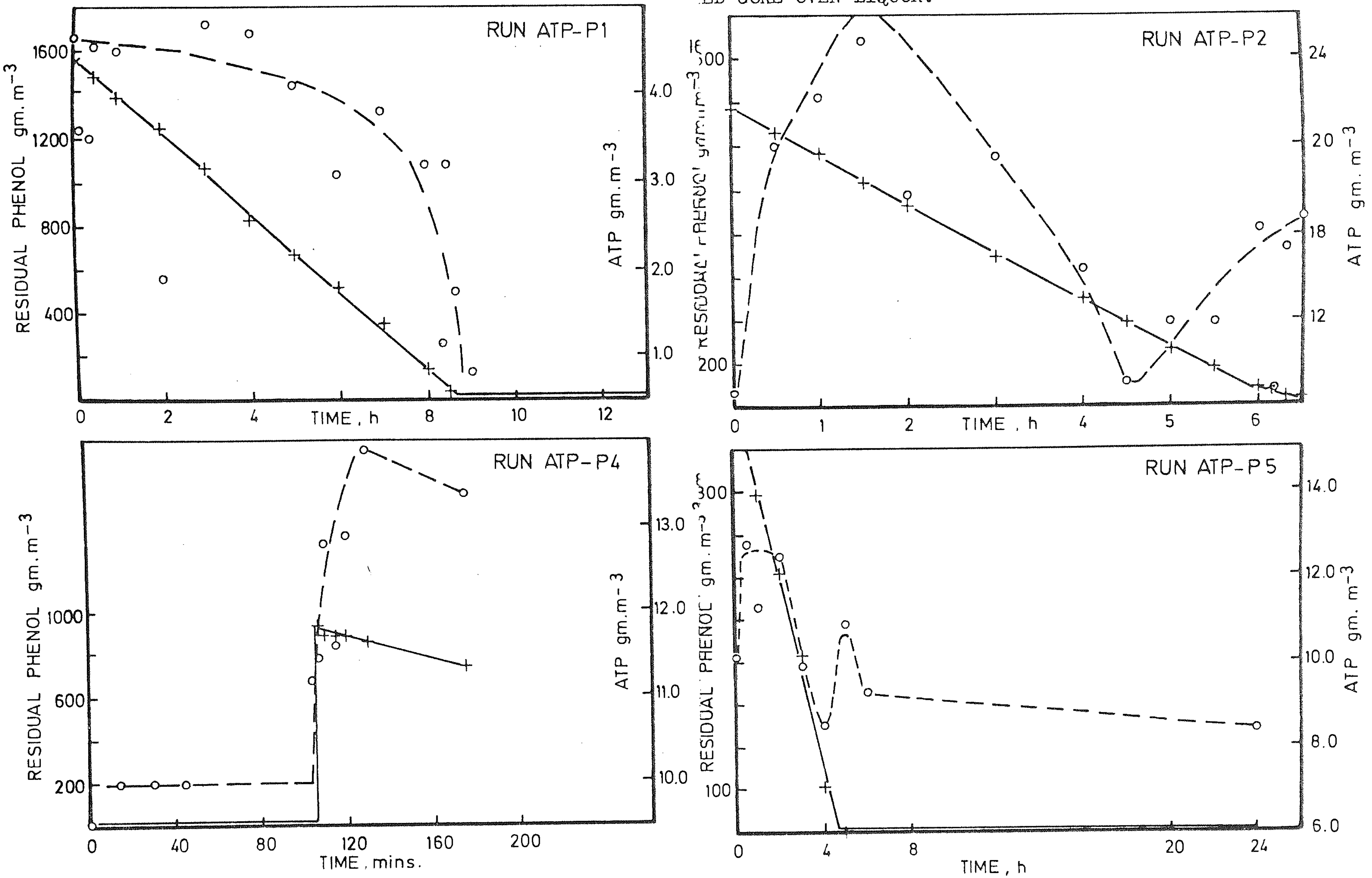
TOMLINS proposed that inperiods of nutrient sufficiency in sludges treating phenol, theavailable substrate was converted to storage compounds. This required the expenditure of ATP, and was thus assumed responsible for the decline in ATP concentration observed following phenol addition to the sludge. These storage compounds it was suggested were then utilised when the organisms experienced nutritionally less favourable conditions. The metabolism of these storage compounds were assumed to generate ATP which was responsible for the recovery in the ATP level observed in the sludge. TOMLINS two stage system was represented thus;



TOMLINS scheme on first examination appears reasonable in principle in that it explains the continuing rise in the sludge ATP level that he observed in batch experiments beyond the period that phenol was detectable in the sludge supernatent. (This behaviour can be seen by inspection of TOMLINS results for these batch experiments which have been presented in Figures 12.10 to 12.12). The continuing rise in ATP is explained in the model by metabolism of stored products.

However, although reasonable in principle it is suggested that some of the basic assumptions made in the mathematical model proposed, to explain the storage process were not consistent with the normally accepted behaviour of living organisms or with the findings and proposals made earlier in this project.

FIGURE 12.10 GRAPHICAL REPRESENTATION OF THE RESULTS REPORTED BY TOMLINS ON THE ATP BEHAVIOUR OF ACCLIMATISED BATCH REACTORS OXIDISING PHENOL IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR.



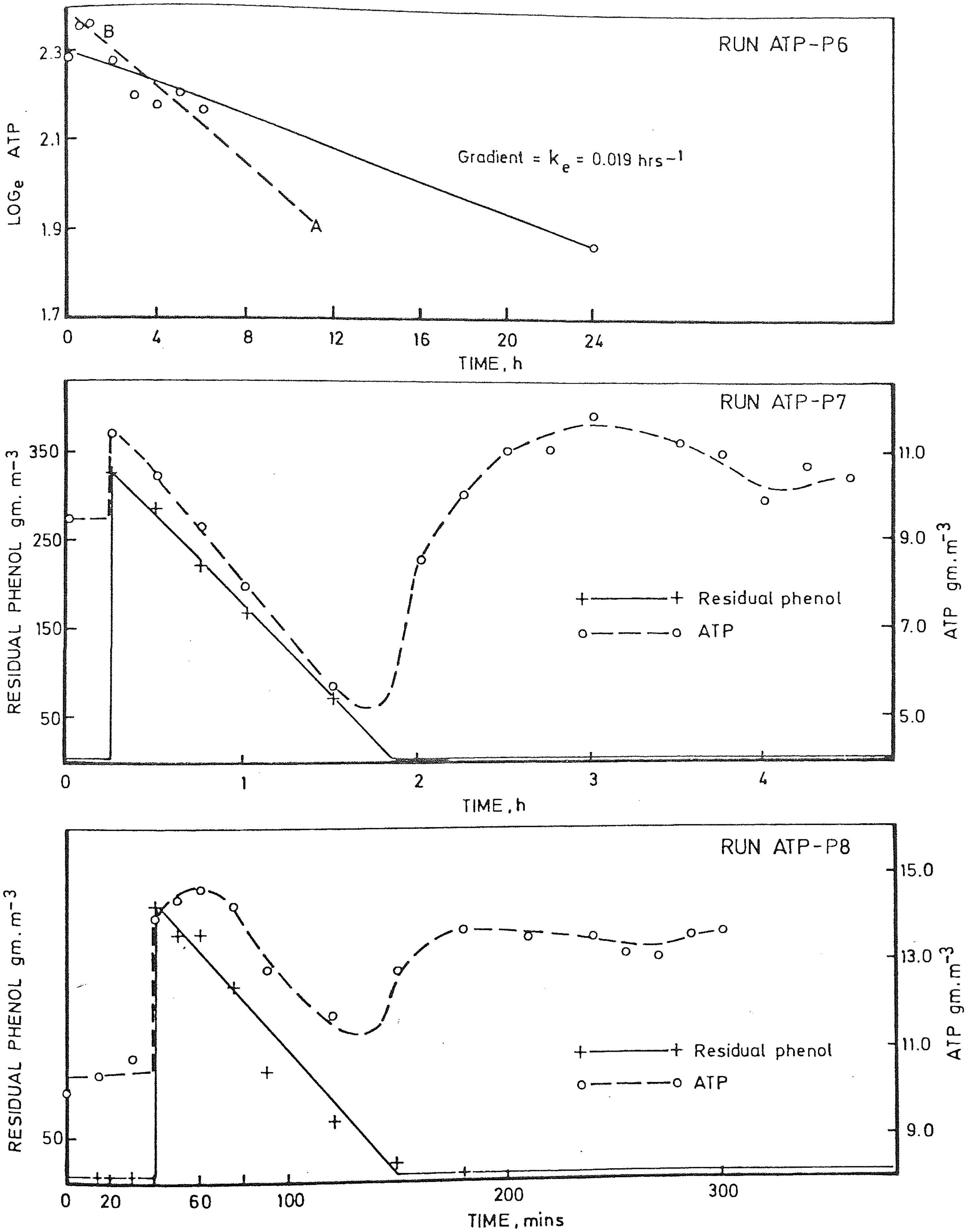


FIGURE I2.II GRAPHICAL REPRESENTATION OF THE RESULTS REPORTED BY TOMLINS ON THE ATP BEHAVIOUR OF ACCLIMATISED BATCH REACTORS OXIDISING PHENOL IN IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR.

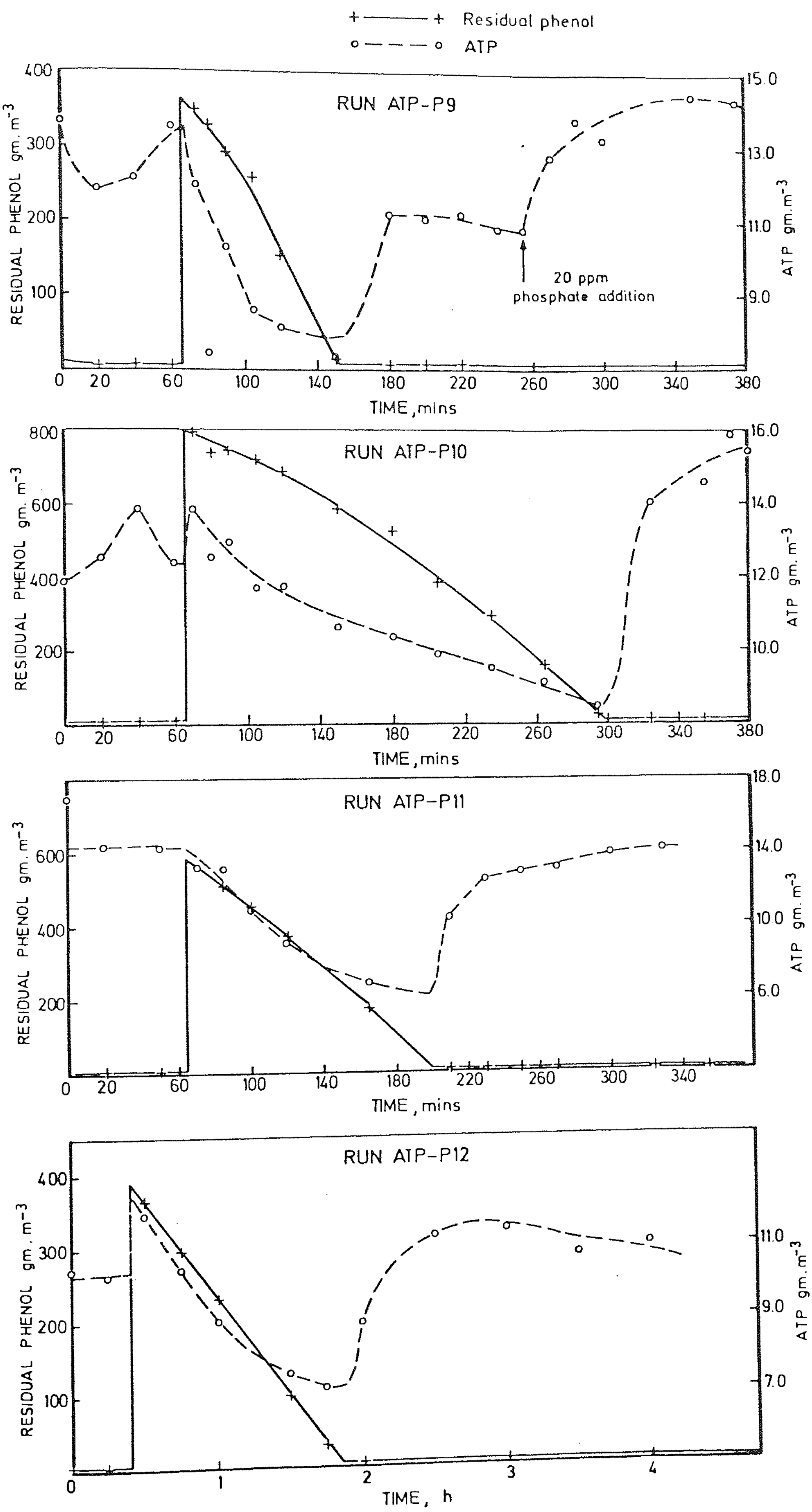


FIGURE I2.I2 GRAPHICAL REPRESENTATION OF THE RESULTS REPORTED BY TOMLINS ON THE ATP BEHAVIOUR OF ACCLIMATISED BATCH REACTORS OXIDISING PHENOL IN A BASAL MEDIUM OF TREATED COKE OVEN LIQUOR.

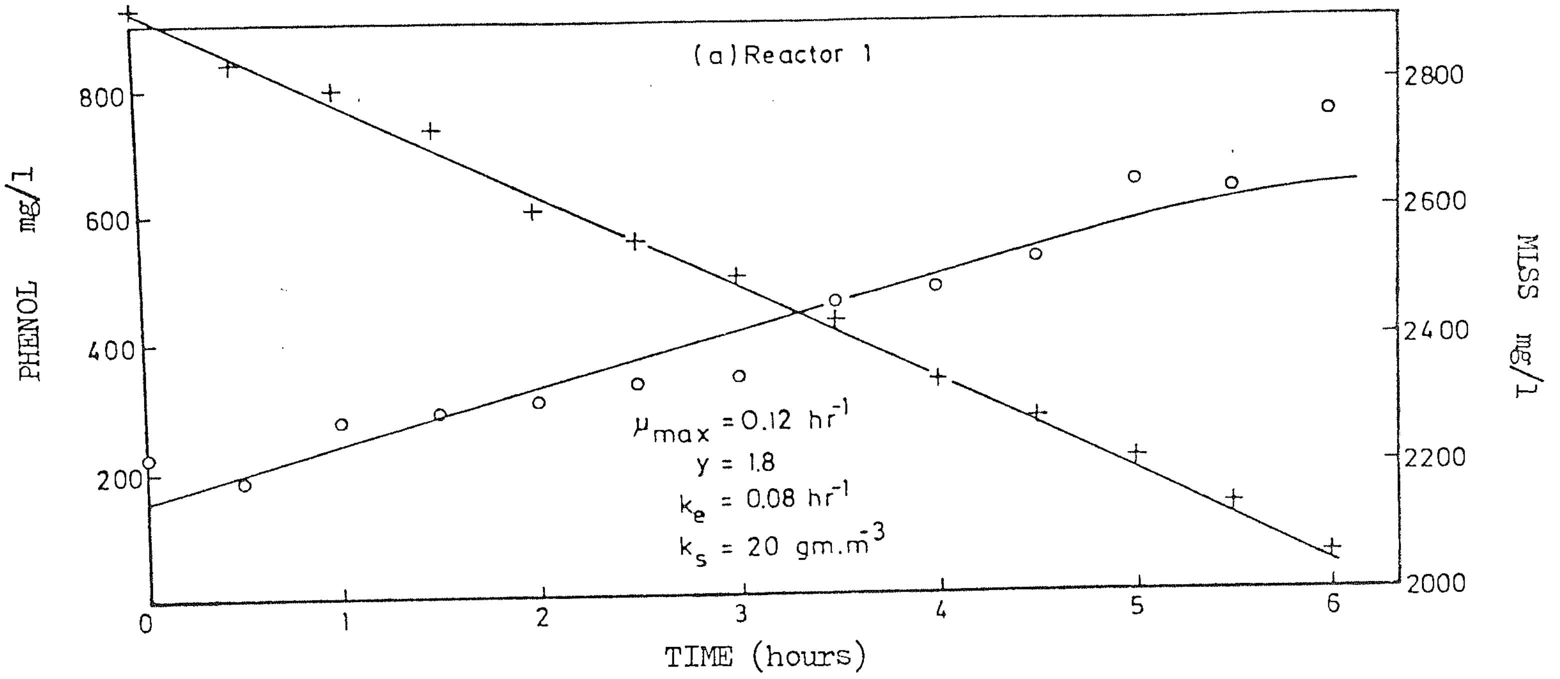
The model assumed that during periods of nutrient sufficiency substrate was lost from the system solely by conversion to stored products within the sludge organisms, and that the active cell concentration remained approximately constant throughout this period. As the experiments reported in TOMLINS thesis showed a large and continuing increase in biomass during the batch degradation of phenol solutions, the implication was that the observed increases in biomass were solely due to the accumulating mass of storage product within a fixed cell population.

The results of one typical batch experiment reported by TOMLINS in which phenol and MLSS were monitored is recorded in Figure 12.I3.

The increase in biomass with substrate removal shown in these experiments, followed the general pattern that would be expected by a bacterial population metabolising substrate. This does not of course preclude substrate storage according to the general scheme outlined, but is suggested that the major and most likely reason for the observed increase in biomass would be cellular reproduction. The assumption that substrate storage will occur without cellular reproduction in conditions of substrate excess is not it is suggested a simplification that can be reasonably accepted in the light of the behaviour normally observed by organisms in such conditions.

The storage model also assumed that the initial steady state

FIGURE 12.13 RESULTS OF A TYPICAL BATCH EXPERIMENT REPORTED BY TOMLINS IN WHICH PHENOL AND MLSS WERE MONITORED.



ATP content of the batch sludge in the starved condition before phenol addition could be represented by the ATP content of the sludge at that time. This assumption is consistent with that made earlier in the project, that the ATP content of a cell in endogenous conditions was a constant and could therefore be used as a measure of biomass. However, the model then went on to consider ATP as just a measure of biomass throughout the batch degradation of phenol. It was obvious from the experiments with batch sludges treating thiocyanate described earlier in this project, and from TOMLINS own similar experiments, that ATP was a measure of activity rather than just biomass in conditions in which substrate was available to the sludge organisms. (ATP can only be used solely as a measure of biomass in rather special circumstances, in endogenous conditions, or when the substrate conditions remain constant in the systems under consideration. Neither of course would be applicable to sludges treating phenol in batch mode).

It is suggested that the behaviour of TOMLINS batch sludges treating phenol may be explained more in terms of substrate inhibition than by the principle of storage whereas his mathematical storage model assumes the opposite to be true.

It is not possible at this stage of the discussion to dismiss the concept of storage entirely. It is possible however to challenge the need for the model to explain the observed ATP behaviour even further. Two reasons that may be put forward for justifying the need for such a model may be;

1. To provide an explanation for the continuing rise in sludge ATP in the absence of phenol following degradation of a batch of substrate.

2. To explain the large drop in sludge ATP level recorded following phenol addition at the start of TOMLINS batch experiments. (As the sludges were reported starved of substrate before the start of the experiments it might be assumed from the discussions earlier in this project that the cells contained the minimum level of ATP corresponding to endogenous conditions. Some new explanation would therefore be required to explain a fall in ATP level below what was assumed to be the minimum value).

It is suggested that better explanations than those offered by the storage model, may be put forward to explain 1. and 2. above.

It was evident from the ATP/substrate relationship shown in Figure 12.9, that the maximum sludge activity as measured by ATP was obtained at very low phenol concentrations, at a level of about 2mg/l in this case. Mono-phenol concentrations are difficult to measure accurately in coke oven liquors at such low levels due to interference in the assay by other organic compounds typically found in coke oven liquor. It would therefore have been difficult to ensure that a zero phenol concentration existed in the so-called endogenous or starved conditions employed in the experiments described. In addition, even in the absence of mono-phenol, the organisms responsible for its metabolism may also be able to metabolise other materials present in the sludge medium, including those derived from the liquor and the breakdown products of lysed sludge organisms. The organisms may of course still be metabolising metabolic intermediates in the breakdown of phenol which may have accumulated intra- or extracellularly. Figure 12.[14] shows various metabolic pathways for the degradation of phenol, and the large number of potential intermediates that may accumulate.

Thus it can be seen that there may be several ways in which the sludge organisms may display appreciable metabolic activity in the absence of phenol. This might also logically lead to challenging the concept of steady-state endogenous metabolic conditions in these systems. It is suggested for the reasons outlined, that so called endogenous conditions are unlikely ever to display steady-state activity in reality, particularly when such a sensitive measure of cellular activity as that offered by ATP is used. The concept does offer a convenient simplification of the system however, which has advantages in modelling the system, as will have been evident from kinetic work described earlier in the project.

It is convenient to project a constant ATP level for a sludge in endogenous conditions, but there is abundant evidence that constant ATP levels are not found in reality in starved sludges. In fact wide fluctuations in the ATP content of sludges experiencing starvation conditions were a feature of all the

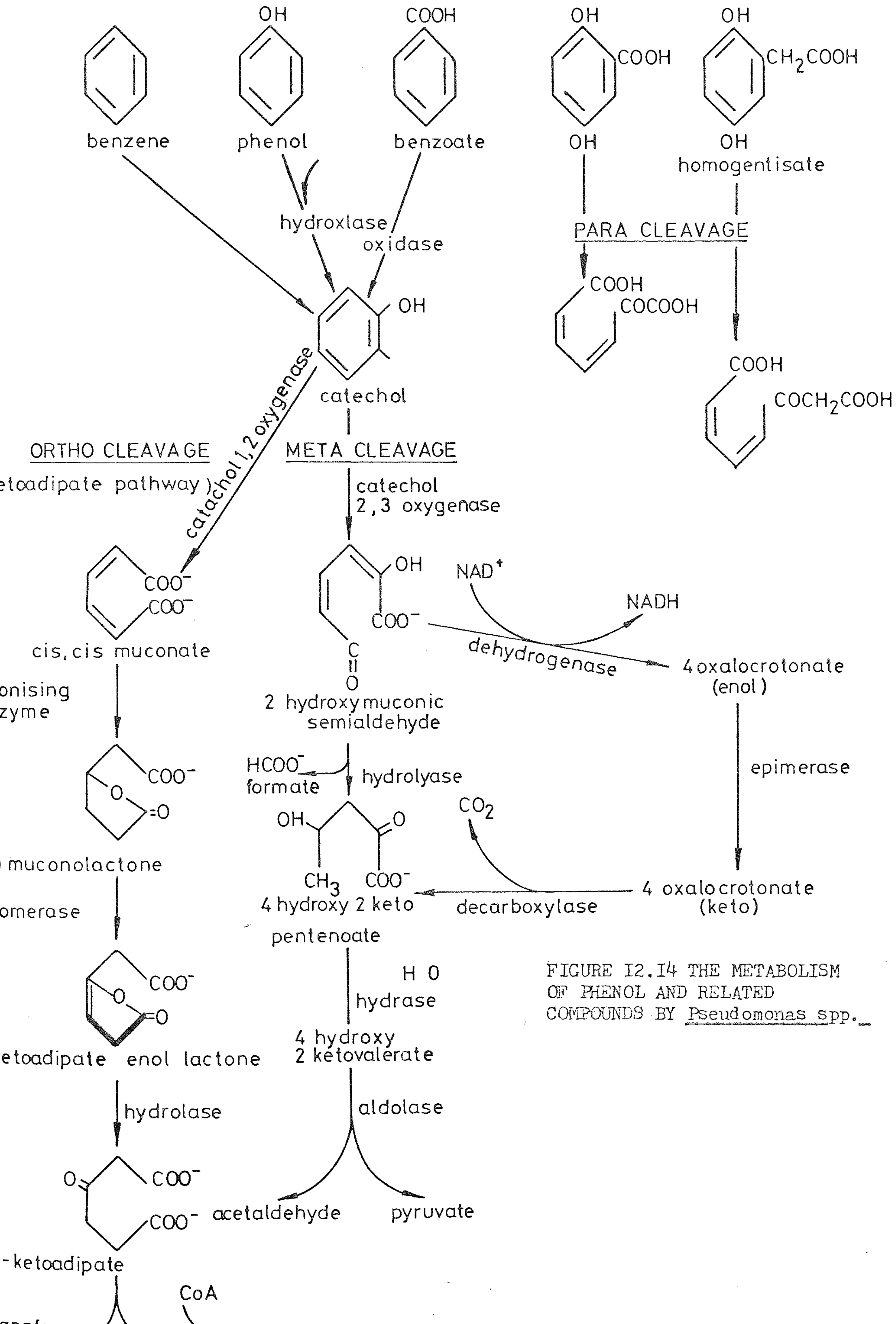


FIGURE 12.14 THE METABOLISM OF PHENOL AND RELATED COMPOUNDS BY *Pseudomonas* spp.

experiments conducted by TOMLINS and in this project, regardless of whether phenol or thiocyanate-treating sludges were considered.

It would be convenient to be able to explain the ATP behaviour of sludges treating phenol solely in terms of substrate inhibition. If ATP represented a measure of growth rate as proposed in Chapter 4, and the growth rate was subject to substrate inhibition of the type described by HALDANE, then the relationship between ATP and phenol shown in Figure 12.15 would be expected. Evidence has already been provided that this is the type of ATP/substrate relationship found. Assuming that this is the only relationship governing the ATP behaviour of the sludge, then the predicted behaviour of a batch activated sludge treating phenol would be of the type shown in Figure 12.16 . It is the trend in ATP behaviour rather than the precise location of the curve that is important, the precise location being dependant upon the values chosen for the kinetic constants in EQUATION 12.1. If the explanations offered earlier to explain 1. and 2. are accepted, the only difference between the trend shown in Figure 12.16 and that shown in TOMLINS experiments is the rapidity of the decline in the ATP pool following phenol addition. TOMLINS experiments typically showed a gradual decline in the pool, over a period greater than one hour, whereas consideration of substrate inhibition alone would require the immediate reduction in the ATP pool to the minimum value.

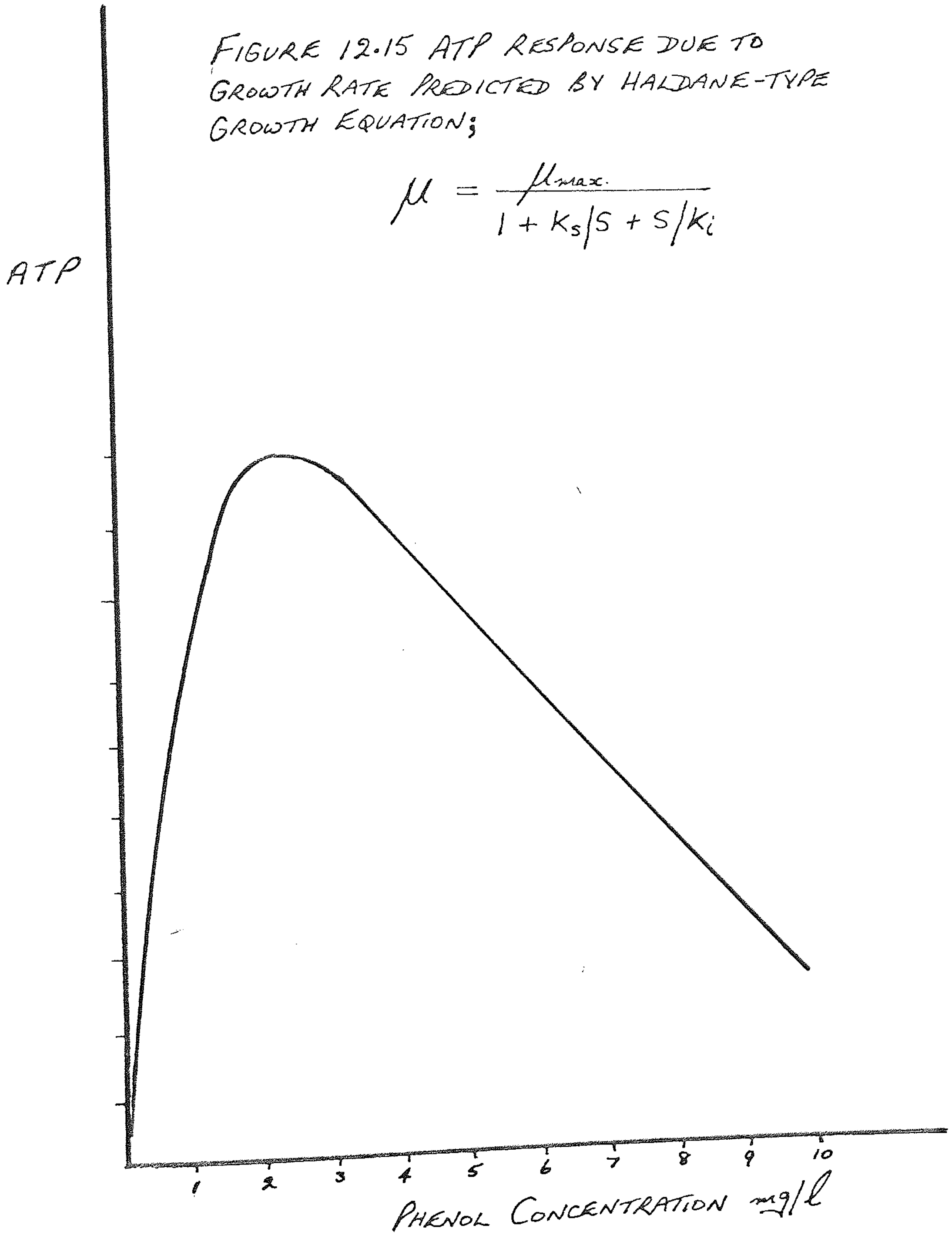
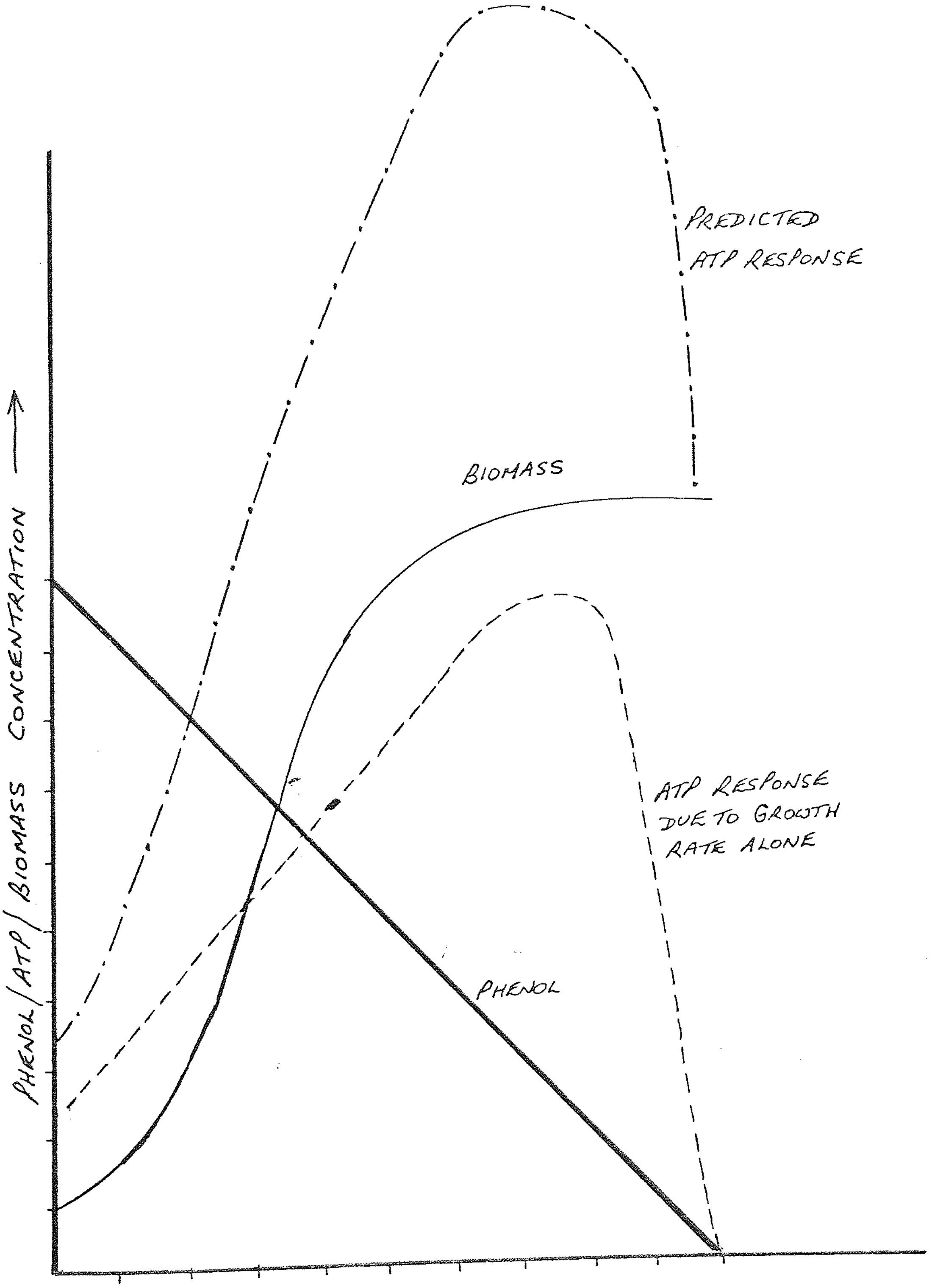


FIGURE 12.15 ATP RESPONSE DUE TO GROWTH RATE PREDICTED BY HALDANE-TYPE GROWTH EQUATION;

$$\mu = \frac{\mu_{max.}}{1 + K_s/S + S/K_i}$$

FIGURE 12.16. PREDICTED BEHAVIOUR OF BATCH SLUDGE TREATING PHENOL WITH ATP AS A MEASURE OF BIOMASS AND GROWTH RATE, USING THE HALDANE-TYPE GROWTH EQUATION. (NO STORAGE MODEL).



It is not possible at this stage therefore to dismiss the need for a storage or similar concept, but it can be seen from Figure 12.16 that substrate inhibition will explain the major features of the ATP behaviour observed.

There are also difficulties in explaining the decline and recovery behaviour in the ATP pool following multiple substrate additions in experiment E11 solely in terms of substrate inhibition.

The most important finding so far then from the examination of the ATP behaviour of batch sludges treating phenol is that ATP behaviour seems to be related to substrate concentration in a similar manner to that found for thiocyanate treating sludges, and that it would appear that the concept of ATP as a measure of growth rate may be applicable to both. The data from this project upon which the relationship between ATP and phenol concentration is based however, was based on limited data from the experiments described in Chapter 7. As with the results of the batch sludges treating thiocyanate there were dangers in assuming this relationship on the basis of the results of batch experiments of the type described in Chapter 7. Experiments analogous to those performed for the thiocyanate treating sludge in Chapter 6 were therefore performed for the phenol system and were described in Chapter 8. These are now considered.

12.3.3. The Significance of the Initial ATP Response of Batch Sludges to Different Phenol Loadings (Chapter 8).

The design and principle of the experiments described in Chapter 8 were the same as those described in Chapter 6 for the thiocyanate system.

The most important finding from this experiment was that the relationship between the ATP content of the sludge per unit biomass and substrate concentration showed two peaks in activity, as shown in Figure 8.23. The possibility existed therefore that the ATP behaviour observed reflected the activity of 2 distinct populations of organisms. However, the distinction between these two peaks was less obvious than for the thiocyanate treating sludge. It may be possible to approximate the curve in Figure 8.23 by a single curve showing a gradual decline at phenol concentrations greater than 40mg/l. It would be possible to interpret the behaviour as that approximating to that of a single population of organisms exhibiting substrate inhibition above that substrate level. Neither the first peak occurring in the concentration range 0-50mg/l or the single peak that would be produced by the above approximation produced a peak in activity as low as that shown for the work described in Chapter 7.

12.3.4 ATP and Phenol Removal in the Continuous Reactor Study (Chapter 9). A Comparison with the Results of Batch Experiments.

The results of examining the ATP/phenol relationship in this study was that this indicated that maximal activity occurred at a phenol concentration of about 2mg/l and that activity was inhibited at higher concentrations consistent with the phenomenon of substrate inhibition.

Precise definition of the curve at very low phenol concentrations due to errors in assaying these low values, and at higher concentrations due to limited data was not possible, but the trend shown was obvious. The ATP/substrate relation shown was very similar to that observed from the results of Chapter 7 already considered. It was not possible to check for the existence of the twin-peak activity found in Chapter 8 and recorded in Figure 8.23, as reactor phenol concentrations greater than 21mg/l were not found during the continuous reactor study. The observations made in the previous section regarding comparison of the ATP/substrate curve produced in Chapter 7 with that produced in Chapter 8 also apply to the comparison of the relationship found in Chapter 9 with that found in Chapter 8.

12.3.5 Respirometric Testing of Phenol Removal

These tests were, as described earlier, conducted on samples of sludge taken from the continuous reactor treating coke oven liquor.

The distinctive feature of these results was that the OUR/substrate relationship showed maximal activity in the 2-10mg/l phenol range, with a decline in activity at greater phenol concentrations. These results were similar to those obtained by HIAM in respirometric tests on sludge taken from a full-scale plant treating coke oven liquor. (These results were recorded in Figure 12.17).

As before it is possible to interpret the decline in sludge activity at greater phenol concentrations as substrate inhibition .

The results of these tests were therefore consistent with those reported for ATP earlier in showing maximal activity at a low phenol concentration, and substrate inhibition at higher levels. The OUR/substrate curves were most similar in behaviour to the ATP/substrate behaviour found in Chapter 7 and there was no evidence of two peaks in OUR/phenol relationship, but the phenol concentration range at which Figure 8.23 might suggest this was evident was not considered in these studies.

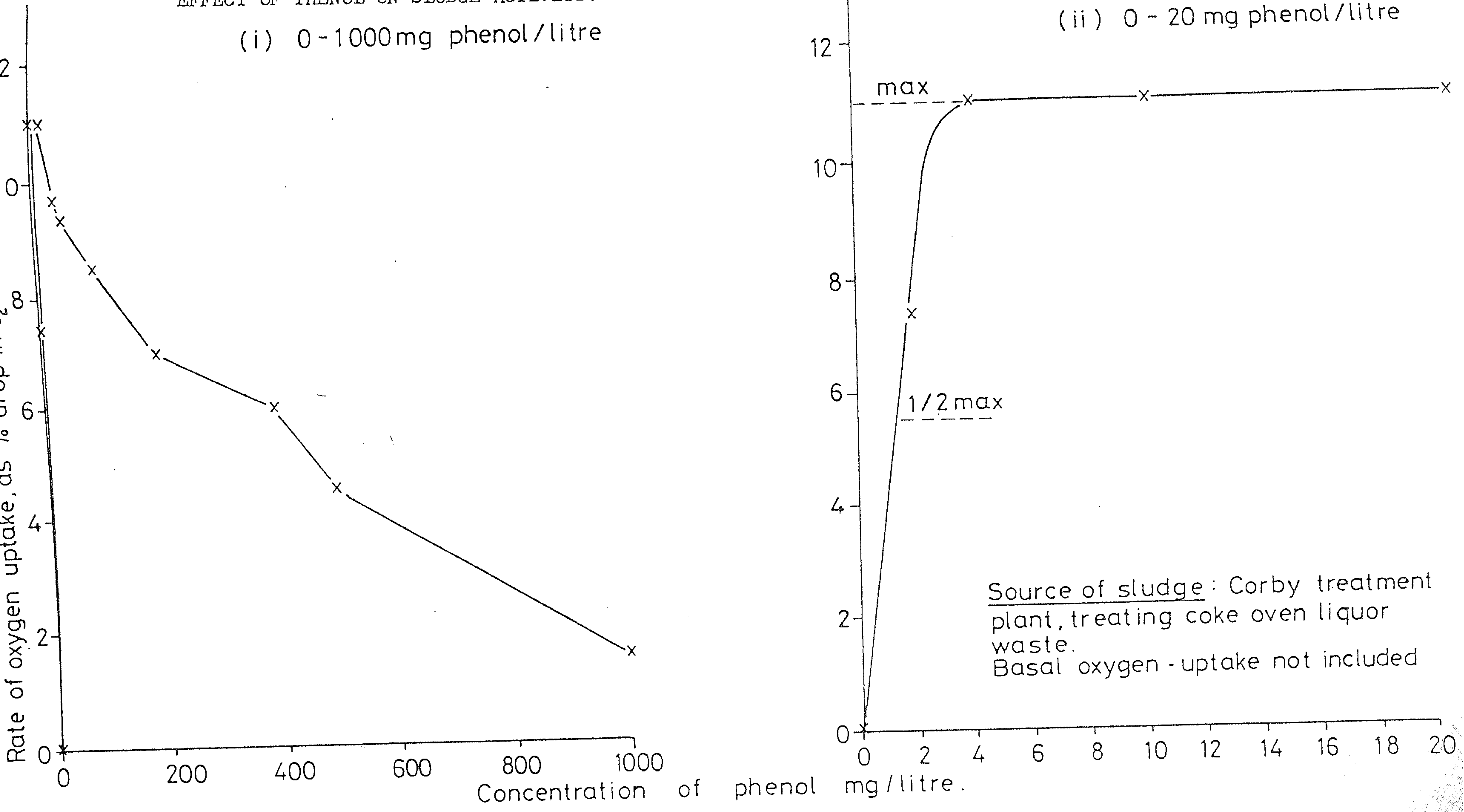
It may be concluded that the OUR behaviour of the sludges was very similar to the ATP behaviour of sludges with respect to phenol concentration.

12.4 Discussion and Comparison of the Findings for Phenol and Thiocyanate Degrading Sludges

12.4.1 Qualitative Explanations of the Significance of ATP

It was proposed in Chapter 4 that ATP represented a measure

FIGURE I2.I7 RESULTS OF RESPIROMETRIC STUDIES (FROM BSC INTERNAL REPORT)
EFFECT OF PHENOL ON SLUDGE ACTIVITY.



of activity, (or more specifically a measure of growth rate), and active biomass. The proposal that it represented a measurement of growth rate meant that the relationship between ATP and thiocyanate concentration, for a given sludge and conditions, would be described graphically by a rectangular hyperbola analogous to the relationship between growth rate and substrate concentration given by MONOD kinetics. This proposal had to be modified to explain the ATP behaviour of the batch sludges treating thiocyanate. That behaviour was explained by proposing that there were two populations of organisms each with a distinct range of activity relative to thiocyanate concentration, and each exhibiting substrate inhibition. The experiments described in Chapter 6 were designed to check this ATP behaviour whilst removing many of the other factors that may have caused the observed variations in activity. These experiments confirmed the ATP behaviour described in Chapter 4. Respirometric tests described in Chapter 10 showed OUR behaviour similar to that for the peak found in the lower concentration range in the results of Chapters 4 and 6.

The respirometric tests did not confirm the existence of the second peak in activity but there was insufficient data for the existence of this second to be adequately tested.

The results of Chapter 7, for the phenol-treating sludge showed ATP behaviour that could be explained by postulating a role for ATP as a measure of growth rate, with the system exhibiting substrate inhibition. The results of Chapter 8 showed a more complicated ATP behaviour which showed two peaks in activity, but which could easily be approximated to a single activity peak exhibiting substrate inhibition. The continuous reactor study showed a relationship between reactor phenol and ATP very similar to that observed in Chapter 7.

The respirometric tests of Chapter 10 showed OUR behaviour that was very similar to the ATP behaviour observed in Chapter 7 and 9.

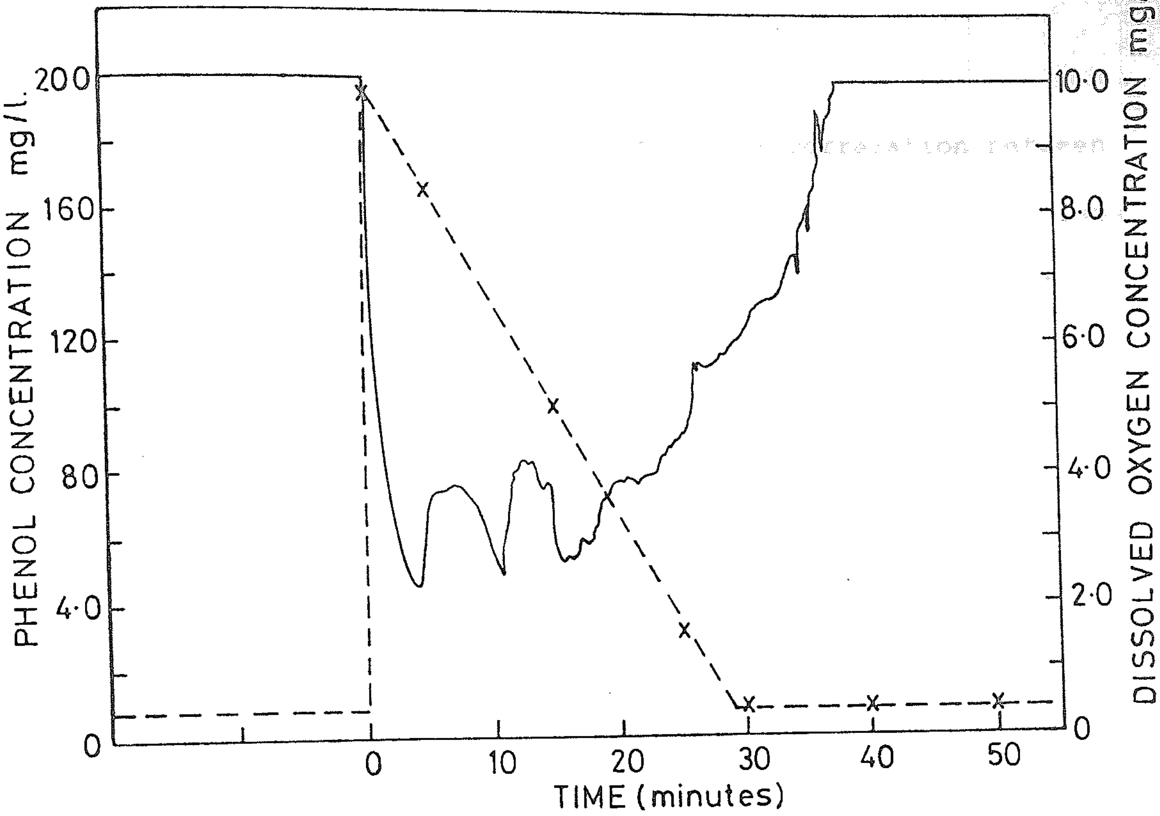
The ATP behaviour of sludges treating phenol and thiocyanate could therefore be regarded as consistent with its postulated role as a measurement of growth rate with the modifications described.

A very important feature of the results discussed so far is the very close similarity in the results obtained using OUR as a measure of activity and those using ATP. In fact in the continuous reactor study described in Chapter 9 a linear relation between ATP and OUR measurements of the reactor sludge was found with a correlation coefficient of 0.97.

Other evidence of a correlation between oxygen uptake and ATP also exists elsewhere. For example, the fluctations in ATP observed in experiment E11 described in Chapter 7 were associated with similar fluctuations in dissolved oxygen level (and also changes in redox potential). As the air supply rate in that experiment was constant the fluctuations in D.O may be assumed to be indicative of fluctuations in OUR by the sludge.

TOMLINS in his thesis showed a graph indicating the typical change in D.O that occurred in batch sludges treating phenol. Unfortunately he did not show this behaviour for any of the batch experiments in which he monitored ATP. If the trend in D.O behaviour in such batch experiments is compared with the typical ATP behaviour of a sludge treating phenol, as shown in Figure 12.18 , it can be seen that they are broadly similar. Assuming, as before, a constant air flow rate in the experiment this correlation could be interpreted as providing some additional evidence for a correlation between OUR and ATP.

From a knowledge of the biochemistry of organisms the close relationship between OUR and ATP found in these results is not unexpected in view of the fact that ATP generation in the cell is intimately linked to the respiration of the cell. Without persuing a detailed discussion of cellular biochemistry it must be noted that ATP generation is cells is not necessarily dependant upont the oxygen uptake of the cell and oxidative phosophorylation. In activily photosynthesising organisms of course, ATP is generated with the net evolution rather than uptake of oxygen. In other organisms ATP generation is not linked to oxidative phosphorylation and oxygen uptake, for example in anaerobic or oxygen limiting conditions where ATP generation by substrate phosphorylation may occur and a variety of compounds other than oxygen may be used as the



KEY
x Phenol Data
– – – Plot Through Phenol Data.
o ATP Data
—·—·- ATP Response Predicted by Tomlins Storage Model
——— D.O. Conc. (Continuously Monitored)

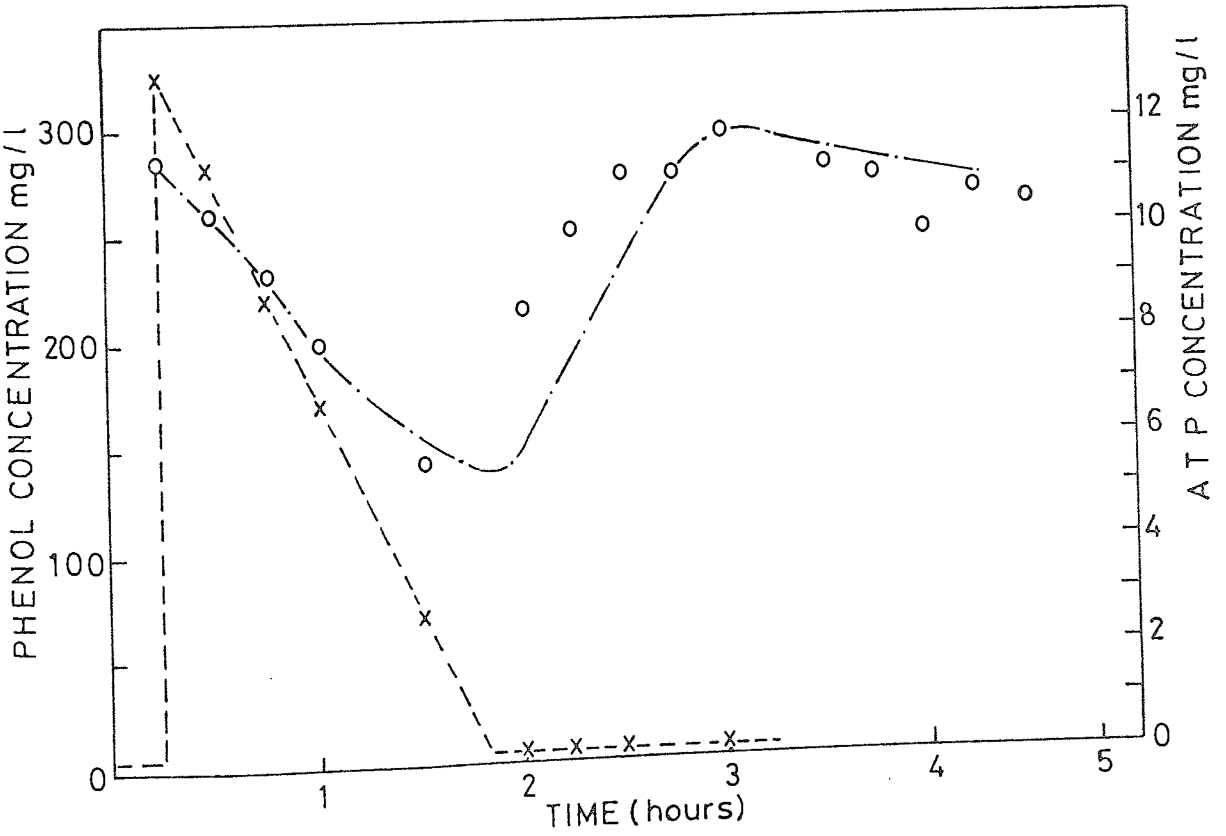


FIGURE 12.18 Plots of Data Derived From Two Similar Experiments Reported by Tomlins for the Oxidation of Phenol (in a Basal Medium of Treated Coke Oven Liquor) in Acclimatised Laboratory Scale Batch Activated

electron donor. Nevertheless, the close correlation between the oxygen consumption of the aerobic heterotrophic organisms found in the activated sludge process in non-oxygen limiting conditions, and ATP generation and therefore ATP content of the organisms, would be expected.

12.4.2 The Development of Equations to Explain the Quantitative Significance of ATP

BENEFIELD, LAWRENCE, and RANDALL(153) have described the following equation to relate oxygen utilisation to microbial growth;

$$\frac{dO_2}{dt} = a\frac{dX}{dt} + bX = OUR \qquad \underline{\text{EQUATION 12.2}}$$

where $\frac{dO_2}{dt}$ = rate of oxygen utilisation

a = oxygen required per unit of biomass produced

b = oxygen required per unit of biomass for the maintenance of life per unit time.

It will be noted that EQUATION 12.2 related oxygen utilisation or uptake rate to the biomass concentration, X, and to the activity of the biomass in terms of the rate of change of biomass.

It was proposed earlier in the project that ATP represented both a measure of biomass and activity. In view of this and the relation between oxygen uptake and ATP generation in aerobic heterotrophic cells, and the similarity in sludge OUR and ATP behaviour reported earlier, an equation similar to 12.2 may be proposed to explain the significance of ATP in sludge;

$$ATP = e \frac{dX}{dt} + f X \qquad \text{EQUATION 12.3}$$

where ATP = ATP concentration (ML^{-3})

e = ATP content per unit rate of change of biomass (T^{-1})

f = ATP content/unit biomass associated with the maintenance of life (dimensionless ratio)

This equation can be modified to include the proposed relationship between ATP and sludge growth rate.

Specific growth rate can be described by the equation

$$\mu = \frac{dX}{dt}/X \qquad \text{EQUATION 12.4}$$

so that ATP can be related to growth rate by dividing EQUATION 12.3 by X, giving

$$\frac{ATP}{X} = e\mu + f \qquad \text{EQUATION 12.5}$$

where $\frac{ATP}{X}$ = the ATP content per unit biomass

This relationship between $\frac{ATP}{X}$ and net growth rate μn, which is related to specific growth rate U by

$$\mu = \mu_n + K_d \qquad \text{EQUATION 12.6}$$

where K_d = the decay coefficient

was demonstrated in Chapter 9, where a linear relationship between the two was shown with a correlation coefficient of 0.92.

EQUATIONS 12.3 and 12.5 therefore effectively quantitatively describe the significance of ATP in the activated sludge process, and include the orginal proposals that ATP represented both a measure of biomass and activity, or growth rate, made early in the project.

To emphasise that ATP was a measure of activity and biomass, rather than just biomass a graph relating sludge ATP concentration per unit sludge volume to the MLSS concentration in Chapter 9 showed a poor linear correlation.

12.5 A Further Analysis of the Batch Results Reported by TOMLINS

The evidence supporting the proposal of EQUATIONS 12.3 and 12.5 was of course mainly derived from the experimental work conducted in this project. It would be useful, and may provide further insight into the ATP behaviour of sludges treating liquor components, if the results of TOMLINS experiments were shown to support the findings from this project. The following brief analysis of

TOMLINS results was able to do just that.

From EQUATION 12.3 it can be seen that the sludge ATP pool should be directly proportional to the rate of change of biomass, $\frac{dX}{dt}$.

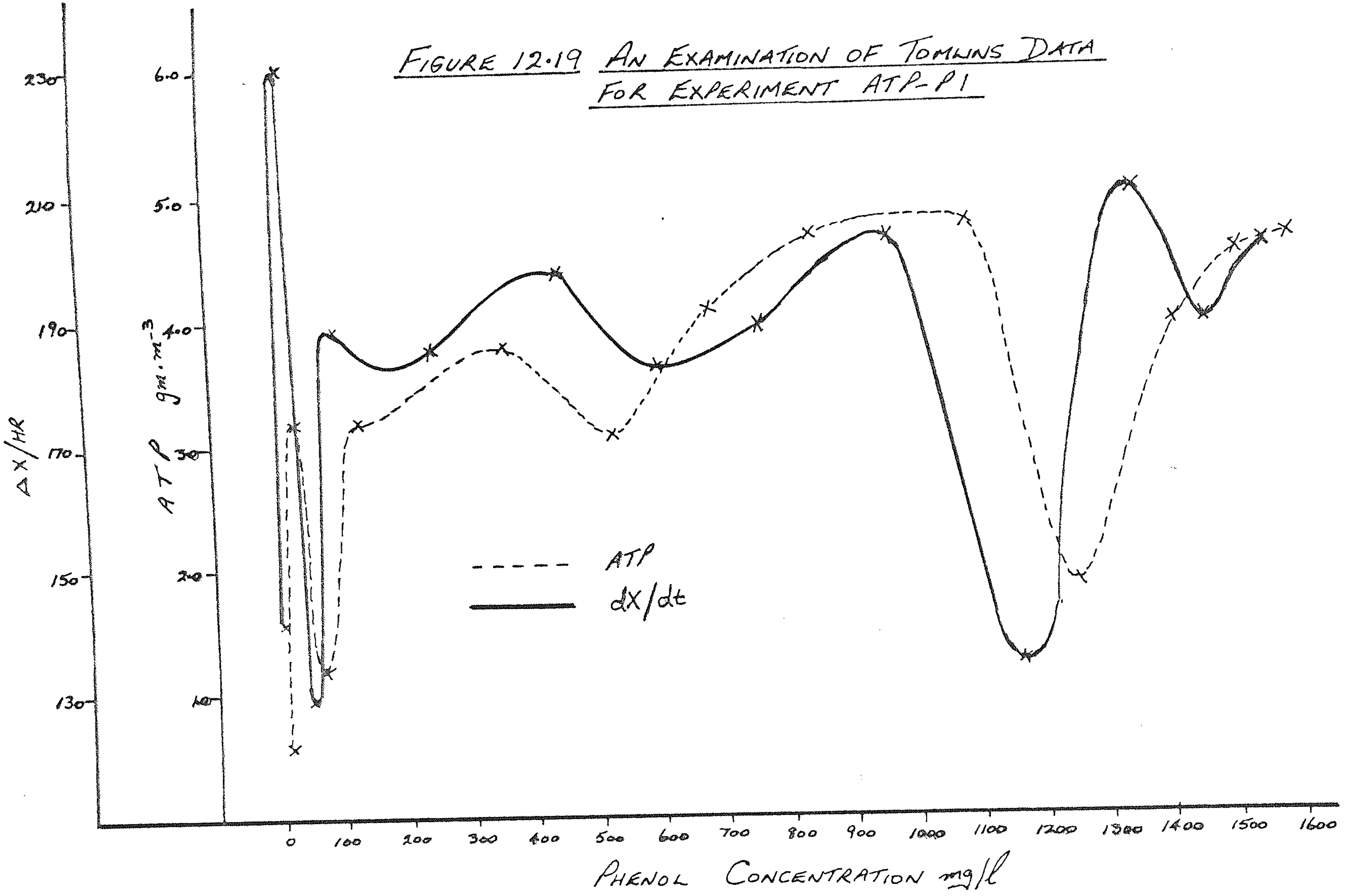
There were only two experiments with phenol-treating sludges in which TOMLINS monitored both ATP and MLSS, runs ATP-P1 and ATP-P2. Values for $\frac{dX}{dt}$ were determined for each of these experiments by calculation of the change in biomass between successive MLSS determinations per unit time. A graph was plotted in Figure 12.19 relating ATP concentration/unit volume, and $\frac{dX}{dt}$, to the phenol concentration in the sludge during batch experiment ATP-P1. This showed that the behaviour of the $\frac{dX}{dt}$ curve was very similar to that of the ATP curve.

These results not only supported the relationship shown in EQUATION 12.3 but also indicated that the fluctuations observed in the ATP behaviour of batch experiments were real rather than the product of experimental error. (ATP and MLSS determinations were carried out completely independant of each other in TOMLINS experiments).

These results did also show a decline in ATP level at a phenol concentration of about 50mg/l similar to that reported in experiment 8 in this project.

To check the relationship between growth rate and sludge ATP concentration/unit volume, estimates of the growth rate during

FIGURE 12·19 AN EXAMINATION OF TOMLINS DATA FOR EXPERIMENT ATP-P1



the experiment were made by dividing each value for $\frac{dX}{dt}$ by the estimated MLSS value for the time concerned, in accordance with the relationship

$$\mu = \frac{dX}{dt}/X$$

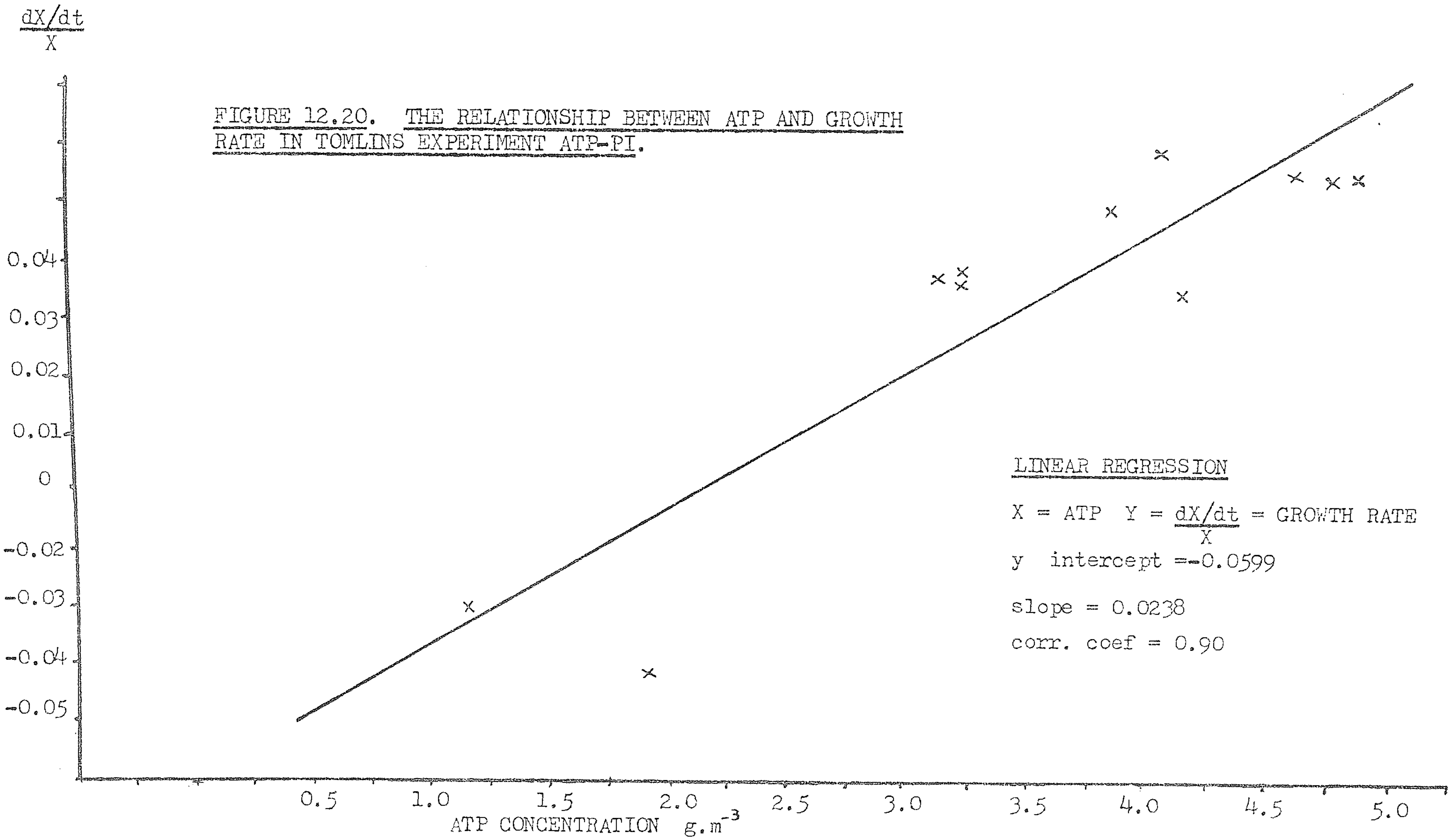
These results were shown in Figure 12.20 and showed a linear relationship between μ and ATP with a correlation coefficient of 0.90.

The results of experiment ATP-P2 were analysed in a similar manner, and a graph relating ATP, and $\frac{dX}{dt}$, to phenol concentration plotted in Figure 12.21.

A graph was also plotted relating these two parameters to the time period in the batch experiment, in Figure 12.22 . The results of these two graphs also indicated that $\frac{dX}{dt}$ and ATP were closely related, although this relationship was less obvious at very high phenol concentrations. The same marked decline in activity in terms of ATP and $\frac{dX}{dt}$ was again observed at a phenol concentration of 50mg/l. The possibility that this decline in ATP activity observed at this concentration in runs ATP-P1 and ATP-P2, and in the experiments conducted in Chapter 8 in this project, was due to an artifact of the ATP assay procedure could be dismissed because of the behaviour of the $\frac{dX}{dt}$ curve in TOMLINS experiments.

The errors involved in this type of analysis of TOMLINS results, and particularly in the determination of growth rate values, are

FIGURE 12.20. THE RELATIONSHIP BETWEEN ATP AND GROWTH RATE IN TOMLINS EXPERIMENT ATP-PI.



LINEAR REGRESSION

X = ATP Y = $\frac{dX/dt}{X}$ = GROWTH RATE

y intercept = -0.0599

slope = 0.0238

corr. coef = 0.90

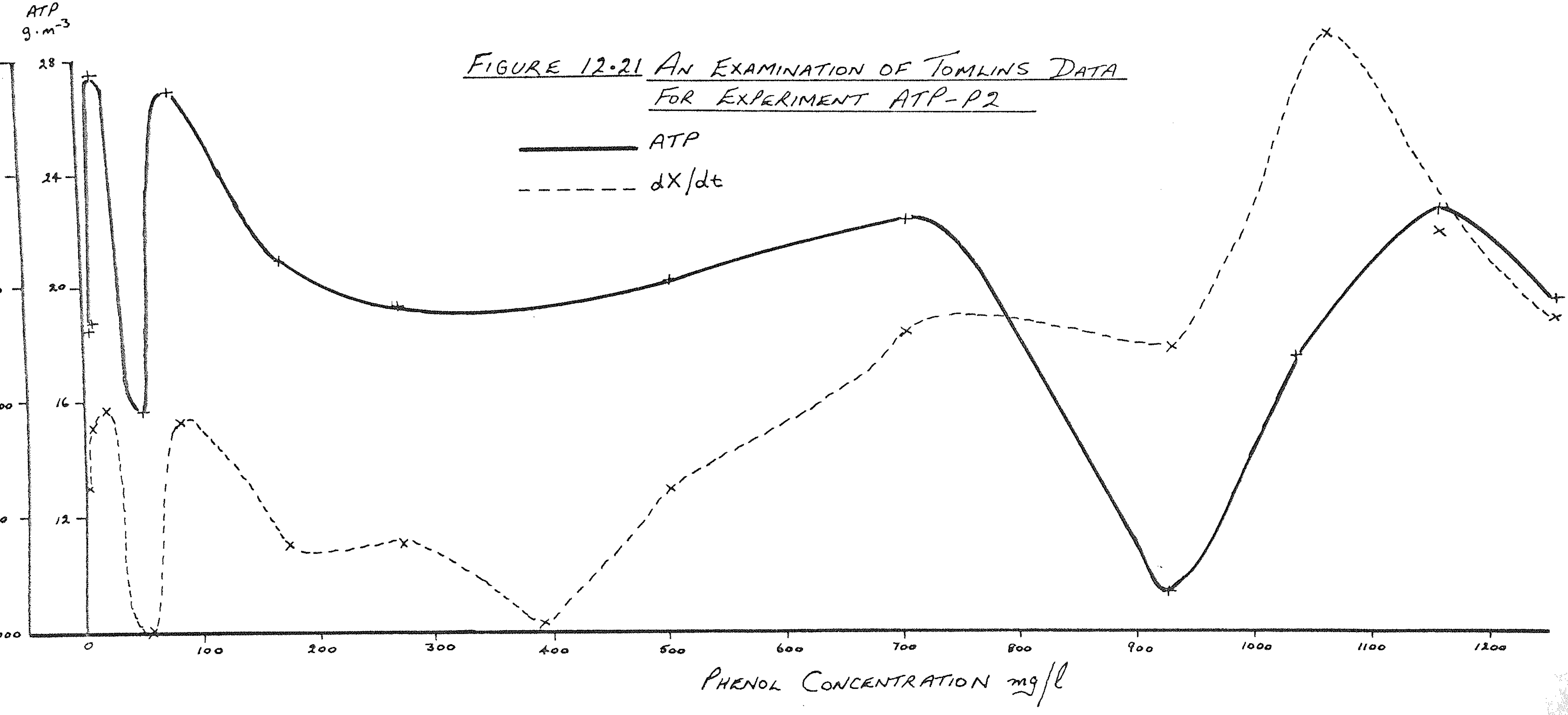
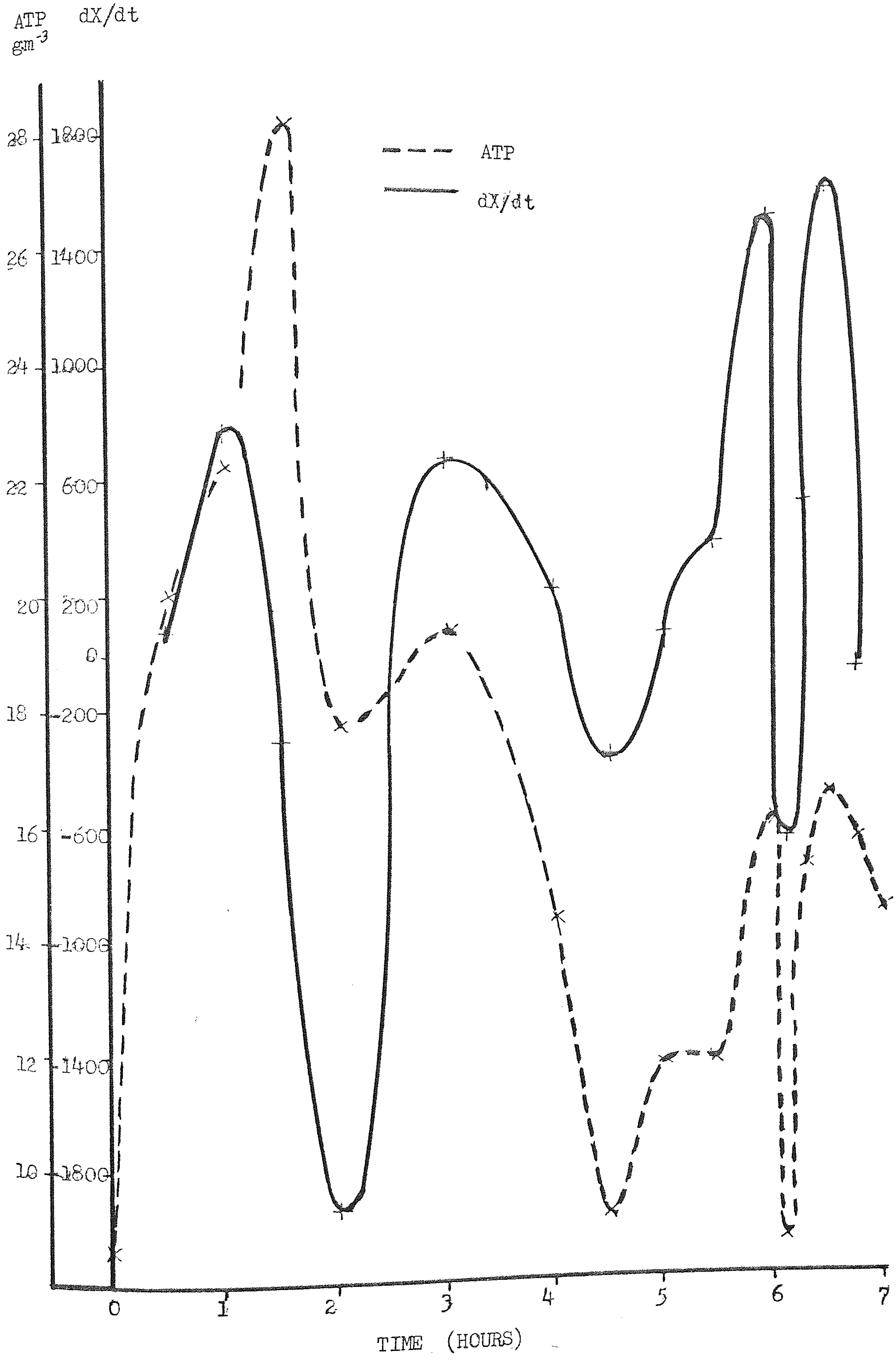


FIGURE 12·21 AN EXAMINATION OF TOMLINS DATA FOR EXPERIMENT ATP-P2

FIGURE 12.22 AN EXAMINATION OF TOMLINS DATA FOR EXPERIMENT ATP - P2



potentially large, but the trends shown in the above Figures were so clear as to dismiss the possibility that they were an artifact, the product of these potential errors.

The results of these analyses have important consequences. They may of course be used as additional evidence supporting the relationships proposed in EQUATIONS 12.3 and 12.4. They also indicate that the fluctuations observed in ATP behaviour in the batch experiments were real rather than the product of experimental error.

In addition, this behaviour suggests that it may be necessary to regard such batch sludges treating phenol, and thiocyanate as containing at least 2 distinct populations of organisms, and 3 in the case of TOMLINS sludges treating very high phenol concentrations. In the case of phenol removal it may be possible to approximate this apparent bi-population behaviour to the behaviour of a single population exhibiting substrate inhibition, in the phenol concentration range of 0-300mg/l.

In reality it may therefore be necessary to regard batch sludges as containing multiple populations of organisms and that the ATP/ substrate concentration behaviour observed represents the net result of their interaction in terms of activity and metabolism of the substrate.

Still other explanations may be put forward to explain the different peaks in activity which are not dependant upon the existence of distinct populations of organisms. These will not however be discussed because confirmation of the existence of distinct populations of organisms in the systems is now less important than it was earlier in the project, and less important than the confirmation of the relationships shown in EQUATIONS 12.3 and 12.4.

It is important to note that the ATP and OUR results obtained using sludges from a continuous reactor treating coke oven liquor failed to show behaviour that could not be explained by a single population of organisms treating the individual component and subject to substrate inhibition. It is true that the substrate concentration ranges considered were not such that the existence of distinct multiple populations metabolising each liquor component could be excluded however, and this may form the subject of research by other workers. In the absence of positive evidence to the contrary from continuous reactors, laboratory or full-scale, the metabolism of phenol and thiocyanate components of liquor will each be assumed to be due to a single distinct population of organisms as originally proposed in the project. In so doing, and although the low growth rate observed in the batch sludges treating thiocyanate indicate that removal was autotrophic, the possibility of significant heterotrophic removal can not be ignored.

Before proceeding to consider the significance of EQUATIONS 12.3 and 12.4 to the control of the activated sludge process in the following chapters, a few other useful observations may be made.

One important consequence of the analysis of the results of runs ATP-P1 and ATP-P2 is that because of the obvious close relationship between ATP and $\frac{dX}{dt}$ it would now seem possible to dismiss completely the need for a storage model to explain the ATP behaviour of batch sludges treating phenol, and instead to interpret the behaviour in terms of the net results of the activity of multiple populations of organisms.

Another important observation is that the results of batch experiments, particularly those of the type used by TOMLINS for sludges treating phenol, appear inherently unreliable for the determination of the value of kinetic parameters which will be useful in describing the behaviour of sludges in a full-scale plant treating the waste in a continuous mode. TOMLINS used a higher concentrations of substrate than are ever found a full-scale plant treating coke oven liquor, and this observation merely confirms what might be expected intuitively when the treatment of the slduge used to derive kinetic parameters is so different from that of the sludge that it is supposed to model.

12.6 The Significance of Other Parameters Monitored in the Project

A number of parameters other than ATP were monitored during the experiments described earlier. Some of these, including phenol, thiocyanate, ammonia, MLSS and OUR have already been used extensively in conjunction with the evaluation of ATP data. Others, including temperature and pH have not been used quantitatively in the analyses that have been reported but served mainly to illustrate that experimental conditions with regard to these were consistent in successive experiments, and that the observations of changes in sludge ATP levels could not obviously be attributed to changes in pH and temperature which in general showed little variation in experiments.

Other parameters, including redox potential, and measurements made using thiocyanate and sulphide electrodes were recorded but received little or no mention in subsequent analysis and discussion of ATP data. This was because the results obtained using these parameters gave little or no insight into the significance of ATP in the treatment system, which was the object of including their use in the experiments. Changes in redox potential in experiment E11 described in Chapter 7 were coincident with changes in sludge ATP concentration, but these changes were small, as were changes in dissolved oxygen concentration during the same period. The use of dissolved oxygen measurements in conjunction with OUR determinations has already been described, and was valuable in helping to determine the significance of ATP in the treatment system. The use of redox potential was not persued because of the limited time available for the project, other subjects were seen as warranting priority.

One important fact that was gained from the observation of parameters other than ATP as a measure or reflection of sludge activity, including redox potential and OUR was that none of these was as sensitive to changes in sludge activity as ATP. This observation alone justified the investigation of the use of these other parameters.

12.7 ATP and Growth Kinetics

In the discussion presented earlier, it was shown that ATP represented a measure of both activity and biomass, and EQUATIONS 12.3 and 12.5 defined the relationship between these. The aim of the project was to determined the signficance of ATP in the treatment system and it may be concluded that that significance is embodied in those 2 fundamental equations.

(12.3) $ATP = e\frac{dX}{dt} + \beta X$

(12.5) $\frac{ATP}{X} = e\mu + \beta$

The full significance of ATP will of course only be realised in the application and development of those equations, and the work described in the following chapter represents the application of those equations to the control of the treatment system and to sludge settlement.

It can be seen that simple manipulation of EQUATION 12.5 and its use in conjunction with the equations described in Chapter 11, will enable the determination of all the required constants for modelling purposes. Usual inspection of figures showing ATP/substrate relationships enables an approximation of the value of K_s, and examination of the data for phenol removal derived from the continuous reactor study described in Chapter 9 produces values for K_s, $\mu_{max.}$, and K_d closely approximating to those obtained from the pilot plant study in Chapter 11.

The use of the ATP data presented earlier for the determination of kinetic constants was unfortunately not possible within the time constraints of the project, and the results of this application of ATP will be reported elsewhere.

The significance of EQUATIONS 12.3 and 12.5 to the control of the activated sludge process is now considered in the following chapters.

CHAPTER 13

THE USE OF ATP AS A CONTROL PARAMETER IN THE ACTIVATED SLUDGE TREATMENT OF WASTEWATERS

Introduction

13.1 Present Control Methods
    13.1.1 Substrate Utilisation Rate U
    13.1.2 Sludge Age
13.2 The Application of ATP to Process Control
13.3 Proposed Control Strategy using ATP
13.4 Discussion

THE USE OF ATP AS A CONTROL PARAMETER IN THE ACTIVATED SLUDGE TREATMENT OF WASTEWATERS

Introduction

One of the important observations made in the literature review in Chapter 2 was that although several workers had postulated the role of ATP as a measure of activity in mixed cultures of organisms and in activated sludge, none had defined or quantified that role in terms which had more than limited practical value. On the basis of the studies with laboratory scale continuous and batch activated sludge reactors treating coke oven liquor or major components thereof, it was possible earlier in the project to postulate a precise definition for the role of ATP in such systems as both a measure of living biomass and activity. This was defined by the relationship described in the following equation;

$$ATP = e\frac{dx}{dt} + fX \qquad \text{EQUATION 13.1}$$

where ATP = ATP concentration $(M.L^{-3})$

X = microorganism concentration $(M.L^{-3})$

$\frac{dx}{dt}$ = change in microorganism concentration $(M.L^{-3}T^{-1})$

e = ATP content per unit rate of change of biomass (T^{-1})

f = ATP content per unit of biomass associated with the maintenance of life per unit time. (dimensionless ratio)

This equation forms the basis for proposals for the use of ATP as a control parameter for the activated sludge process. These proposals are described with reference to treatment of wastewaters in general, and then with particular reference to treatment of coke oven liquors.

It is believed that these proposals enable ATP to be used in a quantitatively precise manner which has hitherto not previously been possible, and represents a significant improvement over the purely qualitative or semi-quantitative uses previously described.

13.1 Present Control Methods

Before considering the use of ATP in the control of the activated sludge process it would be useful to briefly consider the various types of control methods presently used. Although there are a large number of these, the most common can be regarded as being based on just two methods, control of substrate utilisation rate, and control of sludge age.

13.1.1 Substrate Utilisation Rate U

This method depends on the maintenance of a constant value for U, also known as the food to microorganism (F:M) ratio, or process loading factor. For a complete mix reactor the substrate utilisation rate can be represented by

EQUATION 13.2

$$U = \frac{F\ (S_i - S)}{VX}$$

Where S_i = influent substrate concentration

S = effluent substrate concentration

F = influent wastewater flow rate

X = microorganism concentration in reactor

V = volume of reactor

12.1.2 Sludge Age

This method depend on maintenance of a constant sludge age, θ_c, also termed Solids Retention Time, SRT.

The equation for θ_c is dependant upon whether sludge is wasted from the recycle line or from the reactor.

If wastage is from the recycle line, θ_c is calculated from;

$$\theta_c = \frac{VX}{F_W X_R + (F - F_W) X_e} \qquad \text{EQUATION 13.3}$$

and if from the reactor;

$$\theta_c = \frac{VX}{F_W X + (F - F_W) X_e} \qquad \text{EQUATION 13.4}$$

where F_W = wasted sludge flow rate

X_R = return sludge concentration

X_e = sludge concentration in treated effluent from final clarifer

If the treatment system is operated satisfactorily X_e is small, and EQUATIONS 13.3 and 13.4 can be approximated to 13.5 and

FOR SLUDGE WASTED FROM RECYCLE LINE $\theta_c = \frac{VX}{F_W X_r}$ EQUATION 13.5

FOR SLUDGE WASTED FROM REACTOR $\theta_c = \frac{V}{F_W}$ EQUATION 13.6

The equations for both control methods make the assumption that;

1. Waste stabilisation by the microorganisms only occurs in the reactor.

2. The volume used in the calculations includes only the volume of the reactor.

The control of sludge age in steady-state conditions also involves control of the substrate utilisation rate since it can be shown in such conditions that;

$$\frac{1}{\theta_c} = UY - k_d \qquad \text{EQUATION 13.7}$$

where Y = substrate yield coefficient

k_d = endogenous decay coefficient

13.2 The Application of ATP to Process Control

The mass balance for substrates, S and biomass, X, for a complete-mix activated sludge system, to which assumption 1 and 2 are applied, can be represented by;

$$V \frac{dS}{dt} = FS_i - FS - \frac{XV}{Y}$$ EQUATION 13.8

$$V \frac{dX}{dt} = (\mu - Kd)\ VX - F_w X$$ EQUATION 13.9

where μ = microorganism growth rate.

In steady-state conditions $\frac{dx}{dt} = 0$, and $\frac{ds}{dt} = 0$, and EQUATIONS 13.8 and 13.9 can be simplified to give;

$$S = S_i - \frac{\mu X}{YD}$$ EQUATION 13.10

$$\mu - Kd = \frac{Fw}{V} \quad (= \frac{1}{\theta_c})$$ EQUATION 13.11

If EQUATION 13.1 is divided by X the following equation is obtained;

$$\frac{ATP}{X} = e\mu + f$$ EQUATION 13.12

as $\mu = \frac{dX}{dt}/X$

EQUATION 13.10 can be solved for μ to give

$$\mu = \frac{(S_i - S)\ YD}{X}$$ EQUATION 13.13

and this resulting equation can be substituted for μ in EQUATION 13.12 to give

$$\frac{ATP}{X} = e \; \frac{(S_i \; - \; S) \; YD}{X} + f \qquad \underline{\text{EQUATION 13.14}}$$

EQUATION 13.14 can be solved for S, the effluent substrate concentration to give;

$$S = S_i - \left(\frac{ATP}{X} - f\right) \frac{X}{Y.e} \qquad \underline{\text{EQUATION 13.15}}$$

EQUATION 13.14 can also be rearranged to give

$$\frac{F(S_i - S)}{VX} = \left(\frac{ATP}{X} - f\right) \frac{1}{Y.e} \qquad \underline{\text{EQUATION 13.16}}$$

It will be noted that the lefthand side of this equation is equivalent to U in EQUATION 13.2 so that it could be rewritten as

$$U = \left(\frac{ATP}{X} - f\right) \frac{1}{Y.e} \qquad \underline{\text{EQUATION 13.17}}$$

Substituting for U in EQUATION 13.7 the following relationship is obtained

$$\frac{1}{\theta_c} = \left(\frac{ATP}{X} - f\right) \frac{1}{e} - Kd \; \left(= \frac{Fw}{V}\right) \qquad \underline{\text{EQUATION 13.18}}$$

In EQUATIONS 13.15 and 13.18 the term ATP/X can be regarded as a parameter defining the activity of the treatment system in removal of substrate. The terms f, Y, e and K_d are metabolic constants and may be determined experimentally. S_i, ATP and X are process variables which can be measured, and F and F_w, and therefore θ and θ_c, can be controlled by the plant operator. In both equations therefore all of the quantities involved are constants or variables which can be determined and/or controlled by the plant operator. EQUATIONS 13.15 and 13.18 therefore form the basis of a control strategy which enables prediction of steady-state effluent substrate levels from determinable parameters.

13.3 Proposed Control Strategy using ATP

The control strategy that can be formulated on the basis of EQUATIONS 13.15 and 13.18 will be applicable to the activated sludge treatment of wastewaters in general. The treatment of carbonised wastes behaviour involves a more complex ecosystem which characterises sludges treating domestic wastewaters for example, and a number of additional factors need to be considered in modelling their treatment. The general control strategy, applicable in principle to the activated sludge treatment of any wastewater, is considered first however before outlining the additional factors that need to be considered for the treatment of coke oven liquors.

The proposed control strategy can be outlined as follows:

1. Classical design equations are used to choose a range for the sludge age value within which effluent of the desired quality should be achieved.

2. Influent flow, influent substrate, and reactor MLVSS and ATP are measured periodically. Prior experience will dictate the frequency with which these measurement need to be made.

3. The hydraulic residence time is calculated from the influent flow rate and reactor tank volume.

4. From EQUATION 13.15 the desired ATP/X for the existing hydraulic residence time and influent substrate conditions is calculated on the basis of the desired value for the effluent substrate concentration.

5. Adjustment of the ATP/X value can be achieved by appropriate alteration of the flow to waste (using EQUATION 13.18) or by alteration of the sludge recycle rate.

13.4 Discussion

The strategy outlined in steps 1-5 is applicable to activated sludge plants in general. Before discussing the more detailed application of this strategy, the additional factors involved in the treatment of coke oven liquors are considered.

The mass balance EQUATIONS 13.8 and 13.9 and the subsequent equations derived from them are based on MONOD kinetics and the assumption that there is a single homogenous population of organisms responsible for the degradation of the waste. The modelling of the treatment of coke oven liquor however requires the consideration of more than one population of organisms.

The approach that was adopted and found useful in the work described earlier in this report was to regard the waste liquor as containing three main components, phenol, thiocyanate and ammonia, each of which was assumed to be associated with a distinct population of organisms responsible for its degradation. Heterotrophic bacteria were assumed responsible for phenol oxidation, and separate and distinct autotrophic populations of bacteria responsible for thiocyanate removal and nitrification. As nitrification is not a treatment objective in most single stage complete-mix activated sludge plants treating this waste, nitrifying organisms need not be considered here. At its simplest therefore the system can be regarded as containing one population of autotrophic bacteria responsible for thiocyanate removal and one heterotrophic population responsible for phenol removal, with both behaving completely independantly of the other.

With these assumptions, the equations described earlier now need to be applied to each of the two populations outlined. Although there are two distinct substances, thiocyanate and phenol, the total biomass in the reactor will be given by:

$$X = X_T + X_P \qquad \text{EQUATION 13.19}$$

Where X_T and X_P represent the biomass of thiocyanate and phenol degrading organisms respectively.

Similarly, the total ATP measured in the reactor will be given by:

$$ATP = ATP_T + ATP_P \qquad \text{EQUATION 13.20}$$

Where ATP_T and ATP_P represent the level of ATP due to the activity and concentration of thiocyanate and phenol-degrading organisms respectively.

The growth characteristics of autotrophic and heterotrophic organisms are typically very different and this is reflected in the value of the constants used in the kinetic equations. An important consequence of this is that satisfactory autotrophic removal of thiocyanate required a much larger solids retention time in the system than does heterotrophic phenol removal. This can be explained as follows.

θ_c^m is the term used to represent the lower value of θ_c at which complete failure to remove a substrate occurs, and represents the minimum biological solids retention rime at which $S = S_i$. Below θ_c^m the population of organisms concerned are removed from the system at a rate greater than their growth rate, so that eventually no organisms of that population remain in the system, and removal of the corresponding substrate ceases.

If $S_i \gg K_s$ (and K_s is low for both of the populations concerned) then the limiting value for θ_c is given by:

$$\left.\theta_c^m\right]_{\lim} = \frac{1}{\mu_{max.} - K_d} \qquad \underline{\text{EQUATION 13.21}}$$

As the value for $\mu_{max.}$ is typically smaller for autotrophic than heterotrophic organisms, it follows that in a system in which removal of phenol and thiocyanate components is required the value for θ_c necessary to achieve thiocyanate removal will be the deciding factor in the operation of the plant. To achieve removal of thiocyanate to a satisfactory level the value chosen for θ_c will of course need to be somewhat greater than the limiting value.

Another complicating factor in this system is that both phenol-removal and autotrophic thiocyanate-removal exibit substrate inhibition but this factor can be included in the model of the system by using a substrate inhibition equation of the HALDANE form to describe the growth of the organisms involved:

$$\mu = \frac{\mu \text{max.}}{1 + K_s/S + S/K_i} \qquad \underline{\text{EQUATION 13.22}}$$

where μ = the observed growth rate

μ max = the maximum growth rate in the absence of inhibition

K_s = the saturation constant

S = the limiting substrate concentration

K_i = the inhibition constant

The inclusion of two populations in the model of the treatment system complicated the application of the control strategy because of the difficulty in distinguishing between biomass and activities of autotrophic and heterotrophic organisms as reflected by ATP levels in the reactor. This is compounded by the fact that autotrophic bacteria would be expected to form only a small percentage of the total active organisms present in the sludge so that the percentage contribution of autotrophic bacteria to the total ATP pool would be small. Assuming this to be so, ATP measurements of the system would be expected to be considerably less sensitive to the activity of the autotrophic bacteria than to heterotrophs. This would be unfortunate as this is the area of treatment most susceptible to failure.

Although there may be practical difficulties in doing so, it is possible to distinguish between the activities and biomasses of the autotrophs and heterotrophs in the treatment system. The biomass of any component population of the sludge can be determined from the following relationship which has been determined by a rearrangement of EQUATION 13.13 and substitution for μ in the resultant equation using the relationship for μ derived from EQUATION 13.11.

$$X = \frac{(S_i - S)\,YD}{1/\theta_c + K_d} \qquad \text{EQUATION 13.23}$$

The biomass responsible for autotrophic thiocyanate removal for example could be calculated using this equation by determining influent and reactor thiocyanate levels. The remaining factors in the equation are either known operational variables or kinetic constants that can be obtained from laboratory experiments. If the values for e and f for the autotrophic population were also determined in the laboratory, then the contribution of this population to the total ATP pool of the system could be determined.

One of the advantages of the control strategy outlined using EQUATIONS 13.15 and 13.18 for a single population is that it is not necessary to know the effluent substrate concentration, which may be difficult to determine accurately at low concentrations, for successful control of the process. A bi-or multi-population approach however, requires the accurate determination of one or more effluent substrate levels, complicating and introducing potentially large errors into the application of the model. However, a potentially more serious complicating factor in the practical application of this control strategy lies in the current confusion regarding thiocyanate removal in the system.

Although the initial model of the system assumed that oxidation of thiocyanate was solely due to the action of autotrophic bacteria, it was noted in Chapter 3 that heterotrophs able to utilise phenol and thiocyanate simultaneously have been isolated. For example, STAFFORD and CALLELY have isolated such a bacterium, a pseudomonad similar to Pseudomonas stutzeri. and HIAM later isolated a similar organism from an activated sludge plant treating carbonisation waste, and this organism resembled Pseudomonas putida. The ability of heterotrophs to remove thiocyanate (using it as nitrogen source) is important, but there is no conclusive evidence that heterotrophic organisms play a significant role in removal of the component in full-scale plant treating the waste. The relative importance of autotrophic and heterotrophic removal in the treatment system will have important consequences for the use of ATP as a control parameter in the system, and this is an area that requires further investigation.

If it is shown that ATP levels in the reactor reflect mainly the activity of heterotrophic organisms in phenol removal, as seems likely, then even before the situation pertaining to thiocyanate removal has been resolved, it should be possible to use ATP to control the behaviour of the plant with respect to phenol removal.

It will be evident that a potentially very complex ecosystem is involved in the activated sludge treatment of coke oven liquors.

In reality the system involves a complex mixture of interacting bacterial populations, in which the removal of any one liquor component directly or indirectly influences the removal of any other. This has been described in more detail elsewhere. However, to take one example, phenol removal, as well as being subject to substrate inhibition, has also been shown to be influenced by the ammonia concentration; KOSTENBADER and FLECKSTEINER (129) have indicated that high ammonia concentrations reduce phenol removal. More recent work by NEUFELD and VALIKNAS (130) has indicated that phenol degradation is inhibited by thiocyanate and that this becomes significant to the design and operation of a plant treating liquor when low effluent phenol concentrations are required. However, the simple approach that has been adopted here seems to offer a close enough approximation to the real situation to be practically useful in the control of the process.

The problems outlined arise due to the complexity of the ecosystem involved in the treatment of coke oven liquors, and there should be no such difficulties in the application of the control strategy described for treatment of simpler wastes. Despite the problems, the equations described earlier are still seen as providing the basis for deciding the appropriate strategy for responding to a number of the potential causes of the deterioration or complete failure in the performance of treatment plant with respect to the removal of one or more of the liquor components. Some possible causes of unsatisfactory performance are;

1. Hydraulic overloading

2. Increase in influent strength to a level that gives rise to substrate inhibition in the reactor

3. Failure to maintain adequate levels of phosphate in the reactor

4. Inadequate pH control in the reactor

5. Toxicity due to minor components of liquor such as cyanide or higher organic compounds.

The consequences of increasing the hydraulic load to a treatment plant can be predicted from EQUATION 13.15 and this equation can be used to define the conditions of flow that the system can tolerate whilst maintaining an effluent substrate level falling within the desired range. The plant can therefore be designed and operated so that flows to the works are balanced and excess flows are diverted.

Excessive substrate loading will lead to an increase in effluent substrate levels, as can be seen from EQUATION 13.15. From EQUATION 13.12 it can be seen that ATP/X is directly related to growth rate, and the latter is subject to substrate inhibition as described by EQUATION 13.19. An increase in influent substrate concentration which raises the reactor substrate concentration above a critical level, determined by the value of K_i, will therefor cause substrate inhibition. An appropriate response to an excessively strong feed to the plant is to dilute the influent with water or good quality effluent. In this case EQUATION 13.15 can be used to predict the quantity of diluent required and the consequences of this increased influent flow at the new reduced feed strength.

A large number of other factors, including temperature, pH, phosphate deficiency, and high levels of toxic metals and toxic organic compounds, can influence the activity of the microorganisms present in an activated sludge plant. Fortunately ATP has been shown to be a good indicator of the effects of these on the performance of the plant. The simple equations presented can be modified to take account of these, but this has not been done here. The main reason for this is that, although these other factors may be responsible for the poor performance of plants treating waste liquors, in general experience indicates that most such system behave unsatisfactorily due to inadequate attention to the factors already included in the equations presented. Where plant failure has occurred due to a failure in pH control or phosphate dosing apparatus, for example, the reasons for, and the appropriate response to, poor plant performance are so obvious that further complication of the model is unnecessary, and may at this stage deter the practical application of the proposed strategy.

CHAPTER 14

SLUDGE SETTLEMENT

AN HYPOTHESIS POSTULATING THE SIGNIFICANCE OF ATP AND OUR

Introduction

14.1 Literature Review

14.2 Discussion

14.3 An Hypothesis to Explain Qualitatively the Proposed Relationship between Sludge Settlement and Activity

14.4 A Mathematical Model of the Kinetics of Sludge Settlement to Describe the Postulated Relationship between Sludge Settlement and Activity

14.5 Validation of the Model using Data from a Laboratory-Scale Plant Treating Coke Oven Liquor

- 14.5.1 Sludge Settlement in the Continuous Reactor Study Described in Chapter
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14.6 Conclusion

SLUDGE SETTLEMENT

AN HYPOTHESIS POSTULATING THE SIGNIFICANCE OF ATP AND OUR

Introduction

The efficient treatment of a waste in the activated sludge process requires not only the adequate bio-oxidation of the settled waste in the reactor, but also the separation of the biological solids produced in the oxidation process in the subsequent sedementation stage. This project has mainly been concerned with the design and operation of the oxidation stage of the activated sludge process. The design and operation of secondary sedimentation however, is equally important as failure to settle the sludge properly will produce an unsatisfactory final effluent, high in suspended solids and biological oxygen demand, as well as possibly depriving the oxidation stage of adequate levels of microbes in the recycled flow to enable continued efficient biological treatment.

Investigation of the design and operation of sedimentation tanks was neither practical or an objective of the project, and was therefore not carried out. For this reason the work outlined earlier concerning the reactor design and operation assumed appropriate performance of the secondary sedementation stage to achieve the desired objectives for effluent and recycle flow solids levels. However, it is still possible to make a valuable contribution to the understanding of sludge settlement problems by examining the results of other workers, and linking them to ideas and results described earlier in the project.

It is suggested here that the sedimentation of sludge in the final settling tanks is not just a physical phenomenon devoid of the influence of the performance of the preceding oxidation stage, but is instead intimately related to the latter. This chapter briefly reviews those factors shown to affect the flocculation of microorganisms generated in the treatment system, and puts forward an hypothesis linking flocculation and sludge settlement with the performance of the bio-oxidations stage, and indicates the potential of using ATP and OUR as a contol parameter having overall significance to the activated sludge process.

14.1 Literature Review

Complete and rapid microbial oxidation of substrates by cultures of free-floating bacterial cells are well-known, but these are of little value in wastewater treatment. The ability of microorganisms to form flocs is vital for activated sludge treatment of waste. The floc structure enables not only the adsorption of soluble substrates typical of the simple chemically defined media used in laboratory work, but also the adsorption of the colloidal matter and macro-molecules additionally found in, and characteristic of, most wastewaters. The adsorbative capacity of flocs therefore facilitates the oxidation of these more complex wastewaters.

The ability of flocs to settle in wastewater in a relatively short time in quiescent conditions is also very important. Without this ability offered by the structure of the floc, the biomass produced as a result of oxidation of the waste would pass to the watercourse receiving the effluent, and therein a large proportion of the potential pollution load that the original untreated waste represented.

Sludge settling characteristics are generally measured in terms of the Mohlman Index of Sludge Volume Index (SVI). One of the major problems with activated sludge plants of any kind, (whether treating coke oven liquors, domestic, or other wastewaters), is the apparent unpredictable nature of sludge settlement, which when it rapidly deteriorates gives rise to the phenomenon known as "sludge bulking". The poor settling indicated by bulking produces an unsatisfactory effluent, and complete process breakdown if appropriate steps are not quickly taken to reduce the loss of solids from the system. "Bulking" generally occurs when SVI values are greater than 150-170.

A number of theories have been advanced to explain the causes of poor settlement, but it is evident that further work is required in this vital area before a satisfactory insight into the problem can be gained.

One popular theory, supported by work by PIPES (154)(155)(156) and others is that sludge bulking can be attributed to the predominance of filamentous microorganisms such as <u>Sphaerotilus natans</u> in the sludge. However, these claims are disputed, and work by PAVONI, (158) and BUSCH and STUMM (159) suggest that sludge settling, flocculation and the types of extracellular polymers present, are all related in some way. Many workers, for example

FINSTEIN and HEUKELEKIAN,(160) would claim that the presence of filamentous organisms accompanying bulking was merely a symptom rather than a causative factor. It can be reasoned that the nutritional and other environmental conditions that promote the physiological changes in microbial surfaces which in turn affect flocculation, are the same conditions which favour filamentous growth.

PIPES(156) has defined several categories of poor settling sludge, and has also described a condition which he terms "zoogleal bulking" and suggests that it might be related to a deflocculation phenomenon.

Whatever the significance, if any, of filamentous growth to sludge flocculation, work by FORSTER,(161) and KAYO(162) suggests that the surface of sludge microorganisms and the sludge matrix are important in the flocculation process. Even accepting the the filamentous concept, work by PIPES(163) suggests that surface charge may well be a deciding factor where bulking is concerned, since it will influence whether filaments either protrude and therefore hold particles apart, or coil around them and permit a close packing. The apparent importance of the nature of the surfaces of the sludge microorganisms and matrix, and their interactions, has stimulated considerable research into the nature of the extracellular polymers produced by bacteria and their significance in flocculation, and this has been reviewed by FORSTER(164).

FRIEDMAN,(165)(166) WILKINSON,(167) and others have been involved in detailed examination of bacterial exopolymers, including consideration of their composition and the nutritional conditions which appear to stimulate their formation. Whilst these exopolymers are generally polysaccharides in the case of pure bacterial cultures, the matrix of activated sludges is more complex. It has been shown to contain in addition the various products of cell lysis, including RNA, DNA,(168) (169) (170) proteins,(169) (170) polyesters, and poly-β- hydroxybutyric acid,(170) and the products of the members of the microbial community associated with the sludge, in particular the protozoa. Each of these classes of polymers has been indicated as having some possible role in the floc formation and flocculation, and more will be said later of TENNY and VERHOFF'S (170) suggestion that flocculation is mainly an ionic binding process involving transport enzymes at the active sites of the microorganisms. The majority of work has been involved in investigation of bacterial polysaccharides. Bacterial capsular polysaccharides have received much interest because of their role as antigens, as have soluble polymers because of their commercial value. Several workers, including FRIEDMAN,(165) FORSTER,(161) WALLEN and DAVIS (172) and VEDA and EARLE,(173) have postulated a role for capsular polysaccharides and for extracellular polysaccharide fibrils in microbial flocculation. However, despite the considerable information available for some extracellular polysaccharides regarding the conditions that promote their formation, their saccharide content, and the knowledge that there appears to be some relation between the polysaccharides content of an activated sludge and its settling properties, their role in flocculation is little understood.

FORSTER(164) examined the variations in molecular weights of activated sludge "slime" polymer. Fractionation of the polymer using gel filtration indicated that there were two distinct molecular weight species in the polymer, one $>$100,00 and one varying between 26,000 and 78,000. He reported that this last component appeared to become more polymeric as the SVI increased. Ultrafiltration studies indicated the existance of a further significant fraction of molecular weight $<$ 10,000. Although these fractionation studies were very preliminary in nature he was able to suggest by way of conclusion that those polymers in the molecular weight range 20,000-90,000 had the greatest influence on sludge settling behaviour.

The work of PAVONI,(158) therefore indicates that sludge surface polymers have a significant affect on settlement, and FORSTER'S (164) work indicates that the molecular weight of these polymers appear to vary with settlement. Other work by FORSTER (175) has shown that SVI is linearly related to surface charge, and the work of STEINER,(176) and RIDEAU and MORFAUX (177) indicates that the polysaccharide material in the polymers appears to influence settlement and surface charge. In addition, FORSTER and LEWIN (178) have indicated that settlement is affected by metals and water bound to sludge polymers, and SETTER (179) that the cationic exchange capacity of sludge is related to its settlement. More recently FORSTER and DALLAS-NEWTON (180) have suggested that settlement properties are related to nutritional conditions. Various workers (181) have indicated that excesses or deficiences of BOD, ammoniacal nitrogen, and ortho-phosphate are associated with sludge bulking. FORSTER and DALLAS-NEWTON (180) examined data from a pilot activated sludge plant treating domestic sewage, and by regression analysis showed that all three of these nutrients, had a marked affect on SVI. A logarithmic type relationship between SVI and each individual nutrient, and for two new factors, the nutrient product (NP) and specific nutrient product (SNP), was found, with correlation coefficients between 0.60 and 0.80. NP was defined as;

EQUATION 14.1

$$\text{NUTRIMENT PRODUCT} = (\text{BOD}) \times (\text{AMM.N}) \text{ X } (\text{PO}_4)$$

and SNP is

EQUATION 14.2

$$\text{SPECIFIC NUTRIMENT PRODUCT} = \frac{\text{FLOW}}{\text{Kg}} \times \frac{(\text{NP})}{\text{MLSS}}$$

FORSTER, (182) and FORSTER, SURENDRA and WATT (183) have also shown that OUR is linearly related to SVI.

The main factors shown to influence sludge settlement, generally as measured by the SVI value, are summarised in Figure 14.1.

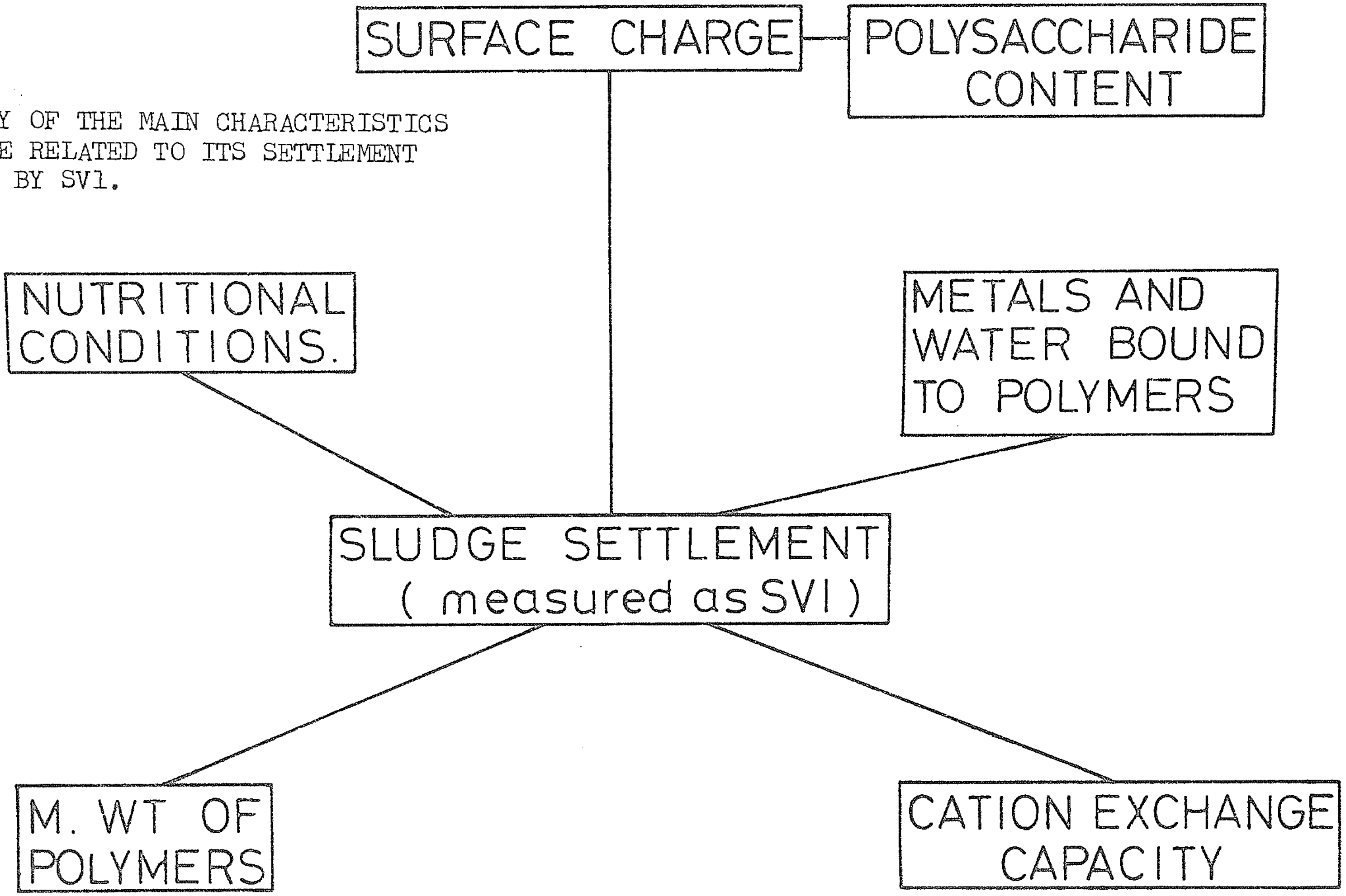


FIGURE 14.1. A SUMMARY OF THE MAIN CHARACTERISTICS OF A SLUDGE SHOWN TO BE RELATED TO ITS SETTLEMENT PROPERTIES AS MEASURED BY SV1.

14.2 Discussion

There is abundant evidence that the settlement properties of activated sludge are related to its chemical structure and nature, especially with regard to the surfaces of the sludge matrix and component microbes. That settlement has been shown to be related to various physico-chemical factors such as cation-exchange capacity, metal and water-binding properties and surface charge, is not unexpected as these can be regarded as manifestations of various properties associated with the sludge's chemical nature. Although much of the present research into sludge settlement is concerned with gaining a detailed understanding of the chemical structure and nature of sludge and its various physico chemical properties, it seems highly unlikely that such work alone will lead to a practical solution to settlement problems experienced in the day-to-day operation of a full-scale treatment plant.

It would seem that there has been a failure to date to understand the basic principles governing the settlement of activated sludge flows, despite the filamentous and various other theories. The most basic principle involved, it is suggested, is very simple: it is that the settlement behaviour of a sludge is dependent upon the biological activity of that sludge. This is derived from what is proposed as the First Law Of Biological Treatment: that "The behaviour of the biological solids in any biological treatment plant, at whatever stage in the treatment, will be dependent upon the biological activity of those solids."

Given the relation between sludge settlement and the various properties associated with its structure, the postulated relationship between settlement and sludge activity seems logical. One would expect that the chemical nature of the sludge would be dependent upon the types and behaviour of the organisms that produce and compose it, since it has been well-established from pure culture work that different organisms produce different polymers, and that behaviour, in terms of polymer production by the same organisms (particularly exopolymers) varies with culture conditions. The chemical nature of the sludge floc would therefore be expected to be dependent upon the nutritional and other environmental conditions operative in the plant, and ultimately dependent upon the activity of the inhabiting organisms.

The concept of sludge "activity" has already been considered at length earlier in the thesis with regard to ATP measurements, and the correlation of the latter with another measure of activity, OUR. Qualitative reference to sludge activity in the terms so far described in the context of sludge settlement have, of course, little practical value. As elsewhere in this thesis, successful application of the concept of activity will be dependent on the degree to which all of the factors involved can be related in a mathematical model, and quantified.

From the above brief review of research into sludge settlement, it will be recalled that FORSTER and DALLAS-NEWTON suggested that settlement was related to nutritional conditions. Also, it will be recalled that FORSTER and co-workers demonstrated a linear relationship between OUR and SVI and in separate work, a logarithmic relationship between SVI and the concentration of various nutriments, and also the nutriment product, as defined earlier. There was therefore an implied relationship between settlement and the activity of the sludge, as measured by OUR, and the concentration of nutriments. However, no attempt was made to link OUR relationship to the observed correlation between SVI and nutriment concentrations, or to link either to the growth kinetics of the system.

It will be recalled from BENEFIELD, LAWRENCE and RANDALL'S work (153) described earlier in the thesis that the following equation was used to relate oxygen utilisation to microbial growth;

EQUATION 14.3

$$\frac{dO_2}{dt} = a.\frac{dX}{dt} + bX = OUR$$

where $\frac{dO_2}{dt}$ = rate of oxygen utilisation (mass volume^{-1}. time^{-1}).

a = oxygen required per unit of biomass produced

b = oxygen required per unit of biomass for the maintenance of life per unit time.

And substrate utilisation and microbial growth rates were related by the following equations developed from Monod kinetics;

EQUATION 14.4

$$\frac{ds}{dt} = -\mu \frac{X}{Y}$$

$$\frac{dX}{dt} = \mu X - K_d X$$ EQUATION 14.5

where $\frac{dS}{dt}$ = rate of substrate utilisation (mass volume^{-1}. time^{-1})

X = biomass concentration (mass volume^{-1})

Y = yield coefficient (mass biomass produced. mass substrate utilised^{-1})

$\frac{dX}{dt}$ = change in biomass concentration (mass volume^{-1}. time^{-1})

Kd = microbial decay coefficient (time^{-1})

μ = specific growth rate (time^{-1})

μ max. = maximum specific growth rate (time^{-1})

S = substrate concentration (mass volume^{-1})

K_s = saturation constant, related to substrate (mass volume^{-1}).

It will also be recalled that the following equation was proposed to indicate the significance of ATP measurements in the treatment system;

$$ATP = e\frac{dX}{dt} + f\ X$$ EQUATION 14.6

where ATP = ATP concentration (M.L^{-3})

e = ATP content per unit rate of change of biomass (T^{-1})

f = ATP required per unit of biomass for the maintenance of life (dimensionless ratio)

Rearrangement of EQUATION 14.3 gives;

$$\frac{dX}{dt} = \frac{(OUR) - bX}{a}$$

EQUATION 14.7

and rearrangement of EQUATION 13.6 gives;

$$\frac{dX}{dt} = \frac{(ATP) - f\ X}{e}$$

EQUATION 14.8

A dynamic model for a complete-mix activated sludge plant can be developed by considering the material balance of substrate and biomass in the treatment system, and a number of such models, and design models derived from them, were described earlier in the thesis.

Differential equations consisting such a dynamic model and change in biomass and substrate in a complete-mix plant were described in Chapter 3, and are summarised below;

FOR THE CHANGE IN BIOMASS:

$$\frac{dX}{dt} = D \alpha X_R + (\mu - K_d)\ X - D\ (1 + \alpha)\ X$$

EQUATION 14.9

FOR THE CHANGE IN SUBSTRATE:

$$\frac{dS}{dt} = D\ (S_i - \alpha S) - D\ (1 + \alpha)\ S - \mu \frac{X}{Y}$$

EQUATION 14.10

Equations 14.8, 14.9, and 14.10 can therefore be used as the basis for using ATP as a control parameter in the modelling of the treatment process.

Proposals for the specific use of ATP as a control parameter were discussed earlier, and several workers have outlined control strategies using OUR. As there has been shown to be a linear correlation between OUR and SVI, and as the role proposed for ATP is in many ways analogous to that of OUR, it should be possible to relate these two parameters and the equations outlined earlier, (which together describe the activity of the sludge and its relation to the operation of the treatment system), to the settlement properties of the sludge produced. Before attempting to define this relationship in a mathematical form however, an hypothesis is put forward and discussed to explain why the relationship proposed should exist.

14.3 An Hypothesis to Explain Quantitatively the Proposed Relationship between Sludge Settlement and Activity

To explain the proposed relationship between the activity of the sludge and its settlement properties let us start by supposing that flocculation of sludge particles occurs due to the attraction betweem, and attachment of, long chain polymers between floc particles. Suppose also that these natural biopolymers form part of the floc particles and that their quantity and nature are directly related to the biological activity of the flocs. TENNEY and VERHOFFS suggestion that these correspond to transport enzymes would seem plausible as their nature and concentration is likely to be directly related to the activity of the cells present in the floc particles. It is not necessarily supposed that flocculation only involves direct linkage and interaction between transport enzymes of adjacent floc particles. As well as being directly involved in inter-floc binding, they are also seen as loci on the floc particles to which other biopolymers bind mediating the flocculation of particles.

Binding between other biopolymers, such as DNA and RNA, in flocculation seems likely but it must be remembered that many of these will only be present as the result of lysis of the cells. All of the polymers present in sludge are therefore seen as influencing settlement, but transport enzymes are assumed to be the most important because of their protein nature and their role as binding agents in enzyme catalysis.

Transport enzymes are of course structurally located on the outer surface of cells and unlike other polymers such as DNA, RNA, and intracellular proteins, would be expected to influenceflocculation as components of living cells in the floc, the potential activity of the others being limited to lysed cells. As specific structural components of cell membranes, transport enzymes might be regarded as more stable than intracellular polymers when cell death and lysis occurs, and therefore more likely to influence flocculation than other polymers, even after cell death. Reports by various workers who have conducted research into transport enzymes using ruptured cells would suggest that the structural and functional integrity of these proteins was likely to a large degree to be maintained in flocs following cell lysis.

The apparent stability of these proteins suggests that once formed they will continue to influence the settlement properties of the sludge for as long as it remains in the treatment system. KRAUS (184) and others have made the assumption that the settlement properties of flocs as measured by SVI are determined when they are formed and that these remain unchanged whilst they remain in the treatment system. Their assumption could be seen as being consistent with the proposals outlined here. The other assumption made by KRAUS, that the settlement properties of the total sludge represents the weighted average of the various components of the sludge is also seen as being consistent with the hypothesis presented here. Here it is proposed that the "components" correspond to the flocculation active centres on the flocs represented by the transport proteins, and that the settlement properties reflect their total number, nature, and distribution in relation to the total number of flocs present in the sludge.

The settlement properties of the sludge are therefore seen as very much representing the activity history of the sludge particles.

Before continuing to discuss further and consider the consequences of the main hypothesis, that sludge settlement is directly related to sludge activity, it is worth noting that that hypothesis does not require that the transport proteins be the main polymers influencing flocculation. All that is required is that the polymers responsible for flocculation reflect the activity of the flocs as described. It seems however that the transport proteins are the polymers most likely to fulfil that role.

The observation by FORSTER, SURRENDA, and WATT of a correlation between decreased settleability of sludge (i.e. increase in SVI) with increasing sludge activity as measured by OUR is consistent with observations by many workers that microorganisms in general are dispersed during periods of high growth rate (i.e. high activity) and that agglutination occurs at lower growth rates.

TENNEY and VERHOFF who proposed that transport enzymes served as flocculating polymers reported that auto-flocculation of microbial cultures does not occur until the endogenous phase of growth (i.e. low activity) is reached, but that flocculation with synthetic polyelectrolytes is most efficient during the declining growth phase (i.e. a much higher level of activity). They suggested that the transport enzymes acted as the site of action for binding by synthetic polyelectrolytes in the declining growth phase but that autoflocculation occurred during the periods of lower activity corresponding to endogenous growth due to the lysis of cells and the release of some of these proteins where they served as the flocculation agent.

TENNEY and VERHOFF'S proposals for the role of transport proteins in cell agglutination, KRAUS'S proposal that the settlement properties of flocs are determined when they are formed and remain unchanged, and FORSTER et al's observation of the correlation between sludge settlement and OUR, can be modified, adapted and combined to form the basis for a unified approach to explain sludge settlement in terms of sludge activity. This approach may be summarised as follows. Sludge settlement properties are dependant upon the activity (both present and historical) of the sludge. This is reflected in;

1. the total number of transport protein sites on the flocs
2. the nature and type of transport proteins present.

The importance of these can be seen from the following discussion.

As transport enzymes typically exhibit substrate specificity, species of organisms metabolising different substrates could be assumed to have different transport proteins. One would expect different transport proteins to be associated with different properties with regard to influencing settlement. For similar species of organisms metabolising broadly similar substrate however, these differences are assumed small and not significant.

Where very different substrates are involved, particularly where the mode of nutrition is very different, for example where heterotrophic and autotrophic metabolism is involved, such differences would be expected to be significant. (A first approach to verifying the postulated relationship between activity and sludge settlement might therefore be to investigate the correlation between the activity of heterotrophs and autotrophs with settlement). The effect of the activity of different autotrophs on sludge settlement, for example those involved in thiocyanate removal, nitrification, and denitrification, would also be expected to be different and distinguishable.

The settlement properties of the total sludge are seen as representing the weighted average of all the various flocculation active sites as indicated earlier.

A further refinement is needed to the above description to fully explain the postulated relationship between activity and settlement. This is illustrated with the aid of Figure 14.2. The same principles are used to explain the agglutination of free cells, sludge floc formation, and the interactions between flocs that influence the sludge settlement. Bacterial cells are used for convenience to illustrate the principles involved.

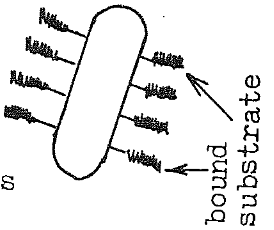
Each bacterial cell is seen as containing a fixed number of transport proteins (flocculation active centres).. During periods of high activity there are high levels of substrate, substrate utilisation is maximal, and all the sites are effectively bound with substrate. As a consequence they are not free to influence flocculation and aid sludge settlement. This explains why in the case of free cells they are dispersed in these conditions, and for sludges settlement of flocs is poor.

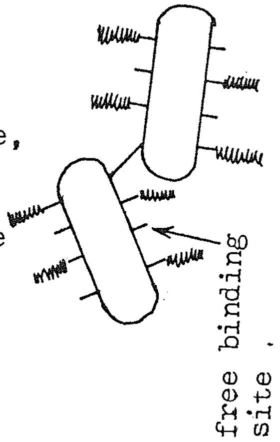
When substrate utilisation is not maximal, in periods of medium activity, at any one time only a proportion of the sites are bound to substrate, and the remaining sites are free to interact and to

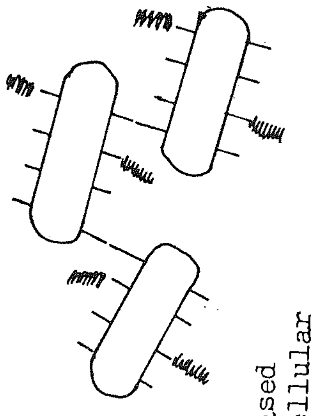
FIGURE 14.2 TO ILLUSTRATE THE PRINCIPLES OF THE POSTULATED RELATIONSHIP BETWEEN ACTIVITY AND SETTLEMENT/CELL AGGLUTINATION FOR BACTERIAL SUSPENSIONS/SLUDGE FLOCS.

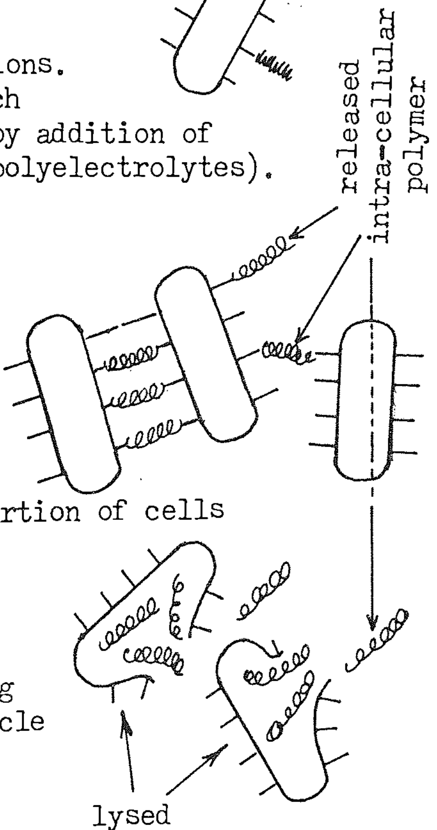
SETTLEMENT/ AGGLUTINATION.	CELLULAR ACTIVITY (Growth Rate)	COMMENTS.
POOR (Sludges have High SVI)	HIGH	Substrate utilisation maximal--all sites effectively bound with substrate. Very little inter-particle binding.
	MEDIUM	Only a proportion of sites bound to substrate, leaving more sites available for inter-particle binding.
	DECLINING	Even more sites available for inter-particle binding, but these are limited by spatial considerations. (Binding much improved by addition of synthetic polyelectrolytes).
GOOD (Sludges have Low SVI).	VERY LOW	Endogenous growth conditions. Large proportion of cells die & lyse releasing large no.s of polymers facilitating inter-particle binding.

↓ IMPROVING SETTLEABILITY/AGGLUTINATION.









influence flocculation. For sludges such conditions are associated with improved settleability. Flocculating ability increases and settlement improves as the cellular activity decrease and more sites become available. Although the free sites are themselves able to interact and therefore improve settlement spatial considerations are seen as a limiting factor in this. Spatial limitations can however be overcome if other polymers are available to bridge, that is form a link betweem, binding sites on different bacteria or floc particles. The sites therefore also act as the loci for the binding of other polymers which then mediate in the flocculation or agglutination process. However, in periods of medium activity the amount of suitable natural polymer available for this purpose is likely to be limited. As a result, although there are more sites available for intercellular or inter-floc binding, and the potential for binding is therefore greater than in periods of high activity, the improvements observed in settleability of sludges and in agglutination of cells in these conditions are less than those possible. The full potential for improved interactions can be realised if extra polymer is added to suspensions of the cells or flocs, and this explains why synthetic polyelectrolytes are so effective in improving agglutination and flocculation in these conditions.

In very low activity conditions a large proportion of the cells die and lyse and a large number of natural polymers are released. These biopolymers are now available to assume a bridging role and facilitate improved cellular and floc interaction, which leads to a further improvement in settlement. This corresponds to the to the autoflocculation condition referred to earlier.

The above hypothesis was proposed to qualitatively explain the postulated relationship between sludge settlement and activity described in the more general hypothesis outlined earlier. It would be useful to verify the role of transport proteins and sludge polymers in sludge settlement as described above but this was neither practical nor an objective in the present project. The above qualitative hypothesis does serve however to strengthen and form a rational basis for the more general hypothesis.

However, for the postulated relationship between sludge settlement and activity to have any practical application to the design and operation of treatment plants, and so that the general hypothesis described may be tested, the relationship needs to be defined quantitatively. The following model was proposed with the aim of fulfilling that objective.

14.4 A Mathematical Model of the Kinetics of Sludge Settlement to Describe the Postulated Relationship between Sludge Settlement and Activity

On the basis of the equations and relationships described earlier in the chapter it was possible to present a simple mathematical model which described the postulated relationship between sludge settlement and activity. Such a model was presented here and enabled the relationship to be quantified and tested using the laboratory-scale reactor data presented earlier in the project.

The first assumption made was that the average SVI of all the sludge organisms was given by;

$$I = \sum_{n=1}^{K} I_n \frac{X_n}{X} \qquad \underline{\text{EQUATION 14.11}}$$

where I = the average SVI of the total sludge organisms

n is a positive integer between 1 and K and each refers to the identity of different populations of sludge organisms

I_n = the SVI attributable to the population of organisms, n, in the sludge

X_n = the concentration of population n in the sludge

X = the total concentration of sludge organisms

This equation was consistent with that used by PIPES to describe the contribution of the SVI of normal and filamentous sludge organisms to the average SVI of a sludge containing both.

The second assumption made was that the sludge volume, SV, attributable to each population of sludge organisms was directly proportional to the activity of the sludge as measured by ATP or OUR, thus

$$SV \propto ATP \qquad \underline{14.12}$$

and that the sludge volume index SVI, given by SV/X, and represented by I_n in the equations, was directly proportional to the concentration of ATP per unit mass of sludge organisms, thus

$$I \propto \frac{ATP}{X} \qquad \underline{14.13}$$

The use of ATP as a control parameter was discussed in Chapter and the equations derived therein were based on the kinetic equations presented earlier in this chapter. The equation proposed earlier in the project to describe the significance of ATP was;

$$ATP = \frac{e\,dX}{dt} + f\,X \qquad \underline{\text{EQUATION 14.6}}$$

If this equation is divided by X the following is obtained;

$$\frac{ATP}{X} = e\mu + f \qquad \underline{\text{EQUATION 14.14}}$$

as

$$\mu = \frac{dX}{dt} \Big/ X \qquad \underline{\text{EQUATION 14.15}}$$

These equations were described in Chapter 13 . From that chapter the following equation was derived;

$$\frac{ATP}{X} = e \; \frac{(S_i - S)\ YD}{X} + f \qquad \underline{\text{EQUATION 14.16}}$$

and from 14.13 therefore it can be deduced that;

$$I \propto e \quad \frac{(S_i - S)\ YD}{X} + f \qquad 14.17$$

or

$$I \propto e \quad \frac{(S_i - S)\ YF}{XV} + f \qquad 14.18$$

since

$$D = \frac{F}{V} \qquad \text{EQUATION 14.19}$$

Before the simple model represented by these equations was tested using experimental data, it was possible to show, as follows, that the empirical relationships between SVI and nutrient conditions, NP, and SNP, described by FORSTER and DALLAS-NEWTON were consistent with this model. It is suggested that the above model offers an explanation for the important observations made by those researchers. This could be seen from the following examination of their findings on the basis of the model.

Examination of the empirical relationship between sludge settlement and nutrient conditions reported by FORSTER and DALLAS-NEWTON

1. The relationship between SVI and the concentration of individual nutrients

The researchers reported a logarithmic type relationship between SVI and the concentration of individual nutrients.

From the relationship shown in EQUATION 14.14 it could be seen that SVI was directly related to growth rate, μ , thus;

$$I \propto \mu \qquad \underline{14.20}$$

From Monod kinetics;

$$\mu = \mu\,\text{max.}\ \frac{S}{K_s + S} \qquad \underline{\text{EQUATION 14.21}}$$

In this equation the relationship between μ and S, and therefore the postulated relationship between I and S, is represented graphically by a rectangular hyperbola (see Figure 14.). As there are two constants in EQUATION 14.21 K_s and μ max., the equation is usually transposed to the form;

$$\frac{1}{\mu} = \frac{S + K_s}{\mu_{\text{max.}} S} = \frac{1}{\mu\,\text{max.}} + \frac{K_s}{\mu\text{max.}} \cdot \frac{1}{S}$$

$$\underline{\text{EQUATION 14.22}}$$

In the Lineweaver-Burk type plot, which uses this equation and was used earlier in the project, I/μ is plotted against $1/S$ to obtain a straight line of slope K_s/μ max. and intercept $1/\mu$ max.

The rectangular hyperbola produced from EQUATION 14.21 can however be approximated by an equation of the form;

$$y = Ax^{n} \qquad \underline{\text{EQUATION 14.23}}$$

where n is positive but less than 1.

and the relationship between I and S can be approximated to a logarithmic type, consistent with the findings of FORSTER and DALLAS-NEWTON of a logarithmic type relationship between SVI and the concentration of individual nutrients.

For example, the rectangular hyperbola shown in Figure 14.3. was produced from EQUATION 14.21 with $\mu_{max.} = 10$ and $K_s = 1$. This could be approximated by a rectangular hyperbola produced from an equation of type 14.22 where $y \equiv \mu$, and $x \equiv S$, and with A = a constant = 5.8, and $n = 0.18$, also shown in Figure 14.3.

EQUATION 14.22 can be written in the form;

$$\log.y = \log.A + n \log x \qquad \text{EQUATION 14.24}$$

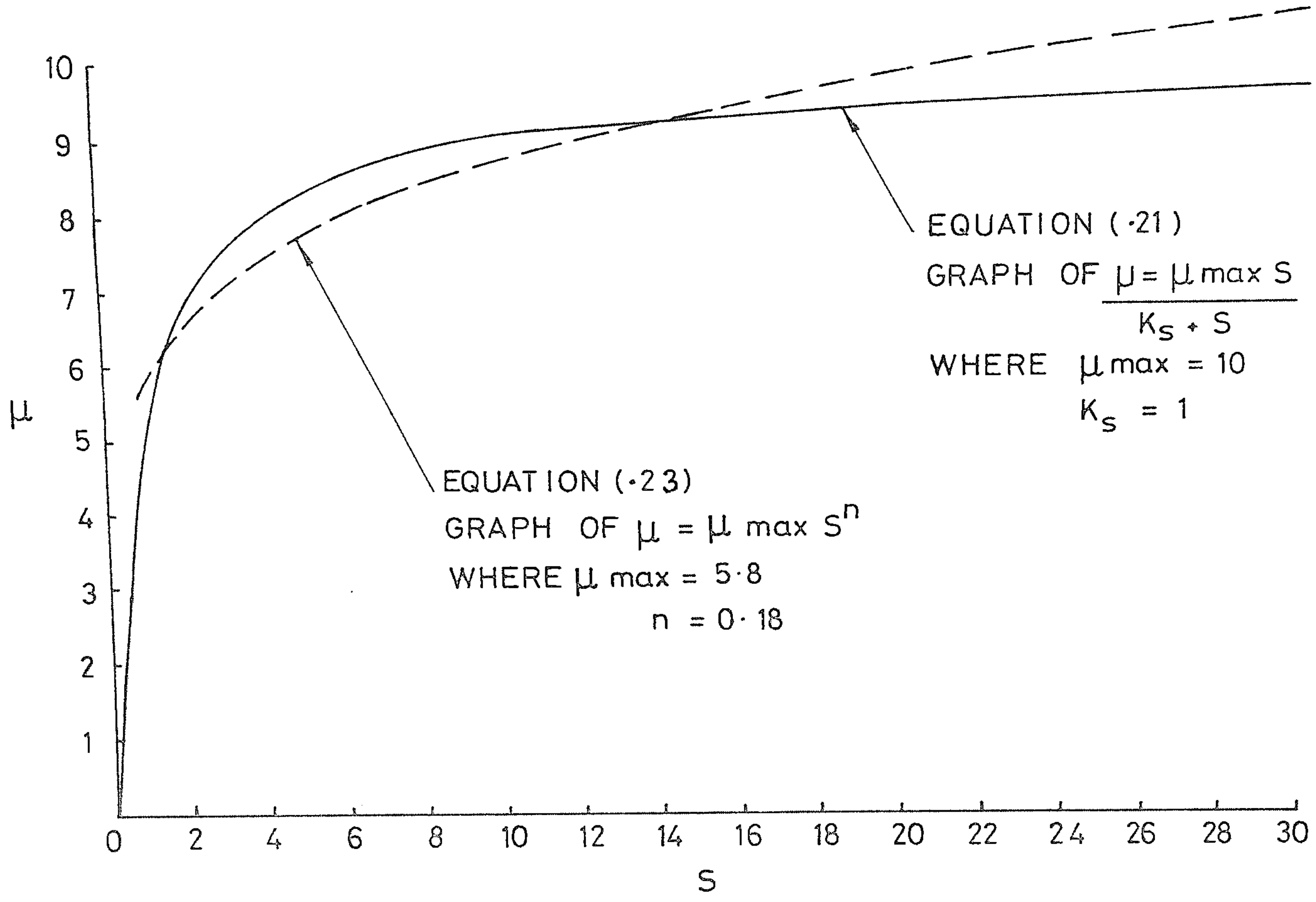
which can be further arranged in the form;

$$Y = nX + \log.A \qquad \text{EQUATION 14.25}$$

where $X = \log x$ and $Y = \log.y$

As A is a constant, log. A is also a constant. A plot of log. y against log x produces a straight line. If μ is substituted for y , and S for x , then a logarithmic relationship between μ and S becomes apparent. Similarly substituting I for y, and nutrient concentration (S) for x , produces a a logarithnic relationship between SVI and nutrient concentrations.

FIG 14.3 TO SHOW THAT AN EQUATION OF THE TYPE $y = Ax^n$ (·23) MAY APPROXIMATE TO THAT OF THE MONOD TYPE $\mu = \frac{\mu \max S}{K_s + S}$ (·21)



2. The relationship between SVI and N.P

The nutrient product, NP, as defined earlier, was equal to the product of the concentrations of three nutrients. If the relationship between SVI and the concentration of individual nutrients was assumed to follow a Monod-type relationship, but approximated by an equation of the type shown in 14.22, then the relationship between SVI and the product of the individual nutrients would be expected to be logarithmic.

3. The relationship between SVI and SNP

From the relationship shown in 14.18, a linear relationship between SVI and flow, F, and between SVI and 1/MLSS or $1/_X$, would be expected. However, the inclusion of NP in the definition of SNP, would from the above considerations produce a logarithmic relationship between SVI and SNP.

The empirical relationships reported by FORSTER and DALLAS-NEWTON therefore appear to be consistent with the model presented here.

14.5 Validation of the Model using Data from a Laboratory-Scale Plant Treating Coke Oven Liquor

The postulated relationship between sludge settlement and activity, as described by the model presented in the previous section, was investigated using the data derived from the continuous reactor study reported earlier in the project. Before doing so however, a brief review was made of the results obtained and conditions employed relating to sludge settlement in that study.

14.5.1 Sludge Settlement in the Continuous Reactor Study Described in Chapter 9.

A wide range of settlement conditions were observed in the study. The SVI values obtained were recorded in Table 14.1 along with other results from the study. On days 1-12, and from day 40 onwards, the SVI values recorded were consistently within the range typically found for sludges from full-scale plants treating domestic wastewater having satisfactory settlement properties. This corresponded to SVI values less than about 140. Unsatisfactory sludge settlement, generally termed "sludge bulking", usually occurs in domestic wastewater treatment plants with SVI values higher than this. This was also found to be the case in the study reported in Chapter 9. Sludge bulking was observed on days 13-21 inclusive, and 24-27 inclusive, as evidenced by the collection of thickened sludge at the top of the settler. (SVI values of greater than 140 were generally observed during these periods). This had the result on some of those days of causing unsatisfactory recycling of sludge from the reactor. In addition, on days 14-18 inclusive significant losses of sludge occurred due to buoyed sludge overflowing the settler, with similar but smaller losses occurring on days 24-27 inclusive.

TABLE 14.1

DATA DERIVED FROM THE CONTINUOUS REACTOR STUDY DESCRIBED IN CHAPTER 9.

DAY	SV1	ATP/X fg/mg	μn DAY^{-1}
1	78.51	1.0144	0.01035
2	84.43	1.0758	0.01806
3	65.69	1.1977	0.01689
4	104.20	1.5474	0.01964
5	108.21	1.5526	0.01779
7	122.34	0.6972	0.01469
8	88.77	1.0412	0.02444
9	85.38	1.0228	0.02004
10	86.66	1.0220	0.02126
11	109.31	1.2377	0.02454
12	113.86	2.0172	0.02750
13	142.65	2.3367	0.03318
14	143.35	2.4874	0.03647
15	173.01	4.0484	-
17	148.54	5.9893	-
18	81.55	3.7920	-
20	145.47	-	-
21	115.64	3.6018	-
23	127.07	2.7490	0.03447
27	97.23	3.6889	-
29	191.87	3.9220	0.03893
30	190.60	3.6674	0.03995
31	203.24	2.9724	0.03552
33	217.47	3.5167	0.03371
34	270.67	4.2581	0.04474
35	249.62	4.8387	0.04390
36	251.28	6.8995	0.04687
40	110.04	6.5757	0.04988
41	120.70	7.2740	0.04972

Inadequate sludge recycling and sludge loss were the cause of the fluctuations in reactor MLSS observed during these periods.

Small quantities (0.05 to 0.1ml) of silicone antifoaming agent (BDH - containing 30% W/W silicone) were added to the reactor on days 19-23 inclusive, and days, 30, 31, 35, and 41. This had the effect of reducing or preventing sludge buoying in the settler when high SVI values were observed. It also had the effect that it became possible to observe higher SVI values than would normally have been possible in the absence of antifoaming agent.

SVI determinations during the continuous reactor study involved determination of the sludge volume after a 10 minute settlement period. Conventionally SVI values are determined using the settled volume (or stirred volume in the case of SSVI determinations) after a 30 minute settlement period, using the prescribed apparatus for the test. Practical difficulties related to time and scale prevented the use of conventional tests used for full-scale plants. However, it was noted that SV_{10} values were used by some staff, at least in monitoring the performance of full-scale plants treating coke oven liquor, and found to be an adequate measure of sludge settlement properties. The use of different conditions for measurement of SV were not regarded as significant in the following investigation.

14.5.2 Model Validation

SVI and Growth Rate

It was proposed in EQUATION 14.20 that sludge settlement, as measured by SVI, was directly proportional to specifc growth rate. Values for net growth rate were recorded in TABLE 14.1. This is related to specific growth rate by the equation;

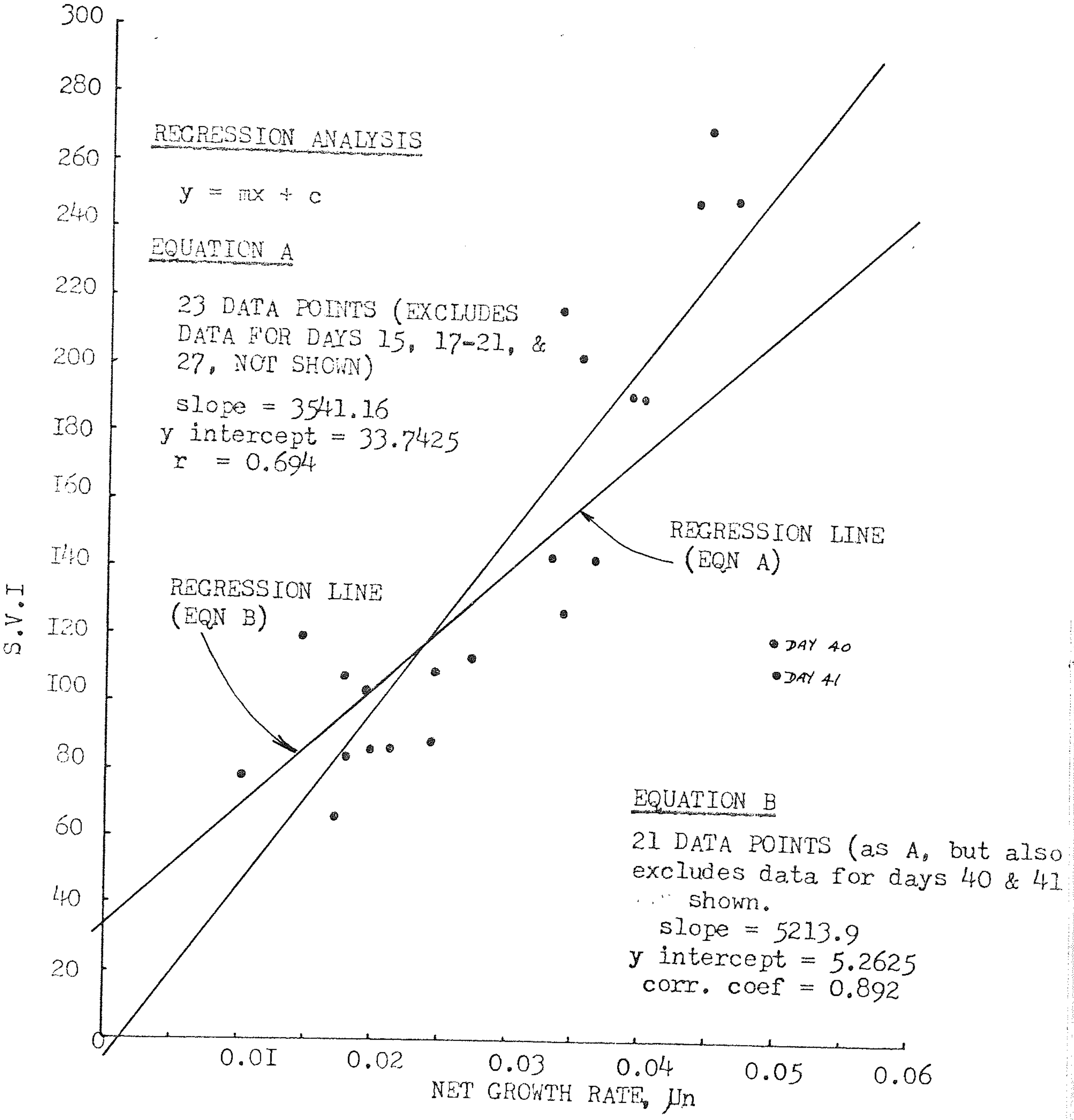
$$\mu_n = \mu - K_d \qquad \text{EQUATION 14.26}$$

where μ_n = net growth rate
μ = specific growth rate
K_d = a constant, the decay coefficient

As K_d is a constant a linear relationship between SVI and net growth rate would be expected if the proposed model applied. A graph was plotted in Figure 14.4, relating SVI to net growth rate.

Data corresponding to days 15, 17, 18, 21, and 27 were excluded from the graph. Large and unknown sludge losses on the first 4 of these days, and smaller losses on day 27, meant that it was not possible to accurately determine the corresponding net growth rates which would have required an accurate knowledge of the solids balance of the treatment system.

FIGURE I4.4 THE RELATIONSHIP BETWEEN SVI AND NET GROWTH RATE.



REGRESSION ANALYSIS

y = mx + c

EQUATION A

23 DATA POINTS (EXCLUDES DATA FOR DAYS 15, 17-21, & 27, NOT SHOWN)

slope = 3541.16
y intercept = 33.7425
r = 0.694

EQUATION B

21 DATA POINTS (as A, but also excludes data for days 40 & 41 [illegible] shown.

slope = 5213.9
y intercept = 5.2625
corr. coef = 0.892

The graph showed a linear relationship, with a correlation coefficient, r, of 0.69. Two data points, those corresponding to days 40 and 41, deviated considerably from the trend shown by the regression line. Excluding this data from the regression analysis, a value for R of 0.89 was obtained for the remaining data points.

14.5.2.2 SVI and ATP

From the relationship proposed in EQUATION 14.13 there should be a linear relationship between SVI and ATP/X. Excluding as before data for days 15, 17, 18, 21, and 27, a graph was plotted relating SVI and ATP/X in Figure 14.5. A linear relationship was found with r = 0.60.

As before, the data relating to days 40 and 41 deviated considerably from the regression line shown. Exclusion of these from the regression analysis produced a value for r of 0.91 for the remaining 21 data elements.

SVI and Substrate Utilisation Rate, U

From the relationship shown in EQUATION 14.18 the following relationship can be derived by removing kinetic constants, e, f, and Y;

$$I \propto \frac{(S_i - S)\ F}{XV} \qquad \underline{14.27}$$

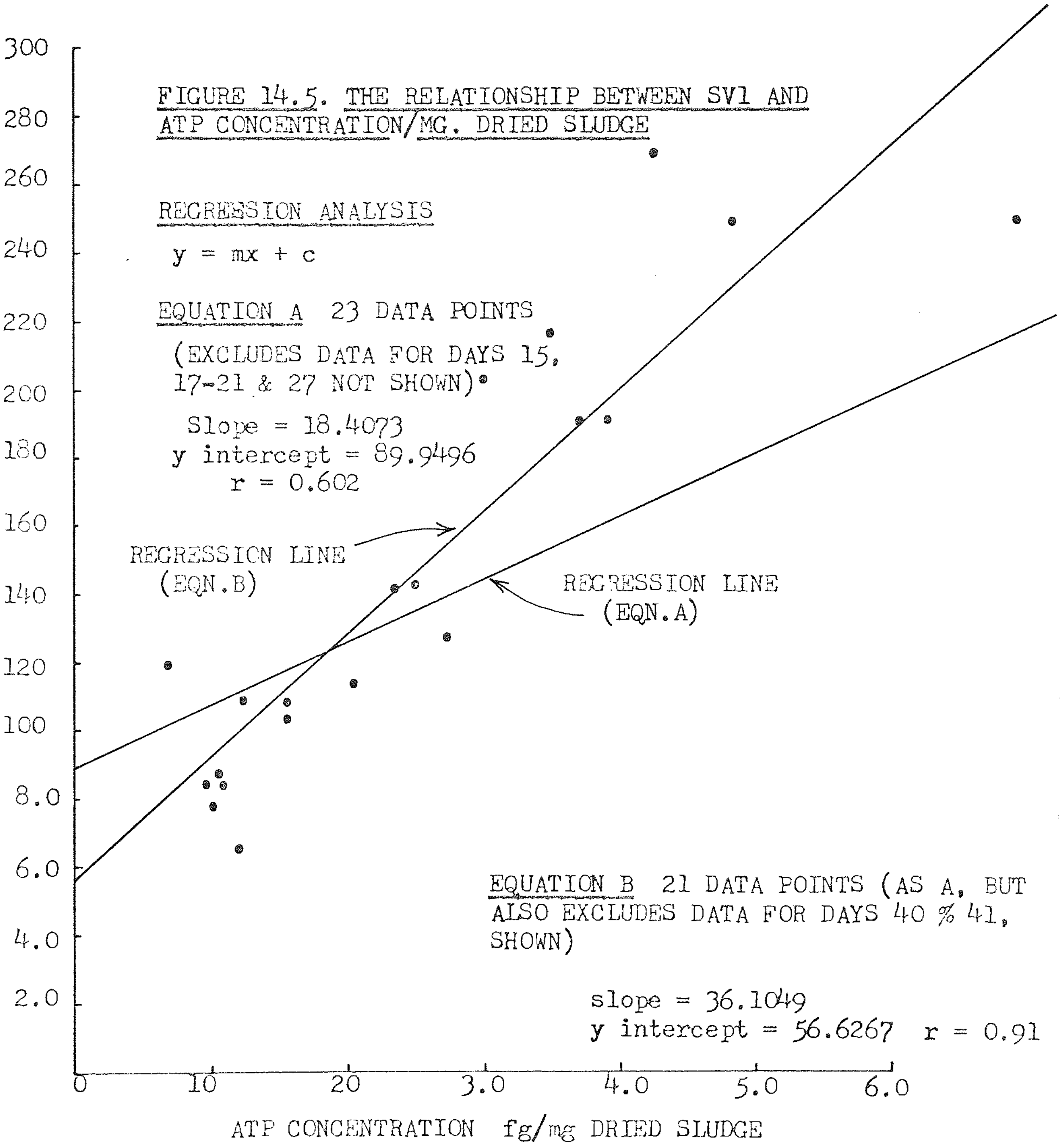


FIGURE 14.5. THE RELATIONSHIP BETWEEN SV1 AND ATP CONCENTRATION/MG. DRIED SLUDGE

REGRESSION ANALYSIS

y = mx + c

EQUATION A 23 DATA POINTS (EXCLUDES DATA FOR DAYS 15, 17-21 & 27 NOT SHOWN)

Slope = 18.4073
y intercept = 89.9496
r = 0.602

EQUATION B 21 DATA POINTS (AS A, BUT ALSO EXCLUDES DATA FOR DAYS 40 % 41, SHOWN)

slope = 36.1049
y intercept = 56.6267 r = 0.91

The latter term is equal to the substrate utilisation rate, U, as defined in Chapter 13. The substrate utilisation rate was determined for phenol and thiocyanate by applying the equation for U to the data presented in Chapter 9 . The results of this analysis were recorded in Table 14.2.

Phenol

A graph was plotted in Figure 14.6 relating SVI to U for phenol removal. Data corresponding to days 15, 17-21, and 27 were excluded for the reasons outlined earlier. The graph showed a linear relationship with r = 0.92.

Thiocyanate

A graph was plotted in Figure 14.7 relating SVI to U for thiocyanate removal. Data corresponding to days 15, 17-21, and 27 were excluded for the reasons outlined earlier. The graph showed a linear relationship with a value for r of 0.77.

(If data corresponding to days 40 and 41 were also excluded, as in earlier sections, r = 0.87).

SVI and OUR

The model described in the previous section was concerned with the relation between sludge settlement and activity, the

TABLE 14.2

SV1 AND U RESULTS DERIVED CONTINUOUS REACTOR STUDY DESCRIBED IN CHAPTER

DAY	SV1	U PHENOL	U THIOCYANATE
1	78.51	0.01307	0.01776
2	84.43	0.01745	0.01823
3	65.69	0.01518	0.01600
4	104.20	0.01976	0.02067
5	108.21	0.01372	0.01745
7	120.34	0.01685	0.01838
8	88.77	0.01826	0.01536
9	85.38	0.01506	0.00985
10	86.66	0.01429	0.00758
11	109.31	0.01904	0.00735
12	113.86	0.06514	0 00756
13	142.65	0.07536	0.00980
14	143.35	0.08575	0.00939
23	127.07	0.07410	0.05880
29	191.87	0.10764	0.06502
30	190.60	0.11030	0.06807
31	203.24	0.10336	-
33	217.47	-	0.06462
34	270.67	-	0.07080
35	249.62	-	0.06949
36	251.28	-	0.07634
40	110.04	-	0.07109
41	120.70	-	0.05238

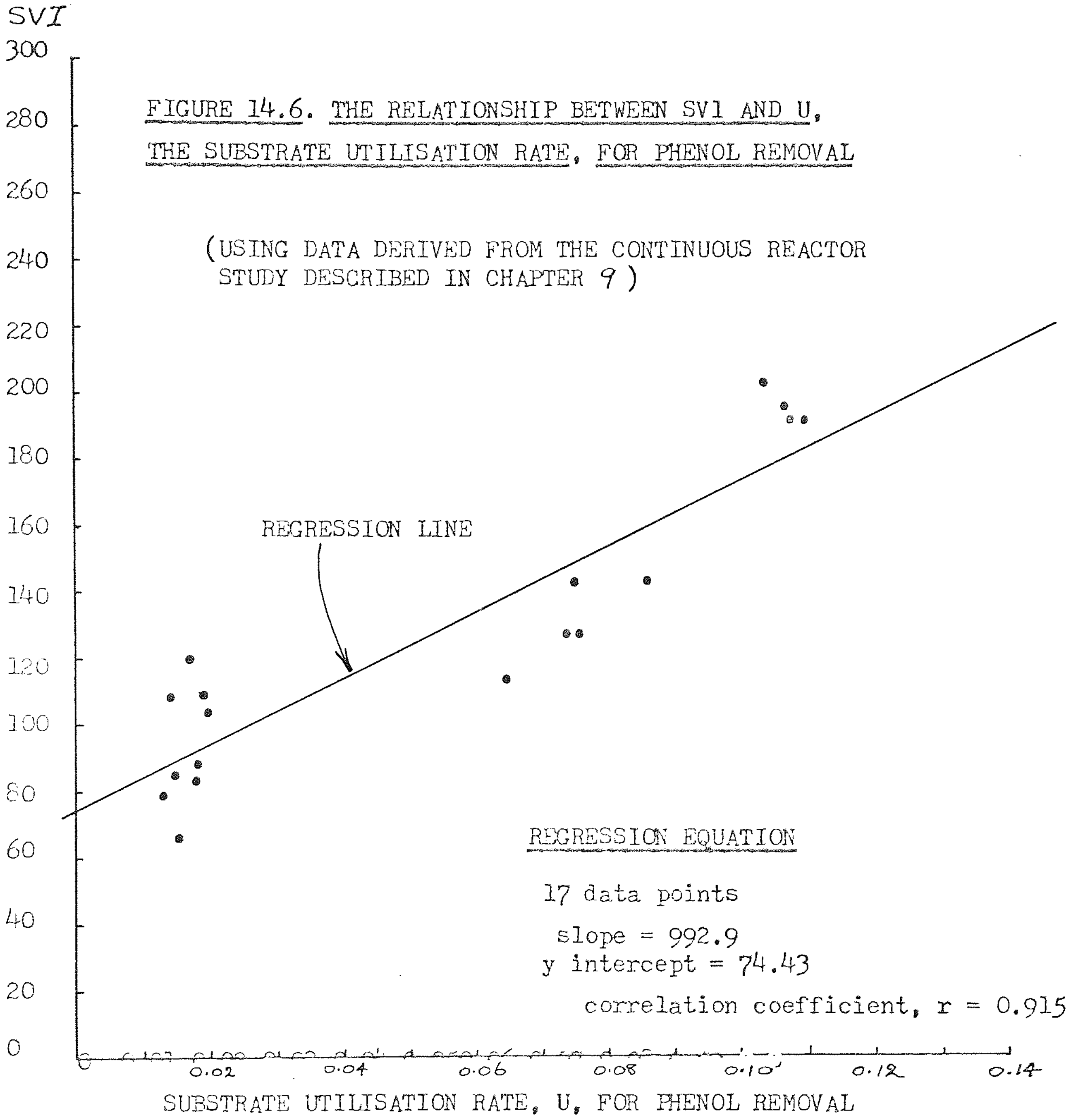


FIGURE 14.6. THE RELATIONSHIP BETWEEN SV1 AND U, THE SUBSTRATE UTILISATION RATE, FOR PHENOL REMOVAL

(USING DATA DERIVED FROM THE CONTINUOUS REACTOR STUDY DESCRIBED IN CHAPTER 9)

REGRESSION EQUATION

17 data points

slope = 992.9

y intercept = 74.43

correlation coefficient, r = 0.915

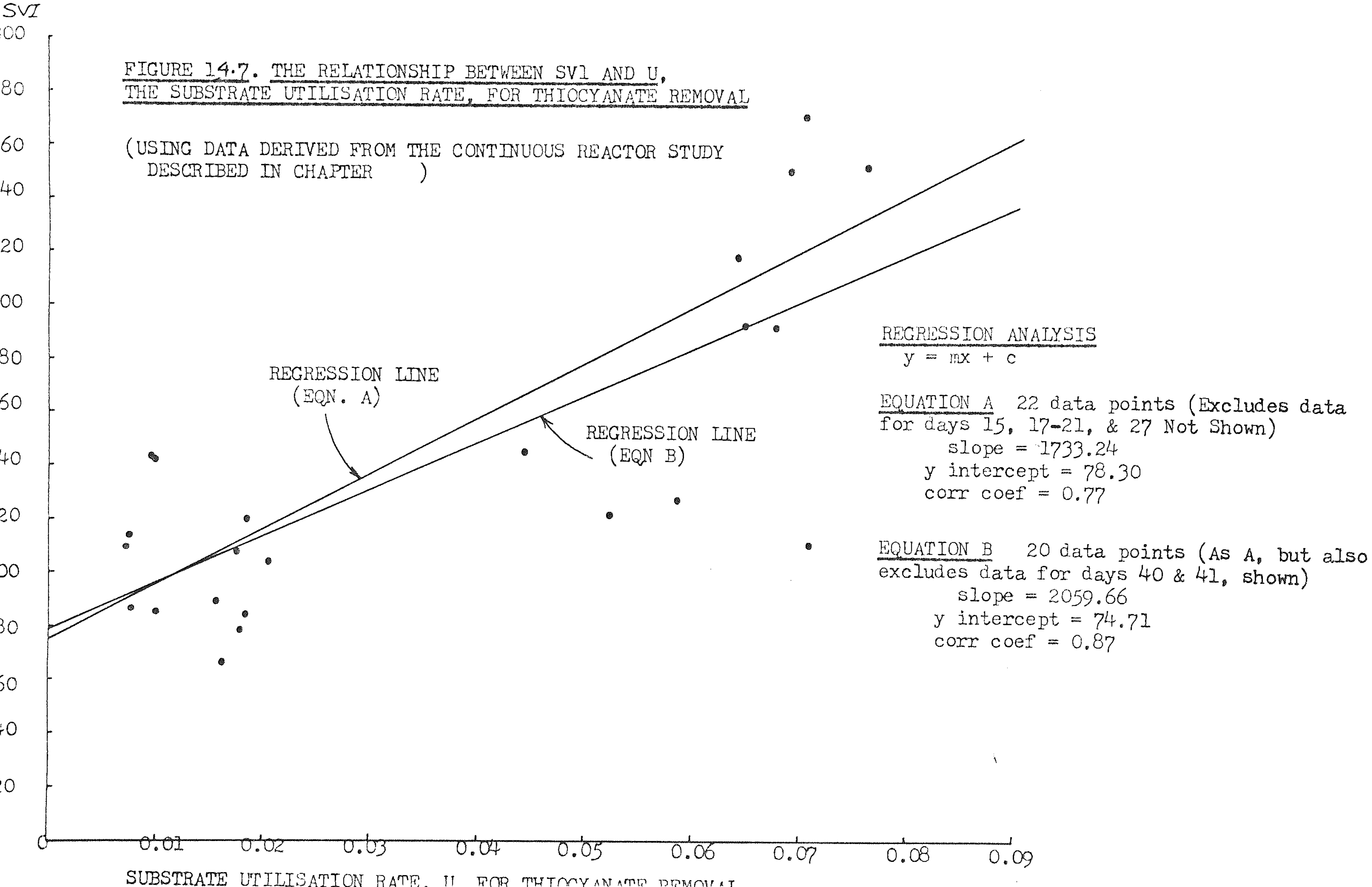


FIGURE 14.7. THE RELATIONSHIP BETWEEN SV1 AND U, THE SUBSTRATE UTILISATION RATE, FOR THIOCYANATE REMOVAL

(USING DATA DERIVED FROM THE CONTINUOUS REACTOR STUDY DESCRIBED IN CHAPTER)

REGRESSION ANALYSIS

$y = mx + c$

EQUATION A 22 data points (Excludes data for days 15, 17-21, & 27 Not Shown)
slope = 1733.24
y intercept = 78.30
corr coef = 0.77

EQUATION B 20 data points (As A, but also excludes data for days 40 & 41, shown)
slope = 2059.66
y intercept = 74.71
corr coef = 0.87

latter being measured in terms of ATP. As the object of the project was to determine the significance of ATP in the treatment system it was natural that the model presented should have been primarily concerned with ATP.

However, it will be recalled that the fundamental equation used to define the significance of ATP in the treatment system, EQUATION 14.6, was very similar in form to that used to indicate the significance of OUR, EQUATION 14.3. It can be seen therefore that a model similar to that developed using ATP, could be produced relating SVI to OUR, and thus a similar role postulated for OUR as proposed for ATP. Thus, using EQUATION 14.3 as the basis for the model, analogous to using EQUATION 14.6, the following relations 14.28, 14.29, 14.30, and 14.31, would be equivalent to those developed using ATP, 14.14, 14.16, 14.13, and 14.18 respectively.

$$\frac{OUR}{X} = a\mu + b \qquad \underline{\text{EQUATION 14.28}}$$

$$\frac{OUR}{X} = a \frac{(S_i - S)\ YD}{X} + b \qquad \underline{\text{EQUATION 14.29}}$$

$$I \propto \frac{OUR}{X} \qquad \underline{\text{EQUATION 14.30}}$$

and

$$I \propto a \frac{(S_i - S)\ YF}{XV} + b \qquad \underline{\text{EQUATION 14.31}}$$

A clear linear relationship between sludge ATP and OUR was discovered in Chapter 10. The relation between SVI and OUR/X would therefore be expected to be similar to that found for that between SVI and ATP/X. Only limited OUR data was available to check this relationship, but regression analysis of the available data, presented in Table 14.3, showed a linear relationship with r = 0.89. The graph relating SVI and ATP/X was shown in Figure 14.8.

14.6 Conclusion

The analysis of the data from the continuous reactor study presented in Chapter 9 , therefore confirmed the model and the general hypothesis described in this chapter. This meant that it was now possible to consider ATP as a parameter having significance both to the activated sludge treatment of coke oven liquor in the reactor, and to the subsequent settlement behaviour of the sludge in the settler. The importance of these results however goes beyond their application to coke oven liquor treatment. The principles used in this chapter to consider sludge settlement and activity could be applied to any activated sludge process treating any wastewater. The most important consequence of the work presented here therefore was that it was now possible to develop it to provide a unified basis for practically controlling activated sludge plants on the basis of activity.

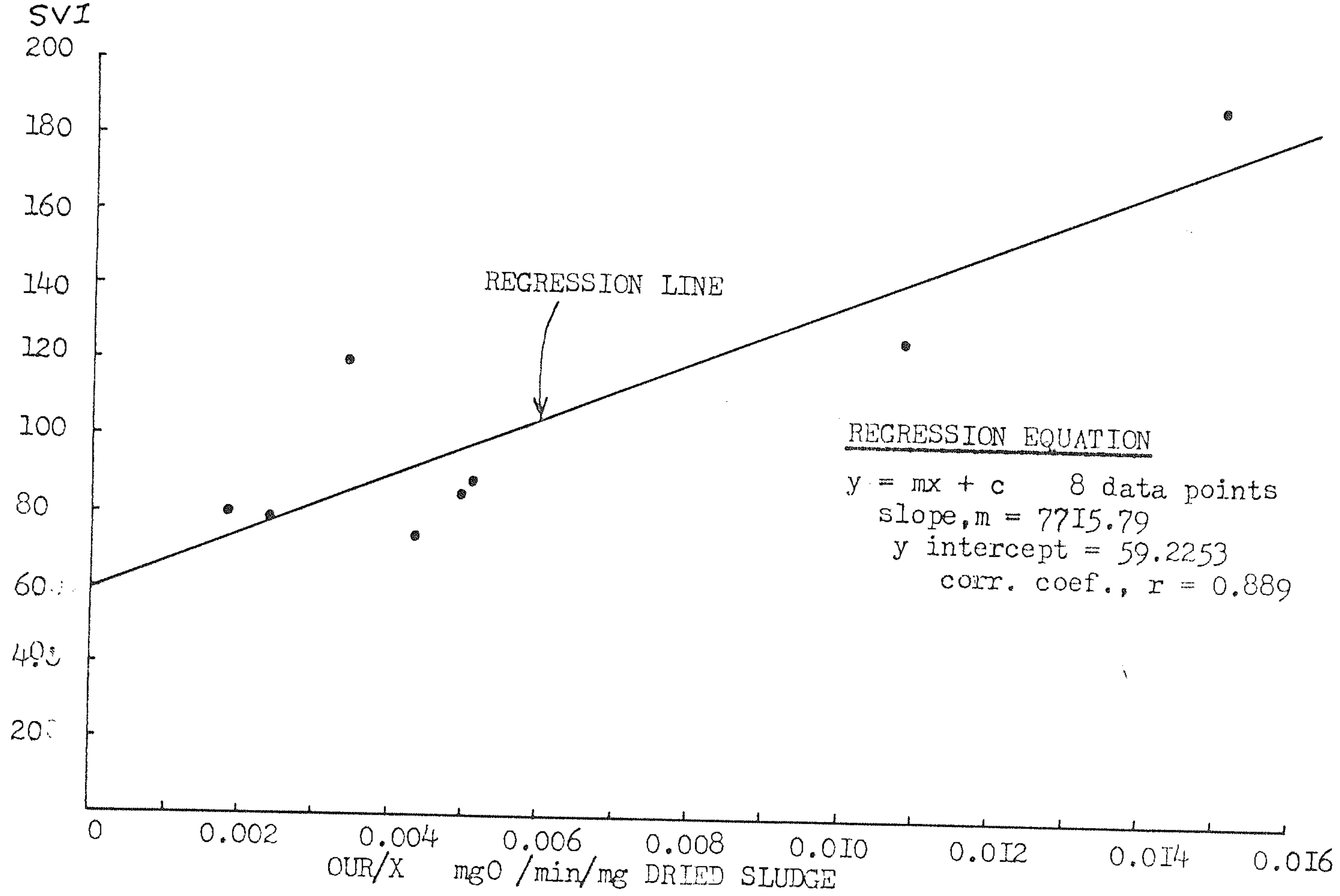
It may be proposed therefore that sludge activity represents potentially the most useful, and the most rational basis, for achieving overall control of the activated sludge process, and

TABLE 14.3

SV1 AND OUR RESULTS DERIVED FROM TEH CONTINUOUS REACTOR STUDY DESCRIBED IN CHAPTER

DAY	SV1	OUR $mgO_2/min/5m\ell$	MLSS (=X) mg/ℓ	OUR/X $mgO_2/min/mgSS$
7	120.34	0.120	7022	0.003418
8	88.77	0.179	6984	0.005135
9	85.38	0.168	6793	0.004946
23	127.07	0.153	2833	0.01080
30	190.60	0.229	3043	0.01505
44	74.24	0.050	2290	0.004367
46	77.92	0.028	2310	0.002424
47	80.00	0.019	2000	0.001880

FIGURE 14.8 THE RELATIONSHIP BETWEEN SVI AND OUR/mg DRIED SLUDGE.



that ATP represents potentially the most useful means of measuring that activity.

CHAPTER 15

FINAL REMARKS

FINAL REMARKS

The aim of the project, to determine the significance of ATP measurements in the activated sludge treatment of coke oven liquors, was achieved. The significance of ATP was fully discussed in the project and no useful purpose would be served by repeating the results of those discussions here. The significance of ATP is embodied in EQUATIONS 12.3 and 12.5 described in Chapter 12, and readers are referred to that chapter for the detailed discussion of the projects findings, and to Chapters 13 and 14 for some applications of those findings to the control of plant treating this wastewater.

It will be apparent that the findings reported in this thesis have importance beyond the treatment of coke oven liquor. The principles developed describing the significance of ATP, and sludge activity in general, have application in the general field of wastewater control, and it may be in areas other than coke oven liquor treatment that these principles find first practical application .

Certainly it will be obvious that the full potential for the use of ATP in wastewater control has yet to be realised both in theoretical terms and in terms of its practical application. Equally obvious is that there remain a number of exciting areas for further study which the limited time allowed for this project meant could not be explored.

The direct measurement of sludge activity forms the most rational basis for the control of activated sludge treatment processes, and biological treatment processes in general. All the evidence indicates that ATP represents the best and most sensitive measure of the overall activity of microorganisms in these systems, and there can be no doubt that ATP will find new and useful application in the wastewater treatment field in the future.

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APPENDIX 1

THE ATP ASSAY PROCEDURE USING THE DU PONT 760 LUMINESCENCE BIOMETER

The assay procedure used was that described in the instruction manual for the above instrument. This has been summarised below along with some useful practical details not included in that publication.

REAGENTS

All glassware used in the preparation of reagents and in the assay procedure was chemically clean, and was either sterilised in an autoclave, or heated in an oven for several hours at 105°C and then allowed to cool in clean or aseptic conditions just before it was required.

Low response (LR) water and MOPS buffer reagents were prepared as described in Chapter 4.

ATP DILUENT

At least 2 litres of this was required in advance of the preparation of the standard ATP solution for the calibration of the instrument. This was prepared as follows:

a) 4.18g MOPS buffer powder (Hopkins & Williams M.Wt. 209.3), 0.744g EDTA disodium salt (Analar), and 4.93g $MgSO_4 .7H_2O$ were dissolved in about 1,800 ml of LR water in a 2 l beaker.

b) The pH was adjusted to about pH 6.5 with NaOH using a pH meter, the solution being constantly stirred. When the reagents had completely dissolved the pH was adjusted to 7.7.

c) The solution was quantitatively transferred to a 2l volumetric flask and diluted to volume with LR water.

d) The solution was distributed into 100 ml capped bottles and sterilised by autoclaving.

e) The pH of a cooled sample of each batch of reagent was checked. The reagent was stored in a refrigerator (2-5°C) until required.

ATP PROCEDURE

119.3 mg of crystalline adenosine 5^1 triphosphate disodium salt trihydrate (Sigma Chemicals), equivalent to 100.0 mg ATP, was weighed accurately and dissolved in 100 ml of ATP diluent in a 100 ml volumetric flask. From this solution four 10:100 serial dilutions were made with ATP diluent using volumetric pipettes and flasks to prepare a solution with a final concentration of 1×10^{-1} g ATP/ml (1×10^8 fg/ml). This solution was immediately placed in an ice bath to keep the ATP stable.

Using a 10 ml glass syringe 0.5 ml aliquots of the final ATP solution were dispensed into clean cuvettes without delay. These were immediately frozen by gently immersing the bottom of the cuvettes in a dry ice/acetone mixture. The cuvettes were capped and stored in a large capped jar containing silica gel dessicant at -18°C.

Individual tubes of standard were removed from the refrigerator immediately before required in the calibration procedure and thawed. 10μl of the thawed and mixed standard solution were injected into the reaction mixture in the Biometer. The standard solution remaining in the cuvettes after calibration of the Biometer was discarded.

REACTION MIXTURE

The reaction mixture was prepared using the reagent kit (760145) supplied by the instrument manufacturers, and included the reagent and cuvettes required for 500 ATP assays. Each kit contained 20 vials of the purified enzyme luciferase and the substrate luciferin combined in a lyophilised powder, and a vial of twenty buffer salt tablets. It was important that these vials were kept frozen and desiccated. (Reagent kits were shipped from the manufacturers in the USA at the time of the project. It was found to be essential to reject those kits in which the dry ice refrigerant had completely evaporated during shipping, or where the lyophilised powder was visibily contaminated with particles of firefly. Failure to do so resulted in a marked deterioration in the reproducibility of the assay results).

The reaction mixture was prepared by placing 1 buffer-salt tablet in a clean test tube and adding 3.0 mls of LR water. When the tablet had completely dissolved, the entire contents of one vial of enzyme-substrate powder was added and an homogenous solution prepared by gentle mixing. 0.1 ml aliquots of this mixture were dispensed into 25-30 reaction cuvettes, and these were allowed to stand for 10-20 minutes at room temperature to dissipate inherent light.

CALIBRATION

To achieve calibration, standard ATP solution was successively injected into aliquots of the reaction mixture to determine the mean light response of the enzyme. The mean light response was proportional to the standard ATP femtograms (10^{-15} grams), corresponding to the ATP concentration of the standard by suitable adjustment of the sensitivity.

CALIBRATION PROCEDURE (See Figure4.2)

a) The DARK CURRENT switch was set to AUTO ZERO.

b) The INTENSITY dial was adjusted to mid-point by turning it to either end stop and then reversing 5 turns.

c) The RANGE switch was set to ON.

d) The COARSE SENSITIVITY dial was adjusted to midpoint.

e) The EXPONENT wheel was turned to read 5 when the instrument was zeroed.

f) A cuvette containing reaction mixture was inserted with the DRUM in the LOAD position, and the DRUM turned to the EXPOSE position.

g) 0.01 ml of standard ATP solution was injected into the cuvette through the injection port using a 50 ml Hamilton precision syringe mounted in a Shadon Repro-jector. (see Figure 4.3). The syringe was left in place.

h) The COUNT button was then pressed.

i) After the readout had appeared the syringe was removed and the DRUM turned back to the LOAD position.

j) A new cuvette of reaction mixture was inserted into the DRUM and steps f to i inclusive repeated. When 3 consecutive readouts showed a count variation of no more than 0.15 the calibration procedure proceeded to step l.

k) The mean of the three ATP injections was calculated to obtain the mean light response.

l) The DRUM was turned to the LOAD position and the memory cleared by pressing the RESET button.

m) The lamp's intensity was checked by pressing the READ button and holding it down until a new readout appeared.

n) The INTENSITY dial was adjusted until the lamp light intensity corresponded to the mean light response of step l.

o) Between INTENSITY dial adjustments the memory circuits were cleared by pressing the RESET button. Step m was repeated until the desired readout appeared. When this was achieved the calibration lamp matched the intensity of the light flash produced by the standard ATP solution, and the calibration lamp was used thereafter as the reference in the calibration procedure.

p) Leaving the DRUM in the LOAD position, the RESET button was pressed to clear the instrument.

q) Using the RESET and READ buttons and the SENSITIVITY dials, the COARSE SENSITIVITY dial was adjusted to obtain a reading of $1.000 \times 10^8 \pm 0.02$.

r) The COARSE SENSITIVITY dial was locked with the lever on the side, and the final adjustments of the two sensitivity dials, the memory was cleared by pressing the RESET button, and the results of the adjustment checked by pressing the READ button.

CALIBRATION CHECK

The calibration procedure was checked by injecting standard ATP solution as before, but using another freshly thawed solution. The instrument was considered to be properly calibrated if a readout of ± 0.05 the calibration value was obtained. Where excessive variation occurred the calibration procedure was repeated with a new ATP standard.

APPENDIX 2

CYANIDE DETERMINATION

Indicator Electrode

Silver/sulphide ion electrode.

Reference Electrode

Double Junction.

Reagents

Cyanide Solution 100mg/l

0.25g of potassium cyanide was dissolved in water and diluted to 1 litre.

Silver Nitrate Solution

1.70g of silver nitrate were dissolved in water and diluted to 1 litre.

Indicator Solution

100ml of cyanide solution was added to a beaker. The solution was stirred using a magnetic stirrer and the electrodes immersed. Silver nitrate was added until the predetermined 90% equivalence point was reached.

Lead Nitrate Solution

100g of lead nitrate were dissolved in water and diluted to 1 litre.

Sodium Hydroxide

Procedure

10ml of sample was diluted to 100ml with water. 1ml of lead nitrate solution was added. The solution was filtered through a Whatman 541 filter paper. The filtrate was collected in a beaker, and 1g of sodium hydroxide added and dissolved by stirring. 1ml of indicator solution was then added. The electrodes were immersed in the solution and the electrode potential measured. Five 0.1ml additions of 100mg/l cyanide solution were added, the electrode potential being measured after each addition. A Gran plot was prepared from the results from which the cyanide concentration of the smaple was determined.

Standard and reagent blank determinations were carried out for each set of analyses.