SOME ASPECTS OF THE MICROBIOLOGICAL PROPERTIES OF POLYMERS AND COMPOSITE MATERIALS

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SUMMARY

The interaction of microorganisms with glass-reinforced polyester resins(GRP), both under laboratory and simulated operating conditions, has been examined following reports of severe fungal biodeterioration. Although GRP was not previously associated with substantial microbial growth, small amounts of microbial activity would pose problems for products associated with comestible materials. The microbiology of the raw materials was investigated, two ingredients were supportive to microbial populations whilst five materials were biostatic or inhibitory in their action. Production laminate was not susceptible to microbial deterioration or inhibitory to microbes. Incorporation of zinc stearate, one of the supportive ingredients, at 300% manufacturing level or drastic undercuring produced laminate capable of supporting microbial growth but only after a non-biotic stage of degradation. Study of the long-term population dynamics of cisterns of GRP and competitive materials under conditions simulating in-service conditions, monitoring microbial numbers within the experimental vessels and comparing with the populations of the supply water, suggests that the performance of GRP cisterns is slightly superior to conventional competitive materials. An investigation of the biological performance of GRP cisterns in an isolated area of known microbiological hazard was conducted. Severe biodeterioration had been experienced with Preform GRP articles moulded using different production techniques, but substitution of current GRP articles resulted in no recurrence of the problem. All attempts to establish the fungal isolate responsible for the phenomena in cisterns under controlled conditions failed. Scanning Electron Microscopy of GRP surfaces showed that although differences exist between current and Preform laminates, these could not satisfactorily explain the differences in service behaviour. These results and the results of the British Plastics Federation Expert Working Group interlaboratory study are discussed in relation to the original report of gross fungal biodeterioration and, to the design of future testing programmes for the products of industrial concerns.

Biodeterioration Polyester Microorganisms Water Testing

ACKNOWLEDGEMENTS

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CONTENTS

CHAPTER I

General Introduction and Literature Survey

1.1 The Firm

BTR Reinforced Plastics Limited is the largest manufacturer of hot-press mouldings in the United Kingdom. It is an autonomous company working within the BTR group of companies, an international industrial concern. The manufacture of goods as diverse as sports equipment, high pressure hoses, conveyer belting, rubber footwear and boats is carried out within the Group, whose origins lie in rubber moulding.

BTR (RP) were based at Uxbridge, UK now at Gloucester and work solely in glass-reinforced polyester-resins (GRP) producing a range of articles. The mainstay of its operations is the mass productions of cold-water cisterns and large fabricated liquid (usually water) holding tanks. BTR bought the manufacturing rights of the coldwater cisterns from Permali Limited, at that time a separate company, in the early sixties. Production was by the semi-hand lay-up 'Preform' process. BTR gradually changed production on all sizes of cistern to the 'Sheet Moulded Compound' (SMC) process, this being accomplished by 1969. Tank panels, from which sectional tanks are constructed on site have always been an SMC composition moulding.

These production processes are described more fully in 1.5.

Around this backbone the facility for the production of highly specialised mouldings has been developed, many

of these being high-quality, short production run articles. The profit-margins on the specialised mouldings are an attractive inducement to their continuation but this can only be achieved because the majority of capital and operational overheads are carried by the cistern and tank moulding business. Any significant curtailment of tank manufacture would have serious commercial implications, and the company would not survive except in a reduced form.

The ability of the present management to switch production to specialised forms of mouldings is very much a consequence of the events leading up to the initiation of this work, and the diversification process is continuing.

1.2 Historical Background to the project

Tests carried out by the Metropolition Water Board (MWB) during the late summer of 1972 indicated that cold water cisterns, submitted by BTR for approval for use, were capable of supporting fungal growth. This observation was unexpected as the formulation of the cisterns submitted was very little different to the formulation passed several years previously by MWB. Furthermore, over the period of more than eight years marketing experience, no significant in-service complaint had been received that was attributable to fungal growth. Upon making detailed enquiries it was found that MWB had recently increased the severity of the test procedure and would review the nature of the test continuously to keep abreast of demands upon the water distrubution industry.

BTR instituted an immediate search for expert help in the water industry, the chemical industry and the academic institutions, assessing ideas for possible solutions to the problem including biocides, alternative release agents, metallic barrier films, unplasticised-plastic barrier films, removal of zinc stearate after moulding, external release agents and fungi-stats. It was during this period that Peter Grey (Central Research and Development Laboratories, BTR Silvertown, Burton-on-Trent) established contact with the Biodeterioration Information Centre (BIC), originally with regard to use of biocides but then with a literature survey and contract research work.

The contract research work done by C. McShane (B.I.C.) involved the assessment of each constituent of the GRP mix as a nutrient carbon source to fungi. The main findings were the appreciable fungal growth with zinc stearate and marked difference between pigments supplied by two companies, one supporting growth, the others appearing to be fungistatic (McShane, 1973). It was from this work that one set of pigments was discarded and alternatives to zinc stearate investigated. Also set up under the guidance of Dr. Burman of MWB and Drs. Eggins and Allsopp of BIC were the experiments on whole tank testing, detailed in Chapter 7, in which BTR and rival cisterns were directly compared. No growth of fungi was apparent in any of the cisterns and so cisterns were resubmitted to MWB for approval. This application was successful but BTR, mindful of the possibility of a repeat of the episode,

with the possibility of an unsuccessful outcome, set up the industrial side of this Interdisciplinary Higher Degree project to provide a developing steady programme.

1.3 National Water Council AcceptanceProcedure

The testing by MWB, previously described, was an acceptance test which at that time was recognised by the other water authorities as the testing procedure for the whole water industry. Under this testing procedure the product is assessed against the requirements of the Model Water Byelaws. Since the reorganisation of the Water Authorities (1st April 1974) the test has been adminsistered by the NWC. TheCouncil's testing scheme is voluntary in the sense thatits object is to provide advice to the water industry and to manufacturers and users of water equipment as to whether any particular item complies with the Department of the Environment's Model Water Byelaws; the Water Authorities and Water Companies are, on the other hand, the bodies actually responsible for enforcing their byelaws and by and large accept the NWC's advice, so that if an item of equipment has passed their tests and in their view complies with the Model Water Byelaws, the Water Companies usually allow its use in their area. Conversely, if an item of equipment does not pass the tests, the Water Authorities and Water Companies usually do not accept the item in their areas; but the final decision is theirs.

Enforcement of the Byelaws is a matter for the Water Authorities, and the law does not at present allow

them to prohibit the sale of an item which has not been submitted for tests; their only power is to take action for breaches of the byelaws in order to prevent the use of equipment.

The test detailed in Chapter 2.12 and the former test in 2.11 is not the whole MWB (NWC now) test. The tests to which a fitting is liable are:-

1. mechanical
2. toxicological
3. organoleptic and physical (taste, odour, colour and turbidity)
4. microbiological growth (MWB 2.11, NWC 2.12)

The requirements under 2,3, and 4 will be satisfied by the tests contained in the 45th report of the Director of Water Examination of the MWB, sextion 11 (1973)

Although the test now is nationally administered and the detailed tests freely given out as information, in 1972 the MWB were very secretive about their testing procedure and gave details of testing unwillingly and not finally until the British Plastics Federation Microbiological Growth Committee was formed. It was this Committee and the administration of the test being centralised under the NWC which, in the author's view, changed the total outlook of the MWB to the testing procedure from one of keeping manufacturers totally in the dark about exactly what their products had failed to do and the recent establishment of independent testing stations by the MWC, but under guidance by Dr. Burman of MWB, to which manufacturers may submit items. This is an important step

forward in the relationship between the water industry and its suppliers and the contribution of this study and its implications should not be underestimated.

1.4 The Chemistry of glass-reinforced polyester

We may consider the formulation of a GRP as a quantified mixture of compounds from three groups:

1. the polyester resin
2. the additives
3. the glass

In the second group, the additives, are included such compounds as pigments, catalysts, release agents, slip agents and fillers. In many ways glass itself ought to be put down as one of the many additives but as its role in the final fabrication is vital it is usually considered as a separate entity.

1.4.1 Polyesters

An ester is formed by the interaction of an alcohol and an acid. Water is obtained as the other product of this reaction, which is reversible. Ethanol and acetic acid, for example, react to give ethyl acetate:

$$C_2H_5OH + CH_3COOH \rightleftharpoons CH_3CO.OC_2H_5 + H_2O$$

In this simple example of esterification, the reactants are a monohydric alcohol and a monobasic acid. These have one reactive hydroxyl group (-OH) and one reactive carboxylic group (-COOH) respectively, per molecule. In the same way simple esters are obtained by reacting together a monohydric alcohol and a polybasic acid or a polyhydric alcohol and a monobasic acid. Thus

ethanol and succinic acid (HOOC. CH_2.CH_2.COOH) yield diethyl succinate (C_2H_5O.CO.CH_2.CH_2.CO.OC_2H_5), while ethylene glycol (HO. CH_2.CH_2.OH) and acetic acid yield ethylene glycol diacetate (CH_3.CO.O.CH_2.O.CO.CH_3).

The reactions between a polyhydric alchohol and a polybasic acid nearly always results in the formation of a polyester resin.

When a dihydric alcohol is reacted with a dibasic acid, chain molecules are formed having terminal hydroxyl or carboxylic groups which can undergo further intermolecular condensations to give longer chains, or intramolecular condensations giving rings, but which lack the facility for cross-linking and, therefore, do not thermoset. The synthetic fibre 'Terylene' is a polyester of this type derived from ethylene glycol and terephthalic acid.

When a compound containing more than two hydroxyl groups per molecule is reacted with a dibasic acid, the additional hydroxyl groups provide facilities for molecular cross-linking and the resins are thermosetting. The interaction between compounds with two or more hydroxyl groups, and acids with a basicity greater than two, produces similar thermosetting resins - the so-called alkyd resins. The resins are relatively slow hardening and find only limited use in moulding and laminating.

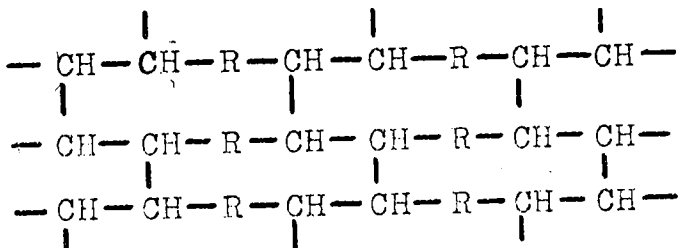
a) Unsaturated Polyesters

A further type of polyester resin may be obtained by reacting together a dihydric alcoholand a dibasic acid, either or both of which contain a doubly-bonded pair of carbon atoms. Thus ethylene glycol reacts

with fumaric acid (HOOC.CH=CH.COOH) giving molecular chains with the general formula:

$$HO.\ (CH_2.CH_2.O.CO.CH{=}CH.CO.O)_nH$$

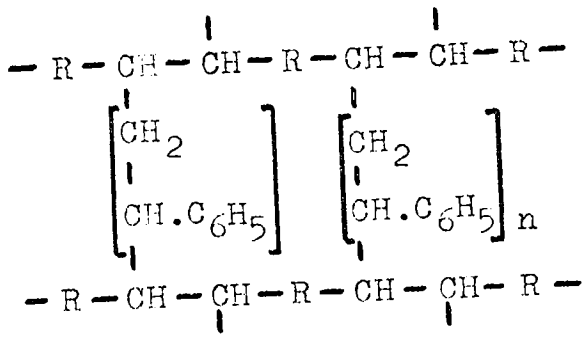
The unsaturated polyesters so formed have the facility for cross-linking through the reactive double bonds, forming structures which can be represented ideally as follows (R represents -CO. O. CH_2. CH_2. O. CO-):



After prolonged heating the resins gel. Cross-linking, however, occurs much more rapidly when organic peroxide catalysts, such as benzoyl peroxide, are present. The ultimate product is a hard infusible mass.

The reactivity of an unsaturated polyester resin may be modified by partly replacing the unsaturated reactant by an equivalent amount of a saturated compound of the same type. Thus, in the ethylene glycol - fumaric acid reaction, if a proportion of the fumaric acid is replaced by an equivalent quantity of succinic acid, the saturated succinyl groups (- $CO.CH_2.CH_2.CO$-) take up random positions in the polyester chains, the average distance apart of double bonds increased, and the number of reative points available for cross-linking reduced.

Unsaturated polyesters usually set more rapidly if mixed with a proportion of one unsaturated monomer such as styrene when the possibility is present of the chains being cross-linked through the monomer, e.g.,



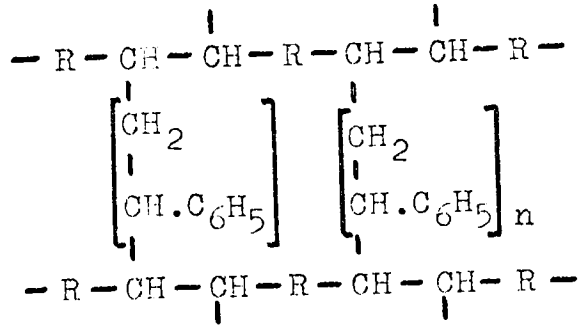
Monomers having more than one double bond per molecule, such as diallyl phthalate are also used in copolimerisation with unsaturated polyester resins. Monomers of this type, having sufficient reactive points per molecule to ensure cross-linking, will themselves thermoset when heated with a peroxide catalyst.

Commercial polyester resins used for the production of reinforced laminates generally consist of a mixture of an unsaturated polyester and a liquid monomer (usually styrene). Since a great number of variations can be made in the composition of the unsaturated polyester and on the character and proportion of the monomer used, a considerable variety of resins with a wide range of properties is possible. The resins, liberating no volatile products during the setting process, can be moulded using very low pressures, and by using promolton in conjunction with peroxide catalysts may be induced to set at room temperatures.

1.4.2 Additives

If the variety of resins alone is enough to give a wide range of properties, the range of additives available to the polymer scientist extends the scope of resins even further.

Perhaps the simplest group of all are the fillers.



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These are blended in to 'expand' the mix in order to decrease the cost/weight ratio. Among the compounds used are woodflour, cornflour, starch, chalk and recycled plastic waste. The degradable nature of starch and the wood flours had tended to produce a shift towards inorganic fillers.

As has been explained already a catalyst is required to induce polymerisation and these are usually liquid organic peroxides.

Self-Colouration of finished articles can be achieved by the use of pigments, usually in a polyester resin base. On polymerisation the pigments base-resin links to the main resin so fixing the colouring particles within the matrix of the article.

Slip and release agents are added to aid extraction of the fabricated article from the mould. These are very often polyethylene and stearate respectivaly. With some processes the release agent is not internal but is externally applied to the moulding tool surface prior to moulding. These external release agents, frequently waxes, or oils, are not strictly additives but since they are often deposited on the moulded surface during processing it is perhaps fitting to mention them here.

1.4.3 Glass

The types of glass used in the reinforcement of plastic articles is 'E' glass, an alkali resistant formulation which is produced in very thin fibres. The form in which the glass fibre is used varies from

'winding', where the glass roving is laid down in a continuous slow spiral on tubular laminates, woven mats where the glass is made into a glass cloth, to chopped fibre mat (CFM) where the continuous roving is chopped into short sections (usually one to two inches in length) and loosely bound into a mat form with binder resin. In Sheet Moulding Compound (SMC) production the roving is chopped into one inch sections but is deposited in the resin/additive mix as it is sheet formed. This process is detailed in 1.5.

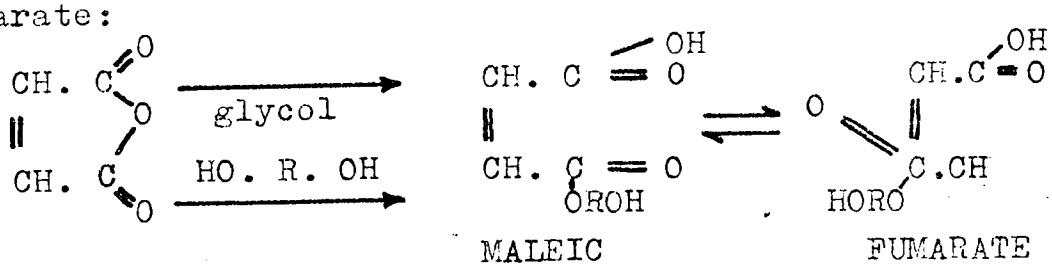
1.4.4 The Polymerisation Process

When a GRP mix is moulded a very complex series of chemical reactions occurs and to attempt to fully define them for all formulations is not possible here. However, generalisations can be made concerning the behaviour of the consitituents and it is these major trends which will be described.

The polyester resin we are concerned with for the majority of the work i.e. the SMC resin, has these principal constituents:

glycol	$HO.CH_2.CH_2.OH$
phthalic acid	$C_6H_4(COOH)_2$
maleic anhydride	CH.C.O.O.O.C.CH

Maleic anhydride is an optical isomer of fumarate:



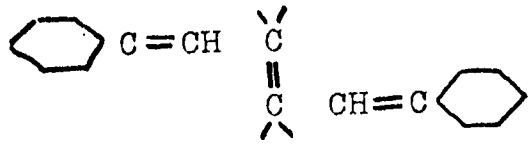
The principal constituents form into chains of the type:

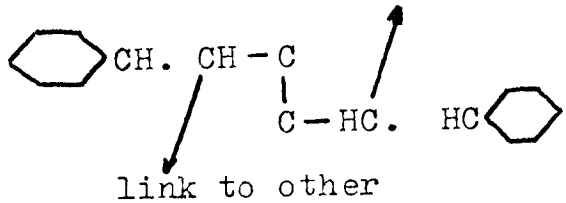
—Ph—G—Ph—M/F—Ph—G—

When styrene is added it tends to align itself to the ethylinic unsaturation point in the polymer,

i.e. 2 styrene/fumarate

1 styrene/maleic





Any residual styrene is formed into polystyrene and anything in the styrene thrown out of solution, i.e. the polyethylene.

The polyethylene together with the stearate will tend to preferentially accumulate at the surface due to their lower surface free energies compared to those of polyester and polystyrane and it is in this fashion they aid in easing the fabricated moulding from the mould tool.

1.5 Glass reinforced laminate production techniques

Of the techniques available for the moulding of glass reinforced plastics only two have any real concern for us. These are the methods employed at some time to produce the cold water cisterns that were the concern of the project. Other manufacturers are currently producing hand layed-up cisterns, but BTR have only used either the semi hand lay-up 'Preform' techniques or the Sheet Moulding Compound (SMC) process.

1.5.1 Preform Fabrication

This process uses a hot-moulding stage where a matching pair of dies are put together to produce the shaped plastic article. Firstly, chopped strand glass is sprayed on the male half of the matched dyes together with a binder resin. The lips and corners are reinforced with fine weave glass cloth and this glass preform allowed to set.

The resin/additive mixture is then poured onto the glass preform and the female tool brought down to mould the article.

In order that the resin/additive mixture is liquid enough to pour the resin/filler ratio must be relatively high and the resulting laminate has a relatively low glass/resin ratio.

On the male tool side of the article, the interior for a cistern, there also tends to be an accumulation of the preform binder resin and release agent from the tool surface, this process being partly 'externally released'.

1.5.2 SMC Processing

This too is a hot-press moulding operation using matched metal dyes but all real similarity ends there.

The resin/additive mixture is made up in large batches of approximately 100 gallons. The resin/filler ratio is much lower than for the Preform process and the resulting mixture resembles a thick clay slurry. This is placed in a hopper which allows a thin film, approximately 5mm, to form on a moving conveyor belt of polyethylene film. Glass rovings are chopped by high speed diamond

cutters and fall at a determined rate onto the sheet of mixture. A second sheet of polyethylene film is put on the surface of the sheet and the polyethylene film/sheet moulding compound sandwich rolled onto storage tubes, or laid in flat batch piles. The SMC may be stored in a cold room for periods of upto 30 days before use.

To mould the article a quantity of SMC, measured linearly from the stock, is placed onto the male mould tool after having the polyethylene film removed, and the matched dies brought together.

The pairs of matched dies are the real focus of the process as it is their design, temperature and closing speed that determines the flow rate of the SMC around the male tool and so ensure a correct distribution of resin, but more importantly of glass, in the finished article.

Due to the low resin/filler ratio and the high proportion of glass in the SMC the resin/glass ratio is very much lower than in the preform process and a stronger, tougher moulding results.

1.6 Literature Review

Research on the biodeterioration of plastic materials started seriously during the later stages of World War 2 when military equipment which had seen service in other theatres frequently became covered with fungal growth when used in tropical areas, often resulting in loss of those physical properties found desirable for successful operation. Intensive research programmes, initially by government departments but very quickly joined by manufacturers, were undertaken resulting in the publication

in the late 1940' s of many such investigations. An excellent comprehensive review of the field of plastic biodeterioration was published by Wessel (1964), in which he collated the results established for the microbiological resistance of polymers and polymeric constituents of plastics. This was complemented by the literature review of MacLachlan, Heap and Pacitti (1966).

Unfortunately the earliest work tended to be confusing as no differentiation was made between breakdown of the polymer and breakdown of additives. However, work on pure polymers was undertaken, notably by Brown (1945) and Abrams (1948) who tried to grow fungi or a fungus on pure polymers. They came to the conclusion that most polymers in use at that time were not susceptible to fungal attack.

The view that pure polymer is resistant to microbiological attack has since been the starting point for many researches and has been reinforced periodically. (Heap, 1965; Wessel, 1964, MacLachlan et al, 1966; Allakherdiev, 1967; andBooth and Robb, 1968). Kestelman, Yarovenko and Melnikova (1972) undertaking fermentation research found that the surface of the majority of polymeric materials remained unchanged. However, Evans and Levisohn (1966, 1968) showed that polyester-based polyurethane could be degraded by fungi and isolated among others Scopulariopsis, Monilia and some bacteria. Other polymers have also been found to support microbiological growth, among them melamine-formaldehyde, cellulose-acetate, cellulose-nitrate and polyvinylacetate (MacLachlan et al (adapted from Wessel), 1966; and van der Toorn, 1969). To these may be added

polyethylene (Nykvist, 1973; Kuster andAzadi-Bakhsh, 1973; and Azadi-Bakhsh, 1972), although support is also gained for the conclusion of Hueck (1973, 1974) that primary biodegradation of polyethylene by micro-organisms is unlikely (Demmer, 1968) and that such biodegradation would have to be preceded by non-biotic degradation to alter the polyethylene matrix structure. Spencer, Heskins and Guillet (1976) recently isolated ten genera of bacteria from cultural conditions including very fine dusts of polymeric material. Polypropylene and polyethylene were ground to an average particle size of 0.020 mm. with a range of 0.003 - 0.080 mm. It is worth remembering that common bacteria are of dimensions approximately 0.001 - 0.002 mm. The difficulties involved in studies of this kind include the measurement of extremely slow biodegradation processes. A possible method of overcoming inaccuracies due to the long time scale involved in degradation experiments was demonstrated by Albertsson and Ranby (1976) using radio-labelled polyethylene. This work followed on from Nykvist (1973) and is particularly sensitive, 0.001% polymer degradation being detected. The state of flux existing at the present time in the field of polyethylene susceptibility may reflect evolutionary changes in microbial enzyme systems, brought about by the problems of recalcitrance posed by man-made high polymers. Evidence exists pointing to the microbial degradation of other polymers once thought to be resistant, such as polypropylene and polyvinylchloride (Azadi-Bakhsh, 1972; and Kuster and Azadi-Bakhsh, 1973). Recent work has tended to be concerned

with the design and production of susceptible polymers. This has occurred since the problems of plastics in the overall refuse system have been highlighted by studies like those of Mills (1973), and the simultaneous upsurge of concern amongst scientists, politicians and the general public alike over mans impact on his environment. Consequently interest has focussed again on the susceptibility of individual components, but this time with the intent of producing the most susceptible components possible in order that the polymer, if a means can be found to initially break it down into its component molecules, e.g. photodegradation, heating, mechanical breakage, will be biodegradable.

Huang et. al. (1976) conducted an extensive exerise to design, synthesise and test the degradability of those polymers. To this end the incorporation of amino-acids in polyesters was attempted with the consequent production of degradable polymer. Bailey, Okamoto, Kuo and Narita (1976) also produced a degradable copolyamide where one of the co-polymers was an α-amino acid.

Fields and Rodriguez (1976) used agar-plate testing to investigate the influence of diol and diacid structure on degradability. Maximum enzymic degradability was shown for diols of four or six carbon structure and diacids of six to ten carbons, with larger units approaching the inertness of high molecular weight polyethylene. BTR SMC GRP uses a two carbon diol, ie. ethylene glycol. This supports the findings of Berk et.al. (1957) who found that the four carbon succinic acid was more degradable than other acids in the two to nine carbon atom range. Fields and Rodriguez

postulated that the relative biodegradability was due to the influence of the distance between ester groups on the template nature of enzyme action. Potts et. al. (1973) described the influence that the structure of individual components may have on the biodegradability of synthetic polymers by microbes. Their main findings were that:

a. aliphatic polyesters and urethanes were the only class of synthetic high molecular weight polymers out of polyesters, polyamides, polyethers and polyolefins found to be degradable.

b. polyesters based on fumaric acid, which is an unsaturated dibasic acid, appear to be utilised more poorly than those based on saturated dibasic acids such as succinic and adipic acid.

c. aromatic structures as exemplified by polyethylene terephthalate render the polyester unassimilable.

d. esters of phthalic acid are not attacked, which supports the finding of Demmer (1968).

The resins used in Cistern manufacture, as described previously in 1.4.4 are derived from maleic/fumarate acid, a two carbon glycol and are aromatic, being esters of phthalic acid.

The hydrolysis of polyesters may be catalysed by esterases, an enzyme very common in fungi (Heap and Morrell, 1968). Esterases are very low in specificity (Fields and Rodriguez, 1976) and the hydrolysis of simple esters and commercial plasticizers by an intracellular esterase system was shown by Williams, Kanzig and Klausmeier (1968).

Evidence for the susceptibility of glass-reinforced polyesters is confused and much of this is possibly due to the many different permutations possible in the constitution and fabrication of a moulding. Tomashot and Hamilton (1956) showed that a slight to moderate fungal growth occurred in pure culture, depending on the type of polyester glass fibre material, combined with some loss of strength. However, the loss in strength was practically identical to that encountered when the moist culture-media alone was allowed to take effect, without fungi.

Connolly (1972) summarised the results of Ventrice (1972), Kwei (1972) and Klein (1972), who examined soil-burial on the properties of styrene-polyesters, casting-resins and laminates. No case of microbial degradation was found after eight years. It is worth noting that the programme was initiated in 1958 using starch-sized glass-fibres.

Marine service of GRP has been reported by Fried and Graner (1966). Large GRP fairwaters were fitted to a U.S. submarine and the results of investigation after eleven years reported. 95% strength and stiffness over five years was reported. Marine fouling occurred on test-panels but was readily scraped off.

The 'Reports on Plastics in the Tropics' (1951-1963) reported in 1962 on low-pressure laminates bonded with polyester resins after exposure in tropical sites. The samples were only affected to any extent at the jungle sites, more so in clearings than undergrowth, and showed

slight growth, if any, which was easily removed. Altogether, the laminates were considered to be very resistant to attack by fungus.

The only work concerning the microbiological testing of GRP in contact with potable water, is that reported by Burman (1976). A 60% failure rate for glass-reinforced plastics (including GRP) is given. It must be remembered that this figure is not based on evidence of degradation of the material but rather on the Biomass-dynamics of the water contained within the cistern.

Generalisations on the behaviour of glass-reinforced polyesters in conditions of microbial hazard are dangerous if taken as more than broad guidelines. This is especially so when trying to relate the established performance of hand layed-up and preform laminates to the future performance of SMC laminates, with their much higher inert filler content and unique resin systems. However, the general conclusion of almost all the studies of GRP's is that they do not support the growth of micro-grganisms, the exceptions being the testing of samples prior to use in the water distribution industry (Burman, BTR cistern rejection certificate, and 1976).

Although authors have suggested that incorporation of a susceptible additive to a plastic will make that plastic inherently more susceptible to attack (Pankhurst and Davies, 1968; Pankhurst, Davies and Blake, 1972), Benicelli (1958) states that 'the nature of the ingredients cannot serve as a final criterion to predetermine the fungal

resistance of the finished compounded materials'.

Apparently, resistance to microorganisms is not a matter of simply adding up properties of chemicals, but in this respect every compound should be considered on its own merits.

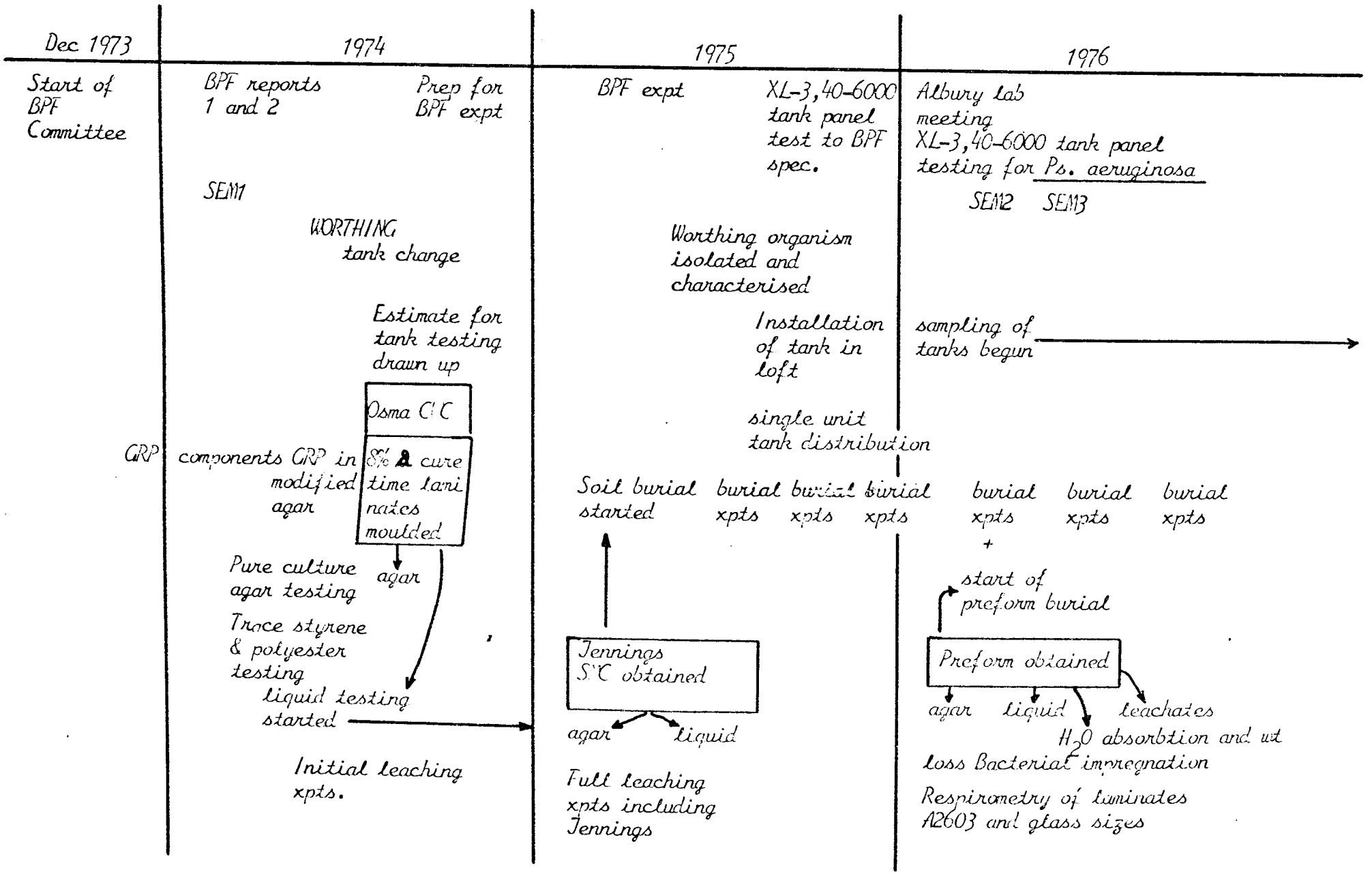
This puts the work detailed in the following chapters into a very specialised niche - it is original research into the microbiological properties of BTR production SMC laminate and specialised derivatives, and as such will determine its suitability as a material for use in contact with potable water.

1.7 Outline of sequential events in this project

The sequence of tests done will be given here so that the results found in the combined sections of chapters may be given a place in time and the influence they exerted on other studies assessed.

The basic premise was that the laminate had been shown to be highly susceptible (Burman-rejection certificate) and indeed the initial agar testing had shown components of the GRP mix to be highly nutritive to microbes. This together with the first SEM study led to a decision to study the microbial interaction with the moulded surface, which led to :

a. long term tank testing
b. Worthing tank change and further study
c. 8% Zn. stearate laminate to be studied
d. low and high cure laminates


Dec 1973
1974
1975
1976
Start of BPF Committee
BPF reports 1 and 2
Prep for BPF expt
BPF expt
XL-3,40-6000 tank panel test to BPF spec.
Albury Lab meeting
XL-3,40-6000 tank panel testing for Ps. aeruginosa
SEM2
SEM3
SEM1
WORTHING tank change
Worthing organism isolated and characterised
Estimate for tank testing drawn up
Installation of tank in loft
sampling of tanks begun
Osma C/C
GRP
components GRP in modified agar
% cure time tani nates moulded
single unit tank distribution
Soil burial started
burial xpts
burial xpts
burial xpts
burial xpts
burial xpts
burial xpts
Pure culture agar testing
agar
Trace styrene & potyester testing
liquid testing started
start of preform burial
Jennings S'C obtained
Preform obtained
agar
liquid
agar
liquid
leachates
H_2O absorbtion and wt loss Bacterial impregnation
Initial leaching xpts.
Full leaching xpts including Jennings
Respirometry of luminates A2603 and glass sizes

The BPF reports 1 and 2 were written for the BPF expert working group to study and discuss, and were a serious attempt to establish the basis for potable water testing that might be carried out by any well-equipped microbiological laboratory. This might then allow manufacturers to establish the effects of any changes in production technique and so help in directing product development. These are appended (Appendix's 3 and 4).

Albury Laboratories are an independent testing station licenced by the NWC to test samples. Albury contacted BTR to ask if they wanted to use their facilities instead of those of the MWB. BTR had not heard of any independent stations and this illustrates the uncommunicative nature of the relationship between NWC/MWB and BTR, if not MWB and manufacturers as a whole.

XL-3 is one of a number of samples of sealant which were tested throughout the period October 1973 to March 1976.

The experiments described in the following chapters were an attempt to determine the susceptibility of SMC GRP to microbial attack, to determine the differences in behaviour between SMC and preform tanks, if any, within the framework of an industrial project where commercial implications, such as publicity, had to be regarded at all times.

1.8 Aims of the Study

Although recognising the value of Denicelli's statement (1958), with regard to the nature of individual

components, it is apparent that to a manufacturer without independant testing resources of his final product, wherever substitution of an ingredient with one of lower susceptibility to microbial attack can be made, it is wiser to do so. This was BTR's position in 1973 and at an early stage it was decided to examine the individual components of the GRP mix, and any future substitutes, for resistance to microbial attack. This would make it possible to eliminate as far as possible susceptible ingredients.

Even if it were possible to completely eliminate all susceptible ingredients, which it is not whilst retaining the SMC process, the changes that occur on moulding are so complex that this could not be taken as definitive evidence concerning the microbiological nature of the laminate. The moulded surface of the finished product must be assessed on its own merits, and not thought of as a product of summing all susceptible compounds and subtracting inhibitory ingredients.

It was probable that no one line of research would provide all the evidence needed to answer the problem for BTR. In this light a multidisciplinerary approach was adopted, concentrating effort on four fronts:

a. The microbiological testing of laminates, both production SMC and any variations necessary, to elicit information concerning the changes in laminates during degradation. The subsequent testing of any proposed solutions to be carried out together with the devising of future testing schemes for articles

in contact with potable water.

b. The long-term testing of cisterns to study the population dynamics of the held-water and the laminates surface, to quantitatively and qualitatively define the organisms or groups of organisms present.

c. The investigation of any known incidents of microbial growth in GRP tanks and cisterns. Any deteriogen found might then be used in the microbial investigations (1. above) in order to determine its role in the degradation of SMC GRP, and its characteristics defined in order to elucidate its nutritional requirements and enzymic capabilities.

d. Representation on the British Plastics Federation Expert Working Group, firstly to keep abreast of developments, secondly to be involved in the formulation and testing of any experimental testing programme and thirdly to publish the results of the three areas of research outlined above.

CHAPTER II

2 MATERIALS AND GENERAL METHODS

2.1 GRP components and laminates

Cold water cistern (CWC) components and laminate moulded frommixes of these compounds were used for the major part of this project. The individual components were obtained from the bulk storage facilities at the factory site and were used experimentally in their manufactured state with no attempt at grading or purification. They were stored in small glass jars at room temperature in the laboratory.

The laminates used in this study were of two basic types, differentiated by the manufacturing moulding process involved. These were the Sheet Moulding Compound (SMC) process and the earlier Preform process. Laminate samples of these two types were obtained by cutting into manufactured cisterns in such a manner as to separate the four sides and the base. All lips and radii were removed, edges straightened and the five resultant flat sheets marked with identification numbers. Further processing prior to testing was carried out at the Biodeterioration Information Centre and is detailed in 2.2.

Laminate samples obtained in this way were Osma CWC, Jennings CWC and Preform CWC.

Special GRP laminate was also produced but unlike production formulations they were moulded up directly into flat sheet, rectangular in shape on an existing BTR tool. The first of these special formulations was production CWC SMC with additional zinc stearate to give

a final zinc stearate content of 8% w/w. This was produced in the laboratory at Uxbridge in the form of a single batch of dough moulding compound. Moulding was carried out using the mould temperatures and cure time designated for cistern production. The second specialised mouldings were a series of laminates of production SMC differing only in the cure time given in the press. The range of cure time utilised was from 10 to 120 seconds.

Two types of tank panel laminate were tested. These were production tank panel and a formulation based on Freemans 40-6000 resin. The samples were obtained by cutting a rectangular piece out of the main face of a moulded tank panel.

Some testing was done on materials which form part of a raw material used in the manufacturing process at BTR. These were glass size emulsions applied by Fibreglass Limited, in the production of their type 11 and 12 rovings. These were kindly supplied by Fibreglass Limited and were used as supplied.

Preform binder resin of the type employed by BTR in the production of preform cisterns is no longer produced on a commercial scale. BP chemicals however kindly supplied a small sample produced to the original specification.

2.2 Conversion of laminate into test pieces

Testing of moulded laminate was carried out on both solid block samples andpowdered samples. The dimensions of the test blocks used varied in the different

experiments and will be described in the relevant sections.

Generally the sheet laminates were converted into test blocks on a small band saw. To obtain powdered laminate small blocks were fed into a hammer mill and the powder collected in clean test tubes, sealed and labelled. Part of the powdered samples was placed in a ball mill jar and ball-milled for 48 h.

2.3 Agar plate visual growth assessment

Test material combined with a mineral salts agar medium in petri plates was inoculated, incubated and growth assessed visually at intervals. The mineral-salts agar used was that of Eggins and Pugh (1962). A modified medium was also used, modification of the E & P salts medium being the deletion of carbon containing constituents i.e. yeast extract and L-asparagine. Where the test material was a powder or liquid they were incorporated in agar at 40°C using a Waring-blender. (15 seconds). Laminates were placed on semi-liquid agar, which had been poured into petri-plates, and allowed to fuse with the agar. Where trace amounts of styrene or polyester resin were incorporated with the test material, this was achieved by adding the styrene or polyester to the cooled agar prior to portioning and blending. Various inocula were employed and will be detailed in the relevant section together with incubation temperature and time. Visual assessment was scored on a scale of 0 to 4 being a measure of the area of agar surface covered by growth (ASTM G21, 1970).

2.4 Liquid culture technique

To study the effect of GRP onthe production of biomass, sterile E & P medium and modified E & P medium was prepared as before but without addition of agar. Twenty five millilitres of medium was dispensed into conical flasks and covered with cotton wool plugs. The flasks had previously been prepared by the addition of the test material. The flasks were each inoculated and incubated for a period. At the end of the incubation period the biomass was filtered off through weighed filter discs and dried overnight in an oven set at 100°C. They were weighed again and the original weight subtracted to determine the weight of bios produced. It was realised that with powdered test materials, the weight of these might affect the calculation of weight differences. The weight of the powders were determined for a 25 ml medium after drying as before and this weight taken into account in the calculation of biomass.

2.5 Extraction of test blocks with cold water

Blocks were placed in conical flasks and 100 ml distilled water added. A cotton wool plug was used to cover the flask and they were allowed to stand on the bench top for the duration of the extraction. When water uptake or loss in weight as a result of extraction was to be known the blocks were chosen randomly from a selection of blocks. The blocks which were not extracted with water were dried in the oven at 90°C for 24 h, the weights before and after drying being used to determine the water content of the block. The mean water content

could then be determined and used as a correction factor for the weights already determined for the blocks undergoing extraction.

2.6 Extraction of test blocks with boiling water

More severe extraction in boiling water was carried out in two ways:

a) with the soxhlet leaching apparatus

b) with a refluxing apparatus

Again, if weight determinations were to be made random blocks were used to determine the water content of the test samples.

Test blocks were placed in the soxhlet chamber above the water flask in the soxhlet apparatus and in the water flask when refluxing. Extraction was carried out for a period of time which will be detailed in the approxpriate section.

2.7 Extraction of ground and ball milled laminate

All extractions were carried out using the soxhlet apparatus, the test sample being contained within a filter thimble in the central chamber of the apparatus. When loss in weight as a result of extraction was to be known a portion of the powder was weighed, dried overnight in the hot oven at 90°C and weighed, and the water content found. This could then be used to determine the corrected dry weight of the sample undergoing extraction.

2.8 Source of Soil

The soil used in this work was obtained from Broome farm, Broome, Worcs. It was removed from the same site as samples used by Mills (1973). Its water holding

capacity was determined by the method described in the IRG document No. 208. In all the soil burial studies described the soil was maintained at this capacity, namely 34%. It was checked periodically by weighing the culture containers and spraying the soil surface with sterile distilled water to make up for any loss.

2.9 Culture containers for soil burial

Rectangular plastic containers (50 cm x 20 cm x 20 cm length x width x depth) were employed for soil burial. Each container was filled with 18 to 20 kg of soil, a container of sterile distilled water placed on the soil surface to maintain high humidity in the chamber, and a plastic lid fashioned from polythene sheet sealed around the lip of the container. Thirty six blocks were placed in each container. All blocks were at least 3 cm apart and the upper surfaces not less than 4 cm below the soil surface. The soil was not amended with nutrients and reliance was placed on the natural soil microflora for infection. Each batch of soil was used once only. The containers were placed in a constant temperature room maintained at 30°C throughout the incubation. The period of incubation will be mentioned in the specific sections of the thesis.

2.10 Warburg Respirometry

A large part of this study has involved the use of manometric techniques to measure the uptake of oxygen and hence of aerobic microbial activity. Details of experimental procedure are given in the specific experiments. Warburg respirometers in a circular water

bath were employed in all the determinations of oxygen uptake, the temperature of the water bath being maintained at 30°C throughout.

The central well of the flasks contained 1.0 mls of 20% potassium hydroxide and a roll of filter paper to increase the absorbing surface. A thermobarometer was included in the determinations and this measured the changes caused by variation in atmospheric pressure. A correction from the thermobarometer was applied to the experimental readings so establishing the pressure change in the experimental system due to the oxygen uptake in the reaction flask alone.

2.11 "British Plastics Federation" Test Method

This method was adopted by participating laboratories in a programme designed to investigate the parameters that affect microbiological growth on GRP materials. The counting procedures were laid down and based on those found in the Bacteriological Examination of Water Supplies (No. 71).

100 ml of soil extract was added to all experimental GRP vessels and the glass beakers acting as controls. The soil was that detailed in Chap. 2.8 and was sent out to the participating laboratories by the author. A further 650 ml of water was added, the vessels covered with aluminium foil and incubated without light at 30°C. The water was changed after 3 and 4 days alternately, 10 ml of the water being allowed to remain through each change and a fresh 740 ml being added. Visual observation of any apparent growth or surface film on the

vessels was undertaken just prior to water changing and the count out on the water to be discarded at the change.

Coliform counts and 3 day 22°C and 48 hour 37°C colony counts were carried out by the method specified in Report 71. E. coli counts are carried out only if coliform count persists. Fluorescent pseudomonads were counted using Kings B broth with 4 mg/ml erythromycin by an MPN procedure using replidishes. Five replicates of five dilutions may be accommodated in a single replidish, incubation being at 22°C for 3 days. The cultures are examined under ultra-violet light for fluorescence and the numbers/ml found using McGrady's tables (given in Report 71).

Fungi and yeasts were counted using a membrane filtration technique. 100 ml and 10 ml quantities of water are filtered through sterile membrane filters. The membranes are cultured on Martin's rose bengal agar but with 100 mg/ml kanamycin and incubated at 22°C for 7 days. Colonies are counted and differentiated as fungi or yeasts on colony appearance.

Termination of the test is consequent on the maintenance over three consecutive counts of either less than tenfold increase in counts or greater than tenfold increase, the former being considered a pass, the latter a fail. Also certain absolute limits were put on the counts below which the count could be ignored ranging from 0/100 ml for coliforms to 10000000/100 ml (10^7/100 ml) for 22°C colony count.

The types of sample and waters used are detailed in the relevant section.

2.12 Current NWC test method

This test is very similar to that used in the BPF trial, the differences involving the evolution of the fluorescent pseudomonad count to a count of Pseudomonas aeruginosa and the treatment of sheet laminate samples.

Pseudomonas aeruginosa was counted by using membrane filters on Kings's A broth (Drakes modification with 0.05% cetrimide). Membranes were plàced on pads saturated in this medium, incubated at (37°C for 48 hours)* and green colonies fluorescing under U.V. are counted. (* or 42°C ± 0.5°C for 24 hours)

If in doubt they may be confirmed by caesin hydrolysis on milk agar or by subculture of individual colonies. Confirmation was by imprinting in this laboratory.

Samples not in the form of a container are put in a glass beaker of approiate size to take the sample whole and submerge it totally with the water. The sheet laminate samples were cut as detailed in II (ii) to a block of dimensions 50 mm x 20 mm. In certain experiments a glass slide was placed in the control vessel in order to equalise the surface area available for growth.

CHAPTER III

3. MICROBIOLOGICAL TESTING

3.1 Introduction

Numerous test methods for plastics have been developed by workers in the field but the test criteria used are generally of a limited number. These are of two main types; firstly, methods relying on direct measure of microbial activity:

1. visual evaluation of microbial growth
2. biomass production
3. respirometric techniques
4. measurement of pH changes

and secondly, those methods relying on indirect measure of microbial activity by measurement of physical properties of the material under test. These are:

1. wt. loss of test piece under attack
2. changes in mechanical properties of test piece
3. changes in electrical insulation properties of test piece

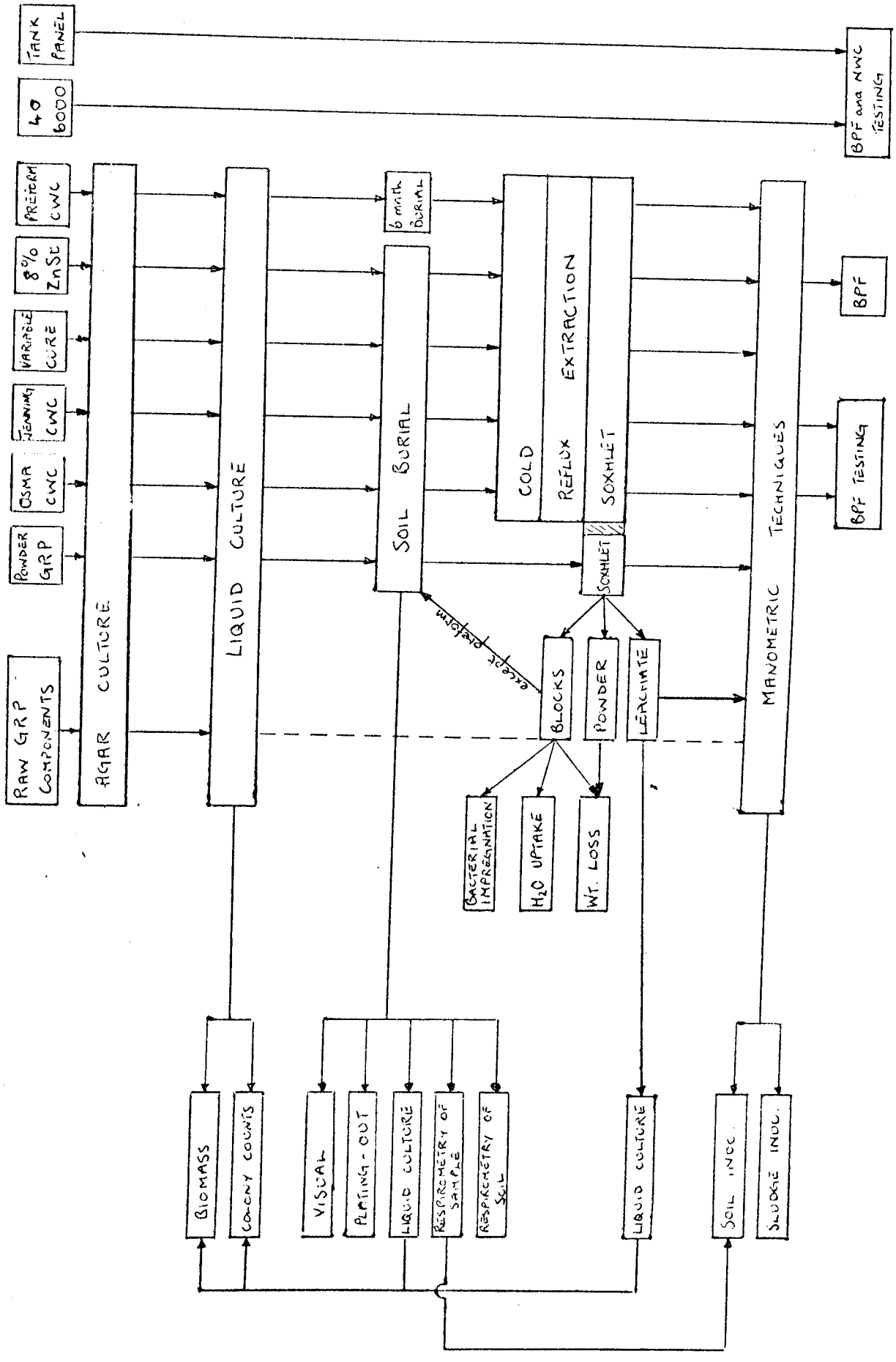
The experiments described in the following chapter rely more heavily on the first category of test criteria just described, and the outline of these tests and the materials tested by each of these tests is given in Figure 1.

3.2 Agar Plate Methods

3.2.1 Introduction

The literature contains many references on the subject of plastics testing using agar culture techniques, although the majority of papers are concerned with the testing of plasticised PVC or plasticisers themselves

Figure 1 Scheme of testing for materials



(Berk, 1950; Klausmeier and Jones, 1961; Burgess and Darby, 1964, 1965; Hazeu, 1965; Hitz, Merz and Zinkernagel 1967). Other plastic materials have been tested using agar plate methods, including Nylon (Gray, 1945; Nigam, 1965; Rogers and Kaplan, 1971), Polythene (Allakherdiev, Martirosova and Tariverdiev, 1967) and Polyurethane (Evans and Levisohn, 1968), also have biocides (Dolezel, 1967).

Because of the methods proved record with many plastic materials and associated compounds it is a very useful screening procedure for assessing the potential biodegradibility of plastics and the petri-plate method has become a standard test method for plastics (I.S.O. R846 - 1968 (E) and ASTM G21 and ASTM G22, 1970).

3.2.2 Methods

All materials which came under investigation during this project at some time or other went through agar testing with the exception of tank panel and 40-6000 resin laminate. They were examined by the methods described in Chap.2, and by two modifications described below.

Solid sheet material tested as described in Chapter II were found very difficult to assess and a modification of the technique was applied as defined in ASTM G22. In this method the sheet material was placed on semi-molten agar and allowed to fuse. Agar, seeded with soil organisms, is then poured over the pre-poured agar and specimen block and allowed to gel. The agar used in all cases was modified E & P agar

(detailed in Chapter II).

Constituents of GRP mix were tested in agar with incorporation of trace styrene and polyester resin. A modification of this technique was also applied. In this method, the plates of mineral salts agar with incorporated test material were exposed to styrene and polyester resin vapour by placing in the bottom of the incubator an open container holding the requisite liquid.

Inoculation of plates was by one of three methods:

1. exposure of open plates to the atmosphere for 30 minutes
2. spraying of the agar surface with a suspension derived from clent soil (source of soil given in Chapter II)
3. spraying of agar surface with a known fungal spore suspension derived from isolates obtained from (1).

The results are presented under three headings concerning the inoculum.

All incubation was at 30°C and examination of the plates took place after 5 days for the soil inoculated samples and 21 days for all plates.

Controls in all experiments were agar plates prepared, inoculated and incubated in precisely the same manner as the test plates except for the deletion of any test material.

Assessment, as previously stated was on a scale 0 - 4.

Observed growth on specimens	Rating
None	0
Traces of growth (10%)	1
Light growth (10 - 30%)	2
Medium growth (30 - 60%)	3
Heavy growth (60 - 100%)	4

Light cobwebby growth covering all specimen but not obscuring the specimen was rated as 2.

Chemical composition GRP mix components given in Appendix 1.

3.2.3 Results

3.2.3.1 Inoculation by atmospheric exposure - replicated 10 x

a. Modified E & P agar with 1% material incorporated or sheet laminate

0	1	2	3	4
Maglite D	control	40-2386	RCR 2	zinc
29 B 50	LR 13921	40-2397	[ground GRP's]	stearate
styrene	1927	[8% ZN st CWC]	[ball-milled GRP's]	
	BLR 2			
	7ML	[low cure CWC (10s)]		
	alkathene			
	triganox C			(glucose)
	britomya M			
	osma CWC			
	[Jennings CWC]			
	Preform CWC (200s)			
	high cure CWC			

It was found in the agar testing range of experiments that E & P salts agar gave higher control assessments than the modified E & P salts series.

The assessment group for controls was found to be 2 generally and most of those shown in column 1 above were assessed also as 2, except Osma CWC and high-cure CWC which remined in 1. Those found in column 0 above were also found in 0 with E & P agar. Due to the general similarity of the results obtained on the two media only modified E & P results will be tabulated any significant deviation being noted after the appropri--ate table.

b) Modified E & P agar incorporating trace polyester resin (0.1%), test material (1.0%).

0	1	2	3	4
Maglite D	control	LR 13921	RCR -2	zinc
29 B 50	BLR 2	1927	[ground grp CWC]	stearate
styrene	7 ML	alkathene		
	triganox C	40-2386	[ball-milled CWC]	
	britomya M	40-2397		
	Osma CWC	[preform CWC]		
	[Jennings CWC]			
	[8% Zn st CWC]			(glucose)
	[highcure CWC]			
		[low cure CWC]		

c) Modified E & P agar incorporating trace polyester with test material (1%) or sheet laminate

As above except that preform CWC reverted to column 1

and alkathene and resin 40-2386 moved to column 4.

d) Modified E & P agar incorporating test material laminate exposed to polyester resin vapour

As 0.1% trace polyester resin except:-

1) 1927, alkathene in column 1

2) control in column 2

e) Modified E & P agar incorporating trace styrene (0.1%) with test material (1%) or sheet laminate

As for test material agar plates shown in (a)

f) Modified E & P agar incorporating trace styrene (0.01%) with test material (1%) or sheet laminate

As for (a)

g) Modified E & P agar incorporating test material (1%) or sheet laminate exposed to styrene vapour

As for (a)

3.2.3.2. Inoculation with spray of suspended Clent soil organisms

- replicated 10 x

a) Modified E & P agar with 1% test material incorporated on sheet laminate

Again, E. & P agar showed a higher control assessment so condensing column 2,3, and 4 without altering the relevant positions of test materials so again only the tabulated results for modified E & P salts agar are presented.

0 maglite D, 29 B 50, styrene

1 control, LR 13921, 1927, BLR 2, 7ML, alkathene, triganox C, 40-2386, 40-2397, britomya M, Osma CMC, Jennings CMC, Preform CMC, High cure CMC.

2 *ground CIC's, ball milled CIC's, 8% Zn. St. CIC, low cure CIC (10s).*

3 *RCR 2*

4 *zincstearate*

b) Modified E & P agar incorporating trace polyester resin (0.1%) and(0.01%) with test material and sheet laminate

Both these series of tests gave similar results and were different to the table above in that the control and all others in column 1, except Osma CWC, were assessed in column 2.

c) Modified E & P agar incorporating trace styrene (0.1% and 0.01%) with test material or sheet laminate.

Both concentrations of trace styrene gave results as table (a).

3.2.3.3 Inoculation with pure fungal spore suspension - replicated 10 x

a) E. & P salts agar

Organism	GRP component		
	40 2386	40 2397	zinc stearate
Trichoderma viride	3	3	4
Chaetomium spp	3	4	4
Aspergillus niger	2	2	2
Aspergillus fumigatus	0	2	2
Aspergillus spp	0	0	1
Penicillium spp	0	0	1
Paecilomyces spp	0	1	1
Control	0	0	0

b) E & P salts agar - *modified*

Organism	GRP component		
	40 2386	40 2397	zinc stearate
Trichoderma viride	3	3	4
Chaetomium spp	3	3	4
Aspergillus niger	2	2	2
Aspergillus fumigatus	2	2	3
Aspergillus spp	1	0	2
Penicillium spp	0	0	2
Paecilomyces spp	2	2	2
Control	0	0	0

3.2.4 Discussion

It will be seen that most of the GRP components and sheet laminates show no capacity to act as nutrients, maglite D, 29 B50 and styrene consistently inhibiting growth, and zinc stearate, RCR 2, ground and ball milled GRP's, 8% zinc stearate and low cure CWC show consistent encouragement of growth.

Incorporation of trace styrene or exposure to styrene vapour with other components showed no detectable effect, trace polyester resin both incoporated and as vapour showed slight encouragement of growth but did not overcome the inhibitory effects of maglite D, 29B50 or styrene.

Pure culture studies showed a marked difference between the activities of Chaetomium spp., Trichoderma viride, Aspergillus niger and the other organisms. This is supported by work on the enzymic characteristics of certain fungal species, where Chaetomium.sp. and Aspergillus niger were found to have high enzymic activity over a complex enzymic apparatus (Lazar, 1974)

3.3 Liquid culture methods

3.3.1 Introduction

Once more, the literature contains many references to the testing of plastics in some form of liquid culture and again the main use of these has been to test plasticised PVC (Hitz and Zinkernagel, 1967; Booth and Robb, 1968; Booth, Cooper and Robb, 1968; Kestelman and Vilnina, 1971; Kestelman Yarovenko and Melnikova, 1972). Incorporation into nutrient salts has been used to test plasticisers (Williams, Kanzig and Klausmeier, 1969) and some general biodegradability methods have been developed (Sharpe andWoodrow, 1972).

The assessment techniques are usually weight loss combined with physical parameter testing (Booth and Robb, 1968; Booth et al, 1968; Kestelman et al. 1972) but other estimations are biomass production (Kestelman and Vilnina, 1971) and production of ammonia from endogenous protein (Sharpe and Woodrow).

Biomass production was the assessment used for this study in the form of colony counting technique and dry weight of biomass after filtration of culture medium. Turbidity was not considered for use in this study due to the heterogenous nature of the inoculum and the consequent difficulty in producing a standard growth versus turbidity curve.

3.3.2 Materials and Methods

The materials tested in this way are shown in Fig 1.

Table 1 Biomass production with test materials expressed as mean ± standard deviation

Material	biomass mg	Material	biomass mg
Control	8.6 / 1.94	britomya - M	8.7 / 1.19
13921	7.2 / 1.38	Osma sheet	8.3 / 1.27
1927	9.1 / 0.98	Jennings	8.9 / 1.32
BLR2	8.4 / 1.72	8% zn.st.	9.5 / 1.23
maglite D	0.9 / 2.06	Undercured	10.6 / 1.82
7 ML	6.9 / 1.92	Overcured	9.3 / 1.62
Alkathene	7.6 / 1.62	preform	10.1 / 1.70
RCX 2	18.4 / 1.29	Osma ball-milled	7.9 / 2.96
zinc stearate	39.2 / 2.38	Jennings	10.7 / 1.83
toganox - C	6.2 / 1.90	8% zn.st.	17.9 / 1.66
29 B 50	0.8 / 2.30	undercured	10.8 / 1.49
40 - 2386	11.0 / 1.98	overcured	9.5 / 1.51
40 - 2397	8.7 / 1.80	preform	8.1 / 1.83
styrene	1.2 / 2.68		

Undercured laminate (10 second cure)
Overcured laminate (200 second cure)

Table 2 Colony counts with test materials expressed as mean of 4 plates

Material	10-1 dilution	10-2	10-3
Control	T.N.C.	79	9
13921	T.N.C.	84	17
1927	T.N.C.	93	52
BLR 2	T.N.C.	88	13
maglite D	194	25	-
7HL	T.N.C.	51	7
alkathene	T.N.C.	63	11
RCR 2	-	T.N.C.	209
zinc stearate	-	T.N.C.	272
triganox - C	T.N.C.	59	10
29 B 50	127	-	-
40 - 2386	T.N.C.	107	12
40 - 2397	T.N.C.	121	11
styrene britomya - M	408	32	-

The assessment of growth using dry weight measure of biomass has been described in 2.4. Also used in the initial stages of the study was a viable count method. The method was similar to that outlined in 2.4 until the end of incubation. Serial dilutions were obtained of the culture media and pour plates formed with nutrient agar at 40°C. The plates were allowed to gel, incubated at 30°C for 5 days and then counted. Counts given are the mean of four pour plates used at each dilution.

Inoculation of flasks was from a fresh Clent soil suspension and incubation of the flasks was at 30°C for 21 days, with 4 mm discs of mycelium fromthe seven fungal isolates used in the agar plate pure culture studies.

3.3.3 Results

Biomass results are the mean of 5 flasks and also given is the standard deviation of the mean. - Table 1

Counting results are the mean of 4 plates. - Table 2

3.3.4 Discussion

From table 1 it is evident that the test materials may be divided into three groups. The largest group are those which cannot be distinguished from the control. On either side of these are two small groups of test materials which inhibit growth and test materials which actively support growth. It can readily be seen that none of the sheet laminates is in one of these smaller groups, except for ball-milled 8% zinc stearate sheet laminate.

Table 2 shows the results obtained from the colony counting assessment which was only carried out

on the mix component test materials. Again, there was a differentiation of the materials into the three groups described above but with less sharply defined boundaries.

3.4 Soil-burial of sheet laminates

3.4.1 Introduction

Burial of materials in soil is used by many workers in many areas to provide specimens for further evaluation. In the plastic testing field it has been used in this way by many researchers on a wide range of materials, including plasticised PVC (Klausmeier and Jones, 1961; Booth and Robb, 1968; Booth et al. 1968; Cavett and Woodrow, 1968 Wendt, Kaplan and Greenberger, 1970), cross-linked polyethylene (Bebbington, 1972), casting resins (Ventrice, 1972) and reinforced plastic laminates (Ventrice, 1972; Kwei, 1972; Klein, 1972). The trends in the behaviour of plastic materials after soil burial are described by Connolly (1972) and among his findings is 'styrene polyesters - have been essentially unaffected'

This investigation was designed toprovide further information on the behaviour of glass reinforced polyester laminates on soil burial, to provide if possible decayed specimens for further evaluation and to determine if organisms could be found associated with such changes.

3.4.2 Materials and Methods

The materials tested in this way are depicted on figure 1. The 'powdered GRP's' were in this case ball-milled samples contained in pouches made of glass cloth. The glass cloth was also buried to determine whether it was

able to support microbial populations, and if so for those results to be weighed against any achieved for the ball-milled specimens. The laminates ball-milled and to buried in this way were as follows:

1) Osma CWC
2) Jennings CWC
3) 8% Zn. stearate CWC
4) Undercured CWC (10s)
5) Overcured CWC (200s)
6) Preform CWC - 6 months burial only

The blocks of sheet laminate were cut from the sheet material described in 2.2 into pieces 50 mm x 30mm. Half of the blocks were wrapped in glass cloth held by adhesive-glass tape.

All samples were buried as described in 2.9, and samples withdrawn at three month intervals, two of each laminate type.

Smaller samples, 20 mm x 10 mm, which had previously been extracted as described in 2.6, were also buried. The exception was preform laminate. Again these were with-drawn at intervals, this time of 13 weeks.

The withdrawn samples were cleaned, examined for visual deteriorations, then cut into three segments. The first segment was put into the hammer mill, which had been cleaned with alcohol, and the ground dust collected directly in a petri-plate. Nutrient agar was poured on (at 40°C), the plates left to gel after thorough agitation to disperse the particles, incubated at 30°C

for 7 days and counted.

The second segment was placed in 25 ml of nutrient broth in a conical flask, incubated at 30°C, filtered and dried and the biomass determined.

The third segment was placed in a Warburg flask containing a nutrient salts solution and glucose (2.5 ml) and 0.5 ml of 20% potassium hydroxide in the central well. They were then placed in the water bath and incubated for 12 hours, with readings every hour.

Soil was also withdrawn at each sampling date from two places. The first was from the soil surrounding an 8% zinc stearate sheet laminate that was to be sampled and the second lot of soil from an area where no sample was buried. 1g of each soil was placed in Warburg flasks containing a glucose-nutrient salts medium and incubated for 12 hours, reading every hour.

3.4.3 Results

Visual examination of sheet laminate showed no surface discolouration or apparent colonisation ordecay. On samples withdrawn after 12 months deterioration of the edges was apparent with the resin/glass bond being weakened sufficient to allow 'crumbling' of the resin from the glass fibres with the fingers.

Microscopic examination revealed no presence of micro-organisms around the areas affected and it is seen on some extracted samples before burial, suggesting a water 'wicking' effect along exposed fibres from the cutting process.

The glass used to encapsulate the powders and

and laminates again showed no discolouration or the presence of micro-organisms. Pour plate colony counts for test materials are shown in Table 3.

Dry weight of biomass produced from incubation of the test materials in nutrient media is presented in Table 4.

Oxygen uptake after twelve hours for test materials is shown in Table 5.

Oxygen uptake for various soils is presented in Table 6 and Figure 2.

3.4.4 Discussion

The results of this investigation are in agreement with those of Kwei (1972) and Klein (1972). No evidence was found of microbial degradation of any of the sheet laminates and only 8% zinc stearate ball-milled powder gave indication of any microbial association.

3.5 Extractions

3.5.1 Introduction

The inability of sheet laminate to support growth in the experiments so far led to a re-examination of the approach and a return to a form of liquid testing. However, instead of testing the laminate as a carbon source in nutrient salts at slightly above ambient temperatures, a method of leaching in boiling water was examined. It was postulated (Mallorie - per communication) that boiling water exposure would probably not be too different from cold water in the material that was extracted, just more rapid. To test this suggestion and to examine the biological significance of extraction

Table 3 Pour-plate colony counts for test materials at various periods of burial

Material			Incubation period (months)					
			3	6	9	12	15	18
LAMINATES	Osma CWC	bare	0	1	0	4	0	5
		covered	0	8	0	8	1	0
	Jennings CWC	b	0	0	9	8	0	9
		c	9	1	5	0	0	0
	Undercured CWC (10s)	b	0	0	7	0	8	0
		c	6	0	5	3	0	6
	Overcured CWC (200s)	b	0	6	0	0	5	9
		c	1	2	0	5	1	0
	8% Zn.st CWC	b	0	0	4	0	9	0
		c	7	0	0	9	0	0
	Preform CWC	b	0	2	/	/	/	/
		c	0	0	/	/	/	/
	Osma CWC	Refluxed Extracted	0	5	0	2	0	5
	Jennings CWC		1	0	0	4	0	0
	Undercured CWC		0	0	8	2	3	0
	Overcured CWC		1	0	0	5	8	0
	8% Zn.st CWC							
POWDERS	Osma CWC		0	5	3	0	2	0
	Jennings CWC		0	4	0	6	0	0
	Undercured CWC (10s)		0	3	9	0	17	4
	Overcured CWC (200s)		0	0	6	0	5	1
	8% Zn.st CWC		5	3	17	38	52	30
	Preform CWC		4	7	/	/	/	/

Table 4 Dry weight of biomass produced from test materials after various periods of burial after deduction of control

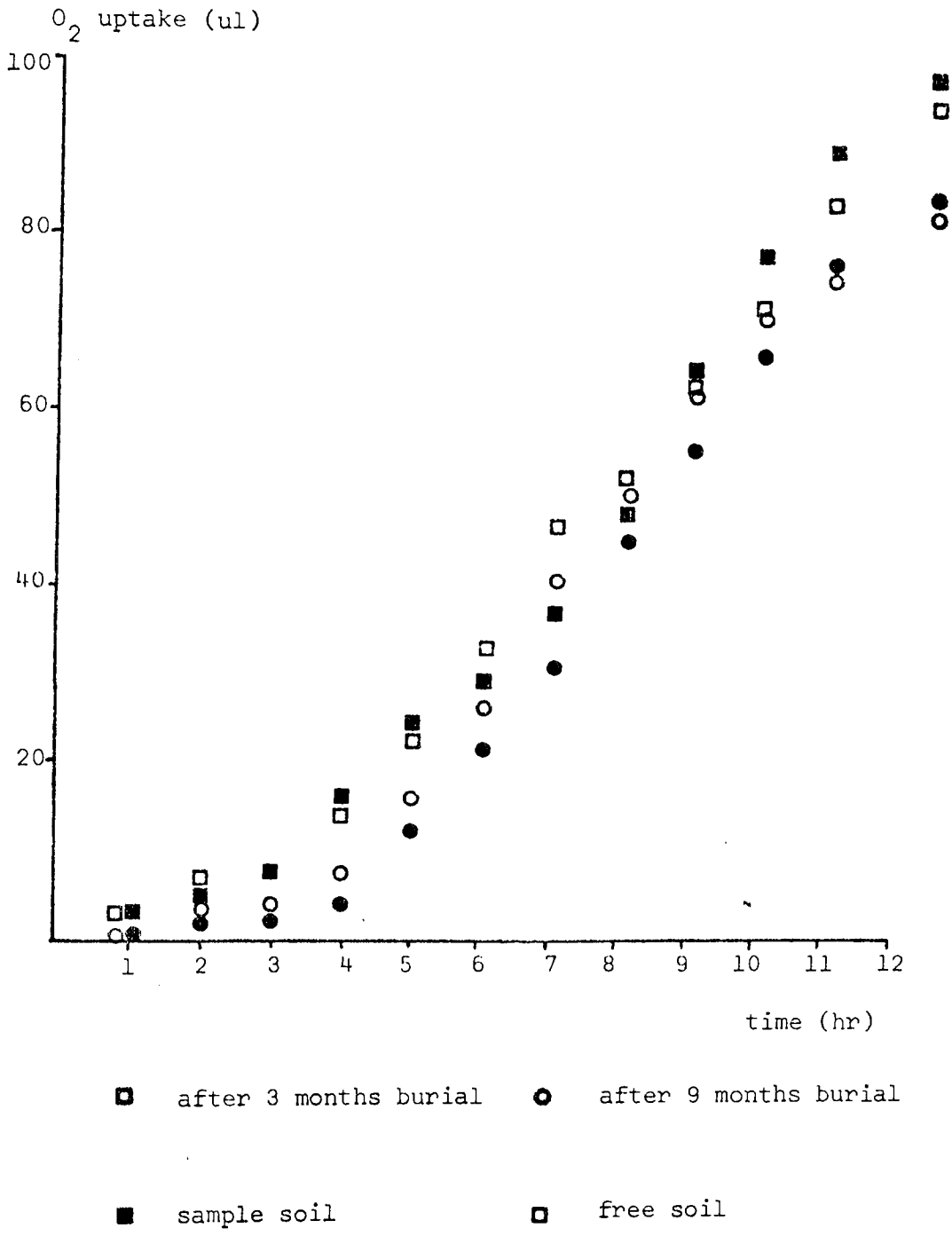
Material		Incubation period (months)					
		3 mg	6 mg	9 mg	12 mg	15 mg	18 mg
Osma CWC	bare	2.2	1.4	0	0	0.2	0
	covered	0	0	3.1	0	0	0.7
	extracted	0	0	0.1	6.1	1.3	0
	powder	1.1	1.7	0	0	1.6	0.8
Jennings CWC	b	0	5.3	0	0	7.2	0.4
	c	3.7	0	2.9	5.7	0	0.3
	e	0	2.1	0	1.7	2.4	0
	p	0	1.5	2.0	1.4	0	1.1
Undercured CWC (10 second)	b	1.6	0	2.0	0	7.2	0.8
	c	0	8.0	0	1.5	0	3.2
	e	0	0	1.1	7.9	0	3.7
	p	4.3	4.7	5.9	6.4	0	2.3
Overcured CWC (200 second)	b	5.2	1.1	0	0	4.9	0
	c	0	1.7	4.6	7.2	0	3.8
	e	6.0	0	0	6.9	0	2.9
	p	0	8.3	4.2	0	0.7	0.3
8% Zn.st CWC	b	2.1	0	3.7	5.3	4.1	3.2
	c	2.7	3.1	2.2	0.7	0	0
	e	2.2	0	1.9	2.5	3.9	1.9
	p	5.7	8.9	8.4	5.1	7.3	2.8
Preform CWC	b	6.1	0				
	c	0	0.8				
	p	0	5.9				

If experimental biomass less than control biomass - 0 registered

Table 5 Oxygen uptake after 12 hours for test materials after various periods of burial

Material		Incubation period (months)					
		3	6	9	12	15	18
Osma CWC	bare	0	0	0.2	0.4	0.15	0.5
	covered	0	0	0	0.15	0	0.2
	extracted	0	0	0.15	0	0	0
	powder	0.2	0	0.15	0	0	0.15
Jennings CWC	b	0	0	0	1.2	0	0.3
	c	0	0.6	0	0	0	0.3
	e	0.9	0	0.9	0	0.15	0.15
	p	0	0.6	0	0	0.3	0
Undercured CWC (10s)	b	0	0.5	0	0	0	0.3
	c	0	0.15	0	0.5	0	0.5
	e	1.0	0	0	0	1.2	0.15
	p	0	1.2	0	0	0	0.6
Overcured CWC (200s)	b	0	0	0	0.5	0	0.15
	c	0.6	0	0.5	0	0	0
	e	0	0	0.3	0	0.3	0
	p	0	1.5	0	0	0.5	0.6
8% Zn.st CWC	b	0	0.15	0.3	0	0	0.3
	c	0	0.8	0	0.9	0	0.6
	e	0	0	2.1	0.3	1.8	0
	p	10.7	30.1	26.8	52.1	56.1	49.8
Preform CWC	b	0	1.2				
	c	0	0				
	p	1.5	0				

Fig 2 Oxygen consumption of sample-surrounding soil and free soil at 30°C



of materials already seen in the laboratories at Uxbridge, a programme of extraction was initiated with both the leachate being further tested and the leached material. Other plastics have been tested in this way (Evans and Levisohn, 1968) but not at elevated temperatures.

3.5.2 Materials and Methods

Materials tested are shown in Figure 1 and the methods given in 2.5.7. Sheet laminate was cut on the bandsaw into pieces 20 mm x 10 mm for extraction and 10g of test material with 150 ml distilled water were used in all cases.

Leachates were tested either by liquid culture or by respirometry. For liquid culture assessment the leachate was added to an equal amount of double strength mineral salts liquid media, dispersed into conical flasks in 25 ml portions and inoculated with a fresh suspension of soil organisms and 4 mm discs of hyphal preparations of seven fungal isolates detailed in 3.2. The respirometric studies involved a similar mixing with mineral salts, portioning into Warburg flasks, inoculating with soil suspension or a sewage sludge and determining oxygen uptake.

The leached material was tested in various ways. Some were buried in soil as previously described, others were used to determine weight loss, water uptake and the possibility of bacterial impregnation.

Weight loss was determined by drying the sample in the oven at 90°C to constant weight. Water uptake

involved weighing the sample after leaching, drying at 90°C to constant weight.

Table 6 Oxygen uptake after 12 hours for various soils recovered after various periods of burial trials

Incubation period (months)	Material	
	soil from around 8% Zn.st sample	soil from a point with no sample
3	98.3µl	95.9µl
6	99.7µl	101.2µl
9	81.0µl	80.1µl
12	79.1µl	81.3µl
15	72.4µl	71.2µl
18	70.7µl	71.1µl

Bacterial impregnation was attempted with Osma and Preform laminate after extraction for 30 h. The samples were placed in a dessicator, subjected to a vacum for 30 minutes, then a bacterial suspension of known composition and number was allowed into the vessel to cover the samples and left for 60 minutes. Laminate samples were extracted, rinsed quickly in sterile distilled water, passed through the hammer mill and the powder collected in petri plates from wich pour plates were made using mutrient agar. Some samples were placed in Warburg flasks containing 2.5 ml of glucose-nutrient salts and incubated for 12 hours to assess oxygen consumption.

Leaching time (except cold water) was 30 h unless stated otherwise.

3.5.3 Results

Respirometric studies of leachates are given in the section on manometric techniques. Results for liquid culture are given in Table 7.

Weight loss and water uptake is presented in Table 8 and Figure 3, Table 9 and Figure 4 and Table 10.

All attempts to impregnate with bacteria failed, no counts being registered in agar pour plates and no uptake of oxygen being observed after 12 h.

3.5.4 Discussion

Using hot water extraction, either refluxing or soxhlet, it was possible to determine weight loss of the test samples after varying periods of leaching, the water uptake by the test sample and from there the % water in a soaked sample. The readings obtained may be

Table 7 Biomass production on 30 h reflux leachates of laminate material

Material	Biomass mg	Standard deviation of mean mg
Osma CWC	0.12	0.96
Jennings CWC	0.60	0.81
8% Zn.st. CWC	2.85	0.47
Undercured CWC (10s)	5.68	0.58
Overcured CWC (200s)	0.25	1.21
Preform CWC	0.90	0.79

Table 8

% weight gain with time for SMC Osma CWC

Extraction treatment		Time (h)														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Reflux	% wt gain	1.43	1.72	2.06	2.52		2.94				4.25	4.51	4.67	4.78	5.03	5.19
	% wt gain	1.58	1.94	2.17	2.60		3.06				4.31	4.58	4.78	4.82	5.10	5.22
Soxhlet	% wt gain	1.25	1.49	1.80	2.12		2.63				3.71	3.98	4.14	4.37	4.63	4.82
	% wt gain	0.99	1.22	1.52	1.87		2.44				3.49	3.81	3.96	4.30	4.50	4.68

Cold Water Extraction for 6 months

% H_2O uptake 1.02%

% wt. loss 0.09%

Fig 3 % weight gain with time for SMC Osma CWC

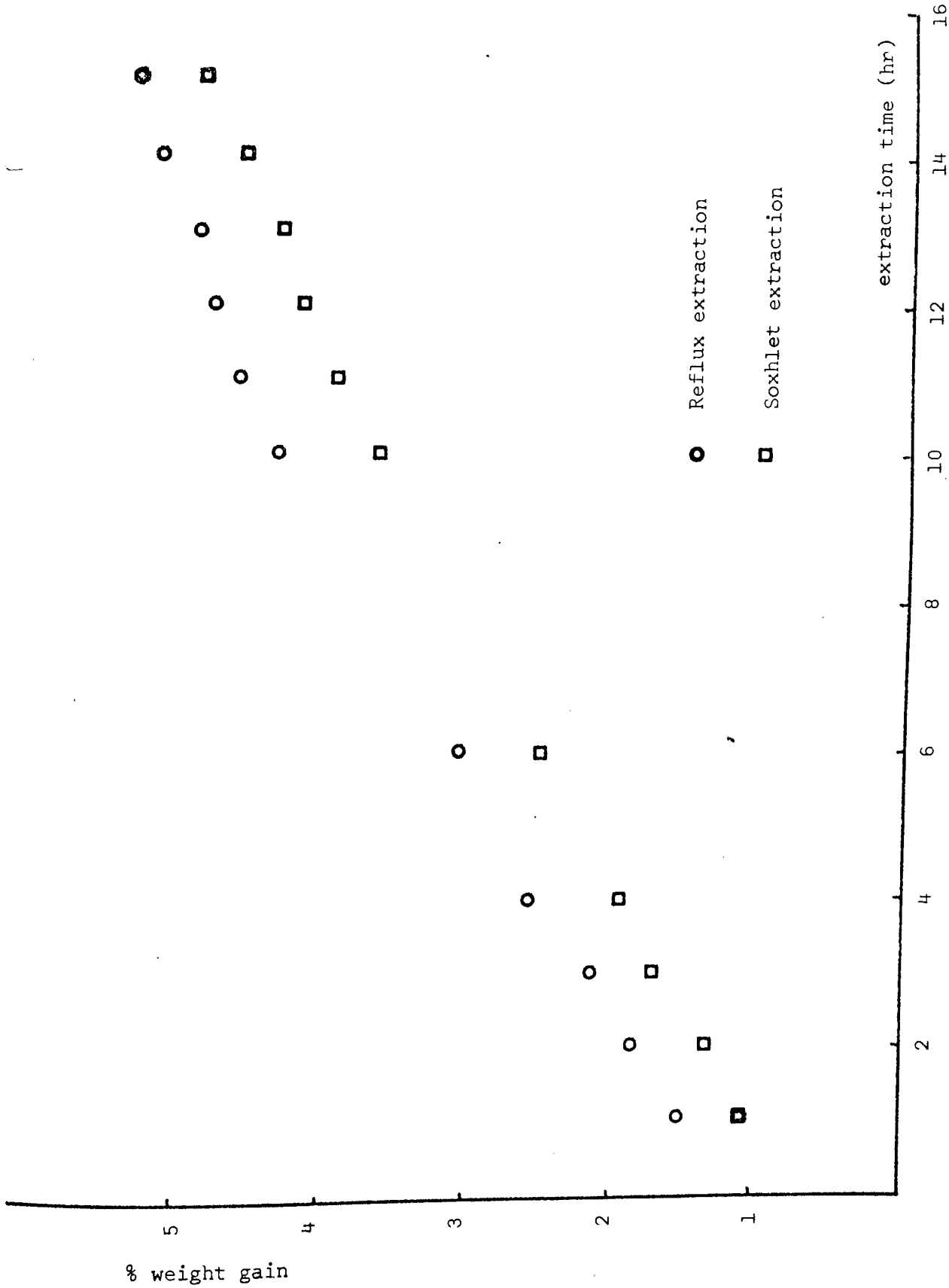


Table 9

% H_2O uptake and % wt gain for preform and SMC laminate

Material refluxed		Time (h)														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Osma CWC	% H_2O	1.51	1.84	2.12	2.56		3.00		3.71		4.28	4.55	4.73	4.80	5.07	5.21
	% wt loss	0.15	0.20	0.22	0.32		0.52		0.68		0.80	0.92	1.00	1.06	1.19	1.25
Preform CWC	% H_2O	2.15	2.20	1.89	1.70	2.34	2.52	2.84	2.65	2.70	3.00	3.33	3.20	3.13	3.11	3.26
	% wt loss	0.27	0.36	0.41	0.31	0.55	0.58	0.80	0.68	0.82	0.95	1.12	1.10	1.24	1.25	1.40

Fig 4 % water uptake & % weight loss of Preform CWC and S/C CWC

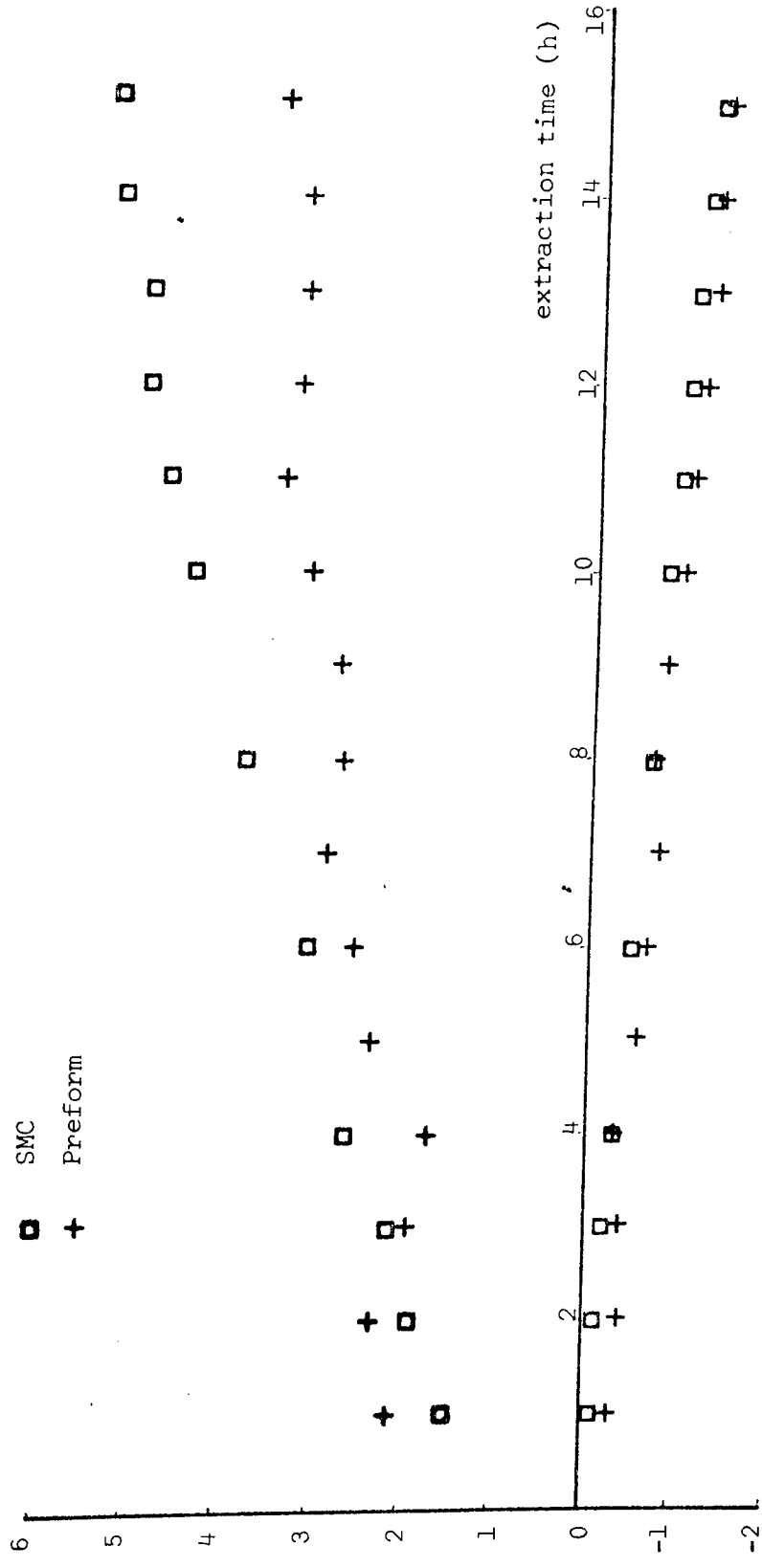


Table 10 % water uptake and % wt loss of laminates after 15 h reflux

Material		% wt of test laminate	standard deviation of mean
Osma CWC	% water	5.21	0.221
	% wt loss	1.25	0.030
Jennings CWC	% water	5.32	0.085
	% wt loss	1.28	0.042
8% Zn.st CWC	% water	5.29	0.042
	% wt loss	1.27	0.030
Undercured CWC (10s)	% water	16.73	0.20
	% wt loss	4.26	0.14
Overcured CWC (200s)	% water	4.99	0.014
	% wt loss	1.09	0.028
Preform CWC	% water	3.26	0.226
	% wt loss	1.40	0.113

compared to those found on extraction in cold water shown under Table 8. This indicates that the water uptake and weight loss after 6 months cold extraction are less than the respective values obtained after 1 hour of soxhlet or reflux extraction. From Table 7 we may see that using a Students-t test for comparison of means for Osma, Jennings, 8% and Overcured CWC, there is no significant difference between the mean values found. Undercured CWC took in more water and lost more weight than the other materials and preform, although taking in significantly less water, lost the same amount of material as the other SMC materials (no sig. diff).

The variations between preform samples was marked and comparison with SMC (except undercured) shows this. This is perhaps a reflection of the more homogenous nature of SMC laminate from area to area and between mouldings.

Biomass production on the leachates are shown in table 7. Using Students-t test to evaluate differences between means it was found that the nutritive properties of undercured laminate was significantly greater than 8% zinc stearate laminate which itself was significantly greater than the other laminates which could not be distinguished.

3.6 Manometric techniques

3.6.1 Introduction

Some of the results from respirometric studies have been shown already in the section on soil burial and referred to in the previous section on Extraction. Although respirometry has become an accepted technique in plastics testing, especially for rapid screening, since Burgess and Darby (1964, 1965) adapted a mildew screening method (Siu and Mandels, 1950; Mandels and Siu, 1950 and Siu, 1951), its use in this investigation has involved other aspects of testing procedure, especially as a detector of very small amounts of decay in test pieces.

3.6.2 Materials andMethods

Figure 1 shows the materials which underwent respirometric analysis. The six laminates were tested in ball milled and sheet (5mm x 5mm) form, the GRP components being tested in their 'raw' state, as received from the manufacturer. These compontents were the two glass size suspensions and preform binder resin.

Before the experiments were initiated the flasks and manometers were assigned in pairs andthe manometer constant for each pair calculated according to the equation below:-

$$K = \frac{V_g\ (^{273}/_T) + Vf\alpha}{Po} \qquad (1)$$

where V_g = volume of gas in reaction flask and manometer in μl.

V_f = volume of liquid placed into reaction flask, in µl

T = temperature in reaction system in ^oK

= gas absorption coefficient in ml gas/ml liquid at T^oK

Po = standard pressure = 760 mm Hg

= 10,000 mm Brodie column

This constant is used in the calculation of the volume of gas absorbed according to the equation:-

$$x = K.h \qquad \text{(2)}$$

where x = gas envolved or absorbed, in ul referred to standard conditions (760 mm Hg, 273^oK)

K = manometer constant

h = manometric difference = pressure difference in mm as read on manometer after barometric correction.

Where the sample was a liquid it was accurately added to the reaction flask using a pipette. With sheet laminate or powder a weighed amount was added, the density having been determined separately by immersion, and the volume for Vf in equation (1) above calculated.

Inoculation was either by soil organisms or by a suspension of domestic sewage activated sludge. The soil inoculum was produced from the mixing of equal amounts of 48 h soil cultures in E & P cellulose, E & P glucose, Nutrient broths and tap water, just prior to use.

3.6.3 Results

The 'glucose' flask, added toeach run to check initial inoculum viability, usually evolved approximately

95-110 µl in the first hour and was subsequently ignored.

Table 11 gives the amount of oxygen consumed in ul after 24 h incubation, the figure for soil being the mean of five flasks ± standard deviation of the means. Only two runs were made using the sewage inoculum and values obtained are indicated in the relevant column to its control. Table 12 gives the conversion of these results expressed as percentage of control value.

3.6.4 Discussion

From the results of soil inoculum oxygen uptake, using students-t test for comparison of two means of small populations, it is possible to detect significant (95% level) differences between test materials, and between test materials and the endogenous soil inoculum respiration. From this analysis we find that 8% zinc stearate leachate and undercured ball-milled powder and leachate are the only laminate derived test materials to differ significantly from the control in respiration. 8% zinc stearate leachate and undercured powder cannot be distinguished but are both significantly different from the undercured leachate. These materials showed a greater repiration than the control. However, three materials showed a slower respiration rate than the control, the two glass sizes and preform resin binder. It would seem that these materials or a component(s) of these materials are inhibiting microbial activity. These patterns were also obtained with sewage inoculum.

3.7 BFP and NWC testing

3.7.1 Introduction

Table 11 Amount of oxygen consumed, in μg by organisms incubated for 24 h with the test material

Materials		Soil	Sewage	
Osma CWC	sheet	35.46/7.74	224.3	
	powder	39.18/7.07	357.1	
	leachate	32.48/5.79	345.0	
Jennings CWC	s	38.24/7.48	348.5	
	p	37.40/8.31	301.2	
	l	34.16/7.31	351.6	
8% Zn.st CWC	s	25.08/7.94	343.7	
	p	30.14/7.74	391.6	
	l	50.26/5.34	341.9	
Undercured CWC (10 second)	s	40.92/7.68	394.1	
	p	61.04/8.31		7322
	l	104.86/2.35		7674
Overcured CWC (200 second)	s	30.78/4.76		240.2
	p	25.46/6.78		267.1
	l	22.44/7.02		247.7
Preform CWC	s	35.50/4.74		223.1
	p	34.72/6.26		304.0
	l	35.30/1.94		251.8
Glass size	FGRE 11	20.34/6.03		207.4
	FGRE 12	3.78/7.52		177.7
Preform binder resin	A 2603	-10.08/6.96		163.2
CONTROL		33.02/4.74	337.2	263.1

Table 12 Amount of oxygen consumed by organisms incubated for 24 h with test materials expressed as a % of the control

Material		Sewage
Osma	s p l	87.28 105.90 102.31
Jennings	s p l	103.35 89.32 104.27
8% Zn.stearate	s p l	101.93 116.13 101.39
Undercured (10 second)	s p l	116.87 >122.39 >256.18
Overcured (200 second)	s p l	91.30 101.52 94.15
Preform	s p l	84.80 115.55 95.71
Glass size	FGRE 11 FGRE 12	78.83 67.54
Preform binder resin	A 2603	62.03
CONTROL		100

Following the 'publication' of the testing method for materials in contact with potable water by the Metropolitan Water Board Microbiology Testing Laboratory, some testing of this type was carried out on standard BTR laminates and some experimental resin system laminate. This test method was eventually published by the National Water Council contained within 'Acceptance of Water Fittings'.

3.7.2 Materials and Methods

The materials tested were cut into pieces 3" x 1" and two of these samples pieces used in1 litre glass beakers. Controls were similar beakers containing two clean slides. With Osma CWC two pieces had all cut edges covered with araldite epoxy resin to assess any change in behaviour compared to normal test pieces. As a further control a beaker was set up containing two araldite strips, cast on waxed glass and subsequently cleaned, as test material.

3.7.3 Results

Using a mixed sewage/soil inoculum it was found that three consecutive counts of less than ten times control counts was achieved in all counting categories by all materials. Coliforms and fluorescent pseudomonads were eliminated within three weeks and the other counts held at a pass level, in all cases less than six weeks.

No evidence was found of any support of microbial growth by any material whether araldite coated on exposed edges or not.

The above tests when repeated on tank and40-6000

panel with the Pseudomonas aeruginosa count in place of fluorescent pseudomonads gave similar results, the time taken for three consecutive counts (37°C) being at seven weeks to terminate the procedure.

3.8 Uxbridge installation of preform tank from Worthing

3.8.1 Method

The tank was connected to a water supply and controlling ball value and emptiedthree times/week.

Observations were made on the growth at monthly intervals.

3.8.2 Results

The growth lost its 'fluffy' nature within 4 weeks and gradually tended to lose its dark brown colour, lightening to a rusty brown, and its apparent 'depth of film', and disappearing in places. After 12 months it was disconnected and taken to the BIC for the SEM work.

3.9 Summary of microbial testing results and Discussion

Although discussion of the results of individual sections has been briefly attempted, a full summary of all the results in this chapter is necessary.

The microbial testing has moved from agar testing with visual, and necessarily subjective, assessment through liquid testing with the beginnings of more accurate assessment to respirometric techniques, combined with extraction experiments, to give accurate objective assessment of biodegradation. Although the evaluation techniques have become more sophisticated this study has not shown any evidence to suggest that normal

production laminate can support growth of micro-organisms. It is also worth stressing that apart from the visual assessment agar testing, laminate in sheet form has not been shown to be degraded by liquid testing or by respirometry of the sample itself. Leachates have been shown to support growth using sheet laminate as the extracted material, but again it was only undercured, highly undercured, laminate and 8% zinc stearate sheet which was found to support growth.

This evidence is supported by the 'BPF and NWC tests) carried out in this laboratory which showed a satisfactory behaviour for all laminates tested and by the BPF testing described separately in 7, in which the laminates tested could not be differentiated by the test and where all three were failed on 'surface growth' which seemed to be a factor brought about solely by the inoculum since glass control beakers also suffered similarly.

Water uptake and weight loss experiments showed the marked difference in behaviour under testing between undercured laminate and all others. SMC laminates, with that exception, had performed similarly to laminates tested at Uxbridge (Mallorie, personal communication), and the characteristics of laminates may be summarised as follows:-

Mean % water uptake for SMC (normal or long cure)	5.20
Mean % water uptake for undercured SMC	16.73
Mean % water uptake for preform	3.26

Mean % wt loss for undercured SMC 4.26

Mean % wt loss SMC and preform 1.26

all figures after 15 h reflux in water

Following reflux extraction, although it was found possible to impregnate the blocks with water using a vacuum/soak procedure, it was not possible to detect bacteria in the sample when a bacterial solution replaced the water. From this it may be postulated that the points of entry for water into the laminate are too small to allow entry by bacterial cells, so effectively ruling out colonisation of the interior matrix.

Six materials were found to inhibit activity of micro-organisms:-

1. the two glass sizes
2. the preform binder resin
3. styrene
4. maglite D
5. 29 B 50

Two materials consistently support microbial activity, these are zinc stearate and RCR 2. However, the incorporation at manufacturing levels into laminate does not make the laminate susceptible to microbial attack. It is only when zinc stearate is added at 8% to the mix that the laminate will support growth, and then only after severe leaching, compared to service conditions, or severe degradation of the laminate structure by ball-milling.

CHAPTER IV

CHAPTER IV

4 THE LONG TERM TESTING OF FABRICATED CISTERNS UNDER CONDITIONS SIMULATING IN-SERVICE CONDITIONS

4.1 Introduction

When BTR took over the manufacture of GRP cisterns from OSMA they acquired moulds, patents, GRP formulations and a marketing outlet. Along with this went another set of commodities:- the foundations of a market, customer acceptance of a known product of accepted quality and perhaps above all, the belief that the product was suited to its application. When the MWB, on behalf of the NWC, issued a notification of failure for the GRP cistern it was not just the commercial viability of the company that was challenged, it was also this belief in the suitability of the material. BTR had not initiated research into the long-term biological behaviour of their cisterns with a conventional test programme but a test programme of a much larger scale had been under way for many years. This was the marketing of the product and results were obtained daily by the company. These were that apart from the Worthing area the preform cistern had no in-service complaints against its name, as was also the case with SMC cisterns. The scale of the programme is enormous:- over one million cisterns in use; biological failures, excluding Worthing, nil.

It was against this background that the project was originated and very quickly a pattern of research developed, designed to produce results which might be used as 'ammunition' in the 'fight' against the NWC

test programme. Direct involvment with the formulation of a test programme for all plastics which was taking place within the BPF expert working group was the first line of effort. The second was the initiaton, development and assessment of other forms of microbiological testing. The third direction of effort was into investigations of the only customer complaint of in-service failure, the Worthing area, and the fourth was in the direction of assessment of in-service long-term biological characteristics of the cisterns.

The overall form of the research in this direction was greatly influenced by considerations of commercial strategy. Perhaps the simplest scheme for this part of the project was for the Water Boards to be contacted, their help elicited, and direct surveys made of all cisterns in designated areas. Another suggestion was to publicise the problem, research all feedback, and establish a test programme with co-operative parties. However, scare and scandal that such programmes may lead to was an over-riding factor, even down to not even limiting the publicity exercise to BTR group personnel.

Also of importance was finance. To establish a single cistern at a testing site is not cheap. Besides the cost to BTR of the cistern are other expenses, namely the high cost of labour in plumbing-in the tank to water inlet and outlet systems. It was estimated that the average cost of each operational tank would be £20. In practice it was just over £30.

With these considerations as parameters a test scheme evoloved, and in final shape was to establish a multi-tank test rig at the BIC, to assess behaviour of different tanks to one type of water, and a small number of single tank units dispersed around the county in order to try and compare the effects of different waters on the tank surface growth, if any.

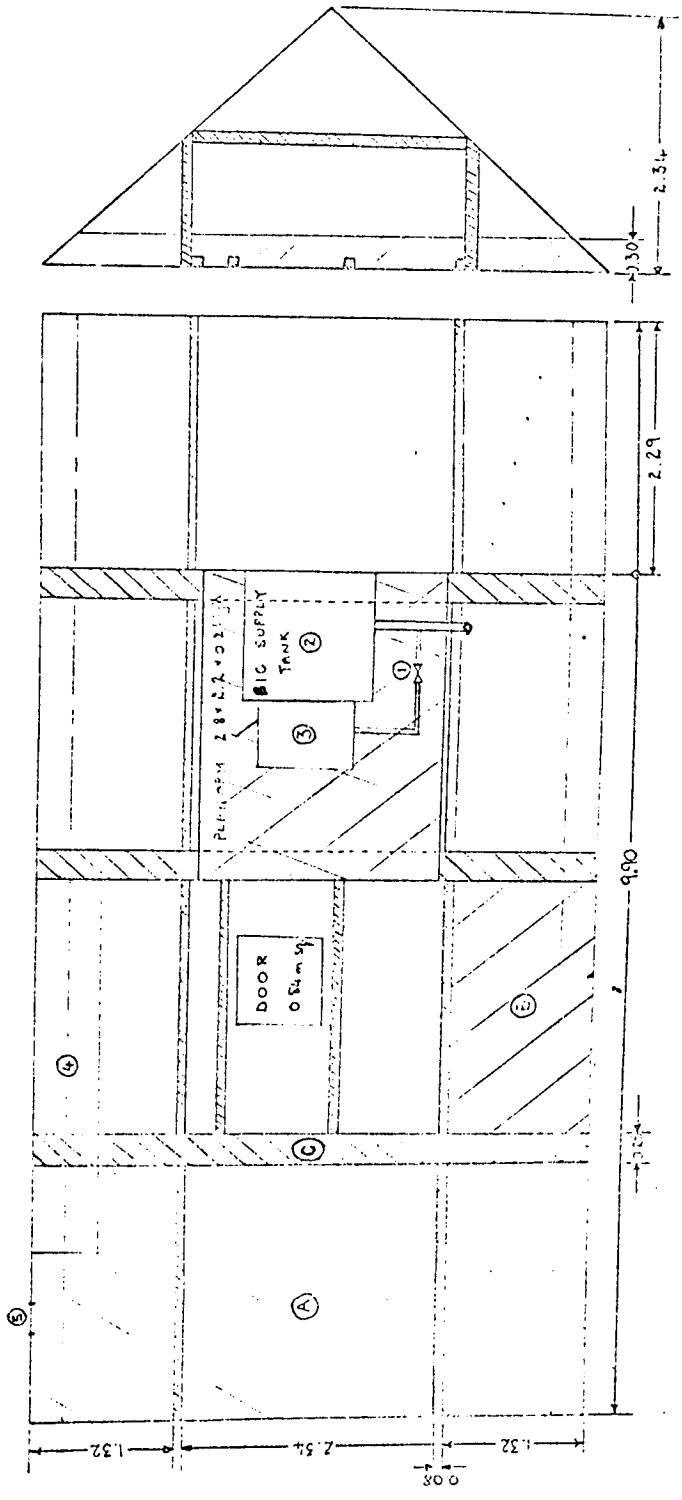
4.2 Installation of the test tanks at the test sites

The multi-tank test rig has been erected in the loft space of the BIC. The space available is shown in Figure 5. Twenty tanks were planned for area A. Three concerete beams run across the loft and between two of them a platform had been built to take the cold water supply cistern (2) and the central heating header tank (3). Mains water is delivered through a 50mm dia pipe to the cold water tank and the supply for the test rig taken just before the stopcock (1).

Contractors were used to install lighting and a ladder to the loft space. Further work involved the construction of platforms to support the test tank using wooden beams set into the end wall and resting on the concrete beam (c), with chipboard placed over to give a flat base. This, and the laying of cold water mains up to the tanks, was contracted out.

Work involved in finishing the installation involved the laying of an electricity supply to the loft for the time clock, the plumbing of the outlet and over-flow systems, and the connection of the time clock to

Figure 5



PLAN OF BIC LOFT SPACE

the solenoid valves. A schemetic representation of the system is shown in Figure 6. Both outlet and overflow systems are taken through guttering to join the BIC rainwater system at point 5.

Each tank was provided with a solenoid operated valve on the outlet side which was switched on for two periods of 30 minutes each every 24 hours. The tanks were regulated on the outlet side to consume daily 150% of their effective water capacity. Since the outlet systems of the tanks are not interconnected due to the 'open' nature of the plumbing system and the use of non-return valves it was hoped to cut out cross-contamination between tanks by other than aerial routes.

The sites for the individual tank units were BTR group premises at four locations. Tanks and ballcocks were delivered to the site and the plumbing in to a system done by the maintenace department responible for each site. All tanks were plumbed in using traditional materials and techniques. The placement of tanks was left to the groups concerned, the only criteria being that the cistern should get changes of water and not remain static. The four sites which became operational were:-

BTR Reinforced Plastics Ltd., Uxbridge

BTR Silvertown Factory, Burton-on-Trent

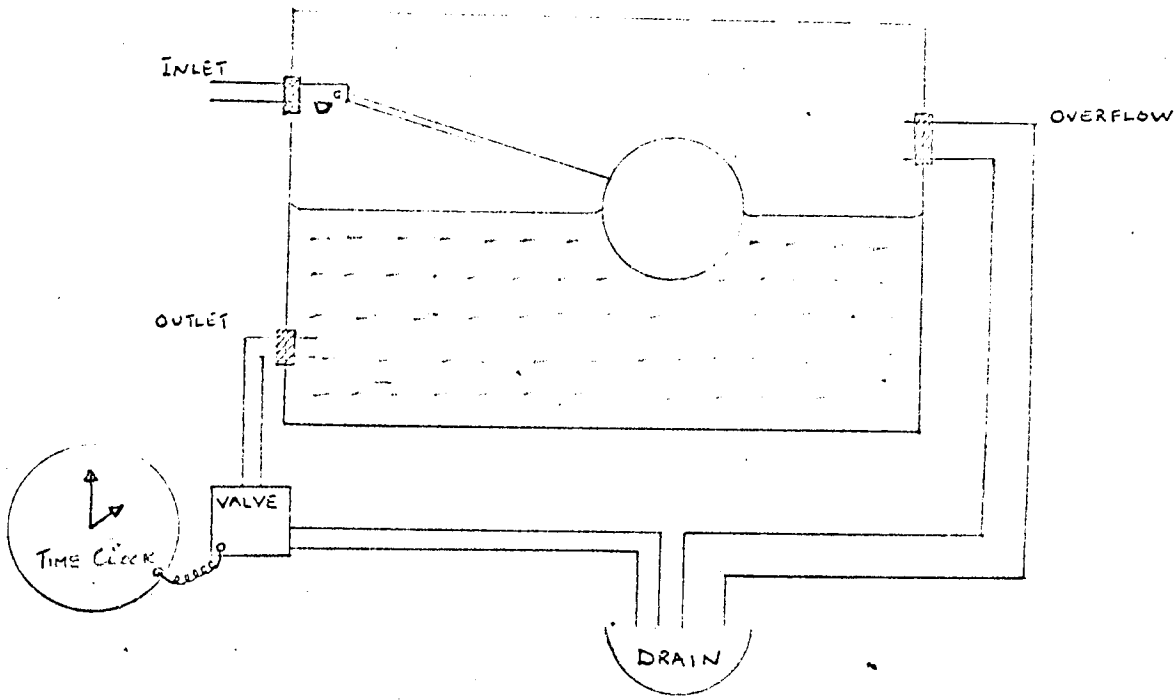
Hiflex International, Salisbury

BTR Hose Ltd., Faringtон

It may be consdered that another site is

38 Cranleigh Road, Worthing

Figure 6 Schematic representation of tank system



4.3 Materials and Methods

4.3.1 Cisterns

All tanks used at single tank unit sites were BTR GRP tanks as supplied to Osma Ltd., complete with lids. They were moulded from stock SMC strictly according to the specification data as regards cure time and temperature. All sites were asked to clean the tank of any cutting dust, pipeseal etc. before filling with water.

The tanks used in the multi-tank rig at the BIC were as follows

1. Two galvanised steel cisterns (18" x 12" x 12") one with lid
2. Two polypropylene cisterns (18"x12"x12") one with lid
3. Two polythene cisterns (18"x12"x12") one with lid
4. Two polythene cisterns (18" dia x 16" dia x 10") one with lid
5. Four BTR 'Osma' cisterns (19"x14"x13") cure time 55 seconds, two with lids
6. Four BTR 'Osma' cisterns - cure time 80 seconds, two with lids
7. Four BTR 'Osma' cisterns - cure time 300 seconds, two with lids

All were cleaned of debris before filled with water.

4.3.2 Sampling procedures

Sterilised 200 ml bottles were sent from the BIC to the single-tank test sites through the GPO.

An operator at the site removed the lid from the tank and noted any visible signs of discolouration or growth. The bottle was rinsed in tap water, then held under the tank water surface and the cap removed. After filling the cap was replaced, the bottle brought out of the water, dried, repackaged and sent bact to the BIC. All operators were asked towash their hands and thoroughly rinse them immediately prior to the sampling procedure.

Sampling of the multi-tank rig was from the outlet of the tanks in question, the samples being collected in 200 ml bottles similar to those sent out to the BIC sites as detailed previously. Bottles were placed under each outlet, the solenoid valves switched on by manual over-ride of their time clock, and switched off when all the bottles were full. The sample bottles were capped and taken to the laboratory for examination.

4.3.3 Microbiological testing procedures

All samples were tested by the methods detailed in 2.11 for fungal loading and bacterial colony counts at 22°C and 37°C. Those from the multi-tank test rig were also tested for fluorescent pseudomonad counts and coliform counts as detailed in 2.11. Cell counts were also made using a counting chamber examined under the light microscope.

From the millipore filters used for evaluating fungal loading, individual colonies were picked off and cultured on 2% malt agar for identification. The plates were incubated at 25°C for 7 days without illumination, examined and indentified. If growth was not sufficient

to allow indentification they were incubated further under similar conditions.

4.4 Results

The multi-tank test was sampled for 40 weeks and on only three occasions were fluorescent pseudomonads recorded, twice in galvanised steel cistern and once in a polythene cistern. On all occasions the count was zero on the next date of sampling.

On no occasion was a coliform count recorded from any cistern in the test rig.

The mean and standard deviation for 22°C, 37°C and fungal counts is given in Table 13 for the period of observation.

Table 13 Multi-tank microbiological counts

Shown on page 87

Using students t-test for comparison of means of two small samples it is possible to analyse significant differences. For the means above, $n>30$, therefore we use the comparison of means of two large samples.

Table 13 Multi-tank rig microbiological counts

Tank material		22°C ml	37°C ml	Fungi 100 ml
Undercured GRP (55 s)	Lid	* 223214/41328	10274/2094	* 1194/317
	No lid	*204621/45378	* 8997/2786	1076/472
Normal GRP (80 s)	Lid	93026/44157	7630/1911	1034/490
	No lid	*164633/56078	7842/3271	942/399
Overcured GRP (300 s)	Lid	*137451/29007	8006/2841	923/243
	No lid	*136906/31478	7542/2227	971/209
Galvanised steel	Lid	*167237/43781	9237/5612	*1217/236
	No lid	132614/39919	*9178/4017	*1609/627
Polypropylene	Lid	*178410/29193	*11364/3421	872/507
	No lid	*192431/45016	*11621/4709	901/362
Polyethylene	Lid	*183214/43927	*9841/3780	*1108/423
	No lid	*192331/37219	7762/3943	1064/553
Mains water supply		112710/63195	7300/3240	855/633

* differs from mains water supply mean

$$\Rightarrow \quad d = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{S1^2}{n_1} + \frac{S2^2}{n_2}}}$$

where: for two means to be equal at $p = 0.05$

$$d \leqslant 1.960$$

Figure 7 shows the counts over the period for the standard manufacturing cistern with lid in the test rig.

The comparative counts from the single-tank units are shown in Table 14.

Table 14 Single-tank units microbiological counts

Tank Site	22°C	37°C	Fungi
Salisbury	131000/50923	8647/3231	1007/2372
Leyland	167250/48736	9768/4013	1489/1992
Uxbridge	173500/41236	9126/4127	924/4897
Burton-on-Trent	121500/41300	10061/2978	1516/2792
Worthing	55591/31442	7489/3868	2025/4846

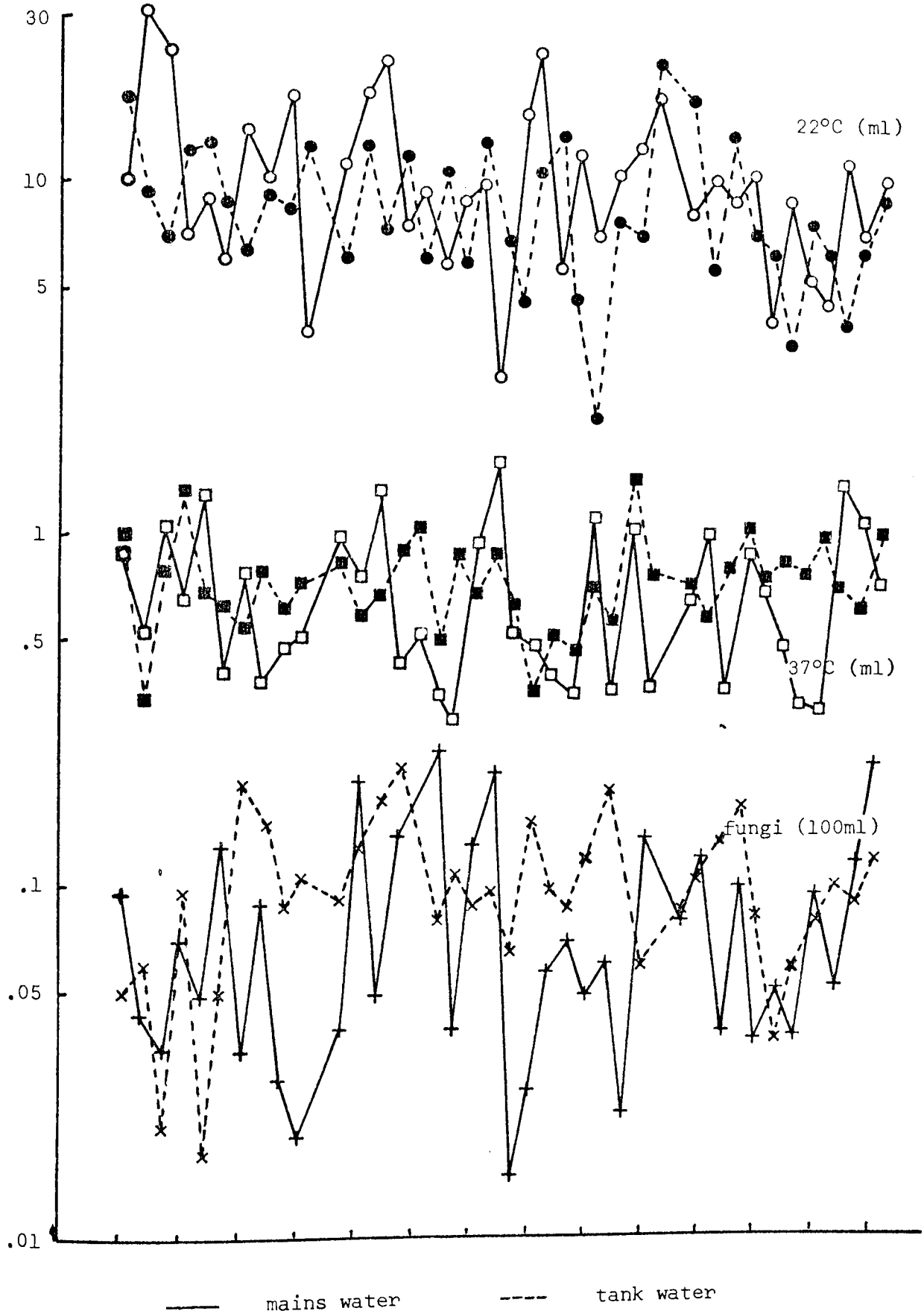
4.5 Discussion

The microbiological levels in the water contained in cisterns has been looked at over a 40 week period with testing once per week.

The results suggest that the water is consistent with its specification on coliforms and is also free of fluorescent pseudomonad contamination. The levels of the other group of organisms tested for varied quite markedly but even so it was possible to determine where

Fig 7 Organisms present in Osma tank at various sampling times

(x 10000)



the populations found in tanks were significantly different to those in the supplied water. This may be summarised as follows:

1. 22°C - 10 tank types differed out of 12
2. 37°C - 6 out of 12 differed
3. Fungi - 4 out of 12 differed

and also by:-

1. Undercured GRP - 5 cases of 6 differed
2. Steel, polypropylene andpolythene - 4 our of 6
3. Overcured GRP - 2 out of 6
4. Normal GRP - 1 out of 6

In no section does the experimental mean approach 10 x mains water mean (re BPF and NWC assessment) and in fact the greatest difference was just under 100% of the supply water mean. (Undercured with lid - 22°C)

The information from the single tank units suggest that on a direct comparison Worthing water supplied to the tank is low in 22°C organisms (50,000 ml compared to approximately 140,000 ml) and approximately alike in 37°C and fungal populations. However the other counts are approximately similar and cannot be distinguished by analysing for differences between the means for the different areas. No incident of microbial growth has been reported form any of the sites.

It is suggested that the single tank units are kept in operation whereever possible and periodic checks made on the visual appearance of the cistern, a system requiring very little time, almost negligible costs

since the capital outlay was in setting the installations up, and will continue to yield results concerning the behaviour of BTR cisterns.

CHAPTER V

5. [illegible] LAMINATES

5.1

[illegible] work described in this section was performed [illegible] periods during the project to investigate [illegible] speculations as to the surface morphology [illegible] moulded laminates.

The first attempt to utilise the scanning electron microscope (SEM) came soon after initial microbial testing (3.2) had confirmed the ability of zinc stearate to act as nutrient for micro-organisms which have been described earlier (Holmes, 1973). A series of special laminates was examined to determine the effect on surface morphology of variations in cure temperature, mix composition and the presence or absence of zinc stearate. Attempts were made to establish whether preferential accumulation of zinc stearate at the moulded surface was occurring, a suggestion had been put forward to account for its mechanism as being a mould release agent.

In the second instance DMO and preform [illegible] were examined in order to compare the surface [illegible] of these moulded laminates. This study [illegible] the different behaviours of DMO and p[illegible] led in the warping area (described [illegible] the observations of water uptake [illegible] and the concomitant dimensional [illegible] The samples were chosen to [illegible] what changes in surface [illegible]

5. Scanning Electron Microscope studies on ...

La...

5.1

... in this section was performed ... method during the project to investigate ... speculations as to the surface morphology ... moulded laminates.

The first attempt to utilise the scanning electron microscope (SEM) came soon after initial microbial testing (3.2) and concerned the ability of zinc stearate to act as nutrient for micro-organisms which have been described earlier (Lemane, 1973). A series of special laminates was examined to determine the effect on surface morphology of variations in cure temperature, mix composition and the presence or absence of zinc stearate. Attempts were made to establish whether preferential accumulation of zinc stearate at the moulded surface was occurring, a suggestion had been put forward to account for its mechanism as being a mould release agent.

In the second instance SMC and preform... were examined in order to compare the surface... of these moulded laminates. This study ... the different behaviours of SMC and pr... led in the Worthing area (described ... the observations of water uptake ... and the concurrent dimensional ... The samples were chosen to ... what changes in surface ...

5. SCANNING ELECTRON MICROPCOPE STUDIES OF GRP LAMINATE SURFACES

5.1 Introduction

The work presented in this section was performed at two distinct periods during the project to investigate separate speculations as to the surface morphology of moulded laminates.

The first attempt to utilise the scanning electron micropscope (SEM) came soon after inital microbial testing (3.2) had confirmed the ability of zinc stearate to act as nutrient for micro-organisms which have been described earlier (McShane, 1973). A series of special laminates was examined to determine the effect on surface morphology of variations in cure temperature, mix composition and the presence or absenceof zinc stearate. Attempts were made to establish whether preferential accumulation of zinc stearate at the moulded surface was occuring, as this suggestion had been put forward to account for its mechanism in being a mould release agent.

In the second instance SMC and preform surfaces were examined in order to compare the surface appearance of these moulded laminates. This study followed firstly, the different behaviours of SMC and preform tanks installed in the Worthing area (described in 6), and secondly, the observations of water uptake of laminates on soaking and the concurrent dimensional changes (described in 3.5). The samples were chosen in order to attempt to establish what changes in surface appearance occur on exposure to

water and to compare what changes in surface appearance occur on exposure to water and to compare these with the surface appearance of samples exposed to the action of a fungal organism overgrowing it.

The studies were performed at the Centre for Materials Science, Birmingham University.

5.2 Materials and Methods

The laminates examined at the first session of SEM work were all based on formulation SC 193 and produced by Uxbridge. The samples were cured between 'Mylor' polyester film at either 135°C or 155°C and did not contain glass. Details of the mixes are as follows:-

Mix			
1	Standard SC 193	no pigment	
2	SC 193	"	no polythene
3	SC 193	"	" no zinc stearate
5	SC 193	"	" 5% zinc stearate
6	SC 193	"	" 7½% zinc stearate

After moulding samples of each mix were exposed to tetrachloroethylene at 65°C for 2 or 10 minutes. This was to etch from the surface zinc stearate.

The second set of samples examined with the SEM were based on three laminates and were as follows:-

Sample 1 cold water cistern SMC in manufactured condition

2 CWC SMC after exposure to water

3 CWC preform in manufactued condition

4 CWC preform after exposure to water

5 Worthing preform CWC above water line

6 Worthing preform CWC below water line- surface cleaned

7 Worthing preform CWC below water line - no cleaning

All samples were cut from the panels of cold water cisterns, the cisterns referred to as Worthing preform being the tank removed from a household in the area as detailed in 6.

All samples were cut to a size 10 mm square and mounted on SEM stubs. Each was then coated with approximately 500 Å gold to render the surfaceconductive, cleaned in an ultrasonic bath (if stored) and placed in the chamber of the microscope. The surfaces were examined and photographs taken of areas of interest. Ortec analysis of the surface was also undertaken at certain points in the examination to show the elemental composition of the surface.

5.3 Results

It can be seen from the plates presented that there are no significant differences between the unetched surfaces of Mixes 1, 2 and 3 (Plates 1 - 6), and variations in cure temperature have no apparent effect on surface morphology either (Plates 3 - 6). Likewise etching in tetrachloroethylene at 65°C for either 2 or 10 minutes produces no significant change in the surface of Mix 1 (Plates 1, 7 and 8), although mixes 5 and 6 which are rich in zinc stearate are severely etched (Plates 9 -12).

From the second series of plates it can be seen that the effect of water contact has, in all samples, been to show surface deterioration. Baring of fibres close to the surface occurs and pitting of the resin-rich areas. All the samples suffered from contamination by debris, probably from the 'cutting out' procedure and indicates insufficient cleaning prior to gold coating. Atomic number contrast (back scatter electron mode) showed no real difference between the surface and the debris supporting the idea that the debris was from the cutting operation.

Ortec analysis of samples is summarised in Table 15.

Table 15 Ortec analysis of laminate samples and X-ray mapping

Elements	1		2	3	4	5	7		
	surface	debris				debris	resin	scale	organic growth
Calcium	2434	2461	2732	Lower than as (3) SMC		Concentrations found in debris	985	1282	very low
Silica	Shows as a peak if glass strand in analysed area						1418	395	
Titanium	90	197	230	slight	slight	DISTINCT (uniform spread) 70	14	98	-
Zinc	-	133	-	slight	slight	-	-	-	-
Sodium	-	-	-	-	-	-	trace	trace	-
Potassium	-	-	-	-	-	-	90	-	-
Iron	-	-	-	-	-	-	-	-	very heavy

slight < 20
trace not countable

A summary of the results obtained for the seven samples is given and reference made to plates having a special significance to the points outlined.

Sample 1 Glass fibres markedly visible beneath surface i.e. very close to surface (Plate 13)
some depressions but no pits (plate 14)
no surface zinc
debris does contain zinc

Sample 2 'eruption' of glass fibres - exposure of interior matrix
pitting - no zinc in pits (Plates 15, 16)

Sample 3 glass fibres not as close to surface as in sample 1
rougher surface than SMC - many depressions (Plate 17)
lower calcium and titanium than SMC
trace zinc on surface.

Sample 4 'eruption' of glass fibres (Plate 18)
some pitting

Sample 5 etching and channeling of surface (Plates 19, 20)
debris titanium levels same as surface
calcium concentrations in debris

Sample 6 eruption of fibres similar to sample 2 (Plate 21)
some pitting

Sample 7 surface showing cracking, dissimilar to sample 2 in that often cracking and glass fibre direction are opposed at 90°C (Plates 22,23,24)

some evidence of existince of organic growth on surface (Plates 25, 26, 27).

5.4 Discussion

The information gained from the SEM study has been in two complementary forms, firstly the visual presentation of the topographical features of laminate surfaces and secondly, from adaptation to the mode of operation of the election beam recording, elemental analysis of the samples' surfaces.

From the first session it may be seen that variation in cure temperature (135°C or 155°C) had no discernable effect on surface morphology of the laminate. This helps to eliminate one variable from the manufacturing process in that although mould face temperatures are not checked between each pressing operation it is unlikely that they would vary outside the range 135-155°C. From this it would appear that if the cistern submitted for NWC approval which failed, thus eventually setting up this study, did so because it was incorrectly processed in some way, it is unlikely that the peculiar fault in processing was of the nature of cure temperature.

Also seen to have no significant effect was the depletion of polythene and zinc stearate from the mix and the addition of abnormally high concentrations of zinc stearate. The unetched surfaces of all mixes showed a very similar appearance. However, etching of laminates rich in zinc steatate effected severe pitting of the surface, in contrast to the appearance of surfaces of laminates with either normal levels of zinc stearate

(about 1%) or a complete absence of zinc stearate. The etched zinc stearate rich surfaces also contrast with the appearance of both preform GRP and SMC GRP after contact with water only and with water and overgrowing organism. This suggests that water alone does not extract zinc stearate from the surface, so providing organisms with a nutrient source and that the overgrowing organism found in Worthing has not derived its nutrition by some mechanism of stearate extraction either.

The second series of studies, apart from providing supporting evidence for the factual appearance of the reference surfaces also serve to illuminate some differences between preform GRP and SMC GRP. It has been shown that points of similarity exist, in that the apparent effect of water has been to show similar surface deterioration, that is:-

1. 'eruption' of glass fibres
2. pitting of the resin-rich surface

But, differences have also been shown:-

1. the newly moulded surface of preform GRP is rougher than SMC surface
2. direction of cracking differs. Preform is at 90°C to fibre direction, SMC is usually along a fibre path

These findings are complemented by differences found in the composition of the surfaces. Preform GRP has a lower concentration of calcium overall, a lower surface concentration of titanium and very low amounts of zinc. The findings on these are compatible with an

overall lesser concentration of fillers in preform GRP with a corresponding greater proportion of resin and glass. However, one of the most interesting recordings is that for zinc on sample 1. This shows zinc in the debris, which is thought to be cutting dust and so likely to have the same composition as the bulk structure, and an absence of zinc from the surface. It would seem that if zinc stearate is preferentially accumulated at the laminate surface it is orientated with the zinc in the bulk structure and the stearate group on the surface. This is the orientation that theoretical predictions based on lowest free energy states for surfaces would suggest (P. Grey, personal communication).

What then of the radical differences in the behaviour of the two types of tank in the Worthing area? It would not seem to be a difference in surface roughness that is in question. Both types of laminate show similar surface disruption with the creation of irregular, rough surfaces and deep crevices and channels. This is illustrated in Plate 15 where the long channel created by the complete removal of a glass fibre is in excess of 1100 μ in length and approximately 9 μ wide and deep. It can be argued that the preform surface as moulded is rough and irregular so providing the colonisers with something to hang on to, but examination of SMC GRP after contact with water shows the creation of a more severely disrupted surface. The rougher moulded surface of preform may be a help in establishing initial colonisation rapidly

but where is the similar but lagging behaviour of the SMC surface?

However, there is an aspect of surface change which is most interesting. This is the channeling observed on preform surface above the water line. (Plates 19 and 20). On plate 19 there is evidence of two channels coming together and running along the surface. The channeling would appear to be caused by removal of material not deposition of material on surrounding areas. The whole picture assumes more significance when correctly orientated to the water line. When this is done it is seen that the two separate channels are running together in the same way that droplets of condensation run down a surface, leaving tracks that get wider as the paths intersect and the flows join. Subsequent visual examination of the cistern that the sample was removed from reveals that at the water line the whole surface is at the same level as the channeling. This suggests solubilisation of a surface layer of the preform type, and the possiblility that it is this layer that is nutrient to the Phialophora species. Thus on samples 4, 6 and 7 we may not be seeing the effect of water on the moulded surface but the topography of a secondary surface exposed by the removal of the original surface layer.

One other possibility is also illustrated. The glass fibres have been shown to be exposed to the water and it is known that the glass is coated with size. It is also known that the glass size of SMC glass is different to that of preform glass. Perhaps the difference in

in-use characteristics is dependant to some extent on the glass-size.

Plate 1 Unetched surface of Mix 1 moulded at 135°C

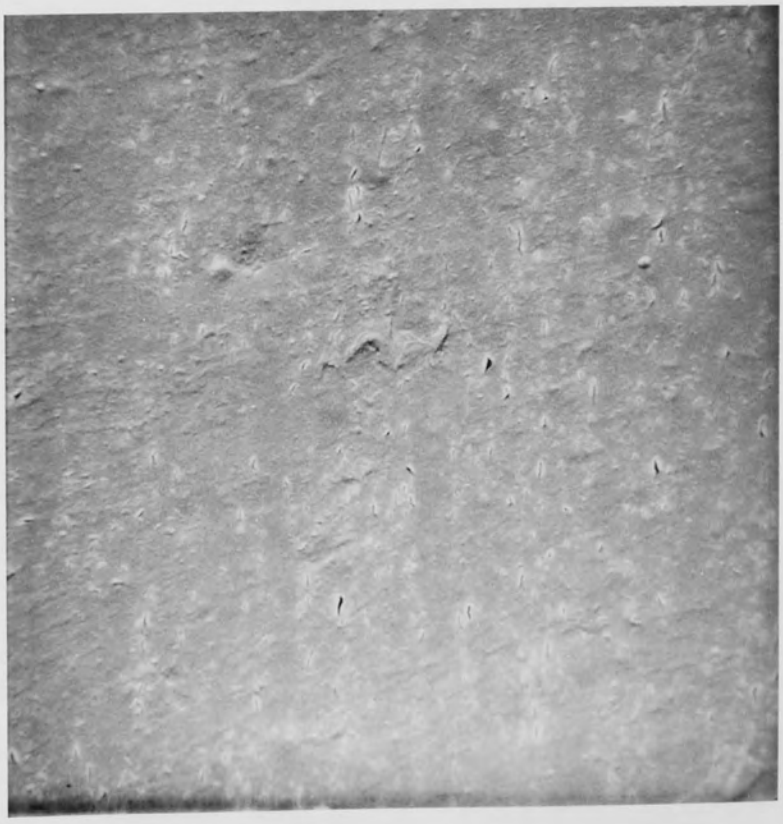
x1095

Plate 2 Unetched surface of Mix 2
moulded at 155°C
x1000

Plate 3 Unetched surface of Mix 3
moulded at 135°C
x1050

Plate 4 Unetched surface of Mix 3
moulded at 135°C
x5100

Plate 5 Unetched surface of Mix 3
moulded at 155°C
x1100



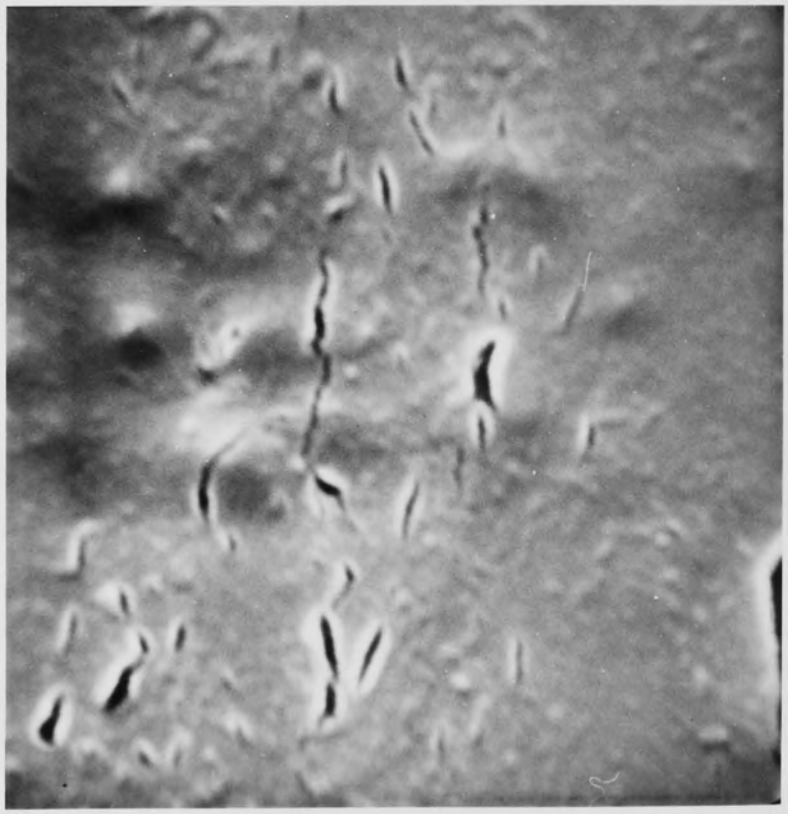
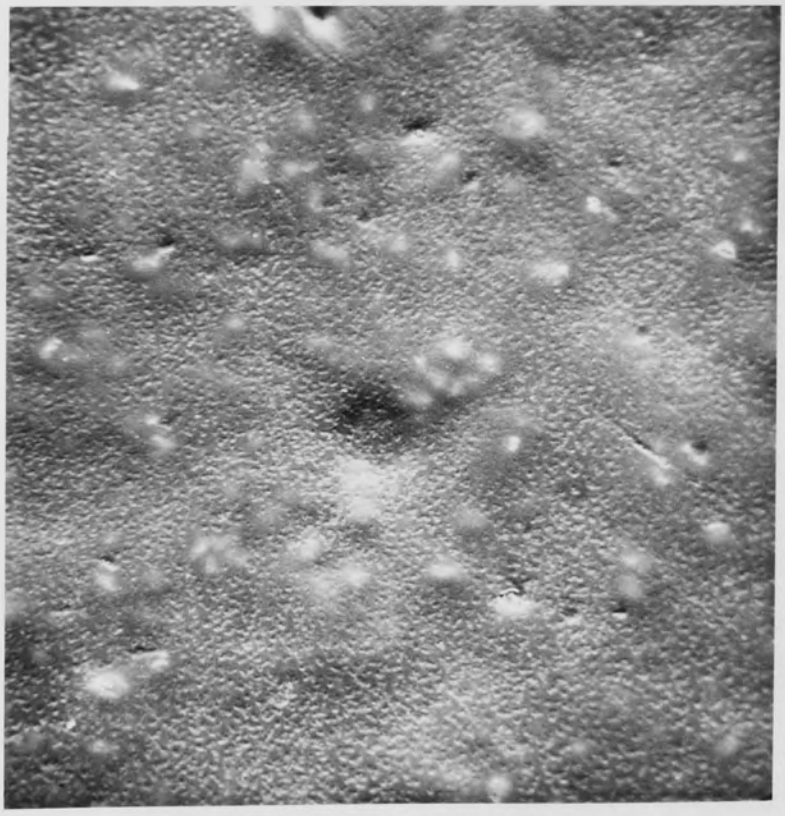


Plate 6 Unetched surface of Mix 3
moulded at 155°C
x5500

Plate 7 Surface of Mix 1 - 2 minute etch
moulded at 155°C
x1090

Plate 8 Surface of Mix 1 - 10 minute etch
moulded at 135°C
x1050

Plate 9 Surface of Mix 5 - 10 minute etch
moulded at 155°C
x1060



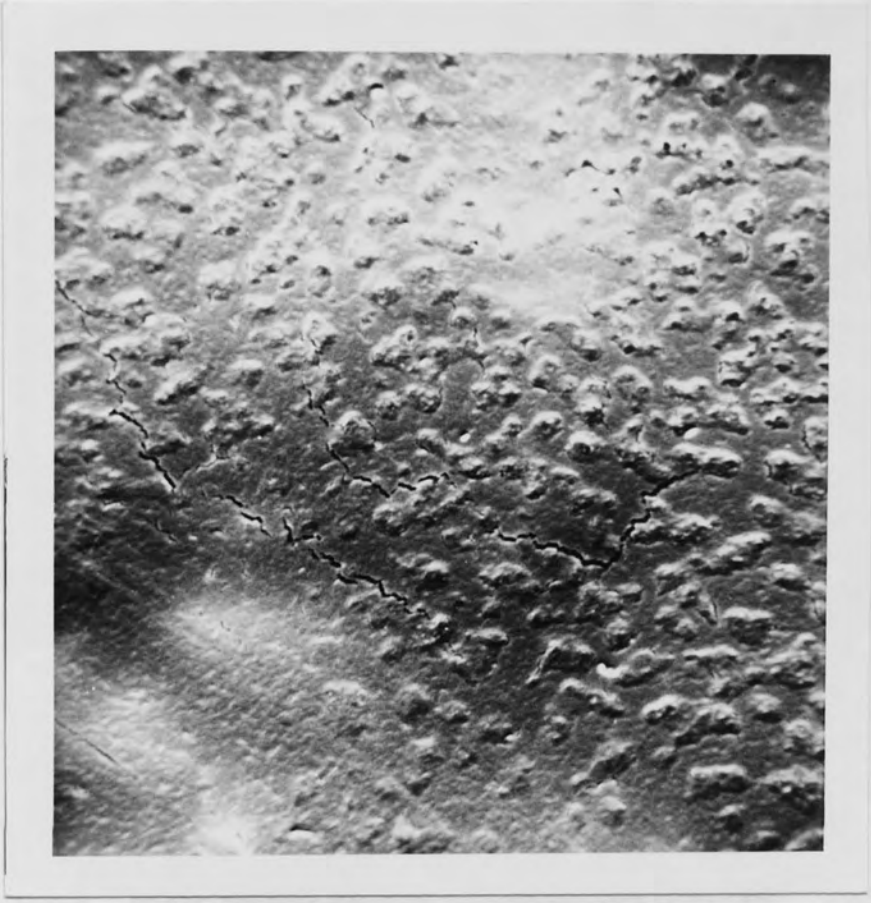


Plate 10 Surface of Mix 5 - 10 minute etch
moulded at 155°C
x5150

Plate 11 Surface of Mix 6 - 10 minute etch
moulded at 155°C
x1050

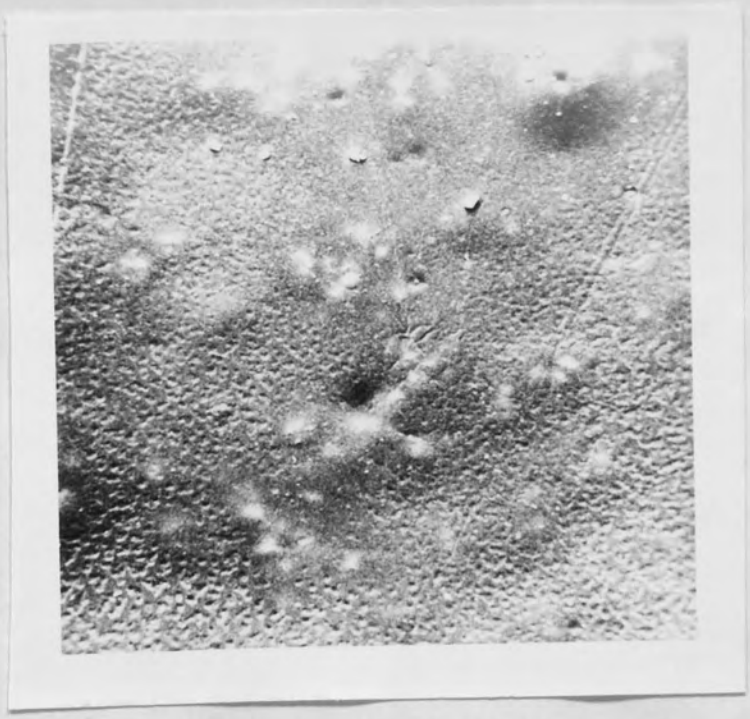


Plate 12 Surface of Mix 6 - 10 minute etch
moulded at 155°C
x5300

Plate 13 SMC CWC in unexposed condition

x95

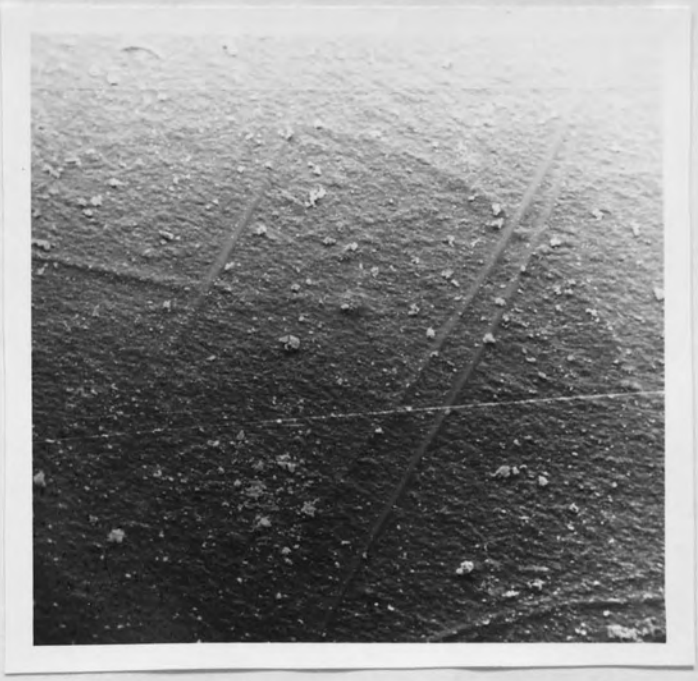


Plate 14 SMC CWC in manufactured unexposed condition x1900

Plate 15 SMC CWC after exposure to water

x98

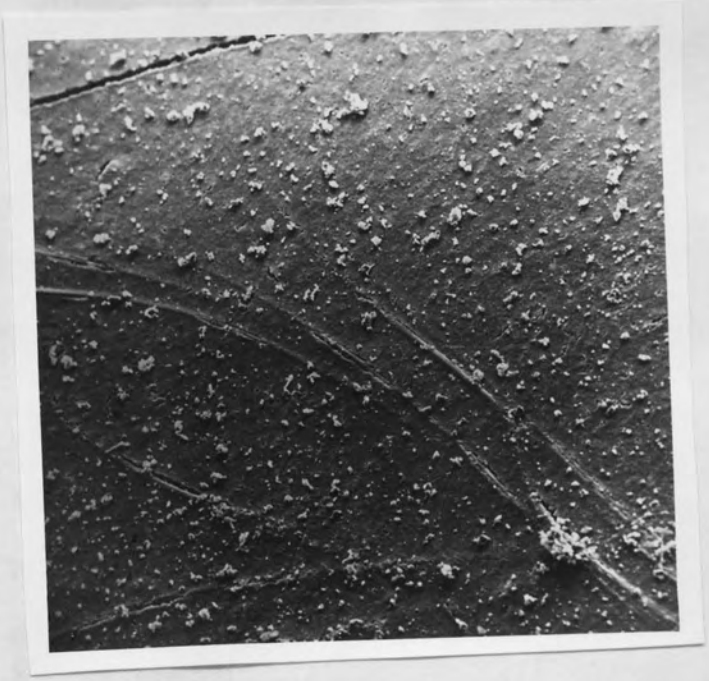


Plate 16 SMC CWC after exposure to water

x920

detail of 'stress cracking' around glass fibre

Plate 17 Preform CWC in unexposed condition

x1000

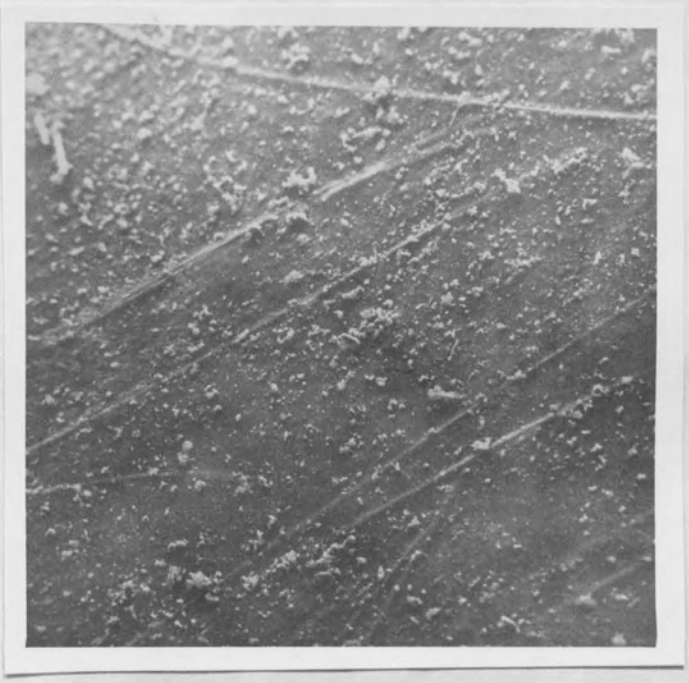
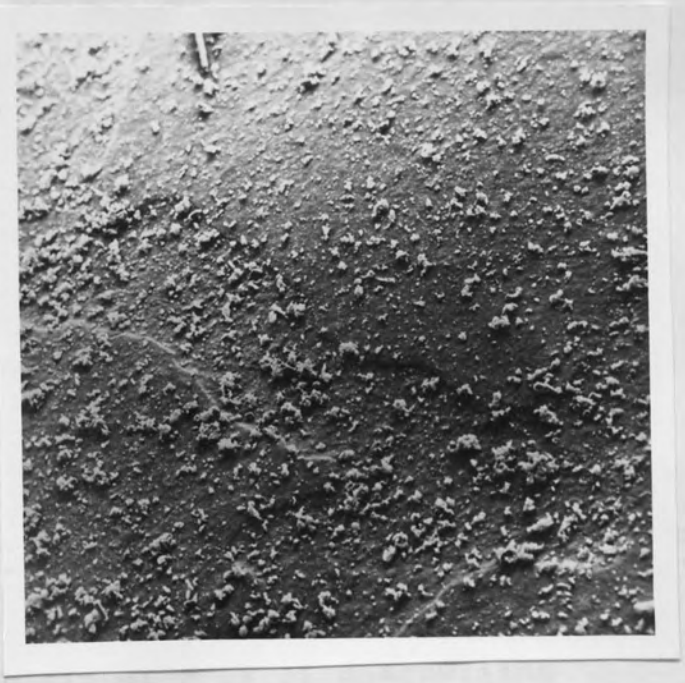


Plate 18 Preform CWC after exposure to water x105

Plate 19 Worthing preform CWC above water line x100



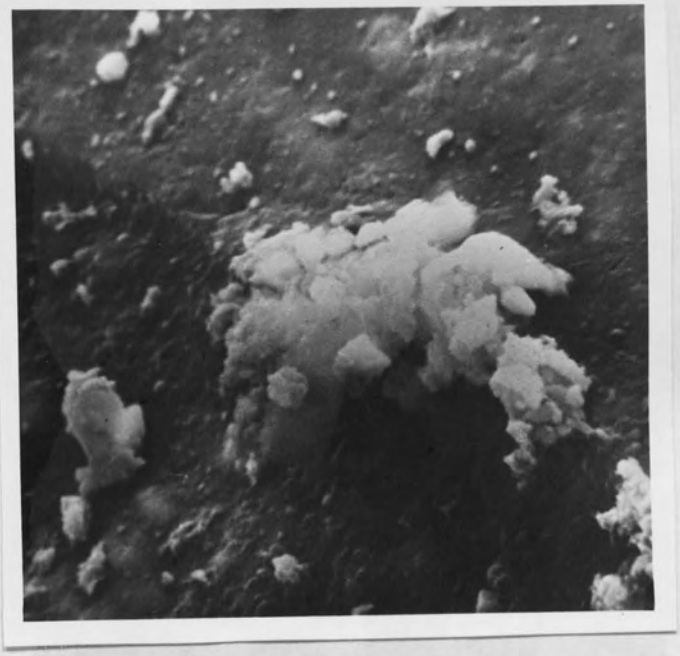


Plate 20 Worthing preform CWC above water line

x2000

detail of edge of channelling

Plate 21 Worthing preform CWC below water line-
surface cleaning

x100

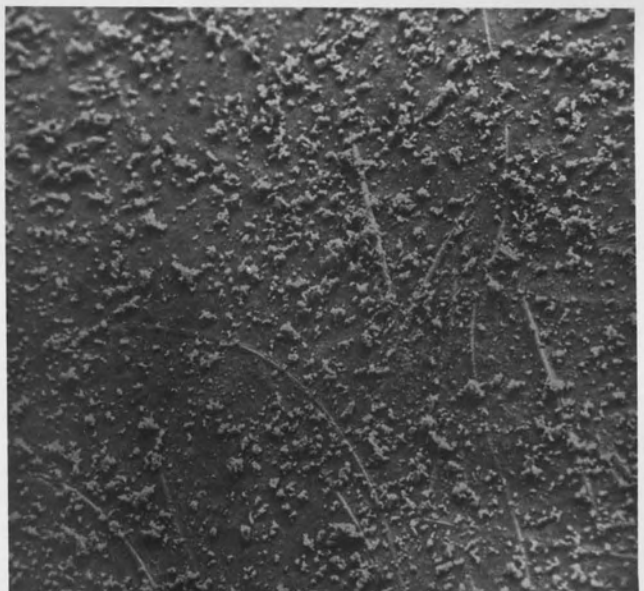


Plate 22 Worthing preform CWC below water line-
no cleaning

x53

Plate 23 Worthing preform CWC below water line x1150

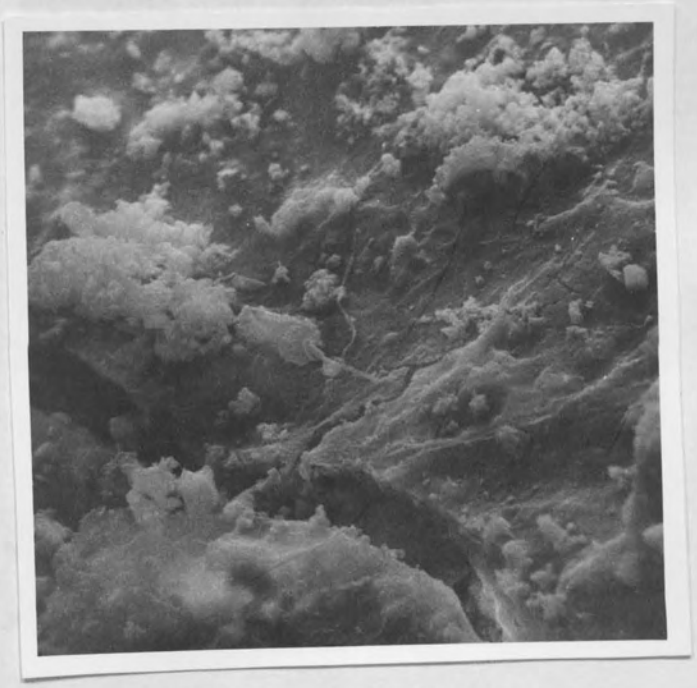
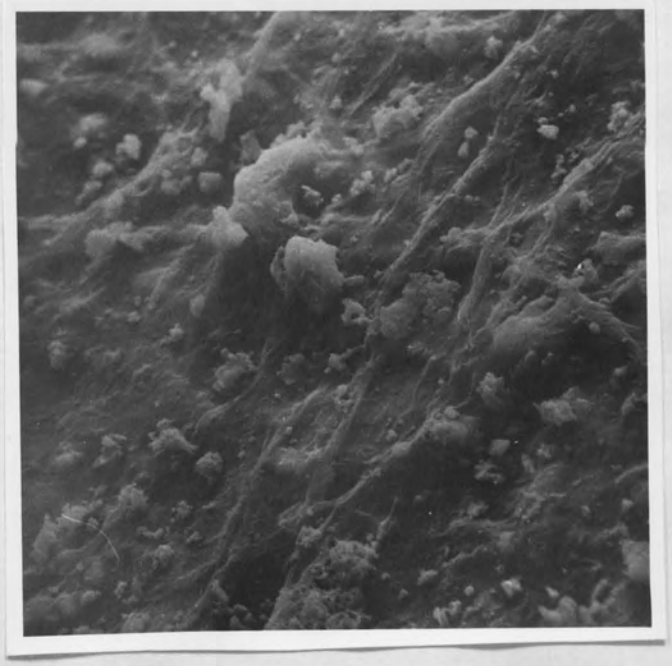


Plate 24 Worthing preform CWC below water line

x230

Plate 25 Worthing CWC showing microbial growth x1150



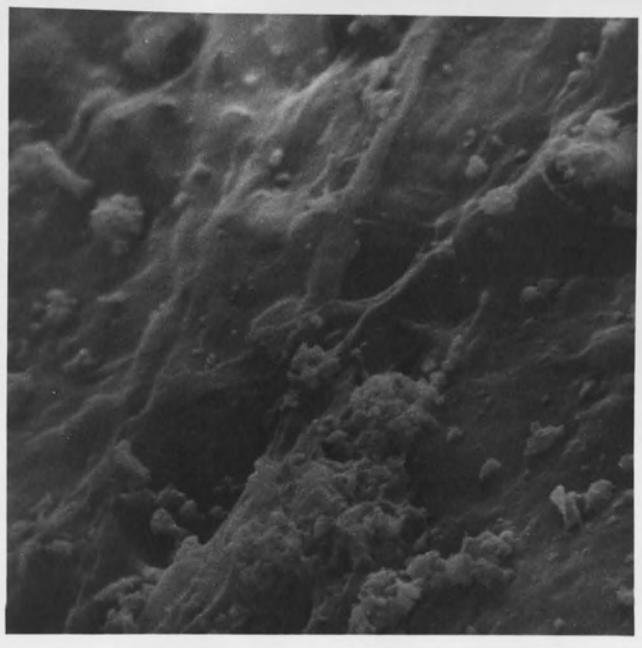
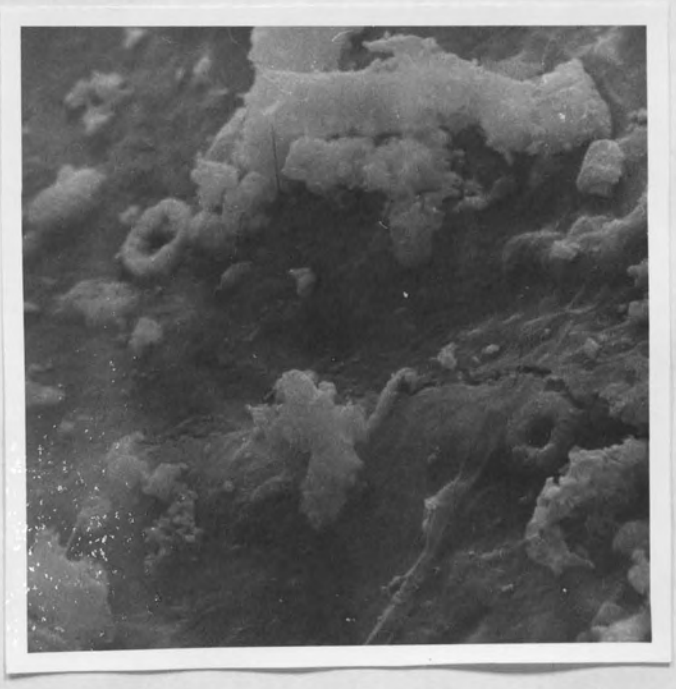


Plate 26 Worthing CWC showing detail of microbial growth

x2300

Plate 27 Worthing CWC showing microbial growth over surface of GRP

x2100



CHAPTER VI

VI A PARTICULAR INCIDENT OF MICROBIOLOGICAL GROWTH IN THE WORTHING AREA

6.1 Introduction

Through one of the marketing outlets for BTR cisterns, reports came back of complaints regarding the behaviour of cisterns after installation. This was of special interest since not only was there the possibility of isolating a possible deteriogen with a substantially documented background, but also because it was at the time, and has remained to date, the only case of 'failure' received by BTR. For these reasons the marketing distributor was contacted and permission sought to study the problem further. The distributor proved willing and took the necessary steps themselves to arrange an initial meeting between the Water Board, BTR and the distributor. Set out below are the results of that arrangement and a short history of the problem pieced together from files handed over by the distributor and from personal discussions with one of their employees who had been involved from a time shortly after the initial complaint was received.

6.2 Historical background to the problem

The distributors were informed in late 1965 by the Worthing Water Board of problems involved in installations of GRP cisterns. The subject had arisen following examination of a cistern placed in an airing cupboard. Talks were held with the Water Board as a result of which the county's Public Health Laboratories were brought in by the distributors to examine the problem, to identify the cause and if possible suggest a remedy. The laboratory

was involved in various contract research until June 1967. During this period the work revolved around the release agent used in the manufacturing process. For the preform process an internal release agent was a constituent of the GRP mix, and an external release agent, essentially a wax spray, was applied to the mould to avoid any difficulty in separating the fabricated tanks from the steel mould by means of which it was fabricated. The results of these tests were that the internal release agent was found to inhibit the growth of moulds whereas the external release agent did not inhibit the growth of moulds in agar culture. When fabricated tanks were tested only those which had been formed on a freshly sprayed mould supported the growth of moulds. The finding appeared to explain the fact that trouble was only associated with a very small proportion of the tanks in service.

In February of 1968 however, the laboratory was again called in to investigate a growth of mould in a replacement cistern for the premises in Worthing which were concerned previously. The whole of the inside surface of the cistern was coated with a dark mould growth up to the water mark, which was found to consist almost entirely of an unusual species which they sent to the Commonwealth Mycological Institute for indentification. CMI indentified the fungus as a species of Lecythophora Nannf.. a genus which is now considered to be in the genus Phialophora Medlar. Futher tests were carried out with the isolate which showed it capable of growing in distilled water and Worthing water, with or without various GRP ingredients.

The internal release agent was again found to show an inhibitory effect, even when incorporated in culture media. A further experimentwith various metal powders showed that Zinc turnings had no significant effect on mould growth. Silver chloride, Copper turnings and Copper sulphate were very slightly inhibitory and catarlyn silver completely inhibited growth on plastic tank material.

However, the rapidity at which Phialophora colonised replacement tanks (less than one month) at the initial premises, and the occurance of the same complaint at other addresses in Worthing led to their complete ban by the Worthing Board. A small number were allowed to stay for the Water Board to examine further, and the inspectors asked to 'keep an eye on them'.

6.3 Involvement of BTR personnel in the Worthing affair

The distrbutors, as stated earlier, set up a meeting between the Worthing Water Board, BTR and themselves. A representative of the distributor (also referred to earlier), the then Technical Manager of BTR (I. Whitney) and the author went to Worthing to talk with the Chief Water Engineer about the behaviour of the tanks. Unfortunately, Mr. Attle, the Water Engineer, could not attend the meeting and instead arranged for the party to visit a number of premises where the cisterns were installed. This was done in the company of a water board inspector who held the list of properties, Mr. Bayne.

The first house visited was the home of a water board employee who was obviously interested by the

problem, but not concerned by it. The cistern was a preform type, installed for four years. On the sides was a dark brown fluffy growth which appeared to be fungal. Two other cisterns were seen, one very similar in condition to the first, the second having a less dense growth. Mr. Payne confirmed that the other installations he had inspected were much the same, all having similar brown growth. He said he had not come across it on any other type of cistern and also confirmed the speed at which tanks were colonised - one month for coverage of all sides up to water level.

Following the visit the decision was taken to undertake some sort of research programme in the Worthing area if the Water Board were agreeable, and as a first stage to arrange for the installation of an SMC cistern so that the dynamics of colonisation might be studied and the deteriogenic nature of the organisms confirmed.

6.4 Installation of replacement SMC cistern at a property in the Worthing Water Board Area and its subsequent examination

6.4.1 Microbiological Sampling

Immediately prior to disconnetion of the preform cistern in the replacement procedure, samples were obtained of the brown fluffy growth on the sies of the tank. Pre-sterilised universal bottles were submerged in the cistern water and the tops removed allowing the bottles to fill with water. A piece of the presumed fungal growth was scraped from the cistern surface with a spatula and guided into the univeral bottle. The top was screwed on

the bottle and the bottle lifted out. Six samples were taken from points around the four internal sides of the cistern in this fashion.

The samples were examined at the BIC and the results are presented in 6.4.4.

6.4.2 Replacement

The nature of the installation is shown in Plate 28. The original cistern was a 25 gallon preform type complete with the standard GRP lid. Enclosing the cistern was polystyrene sheet insulation. The condition of the cistern prior to replacement is shown in Plate 29, with brown growth up to the water line. This tank was removed from the domestic pipework and a 25 gallon SMC cistern installed in its place (Plate 30). The actual plumbing was done by one of the distributors engineers, a specialist in plumbing applications. Care was taken to ensure that the cistern was as clean as possible and free from grease, GRP cutting dust, polystyrene granules from the insulating sheets and PTFE tape used to seal the threads. A lid was fitted and the water supply reconnected and the cistern allowed to fill.

The preform cistern which had been removed was placed in a large plastic bag, with some water to keep the atmosphere moist, and taken to BTR Uxbridge where it was reconnected to a water supply and outlet plumbing in a test rig. Throughput in its domestic situation had been estimated at 30 gallons/day and arrangements were made to give the tank in the test rig one complete change of water every day.

This work took place on 9th May 1974 and the property concerned was the first to be visited on the previous journey to Worthing detailed in 6.2, belonging to Mr. Stone of Cranleigh Road, a water board employee. Arrangements were made to return at intervals to inspect the installation and take samples of any growth on the cistern and the water.

6.4.3 Inspection and Sampling

Mr. Stone agreed to keep a daily watch on the replacement cistern and contact the author immediately any discolouration of the tank occured. It was anticipated that from previous experiences ofGRP tank installations this would be within one week. When after three weeks no contact had been made by Mr. Stone, the author rang him and was told that the tank remained 'clean'. Therefore a visit was made on June 6th 1974, to the premises and the condition of the tank is shown in Plate 31. No growth of any sort is visible, the only noticeable difference from the day of installation being the presence of a sandy sediment on the base of the cistern. A sample of mains water was obtained direct from the ball valve outlet after first cleaning the mechanism with alcohol. A similar sample was taken from the cistern water.

Similar visits were made on 10th July, 15th August, 24th October, 19th December 1974, three occasions in 1975 and three up to June 1976. On each occasion samples of mains and tank water were taken and a visual examination made of the cistern. On the occasion of 24th October 1974, after visiting the premises, a visit was made to the

borehole which supplied the premises, marked BROADWATER ◬ on figure 8. A sample of water was taken from the wellhead and taken, along with the samples from the cistern, back to the BIC.

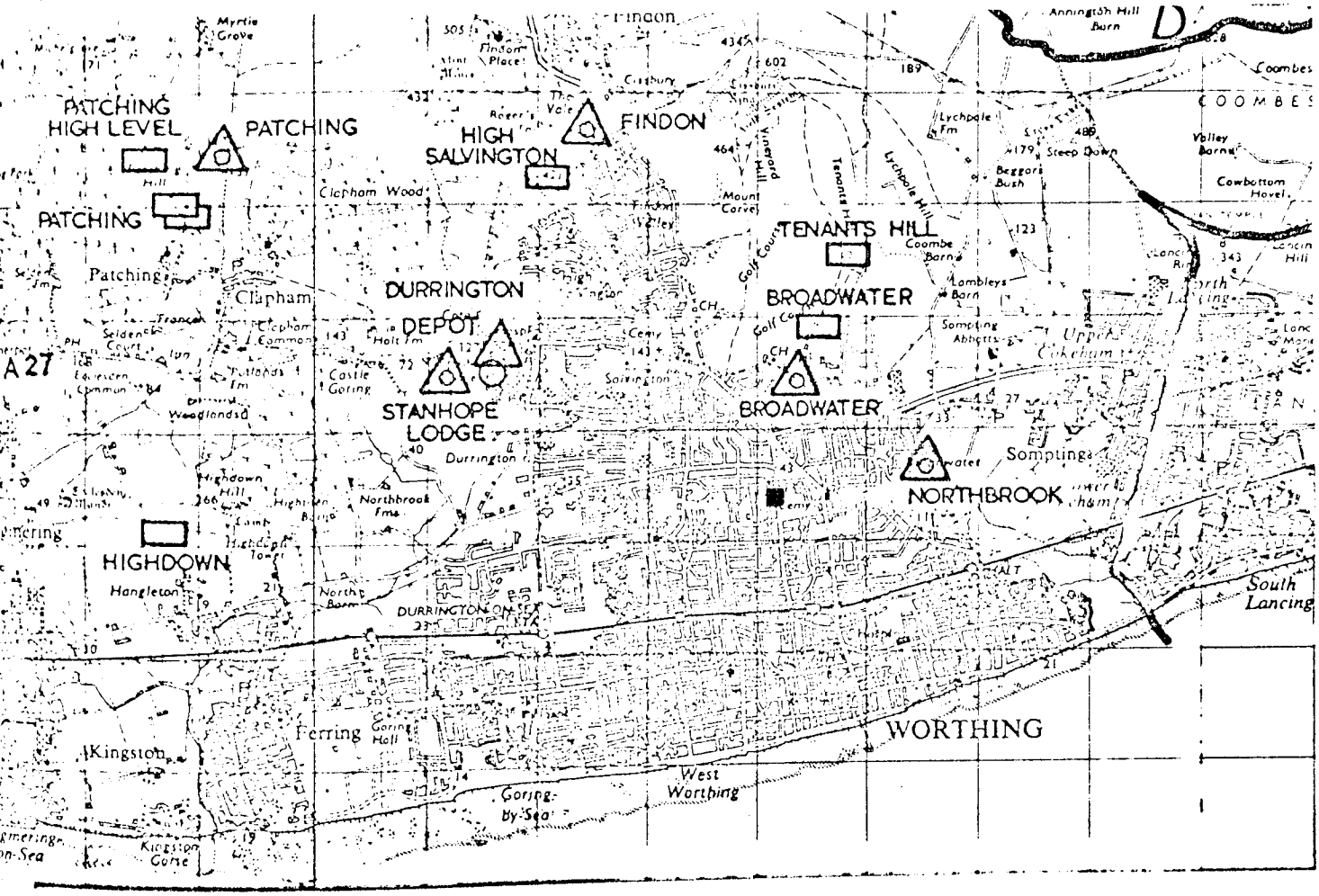
6.4.4 Results of examination of water samples

The samples taken from the side of the preform cistern removed from the premises were examined microscopically and found to consist of fungal hyphae showing flask shaped phialides with or without a discernable collarette, (Plate 32). The sample was plated out on 2% malt agar (Wang , 1965) where it proved very difficult to isolate, being overgrown by other organisms presumably present in the water, mainly Penicillium spp. and bacteria. Prolonged washing in distilled water with kanamycin (100 µg/ml) followed by plating out on media also containing kanamycin at the same concentration eventually proved successful and the isolate then grown on a number of agars and at different temperatures to characterise its growth characteristics (Wang , 1965; Schol-Schwarz, 1970; Cole and Kendrick, 1973).

These tests have shown the isolate to be able to grow on all common laboratory media, although slowly. The most rapid growth rate was on malt agar and produced a colony of 34 mm ± 1.7 mm (s.d.) diam. after 15 days at 25°C. The isolate showed a range of colouration on the different media from pale green to light browny grey. (Figure 9)

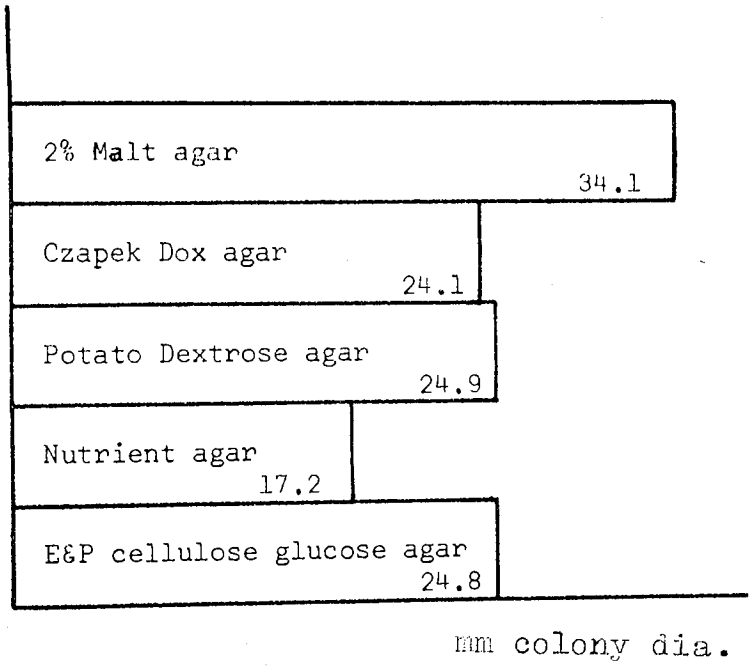
Optimum growth temperature was found to be 31°C

Fig 8 Boreholes and Wells in the Worthing Area



■ Replacement cistern

Fig 9 Growth of isolate on laboratory media after 15 days at 25°C



with a sharp cutailment of activity between 40° and 45°C and no apparent growth at 5° or 50°C. (Figure 10).

Microscopic examination of the isolate has shown septate hyphae, hyaline when young to green; on ageing swollen cells occur.

Phialides are flask shaped, mostly ellipsical occuring singly laterally on hyphae with collarette. The conidia are hyaline to subhyaline, more or less ellipsoidal but slightly apiculate at one end with thin walls and one or two oildrops. In older cultures condidia are thick-walled, darker and more regular (Plate 33). It would appear that the isolate is a Phialophora sp. and is the organism found on initial microscopic examination of the preform cistern growth.

The water samples from the inspection visits were tested for presence or absence of 'tank isolate' and by the methods detailed in 2.11, for fungal and bacterial loading. The results are presented in Table 16 and Figures 11 - 14.

6.5 Reinfection of Preform and SMC cisterns with the isolate in the multi-test tank rig

In order to examine the properties of the suspected deteriogen further and to fulfil the third part of Kochs postulates i.e. reinfection of host substrate with isolated organism to reproduce phenomena of biodeterioration under controlled conditions (Hueck, 1974) a preform cistern and an SMC cistern were attached to the test-rig at the BIC. A full description of the test system may be found in Chapter 4. The cisterns were inoculated with

Fig 10 Growth of Worthing isolate at various temperatures on 2% malt agar

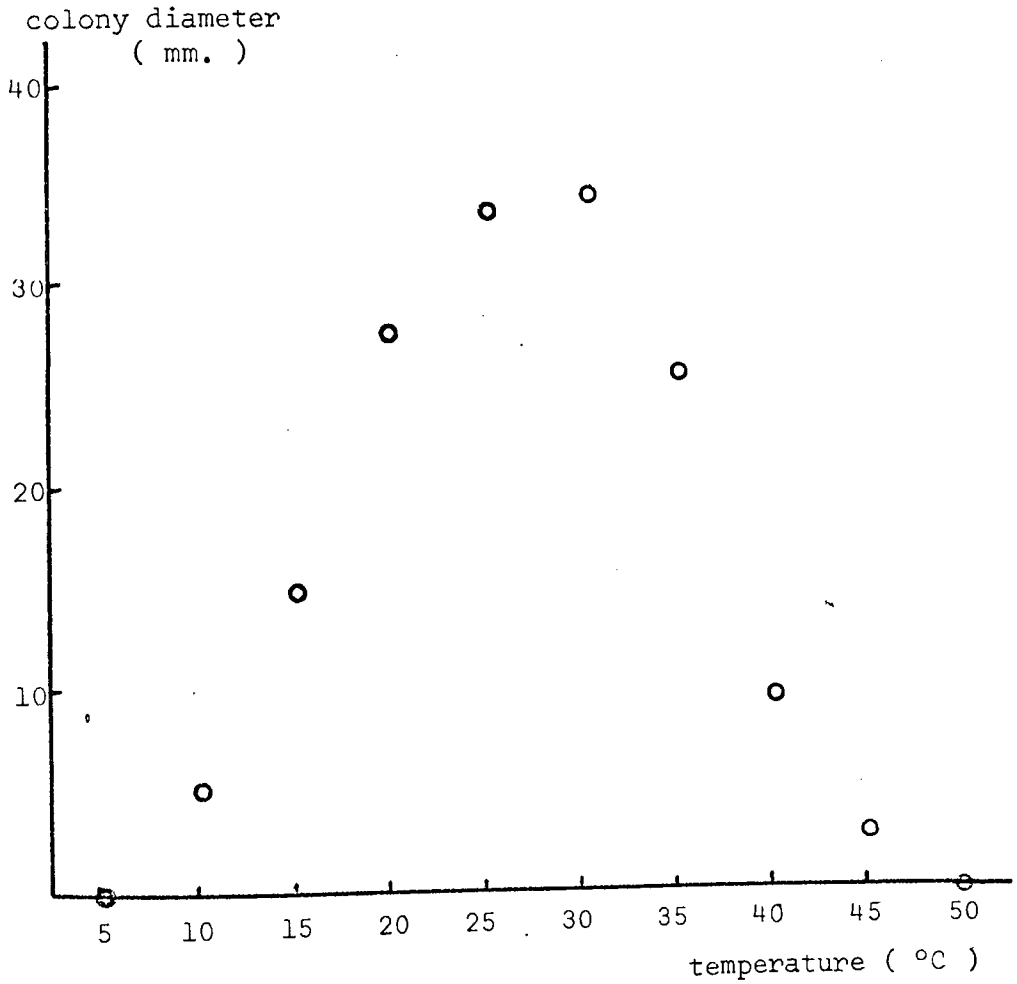
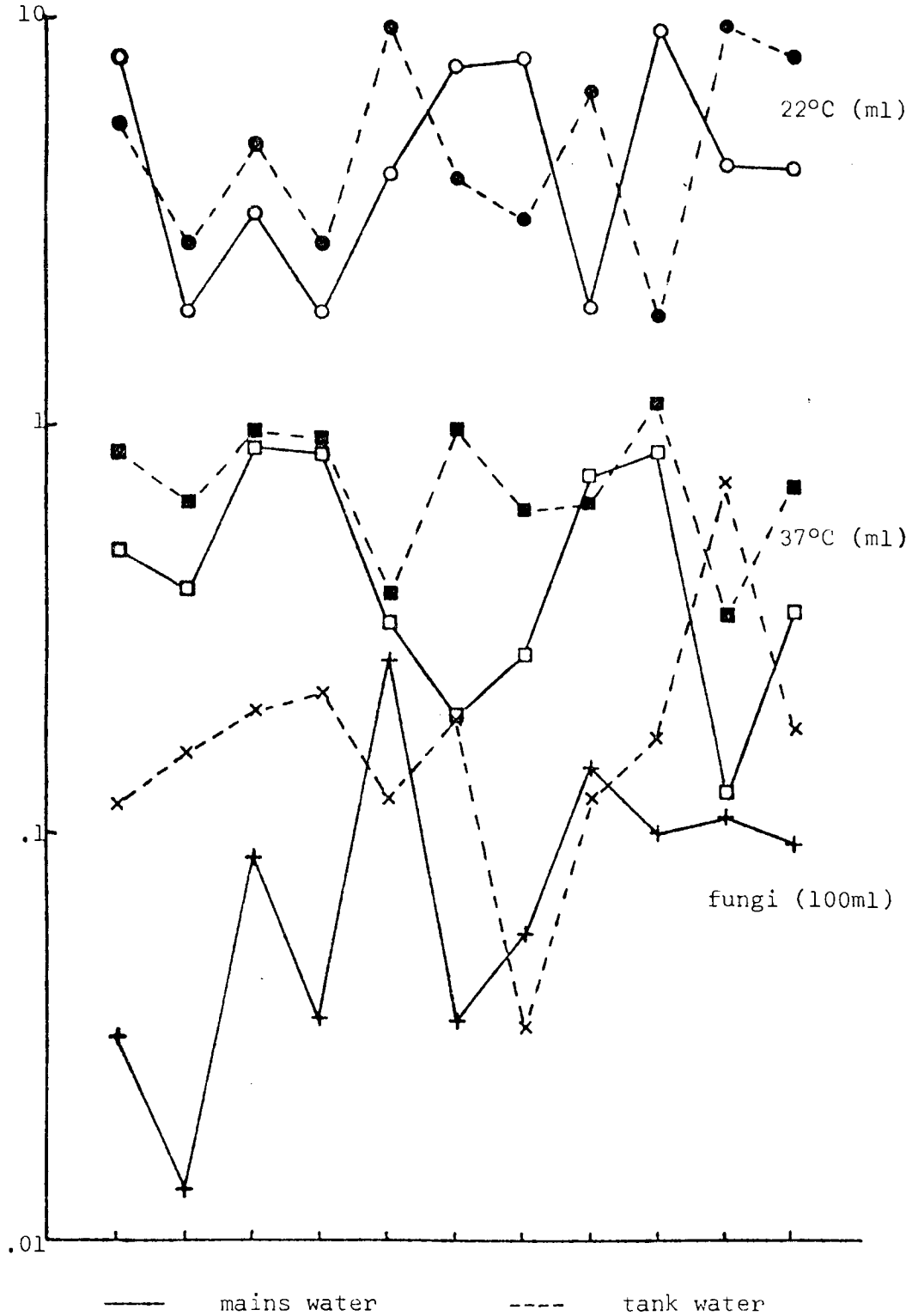


Table 16 Worthing samples - colony counts and isolate presence

DATE	WATER SAMPLE	TANK ISOLATE	FUNGI 100ml	COLONY COUNTS /ml	
				22°C	37°C
6.6.74	mains	no	320	57000	4830
	tank	yes	1190	80900	9070
10.7.74.	mains	yes	130	28300	3930
	tank	yes	1540	19000	6390
15.8.74	mains	yes	870	33600	8630
	tank	yes	1990	51400	9540
24.10.74	mains	yes	340	27900	8870
	tank	no	2170	19300	9120
	bore-hole	no	10	2790	1440
19.12.74	mains	no	2660	41600	3250
	tank	yes	1180	96400	3600
26.2.75	mains	no	340	78000	1930
	tank	yes	2020	41900	9870
25.6.75	mains	yes	560	81000	2700
	tank	yes	320	32800	6210
20.11.75	mains	no	1470	19300	7510
	tank	no	1230	68500	6470
20.1.76	mains	no	990	95200	8920
	tank	no	1690	18700	11700
25.2.76	mains	no	1100	43800	1180
	tank	yes	7140	98700	3360
4.3.76	mains	yes	950	43800	3570
	tank	yes	1810	84000	7050

Fig 11 Trends in microbial numbers in Worthing samples

(x 10000)



Figures 12-14 Scatter diagrams of paired samplecounts

Degrees of Freedom 9

to be correlated at 5% level

$-0.602 > r > 0.602$

Fig 12 Correlation of Fungi and Yeasts in Worthing water samples (100ml)

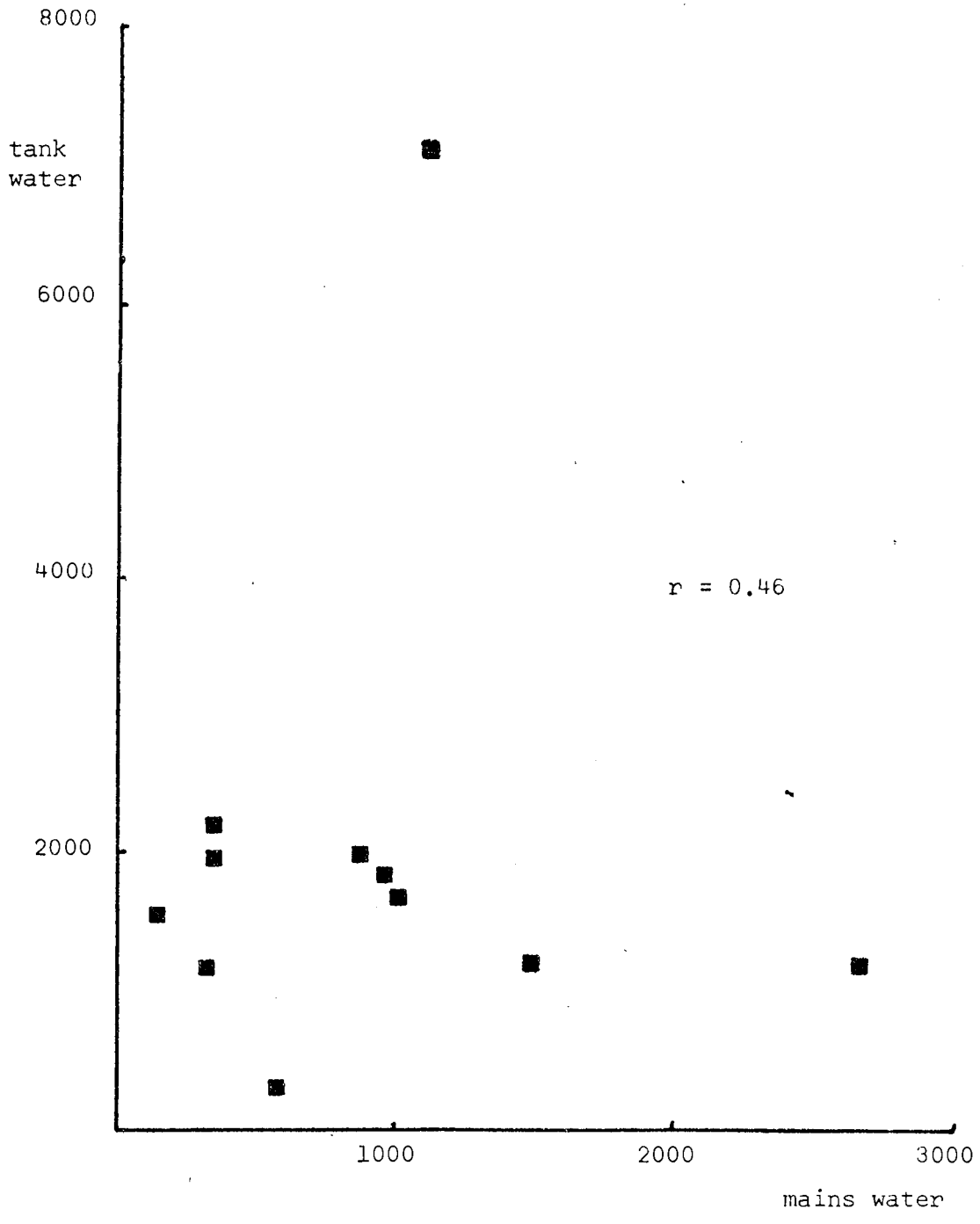


Fig 13 Correlation of 22°C counts in Worthing water samples (ml)

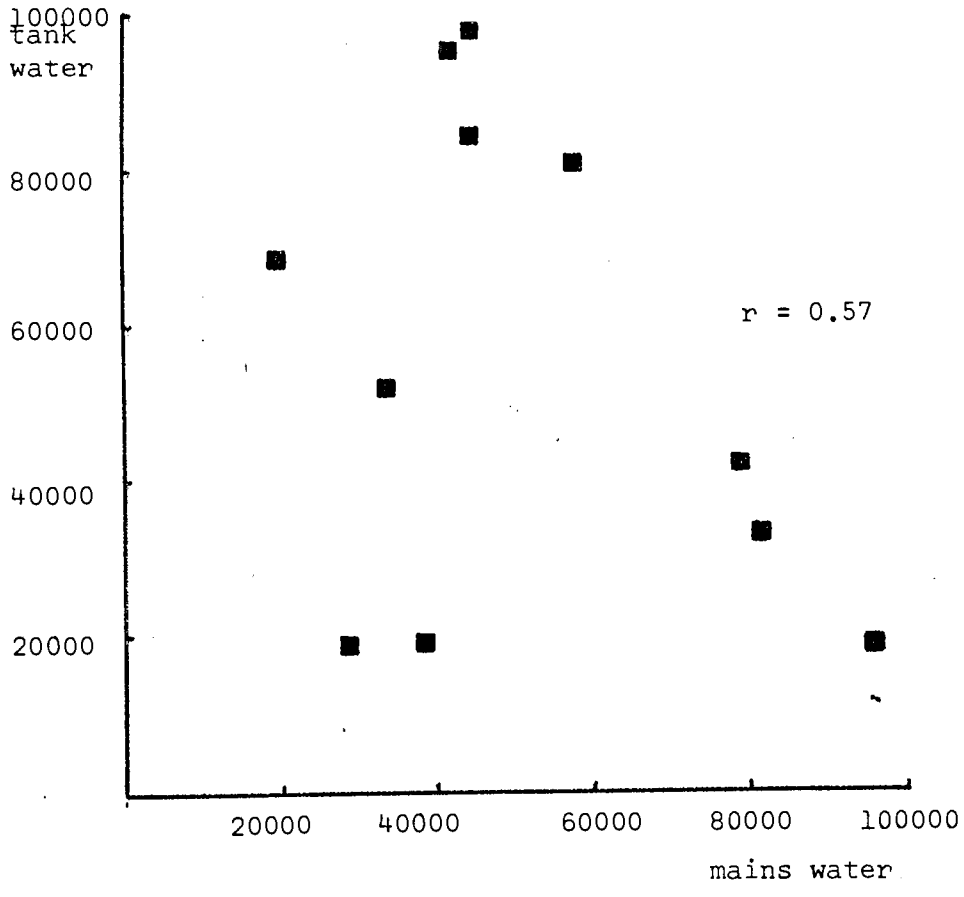
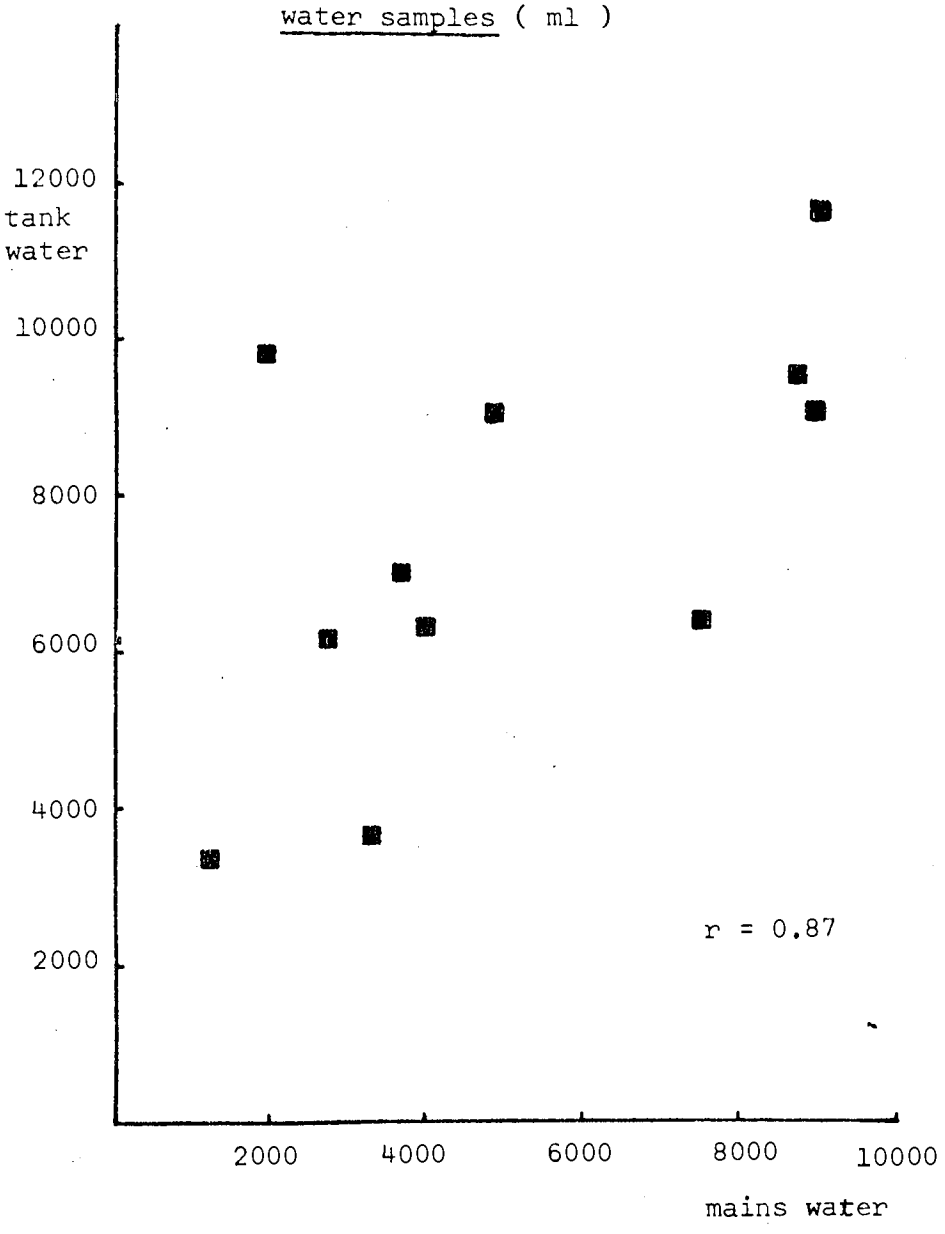


Fig 14 Correlation of 37°C counts in Worthing water samples (ml)



growing cultures of the Worthing isolate and the microbiological water content and the visual appearance monitored.

6.5.1 Material and Methods.

The SMC cistern was one of normal cure time prepared to an identical specification to those detailed in 4.3.1 (6). The preform cistern was one of a small quantity found at BTR (Uxbridge) and used for studies detailed in Chapters 3 and 4. It was estimated that the cisterns were produced in late 1969, among the last to be made before the cisterns became a totally SMC product. Both cisterns were provided with lids of current manufacture.

The inlet, outlet and overflow systems are detailed in 4.2. and the two cisterns replaced one of the pairs of overcured SMC cisterns in the long-term testing experiment.

Inoculation was with 72h shake-flask cultures of the Worthing isolate in one-tenth strength malt broth, 50 ml inoculum per cistern.

Daily following inoculation samples were removed and the fungal load determined using the methods detailed in 2.11. The proportion of the fungal numbers supplied by the isolate was determined.

6.5.2 Results

The first attempt to reintroduce the Worthing isolate showed a complete washout of the organism from both types of cistern within 7 days. On that occasion water throughput was 100% daily and was started on the

day following inoculation.

Subsequent attempts attempted to give the organism time to become established before water-changing began. It was found that if water throughput was 100% daily, following upto a 6 day delay in starting water changing, washout occurred within a maximum of 10 days. Regulation of water throughput down to 20% daily lengthened the washout time to a maximum of 23 days with a steady downward trend in numbers apparent.

6.6 Respirometric studies with the Worthing isolate

The isolate was tested for its ability to utilise preform binder resin and the two glass sizes, using the Warburg respirometer.

6.6.1 Method

The binder resin or glass size was incorporated in an E & P nutrient salts medium at either a 1%, 0.1%or 0.01% level. 10 ml of this media was dispensed into Warburg flasks and 0.1 ml of a 72h E & P glucose-cellulose shake flask culture of the isolate added. Two controls were used:

a. flask containing inoculum with 10 ml E & P nutrient salts medium without test material

b. flask containing inoculum with 10ml E & P nutrient salts medium + glucose (0.1%)

An uninoculated flask containing 10.1 ml of E & P salts medium was used as a thermobarometer.

6.6.2 Results

The 0.1% glucose flask was used as a positive control of organism viability. Typical oxygen consumption

was 55-70µl/hr and after the first hour it was not read further. All other flasks were read for 12 hours. The second control flask provided a basal respiration rate for the organism on the nutrient salts medium alone.

The results may be summerised as follows:-

a. All three test materials totally inhibited respiration when incorporated at the 1% levels.

b. All three test materials depressed respiration rate when incorporated at the 0.1% level, i.e. respiration was lower than the second control flask but showed a greater change than the thermobarometer.

c. At the 0.01% level, the respiration rate was not significantly different to the basal rate.

A second series of determinations were made using organism which had been repeatdly grown with 0.01%of test material incorporated in the growth media (53 x 120h cultures) in order to determine whether adaptation might occur. Results were as determined in the first series.

Klausmeier (1966) reported that some organisms could not use plasticizer as sole carbon source but would grow in the presence of added nutrient. A third series of experiments was run using the 'adapted' organism as inoculum. This involved a comparison of flasks containing nutrient salts medium with 10 moles of glucose with similar flasks containing added test material (1mg).

No increase in respiration was observed in flasks containing test material and depletion and subsequent curtailment of respiratory activity occurred simultaneously.

6.7 Reorganisation of Water Authorities

April 1st, 1974, saw the demise of the Worthing Water Board under the general reorganisation of local authorities and water undertakings. England and Wales were reorganised in the water industry so that control was established under 10 regional authorities. Worthing Water Board became part of the Southern Water Authority and as such accepted the SWA's decisions regarding acceptable water fittings. The ban on BTR's GRP tanks therefore lapsed and marketing could begin again in the area. If that situation had developed then not one SMC tank would exist in the Worthing area, but more, as replacements or in new developments. This would have enabled a more positive conclusion to be drawn about the relative susceptibilities of preform andSMC cisterns to attack by the organism peculiar to the area.

6.8 Discussion and Recommendations

Biodeterioration in general may be summarized by the equation:

substrate + organism → substrate' + organism'

Thisexpression requires that a cause-effect relationship be established between a particular substrate and a particular organism, Hueck (1965) applying Kochian principles, andmay be summarised as follows:

a. The biodeteriogen, in such numbers and distribution to explain the phenomena, must be present in the close vicinity of the material displaying the phenomena of biodeterioration.

b. isolation of the biodeteriogen from the

material and subsequent growth in pure culture must be possible.

c. Using the isolated pure culture, it must be possible to reproduce the phenomena or a comparable phenomena.

Some workers would insist that steps (b and c) should again be possible after (c) and direct comparison made of the isolates and the biodeterioration phenomena at each stage.

In this investigation of a particular incident of suspected biodeterioration in Worthing it is obvious that principle (a) has been shown. It is believed that principle (b) was also shown. However it was not possible to show principle (c) using the test-rig at the BIC and the replacement SMC cistern did not exhibit the biodeterioration phenomena although it is known that the phenomena was repeatedly shown at one premise with replacement preform cisterns,and with preform cisterns at a number of sites on installation. It is possible that the preform cistern used in the reinoculation experiment (6.5) had changed its characteristics during long storage and was not characteristic of the newly moulded preform cistern. One suggestion was a change in the nature of the binder resin with time, but the inability of the isolate to utilise the unpolymerised binder resin as nutrient questions whether the binder resin should be implicated in the original phenomena. This information must be assessed in the light of the known change in the surface of preform GRP with time seen in the Scanning Electron Microscope studies (Chapter 5)

If it is assumed that the repeated growth of the organism in preform cisterns and its reappearance in replacement preform cisterns (6.2) fulfils principle (c) and we accept that a valid phenomena of biodeteriogen was seen, then since the presence of the isolate in mains water was repeatedly shown, it would appear that the phenomena was not associated in any way with SMC GRP.

In this chapter it can be seen how great the benefit can be to the manufacturer of his awareness of the effect on subsequent in-service performance of changes in his manufacturing process, and the importance of being able to assess this himself. Also, as a by-product of this microbiological investigation, the commercial importance of keeping up with changes in legislation and governing bodies can be emphasised. This is illustrated by the failure of the marketing side of BTR to assess the new atmosphere prevailing in Worthing after the reorganisation of the water industry, and led to a gap of over twelve months between the cessation of the ban and their awareness of this change, brought to their attention by this investigation.

In this particular case the change in manufacturing process was quite drastic i.e. the changeover from preform production to SMC manufacture, and the subsequent change in service performance extreme. Preform cisterns had a history of complete failure to withstand overgrowth by Phialophora . Indeed various sources gave, independently, figures of less than one month from installation to complete coverage. The substituted SMC cistern has now

been in service for more than 27 months and shows no sign of any discolouration or fungal growth on the tank surface as can be seen from Plate 34 taken after 22 months service.

From this it seems that recommendations may be given to both sides in this incident and to manufacturers and controlling bodies in general.

The first, for the manufacturer to ensure that he is aware of the criteria by which his product is assessed and very fully informed of particular aspects of testing procedure if it is found that his products fail in this respect. If possible he should test any changes in product to ascertain whether the previous fault has been removed, even if he must then submit the article to the governing body for testing. Failure to do so may lead to a similar situation to that found in Worthing. The ban ended on 31st March 1974 (although BTR were not made aware of this and did not themselves realise it) but the possibility exists that successful marketing in the Area could have taken place from a time very soon after the change to SMC procedure if the Water Board had been informed of this change in product specification and a test installation made. Finally the manufacturer should keep the governing body informed of changes in product specification and should seek to re-evaluate the validity of the previous failure with this in mind.

The governing body has responsibilities and obligations too, and two recommendations will be made here. Firstly, any testing procedure should be published

or otherwise made available to the manufacturer and standardised where-ever possible to enable the manufacturer to test his products, either in his own laboratories or using independant test houses, to evaluate the effect of changes he may wish to make. Secondly, if the governing body do fail an article and have the power to withold a market from the manufacturer they should also act responsibly towards attempts by the manufacturer to effect a remedy and shouldmake any ban a specific action, relating only to that precise article and not to a similar article of different composition.

In effect, the importance of communication between organisations is illustrated and the consequence of a failure by either party may be commercially significant. The work carried out by the County's Public Health Laboratories may also be examined. Their first and major conclusion was that the external release agent was biodegradable and that it was traces of this remaining on the surface of the tank which promoted mould growth. Also, since only a small proporation of tanks would be fabricated directly after release agent had been applied to the steel mould this would explain the very small number of customer complaints. However, in the Worthing area it would seem that every preform tank developed mould growth so adding a further dimension to the problem. There are a number of possible explanations:-

1. The organism found specifically in the Worthing area is well adapted to utilise even trace amounts of external release agent

remaining on all fabricated cisterns.

2. The organism, although capable of utilising release agent, was deriving nutrition from another component of the laminate.

3. The organism was using the tank surface as an attachment point and deriving nutrition from the constant passage of fresh water through the cistern.

The results of the Public Health Laboratory show that (1) and (3) may be possible but that no work was reported on (2). If we examine the case for growth being supported by external release agent we again have a number of choices.

Either all the tanks in the Worthing area were those fabricated after release agent had been applied to the mould. If release agent was applied every 5 tanks moulded then the probability of any tank being the article fabricated directly after release agent application is 0.2 (20%). For two random tanks to be the appropirate mouldings is $(0.2)^2$, which is 0.04 (4%). For three tanks the probability is $(0.2)^3$, which is 0.008 (0.8%). On our first visit to Worthing we inspected three tanks all exhibiting growth, were not allowed to see tanks at two other premises, and the accompanying inspector had a list of at least ten addresses. The probability for five and ten tanks are 0.00032 (0.32%) and 0.0000001 (0.00001%) respectively. In round figures these represent one chance in 3000 and 10 million respectively. Or the tanks in the area were a normal distribution ranging from those

fabricated just after application of release agent to those fabricated just before application to the steel mould. The second of these possiblities seems more likely, which means that the organism must be able to conserve its energy source to last at least 6 years or more. For this alone to be the explanation for the differences in behaviour noted between preform cisterns and SMC cisterns does not seem probable.

This leaves two further possibilities both requiring comparative work on the two types of material. If the organism is able to utilise a component of preform yet seems unable to utilise any part of SMC GRP then defining differences in mix composition, and their respective showings in microbiological testing, may help us to explain the behavioural differences. Alternatively, if the GRP is acting merely as an unutilised, colonised substrate then perhaps it is differences in surface morphology which explain the extremes of behaviour exhibited in service.

For these reasons it wasthought necessary and desirable to study the surface morphology of laminates using the Scanning Electron Microscope and to do side-by-side microbiological testing of the two laminates.

One final possibility is that the behaviour of the interaction between the organism and preform surfaces is a culmination of all three possibilities. The organism respires actively using the release agent and in doing so colonises large areas of GRP surface. Gradually as the release agent is used up the organism is able to utilise

a component of the plastic, perhaps only made available by its previous activity or that of the water itself, as part of its nutritive requirements, and derive the remainder from the water itself. Perhaps the other studies of preform and SMC laminate indicated will help in elucidation of the final picture.

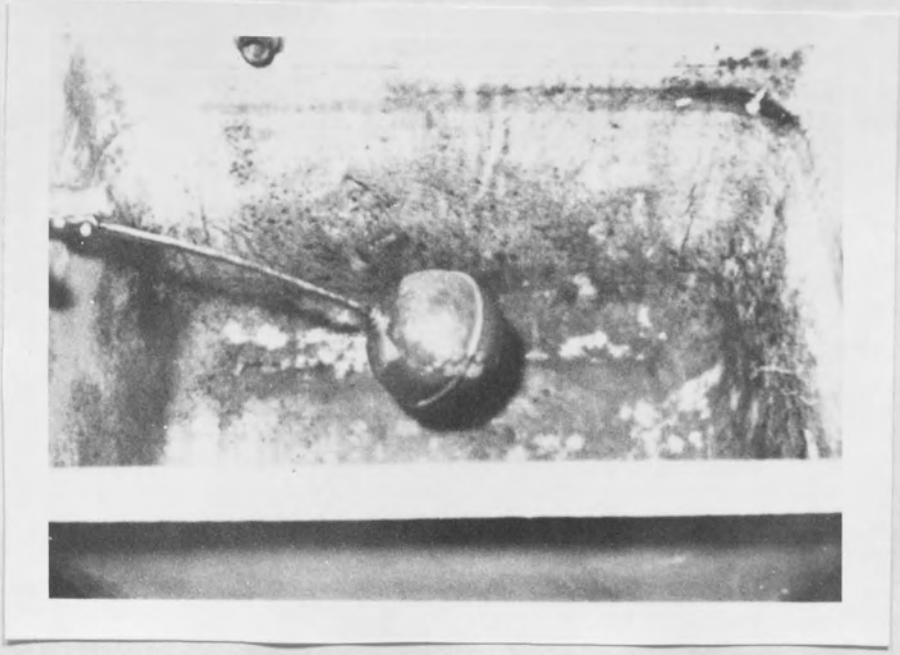
The Source of the fungal inoculum was originally thought to be the wood usedin the surrounding structures in which the tanks were housed. Again an examination of the probability of that being the case is desirable. Although Phialophora has been associated with pulp-mills and timber deterioration, its occurrence in this country is still infrequent. For the organism to be found in all types of housing in Worthing postulates that all the timber used in that area is infected even though the properties concerned were built by many different firms and cover a long period of time. If this is the case, then why should Worthing be so exceptional in its timber infection frequency and if it is no different to that found elsewhere in the country then why is there not a widespread problem associated with GRP tanks. Evidence from the sampling of mains water at the property in Worthing suggests that the inoculum is brought in via the supplied water, and that contamination of water supplies occurs after leaving the borehole. It is interesting to note therefore that the Commonwealth Mycological Institute hold a culture of Phialophora cinerescens isolated from wilted Dianthus caryophyllus at Littlehampton, Sussex. Many market gardening concerns surround the Worthing urban area

and they may possibly provide a reservoir of Phialophora to repeatedly infect the water supplies.

The trends in microbial numbers are illustrated in figure 11 and it may be seen from this that only of the 37°C counts does any real similarity exist between the two graphs of mains water numbers and tank water numbers. This is confirmed by figures 11-14, scatter diagrams for pairs of samples giving the correlation coefficient. It will be seen that only the 37°C correlation is significant at the 5% level. Comparison of the means for each pair of samples shows no significant difference between them. It may be that the independance of the 22°C and fungal counts from the mains water counts, within the nature of the comparable population numbers, is a reflection of a more evolved ability to resist transitory fluctuations in the mains water activity than the 37°C organisms. However, the real test of that would be an examination of the correlation achieved over successive days of sampling not isolated samples at infrequent intervals.

Plate 28 The Worthing Installation

Plate 29 The cistern prior to replacement



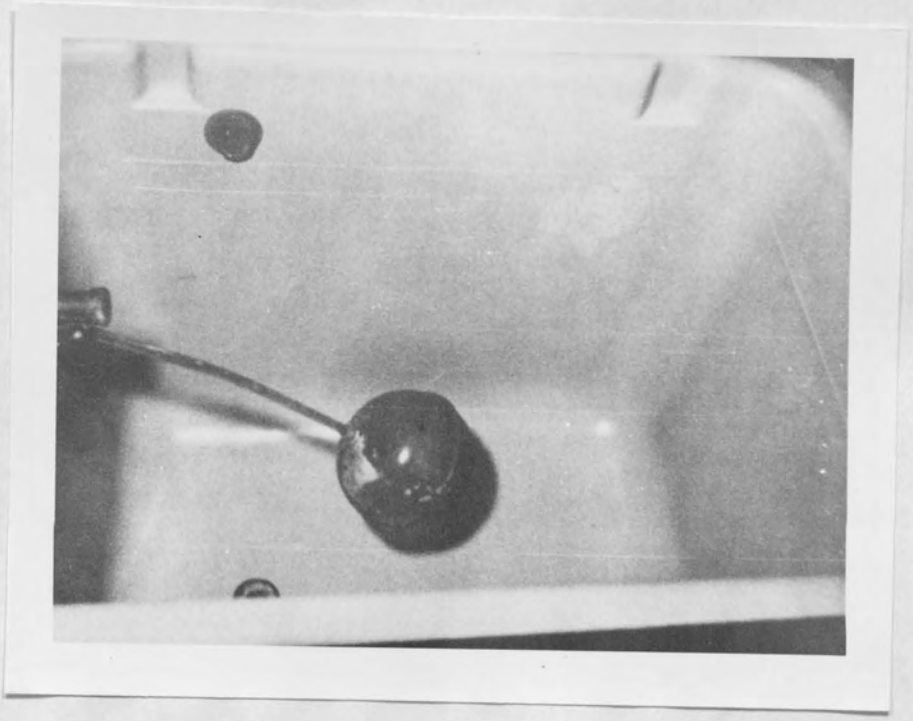
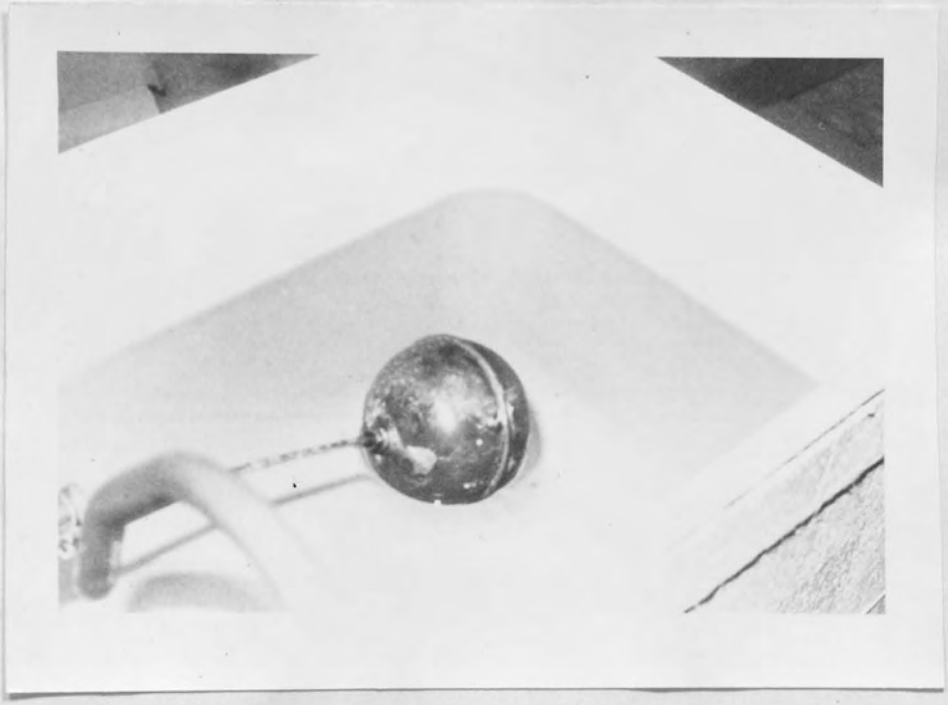


Plate 30 Replacement SMC cistern

Plate 31 Replacement SMC cistern after 3 weeks



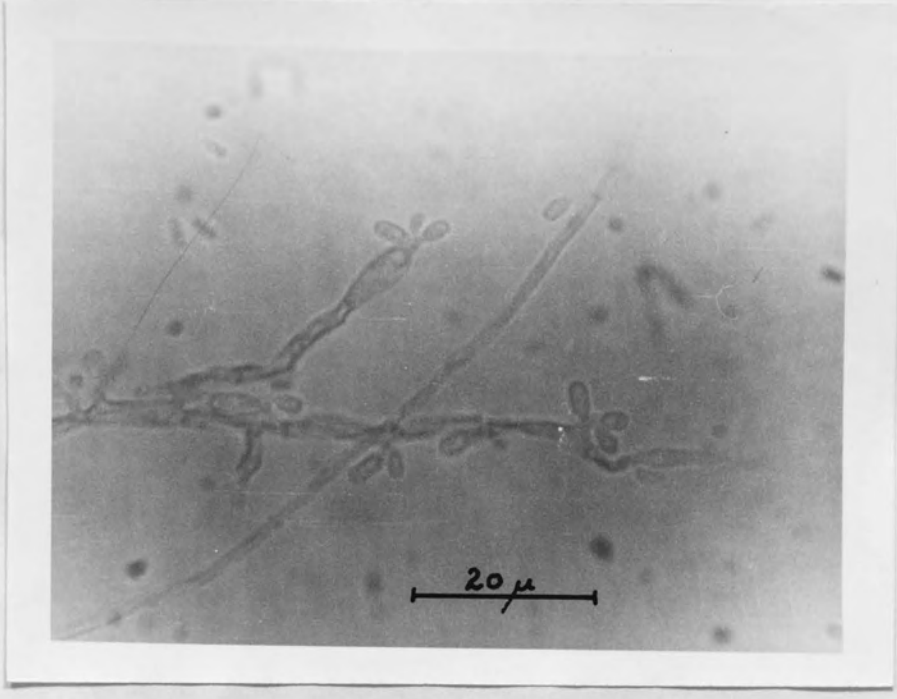
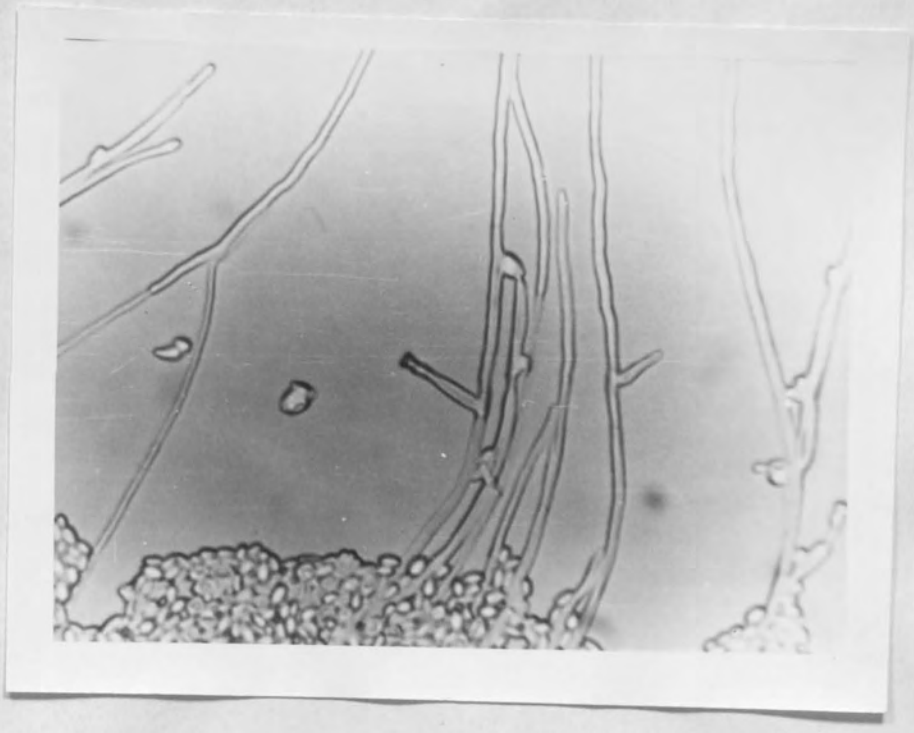


Plate 32 Worthing isolate

Plate 33 Worthing isolate- older culture



20μ

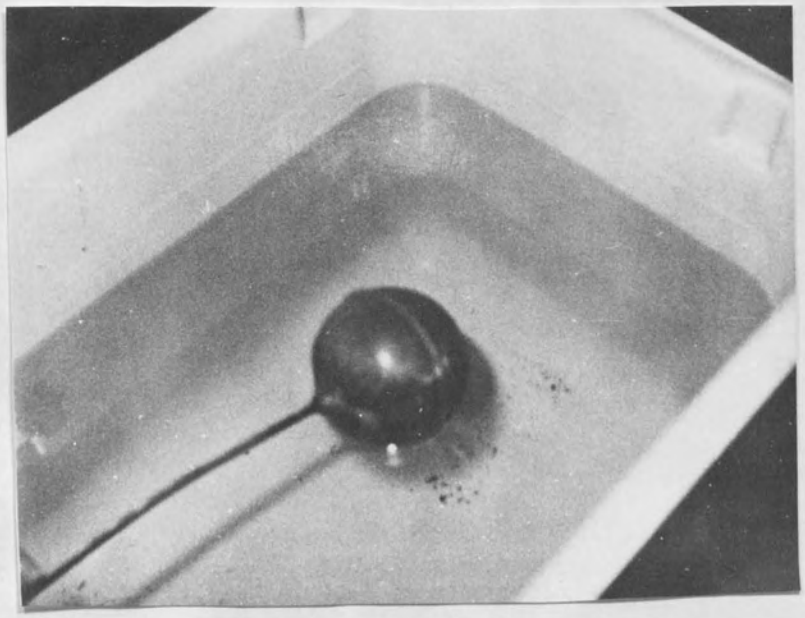


Plate 34 Replacement SMC cistern after 22 months

Chapter VII

7 Cooperative experimentation

7.1 Introduction

Cooperative experiments to establish testing techniques suitable for use in any normally equipped scientific laboratory are an established procedure in many fields. In the broader biological area, there are groups meeting at intervals to discuss common problems and experimental data, for instance the International Biodeterioration Research Group and the International Research Group for Wood, from whose documentation the method for establishing the water-holding capacity of soil (Chapter 2) was taken. In the plastics field Dolezel (1967) and Klausmeier(1970, 1970 b, 1972a, 1972b) reported interlaboratory testing methods and results. The interlaboratory tests described here are those which occurred jointly between BTR/BIC and MWB (TWA). Before 1973, when the author became involved in this project, two such experiments had been under-taken, one at Uxbridge at the BTR site and the other in Central London at BTR premises. These involved the setting-up of tanks to MWB specifications and their subsequent monitoring . Although the MWB were critical of the tests at Uxbridge, due to presence of free styrene in the factory atmosphere, which led to the tests in Central London, no evidence to support the Metropolitan Water Board's observations of 1972 was found in either location. It was the general dissatifaction with the MWB test method and the fact that it was not possible to

reproduce the 'gross fungal growth' claimed by MWB that led BTR to intiate and sponsor this project.

However, with the formation of the British Plastics Federation Committee to establish an agreed and acceptable test method for materials in contact with potable water, a joint interlaboratory experiment was proposed using the test with which the original observations on the support of fungal growth were made. It is that test and the nature of the results together with a description of the activities of the committee since 1976 that is described here.

7.2 Materials and Methods

The methods used were those detailed in 2.6, being the test employed at that time by TWA laboratory.

For reasons of economy and availibility the containers were Permali fuse box covers, a squareshallow tank of dimensions 250 mm sq. x 100 mm. The mouldings were fabricated at BTR, Uxbridge, the tool being loaned by Permali (at that time a separate compnay).

The materials used in their fabrication were not known by the author and details were not made available to other participating laboratories, but the requirement was that as far as possible one set of containers should be moulded from almost standard BTR SMC CWC (cold-water cistern compound), one set capable of supporting heavy microbial growth and composed probably by incorporation of abnormally high incubation of zinc- stearate and poly-thene and of short cure-time and the third set from SMC compound having a very low level of all

components, implicated by any party in microbiological growth, compatible with the production of high-quality mouldings i.e. good, standard, poor

The containers for the test were dispatched to each laboratory directly from Uxbridge marked A, B or C and were tested as found with glass beaker (2l.) controls.

Three types of water were tested; distilled water, tap water and the artificial (MWB) water.

7.3 Results

The official summary of the results from all participating laboratories was composed by Dr. Burman (TWA). It is appended (Appendix 2).

The BIC failed all the tanks, but only on visible growth criteria. This was common to all laboratories and would seem to be a consequence of using a soil inoculum. This interpretation is confirmed by Dr. Burman who stated that 'he had never come across this before'. On all other testing requirments all the tanks passed and the full BIC results are presented in tables 17-34.

7.4 Discussion

The results obtained at the participating laboratories varied widely and in some cases there was total disagreement e.g. BIC and North Surrey - visible growth. However there was general criticism of this aspect of the soil inoclum and if rejections for visible growth are disregared a much clearer picture emerges.

BIC and North Surrey had identical assessment of all tanks in all waters except for one instance - fungal growth in synthetic water/tank A at North Surrey. ICI

were the only laboratory to fail on 37°C counts and pseudomonads, and together with a TWA rejection of a single tank, on 22°C counts. This may have been due to exceptionally low control counts at 22°C and 37°C. TWA were the only laboratory (apart from single incident at North Surrey) to fail on yeasts and fungi.

At first sight the results seem disappointing both for the seemingly erratic results the test gives between laboratories and also for BTR since all mouldings failed at all the laboratories, with the exception of North Surrey who only failed moulding A. However, if the above points are noted and applied a different pattern emerges.

Disregarding visible growth failures, the total rejections at BIC and North Surrey is 1 out of a possible 108 and for all four laboratories is 30 out of a possible 234 (13%). Since one of the mouldings was deliberately conceived to be poor in that it should be susceptible to microbial utilisation then one might expect a minimum of 16% failure if growth only failed this moulding in 50% of cases.

If ICI 22°C and 37°C rejections are disregarded due to their very low control counts then total rejections are 19 out of a possible 216 (9%) and finally if synthetic water results are disregarded due to the exceptionally heavy growth found in controls by the laboratories, then total rejections are 17 out of a possible 150 (11%).

The optimism that these results may have brought to BTR with regard to their products, might also be shared by the Expert Working Group ofthe British Plastics Federation Committee, in that a basis had been established for believing that a test reproducible between laboratories and within laboratories was possible. The test also highlighted areas of difference and directed the research to explore more fully the critical nature of the assessment of test results to distinguish those materials inherently susceptible to microbial utilisation.

The greatest weakness of the first interlaboratory experiment was that although material was provided that was consistently acceptable on assessment, no control material was provided that consistently failed. This may be due to either the test not being critical enough to distinguish such a material or alternatively even poor quality SMC GRP is not susceptible to decay. The microbial assessment of sheet laminate described earlier in Chapter 3 confirms that the latter may be the case, although these results were not available when the BPF experiment was being planned and the result was unforseen. A logical next step would be to test two related materials of known composition one of which consistently passed the other consistently failing when regularly tested by TWA laboratories. This is exactly what the test panel decided to do in late 1976. By that time the BIC was being sponsored by BPF directly as the offical sponsorship by BTR was terminated.

The material chosen was polyvinyl chloride, with or without plasticiser, with glass as the control. The materials were in the form of 6"x2" sheet in glass beakers and this simplification aided replication of the test system at low cost. Inoculation was of two types, either river water or mixed fungal pure cultures, and glass distilled water for water changes. The test procedure drawn up by the BPF is appended (Appendix 3).

Although the results again varied within and between participating laboratories a clear difference in assessment was possible between unplasticised PVC (UPVC) and plasticised PVC (pPVC). A persistent anxiety concerns glass controls which may vary widely within a single laboratory. Indeed instances of 10 fold differences between one control vessel and another are found.

This high level of variability has again prompted thought of a different form of testing and here various methods may be applicable. The method should reinforce or replace the results derived from the assessment of microbial populations, and should be reliable, simple and readily interpretable.

The first and most widely known of these alternative test methods is the COD determination. A refinement of that principle leads to Total Organic Carbon (TOC) determination which in essence is a chemical BOD determination. Both of these would distinguish between microbial growth resulting from indigenous carbon in the inoculum and that eluted, if any, from the material under

examination. However, polymeric substances such as polysaccharides, starch and cellulose would not be directly accounted for in a TOC value. The present acceptance procedure of the National Water Council contains safeguards in that a submitting manufacturer is obliged to submit an inventory of primary ingredients. At this stage any substances that the TOC would fail to detect might be picked up, but this relies solely on the information supplied by a manufacturer. The folly of one unscrupulous manufacturer demonstrated by subsequent in-service failure might condemn all manufacturers in the eyes of the testing authorities, and although cellulose and starch are no longer widely used in the plastic industry as fillers, the TOC would fail to pick it up and show an inherently susceptible material.

A similar problem may arise if a polymer is specified that previous reports had shown to be non-biodegradable. On submission of the list of ingredients to the NWC by the manufacturer, no rejection would be issued based on previous evidence. If that polymer however, was biodegradable but only over a long time period, then the short incubation period of the NWC/TWA microbiological test nor the alternative chemical methods would 'pick-up' the potential microbiological hazard. Polymers previously thought to be non-degradable but shown to be degradable on long incubation by Azadi-Bakhsh, 1972 and Kuster and Azadi-Bakhskh, 1973 are polyproplene, and polyvinylchloride.

A very precise method for determing microbial growth is with an Adenosine triphosphate (ATP) photometer. The determination is extremely rapid compared to normal quantitative biological methods and very sensitive.

These alternative methods, whilst offering many advantages and attractions, all fail in one regard. The Water Authorities are obliged to deliver water with a defined coliform count, they feel obliged to deliver water not containing Pseudomonas fluorescens on health grounds (although this is debateable - Strang, 1977) and believe that the presence of fungi, in producing a 'musty' taste, would mean a failure to deliver ' pure and wholesome water' - their legal requirement.

It is for these reasons that any future test may reasonably be expected to contain a coliform determination, a fungal determination and, until expert medical evidence to the contrary, a Pseudomonas fluorescens determination. If the ATP photometer or TOC detmination proves reliable and accurate then it may well be employed to back-up the specialised microbiological requirements laid down by TWA.

Tables 17-34 Microbiological sampling of GRP containers inoculated with soil and replenished with tap-water, artificial tap-water or distilled water.

Coliforms
Fungi
Yeasts
] expressed as numbers/100 ml.

$37^{o}C$ count
$22^{o}C$ count
Fluorescent pseudomonads
] numbers/ml.

Waters 1 Tap water
2 Distilled water
3 Artificial water

Inoculum Clent Soil (a)

Table No. 17

Sample Reference A — Tap Water

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
1	0	0	375	1350	>300000	171000	2250	140	TNC	TNC	TNC	TNC
2	0	0	1290	1410	33000	127500	550	1150	"	"	"	"
3	.5	1	820	670	104500	134000	16000	2700	"	"	"	"
4	0	0	770	705	111000	108500	18000+	4300	"	"	"	"
5	0	0	1050	760	92500	103500	16000	1750	"	"	"	"
6	0	0	675	725	93000	110000	1700	16000	"	"	"	"
7			1940	945	>300000	186500	1750	16000	"	"	"	"
8			2385	1075	217500	236000	500	2250	"	2000	"	2600
9			2325	960	>300000	210000	120	5000	"	230	"	110
10			>3000	1230	"	>300000	3400	3400	260	40	1000	3500
11			"	2220	"	"	600	1900	TNC	170	1300	TNC
12			"	2835	"	"	250	1700	370	130	485	"
13			"	>3000	"	"	1900	2000	TNC	290	TNC	"

Table No. 18

Sample A — Tap Water

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
14			>3000	>3000	>300 000	>300 000	5000	3500	TNC	TNC	TNC	TNC
15			"	"	"	"	1600	8000	"	"	"	"
16			"	"	"	"	7000	9500	"	"	"	"
17			"	"	"	"	16000	8000	"	"	"	"
18			"	"	"	"	2800	11000	"	"	"	"
19			"	"	"	"	1100	16000	"	"	"	"
20			"	"	"	"	400	16000	"	"	"	"
21			"	"	"	"	1750	9000	"	"	"	"
22			"	2465	"	"	5000	3500	"	"	"	"
23			"	2960	"	"	8000	1300	>1000	>1000	"	"
24			"	2050	"	"	8000	2500	"	"	"	"
25			"	1870	"	"	5000	4250	"	"	"	"
26			"	2375	"	"	7000	3500	"	"	"	"

Table No. 19

Sample B *Tap Water*

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
1	4·5	0	2050	1350	221000	171000	16000	140	TNC	TNC	TNC	TNC
2	0	1	245	1410	145500	127500	16000	1150	"	"	"	"
3	0	1	176	670	197500	134000	1400	2700	"	"	"	"
4	0	0	1340	705	127000	109500	5500	4800	"	"	"	"
5	0	0	1550	760	195500	103500	5000	1750	"	"	"	"
6	0	0	1105	725	182500	110000	5500	16000	"	"	"	"
7			985	945	7300000	186500	2500	16000	"	"	"	"
8			870	1075	"	236000	2500	2250	1570	2000	"	2600
9			805	960	"	210000	2200	5000	150	230	420	110
10			865	1250	"	7300000	1900	5400	250	40	30	3500
11			1450	2220	"	"	700	1900	110	170	150	TNC
12			1865	2855	"	"	400	1700	40	150	520	"
13			2650	73000	"	"	1150	2000	290	290	TNC	"

Table No. 20

Sample B *Tap Water*

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
14			73000	> 3000	>300 000	>300 000	9000	3500	250	TNC	TNC	TNC
15			2955	"	"	"	11000	8000	TNC	"	"	"
16			73000	"	"	"	8000	9500	"	"	"	"
17			"	"	"	"	9500	8000	"	"	"	"
18			"	"	"	"	16000	11000	110	"	"	"
19			2765	"	"	"	18000 +	16000	TNC	"	"	"
20			73000	"	"	"	"	16000	"	"	"	"
21			"	"	"	"	16000	9600	"	"	"	"
22			"	2465	"	"	16000	3500	"	"	"	"
23			"	2960	"	"	11000	1500	> 1000	> 1000	"	"
24			2340	2050	"	"	9000	2520	"	"	"	"
25			2890	1870	"	"	16000	4250	"	"	"	"
26			73000	2375	"	"	16000	3500	"	"	"	"

Table No. 21

Sample C — Tap Water

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
1	0	0	5200	1850	198 000	171 000	170	140	TNC	TNC	TNC	TNC
2	0	1	2150	1410	69 000	127 500	18000+	1150	"	"	"	"
3	0	1	2675	670	182 500	134 000	18000+	2760	"	"	"	"
4	0	0	2950	705	175 500	108 500	18000+	4500	"	"	"	"
5	0	0	1495	760	184 500	105 500	16000	1750	"	"	"	"
6	0	0	1825	725	108 500	110 000	18000+	16000	"	"	"	"
7			2775	945	165 500	186 500	5000	"	"	"	"	"
8			5150	1075	7500 000	236000	8000	2250	"	2000	1010	2600
9			73000	960	"	210 000	1600	5000	450	250	50	110
10			"	1250	"	7500 000	2800	5400	450	40	1820	3500
11			"	2220	"	"	220	1900	290	170	840	TNC
12			2980	2835	"	"	400	1700	320	130	1150	"
13			73000	73000	"	"	2000	2000	TNC	290	TNC	"

Table No. 22

Sample C — Tap Water

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
14			73000	73000	730000	730000	2500	3500	TNC	TNC	TNC	TNC
15			"	"	"	"	2500	8000	"	"	"	"
16			"	"	"	"	5000	9500	"	"	"	"
17			"	"	"	"	8000	8000	"	"	"	"
18			"	"	"	"	7000	11000	"	"	"	"
19			"	"	"	"	5000	16000	"	"	"	"
20			"	"	"	"	11000	"	"	"	"	"
21			"	"	"	"	16000	9000	"	"	"	"
22			"	2465	"	"	"	5000	"	"	"	"
23			"	2960	"	"	"	1300	>1000	>1000	"	"
24			"	2050	"	"	8000	2500	"	"	"	"
25			"	1870	"	"	11000	4250	"	"	"	"
26			"	2375	"	"	9000	3500	"	"	"	"

Table No. 23

Sample A *Distilled Water*

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
1	2.5	2	1940	915	7300 000	121 500	16000	1100	TNC	TNC	TNC	TNC
2	0	0	3510	770	"	105000	550	4850	"	"	"	"
3	0	1	73000	570	"	91000	9000	1400	"	"	"	"
4	0	0	2345	550	"	101 000	16000	3500	"	"	"	"
5	0	0	2100	695	209 500	88 500	16000	3500	"	"	"	"
6	0	0	1265	660	243 500	90 500	16000	9000	"	"	"	"
7			2345	950	7300 000	241 000	8000	800	"	"	"	"
8			2935	875	"	7300 000	3500	5000	2650	2650	73000	1910
9			73000	1165	"	"	100	400	70	70	340	140
10			"	2380	"	"	40	4500	70	70	530	3500
11			"	2470	"	"	140	3500	90	90	330	2470
12			"	73000	"	"	400	600	50	50	480	260
13			2770	"	"	"	3500	5000	TNC	TNC	TNC	3160

Table No. 24

Sample A *Distilled Water*

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
14			> 3000	2765	>300 000	>300 000	3500	1750	TNC	1010	TNC	TNC
15			2910	>3000	"	264 500	3500	7000	"	TNC	"	"
16			2855	"	"	287 000	1100	9000	"	"	"	"
17			2760	"	"	>300 000	2500	8000	"	"	"	"
18			>3000	2865	"	"	3300	16000	"	"	"	"
19			"	>3000	"	"	7000	"	"	"	"	"
20			2945	"	297 500	"	11000	18000 +	810	"	"	"
21			>3000	2840	>300 000	"	4250	11000	TNC	"	1100	"
22			"	>3000	276 500	"	9000	5000	"	"	850	"
23			"	"	>300 000	291 000	16000	7000	>1000	> 1000	785	"
24			2680	"	291 000	>300 000	"	9000	"	"	TNC	"
25			2815	"	>300 000	"	"	9000	370	"	320	"
26			>3000	"	"	"	5000	7000	460	"	890	"

Table No. 25

Sample B *Distilled Water*

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
1	0		510		171 000		18000 +		TNC		TNC	
2	3		270		46 000		16000		"		"	
3	0		615		168 500		18000 +		"		"	
4	0		730		163 500		16000		"		"	
5	0		675		154 000		8000		"		"	
6	0		990		161 000		4250		"		"	
7			1705		283 000		5000		"		2910	
8			2455		7500 000		2500		"		820	
9			2910		"		160		71000		750	
10			73000		"		20		570		16260	
11			"		"		240		450		TNC	
12			"		"		80		170		"	
13			"		"		350		TNC		"	

Table No. 26

Sample B *Distilled Water*

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
14			> 3000		> 300 000		425		TNC		TNC	
15			"		"		80		"		"	
16			"		"		50		"		"	
17			"		"		90		260		"	
18			"		"		70		TNC		"	
19			"		"		350		"		"	
20			"		"		1600		430		~ 12000	
21			"		"		175		480		"	
22			"		"		425		470		"	
23			2640		"		8000		320		"	
24			2890		"		1600		TNC		"	
25			> 3000		"		3500		570		~ 9500	
26			"		"		3500		TNC		"	

Table No. 27

Sample C *Distilled Water*

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
1	1		3150		252 000		700		TNC		TNC	
2	0		1740		64 000		16000		"		"	
3	0		675		190 500		9000		"		"	
4	0		920		194 500		16000		"		"	
5	0		1005		123 500		8000		"		"	
6	0		1135		107 000		3500		"		"	
7			1215		>300 000		9000		"		"	
8			1275		207 000		5000		1640		>3000	
9			1510		291 000		160		225		890	
10			1765		>300 000		700		290		1090	
11			2450		"		220		190		> 3000	
12			2970		"		260		1230		2990	
13			> 3000		"		1400		TNC		TNC	

Table No. 28

Sample C — Distilled Water

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
14			75000		>500 000		5000		TNC		TNC	
15			2470		"		3400		"		"	
16			73000		"		1900		"		"	
17			2960		"		550		"		"	
18			73000		"		1750		1600		> 4000	
19			2985		"		8000		"		TNC	
20			2540		"		5000		"		"	
21			2815		232 500		4250		"		"	
22			> 3000		274 500		2250		"		> 3000	
23			2765		256 000		3500		> 1000		TNC	
24			2660		>500 000		3500		"		"	
25			2845		"		5000		"		"	
26			2510		"		3500		"		"	

Table No. 29

Sample A *Artificial Water*

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
1	0	2	920	1845	7300 000	130 500	18000 +	140	TNC	TNC	TNC	TNC
2	0	1·5	850	1475	"	267500	200	1300	"	"	"	"
3	0	3	1050	1360	"	109000	170	160	"	"	"	"
4	0	0	1115	1515	"	32500	850	140	"	"	"	"
5	0	0	1265	1460	"	91000	160	120	"	"	"	"
6	0	0	805	1090	"	88500	250	150	"	"	"	"
7			1310	2185	"	7 300 000	500	140	"	1090	"	"
8			945	2770	"	274 000	500	250	"	210	"	7 30 000
9			1655	7 3000	"	7 300 000	900	400	"	150	"	"
10			2370	"	"	"	260	18000 +	1470	120	8140	12400
11			2755	"	"	"	1900	1000	40	110	4170	TNC
12			7 3000	2875	"	"	16000	1500	40	370	4220	"
13			"	7 3000	"	"	16000	1950	100	TNC	TNC	"

Table No. 30

Sample A — Artificial Water

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
14			> 3000	73000	7300 000	7300 000	16000	2500	TNC	TNC	TNC	TNC
15			2945	"	"	"	18000 +	1400	"	"	"	"
16			73000	"	"	"	16000	1100	"	"	"	"
17			2965	"	"	"	18000 +	1750	"	"	"	"
18			73000	"	"	"	18000 +	1200	"	250	"	"
19			"	2790	"	"	11000	500	"	TNC	"	"
20			"	73000	"	"	8000	425	"	"	"	"
21			2870	"	"	"	11000	250	"	260	~ 9000	"
22			73000	2615	"	"	9000	350	"	TNC	TNC	"
23			"	> 3000	"	"	9000	950	> 1000	> 1000	"	"
24			"	2935	"	"	5500	1100	"	"	"	"
25			2735	2765	"	"	3500	1400	"	"	"	"
26			2940	2870	"	"	3500	1100	"	"	"	"

Table No. 31

Sample B — Artificial Water

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
1	1		1810		7800 000		16000		TNC		TNC	
2	0		73000		"		18000 +		"		"	
3	0.5		2105		"		"		"		"	
4	0		2090		"		"		"		"	
5	0		1415		"		16000		"		"	
6	0		1575		"		9000		"		"	
7			895		"		2500		"		"	
8			1455		"		3500		2940		2310	
9			2520		"		1600		410		2500	
10			2995		"		7000		1610		6520	
11			73000		"		2800		200		TNC	
12			"		"		1100		70		"	
13			"		"		8000		20		"	

Table No. 32

Sample B *Artificial Water*

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
14			> 3000	> 3000	>300 000	>300 000	5000	2500	70	TNC	TNC	TNC
15			"		"		5500		90		"	
16			"		"		9000		55		"	
17			"		"		4250		40		"	
18			2870		"		3500		10		~ 52 000	
19			73000		"		3500		25		TNC	
20			2580		"		9000		70		"	
21			73000		"		5500		35		"	
22			"		"		16000		90		"	
23			"		"		16000		145		"	
24			"		"		3500		35		"	
25			"		"		2000		45		"	
26			"		"		2750		20		"	

Table No. 33

Sample C *Artificial Water*

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
1	2		1320		>300 000		1150		TNC		TNC	
2	0.5		2600		"		5500		"		"	
3	0		1745		"		9000		"		"	
4	0		1930		"		8000		"		"	
5	0		1470		"		16000		"		"	
6	0		1210		214 500		"		"		"	
7			1655		>300 000		"		"		"	
8			1985		"		700		1550		"	
9			2360		"		80		190		"	
10			>3000		"		7000		550		"	
11			"		"		160		90		"	
12			"		"		260		140		28 280	
13			"		"		5000		370		TNC	

Table No. 34

Sample C — Artificial Water

Sample No.	Coliform		37°C Count		22°C count		Fluorescent pseudomonads		Fungi		Yeasts	
	T	C	T	C	T	C	T	C	T	C	T	C
14			> 3000		> 300 000		8000		TNC		TNC	
15			"		"		2500		"		"	
16			"		"		1200		250		"	
17			"		"		3500		TNC		"	
18			"		"		1400		"		"	
19			2910		"		3500		"		"	
20			2745		"		5500		"		"	
21			> 3000		"		1300		"		"	
22			"		"		1300		"		"	
23			2795		"		1750		> 400		"	
24			> 3000		"		950		"		"	
25			"		"		2000		"		"	
26			"		"		2500		"		"	

CHAPTER VIII

Discussion and General Conclusions

The studies described in previous chapters were designed to examine fundamental questions regarding the susceptibility to microbial degradation of SMC GRP as moulded by BTR Reinforced Plastics Limited, and the behavioural differences in-service between 'Preform' and 'SMC' cisterns in an area of known biological hazard. Advancement of knowledge in any subject usually proceeds in a stepwise manner, with the successive addition of small items of new knowledge to the sum of previous knowledge. The research process designed to elicit new information necessarily uses the body of known information to formulate working hypotheses and subjects the results produced to strict scrutiny to ensure that as far as possible the phenomenon examined has not been erroneously recorded or that artifacts have interfered with the process in any way. Only after successfully negotiating the rigours of this process are most experimental results finally accepted as valid and incorporated in the total body of knowledge to be used in the formation of future hypotheses.

It is inevitable that in a few instances, due to either insufficient rigour or the workings of chance, erroneous 'facts' will be temporarily added to the sum of information. Discovery of the mistake may occur with therepetition of the original experiments or by further

research producing rigorously accepted results which cannot be matched with the previous work. One obvious way to eliminate as far as possible a chance occurrence interfering with the recording procedure is to replicate the test-system or repeat the experiment re-using the test-system. It is fair to say that the majority of workers adopt this approach if possible, and replication of some form is present in nearly all widely used test methods.

In this light, perhaps the origins of this investigation may be commented upon in isolation from the results of the investigation they were responsible for.

The Metropolitan Water Board issued a notification of failure, their criteria being 'gross fungal growth' in a single cistern submitted for test. No similar cistern was requested from BTR for comparison purposes, the original cistern was not scrupulously cleaned and re-tested, nor was it retained in its contaminated condition for BTR to examine for themselves. In this respect replication was totally lacking from the test-system yet, the evidence of a single instance invoked serious statutory limitations on BTR which if followed through would have led to cessation of all cistern and sectional-tank manufacture. BTR managed to obtain a provisional acceptance, strictly limitedto the period required to re-submit the cistern to the test, and in conjunction with the MWB and

BIC established replicated test cisterns at Uxbridge and later in Central London (these joint tests are described further in Chapter 7). At no time was the original MWB observation confirmed. Had the total observations been critically examined at that stage there must have been doubts concerning the MWB demands for the investigations reported here.

In Chapter 3 it was shown that two ingredients of the GRP consistently supported growth; zinc stearate and titanium dioxide (RCR-2). Neither of the two polyester resins was found to be capable of supporting microbial growth. From a consideration of their chemistry this finding is in agreement with that of Demmer (1968), who showed that esters of phthalic acid were not attacked by five test fungi, and Potts at. al. (1973) who showed that aliphatic polyesters were the only polyesters capable of being degraded by microorganisms. They also showed that polyesters based on fumaric acid, an unsaturated dibasic acid, were utilised more poorly than those based on saturated dibasic acids such as succinic and adipic acid which are commonly used in plasticizer and polyurethane polyesters. They further showed that aromatic structures as exemplified by terephthalate render the polyester unassimilable. The support by stearate was not unexpected, but pure titanium dioxide one would not expect to be capable of supporting microbial growth. However, it is known that the RCR-2 is a 'coated powder form' of titanium dioxide and, although the manufacturer could not release

details of the coating material in this case, they are very often low molecular weight organic compounds designed to aid mixing and wettability (Mallorie - personal communication). Five constituents of GRP were found capable of inhibiting microbial growth when tested in isolation but, as was later shown, incorporation of these materials at manufacturing levels did not produce a susceptible or inhibitory laminate. Heap and Morrell (1968) postulated that crosslinking agents are active fungicides and hence diminish attack and the crosslinking agent in SMC was one of five compounds capable of showing inhibition.

Although BTR did eliminate one range of pigments from the mix after they were shown to support microbial growth, and a fungistatic pigment range used instead, this should be taken as the logical response of a responsible manfacturer to the information presented to him.

It was shown that in liquid testing or respirometric studies of samples, laminate in sheet form was not attacked by microbes. SMC laminate was unaffected by soil burial and its leachate not capable of supporting microbial growth. This is in agreement with the burial studies of Kwei (1972), and Klein (1972) and Ventrice (1972) where glass-reinforced polyester resin systems were essentially unaffected, no microbial degradation being found after eight years, even though some contained starch-sized glass fibres. Evens and Levisohn (1968) did show the

growths of fungi viz., Mesobotrys, Metarrhizium, Monilia, Scopulariopsis and a few bacteria in the aqueous leachate of polyester but no chemical formulation of the polyester was given. It is highly unlikely that the polyester was similar to the SMC polyester resin system used in cistern manufacture as Evens and Levisohn were investigating Polyester-polyurethanes.

Degradation was observed on 8% zinc stearate laminate and undercured laminate in a number of tests. Undercured laminate was the more susceptible of the two and supported growth particularly if ball-milled or as leachate. Ball-milling of laminate material generally made the material more susceptible to attack in all forms of testing, thus supporting Hueck (1973,1974), who stated that non-biotic degradation of polymer made the material more susceptible in secondary microbiological degradation.

The results obtained from laboratory testing of laminates are supported by the BPF/NWC style testing. Of the laminates subjected to this scheme of testing none failed. The BPF cooperative experiment (chapter 7) and the two experiments on cisterns preformed prior to October 1973 in conjunction with Dr. Burman (MWB) also support the findings of the laboratory tests. However, they demonstrate too, the failure of the MWB to support its original claim that 'gross fungal growth' was supported by the BTR SMC cistern.

Although it was shown that material is lost when GRP is in contact with water, the weight loss and water uptake experiments demonstrate that:

a. degradation of the bulk structure is occurring and low molecular weight products are probably being formed by hydrolysis and leached.

b. that except with 8% zinc stearate and highly undercured laminate, this material does not support microbial activity.

c. the amount of this material leached out under normal operating conditions would be very small and could not be detected after three months by weight loss and was 0.09% after 6 months.

In chapter 4 it was demonstrated that the behaviour of GRP tanks in simulated operating conditions is very similar to competitive materials and perhaps slightly better in that the means of populations of microbial groups studied were found to differ significantly from the incoming water and in no case does the level of the population in the tank approach the 10x criterion used for BPF, NWC, testing.

Comparison of tanks in different locations suggests that Worthing water effects the 22°C population in some way as it was found to be significantly different to the

mean 22°C figure for other areas. Further sampling may help to establish whether the Worthing fungal population is significantly different. At the moment the standard deviations of the means and the problems of analysing small samples (less than 30 readings) do not allow us to suggest a difference statistically although again the Worthing mean 'appears' to be different from the others.

The SEM studies detailed in Chapter 5 showed that cure temperature difference (135 or 155°C) had no discernable effect on surface morphopogy of the moulding. If the 'Worthing problem' is dependant on surface morpholgy it would not therefore appear to be dependant on mould temperature. We might also believe it possible to eliminate mould temperature as a manufacturing variable contributing to the 'failure' of the submitted cistern, and also surface cracking due to transit or mechanical testing when the colonisation patterns of leached GRP (Chapter 3)are examined. The studies also showed that abnormally high concentrations of zinc stearate and the absence of polyethylene and stearate had no effect on surface appearance. However, when an etching agent was applied the laminates rich in stearate were found to undergo significant 'pitting' or shallow depression forming. This is in contrast to the appearance of surfaces of laminate with none or manufacturing levels of stearate and also in contrast to the appearance of laminate of preform or SMC after contact with water only and water plus organisms.

This suggests that water alone does not remove zinc stearate from the surface, so providing organisms with a carbon source, and that the organism found in Worthing has not derived its nutrition by some mechanism of stearate extraction.

Comparison SEM studies of SMC and preform surfaces show that the effect of water is very similar, both laminates showing pitting of resin-rich areas and the 'eruption' of glass caused by stress cracking of resin overlaying the glass due to the water ingress. The findings for zinc in the bulk structure of SMC but not on the surface is what one might theoretically predict from the study of surface free energy states.

The difference in the behaviour of the tanks in the Worthing area may be related to the 'channeling' noted on one sample taken from above the water line under SEM examinaton. This, and subsequent examination of the cistern, suggests the solubilisation over the tanks life of the surface of the preform tank and the possibility that it was of nutrient value to organisms. Microbial testing of the preform binder resin showed this substance to be resistant to microbial attack as also were glass sizes, another possible nutrient source suggested from the SEM.

Chapter 6, the investigation of the Worthing phenomenon showed the situation that can easily develop if dialogue between industry and the controlling body is stopped. It also showed the outstandingly different behaviour of SMC cistern in an area of known problems for GRP articles. Comparison may be made of complete fungal coverage in less than one month for preform cisterns to the complete absence of fungal deterioration in the replacement SMC cistern.

Taking all of these points into consideration, this project has shown that under no circumstance of testing is manufacturing grade SMC susceptible to microbial attack. Mouldings have been susceptible or have provided susceptible test materials but only where the laminate is either greatly undercured or a total of 8% stearate is in the composition and even then the necessity of a non-biotic stage of decomposition is shown. This has been provided by ball-milling or severe leaching.

This finding is in direct opposition to the recently quoted (Burman, 1976) figure of 60% failure for glass-reinforced plastics and to the original failure of the SMC GRP cistern submitted to Dr. Burman at the Metropolitan Water Board Laboratories. It was pointed out in the introduction that the commutations possible in GRP mix formulations are very large indeed and that the study of individual components will not lead to an

implicit knowledge of the 'whole'. Therefore one should set the 60% failure rate for all GRP articles tested by MWB and the failure rate achieved, jointly by this author and Dr. Burman himself, using BTR SMC GRP in Chapter 7. It was 13% maximum and included 33% of moulding deliberately conceived and designed to be failures.

Unfortunately, it is almost impossible to determine the cause of the initial SMC GRP cistern failure at the MWB laboratories in 1972, but this study has ruled out many variables such as cure-temperature and cure-time. The lack of customer in-service complaints lends support to the idea that the SMC production batch from which the tank was moulded was satisfactory, and the service record of over 1 million cisterns stands as testimony to the general high standard of the product.

Is it possible that the original MWB test result was a capricious event? If so, can it be determined ?

All the tests outlined in 1.3 are applied to a fitting submitted for testing and these occur in two phases:

a. mechanical testing

b. toxicological, organoleptic, physical and microbiological growth testing.

Only one fitting is submitted by the manufacturer and it was found that the mechanical testing was the first phase. This leads to a consideration of the deleterious effects any cracks or deterioration of the surface might have on the secondary phase of the submittal procedure. It was shown in Chapter 3, however, that severely surface-degraded laminates could not be distinguished from undegraded control samples in their ability to support microbial growth.

One possibility remains and is connected with both the mechanical testing and the initial stages of the microbiological procedure. This is the question of contamination. The SMC cistern was submitted six months before BTR was notified of its failure. Of that time only five weeks at most was spent at the MWB Laboratories for the secondary testing. This may be deduced from the MWB testing schedule (2.11). It is possible that during its period at the mechanical testing laboratory it was contaminated with organic debris, perhaps mineral oils, On arrival at MWB samples are tested with only a quick preparatory rinse (Burman - personal communication). If contamination with oil had occurred this rinse might only serve to redistribute the oil over the cistern surface, leading to the fungal growth phenomena on subsequent microbiological testing.

The inability of production SMC laminate to support microbial growth in any section of the microbiological

testing producedure leads to further avenues of thought. Perhaps the tests were not sensitive enough? This is not to say that a positive reaction in a more sensitive test would of itself lend credence to the original report of 'huge fungal growth', but does a more sensitive system exist or might it be devised. Mention has already been made of ATP determinations in Chapter 7 but the capital investmet required appears to have limited its availability and hence usage. Albertson and Ranby (1976) following on the work of Nykvist (1973) have developed a radiochemical technique to examine the degradation of polyethylene. The method, utilising C_{14}, is sensitive to 0.001% of the polymer sample. In the early stages of the investigation of SMC a detailed review of the possibility of adopting a similar radio-isotopic approach was conducted. However the general technique would seem much more suitable to the investigation of homopolymeric materials, such as polyethylene and polystyrene, rather than such a complex polymerised structure as GRP as it would require the seperate labelling of many of the constituents and, hence necessitating the testing of a large number of laminates each containing a different radio-labelled compound with a consequent increase in cost.

Alternatively, perhaps the sensitivity of the test methods employedwas sufficient and in effect SMC GRP may be considered a 'recalcitrant structure'. It is reasonable to suggest that 'recalcitrant molecules' are

possible, even biologically desirable (Iamanna,1976), but there exists also the capacity of organisms to adapt to environmental conditions and we should be aware that the present state of the literature and of scientific thought on polymeric materials may be a reflection of slow continuous mutation of organisms to the increasing environmental impact of plastics. However, there exist limits to biological adaptation, a function of genetic phenomena, and the study described in Chapter 6 presents information concerning one organism that had no capability to adapt from one type of GRP structure to another i.e. preform versus SMC GRP. The aim of the microbiological testing described in Chapter 3 was to determine whether the laminate or its constituents were sentitive to micro-organisms and represents an evolving study using progressidely more sensitive test-methods. In contrast, the MWB/NWC test procedure concentrates not on showing active utilisation of the test material,but on testing the levels of representative groups of organisms within the contained water, a feature which the studies in Chapters 4 and 7 were concentrated upon.

The slightly superior performance of BTR SMC cisterns as compared to competitive materials has already been noted, but it was also demonstrated that none of the cisterns undergoing microbial dynamics testing at the BIC showed support for the normal flora of tap water. In this situation is it realistic to fail materials for support

of coliforms or of Pseudomonas aeruginosa only, when the Water Authorities themselves are obliged to ensure that the materials in-service will not come into contact with these organisms. It may be argued that no system is foolproof and that any water distribution system risks a seriouscontamination incident. Following this reasoning, then a material capable of supporting these organisms would become a potential health hazard.

The NWC/TWA scheme of testing would appear to guard against this possibility, but close examination reveals serious flaws even in these points of the standard. Firstly, the testing is of limited duration - carried on until three successive counts are below ten times the control count or three successive counts above ten times the control count. There is no safeguard against a material apparently not able to support growth initially becoming conditioned by service and at a later stage being supportive. Secondly, the water throughput in the test-vessels is very low i.e. a long retention period. If a material is slightly supportive to growth this would tend to allow organisms time for adaptation and hence increase populations which in normal situations would not be in contact with the material long enough to be affected. For example, when a cistern is tested it has two changes of water per week. In most service conditions average retention time is roughly twelve hours i.e. 14 water changes per week. In an article such as a pipe joint not only does the flow of

water through the article have a self-cleaning effect but the volume of liquid in proximity to the article at times of standing is low. Both of these considerations apply to a contamination hazard of the water distribution network and relate directly to the fundamentals of the test-system. In the event of contamination of a very high-level the Water Authorities would surely advise all users to thoroughly flush their systems. Such low retention times as would ensue bear no relevance to the twice weekly changes incorporated in the test.

From these considerations, it is possible to lay down an outline of a possible test-routine for materials in contact with potable water. As a background to the proposal it is assumed that the Water Authorities will continue to operate routine microbiological sampling of their distribution system to monitor levels of coliforms, Ps. aeruginosa and any other organism they feel it necessary. The test should be rapid, reproducible and if possible, at low cost. It is suggested that sample material is placed in glass containers with identical glass controls (as for the latest BPF interlaboratory testing) and the water, after inoculation with a dilute sewage suspension, be analysed using the ATP determination. A second alternative would be to test the water for fungi and 37°C populations as these two tests have proved to be the main causes of failure of materials in previous testing (HMSO, 1973).

One recommendation that may be made to BTR and to all other manufacturers of cisterns and containers for liquids is one of general cleanliness. A lack of extraneous material within an environment implies a lack of both diversity and quantity of nutrients for microbial utilisation. Any scheme which tends to limit the absolute number of organisms within an invironment will tend to diminish the rate of appearance of mutants able to attack the material and the quantity of extracellular enzyme or metabolic by-products which may be able to render the material more susceptible to microbial attack. For these reasons manufacturers should strenuously urge those responsible for all new plumbing applications to clean out cisterns of cutting swarf, work debris etc and in order to cut down the input of atmospheric dust, carrier of both nutrients and organisms, supply cistern and lid as a single unit - a marketing policy of sound scientific worth.

Although BTR are still in the position of submitting new cisterns and tank panel materials to the National Water Council, the change in the relationship between BTR as a manufacturer and the Water Authorities is significant. BTR can now be more confident in the behaviour of their laminate material and the continuation of the BPF Expert Working Group enables concerned industrial organisations to be involved in the development of an effective test procedure.

The involvement of this project in not only microbiological testing but also the BPF committee, SEM studies and the investigation of a known instance of biological fallibility of GRP, illustrates the problem solving potential of the interdisciplinary approach to research.

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Appendix 1

LR 13921 1927	Pigments
OMYA BLR2	$CaCO_3$
MAGLITE D	Magnesium oxide
SNOWCAL 7ML	$CaCO_3$
ALKATHENE 73-03-00	polyethylene
TIOXIDE RCR2	Titanium dixoide
ZINC STEARATE	
TRIGANOX C	tertiary-butyl perbenzoate
TRIGANOX 29B50	benzoyl peroxide
RESIN 40-2386 RESIN 40-2397	Stypol resins
STYRENE	Styrene
BRITOMYA M	$CaCO_3$

c/Microfiche/2/75

APPENDIX 2

Director of Scientific Services
H Fish OBE, BSc, FRIC, FIPHE, FIWPC

Thames Water

New River Head, Rosebery Avenue,
London EC1R 4TP.

Telephone 01-278 2300 Ext 105

Date 11th November, 1975.

Your Ref.

Our Ref. NPB/ff

Please reply to: Manager, Metropolitan Water Services.

BPF Reinforced Plastics Group

Microbiological Expert Working Group

Report on Test Programme

Assessment of Acceptance of Samples

Laboratory : T.W.A.	Samples		
	A	B	C
Distilled Water			
Coliforms	A	A	A
37°Count	A	A	A
22°Count	R	A	A
Pseudomonads	A	A	A
Fungi	R	R	R
Yeasts	R	A	A
Visible Growth	R	A	A
Synthetic Water			
Coliforms	A	A	A
37°Count	A	A	A
22°Count	A	A	A
Pseudomonads	A	A	A
Fungi	A	A	A
Yeasts	R	A	A
Visible Growth	R	R	R
River-derived tap water			
Coliforms	A	A	A
37°Count	A	A	A
22°Count	A	A	A
Pseudomonads	A	A	A
Fungi	R	A	A
Yeasts	R	A	R
Visible Growth	R	R	R
Well Water			
Coliforms	A	A	A
37°Count	A	A	A
22°Count	A	A	A
Pseudomonads	A	A	A
Fungi	R	R	R
Yeasts	R	A	R
Visible Growth	R	R	A

Assessment of Acceptance of Samples

Laboratory : Aston University

	Samples		
	A	B	C
Distilled Water			
Coliforms	A	A	A
37°Count	A	A	A
22°Count	A	A	A
Pseudomonads	A	A	A
Fungi	A	A	A
Yeasts	A	A	A
Visible Growth	R	R	R
Synthetic Water			
Coliforms	A	A	A
37°Count	A	A	A
22°Count	A	A	A
Pseudomonads	A	A	A
Fungi	A	A	A
Yeasts	A	A	A
Visible Growth	R	R	R
Tap Water			
Coliforms	A	A	A
37°Count	A	A	A
22°Count	A	A	A
Pseudomonads	A	A	A
Fungi	A	A	A
Visible Growth	R	R	R

Assessment of Acceptance of Samples

Laboratory : I.C.I.	Samples		
	A	B	C
Distilled Water			
Coliforms	A	A	A
37° Count	R	R	R
22° Count	R	R	R
Pseudomonads	A	R	A
Fungi	A	A	R
Yeasts	A	A	A
Visible Growth			
Synthetic Water			
Coliforms	A	A	A
37° Count	A	A	A
22° Count	A	A	A
Pseudomonads	A	A	A
Fungi	A	A	A
Yeasts	A	A	A
Visible Growth			
Tap Water			
Coliforms	A	A	A
37° Count	A	R	R
22° Count	R	R	R
Pseudomonads	A	R	A
Fungi	A	A	R
Yeasts	A	A	A
Visible Growth			

Assessment of Acceptance of Samples

Laboratory : North Surrey	Samples		
	A	B	C
Distilled Water			
Coliforms	A	A	A
37°Count	A	A	A
22°Count	A	A	A
Pseudomonads	A	A	A
Fungi	A	A	A
Yeasts	A	A	A
Visible Growth	A	A	A
Synthetic Water			
Coliforms	A	A	A
37°Count	A	A	A
22°Count	A	A	A
Pseudomonads	A	A	A
Fungi	R	A	A
Yeasts	A	A	A
Visible Growth	A	A	A
River-derived Tap Water			
Coliforms	A	A	A
37°Count	A	A	A
22°Count	A	A	A
Pseudomonads	A	A	A
Fungi	A	A	A
Yeasts	A	A	A
Visible Growth	A	A	A

APPENDIX 3

REINFORCED PLASTICS GROUP - Microbiological Expert Working Group

Test procedure for 2nd. stage experimental programme .

1. Samples

Samples shall be of sheet material cut to size 6" x 2" and shall be of the following materials a. Plasticized PVC. Preferably with a phthalate plasticizer or failing that a sebacate plasticizer at the maximum concentration at which these are normally used for flexible tubing.

b. Unplasticized PVC of any make that is used for cold water pipes.

c. Glass sheet as controls.

It was agreed that BPF would approach ICI for the supply of the PVC materials.

2. Sample Containers

The samples should be placed in glass beakers or wide-mouthed flasks of 1.5 or 2 litre capacity which have been cleaned with Decon 90 (Decon Labs. Ltd.). A 3% - 5% (v/v) solution should be used with steaming (or boiling) for 2h. followed by rinsing with tap water and finally distilled water. The higher concentration should be used on glassware that has been previously used with samples that support growth.

3. Initial Setting Up

The three materials should be set up with distilled water, with two types of inocula, and should be replicated at least 3 and preferably 5 times. This would require a total of at least 18 containers or at most 30 containers.

The samples should therefore be distributed in the appropriate number of flasks. Half of the flasks should have inoculum A added and half inoculum B.

Inoculum A: Either 1. Sewage effluent, preferably from a mixed domestic/industrial source, which is of a quality that the water course to which it discharges is suitable for abstraction for treatment for drinking water below the effluent discharge, or 2. River water that receives both mixed sewage effluent and land drainage and which is suitable for abstraction for drinking water. The inoculum should be standardised on its content of coliform organisms and Pseudomonas aeruginosa. A volume of inoculum should be chosen to contain between 100 and 1000 coliform organisms and Ps. aeruginosa

must be shown to be present. This will require some preliminary counts to determine its suitability.

Inoculum B: For the purposes of this trial 6 cultures will be supplied of organisms recently isolated from growth tests and which have been grown on a number of different materials. These will be identified as follows:-

TWA 1 Pseudomonas aeruginosa, pyocine type 29
TWA 2 Pseudomonas aeruginosa, pyocine type 31
TWA 3 Citrobacter freundii
TWA 4 Aeromonas formicans
TWA 5 Unidentified yeast-like organism A
TWA 6 Unidentified yeast-like organism B

Insufficient progress has been made on isolation of other fungi to be able to recommend suitable strains for use in the current exercise.

Suspensions of these organisms should be prepared as follows. The cultures of TWA 1-4 can be maintained on nutrient agar. TWA 5&6 can be maintained on malt agar. Initial subcultures should be made into peptone water and incubated at 30°C for 24 hours for TWA 1-4 and at 25°C for 3 days** For TWA 5&6. After the specified time subcultures should be made into tubes of the same medium using a standard inoculum, either a straight wire or a standard loop, and these should again be incubated for the specified time. These cultures should then be subcultured to nutrient agar and malt agar slopes respectively, using a standard drop of 0.05ml. After further incubation for the appropriate time at the specified temperature the growth should be washed off in normal saline, centrifuged and resuspended in normal saline. Serial dilutions should be prepared and the no. of viable cells counted by poured plates on the same medium.

A quantity of each suspension should be used for the final inoculum B to contain between 100 and 1000 organisms.

The volume in all the flasks should be made up to 1l. with glass distilled water which has been stored in glass containers.

Each flask or beaker should be covered with aluminium foil or with a glass beaker and incubated at 30°C in the dark.

** The temperature and time are not critical so long as the same technique is used each time. Temperatures from 20-30°C are satisfactory, selecting a time that gives maximum growth.

4. Growth assessment procedure

After 3 or 4 days, 10 ml of the water from each flask should be withdrawn and set on one side in sterile containers for re-inoculation. Enumeration of coliform organisms, Ps. aeruginosa, fungi, yeasts and bacteria capable of growth at 22°C and 37°C should be carried on the remaining water.

Each flask should also be examined for visible growth which could be manifest as films of microbial slime on the surface of the sample or as aggregates of organisms suspended in the water or as turbility. All such visible phenomena should be confirmed as microbial by microscopical examination.

The residual water should be discarded, the 10 ml volume set aside to be put back in the flask and the volume made up to 1l with glass distilled water. This incubation and enumeration procedure should be repeated at intervals of 3 or 4 days (ie. twice weekly) as long as is necessary to complete the test. If this work load is excessive, the enumeration procedure should be carried out once a week, but the water changing procedure must continue twice a week.

5. Interpretation

The criteria required for passing these tests are that the counts in the test flask should not exceed 10 times the counts in the control flask. The test should therefore be continued as long as the counts in the test flasks are declining or until they come within the tenfold limit. If counts are increasing the test can be stopped if they reach and remain above the 10 fold limit. Samples should not be deemed to have failed the test unless counts have remained above the 10 fold limit for three consecutive estimations.

Ps. aeruginosa and coliform organisms should be absent from 100 ml in 3 consecutive estimations.

The presence of a visible film on the sample or turbidity in the water which is confirmed on microscopic examination as growth, also constitutes a failure.

Enumeration techniques

(a) Coliform organisms may be counted by the standard membrane filtration technique or the standard multiple tube method in "The Bacteriological Examination of Water Supplies", Reports on Public Health and Medical Subjects No. 71, London, HMSO 1970 (usually referred to as Report 71).

(b) Colony counts at 37°C and 22°C are counted by the standard agar plate count procedure using yeast extract agar, also given in Report 71.

(c) Ps. aeruginosa. A volume of 100 ml of the test water is filtered through a sterile standard bacterial membrane of pore size 0.45 u which is then placed face upward on a previously poured Kings A agar petri plate. This is incubated at 41.5 ± 0.5°C in a closed container for 24h. Colonies which are flat in appearance, approximately 1-2 mm in diameter, green in colour and fluorescent under ultra-violet light are counted as Ps. aeruginosa. They may be confirmed by subculture from the membrane to milk agar + 0.03% centrimide, incubating at 41.5 ∓ 0.5°C for 24h and examining for fluorescence with a clear zone of casein hydrolysis.

(d) Fungi and Yeasts. A volume of 100 ml of the test water (or less if numbers are high) is filtered through a sterile standard bacterial membrane of pore size 0.45 u which is then placed face upwards on a previously poured Martin's Rose Bengal agar plate containing kanamycin (100 ug/ml). This is incubated at 22-25°C for 7 days. Colonies of fungi are easily recognised and counted. Colonies of yeasts are usually raised, smooth and shiny. The only other organisms which occasionally grow on this medium are aerobic sporing bacilli which are usually small, dry and wrinkled. If in doubt, they may be confirmed by microscopical examination.

These techniques are intended as reference methods and are not intended to preclude the use of other techniques that can be shown to give results that are not significantly different, if this is more convenient in any participating laboratory.

British Plastics Federation

APPENDIX 4

Expert Working Group of the
Microbiological Growth Sub-Committee

Report by B.I.C. for discussion
on 15th February 1974

Biodeterioration Information Centre.
British Plastics Federation - Expert Working Group
of the Microbiological Growth Sub-Committee.

APPENDIX 5

A POSSIBLE PROCEDURE FOR THE FORMULATION OF AN INTERIM TEST FOR MICROBIOLOGICAL GROWTH ON PLASTIC MATERIALS