The Kinetics of Mould Growth in Aerobic Tower Fermenters

by

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A

THESIS

Submitted to

THE UNIVERSITY OF ASTON IN BIRMINGHAM

in partial fulfilment of the

requirements for the award of

the degree of Doctor of

Philosophy

October 1972

DECLARATION

I hereby declare that the whole of the work now submitted in this thesis is the result of my own investigations except where reference is made to published literature and where assistance is acknowledged.

.....

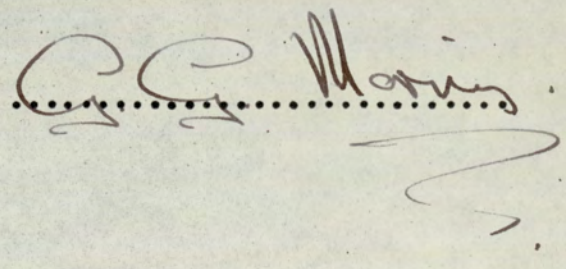
Candidate.

.....

Director of Studies.

CERTIFICATE.

I hereby certify that the work embodied in this thesis has not already been submitted in substance for any degree, and is not being concurrently submitted in candidature for any degree.



ACKNOWLEDGEMENTS.

I would like to thank.

DR. R.N. GREENSHIELDS for guidance and supervision during the course of this work.

DR. E.L.SMITH for help and advice as to the mathematical and chemical engineering content of this work.

MR. A. JAMES for his assistance in ellucidating some of the physical morphological characteristics of Aspergillus niger (ML)

MR. J.SHAYEGAN for his assistance in some of oxygen transfer (air volume retention) work.

MR. R. COCKER and THE UNIVERSITY VISUAL AIDS SECTION for their photographic assistance.

MY COLLEAGUES in the Fermentation Technology

LABORATORY for allowing me to do some work

MRS. A. HALFORD for typing the thesis.

THE DIRECTORS OF TATE & LYLE LTD. PROFESSOR A.J.VLITOS MR.F.K.E.Imrie for permission to carry out the research, publish results, and for financial assistance.

List of Abbreviations Used.

S.T.R.	Stirred Tank Reacter.
T.F.	Tower Fermenter.
O.T.R.	Oxygen Transfer Rate.
S.G.V.	Superficial Gas Velocity.
K.L.A.	Oxygen Transfer Coefficient (hr^{-1})
K.	Oxygen Transfer Rate (millimoles O_2/l/hr)
A/R	Aspect Ratio
r.p.m.	Revolutions per Minute.
cm	Centimetre
mm	Millimetre
μm	Micro ($x10^{-6}$)
ml	Millilitre
l	Litre
min	Minute
hr	Hour
Kg	Kilogram
g	Gram.
mg	Milligram
w/v	weight per unit volume
v/v	Volume per unit volume
p.s.i.g	Pounds per square inch of gravity.
W/M^3	Watts per $metre^3$
Vg	Superficial gas velocity
g	Gravitational constant
H.P.	Horse power
N.Re	Reynolds Number
N	Stirrer speed
D.I	Impellor diameter

L	Liquid viscosity
Np	Power number

I N D E X

Section 1

INTRODUCTION

Section 2

METHODS and MATERIALS

Summary.

This study deals with the growth of fungi on low grade agricultural carbohydrate sources in tower fermenters. - The tower or tubular fermenter was chosen as the vessel in which to grow the fungi because of simplicity, ease of construction, low capital and working costs.

The work carried out attempted to present the relevant running data required to design and build a tower fermenter for the growth of fungi. Due to the nature of the fermenter it was found that filamentous fungi were able to grow in the colony form. The mode of formation and the kinetics of colony growth were studied together with their relevancies to this particular project. Medium optimisation was also investigated in relation to the production of large amounts of protein rich biomass. The nutritional aspects of the fungal end product was studied, in particular the concentrations of amino acids in the mycelium. Conclusions were therefore reached as to the suitability of the system and the fungus for the production of fungal biomass.

SECTION 1

INTRODUCTION

1. The World Food Situation.

1.1. Population and Food Consumption.

The world population is increasing at a phenomenal rate. If present trends continue it is expected that it will reach the alarming figure of 6,000 million by the year 2000. (Gray 1966).

One of the major problems arising from this population explostion is the increasing requirements for food and energy. Science and technology are at present striving to avoid the catastrofic situation which will be impending by the end of the century. The present opinion is that with such foresight action has been taken in sufficient time, and the next decade or so will see a reversal in some of the present trends of increasing population, pollution and the depletion of natural resources.

Research and development into food products in particular have made great advances in the last two or three decades not only in conventional food manufacture (Pyke 1970) but also in the more exciting areas of novel food and feed production.

1.2. Advances in Conventional Food Production.

During the last century tremendous strides have been made towards increasing the efficiency of conventional crop growth, both in terms of the labour force and in the yields attainable. For example, new corn hybrids have boosted yields from 22 bushels per acre to as much as 125 bushels per acre (Gilbert and Robinson, 1957). It has also been pointed out by Sir John Russell (1962) amongst others that there are vast areas of

the world which could be declared suitable for the more conventional food crops, if we were to cultivate some of the more remote areas of the globe including North America, Northern Eurasia and certain parts of the tropics. Gilbert (1964) states that at present just 50% of the worlds farmers produce 90% of our food and this output could be improved by the full use of existing agricultural science. Pirie (1969) writes that improvements in agricultural production can be brought about by better irrigation and drainage systems, the use of fertilisers, introduction of better plant strains and the control of, pests and predators. A final remark on the potential of conventional food resources must lie with Kellogg who makes the point that if the political and economic barriers to effective soil use were to be removed on even greater increase in food output could be achieved. However, it must be remembered that there is a maximum rate of growth and yields attainable, after which the law of diminishing returns comes into operation, other methods of food production are then required.

1.3 Present Food Potential

It is already apparent in many countries of the world that there is a food problem at present. These are inevitably the developing countries which do not have the technology to cope with their population demands. If we were to consider the food problem in these countries solely from a calorific stand point however, the conclusions might be very misleading. In some of these countries where carbohydrates (or the ability to produce them) are relatively abundant, they are injested as the main energy of the diet and may even contribute more than four-fifths

of the energy. Gray (1962) has suggested that there is sufficient carbohydrate and fats for a world population of years to come, however the deficiencies lie in the lack of protein. For a well balanced diet it is necessary to have all three in the correct proportions. It is estimated by the joint F.A.O/ W.H.O expert group on protein requirements (1965) that the body requires 0.7g./Kg body weight day of protein to satisfy the needs of most people.

F.A.O - Food and Agricultural Organisation.

W.H.O - World Health Organisation.

2. Novel Food Products.

Research and development is now entering a new era in food production, it is considering what is known as novel food products. Amongst these more novel food forms are considered the farming of wild animals never before used for human meat consumption, the production of leaf protein extracts (Pirie 1942), and the cultivation of microbial biomasses. It is the latter methods of fermentation which concern us here.

2.1 A case for microbial foods and feeds.

There are basically three forms of microbial protein which could be utilised as a food form they are, algae, bacteria and fungi. The use of microorganisms for food and feed production has many advantages. Amongst the most obvious is that of rapid growth. A favourite quote by armchair scientists, is that if a bacterium divides every 20 minutes then in two days one cell could produce (2.2×10^{43}) cells. Even though the weight of a single cell is only 10^{-12} gm. the total weight of this biomass would be 2.2×10^{25} tons. (Ghose, TK.1969). The attractiveness of rapid mass doubling is further emphasised when it is realised that far smaller land masses are required for the production of microbial food. (TABLE 1.)

TABLE 1.

Mass Doubling Times (Humphrey, 1969)

Maximum rates are compared.

Organism.	Time for doubling in mass
Bacteria and Yeast	20 - 120 hrs
Mould and Algae	2 - 6 hrs
Grass and some plants	1 - 2 weeks
Chickens	2 - 4 weeks
Hogs	4 - 6 weeks
Cattle	1 - 2 months
People	3 - 6 months

As well as having a rapid growth pattern microorganisms are extremely efficient converters of substrate into cell mass for example, fungi are able to give a 50% conversion efficiency of carbohydrate into cell mass. A conversion of over 20% can be quoted for carbohydrates into proteins by yeast and bacteria. By contrast the efficiency of cattle is less than 2% (Gilbert and Robinson 1957).

A very attractive aspect of microbial biomass growth is that of the wide substrate utilisation capacity that these organisms possess. Reports have been made of microbial biomass production from carbohydrate sources originating from urban, industrial and agricultural wastes (Morris 1972). Any process that is able to utilise a countries own waste products must be of vast economic importance. (Both in terms of pollution and microbial biomass production). Notable amongst yeast biomass production is their growth on petroleum hydrocarbons

(Champagnat 1963). Gas and oil companies are also exploiting the possibilities of using methane and other associated gases for bacterial culture.

The selection of microorganisms for particular processes usually involves an initial screening programme out of which a number of the most promising are selected. When dealing with microorganisms it is again possible to improve the strains obtained by various selective proceedures, and of course by mutation. Due to the rapid growth and reproduction of these organisms a mutation programme can yield results relatively quickly. By careful screening it would be possible to obtain microorganisms which were rich in certain amino acids (especially the sulpher containing essential amino acids). Two or three organisms which have complementary amino-acid profiles could then be blended to give a product of very high biological value.

The possibilities for fully continuous processes are many-fold when considering the production of microbial biomass. The advantages are obvious and include, greater yields with time and the utilisation of a smaller land area. Olsen and Slur (1960) describe a yeast production unit in the U.K. which produced about 300 tons of yeast per week. Butterworth (1966) discusses the various merits of batch and continuous fermentation and concludes that there are advantages to be gained by utilising continuous systems for the production of simple products such as microbial biomass.

There are many reviews available in the literature on microbial feed and food production dating back to the early 1950's It is sufficient here to list them so that for further details they may be consulted. Thatcher, (1954), Gilbert and Robinson, (1957).,

Rose, (1961)., Bunker, (1963), Mateles and Tannenbaum, (1968a)., Lipinsky, Kinno and Litchfield, (1969), Ghose, (1969), Snyder, (1970) and Vilendrich and Akhtar (1971). Amongst the books which should also be consulted is that by Mateles and Tannenbaum (1968b).

3. The use of Fungi as Microbial Food

3.1. Advantages in the use of fungi

Possibly one of the greatest disadvantages in producing microbial food for human consumption could be its non-acceptability. The acceptability of any novel foodstuff depends on many interelated factors; taste and appearance (Saeki, 1966) possibly being of greatest importance. Texture and digestability will also present a selling point whilst psychological and aesthetic factors are inevitable variables. Fungi are possibly the organisms that will most readily accepted for human consumption, primarily because of the fact that they have long been known in the food industries. The fungi of the basidiomycete species have been part of the human diet for many hundreds of years. Yeasts (unicellular fungi) are also well known, not only in the brewing industry but also as a medicinal source of vitamin.

Perhaps the greatest advantage that the filamentous fungi offer to the fermentation process in particular however, is that of their separation from the spent media. Separation, which also affects harvest yield, is one of the key processes where the economics of production could be seriously altered. By virtue of their morphology the filamentous fungi facilitate easy separation and avoid costly centrifugation processes.

The fact is not often appreciated that large amounts of microbial feeds and foods are already being produced in many parts of the world. Delbruk (1910) originated the idea of growing yeast as food for direct human consumption, (Peppler 1970) and it was estimated (Peppler 1967) that over 310,250 tons of bakers and dried yeast were being produced annually.

3.2 Unicellular fungi (yeast) as a source of proteinaceous foodstuff.

Among the fungi which have been considered for their use as food and feeds the unicellular yeasts have been used more than any other organism.

Food and fodder yeast provide high protein products that are also a rich source of the Vitamin B complex, among the desirable characteristics of a food and fodder yeast are its high nutritive value, agreeable flavour, stability in culture, capacity for high growth rate and yield, ability to assimitate diverse sources of carbon and nitrogen, good appearance and ease of processing after harvest. The best economics are however associated with Torula yeast, also known as Candida utilis or Torulopsis utilis. Candida arborea and Oidium lactis have also been utilised to produce feed yeast on a commercial scale. Although few of the food yeast factories in operation cultivate strains of Saccharomyces cerevisiae, it should be noted that there exists an almost unlimited capacity for producing this yeast in the brewing process and of the 39,000 tons produced each year in the U.K practically all of it is used for feed or in the manufacture of yeast extracts (Bunker 1955).

The abilities of yeast to utilise various diverse forms of nutrition is seen in the variety of substrates used for their cultivation. Dried Torula yeast is made exclusively in propagators installed and operated adjacent to a paper mill. The yeast C.utilis is grown on spent sulphite pulp mill waste semi-continuously in modified Woldhoff - type fermenters (Wiley 1954). More recent workers on the production of yeast from sulphite waste liquors have been Lenushina et al (1966), Oh, (1966) and Ivanyukovich (1968)

Whey is another material on which yeast is grown commercially. It is a byproduct of the dairy industry. The industry has an estimated 400,000 tons of whey solids available annually from all cheese manufacturing. They Whey is utilised by using a lactose-fermenting yeast in order to cultivate large masses of that yeast. Powell et al looked into the batchwise production of Saccharomyces fragilis in cottage cheese whey.

Tviet (1960) in a paper on microbial food production made special reference to the potential of the new 'Symba-Yeast Process. It is stated that tropical plants, such as cassava, manioc and tapioco contain huge reserves of starch, which by the addition of a cheap nitrogen source may be used as a substrate for microbial growth. The Symba Yeast Process works by employing a two-organism system. C.utilis is grown together with Endomycopsis fibuliger, the latter breaking down the starch into an utilisable carbohydrate substrate available to the yeast.

As it has already been stated yeasts have also been cultivated with great success on hydrocarbon sources and they seem to be most expeditious for protein manufacture, containing considerable amounts of essential amino acids and B vitamins. This line of research was actually started at the beginning of the century but has received little attention until the last decade. Yeast growth on hydrobarbons serve a dual purpose, the yeast utilises the paraffins with an unbranched chain (these are the molecules that make the oil thicker and are therefore rather troublesome), and also offers the possibility of protein production by the yeast. The oil is simply a source of carbon, nitrogen, phosphorous and sulphur, the other essential elements must be added.

Production of the yeast Candida lipolytica by Champagnat

(1963, 1964, 1964) on a hydrocarbon source was accorded great public acclaim. Treccani (1964) gives an useful review on the microbial degradation of hydrocarbons. Shacklady (1969) reports that a factory in Grangemouth, Scotland, will soon be producing 4000 tons of yeast a year which has been grown solely on hydrocarbons (This plant is now actually in operation). Lipinsky (1968) discusses the merits and otherwise of the use of hydrocarbons compared with carbohydrates and concludes that it is geography that rules the situation as to which new material to use.

Appart from protein production yeasts have also been cultivated as a source of fats and sterols. Woodbines' (1959) review deals comprehensively with all aspects of microbial fat production including that by yeasts. Extensive reviews on food and feed yeasts are given by both Prescott and Dunn (1959) and Peppler (1966).

3.3. Filamentous fungi as a source of proteinaceous foodstuff.

The filamentous fungi as such have not been grown extensively in submerged culture for human consumption. There are a few reports however of their use, for example Frazier (1958) states that Geotrichum candidum was grown by the Germans during World War 11. Gilbert and Robinson also report that this same mould was grown on waste sulphite liquor and the dried product used in sausage meat manufacture.

Mushrooms, of course, have been recognised and used as food an flavouring for many years. The Ancient Asian civilisations, the Chinese and Japanese, were possibly the first to recognise certain edible filamentous basidiomycetes. Cultivation however, began during the eighteenth century in France whence it spread to the remainder of

Europe and later to N.America. Nowadays the fruiting bodies of many of the basidiomycetes including the species of Boletus, Morchella and Tuber are still considered delicacies whereas the cultivation of the Agaric species (both campestris and bisporus forms a large and important industry. The scope of the mushroom industry has been described by Stoller (1954) and by Robinson and Davidson (1959). The American mushroom industry is seen to be particularly productive at present.

The convential method by which mushrooms are grown (e.g. Agaricus bisporus in particular) is on large areas of compost bed. This process is both expensive and space consuming and since a large proportion of the final product is pulped and made into flavouring for such things as soups there is seen to be a large scope for their submerged cultivation (i.e.) the production of just the mycelium itself. Humfeld (1948), Humfield and Sugihara (1952) and Block et al (1953) report on pleasant but uncharacteristic flavours being produced via this technique. An extensive review containing many references especially on mushroom culture is given by Litchfield (1964).

Despite the many advantages attributable to the basidiomycetes their growth rate is considerably slower than that of the 'lower' filamentous fungi. Although relatively few of these fungi have actually been used for food production their potential has nevertheless been recognised for sometime. Perhaps it was the antibiotic industries that stimulated interest in the use of fungi grown in submerged culture as a food source because of the extremely large quantities of waste mycelium available after the fermentation which could be around 8-10% the volume of the fermenter. Many workers have since suggested in the literature the use of waste

mycelium from industrial fermentations, Pathak and Seshackri, (1965), Zdzislaw, (1967)., Aidinyan, (1967). Korats, (1969)., Yano, Horii and Ozaki, (1969)., as a source of foodstuff.

Even in 1920 Pringshiem et al. was experimenting on the use of proteinaecous moulds for the enrichment of straw fodder and in 1923 Ivanov described the nature of the fungal protein after hydrolysis. Aspergillus Oryzae was one of the first fungi in which a certain amount of interest was taken from the point of view of producing proteins from fungi Takata (1929) showed the mycelium to contain 38g% crude protein and was also a rich source of the B. vitamins. Skinner (1934) reviewed all the earliest studies that had been undertaken on "Proteins from Moulds" - Skinner also observed the high protein content of Penicillium flavo-glaucum.

Peukert (1940) discussed the possibility of growing mould mycelium as a source of protein fodder. He noted it as being an appertising form of protein and that in the dry state it was brittle and easily pulverised.

In 1941 Fink and Shmidt were granted a patent for a biological process for the production of protein rich food. A mylolytic and cytolytic fungi were grown on loose substrata containing starch and/or sugar to which were added nitrogenous materials. Fink's fungus protein flakes were fed to ruminants and swine by Marigold et al (1941) where they gave evidence as to the foods biological value.

In 1962 Gray and his Coworkers started a series of papers on their work with the Fungi Imperfecti as a potential foodstuff to meet the protein deficit problem. He restricted his efforts to this one ubiquitous class of moulds and investigated a variety

of carbohydrate sources for the growth of these fungi including, cassava, manioc, raw sugar cane juice, rice, sugar beets and pulp. As well as this initial screening programme, Gray and his associates also investigated a variety of ways in which the fermentation process could be made into a viable economic proposition. Gray, Pinto and Pathak (1963) substituted sea water for fresh water in order to overcome the high cost or scarcity of large quantities of fresh water. Another method of cost reduction was to avoid the costly sterilisation process by the use of protected fermentations. Gray and Abau - El - Seoud (1966) achieved this by lowering the P H of the initial medium. Much of the work carried out by Gray however was in surface culture using the fungi as a source of protein enrichment mixed with the already high carbohydrate containing substrates.

Spicers work (reported by Coppock 1970) is noteworthy since it is claimed that Penicillium notatum grown on a substrate of starch and ammonium salts produced a product estimated to contain 50-55% protein. The moulds protein also had an adequate amino acid profile being appropriate to human requirements.

4. Production of Fungal Foods by Fermentation.

A controlled environment is a prerequisite for the economic production of fungal biomass. Within this closed system the organism must be provided with both the essential chemical nutrients and also the appropriate physical conditions under which it can survive and grow. These are parameters prescribing the medium and fermenter respectively. Practically these can not be separated theatrically however, they may be dealt with on their own.

1.1 Nutrient Requirements

Quantitatively the most important elements required by living cells are carbon hydrogen, oxygen, nitrogen, sulphur, and phosphorus. A wide range of other elements and compounds is also required in much smaller amounts. All fungi require a relatively complex media in order to grow at their maximum rate. Their carbon source which contributes to the bulk price of medium is usually supplied in carbohydrate form. Fungi degrade and utilise a wide variety of carbon substrates and this seems to be of particular advantage in their growth as a food source. (Morris 1972). These carbon substrates are usually in the form of carbohydrates and there are various parameters to which they must comply, the main ones are tabulated below. The majority of these parameters are interalated and must form the basis of an economic proposition.

(1) Price

(2) Availability and abundance. (including seasonal variations).

(3) Toxicity

(4) Carbohydrate content

(5) Fungal utilisation.

As it has already been dated the ubiquitous mould utilises such diverse forms as wood waste, milk whey and molasses.

The sources of nitrogen (which can also affect the process cost) utilisable by fungi display an even wider diversity than the sources of carbon. Microfungi, for example will utilise nitrogen as inorganic ammonium salts, the sulphate being the cheapest and most commonly used. Nitrate nitrogen as sodium nitrate or combined with ammonia as ammonium nitrate is also widely used. Another good source of nitrogen is urea. Although many organisms will utilise inorganic nitrogen exclusively they invariably grow faster if at least supplemented with organically based nitrogen. There are many commercially available forms, including corn steep liquor, yeast extract distillers solubles, soya bean meal and bacterial peptone (industrial grade). A process whereby a high protein content is required in the mould mycelium invariably uses a high nitrogen content in its media, or at least a low C.N ratio. Hilpert et al (1937) recognised that the composition of the mycelium altered substanially by different concentrations of the nitrogen source. In addition to lowering the nitrogen content of the mycelium the low nitrogen medium caused a reduction in the total carbon content of the mycelium.

Microorganisms, fungi in particular require other nutrient supplies, for example, sulphur, phosphorous metal ions and even some vitamin source. Many of the carbon and nitrogen sources already described contain most of the minor nutrients essential for successful growth. By judicious blending of materials from various sources the requirements for the minor elements can often

be satisfied, at the same time supplying the necessary major inorganic elements.

4.2 Physical Requirements

The physcial requirements for a fermentation are also 'reactor dependant' as well as being 'medium dependant' to a certain extent.

The main physical parameters which are characterised by the fermenter design are, oxygen transfer, mixing and temperature control.

Oxygen is essential to the growth of all fungi and is utilised at a rate according to zero order kinetics above a certain value, (C.crit). This value is characterised by the mould below which it's growth rate is limited. It is therefore obvious that the design characteristics of such a fermenter must incorporate a means of imparting high levels of oxygen into the liquid.

Mixing, which in most fermenters is closely allied to aeration, is perhaps of greater significance when running continually. However, mixing in batch systems so as to avoid localised stating effects and build-up of undesirable metabolites. Temperature control is essential in the kind of fermentations where the mould requires an optimum value for maximum growth or metabolites production. As the rheology of mould suspensions, change during the course of a fermentation large amounts of heat are evolved and therefore some form of compensation is necessary.

4.3. Fermenter Design

The function of a fermenter is to provide a controlled environment in which microrganisms can grow and reproduce. Basically this means providing the fermenter with the necessary

equipment whereby the physical and chemical factors can be established and maintained. It is necessary to sterilise the vessel and its connections to and from other vessels, and the medium and air entering the vessel. Provision must also be made for sampling and material transfer.

These are the fundemental bases on which all fermenters are built. The next consideration is to what purpose is a fermenter to be put. A bench or laboratory fermenter can almost be of a very basic design and act as a controlled environment chemostat, whilst pilot and commercial scale fermenters should always be purpose built. If the reaction vessel is to be used to study microbial metabolism only, then versatility is essential. On the other hand however, if a specific reaction is to take precedent then the fermenter aught to be designed with this reaction in mind.

The culture of fungi, for instance, requires a highly aerobic reaction vessel. The chemical engineer may then present design characteristics capable of allowing maximum aeration to the organism. This can be done initially by considering a two phase air/water system and using such techniques as those of Cooper et al (1944) in which the relative conversion rates of sulphite into sulphate in a catalysed sodium sulphite solution are measured. Then proceeding into a variety of air/medium/organism systems under which actual fermentation conditions are simulated. These techniques are discussed by Tuffile and Pinho (1970). Informative reviews on oxygen supply and demand in aerated cultures have been made by Steel (1958)., Brandl et al (1966)., Phillips and Johnson (1961) and Solomons (1901), Hospodka (1966) also gives an useful review with many references. Experimentation is then taken into

the final stage where respiratory demands of the organism are considered. Resistances encountered in the supply of oxygen to the individual microbe is discussed by Blakebrough and Hamer (1963). The effects of the design characteristics on the mould itself must also be taken into consideration. Of particular importance in mould fermentations is the effect of reactor design of the morphology and rheology of the mould. The rheology of the broth can be altered by merely altering impeller size or omitting baffles. Oxygen demand in mycelial pellets, a characteristic morphological variation in moulds, is discussed by Phillips (1966)., Yoshida et al, (1967), Pirt, (1966). In these papers various aspects of oxygen transfer into the mould colony are discussed together with figures for the distance oxygen can travel into the colony. These figures vary considerably and no consolidated work of this kind has yet been attempted.

As it can be seen the transfer of molecular oxygen to the microorganism involves a number of distinct steps all of which have their own resistances. Factors which determine both power requirements and the oxygen transfer coefficient in fermenters include tank geometry, impeller speed, air spraging rate and physical properties of the culture. This is an important set of estimations when coming to evaluate any fermentation system. Various correlations available for estimating power consumption and the oxygen transfer coefficient are discussed by Richards (1961).

4.4. Fermenter Types.

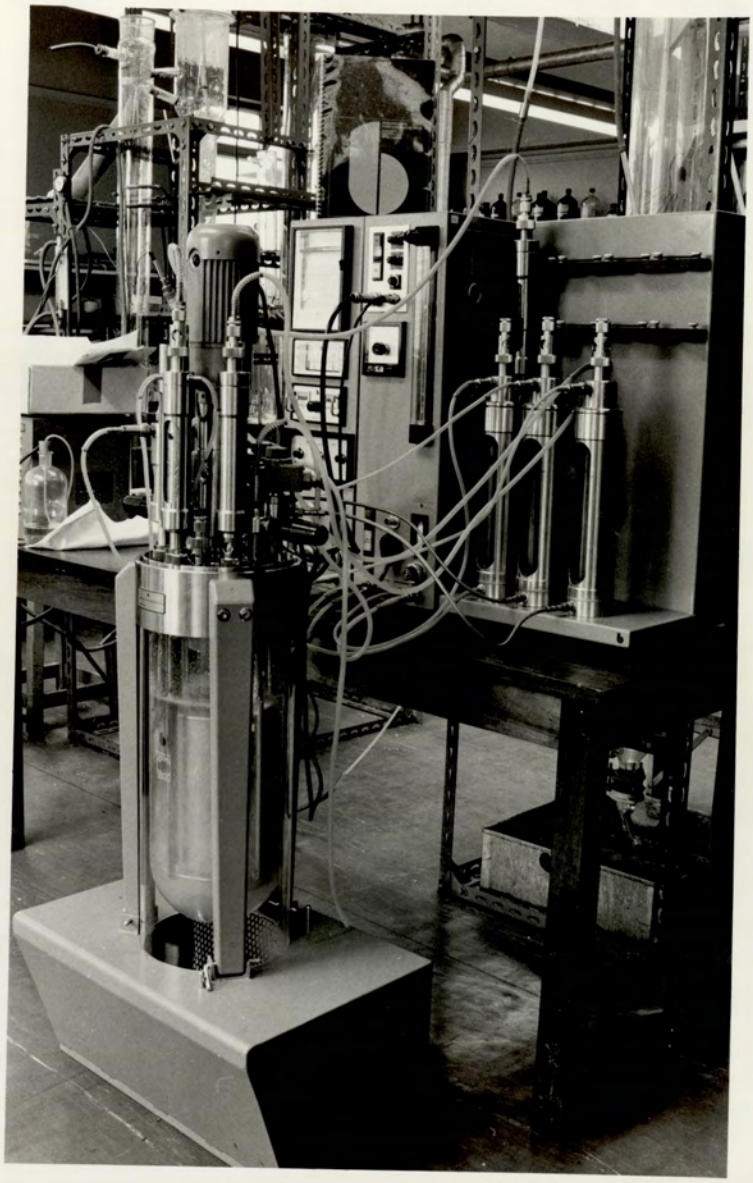
The requirements of particular fermentations has led to the production of a variety of different fermentation vessels. The design characteristics required by the organism were not

always taken into account. This attitude led to the adoption of one basic fermenter design which has become modified and mutilated with little forethought of use. The general design of those vessels is that of a stirred jar into which sterile air is added beneath the impeller, aeration and agitation being maintained in this fashion. Chemical engineering adaptations of this basically crude fermenter have varied the impeller/ propeller system and added baffling. (Plate 1)

As it has been stated the most widely used commercial fermenter is the stirred, baffled, upright cylinder with operation, the main features of which are illustrated in diag.1. This type of vessel can be produced in a range of sizes from 1 litre up to many thousands of gallons. There is a great deal of information available in the literature as to the smaller scale fermenters. Fermenter design has been reviewed by Elsworth, (1960) Deindoerfer, (1963) and Blakebrough(1967). Information on pilot and production scale equipment is sparce however, and where available include very little detail. Chain and his colleagues are obvious exceptions to this. Chain et al (1954) and Paladino et al (1954) - both publications give good working drawings and photographs of the fermentations involved. There are even fewer publications given on plant scale culture vessels.

The operation of these 'pots' is usually quite complicated and their technology has been built up over a number of years hence variations on these forms were inevitable. The vortex fermenter is similar to that already mentioned except in that it has no baffles or sparger. Aeration is achieved by allowing a deep vortex to form around the position of the impeller shaft. These forms usually suffer from the fact that a large volume of

PLATE 1



BIOLOGICSHE VEFARENSTECKNIK .
Laboratory Stirred Tank Fermenter (10 litres volume)

of the fermenter is occupied by the vortex alone, poor oxygen transfer rates have also been reported for these vessels. Vortices are also difficult to form in larger vessels (especially when viscous mould mycelium is present). The Waldhof fermenter embodies features of both types already mentioned in that it is sparge aerated and partially baffled. A vortex is then produced by the effect of having a draught tube around the impeller. This unit has been used successfully in the production of food yeast, but has not been adopted in many other cases.

Some more enterprising research workers have developed other novel systems for their processes. Horizontal rotating drums have been used, especially in the gluconic acid fermentating, but mass transfer rates have been found to be low compared with baffled sparged vessels. Ugolini (1960) overcame this to a certain extent by using a stationary laboratary fermenter of 18 litre capicity fitted with rotating aeration devices. By using a sheet of stainless steel rolled into a spiral on which a fresh film of liquid is continuously exposed to the air, oxygen diffusion is in the same order as shqke flasks, but the shearing action is minimised.

Shake flasks themselves have been used extensively in a variety of fermentation process. They are usually employed as a form of (rough) screening technique (especially in the antibiotic industries) before venturing further into stirred fermenters. Shake flasks suffer from the fact that scale-up is wirtually impossible over about 500 ml. and that they are often quoted to bear very little relationship to other vessels used for the same fermentation.

Other fermenter variations where no mechanical agitation s

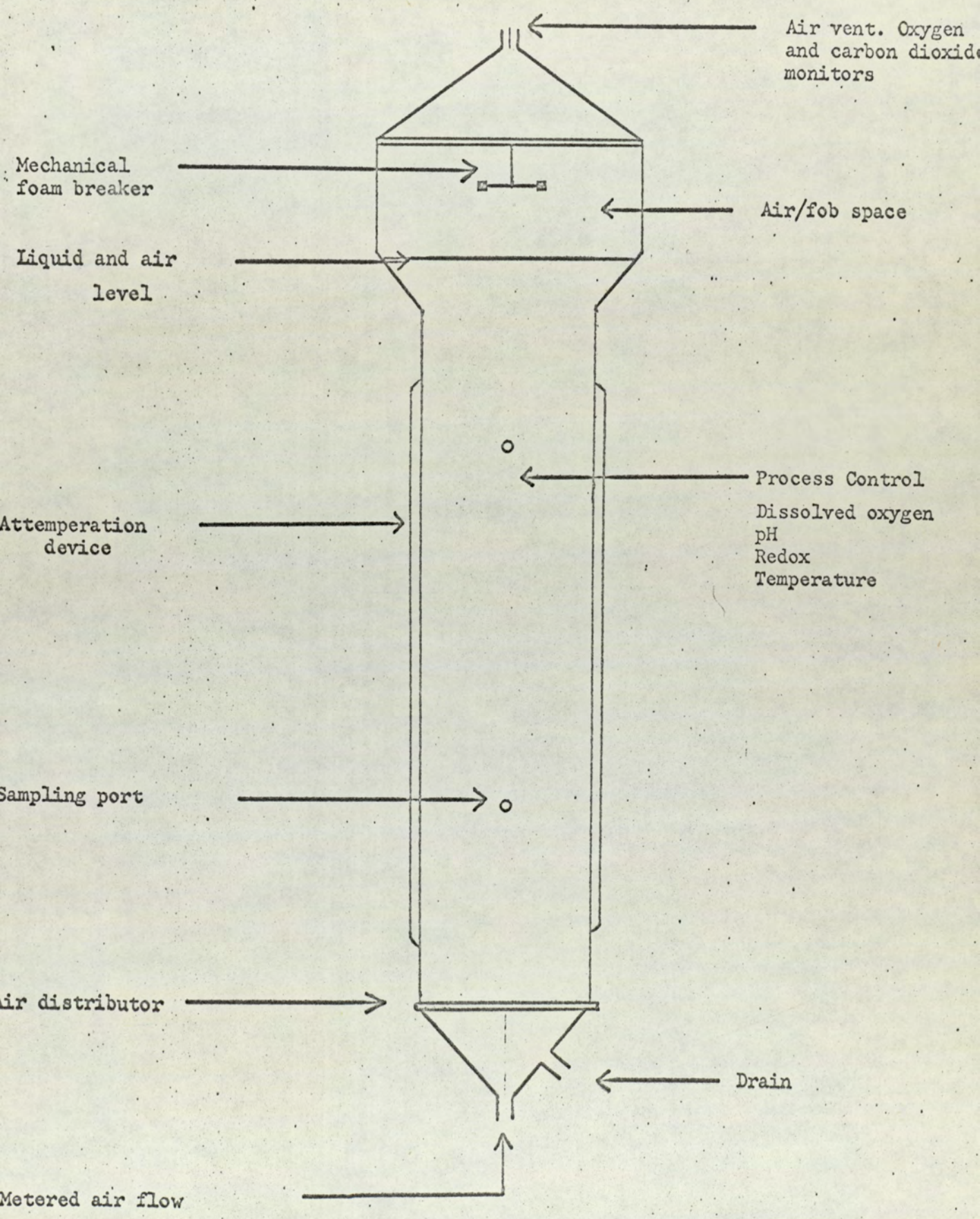
is employed in order to achieve aeration are in the air-lift and tower fermenters. The air-lift fermenter was first developed for teaching purposes by Lundgren and Russell (1956). It is both cheap and easy to construct and high levels of oxygen transfer have been reported for such vessels. As a means of aerating it uses liquid lift and circulation tubes in which volumes of air and media are passed together into the main fermentation chamber The 'air-lift fermenters' uses are perhaps somewhat limited. Success in the growth of single celled organisms might be achieved however. Sharkov (1950) describes this system for the continuous cultivation of C.utilis. In France the "Lefrancois" system for continuous food yeast production works on a similar principle.

The tower or tubular fermenter belongs to a new technology in fermentation (Royston) although the basic idea has been in evidence for some time in the chemical industries. It is again a non-mechanically stirred fermenter and is capable of high oxygen transfer rates. (It will be dealt with in more detail later) Diag. 2.

Some attempts have been made to use other than stirred and aerated vessels, Freeman (1961) described a sponge fermenter in which nutrient-agar was supported on cellulose sponges which offer a large surface area in a small volume. Such equipment, however, suffers from basic disadvantages such as lack of p.H control, lack of flexibility (unsuitable for microfungi), and really represents a novel idea rather than a practical solution. Porter and Nack (1960) came to the conclusion that overall, surface methods were more costly and less easy to operate than submerged ones.

DIAGRAMMATIC REPRESENTATION OF A TOWER FERMENTER

DIAG 2



4.5 Materials of Construction.

Almost invariably the actual bodies of the fermenter units have been constructed from either stainless steel or glass. Glass being mainly used in smaller vessels or as sighting ports on the larger vessels. The material of construction of fermenters must have the following attributes, smooth, mon-toxic, more or less corrosion proof, rigidity, capable of with standing extremes of temperature $5^{o}c$ - $120^{o}C$, pressurised as required. Glass and stainless steel fulfills all these requirements when used in stirred vessels. Both materials have the disadvantage of being extremely expensive (Solomons 1969). However, when considering non-mechanical agitation vessels, such as the air-lift and tower fermenters, which do not have such a great demand on absolute rigidity because of the lack of impeller shafts etc.it might be possible to use cheaper materials of construction. Rigid polythene for example is cheap and easy to construct but can only with stand $80^{o}C$ of heat. Therefore it would be unsuitable for most fermentations which require complete sterility. Polypropylene however, can withstand autoclaving at 15 p.s.i.g for 20 mins. And its chemical resistance is rather better than polythene and can be most useful for fermentation work. Tower Fermenters up to 2000 litres capacity have been built in this material and operated successfully (Greenshields 1972). A 1000 litre tower was operated in the authors laboratories. These fermenters were constructed at a fraction of the cost attributable to a stainless steel vessel of the same size.

Sterilisation of media and equipment may not necessarily be so exacting for the production of fungal biomass as it is

for antibiotic production with the use of fungi, especially the Aspergilii. a low p H is attained during the fermentation which discourages any large scale infection to take place. A form of semi-continuous cultivation may be envisaged whereby a little actively growing mycelium is left behind in the fermenter and fresh medium poured onto it.

5. The Tower Fermentation System.

5.1 History and Description

The tower or column reacter has long been knownto the chemical engineer. It has only been during the lasty twenty years that the tower fermenter has been considered as a suitably controlled environment for microorganisms. Unlike most fermenters it was initially designed for a continuous process. The continuous process in question was the production of beer. The final patent rights for this process were granted to Shore and Watson of the A.P.V Company Ltd., (1963). One of the main objectives of the inventers was to provide a method and an apparatus which was readily adaptable to different fermentation requirements. A further objective was to provide an apparatus which was simple and therefore have little liability to derangement, which required relatively little grond space for its accomodation. Subsequent development on pilot and production scale fermenters showed this to be a viable proposition, Royston (1966), Klopper et al, (1965), Shore, (1968)., Ault et al (1969). Research and development continued along these lines until a production scale fermenter was placed in the Mitchells and Butlers brewery in Birmingham (Plate 1). Another large brewer (Bass) is also using a commercial tower fermentation system successfully. Greenall Whiteley are at present also installing tower fermenters for beer production.

As well as beer and lager production which are basically anaerobic processes the tower fermenter has also been exploited for the production of vinegar charging wort (Greenshields and Smith 1971). The production of metabolites under aerobic conditions has also been considered, for example the production of

acetic and citric acid. The acetic acid fermentation gives a growth associated metabolite and was carried out continuously (Jones 1970) whilst the citric acid fermentation was batch only (Davies 1971). A nutural progression from metabolites was on to the growth of microbial biomass using Yeast (Greenshields and Smith 1971). Bacteria (Pannell 1972) and Fungi (Greenshields et al 1971).

As with the metabolite process the production of microbiol biomass requires high oxygen tensions which have to be provided for by the fermenter design. The design characteristics as far as oxygen transfer is concerned must therefore be of prime importance. The techniques at hand for the assessment of oxygen transfer efficiency must therefore be exploited in order that optimal conditions may be achieved. Despite the fact that each process requires its own particular fermenter configuration a generalised diagram may be made as to the design required for the growth of fungi. This only differs slightly in its overall pattern from the beer fermenters in that the columns have a special means of aeration and a mechanical foam breaker fitted into the expansion chamber section.

By the use of the tower fermentation system it is hoped certain advantages may be obtained over the more conventional stirred tank reacter. The advantages envisaged are in the materials and methods of construction, space for construction, simple operation and low 'down' times, smaller energy requirements and the facilitation of morphological control of organisms to some advantage.

5.2. Kinetics of Microbial Growth in Tower Fermenters

A great deal of the work on microbial growth kinetics has

been carried out on bacteria. The classical methods by which the growth of bacterial cultures are studied is by dividing the growth cycle into a number of distinct phases, as described by Monod (1949) and Buchanan (1918). These particular phases being the lag, acceleration, logarithmic, retardation, stationary and death phases. The logarithmic phase being the most important as far as the production of cell biomass is concerned.

The specific growth rate, which can be defined as the increase in microbial mass per unit time per unit microbial mass, can be written thus in the logarithmic phase of growth

$$\frac{d\ X^{o}}{dt} = \mu\ X^{o}$$

Where X^{o} is the concentration of organism, in dry weight/litre t is time in hour and μ is the specific growth rate during the logarithmic phase.

Monod (1942 and 1949) adapted the equations of the relationship between enzyme activity and substrate concentration to microbial growth

K is the Michaelis and Menton constant, μ max is the maximum specific growth rate, and Xs is the concentration of limiting nutrient.

The Monod equations are those on which most microbial kinetics are based. Luedeking (1967) reviews and describes more recent kinetic applications to bacterial culture which involve the complexities of the system as a whole.

The growth of fungi in submerged culture follow the same phases as do the bacteria. However, it is usually found that even

with filamentous culture (in high speed stirred fermenters) that the length of the logarithmic phase is shortened due to the fact that fungi are not unicellular but form filaments. In order to give maximum yields of fungal biomass in the shortest time the logarithmic phase has to be as long as possible. Moulds in submerged culture however can take on a variety of morphological forms; these forms affect their growth kinetics. The morphology that the mould takes is that of colonial forms. Foster (1949) and Camici et al (1952) were amongst the earliest workers to describe the morphological variations seen in the submerged culture of the fungi. Burkholder and Sinnott (1945) also observed that many moulds formed mycelial pellets in shake flask culture. Emerson (1950) was probably the first to describe the growth kinetics of these colonies. Emerson used a strain of Neurospora grown in liquid culture and concluded that "there is a cube root phase of growth of Neurospora pellets that corresponds to the logarithmic phase of unicellular organisms" and suggested that these kinetics resulted from pellets increasing in diameter at a constant rate. Various other workers have also obtained cube root kinetics with several moulds in submerged culture, Marshall and Alexander (1960)., Pirt (1966) and Trinci (1970). The cube root growth is explained by the fact that the diameter is increasing at a constant rate and Pirt (1966) suggests that this is due to oxygen limitation and so growth is proceeding on the outside of the colony only. He describes the cube root growth by the equation

M = mass of mycelium/ cm^3 at time t and μ is a constant

Tower fermenters have a distnict advantage over stirred reacters in that the micrcorganisms within them are able to develope their own morphology without disruption by the impeller blades (Greenshields et al 1972). By developing their own morphology it is meant that they are able to form aggregates. These microbial aggregates might in turn have specific advantages over 'looser' morphological forms of the organism. For instance, the flocculent character of the yeasts grown in tower fermenters for beer production, by virtue of their flocculent nature they are able to be retained within the tower and not washed out with the beer. Also during the continuous acetification process using tower fermenters (Jones 1970) loose irregular flocs of Acetobacter spp. were formed. These flocs also served as protection against wash-out. Filamentous fungi usually formed pellets which were either compact or loose depending on culture conditions and reflecting the morphology of the mycelium. Various fungi have been tested (Alagaratnam (1971) Daunter 1972) most of which gave compact pellets (except Fusarium oxysporum which had a yeast-like morphology). It should finally be pointed out that combinations of organisms as occur in symbiotic cultures afforded by natural ecosystems allow unusual but predictable morphology in towers. A preliminary slide of an activated sludge tower system (Chesson 1972) revealed that yeasts and bacteria are assisted in flocculation or pellet formation by various fungi thus giving conditions which favour the tower fermenter.

Aspergillus niger (M1) was the organism chosen for biomass production. Broadly speaking there are two forms that the morphology can take, these are (1) a purely filamentous variety (11) a compact or

colony form. In tower fermenters it was found to be possible to grow and control any particular morphology of A. niger. Control of the morphology, especially the non-desruption of the colony form is facilitated in the tower fermenter since it imparts relatively little shear force on the liquid in its method of aeration and agitation. This contrasts vastly with the stirred fermenters which use high impeller speeds 500 - 1000 r.p.m in order to maintain oxidation levels, this results in the mould exibiting a filamentous morphology only. This means that the mode of growth attributable to the mould is a function of both the culture conditions and the fermenter. For this reason the kinetics of both the tower and the moulds have to be studied together.

Pellet formation and morphological variation form an important aspect of this work since it is believed that when the pellet form is grown in tower fermenters it has certain advantages over the filamentous variety. The experiments, results and discussion that follow in Section 1V are devoted to the formation and differentiation of pellet culture and to the advantage and disadvantages of gowing pelleted mould mycelium in submerged culture.

SECTION 2

THE FERMENTERS.

2.1.1. Glass Tower Fermenters.

A variety of glass tower fermenters were used which had volumes rangeing from 1 l to 60 l. The small volume fermenters consisted of glass jointed section (B60 - 8.5 cm dia; B100 6 cm dia). The larger columns were assembled using lengths of Q.V.F ± ½" glass tubing sealed together by using neoprene rubber gaskets (see table 2)

2.1.2. Plastic Tower Fermenters (High Density Polypropylene).

One of these fermenters were in use which had a working volume of between 500 and 750 litres. This was assembled in two sections (stem portion and the expansion chamber) using rubber gaskets and stainless steel bolts.

TABLE. 2

Dimensions of Tower Fermenters Used.

Working Volume	Diameters		Length.	
	Long Section.	Expansion Section.	Long Section	Expansion Section.
1 litre	6 cm	9 cm	30 cm	12 cm
10 litre	8.5 cm	12 cm	150 cm	30 cm
50 litre	15 cm	30 cm	210 cm	38 cm
500-750 litres	30 cm	200 cm	4,800 cm	150 cm

By underfilling the fermenters other volumes were also available.

Sampling Procedures

Samples from tower fermenters were usually taken from one sampling part in the lower half of the column. This was done

by increasing the air rate momentarily so as to ensure some degree of homogeneity in the sampling. In the stirred tank fermenter samples were always extracted from just below the impellor blades. This was done by putting the vessel under a slight pressure leaving the sample part as the only exit.

2.1.3 Stirred Tank Fermenter.

The stirred tank fermenter used for comparison purposes was the smallest model produced by Biologische Berpahrenstechnik [3]. The total capacity of the tank being 15 litres and having a working volume of 10 litres. This being a top drive fermenter.

@ The fermenter

Except where stated this fermenter was run with three six bladed open turbine impellers. The first situated as low as possible down the shaft close to the air supply, the second approximately 30 cm higher, and the third was situated in the head space and used as a form of mechanical foam breaker. A ½ H.P motor was mechanically coupled on to the drive shaft. This was capable of producing shaft speeds of just under 1000 r.p.m. The fermentation tank hadafour baffle system, these being set equidistant from each other. These baffles were hollow so that the flow of attemperated water through them ascerted temperature control on the system. The sterile air supply entered the media through a three orifice nozzle just below the first impellor. Foam control was possible by the use of a sensitive foam probe coupled to a pneumatic valve system which released specific amounts of anti-foam into the medium. p H was monitered and recorded by the use of a p H probe 'in situ'. Control of p H was also on a pneumatic

valve system. Temperature was monitered by a long thermometer inserted in a stainless steel casing inside the fermenter. A specific air rate was maintained by the use of a rotameter.

(b) Fermentation Procedures.

Sterilisation was performed 'in situ', all accessories (air filters, addition valves, resevoirs and sampling lines) were autoclaved separately. The sterilisation procedure involved passing steam under pressure through the baffle/attemperation system. A temperature of 115°C was held for 30 min.

Sampling was done by holding the tank under a slight pressure with the sample part as the only outlet. The sample line took its material from near the air inlet, just below impellor 1.

2.1.4. Shake Flasks.

Shake flasks of volumes 100-500 ml were used in certain experiments.

(a) Rotary Shaking

100 ml flask were used on a 150 flask rotating table. These flasks were baffled in order to accentuate aeration. They were all run at ambient temperature.

(b) Reciprocating shaking

A Gallen Kamp[4] reciprocating shaker was used which took flasks of 500 ml and was capable of oscillations at 500/minute. These were not necessarily baffled.

2.1.5. Vortex Fermentation Jar.

This form of submerged culture was used occasionally in work on the morphology of A.niger.It employed a magnetic stirrer

placed inside a two litre flat bottomed flask. Aeration was achieved by a vortex effect set up by the magnet revolving at speeds up to 400 r.p.m.

2.1.6. Race Plates

Race plates were used as a rough method of assessing growth rates in various conditions. This surface culture procedure gave indications only, as to parameter optimisation. Inoculation was by spores at the centre of the plate. The colony diameters were measured at predetermined intervals. A plot was then made of the square of the radius against time. (The square of the radius is directly proportional to the area). The plates used were fabricated out of a plastic material and were 9 cm in diameter.

2.1.7 Air Distributors.

The most common form of air distributor used in the glass tower fermenters were composed of sintered glass discs which varied in maximum pore diameter between 1 & 250 μ.m. These were porosity grades 0-5 (GallenKamp). Sinters occasionaly used on the 50 litre glass fermenters were made of sintered P.T.F.E and sintered stainless steel. These had pore sizes between 10 & 30 μ.m. The air distributor in the plastic tower however was not interchangable and was composed of a squat cylinder the same diameter as the tower. This distributed the air by means of holes drilled in the uppermost surface of the cylinder which were 1/3" diameter.

2.1.8 Additional Fermenter Equipment.

Sterile Air Supply.

The air supply for the 1-50 litre fermenters was sterilised by passing through a sterile cotton wool pre-filter connected

in line with a Biotex laboratory scale glass fibre paper filter. The quantity of air supplied was metered with Gapmeters [2] (G.A. Platon Ltd.) For the larger tower fermenter a Gelman [6] paper filter was used (Model 12505-1) Particle size 0.45 m.

(b) Antifoam.

When a chemical antifoaming agent was required Silcolapse 437[R] (I.C.I Ltd) [7] was used. This contains 30% silicone as methyl polysiloxane and was diluted by the addition of 2 parts of water to 1 part of antifoam emulsion before sterilising by autoclaving. It was confirmed by the manufacturers that the emulsifiers were conionic and should not interfere with the ion - exchange resin in the amino acid analysers.

Where possible a mechanical foam breaker was used. This consisted of a high speed impeller situated in the expansion chamber section of the fermenter. This was found to have several advantages over the use of chemical antifoams.

(c) Temperature control

Temperature control was maintained by means of thermostatically controlled element wraps on the item positions of the fermenter or where possible by circulating water around water jackets fitted on the stem portion of the fermenter.

(d) Dissolved oxygen measurement.

Dissolved oxygen concentration was monitered by means of a 'New Brunswick' [8] dissolved oxygen electrode and recording system. This method has been described by Johnson M.5 Barowski J. and Engblom, C.

2.2. ANALYTICAL PROCEDURES.

2.2.1. Sugar content (Reducing and Non-reducing Sugar).

These were determined by the method of Lane and Eynon (1923). Non reducing sugars were determined after hydrolysis with concentrated hydrochloric acid at 60°C for approximately 5 minutes. Reducing sugars were determined prior to hydrolysis.

2.2.2. Nitrogen content..

Total nitrogen contents were determined by the Kjeldahl method. The catalyst used for digestion was a 100:40: 1 mixture of potassium sulphate and copper sulphate and sodium selenate. The ammonia released was distilling into 4% boric acid solution which was then back titrated against 0.01N-Hydrochloric acid. The indicator was a mixture of equal volumes of 0.2g%w/v Methyl Red and 0.1g%w/v Methylene Blue in obsolute alcohol. The distillation was carried out in a Markham still using 40%w/v sodium hydroxide.

2.2.3 Ammonia content

This procedure was also carried out using a Markham still, the ammonia was driven out of solution by magnesium oxide powder. Determination was as in (111).

2.2.4 Nitrate and Nitrite content.

This procedure was also carried out using a Markham still. The ammonia was first liberated as above, the nitrate and nitrite were then converted to ammonia by the use of Derardos Alloy and 40% sodium hydroxide. Determination was as in (111).

2.2.5 Protein content.

These were determined using three methods of analysis

(a) This can be estimated by multiplying the Kjeldahl nitrogen by a factor of 6.25 (This assumes protein contains 16g % nitrogen, as it does in the majority of biological material).

(b) Total protein was also estimated by enumerating the amino acids separated during amino acid analysis.

(c) The Lowry method of estimation was also used for protien determination. This is the method of Lowry et al (1951) which is the measurement of proteins with the Folin phenol reagent after alkaline copper treatment.

2.2.6 Amino-nitrogen.

This was determined by the use of E.E.L Amino Acid analyser.

2.2.7 Amino acid analyses.

These were determined by the use of an E.E.L 194 amino acid analyser, which operates on the principle described by Spock-man, Stein and Moore (1958). The protein samples were prepared for analysis by hydrolysing a known weight of sample with 6N hydrochic acid at 120°C for 6 hours in an inert atmosphere of nitrogen The pool amino acids were released from the mycelium by boiling with water for 10 mins of 100°C

2.2.8 Methods for assessing the rate of oxygen transfer into solution.

(a) Sulphite Oxidation Method.

The method used was that of Cooper et al (1944) which measures the maximum rate of solution when the dissolved oxygen

concentration is very nearly zero. The sodium sulphite concentration at the beginning of the run was approximately 0.3% w/v. The catalyst used was 0.025g w/v of copper sulphate. The sulphite is removed from the fermenter at predetermined intervals and added to 0.5.N-Iodine solution which is then back titrated with 0.1.N-Sodium thiosulphate. The rate of conversion of sodium sulphite to sodium sulphate was then calculated. This gave a measure of the rate of oxygen solution (Catd.) With the concentration of chemicals used it was possible to limit severe foaming in the majority of cases. All samples were taken from the base of the fermenter just above the sinter, it had been established that due to the efficiency of mixing in the fermenter no difference in tiration values could be seen at any level. The sulphite oxidation method used here gave reproducable results, and was useful on a comparative scale, the results however in this air/water/sulphite system gave higher oxidation values than other methods employed using air/media/mould systems.

(b) Polarographic gassing-out method.

The method used is that of Wise (1951). The polarographic method can be used to determine oxygen concentration in the presence of variables which invalidate the use of the sulphite system due to poisoning e.g. mycelium and proteins.

The fermenters are de-gassed using mitrogen gas. When the system is switched back to the air supply the rate of oxygen solution is calculated by means of the New Brunswick dissolved oxygen recorder.

Evaluation of K.L.A. was done as shown in Appendix 11 of Materials and Methods.

2.2.9 Oxygen uptake by mould mycelium.

A manometric method was employed for the determination of respiration rates in mould mycelium. A 'Warburg' apparatus was used. The carbon dioxide so produced in this experiment was absorbed by alkali and the oxygen uptake measured directly. In the constant -volume instrument, the observed pressure change (h) is related to the volume change causing it (v) by the flask constant (k).

$$\therefore \quad v = h \times k$$

The method used for the determination of the flask constant for oxygen was the Ferricyanide - Hydrazine method of Michaelis and Rona (1930).

As far as possible the weights of mould employed during these determinations were estimated after use as respiration material, this minimised errors due to sampling.

2.2.10. Cell - mass (Dry-weight)

The dry-weight of mycelium in the fermentation broth was estimated by filtering the mould, and washing several times with distilled water until the washings were clear. The filtered mould was then dried in an oven at 105°C to constant weight.

2.2.11 Spore counts.

Spore counts were made using a 'Thoma' haemocytometer slide (Depth 0.1 mm. Area 1/400 mm^2) Dilutions were made where necessary.

2.2.12. Pellet Numbers.

Pellet numbers were counted manually from a predetermined volume of fermentation broth.

2.2.13 Fat Analysis.

The method used for the estimation of fats was that of Soxhlet. Here the fat is extracted with ether, , dried, and weighed, the percentage thus calculated.

2.2.14 Estimation of Nucleic acids.

This was performed by suspending ground-up mould in ice-cold ethand and allowing to extract for ½ - 1hr at 0°C, then extracting three times with ethanol - ether (3:1) at 90°C - for 3 min. The residue being dried in-vacuo. 100 mg of this preparation is weighed out, the procedure is then according to Ceriothi, R (1955).

2.2.15. Paper Chromatography of Fermented Media

The identification of the organic acids produced during fermentation performed by means of chromatography. The methods of Frohman et ol. (1951) and Isherwood (1946), modified by Davies (1971) were used. The solvent system used was:-

Butan - 1 - ol	18)	
Formic Acid	4)	Parts by volume.
Water	3)	

Whatmans 3mm Chromotography paper was used, spots being applied in 10 ml. The chromatograms were run for 12 hours in an insulated glass tank using descending chromogography. The indicator base detection system was:-

Bromophenol blue 0.35 g w/v) In 1 litre of Ethanol

Methyl red 0.15g w/v)

p.H = 8.5 adjusted with formic acid or ammonium hydroxide.

2.2.16 Viscometric Determinations of the Fermentation Broth.

A Feranti portable viscometer was used for all deter-

minations. The Ferranti model is a coaxial cylinder viscometer which consists of a rotating outer cylinder with a second cylinder located coaxially within it. The inner cylinder is free to rotate against a calibrated spring with a pointer to show the angular deflection. The relative distance between these discs was varied as required. The more viscous the fermentation broth became the larger the space between the discs had to be. For broths of high viscosity (those containing appreciable quantities of myceluim) holes were drilled in the viscometer disc heads so as to promote flow between them. In this experiment re-calibration of the viscometer was necessary, this was done with liquids of high viscosity. The liquids used were castor and rape oils.

2.3 THE ORGANISM.

2.3.1. Culture and storage.

The culture used was Aspergillis niger (M.1) which was obtained from TATE and LYLE LTD. This culture was isolated from rotting carob beans and was selected on the basis of its high yields, protein content, carbohydrate conversion effeciency and its fast growth rate.

All cultures of A.Niger were stored on beet molasses agar in universal bottles at 4°c. The organism was subcultured at frequent intervals, not exceeding one month.

2.3.2. Preparation of inocula.

The A.niger was subcultured on agar slopes in 5 fluid ounce flat bottles prior to preparation of inocula. The medium used for this was identical to that used for maintaining the fungus. Various types of inocula were used.

(a) Spore inoculum.

When a spore inoculum was required, the spores were removed from the agar slopes with a small quantity of sterile 0.05% v/v. 'Tween 80' in water. This suspension was then shaken up with glass beads so as to prevent the majority of the spores from sticking.

(b) Liquid surface mat

500 ml quantities of the growth medium were autoclaved in 2 litre flat bottomed flasks at 15 p.s.i.to 15 minutes. On cooling these were inoculated with a spore suspension obtained by shaking up a small quantity of sterile 0.05 v/v 'Tween 80' with a culture grown on agar slopes in flat bottles. The flasks were incubated at 29°C without agitation for 9 days after which time a surface mat was obtained. For inoculating a fermenter the surface mat and

media were aseptically transferred into a sterile waring blender and mascerated for 20 seconds. The suspension so obtained was used as the inoculum.

(c) Actively growing mycelium

In order to obtain actively growing mycelium a small scale fermenter 1-10 litres was prepared by inoculating by either methods (1) or (11) above. During the active growth period the mycelium was aseptically removed from the fermenter and transferred into a sterile waring blender and mascerated for 6 seconds only. This mycelial suspension was then used as the inoculum at a give rate in one of the larger fermenters.

2.4. THE MEDIA.

2.4.1. Composition of Molasses used.

Beet molasses was used as the base media for all experiments carried out. This was obtained from the British Sugar Corporation refinery at Kidderminster. Three different batches were used and the analyses of these are as follows (table).

TABLE. 3

	Batch 1	Batch 11	Batch 111.
● Percentage solids	80.2	82.5	80.5.
■ Percentage reducing sugars	50.8	51.6	52.1.
◀ Percentage nitrogen	1.01	1.20	1.09
p.H 10% w/v solution	6.8	6.8	6.7

● Determined by drying to constant weight at 105°c

■ Determined by method of Lane and Eynon (1923) after hydrolysing with concentrated Hydrochloric acid of 60°C for approximately five minutes

◀ Determined by Kjeldahl method.

2.4.2. Defined Media.

A sucrose - based defined media was used, this consisted of the following reagents.

Sucrose 5 g % w/v (Bulk Chemical Ex.Tate & Lyle Ltd).
Sodium dihydrogen phosphate 0.1g% w/v B.D.H 14
Ferrous sulphate 0.03g % w/v B.D.H 14
Magnesium sulphate 0.03g % w/v Hopkins and Wms. 13
Zinc chloride 0.03g % w/v B.D.H. 14

Nitrogen sources as required.

Ammonium sulphate, Hopkins and Wms. 13.
Potassium nitrate, Fisons 15
Yeast Extract, Oxoid 16.

2.4.3 Media Preporation

The basic beet molasses was broken down with tap water to the required concentration. Any additional chemicals were of laboratory grade.

2.5. PREPARATION OF FERMENTERS.

2.5.1. Tower fermenters.

The tower fermenters were sterilised by free steaming for up to 24 hours, where possible a slight pressure was placed on the fermenter so as to raise the temperature over 100°C. The fermenters were then filled by the use of peristaltic pumps whilst keeping the tower under a positive air pressure, so as to avoid contamination.

2.5.2 Stirred tank fermenter.

The media for this fermenter was sterilised 'in situ' by passing steam under a 10 p.s.i pressure through the attemperation coils. This raised the temperature up to 115°C, this was held for 30 minutes.

2.5.3. Inoculation of Fermenters.

Where it was possible all inocula were added to the fermenters by use of peristaltic pumps or by gravity feed from a pre-sterilised glass container.

SECTION 3

SECTION 3.

Parameters Prescribing the Tower Fermenter.

When moulds are grown in submerged culture the transfer of oxygen into solution is of prime importance. The reasons for this are many. Paramount are the facts that moulds are obligate aerobes and that the low solubility of oxygen in water limits the reservoir of dissolved oxygen the organism can water limits the reservoir of dissolved oxygen the organism can draw upon. This problem is aggravated in the production of microbiol biomass due to the large volumes of mould grown in the fermenters and by the use of complex media and elevated temperatures which considerably lower oxygen solubility levels. Failure to maintain an adequate dissolved oxygen level normally results in a suboptimal fermentation (with respect to biomass levels).

This section considers the parameters, with respect to the fermenter, that affect the aeration and agitation within the system. Three well known techniques were used to ascess the mode and efficiency of oxygen transfer into solution, (a) Sulphite oxidation (b) Static gassing-out (c) Hold-up or air volume retention. Technique (c) was used in conjunction with both (a) and (b).

The most widely used technique was the electrolyte system involving catalysed sodium sulphite solution. The sulphite oxidation method characterises the performance of a piece of equipment under specified reproducible conditions. If the apparatus is to be used for a variety of applications empirical factors will at least allow for an approximate prediction of its capacities as an oxygen absorber. Thus for intitial design procedures this may be sufficient. However figures quoted for oxygen absorption rates may not be taken as those that will be encountered

by organisms in submerged culture, since media properties differ from those of sulphite solution.

Aeration and agitation is achieved in tower fermenters by passing air across an air distributer at the base of the column. The air bubbles so formed pass up through the liquid, gaseous exchange occuring in this way. The efficiency of oxygen transfer in this system is a product of various operational and design parameters. These will be discussed further in the text.

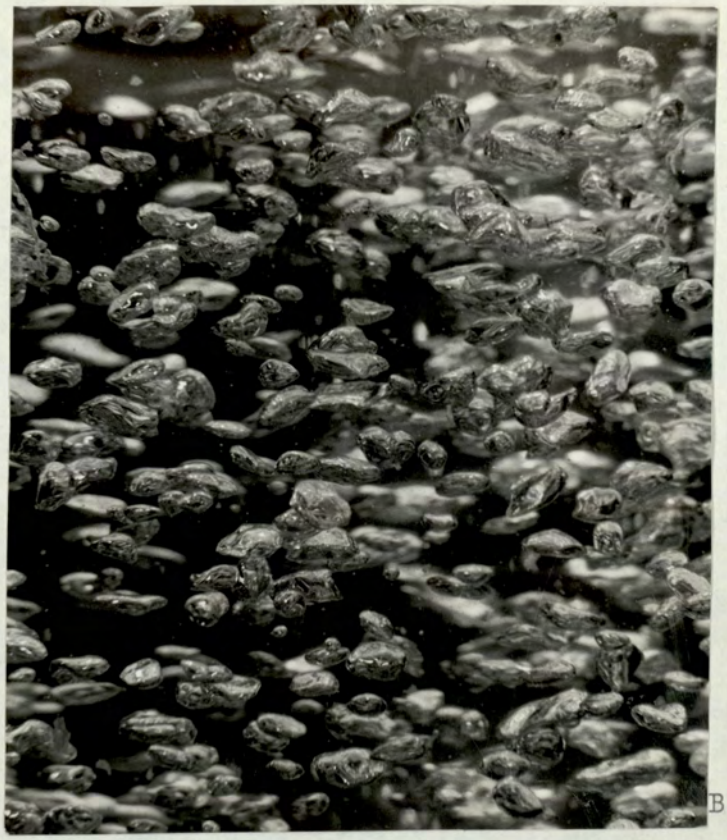
3.1. Superficial - Gas Velocity.

In the system studied a relationship was seen to exist between 'hold-up' volume, oxygen transfer rates (O.T.R.) and the superficial gas velocity (S.G.V.)

Air flow into tower fermenters may be characterised by three general flow patterns. In the first instance, low superficial gas velocities (- 1cm/s) produce 'bubbly flow' which is described as the air bubbles travelling up the column in nearly straight lines and producing very little back-mixing (Plate 3) Secondly, at higher superficial gas velocities (- 3cm/s) a kind of 'turbulent flow' exists in which there is a deal of back-mixing in evidence, (Plate 4). In the third case at still higher superficial gas velocities (- 5cm/s) a 'slug-flow' is pronounced. (Plate 5). This is also known as 'flooding'.

During bubbly flow the size of the air bubbles are more or less uniform and to a large extent a function of the liquid and air distributor. When these bubbles exceed diameters of around 4-5 mm. They cease to be rigid and assume a floppy shape which is continually changing. This transient shape must be of advantage to the oxygen transfer driving force. In turbulent flow conditions these bubbles may still exist, but there is also a second kind present. These are much smaller being less than 1 mm in diameter and have a rounded rigid shape and give a large air surface to volume ratio, this facilitating oxygen transfer. The small rigid bubbles are formed during turbulent flow irrespective of the liquid or air distributor in the system but can be accentuated in terms of numbers when a fine air distributor (sinter) or culture media is present. These bubbles are therefore thought to be formed both by the fragmentation of larger bubbles and formation through the smaller holes in a sinter plate. In 'slug-flow'

PLATE 2



BUBBLY FLOW.

PLATE 3a

TURBULENT FLOW.

PLATE 3b

TURBULENT FLOW

PLATE 4

SLUG FLOW

there is an even greater heterogeneity in terms of bubble size. The slugs themselves can be in the region of 20 cm. dia. depending on such fermenter conditions as liquid composition, flow rate and air distribution. Slug flow in smaller diameter columns is characterised by single large bubbles travelling up the column and occupying its entire area. This is more likely to occur in columns that are less than 30 cm. diameter. Various other size of bubble also exist in this region, down to the smaller rigid spheres.

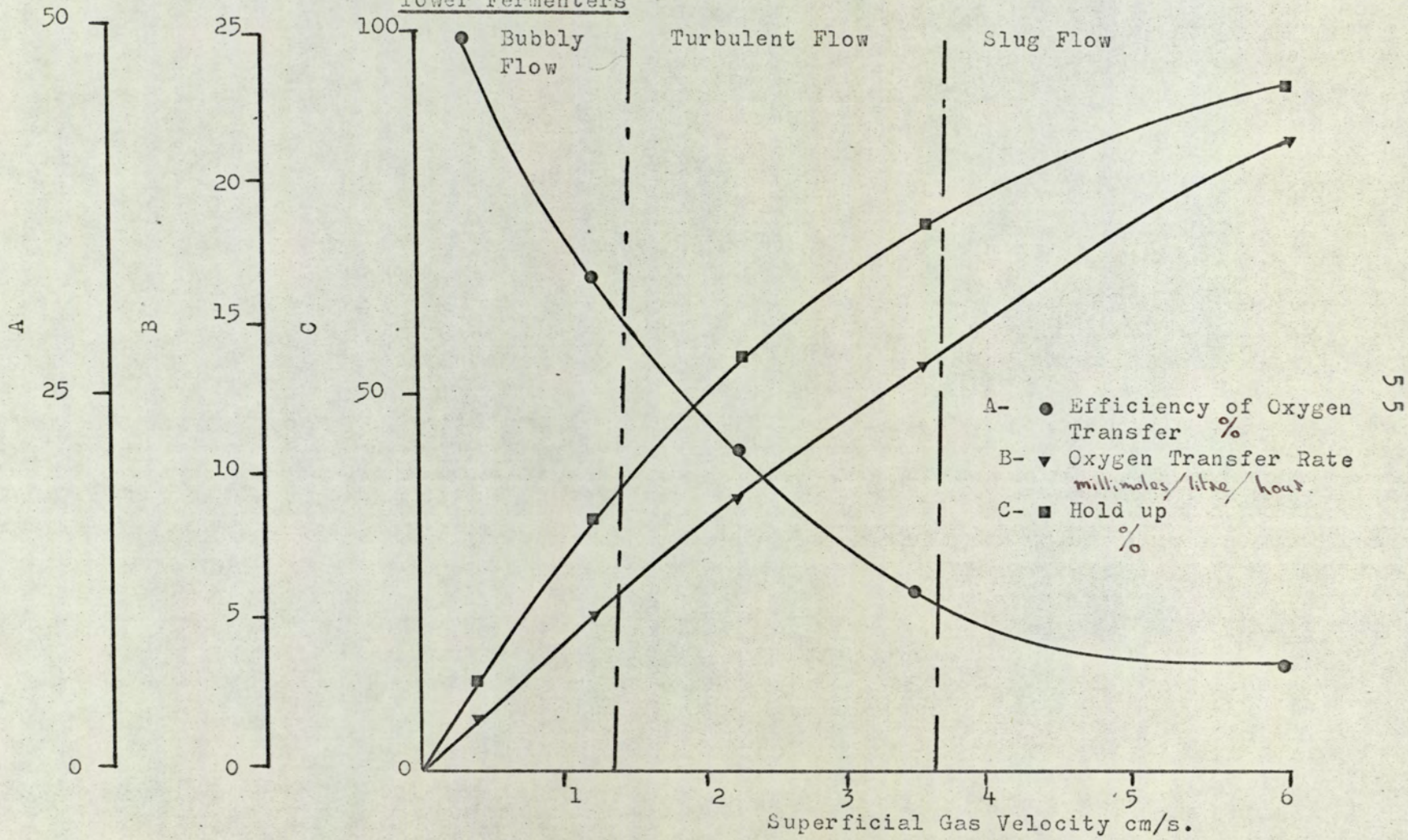
These three types of air/liquid interaction can be further classified by the use of methods for determining oxygen transfer efficiency (Graph 1).

During bubbly flow and using the sulphite oxidation technique, the oxygen transfer rate into solution was found to increase almost linearly with increase in gas flow rate, this proportionality being dependent on the air distributor.) It is in this region where the greatest efficiency of oxygen transfer occurs. During turbulent flow the oxygen transfer rate reaches a maximum point despite the fact that efficiency is falling. Under slug flow conditions however, as well as a decline in efficiency a slight fall is also observed in the rate of oxygen transfer. When air hold-up or air volume retention is monitored alongside the sulphite value an almost parallel condition is noted. The decline in the sulphite value at high flow rates is thus explained by the fact that large bubbles, with a relatively low surface area to volume ratio , move quickly up the column thus providing little time for oxygen transfer

It is only possible to discuss superficial gas velocities in the most general terms since there are many parameters that are interelated with it, such as the air distributor porosity, its area and the aspect ratio of the column.

GRAPH 1

Generalised Relationship between Flow Behaviour and Oxygen Transfer in Tower Fermenters



3.2. Air Distributor.

In tower fermenters where there is no mechanical agitation used to break up the air bubbles the air distributor must be considered as an integral part of the fermenter. The aeration is affected markedly by the air distributor, this is contrary to the affects observed in stirred fermenters where the form of air orifice does not appreciably alter aeration characteristics. Both porosity and size of air distributor affect aeration in tower fermenters. These are combined with the affect imposed by the liquid phase composition on flow behaviour and bubble formation.

3.2.1. Air Distributor Porosity.

The porosity of the air distributor was varied in order to investigate it's affect in the fermenter as to oxygen transfer. (Grades 1, 2 and 3 - Gallenkamp - were used). A variety of materials were also examined for their use as air distributors in tower fermenters. They were, sintered glass, stainless steel and sintered plastic (P.T.F.E.). Perforated or drilled sheet stainless steel was also used. Most of the work on porosity was carried out in the 8.6 cm diameter laboratory fermenter which employed the sintered glass discs.

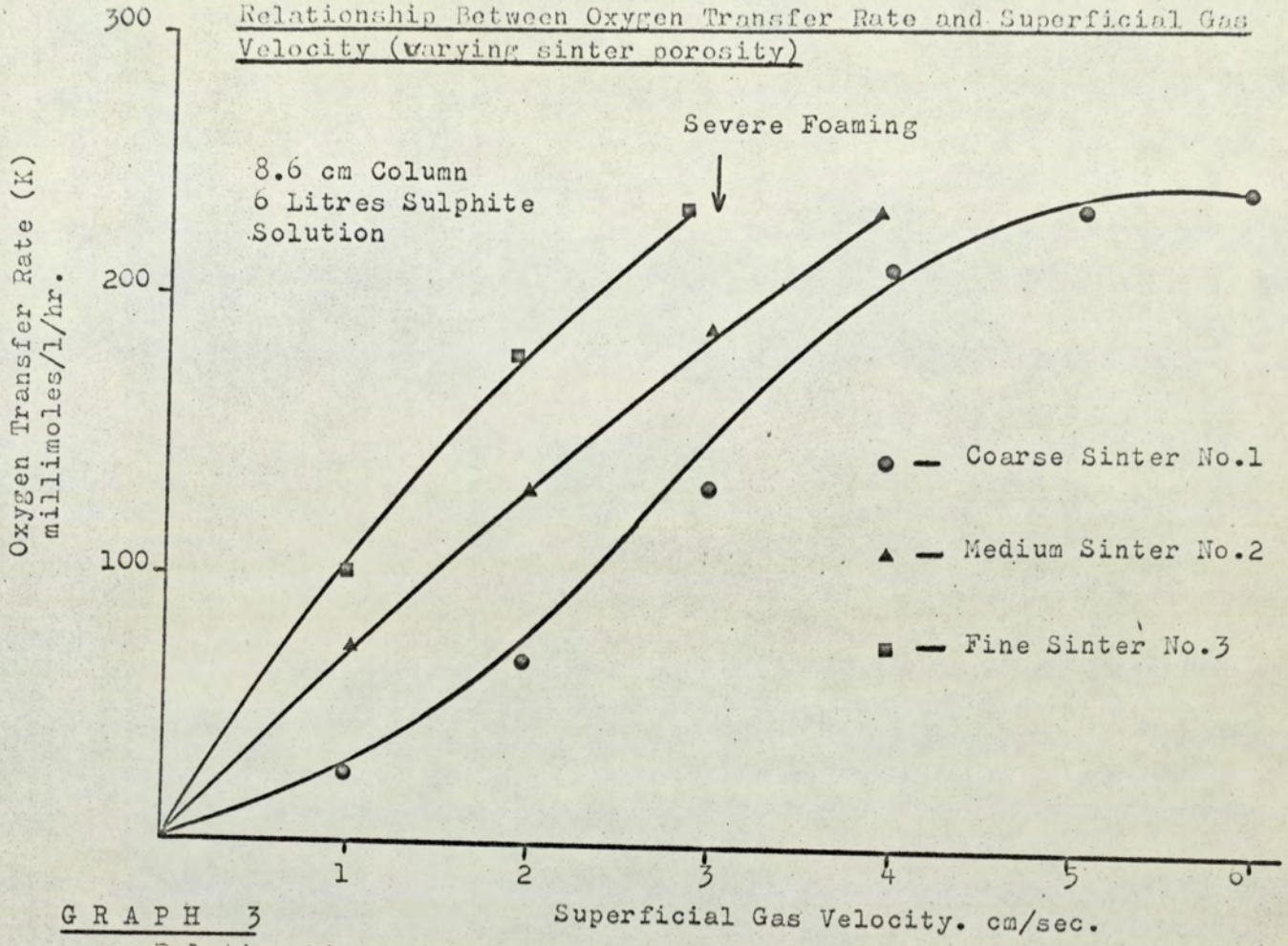
The sinter or air distributor in a tower fermenter effects a partition between the liquid and gas phase in the column. When the pressure of the gas in sufficient to overcome the hydrostatic head of liquid air will then pass through the sinter and enter the liquid. The purpose of the sinter is therefore in the formation of bubbles in the liquid phase. The size and number of bubbles present will be a function of both the air distributor and the

liquid present. In general it may be said that at extremely low superficial gas velocities only a few pores in the sinter plate come into operation, (those being the largest); their numbers increase with the gas flow rate up to the point where all pores are active. By increasing the gas flow rate even further the holes that were yielding bubbles in the first place are by now producing larger ones. Although it is stated throughout this text that the sinter porosity affects air bubble formation their actual size is 2 or 3 orders of magnitude larger than the pores themselves.

When using the sintered glass discs in the laboratory columns it was noted that when operating in air/water systems there was little variation in bubble size and air retention volume between the sinter grades. The average size of the air bubbles being 4 mm dia. this phenomenon is due to the low electrostatic potential of water. When molasses or sulphite solutions were used in the columns however a marked difference in bubble size air retention volume and oxygen transfer was noted. This was in spite of the media having a lower surface tension than that of water. The electrostatic potentials of media and sulphite solutions was however much higher than water. Thus they fascilitate small bubble formation. Graph 2 shows the relationships between oxygen transfer rate using sulphite solution and the gas flow rate using three sinter porosities. The differences in the sulphite values are more marked at low gas flow rates. This can be explained by the fact that the range of pore size is greater in the coarser sinters. Therefore, at low gas flow rates using coarse sinters bubbles will be formed through the larger holes first and thus total utilisation of the air distributor is denied up until the higher gas flow rates. This is less likely to occur with the

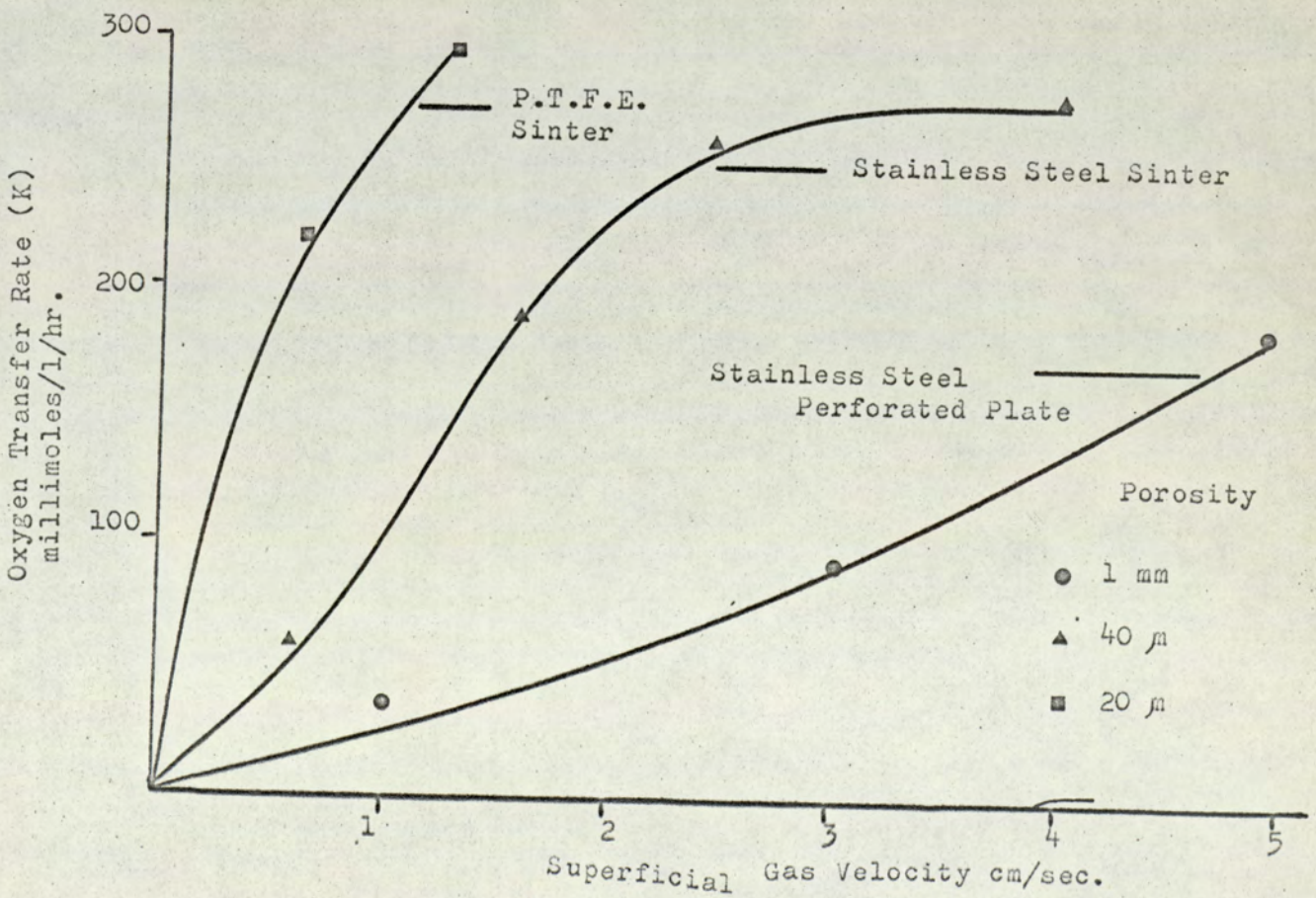
GRAPH 2

Relationship Between Oxygen Transfer Rate and Superficial Gas Velocity (varying sinter porosity)



GRAPH 3

Relationship Between Oxygen Transfer and Superficial Gas Velocity in 15 cm. dia. Column



finer sinters (or at least will occur only at low gas flow rates).

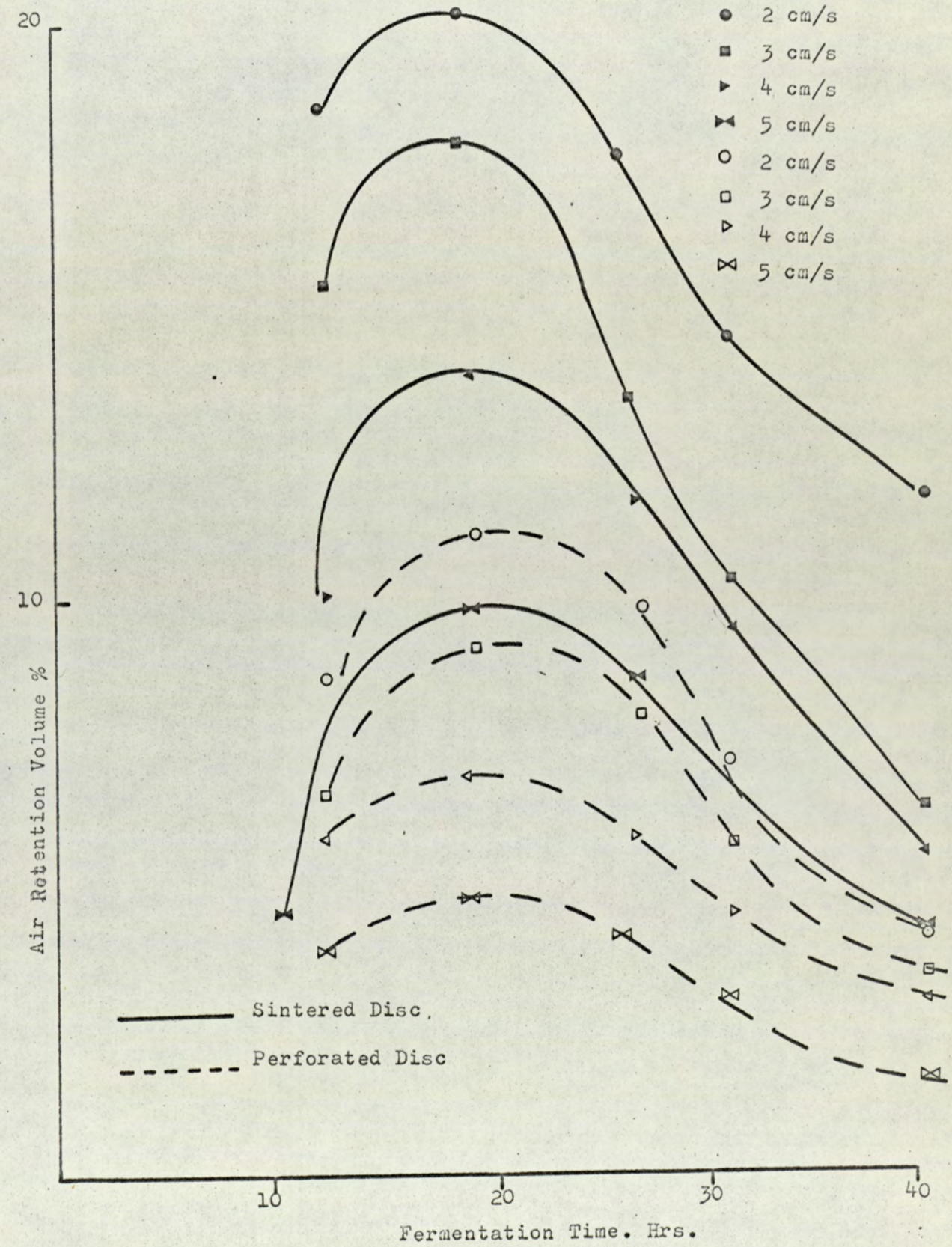
Three air distributors were also tested in the 15 cm diameter column the results of which are given in Graph 3. Fermentations were also carried out with these air distributors and their performances as to air retention volumes were monitered throughout the runs. Graph 4 shows how the air retention volumes change during similar fermentations using two forms of air distributor. (See also page 129)

The choice of air distributor does not entirely depend on the efficiency of oxygen transfer however. One must also consider the energy required to produce these aeration values. For instance, the pressure drop across the No.3 glass sinter was about three times that across the No.1 sinter. The pressure drop across some of the perforated plates was almost negligable. A balance must therefore be drawn between the oxygen requirement and the energy expenditure of the system. In addition it must be pointed out that it was observed during actual mould fermentations that the mycelium adhered to the sinters, this effectively reduced the sinter area. It was noted that this growth was more marked on the finer porosity sinters and was entirely absent in the drilled plate air distributor. The reason for this being that the shearing action of the air passing through the holes was so much greater in the drilled plate. The turbulence was also greater in the latter type aerater. Thus a coarser grade air distributor might be advantageous for the later stages of mould growth.

The choice of air distributor is especially important when considering the scale-up of a process. It is convenient to use sintered glass discs in laboratory fermenters up to 20 litres capacity. In larger columns however it is of advantage to use perforated plates. Very little work was actually performed on this

GRAPH 4

Variation in Air Volume Retention During Fermentation



type of aerator, but when using the fixed plate in the plastic 30 cm. Column (holes 1/16" dia) the oxygen transfer was considered to be too low for efficient mould growth under the conditions tested, Graph 6. Assuming the oxygen uptake rate to be approximately 50 millimoles/litre/hour (With a mould concentration of 20g/litre) then the S.G.V would have to exceed 6 cm/sec in order to avoid oxygen limitation. It is therefore evident that the pores are too big for this size of fermenter. The perforated plate in the 15 cm column was also tested for its oxygen transfer efficiency, Graph 6, and was shown to give higher values, because of the smaller holes in the plate.

As far as material of construction is concerned it was noted that for the smaller columns (less than 10cm dia.) the glass sinters were adequate. However in the larger column (15 cm.dia.) a more robust air distributor was required, for instance sintered P.T.F.E. or stainless steel were found to be more suitable.

This was beacuse of their robustness and ability to withstand the greater pressures encountered in the larger column. It was also shown that perforated plates were of greatest advantage when high densities of mycelium were present. Therefore in the pilot and production scale columns stainless steel perforated plates should be employed.

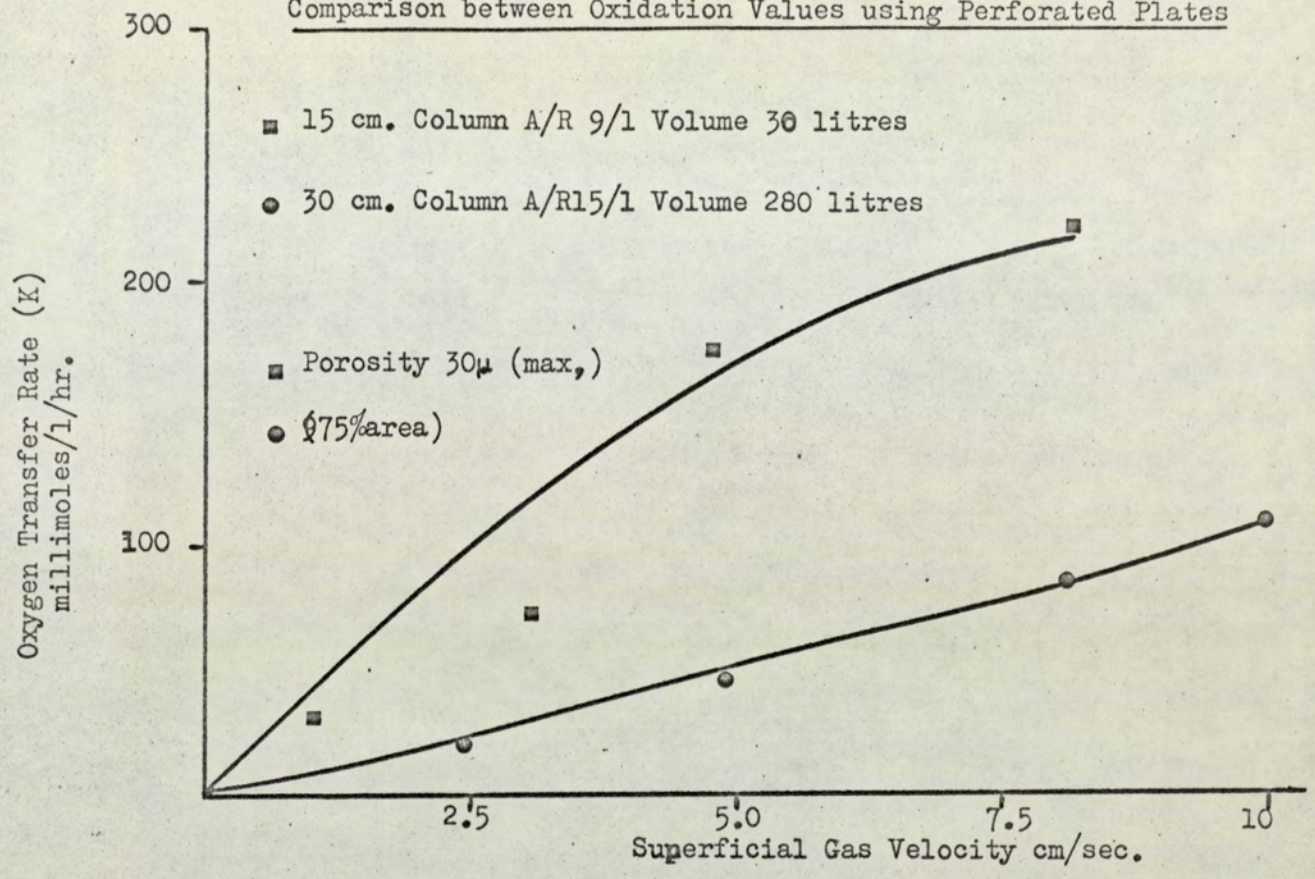
3.2.2. Area of Sinter.

The effect of the sinter area on oxygen transfer was investigated using the 8.6 cm.dia. column. The sinter area was varied by using 'araldite' adhesive to block off sections of the sinter.

Graph 5 shows how the reduction in the sinter area affects

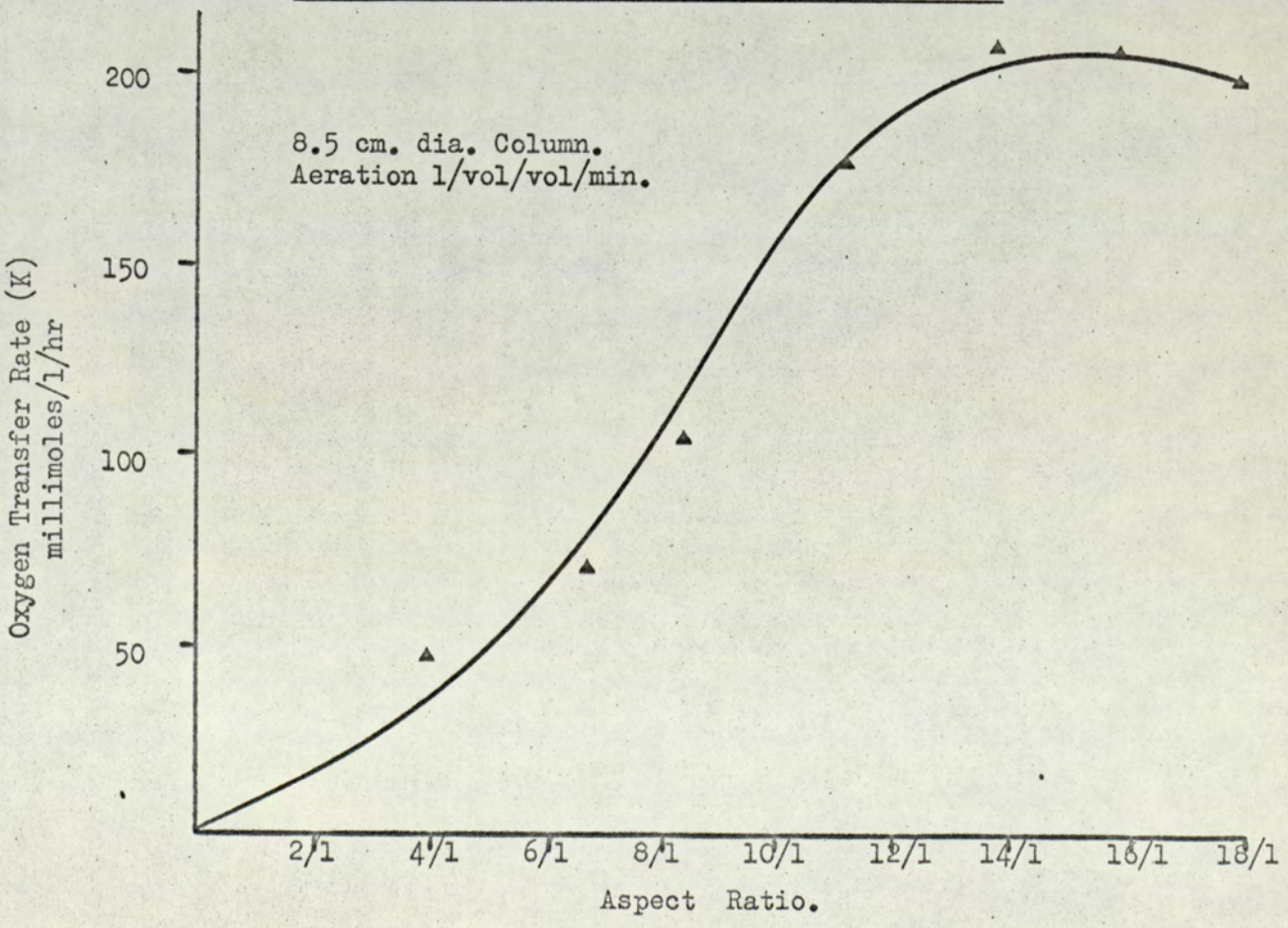
GRAPH 6

Comparison between Oxidation Values using Perforated Plates



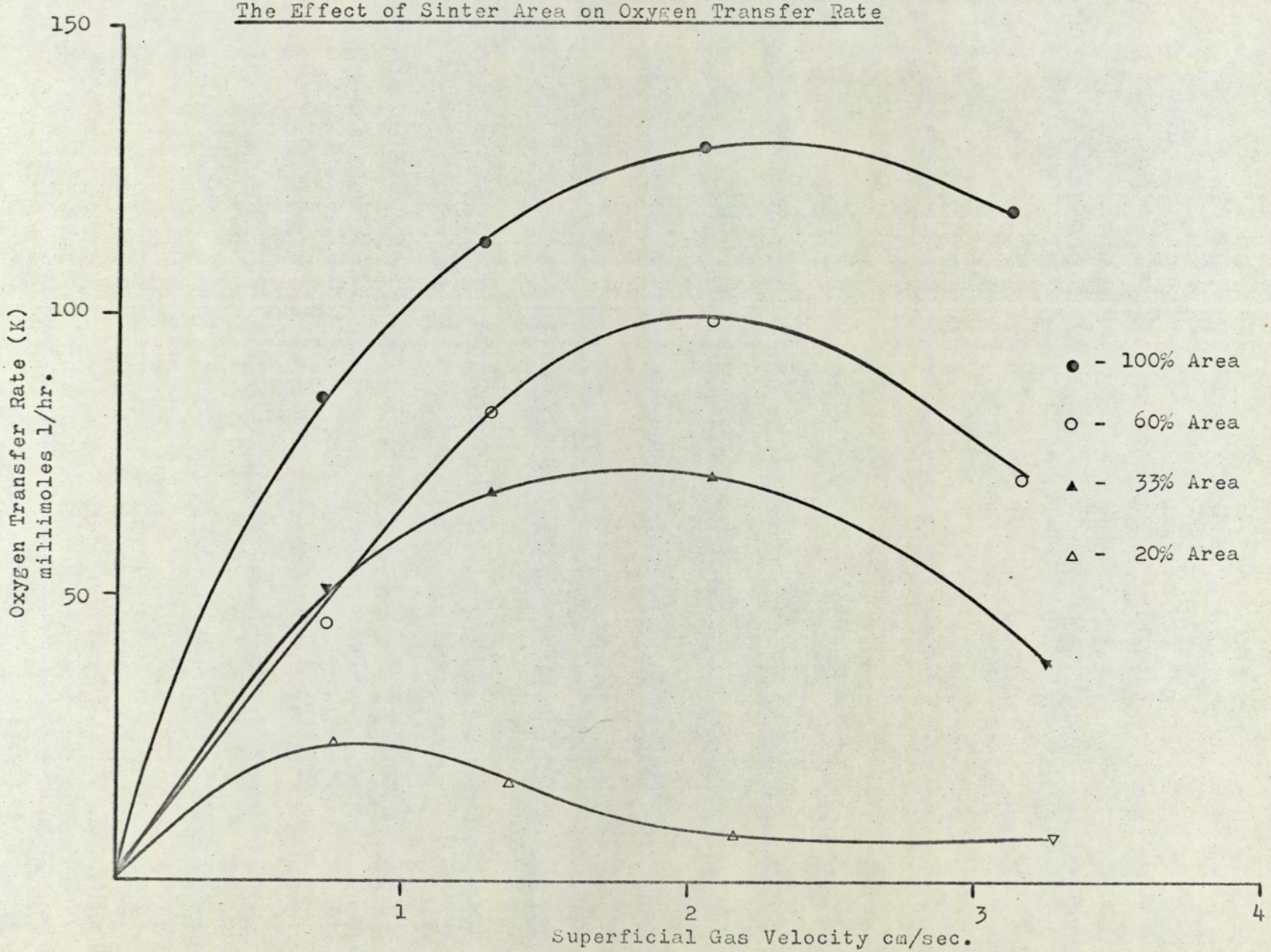
GRAPH 7

The Affect of Aspect Ratio on Oxygen Transfer.



GRAPH 5

The Effect of Sinter Area on Oxygen Transfer Rate



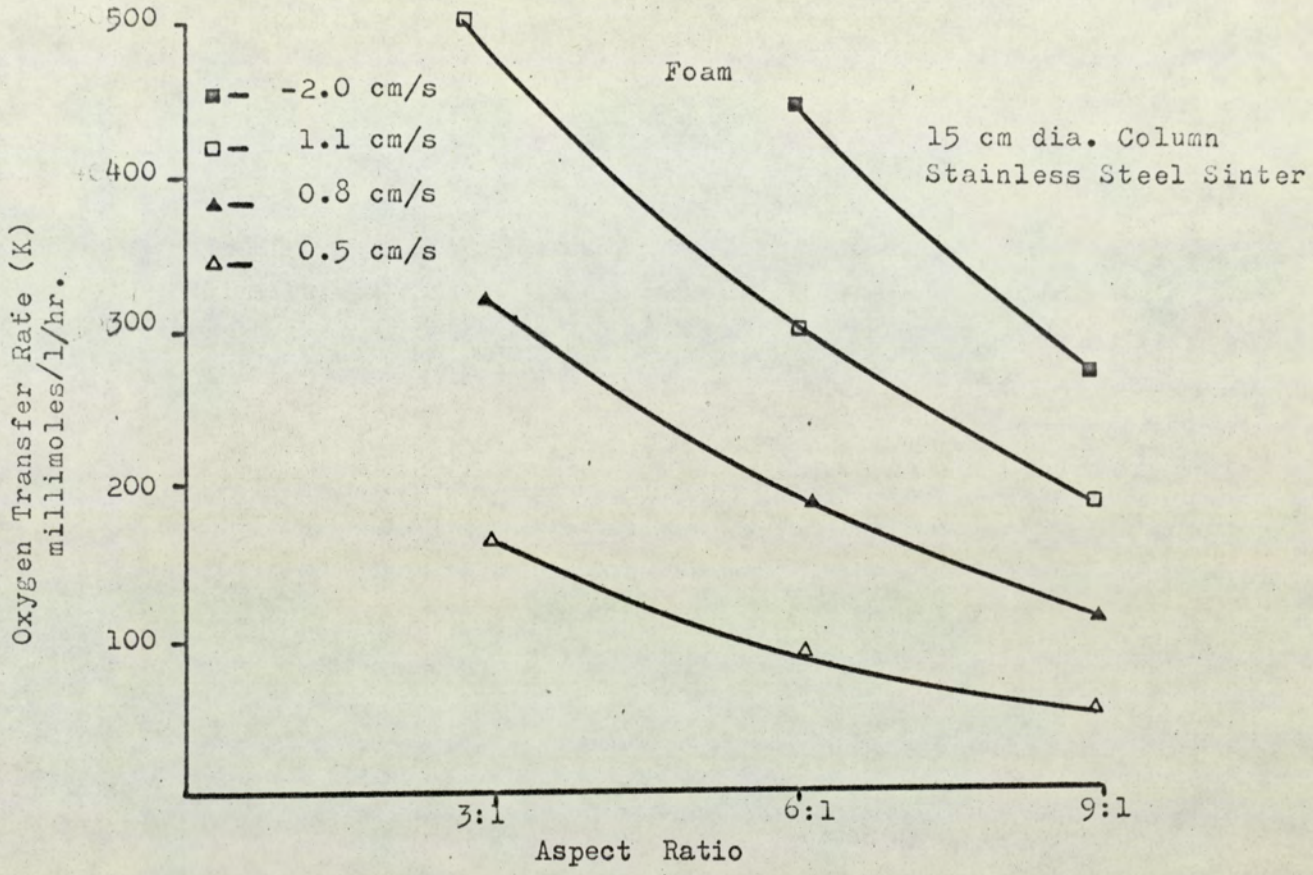
3.3. Tower Fermenter Configuration.

If we were to consider a single air bubble travelling up through a column of liquid and continually releasing its oxygen into solution then it is obvious that the driving force for oxygen transfer will become smaller as the bubble reaches the upper extremeties of the column. The efficiency of oxygen transfer E will necessarily be greatest in an infinely tall column. Under these conditions E will approach 100% exponentially. However oxygen transfer per unit time K and the mass transfer coefficient K.L.A will optimise at a particular column height. Any further increase in height will necessarily reduce the oxygen transfer rate. (Graph 7). [See Page 64]

This is again a very generalised picture of the effect of aspect ratio on oxygen transfer. From graph 8 it is seen that the air rates considered are equivalent in volume to that of the liquid in the fermenter. Thus it shows that there is a particular advantage in oxygen transfer rate at this aspect ratio. From Graph 8 however, in which the same S.G.V. is maintained at the same level despite the liquid volume variation, it can be seen that the lower the column height the better it is for mass transfer. This is because the oxygen partial pressure in the bubbles is approaching that of air (0.21 Atmos). Therefore the driving force is maximum under these conditions. If the efficiency of oxygen transfer were to be plotted alongside these values there would be a slight tendency for the higher efficiencies to be recorded in the taller columns. In this column (15cm dia.) fitted with a full diameter sinter (30 pore size) the difference is not very marked since very high oxidations resulted (some of the efficiencies

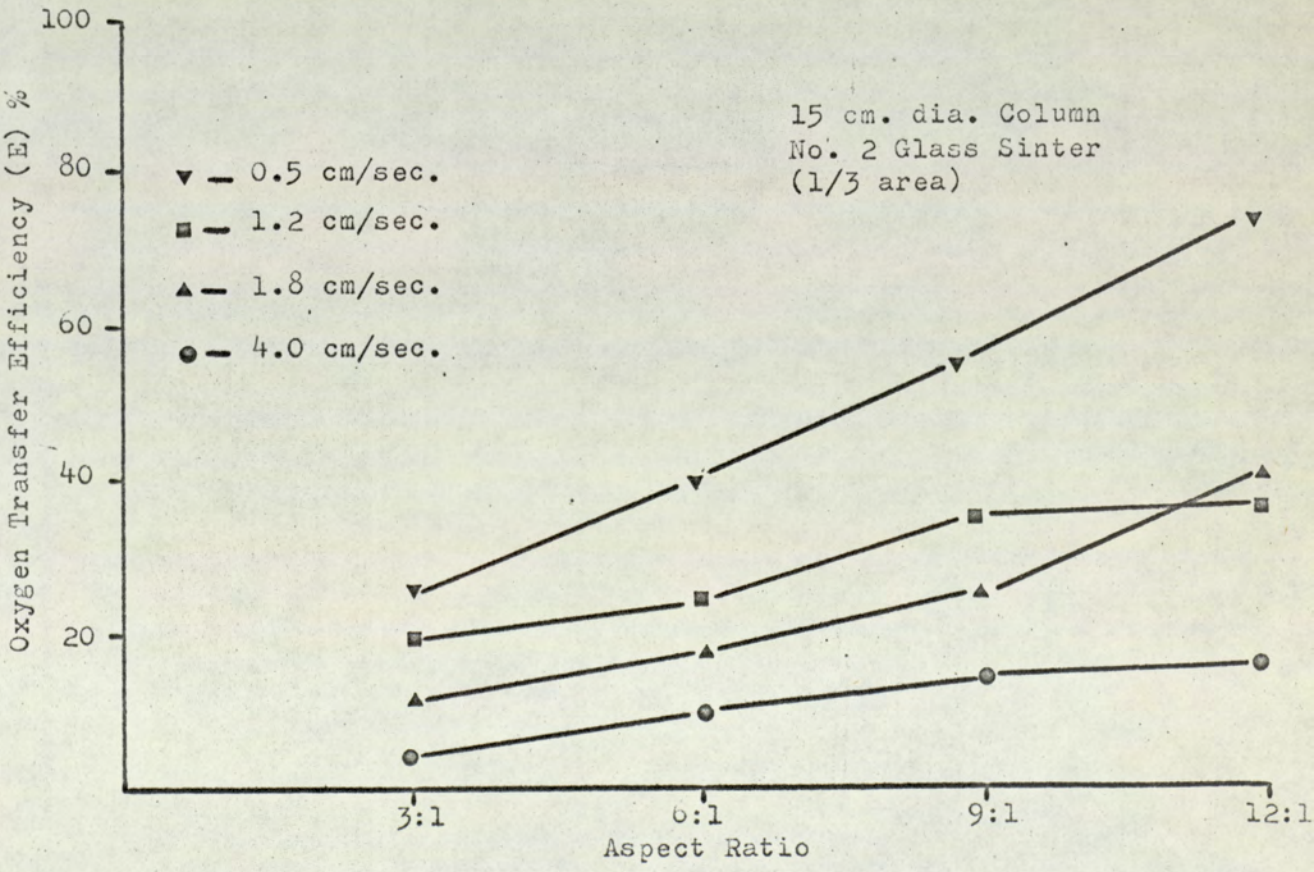
GRAPH 8

The effect of Aspect Ratio on Oxygen Transfer
(constant gas flow rate.)



GRAPH 9

The effect of Aspect Ratio on Oxygen Transfer
Efficiency at Various Gas Velocities



approached 100%). If the efficiencies of a similar test in the same column using a 1/3 area glass sinter (45μ pore size) are plotted it can be seen that this phenomenon is accentuated. Graph 9.

The results reported so far in tower configuration have been taken from columns exceeding 15 cm. dia. Smaller diameter columns are also worthy of note, since it was possible to reach high oxygen transfer rates in them. In these columns (6 cm.dia). it was possible to put 6 times the liquid volume of air through a fine sinter (No.3) thus attaining high oxygen transfer rates. Table5 shows these results in which it can be seen that this is an inefficient mode of aeration (i.e. as far as oxygen utilisation is concerned).

TABLE.5.

Oxygen Transfer in 6 cm.dia Columns at Various Aspect Ratios.

Aspect Ratio - 3: 1 Liquid Volume - 500 ml No.3 Sinter

Air Rate ml/min	K.Millimoles/l/min.	Efficiency of O_2 Transfer
1,600	430	34.7%
6,400	1470	20.5%

Aspect Ratio - 6:1 Liquid Volume - 1000ml. No.3 Sinter

Air Rate ml/min	K.millimoles/l/min	Efficiency of O_2 Transfer
1,600	315	50.0%
6,400	435	12.1%

These smaller tower fermenters however are not suitable for mould growth because of their limited volume which promotes clogging.

At this juncture the differences between column (or liquid height) and aspect ratio should be pointed out. When con-

sidering liquid depth alone it is seen that pressure increases are recorded which in turn increases oxygen solubility, however variations in aspect ratio can affect the degree of mixing and turbulence in the column and hence oxygen transfer. In general it may be said that in tall columns with low air flow rates induced turbulence can actually decrease oxygen transfer.

In order to specify a particular column height optimum for oxygen transfer one must consider the variations in air distributer and liquid composition. These two variables will affect surface area of air/water ratio and the bubble residence time respectively. So in consequence the larger the surface area the air presents to the liquid the shorter the height of column must be for maximum oxygen transfer. If, for instance, as it has been shown that decreasing the sinter pore size produces a smaller range of bubbles sizes then the liquid height must also decrease in order to cope with it. If, on the other hand the area of the air distributer were to be increased in relation to the column diameter then an increase in liquid height would provide an increase in the rate of oxygen transfer at higher flow rates.

In order to specify a particular column configuration for mould growth it is necessary to consider the effect of the high densities of mycelium of the residence time of the air bubbles in the column. On the whole it was seen that with increasing mycelial density the bubbles travelled slower in the liquid. Therefore the columns chosen for the biomass production work on a laboratory scale were a compromise between the mould and non-mould systems.

3.3.2 The Expansion Chamber Section.

During the production of beer in tower fermenters the

expansion chamber is used to house the settling zone and also to provide additional volume to the fermenter, for degassing and defoaming. At first this also seemed an attractive proposition in the production of fungal biomass. The effect of utilising part of this expansion chamber for this latter purpose was evaluated by oxygen transfer techniques.

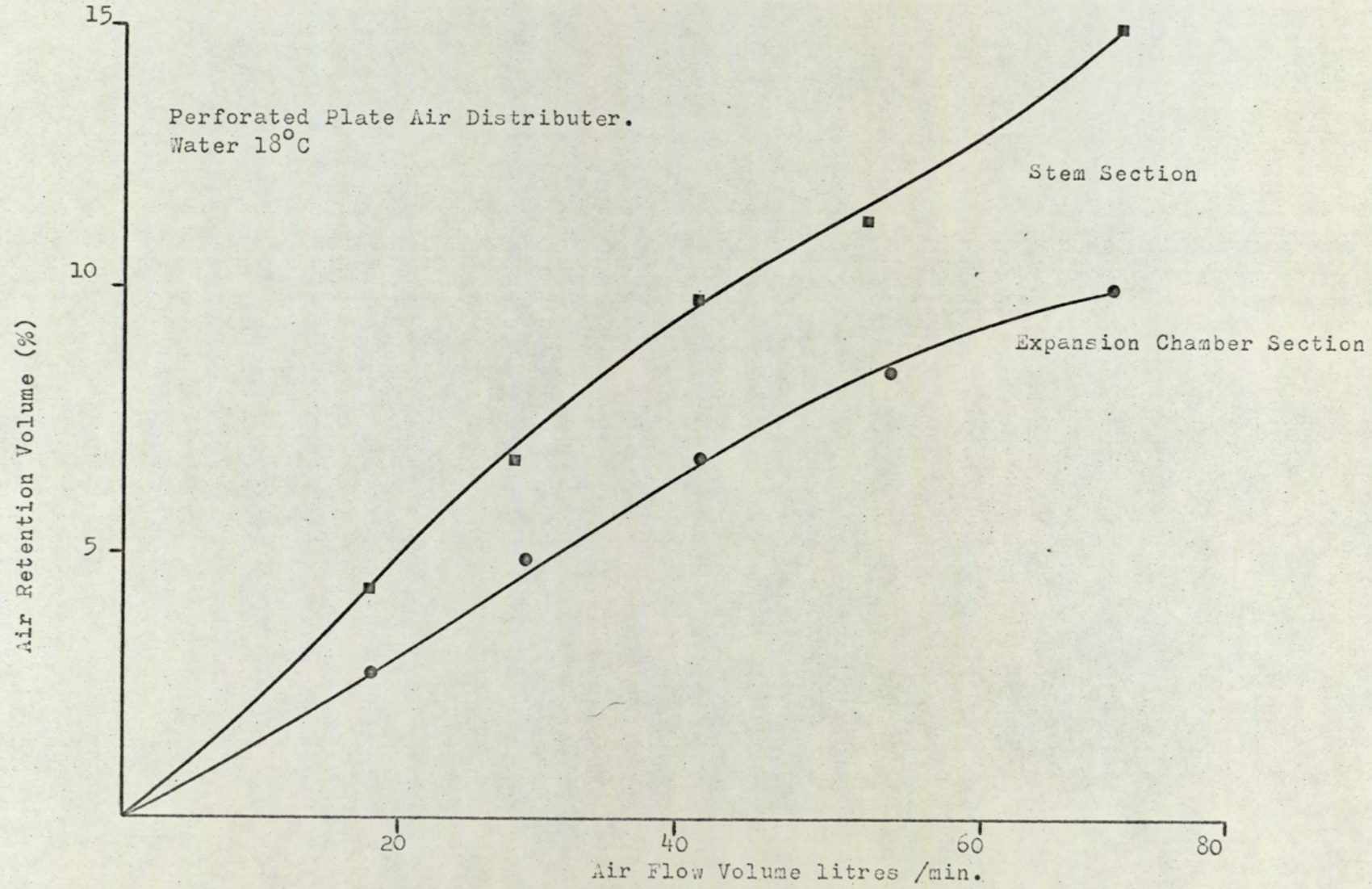
At low air rates (1.3 cm/s) a stream of air bubbles rise up the column and into the expansion section this causes very little turbulence and circulation of the air within this section. It requires a far higher air rate (- 6cm/s) to cause turbulent conditions within the expansion chamber of the fermenter than within column itself. Measurement of hold-up volume showed no advantage in Operating in this region of the column. (Graph 10). A depression in the hold-up relative to the flow rate was encountered. Lowering of the oxygen transfer rates were also shown when the expansion section contained liquid.

When the expansion section is being used as an integral part of the fermenter, effectively there is a reduction in the area of the air distributer to this section relative to the rest of the column (As it has already been shown the reduction in the sinter area reduces oxygen transfer). There is also combined affect of the oxygen in the air being at a lower concentration and having less driving force by the time it reaches the area of the expansion chamber. The relative superficial gas velocity to that volume of the fermenter is lower than that to the rest of the column thus explaining the vast differences in the flow charecteristics and oxygen transfer in this zone.

Although the extra length is available in the fermenter for increased oxygen transfer efficiency it also contains a

GRAPH 10

Relative Hold-up Values in Stem and Expansion Sections of 15cm dia. Column



greater volume of liquid than does a similar length of the stem portion. In fact with fermenters used in this experiment the expansion section was twice the diameter of the stem section therefore it contains four times the volume for an equal height of liquid.

If the extra liquid volume is required in a tower fermenter it would be plausible to attach a slightly expanded section on to the bottom of the column rather than the top.

3.4. The Effect of Antifoam Agents.

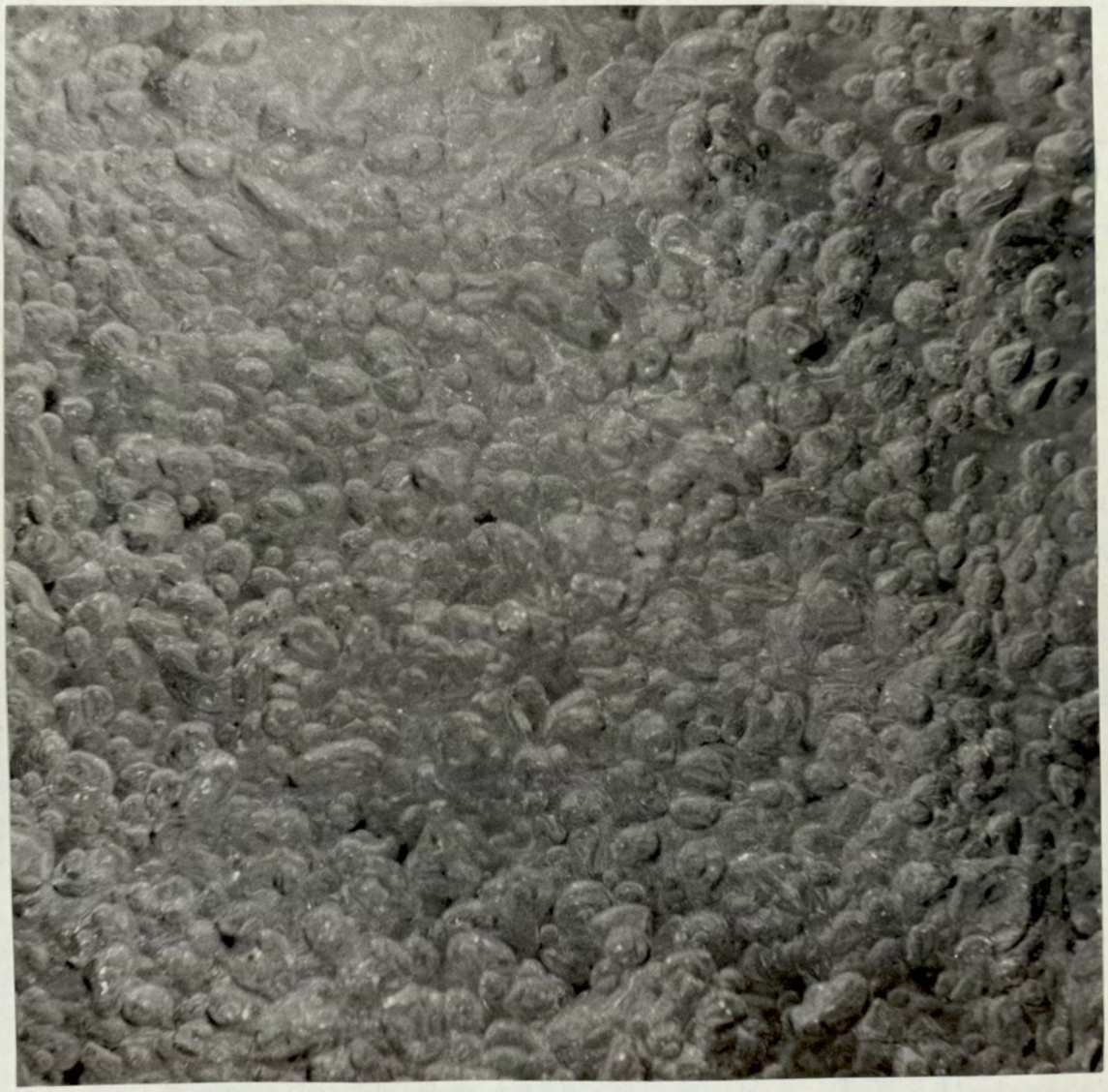
This sub-section indirectly affects fermenter design in that the use of fitted mechanical foam breakers were found to be necessary on the tower fermenters.

Bubble size in tower fermenters is governed by two liquid properties. Firstly there is the electrostatic potential at the gas-liquid interface, this produces smaller bubbles in electrolyte solution. Secondly there are the surface tension properties of the liquid, in general when the surface tension is high smaller bubbles ensue.

The electrostatic potential in media and in sulphite solution enhances small bubble formation and falcilitates oxygen transfer. The effects of this high electrostatic potential can be nullified however by reducing the surface tension properties of the liquid. This is the antifoam effect. The antifoam causes coalescence of the air bubbles into large 'slugs' which travel quickly up the column (it is also in evidence at low flow rates 1 cm/sec). This effect is demonstrated in Plates 6 and 7. Three forms of chemical antifoam were used namely silicone, octadecanol, and corn oil. The silicone was by far the most effective in breaking foam and causing air bubble coalescence.

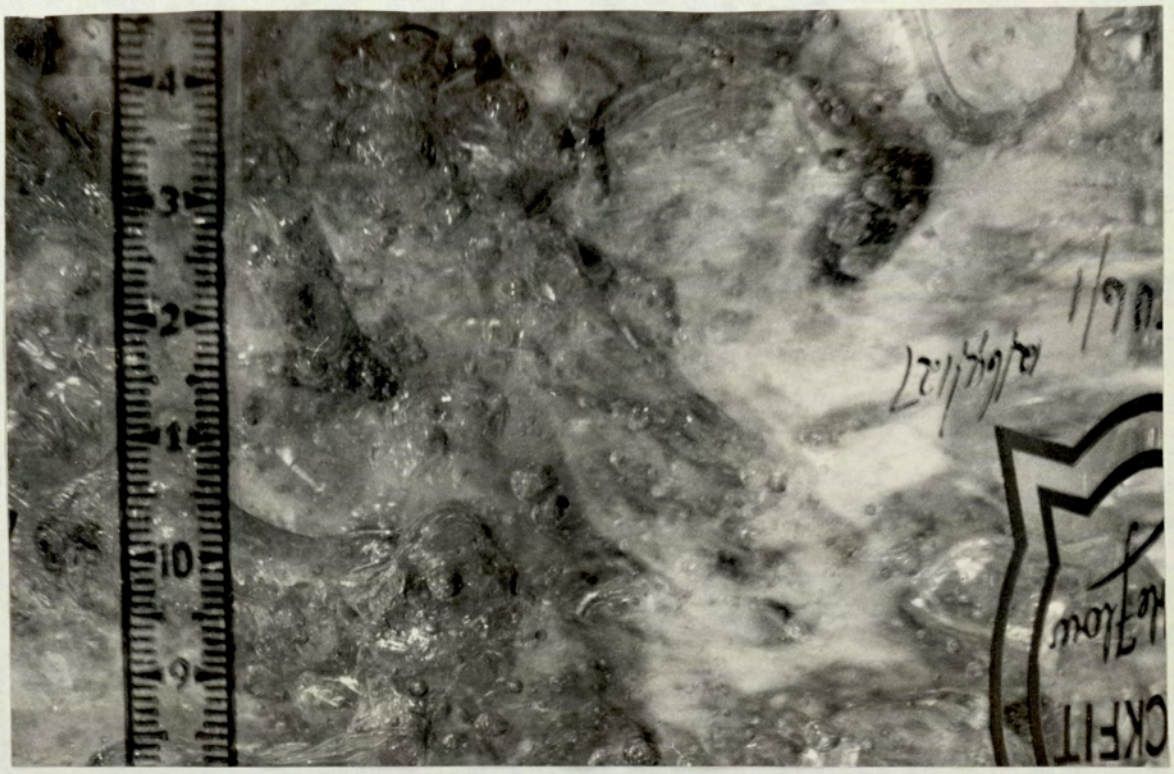
The deleterious effect of adding antifoam to tower fermenters can be shown by using hold-up and gassing-out techniques. Graph 11 shows the hold-up in clean water and that when 0.5 ml of silicone antifoam has been added. Graph 12 shows the oxygen transfer into molasses media with and without antifoam. This confirms studies by other workers, Deindoerfer and Gaden (1955), Solomons and Perkin (1958) showed the effect of anit-foam addition e.g., 0.25% of 3% Alketerge (in lard oil reduced the O.T.R by 50%

PLATE 5



Aerated Tower Fermenter (Air/Water System)
Without Antifoam.

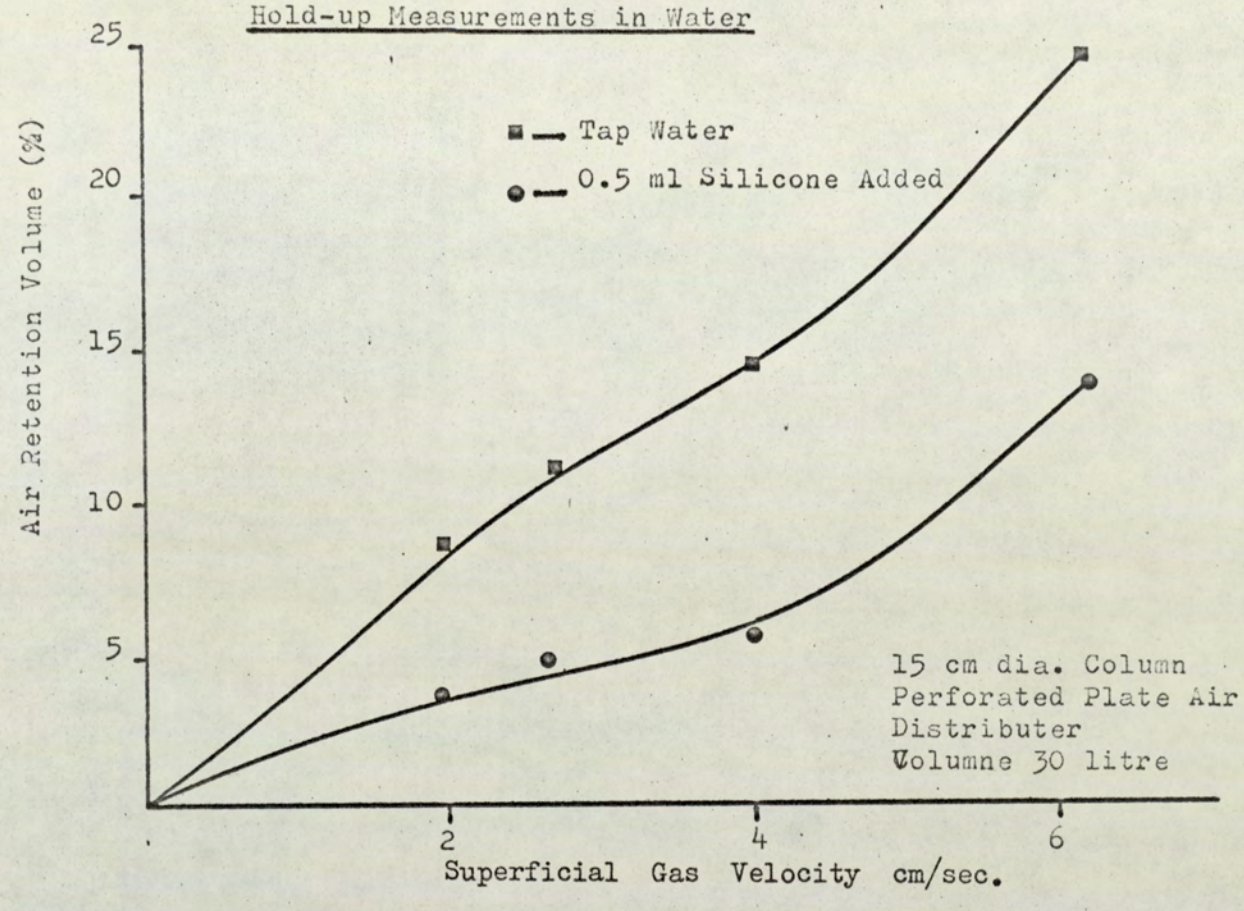
PLATE 6



Aerated Tower Fermenter (Air/Water System)
0.5 ml. Antifoam Added.

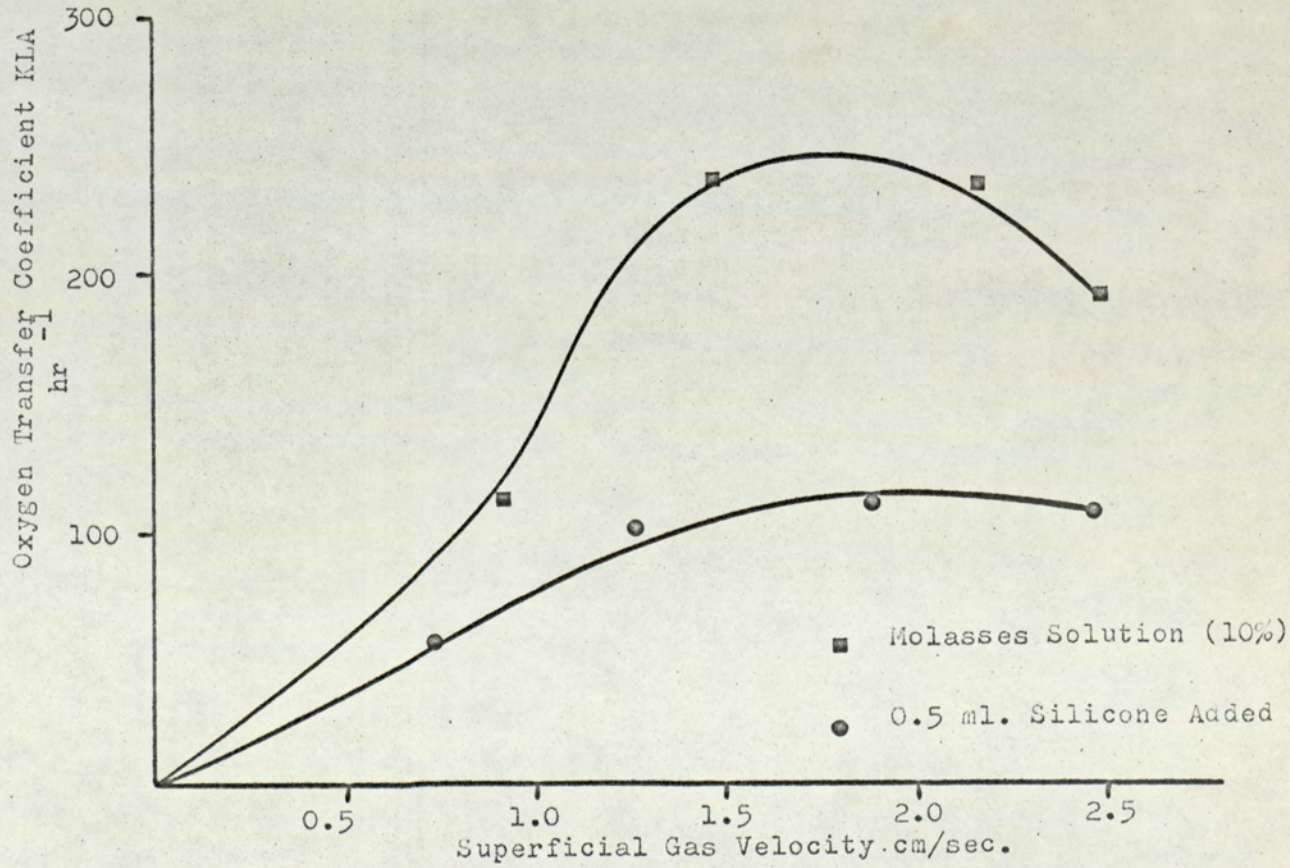
GRAPH 11

Hold-up Measurements in Water



GRAPH 12

Oxygen Transfer Measurements in Molasses Solution



Elsworth et al (1958) stated that sudden drops in the oxygen tension in culture utilising most of the oxygen available should be avoided and advocated the continuous rather than intermittent addition of antifoam to fermentations.

During most fermentations a certain amount of foaming is always encountered which can be destroyed by antifoam agents at the risk of deleteriously affecting oxygen transfer. The foaming is partly a result of increasing surface tension (metabolic activity) as the fermentation progresses (See page 187) The foaming can be overcome by fitting a mechanical foam breaker into the expansion chamber of the column (See page 34). which obviates the use of chemical antifoams. Contrary to a stirred fermenter, in which the effects of antifoam are reduced after a time because of the shearing action of the impellor blades, the tower fermenter is rather prone to the antifoam effect and does not recover very readily. Thus in the production of biomass in batch culture which gives high yields of mycelium in short times the use of antifoam is to be avoided.

3.5. Comparison with Stirred Tank Fermenters.

A comparison of the oxidation rates in the 8.6 cm. column and in a stirred tank fermenter was made. The two systems have a similar working volume and so lent themselves to this excercise. Graph 13 shows the relationship between oxygen transfer rate and the stirring speed in the S.T.R. Figures are also quoted on this graph for the optimum values obtained in the tower fermenter. It is, however, extremely difficult to compare the systems under these conditions since they have vastly different aeration devices. An attempt was therefore made to calculate the energy requirements for the oxygen transfer reaction of both fermenters to form a basis for comparison.

Oxidation in Tower Fermenter.

For small scale tower fermenters the power input per unit volume (p/v) can be calculated with reasonable accuracy by the following formula.

$$\left(\frac{P}{V}\right) = Vg.\ g.\ Cl.$$

where Vg = Superficial gas velocity

g = Gravitional constant

Cl = Liquid phase density

$\therefore$ P/V for $*_1$ in Graph 13

$= 262\ W/M^3$

or 1.59 H.P / 1000 GAL.

Average design figures are in the region of 10 H.P /1000 Gal.

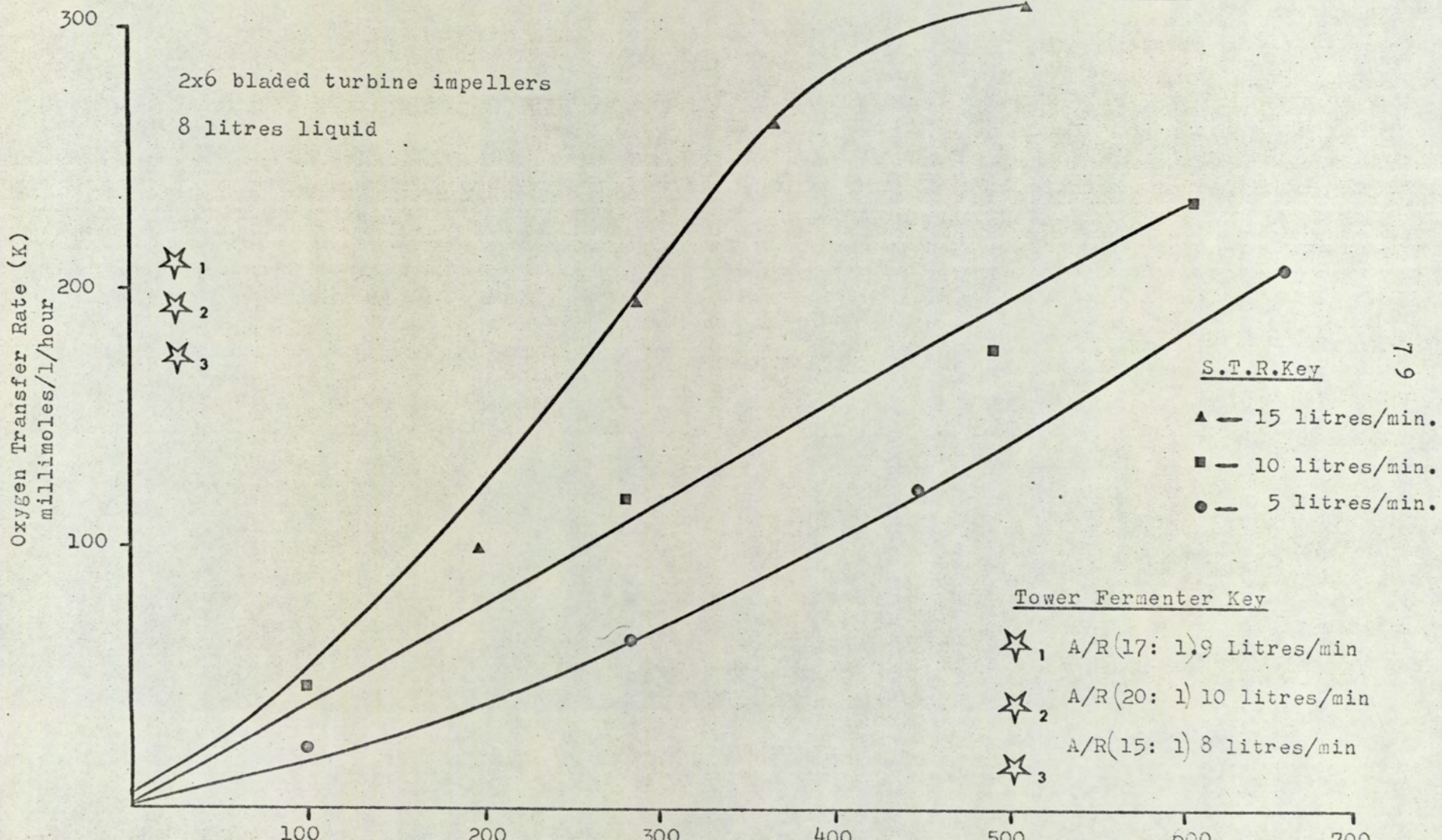
Oxidation in stirred tank reacter.

If we were to take an equivalent oxidation rate figure from the S.T.R. it would be:-

From Graph 13 - 550 r.p.m and 10 litres of air/min.

GRAPH 13

Stirred Tank Fermenter-Oxidation Rates (Showing Relation with Tower Fermenter)



The Reynodls Number (NRE) must first be computed from the formula:-

$$NRE = \frac{N.D_1^{2}.PL}{L}$$

where N = Stirrer Speed

DI = Impellor diameter

L = Liquid viscosity

$$\therefore \quad NRE = \left(\frac{550}{60}\right) \times \frac{12^2 \times 1.03}{0.01}$$

$$= 136000$$

The Power Number (Np) is now obtained

Holland and Chapman p. 78) fig. 4.4.

Np = 6 from fig. 4.4

It must be noted however that the figure quoted for Np is only accurate for standard tank configurations.

$$\text{Now} \quad Np = \left(\frac{P}{PL.N^3.DI^5}\right)$$

where P is the power required by the impeller

$$\therefore \quad P = 6.0 \times 1.03 \times \left(\frac{550}{60}\right)^{3} \times 12^{5}$$

$$P = 104 \text{ W.}$$

This is the power required by the turbine when there is no aeration.

$$\text{Now } Pgas = 0.08 \left(550 \times \frac{12}{2.54 \times 12} \right)^{3} \left(\frac{104}{7.46 \times 10^{2}} \right)^{2} \left(\frac{5.1}{28.1} \right)^{0.56}$$

$$Pgas = 0.08 \left(12.16 \times 10^{-2}\right)^{0.45}$$

$$\therefore \quad P_{gas} = 0.031 \text{ H.P.}$$

$$\therefore \quad \left(\frac{P}{V}\right) = \frac{0.031 \times 7.46 \times 10^{2}}{8.5 \times 10^{3}}$$

$$= \frac{2718}{M^3} \text{ W}$$

or 16.57 H.P. / 1000 GAL.

=

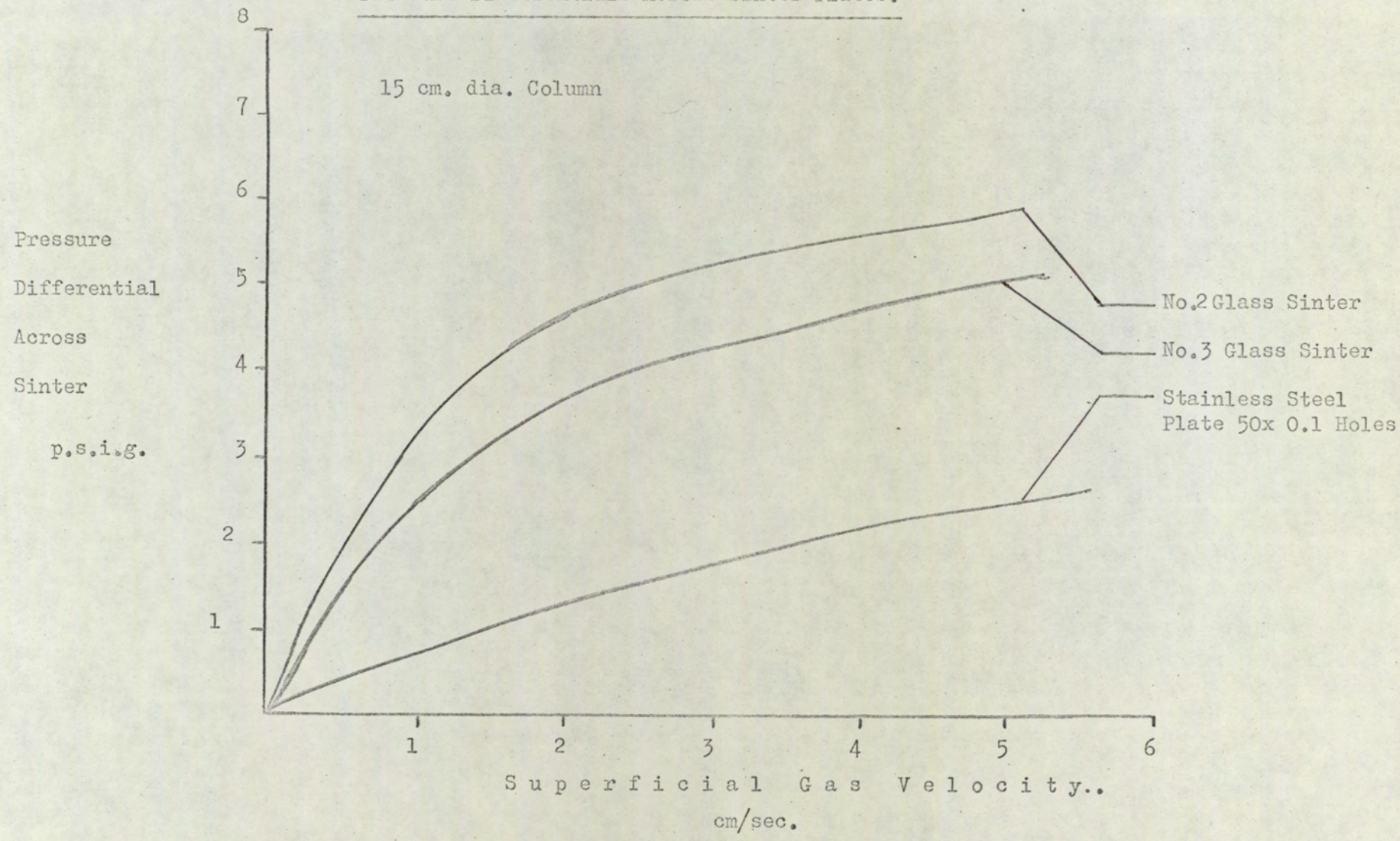
Additional power from air flow = 26.3 W/M^3

These results give comparisons as to the power dissipation into the liquid alone, therefore this is a basis for calculating the energy required to complete a certain reaction within a particular fermenter. The results do not consider the power losses encountered at the air distributor or in the motor and shaft bearings of towers and S.T.R's respectively. In order to get a true cost comparison of any process involving these systems a total energy expenditure balance would have to be made. This costing would include calculations of the work done by both the air compressors and by the stirrer motors. The best method by which this could be carried out is by metering both systems at their mains electricity supply.

It is noted that the main loss of energy, apart from the reaction system in tower fermenters, is the sinter plate or air distributer. An indirect estimate of energy expenditure due to gas passing across a sinter plate can be made by measuring the pressure drop across it. Graph 14 shows a plot of pressure differential between both sides of a sinter against the superficial gas velocity. This graph reveals that once a certain amount of energy has been expended for the initial pressure drop very

G R A P H 1 4

Pressure Differentials Across Sinter Plates.



little extra is required for incremental increases in gas flow rate. This small change in pressure is explained by the fact that there is an increasing number of pores through which gas flows as the gas velocity is increased. It must also be pointed out here that with the increase in scale of a tower fermenter the use of sinters will be obviated and the perforated plate air distributor will be used. These plates create a far smaller pressure drop due to the fact that they have larger holes and have a greater percentage of free space than do the normal sinters. The drilled holes in the perforated plate are necessarily all approximately the same size hence if the pressure differential against superficial gas velocity were to be plotted, a straight line graph would be obtained. Nevertheless, the relative pressure drops across air distributors in large columns would still be much smaller than those across the laboratory columns.

3.6. Conclusions.

From the foregoing results it has been shown that the tower fermenter can be an efficient piece of apparatus for the transfer of oxygen into solution. It has also been shown to be economical in terms of space consumption per unit volume and in that the reaction power requirements for this kind of system compared to average design figures quoted in the literature are very low.

As it has already been stated the design specifications of the aerobic tower fermenter must firstly consider the type of reaction involved and also the organism used. Once figures for oxygen requirements of the particular organism have been calculated the design of the fermenter can be worked out accordingly. In conclusion to the results obtained in this survey a column design is proposed which is considered optimal for the production of fungal biomass. The column envisaged would be composed of a tube section (height and diameter ratio determined in relation to the air distributor) which would be enlarged at its base. The base enlargement comprising of the bottom 1/3rd of the column would taper into the normal size of the stem. It is claimed that this structural deviation would have many advantages over the conventional 'A.P.V' design (Brit.Pat. 1,263, 059).In that (a) an enlarged air distributor area relative to the cross section area of the stem portion would provide for high mass transfer characteristics and greater efficiency since the flooding zone will occur at far higher superficial gas velocities than with the smaller air distributors. It is also claimed that air volume hold-up will be greater in such a system; (b). With the extra volume achieved in the lower section of the fermenter full advantage is taken of the hydrostatic pressure

imposed on this region and this greater oxygen solubility could be achieved (c) The extra volume would ensure that a greater amount of organism is present in the region of high oxygen tension than there would normally be in an uniform diameter column. This is exaggerated by the fact that there is always a slight heterogeneity as to the organism concentration toward having a greater amount at the base of the column (d) The increase in volume at the base of the fermenter would compensate for the fact that inefficiency is caused by having liquid enter the expansion chamber section of the column. Thus in a batch system, where total volume utilisation is all important this would be of some considerable advantage and (e) due to the fact that the superficial gas velocity is increasing as it travels up the column (because of the decrease in area of tube) increased turbulence will be induced thus minimising heterogeneity A recycling tube for the mould only from the top to the bottom of the column might also be envisaged this minimising the effect of oxygen starvation in the upper reaches of the fermenter.

SECTION 4

4. Morphological Variations In Aspergillus Niger.

Pellet formation and morphological variation forms an important aspect of this work since it is believed that when the pellet form is grown in tower fermenters it has certain advantages over the filamentous variety. The experiments, results and discussion that follows are devoted to the formation and differentiation of pellet culture and to the advantages and disadvantages of growing pelleted mould mycelium in submerged culture.

The morphology of A.niger was studied under submerged conditions in four fermentation systems. These were (1) Shake flasks - rotatary and reciprocating (11) Vortex fermentation jar (111) Tower fermenters (1V) stirred tank fermenter.

4.1 Control of Morphology.

The main experiments carried out on the control of A.niger morphology were done in the tower fermenter. It was found that there were two main parameters which affected pellet formation in these fermenters.

Form of inoculum.

Agitation during the germination phase of mould.

These are parameters which can affect colony formation within the bounds of this project.

4.1.1. Form of inoculum.

In general there are two kinds of inoculum that can be used, namely spores and actively growing mycelium. Within these two groups the variation in size contributes largely to the morphology that ensues.

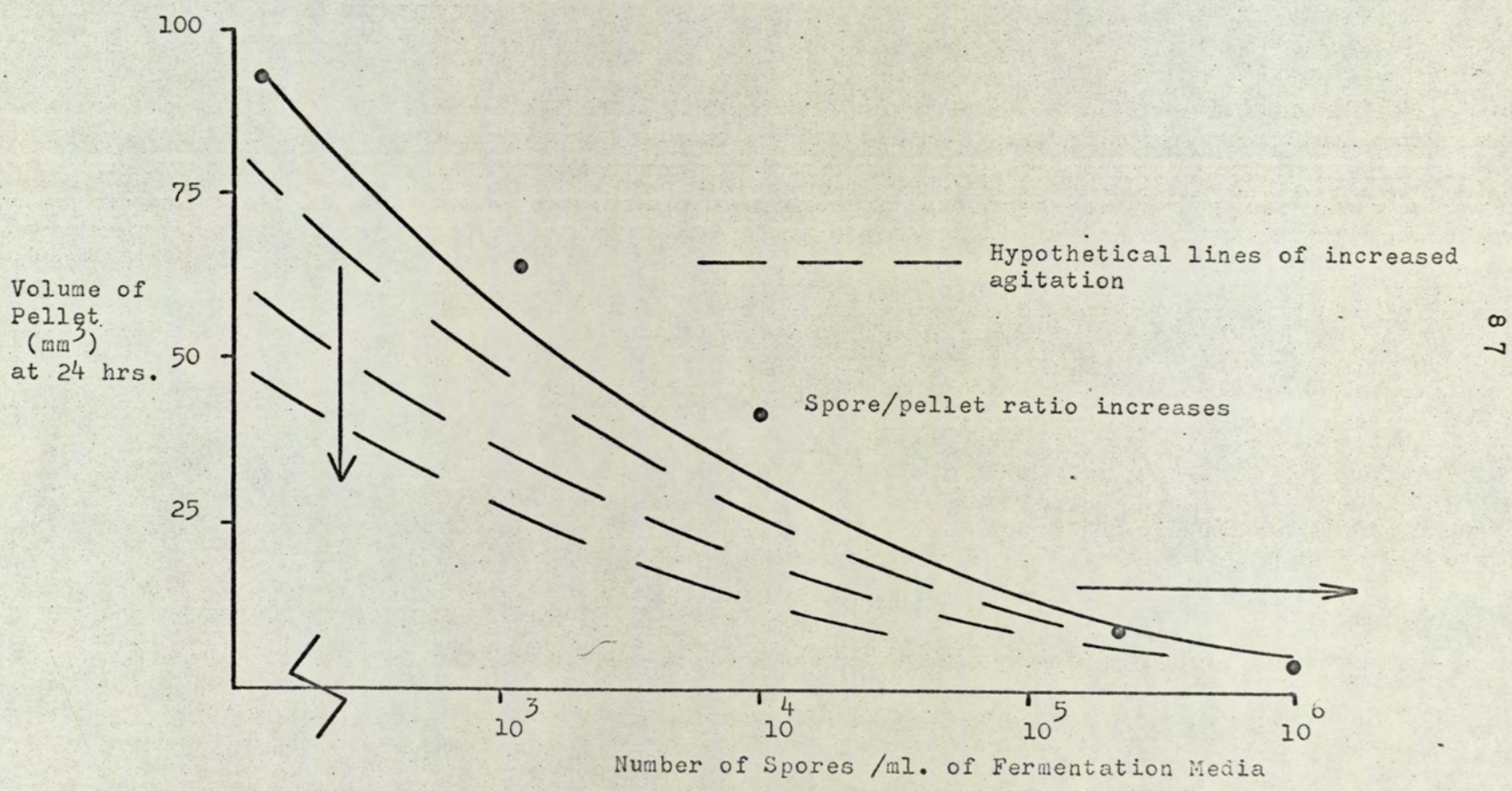
An experiment was thus performed to determine the effect of spore numbers on, the form of morphology, size of pellet, and the number of spres constituting a pellet under given conditions.

Spore counts were done 4-6 hours after inoculation; thus catching the onset of germination. Only the spores that had begun to germinate were counted to have an accurate figure of the spores responsible for biomass production. This would therefore avoid erronous counting of the vast numbers of spores which were stranded at the upper reaches of the column due to foaming and media splashing.

Graph (15) shows the relationship between pellet volumes at 24 hours and spore inoculum size. It can be seen that by increasing the initial spore count beyond certain values the pellet size decreases only slightly. It is in this area that

GRAPH 15

The Effect of Inoculum Size on Pellet Volume



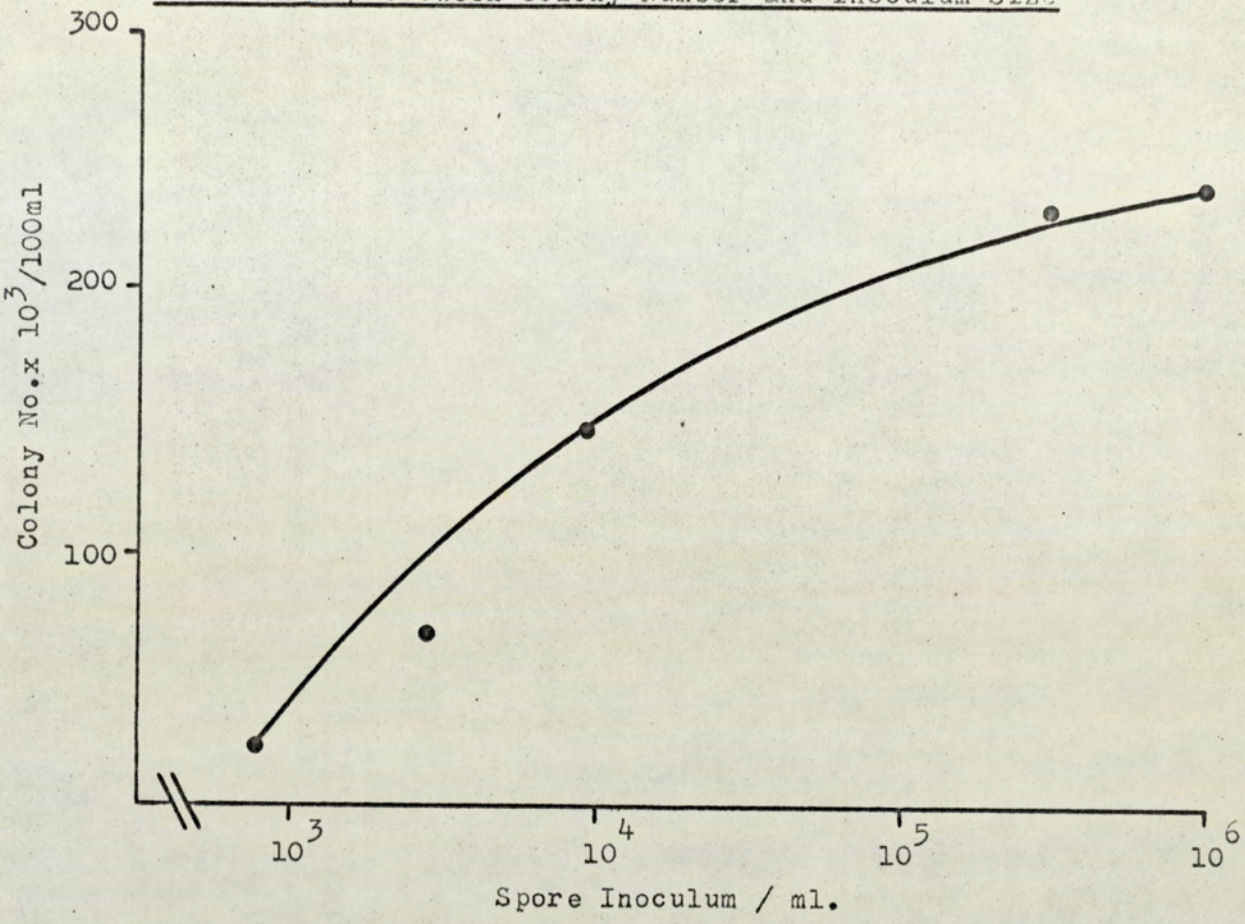
spore/pellet ratio increases considerably. The number of pellets per unit volume is also seen to increase up to this point. Up to this stage the spore/pellet ratio remains reasonably constant Graphl5 &1 6

Surfactants, for example detergents similar to 'Tween 80' are often used in fermentations and their uses were found to be advantageous in this case. This action of 'Tween 80' had the effect of allowing spores to move about singly in the pregermination stages of fermentation i.e. prevented initial sticking of the spores. Experiments with and without the use of 'Tween 80' were carried out in identical shake flasks and it was found that flasks containing no surfactants gave a small number of pellets of varying sizes. However in the flasks containing 'Tween 80' there were seen to be a larger number of small pellets giving more uniform sizes. This suggests that the action of 'Tween 80' kept the spores apart in the inoculum (this was also substansiated by microscopy) and allowed random numbers of spores or hyphae to come together in the culture vessel, thus giving an uniform size of pellet. This contrasts sharply with the flasks which contained no detergent, since in the inoculum spore-clumps must contain a wider distribution of spore numbers. These in turn give rise to pellets which are related in size to the spore clump from which they are formed.

When an inoculum of actively growing mycelium is used, the type of morphology which ensues is again largely due to its size. In general it may be said that if the inoculum produces a dry weight in the fermenter immediately after inoculation of less than 0.08 g % w/v then the mycelial strands will round off and produce colonies. Whilst on the other hand an inoculum of over 0.10 g% w/v gives rise to the same morphological form of the

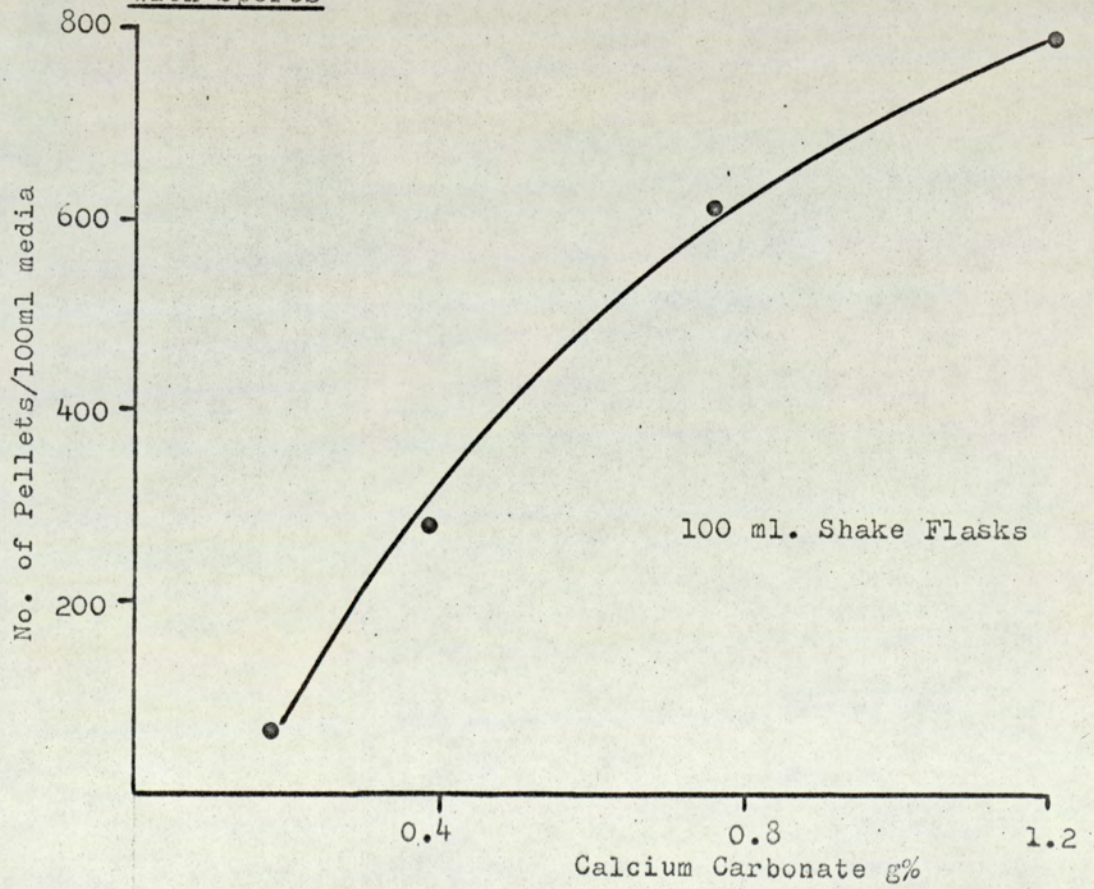
GRAPH 16

Relationship between Colony Number and Inoculum Size



GRAPH 17

Effect of Inert Nuclei on Fermentation Broth Inoculated with Spores



mould as the inoculum. However, the pellets produced by using an actively growing mycelial inoculum vary in size to a far greater degree than do those produced from spores. This again is dependant on the degree of agitation afforeded to the fermentation broth in these pre-growth stages.

Nuclei present in the media also have a great bearing on the size, shape, and number of colonies formed for a given inoculum. These nuclei are usually inert substances, insoluble in the media, and act as anchors for the spores to hang on to before even germination begins. The result of having nuclei or foreign bodies present in the fermenter is that the number of pellets is reduced and an increase in the average size of the colony is observed. Therefore the spore/pellet ratio also increases.

An experiment was performed in order to assess the effect of having this form of inert nucleus present in the media. Calcium carbonate was used as a source of insoluble nuclei and it was shown that the size and number of colonies formed were proportional to the concentration of $CaCo_3$ used. (Graph 17). This evidence may be especially important in work concerning the production of filamentous fungi from agricultural wastes because of the variety of inert substances present in such media. For example 'Carob Bean' extract used as a fermentation media contains many small particles on to which spores were seen to agglutinate. Even small cotton wool fibres accidently present in the media gave anchorage to the spores.

4.1.2. Agitation during the initial stages of fermentation.

In tower fermenters it is the air alone which provides both aeration and agitation to the system. There are no moving parts within the fermentation liquid. During the stages following

inoculation in tower fermenters the superficial gas velocity is low (approx. 0.5 cm/sec) such that 'bubbly' flow patterns exist. This provides very little agitation but sufficient oxygen transfer is maintained for the level of myceluim present. Spore agglutination or hyphal entanglement can easily take place, even in pilot scale fermenters.

An increase in the agitation during the post germination stages however, helps to keep hyphae apart for longer periods so that they can develop independently of other colonies (Graph 15). A high degree agitation in the later stages of fermentation, that is, when colony fermation has begun causes a greater degree of mycelial fragmentation thus providing new growing points and the formation of satellite colonies.

The effects of agitation rates on colony formation can be demonstrated by using reciprocating shake flasks and controlling the number of oscillations/min, thus giving some measure of the degree of agitation. Three oscillation rates were used and the same spore inoculum placed in each. This was a rather ill defined experiment as it was only possible to describe the oscillation rate by the arbitary figures on the shaker control.

TABLE 6

Speed	No of Colonies	Size of Colonies.	Description.
Slow	12	6-8 mm dia	Smooth colonies
Medium	61	3-5 mm dia	Filamentous Colonies.
Fast	79	2-3 mm dia	Filamentous Colonies.

All observations taken 24 hours after inoculation.

Although the experiment is by no means definitive the observations are however valid in that in the 'slowly' oscillating flasks a small number of large pellets were formed whilst in

the 'quickly' oscillating flask there were numerous small pellets. The form of the colonies also changed in as much as there was an increase in the 'hairy ragged' variety as the oscillations of the shaker increased. In flasks which gave a very high degree of agitation an almost purely filamentous morphology ensued.

In the stirred tank fermenter, the aeration and agitation is accomplished by passing air into the liquid through an open ended pipe or sparger situated immediately beneath a six bladed turbine impeller, which gives a high degree of agitation together with a large shear force. These two forces could not be separated under experimental conditions, and so it is seen that they had the dual effect of preventing excessive spore agglutination during the immediate post inoculation stages and also the disruption of the colony form during the post germination stages. The result of this combination was to produce a filamentous form of morphology of a similar nature to that produced in the tower fermenter when the inoculum consisted of actively growing mycelium greater than 0.1g% w/v

It is interesting to note that as the level of mycelium approached 2g% w/v dry weight and the apparent viscosity was around 1000 centipoise the agitation rate (and shear force to the bulk of the fermenter) dropped off considerably. During this period small (1mm dia.) densely packed pellets of mycelium were in evidence. These also formed in the towers when filamentous mycelium was grown to these proportions.

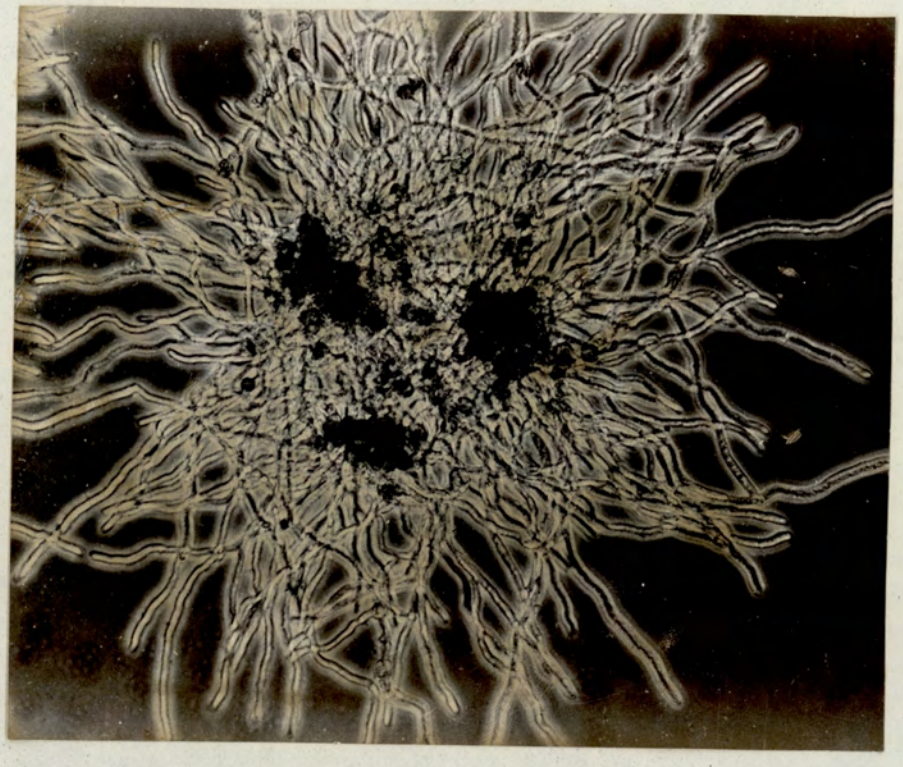
A similar situation was noted in the vortex fermentation jar except that some colony formation was still in evidence. This was due to the fact that the shear force exerted on the liquid/ mycelial broth was lower than in the S.T.R because the vortex fermentation jar did not contain baffles.

4.2. Theory of Colony Formation.

From the foregoing results a theory of pellet formation may be postulated. It was noted that when a tower fermenter was inoculated using spores that in the pregermination stages the individual spore counts were becoming smaller and the incidence of agglutination greater at each successive sampling. Thus, spore agglutination is the first contributory factor to pellet formation. It has also been noted that when no attempt has been made to disperse the spores prior to inoculation a hetergeneity in relation to size of pellet ensues. When spores are not allowed to agglutinate at all colonies form which are not perfectly spherical in shape. This is only true of the earlier stages of the fermentation, as time proceeds the irregularities tend to disappear.

The stage in pellet formation which is of greater significance is the entanglement of spores and young hyphae, which occurs immediately after germination has taken place.

Plate 5



(Mag. x 300)

Plate 5 , shows three areas of spore agglutination these being attached to each other by entwind hyphal threads.

If however, according to the theory, the agitation were to be increased immediately prior to this stage three separate fungal colonies would have formed. Also if a greater amount of surfactant was used the degree of spore agglutination would be smaller thus giving rise to more colonies. It is suggested

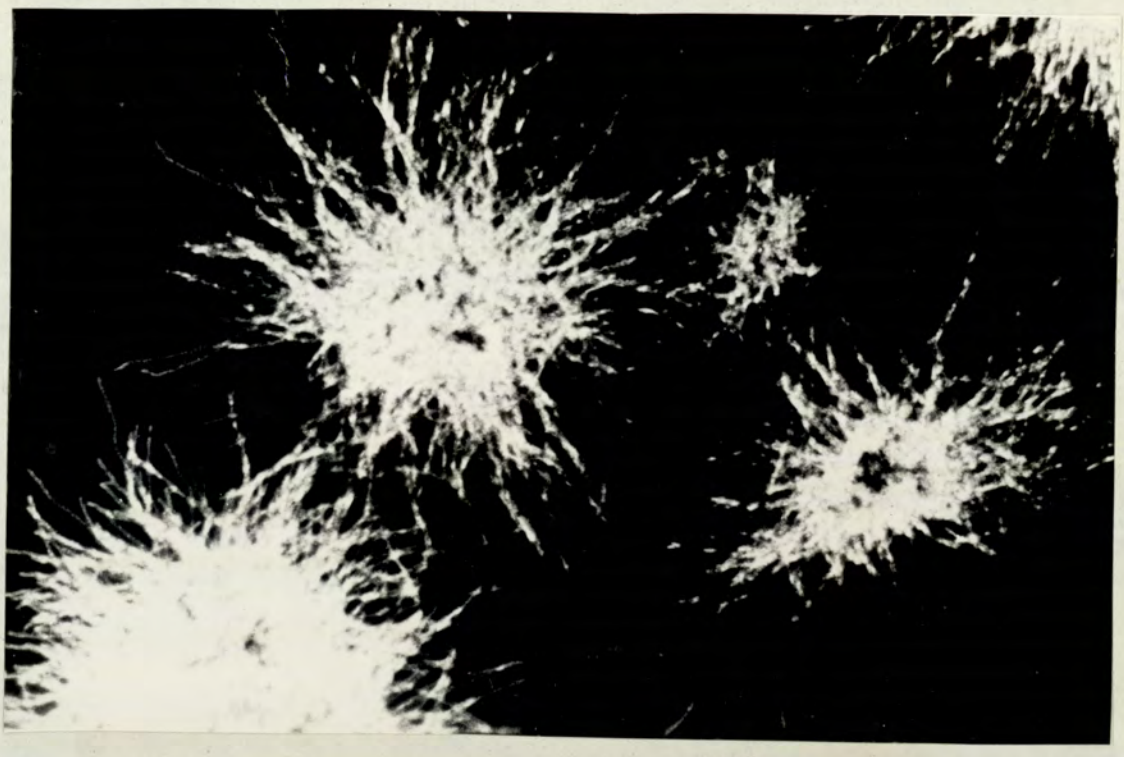
that in stirred tank fermenations agglutination of the spores still takes place to a certain degree but it is the shearing action of the impellor blades on the young mycelium that causes a filamentous morphology to develop.

Another interesting point to note is the sticking of young hyphae just after germination (Plate 5). This is not hypha l entanglement as the hyphae one are still too short for such an effect to take place. The hyphae at this stage are under 10 μ in length (This is approximately twice the diameter of the swollen spores).

When a low inoculum of actively growing mycelium is used in tower fermenters, pellets are again formed, thus showing hyphal entanglement to be of prime importance. These pellets are formed due to the swirling action of the media and the fact that there are so few pieces of mycelium per unit volume of media. It is usually observed that heterogeneity in relation to size increases as the size of inoculum decreases. However in a heavy mycelial inoculum which has a very high apparent viscosity no individual peices of mycelium are able to form into colonies and so growth continues in the same manner.

The prime direction of growth in the pellet is away from the central mass. (Plate 6). This growth continues at a specific rate until it's immediate environment becomes restricted due to other colony growth.

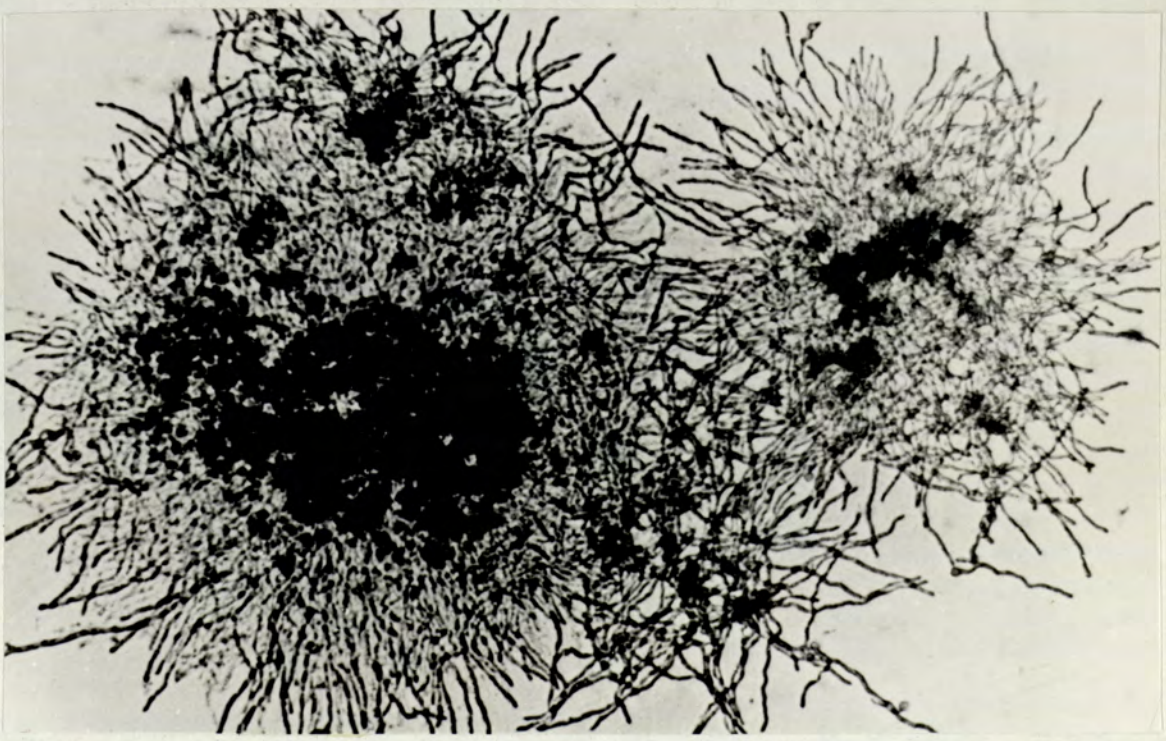
PLATE 6



Mag. x 50

At the same time intermediate hyphae are also growing, branching and entangling in the inner regions. (Plate 7).

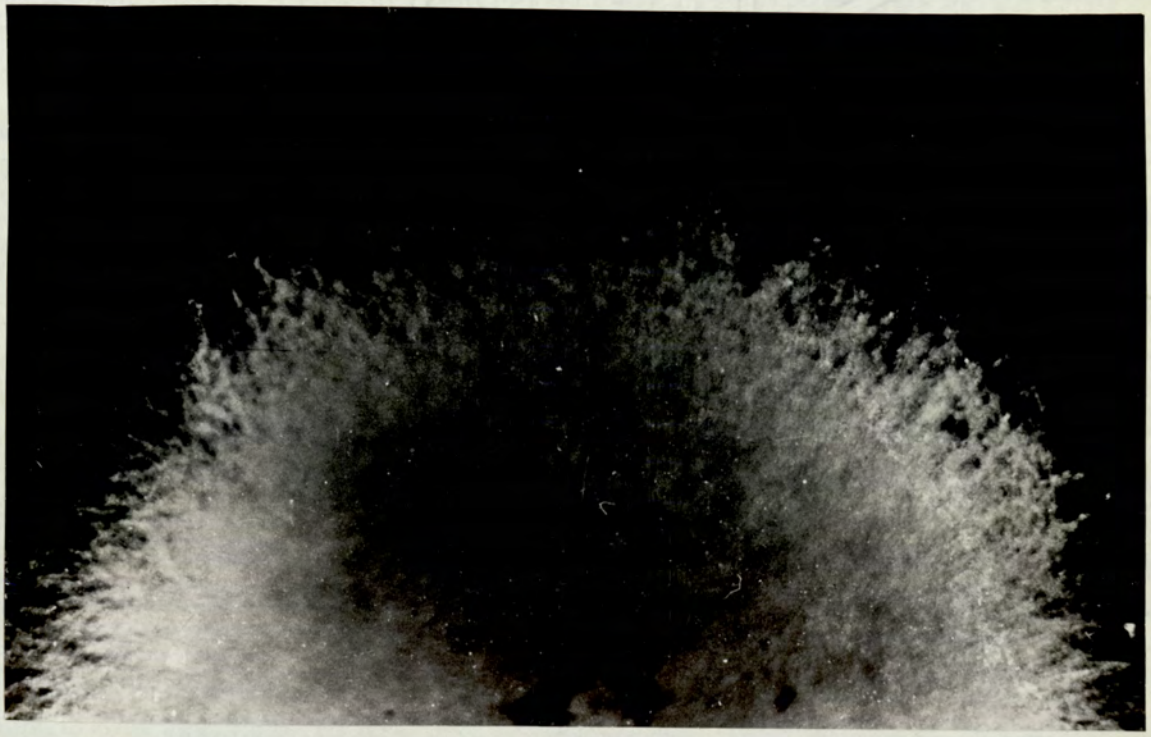
PLATE 7



Mag. x 250

The net result of this growth is that the inner potion of the colony fills up with mycelium (Plate 8).

PLATE 8.



(Mag. x25)

The ratio between the outer perimeter growth region and the inner section decreases as the colony grows (Graph 18). Therefore it can be said that the filamentous pellets are an intermediate stage toward the formation of the closely packed colony seen in Plate 8 . It should be possible by controlling the packing densities of the pellets and the growth rates of mould to obtain a particular morphology at any time during the linear growth phase of the fermentation. It is again postulated that it is the rate of growth which determine the overall form the pellet

will take after the intial pellet formation has taken place. Many workers, Foster, (1951), Martin and Waters, (1954), Steel et al (1953) have stated in the literature that various conditions in the media cause formation of pellets, for example, extremes in p H values quoted by Steel et al (1953) were said to be responsible for pellet formation. It is suggested that the p H values are responsible for changes in the rate of growth of the fungus which in turn influences the morphology. In producing citric acid in tower fermenters the enzyme and partial growth inhibiter, potassium ferricyanide, was used by, Martin and Waters, Steel et al and Davies (1969). This inhibitor had the effect of producing short stumpy hyphae which were highly branched, this naturally led to pellet formation, especially when cultured in tower fermenters.

From this general theory it would seem possible that a pellet will grow in tower fermenters to any size if the packing density of the other colonies within the fermenter is not too great. This is in fact true up to a point; colonies of 38 mm dia. have been grown, (Plate 9) but at this point they became extremely fragile and even at low aeration rates in tower fermenters they tend to fragment. However, in these large diameter pellets autolysis takes place in the central regions due to lack of nutrient transfer (Plate 9) and this is obviously disadvantageous to biomass production.

PLATE 9

3 8 mm. pellets grown in an aerated tower fermenter.

4.3. Kinetics of colony formation

The kinetics of the growth of fungal pellets has been described in part by Trinci (1970). He describes the increasing radius of the pellets to be linear with time and their dry weights to follow cube-root kinetics. Although no work was done on isolated pellets, it was noted that during actual fermentation runs in tower fermenters which produced pellets these same trends were applying in at least the first part of the fermentation where pellet concentrations were low and there was free space available for growth.

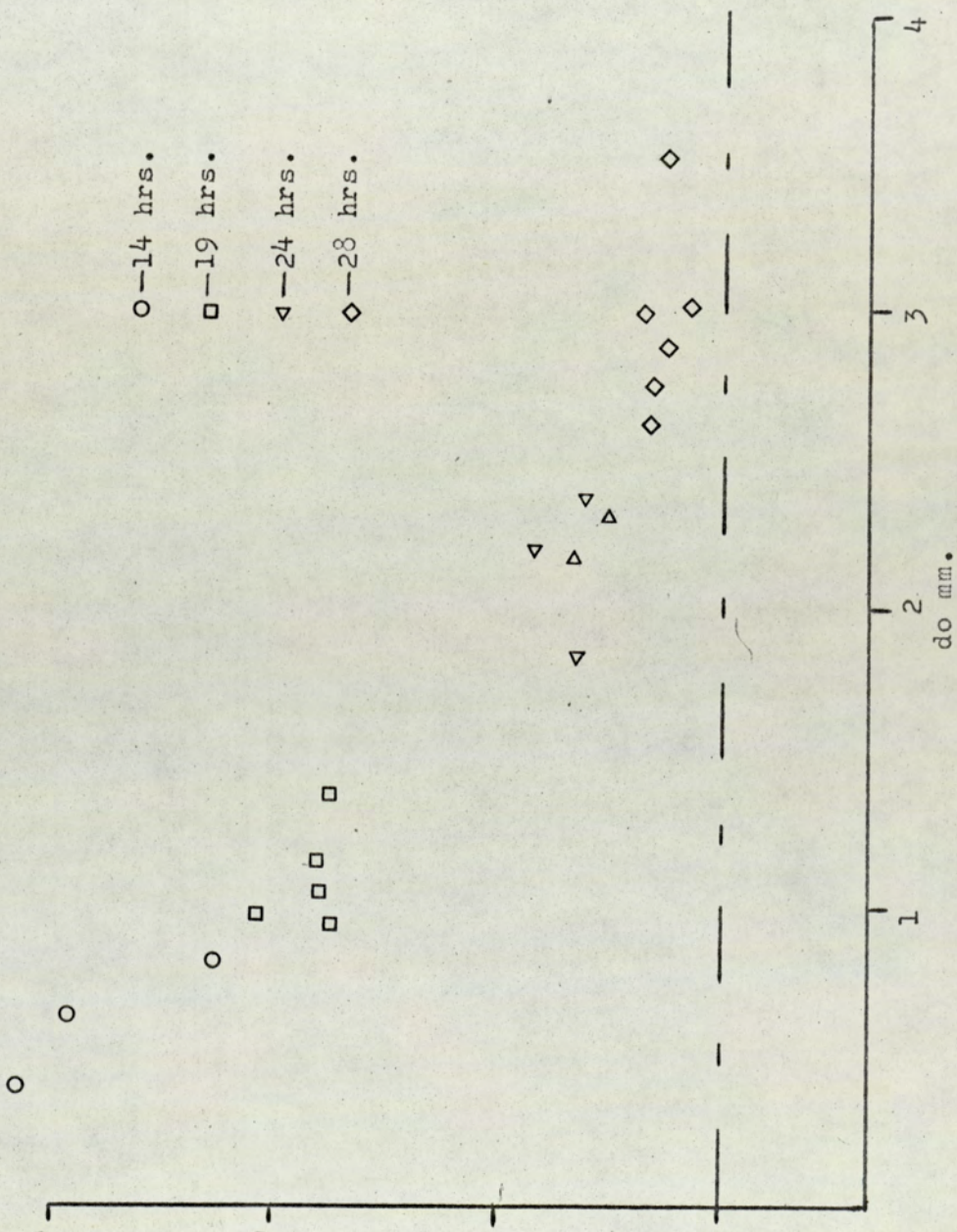
This work however, is chiefly concerned with the area of the pellet which governs maximum biomass production. It is obvious from microscopic examination that there are two different zones of growth in an actively growing colony of A.niger. There is the inner zone of densely packed mycelium which contributes largely to the weight of the pellet and an outer zone which exibits diffuse growth. Cytological observations have also shown differences in the inner densely packed zone when the pellets are old and large. On this basis the inner zone, can further be subdivided into the centremost region whose hyphae are by now devoid of oxygen and nutrient supply and therefore may be on the point of autolysing and the region on the outside of this which has an energy supply in excess of that required for maintenance metabolism and may be exibiting profuse branching. There is no defined boundary between the regions in non autolysing pellets, but a large zone of transition exists where there is a gradient in the cytological variation of the hyphae. Since it has been suggested that the inner zone contributes mainly to the weight of the pellet, and the outer zone decides the final volume of the pellet, it is the relationship between the two zones of growth which is of interest here.

Pirt (1966) suggested that if oxygen could enter the colony by simple diffusion alone, growth of the pellet would be restricted to a peripheral zone 77μ wide. In the active growth phases of A.niger (M1) however, the zone (designated $\frac{do-di}{2}$, where do is diameter of pellet and dl is diameter of the inner zone) is over 200μwide. It is now suggested that oxygen transfer in this region is fascilitated both by simple diffusion and also by the action of eddy currents. This is because of the very loose texture of the peripheral zone of the pellet (Plate 6). The lengths of these hyphae are such that movement is possible due to the agitation of the media outside, this setting up slight currents in the outer regions of the colony. It is now conceded that simple diffusion into the inner zone (diamter designated d_1) may be restricted to an extent of only 77μ. This is due to the much denser nature of the colony in this area. Therefore we may have a much larger zone of mycelium which has an adequate supply of oxygen than was at first indicated.

Twelve to fifteen hours after inoculation with spores, the pellet form is easily recognisable to the naked eye, from this point the do/d_1 ratio approaches unity with time. Here we may consider two concentric colonies growing outwards at a specific rate. The inside diameter will approach the outer. This was shown experimentally (Graph 18). The pint at which do stops increasing is where the packing density of colonies within the fermenter has become too great. The further increase of do is therefore hampered by both the fermenter itself and by the other colonies. When a large spore inoculum is used which gives rise to a number of colonies then do is seen to stay the same size throughout the growth phases of the fermentation. (Graph19). This is because of the relative free space in the fermenter being so low. This situation seems to exist when the free

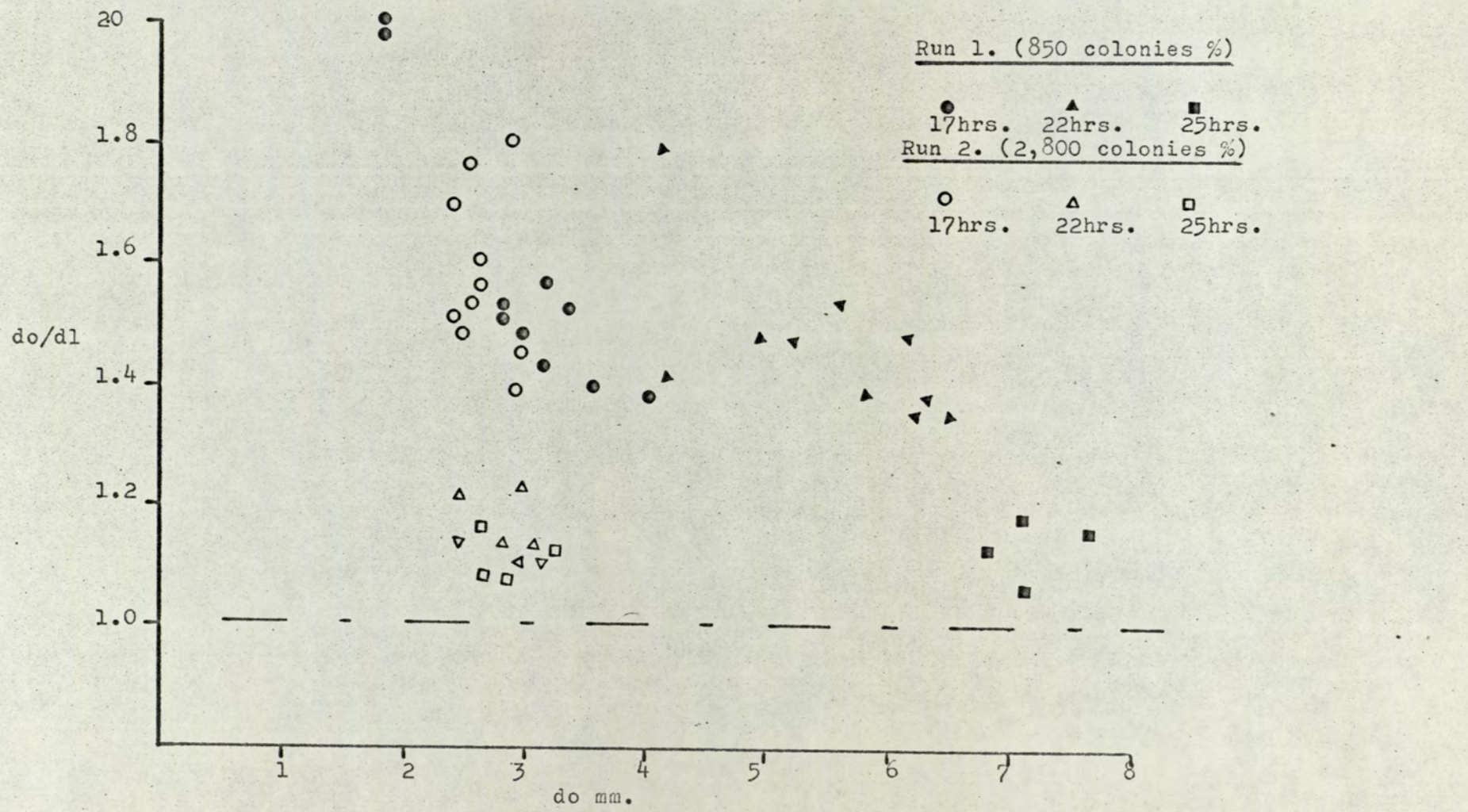
GRAPH 18

Relationship Between Internal and External Diameter of Colonies of Various Time Intervals



GRAPH 19

Relationships Between the Internal and External Diameter of Colonies at Various Time Intervals

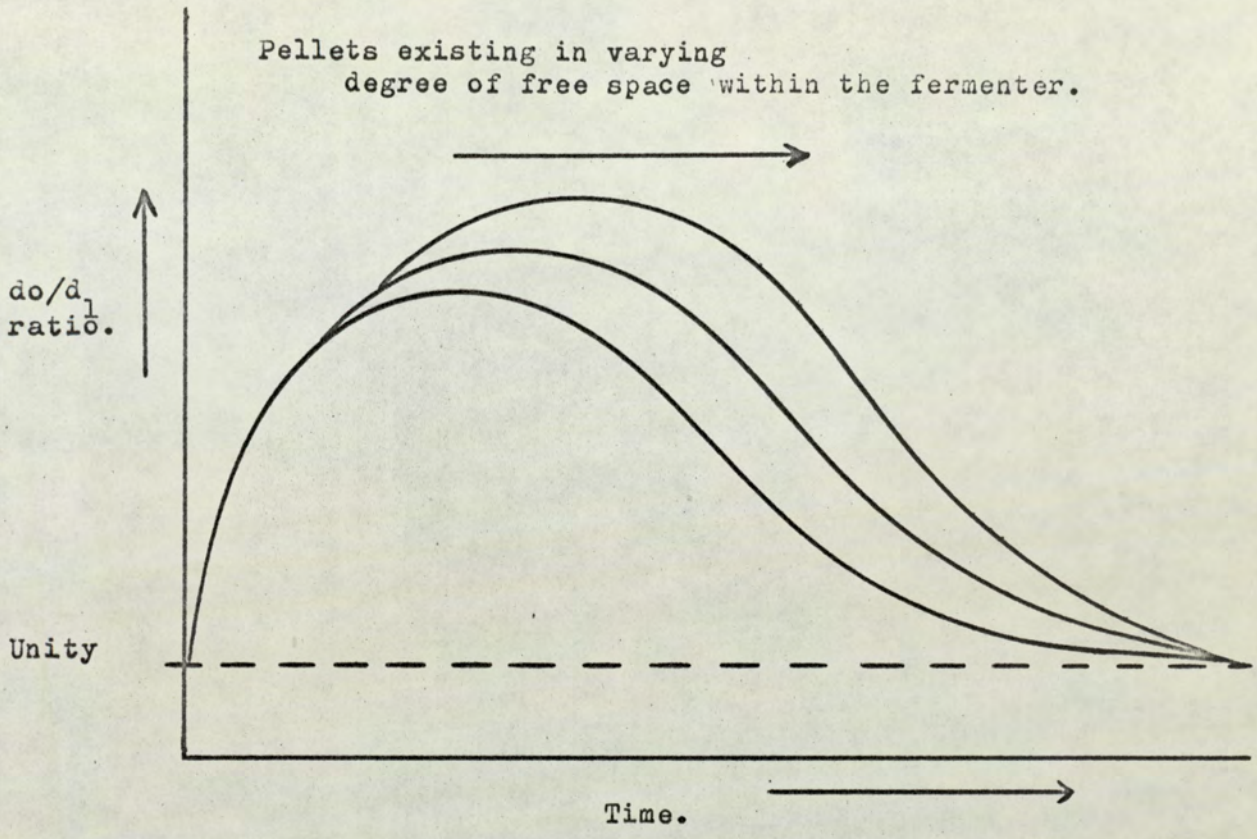


space is approximately 70% of the fermenter volume. In this instance do/d_1 approaches unity due only to growth of the inner zone. However, when less spores were used fewer colonies formed (spore/pellet ratio similar), these exist in relatively free space, which in this case was approximately 90% of the fermenter volume. Thus, do was able to increase at a faster rate and the do/d_1 ratio would take an increased time to approach unity. It is seen that by this mode of growth a less dense pellet is formed. It must be emphasised at this point that the concept of free space within a fermenter is also a function of agitation of the media, since at high agitation rates pellet movements will necessarily restrict their own development.

The general aim of this excercise is to produce large volumes of mould biomass in the tower fermenter. Postulations may now be made as to the mode of producing high dry weights of organism in the colony form. It has been shown, by studying the hydrodynamics of these pellets, that it is possible to predict their relative densities. These densities have a direct bearing on the dry weights of a particular pellet. (The densities were estimated by measuring their terminal velocities in water).

As a general rule it may be said that the very dense pellets have a low specific growth rate (their diameters following cube root kinetics for most of the fermentation). These pellets have short highly branched hyphae that form a compact interwoven mat of mycelium. The less dense pellets have a higher specific growth rate and exibit less frequent branching in the outer regions of the colony. It is believed that these densities may be predicted to a certain extent by the size and number of the colonies within the fermenter volume and also by controlling the growth rate of the organism (Graphic Model).

GRAPHIC MODEL OF PELLET GROWTH.



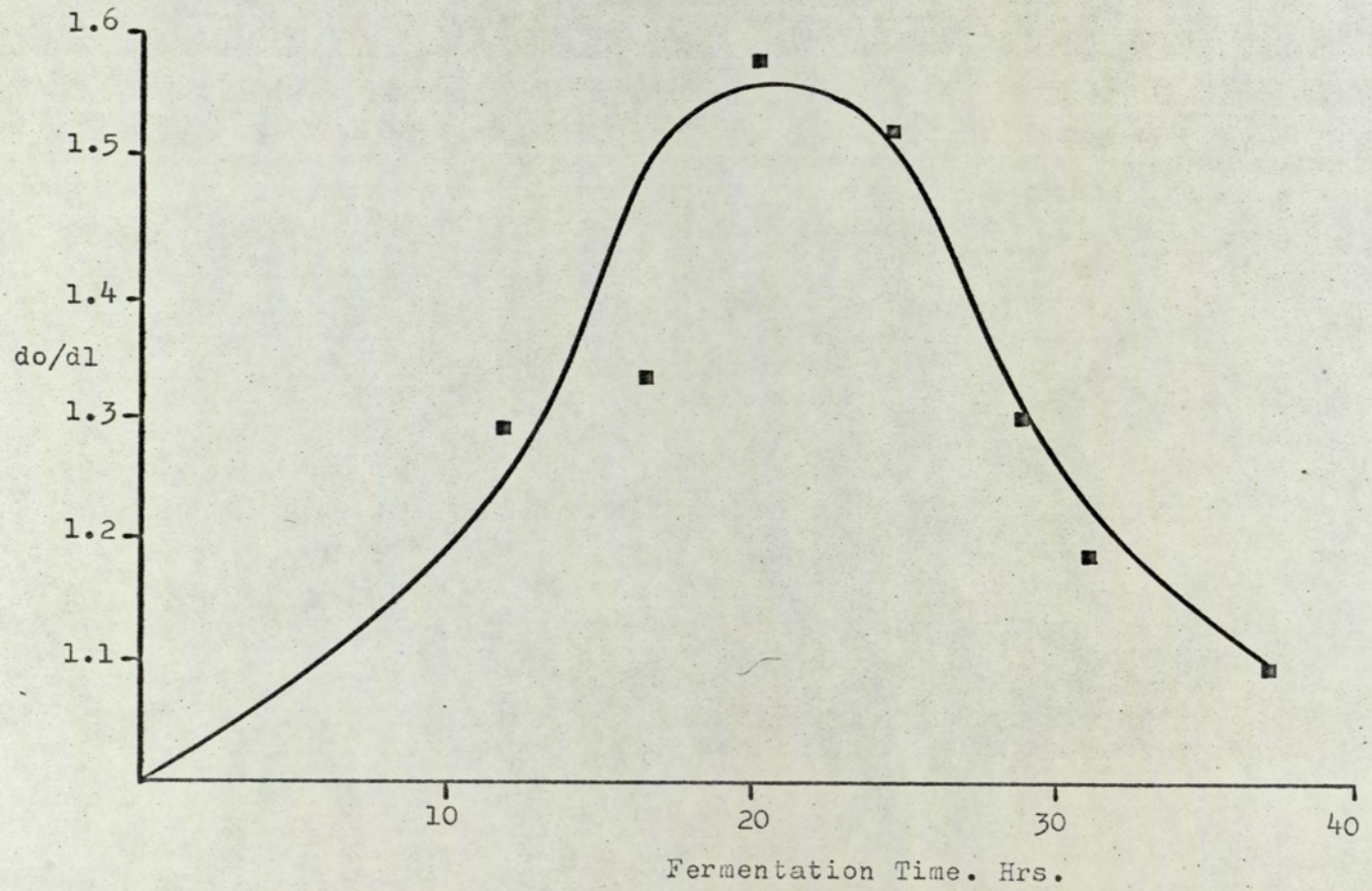
The peak heights will necessarily depend on (1) the specific growth rate of the organism. (11) The degree of spore agglutination or hyphal entanglement (which are directly affected by surfactats and agitation).

It can therefore be shown diagrammatically that there is an optimum peak height for pellet growth and density. The actual distances between the two zones of growth should be controlled so as, (a) not to exceed that distance over which mass transfer can operate effectively, and (b) should not be so small as to hinder further development of the colony.

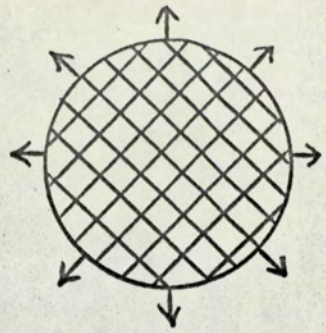
Graph 20 , which is taken from actual fermentation, gives some reality to the model.

GRAPH 20

Variation in the External and Internal Diameter Ratio with Time



a

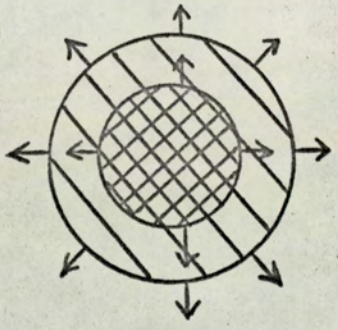


Discrete colony with small do/d_1 ratio.

Growth is in one direction only.

Specific growth rate will be depressed.

b



Filamentous pellet with large do/d_1 ratio

A larger do/d_1 ratio may limit

mass transfer of oxygen and nutrients.

Case (a) might be achieved by the use of a very high inoculum or else by inhibitors of the type used in the citric acid fermentation (Martin and Waters) or extremes in p H values (Steel et al). Case (b) on the other hand is achieved with isolated pellets and very little agitation, or when they are very young.

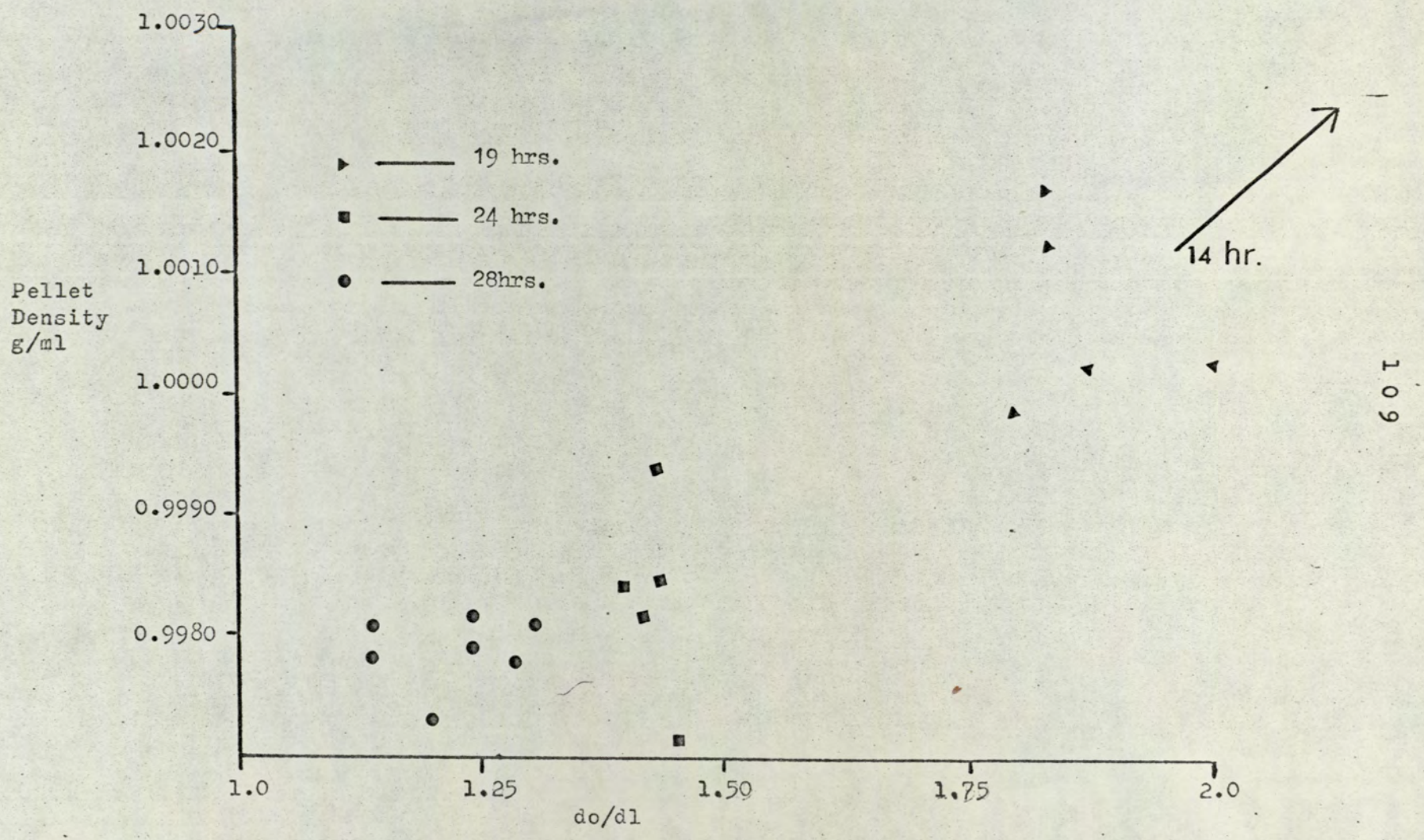
From Graph 21 which shows the density variation in relation to the do/d_1 ratio during a fermentation run, it can be seen that the trend is toward the denser colonies having the largest ratio. When the pellet density is plotted against the external diameter (do) however, (Graph 22) it can be seen that the less dense pellets are in fact the largest. Graph 23 confirms this to a large extent although this fermentation had far more irregular sized colonies. This irregularity was caused by the fact that no surfactant (Tween 80) had been used to disperse the spores prior to inoculation. Since the size of the colonies vary it also follows that the specific gravity of the hyphae will also vary and so produce different density figures. It should also be pointed out that irregular sized colonies in a such a system will have different age distributions of mycelium.

The mycelial or colony age affects the density in respect to changes in cell specific gravity and also autolysis. It is believed however, that cell breakdown is not merely a continuation of changes in cell specific gravity. Autolysis occurs when the supply of essential nutrients to the cells are completely cut off, whilst reduction in specific gravity is a natural phenomenon of cell ageing. Graph 24 shows how the cell specific gravity varies as the fermentation proceeds. Therefore when considering the overall density of a pellet it is important to note at which point there is a turn-over in the cell specific gravity.

Autolysis on the other hand occurs in colonies whose diameters have become so large as to restrict mass transfer (Plate 9) or have become so dense that even in the smaller colonies 5 mm dia. mass transfer of oxygen or nutrients is restricted. (Plate 8). Colonies of the first kind are similar to those shown in Graph 18 and have had near optimal growth conditions

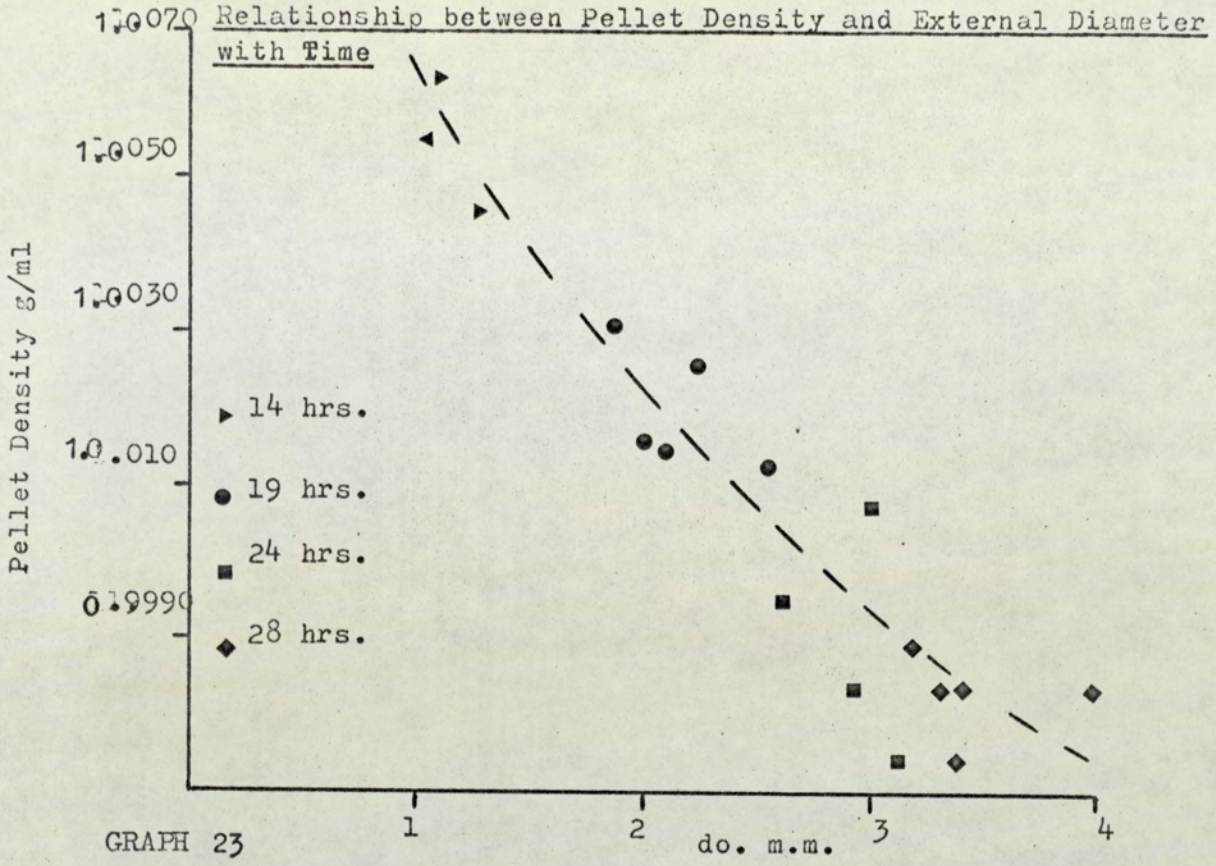
GRAPH 21

Variation in Pellet Density during Fermentation



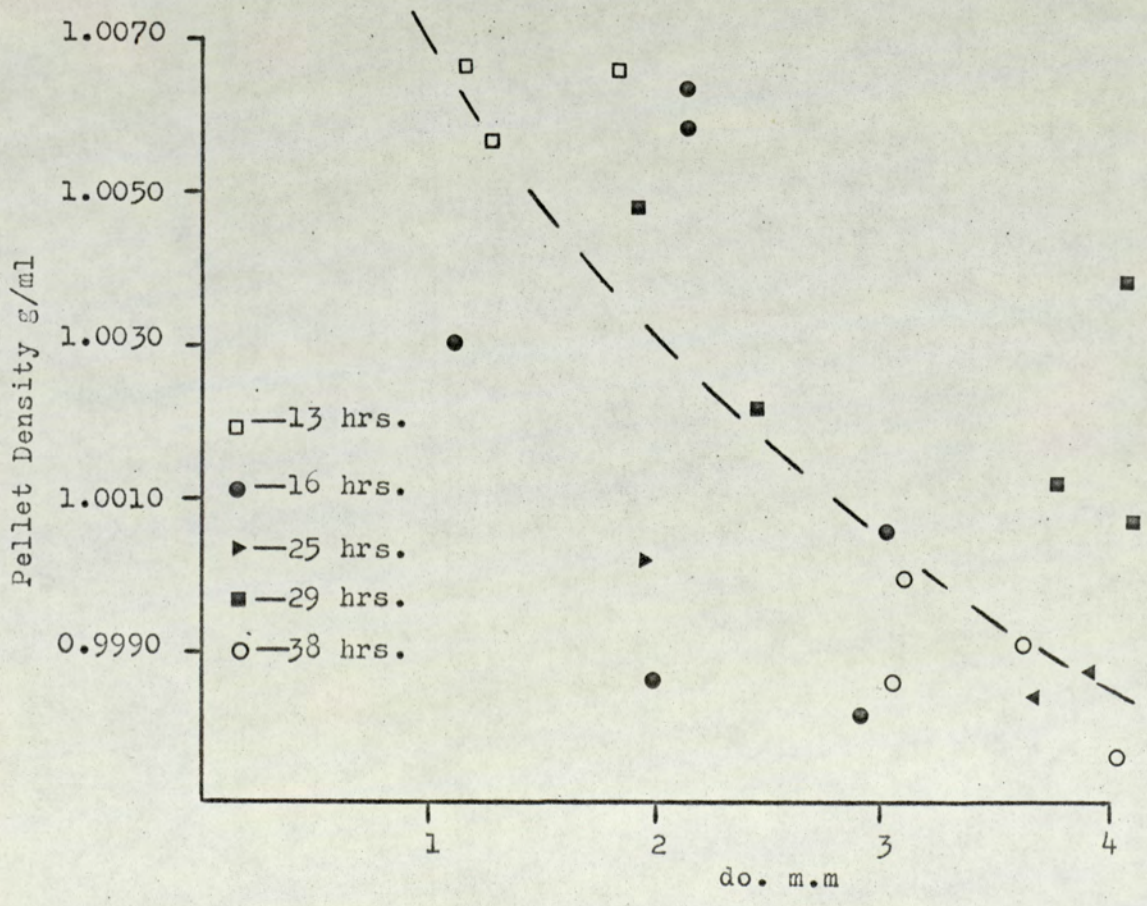
GRAPH 22

Relationship between Pellet Density and External Diameter with Time



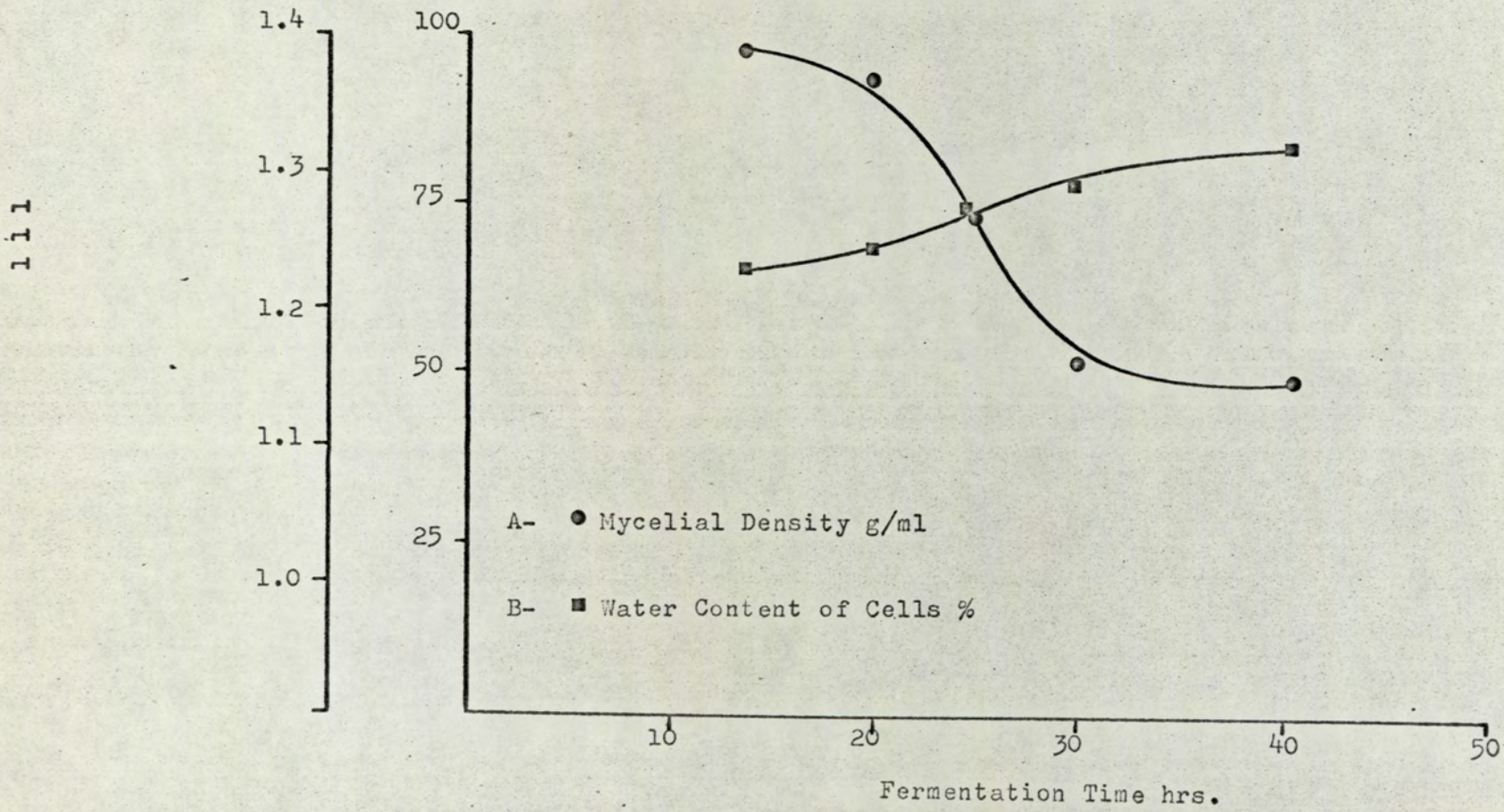
GRAPH 23

Relationship between Pellet Density and External Diameter



GRAPH 24

Relationship Between Cell Density and Water Content



whilst the denser variety were grown in suboptimal conditions (high C:N ratio, low temperature). It was noted that under sub-optimal conditions of growth the frequency of branching along a primary filament was more intense than that under optimal conditions. The reasons for this are not understood but it is considered to be an important aspect in determining ways of producing large amounts of biomass per unit volume with pelleted mycelium.

If we were to consider a mould pellet which contained such an amount of dense mycelium on its outer perimeter so as to restrict mass transfer into the central portion autolysis would occur and a space would form in the middle of the pellet. Now if the density measurements for this type of colony were to be corrected on the assumption that the autolysed centre was still mycelial in nature a situation would be reached whereby the density of the pellet as a whole would approach the density of a single cell. This would then mean that there would be very little water associated with the pellet structure itself and that the colony would consist of very dense, compact, interwoven hyphae. Under this condition the colony voidage (amount of extraneous water associated with the colony) would approach zero. This is the ideal situation for the production of fungal biomass by using pelleted mycelium. See table.

TABLE. 7

	e	X	Mw	M
(a) Fast growing loose pellets	0.9883	0.23	0.819	1.17
(b) Slow growing dense pellets (autolysed)	0.9878	0.24	0.819	1.22
(c) Slow growing dense pellets (assuming no autolysis).	0.9750	0.50	0.819	2.50

Where e = Colony Voidage - volume fraction.
X = Dry weight g% of Colony.
Mw = Mycelial Water.
M = Mycelium weight g% of Colony.

These results suggest that it would be advantageous as far as growing large volumes of biomass are concerned, to produce very small, dense pellets which exhibit prolific branching and would also have a slightly reduced linear growth rate. This was actually achieved to a certain extent by growing A.niger (M1) on defined sucrose based media and using ammonium sulphate as a nitrogen source. The morphology was that of very small disc shaped colonies which were relatively dense (approx. 1.020 g./ml). A dry weight of 15g/litre was achieved in 50 hours. It was also observed that oxygen limitation (with respect to the medium) was not in evidence and there was apparently no other restriction to further growth.

4.4. Fluidisation Phenomena.

As a conclusion to this section it was decided to calculate the maximum possible amount of biomass that could be achieved in these fermentation systems. This was done by using data obtained by James (1972) on the fluidisation behaviour of the pelleted mycelium.

James' results revealed that the minimum possible voidage in a packed bed of colonies was 85%. By experimentation it was shown that the dry weight of mycelium was 70% that of its wet weight.

Now, in this calculation it is assumed that there is no immobilised water associated with the colony itself. This is due to the fact that they are continually being agitated and it is assumed that all the immobilised water is continually being replaced. It is admitted that this is an over simplification since some of the water will always remain immobllised in the dense central core of mycelium.

By measurement the specific gravity of the dry mycelium was found to be approximately 1.24.

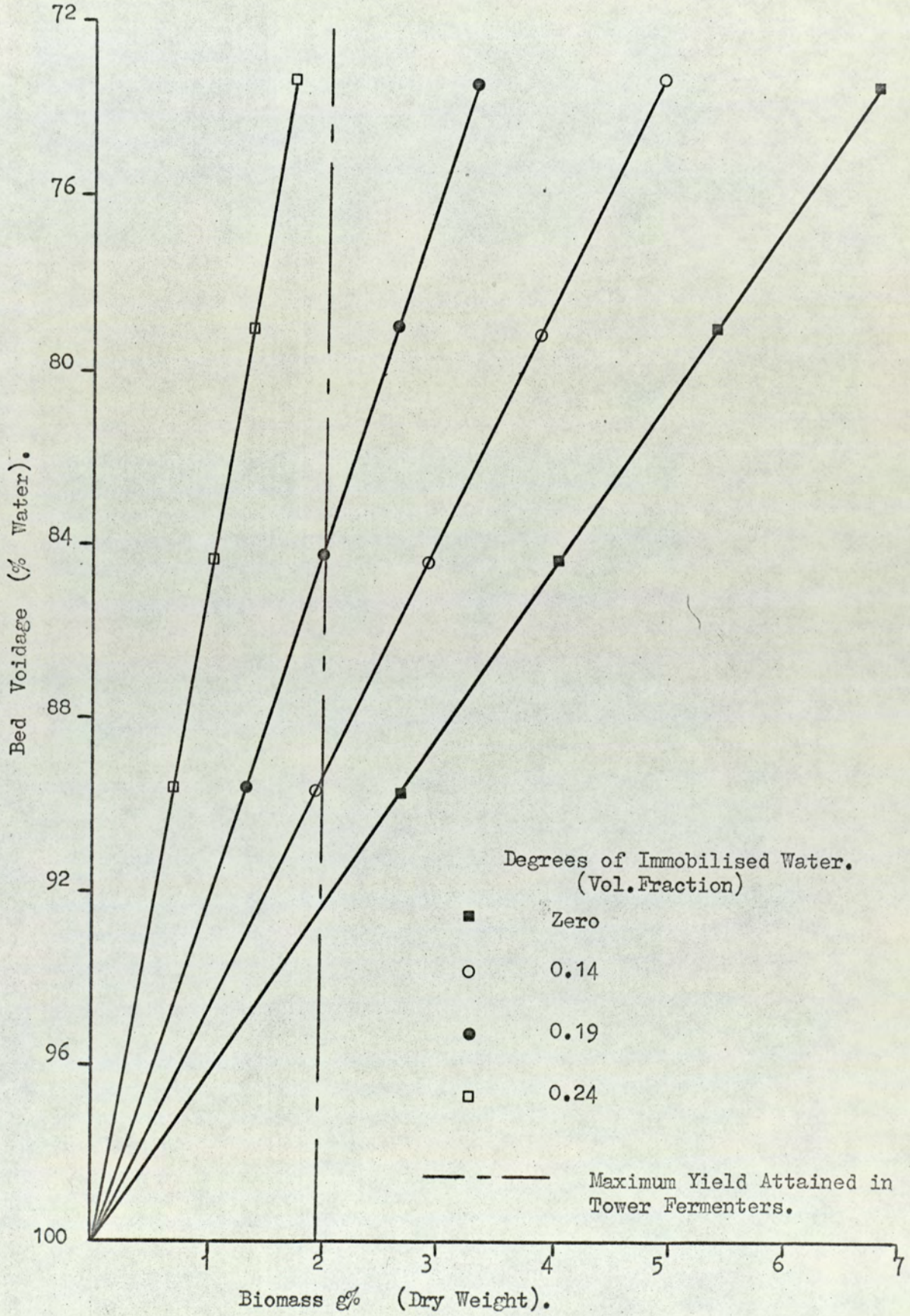
These results can be summarised by using Graph 25 . The line shown on this plot is that assuming the immobilised water to be zero. If an the other hand there is some of this water present in the colonies then the line will move toward the right.

It can therefore be seen from the graph that the maximum possible dry weight of organism achievable by the use of pelleted mycelium is in the region of 2g% w/v. This has already been achieved using pellets in the tower fermenter.

By the use of other than rounded colonies, for example disc shaped ones as mentioned earlier in this section, they could assume an even lower packed bed voidage. This fact being true a

GRAPH 25

Estimated Biomass Yield from Pelleted Mycelium.



greater dry weight might be acheived using this morphology. As far as the filamentous morphology is concerned it is considered that a greater packing density would also be achieved by its utilisation.

4.5. Conclusions.

From the results presented it is now possible to determine the type of morphological variant that will ensue under a particular set of culture conditions. It is also possible to grow and to control any colony size that is required, suggestions have also been put forward of how to produce the highest dry weight of mycelium per unit volume. It must be concluded therefore, that by the use of mycelium in the colony form in tower fermenters for the production of biomass, there are a number of variables that must first be considered. It is accepted that a fine balance must be maintained between a three parameter system in which growth rate, pellet density and possible autolysis play the major roles. The economics of the process will prevail when the decision is made on whether to produce a large volume of mycelium over a long period of time or smaller volumes over a shorter period.

SECTION 5

5. The Affect of Morphological Changes on the Fermentation.

It has already been stated that the use of the tower fermentation system for the growth of moulds facilitates the control of the moulds morphology. This control can be put to good use in the tower since it is considered that there are several advantages in growing the mould in pellet form. It is believed that there is an optimum morphological form of this fungus which would give the best yields in terms of growth and carbohydrate consumption, in terms of the energy required for aeration and agitation of the fermenter, and also in terms of maintaining the fungus in a state of active growth. The optimum morphology, or more specifically the pellet size, is not the same for all the above parameters and once again a balance must be made in choosing the form in which the organism is grown.

5.1. Oxygen Utilisation and Requirements of A.niger.

There are many parameters that can affect the respiration in mould mycelium. Some of these parameters involve the medium constituents, therefore as far as possible these were kept constant. The parameters in relation to the fungal mycelium however, especially the morphology were studied in some detail.

In general there are three main parameters that can affect oxygen uptake in A.niger in relation to its morphology. These are:- Age of Mycelium

Mass Transfer Difficulties

Changes in the Direction of Metabolism.

When a fungal cell ages many changes occur the specific growth rate decreases, the cell wall becomes thicker and the cells vacuolated. The cell now relies on a smaller amount of energy, termed the energy of maintenance and is probably used for such functions as turnover of protein and nuceic acids, osmotic regulation and p H control. (Bainbridge 1969) This means that the specific utilisation of oxygen drops far below the value for young actively growing cells. Therefore in batch culture specific oxygen utilation drops throughout the later fermentation stages.

When the specific oxygen utilisation of the mould drops below the requirement for maintenance metabolism then catabolic changes occur within the fungal cell. If this situation is allowed to occur signs of autolysis will appear in the cells, leading to the eventual break down of the walls. Mass

transfer difficulties are usually responsible for this condition, either mass transfer into the medium or the restriction in the transfer of materials into the cells. It is usually this last difficulty that can occur with pelleted mycelium. Particularly when pellets are of such a diameter (or density) as to restrict the transfer of oxygen.

Oxygen utilisation may also vary in cases where a change in the direction of metabolism occurs. This change in the metabolic direction is away from the carbohydrate utilisation for the production cellular material toward producing extracellular metabolites, thus lowering the carbohydrate conversion efficiency. In the A.niger fermentation in particular various organic acids e.g. citric, gluconic and oxalic acids may be produced in considerable quantities under the correct conditions (usually highly aerobic fermentation). It is normal that such metabolic changes take place in mature mycelium (since it is considered that they are non-growth associates metabolites).

The first two cases (Mycelial age and mass transfer difficulties) probably represent the main conditions in which oxygen uptake is reduced, whilst organic acid production is of secondary importance.

An experiment was performed in which both filamentous and pelleted mycelium were grown side by side in a stirred tank reacter and tower fermenter respectively. In the filamentous case the assumption was made that there was relatively little mass transfer restriction toward the vicinity and into the cells of the mycelium (This assumption is reasonable since the 'Warburg' respirometer was used to assess the oxygen uptake rate and the mass of mycelium could be controlled to avoid the mass transfer restrictions which would be imposed by direct readings taken

from the fermenter). In the experiment performed it was noted that the growth rates in both fermenters were similar, and it is suggested that the rate approached μ max. From Graph 26 which illustrates the changes in specific oxygen utilisation during the course of the fermentation it can be seen that maximum utilisation occurs just after the onset of germination. This shows that the young cells use more oxygen than the older ones. The peak oxygen utilisation is around 200 ml/g/ hour. This figure then tails off considerably during the phase of linear growth until it levels off at around 20-30 ml /g/ hour. The reason for this decline in specific oxygen utilisation is that the ratio of the volumes of young cells to old cells is reducing throughout. Graph 26 a.

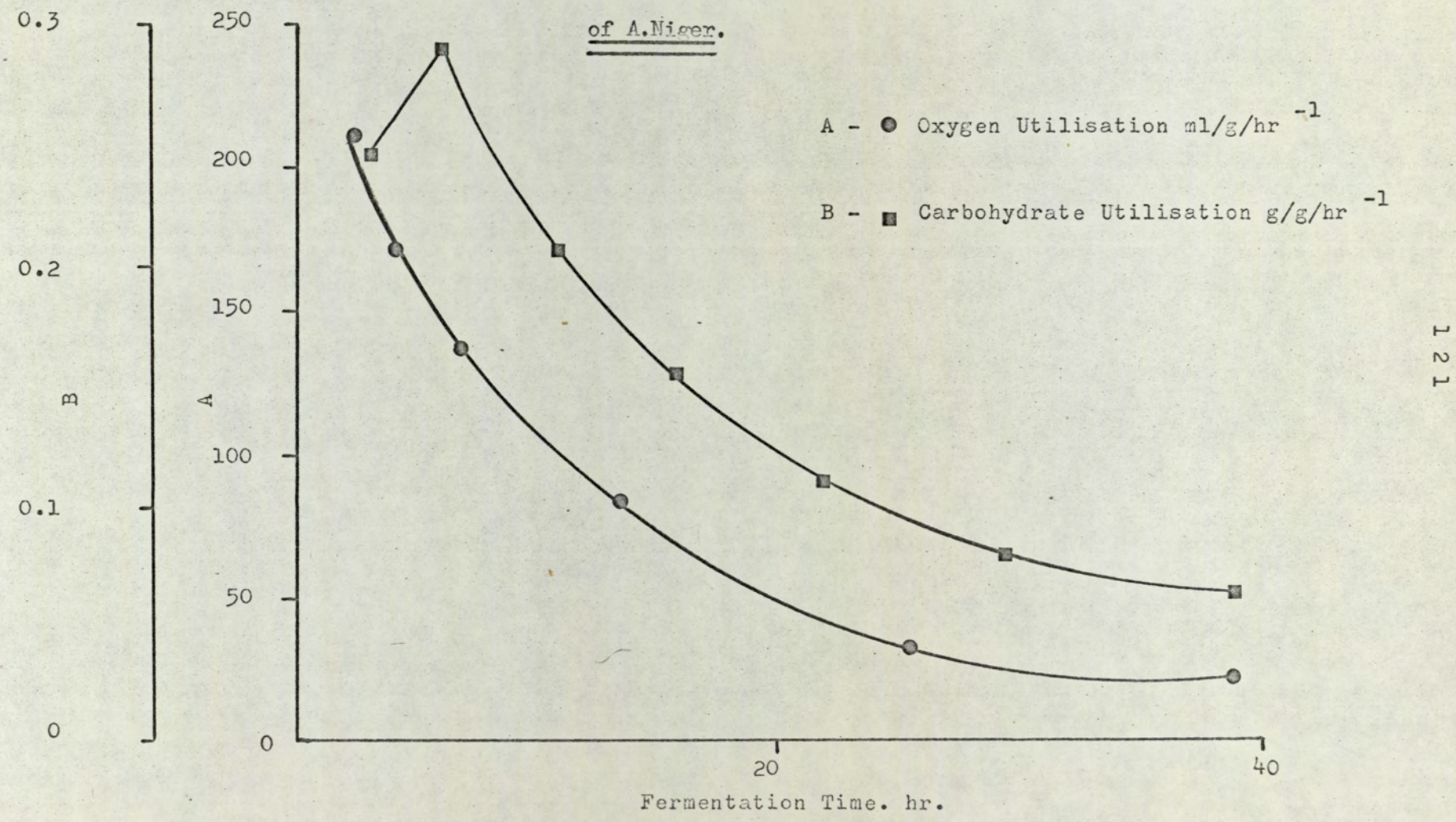
Graph 26b. also shows how the oxygen transfer in pelleted mycelium behaved throughout the fermentation run. This graph presents us with figures which reveal that the pellets are using less oxygen than corresponding amounts of filamentous mycelium.

In order to show that the oxygen tensions within the respective fermenters had no affect on the oxygen utilisation in the 'Warburg' respirometer a flask of mycelium was kept anaerobic for eight hours. When the oxygen utilisation was checked before and after the time given no significant differences were recorded. A corresponding test was carried using high oxygen tensions in the S.T.R, no differences were seen.

The results shown here appeared to be reasonably consistent over a number of runs. However the lower oxygen utilisation rate at the beginning of fermentation for pelleted mycelium can not be completely explained. A probable explanation being that errors might have arisen due to the small

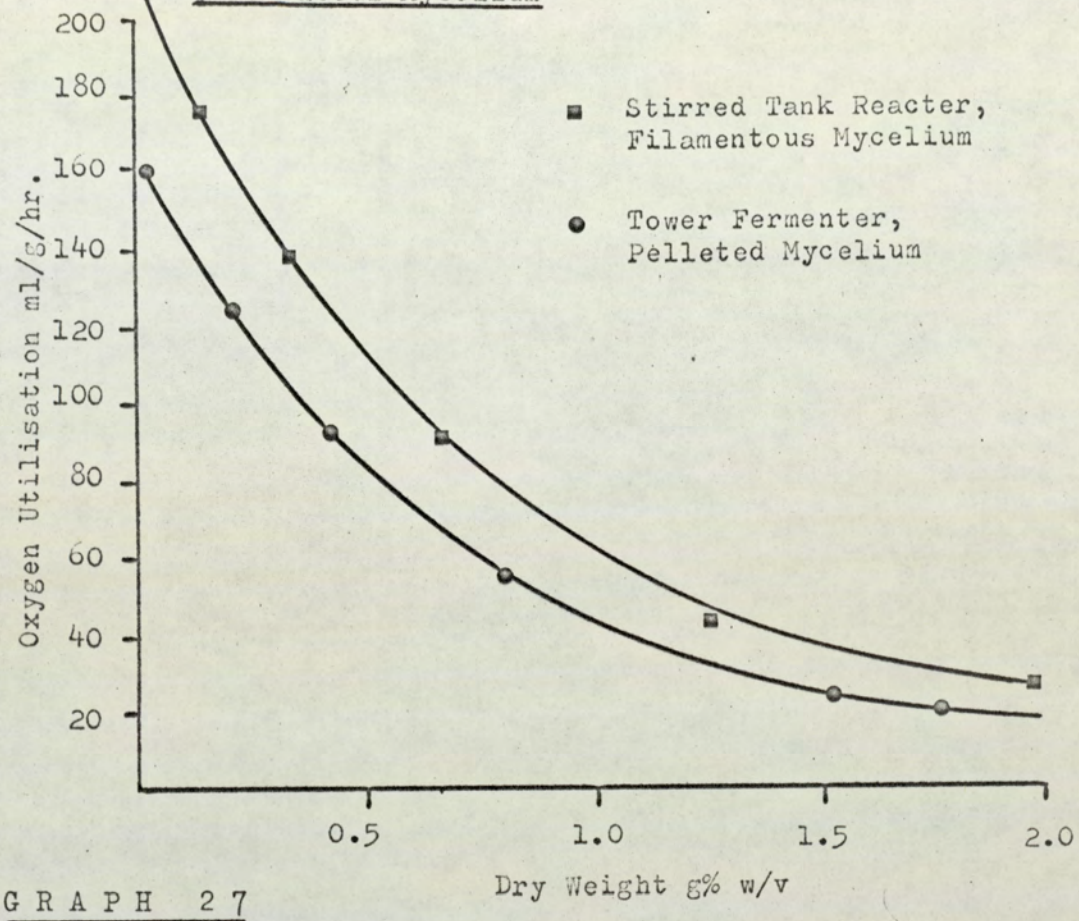
GRAPH 26a

Specific Oxygen and Carbohydrate Utilisation during Growth of A.Niger.



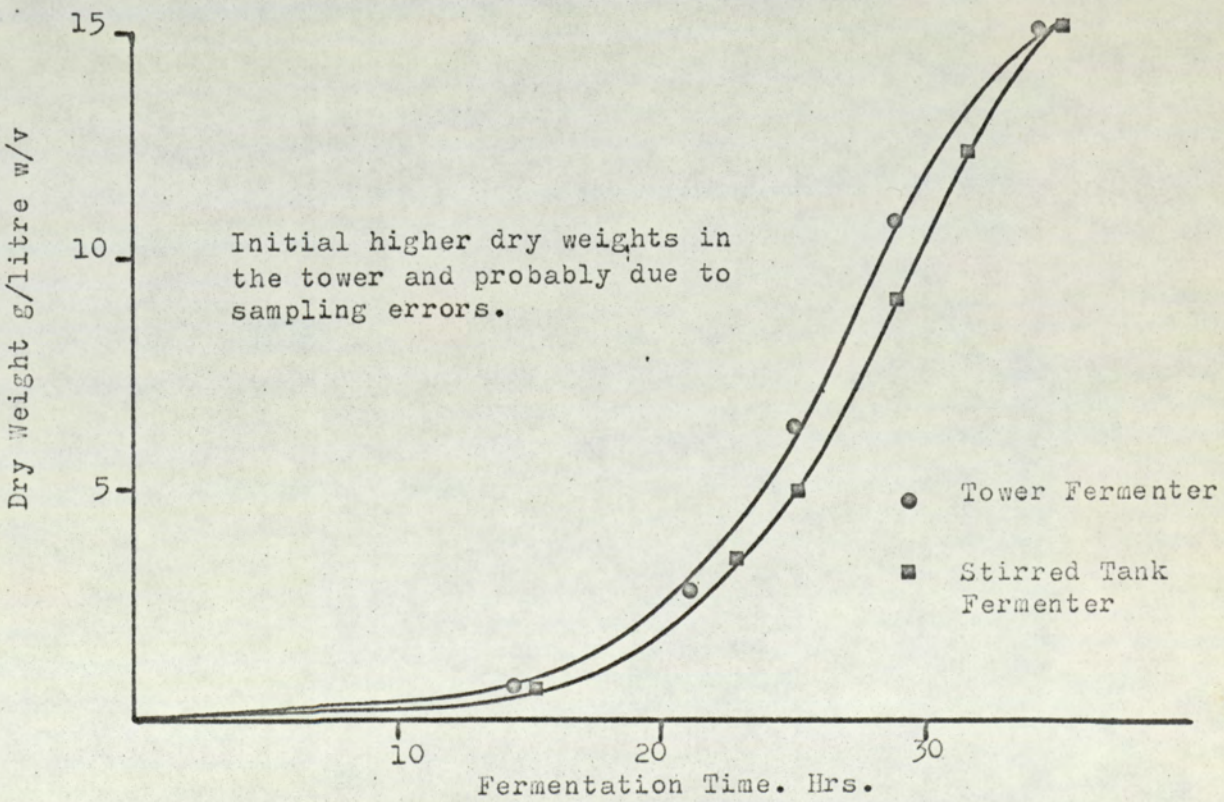
GRAPH 26b.

Comparisons between Oxygen Utilisation in Pelleted and Filamentous Mycelium



GRAPH 27

Growth of A. niger in tower fermenters (Pellets) and Stirred Tank Reacters (Filamentous)

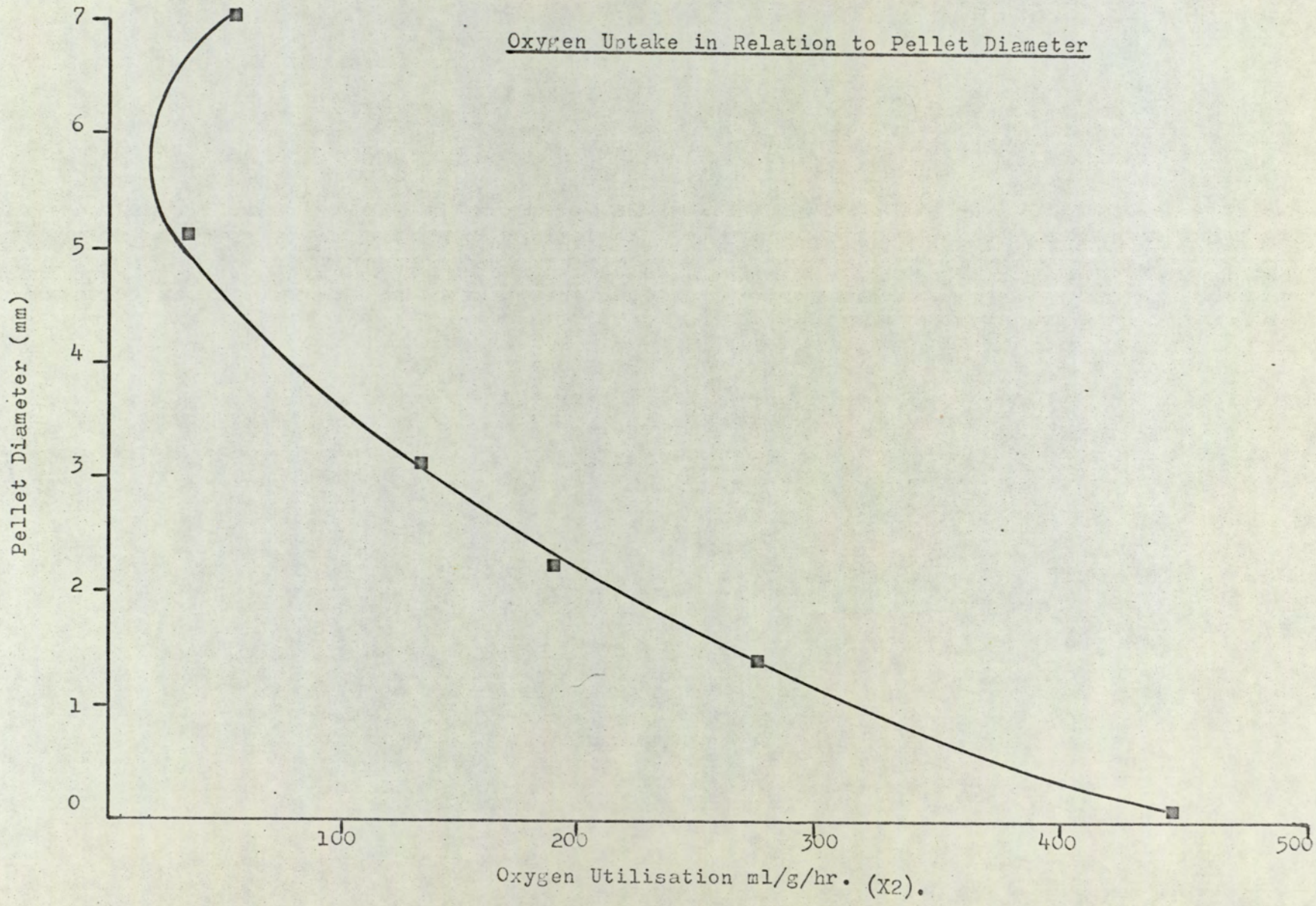


amounts of mycelium used at this juncture.

Mass transfer restrictions (due to pellet formation) of oxygen into the vicinity of the mycelium can be described by Graph 28. This graph shows the relationship between pellet diameter and oxygen uptake. These figures reveal how the oxygen utilisation rate is lowered as the diameter of the colony increases. From the figures on graphs 28 and 26 it can be seen that difficulties in oxygen transfer begin to appear between 3 - 4 m.m. diameter pellets. (This is taking into account that the largest diameter pellets necessarily consist of the oldest mycelium). The pellet diameters do not take into account the relative densities of the different cores of mycelium. Therefore with different density pellets grown under other culture conditions another set of oxygen utilisation figures will be obtained. The density relationship is demonstrated by the fact that the oxygen utilisation curve begins to increase again with pellet diameters of 6-7 mm. This can be explained by the fact that autolysis had begun in these larger colonies, so producing less dense, loose colonies. In fact the figure quoted for these colonies is approximately that of filamentous mycelium at that age (20-30 ml O_2/g mould) thus showing mass transfer restrictions are almost absent.

Since the filamentous mycelium has a greater oxygen uptake than the pelleted mycelium and also has a similar growth rate Graph 27. Then the oxygen taken up out of solution must be channelled off in some direction other than cell production. With the knowledge of the Aspergilli in the organic acid producing field it was conceivable to suggest that the oxygen was being used for the production of

GRAPH 28


Oxygen Uptake in Relation to Pellet Diameter
Pellet Diameter (mm)
0
1
2
3
4
5
6
7
100
200
300
400
500
Oxygen Utilisation ml/g/hr. (X2).

such acids. The metabolic chain is the 'Tricarboxylic Acid Cycle', which ends in the electron transport or respiratory chain where oxygen acts as the final hydrogen acceptor. Graph 29 shows the changes in the p H value of the media in both tower and stirred tank fermenters growing pelleted and filamentous mycelium respectively. The sharp drop in the p H of the S.T.R media compared to that in the towers, can clearly be seen and also the value to which the fermentation proceeds. It was subsequently shown that sharper drops could be ascribed to the pelleted mycelium, as long as it was grown in a very diffuse form. The dropping in the p H values observed during these fermentations can be ascribed basically to two functions (a) the production of organic acids by the mould and (b) the utilisation of the NH^{+}_{4} ions from the $(NH_4)_2$ SO4.molecule, used as a supplementary nitrogen source, thus leaving behind the negative radical SO_4 = which in turn affects acidity and p H. It is suggested that the p H drop in pelleted mycelium is due mainly to the utilisation of the ammonia ion whereas in filamentous mycelium under highly aerobic conditions there is a combination effect between the sulphate radical and organic acid production.

Another fact supporting the theory that the oxygen is being used for other metabolic pathways, other than those concerning cell production is seen in the conversion efficiency of the respective mycelial forms. A slightly lower conversion efficiency of carbohydrate into mycelium is noted when using the filamentous morphology than there is when using pellets .

GRAPH 29

Variation in pH During Fermentation Using Pelleted and Filamentous Mycelium

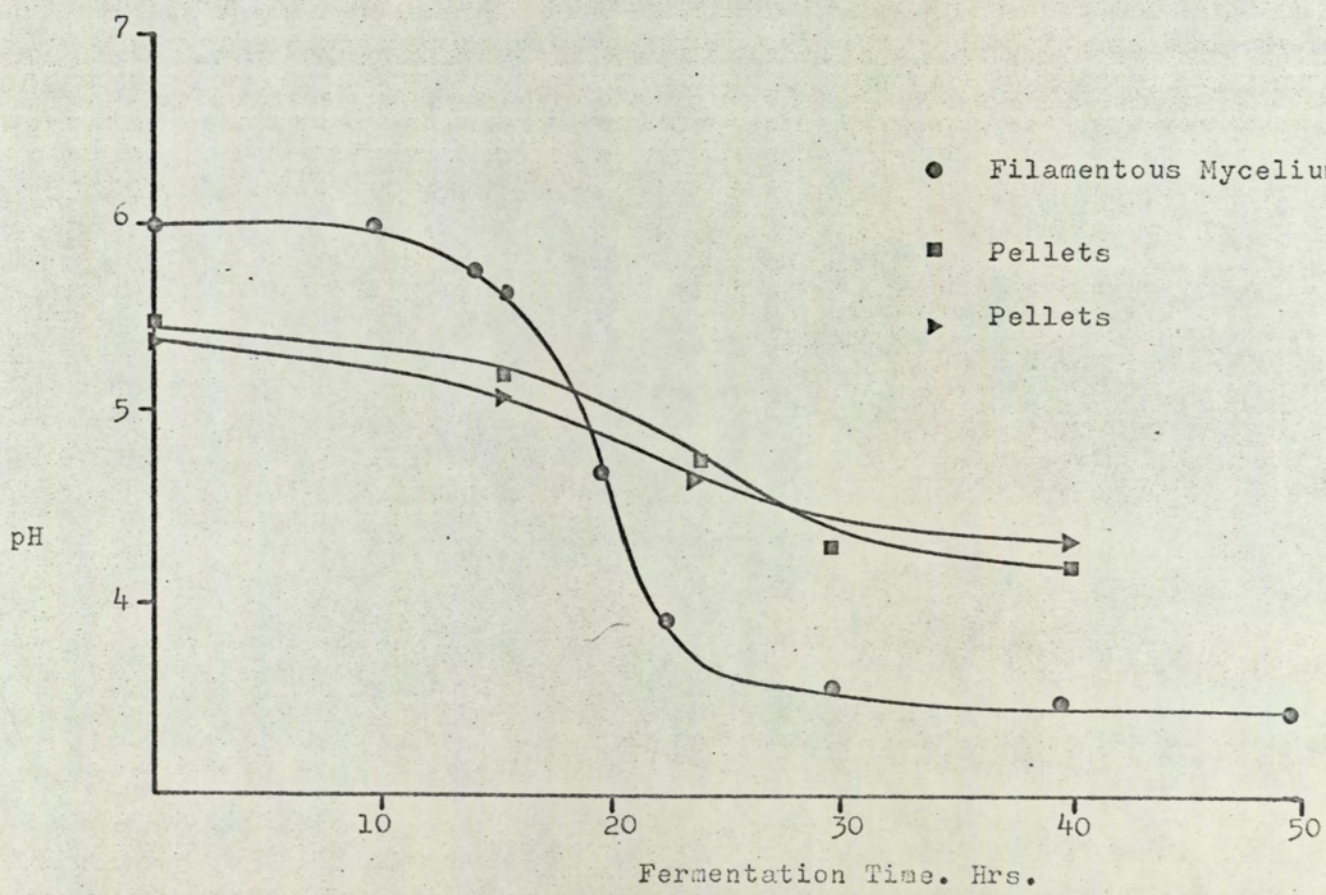


TABLE. 8

Relative Carbohydrate Conversion Efficiencies of Pelleted and Filamentous Mycelium.

MORPHOLOGY	DRY WEIGHT	CARBOHYDRATE UTILISATION	CONVERSION EFFICIENCY.
Filamentous	1.8 g% w/v	4.1g% w/v	44%
Pelleted	1.75 g%w/v	3.7g% w/v	47%

CONVERSION EFFICIENCY = $\frac{\text{Carbohydrate Consumed} \times 100}{\text{Mycelium Produced.}}$

The volumes occupied by the mould and medium were taken into account when calculating sugar consumption.

Analysis of the spent media in both systems was also made. The analysis comprised of paper chromatographic procedures in order to assess what organic acids were present. The chromatograms showed that the amounts of citric acid produced was very low in both systems, however oxalic and gluconic acids seemed to dominate in the media taken from the filamentous mycelium.

The results obtained were purely qualitative, more useful data may be obtained by using quantitive methods of analysis.

5.2.1. THE RHEOLOGY OF MOULD SUSPENSIONS.

As fermentations proceed the rheology of the broth changes considerably, from being a clear liquid with a viscosity just exceeding that of water into a thick pulpy mass of organism

which has has apparent viscosities in the order of 800-1000 centipoise. The viscosity of the fermentation broth increases logarithmically as does the dry weight within the fermenter. For this reason vast increases in the power requirements needed for aeration and agitation will be encountered in any mould fermentation. The main reason why moulds are so much more viscous in native than other single cell organisms is because of the long intertwinning and branching hyphae which are characteristic of most fungi. It is therefore evident that morphology as well as cell mass influences changes in the rheology of microbial suspensions.

The tower fermentation system (in the work already presented) has been shown to be capable of changing and controlling the morphology of moulds under submerged culture conditions. It is therefore possible, by using the tower system that the rheology of mould suspensions might be controlled to advantage. The advantages envisaged are higher degrees of aeration and agitation per unit power per unit mould concentration.

As it has already been stated the rheology of the fermentation is in a state of change. In general during a fermentation involving the production of mould biomass there of the fermentation broth, these are:-

(1) Changes in media characteristics
(also involving detergents and antifoams)

(2) Changes in the moulds morphology.

(3) Changes in biomass volume.

5.2.2. Changes in Media Characteristics.

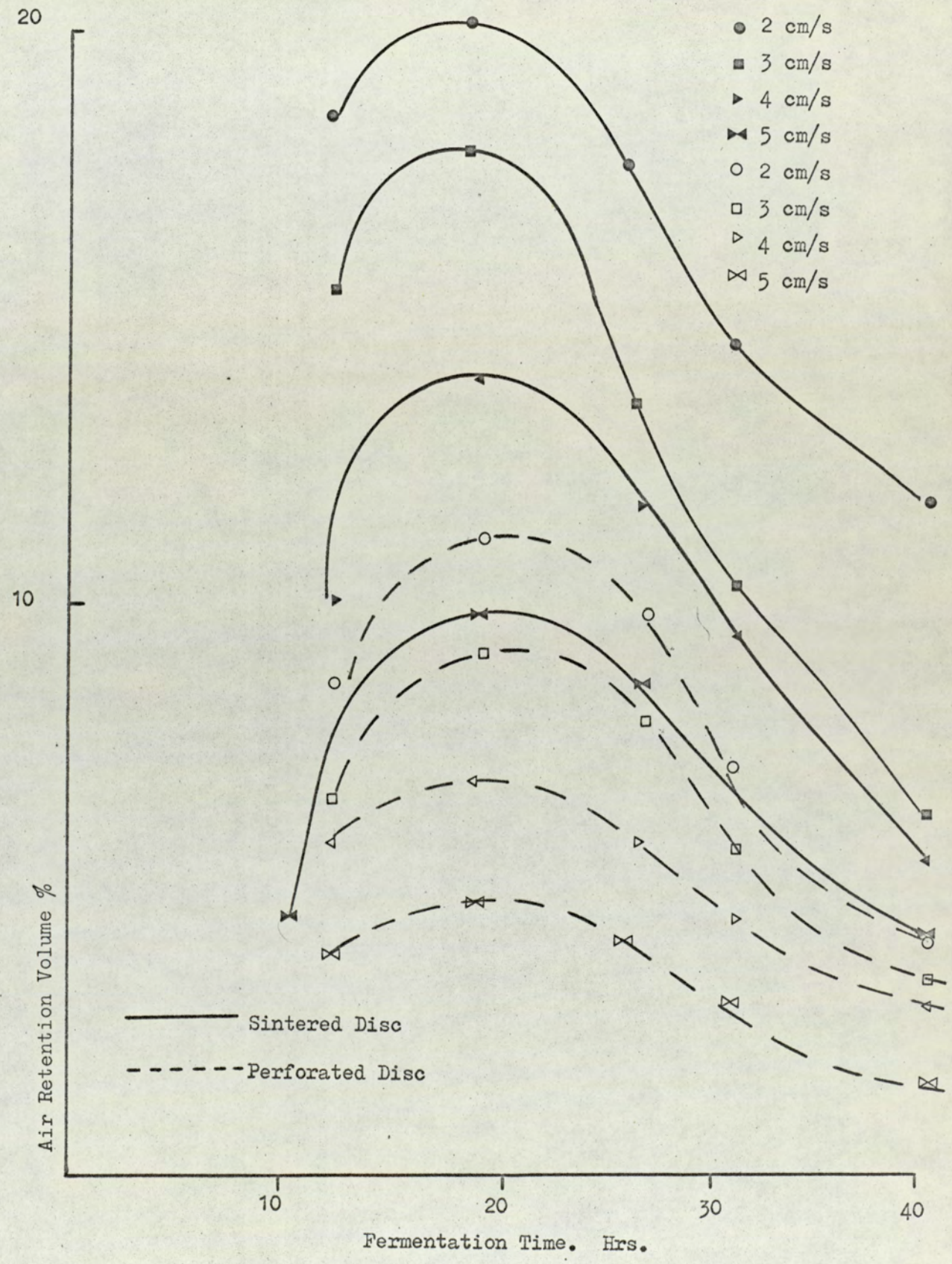
Changes in media characteristics, during the batch

culture of A.niger are continually taking place. The pre-inoculation procedures such as p H adjustment alter the surface tension and electrostatic potential of the media. Inoculation, in which detergents (Tween '80') are used, represents another significant surface tension change in the media. Changes in media composition during the course of the actual fermentation also affect the characteristics of the liquid phase considerably. This is due to nutrient utilisation and cell excretions. The control of foaming by the use of chemical antifoams represents yet another change in surface tension and thus the nature of the liquid phase. Due to the fact that tower fermenters have no impeller/propeller system these changes in media composition represent a marked difference in aeration/agitation characteristics. These differences are not often appreciated in stirred tank fermenters since the agitation system allows a more consistent environment to be maintained. A greater knowledge of media rheology in tower fermenters is thus required. It was possible with the information acquired from the following set of results to characterise the media changes more precisely and to make suitable adjustments to the tower system in order to allow for them.

In order to characterise some of these rheological changes in the media during the course of a fermentation, experiments were carried out which measured the hold up volume and surface tensions encountered. Graph 30 shows the hold-up volume related to the fermentation time at various superficial gas velocities (this graph also shows the differences between the forms of air distributor used). It can be seen that the hold-up volume increases steadily during the initial phases of microbial growth until it reaches a peak at approximately 17 hours

GRAPH 30

Variation in Air Volume Retention During Fermentation

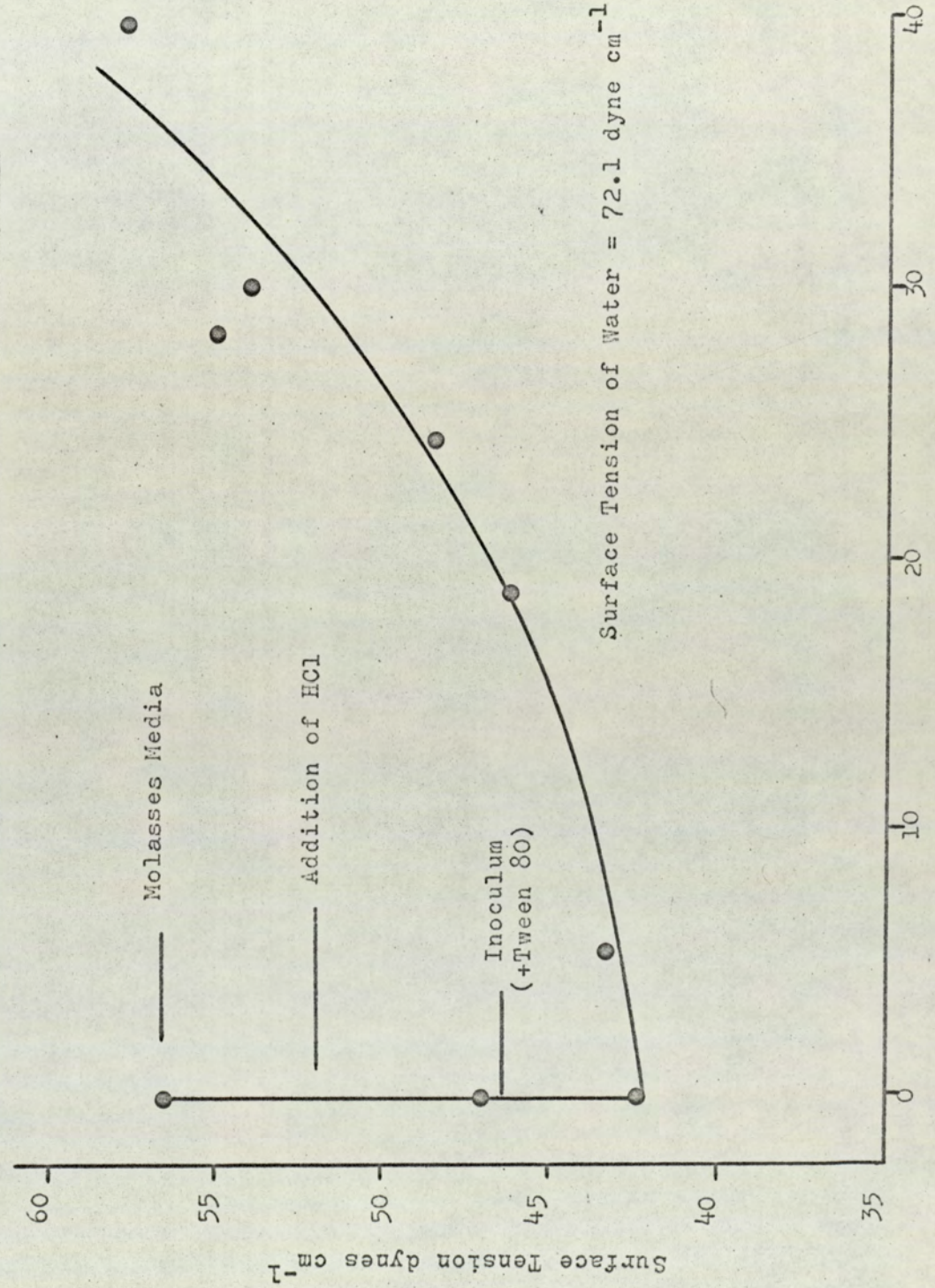


(approximately 1gm/l. mould) then begins to decline. The initial increases in hold-up volume can be explained by inspecting Graph 31 which is a plot of the surface tension relative to the fermentation time. This shows that the surface tension is continually increasing during the run. By increasing the surface tension within a column of liquid it has been shown that it has the effect of decreasing bubble size. The smaller the size of bubble that is travelling up a column of liquid the longer it's residence time within that liquid tends to be. Since these bubbles have a longer residence time they will tend to accumulate in the column and so give a higher air volume retention values. In the later stages of the fermentation a gradual decline in hold-up volume is observed due mainly to the increasing biomass present in the fermenter. This mould volume is thought to outweigh the surface tension effect in determining hold-up volumes. (This will be discussed in more detail in Section 5.2.4). It will be noted from Graph 31 that the surface tension continues to increase during the later stages of fermentation. This is probably due to the fact that nutrient utilisation is taking place and the media is approaching the surface tension of water. It is interesting to note that the surface tension increases more rapidly as the media volumetric utilisation rate increases. The effect of cell excretions (organic acid production) on the surface tension is to lower it. However the media utilisation causes a greater size in surface tension and so the general trend is in an upward direction.

In the pre-inoculation stage of the production of microbial biomass using molasses the p H of the media is adjusted by using hydrochloric acid, which has the effect of decreasing surface tension. A reduction in bubble size occurs and is

GRAPH 31

Changes in the Surface Tension of the Media during Fermentation



probably due to the increase in the electrostatic potential of the media. During inoculating it was seen that the action of 'Tween 80' surfactant had the same effect and this also decreased bubble size. In the period between 15 and 20 hours in a spore inoculated fermentation foaming takes place. This is also the time of greatest hold-up volume in the column. It is reasonable to assume that the increases in surface tension in the liquid are responsible for these factors. A silicone based antifoam was used to control the foam in some instances. This surfactant had the effect of decreasing surface tension, which in turn caused coalescence of the air bubbles, this reducing the hold-up volume in the fermenter. (It was shown that tap water including antifoam at a concentration of 0.005 % w/v reduced the surface tension from 72 dyne cm^{-1} to 50 dyne cm^{-1}, which is a considerable increase compared to those shown during a fermentation). The effects of anitfoam have been shown to be deleterious on the aeration charateristics and its use should be avoided as far as possible. A more detailed account of its action is given in Section 3.

From the discussion it is evident that media characteristics play an important role in the aeration and agitation of tower fermenters. It has been noted that differences can even occur in the bubble flow patterns in the fermenter between media that has been autoclaved and that which has been pasteurised. It is this necessary to control the mould morphology since it is believed that the growth of the pellet form can compensate for some of the deleterious effects encountered when medium rheology is changed.

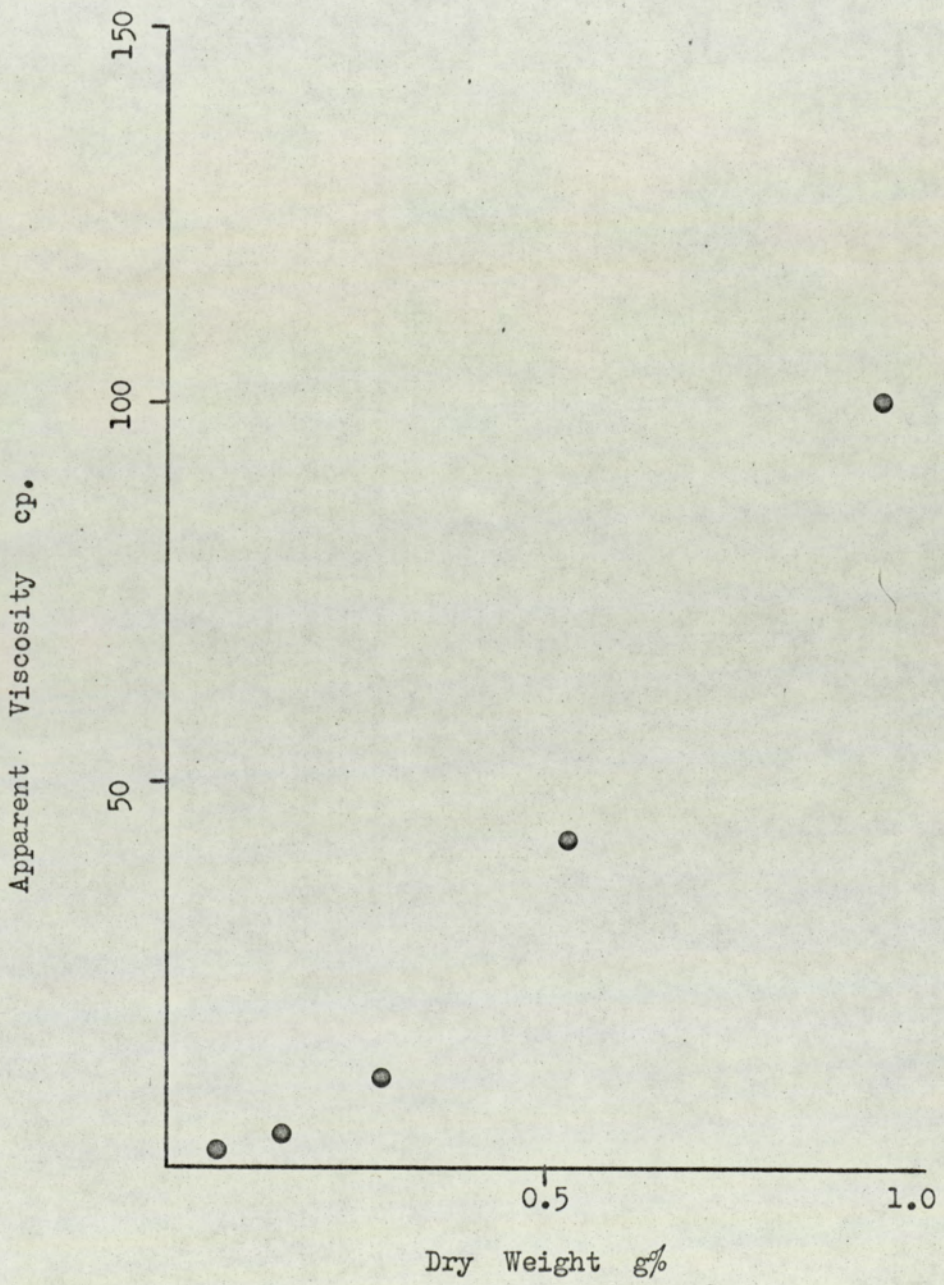
5.2.3 Morphological Changes During the Fermentation

It has been shown in Section 4 that mould morphology changes throughout the fermentation, The mould pellet changing from being small and filamentous, (Plate 5) to being a little larger and smoother in the later stages of fermentation, (Plate 7) Such changes can affect the air flow behaviour in tower fermenters. The variations in aeration characteristics are also reflected in the viscosities encountered during an A. niger fermentation. Since not only is the viscosity related to the power requirements for agitation but also, in tower fermenters, changes in the aeration can also be detected.

The apparent viscosity of a non-aerated fermentation broth was calculated throughout the course of a run. The results obtained from these measurements can be seen in Graph 32 . which shows the apparent viscosity of the fermentation broth increasing with time. This is entirely as expected since the volume of mycelium within a fermenter is continually increasing as time proceeds. If we were to look a little further into this situation it can be seen that there are two variables present, namely volume changes and morphological changes. By taking samples of the fermentation broth of predetermined intervals and varying the amount of mould in each batch by successiwe concentrations then determining the viscosity of each sample; a 'picture' of the whole fermentation is built up such as shown in Graph 3 3 . This graph is a double log plot of the apparent viscosity against the dry weight. The dotted lines on the graph are the results of Takahashi (1970). The line on the extreme left is the apparent viscosity of filamentous mycelium and that on the extreme right is of smooth pellets. A series of lines was obtained from the

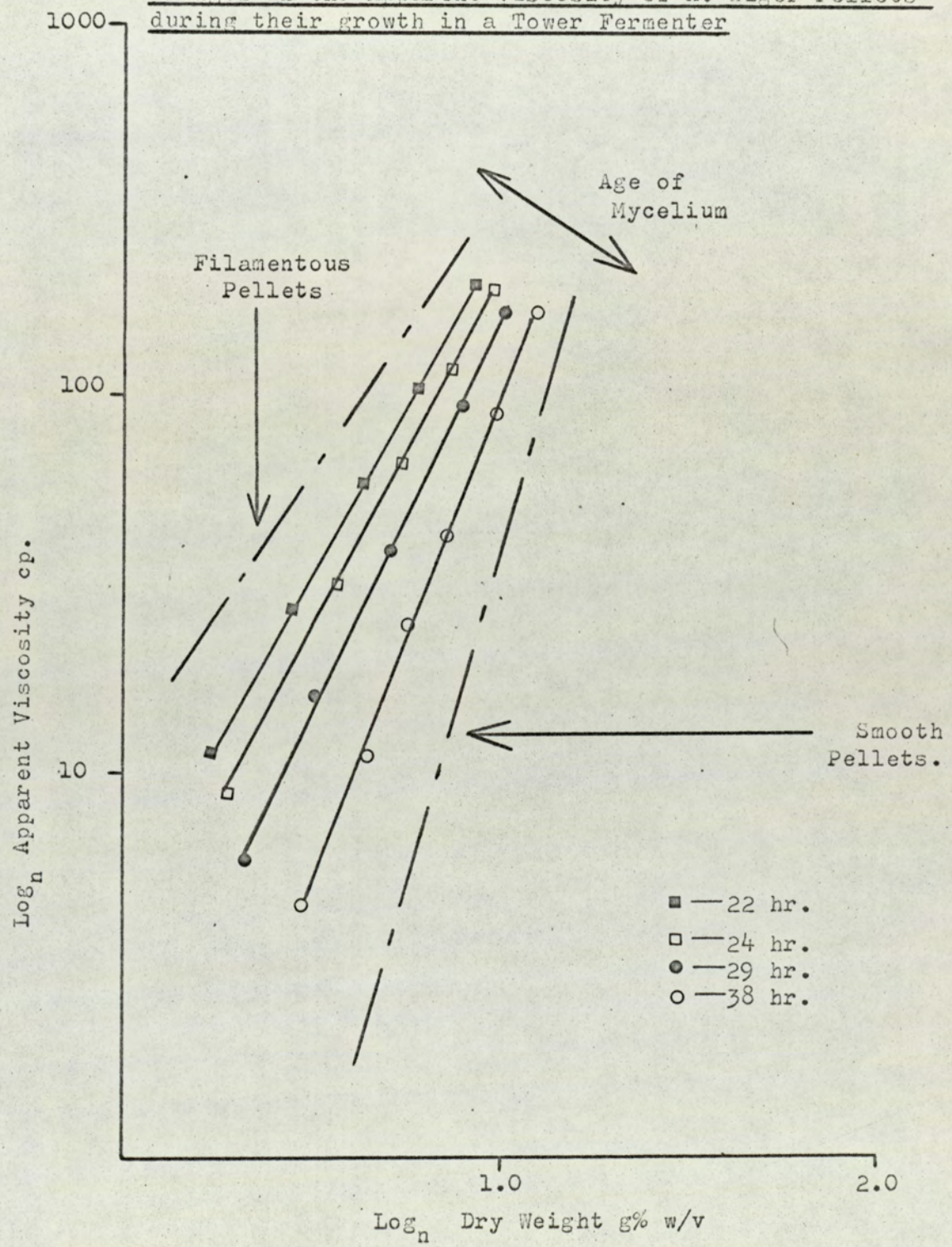
GRAPH 32

Changes in the Apparent Viscosity of the Fermentation Broth with Time.



GRAPH 33

Changes in the Apparent Viscosity of A. niger Pellets during their growth in a Tower Fermenter

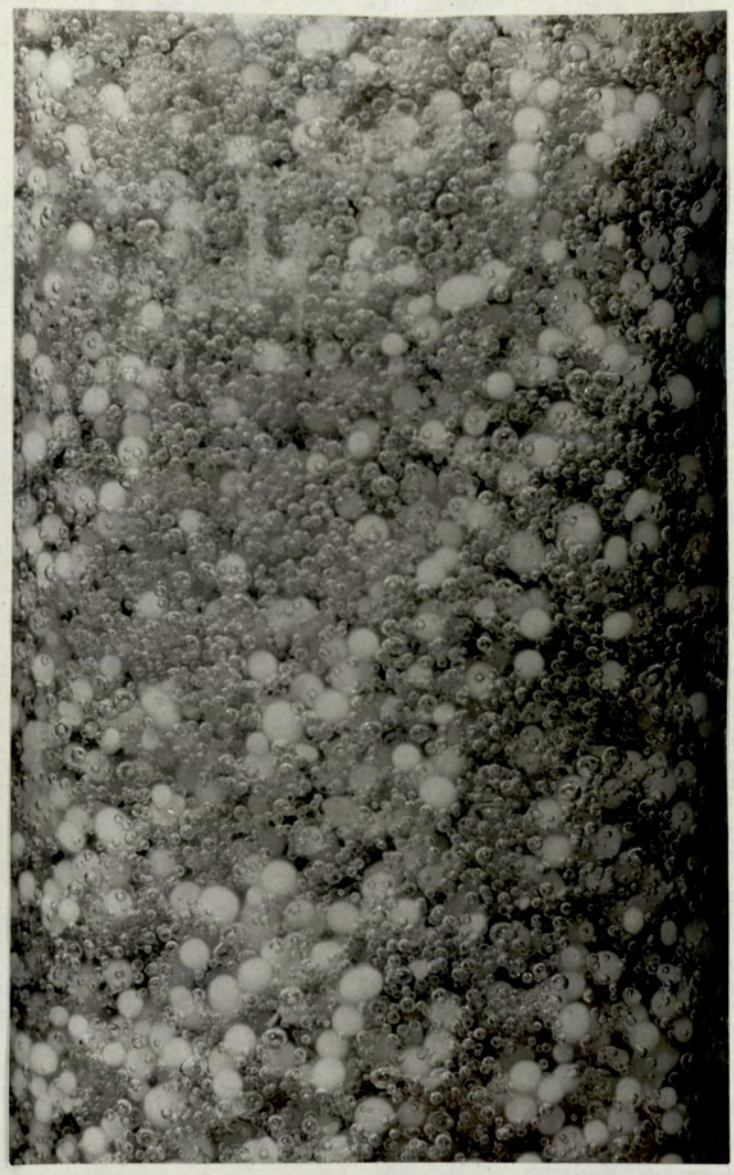


A.niger fermentation which lay in between those of Takahaski. These lines show a gradual movement from left to right as the fermentation proceeded and ended up fairly close to the smooth colony dotted line. In fact this last line represented colonies which had a do/dl ratio which was approaching unity. The morphology/viscosity change during fermentation is a natural progression of organism and liquid system.

Since the changing morphology has such a marked affect on the viscosity of the system it is reasonable to suppose that this change will also affect such things as aeration effeciencies and hold-up volumes within the fermenters. Usually it was shown that by adding any amount of solids to the air/liquid system in the column had the effect of causing slugging to occur at a lower superficial gas veocity (see again Graph 1) than it would normally do so. An experiment was performed in the laboratory glass columns (8.6 cm dia) in order to assess the effect of morphology on gas hold-up volume. From Graph 3 4 it can be seen that the filamentous mycelium lowered the hold-up appreciably for the same volume of pelleted mycelium. In fact reductions of between 20 and 30% could be seen in the hold-up volume using superficial gas veocities of between 3 and 6 cm/s. This could mean that significant differences in oxygen transfer efficiencies might be recorded for these systems. This hold-up figures show that it is possible to have more air in the system at any one time when the pelleted mould is used than it is when filamentous mycelium is present. Plate 1 0 illustrates this point well. In this photograph we can see that there is an uniform size of air bubble 2-3mm in diameter and no slugging is in evidence here. From the photograph an idea can also be gained of the amount of

air that is in the system

Plate 10

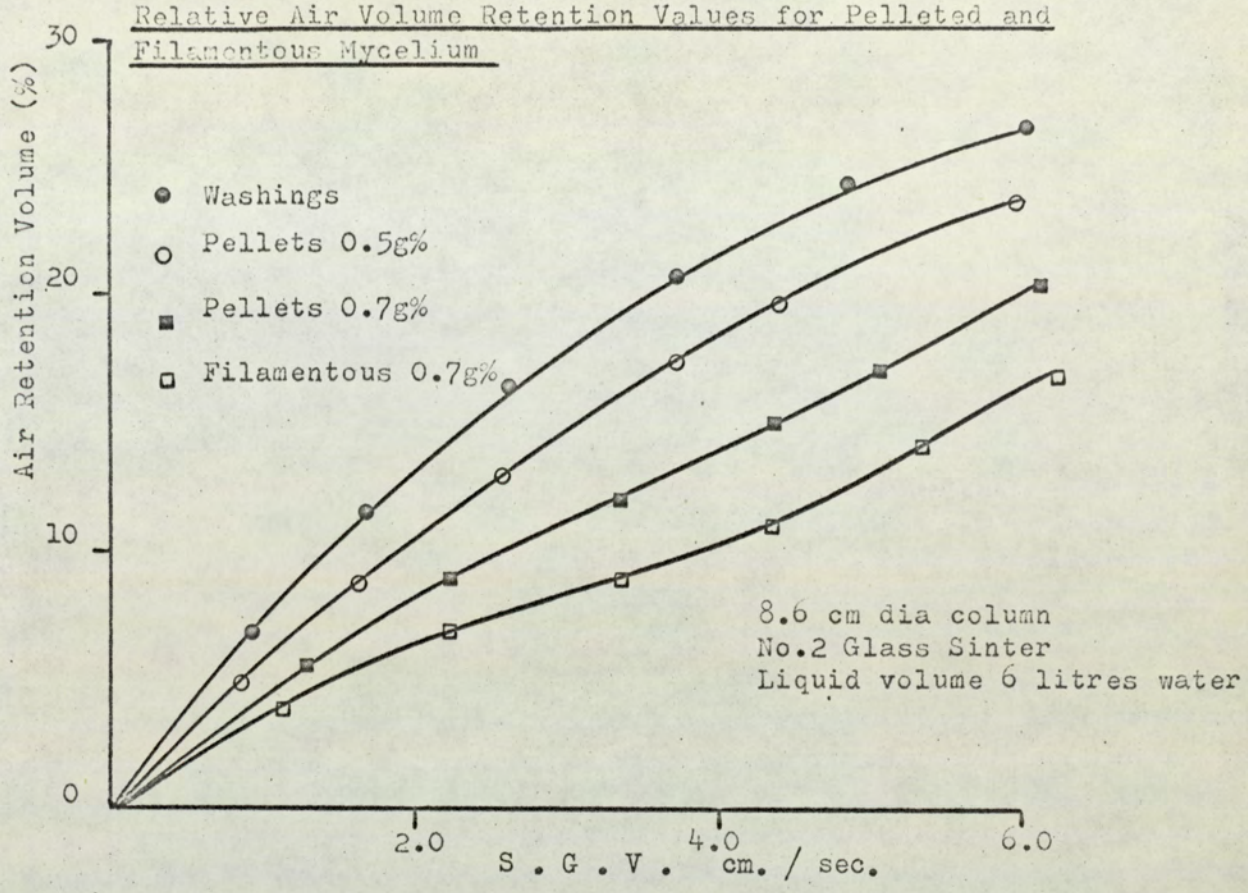


Aeration approximately 2-3 cm/sec - 8.6 cm glass column containing smooth mould pellets.

Oxygen transfer into such systems is clearly affected by the mould/morphology. Experimentation involving gassing out procedures were carried out in order to ellucidate to what extent the morphological variation affects the oxygen transfer rate into solution from Graph 35 it can clearly be seen that higher oxygen transfer coefficients can be obtained by the use of pelleted mycelium rather than that of the filamentous variety.

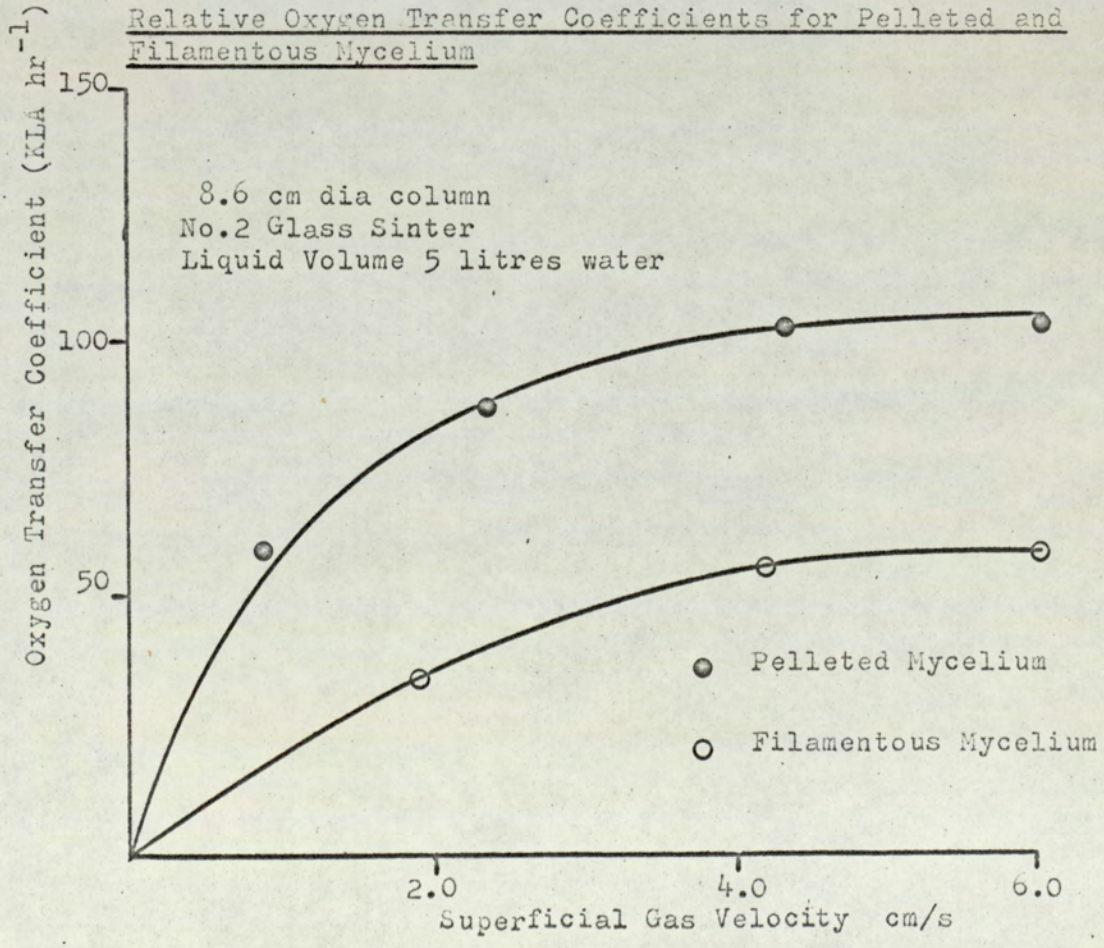
GRAPH 34

Relative Air Volume Retention Values for Pelleted and Filamentous Mycelium



GRAPH 35

Relative Oxygen Transfer Coefficients for Pelleted and Filamentous Mycelium



The experiment described here was performed using tap water and mould mycelium which had been washed clean of all other substances. Therefore it can be stated that it is the morphology of the mould present that is the contributing factor to the variation seen in oxygen transfer coefficients. There is almost a 50% reduction in the KLA value obtained for the filamentous mycelium relative to the pellets. The pellets used were of the smooth variety with a low do/dl ratio. Various lines of oxygen transfer coefficients might be imagined decreasing with increases in the do/dl ratio until the filamentous form is reached. Since these tests were carried out in ordinary tap water it was deemed necessary to carry out a certain amount of experimentation using actual media. The liquid used in these tests was 10% molasses solution. The results show (Table 9) that the difference between the two morphologies was not so marked as that shown when the tests were performed using tap water. The figures obtained tended to reveal that at low superficial gas velocities there was little or no differences between the KLA values of the two morphologies.

TABLE. 9

Relative changes in the Oxygen Transfer Coefficient for pelleted and filamentous mycelium.

Morphology	Superficial Gas Velocity	KLA hr^{-1}
Filamentous	1 cm/s	250
-ditto -	2 cm/s	200
-ditto-	3.5 cm/s	300
-ditto-	4.3 cm/s	390
Smooth pellets	1 cm/s	150
-ditto-	1.9 cm/s	310
-ditto-	3.5 cm/s	450
-ditto-	4.3 cm/s	500

All measurements taken in 10% molasses solution at 30°C in the 0.6 cm dia column.

At higher superficial gas velocities however advantages could be seen by using the pelleted form of the mould.

It must be noted that the values for the oxygen transfer coefficient in media are much higher (x 5) than those quoted for water. This is because the electrostatic potential of the molasses solution is higher than water, therefore smaller bubbles, which necessarily increase the K.L.A ensue. This is consistent with the fact that there is little difference at low superficial gas velocities between the two morphologies. However, the presence of filamentous mycelium at higher gas velocities created what is known as a 'slug' flow situation, and therefore reduced the oxygen transfer efficiency into solution.

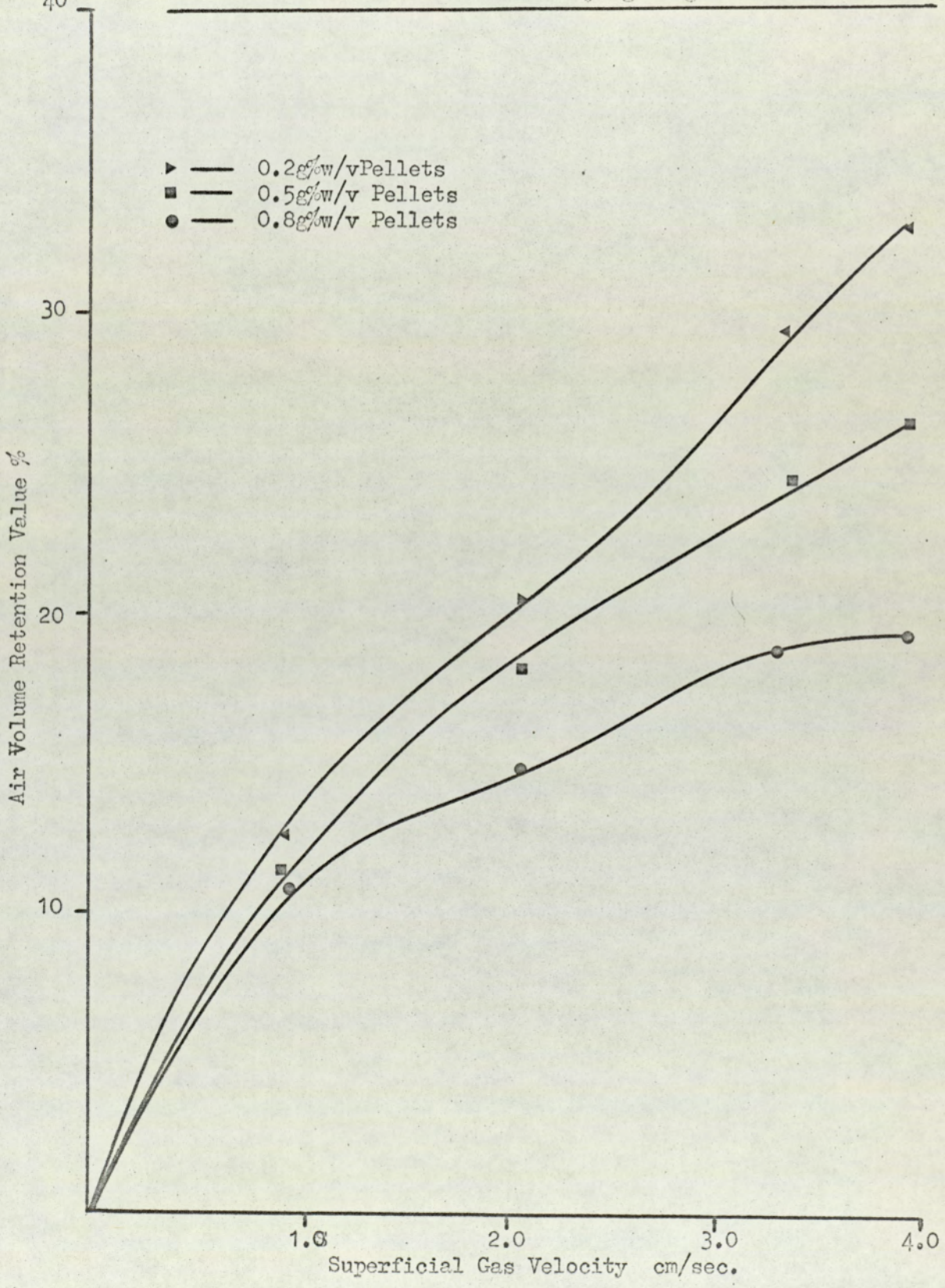
5.2.4. The Affect of the Volume of Mould Present on the Rheology of the Fermentation Broth.

It has been shown in Graph 30 which illustrates the changing hold-up volume during a specific fermentation. The hold-up begins to decrease after about 18 hours fermentation despite the fact that surface tension (which affects bubble volume) is increasing considerably. It can therefore be demonstrated to what extent the mould volume outweights all other effects. Further by studying graphs and more evidence can be gained. The decreasing air volume retention values due to increasing biomass volume can be explained by the presence of mould creating 'slug flow' at lower superficial gas velocities than would normally occur.

During an A.niger fermentation the mycelium is con-

GRAPH 36

Air Volume Retention Value for Varying Weight of Mould Pellets.



tinually increasing in volume with time and thus the opportunity for mass transfer of oxygen into solution is continually declining. A position is reached when the mould is about 1.5g% w/v of the fermentation broth where it can be noticed that there is a mass of tiny air bubbles percolating slowly up through the mould. (In the absence of antifoam). These bubbles are often observed to be moving in a downward direction. This may, be explained by the fact that there are 'slugs' of air (due to coal escence) rising quickly up the centre of the column causing transient vacuums behind the bubble, thus drawing the small air bubbles into them. It is suggested that the majority of these tiny air bubbles lay close to the walls of the fermenter (Plate 11). It is obvious that some of the bubbles are drawn into the bulk of the broth by the swirling action of the liquid (S.G.V = 2-3 cm/s. in the 15 cm dia. column). The magnitude of the affect of these bubbles on oxygen transfer is therefore uncertain. Due to their extremely long residence time in the fermenter the oxygen driving force is low. It is known by the use of a dissolved oxygen probe, it can be demonstrated that oxygen transfer drops off sharply when these bubbles are absent. If the air flow is increased to beyond 6 cm/sec at this point considerably more agitation occurs and many of the bubbles are removed from the system, whilst returning to a lower air flow rate allows them to reform. This last point may be of value in replacing deoxygenated bubbles in the system.

5.3 Conclusions.

In conclusion it may be said that pelleted mycelium uses less oxygen than the filamentous form because of the three factors mentioned at the beginning of the section viz mycelial age, mass transfer difficulties, changes in metabolism. Thus there is a limiting supply of oxygen to the centre of the pellets (even in colonies 2-3mm dia.). Since there is an age gradient in the colony from the centre outwards this is not an oxygen limitation below the maintenance level but only a limitation for organic acid production. This fact can clearly be shown when using the filamentous mycelium where no mass transfer difficulties are encountered and acid production is enhanced. Other advantages in utilising the pellet form of the organism must lie in the fact that increased aeration and agitation efficiencies may be maintained a high levels of dry weight.

SECTION 6

SECTION. 6.

Parameters Prescribing the Medium that Affect Mould Growth.

There are many parameters, chemical and physical that can affect the growth of moulds in submerged culture. The prime objective of the following experiments was to establish an optimum for economic biomass production in terms of growth rate, yield determined as highest weight of product obtainable, protein content and carbohydrate conversion efficiency. Although other parameters such as fat, carbohydrate and nucleic acid contents are also important in the consideration of fungal feeds and foods, the main assessment was made with the former set of parameters in mind.

A variety of medium costituents were used during the experiments, the conditions of culture will be stated at the beginning of each experiment. The basis of the media being beet molasses supplemented where necessary by ammonium sulphate as a nitrogen source and sodium dihydrogen phosphate as a source of phosphate.

During the investigation of most of the parameters a combination of fermentation systems were used, surface culture in terms of race plates and submerged culture in terms of shake flasks and tower fermenters. The race plates and shake flasks were used in the primary screen for assessing the various parameters and have a wider bearing than the work done a tower fermenters. The tower provided the finer details in the optimisation of the many interelated parameters. Only where relevant will the results of race plates and shake flasks be reported.

The parameters optimised experimentally were:-

(1) Carbohydrate concentration.

(11) Carbon nitrogen ratios.

(111) Form of nitrogen supplement

(1V) Phophate concentration.

(V) p.H

(V1) Temperature

(V11) Dissolved oxygen.

All fermenters were pitched with a spore inoculum to give pelleted mycelium (See Section 4).

6.1. Carbohydrate concentration.

Media Constituents.

Beet molasses (at varying concentrations)

C:N ratio adjusted to 15:1 using ammonium sulphate.

Sodium dihydrogen phosphate 0.1g %p

p H adjusted to 6.5.

Temperature 28°C

Aeration 1 vol/vol/min.

Fermenter used

8.6 cm dia glass tower fermenter.

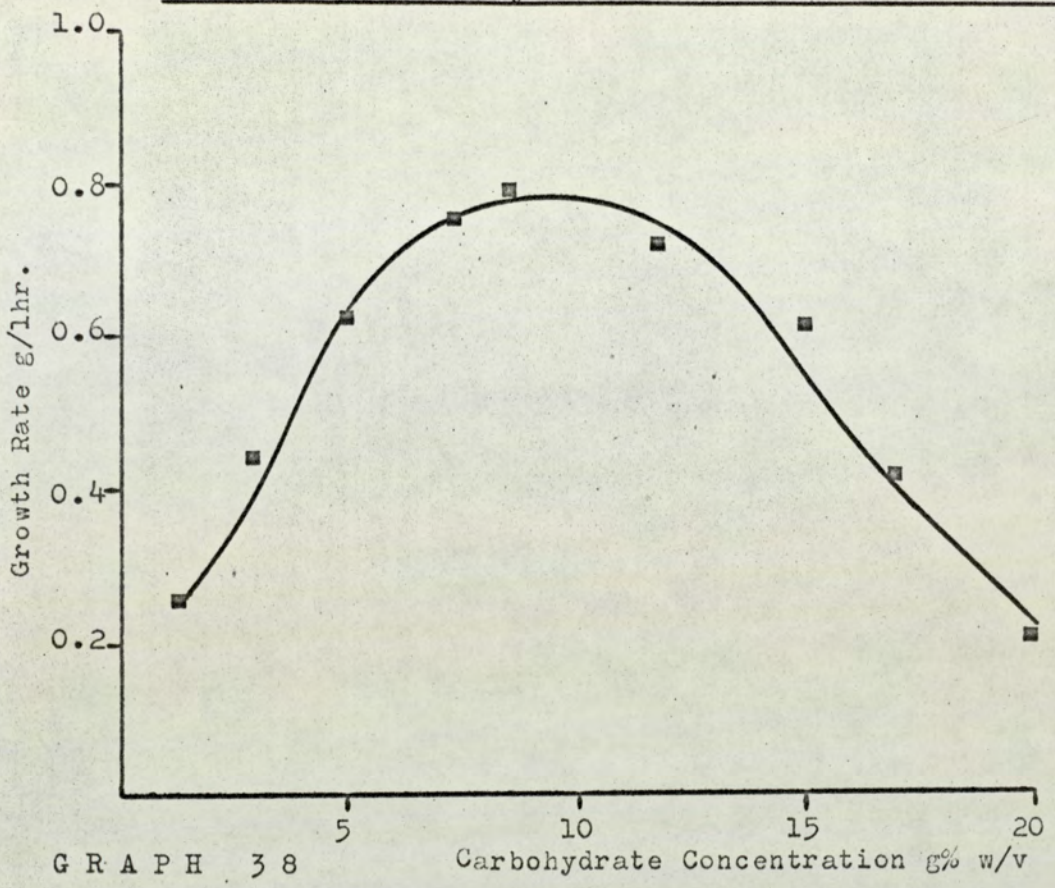
No.2. Sinter (glass(. Occupying the full cross-sectional area of the fermenter.

Graph 3 7 shows how the growth rate varied as to the particular carbohydrate concentration used in the media. It was found that A.niger exhibited a wide tolerance to sugar concentration Graph 37 shows that there is no marked change in growth rate between 5 and 12g%. The apparent optimum lay between 7 and 8g%. However, due solely to economic consideration a slightly lower concentration might be advantageous when considering water and efflu ent factors. The protein concentration did not vary significantly over this optimum range of carbohydrate concentration.

A plot of reducing sugars (sucrose being inverted into the reducing sugars glucose and fructose) was also made in order to show what part of the fermentation the hydrolysis took place. It can be seen that a large part of the lag phase is devoted to the inversion of the sugar (present extracellular enzymic action) whilst relatively little is being utilised in metabolism. Experiments on race plates using beet molasses and supplementing with a variety of sugar sources (e.g. fructose, glucose, sucrose, lactose) were carried out. It was

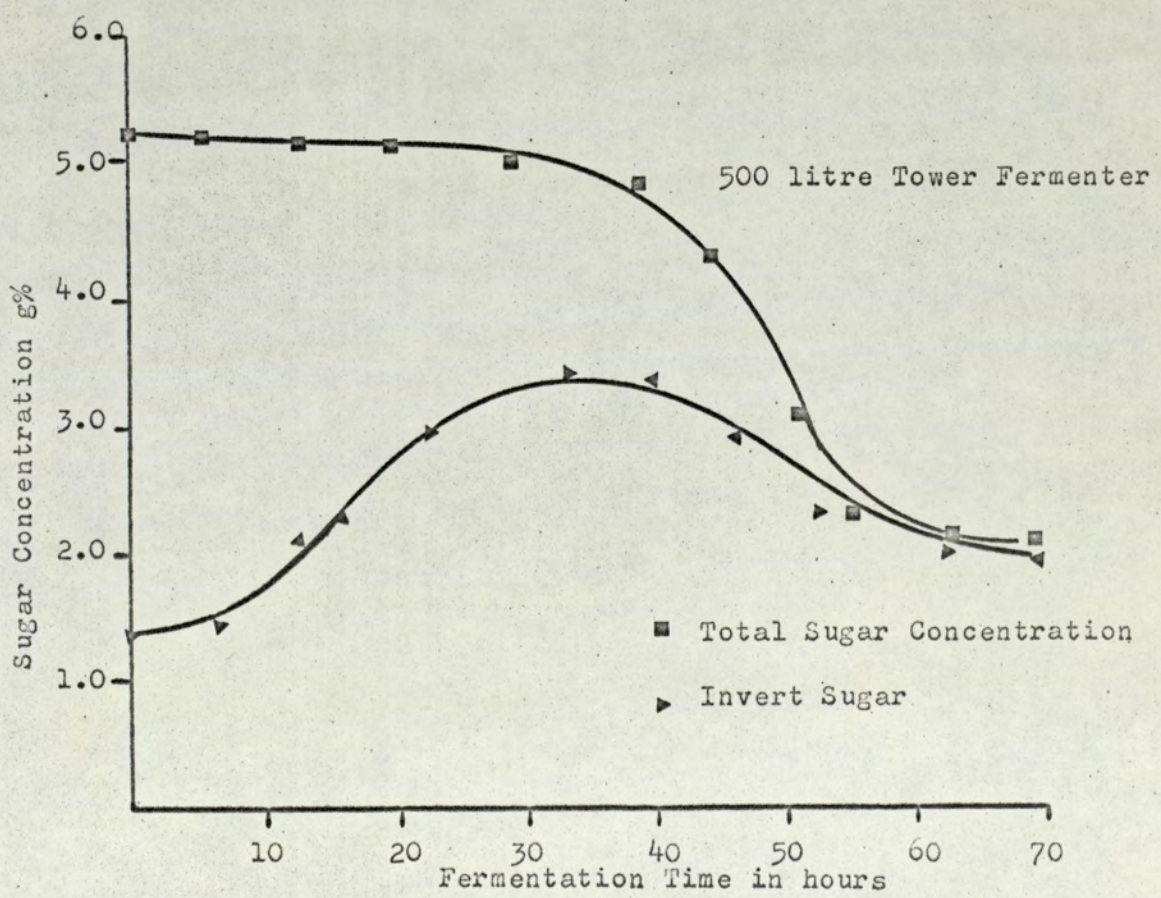
GRAPH 37

The Effect of Carbohydrate Concentration of Growth Rates



GRAPH 38

Relationship Between the Relative Quantities of Invert and Total Sugar Concentration During an A. niger Fermentation



found that these sugars had no significant effect on the growth of the organism.

6.2 Carbon Nitrogen Ratios.

Media Constituents.

Beet Molasses 10g % w/v

C:N ratio adjusted as necessary using ammonium sulphate

Sodium dihydrogen phosphate 0.1 g% w/v

p H adjusted to 6.5

Temperature 28°C

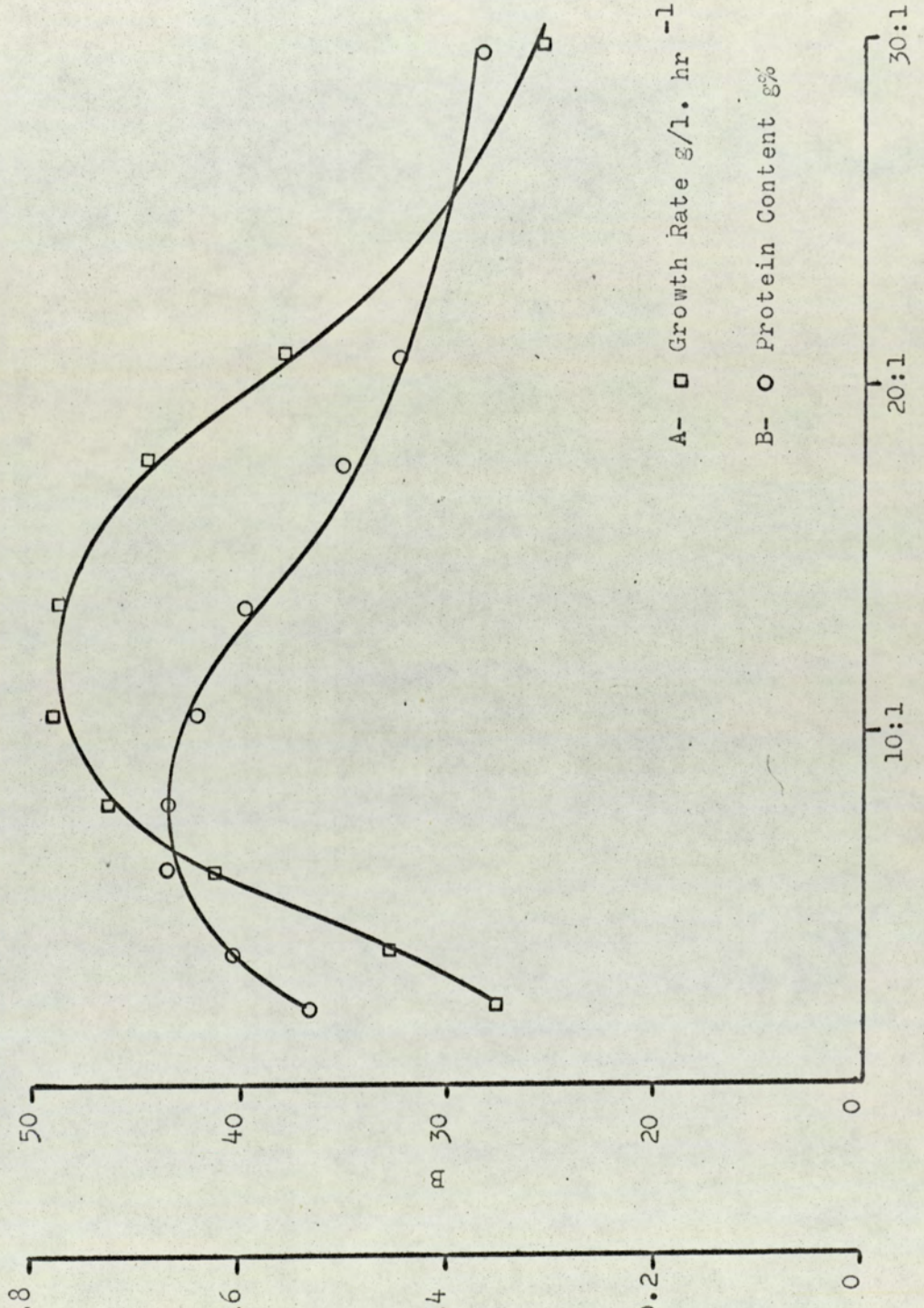
Aeration 1 vol/vol/min.

(N.B Unsupplemented beet molasses has a C:N ratio of 20:1 in order to obtain higher ratios the molasses was supplemented with sucrose).

Supplementation of a 5g% carbohydrate media by various quantities of ammonium sulphate was made in order to vary the C:N ratio. The graph illustrates the change in growth rates and protein contents during these experiments. The optimum growth rate lay between C:N ratios of 7:1 and 13:1 whilst the optimum protein content is around 6:1. At higher C:N ratios however a great deal of fat was seen to be deposited in the mycelium in place of protein. (Graph 38).

GRAPH 38

Relationship Between Growth Rate, Protein Content and C:N Ratio



6.3 Form of nitrogen supplement

Shake flask cultures containing defined media were used in order to assess the modifications which could be made in terms of the nitrogen source. From the results of these defined media experiments it was concluded that whilst A.niger was capable of growing on a variety of nitrogen sources including NH_4 + NO_3-. NH_2+ it grew best on mixed amino nitrogen sources and the least growth obtained using nitrate.

On examination of the nitrogen uptake from a complex molasses based media the following results were obtained. Ammonia as $(NH_4)_2 SO_4$ was the only supplementation.

TABLE. 10

Time (hr)	Total N in Medium.	NH_3 in Medium	NO_3 & NO_2 in Medium	Organic N in Medium.
0	0.387	0.137	0.0518	0.192
12	0.362	0.120	0.0510	0.191
18	0.302	0.090	0.0480	0.166
30	0.327	0.110	0.0480	0.174

All quantities in g %

As it can be seen it is the NH_3+ and NH_2+ nitrogen forms that are being used more readily under these conditions.

Experimentation was taken a stage further and an attempt was made of providing a nitrogen balance for this fermentation . In this study the total nitrogen, ammonia nitrogen and the amino acid nitrogen were monitered throughout the fermentation as well as the mycelium nitrogen. The experiment was carried out in a 50 litre tower fermenter.

Initial nitrogen analysis

Total nitrogen	NH_4+	NH_2+*	NO_3-	Bound N_2*
0.254	0.101	0.021	0.051	0.081

All quantities in g %

* Amino nitrogen estimates as 15% of total amino acids.

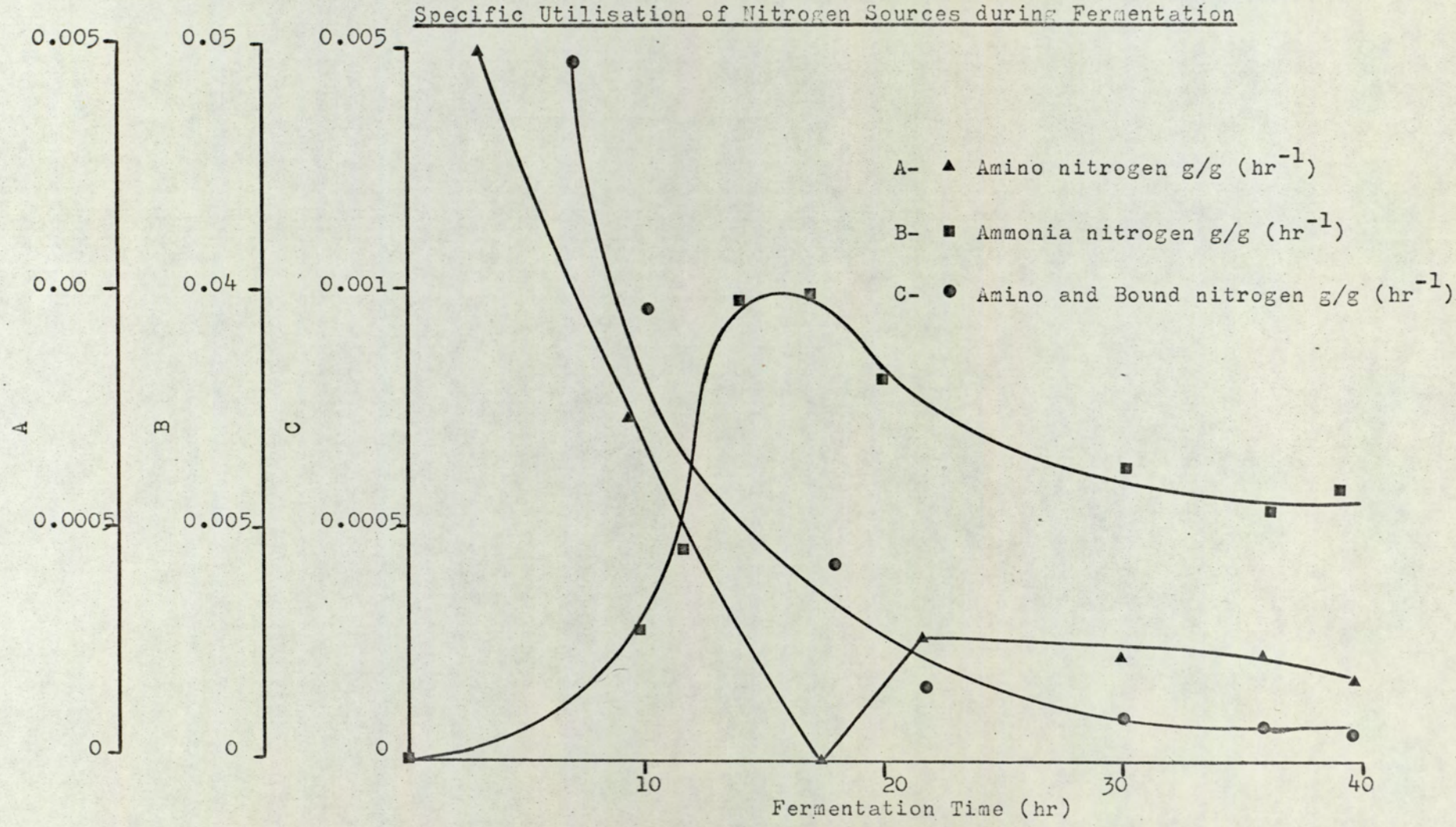
. Bound nitrogen estimated by difference - probably in the form of proteins.

From previous experiments it was assumed that the assimilation of nitrate and nitrite was negligable.

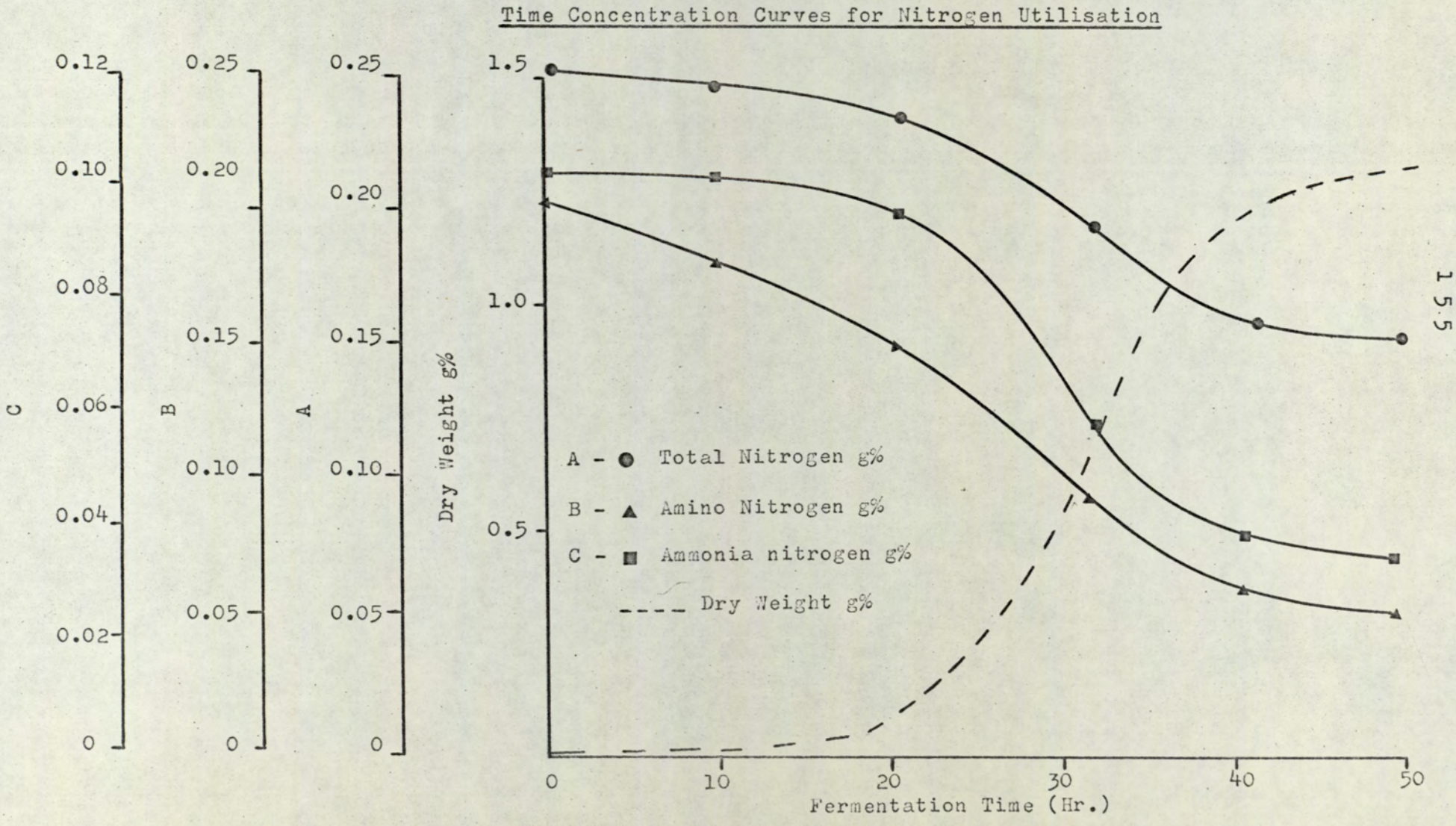
From the graph of specific amino acid utilisation (Graph 39) it can be seen that it is the NH_2^+ ions that are amongst the first to be assimilated. This takes place immediately after germination has begun. The trace of amino acid and bound nitrogen (in the protein form) confirms this. However during the log phase of growth this trace reaches zero and then raises again; this is possibly due to the lack of available amino acids. Proteolysis, via extracellular enzymes then break down the bound protein thus serving as an extra amino acid pool which is utilised at a specific rate. From the analysis of the amino acid profiles of the media with time, table (11), it is seen that glutamic acid is the only amino acid to be utilised to any degree. The total amount used in the first 16 hours corresponds closely to the total amino nitrogen utilised. This is consistent with the fact that glutamine is central to a number of cellular transamination reactions (See section 7)

The ammonia utilisation trace is different again in that it is not utilised to any great extent until the growth of the organism is well into the log phase. This shows that there is a preferable utilisation of amino nitrogen taking place in the growth phase immediately after germination. When A.niger was grown on a defined sucrose ammonium sulphate based media (ammonium sulphate being the only nitrogen source) it was shown that the log phase of growth was much longer. In fact the log phase was approximately 8 hours longer than when mixed amino nitrogen sources were present.

GRAPH 39


Specific Utilisation of Nitrogen Sources during Fermentation
A
B
C
0.005
0.00
0.0005
0
0.05
0.04
0.005
0
0.005
0.001
0.0005
0
A- ▲ Amino nitrogen g/g (hr-1)
B- ■ Ammonia nitrogen g/g (hr-1)
C- ● Amino and Bound nitrogen g/g (hr-1)
10
20
30
40
Fermentation Time (hr)

GRAPH 40


Time Concentration Curves for Nitrogen Utilisation
C
B
A
0.12
0.10
0.08
0.06
0.04
0.02
0
0.25
0.20
0.15
0.10
0.05
0
0.25
0.20
0.15
0.10
0.05
0
Dry Weight g%
1.5
1.0
0.5
A - Total Nitrogen g%
B - Amino Nitrogen g%
C - Ammonia nitrogen g%
Dry Weight g%
0
10
20
30
40
50
Fermentation Time (Hr.)

On examining TABLE11 which is an amino acid profile of the molasses based media during a fermentation it can be seen that there is an actual increase in some of the amino acids at the onset of the growth phase of the organism. A possible explanation of this fact is that proteolysis of the bound nitrogen portion (protein) of the molasses is taking place. Although shock excretion of amino acids is known in yeasts it has not been demonstrated in the filamentous fungi, thus leaving proteolysis as the explanation to the increase in the levels of some of the amino acids in the media. (N.B. There was no corresponding lowering of the amino acid level in the mycelium during this period). An experiment was devised in order to investigate the bound nitrogen portion (protein) of the molasses further. An acid hydrolysate of the media was prepared using the same method as for the hydrolysis of mycelium. The medium hydrolysate was then analysed for its amino acid composition. The increases in the amino acid concentrations corresponded closely to that estimated as the bound nitrogen fraction of the molasses.

In conclusion it may be said that care must be excercised when choosing the form of the C:N ratio because of the varied nitrogen utilisation ability of the organism. This is especially true when considering a complex media such as molasses.

It has already been shown that a complex mixture of amino acids (such as exist in molasses) enhances the rate of fermentation. Moreover it is true that it releases more carbohydrate for fermentation because of the fact that amino acids use their own carbon skelétons for cellular syntheses, thus giving a greater conversion efficiency of carbohydrate into cell biomass. This fact was clearly shown during a fermentation when ammonium sulphate was used as the only nitrogen source. It was seen that the

conversion efficiency of carbohydrate into biomass was only 27% which was almost half that achieved when using amino nitrogen sources. Among organic nitrogenous compounds amino acids are the most frequently available as substrates for micro-organisms. Since these compounds are at an oxidation level lower than that of carbohydrates, they are potentially useful as sources of energy for both aerobic and anaerobic microorganisms. The main pathways followed during the catabolism of amino acids are described in the reviews by Greenberg (1961) and Barker (1961).

TABLE. 1 1

Amino acid composition of medium at various stages of fermentation.

g amino acids/100 ml of media.

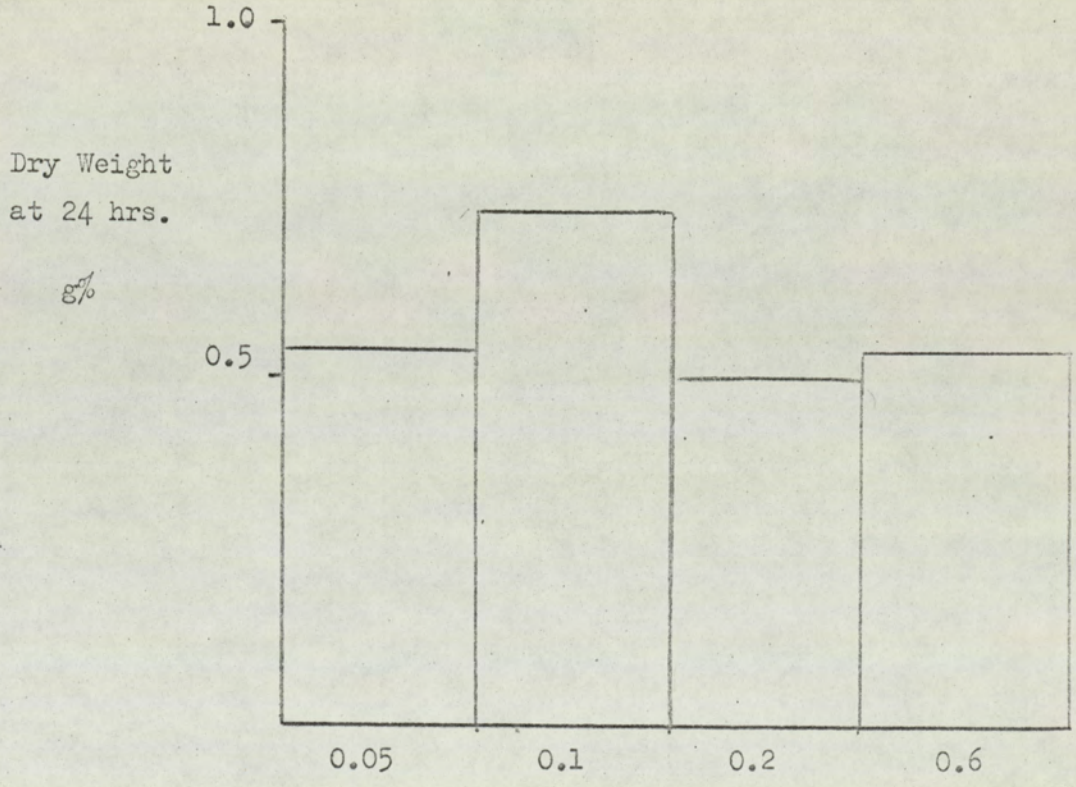
Amino acid	0hrs	16hrs	19hrs	23hrs	26hrs	37hrs	43hrs	47hrs
Aspartic	.026	.032	.014	.023	.026	.017	.008	.010
Threonine	.005	.015	.001	.003	.001	Trace	.001	.030
Serine	.045	.061	.026	.034	.003	.001	.001	.024
Glutamic	.049		.039	.041	.049	.025	.022	.024
Proline	.001	.001	.002	.008	.001	.001	.001	-
Glycine	.005	.016	.014	.004	.005	.005	.002	.016
Alanine	.012			.006	.005	.009	.005	
Cystine	Trace	Trace	Trace	-	-	-	-	-
Valine	.008	.006	.006	.006	.004	.004	.003	.002
Methionine	-	Trace	-	-	-	-	-	-
Isoleucine	.016	.010	.013	.009	.007	.001	.001	.002
Leucine	.014	.011	.010	.005	.002	.001	.001	.001
Tyrosine	.021	.020	.024	.012	.009	.013	.008	.031
Phenylalanine	-	-	-	-	-	-	-	.019
Total	0.202	0.172	0.149	0.145	0.112	.077	.053	0.108

6.4 Phosphate Concentration.

The optimum phosphate concentration was evaluated in fermentations carried out using 500 ml shake flasks. Four concentration levels of phosphate were tested, those were between 0.05 and 0.60 g% (Figures pertain to weight of sodium dihydrogen phosphate added).

C:N ratio 4.1

Media as in preceeding experiments.



The results suggest that an optimum value of phosphate addition is 0.1 g % when molasses medium is used. It must be noted that molasses also contains a certain amount of phosphate.

6.5 p H value

Molasses solutions have been used in the majority of experiments and it was noticed that there was very little p H change during fermentation (Especially during runs which contained pelleted mycelium). This is because of the high buffering capacity of such complex media. In spite of relatively large amounts of acid being produced the p H during most fermentations only dropped about two units. Similar fermentations on defined media however dropped approximately four units of p H. It is advantageous for the production of biomass to start the fermentation at a low a p H value as possible. This would therefore avoid costly sterilisation processes and minimise contamination.

The optimum p H conditons for germination and growth of A.niger M4 were initially tested in shake flasks. p H values between 2.4 and 9.8 were used. It was noted that at the extreme p H values no germination took place after 48 hours and the fastest germination time under these conditions was seen to be between 4.8 and 6.0. Those at 6.0 gave the longer hyphae at this time, and alkalinity beyond p H 6.0 has a slight depressant effect. Values of around p H 5.5 are therefore claimed to be optimal for germination for these conditions.

Experiments on p H levels in tower fermenters were conducted in the 1 litre vessels. These fermenters were all inoculated with macserated mycelium thus cutting out the germination stages altogether. The results are summarised in Table12.

Again, p H values of around 5 seem to be consistent for optimum growth conditions. No growth was observed at p H 1.7 or 8.5, this may be due to the fact that a large amount of acid/alkali must be added in order to obtain these values and since they are highly dissociated in solution the redox value of the media

will change considerably.

TABLE. 12

p H of media	Fluid p H	Dry Weight
1.7	1.8	negligable growth
2.2	2.7	0.35 g%
4.1	3.7	0.59 g%
6.3	3.8	0.55 g%
6.8	3.8	0.54 g%
8.5	8.4	Negligable growth

6.6 Temperature

Media Constituents

Beet Molasses 10 g% w/v

Ammonia Sulphate 0.50 g% w/v

Sodium dihydrogen phosphate 0.1 g% w/v

p H 6.5

Aeration 1 vol/vol/min.

Experiments carried out in 8.6 cm (10 litre) glass tower fermenters.

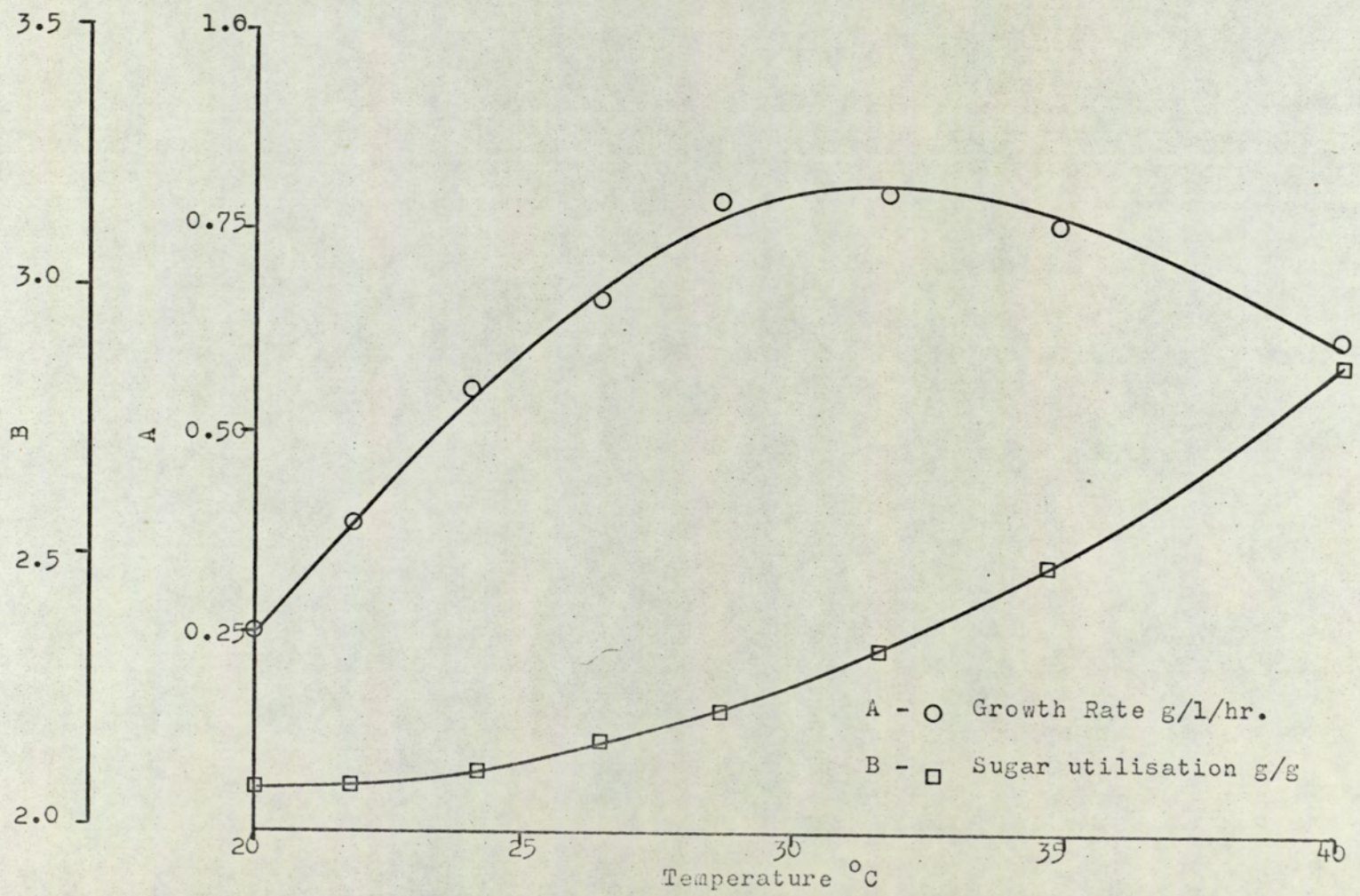
The range of temperature studied was 20 to 40°C in submerged culture, however a wider range of experimentation was undertaken using race plate methods. Graph 4 1 shows that there is an optimum value for growth rate between 27°C and 33°C for A.niger. At temperatures as low as 4°C can survive and even grow. At 10-15°C it is possible to measure their growth response on race plates. The upper limit for substantial growth of A.niger is around 45°C

Of economic importance is the fact that at elevated temperature the whole fungal metabolism is increased, giving rise to a greater utilisation of carbohydrate. However, this may not necessarily be channelled into biomass production.

Those countries which require protein additions in their food intake are frequently those where high temperatures prevail and cooling systems can become expensive. The wide tolerance of A.niger to temperature variation might be significantly advantageous for its choice as a biomass producing organism. The use of the tower fermenter is also advantageous due to the fact that it has a large surface area to volume ratio available for cooling.

GRAPH 41

The Effect of Temperature on Mould Growth Rate



6.7 Dissolved Oxygen.

Aspergillus niger is an obligate aerobe therefore when grown under submerged conditions it requires a continual supply of dissolved oxygen. This is provided in the tower fermenters via the sterile air supply. Since it was not possible to control the level of oxygen in solution in the fermenters, largely empiracal experiments were carried out. Three stages in oxygen uptake have been investigated during the course of this work. The first, mass transfer of oxygen from the air into solution was dealt with in Section 111, secondly, the oxygen in solution will be discussed in this section whereas in the third place the transfer of oxygen in the mycelium is largely considered in Section V.

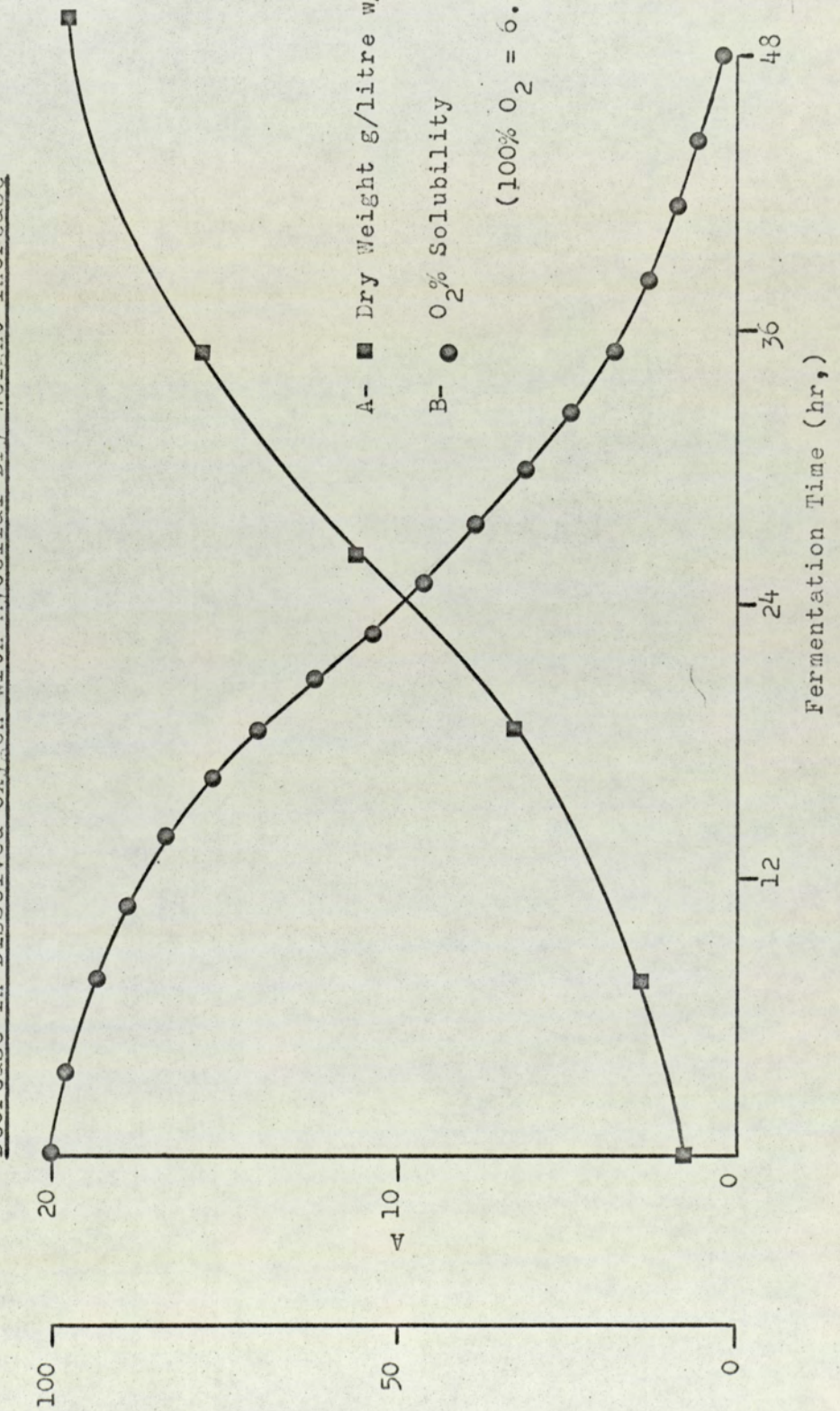
From the experiments done so far it seems that the limiting factor in terms of growth and final mycelial density is the dissolved oxygen concentration in the media. This is shown particularly well in Graph 42 which is a plot of the decreasing amount of dissolved oxygen with the increase in mould concentration.

An increase in the dissolved oxygen concentration in solution above the critical value, should bring about an increase in growth rate or at least give a higher yield of product. The affect of increasing the levels of dissolved oxygen on product level was shown clearly when two identical fermentations were run. One with a stable air flow through the fermenter and the other where the air flow was increased as the level of dissolved oxygen fell in the fermenter, (shown on the dissolved oxygen meter). Graph 43 shows this effect.

The increased air rates however brought about other problems such as severe foaming. These could be easily controlled by the use of chemical antifoam agents. Although these agents had no direct affect on mould metobolism their surfacant effect indirectly

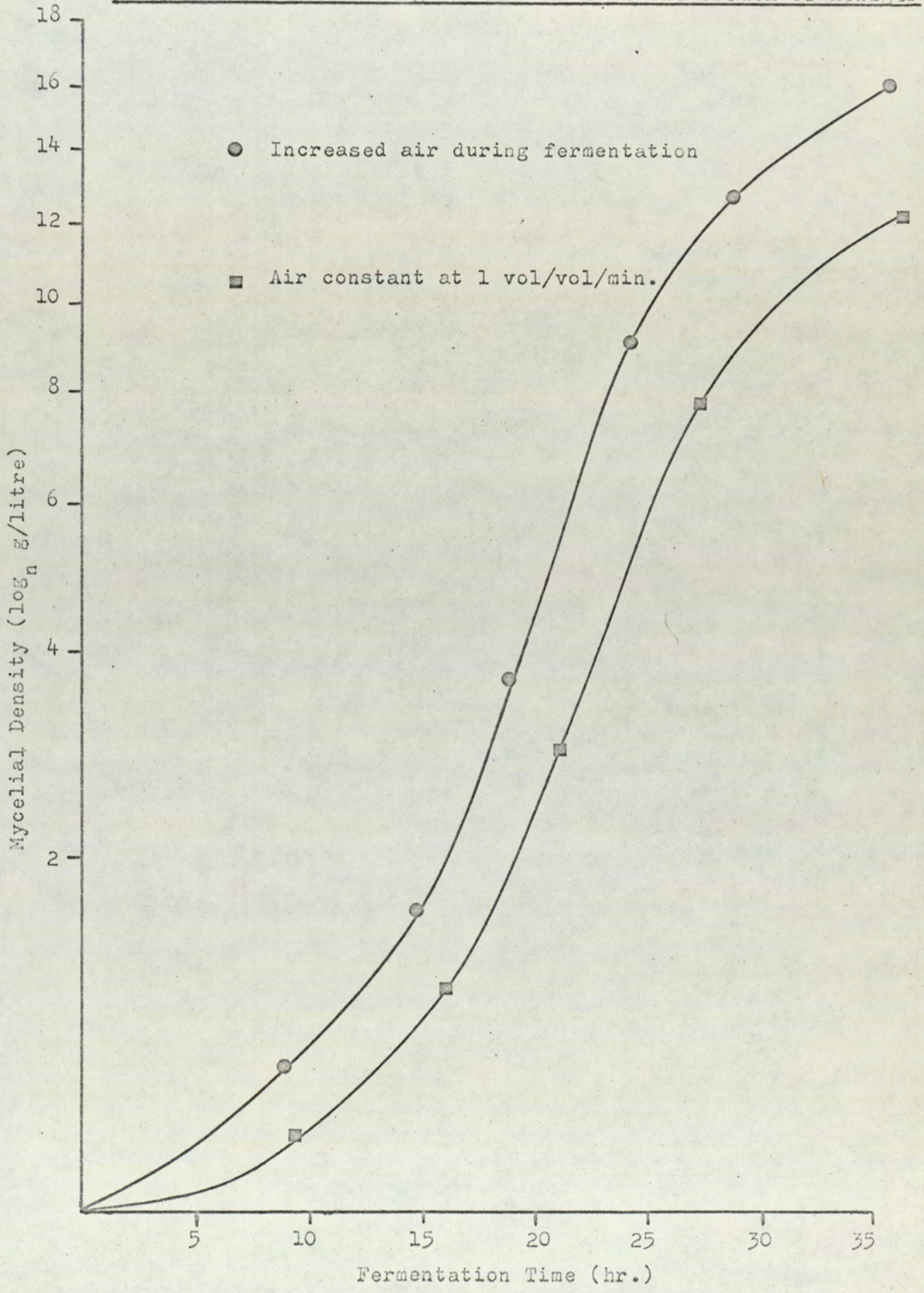
GRAPH 42

Decrease in Dissolved Oxygen with Mycelial Dry Weight Increase



GRAPH 43

The Effect of Increasing the Air Rate on the Growth of A.niger



retarded growth by severely decreasing the amount of oxygen in solution. This is clearly shown in graph 11 (Section 3). The dissolved oxygen content drops because the mass transfer into solution is lowered due to the fact that the antifoam decreases the surface tension of the liquid which in turn causes coalescence of the air bubbles. This means that the ratio of the surface area of the bubbles to the volume of liquid is substantially reduced. It was also noted that the gas hold-up in the fermenter is reduced due to the fact that large bubbles pass quickly through the column, whilst smaller bubbles have a long residence time. The use of chemical antifoams can however be obviated by fitting a high speed mechanical foam breaker into the expansion chamber of the fermenter With the use of a foam breaker in the 8.6 cm tower fermenters the final dry weight of mould was increased by some 50-100%. Coalescence of air bubbles is also caused by the filamentous mould morphologies and by sinter blockage (area reduction of air distributor) - See also Sections 3 and 5.

If it is to be assumed that it is the oxygen that is the limiting factor to mould growth then the point at which the mould leaves the linear phase of growth is the critical level of dissolved oxygen for the mould under these particular conditions. The critical level of dissolved oxygen is the concentration where oxygen utilisation is no longer a zero order reaction but probably follows first order kinetics. From the experiments performed the critical level was revealed to be in the order of 3 mg/ml oxygen.

6.8 Interelationships of Media Parameters.

When assessing the effect of any one parameter on a fermentation system it must also be borne in mind how the variations in the prime parameter affect other secondary parameters. Examination of parameters in isolation can be misleading and give a false impression of the situation at hand. For instance when the carbohydrate concentration of the media is altered, some of the other immediate alterations are in the oxygen solution rate, bubble size, osmotic pressure and viscosity.

The oxygen solution rate will be influenced by any dissolved matter in the media and also by its temperature. The bubble size is also altered by the above two parameters but in addition it is affected by p H and the presence of any surface active agents such as detergents and antifoams. Bubble volume is of special importance in tower fermenters where there is no impellar to break them up. Osmotic pressure is in relationship to constituents dissolved in the media whilst viscosity is a confirmation between dissolved solids and also those that are in suspension Therefore it may not be altogether advantageous, especially when using a tower fermenter to place a prescise optimum value to those shown in the graphs . In fact, it would be profitable to lower the carbohydrate value, thus achieving complete utilisation, and modifying bubble size by the use of detergents. This would give the double advantage of increasing the oxygen solution rate by decreasing dissolved components of the media, and also increasing the interfacial air/water area in the fermenter.

Due to the column design the various parameter interelationships have become far more complicated in the tower fermenters than they would be in stirred fermenters. For this reason the above experiments can give no more than an indication as to optimum media.

conditions. Much more exhaustive tests must be carried out under steady state conditions in order to draw any firmer conclusions than have been laid down here.

6.8 Conclusions.

In conclusion there may be calculated values for the conversion of carbohydrate material into cell protein.

Assuming a 48% conversion efficiency of carbohydrate in cell mass

Then 1g Carbohydrate $\bar{=}$ 0.48g Cells.

Assume also a protein level of 35%

Then 1g Carbohydrate $\bar{=}$ 0.168g Cell Protein.

Working at the maximum doubling time of 4 hrs and starting with 1g of cell mass one could achieve approximately 21g cell protein in 24 hrs.

This however is working at a maximum rate of efficiency. To achieve this rate the carbohydrate concentration should be in the order of 7g%. If we are to assume a 2g% cell yield then this would leave behind 3g% carbohydrate which could possibly be re-circulated or become an effluent factor. As it has already been suggested a lower carbohydrate value would be of greater value to the economics of the fermentation.

Starting from a spore suspension in the tower fermenter mycelial yields of between 1.5 and 2.0 g% dry weight can be produced in 30-36 hrs. Using a mycelial type inoculum this may be achieved in less than 24 hrs.

SECTION 7

SECTION (7)

A mino Acid Analysis of the Product.

The general aim of producing large amounts of fungal biomass is for use as feedstuff, either for human or animal consumption. A mino acid analyses were performed in order to provide information as to the nutritive value of this product.

7.1. Preliminary Feeding Trials.

Preliminary feeding trials have also been carried out by TATE and LYLE LTD. (Imrie et al.) on *A.niger* grown on molasses based medium. These feeding tests were performed basically to provide information as to the toxicity of the organism. These trials were carried out using rats. The rats were fed on the experimental diet after weaning and their body weight recorded. The results can be seen in Graph 43. The diet on which they were fed in shown in Table 13. These results show no unfavourable effects and can be used as a basis for assuming there will be no toxins produced by this organism.

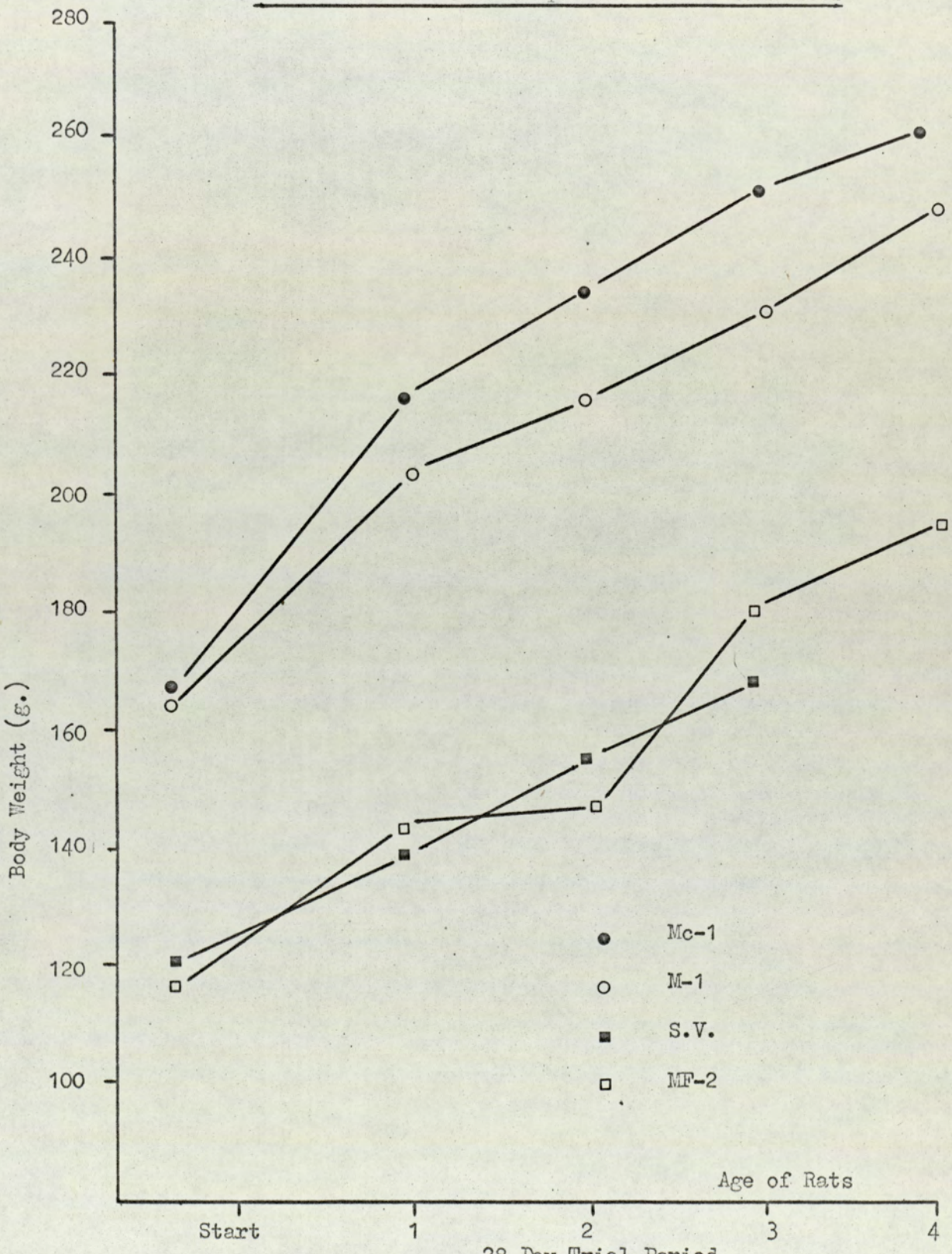
TABLE. 13.

Composition of the Experimental Diets.

Ingredients	Diet %			
	M-1	MF-2	SV	Mc-1
Wheat Meal	20	20	2J	
Barley Meal	40	40	42	
Soybean Meal	7	15	25	
AS - M1/Oven Dried	25	-	-	
AS - M1/Freeze Dried	-	17	-	
Maize Oil	3	3	3	
Minerals	4	4	4	
Vitamins	1	1	1	
B 12	0.5	0.5	0.5	
Total Protein (Kjeldahl)	15.9	16.1	17.4	21.7
Dry Matter	96.5	92.0	93.2	89.6

GRAPH 43

Growth of Rats Fed on Experimental Protein Diets



Mc-1; M-1; S.V; MF-2; Refer to specific diets. (See Table 13)

7.2 Evaluation of the Mould Protein by Amino Acid Analysis

It is extremely difficult to estimate the biological value of mould proteins merely by using the results of superficial feeding trials. The nutritional value of protein has been discussed by Goldblith (1968). The methods used for these evaluations included amino acid analyses of the product. It was decided to use some of these methods in this investigation.

A large amount of this thesis has been concerned with the production of pelleted mycelium in tower fermenters whilst in stirred tank fermenters it is usually the filamentous morphology that ensues. Since the different environment produces such marked changes in the morphology it is reasonable to assume that variations might also take place in the protein make-up of the mould. An investigation was therefore carried out in order to reveal any differences between the microbial protein produced in the two systems. Basically, analyses were performed in order to show that the morphological variations of the mould did not have any deleterious effects on the nutritive value of the fungus.

7.2.1. Protein and Amino-Acid Content.

The methods of protein and nitrogen estimation of Lowry and Kjeldahl were used. The methods provided no positive conclusions as to the relation of protein and nitrogen values between the two morphological forms (pellets and filaments). The Lowry protein estimation did not give a great enough insight into the biological value of the mould under the optimum condition of fermentation used (see section 6). The following results for protein measurement were recorded in tower fermenters.

Kjeldahl Protein (Nitrogen x 6.25) = 38.25g %
Lowry Protein = 30.05g %

Nitrogen in Cell Walls = 3.06g %

Nitrogen in Cytoplasm . = 2.53g %

The amino acid profile was also determined and can be seen in Table 14

TABLE.14 Amino Acid composition of Mould Mycelium Grown in Tower Fermenters.

Amino Acid.	g % Total Protein.	g % Dry mycelium	g/14 g NH_2 Nitrogen
Asparagine	14.1.	5.10	12.30
Threonine.	3.5	1.27	3.07
Serine *	18.3	6.62	16.02
Glutamine*		- ditto -	
Proline	1.2	0.43	1.04
Glycine *	10.7	3.87	9.37
Alamine *		- ditto -	
Valine	4.8	1.74	4.21
Methionine	1.0	0.36	0.87
Iso-Leucine	8.1	2.93	7.09
Leucine	9.5	3.44	8.32
Phenylalanine	6.9	2.50	6.05
Lysine	7.5	2.72	6.58
Histidine	2.3	0.83	2.01
Arginine	8.0	3.00	7.26

Pellet size 4 mm dia.

amino Acids 36.2 g%

Units refer to g. of amino acid.

* Denotes poor separation.

It was found that the amino acid profile changed both with morphology and the age of the culture itself. A superficial survey was therefore made in order to determine what the main parameters that affect the relative quantities of amino acids within the mould mycelium (with respect to this project).

7.2.2. Amino Acid Profile With Respect to Morphological Variations

In the initial experiment two different morphologies of A.niger (M_1) were grown in separate systems on identical media - viz. pelleted and filamentous mycelium.

Media composition.

Beet Molasses 10 g % w/v
Ammomium Sulphate 0.5 g % w/v
Potassium dihydrogen phosphate 0.1 g % w/v
p.H of media 6.5.

Tower Fermenter.	Stirred Tank Fermenter
8 litre working volume.	10 litre working volume.
8 litres air/min	5 litres air/min
(No.2 glass sinter)	500 r.p.m. stirrer.

Tables 15 and 16 show these results.

TABLE 15

Amino Acid Profile of Free-flowing Mycelium (Stirred Tank Fermenter).

Time	17hr	21hr	36hr.	42hr.
*Asparagine	20.2	18.3.	16.4	17.8
Threonine	4.7	4.3	4.9	4.5
Serine	8.7	6.2.	7.4	6.4
*Glutamine	21.1	19.3	15.4	16.7
Proline	2.3	1.6	2.7	0.5
Glycine	6.9	5.8	5.1	6.2
*Alanine	8.0	7.7	6.9	7.1
Cystine	-	-	-	Trace
Valine	3.5	2.6	3.2	3.3
Methionine	1.o	1.1	1.0	1.5
Iso-Leucine	2.9	2.0	2.8	2.6
Leucine	7.3	5.3	6.2	6.7
Tyrosine	1.9	3.4	3.8	3.7
Phenylalanine	1.1	7.8	18.0x	9.2
Lysine	4.9	6.9	3.9	6.1
Histidine	1.9	1.7	1.1	2.0
Arginine	3.4	5.9	1.3	5.7

Amino acids given in g% total protein.

* - Denotes primary compounds in amino acid metabolism (See diag.)

x - Suspect amino acid value.

TABLE 16a.

Sizes of Pellets at Various Time Intervals

Time	Diameter in m.m.
20.5	1 mm
25	2 - 3 mm
28	4 mm
90	10 mm - signs of autolysis in the centre of colony.

Refers to pellet size at each sampling.

TABLE.16b

Amino Acid Profile of Pelleted mycelium (Tower Fermenter)

Time	20.5hr.	25hr.	28hr.	90hr.
*Asparagine	9.8	8.2	14.1	7.8
Threonine	4.0	3.6	3.5	4.1
Serine	4.9	6.3	18.3°	15.1°
*Glutamine	12.9	14.1		
Proline	0.7	0.6	1.2	0.8
Glycine	4.3	4.2	10.7°	4.2
*Alanine	4.5	4.7		6.0
Valine	3.7	3.7	4.8	3.5
Methionine	1.3	1.3	1.0	1.0
Iso-Leucine	2.3	2.4	8.1x	3.5
Leucine	6.0	7.9	9.5	6.0
Tyrosine	3.6	2.9	3.8	16.7x
Phenylalanine	11.5	10.0	6.9	4.4
Lysine	13.4	8.2.	7.5	13.3
Histidine	3.8	2.2	2.3	3.1
Arginine	13.1	7.4	8.0	5.2

Amino acids given in g% total protein.

* Denotes primary compounds in amino acid metabolism (see diag.)

o Denotes poor separtion.

x Suspect amino acid value.

The main differences observed in the amino acid profiles of mould grown in stirred and tower type fermenters is that in stirred culture two out of the three precursors to protein build-up (amino-acid) interchange Diag3 Asparagine and Glutamine make up 40% of the total protein, whilst in tower fermenters Asparagine and Glutamine represent less than 25%. The third precursor Alanine is half their values in both cases. It would therefore seem likely that pathway B and C predominate in both morphological forms. This give a clue as to what nitrogen source is being utilised more rapidly by the mould under these conditions, since it has been suggested that when inorganic nitrogen is utilised, alanine is formed and when organic nitrogen is utilised glutamine is formed.

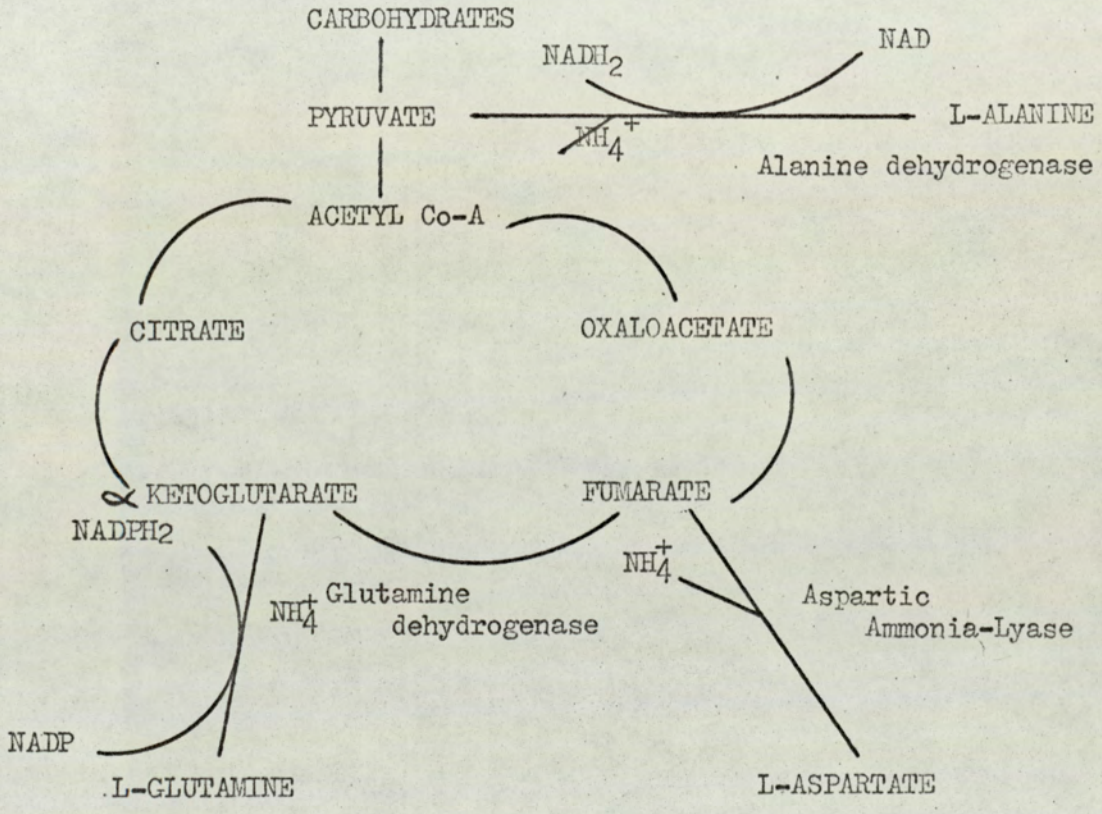
An explanation as to the differences in the levels of the three precursors may also lie in the fact that in colonies as large as those formed in the experiment, remobilisation of the protein could have been occurring in the central portion and also mass transfer of materials into the central region may have presented some limiting factors as to protein build-up.

It can be seen that there is a wider spectrum of amino acid values in the pelleted mycelium which do not rely on the precursors Asparagine and Glutamine to give a high protein value, it is also noted that no specific amino acid is either missing* or has an uncommonly low value when this form of mycelium is produced. Even in the 1 cm pellets no particular disadvantage can be recorded. However in the larger pellets a greater degree of hydrolysis was noted for the same age of culture.

* Except tryptophane and cystine which are broken down during the rigorous acid hydrolysis. Methionine is also broken down to some extent.

DIAG. 3

INCORPORATION OF AMMONIA BY MICROORGANISMS.



[FROM MORTENSON, (1962).]

7.2.3 Pool Amino Acids.

The colour reaction for the Lowry estimation of protein is dependant on the fact that there are peptide linkages present. No colour is obtained with this reaction if the amino acids are in an unbound form. It is well known that there is always a pool of free amino acids present in mould mycelium. Therefore these amino acids will not register as part of the protein pool whilst using the Lowry method of analysis. These unbound proteins nevertheless contribute to the nutritive value of the food form. Fluctuations have been described by several investigators (see references cited by Holden, 1962, also Ras and Ventkataraman 1952, Meyers and Knight, 1961, Chattaway et al, 1962). However, it is particularly important in this project to ascertain the degree of this amino acid pool since it could contribute to the reported protein value.

The amino acid pool was therefore investigated in relation to both the age of the fungus and also to the various C.N. ratios used in the medium. The main results of this investigation are summarised in Tables 17 and 18

From Table 17 it is seen that the accumulated amino acid level reaches a plateau and then tails off after maximum dry weight has been achieved whereas the pool amino acid tend to increase as the fermentation proceeds. The relative proportions of pool amino acids is particularly high after maximum dry weight achievement. This is to be expected since remolbilisation of protein is occuring in this area.

In Table 18 it is seen that as the carbon to nitrogen ratio decreases not only does the accumalated amino acid level rise, but also the level and proportions of pool amino acids raise. From the two samples tested it seems as if there might be some correlation

TABLE 17.

Time	17hrs.	21hrs.	36hrs.	42hrs.
Amino acids g% of mycelium	21.9	32.6	28.5	26.6
Pool amino acids g% of mycelium	0.40	0.51	0.95	1.61
Pool amino acids g% of Total amino acids	1.8	1.6	3.3	6.1

Mycelium grown in tower fermenters.

TABLE 18.

C:N	(a) Amino Acids	(b) Pool amino acids	Pool amino acids % of Total.	Lowry Protein	(a) - (b)
20:1	21.0	0.50	2.4	21.0	20.5
12:1	29.5	1.39	4.7		28.1
6:1	41.0	8.60	21.0	30.1	32.4

Mycelium grown in tower fermenters.

TABLE. 19.

Essential Amino Acids in Protein.

Amino acid	%in F.A.O. Reference Protein.		% in A.niger Mycelium.
Leucine	4.8		9.5
Iso-Leucine	4.2		8.1
Valine	4.2		4.8
Lysine	4.2		7.5
Methionine	2.27)	) 4.2	1.0 *
Cystine	2.0)		-
Phenylalanine	2.8		6.9
Threonine	2.8		3.5
Tyrosine	2.8		3.6
Tryptophane	1.4		Destroyed* during Hydrolysis

* Destroyed to some extent during hydrolysis.

between the 'Lowry protein' estimation and the figures obtained for the accumulation of amino acids minus the pool amino acids. It is therefore suggested that protein analysis by summation of amino acids might be one of the best methods of estimation. This however suffers from the disadvantage that very few workers estimate and report protein in this way.

Amino acid analyses gives a relatively clearer picture than total protein estimations of the nutrition value of the fungal mycelium and how it varies as to the fermenter conditions which prevail. However the final tests as to the actual value of a new food is in large scale feeding trials. Theoretical calculations for protein quality of a feed or food is difficult. In the absence of such data, at present it may be sufficient to compare the fungus grown with that of a F.A.O. reference protein.

The figures quoted for the F.A.O reference protein have only the essential amino acids. Most animals are able to synthesise the rest via their own metabolism. The amino acid profiles of various fungi are relatively similar in that there may be general deficiencies in the sulphur containing amino acids cystine and methionine as compared to the F.A.O reference pattern. TABLE shows values of the F.A.O reference protein amino acids and this can be compared with A niger (M.1) grown in tower fermenters supplemented molasses medium.

There has been little work done on the nutritional value of fungal proteins in the feeding of animals or man. By summarising the reported work it may be said that if A.niger were to be substituted to 100% of the diet then deficiencies would be noted (in the sulphur containing amino acids cystine and methionine) however smaller substitutions would reveal it to be an useful supplementary protein source.

SECTION 8

Discussion and Conclusions.

8.1 Conclusions.

This work was a study of the feasability of producing large amounts of filamentous fungi in tower fermenters. The fungal mycelium thus produced being utilised as a foodstuff, either for animal or human consumption. One of the most important aspects of producing foodstuffs from fungi is the fact that it must be shown to be both an economically viable proposition and operationally simple. This is because of the relatively unsophisticated nature of the product. It was therefore decided to carry out experimentation involving the tower fermenter and to derive sufficient information to be able to assess the technical and economic feasability of such a process.

In the first instance experimentation was directed at the fermenter itself. The design characteristics optimisation was carried out basically with the parameters of oxygen transfer in mind. Oxygen transfer rates are frequently quoted as being the limiting factors in biomass production (Solomons 1961). Whilst working with air/water systems it was soon realised that there were optimum air volume throughputs (and superficial gas velocities) beyond which there was little or no increase in oxygen transfer rate. A similar optimisation value was detected in the column aspect ratio experiments. From the point of view of oxygen transfer conclusions were therefore as to a particular column design, namely a tower fermenter with an enlarged sinter area and specific aspect ratio requirement. It was also deemed un-necessary to utilise the expansion chamber section in this context since this resulted in lower oxygen transfer efficiencies being attained.

The apparent power required to transfer oxygen into solution in standard stirred tank and tower fermenters was compared. It was shown that the power required by a tower was considerably less than a stirred fermenter. Thus economical fermenter running could be achieved by the use of these columns.

The question of foam breaking was also reviewed briefly in relation to the tower fermenter and it was shown that chemical antifoams had deleterious effects on the oxygen transfer capability of this system. It was concluded that mechanical foam breakers, fitted into the expansion section of the column, could profitably be employed in this connection. The mechanical foam breaker avoided the air bubble coalescence phenomenon created by chemical antifoams.

The particular fungus chosen for this study was Aspergillus niger (M1). It had been previously selected for high yields, growth rate, protein production and its economic carbohydrate conversion efficiency. Preliminary investigations on the growth of this organism in tower fermenters revealed that it was capable of growing in many morphological forms. Further experimentation showed that the size and shape of these forms could be controlled. This advantage was realised as far as the production of microbial biomass was concerned. Morphological control was achieved by directing the spore inoculum and the degree of agitation within the column, both of which were found to be the main contributory factors toward pellet formation. It was clearly shown that the growth of certain colony forms of A. niger had advantages over the filamentous variety. The advantages lay mainly in the facts that oxygen transfer rates into solution were enhanced and that the viscosity (hence the power required for mixing) was considerably lower in pelleted than in filamentous mould cultures. It was shown that filamentous mycelium caused the coalescence of air bubbles at lower superficial gas velocities than corresponding amounts of pelleted mycelium. Secondary advantages accomplished by the use of pellets lay in the fact that mass transfer difficulties (mainly of oxygen) occured toward the centre of the colony. This meant that the central core of mycelium was receiving the minimum oxygen concentration for maintenance metabolism and no excess for organic acid production. By studying the fluidisation behaviour of these pellets it was concluded that the maximum theoretical dry weight attainable in these columns was approximately 2 g% w/v. It is conceded that the

filamentous mycelium is able to occupy a greater volume of the fermenter, however in practice this could not be achieved in towers because of the resistance to oxygen transfer observed using this morphology.

The 'A.P.V. Tower Fermenter' was originally designed as a continuous metabolite producing system. However, the production of fungal mycelium in towers was successful on a batch regime. With the morphological form presented here (pelleted mycelium) it would not be convenient to run a continuous mycelium producing system. In a continuous system it would be necessary to revert to the filamentous form of the organism and thus lose the advantage gained by the use of pellets. The reason being, that as the colonies grow larger with time, mass transfer difficulties would become apparent and autolysis of the central portion would occur.

Using the preconceived ideas of the pellet size required for optimum production of microbial biomass further experimentation was carried out in order to maximise yields and protein formation within the fermentation. From the results achieved however it was calculated that it was not practical to run the fermenters at the optimum parameters. It was concluded that due to water and effluent factors involved the more desirable levels of media concentration to use would be slightly below maximum and thus avoid post fermentation expenditure.

A more refined assessment of the biological value of the microbial product was also made. This was done mainly in terms of the amino acid content of the mycelium. Comparisons were made between mould grown in differing morphological forms. It was seen that no deleterious affects could be noted in the pellets in relation to filamentous mycelium. On the contrary estimates suggested a more balanced amino acid profile might be gained by using the colony form of the organism. No satisfactory explanation could be given for this fact. Figures quoted for essential amino

acid profile of the mould grown under these conditions were found to compare favourably with those laid down by the F.A.O. protein group.

It is considered that amino acid analysis gives a clearer picture of the biological value of microbial protein and is preferred to any of the techniques used to estimate total protein concentrations. It is also believed that more emphasis should be placed on the amino acid pool of any organism since this is the portion that is most readily available during consumption by animals. Results showed that this amino acid pool was variable, investigation of which may yield further interesting data.

On considering the nutritional aspects of the mould A. niger (M1) it must be realised that a 100 % replacement in the diet might prove detremental due to inbalance in certain of the sulphur containing amino acids. However substitutions at a lower level e.g. 40-50% would reveal this mould to be a useful protein source.

8.2 Final Discussion.

In retrospect it may be said that fungi were probably the most difficult microorganisms to attempt to grow in large masses in the tower and it is felt that the use of either yeast (Greenshields et al 1972) or bacteria (Pannell 1972) might be more fruitful, especially in continuous culture. Single celled suspensions give rise to lower apparent viscosities and enhancement of oxygen transfer is made possible. Both yeast and bacteria have higher specific gravities than fungal cells. Importance is therefore attached to the fact a combination of both system and organism be studied carefully.

It is obvious that the tower system lends itself to the continuous production of metabolites. This may lead to the use of the columns as microbiological degradation systems or waste treatment plants. Advantages could be gained from the fact that the tower fermenter, of the correct design can maintain high oxygen tension levels and therefore can reduce the biological and chemical oxygen demand of effluents (Chesson 1972). It is considered that research and development in this direction should be on a specific basis. For example a tower fermenter unit could be used to deal with brewery, cannery, refinery or maltster effluents which produce weak carbohydrate containing liquids. Once a suitable ecological balance has been attained in these columns it would be possible to run in series thus fermenting the effluent completely.

One of the greatest difficulties in running fermenters continuously is that of wash-out of the organism. In tower fermenters this can be overcome by using microbial films (Atkinson 1972) or even enzymes adhering to spheres (Emery 1972). The spheres being of such a composition as to prevent washout below the fermentation rate required by the reaction.

Multiple specific reactions may be kept apart in the tower fermenters by the use of dialysis type membranes, here the rate of flow could be

automatically controlled by the rates of the preceeding reactions. Thus columns may be set up whereby conversions of two or three products may be accomplished in the one column. This may be achieved even though either substrate or product may be inhibitory to the other.

Whilst many practical and theoretical problems still remain unresolved in the field of 'tower fermentation', it is obvious that there is considerable versatility associated with this system that is yet to be exploited.

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APPENDIX 1 FOR MATERIALS AND METHODS.

1. James A Jobling and Company Limited,
Newstead Industrial Estate,
Trentham Stoke on Trent St4 8JG.
Staffordshire.
England.

2. Cago Ltd. (Plastic Fabrications).
59 Burlington Street,
Birmingham.6.
Warwickshire.

3. Biologische Verfahrenstechnik,
Postfach 4000
Basel 3.
Switzerland.

4. A.Gallenkamp and Co. Ltd.,
P.O.Box. 290.
Technico House,
Christopher Street,
London.E.C.2.

5. G.A.Platon Limited,
281 Davidson Road,
Croydon,
Surrey.

6. Gelman Instrument Company,
Ann Arfor,
Michigan,
U.S.A.

7. Imperial Chemical Industries Limited,
Stevenson,
Ayrshire.

8. New Brunswick Scientific,
New Brunswick,
New Jersey,
U.S.A.

9. Evans Electroselenium Limited,
Halsted,
Essex.

10. Ferranti Limited,
Moston,
Manchester, M10. OBE.

11. Tate & Lyle Ltd.,
Group Research & Development,
University of Reading,
P.O.Box. 68.
Reading. RG6 2BX.

12. British Sugar Corporation,
Sugar Refinery,
Kidderminster,
Worcestershire.

13. Hopkin & Williams Ltd.,
Chadwell Heath,
Essex.

14. The British Drug Houses Ltd.,
B.D.H. Laboratory Chemicals Division,
Poole.

15. Fisons Scientific Apparatus Ltd.,
Loughborough,
Leicestershire.

16. Oxoid Limited,
London.S.E.1.

APPENDIX 11 MATERIALS AND METHODS.

The following appendix illustrates the method used to evqluate the results of the gassing out procedure. The results obtained had to be corrected for the lag in probe response time.

Experiments show that the response of the probe to a step-change in CO_2 is exponential in form: in mathematical terms:

$$\frac{dCr}{dt} = k_2 \quad (Co^*_2 - C_{le}) \qquad 1.$$

where k_2 is a constant and Co^*_2 indicates that the solution is saturated with air, Integrating equation. (1):

$$\frac{(Co^*_2 - Cr)}{(Co^*_2 - Cro)} = e^{-k_2 t} \qquad 2.$$

where Cro is the probe reading at time t = O. Some care must be taken over the combatability of units: it is probably most convenient to equate Co^*_2 to Croo, i.e. the probe reading at equilibrium. Consequently

$$\left(\frac{Croo - Cr}{Croo - Cro}\right) = e^{-k_2 t} \qquad 3.$$

Equation (3) was in fact used to evaluate k_2 experimentally. In tap-water at 30°C, k_2 was found to equal 1.7 min $^{-1}$: in molasses media, k_2 was 1.5 min $^{-1}$. In practical terms this means that 90% of full scale deflection of the probe-meter occurs in about 80 seconds.

During an aeration experiment c_{o2} is not constant but varies with time depending on the aeration efficiency. Mathematically

$$\frac{dCo_2}{dt} = (K_L A) \quad (Co^*_2 - Co_2) \qquad 4.$$

$$= k_1 \quad (Co^*_2 - Co_2)$$

where k, is a constant and equal to (k,a). Equation (4) has the same form as equation (1) and on integration:

$$\int_{0}^{CO_2} \frac{dCo_2}{(C^*_{o2} - C_{o2})} = k_1 t, \text{ with } C_{02} = 0 \text{ at } t = 0.$$

$$\text{or } - \frac{(Co^*_2 - CO_2)}{C^*_{o2}} = k_1 t$$

Rearranging: $C_{o2} = C^*_{o2} (1 - e^{-k,t})$ 5.

The differential equation for the probe is in this case:

$$\frac{dCre}{dt} = k_2 (C_{02} - C_r) \quad 6.$$

On substituting for Co_2 using equation (5)

$$\frac{dCr}{dt} + K_2 C_r = k_2 C^*_{0\,2} (1.e.^{-k,t}) \quad 7.$$

Using the D operator method for solving equation 7.

$$(D + k_2) Cr = k_2 C^*_{02} (1-e^{-k,t})$$

Complementary Solution: $C_r = Ae^{-k_2 t}$ where A. is the arbitrary constant

Particular Integral: $C_r = C^*_{02} - C^*_{02}\left(\frac{k_2}{k_2 - k_1}\right) e^{-k,t}$

using the boundary condition $Ce = C_{ro}$ at $t = 0$,

the complete solution is:-

$$\frac{Cr}{C^*_{0c}} = 1 - \left(\frac{k2}{k2 - k1}\right) \left\{ e^{-k,t} - e^{-k_2 t} \right\} - e^{-k_2 t} \left(1 - \frac{Cre}{C^*_{02}}\right)$$

In terms of the probe-reading C^*_{02} can be put equal to $Cr\infty$.

Consequently.

$$\left(1 - \frac{Cr}{Cro\infty}\right) = \left(\frac{k2}{k_2 - k_1}\right) \left(e^{-k,t} - e^{-k_2 t} \right) + e^{-k_2 t} \left(1 - \frac{Cro}{Cro\infty}\right)$$

8.

Equation (8) can now be used to evaluate k, from experimental data.

C_{ro}, $C_{ro\infty}$, k_2 are readily obtained from the response-curve, and k_1 can be computed for a selected value of Cre at a known value of t. This is most conveniently done using a graphical solution-method. After inserting the data into equation (8), a relationship of the form:

$$B - C k_1 = e^{-k_1 E}. \qquad 9.$$

is obtained, where B.C and E are numerical constants. Plots of $(B-Ck_1)$ and $e^{-k_1 E}$ as functions. of k_1 then lead to the desired value for k_1, or $(k_1 a)$. It should be noted that the line $(B-Ck_1)$ intersects the curve $e^{-k_1 E}$ at two points, viz $k_1 = k_2$ and the true value of k_1.

A P P E N D I X III

THE PRODUCTION OF FUNGAL BIOMASS IN AEROBIC TOWER FERMENTERS

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as presented at the First International Mycological Congress, Exeter, September 1971.

1. The tower or tubular fermenter can be described as an elongated non-mechanically stirred fermenter with an aspect ratio (height to diameter ratio) of at least 6:1 on the tubular section or 10:1 overall through which there is a uni-directional flow of medium or gases[1]. It can be used for batch, semi-continuous and continuous fermentations and can be classified using Herberts' criteria[2] of continuous systems, as a partially closed heterogenous tubular system. This was clearly demonstrated when this type of fermenter and its associated system was successfully used for the continuous production of ale and lager beer on laboratory[3,4], pilot[5] and commercial scales[6,7,8]. Such application including that when applied to the similar fermentations of cider, wine, whiskey-wash and vinegar charging-wort are growth-associated metabolite fermentations involving yeast under anaerobic or partially aerobic conditions. Its application in a fully aerobic situation has obviously been considered. Initial work has indicated that the aerobic conversion of ethanol to acetic acid by acetic acid bacteria in vinegar manufacture is feasible and continuous runs have been obtained on a laboratory scale[8,9,10,11]. Moreover, the production of yeast biomass in a flocculent condition is also possible on batch and semi-continuous systems and have been accomplished on laboratory, pilot and commercial scale[8,12]. This process is also being considered for non-flocculent yeast in a continuous system.

The work now presented here is the extension of this application to the filamentous fungi, particularly for the production of biomass - the production of fungal metabolites has been considered[8] but is the subject of further study and will not be dealt with here. The fermenter design[9] is shown in Diagram I. This does not materially differ from that used for anaerobic fermentations[8] except that (i) a means for aeration is provided at the bottom of the tower, (ii) the expansion chamber and thus the air/fob space is larger, (iii) some means is provided to prevent foaming and (iv) if continuous, the overflow and separator is of a different design.

The experimental fermenter vessels were made of glass, high density polythene, polypropylene or stainless steel and were sterilised both chemically (available chlorine) and by steam (up to 5 p.s.i). The sinters were made of glass, P.T.F.E., sintered stainless steel and occupied the whole fermenter tube cross-section and thus effectively held up the fermentation liquid.[8] For larger vessels a simpler aerator was used made of stainless steel and which sat on the bottom of the fermenter.

Four sizes of fermenter were used:-

(a) Experimental tubes 1 to 5 litre. 1'0" to 4'0" x 3" diameter.

(b) Laboratory scale (small) 10 to 20 litre. 4'0" to 8'0" x 4" diamete

(c) Laboratory scale (large) 50 litre 12'0" x 6" diameter.

and (d) Pilot scale 500 to 1000 litre 15'0" to 25'0" x 1'0" diameter.

2. The fungi were grown in the tower fermenters on two media - one based on a supplemented aqueous extract of carob bean (Ceratonia siliqua) and the other based on supplemented molasses.

Normally appropriate quantities of nitrogen sources were added in the form of inorganic ammonium salts or as glutamic acid to give the required Carbon : Nitrogen ratios.

Some preselection of the fungi was made using necessary criteria to ensure the most economic and suitable biomass production. This involved:

(i) A primary selection based on literature survey using the following criteria:

(i) Protein content

(ii) Protein extract

(iii) Toxicity

(iv) Growth rates

(v) Calorific value

(vi) CHO conversion efficiency

followed by:

(ii) A primary screening using race plates from which growth rates were ascertained,

then (iii) A secondary selection using experimental tubular fermenters.

Out of some forty fungi selected, the Fungi imperfecti appeared to grow best. - Table I.

The Basidiomycetes were slower growing and although several were tried the results were not promising. It was possible that the nutritional requirements were not satisfied by the media used. The results are shown in Table 2 together with those typical of the Phycomycetes tested. The phycomycetes grew easily, but biomass yields were too low for further consideration of economic biomass production.

3. It was decided to make a detailed study of the batch growth of Aspergillus niger in various sizes of tower fermenter to ascertain its growth kinetics in terms of (a) parameters which prescribe the fermenter, and (b) parameters which prescribe the medium in an attempt to establish the optimum for economic biomass production in terms of growth rate, yield, protein content and carbohydrate conversion efficiency.

(a) Parameters which prescribe the Fermenter.

(i) Aeration rate

Preliminary experiments in laboratory scale towers (10 litre) were performed to determine optimum conditions of aeration and agitation relative to the volume of air passed through the fermenter. In addition the optimum height to diameter ratio was calculated from this data. In tower fermenters oxygen transfer and agitation is caused by the air itself. Within the limits of the fermenters used, oxygen transfer efficiency and agitation increased linearly with aeration rate. Oxygen transfer rates were determined using the sulphite oxidation method of Cooper and co-workers which measured the maximum rate of solution when dissolved oxygen concentration is very nearly zero.

Graph 1 showed that an aeration rate of between 1.5 to 2 volumes of air per volume of liquid are near the optimum for oxygen transfer in this fermenter.

It should be noted that in laboratory scale fermenters it is impractical to use this optimum aeration rate and values of just below 1 vol/vol/min are generally used.

(ii) Aspect ratio

The aspect ratio of the laboratory scale tower (10 litre, 9 cm. diameter) was changed by varying the height of the liquid and hence its volume, whilst keeping the aeration rate per volume of liquid constant. Graph 2 shows that using these conditions, it can be seen that an aspect ratio of 16:1 appears to be optimal for a tower diameter of approximately 9.0 cm.

(iii) Foaming

It has been observed that during actual fermentation runs the addition of an antifoam agent had a deleterious effect on the dissolved oxygen concentration within the fermenter[11]. It caused coalescence of the air bubbles making mass transfer of oxygen into solution far less effective. The 'antifoam effect' was greater with greater mould concentrations, a stage being reached where its effect was irreversible. For this reason, a mechanical foam breaker was fitted into the expansion chamber of the

increase in the dry weight.

(b) Parameters which Prescribe the Medium

Under submerged conditions the morphology of A.niger varies from diffuse growth through a range of pellet sizes up to several millimetres in diameter[14]. Control of a particular morphology was found to be advantageous. The tower fermenter facilitated this control, unlike a stirred fermenter, it presents relatively little shear force to disrupt the colony form. The morphological form of the organism used was selected by calculating (1) Respiratory demands and requirements; (2) Viscosity of the fermentation broth and (3) Oxygen transfer into the fermentation broth.

The mould morphology within the tower fermenter is dictated largely by the form and size of the inoculum used, secondly by the degree of agitation a few hours after seeding the fermenter and thirdly by the method of preparation of the inoculum.

Graph 3 shows the relationship between the size of pellet and the spore inoculum used.

The respiratory demands and requirements of the organism are at present being studied. It must be ascertained whether the oxygen is breaking down carbohydrate to be channelled into biomass production alone or whether there is an accumulation of metabolites. It is suggested therefore that a particular morphology may be optimal for both oxygen consumption and carbohydrate utilisation. Graph 4 shows the decline in oxygen uptake per gram of mould in relation to the size of the colonies. By computing the maximum growth rates together with oxygen consumption for the organism an optimum figure for pellet size may be calculated for particular circumstances and particular fermentation vessels.

Experiments concerning the viscosity of a fermentation broth have shown a relationship between the degree of viscosity and the size of pellet formed. It can be seen from Graph 5 that there will be less energy required to agitate a broth containing descrete pellets as there is for a diffuse mycelial culture. It is also true to say that a

viscous broth causes a greater tendency towards coalescence of air bubbles, thus creating a situation where it might be possible to aerate a broth containing pellets more efficiently than it is to aerate a broth with filamentous organism.

The optimum media conditions and constituents for the production of cell biomass and protein content were determined experimentally for A. niger, the basis of the media being beet molasses which was supplemented where necessary by ammonium sulphate as a nitrogen source and sodium dihydrogen phosphate as the source of phosphate.

(i) Carbohydrate concentration:

It was found that A. niger exhibited a wide tolerance to sugar concentration. Graph 6 shows that there is no marked change in growth rate between 5 and 12 g%. The apparent optimum lay between 7 and 8 g% However, due solely to economic consideration a slightly lower concentration might be advantageous when considering water and effluent factors. The protein concentration of the organism did not vary significantly over this optimum range of carbohydrate concentration.

(ii) Carbon-Nitrogen ratios:

Supplementation of a 5 g% carbohydrate media by various quantities of ammonium sulphate was made in order to vary the C:N ratio. Graph 7 illustrates the change in growth rates and protein contents during these experiments. The optimum growth rate lay between 7:1 and 13:1 whilst the optimum protein content is at 6:1. At higher C:N ratios however a great deal of fat was seen to be deposited in the mycelium.

(iii) Temperature:

The range of temperature studied was 20 to 40°C. Graph 8 shows that there is an optimum value for growth rate between 27°C and 33°C for the A. niger. Of economic importance is the fact that at elevated temperatures the fungal metabolism is increased, giving rise to a greater utilisation of carbohydrate. However, this may not necessarily be channelled into biomass production.

This study gives an indication of the behaviour of A.niger in a tower fermenter but can only provide a useful guide to the behaviour of any other filamentous fungus in such a vessel. It should also be pointed out that the medium is also unique in this respect and the use of molasses medium for this study can only give a guide to other organism on other media.

Scale. As previously indicated aerobic tower fermenters were constructed from laboratory up to pilot scale. Preliminary experiments have shown that the findings in the 5 litre laboratory scale tower fermenters were applicable on a 10x scale-up in fermenter size (i.e. volume) to 50 litre and again on a further 10 or 20x scale-up in fermenter size to 500 and 1000 litre. Moreover the design of such fermenter vessels could be simplified enabling cheap polypropylene fermenters to be used which gave high concentrations of mould biomass economically. If, therefore, fungal biomass is required for whatever purposes, food, protein, biochemicals or enzymes, in developing countries, then a cheap fermenter is required. The tower fermenter is such a fermenter because of its simplicity of design and construction, its advantageous kinetics and its flexibility.

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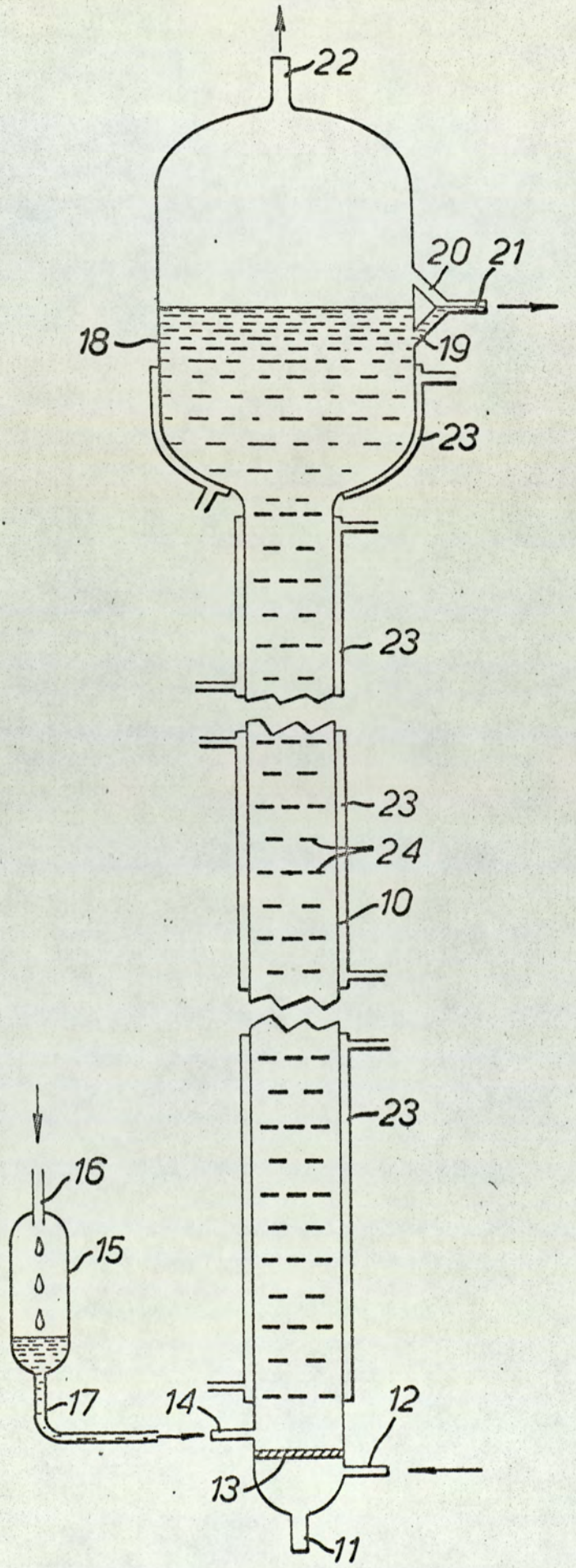
DIAGRAM 1

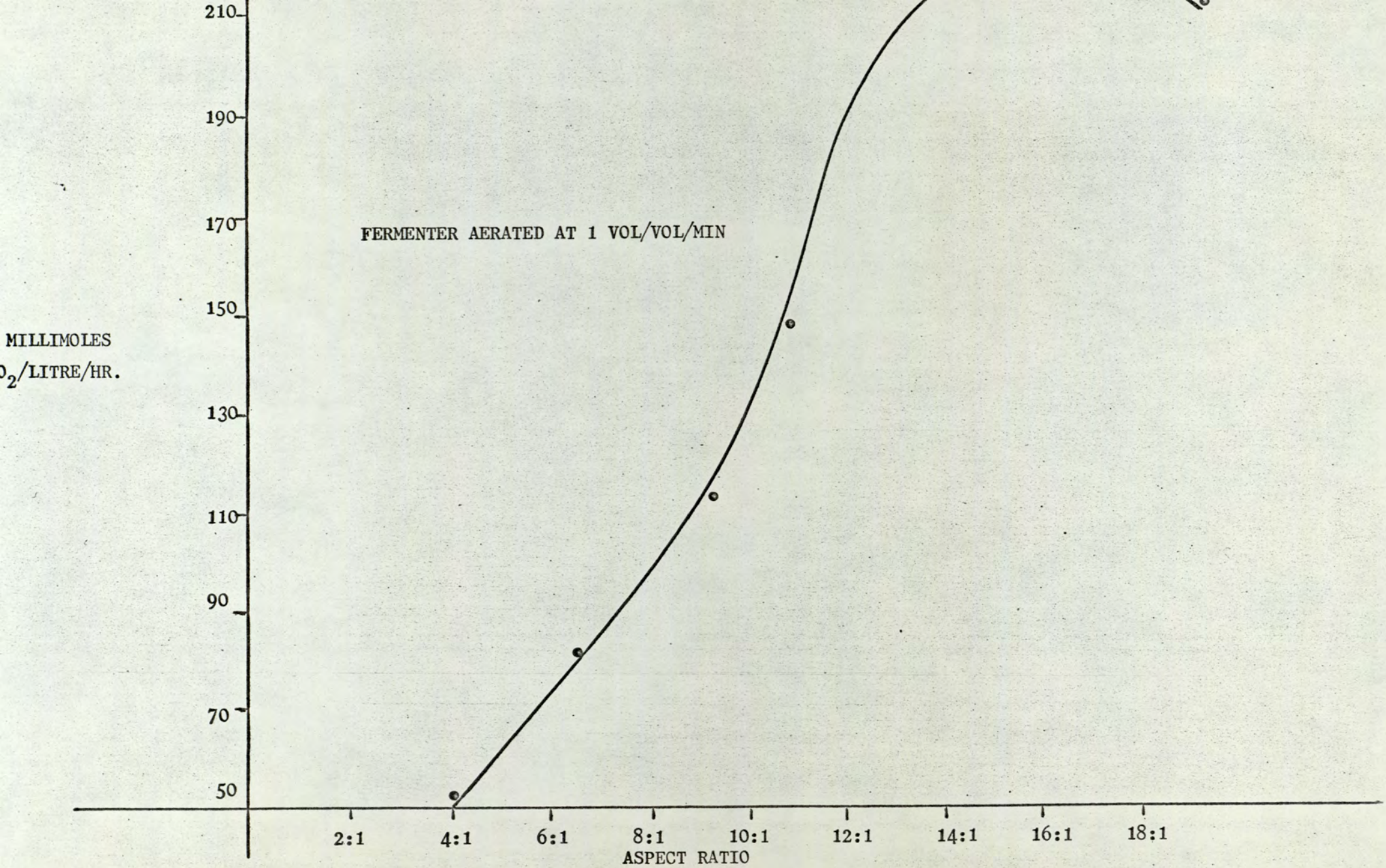


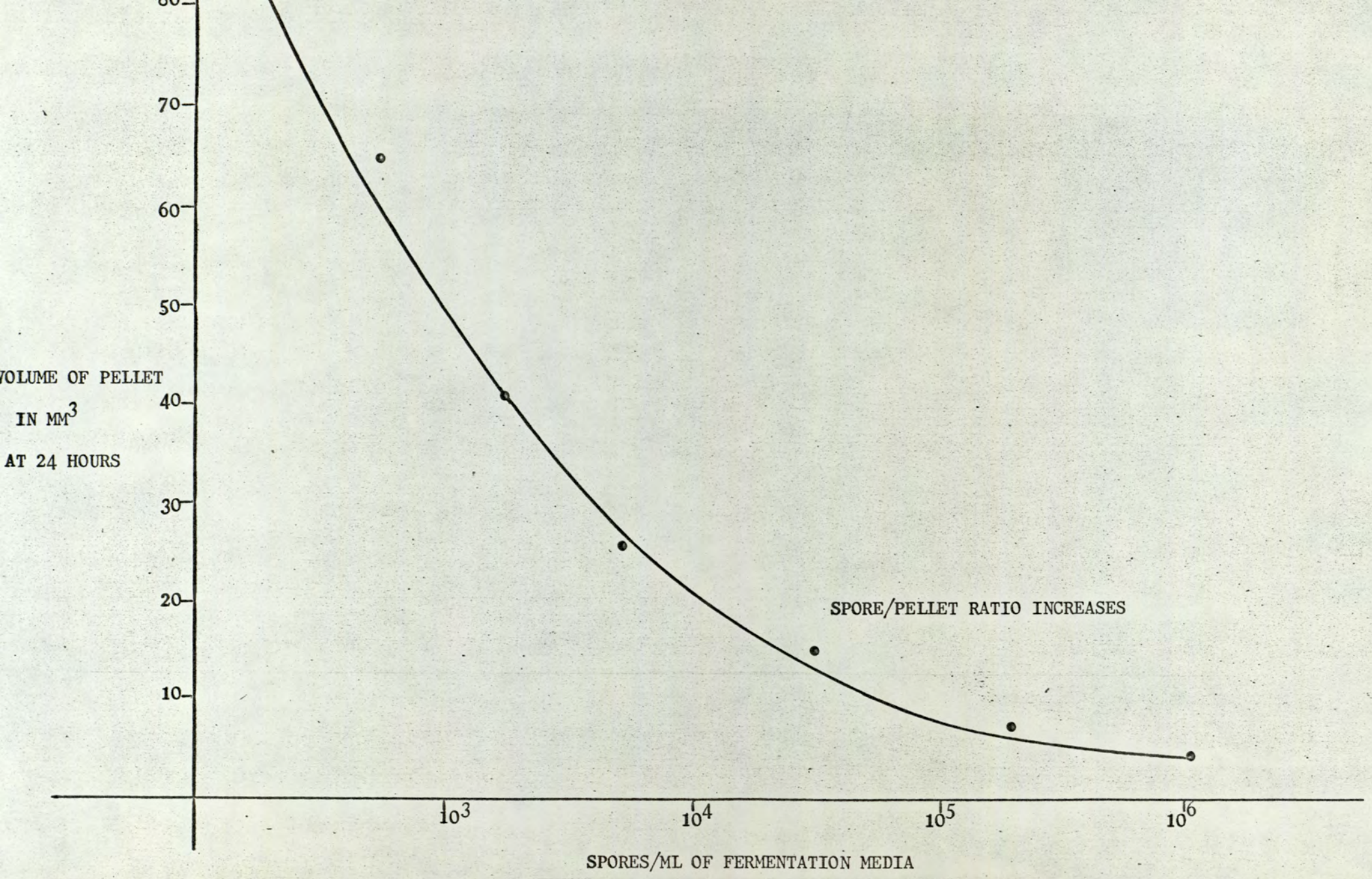
	Organism		Medium	Scale (l)	Time maximum yield (hr)	Yield (g%)	Protein (g%)
☐	1. *Aspergillus luchuensis*	(RA)	Molasses	50	112	1.62	42.7
☐	2. *Aspergillus niger*	(RA)	Molasses	50	90	1.20	37
	3. *Aspergillus oryzae*	(RA)	Molasses	50	120	0.87	34.0
	4. *Aspergillus sydowi*	(RA)	Molasses	5	40	0.75	43.5
☐	5. *Cladosporium cladosporoides*	(BD)	Carob	1	42	1.0	30 - 36
☐	6. *Cladosporium cladosporoides*	(RA)	Molasses	5	30	0.72	36.5
	7. *Cochliobolus sativus*	(BD)	Carob	1	64.8	0.51	35.35
	8. *Fusarium oxysporum*	(RA)	Molasses	5	42	1.29	52.1
	9. *Heterocephalum aurantiacum*	(RA)	Molasses	5	44	0.61	38.5
	10. *Mycogone spp.*	(BD)	Molasses	1	41.9	0.70	45.9
☐	11. *Penicillium chrysogenum*	(RA)	Molasses	50	72	1.2	36.0
☐	12. *Penicillium notatum*	(RA)	Molasses	50	96	1.2	39.2
☐	13. *Spicaria elegans*	(BD)	Carob	1	40	1.0	30

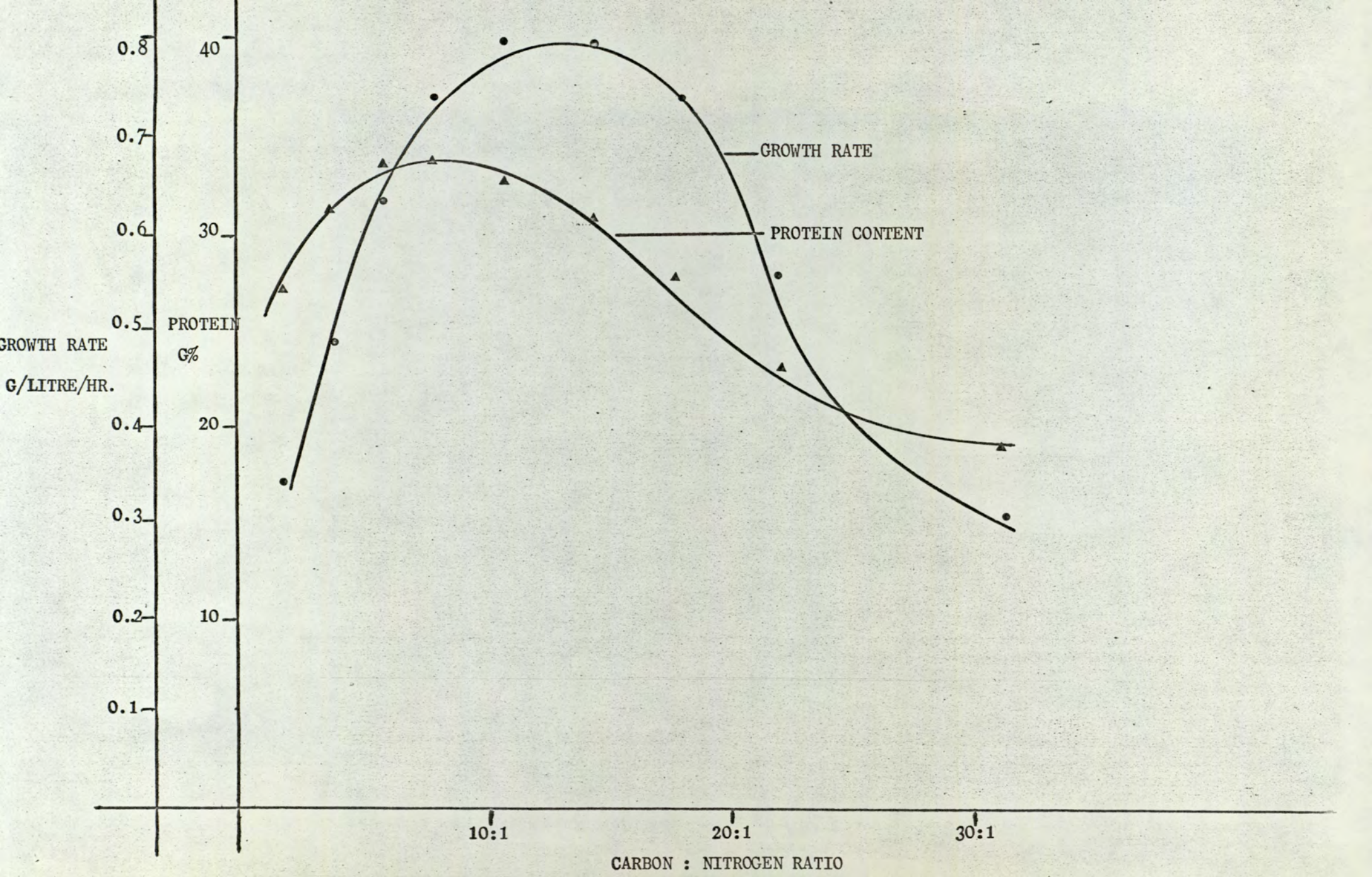
	Organism		Medium	Scale (l)	Time maximum yield (hr)	Yield (g%)	Protein (g%)
1.	*Lenzites trabea*	(RA)	Molasses	2	96	0.67	53.2
2.	*Polyporus tulipferus*	(BD)	Carob	1	121	0.58	44.01
3.	*Poria latemarginata*	(BD)	Carob	1	121	0.55	51.75

GROWTH OF PHYCOMYCETES IN TOWER FERMENTERS

	Organism		Medium	Scale (l)	Time maximum yield (hr)	Yield (g%)	Protein (g%)
1.	*Mucor racemosus*	(RA)	Molasses	50	90	0.94	43.4
2.	*Rhizopus oligosporus*	(RA)	Molasses	50	76	0.65	39.2
3.	*Rhizopus oryzae*	(RA)	Molasses	5	42	0.72	38.6


210
190
170
150
130
110
90
70
50
FERMENTER AERATED AT 1 VOL/VOL/MIN
MILLIMOLES
O_2/LITRE/HR.
2:1
4:1
6:1
8:1
10:1
12:1
14:1
16:1
18:1
ASPECT RATIO


70
60
50
VOLUME OF PELLET
IN MM3
AT 24 HOURS
40
30
20
10
SPORE/PELLET RATIO INCREASES
10^3
10^4
10^5
10^6
SPORES/ML OF FERMENTATION MEDIA


0.8
0.7
0.6
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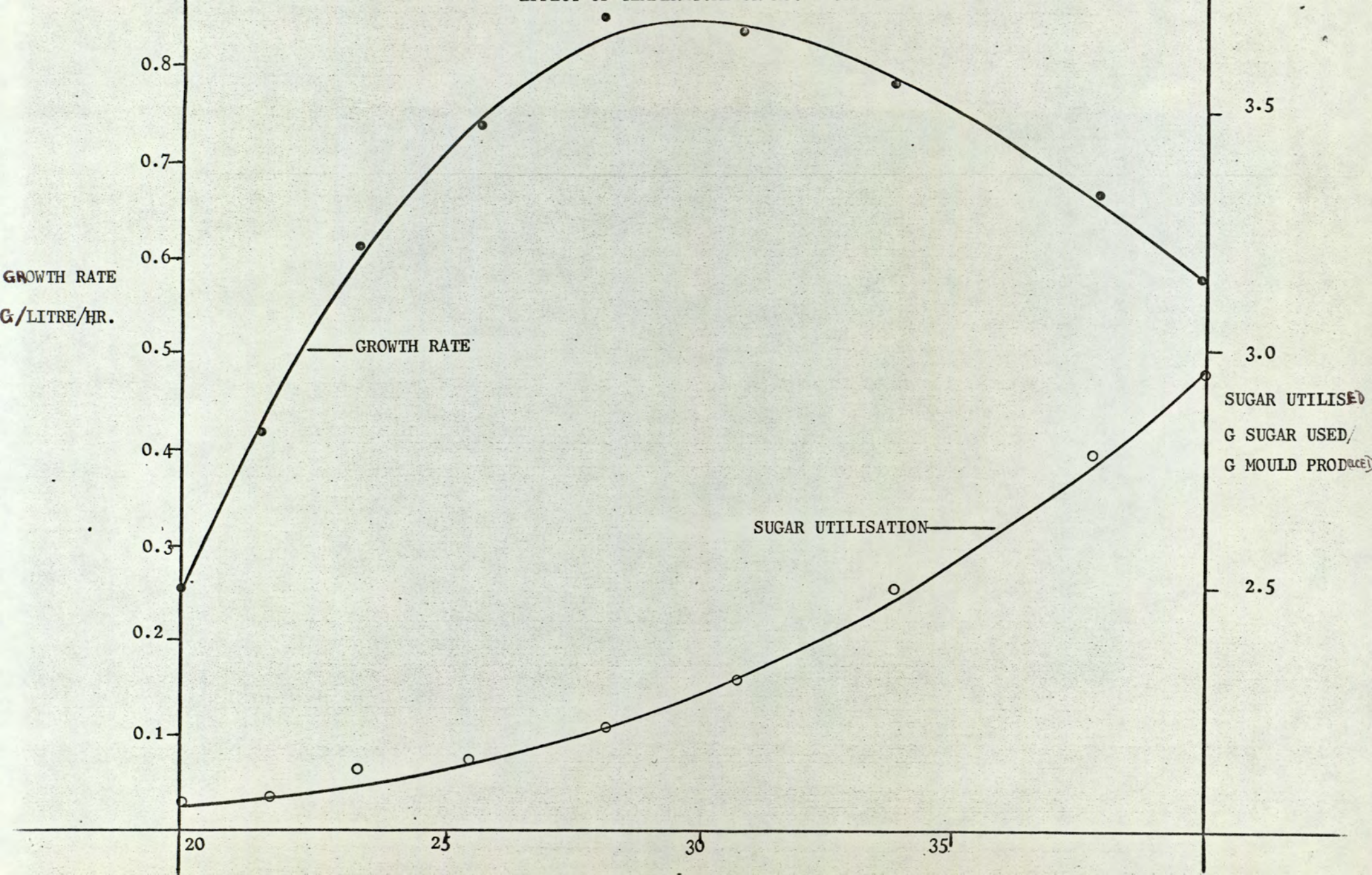
GROWTH RATE
G/LITRE/HR.
40
30
20
10
PROTEIN
G%
GROWTH RATE
PROTEIN CONTENT
10:1
20:1
30:1
CARBON : NITROGEN RATIO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GROWTH RATE
G/LITRE/HR.
GROWTH RATE
SUGAR UTILISATION
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3.0
2.5
SUGAR UTILISED
G SUGAR USED/
G MOULD PRODUCED
20
25
30
35

THE GROWTH AND MORPHOLOGY OF MICROORGANISMS IN TOWER FERMENTERS

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presented at the IVth International Fermentation Symposium.

General Paper Session - 12 Mass Transfer. Paper 11.

24th March 1972

Mr. Chairman - Ladies and Gentlemen.

The tower fermenter has been described[1] as an elongated non-mechanically stirred vessel with a high aspect ratio containing micro-organisms suspended in medium and through which there is a uni-directional flow of medium and/or gases.

SLIDE 1 shows the basic designs of an aerobic[2] and an anaerobic[3] tower fermenter with aspect ratios (height to diameter ratio) of at least 6:1 on the tubular section or 10:1 overall.

Such fermenters have been used for batch, semi-continuous and continuous fermentations on laboratory and pilot-scales both for the production of metabolites and biomass[4,5]. Moreover, their successful application has been made on commercial scale for the production of ale, lager, and vinegar charging-wort[5,6,7,8]. SLIDE 2a b.

A variety of industrially important micro-organisms (yeasts, moulds and bacteria) have therefore been cultured in tower fermenters under aerobic or microaerophilic conditions on a variety of media and their morphological form and growth characteristics studied.

Since the mixing and agitation in tower fermenters is accomplished by the aeration and/or release of fermentation gases, relatively little shear

force occurs to disrupt the form of the micro-organism. A colony form reflecting the micro-morphology of the organism develops which is markedly influenced by the various fermentation conditions.

Most micro-organisms grow in the form of aggregates rather than diffuse growth. Single-celled or chain-forming organisms formed flocs or compact colonies whilst filamentous types gave various forms of pellets depending on their concentration and culture conditions.

Brewing yeasts, Saccharomyces spp., in continuous culture could be divided into three groups, (I) non flocculent, (II) flocculent, depending on medium concentration and dilution rate and (III) flocculent independent of medium concentration and dilution rate. Saccharomyces cerevisiae Type I yeasts rarely attained more than 3 g% w/v expressed as centrifuged wet weight in the tower whether under aerobic or micro-aerophilic conditions and are rapidly washed from the tower at all dilution rates in continuous culture. SLIDE 3 illustrates the light aggregation found. Whereas under brewing conditions (micro-aerophilic), type II yeasts (called physically-limited yeasts) gave light powdery flocs up to 0.3 cm. diameter and had concentrations up to 25 g% w/v. Sedimentation of these flocs gave the heterogeneity essential to the fermentation characteristics of beer fermentation. At any particular specific gravity of the medium there is a critical volumetric efficiency (space-velocity i.e. volume of wort per unit time per fermenter volume) which if exceeded causes a complete wash-out of the organism. This critical speed is dependent on yeast strain, fermentation temperature and media composition. SLIDE 4 and 5 shows typical flocs of this yeast type, whilst SLIDE 6 shows the microscopic character of the floc (40% free space) and SLIDE 7 the appearance in the fermenter. Type III on the other hand, fermentation limited yeasts attained higher concentrations (25 - 45 g% w/v) having heavy 'sticky' flocs up to 1.3 cm. diameter which often aggregated into massive clumps or plugs. These are retained at all but very high volumetric efficiencies but at any particular specific gravity of the medium, the fermentation efficiency is dependent on volumetric efficiency. SLIDE 8 shows the microscopic character of the floc, the yeasts are somewhat compressed and have only 25% free space.

SLIDE 9 shows the appearance of the yeast in the fermenter. Saccharomyces carlsbergensis (lager yeasts) gave characteristic flocs which varied between type II and type III forms depending on temperature conditions, the volumetric efficiency and the fermentation efficiency. The Type II form appears generally at the early stage of fermentation whereas the Type III form appears later. SLIDE 10 exhibits both types.

In aerobic culture for biomass production, Saccharomyces spp. of the type II and III forms grew as compact erythrocyte-shaped colonies which could grow up to 1.0 cm. in diameter. These forms were very friable but gave cell concentrations on settling of up to 85 g% w/v. SLIDE 11 clearly shows such a colony (0.5 cm. diameter).

Bacterial cultures were normally diffuse and non-flocculent in character particularly if spherical or discreet as in SLIDE 12 which shows a Serratia spp. Although rod-shaped varieties gave loose irregular flocs with approximate diameters up to 1.0 cm after prolonged continuous culture. SLIDE 12 shows the initial development of a bacterial floc of this type. This has enabled tower continuous acetification in vinegar manufacture using Acetobacter spp. Experimental laboratory and semi-pilot scale tower acetators have been run continuously with 80 - 90% conversion of alcohol to acetic acid at volumetric efficiencies of 1.0 day^{-1}. [2,9,10,11] However, the concentration of organism and the fermentation efficiency was entirely dependent on aeration although the fermentation shows some heterogeneity depending on the aspect of ratio of the fermenter.

Filamentous fungi usually formed pellets which were either compact or loose depending on culture conditions and reflecting the morphology of the mycelium. Various fungi were tested SLIDE 14 shows the Fungi imperfecti most of which gave compact pellets (except Fusarium oxysporum which had a yeast-type morphology). SLIDE 15 lists the best growing Basidiomycetes and Phycomycetes which gave similar morphological forms. Aspergillus niger was chosen for particular study[4,5] and under submerged conditions varied from diffuse growth through a range of pellet sizes up to several millimetres in

diameter. Isolated pellets could be grown up to some 3 cm. in diameter. In certain fermentations control over morphology is advantageous and the tower fermenter facilitates this control[5,12,13,14]. The morphology of a pellet is largely dictated by the form and size of the inoculum used to seed the fermentation, the degree of agitation a few hours after seeding the fermenter, the method of preparation of the inoculum and the growth conditions.

SLIDE 16. Filamentous colony-form.

SLIDE 17, 18, 19, 20, 21, 22. Development of soft-colonies.

SLIDE 23. Large isolated colonies up to 3 cm. diameter.

These slides illustrate the development of soft 'colonies' up to 0.5 cm. diameter and are generally obtained with spore inocula of between 10^2 to 10^6 spores per ml.; the colony size after 24 hr. reflecting the number of spores. Such inocula germinate and then aggregate to form a dense clump, this grows rapidly forming in the first stages a light 'hairy' pellet but at later stages it becomes more dense internally as well as increasing in volume appearing smoother in character. Finally, in isolated culture, such colonies lyse in the centre and become hollow with a convoluted surface.

Spore-inocular below 10^2 spores per ml. tend to give filamentous forms or may not germinate at all, while in excess of 10^6 spores per ml. tend to give dense filamentous growth. Mycelial (homogenised) inocula give filamentous growth above concentration of 0.08 to 0.10 g% w/v but may form loose aggregates whilst below this concentration smooth aggregates are formed. Where the growth conditions are controlled, for example, in the use of inhibitors to modify metabolism as in the case of citric acid production using ferrocyanide, then often a restricted growth occurs giving rise to smooth and hard pellets with hollow centres due to lysis[13, 14].

SLIDE 24 shows such pellets which develop up to 1 cm. diameter.

SLIDE 25 shows pellets cut open to reveal lysed hollow interior.

Finally it should be pointed out that combinations of organisms as occur in symbiotic cultures afforded by natural ecosystems allow unusual but predictable morphology in towers. A preliminary study of an activated sludge tower fermentation system has revealed that yeasts and bacteria are assisted in

flocculation or pellet formation by various fungi thus giving conditions which favour the tower fermenter.

SLIDES 26 and 27 show Actinomycete fungus clearly providing a 'former' for various yeasts and bacteria to form flocs in a tower fermenter

For chemical engineering design purposes it is not possible to predict the overall essential parameters which prescribe flocculation of all micro-organisms, thus each must be considered separately. However, flocs can be considered as close-packed spheres and the effects due to diffusion on cell-growth and metabolite production estimated, whilst fluidisation-sedimentation behaviour predicted in semi-quantitative terms related to superficial-velocity and floc-concentration[5]. Growth rate equations similar in mathematical form to the Michaelis-Menten equation are not applicable to pellet formation particularly in the case of filamentous forms. However, sedimentation phenomenon appears to resemble 2nd. order kinetics[5, 15] whilst the fermentation race processes are probably zero-order kinetics[5, 7].

Comparisons between stirred tank reactors and tower fermenters have shown that the tower system is a more efficient aerator when operating at 1 volume per volume per minute and since the power input of the stirred tank is an order of magnitude greater[16, 17, 18], the high shear rates obtained prevent aggregate formation of the pellet-type and result in the more familiar filamentous form. The lower 'viscosities' encountered with pellet forms may well improve mass transfer characteristics. There are almost no significant differences in growth rate and substrate utilisation efficiency between the two fermentation systems but the key rate-processes are only affected at high cell concentrations. Calculations show that the control over morphological form allowed by the tower fermentation system provides flexibility in determining growth rate, biomass concentration, mass-transfer of oxygen and metabolites.

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The Growth and Morphology of Microorganisms in Tower Fermenters

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SLIDE INFORMATION

SLIDE 1: Basic design of an aerobic and an anaerobic tower fermenter.
Taken from original Patents. Royston Brit. Patent:929,315.
Greenshields. Brit. Patent: 1,263,059.
Differences in aeration means, separation devices and foam control.

SLIDE 2: Commercial Tower fermenter for beer production. 10 metres x 1 metre.
Diagram 2(a) Photograph 2 (b)

SLIDE 3. Non-flocculent yeast. Magn. x 900. Saccharomyces cerevisiae showing only light aggregation.

SLIDE 4. Development of physically-limited type II. Saccharomyces cerevisiae colony.

SLIDE 5. Large colony of Type II. S. cerevisiae.

SLIDE 6. Type II x 900 showing packing.

SLIDE 7. TYPE II yeast light flocs 0.1 to 0.3 cm. diameter.

SLIDE 8. Type III yeast. S. cerevisiae fermentation-limited showing dense packing 'hexagonal' benzene ring yeast cells.

SLIDE 9. Type III. sticky flocs 1.3 cm. diameter in 'plug' form.

SLIDE 10. Type II and III. Saccharomyces carlsbergensis in tower fermenter showing floc.type.

SLIDE 11. S. cerevisiae colony-form in aerobic tower. Erythrocyte-shaped colony 1.0 cm. diameter.

SLIDE 12. A serratia spp. - not flocculent.

SLIDE 13. Activated sludge bacteria forming small compact pellet-type colonies.

SLIDE 14. Table of Fungi imperfecti cultured in tower fermenters.

SLIDE 15. Table of Basidiomycetes and Phycomycetes which grew best in tower fermenters.

SLIDE 16. Aspergillus niger 72/4 Filamentous colony form.

SLIDES 17. 18. 19. 20. 21. 22. Development of A. niger M.1. soft colony form up to 0.5 cm. diameter.

SLIDE 23. A. niger M1. Large isolated colony with hollow centre. 3 cm. diameter.

SLIDE 24. A. niger 72/4. hard, smooth citric-acid producing pellets in ferrocyanide-molasses medium.

SLIDE 25. Pellets as in Slide 24 cut open to show hollow centres.

SLIDE 26. Actinomycete fungus providing a 'pellet-former' for yeasts and bacteria in activated sludge tower.

SLIDE 27. Clearer detail of SLIDE 26.

AERATION IN TOWER FERMENTERS CONTAINING MICROORGANISMS

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The tower or tubular fermenter can be described as an elongated non-mechanically stirred fermenter with an aspect ratio (height to diameter ratio) of at least 6:1 on the tubular section or 10:1 overall, through which there is an unidirectional flow of medium or gases[1]. This type of fermenter has been used successfully anaerobically or microaerophilically for the continuous production of ale and lager on laboratory[2], pilot[3] and commercial scales[4,5,6]. Likewise, it has also been exploited for the production of vinegar charging wort[6]. The production of metabolites under aerobic conditions has also been considered, for example, the production of acetic[7,8,9] and citric acid[6,10] and for the growth of microbial biomass using yeast[6], bacteria[11] and fungi[12,13].

Biomass production requires relatively high oxygen tensions in order to reach maximum growth rates and yields. The work presented here will cover the aeration characteristics of the tower fermenter during the growth of fungi.

The fermenter design for the growth of fungi[12] is shown in diagram 1.

AERATION IN AIR/WATER AND AIR/MEDIA SYSTEMS

Aeration is achieved in tower fermenters by passing air across a sintered or perforated plate at the base of the column. This produces swarms of air bubbles which travel up through the liquid, gaseous exchange occurring in this way. Oxygen transfer in tower fermenters containing liquid alone is affected by a variety of factors, most of which are well documented[14,15]. Oxygen transfer in these columns which contain quantities of microoganisms has received considerably less attention[16]. An investigation was carried out in order to characterise some of the parameters affecting oxygen transfer and relate them to situations which occur during fermentation.

In most of the systems studied a relationship was seen to exist between 'hold-up' volume or the air retention volume and the actual oxygen transfer rates. These hold-up measurements were substantiated by mass transfer experiments involving sulphite oxidation[17] and static gassing out procedures[19].

Air flow into tower fermenters may be characterised by three general flow patterns. In the first instance, low, superficial gas velocities (1 cm/S) produce 'bubbly flow' which is described as the air bubbles travelling up the column in nearly straight lines and producing very little back-mixing. Secondly, at higher superficial gas velocities (3 cm/S) a kind of 'turbulent flow' exists in which there is a deal of back mixing in evidence (Slide 2). In the third case at still higher superficial gas velocities (> 5 cm/S) a 'slug-flow' is pronounced (Slide 3).

During bubbly flow the size of the air bubbles are more or less uniform and to a large extent a function of the liquid and air distributions. When these bubbles exceed diameters of around 4 - 5 mm.

these cease to be rigid and assume a floppy shape which is continually changing. In turbulent flow conditions these bubbles may still exist, but there is also a second kind present. These are much smaller being less than 1 mm in diameter and have a rounded rigid shape. The smaller rigid bubbles are formed during turbulent flow irrespective of the liquid or air distributor in the system but can be accentuated in terms of numbers when a fine air distributor (sinter) or a culture media is present. These bubbles are therefore thought to be formed both by the fragmentation of larger bubbles and formation through the smaller holes in a sinter plate. In 'slug flow' there is an even greater heterogeneity in terms of bubble size. The slugs themselves can be in the region of 20 cm. dia. depending on such fermenter conditions as liquid composition, flow rate and air distribution. Various other size of bubble also exist in this region, down to the smaller rigid spheres. These three types of air/liquid interaction can be further classified by the use of methodsfor determining oxygen transfer efficiency (Graph 1).

During bubble flow and using the sulphite oxidation technique, the oxygen transfer rate into solution was found to increase almost linearly with increase in gas flow rate,(this linearity being dependent on the air distributor). It is in this region where the greatest efficiency of oxygen transfer occurs. During turbulent flow the oxygen transfer rate reaches a maximum point despite the fact that efficiency is falling. Under slug flow conditions, however, as well as a decline in efficiency a slight fall is also observed in the rate of oxygen transfer. When air hold-up or air volume retention is monitored alongside the sulphite value an almost parallel condition is noted. The decline in the sulphite value at high flow rates is thus explained by the fact that large bubbles, with a relatively low surface area to volume ratio, move quickly

AERATION INTO MICROBIAL SUSPENSIONS

When air is passed into a column containing varying amounts of solid substances such as microorganisms a three-phase situation is set up, thus adding another parameter to consider when discussing oxygen transfer in these vessels. A great number of organisms have been grown with success in the tower fermenters including yeasts, bacteria, moulds and even mixed cultures of these three[20]. Because of their filamentous nature which gives high viscosities, the moulds present the greatest barrier to oxygen transfer in microbial systems. At densities of about 1.35 g% w/v dry weight reductions in oxygen transfer of over 85% have been noted in mould fermentations[18] compared to much lower figures for yeasts and bacteria.

The decreases noted in the oxygen transfer rate are consistent with the reduction in the hold-up values calculated for incremental increases in mould weight (Graph 2). This is also shown to be true during an actual fermentation run where the mould biomass is increasing with time (Graph 3).

In Graph 3 we can see the actual decrease in air retention volume as the quantity of mould is increased in the fermenter.

Increasing quantities of mycelium in tower fermenters cause coalescence of air bubbles at lower air rates. Larger bubbles are formed, thus reducing the residence time. This in turn reduces the hold-up and the oxygen transfer therefore falls off. Small rigid air bubbles are still present even at quite high flow rates (5 cm/S). These bubbles percolate slowly up the column or may even be seen to move in a downward direction due to the fact that the large air slugs moving quickly up the column cause transitory vacuums behind them. The effectiveness of these small bubbles in mass transfer is uncertain.

MORPHOLOGICAL CHANGES AFFECTING AERATION

It is evident that the oxygen transfer rate into solution will be decreased substantially by the use of high densities of filamentous mould mycelium. This is reflected in the very high viscosity of the fermentation broth. Since all fungi are obligate aerobes it is a prerequisite of their fermentations that an adequate supply of oxygen is maintained. This is especially critical in high density systems. In the tower fermenter it has been shown that lower viscosities, greater hold-ups and increases in mass transfer characteristics are possible by altering the morphology of the organism. Thus, fungi which can be grown in a colony form may allow an enhancement of the oxygen transfer into solution per gram of organism present. In their method of aeration and agitation tower fermenters impose very little shear force on the medium or the organism present, thus facilitating the control of the morphology[11,12,13]. The morphology of moulds, Aspergillus niger in particular, range from filamentous threads through degrees of hairy pellets into smooth rounded colonies. The colony form of A. niger has been grown from diameters of a few m.m. up to 35 m.m in diameter.

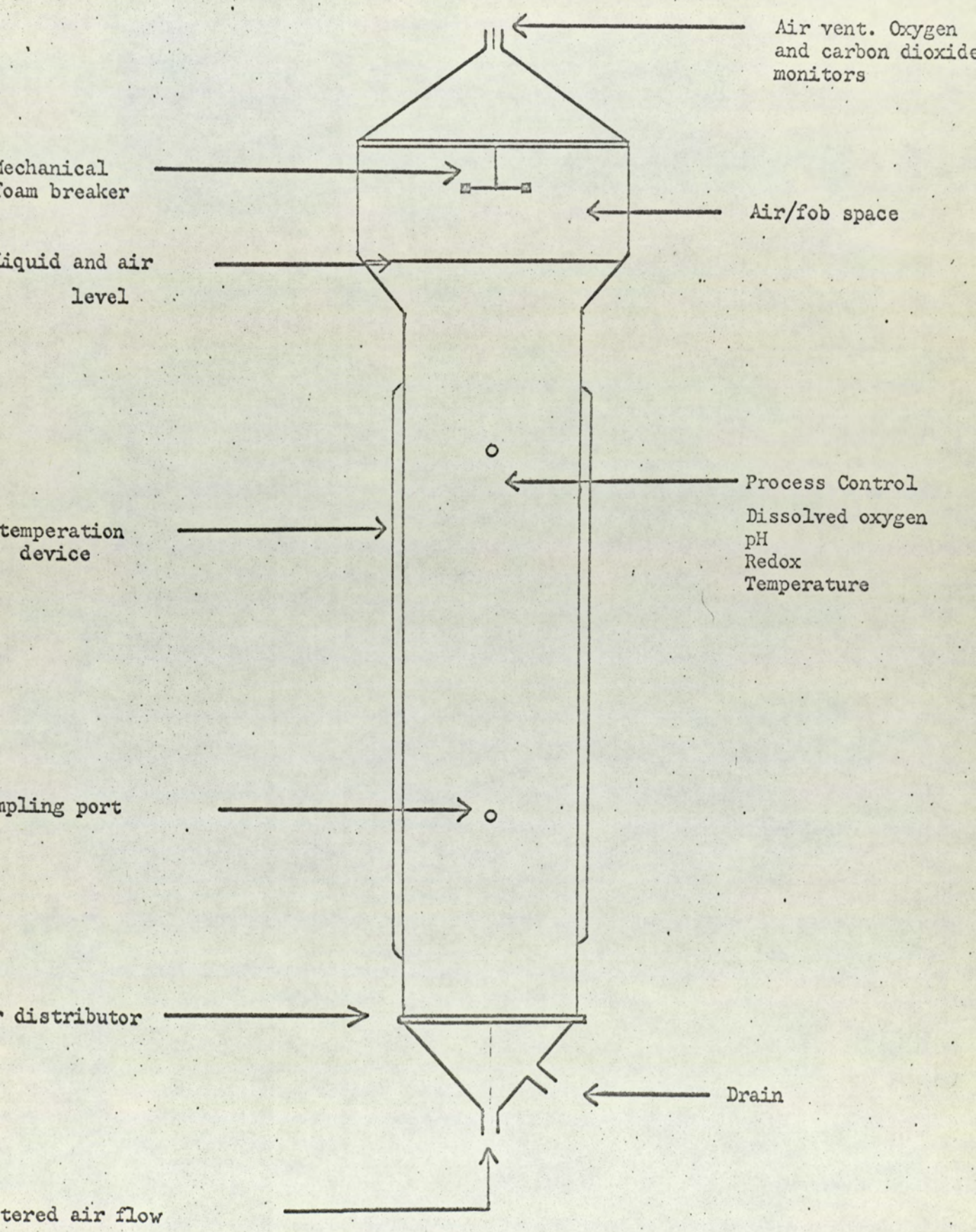
During mould fermentations in tower fermenters the morphology of the organism varies considerably from that of filamentous pellets into a smooth rounded pellet. Thus the viscosity also varies, as shown in Graph 4. The whole rheology of the fermentations change when the morphology is changed. This is reflected in changes in the hold-up and differences in oxygen transfer seen between the filamentous and the colony form of the organism (Graph 5 and Graph 6).

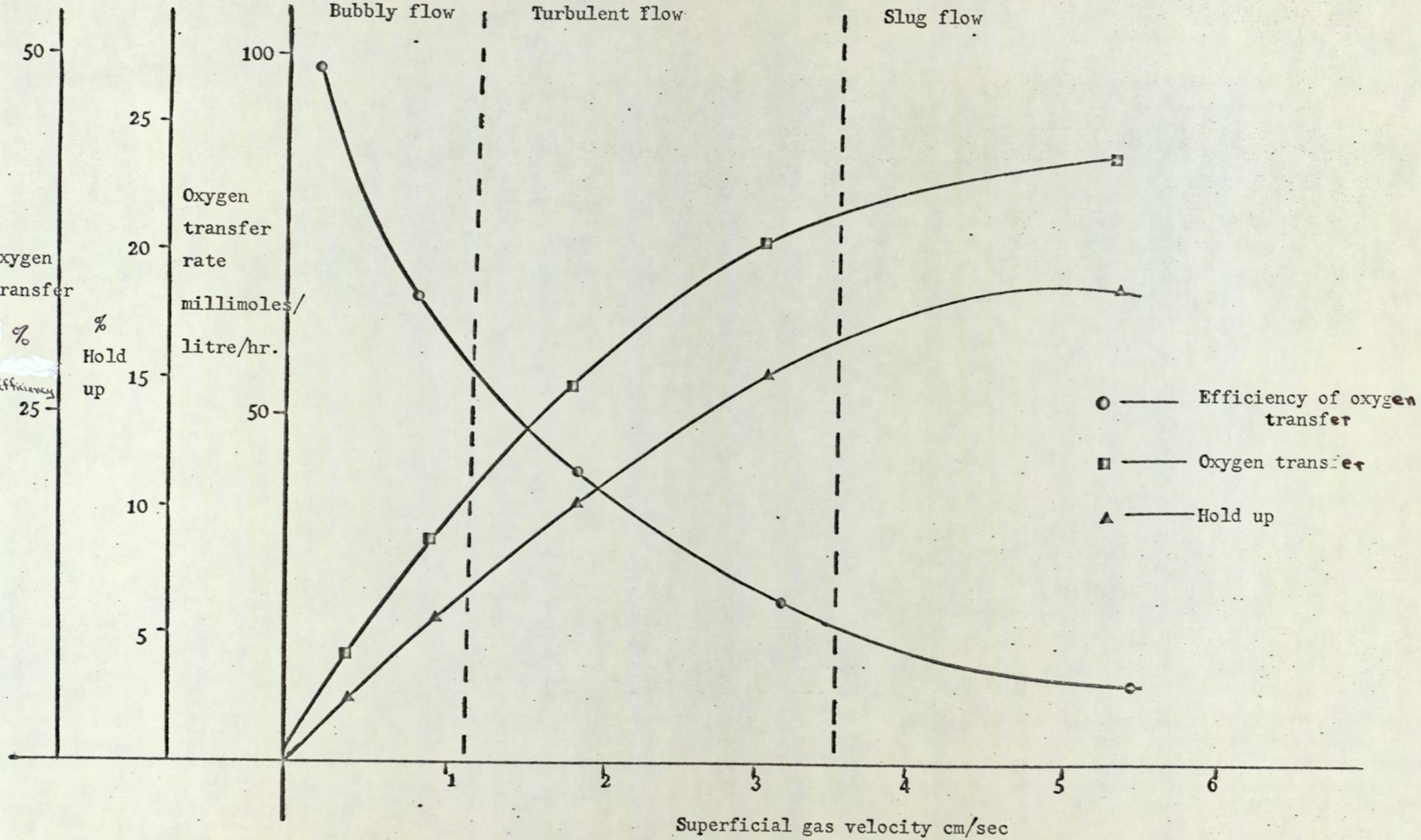
It can be argued however that by use of the colony form of the organism, mass transfer is being restricted into the central region of the pellet. With the organism used (A. niger) the deleterious effects can be avoided to a large extent by controlling the morphology and growth rate at any particular stage in fermentation.

By the use of the optimum pellet conditions[11] it was shown that little or no changes in the relative growth rates of pelleted and filamentous mycelium could be detected up to concentrations of around 2 g% w/v dry weight. This does not in fact prove that mass transfer barriers are entirely absent. However, since it is the actively growing mycelium that utilises the largest specific amounts of oxygen and carbohydrate materials (Graph 7) and these necessarily account for the outer perimeter of the colony, it followed that mass transfer is progressing into the inner portion of the colony at a rate equivalent to the maintenance energy requirement of the organism. It is therefore suggested that the pelleted form of A.niger utilises less oxygen than the filamentous form. This is clearly reflected in the facts that a greater conversion efficiency of carbohydrate into cell biomass is obtained using pellets and also the fact that oxygen is being utilised for organic production (Graph 8).

Comparison of the growth of A.niger in the tower and stirred fermenter in terms of its growth rate under similar fermentation conditions showed little difference. However, specific differences due to the mode of aeration gave advantages of simpler fermenter construction and possibly of lower energy requirements. In addition the tower gives the biological advantage of allowing the development of morphology of the micro-organism and the facility of its control.

DIAGRAMMATIC REPRESENTATION OF A TOWER FERMENTER




Bubbly flow
Turbulent flow
Slug flow
50
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ransfer
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fficiency
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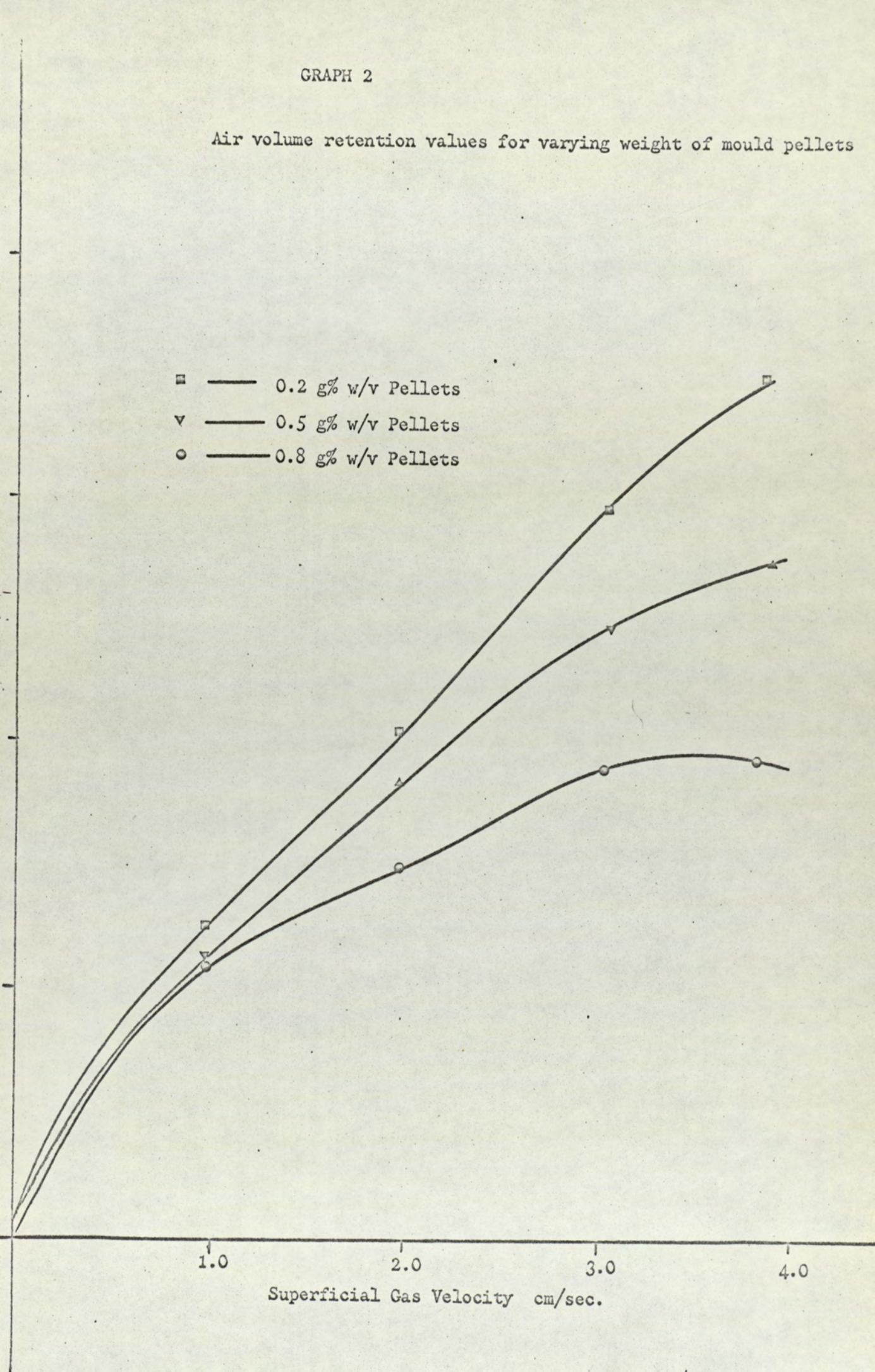
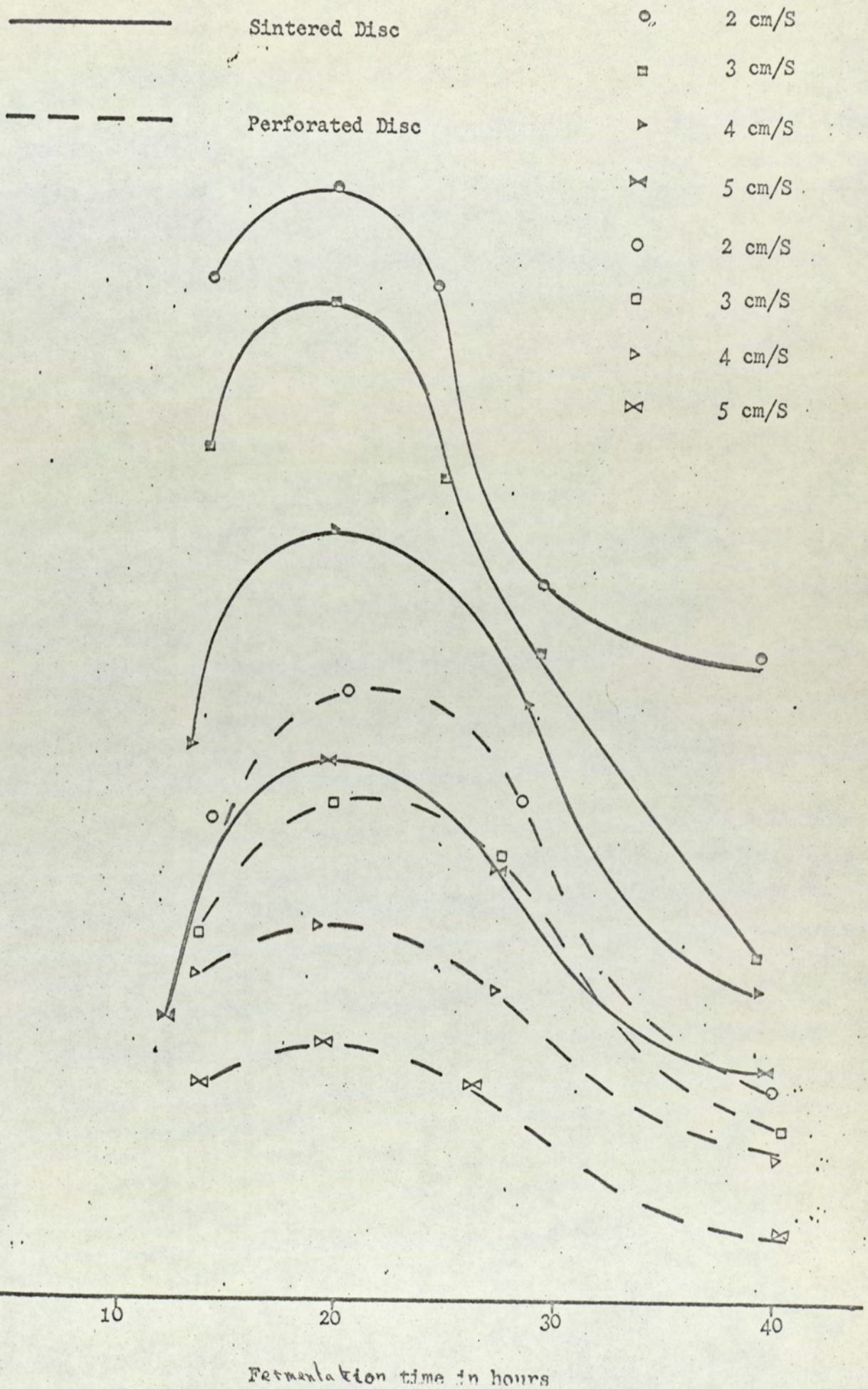
Hold
up
15
10
5
100
50
Oxygen
transfer
rate
millimoles/
litre/hr.
20
Efficiency of oxygen transfer
Oxygen transfer
Hold up
1
2
3
4
5
6
Superficial gas velocity cm/sec

GRAPH 2

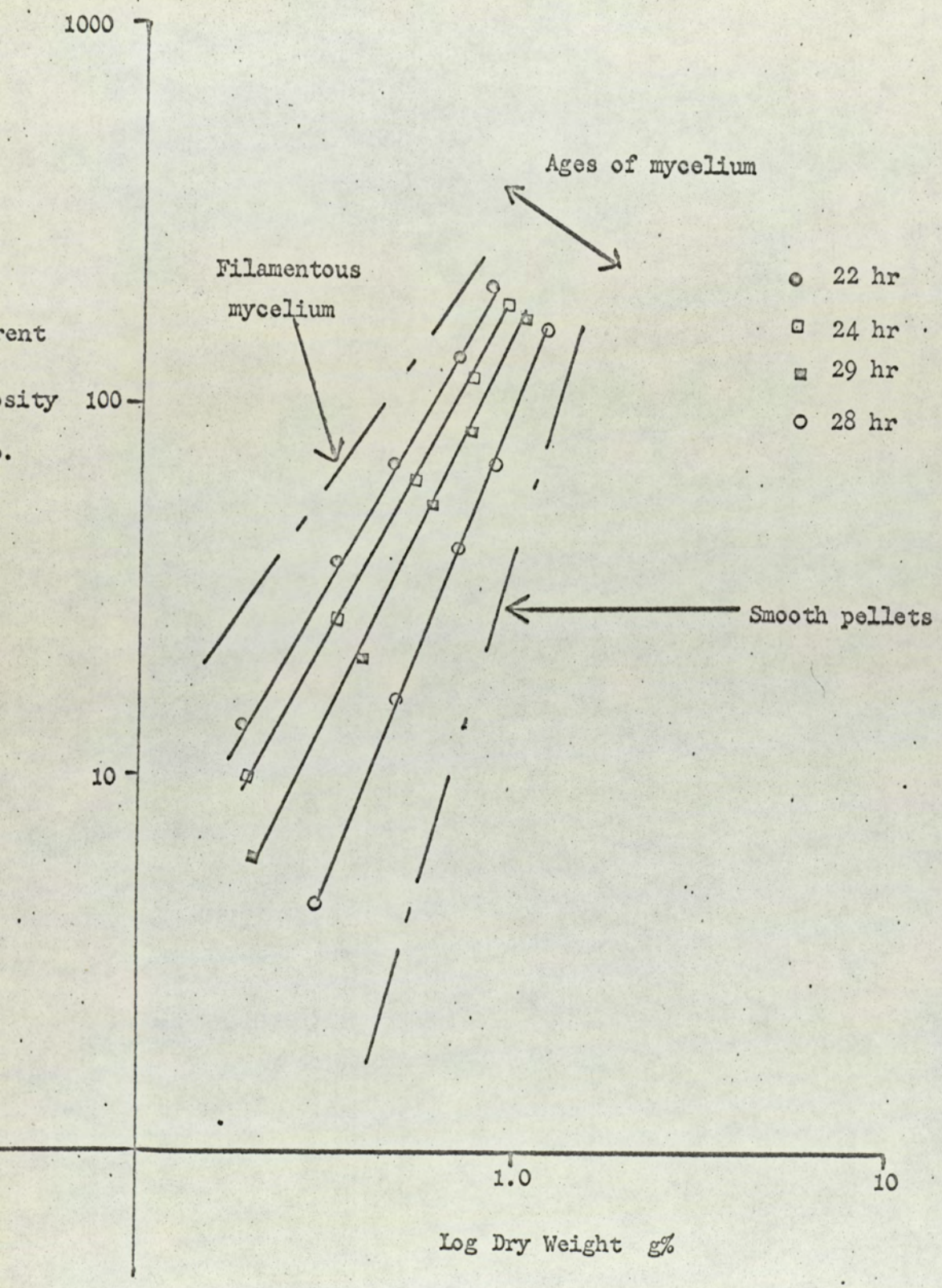
Air volume retention values for varying weight of mould pellets

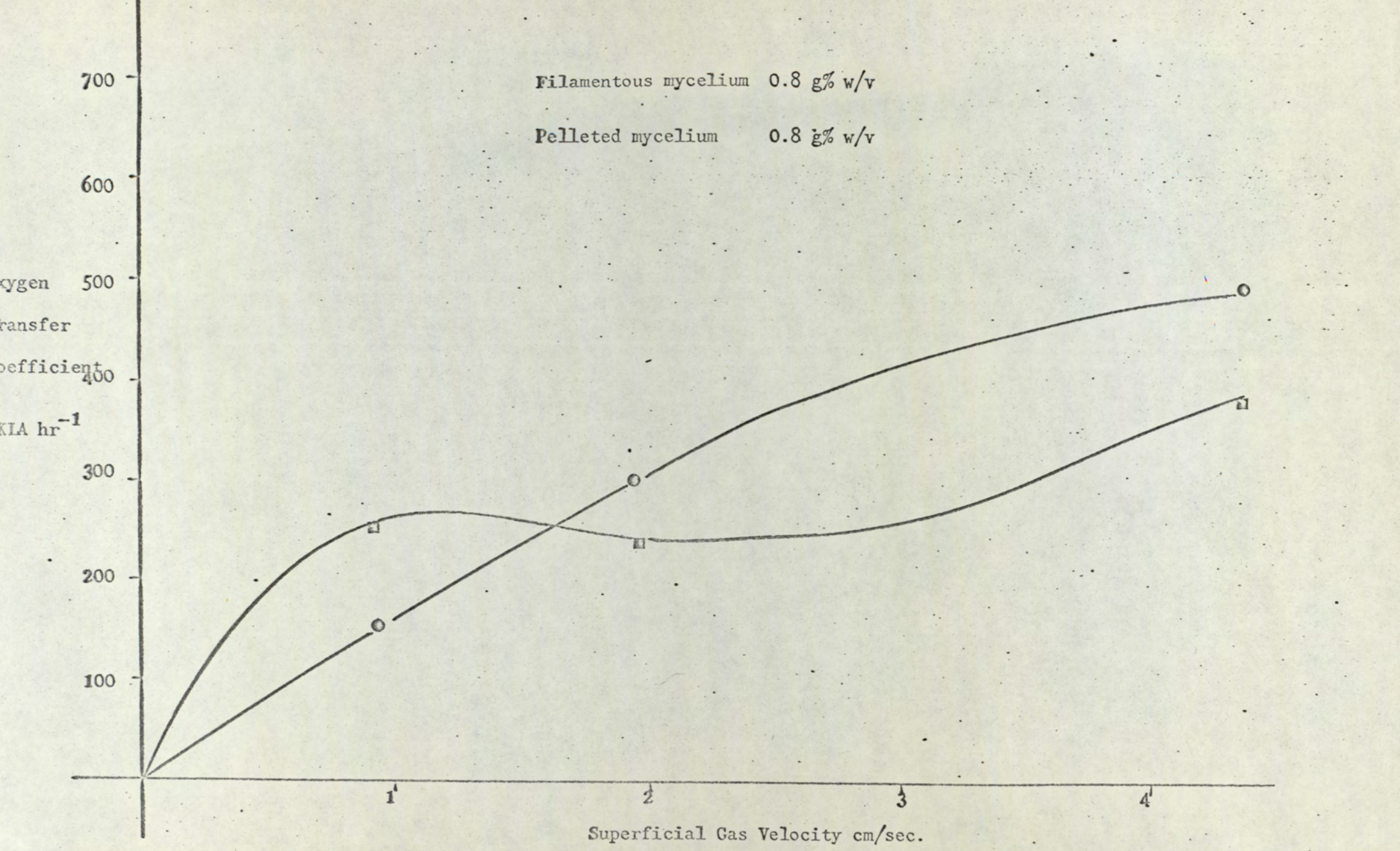


Variation in Air Volume Retention during Fermentation Runs



Changes in the viscosity of A. niger pellets during their growth in a tower fermenter




Filamentous mycelium 0.8 g% w/v
Pelleted mycelium 0.8 g% w/v
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1
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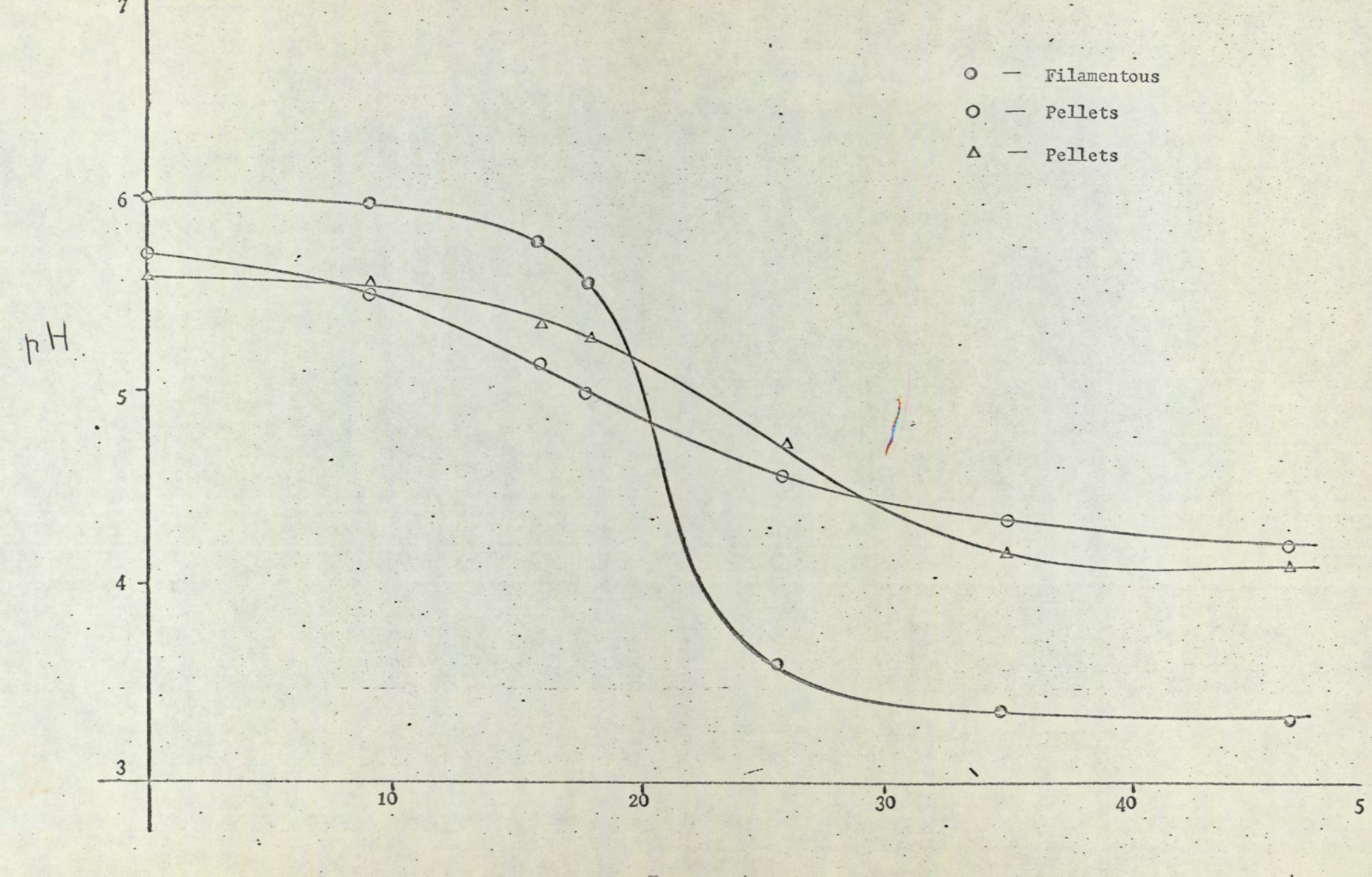
4
Superficial Gas Velocity cm/sec.


7
6
5
4
3
pH
10
20
30
40
5
Filamentous
Pellets
Pellets

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