STUDIES ON THE COMPLETELY MIXED ACTIVATED SLUDGE TREATMENT OF FELLMONGERY AND TANNERY LIME-SULPHIDE EFFLUENTS

by

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CHAPTER I

GENERAL INTRODUCTION

Industries producing highly polluted waste waters are having to purify their effluents to meet with ever increasing requirements laid down by water authorities. The South African Water Act of 1956 has prescribed a very high standard to which waste waters must conform before discharge into a South African water course. Enforcement of these standards falls under the jurisdiction of government authorities such as the Department of Water Affairs. Similarly, municipalities and other local authorities set standards with which trade effluents must comply before discharge into public sewers for treatment in a municipal sewage works. These local authorities are empowered to recover from the trader the additional costs incurred in treating trade effluents. Costs are usually levied in respect of volume, oxygen demand, settleable solids and the production of secondary sludge. In recent years, these standards have been enforced to an extent where the survival of several industries has become dependant on whether these industries are able to purify or dispose of their effluents in a manner acceptable to the water authorities.

The South African hide and skin industry has been placed under just such pressure. Recently, tanneries in certain areas have had their sewage outlets sealed and are forced to meet the additional cost of trucking their effluent away for disposal elsewhere. As a result there is an urgent need for detailed investigations into improving the efficiency of the various methods used to treat hide and skin waste waters.

Fellmongeries (where sheep skins are pulled and processed) and tanneries (where chiefly cattle hides are processed and tanned) produce the most problematic effluents of the hide and skin industry. The major portion of the waste waters from these undertakings are lime-sulphide

effluents arising from beamhouse operations such as the washing, unhairing and bating of hides and skins. Although the biological treatment of domestic sewage has been extensively studied and well documented, comparatively little is known about the treatment of fellmongery and tannery waste liquors. These liquors differ vastly from domestic effluents being characterised by their high pH, sulphide and organic matter concentrations. Consequently, conditions for the effective treatment of these liquors differs in several aspects from those of domestic sewage treatment. A considerable amount of research into the treatment of tannery effluents by a variety of different processes is currently being conducted and summaries of what has been achieved are given by Eye (1962), Thorstensen (1969) and Van Vlimmeren (1972).

The Leather Industries Research Institute of South Africa has over the past few years been directing research into the application of the activated sludge process to the treatment of fellmongery and tannery effluents. Small-scale pilot plants were constructed at a local fellmongery and tannery (Shuttleworth et al., 1974) in order to obtain data to be used later in the construction of full-scale treatment plants. These studies exposed the need for a fundamental investigation into aspects of the activated sludge treatment of these effluents to supplement the largely empirical research which has already been conducted.

1.1 Construction and Operation of Typical Activated Sludge Plants

In its simplest form the activated sludge process consists of an aeration tank followed by a sedimentation tank. A mixed microbial population grows in the aeration tank and is settled out in the sedimentation tank after which a portion is returned to the aeration tank while the rest is wasted. All of the suspended solids in the aeration tank, including organisms and their debris are collectively known as activated sludge. The mixture of activated sludge and waste water in

the aeration tank is known as the mixed liquor. A great number of modifications of the activated sludge process have been devised and many of these have been given completely different names.

In activated sludge treatment systems the method of mixing the waste liquor varies between two extremes, complete mixing and plug flow. In a completely mixed reactor liquid waste is assumed to be instantaneously and homogeneously mixed with the reactor contents. Thus the effluent from the reactor has the same constitution as the contents of the reactor. Ideal complete mixing is however not attainable in practice, although it may be approached in small aeration tanks (Pike and Curds, 1971). When large volumes of effluent are to be treated, the plug flow mixing regime is usually employed. The plug flow reactor is typically a long channel-type tank where the effluent is introduced at one end and is mixed by air sparges along its length. In such a reactor it is assumed that no longitudinal mixing occurs between adjacent elements of waste water, each element of mixed liquor being analagous to a batch culture simply moving along a time axis. Thus the waste concentration decreases along the length of the tank, while the microbial mass concentration increases from its initial value as a result of assimilation. True plug flow systems are not readily obtainable in practice, most of the mixing techniques being intermediate between the two extremes.

The mechanism of aeration in the reactor tank also serves to mix the waste liquor. Aeration may be provided by mechanical surface aerators or by the injecting of compressed air through sparges at the bottom of the tank. Oxygen uptake is most rapid in the initial stages of aeration and this is sometimes provided for by installing a greater degree of aeration at the influent end of a channel-type aeration tank. Alternatively, the influent is injected at various intervals along the aeration tank (Carrousel system).

It is essential that the sludge be separated from the mixed liquor before discharge of the treated effluent. Without removal by sedimentation of the sludge only that portion of the waste that is catabolised will be removed from the waste stream, leaving a considerable proportion of the organic fraction in the form of microbial mass (Harris and Mitchell, 1973). A proportion of the sedimented sludge may be returned to the aeration tank while the remainder is wasted. If no sludge is recycled, the sludge age (mean solids retention time) will be equal to the hydraulic retention time. The recycling of activated sludge is advantageous in that it allows a higher micro-organism concentration to be maintained in the aeration tank. This will in turn allow a reduction in the hydraulic retention time of the tank without necessarily sacrificing effluent quality. A reduction in retention time is equivalent to reactor volume and hence construction and running costs.

The performance of the activated sludge process in purifying waste water is usually estimated by comparing the influent and effluent after settling with respect to an oxygen demand test. This oxygen demand test may be either biochemical as in the case of the 5-day biochemical oxygen demand test (BOD) or chemical, by determining the reduction of either $KMnO_4$ or boiling $K_2Cr_2O_7$ over a stated time period.

1.2 Ecological and Kinetic Approach

Previous research on the fundamentals of the activated sludge process can be divided into two categories according to the point of view of the investigator. An ecological approach to activated sludge research is adopted by the applied microbiologist while the chemist or engineer usually adopts a kinetic approach.

The objective of the ecological approach has been to determine which organisms play a beneficial role and which organisms are a nuisance. The treatment process is then operated under conditions which encourage

the growth of the desired organisms and discourage the growth of nuisance organisms. The object of the kinetic approach has been to describe the many facets of the activated sludge process as accurately as possible in terms of kinetic equations and then use these equations to design and operate effluent treatment plants.

As has been pointed out by Pipes (1966) certain weaknesses are prevalent in each of these two approaches. Although the kinetic approach has been successful in yielding many valuable concepts and much valuable data, many of the kinetic equations are based on assumptions and approximations which are not entirely accurate. Engineers have often been guilty of ignoring the biological nature of the sludge, treating it as though it were some kind of catalyst which increases in mass when aerated in the presence of organic wastes and decreases in mass after the organic matter has been removed from the waste. On the other hand, the ecological approach tends to be descriptive in nature and hence does not produce the quantitative data needed by engineers on which to base their calculations. Microbiologists have tended to have an aversion to working quantitatively with mixed cultures such as are found in the activated sludge process. However, experience with natural communities and semi-natural populations like those of activated sludge has shown that reproducible behaviour is not the exclusive prerogative of pure cultures (la Riviere, 1972).

There is clearly a need to combine the kinetic and ecological approaches where possible, making the ecological approach more quantitative and to pay careful attention to the biological nature of the sludge when making assumptions in the kinetic approach. Both the ecological and kinetic approaches have been adopted in this dissertation to investigate several important facets of the activated sludge treatment of fellmongery and tannery lime-sulphide effluents. The aim of this research has been to provide solutions to certain practical problems peculiar to the

treatment of these effluents and to establish the design and operation of the treatment process on a sound theoretical basis.

AN INVESTIGATION INTO SOME ECOLOGICAL ASPECTS OF THE ACTIVATED SLUDGE TREATMENT OF FELLMONGERY AND TANNERY EFFLUENTS

CHAPTER II

INTRODUCTION

The ecological approach is concerned with the various micro-organism populations in the sludge, the conditions for growth and flocculation of these micro-organisms and the relationships between them. The environment in the activated sludge plant is unlike any natural aquatic habitat. Considerable selective pressures arising from the intrinsic nature of the reactor operate within the process. Factors determining the dominant organisms in the activated sludge are many. Selective pressures are exerted by the presence of toxic materials in the effluent, the pH value, the concentration of dissolved oxygen in the tank, temperature and the composition of nutrients in the waste water. The community is dominated by heterotrophic bacteria, some aggregated into small flocs and others freely suspended in the liquor. These bacteria together with the saprophytic protozoa form the first trophic level. The second trophic level is filled by the holozoic protozoa which feed on the bacteria and saprobic protozoa. Higher trophic levels may be filled by the smaller metazoa such as rotifers and nematode worms. Fungi, although often present in activated sludge do not appear to fulfil any major role in the process.

2.1 Bacteria

There have been numerous attempts over the past 60 years to determine which bacteria play a significant role in the effluent treatment process. Russel and Bartow (1916) isolated thirteen varieties of non-nitrifying bacteria from activated sludge most of which belonged to the Bacillus subtilis group of aerobic spore formers and demonstrated the importance

of these bacteria in the purification of sewage. Butterfield (1935) first isolated a zoogloea-forming bacterium from activated sludge and identified it as Zoogloea ramigera. When aerated with sterile sewage in pure culture, this bacterium produced flocs similar to activated sludge and was capable of removing a high percentage of oxidisable material. Heukelekian and Littman (1939) found Z.ramigera in 15 different sludges. When aerated in sterile sewage they rapidly oxidised carbohydrates and produced ammonia from gelatin, casein and peptone, forming well organised flocs. This discovery led to the belief that Z.ramigera was the primary organism in activated sludge. According to Hawkes (1963) several of the earlier workers found intestinal bacteria to be present in the greatest numbers and considered them to be the most important organisms. Allen (1944) by homogenising his sludge samples to liberate individual cells from floc particles found the majority of organisms were Gram-negative rods most of which belonged to the genera Achromobacter, Flavobacterium and Pseudomonas. He concluded that the intestinal bacteria were present only as adventitious forms and of little significance in the process. The temperature of the activated sludge process being more suitable to the aquatic types of bacteria than the intestinal types. Furthermore, Z.ramigera did not appear in the list of bacteria reported by Allen.

The important bacteria in activated sludge would be expected to be able to floc by themselves, be formed into floc by other organisms or attach themselves to particles large enough to settle well. An organism not being returned together with the recycled sludge in greater numbers than it is present in the mixed liquor would not be able to establish a significant population in the system. Many investigators therefore looked for bacteria which could flocculate by themselves. McKinney and Horwood (1952) isolated 11 organisms which were capable of floc formation in pure culture when aerated in a synthetic sewage similar

in nature to normal domestic sewage. The floc forming organisms isolated other than Z.ramigera belonged to the genera Escherichia, Paracolobacterium, Nocardia, Bacillus and Flavobacterium. McKinney and Weichlein (1953) in an examination of sludges from two plants treating domestic sewage and a plant treating industrial waste, isolated several additional organisms capable of floc formation including Alcaligenes and Pseudomonas. As a result of these studies they concluded that it was not necessary to have Z.ramigera present in order to form an activated sludge.

Dias and Bhat (1964) found that only Zoogloea and Comamonas were present in significant numbers in the sludges they examined. They suggest that these two bacteria form the physical basis of the floc and that other bacteria are present as associated organisms. They do concede however that the less prevalent species (not isolated by the method they employed) could also have a significant role in sewage treatment.

Pipes (1966) compared the findings of McKinney and Weichlein (1953), Jasewicz and Porges (1956), Rogovskaya and Lazareva (1959), Dias and Bhat (1964) and added Bacillus to the three genera that Allen (1944) found as being significant in effluent purification. Although other bacteria Alcaligenes, Micrococcus, Comamonas, Bacterium and Zoogloea were found in large numbers by some of the investigators, Pipes puts them down to faecal or adventitious organisms. Unfortunately these four investigators used a limited range of media for isolations. Although Dias and Bhat (1964) used sewage agar for their primary isolations, they identified only those organisms that survived transfer to proteose-peptone yeast extract broth.

Halls and Board (1973) reported that Acinetobacter and a yellow pigmented Gram-negative rod (which they were hesitant to identify with any genus) dominated the bacterial flora in a trickling filter irrigated with domestic sewage. They propose that the association between these

two organisms might derive from a requirement by Acinetobacter, (which is not known to be active against polymers such as polysaccharides) for the yellow-pigmented rods to break down polymers into assimilable units. Two types of microbial association were proposed, one dominated by Pseudomonas and the other by an asscciation between achromobacters (Acinetobacter) and Flavobacterium. The type of waste and quality of aeration would determine which bacteria dominate. Poorly aerated, carbohydrate rich wastes were thought to favour Pseudomonas, whilst well aerated, proteinaceous wastes favoured the Acinetobacter and Flavobacterium association. Whether such a simple association is present in the activated sludge treatment process is doubtful.

The nitrifying bacteria Nitrosomonas and Nitrobacter are present in activated sludge under conditions described by Downing, Painter and Knowles (1964). These two organisms are the only ones responsible for converting ammonia to nitrite and nitrite to nitrate so that a simple chemical test determines their activity.

One organism which seldom appears on lists of bacteria in reports of general surveys of treatment processes but which has been isolated several times is Sphaerotilus natans. Lackey and Wattie (1940) isolated 14 strains of Sphaerotilus from different sources and concluded that they behaved so similarly they were all S.natans and any variations were due to the environment. The isolation medium for Sphaerotilus is different from the media used in surveys of bacteria and it is possible that Sphaerotilus could be isolated from many sludges if the proper technique were used. Practically all studies of this filamentous organism have been aimed at solving the bulking problem. Ruchhoft and Kachmar (1941) showed that Sphaerotilus in pure culture can produce growth very much like bulking sludge. It has however, also been shown that sludge can bulk without an overgrowth of filamentous organisms (Heukelekian and Weisberg, 1956).

2.2 Protozoa

It has been known for many years that protozoa play an important role in waste water treatment. Reynoldson (1942) made attempts to use ciliates and particularly the peritriches as an indicator of "ripe" activated sludge. He found an inverse linear relationship between the count of Vorticella and the BOD of the treated effluent. Baines, Hawkes, Hewitt and Jenkins (1953) concluded that although the types of protozoa could be used as a qualitative measure of sludge condition no quantitative relationship held. Generally, when many flagellates are present the sludge is in a poor condition; as the sludge improves in condition ciliates predominate and the flagellates disappear. Curds and Cockburn (1970) suggested that all species of protozoa taken together may serve as an indicator of plant efficiency but stress this indicator should be used with caution so as not to oversimplify an extremely complex ecological situation.

About 228 species of protozoa have been reported to occur in activated sludge plants alone. This includes 17 species of phytoflagellates, 16 of zooflagellates, 25 of amoebae, 6 actinopods and 160 species of ciliates. There would seem to be a succession of the dominant protozoa in a treatment plant as the sludge age increases. Initially rhizopods predominate followed by flagellates, free-swimming ciliates, crawling ciliates and stalked ciliates (Barker, 1949). Grainger (1973) reported a similar succession of protozoa in the treatment of farm waste.

The extent to which protozoa participate directly in the breakdown of organic waste matter has been much debated. Pillai and Subrahmanyan (1942) attempted to show the special role of the genus Epistylis in sewage purification. No less than 6 species of this genus were found in Indian sewage purification installations. These protozoa were present in large numbers, up to 16 000 per ml and were reported to "Come into direct contact with colloids which they collect and carry down in the form of flocs". Epistylis were so necessary for effective purification of the

sewage that when Chironomus larvae which feed on Epistylis invaded the tanks, the process of purification was paralysed. The larvae had to be destroyed and the plant restarted.

Jenkins (1942) challenged the importance of Epistylis. He found sludge completely dominated at times by Paramecium. Also flocculation and oxidation were reported to proceed satisfactorily in the absence of protozoa.

Pillai and Subrahmanyan (1944) reported the separation of bacteria and protozoa and found that the isolated protozoa could bring about practically all the changes associated with purification including a 70% reduction in oxygen demand (PV) of the sewage effluent. They considered the part played by bacteria to be negligible as no purification and clarification took place when protozoa were destroyed by various protozoan specific agents. The contention that bacteria play a negligible part in waste water purification was contested by Barker (1946) and Heukelekian and Gurbaxani (1949). Their opinion was that only a few of the rhizopods and small free-swimming flagellates compete directly with bacteria, for organic matter in the waste. There have been suggestions that a few ciliates might directly remove soluble organic substances from sewage, as certain species may be cultured on organic solutions in the absence of bacteria e.g. Tetrahymena spp. Even though this is so, it is doubtful whether such facultative saprophytes could compete with the obligate saprophytic bacteria. Because of the more favourable surface to volume ratio that bacteria have over protozoa it is reasonable to assume that the bacteria are the primary agents of purification.

Curds, Cockburn and Vandyke (1968) have reported some of the most convincing evidence of highly improved effluent quality as a result of ciliate action. They constructed activated sludge plants in which protozoa free sludges could be grown, so that the effect of subsequent controlled inoculations with ciliates on effluent quality could be

determined. Both synthetic sludge and normal domestic sludge filtered free from protozoa were added to their plants in different experiments. Plants were monitored for periods of 70 - 80 days before ciliates were added resulting in an almost immediate reduction in BOD, suspended solids, optical density and organic carbon content of the effluent. Addition of ciliate protozoa was accompanied by a rapid drop in the viable bacterial count. The very significant drop in numbers of bacteria and non-settleable suspended solids on the introduction of ciliates could be due to two factors. Firstly, the bacteria are food organisms on which the ciliates prey and the ciliates have the ability to flocculate both the suspended matter and bacteria. Secondly, significant decreases in BOD and organic carbon in filtered effluent samples could be attributed to an indirect effect predatory activities have on bacterial populations. Cutler and Bal (1926) and Meikeljohn (1932) suggested that the predatory activities of Colpidium although reducing bacterial numbers, stimulate increased nitrogen fixation by Axobacter chroococcum and ammonia production in soil bacteria respectively. Butterfield (1935) found that similarly when the ciliate Colpidium was added to pure cultures of Z.ramigera the bacterial sludge became more efficient. This phenomenon is apparently due to bacterial populations which are kept from reaching self limiting numbers and so remain in prolonged physiological youth.

Sridhar and Pillai (1974) adopted an enzymatic approach to identify the role played by bacteria and protozoa. They reported that the activated sludge as a whole contained twenty times more protease activity than an equivalent amount of the protozoan Epistylis articulata. The protozoan however contained five times more catalase activity than the activated sludge. As a result of their observations they suggested that bacteria play an almost exclusive role in hydrolysis and decomposition of organic matter but even under intensely aerobic conditions are very limited in the final stages of organic matter oxidation. The dominant protozoan E.articulata with its high catalase activity was responsible for the oxidative properties of the sludge.

2.3 Fungi

Information on the role of fungi in the activated sludge treatment process is relatively sparse. Cooke (1956) in a general survey of polluted waters included samples from several activated sludge plants. He reported that only members of the genera Cladosporium, Margarinomyces, Aureobasidium, Geotrichium and Trichoderma were present in large enough numbers to play a significant role in the process.

Cooke, Phaff, Miller, Shifrine and Knapp (1960) described the yeasts isolated from activated domestic sewage sludge among which the genera Candida, Rhodotorula, Torulopsis and Trichosporon were common. Pipes (1966) proposed that since yeasts are known to flocculate under certain conditions and carry out metabolic activities beneficial to the removal of organic waste, it would not be surprising to discover that they play an important part in the process.

Pike and Curds (1971) state that various workers have reported the predacious fungi Zoophagus, Arthrobotrys and Dactylaria in experimental pilot plants where they were capturing rotifers and nematodes. Geotrichium spp. which may dominate the flora in a plant has been reported to be a nuisance organism causing sludge bulking. When nitrogen and phosphorus are limiting this fungus appears to have a competitive advantage over the microbial population as a whole.

Thanh and Simard (1973) suggested that treatment of domestic sewage by fungi held several advantages. Nitrogen and phosphate compounds are efficiently removed by fungi and the mycelial protein produced is useful as a feed or food product. Among 17 strains of fungi studied Trichothecium roseum was the best with respect to mycelium yield, protein content and efficiency in the removal of nitrogen and phosphate compounds. The low optimum pH requirements for fungal growth (pH 3,5 - 4,0) is likely to make large scale treatment of domestic waste waters by fungi unsuitable.

2.4 Biological Flocculation

One of the factors essential to the performance of the activated sludge process is the effective flocculation of the sludge with subsequent rapid settling and compaction of the sludge. Several theories and proposals have been put forward to account for flocculation. Barrit (1940) found that some strains of bacteria when growing in sludge were held together by a gelatinous matrix that was absent in pure culture. Watson (1945) reported that flocculation caused by the ciliate Balantiophorus minutus involves the production of a mucus secreted within the peristome to which bacteria adhere prior to ingestion. This mucus was found to gradually accumulate in culture fluid and remain in the neighbourhood of the ciliates themselves. The resulting local increase in viscosity of the culture medium caused the entanglement and flocculation of bacteria. In contrast to the view of a few specific bacteria or protozoa being responsible for flocculation by the production of a gelatinous matrix, bacteria belonging to a large variety of genera when aerated in pure culture were shown to be capable of flocculation by themselves (McKinney and Horwood, 1952; McKinney and Weichlein, 1953; Dias and Bhat, 1964).

From experience in treatment plant operations it has been generally concluded that the higher the growth rate of the micro-organisms the lower the tendency for floc formation. McKinney (1952, 1956) reported that when the food to micro-organism ratio approaches starvation conditions certain organisms normally present in activated sludge flocculate rapidly. He suggested that the normal dispersed condition of bacterial cultures was caused by mutual repulsion between negatively charged cell surfaces and that flocculation in older cultures resulted from their lack of energy. Eckenfelder (1966) reporting the work of Anderson (1963, Ph.D. thesis) says that flocculation results from the production of a sticky polysaccharide slime layer to which organisms adhere. This slime formation does not occur during log-phase cell growth and requires excess available

carbohydrate. Many other mechanisms of flocculation have been proposed. McKinney (1952) suggested that mutual attraction between colliding bacteria resulting from van der Waals forces may cause flocculation. Crabtree, McCoy, Boyle and Rohlich (1965) found that the accumulation of poly-β-hydroxybutyric acid (PHB) was associated with flocculation of pure cultures of activated sludge isolates. Dias and Bhat (1964) found that a high proportion of their isolates were capable of PHB accumulation. However, experiments by Angelbeck and Kirsh (1969), Tezuka (1969) and Deinema (1972) did not relate PHB levels to the ability to flocculate. Ionic bridging between bacteria mediated by naturally occurring polyelectrolytes such as humic acid has been proposed by Peter and Wuhrman (1971). They presented evidence that suspensions of dispersed bacteria could be caused to flocculate by adding optimum amounts of cationic polyelectrolytes. In the same way extracellular polymers from activated sludge have been extracted by several investigators and their ability to aggregate bacteria, colloidal suspensions of SiO_2, kaolinite and alumina demonstrated (Harris and Mitchell, 1973).

Clearly the flocculation mechanism is not fully understood and much of the evidence relates to pure cultures producing flocculent growth on laboratory media. The applications of the principles of colloid science to the chemical nature of the surface of sludge bacteria would seem to be the most promising line of investigation into the flocculation mechanism.

2.5 Bulking

A sludge which has poor settling qualities resulting from a loose, flocculent cotton wool-like growth condition is said to be "bulking". Sludge bulking is due to extensive growth of filamentous organisms of which *Sphaerotilus* spp. is often the culprit. Other filamentous organisms such as *Geotrichium*, *Zoophagus*, *Nocardia*, *Beggoiatoa* and *Thiothrix* are also known to cause this condition. Because bulking interferes with plant

operation and effluent quality much research on the controlling of these nuisance organisms has been conducted. In general the lack of maintenance of aerobic conditions with an available carbon source stimulates the growth of fungi and other filamentous organisms (Ruchhoft and Kachmar, 1941). Because of their high surface area to volume ratio, filamentous organisms are thought to survive better under low oxygen tensions than flocculated aerobic growth. Heavy metals like Cu, Ni, Zn and Cr have also been reported to cause bulking (Wheatland, Bell and Atkinson, 1971). Low pH values favouring the growth of fungi may have a similar effect (Hawkes, 1963).

Maintenance of aerobic conditions in the aeration tank, followed by an anaerobic period in the final clarifier, appears to promote growth of facultative aerobic bacteria which are effective in the removal of BOD and produce high density flocs and limit the growth of filamentous organisms (Eckenfelder, 1966).

CHAPTER III

ENUMERATION OF BACTERIAL POPULATIONS IN FELLMONGERY AND TANNERY EFFLUENTS.

Summary

A suitable routine technique for enumerating viable heterotrophic bacteria in the aerobic treatment of fellmongery and tannery effluent was developed. The basic procedure was modified at the different stages of enumeration, the modification yielding the highest numerical value at each stage being adopted. Counts were significantly affected by the isolation medium, medium pH and incubation temperature, but not by the homogenisation technique. Based on this study the most suitable method for viable count determinations in untreated fellmongery and tannery effluents was found to be by dilution and spreading onto nutrient agar at pH 9, followed by 7 days incubation at 30°C. In the case of aerated effluent, nutrient agar at pH 7 should be used followed by incubation for 4 days at 30°C. Where the bacteria have flocculated as in the case of treated effluent, the effluent should be homogenised for 30 sec in an ultrasonic disintegrator prior to dilution and plating.

3.1 Introduction

The study of bacterial populations in activated sludge presents several technical difficulties. Pike and Curds (1971) showed that there is a large discrepancy between total microscope counts of bacteria from sewage treatment processes and viable aerobic plate counts. Less than 1% of the bacteria in a mixed liquor sample estimated by direct microscopic count may be viable on a commonly accepted plating medium. There are a number of reasons why not all of the particles seen on microscopic examination will develop into colonies. Not only is it difficult to distinguish between bacteria and inert particles occurring in the mixed liquor but many of the bacteria may be either dead or unsuited to the culture conditions used.

No single medium will reliably support the growth of all nutritional types of bacteria. Allen (1944) recommended the use of nutrient agar in studying the bacteriology of domestic sewage activated sludge because of its high numerical productivity. Prakasam and Dondero (1967), however, reported that an agar medium containing activated sludge extract was more suitable than nutrient agar and gave higher counts than other media. Pike, Carrington and Ashburner (1972) enumerated activated sludge bacteria on seven recognised isolation media including activated sludge extract and found a casitone, glycerol, yeast extract agar to give the highest counts. Unz and Dondero (1970) demonstrated what is probably a combination between low cell viability and the deficiency of isolation media by succeeding in cultivating only 203 of 1498 bacterial cells micromanipulated from zoogloeal slimes. Dias and Bhat (1964) found that only 8% of 150 activated sludge isolates required neither vitamins or amino acids for growth in a basal medium of glycerol, ammonium nitrate and succinate. In addition to the difficulty of obtaining a completely non-selective isolation medium it has been suggested by Pike and Curds (1971) that conditions may arise in which the phenomenon of substrate accelerated death can occur. This phenomenon has been observed when bacteria which have been growing at near starvation conditions are diluted in a non-nutrient fluid and inoculated onto a comparatively rich growth medium (Postgate and Hunter, 1963).

A further difficulty encountered is that the dominant bacterial form growing on an agar medium may differ from the dominant form in a sludge floc or biological filter slime. Grainger (1973) found that the dominant bacterial form from a biological filter treating farm waste was a spiral rod-shaped bacterium, whereas on agar medium a short straight rod-shaped form predominated. Either the spiral rod-shaped bacterium was not recovered on the isolation medium or its morphological form differed when transferred onto agar.

The gelatinous matrix of the flocs has further hampered efforts to enumerate and identify the indigenous bacteria of the treatment process. Accuracy in the enumeration of bacteria by the plate count technique relies on the assumption that the bacteria exist in suspension as dissociated single cell units. Clearly when flocculation conditions exist this assumption does not hold and the flocs have to be dispersed by homogenising the sample before bacterial examination (Allen, 1944; Williams et al., 1970; Davey and Richards, 1970). Pike, Carrington and Ashburner (1972) compared five homogenising procedures for releasing bacteria from flocs and found an ultrasonic cleaning bath treatment to be the most effective. Yin and Moyer (1968) tested 37 chemical dispersants in order to determine whether any were effective dispersers of bacterial flocs without success.

As the enumeration of bacterial populations plays an important part in the study of effluent purification undertaken in this project, it was necessary to evaluate a procedure for routine enumeration of bacteria in fellmongery and tannery waste waters. These bacterial populations can be expected to have their own unique characteristics in comparison with domestic sewage and other types of industrial effluents.

3.2 Materials and Methods

3.21 Source of fellmongery and tannery effluents

i) Fellmongery effluent

The fellmongery effluent was obtained from a small scale pilot plant installed at a local fellmongery which consisted of:

(a) A 1820 l (400 gal) header tank into which the raw lime/sulphide effluent was pumped, and

(b) a second 1820 l elevated tank into which the alkaline effluent (pH 12-13) from (a) flowed by gravity feed and was neutralised to about pH 9, or treated with manganese sulphate and given a preliminary aeration with compressed air.

(c) A third 2730 l (600 gal) aeration tank fitted with a surface aerator, into which the effluent from (b) flowed at a controlled rate to give various retention times.

ii) Tannery effluent

Tannery effluent was obtained from a larger pilot plant which consisted of:

(a) A square concrete tank with a capacity of 80 kl (17 600 gal), equipped with a motorised stirrer, and used for neutralisation of the lime/sulphide effluent with sulphuric acid.

(b) A second concrete tank of 86 kl (19 000 gal) capacity containing a suspended surface aerator (type BSK aerator, Gigant, 750 mm diameter turbine - E.L. Bateman and Co.), into which the neutralised effluent from tank (a) was pumped at a controlled rate to give various retention times, but usually 24 h.

(c) A third sedimentation tank of 65 kl (14 400 gal) with an overflow weir into a sewer.

3.22 Samples and Collection

Samples (5 l) of raw effluent were obtained before neutralisation from the South African Cape Fellmongers Co., Port Elizabeth, and the King Tanning Co., King William's Town. Activated sludge samples (5 l) were taken from the outlet of tank (c) at the fellmongery and tank (b) at the tannery. All samples were stored at 4°C in a cold room and examined within 24 h of collection.

3.23 Diluents

The standard diluent was 0,85% sterile saline solution. In experiments concerning the effect of the pH value of the plating medium on viable bacterial counts, the diluent was replaced by various buffer solutions.

(a) 0,02 M Tris-HCl buffer (pH 5-8) and

(b) 0,02 M Na_2HPO_4 - NaOH buffer (pH 9-12).

3.24 Media

All media were prepared with distilled water, sterilised at 121°C for 15 min and solidified with 1,5% agar.

(a) Nutrient agar

Made up from dehydrated nutrient agar.

(b) CGY agar (Curtis, Pike et al., 1972).

Containing (g/l) casitone, 5; glycerol, 5; yeast extract, 1.

(c) PGY agar (Tomlinson, Pike et al., 1972).

Containing (g/l) peptone, 0,1; glucose, 0,5; yeast extract, 0,1.

(d) YGT agar

Containing (g/l) yeast extract, 2,5; tryptone, 5; glucose, 1.

(e) ASE agar (Prakasam and Dondero, 1967a).

One litre of mixed liquor collected from the effluent end of the aeration tank was autoclaved at 121°C for 30 min. The suspension was filtered through glass wool and the sediment discarded. The filtrate was made up to one litre with distilled water, agar was added and the filtrate autoclaved again for 30 min.

In experiments concerning the effect of the pH value of the plating medium on viable bacteria counts, nutrient agar was used, distilled water being replaced by 0,2M tris-HCl buffer (pH 5-8) and 0,2M Na_2HPO_4- NaOH buffer (pH 9-12). The pH of the media was rechecked after autoclaving.

3.25 Homogenisation of Effluent Liquor

Samples of effluent liquor were diluted 4-fold before homogenisation by one of the following methods.

(a) MSE ATO Mix Blender (Waring Blender)

The diluted sample (200 ml) was homogenised at ½ speed for various time intervals before the viable bacterial count was determined.

(b) MSE Ultrasonic Disintegrator

A 30 ml aliquot of the diluted sample was treated at 21 kHz in the disintegrator (Type 7100, 100 watt) with a probe of 3 mm tip diameter. The sample was treated in a 50 ml tube with the probe 1 cm from the bottom.

(c) Braun MSK Homogeniser

A portion (30 ml) of the diluted sample and 30 g of sterile glass beads (0,15-0,18 mm diameter) were placed in the special steel container and oscillated with a rotary motion at 2000 rpm.

3.26 Enumeration of Viable Bacteria

The spread plate method (Woods et al., 1970) whereby four separate aliquots (1/300 ml) were plated from each of four appropriate dilutions of the sample in decimal series was used. Colonies were counted from all readable dilution areas on a minimum of three plates and the mean colony count for a particular dilution determined.

3.27 Statistical Treatment of Results

Where appropriate, experiments were designed so that the effects of the experiment on the viable count could be tested by analysis of variance. Sets of data were usually found to be distributed approximately log-normally, and in such cases were transformed to their logarithms before applying analysis of variance. When the F value for treatments was significant the least significant difference, l.s.d. (0,05), was used for specific treatment comparisons. Examples of the statistical treatment are given in Tables 3.1-3.2 and an indication of the l.s.d. (0,05) is shown on the graph of each experiment where applicable.

3.3 Results and Discussion

3.31 Effect of medium on viable bacterial count

The mean bacterial counts at 30°C for fellmongery effluent on the five

Table 3.1 The effect of the medium on the log of the bacteria count of aerated fellmongery effluent

	ASE	CGY	PGY	YTA	NA	Total	Mean
	6,16	5,87	5,84	5,88	6,04		
DAY 1	6,22	5,80	5,75	5,73	5,95	88,78	5,92
	6,08	5,95	5,59	5,92	6,00		
	8,08	6,89	6,38	6,80	6,94		
DAY 2	8,02	6,84	6,38	6,59	6,95	105,19	7,01
	8,17	6,86	6,62	6,73	6,94		
	8,19	7,95	7,21	7,62	8,05		
DAY 3	8,22	7,82	6,95	7,52	8,01	116,28	7,75
	8,22	7,94	7,25	7,38	7,95		
	8,25	7,97	7,48	7,65	8,12		
DAY 4	8,27	8,08	7,63	7,71	8,23	119,18	7,95
	8,22	8,12	7,48	7,73	8,24		
	8,27	7,98	7,48	7,71	8,20		
DAY 7	8,25	8,09	7,61	7,71	8,22	119,34	7,96
	8,22	8,11	7,49	7,75	8,25		
Total	116,84	110,27	103,14	106,43	112,09	548,77	
Mean	7,79	7,35	6,88	7,10	7,47		

Analysis of Variance

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Media	7,3819	4	1,8455	293,74
Days	45,6046	4	11,4011	1814,69
Interaction	2,3176	16	0,1449	23,06
Error	0,3141	50	0,0063	
Total	55,6182	74	0,7516	

1.s.d. (0,05) between means of any two cells $= t\ 0,05 \times \sqrt{\frac{2 \times M.S.E.}{n}}$

$= 2,01 \times \sqrt{\frac{2 \times 0,0063}{3}}$

$= 0,1326$

Table 3.2 The effect of homogenisation on viable colony count of aerated fellmongery effluent

	0 sec	10	20	30	60	120 sec	Total	Mean
Waring blender	8,27	8,68	8,68	8,72	8,63	8,64		
	8,48	8,67	8,60	8,77	8,62	8,57	155,24	8,62
	8,60	8,57	8,77	8,72	8,61	8,59		
Ultrasonic disintegrator	8,47	8,55	8,56	8,71	8,59	7,92		
	8,49	8,58	8,68	8,63	8,64	7,65	152,23	8,46
	8,52	8,62	8,60	8,67	8,57	7,78		
Braun homogeniser	8,59	8,62	8,73	8,69	8,47	7,95		
	8,44	8,70	8,70	8,67	8,52	7,91	152,87	8,49
	8,37	8,66	8,71	8,69	8,52	7,93		
Total	76,23	77,65	78,03	78,27	77,22	72,94	460,34	
Mean	8,47	8,63	8,67	8,70	8,58	8,10		

Analysis of Variance

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Time	2,1958	5	0,4392	93,03
Treatment	0,2794	2	0,1397	29,59
Interaction	0,9221	10	0,0922	19,53
Error	0,1699	36	0,0047	
Total	3,5671	53	0,0673	

1.s.d. (0,05) between means of any two cells = $t_{0,05} \times \sqrt{\frac{2 \text{ M.S.E.}}{n}}$

$= 2,02 \times \sqrt{\frac{2 \times 0,0047}{3}}$

$= 0,113$

media tested are shown in Figs 3.1 and 3.2 in the cases of untreated and treated effluents respectively. A comparison of the mean colony count between the two liquors shows that there was an approximately 100-fold increase in the viable bacterial count during aeration of the liquor. Furthermore, that after up to 7 days incubation the colony counts of the untreated liquor were still increasing whereas after 4 days incubation the colony counts of the treated liquor had reached their maximum.

The most likely explanation of this lag in growth rate is that the untreated liquor was more inhibitory to the bacteria present in it (high pH, high sulphide conc. - Table 3.3) and therefore the bacteria needed a longer recovery time before adjusting to their new growth medium.

A comparison of the mean colony counts between the media shows that NA medium gave the highest counts of untreated liquor and was significantly different from CGY agar from day 3 onwards. In the case of treated liquor, ASE agar gave the highest viable bacterial counts, although these counts were not significantly different from NA medium at days 4 and 7. It will also be noted that counts on ASE agar did not increase significantly after day 2 in the case of treated effluent, probably because the bacteria needed no time to adjust to a new medium.

The mean colony count at 30°C for tannery untreated and aerated liquors on the five media tested is shown in Figs 3.3 and 3.4. There was only an approximate 10-fold increase in colony counts between the untreated and aerated waste liquors and no significant increase in counts after 2 days aeration on most of the media. This was probably because the untreated tannery liquor was not as inhibitory to its bacterial population as untreated fellmongery liquor (lower sulphide conc. - Table 3.3).

The NA medium gave the highest colony counts for both untreated and aerated tannery liquors at day 4, although the difference in counts between NA and CGY media was not significant in the case of untreated liquor.

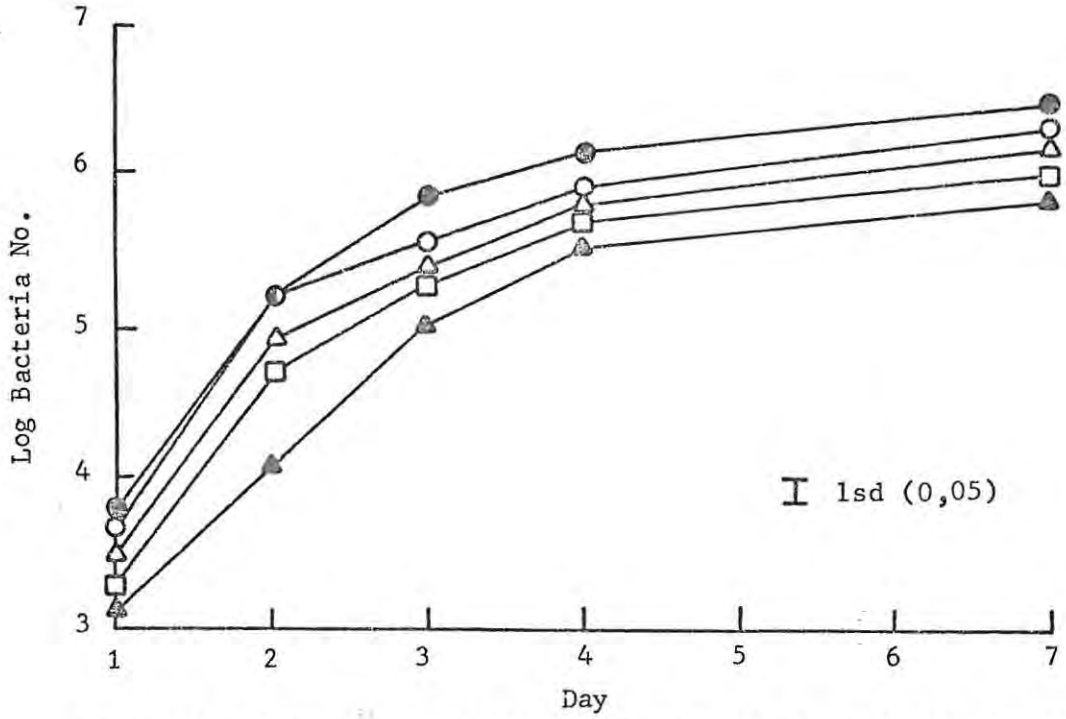


Fig. 3.1. The effect of the medium on the mean of the viable bacterial count of fellmongery effluent before aeration. O—O , CGY; ●—● , NA; Δ—Δ , YTA; □—□ , PGY; ▲—▲ , ASE.

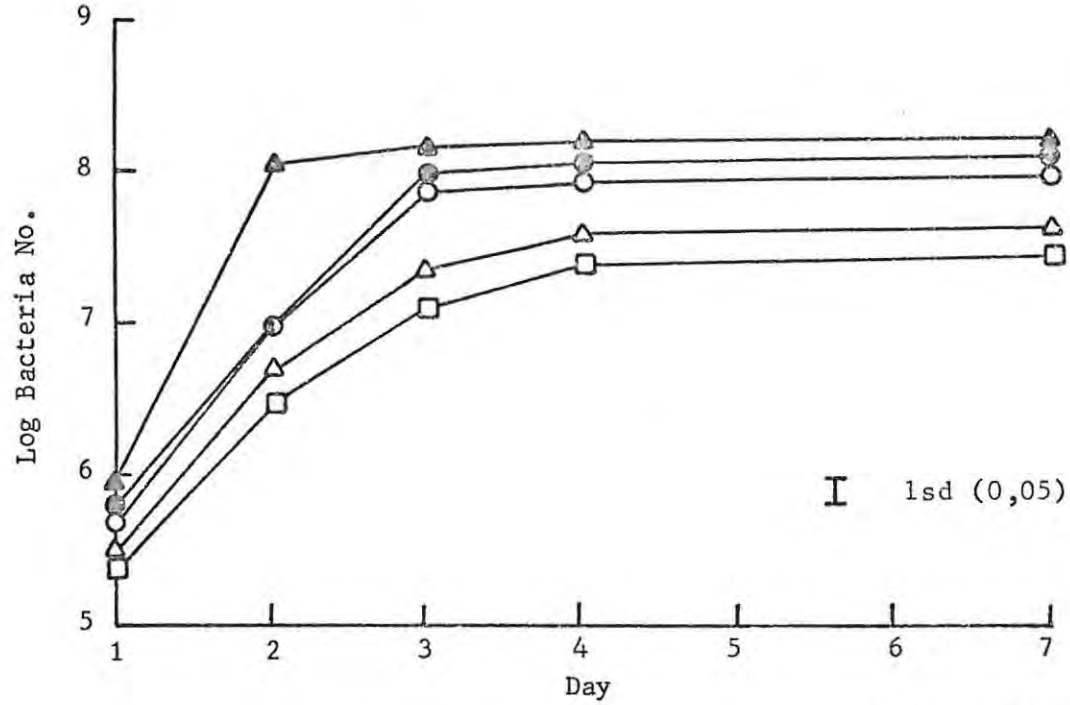


Fig. 3.2 The effect of the medium on the mean of the viable bacterial count of aerated fellmongery effluent.

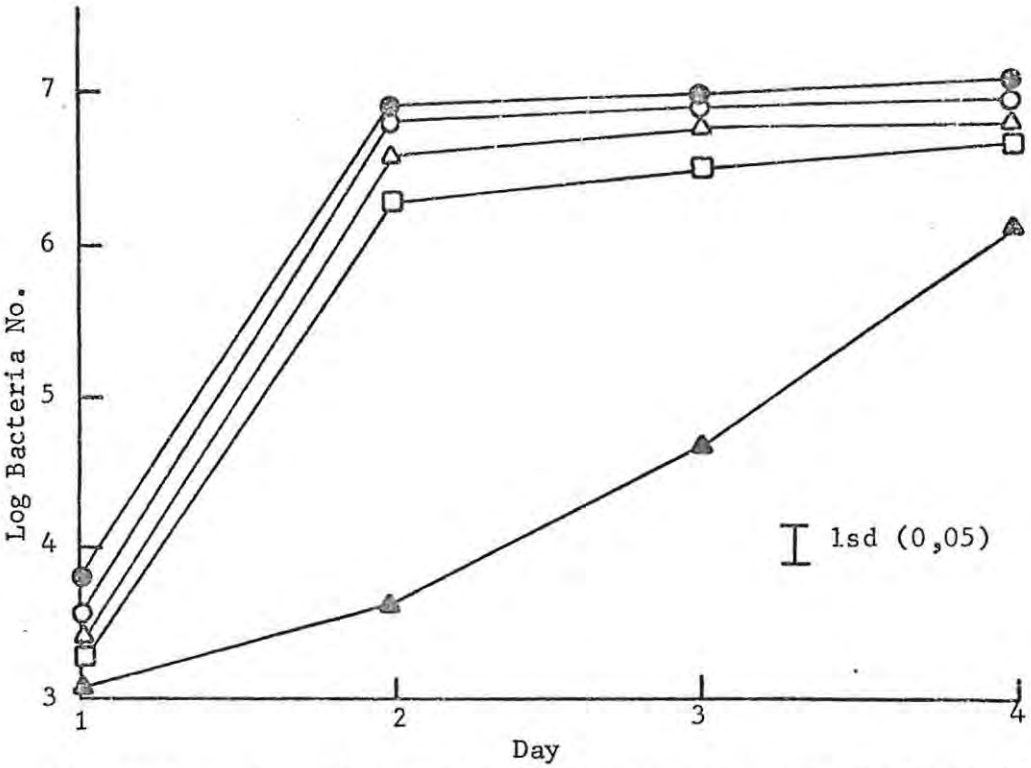


Fig. 3.3. The effect of the medium on the mean of the viable bacterial count of untreated tannery effluent. ○—○ , CGY; ●—● , NA; △—△ , YTA; □—□ , PGY; ▲—▲ , ASE.

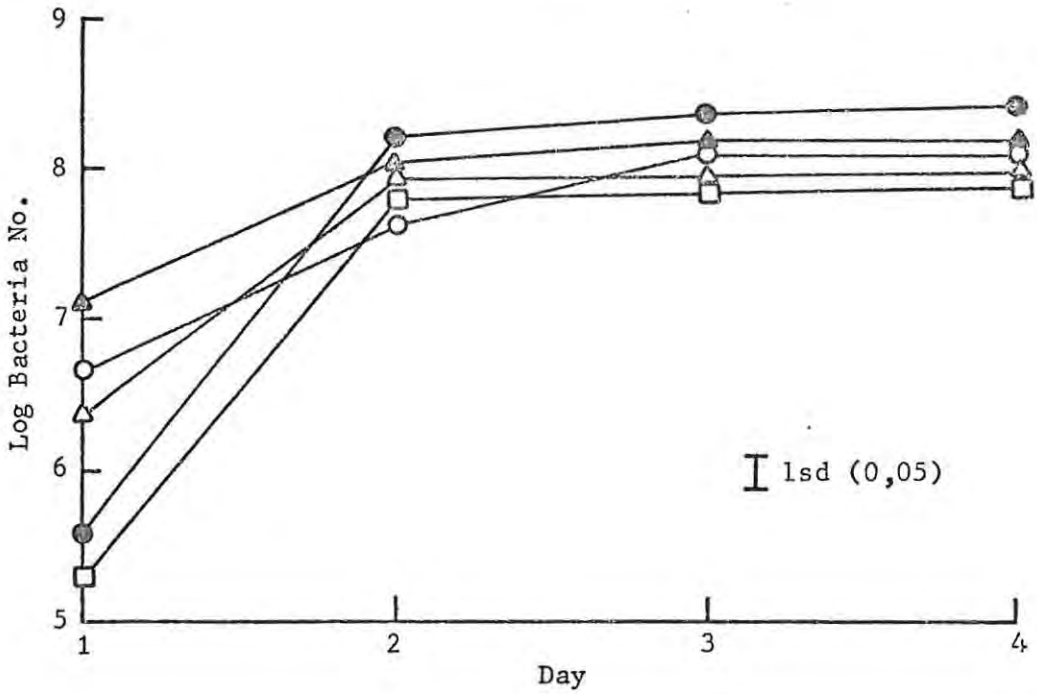


Fig. 3.4. The effect of the medium on the mean of the viable bacterial count of aerated tannery effluent.

Table 3.3 Some characteristics of fellmongery and tannery effluent before and after aeration treatment in the pilot plants

Fellmongery effluent

Characteristic	untreated	aerated
pH	12-13	7-9
4h OA value (mg/l)	1260 (average value)	468 (average value)
Sulphide (Na_2S mg/l)	1000-2000	10-100

Tannery effluent

Characteristic	untreated	aerated
pH	11-13	7-8
4h OA value (mg/l)	1500 (average value)	500 (average value)
Sulphide (Na_2S mg/l)	500-1000	10-20

These values are given as an indication since the final strength of the effluent depends on which effluent liquors are mixed, and the hide or skin load on any particular day.

3.32 Effect of pH on the viable bacterial count

There can be a large fluctuation in the pH of both fellmongery and tannery effluent liquors as the factory discharges them and during the pilot plant treatment process. Untreated liquors may vary from pH 9-13

and treated liquors from pH 7-9. The effect of the pH of the diluents and plating medium on the mean viable bacterial counts for both the fellmongery and tannery liquors is shown in Figs 3.5 and 3.6. The optimum pH for enumerating bacteria in raw factory effluent going into the aeration treatment plant was pH 9 for both fellmongery and tannery effluents. In contrast the optimum pH for bacterial counts in effluent leaving the aeration pilot plant was between pH 6 and 7 for both fellmongery and tannery effluents.

This difference in optimum pH of plating was due to the higher mean pH of effluent entering the aeration treatment plant compared with the pH of effluent leaving the plant, and the acclimation of the bacteria to these conditions.

3.33 Effect of incubation temperature on the viable colony count

The effect of incubation temperatures, ranging from 15-40°C in 5°C intervals, on the mean viable colony count for tannery effluent before and after aeration treatment is shown in Figs 3.7 and 3.8. Colonies were counted on NA at pH 7,0 and 9,0 for untreated and treated liquors respectively.

The mean colony counts did not differ significantly between 30, 35 and 40°C for either liquor after the second day of incubation. Furthermore, there was no increase in colony counts after 3 days incubation at these temperatures. The growth rate at incubation temperatures of 20 and 25°C was markedly slower than at the higher temperatures and the colony counts approximately 3-fold lower than the counts at 30°C after 4 days incubation.

A temperature of 30°C would therefore appear to be the optimum incubation temperature for maximum colony counts. As the temperature of the activated sludge pilot plant lies between 15 and 30°C under normal conditions, this optimum incubation temperature may seem high. However, when it is considered that a large proportion of these bacteria come from the hides of warm blooded animals ($\pm 10^6$ bacteria/gm hide - Woods,

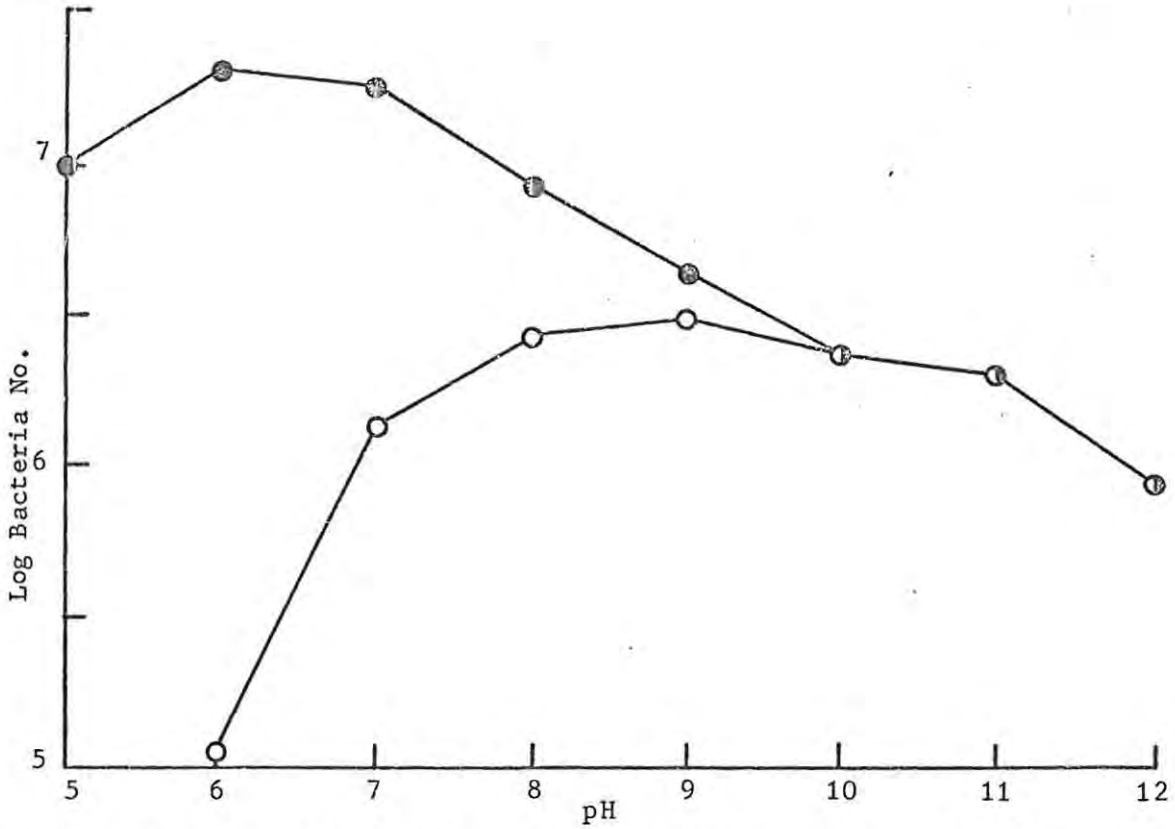


Fig. 3.5. The effect of the pH of the medium on the viable bacterial count of fellmongery effluent. O—O , untreated effluent; ●—● , aerated effluent.

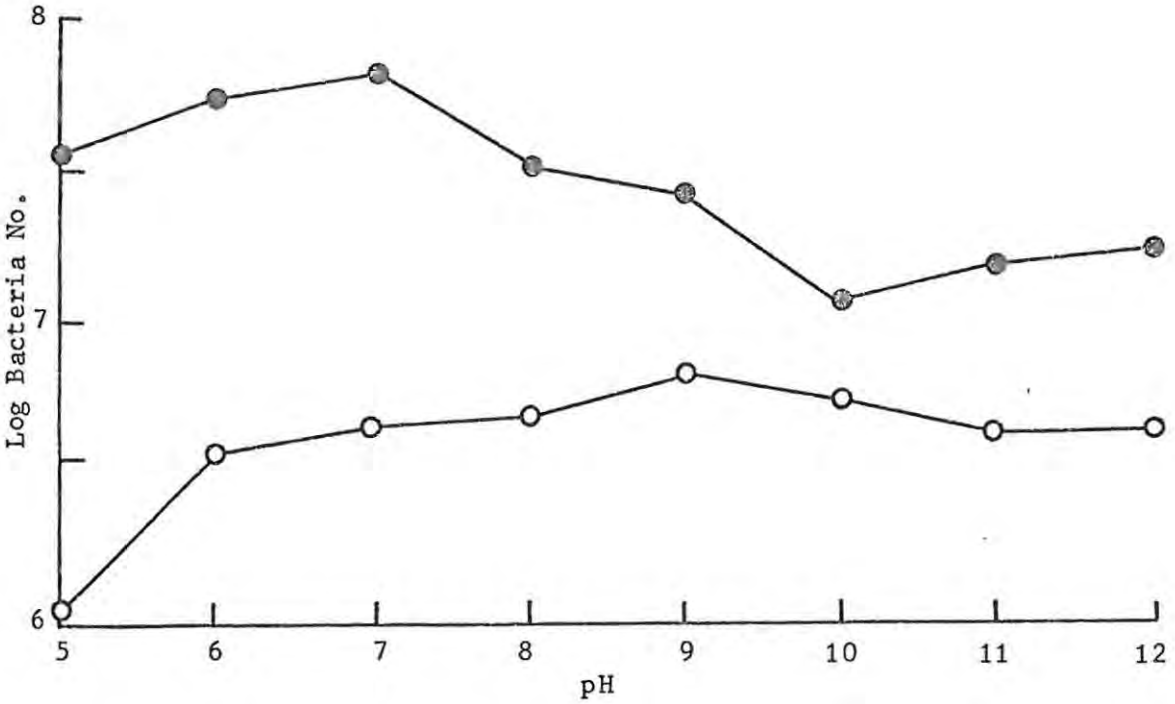


Fig. 3.6. The effect of the pH of the medium on the viable bacterial count of tannery effluent. O—O , untreated effluent; ●—● , aerated effluent.

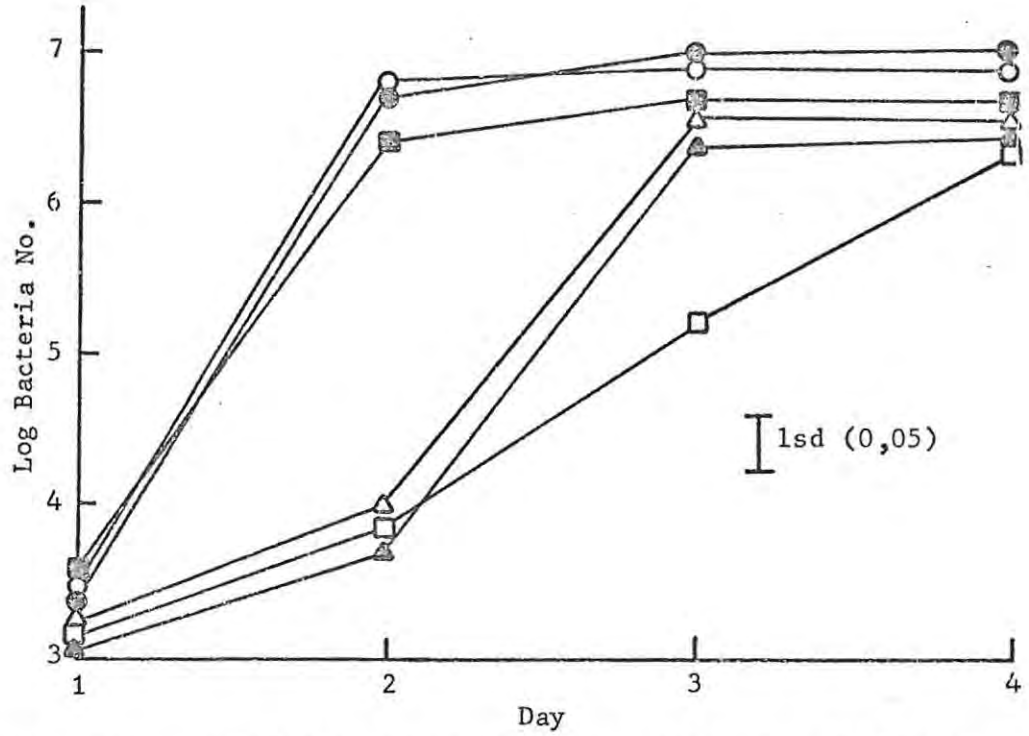


Fig. 3.7. The effect of incubation temperature on the mean viable colony count of untreated tannery effluent. □—□ ,15°; △—△ , 20°; ▲—▲ , 25°; ●—● , 30°; ○—○ , 35°; ■—■ , 40°C.

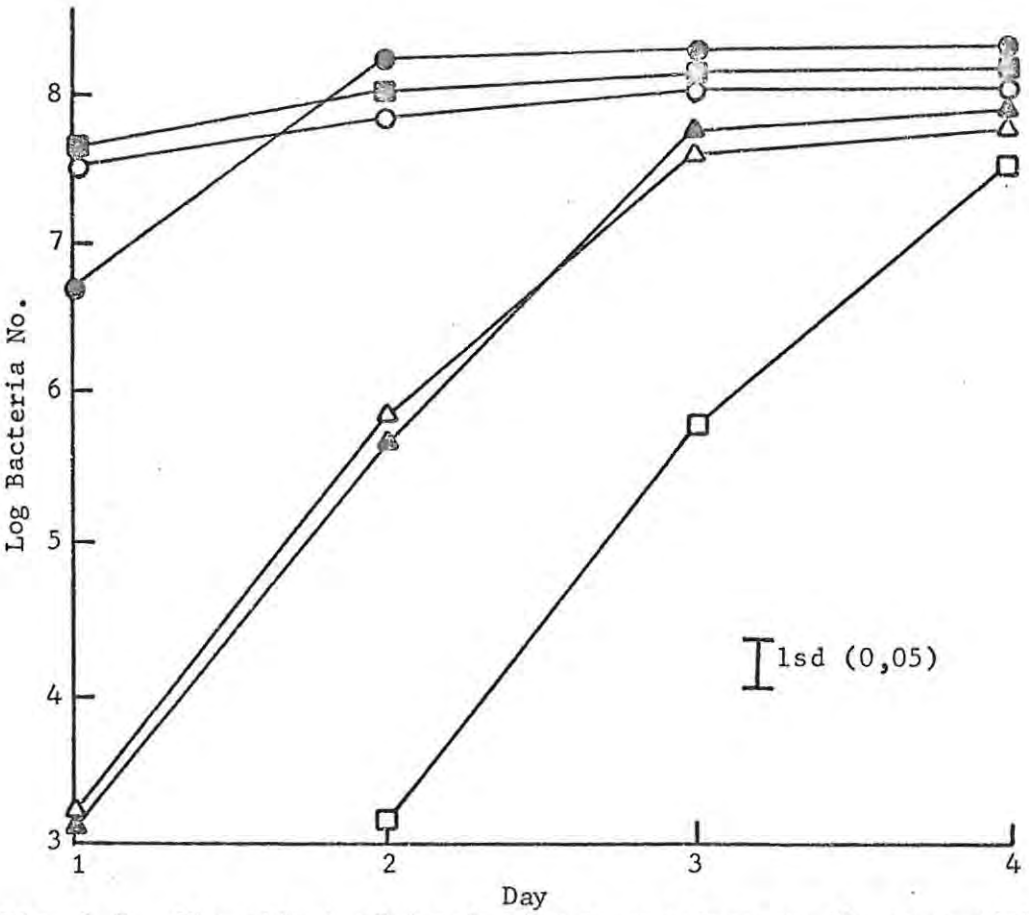


Fig. 3.8. The effect of incubation temperature on the mean viable colony count of aerated tannery effluent.

et al., 1973) this temperature is not unduly high.

3.34 Homogenisation of Sludge Flocs

Progressive homogenisation by the methods outlined above gave initial increases in viable counts up to a maximum value, followed by a decline attributable to the lethal effects of the homogenising technique (Fig. 3.9). Maximum colony counts were obtained after 20 sec treatment in the Braun homogeniser and after 30 sec in the Waring blender and MSE ultrasonic disintegrator. An analysis of the variance shows that there was no significant difference between the maximum colony counts obtained with the three treatments (Table 3.2). Increases in colony count were only 1,7 times the initial counts. This is less than the 3,0-3,5 fold (Pike et al., 1972) and the 3,7 fold increases (Williams et al., 1970) found when domestic sewage flocs were homogenised. The accuracy of the increase obtained is limited by the variability associated with counting the colony forming units in the initial unhomogenised population where the bacteria are not dissociated into single cell units. The increase obtained could also depend on the type of floc aggregation, the efficiency of homogenisation and on the numbers and relative viabilities of freely suspended and floc bacteria (Pike et al., 1972).

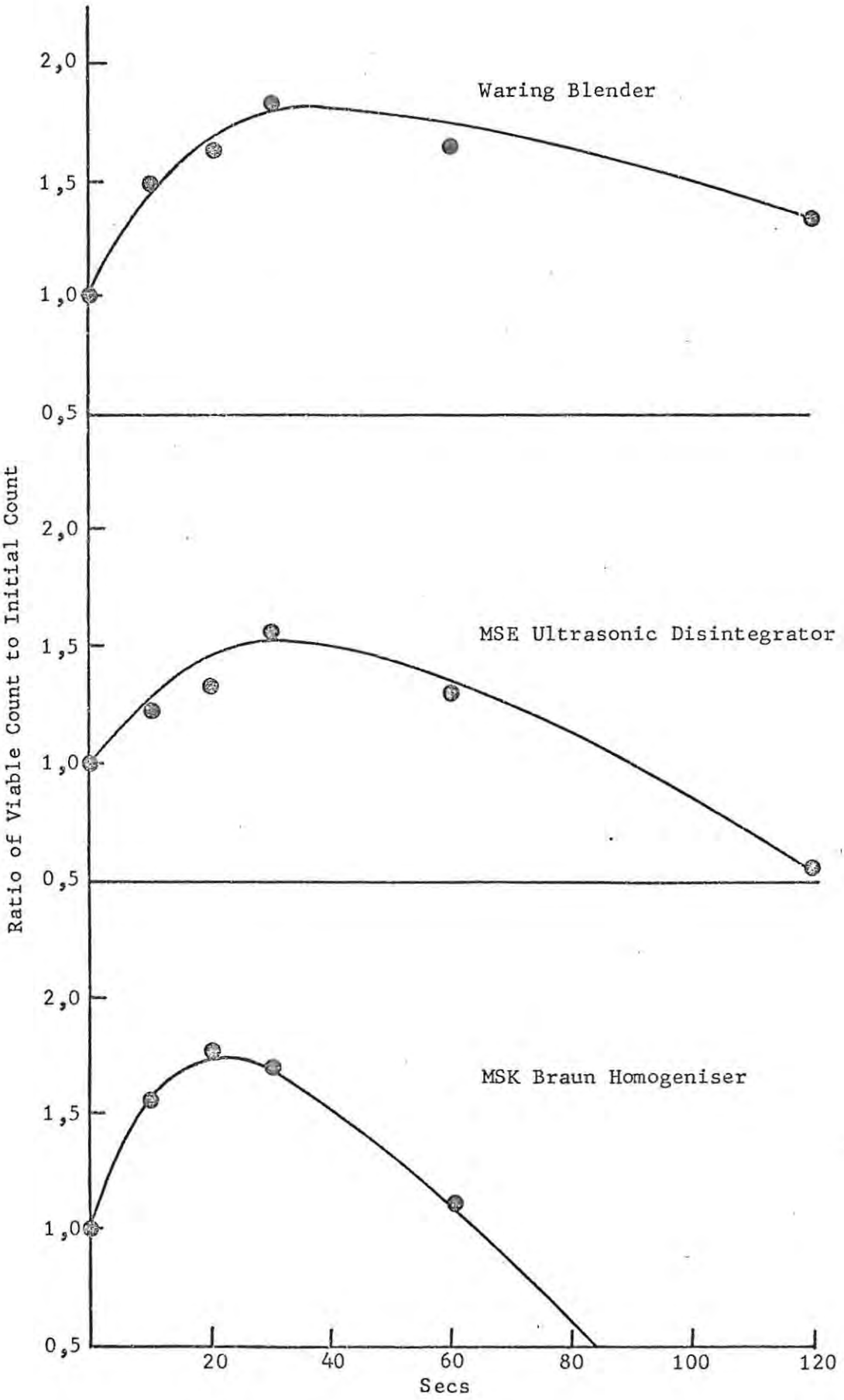


Fig. 3.9. The effect of homogenisation of aerated fellmongery effluent on the mean viable bacterial count.

CHAPTER IV

THE EFFECT OF MANGANESE AND NEUTRALISATION ON THE TREATMENT OF FELLMONGERY EFFLUENT

Summary

In a batch culture laboratory aeration system it was found that acclimated activated sludge micro-organisms contribute considerably to the reduction in oxygen demand (OA value) of lime-sulphide effluents. The degree of neutralisation of the lime-sulphide effluent prior to aeration had an effect on the rate of oxygen demand reduction. The addition of manganese oxidising catalyst to the raw untreated effluent resulted in a rapid removal of detectable sulphide (Na_2S) and an improvement in the rate of reduction in oxygen demand. However, manganese appeared to convert a large portion of the sulphide to an oxidised form having an OA value of its own, which could revert back to detectable sulphide under anaerobic conditions. In contrast, aeration of lime sulphide effluent after neutralisation resulted in the sulphide being lost from the effluent, and therefore should yield an effluent with a lower oxygen demand after treatment.

4.1 Introduction

Fellmongery and tannery waste waters are complex mixtures of sulphide, bisulphide, calcium ions, dissolved albumin, mucoids, mucopolysaccharides, keratin, dissolved and emulsified fats and a variety of other soluble and insoluble organic and inorganic compounds (Cooper, Happich and Naghski, 1973). This results in an effluent with an oxygen demand 10-100 fold greater than that of domestic sewage. There are two characteristics of these effluents which are particularly harmful to the efficient functioning of a biological treatment process, namely the high pH (11-13) and sulphide concentrations (500-2000 mg/l). When considering methods for the lowering of the pH and the removal of the sulphide it is important that the effects these two ecological factors exert on the process is also considered.

4.11 pH

Biological activity is sensitive to changes in pH because the enzymes necessary for the efficient functioning of the vital biochemical pathways

in an organism are pH sensitive. Different organisms have different pH optima and ranges of tolerance. Growth and reproduction of bacteria have been reported to occur above pH 11 (Souza et al., 1974) and at a pH as low as zero in the case of vinegar forming bacteria. Keefer and Meisel (1951) reported that activated sludge could adapt to any pH in the range 6 to 9. However these workers found that the microbial population did not develop into what could be called "activated sludge", if the pH was above 10 or below 5. The treatment of low pH wastes is often hampered by the growth of poorly settling filamentous forms which seem to have a selective advantage at low pH. Pipes (1966) suggests that, on the other hand, high pH wastes entering an activated sludge plant should seldom cause a problem because CO_2 produced by the sludge organisms would rapidly lower the pH to less than 9. The activated sludge floc itself is able to buffer some degree of pH change.

Although micro-organism CO_2 production and the buffering action of the sludge floc may be able to withstand the pH shock loads encountered in domestic sewage treatment, the highly alkaline lime-sulphide effluents may require additional treatment to reduce the pH level to 9 or below. Two methods commonly employed in reducing the pH of lime-sulphide effluents are either the perfusion of effluent with flue gas or the addition of a commercial grade acid such as sulphuric acid. Preliminary investigations showed that the pH of the effluent should be reduced to $\pm$ 9 for the oxygen demand of the effluent to be greatly reduced by the subsequent activated sludge treatment process. However, the large volumes of sulphuric acid needed to neutralise the very large volumes of high pH effluent from an entire factory make this neutralisation costly. The use of flue gas although less expensive may require considerable initial capital outlay before it can be used as a neutralisation method.

4.12 Toxic effluent components

The presence of toxic substances such as heavy metal ions, phenols,

cyanides, thiocyanates, sulphides, thiosulphates, formaldehydes etc. may impair the biological treatment of industrial wastes for a variety of reasons. Nevertheless, the activated sludge process due to its mixed microbial population has an amazing resistance to many such toxins. Sludges capable of oxidising such things as phenol and thiocyanate (Jones and Carrington, 1972; Woodward, Stafford and Calley, 1974) have been developed and some of them are used in treatment plants.

Toxicity measurements and the evaluation of results are complex and difficult tasks (Hartman and Laubenberger, 1968). Toxicity often depends on other factors such as pH, dissolved oxygen, temperature and the presence of other toxic substances. Sometimes the addition of a growth factor such as pyruvate may serve to act as a co-metabolite in the oxidation of a toxic compound as is the case with thiocynate (Woodward et al., 1974). For these reasons toxic levels of various substances may vary in the literature. Hawkes (1966) states that heavy metals are considered toxic at levels of 1-3 mg/l, but that copper levels of up to 100 mg/l have been reported to have no effect on bacteria treating oil refinery waste. Similarly cyanides have been shown to interfere with biological oxidation at concentrations of 1-2 mg/l HCN (Lockett and Griffiths, 1947), whereas it has been found that cyanide concentrations of up to 60 mg/l can be oxidised in bacterial beds without the addition of sewage (Pettet and Mills, 1954). These large variations in the toxic levels of metal ions are probably due to the presence of proteins in the mixed liquor which serve to complex metal ions and so reduce their toxicity. Hartman and Laubenberger (1968) showed that if the amount of activated sludge is increased in proportion to an increase in copper salt, the toxic effect remains the same. The toxic effect of metal ions should not be expressed only in mg/l, but in relation to the biological material in the mixed liquor.

Kampf (1971 a, b) developed a three step method for studying toxic

levels of several chemical compounds in industrial wastes. In the first step, the direct toxic effect can be measured by monitoring the inhibition of microbial respiration in a Warburg respirometer. In the second step the ability of regeneration of the floc is considered and in the third step the adsorptive properties of the activated sludge with regard to the toxic substance can be judged. This technique is ideally suited to toxins such as metal ions and other non-oxidisable toxins. However, in the case of oxidisable toxins such as sulphide the inhibition of microbial respiration cannot be used as the oxidation of the sulphide itself consumes oxygen.

The high sulphide concentrations found in fellmongery lime-sulphide effluents are not only unacceptable to water authorities but detrimental to the treatment process (Van Vlimmeren, 1972). In addition the hydrogen sulphide given off by these effluents is not only extremely poisonous but combines with water to attack concrete, steel and paint (Bailey and Humphreys, 1967). It is therefore necessary that sulphides be removed from the effluent either by precipitation as FeS (Scholtz, 1956) or by oxidising it in an aerator sometimes in the presence of an oxidising catalyst (Eye and Clement, 1972). The indication from preliminary pilot plant investigations was that the efficiency of removal of sulphide by aeration in the absence of an oxidising catalyst is very low at the normal high pH of the effluent. In contrast, if the pH is reduced to pH $\pm$ 9 with sulphuric acid before aeration the sulphide is readily removed by a short pre-aeration period and the oxygen demand of the effluent is greatly reduced by the subsequent activated sludge treatment process.

The addition of manganese to effluent in the form of the sulphate or chloride is relatively inexpensive and several tanneries have been using this method in preference to neutralisation with acid to remove the sulphide. Manganese is known to act as an oxidation catalyst in the removal of sulphide and although the mechanism of the reaction has not been proved

the products are thought to be chiefly thiosulphates together with some sulphates and free elemental sulphur. One of the methods of treating lime-sulphide effluent with manganese is to add manganese to the effluent in a paddle and after aeration allow the effluent to run into shallow aerobic earth ponds (Berg et al., 1967). These workers indicated that a level of 100 mg/l manganese should be effective in removing the sulphide after 5-16 h aeration. However, although manganese catalyst is efficient in the removal of sulphide from effluents, it has been observed in certain South African treatment plants that manganese treated effluent can become offensive due to the return of sulphide. This return of sulphide occurs whenever the effluent treatment ponds become anaerobic.

The laboratory studies reported on in this chapter were preliminary batch culture studies undertaken to determine:

i) the part played by micro-organisms in the reduction of oxygen demand in the treatment of fellmongery lime-sulphide effluent excluding the reduction in oxygen demand due to the chemical effects of aeration, such as the removal of sulphide.

ii) The least amount of neutralisation necessary for the effluent treatment process to continue effectively.

iii) The effects of the addition of manganese catalyst on the effluent treatment process.

iv) Whether the catalytic oxidation of sulphide by manganese is reversible under anaerobic conditions.

4.2 Materials and Methods

4.21 Source of effluent

Fellmongery effluent was obtained from the South African Cape Fellmongers Co., Port Elizabeth. The effluent was a mixture of waste water from the sheepskin soaking and washing process and lime-sulphide

from the unhairing process (lime-sulphide painting). The sheepskin soaking and washing process gives an effluent with a relatively low OA but high chloride content at about neutral pH and containing no lime-sulphide (typical analysis - OA, 90 mg/l; pH 8,4; Na_2S 8 mg/l) while the lime-sulphide effluent contributes the high OA and lime-sulphide content (OA 4 000-7 000 mg/l; pH 11-13; Na_2S 4 000-7 000 mg/l).

4.22 General experimental procedure

Basically the batch culture technique was used. Effluent samples (31) were given extended aeration in plastic containers without the addition of fresh effluent or the removal of treated effluent. Before commencement of aeration, the pH of the effluent was adjusted to the required level with 33% H_2SO_4 or alternatively, a calculated quantity of manganese in the form of $MnSO_4 . 4H_2O$ dissolved in water was added. Unless otherwise indicated aeration was allowed to continue for 24 h in order to remove the sulphide bedore the effluent was seeded with activated sludge ($\pm$ 10% total volume) as prepared below. Samples were withdrawn for analysis after progressive aeration of up to 7 days. Where excessive frothing occurred a small volume (1 ml) of silcolapse 5001 was added.

4.23 Laboratory aeration system

Aeration was provided by an aquarium air pump (Zoobeko, Germany) equipped with a 15 cm air stone. These pumps supplied each container with an approximate rate of aeration of 2 l air/min. This rate of aeration provided a good degree of agitation and mixing of the effluent, while the air was well dispersed into small bubbles by the air stone.

4.24 Preparation of activated sludge seed

Domestic sewage activated sludge was collected from the first maturation pond at the Grahamstown municipal sewage treatment plant. This activated

sludge was aerated in the laboratory together with a small volume of lime-sulphide effluent from which the sulphide had been removed by 24 h aeration at pH 9. The volume of lime-sulphide effluent added was increased over a period of two weeks thus enabling the activated sludge to adapt to the lime-sulphide effluent. At this stage the sludge had a high aerobic bacterial count (10^8/ml) and numerous protozoa with ciliates predominating. The final pH of this sludge seed was 8,2.

4.25 Sterilisation of effluent

The effluent was sterilised either by autoclaving the effluent sample at 121°C for 30 min or by chemical sterilisation (addition of 0,01% acriflavine and 0,05% sodium azide).

4.26 Effluent analysis techniques

(a) Viable bacterial counts were estimated in the mixed liquor after homogenisation by serial dilution and plating out on nutrient agar followed by incubation at 30°C for up to 7 days (Chapter III).

(b) Protozoa counts were estimated using a Pasteur pipette which delivered drops of a known volume, usually 60 drops/ml. A sample of mixed effluent was withdrawn, 8-10 drops distributed over the surface of a glass slide and the protozoa in each drop counted under a low power microscope. Where the protozoa counts became inconveniently large the effluent sample was diluted with a known volume of sterile rain water.

(c) The 4-h oxygen absorbed test was determined using N/80 $KMnO_4$ on mixed liquor after 1 h settling by the method outlined in Ministry of Housing and Local Government, 1956.

(d) Sulphide was determined as sodium sulphide (estimated after 1 h settling) by a method intended for the determination of sulphide in alkaline liquors and unhairing paints (Official Methods of Analysis of the Society of Leather Trades' Chemists, 1965).

(e) The pH was measured after settling using a pH meter.

(f) Optical density (after 1 h settling) was measured using a neutral density filter in a colorimeter.

4.27 Inorganic lime-sulphide effluent

An inorganic lime-sulphide effluent was prepared by diluting fellmongery unhairing paste 100-fold with water, resulting in an effluent having an OA value of 630 mg/l, pH 12,0 and 810 mg/l Na_2S. The unhairing paste was prepared by mixing commercial grade lime and Na_2S with water in the ratio 4:1:5. This effluent preparation was aerated (a) at high pH; (b) after neutralisation with H_2SO_4; (c) after addition of 50 mg/l manganese in the form of $MnSO_4$. $4H_2O$.

4.28 Reappearance of sulphide under anaerobic conditions

To investigate the reappearance of sulphide, fellmongery effluent was aerated at (a) high pH; (b) at a reduced pH; (c) at a high pH in the presence of 50 mg/l manganese; (d) at a reduced pH in the presence of 50 mg/l manganese until all detectable sulphide had been removed. An 800 ml sample from each test was placed in a 1 l ground glass flask and the air in the flask displaced by flushing with a mixture of N_2 and CO_2 prior to sealing the flask. The flasks were allowed to incubate for a week at room temperature in order to allow anaerobic conditions to develop and the facultative anaerobic bacteria to grow before assaying for the return of sulphide.

4.3 Results and Discussion

4.31 The role of micro-organisms in reducing the oxygen demand of lime-sulphide effluents

In order to determine the part played by the micro-organisms in the

reduction of oxygen demand of fellmongery effluent during extended aeration, duplicate samples of effluent were seeded with activated sludge before the micro-organisms in one of the two samples were destroyed by either autoclaving or chemical sterilisation. Chemical sterilisation had the advantage over steam sterilisation in that autoclaving reduced the OA by 44%. This was due to heat precipitation and settling out of proteins and other components which normally contribute to the OA. The rate of oxygen demand reduction of the sterilised effluents was compared with the rate in oxygen demand reduction of similar unsterilised effluents. The oxygen demand decrease obtained in the absence of micro-organisms must be due to the chemical effects of aeration. Oxygen demand decrease over and above this level obtained by aeration of corresponding unsterilised effluent must be due to the micro-organisms present in the effluent.

The addition of sodium azide and acriflavine was an effective means of sterilisation of the effluent although a small number of resistant strains appeared after 3 days aeration (Fig. 4.1). In the case of unsterilised effluent there was a 31% decrease in OA after 1 day and a 79% decrease after 4 days aeration. This decrease in oxygen demand was accompanied by an approximate 100-fold increase in bacterial count after 2 days aeration. Following chemical sterilisation there was only an 18% reduction in oxygen demand after 1 day with a maximum of a 27% OA reduction after extended aeration of 4 days. This 27% reduction in OA is likely to be due to the chemical effects of aeration only, such as the removal of sulphide and various other volatile components from the effluent. The approximately 52% additional decrease in OA obtained when similar unsterilised effluent undergoes extended aeration must be due to micro-organisms.

4.32 The effect of effluent neutralisation before aeration on the effluent treatment process

In order to determine the extent of neutralisation necessary for the

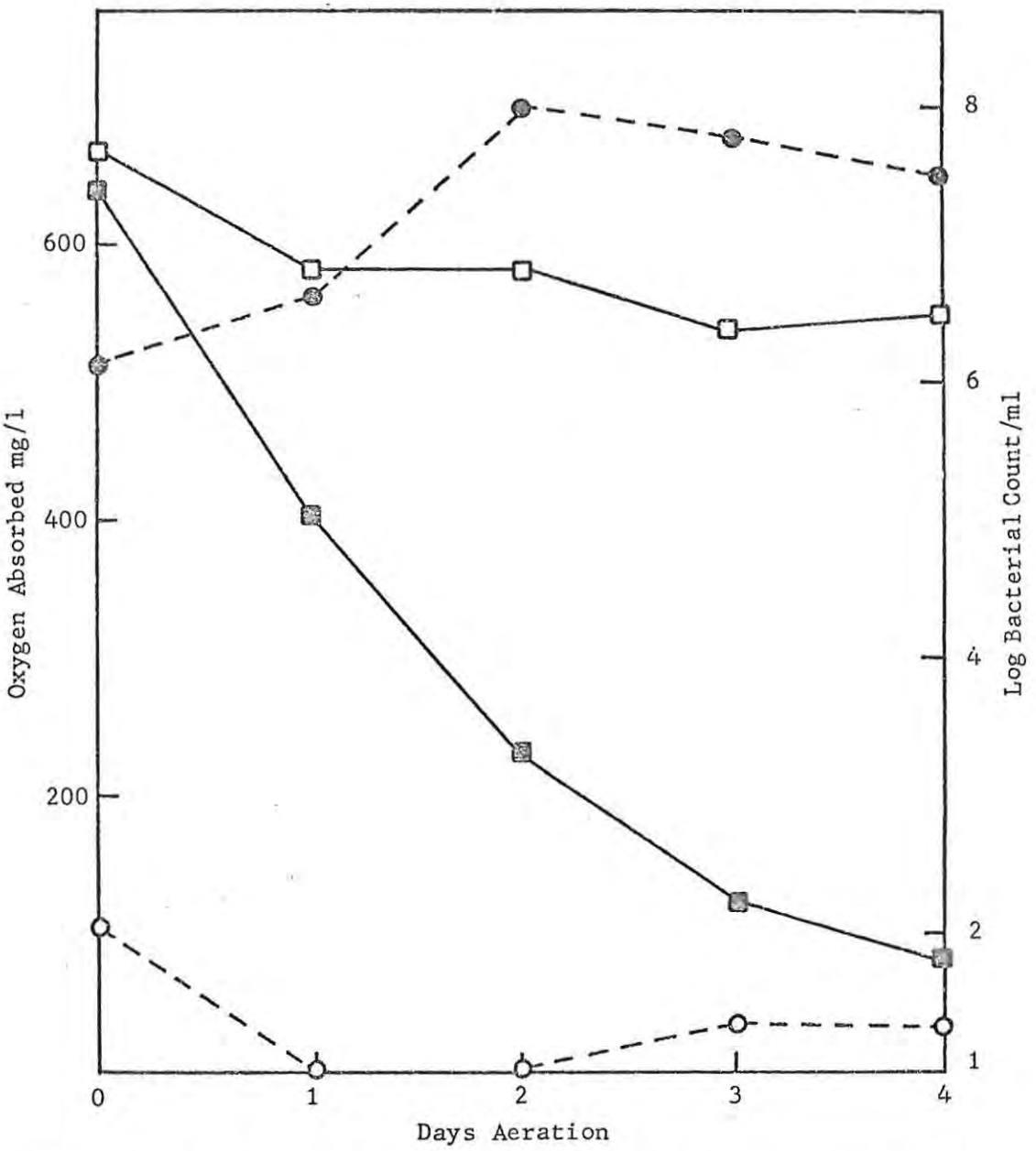


Fig. 4.1. The effect of chemical sterilisation of lime-sulphide effluent on the changes in permanganate value and bacterial count with extended aeration. ▩—▩ OA unsterilised effluent; □—□ OA sterilised effluent; ●—● Bacterial count of unsterilised effluent; ○—○ Bacterial count of sterilised effluent.

effluent treatment process to function effectively, replicate samples of effluent were neutralised to different pH levels before being subjected to extended aeration. This experiment was carried out twice. In the first experiment activated sludge seed was added immediately after neutralisation, whereas in the second experiment sludge seed was added after 24 h aeration when most of the sulphide would have been removed depending on the degree of neutralisation. The reason for this was to determine the effect of a shock load of sulphide on the microbial activity. Changes in OA, pH and bacterial count with progressive aeration were monitored at daily intervals for 4 days and again after 7 days. The results are shown in Figs 4.2 and 4.3.

When the sludge was added immediately after neutralisation, the final pH of the adjusted effluent before aeration was pH 8,0, 9,5, 11,5 and 12,9 (no acid added). When the effluent was adjusted to pH 11,5 or less, the pH of the effluent fell to pH 9 or below after 3 days aeration and to between pH 8 and 8,5 after 4 days (Fig. 4.2a). In the case of effluent which was not neutralised (pH 12,9) the pH of the aerated effluent was still above 11 after 4 days. The results in Fig. 4.2b showed that the greater the neutralisation of the effluent, the greater the rate of OA reduction during the first day of aeration. After the first day of aeration the rate of OA reduction decreased in the cases of the two most neutralised effluents whereas in the other two it increased. This result is readily explained as the greater the initial neutralisation, the more efficiently the sulphide will be removed by aeration and the sooner microbial activity can contribute to oxygen demand reduction. When the initial neutralisation is small, biological activity would be expected to be delayed as the aeration time needed for removal of the sulphide is longer. This effect can also be seen from the changes in the bacterial count with progressive aeration (Fig. 4.2c). The bacterial counts increased rapidly in the neutralised effluents, whereas in the high pH

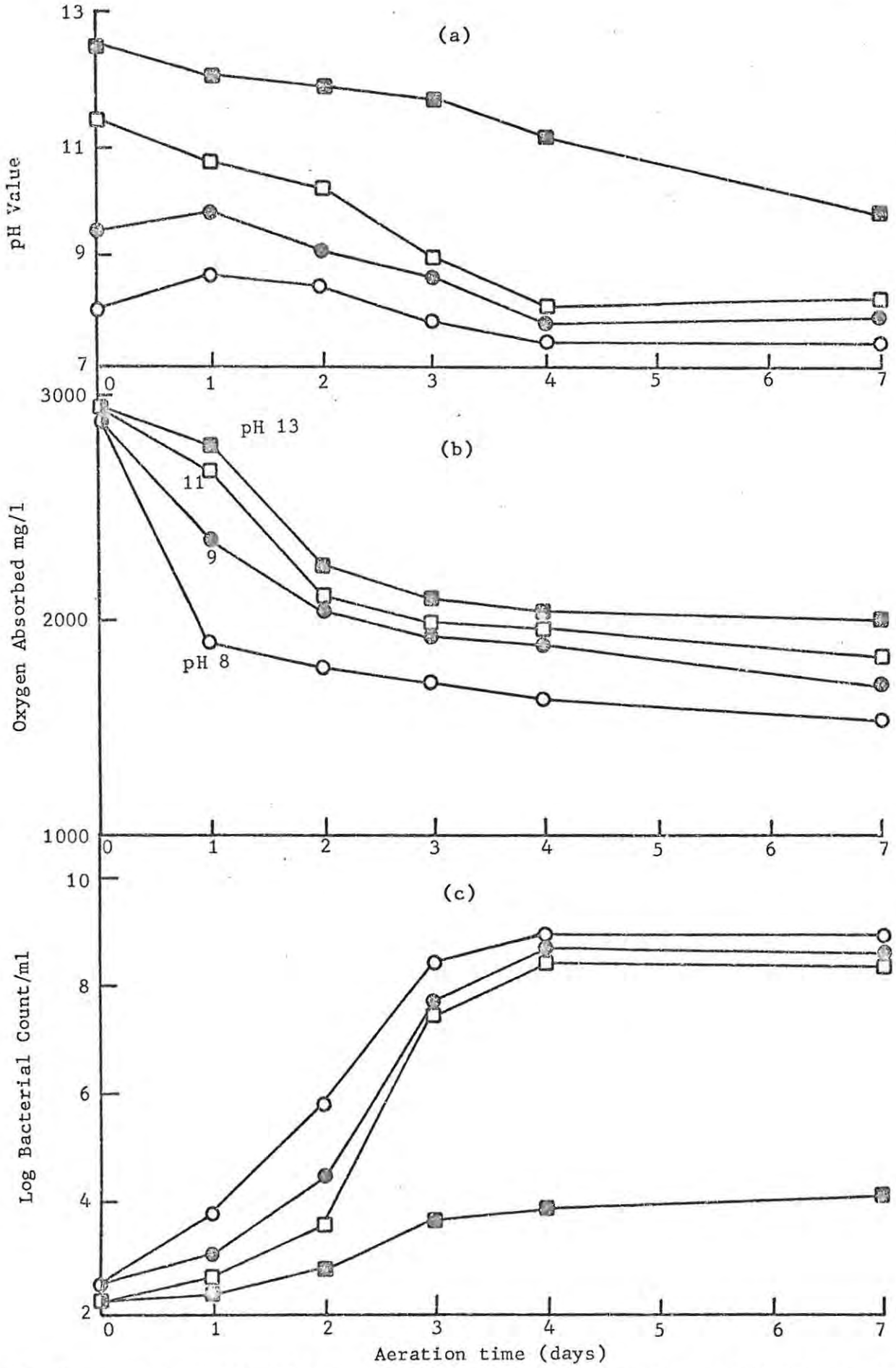


Fig. 4.2. The effect of the extent of neutralisation of lime-sulphide effluent before aeration on subsequent aeration treatment. Sludge added before aeration. Extent of neutralisation - ■—■ pH 12,9; □—□ pH 11,5; ●—● pH 9,5; ○—○ pH 8,0.

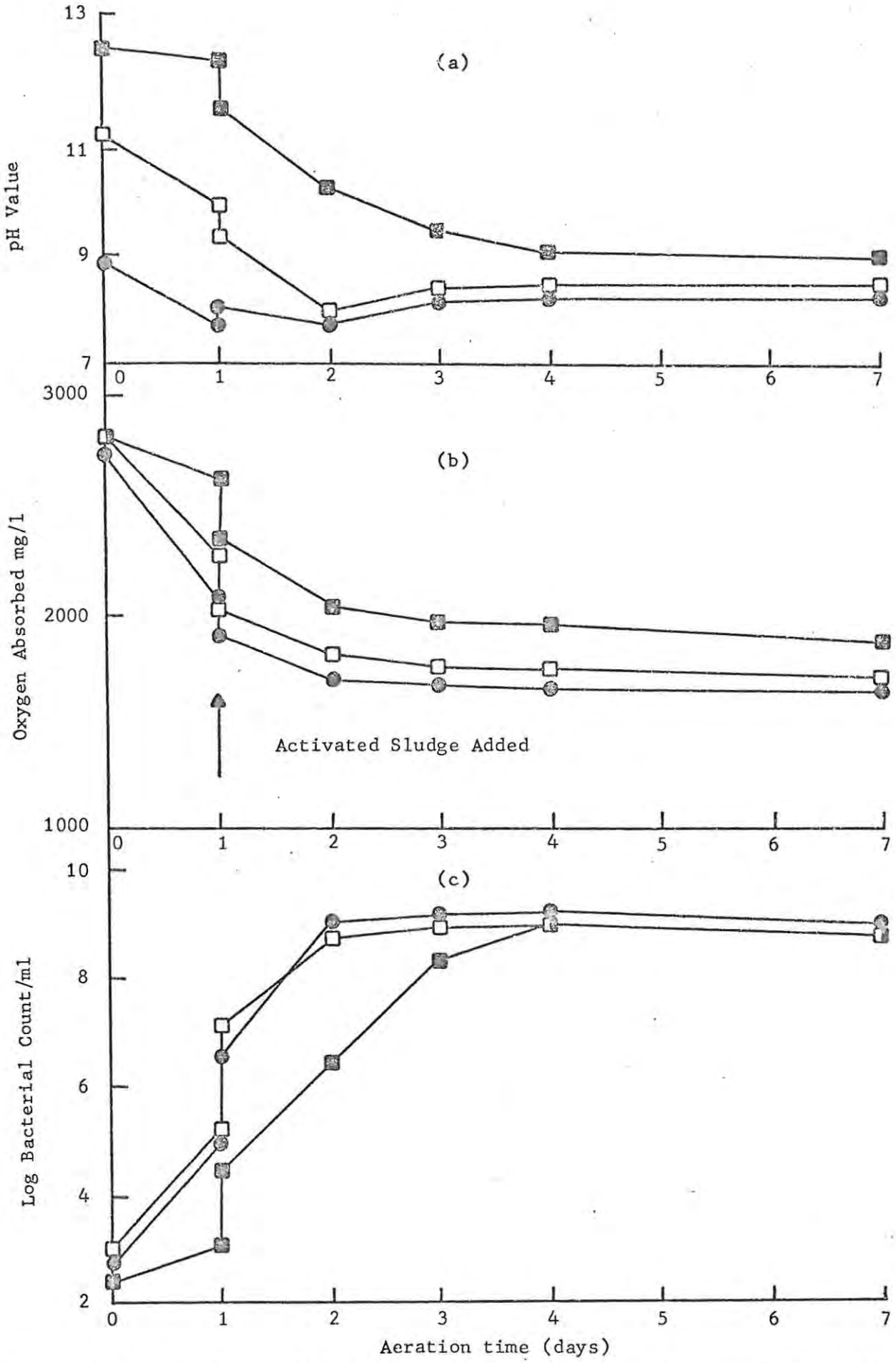


Fig. 4.3. The effect of the extent of neutralisation of lime sulphide effluent before aeration on subsequent aeration treatment. Sludge added after 24 h aeration. Extent of neutralisation - ■—■ pH 12,9; □—□ pH 11,3; ●—● pH 9,0.

effluent the increase in the bacterial count was comparatively small and the final count 10^4-fold lower. The greatest reduction in OA for this strong effluent (OA 2950 mg/l) after 2 days of aeration occurred with the effluent neutralised to pH 8 and amounted to 41%.

In the experiment where the activated sludge seed was added after 1 day of aeration, the initial pH of the effluent was adjusted to pH 9,0, 11,3 and 12,9 (no neutralisation). After 2 days of aeration the pH of the two neutralised effluents was approximately pH 8 and on further aeration stabilised at a level of about pH 8,3 (Fig. 4.3a). The effluent which was not neutralised was markedly more alkaline (pH 10,3 after 2 days of aeration) and never dropped below pH 9,1. The rates of OA reduction for the two partially neutralised effluents (pH 9 and 11,3) were very similar and faster than the rate of OA reduction of the unneutralised effluent (Fig. 4.3b). The effect of neutralisation on bacterial growth was again evident from these results. There was a rapid and similar rate of increase in the bacterial count in the two partially neutralised effluents even before they were seeded with activated sludge. In contrast, the rate of increase in the bacterial count of the high pH effluent was slower than the neutralised effluents up until the time that the effluents were seeded with sludge (Fig. 4.3c). The volume of sludge added to the effluent ($\pm$ 10% total volume) helped reduce the pH and hence the sulphide concentration sufficiently to allow the bacteria to grow rapidly after this stage, thus contributing to an additional reduction in oxygen demand. The greatest reduction in OA occurred in the effluent with greatest neutralisation (pH 9) and amounted to 43% after 2 days aeration.

From the above results it would appear that a small amount of neutralisation before aeration assists greatly in reducing the pH and OA of the effluent while permitting rapid bacterial growth. Applying these results to a continuous flow activated sludge treatment plant it is apparent that the less the neutralisation, the longer the retention time

required to treat the effluent. However, a small degree of neutralisation can be expected to markedly reduce the retention time necessary to oxidise the effluent.

By comparing the rate of OA reduction, pH reduction and bacterial growth between the two experiments, it appears to be advantageous to have a pre-aeration step during which the sulphide is removed, before adding activated sludge and aerating further.

4.33 The effect of manganese oxidising catalyst on the effluent treatment process

Replicate effluent samples were aerated in the absence and in the presence of different concentrations of manganese to determine what effect this oxidising catalyst has on lime-sulphide effluent treatment. This experiment was also carried out in duplicate. In the first experiment activated sludge seed was added prior to aeration and in the second experiment the effluent was seeded after aeration for 1 day. The changes in OA, pH, sulphide and optical density as well as bacterial and protozoa counts of the effluent were monitored at daily intervals for 4 days and again after 7 days of aeration.

The results of the first experiment are shown in Table 4.1. Manganese was effective in removing 100% of the sulphide within 24 h of aeration, even at concentrations as low as 25 mg/l. In contrast there was still $\pm$ 3% of the sulphide left after aeration for 24 h in the absence of manganese. This amount of remaining sulphide would probably have been higher had the initial pH not been made lower than usual by the addition of activated sludge seed. The presence of manganese increased both the rate and the amount of OA reduction. Although the rate of OA reduction did not necessarily increase with increasing manganese concentration, the final level to which the OA was lowered did. Optical density measurements after 24 h indicated that effluent aerated in the presence of manganese was

Table 4.1 Aeration of Fellmongery Effluent - Sludge added before aeration.

(a) Control - No Manganese Added

Aeration time	0 days	1 day	2 days	3 days	4 days	7 days
OA mg/l	1 009,8	786,4	668,8	657,2	644,0	604,8
% redn in OA	0	22	34	35	36	40
pH	11,8	9,4	8,8	8,8	8,8	8,7
Na_2S mg/l	634,0	19,5	0,4	0,4	0,4	0,4
Bacteria/ml	$2,04 \times 10^7$	$9,60 \times 10^6$	$2,73 \times 10^8$	$4,50 \times 10^8$	$1,05 \times 10^8$	$4,24 \times 10^7$
Protozoa/ml	0	0	$1,75 \times 10^2$	$7,12 \times 10^3$	$2,10 \times 10^5$	$1,20 \times 10^6$
Optical density	0,68	0,91	0,96	0,91	0,58	0,49

(b) 25 mg/l Manganese Added

OA mg/l	1 008,2	690,4	604,0	585,6	532,0	436,0
% redn in OA	0	31	40	42	47	57
pH	11,8	9,8	8,9	8,9	8,8	8,7
Na_2S mg/l	639,6	0,4	0,4	0,4	0,4	0,4
Bacteria/ml	$1,98 \times 10^7$	$1,92 \times 10^6$	$3,63 \times 10^8$	$7,50 \times 10^8$	$1,32 \times 10^7$	$1,02 \times 10^7$
Protozoa/ml	0	0	$5,25 \times 10^2$	$2,04 \times 10^4$	$4,40 \times 10^5$	$2,01 \times 10^6$
Optical density	0,70	1,20	0,92	0,72	0,37	0,24

(c) 50 mg/l Manganese Added

OA mg/l	1 007,4	620,8	602,4	561,6	518,4	412,8
% redn in OA	0	38	40	44	49	59
pH	11,8	9,3	8,8	8,8	8,8	8,7
Na_2S mg/l	636,2	0,4	0,4	0,4	0,4	0,4
Bacteria/ml	$2,10 \times 10^7$	$2,73 \times 10^6$	$3,48 \times 10^8$	$3,66 \times 10^8$	$3,31 \times 10^7$	$1,21 \times 10^7$
Protozoa/ml	0	0	$1,08 \times 10^2$	$1,24 \times 10^4$	$8,60 \times 10^5$	$1,48 \times 10^6$
Optical density	0,70	1,20	0,88	0,61	0,26	0,23

(d) 100 mg/l Manganese Added

OA mg/l	1 006,2	642,4	528,8	495,2	464,0	400,0
% redn in OA	0	36	47	51	54	60
pH	11,7	9,8	8,8	8,8	8,8	8,7
Na_2S mg/l	630,5	0,4	0,4	0,4	0,4	0,4
Bacteria/ml	$2,40 \times 10^7$	$3,90 \times 10^5$	$4,95 \times 10^8$	$3,00 \times 10^8$	$6,21 \times 10^7$	$9,45 \times 10^6$
Protozoa/ml	0	0	$3,55 \times 10^3$	$7,90 \times 10^3$	$1,02 \times 10^6$	$3,68 \times 10^6$
Optical density	0,68	1,75	0,82	0,76	0,53	0,19

more turbid than effluent aerated in the absence of manganese. Nevertheless, the final clarity of the manganese treated effluents was better than the effluent without manganese. The addition of manganese had no noticeable effect on the pH or the rate of pH reduction of the effluent, nor did it appear to be harmful to the bacteria or protozoa present even at a concentration of 100 mg/l.

The results of the second experiment in which the activated sludge seed was added after the removal of the sulphide are shown in Table 4.2. These results are very similar to those of the first experiment. There was however a noticeable increase in the initial rate of OA reduction with increasing manganese concentration. This increase in rate with increasing manganese concentration is large up to 50 mg/l manganese but very much less between 50 and 100 mg/l manganese, suggesting that the effluent is becoming saturated with catalyst at about this level. The addition of 50 mg/l manganese gave a catalyst/sulphide ratio of about 0,18 which was close to the most efficient catalyst/sulphide ratio of 0,15 reported by Eye and Clement (1972). In general the rate of growth of bacteria and protozoa appears to be greater when the activated sludge was added after the preliminary aeration to remove sulphide.

4.34 The contribution of sulphide to the oxygen absorbed value

A calibration curve of the oxygen absorbed values of sulphide concentrations from 10 to 560 mg/l Na_2S was constructed (Fig. 4.4). A linear relationship between sulphide concentration and its OA value over the range tested was found (linear regression analysis $y = 3,47 + 0,822x$). However, on the removal of sulphide by aeration this linear relationship no longer holds. This is illustrated from the data in Table 4.1b. After 24 h aeration the sulphide concentration was reduced by 640 mg/l and the corresponding OA reduction was 318 mg/l. From the calibration curve the predicted OA reduction for a 640 mg/l drop in sulphide alone

Table 4.2 Aeration of Fellmongery Effluent Sludge added after 24 hours of aeration.

(a) Control - No Manganese Added

Aeration Time	0 days	1 day Before Seeding	1 day After Seeding	2 days	3 days	4 days	7 days
OA mg/l	1 127,2	863,0	784,0	693,6	627,2	598,4	452,2
% redn in OA	0	23	30	38	44	47	60
pH	12,2	11,6	11,2	9,1	8,8	8,8	8,8
Na_2S mg/l	873,0	46,5	39,7	1,2	0,4	0,4	0,4
Bacteria/ml	$6{,}10 \times 10^{2}$	$8{,}76 \times 10^{2}$	$9{,}30 \times 10^{5}$	$7{,}56 \times 10^{8}$	$1{,}11 \times 10^{9}$	$8{,}98 \times 10^{8}$	$1{,}20 \times 10^{7}$
Protozoa/ml	0	0	$1{,}4 \times 10^{1}$	$1{,}14 \times 10^{2}$	$6{,}62 \times 10^{3}$	$1{,}78 \times 10^{5}$	$5{,}95 \times 10^{6}$
Optical density	0,82	1,00	0,88	0,82	0,74	0,61	0,29

(b) 25 mg/l Manganese Added

OA mg/l	1 126,4	761,8	699,6	621,6	557,6	524,8	449,8
% redn in OA	0	32	38	45	50	53	60
pH	12,2	10,9	10,5	8,7	8,8	8,8	8,7
Na_2S mg/l	875,1	1,8	1,4	0,4	0,4	0,4	0,4
Bacteria/ml	$6{,}96 \times 10^{2}$	$5{,}36 \times 10^{3}$	$4{,}89 \times 10^{5}$	$6{,}00 \times 10^{8}$	$1{,}38 \times 10^{9}$	$1{,}18 \times 10^{9}$	$2{,}44 \times 10^{7}$
Protozoa/ml	0	0	$1{,}9 \times 10^{1}$	$2{,}83 \times 10^{2}$	$4{,}38 \times 10^{3}$	$9{,}68 \times 10^{4}$	$4{,}84 \times 10^{6}$
Optical density	0,82	1,20	1,10	0,82	0,76	0,57	0,18

(c) 50 mg/l Manganese Added

OA mg/l	1 129,2	643,6	583,2	559,2	532,0	503,6	417,6
% redn in OA	0	43	48	50	53	55	63
pH	12,2	10,8	10,4	8,6	8,9	8,9	8,8
Na_2S mg/l	871,4	0,4	0,4	0,4	0,4	0,4	0,4
Bacteria/ml	$5{,}44 \times 10^{2}$	$7{,}68 \times 10^{3}$	$8{,}42 \times 10^{6}$	$4{,}24 \times 10^{8}$	$9{,}09 \times 10^{8}$	$3{,}43 \times 10^{9}$	$6{,}36 \times 10^{6}$
Protozoa/ml	0	0	$2{,}1 \times 10^{1}$	$3{,}30 \times 10^{2}$	$4{,}94 \times 10^{3}$	$1{,}04 \times 10^{5}$	$5{,}24 \times 10^{6}$
Optical density	0,82	1,50	1,40	0,95	0,88	0,64	0,16

(d) 100 mg/l Manganese Added

OA mg/l	1 132,4	626,4	560,0	544,0	521,6	492,0	406,4
% redn in OA	0	45	51	52	54	57	64
pH	12,2	10,6	10,3	8,5	8,7	8,8	8,7
Na_2S mg/l	871,8	0,4	0,4	0,4	0,4	0,4	0,4
Bacteria/ml	$5{,}88 \times 10^{2}$	$8{,}45 \times 10^{3}$	$2{,}85 \times 10^{6}$	$9{,}36 \times 10^{7}$	$7{,}16 \times 10^{8}$	$5{,}42 \times 10^{9}$	$6{,}29 \times 10^{6}$
Protozoa/ml	0	0	$1{,}8 \times 10^{1}$	$1{,}98 \times 10^{2}$	$5{,}66 \times 10^{4}$	$1{,}14 \times 10^{5}$	$5{,}02 \times 10^{6}$
Optical density	0,82	1,50	1,40	0,85	0,74	0,57	0,17

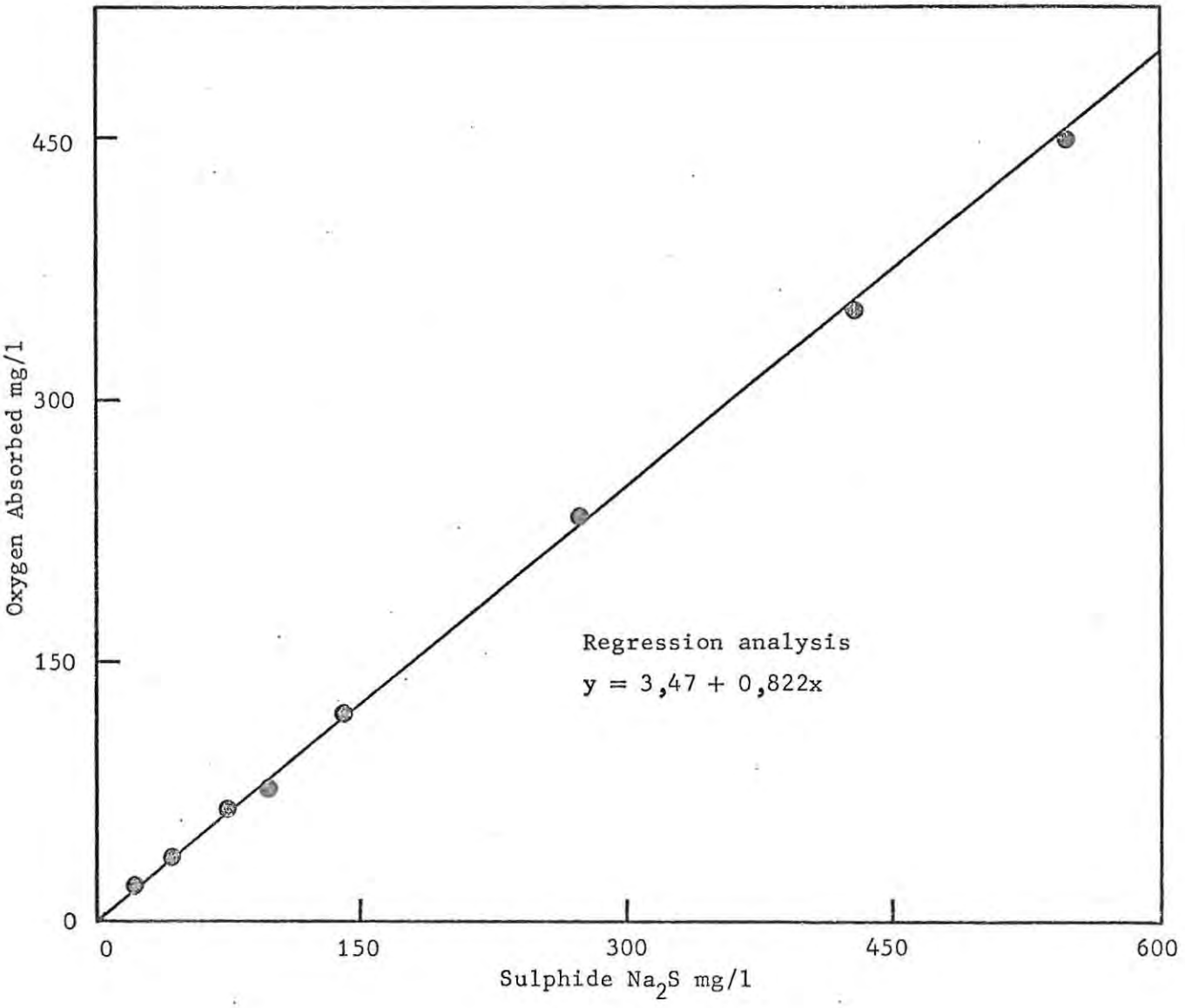


Fig. 4.4. The linear relationship between Na_2S and its oxygen absorbed value.

(excluding any OA reduction due to utilisation of effluent components by micro-organisms) should be 529 mg/l. This observation suggests that although all detectable sulphide was removed in 24 h in the presence of manganese a considerable proportion was converted to a form which still had an OA value. It is therefore important that the methods of removing sulphide be investigated to determine the proportion of sulphide that is converted to a form which is not detectable as Na_2S but still has an OA value.

4.35 Comparison of OA reduction by sulphide removal on aeration at high pH, after partial neutralisation and at high pH after the addition of manganese

The removal of sulphide by aeration was investigated in a laboratory prepared effluent obtained by dissolving lime-sulphide unhairing paste in water. In such a chemical system OA reduction due to biological action can be discounted since there was no organic substrate present. The results of two identical experiments are presented in Tables 4.3 and 4.4. Detectable sulphide (Na_2S) was completely removed within 24 h aeration of the mixture in the presence of manganese, whereas 87% and 96% of the sulphide was removed after 24 h at pH 12,2 and 7,8 respectively (Table 4.3a and b). All three treatments were effective in removing 100% of the sulphide within 2 days. The loss of sulphide from the mixtures was accompanied by a decrease in OA of the mixture, but this decrease varied depending on the conditions of aeration. The OA of the effluent preparations aerated at high and low pH in the absence of manganese was reduced by 77% and 84% respectively. There was only a 36-41% decrease in the OA of effluent aerated in the presence of manganese. A high proportion of the OA value therefore remained behind in solution after catalytic oxidation by the manganese and was not removed by further aeration. As all detectable sulphide was removed within 24 h in the presence of manganese, it appears that manganese oxidised the sulphide to

Table 4.3 Aeration of inorganic lime-sulphide effluent, (a) at high pH; (b) after neutralisation with H_2SO_4; (c) after the addition of 50 mg/l manganese.

a)

Aeration time	0 days	1 day	2 days	3 days	4 days	7 days
OA mg/l	636,0	284,2	163,4	156,2	152,0	149,0
% OA reduction	0	55	74	75	76	77
Na_2S mg/l	806,4	106,0	0,4	0,4	0,4	0,4
pH	12,2	10,3	9,0	9,0	9,0	9,0

b)

OA mg/l	625,2	283,2	128,2	104,8	103,2	102,4
% OA reduction	0	55	79	82	83	84
Na_2S mg/l	800,2	34,7	0,4	0,4	0,4	0,4
pH	7,8	9,2	8,2	7,8	7,8	7,8

c)

OA mg/l	654,0	384,4	408,8	418,4	419,6	418,2
% OA reduction	0	41	38	36	37	36
Na_2S mg/l	816,0	0,4	0,4	0,4	0,4	0,4
pH	12,2	11,8	9,1	9,0	9,0	9,0

a form which remained in the effluent and had an OA value of its own. When the effluent was aerated in the absence of manganese most of the sulphide was expelled as H_2S. The results of the second experiment (Table 4.4) are similar to those described above although the actual OA reductions between the two experiments differed.

Table 4.4 Aeration of inorganic lime-sulphide effluent; (a) at high pH; (b) after neutralisation with H_2SO_4; (c) after the addition of 50 mg/l manganese.

a) Aeration time	0 days	1 day	2 days	3 days	4 days	7 days
OA mg/l	604,4	375,0	196,2	132,0	119,8	116,0
% OA reduction	0	38	68	78	80	81
Na_2S mg/l	801,0	400,4	91,2	0,4	0,4	0,4
pH	12,2	11,5	9,3	9,0	9,0	9,0

b)						
OA mg/l	603,8	302,8	118,0	99,6	96,2	84,8
% OA reduction	0	50	80	84	85	86
Na_2S mg/l	800,8	166,0	15,6	0,4	0,4	0,4
pH	8,7	9,8	7,9	7,8	7,8	7,8

c)						
OA mg/l	604,2	240,0	259,2	260,0	268,6	284,0
% OA reduction	0	60	57	57	56	53
Na_2S mg/l	802,2	0,4	0,4	0,4	0,4	0,4
pH	12,2	11,6	9,9	9,0	9,0	9,0

These results are very significant in that the OA value is often used as an estimate of effluent quality and as the addition of manganese contributes indirectly to this value, its addition as an oxidising catalyst is undesirable.

4.36 The reappearance of sulphide under anaerobic conditions

Reappearance of sulphide after removal may occur in practice if the effluent is run into an oxidation pond which for some reason becomes anaerobic. The effects of the subsequent development of anaerobic conditions following the removal of sulphide by aeration at high and neutral pH in the presence and absence of manganese are shown in Table 4.5.

All four methods of aeration were successful in removing over 99,8% of the detectable sulphide from the effluent within 2 days. After 7 days of anaerobic incubation, sulphide reappeared in the effluent previously aerated at high pH (7,8% of original sulphide), alkaline effluent aerated in the presence of manganese (20,3%) and neutralised effluent in the presence of manganese (23,9%). In the case of effluent aerated at near neutral pH in the absence of manganese only 0,1% of the original sulphide reappeared. It is concluded that the catalytic removal of sulphide by manganese oxidised the sulphide to a state having a considerable OA value which could be reduced again to sulphide under anaerobic conditions. In the case alkaline effluent aerated in the absence of manganese a small proportion of the oxidised sulphide was recovered under reducing conditions. When neutralised effluent was aerated almost all the sulphide was lost as H_2S and only a negligible proportion recovered.

4.4 Conclusions

As a result of these batch culture aeration studies it appears as though adapted activated sludge micro-organisms contribute considerably to the reduction in oxygen demand (OA) of lime-sulphide effluents. Both the partial neutralisation and the addition of manganese oxidising catalyst to raw untreated effluent result in a rapid rate of sulphide removal and a noticeable increase in the rate of OA reduction. Manganese oxidation of the sulphide, although extremely rapid, appears to oxidise the sulphide into

Table 4.5 The return of sulphide under anaerobic conditions after its oxidation or removal by aeration (a) at high pH; (b) after the addition of 50 mg/l manganese; (c) after neutralisation; (d) after neutralisation and the addition of 50 mg/l manganese.

a)	Aeration Time	0 days	2 days	7 days Anaerobic incubation
	OA mg/l	2059,2	1558,4	1464,0
	Na_2S mg/l	1506,7	0,8	117,0
	% Na_2S remaining	100	0,05	7,77
	pH	12,70	9,72	6,73

b)	OA mg/l	1994,4	1668,8	1506,4
	Na_2S mg/l	1496,4	1,17	303,3
	% Na_2S remaining	100	0,08	20,28
	pH	12,68	9,96	6,80

c)	OA mg/l	1960,8	1120,0	1255,2
	Na_2S mg/l	1476,7	1,17	1,95
	% Na_2S remaining	100	0,08	0,13
	pH	7,64	8,34	6,50

d)	OA mg/l	2072,0	1565,6	1111,2
	Na_2S mg/l	1364,2	1,90	327,7
	% Na_2S remaining	100	0,14	23,96
	pH	7,55	7,95	6,30

products which remain in the effluent and contribute to its OA value. Under anaerobic conditions these oxidised products may again be reduced, resulting in an undesirable increase in the sulphide concentration of the effluent. Aeration after partial neutralisation results in the sulphide being lost from the effluent and hence should yield an effluent with a lower oxygen demand. However, as the removal of sulphide by aeration after neutralisation is less rapid than its catalytic oxidation by manganese, a longer retention time may have to be tolerated in a continuous flow treatment plant. Alternatively, the effluent may be treated by two aeration tanks in series, the first to remove the sulphide and the second to enable biological oxidation to take place.

CHAPTER V

THE EFFECT OF THE FEED CONCENTRATION ON THE ACTIVATED SLUDGE TREATMENT OF FELLMONGERY EFFLUENT

Summary

The initial strength of the untreated effluent was found to influence the degree of OA reduction obtained on treatment in both batch and continuous culture. There was a rapid decrease in the efficiency of OA removal by the completely mixed activated sludge (CMAS) process at initial substrate strengths of OA >1500 mg/l. The maximum feed concentration acceptable for treatment by the CMAS process appeared to be limited by the rate at which oxygen could be supplied to the organisms. The efficiency of an acclimated microbial population was demonstrated by the large drop in pH, complete removal of sulphide and a 72% reduction in OA (retention time - 2 days; sludge age-5 days; feed strength - OA 1500 mg/l) which occurred in a single stage reactor without either prior neutralisation or the addition of oxidising catalyst to remove the sulphide.

5.1 Introduction

In the previous chapter the removal of sulphide by aeration in the presence of manganese or after neutralisation with acid was investigated. Both techniques removed all detectable sulphide and improved the quality of the effluent. However, the maximum reduction in OA value using strong lime-sulphide effluent (OA 2800 mg/l) was only 43% and the treated effluent therefore still had a high residual oxygen demand (OA 1600 mg/l). It has been reported (Pipes, 1966) that waste containing high concentrations of soluble organic matter is difficult to treat by the activated sludge process. Hawkes (1963) states that in the aerobic treatment of wastes having a high oxygen demand (BOD >1000 mg/l), oxygen depletion occurs in the immediate environment of the organisms. No matter how efficient the aeration is within the plant, the rate of oxygen uptake would exceed the rate at which it could be transferred from the air through the liquid to the organisms. Hawkes (1963) recommends that such liquors should therefore be diluted with returned effluent or by other means to produce a liquor having a BOD not exceeding 350 mg/l.

5.11 The problem of oxygen supply

The passage of oxygen from the air to the respiratory enzymes within the cell has been divided by Hawkes (1963) into four stages. Atmospheric oxygen must firstly dissolve in the waste water, after which it must be transferred to the surface of the respiring cell, diffuse through the cell wall and cell membrane and finally be absorbed by the appropriate respiratory enzymes. The first two stages of oxygen transfer are controlled by plant design and operation, i.e. the method of aeration and the quality of mixing. The last two stages are governed by the properties of the cells themselves.

High dissolved oxygen concentrations in the mixed liquor do not appear to be necessary for an efficient treatment process. Most micro-organisms in biological oxidation plants are micro-aerophilic requiring only low concentrations of dissolved oxygen. Many workers have found that a dissolved oxygen concentration of less than 1 mg/l is sufficient (Wuhrmann, 1956, Okun and Lynn, 1956). It has been reported (Mishoe, 1972) that even when the rate of aeration is substantially better than normal, a concentration of 1 mg dissolved oxygen/l is difficult to maintain and that only 0,2 - 0,5 mg/l dissolved oxygen is needed to maintain a high effluent quality. Adequate transfer of dissolved oxygen to the flocs is dependant on a good degree of turbulence within the plant. If agitation and mixing are inadequate the dissolved oxygen at the surface of a floc will only be a small fraction of that in the rest of the water (Pasveer, 1956). To ensure saturation of the oxygen-bearing enzymes within the cell it is necessary to maintain the dissolved oxygen concentration at the surface of the cells at as high a level as possible. McNicholas and Tench (1959) reported that increased rates of aeration resulted in increased rates of purification. The enhanced purification was said to be due to a change in species composition of the sludge which were selected for by the higher dissolved oxygen concentration. Presumably the micro-aerophilic species

were replaced by more aerobic organisms which had increased rates of metabolism.

The level of dissolved oxygen in the mixed liquor has an influence on whether nitrification occurs or not. If nitrification is to be encouraged a dissolved oxygen concentration of greater than 2 mg/l appears to be necessary (Downing and Hopwood, 1964; McCarty and Haug, 1972). If nitrification is to be discouraged then a 2 mg/l dissolved oxygen concentration sets the upper limit.

The size and texture of the sludge floc have also been reported to be dependant on the dissolved oxygen concentration (Wuhrmann, 1964). He calculated that flocs with a diameter of 500μ should be supported by a dissolved oxygen concentration of approximately 2 mg/l to ensure the innermost cells an adequate supply of oxygen. A mechanism was suggested by which a low dissolved oxygen concentration could cause break-up of the floc resulting in poor settling properties.

The sludge seems to have a maximum capacity to take up organic matter which is linked to the availability of oxygen and length of aeration. If this capacity is exceeded the rest of the organic matter passes through the treatment system unaltered. Bruce and Boon (1970) have shown that during the first 10 minutes of aeration after mixing sewage with activated sludge, the rate of oxygen uptake by the sludge and the rate of removal of soluble and suspended matter are both very rapid. If at this time the mixed liquor is allowed to settle, the supernatant contains relatively little suspended matter and its BOD is greatly reduced. When this procedure is repeated batchwise a number of times with a high concentration of suspended solids (1500 mg/l) and with intermediate aeration periods of less than 1 h, the sludge progressively loses its purifying capacity and the quality of the supernatant deteriorates. However, if the period of aeration in between organic loads is increased the sludge retains its purifying ability. In the case of domestic sewage and other low substrate

sewages the rate of oxygen uptake often exceeds the rate of nutrient uptake so that oxygen availability is not limiting. However, with some strong industrial effluents the rate of nutrient uptake exceeds that of oxygen which would then become limiting.

The experiments reported in this chapter were designed to determine the effect of feed concentration on effluent quality of the treatment process. In the first set of experiments the batch culture technique was used in order to establish whether the high feed concentration used in the previous experiments was the reason for the low percentage purification obtained in the previous chapter. Once this was established then a set of continuous culture experiments was conducted in order to determine what sort of feed concentration levels the activated sludge process can tolerate. The completely mixed activated sludge reactor (CMAS) used in the continuous flow experiments approximates the large scale activated sludge process envisaged for the treatment of effluent at the tannery or fellmongery.

5.2 Materials and Methods

5.21 Batch culture experiment

Two samples of fellmongery effluent were obtained from tank b of the pilot plant described in Chapter III. A portion of each sample was diluted 4-fold before both the diluted and undiluted samples were aerated. One pair of diluted and undiluted samples was aerated in the presence of 50 mg/l manganese and the other pair after neutralisation with 33% H_2SO_4.

5.22 Continuous flow experiment

a) Source of effluent

A bulk supply of strong fellmongery lime-sulphide unhairing effluent (analysis - OA 6 500 mg/l; pH 13,2; Na_2S 6 280 mg/l) was obtained and stored at $4^{\circ}C$. Twice each day the settled effluent was diluted with tap

water to give an effluent of the required strength before being added to the feed reservoir. Analysis of the feed reservoir was carried out each day together with the analysis of the CMAS reactor.

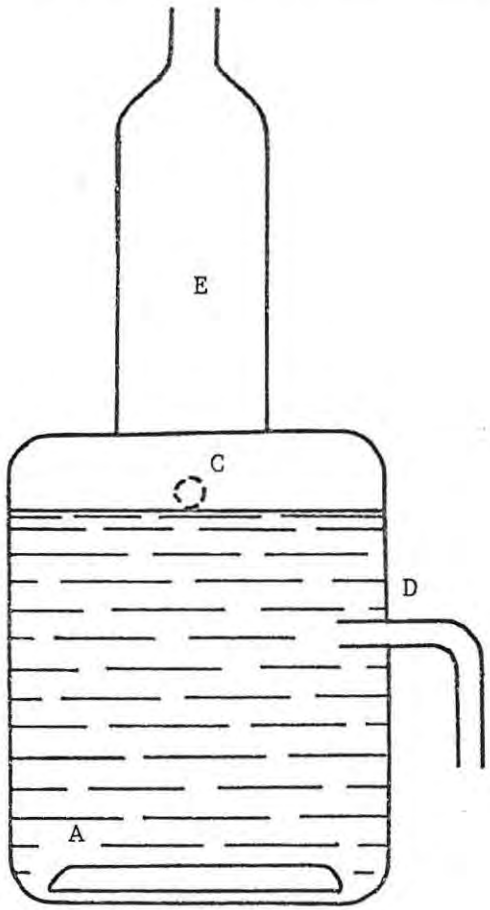
b) Continuous flow apparatus

The CMAS reactor is shown in Figure 5.1. Aeration was provided by an aquarium air pump equipped with a 15 cm air stone. These pumps supplied each reactor with an approximate rate of aeration of 0,45 1/1 reactor/minute. By locating the air stone in the position shown, A, efficient mixing of the effluent was obtained while the air was well dispersed into small bubbles by the air stone. The circulation flow pattern was towards the feed inlet to prevent the feed from being carried out into the separator before adequate mixing had occurred. A vessel with rounded corners was chosen for the reactor to prevent dead spots from developing in the bottom corners. The feed mechanism was by means of a peristaltic pump which provided a continuous supply of feed from a reservoir to the reactor throughout the day. The separator tank was constructed out of a separating funnel with an effective volume of 0,5 1. As the volume of the reactor was 4,5 1 this means the retention time in the separator was 9-fold less than that of the reactor vessel. A quick turnover of effluent in the separator is needed in order to ensure that the sludge does not become anaerobic although the retention time must be long enough for liquid-solid separation to take place.

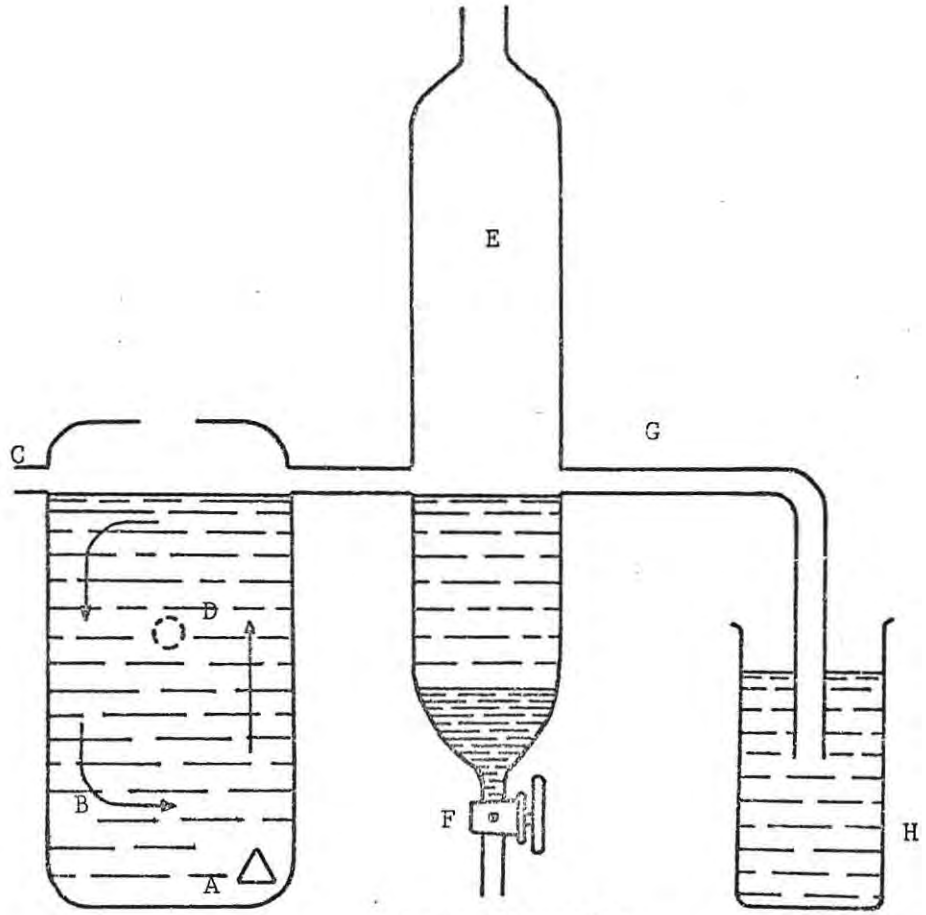
c) Operation

The peristaltic pumps were each calibrated so that the flow rate of each could be adjusted to give the desired retention time in the reactor. The feed pipes were cleaned regularly to prevent filter action which develops when the pipes become partially blocked. The sludge age was adjusted as follows. If, for example, a sludge age of 10 days was required and the volume of the reactor was 4,5 1 then 0,45 1 of mixed liquor must be removed from the reactor every day. This volume was removed in two

Fig. 5.1 A Laboratory CMAS Unit.



Side Elevation



Front Elevation

A	Air stone	D	Sampling port	G	Overflow pipe
B	Current flow	E	Sedimentation tank	H	Treated effluent
C	Feeding port	F	Sludge drainage port		

225 ml portions twice a day. Before each sludge removal all the sludge from the separating tank was drained off and returned to the reactor. Sludge age control in this manner has the disadvantage that it could lead to fluctuations in the steady state sludge concentration, however these fluctuations were found to be small. Steady state was assumed to be established after a period of two sludge ages had passed under each feed state. Samples of effluent were tested regularly to note trends and indicate when steady state was achieved. When steady state was reached duplicate samples were analysed daily for 6 days and tested graphically to determine if the values were normally distributed.

d) Oxygen consumption rate

A one litre flask was filled with the reactor contents and vigorously aerated to a level of about 6 mg/l dissolved oxygen. A mass of feed proportional to the flask to give the same rate as that added to the reactor over a ten minute period was added to the flask. The effluent in the flask was gently stirred on a magnetic stirrer and the decrease in oxygen over ten minutes noted by means of an oxygen probe. This test was repeated each day while steady state conditions prevailed under a particular loading. The normally distributed data obtained were used to measure the oxygen consumption rate per unit time.

5.23 Effluent analysis techniques

Analysis techniques used in addition to those already described in Chapter IV were:

Total suspended solids were determined by centrifuging a 50 ml sample of mixed liquor at 3 000 rpm for 10 min, discarding the supernatent, resuspending in distilled water and evaporating in a preweighed silica crucible overnight. After cooling for 2 h in a desiccator the crucible was again weighed and the total suspended solids calculated.

Volatile suspended solids were determined by igniting the total suspended solids in a furnace at 500°C for 4 h before cooling in a

desiccator and reweighing. The loss of weight on ignition gave the volatile suspended solids.

Settleable solids were determined by volume after settling for 1 h according to the method outlined in Ministry of Housing and Local Government (1956).

Ammonia, inorganic and organic nitrogen compounds were determined by distillation and titration according to the technique described in Methods for Chemical Analysis of Fresh Waters (1969).

Dissolved oxygen was estimated using a Beckman Fieldlab Oxygen Analyser fitted with a dissolved oxygen sensor probe.

5.3 Results and Discussion

5.31 The effect of dilution on the degree of effluent purification obtained in batch culture

The effects of diluting two different fellmongery effluent samples on the reduction in OA are shown in Tables 5.1 and 5.2. Undiluted effluent aerated in the presence of manganese showed a maximum OA reduction of 40%. When this same effluent was diluted 4-fold prior to aeration the percentage OA reduction was increased to 62%. With undiluted effluent aerated after neutralisation, the maximum OA reduction was 50% and this was increased to 81% after a 4-fold dilution of the effluent (Table 5.2). This finding is in good agreement with the results obtained with other very strong wastes (Hawkes, 1963; Pipes, 1966), and fellmongery effluents should, therefore, be diluted with re-cycled effluent or by other means before aerobic biological treatment.

It is interesting to note the effect of manganese discussed in the previous chapter, whereby it appears to be responsible for the conversion of sulphide to a form which contributes to the residual OA of the effluent. In the cases of both the diluted and undiluted effluents a greater reduction

Table 5.1 The effect of diluting fellmongery lime-sulphide effluent on the degree of purification obtained by aeration with 50 mg/l manganese. (a) undiluted effluent (b) diluted effluent.

a)

Aeration time	0 days	1 day before seed	1 day after seed	2 days	3 days	4 days	7 days
OA mg/l	2306,0	1598,4	1433,6	1408,8	1396,0	1383,2	1392,4
% OA reduction	0	30,7	37,8	38,9	39,5	40,0	39,6
pH	12,1	11,8	11,7	9,8	8,8	9,0	8,9
Na_2S mg/l	1490,0	<0,4	<0,4	<0,4	<0,4	<0,4	<0,4
Bacteria/ml	$2,31 \times 10^4$	$6,0 \times 10^3$	$8,4 \times 10^4$	$2,55 \times 10^5$	$1,44 \times 10^8$	$2,25 \times 10^8$	$1,52 \times 10^8$
Protozoa/ml	0	0	0	0	0	0	0
Optical density	1,42	2,00	1,96	1,93	1,8	0,66	0,62

b)

OA mg/l	616,8	420,0	412,0	297,6	280,8	240,0	236,2
% OA reduction	0	31,9	33,2	51,8	54,5	61,1	61,7
pH	11,4	10,6	10,1	8,1	8,1	8,1	8,0
Na_2S mg/l	431,0	<0,4	<0,4	<0,4	<0,4	<0,4	<0,4
Bacteria/ml	$1,35 \times 10^4$	$3,30 \times 10^4$	$7,20 \times 10^4$	$7,52 \times 10^6$	$9,94 \times 10^7$	$1,53 \times 10^7$	$8,62 \times 10^6$
Protozoa/ml	0	0	0	$1,08 \times 10^2$	$1,68 \times 10^3$	$1,50 \times 10^3$	$1,48 \times 10^3$
Optical density	0,44	1,22	1,12	0,32	0,22	0,14	0,13

Table 5.2 The effect of diluting fellmongery lime-sulphide effluent on the degree of purification obtained after neutralisation with H_2SO_4. (a) undiluted effluent (b) diluted effluent.

(a)

Aeration time	0 days	1 day before seed	1 day after seed	2 days	3 days	4 days	7 days
OA mg/l	1967,2	1550,4	1552,0	1507,2	1414,4	1376,8	979,2
% OA reduction	0	21,2	21,1	23,4	28,1	30,0	50,2
pH	7,8	7,5	7,6	8,0	8,1	7,8	7,2
Na_2S mg/l	1700,0	1,9	1,7	<0,4	<0,4	<0,4	<0,4
Bacteria/ml	$2,42 \times 10^3$	$8,42 \times 10^5$	$9,68 \times 10^6$	$3,60 \times 10^8$	$2,88 \times 10^8$	$1,08 \times 10^7$	-
Protozoa/ml	0	0	$5,4 \times 10^2$	$3,9 \times 10^4$	$4,2 \times 10^4$	$7,2 \times 10^4$	$2,4 \times 10^4$
Optical density	1,13	1,30	1,30	1,23	0,96	0,75	0,47

(b)

OA mg/l	505,6	321,6	359,8	294,4	206,4	132,0	97,4
% OA reduction	0	36,4	28,8	41,8	59,2	73,9	80,7
pH	8,0	8,1	8,2	7,7	7,6	7,7	7,7
Na_2S mg/l	392	<0,4	<0,4	<0,4	<0,4	<0,4	<0,4
Bacteria/ml	$8,96 \times 10^5$	$2,44 \times 10^6$	$4,82 \times 10^6$	$1,05 \times 10^8$	$8,11 \times 10^7$	$6,04 \times 10^7$	$8,62 \times 10^7$
Protozoa/ml	0	0	$6,2 \times 10^1$	$3,6 \times 10^3$	$1,1 \times 10^3$	$2,4 \times 10^3$	$4,6 \times 10^3$
Optical density	0,34	0,09	0,10	0,16	0,19	0,21	0,11

in OA was obtained by neutralisation (50,2 and 80,7% reduction respectively) with H_2SO_4 before aeration than by the addition of manganese (39,6 and 61,7% reduction respectively).

5.32 The effects of feed concentration on the completely mixed activated sludge treatment process

The effects of varying the influent substrate concentration on the completely mixed activated sludge treatment of fellmongery lime-sulphide effluent under conditions of constant sludge age (5 days) retention time (2 days) and temperature (18 - 20°C) are shown in Table 5.3.

The activated sludge reactors were able to function with reasonable efficiency (OA reduction >72%) at feed concentrations having an OA less than 1 500 mg/l. Under these conditions the pH was reduced to its usual base level and complete sulphide removal occurred. This enabled efficient activated sludges containing high numbers of bacteria and protozoa to develop. At a feed concentration of OA 2 000 mg/l the treatment process was unable to function efficiently. Although complete sulphide removal occurred, the pH remained high and there was very little activated sludge production as may be seen by the small increase in volatile suspended solids before and after treatment.

The factor limiting the feed concentration acceptable for the efficient functioning of the treatment process to OA 1 500 mg/l appears to be the oxygen supply. There was a rapid increase in oxygen consumption rate with increasing feed concentration up to OA 1 500 mg/l. The oxygen consumption rate at this feed concentration was so great that it was impossible to measure with any degree of accuracy as complete oxygen depletion occurred within a few seconds. The predominance of flagellates and lack of ciliates in the two CMAS reactors with the highest oxygen consumption rates also indicates that an oxygen deficiency is prevalent in these reactors as ciliates are less tolerant to oxygen depletion in their environment than are flagellates

Table 5.3 Effect of feed concentration on the treatment of fellmongery lime-sulphide effluent in a completely mixed activated sludge reactor.

	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
OA mg/l	544,3	105,0	1048,2	240,9	1477,8	410,6	2087,6	1444,3
% OA reduction	-	80,7	-	77,0	-	72,2	-	30,8
pH	11,5	8,1	12,0	8,2	12,3	8,3	12,5	10,7
Na_2S mg/l	499,0	<1,56	997,8	<1,56	1495,1	<1,56	2039,6	<1,56
MLSS mg/l	179	1060	340	1320	518	1957	686	769
MLVSS mg/l	96	944	201	1097	293	1588	385	533
Bacteria/ml	$1,68 \times 10^3$	$3,24 \times 10^7$	$2,24 \times 10^3$	$4,65 \times 10^8$	$1,70 \times 10^3$	$2,49 \times 10^9$	$1,83 \times 10^3$	$7,65 \times 10^7$
Protozoa /ml flagellates x 10^4	0	<0,04	0	5,06	0	12,03	0	0
Protozoa/ml ciliates x 10^3	0	5,94	0	0,04	0	0	0	0
NH_3 mg/l	15,6	80,1	30,4	189,1	44,8	222,7	58,4	20,7
Settleable solids ml/l	5,2	136,0	8,4	132,2	9,8	158,8	15,6	6,0
Oxygen consumption rate mg/l/day	-	366	-	648	-	1500	-	448

Sludge age 5 days Retention time 2 days Temperature 18-20°C

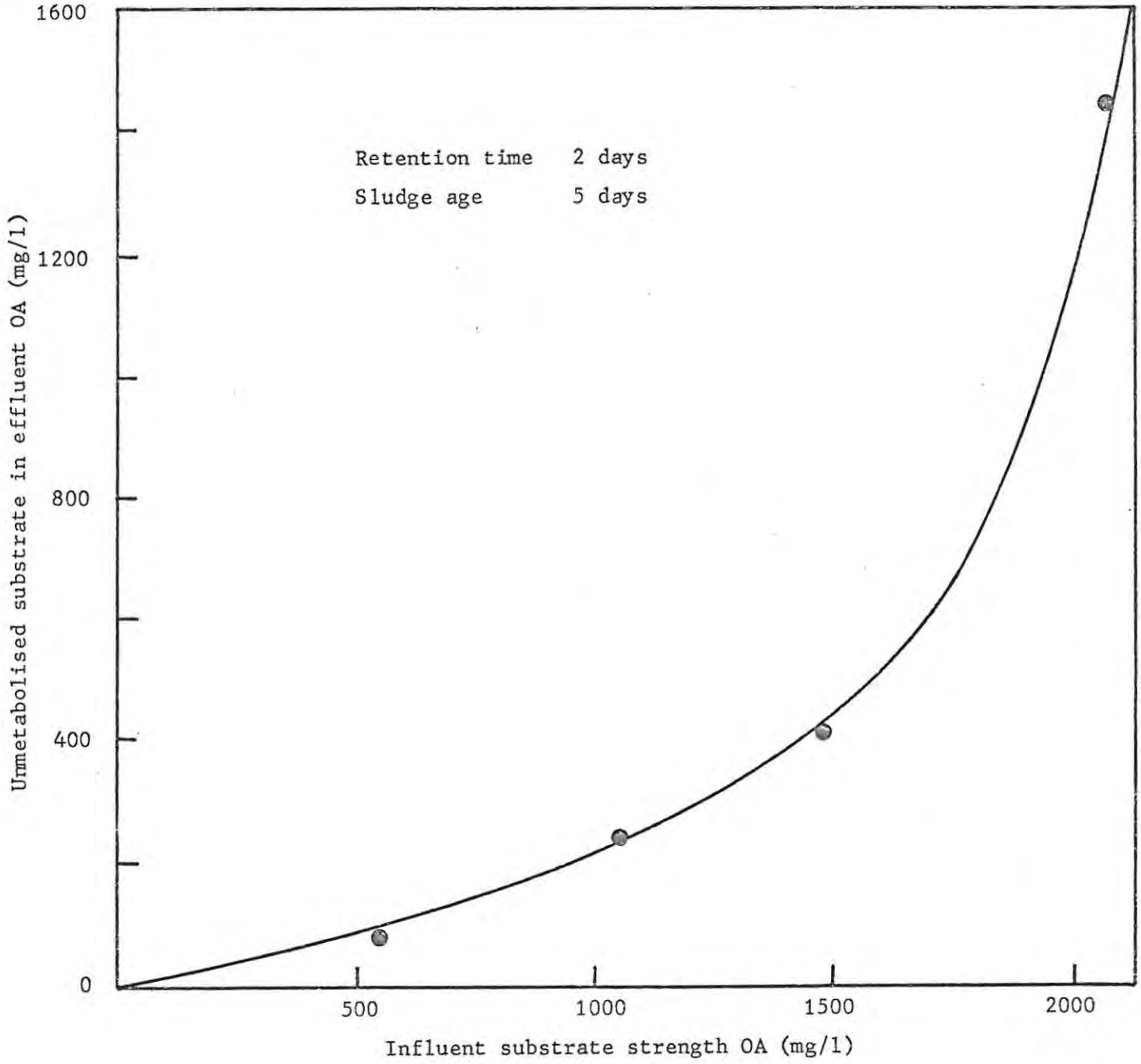
(Hawkes, 1963). There was a steady increase in bacterial count with increasing feed concentration up to OA 1 500 mg/l as would be expected. The mixed bacterial population as found in activated sludge is relatively insensitive to oxygen depletion so that the greater the feed concentration in a reactor, the greater the bacterial population that can be supported.

The decreasing efficiency in OA removal with increasing feed concentration (Fig. 5.2) can be attributed to a number of causes resulting from oxygen depletion. Several workers (Curds, Cockburn and Vandyke, 1968; Curds and Cockburn, 1970) have shown that the production of high quality effluents is dependant on a large ciliate population. Treatment plants containing only free-swimming ciliates produce fairly turbid effluents whereas a plant containing attached ciliates would be expected to produce a highly clarified effluent (Curds, 1971). The reduction in ciliate population with increasing feed concentration is therefore likely to contribute to the deteriorating effluent quality. Another reason for the decreasing quality of the effluent is that as the feed concentration increases the shift in bacterial population from aerobic species to micro-aerophilic species is accompanied by a decreasing rate of metabolism in the latter organisms. This shift in microbial population will have an effect on the kinetics of substrate removal in the activated sludge process. The influent substrate concentration is therefore a variable which must be considered in the design and operation of waste water treatment systems (Grady and Williams, 1975).

The efficiency of the CMAS treatment process even at a feed concentration of OA 1 500 mg/l is remarkable. The versatility of the process is demonstrated by the 72% OA reduction obtained at this feed concentration which occurred in a single stage reactor without prior removal of sulphide either by aeration or catalytic oxidation and without any lowering of the pH. Once adapted to the fellmongery effluent the activated sludge did not appear to be adversely affected by the high

influent sulphide concentration or high influent pH. The microbial population was able to hold the mixed liquor at a sufficiently low pH level for the incoming sulphide concentration to be effectively removed from the reactor.

Fig. 5.2 Effect of feed concentration on effluent quality



CHAPTER VI

AN ENZYMIC APPROACH TO THE STUDY OF THE ACTIVATED SLUDGE TREATMENT PROCESS

Summary

Changes in the levels of certain hydrolytic and oxidative enzymes as well as changes in the numbers of bacteria capable of producing certain degradative enzymes during extended aeration of fellmongery effluent were observed. These observations indicated that the bacterial and flagellate protozoan populations were responsible for the production of the major portion of both the hydrolytic and oxidative enzymes. The enzymic approach was shown to be a potentially useful method for the quantitative biological characterisation of activated sludges.

6.1 Introduction

The objective of the biologist in the study of the microflora of waste water treatment processes should not only be to identify the many bacteria and protozoa present in a particular system, but to continually provide new information to clarify the role of the various micro-organisms present. Essentially three different techniques have been adopted by biologists in approaching this task. One has been to separate the activated sludge bacterial and protozoan populations and then to treat a waste water sample with only one or the other of these populations (Pillai and Subrahmanayan, 1944). At a later stage the other population may be returned to the treatment process and the effect that this has on the process may then be used to obtain an indication of the function of each group (Curds, Cockburn and Vandyke, 1968). The second method of examining the role of the activated sludge organisms is to selectively isolate an organism capable of degrading a particular type of waste. Studies are further continued with the selected organism in pure culture to determine how a specific substrate is degraded and what the products are. This type of investigation yields valuable results but has the disadvantage

that it does not take into account how active the particular organism is within the treatment system.

A third technique which has been used very little by workers in the study of sewage sludges is the adoption of an enzymic approach. Lenhard (1967, 1969) in search of new methods for determining organic degradation activities in sediments of stabilisation ponds showed that the protease, urease and other biochemical activity determinations may provide useful information about the nature of the biochemical changes involved in these sediments. An alkaline phosphotase assay has been used to indicate "surplus" phosphorus uptake by a dilute heterogenous activated sludge culture (Moore, Higgins and Fruh, 1969). Sridhar and Pillai (1974) used the determination of catalase, protease, urease and phosphotase activities in sewage, activated sludge and septic tanks to obtain information on the relationship between the main groups of organisms (bacteria and protozoa) in the treatment process. On the basis of their experiments they suggested that the bacterial population is responsible for the hydrolytic changes in organic matter decomposition and that the protozoan population is responsible for the oxidative changes.

Hankin and Sands (1974) instead of examining the enzyme levels in mixed liquor or culture supernatants (these levels may be extremely low and difficult to measure), used bacterial plate assays to estimate the numbers and percentages of bacteria capable of producing specific enzymes. The numbers of bacteria able to degrade lipids, protein, starch, pectin, lecithin, RNA, DNA, carboxymethylcellulose and alkylarylsulphonate at five stages (raw effluent, primary effluent, mixed liquor, activated sludge, final effluent) within each of four waste water treatment facilities were determined. The numbers of bacteria in all enzyme categories increased sharply at the mixed liquor stage, while bacteria with certain specific enzyme capabilities appeared to be selectively precipitated or destroyed at the activated sludge stage. Although statistical analysis of the

percentage of bacteria able to produce a specific enzyme at each stage within a treatment facility indicated few significant differences, the data provided useful information on the number and percentage of bacteria able to produce specific degradative enzymes.

In the work described in this chapter both the levels of certain enzymes in the mixed liquor and the numbers of bacteria able to produce specific degradative enzymes were investigated. However, instead of examining the enzymic properties of liquors at different stages in a water treatment facility the changes in enzymic properties of the mixed liquor during the extended aeration of effluent in batch culture were followed. A quantity of raw effluent was seeded with a small amount of activated sludge and an attempt made to correlate the cycle of the different groups of organisms during extended aeration (bacteria, saprobic protozoa and predatory protozoa) with changes in the enzymic properties of the mixed liquor.

6.2 Materials and Methods

6.21 Source of effluent

A mixture of effluent from the sheepskin soaking and washing process and lime-sulphide effluent from the unhairing process was obtained from the inflow to tank (b) of the small scale effluent treatment plant described in Chapter III. The effluent was diluted approximately 4-fold (final OA $\pm$ 600 mg/l) and its pH reduced to $\pm$ 11 before aeration.

6.22 General experimental procedure

Fellmongery effluent samples (5 l) were given extended aeration in plastic containers using the laboratory aeration system described in Chapter IV. Aeration was allowed to continue for 24 h in order to remove the sulphide before the effluent was seeded with 250 ml acclimated

activated sludge. Where excessive frothing of the effluent occurred a small volume (1 ml) of silcolapse 5001 was added. Samples for analysis were withdrawn daily during the continuous aeration period of 9 days.

Two experiments were carried out each one in duplicate. In the first the change in the levels of catalase, protease, phosphotase and urease activities with progressive aeration were examined, whereas in the second experiment the numbers of bacteria able to produce certain degradative enzymes were investigated.

6.23 Analysis techniques

The 4-h oxygen absorbed assay, sodium sulphide concentration, pH, protozoa counts (flagellate and ciliate) and total bacteria counts were determined as described in Chapters III and IV.

6.24 Enzyme assays

Duplicate 50 ml samples of mixed liquor were homogenised using an MSE ultrasonic disintegrator. Samples were homogenised at 21 kHz using 5 x 1 min. periods of sonication with 1 min. intervals between in order to keep the temperature of the sample below 30°C. Although sonication did not significantly affect the protease, urease and catalase levels, there was a marked increase in the alkaline phosphotase levels after sonication.

a) Catalase

Catalase activity was determined titrimetrically using an adaptation of the method described by Sridhar and Pillai (1974). An homogenised mixed liquor sample (10 ml) together with 10 ml sodium phosphate buffer (0,2 M, pH 7,0) and 2 ml 10% hydrogen peroxide substrate was incubated at 20°C for a known period so that not more than 50% of the substrate had been decomposed. A control having 10 ml buffer in place of the homogenised mixed liquor was run concurrently with the test. The reaction was stopped

by adding 5 ml 2N H_2SO_4 and the remaining hydrogen peroxide titrated against 0,1N potassium permanganate. Catalase activity was expressed as m moles H_2O_2 decomposed/10 ml sample/10 min. using the relationship:

$$1 \text{ ml } 0{,}1N \text{ } KMnO_4 = 0{,}050 \text{ m moles } H_2O_2.$$

b) Protease

Protease activity was estimated using the synthetic non-specific protease substrate azocoll. The reaction mixture consisted of 15 mg azocoll weighed out into a dry test tube to which 2 ml mixed liquor homogenate and 3 ml Tris-HCl buffer (0,2M, pH 8,0) was added. After 18 h gentle shaking in a water bath at 30°C the reaction mixture was centrifuged at 3 000 rpm for 5 min. and the absorbance read at 520 nm. A mixture consisting of 5 ml buffer and 15 mg azocoll incubated together with the test served as the blank. Corrected absorbance values were converted to mg azocoll released according to the relationship:

$$\text{azocoll released (mg/ml)} = \frac{\text{absorbance} - 0{,}087}{\frac{520}{0{,}502}}$$

Protease activity was expressed as mg/ml azocoll released/2 ml test sample/18 h. The azocoll protease assay was found to be more sensitive than the commonly used non-specific protease assay in which casein is employed as the substrate. The low levels of protease activity found in the mixed liquor were below the optimum regions of activity for the casein-substrate method, thereby making it unsuitable.

c) Phosphatase

Alkaline phosphatase activity was determined using 0,01 M p-nitrophenyl phosphate in Tris-HCl buffer (0,2M, pH 8,0) as the substrate (Garen and Levinthal, 1960). The reaction mixture consisted of 0,2 ml substrate, 1,3 ml Tris-HCl buffer (0,2M, pH 8,0) and a 0,5 ml aliquot of homogenised test solution, all prewarmed by incubation at 35°C for 5 min. A 0,5 ml aliquot of Tris-HCl buffer replaced the 0,5 ml test solution to constitute the blank. Both the test reaction mixture and blank were incubated with

gentle shaking at 37°C for 10 min. after mixing. The reaction mixture was stopped by the addition of 1,0 ml dibasic potassium phosphate (1 M) and the colour allowed to develop for 10 min. before the absorbance was read at 410 nm. Corrected absorbance values were converted to μg/ml p-nitrophenol using the following relationship obtained from a linear regression analysis of the calibration curve:

$$\text{p-nitrophenol } (\mu\text{g/ml}) = \frac{\text{absorbance}_{410} - 0{,}019}{0{,}0268}$$

Alkaline phosphatase activity was expressed as μg p-nitrophenol released/ml test sample/10 min.

d) Urease

Urease activity was measured using a method based on that of Sridhar and Pillai (1974). The reaction mixture consisted of 2 ml substrate (1% urea in water), 2 ml sodium phosphate buffer (0,2 M, pH 7,0) and 2 ml of the homogenised test solution. After 3 h incubation at 20°C the reaction was stopped by adding 2 ml 1 N HCl and the mixture centrifuged at 3 000 rpm for 5 min. A 0,5 ml aliquot of the supernatant was removed and made up to 2,5 ml with distilled water. To this 1,0 ml Nessler's iodine solution was added and the mixture allowed to stand for 15 min. for the colour to develop before absorbance values were read at 400 nm. The corrected absorbance was converted to mg/ml ammonia released by means of the following relationship obtained from a linear regression analysis of a calibration curve prepared using ammonium sulphate:

$$\text{ammonia released (mg/ml)} = \frac{\text{absorbance}_{400} + 0{,}011}{0{,}015}$$

Urease activity is expressed as mg/ml ammonia released/ml test sample/3 h.

e) Keratinase

Keratinase activity was determined by the method of Young and Smith (1975) using ether extracted wool as the keratin substrate. To assay enzyme activity 25 ml 0,025 M borate buffer (pH 9,0) and 2,5 ml 10^{-3} M $MgCl_2$

were mixed with various amounts of mixed liquor and distilled water to give a final volume of 50 ml. Finely cut wool (1 g) was added to each test and incubated at 40°C for up to 14 days. Samples (5 ml) of reaction mixtures were removed at various time intervals, filtered to remove the substrate and the solubilised protein determined using the method of Lowry et al. (1951). Corrected absorbances at 690 nm were converted to mg/ml protein solubilised by the relationship:

$$\text{protein concentration (mg/ml)} = \frac{\text{absorbance} - 0{,}06}{1{,}03}$$

derived from a calibration curve prepared using bovine albumin.

6.25 Bacterial production of enzymes

Samples (30 ml) of mixed liquor were subjected to sonication in an ultrasonic disintegrator in order to release the bacteria from the activated sludge flocs as described in Chapter III. After sonication two appropriate dilutions of the sample were plated onto duplicate plates using the spread plate technique. Incubation for all media was at 30°C and the period of incubation is indicated under each medium. All media were solidified with 1,5% agar.

Bacteria producing proteolytic enzymes were detected on medium containing 0,8% nutrient agar and 0,2% gelatin. After 72 h incubation the plates were flooded with an acidified 15% mercuric chloride solution to precipitate the gelatin. Clear zones around colonies on an otherwise opaque background indicated gelatin proteolysis.

Growth on a medium consisting of 0,5% Tween 20 (sorbitan monolaurate) and the mineral salts solution of Zucker and Hankin (1970) was used to detect the production of lipolytic enzymes. Plates were incubated for 5 days before counting.

The ability of bacteria to degrade detergents was tested on a medium composed of mineral salts solution (as above) and 0,2% sodium alkylaryl-sulphonate. All colonies that grew after 7 days incubation were considered

capable of utilising the detergent, the sole carbon source in the medium (Hankin and Sands, 1974).

Bacteria able to produce amylolytic enzymes were detected on a medium containing 0,8% nutrient agar and 0,2% starch. After 72 h incubation plates were flooded with a 0,3% iodine solution. Yellow zones around colonies in an otherwise dark blue medium showed the production of amylase.

Production of enzymes capable of degrading deoxyribonucleic acid (DNA) was detected on a medium containing 0,8% nutrient agar and 0,3% herring sperm DNA. After 72 h incubation the plates were flooded with 1 N HCl to precipitate the DNA. Production of DNAse enzyme was indicated by a clear zone around a bacterial colony on an otherwise opaque medium.

Bacteria producing enzymes capable of degrading ribonucleic acid (RNA) were detected on a medium containing 0,8% nutrient agar and 0,3% yeast RNA. After incubation for 72 h the RNA was precipitated by flooding the plates with 1 N HCl, RNAse production being indicated by a clear zone around a bacterial colony.

Cellulolytic activity was tested on a medium composed of mineral salts (as above), 0,1% yeast extract and 0,5% carboxymethylcellulose (Hankin and Sands, 1974). After 7 days incubation, plates were flooded with a 1% aqueous solution of hexadecyltrimethylammonium bromide. Clear zones around colonies indicated the ability of bacteria to produce cellulolytic enzymes.

6.3 Results

6.31 Mixed liquor enzyme levels during extended aeration of fellmongery lime-sulphide effluent

The changes in catalase, protease, phosphatase, urease and keratinase enzyme levels are compared with changes in pH, OA, sulphide concentration and micro-organism counts in Figs 6.1, 6.2 and Table 6.1. There was a

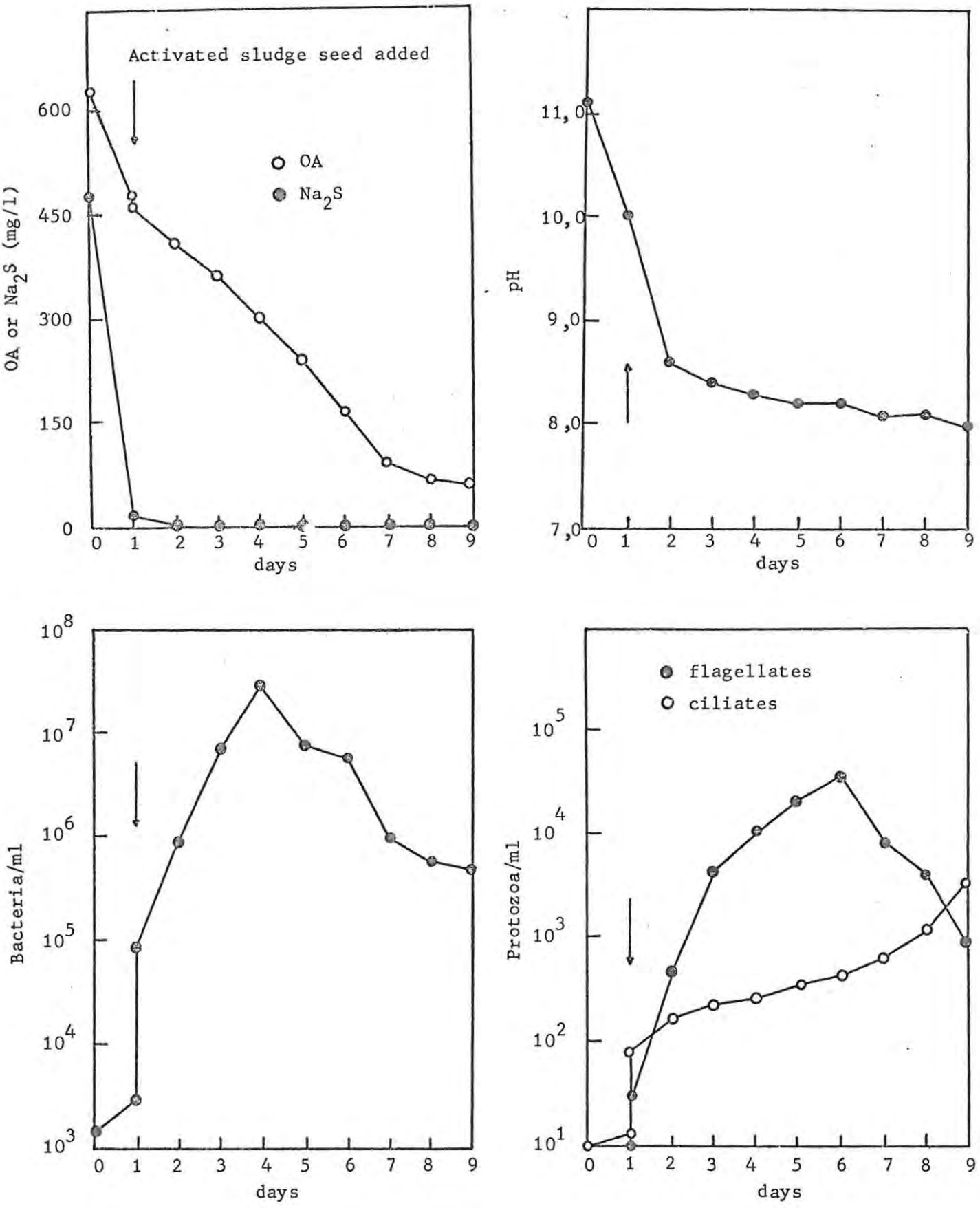


Fig. 6.1. Changes in OA, sulphide, pH and micro-organism counts during extended aeration of fellmongery effluent.

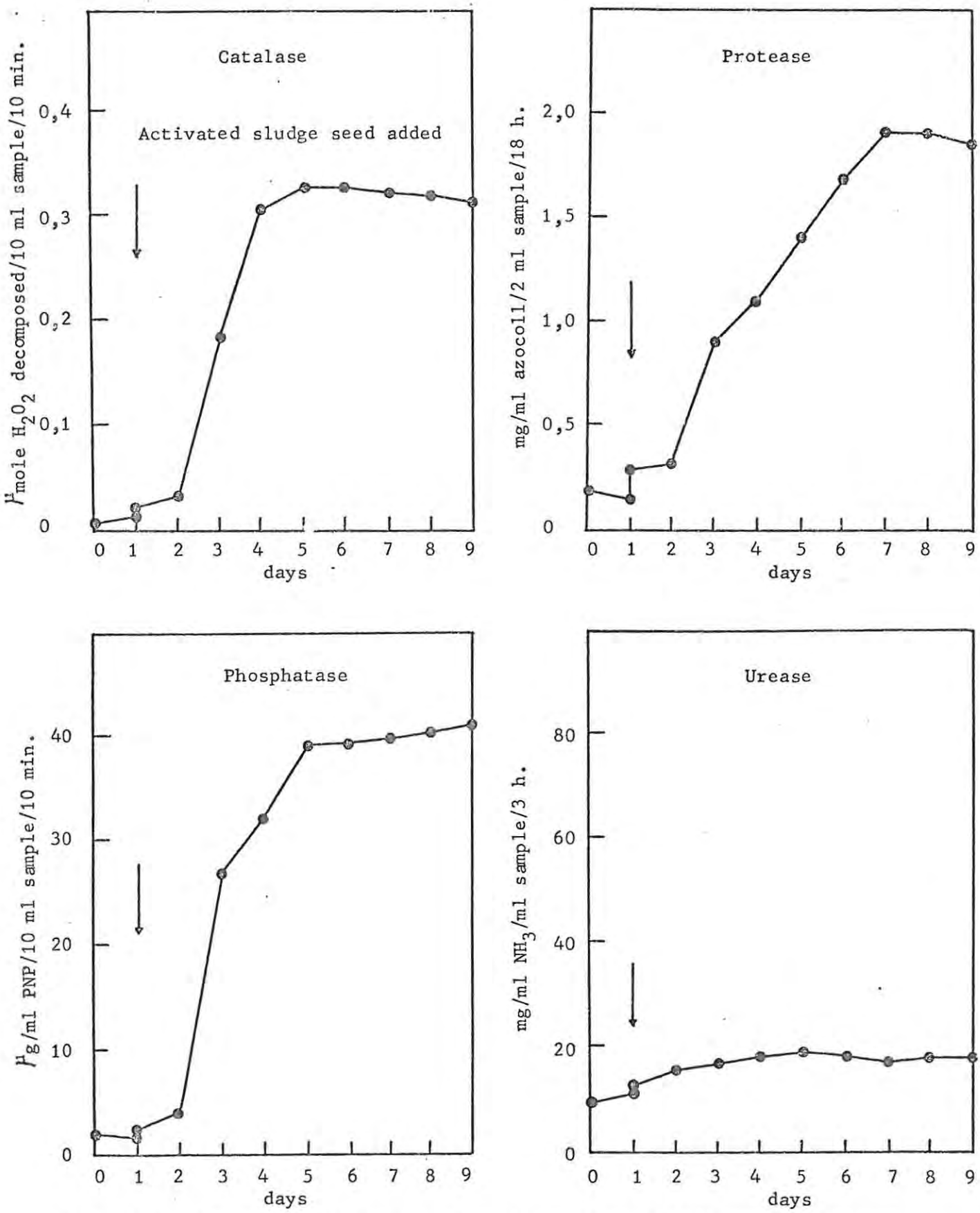


Fig. 6.2. Enzyme levels during extended aeration of fellmongery effluent.

Table 6.1. Keratinase activity of two samples of mixed liquor withdrawn on day 4 and day 9.

Incubation time (days)	Protein released (mg/l)	
	Day 4	Day 9
1	0,019	0,038
2	0,019	0,049
5	0,038	0,038
14	0,029	0,068

rapid decrease in the sulphide concentration and pH value during the first 2 days of aeration after which time these parameters levelled off at their usual base levels. The OA value was reduced fairly steadily for the first 8 days of aeration before levelling off, at which stage an 88% OA reduction had been achieved. The bacterial count increased approximately exponentially for 3 days after the effluent had been seeded with sludge but decreased quickly on further aeration. There was a rapid increase in the number of flagellate protozoa for the first 2 days after seeding, followed by a slower but steady increase for a further 3 days, a peak in the number of flagellates being reached 5 days after seeding. The number of ciliate protozoa increased at a comparatively slow rate for the first 5 days after seeding but this rate increased substantially on continued aeration.

Catalase activity increased sharply between the first and third days after seeding which coincided with the rapid increase in the bacterial and flagellate counts. There was however, no increase in catalase activity coinciding with the rapid increase in ciliate numbers that occurred 5 days

after seeding.

A steady increase in protease activity was maintained for 5 days from the second day after seeding. This increase in activity however, did not correlate with the increase in bacterial count as might be expected and continued even after the flagellate count had begun to decline. The probable explanation for this is that the protease enzymes were steadily produced by the bacteria and saprophytic protozoa and being fairly stable enzymes they gradually accumulated in the mixed liquor.

The alkaline phosphatase activity increased rapidly between the second and fifth days after seeding. There appeared to be a further small increase in phosphatase activity coinciding with the rapid rise in ciliate numbers during the latter stages of aeration.

Urease activity was very limited over the whole period of aeration. This finding is not unexpected as fellmongery effluent is unlikely to contain sufficient urea for there to be a positive selective pressure for urea degrading organisms.

Although fellmongery lime-sulphide unhairing effluent contains keratin, no keratinase activity was found in mixed liquor samples withdrawn after 4 and 9 days aeration. Either there were no bacteria capable of producing this specific enzyme or the level of enzyme was too low to measure by the method employed. As attempts to isolate keratinase producing bacteria using an enrichment culture technique were unsuccessful it is probable that no keratinase producing bacteria were present in the mixed liquor.

6.32 The numbers of bacteria producing specific degradative enzymes during the extended aeration of lime-sulphide effluent

The growth curves of bacteria capable of producing a variety of specific degradative enzymes during 9 days continuous aeration on lime-sulphide effluent are compared in Table 6.2 and Figs 6.3 and 6.4. Corresponding changes in OA, sodium sulphide concentration, pH and total

Table 6.2. The numbers of bacteria producing various degradative enzymes during extended aeration of fellmongery effluent.

Aeration time (days)	0	1 before seed	1 after seed	2	3	4	5	6	7	8	9
OA (mg/l)	608,6	514,0	512,2	457,2	362,2	284,0	150,2	85,0	67,3	51,5	51,0
pH	11,0	9,6	9,6	8,6	8,4	8,3	8,2	8,1	8,0	7,9	7,9
Na_2S (mg/l)	458,3	19,0	11,2	<1,56	<1,56	<1,56	<1,56	<1,56	<1,56	<1,56	<1,56
Ciliates (ml^{-1})	<20	<20	$2,0x10^1$	$5,2x10^1$	$8,4x10^1$	$1,6x10^2$	$2,8x10^2$	$5,3x10^2$	$1,6x10^3$	$2,9x10^3$	$3,0x10^3$
Flagellates (ml^{-1})	<20	$6,0x10^1$	$1,1x10^2$	$2,2x10^3$	$7,2x10^4$	$6,5x10^4$	$2,0x10^4$	$2,9x10^3$	$1,8x10^3$	$9,9x10^2$	$5,0x10^2$
Bacteria (ml^{-1})	$5,7x10^3$	$5,4x10^4$	$4,6x10^5$	$8,2x10^6$	$4,4x10^7$	$3,6x10^7$	$1,4x10^7$	$1,0x10^7$	$9,1x10^6$	$8,6x10^6$	$8,5x10^6$
Gelatin	$4,2x10^2$	$6,4x10^2$	$2,2x10^4$	$4,0x10^6$	$1,2x10^7$	$1,1x10^7$	$6,4x10^6$	$2,1x10^6$	$3,2x10^5$	$1,6x10^5$	$1,4x10^5$
Lipid	$3,7x10^2$	$3,2x10^3$	$4,0x10^4$	$9,4x10^6$	$2,1x10^7$	$8,2x10^6$	$3,3x10^6$	$2,3x10^6$	$2,4x10^6$	$2,1x10^6$	$8,8x10^5$
Alkylarylsulphonate	$2,2x10^1$	$9,6x10^1$	$5,6x10^2$	$7,1x10^3$	$1,3x10^4$	$1,8x10^4$	$4,2x10^4$	$1,0x10^5$	$1,2x10^5$	$1,5x10^5$	$1,9x10^5$
Starch	$1,0x10^1$	$8,2x10^1$	$5,0x10^3$	$8,0x10^4$	$8,2x10^5$	$3,0x10^5$	$8,0x10^4$	$5,0x10^4$	$3,9x10^4$	$2,9x10^4$	$1,6x10^4$
DNA	$1,6x10^1$	$9,5x10^1$	$6,3x10^3$	$2,2x10^4$	$2,4x10^4$	$4,3x10^4$	$4,1x10^4$	$3,5x10^4$	$2,6x10^4$	$3,0x10^4$	$2,2x10^4$
RNA	<10	<10	$6,0x10^1$	$6,1x10^2$	$5,6x10^3$	$3,8x10^3$	$4,6x10^3$	$1,2x10^3$	$8,0x10^2$	$8,0x10^2$	$8,1x10^2$
Cellulose	<10	<10	<10	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	<10	<10	<10

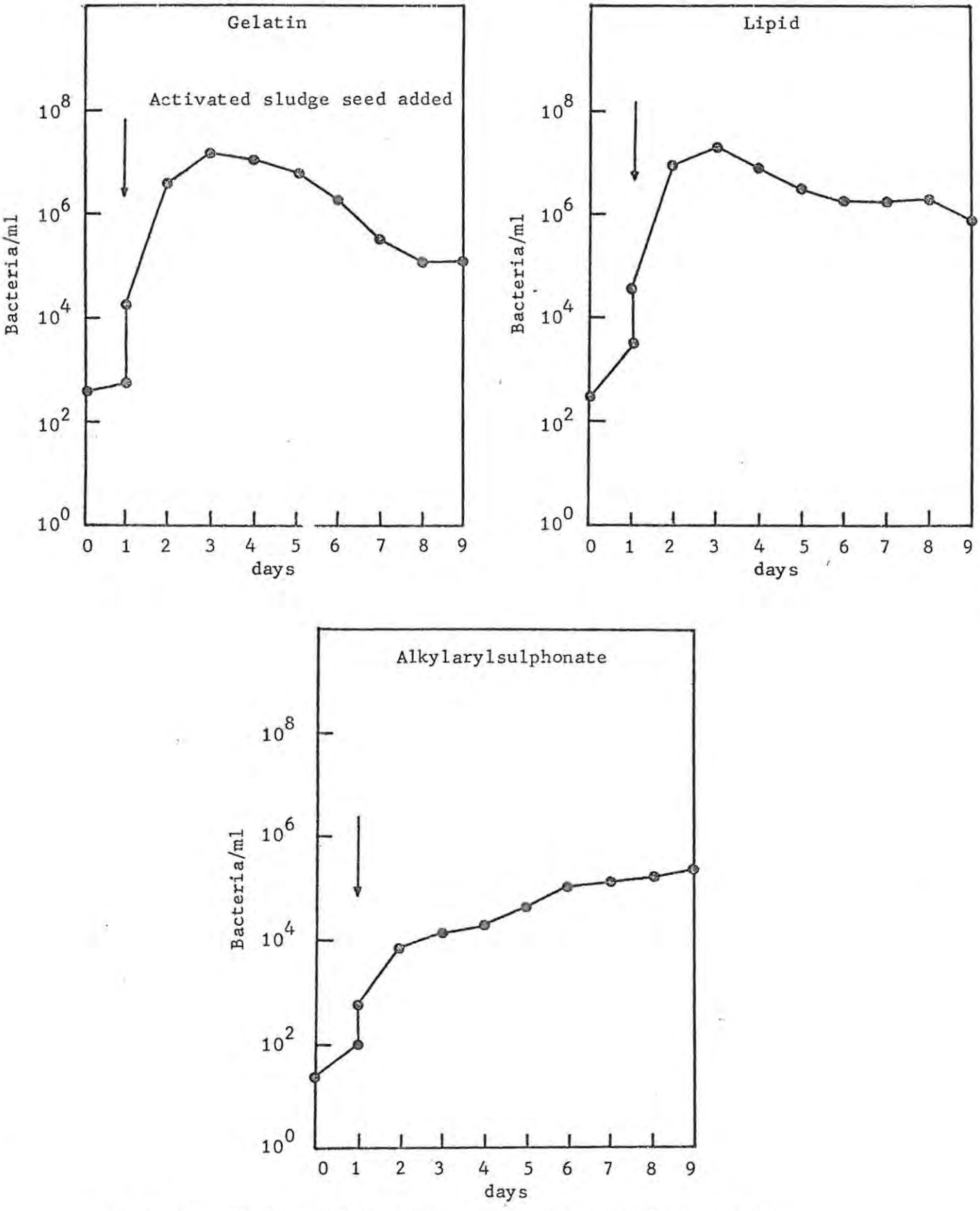


Fig. 6.3. Numbers of bacteria capable of producing various degradative enzymes.

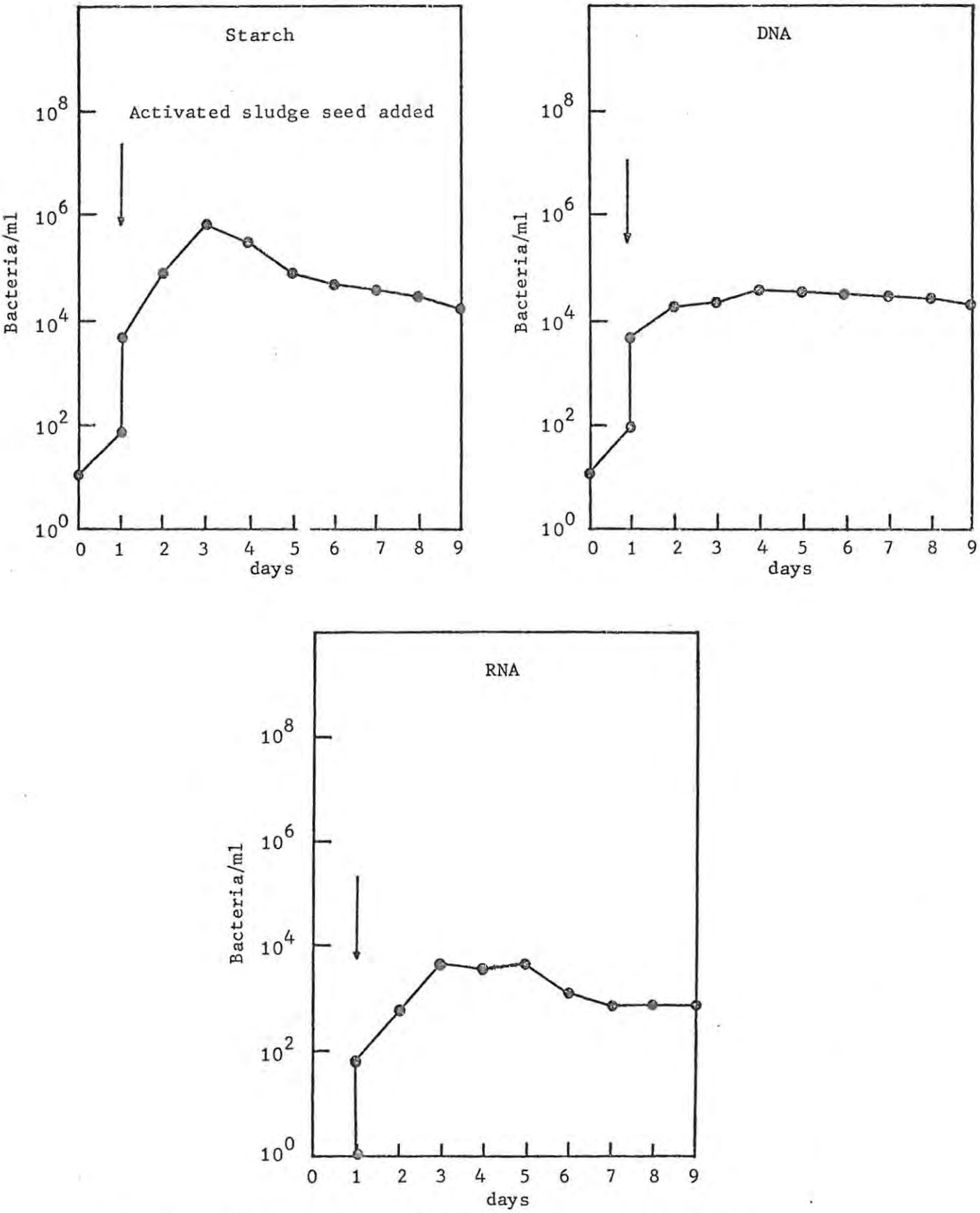


Fig. 6.4. Numbers of bacteria capable of producing various degradative enzymes.

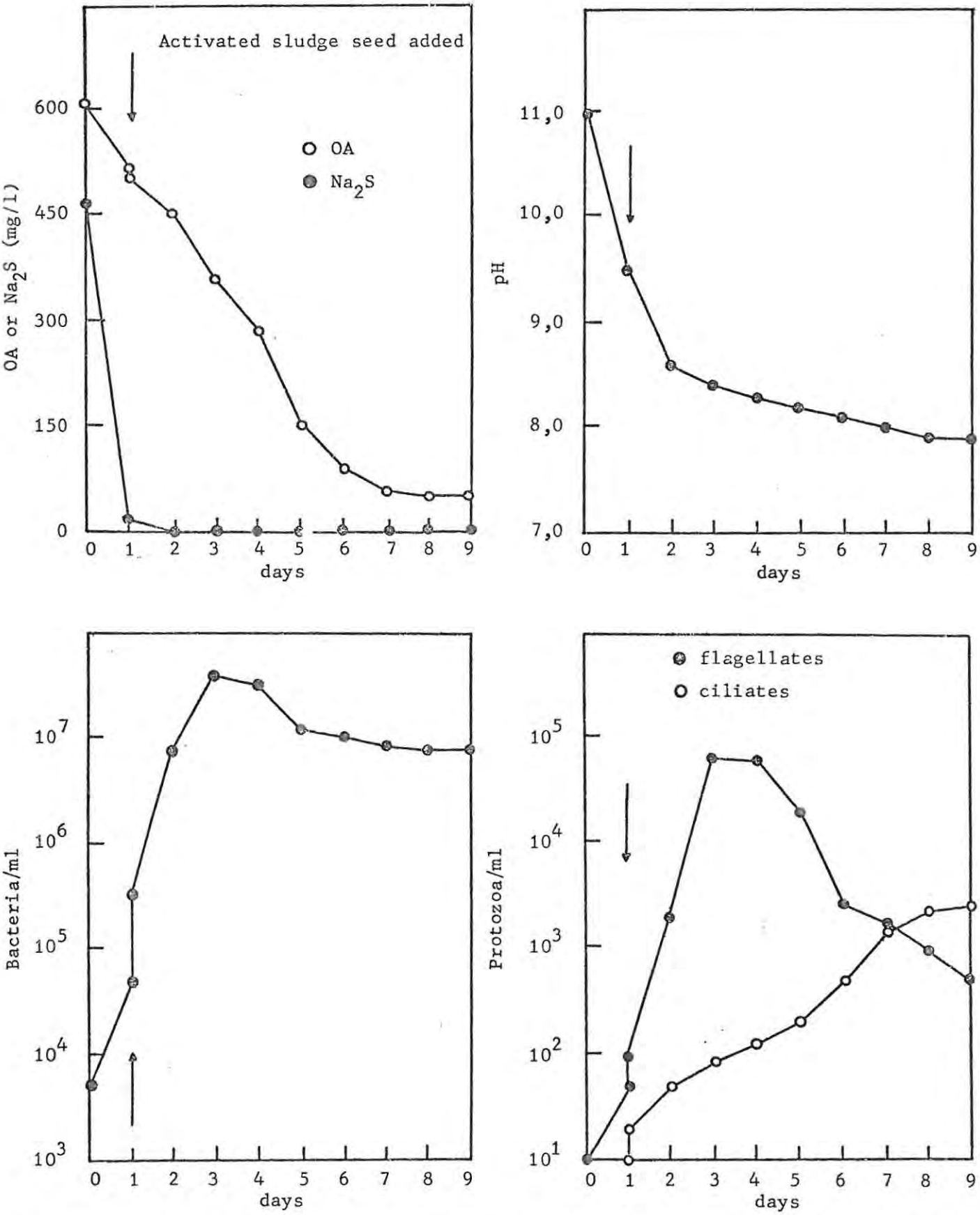


Fig. 6.5. Changes in OA, sulphide, pH and micro-organism counts during extended aeration of fellmongery effluent.

micro-organism counts are displayed in Fig 6 .5 and were very similar to those of the previous experiment.

Bacteria producing enzymes capable of gelatin, lipid and starch utilisation showed similar growth patterns. The rapid increase in numbers during the first 2 days after seeding reached a peak at day 3 and was followed by a steady decrease in numbers on further aeration. Presumably the quantities of substrate in the effluent which stimulated the rapid growth of these bacteria had largely been exhausted after day 3 and the subsequent decrease in numbers was due to a lack of nutrients and the action of predatory ciliates.

The growth curves of bacteria capable of utilising alkyarylsulphonate as their sole carbon source and those producing extracellular DNAase enzymes showed a slow but sustained increase in number for a longer period than the rest. Although the numbers of DNAase producers levelled off after 4 days, the fact that they maintained their numbers in spite of an increasing number of ciliates in the liquor suggests a positive growth rate. The difference in growth pattern between the DNAase producers and the rest is possibly explained by a positive selective pressure for organisms able to utilise cellular material from lysed organisms. When the initial supply of readily available substrate is utilised, endogenous conditions will begin to prevail, favouring the growth of this type of bacterium.

The growth curve of bacteria able to produce extracellular RNAase is intermediate between the two groups already described. An initial rapid increase in RNAase producers was followed by a stationary phase and then a slow fall off in number. The slower fall off in number of these organisms compared with the first group is probably also due to the selective advantage RNAase producers would have under endogenous conditions.

The complete lack of cellulose degrading bacteria is not unexpected as this substrate is unlikely to be found in lime-sulphide effluent in a significant quantity.

The percentage of bacteria able to utilise each substrate before aeration, after 3 days, and again after 9 days aeration is shown in Table 6.3. From this table it will be noted that after 3 days of aeration the percentage of bacteria able to utilise gelatin , lipid and starch are far greater than the rest. However, under the endogenous conditions prevailing after 9 days aeration, bacteria capable of utilising alkylarylsulphonate and those producing extracellular DNAase or RNAase enzymes had either increased or maintained their percentage representation.

Table 6.3. Percentage* of total bacteria degrading a specific substrate at three stages during the treatment of fellmongery effluent in batch culture.

Substrate degraded	Raw Influent	Aeration 3 days	Aeration 9 days
gelatin	7,37	27,27	1,18
lipid	6,49	47,73	10,35
alkylarylsulphonate	< 0,18	0,03	2,24
starch	0,26	1,86	0,19
deoxyribonucleic acid	< 0,18	0,06	0,26
ribonucleic acid	< 0,18	0,01	0,01
cellulose	< 0,18	0,00	0,00

* correct to the nearest one hundredth percent.

6.4 Discussion

The rapid increase in catalase, protease and phosphatase activities in the mixed liquor coinciding with the increase in bacterial and saprophytic protozoan numbers would seem to indicate that these organisms play a large part in both the oxidative and hydrolytic changes occurring during waste decomposition. No evidence was obtained to suggest that the oxidative changes are attributable mainly to the ciliate protozoa as

suggested by Sridhar and Pillai (1974). Although the ciliate protozoa are undoubtedly responsible for the production of high quality effluents, both the bacteria and protozoa appear to be producers of the major portion of the hydrolytic and oxidative enzymes.

An investigation into the microbiology of the activated sludge process by observing changes in mixed liquor enzyme levels is beset by several difficulties. Many industrial effluents although having high concentrations of organic matter are weak nutrient solutions when compared with laboratory media. The numerous substrates occurring in the waste water will give rise to a variety of enzymes but few of them in sufficient quantity to be detected by the methods used to detect the same enzymes in laboratory media.

Counting the number of bacteria able to degrade a particular substrate using a plate technique is a method of detecting the production of enzymes which would not reach a sufficiently high concentration to be determined by a liquid assay. This technique however, also has disadvantages. The detection of the ability of a number of bacteria able to produce a particular enzyme on a plate does not necessarily mean that that enzyme is being produced in the mixed liquor. It is possible that other nutrients are available in the liquor which would be used in preference to those in the plate medium so that there would be no need for the production of that particular enzyme. The plate assay technique has an additional disadvantage when a nutrient source such as nutrient agar is used to supplement the test medium. If the number of bacteria able to utilise the test medium occur in a ratio of less than one in five hundred of those able to grow on the nutrient agar, it is likely that the bacteria utilising the test substrate will not be detected.

Although the enzymic approach to the study of the microbiology of activated sludge has these problems, it is a useful method of sludge characterisation. This approach has the potential of establishing the characterisation of activated sludges on a quantitative basis as

numerical values for enzyme levels and numbers of bacteria producing certain specific enzymes are readily obtainable. As was stressed in the introduction to this dissertation such quantitisation of the ecological approach into the investigation of water treatment systems is essential.

CHAPTER VII

IDENTIFICATION OF SOME OF THE DOMINANT BACTERIA IN FELLMONGERY ACTIVATED SLUDGE MIXED LIQUOR

Summary

A total of 69 bacterial colonies were isolated from a fellmongery activated sludge mixed liquor homogenate on an activated sludge extract medium. Of the 64 isolates which survived transfer to nutrient agar, 37 were identified as belonging to the genus *Pseudomonas* and the remaining 27 to the genus *Acinetobacter*.

7.1 Introduction

A taxonomic characterisation of the bacterial population in a particular activated sludge has many drawbacks. Many isolates have to be selected and identified and the number of strains and subcultures to be handled quickly becomes excessively burdensome. Even then minority classes of bacteria, although important, may not be isolated as completely non-selective laboratory media are impossible to formulate. Prakasam and Dondero (1967a, b, 1970) reported that those bacteria which are isolated are often only identifiable in the broadest of terms due to the incompleteness of taxonomic systems. For these reasons a taxonomic description of the bacteria in an activated sludge can only be qualitative or partially quantitative.

Methods for the characterisation of bacterial populations in sludge other than by classical taxonomic methods have been proposed. Prakasam and Dondero (1967a, b) employed the method of Lochhead and Chase (1943) for describing the population structure. This method entailed dividing soil bacterial populations into a number of categories according to the complexity of their nutritional requirements. Prakasam and Dondero (1967b) proposed that the activated sludge bacterial population be similarly quantitatively characterised according to these nutritional categories and

hence the use of taxonomic characterisation averted. Once the population structure has been categorised in this manner, changes under the influence of stimuli can be used to investigate the adaptability of the population. Activated sludge extract agar was found to be a suitable non-selective medium analagous to Lochhead's soil extract medium and the replica plating technique of Lederberg and Lederberg (1952) was employed to reduce the labour in screening the isolates.

Prakasam and Dondero (1970) developed a similar arbitrary system to characterise the microbial populations of 11 laboratory activated sludges acclimated to a range of aromatic compounds. The bacterial populations in these sludges were characterised by their similarity to each other using Mountford's index and the confidence interval method. Although several deficiencies in this approach are acknowledged by the authors, it does establish the characterisation of the sludges on a quantitative basis.

In the previous chapter the potential of the enzyme approach as a tool in the quantitative characterisation of an activated sludge was discussed. However, in this chapter no attempt has been made to quantitatively characterise the bacterial population in fellmongery activated sludges using either an arbitrary nutritional basis or by means of an extensive taxonomic investigation. However, as the aerobic bacterial populations in a number of different effluents and their sludges have been at least partially characterised taxonomically (Hawkes, 1963; Pipes, 1966; Pike and Curds, 1971), an attempt was made to identify some of the numerically dominant bacteria in fellmongery sludge in order to compare these with those of other sludges.

7.2 Materials and Methods

7.21 Isolation procedure

Mixed liquor samples were withdrawn from the reactor tank of the

laboratory scale CMAS apparatus (Fig. 5.1) operating at steady state under the following conditions: sludge age 5 days, retention time 2 days, feed concentration OA 520 mg/l, temperature 20°C. Samples were homogenised in an ultrasonic disintegrator at 21 kHz for 30 sec to release bacteria from sludge flocs.

Activated sludge extract agar prepared as described in Chapter III was used as the primary non-selective isolation medium. Plates were inoculated with aliquots of homogenised mixed liquor diluted in sterile saline solution (0,85%) followed by incubation for 5 days at 30°C. Approximately 20-25 colonies from a quarter sector of each of 3 plates having a total of 50-100 colonies were chosen, in preference to selecting a few that appeared visually dissimilar. A total of 69 colonies were isolated in this manner and transferred to nutrient agar. All but 5 of the colonies survived the transfer to the new medium (survival 93%). Each isolate was purified by cloning on nutrient agar.

7.22 Identification of isolates

Pure cultures were classified according to Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974), A Guide to the Identification of the Genera of Bacteria with Methods and Digest of Genetic Characteristics (Skerman, 1967) and Identification Methods for Microbiologists (Gibbs and Shapton, 1968). Standard identification procedures as described in Manual for the Identification of Medical Bacteria (Cowan and Steel, 1967) were used. The size of the bacteria and type of flagella were determined by electron microscopy.

7.3 Results

Of the 69 bacterial colonies isolated on activated sludge extract, 64 were amenable to subculture on nutrient agar. These 64 colonies were characterised into 6 strains, 2 of which were classified as Pseudomonas spp.

Table 7.1. Characteristics of *Pseudomonas* Strains in Fellmongery Activated Sludge Mixed Liquor

Strains	1	2
No. of isolates	24	13
Cell morphology		
(a) Shape	Rod	Rod
(b) Length (microns)	2,0-2,5	2,6-3,5
(c) Width (microns)	0,7-1,0	1,0-1,3
(d) Flagella	Thin-single-polar	Large-single-polar
Staining		
(a) Acid fast	-	-
(b) Gram's	-	-
(c) Spore	-	-
(d) Capsule	-	-
Pigmentation	None	Pale orange
Growth		
(a) Obligate aerobe	+	+
Biochemistry		
(a) Oxidative in Hugh and Liefson's glucose medium	+	+
(b) Oxidase production	+	+
(c) Catalase production	+	+
(d) Nitrates to nitrites	+	+
(e) Gelatin hydrolysis	-	-
(f) Starch hydrolysis	-	-
(g) Indole production	-	-
(h) Litmus milk	alkali	alkali
(i) Acid from glucose, sucrose, maltose, lactose, mannose, glycerol	-	-

and 4 as *Acinetobacter* spp. (Tables 7.1 and 7.2). The 2 *Pseudomonas* strains accounted for 54% of the total bacteria isolated (24 isolates of strain 1, 13 isolates of strain 2), whilst the 4 *Acinetobacter* strains

Table 7.2. Characteristics of Acinetobacter Strains in Fellmongery Activated Sludge Mixed Liquor

Strains	1	2	3	4
No. of isolates	8	4	12	3
Cell morphology				
(a) Shape	Oval Rod	Rod	Rod	Rod
(b) Length (microns)	1,3-1,5	1,8-2,3	1,3-1,5	1,2-1,4
(c) Width (microns)	0,7-0,8	0,5-0,6	0,5-0,6	0,8-1,0
(d) Flagella	None	None	None	None
Staining				
(a) Acid fast	-	-	-	-
(b) Gram's	-	-	-	-
(c) Spore	-	-	-	-
(d) Capsule	-	-	-	-
Pigmentation	None	None	None	None
Growth				
(a) Obligate aerobe	+	+	+	+
Biochemistry				
(a) Oxidative in Hugh and Liefson's glucose medium	+	+	+	+
(b) Oxidase production	+	+	+	+
(c) Catalase production	+	+	-	+
(d) Nitrates to nitrites	-	-	-	+
(e) Gelatin hydrolysis	-	-	+	-
(f) Starch hydrolysis	-	-	-	+
(g) Indole production	-	-	-	-
(h) Action on litmus milk	no change	no change	no change	no change
(i) Acid from glucose, sucrose, maltose, lactose, mannose and glycerol	-	-	-	-

accounted for 39% of the remainder (8, 4, 12 and 3 isolates of strains 1, 2, 3 and 4 respectively), see Tables 7.1, 7.2 and 7.3. No attempt was made to identify the 5 isolates unable to grow on nutrient agar as these

Table 7.3. Percentage Representation of the Two Dominant Species in Fellmongery Activated Sludge Mixed Liquor

Genus	No. of isolates	Percentage of total
Pseudomonas spp.	37	54
Acinetobacter spp.	27	39
Strains isolated on ASE agar but not reisolated on NA	5	7

isolates proved difficult to work with and represented only 7% of the total.

7.4 Discussion

Non-pigmented aerobic Gram-negative bacteria which are not polarly flagellate and do not ferment sugars are difficult to identify because the genera in which they may be placed are ill-defined. It has been suggested (Thornley, 1967, 1968) that the genus Acinetobacter should be used provisionally for those non-motile coccoid rods, formerly identified with Achromobacter or Alcaligenes, which are now considered to form a homogenous group that is distinct from those organisms having peritrichous flagella.

A survey of the literature suggests that the 4 genera which tend to predominate in waste treatment processes are Pseudomonas, Flavobacterium, Achromobacter and Alcaligenes (Pipes, 1966; Pike and Curds, 1971). Halls and Board (1973) point out that although Pseudomonas often features prominently in investigations of aerobic treatment processes (Allen, 1944; Jasewicz and Porges, 1956; James, 1964; Adamse, 1968), when pseudomonads are not present in large numbers the flora is often dominated by an association between the achromobacters (Achromobacter and/or Alcaligenes)

and Flavobacterium. In their investigations into the treatment of a synthetic sewage, Halls and Board (1973) found that the flora was dominated by an association between Acinetobacter and certain yellow pigmented Gram negative rods which may have been identified as Flavobacterium in the past. Although hesitant to generalise they suggest that their findings support the view of Adamse (1966 Ph.D. thesis) that when treating a carbohydrate rich waste Pseudomonas will become the dominant organism, whilst processes for the purification of proteinaceous wastes will be dominated by organisms which resemble Acinetobacter. Furthermore that good aeration will establish Acinetobacter and the Gram negative yellow pigmented rods as the dominant flora whereas inadequate aeration and/or a carbohydrate rich waste will favour Pseudomonas.

The finding in this study that the flora of fellmongery activated sludge mixed liquor is dominated by an association between Pseudomonas and Acinetobacter is therefore particularly interesting. It demonstrates that the generalisation that the microflora of treatment processes are dominated by either a Pseudomonas population or an Acinetobacter/Achromobacter/Alcaligenes - yellow pigmented Gram negative rod/Flavobacterium association depending on the nature of the effluent and its aeration, is an over-simplification.

AN INVESTIGATION INTO THE KINETICS OF THE ACTIVATED SLUDGE TREATMENT OF FELLMONGERY EFFLUENT

CHAPTER VIII

INTRODUCTION

8.1 Fundamental Biochemistry

The first objective of the activated sludge treatment process is to provide the aerobic heterotrophic bacteria with suitable conditions for removing organic matter to a level where it can no longer support heterotrophic growth. The second and more difficult objective is to reduce the inorganic substances, phosphates and nitrates so that the photosynthetic autotrophic organisms are restrained from growing and thereby injecting complex organic molecules, which they synthesise from CO_2 and solar energy back into the water. These organic compounds would again serve as an energy source for heterotrophic organisms.

It has been shown that the bacteria are the primary organisms for the removal of organic matter in activated sludge systems. Heterotrophic bacteria metabolise organic waste matter in the effluent to produce protoplasm for the creation of new bacteria, at the same time removing a certain amount of inorganic salts from the effluent. These synthesis reactions require energy which the bacteria obtain by oxidising a portion of the organic molecules being metabolised.

Net Protoplasm Synthesised = Protoplasm Synthesised - Endogenous Respiration

Organic Matter Metabolised = Protoplasm Synthesised - Energy for Synthesis.

In addition bacteria require a small amount of energy to maintain normal functions such as motion and enzyme activation. This basal energy requirement is designated endogenous respiration and is a continuous reaction that results in the breakdown of certain components of the protoplasm even when excess nutrients are available (McKinney, 1962).

The process of aerobic energy abstraction from a complex carbonaceous molecule consists in splitting the molecule which releases CO_2 and H^+.

The CO_2 escapes as gas but the H^+ has to be removed to allow the process to continue. This is achieved by attaching it to a hydrogen ion acceptor which in the activated sludge process is oxygen, yielding water. The energy released is proportional to the oxygen utilised and it is this relationship which enables the oxygen demand to be used as a measure of the energy which can be used for metabolism.

8.2 Oxygen Demand Tests

Several oxygen demand tests are used as an estimate of the amount of oxidisable organic matter (potential microbial substrate) present in a water sample. These oxygen demand tests fall into two categories, biochemical and chemical.

The biochemical oxygen demand (BOD) test estimates the amount of oxygen utilised by heterotrophic bacteria growing on the organic material in the water sample over a 5 day period at $20^{\circ}C$ in the dark. Gaudy (1972) has reviewed the BOD test extensively and pointed out that although the concept of carbonaceous BOD is vitally important to the control and study of water pollution, the applications of the concept do not need to involve "the BOD test". This test has the disadvantage that it is a treatment process itself and is subject to the period of treatment, the initial seed, the presence of any toxic constituents in the effluent, and therefore is highly variable and subject to error.

The chemical oxygen demand tests estimate the organic substrate in the water by oxidising the organic matter either with boiling potassium dichromate (COD) or acid potassium permanganate (OA or PV). The COD test is reputed to oxidise 95% of the carbonaceous compounds including a fraction of unbiodegradable compounds. As the unbiodegradable fraction will not be affected by the bio-process, the change in COD across the treatment system is used as an estimate of the organic matter abstracted from the system. The OA test has a disadvantage that it does not oxidise

the same high percentage of carbonaceous compounds as does the COD test. However the OA test has the advantage that it is not as affected by the presence of chloride and nitrogen in effluents rich in these compounds (Cameron and Moore, 1957). As a result of their speed, simplicity and reproducibility the chemical oxygen demand tests have largely replaced the biological oxygen demand test. Although the COD test has become more widely used than the OA test, in this study the OA test was used in most cases. The reason for this is that in South Africa the OA value is still the major criterion for estimating effluent quality by water authorities and was used in most of the previous research conducted locally.

8.3 Mathematical Modelling

The activated sludge process is a continuous culture process which in contrast to the chemostat is continuously inoculated with organisms present in the incoming effluent and returned sludge. As has already been discussed, the micro-organisms occurring in the treatment process are many and varied as are the different types of organic compounds which constitute the oxidisable substrate. Although this has been understood by research workers, mathematical treatment of the activated sludge process has been largely conducted using continuous culture formulations which strictly only apply to pure cultures growing on a single substrate. Despite the scientific illegitimacy of this, mathematical modelling of micro-organism growth in the activated sludge process has already achieved much success in the field of waste water treatment. Many "constants" vital for the design and operation of activated sludge plants have been derived in this manner. The difficulties encountered in allowing for the mixed microbial population and multi-component substrate will be understood by examining the contributions of various workers to overcome these problems. Curds (1971) divided the micro-organism populations into various groups and considered the growth characteristics of each group

independantly. With the aid of a computer he was able to determine how these different populations interact with each other. However, the values for the various kinetic constants of each population could only be estimated or determined experimentally independantly of the other populations. These constants could be very different when the organisms are grown together due to cross feeding and other population interactions. Jones (1973) investigated how the rate of removal of a 10 component substrate would vary if: (a) it was assumed that all substrates combined as a single substrate and (b) a summation of 10 individual substrate removal rates was made. He used a table of random numbers to determine the kinetic constants and individual substrate concentrations in his hypothetical multicomponent substrate. Although there was a difference in substrate removal rate between the two treatments, such a treatment in practice would not be easy. It would be very difficult to determine what all the substrate components of an effluent are and even more difficult to determine the concentrations and individual rates of removal of each. It is for these reasons that pure culture formulations although not strictly correct have been applied to the activated sludge process and with a good degree of success.

Most of the efforts on mathematical modelling have been applied to the completely mixed activated sludge (CMAS) process. Plants having the plug flow mixing regime may be considered as having several CMAS reactors in series. McKinney (1962) was one of the first to produce a rational consistent kinetic theory describing the CMAS process with sludge recycling. Many of the basic relationships described by McKinney have had a strong influence on subsequent workers even though they may deviate from his original model. McKinney assumed a first order substrate utilisation rate model to develop a relationship in which organic substrate removal was a function of the aeration retention time. Similarly, Eckenfelder (1966) assumed a first order utilisation rate to develop a model which

expressed organic matter removal as a function of the aeration retention time and the concentration of mixed liquor volatile suspended solids in the aeration tank. More recently however particular attention has been paid to the substrate model based on continuous culture work by Monod (1950). This model has first and zero order substrate utilisation rates as special conditions and adaptations of this model have been particularly successful (Lawrence and McCarty, 1970; Marais, 1973; Stensel and Shell, 1974; Woods and O'Callaghan, 1974) in predicting effluent quality and in the design of treatment plants.

8.4 Substrate Model

The substrate model is based on the assumption that the microbial growth rate is limited by the availability of any one substrate and appears to give a greater insight into the physical situation than alternative models. Although the single substrate hypothesis is not directly applicable, in its place a cruder hypothesis might be that the concentration of total biodegradable material may control the mixed microbial population in the same way as a single substrate. Lawrence and McCarty (1970) have used the substrate model to demonstrate the usefulness of the solids retention time or sludge age in the design and operation of the CMAS process. They show how sludge age may be considered a unifying parameter for the description of different treatment processes which employ suspensions of bacteria as their primary organisms. Sludge age is felt to be a particularly useful parameter because of its basic relationship with microbial growth and substrate utilisation. The only true test for this model is in its application.

CHAPTER IX

MATHEMATICAL MODELLING OF THE COMPLETELY MIXED ACTIVATED SLUDGE (CMAS) TREATMENT OF FELLMONGERY EFFLUENT: THE EFFECT OF SLUDGE AGE

Summary

Fellmongery effluent was successfully treated (± 85% OA reduction) in a laboratory scale CMAS reactor with sludge recycle and controlled wasting. Under conditions of constant feed strength, retention time and temperature, effluent quality was found to be a function of the sludge age according to the relationship $1/R_s = YKS - b$. The applicability of the substrate model to describe the CMAS treatment of fellmongery effluent was investigated. A clear distinction between the volatile suspended solids and active microbial mass was made and a method for the independant determination of constants Y and b is suggested. The model was verified as quantitatively describing the treatment aprocess by means of a comparison between the experimental observations and the theoretical predictions that arise from the model.

9.1 Introduction

9.11 Mathematical description of microbial growth kinetics in the CMAS treatment process

Note. Each symbol is defined the first time it is used and a complete list of all symbols and definitions is found on page 124.

Before microbial growth kinetics can be applied to the activated sludge process, it is necessary to have a method of estimating both the substrate and active microbial mass concentration in a given water sample. An oxygen demand determination either chemical or biochemical, can be used as an estimate of the substrate concentration in a water sample as discussed in the previous chapter. The method for determining the active microbial mass is however more complex.

9.12. Estimating the active microbial mass, (X_{av})

The active microbial mass of sludge (X_{av}) cannot be measured directly

but is approximated by either the volatile suspended solids (X_v) or the total suspended solids (X_t) which can be measured directly. As X_v is a more reliable substitute for X_{av} than X_t, X_v will be used to approximate X_{av}. It is important to consider the difference between X_{av} and X_v before substituting the one for the other. The volatile suspended solids are composed of three fractions:-

$$X_v = X_{av} + X_e + X_{io} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where X_e = endogenous residue and X_{io} = inert organic mass.

The active microbial mass accumulates in the CMAS treatment process with sludge return according to the relationship given by Lawrence and McCarty (1970):

$$X_{av} = \frac{Y(S_i - S)}{1 + bR_s} \quad \frac{R_s}{R} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where Y = growth yield coefficient, being mass of organisms synthesised per mass of substrate utilised (mg/mg)

S_i = unmetabolised substrate in the influent (mg/l)

S = unmetabolised substrate in the effluent (mg/l)

R_s = sludge age or solids retention time (days)

defined as: $\frac{\text{mass of sludge in reactor}}{\text{mass wasted/day}}$

R = hydraulic retention time (days)

b = micro-organism decay coefficient (day^{-1})

The endogenous residue accumulates in the sludge mass as a result of endogenous respiration. A fixed fraction of the micro-organisms is assumed to die each day and lyse their contents back into the surrounding liquid to serve again as substrate for surviving organisms. However, some constituents of the cell have been reported to be not readily biodegradable and remain behind as part of the sludge mass (McKinney, 1962). This fraction believed by Symons and McKinney (1958) to be a complex polysaccharide has been estimated to be as much as 18-23% of the cell mass

(Marais, 1973). It has since been shown (Obayashi and Gaudy, 1973) that the extracellular polysaccharides produced by several common waste water organisms are amenable to biological degradation and should not be cited to disprove the theory of total biological oxidation. Nevertheless a proportion of microbial cellular constituents appears to remain behind as an endogenous residue and must be accounted for in a mathematical model. The proportion of endogenous residue remaining probably varies from sludge to sludge. A residual fraction of less than 10% of the cell mass is suggested by the experimental results reported here. Assuming a residual fraction of 10% the endogenous residue accumulates according to the relationship:

$$X_e = 0{,}1 \, b \, X_{av} \, R_s \quad \ldots\ldots (3)$$

A fraction of the organic solids entering the reactor is unbiodegradable and accumulates in the sludge mass according to:

$$X_{io} = X_{ioi} \, R_s / R \quad \ldots\ldots (4)$$

where X_{ioi} = inert organic solids in the influent. Later in this chapter evidence will be presented to show that almost all the organic solids in settled fellmongery effluent are able to be utilised as nutrients and that the inert organic fraction in these effluents is likely to be small. It will be noted from the formula that at high sludge ages the inert organic fraction accumulating in the sludge may become significant. However, as the contribution of X_{io} to the volatile suspended solids is very difficult to ascertain, this fraction will be treated as negligible for biological treatment processes with low sludge ages. Accepting this eq. (1) becomes:

$$X_v = X_{av} + X_e$$

Substituting for X_e from eq. (3)

$$= X_{av} + 0{,}1 \, b \, X_{av} \, R_s$$

$$= X_{av} (1 + 0{,}1 \, b \, R_s) \quad \ldots\ldots (5)$$

The volatile suspended solids determined experimentally must therefore be divided by $(1 + 0{,}1\ bR_s)$ to give an estimate of X_{av}.

Substituting for X_{av} from eq. (2)

$$X_v = \frac{Y(S_i-S)}{1 + bR_s} \quad \frac{R_s}{R} \quad (1 + 0{,}1\ bR_s) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

9.13 The yield constant, Y

In a reactor a certain mass of sludge will be produced each day for the mass of substrate utilised. This is a net yield and is influenced by endogenous mass loss, endogenous residue and inert materials in the influent. The net yield is not a constant but depends on the sludge age.

The numerical value of the yield constant is dependant on both the parameter used to measure the mass of organisms (volatile or total suspended solids) and the method of measuring the substrate. With regard to the parameter for measuring substrate strength, it has been pointed out by Marais (1973) that the same mass of organisms is synthesised for the same substrate utilised irrespective of the method of measuring the substrate. Consequently one can write:

$$\text{microbial mass synthesised (mg/l)} = Y_{BOD}\ \Delta\ BOD = Y_{COD}\ \Delta\ COD$$

The Y value simply changes to allow for different methods of substrate evaluation. Thus several values for Y are obtainable depending on the parameter used to measure the substrate concentration and mass of sludge.

i.e. $Y_{BOD\ VSS} = Y_{bv}$: $Y_{BOD\ TSS} = Y_{bt}$

$Y_{COD\ VSS} = Y_{cv}$ and $Y_{OA\ VSS} = Y_{ov}$

where VSS = volatile suspended solids (mg/l)

TSS = total suspended solids (mg/l)

9.14 Sludge utilisation rate (SUR)

This is a parameter which can be obtained experimentally and is very

useful in the development and verification of the mathematical model. The sludge utilisation rate is defined as follows

$$SUR = \frac{\text{Mass of substrate utilised per day}}{\text{Mass of sludge}}$$

Expressing SUR in terms of BOD and volatile suspended solids

$$SUR_{bv} = \frac{(S_{bi}-S_b) \quad Q}{X_v \; V}$$

$$= \frac{S_{bi}-S_b}{X_v \; R}$$

S_{bi} = BOD of influent (mg/l)

S_b = BOD in CMAS reactor (mg/l)

X_v = volatile suspended solids (mg/l)

Q = effluent flow rate (l/day)

V = volume of reactor (l)

R = retention time = V/Q (days)

The disadvantage of the sludge utilisation rate is that it has six values as the substrate can be measured as BOD, COD, or OA and the sludge mass as VSS or TSS. A value which is independant of these measurements and is related to the SUR is the sludge age (R_s).

Expressing SUR in terms of active volatile mass and unidentified s :

$$SUR_{sav} = \frac{S_i-S}{X_{av} \; R}$$

Substituting for X_{av} from eq. (2)

$$SUR_{sav} = \frac{S_i-S}{R} \quad \frac{1 + b\,R_s}{Y(S_i-S)} \quad \frac{R}{R_s}$$

$$SUR_{sav} = \frac{1 + b\,R_s}{YR_s} \quad \text{.......................................} \quad (7)$$

Multiply by Y reduce and transpose

$$1/_{R_s} = Y(SUR_{sav}) - b \quad \text{.......................................} \quad (8)$$

A plot of $1/_{R_s}$ vs SUR_{sav} defines a straight line with intercept on $1/_{R_s}$ = b and slope = Y (Fig. 9.1). The linear relationship is only valid if the SUR is based on active mass. When SUR is expressed in terms of X_v

instead of X_{av}, X_v must be divided by $(1 + 0{,}1\ bR_s)$ to obtain an estimate of X_{av} (eq. 5).

Expressing SUR in terms of X_v:

$$SUR_{sv} = \frac{S_i - S}{X_v R} = \text{SUR experimental}$$

Substituting eq. (6) for X_v and simplifying

$$SUR_{sv} = \frac{1 + b R_s}{(1 + 0{,}1\ bR_s)YR_s} = SUR_{theoretical} \quad \ldots\ldots\ldots\ldots\ldots (9)$$

i.e.

$$SUR_{exp} = \frac{S_i - S}{X_v R} = \frac{1 + b R_s}{(1 + 0{,}1\ bR_s)YR_s} = SUR_{th} \quad \ldots\ldots\ldots\ldots (10)$$

9.15 Sludge age and effluent quality

Lawrence and McCarty (1970) have related the microbial growth kinetic equations as developed for pure cultures by Monod (1950) to the sludge age via the relationship:

$$1/R_s = YU - b \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (11)$$

where $$U = \frac{K_m S}{K_s + S} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (12)$$

U = specific substrate utilisation rate (day^{-1})

K_m = maximum substrate utilisation rate (day^{-1})

K_s = half-rate constant, substrate concentration

where $U = \frac{1}{2} K_m$ (mg/l)

There are two extreme cases of eq. (12), when S is very high ($S \gg K_s$), eq. (12) is approximated by:

$$U = K_m$$

However, this situation has little application in the activated sludge process where the objective is to reduce the substrate concentration in the mixed liquor to as low a level as possible.

In the extreme case when S is small with respect to K_s ($S \ll K_s$), eq. (12) approximates to:

$$U = \frac{K_m}{K_s} S = KS \quad \ldots\ldots (13)$$

where $K = K_m/K_s$ or substrate conversion rate $(l/mg\text{-}day)$. As pointed out by Marais (1973), this approximation is usually valid for an efficient CMAS treatment process where the unmetabolised substrate in the reactor is small. Substituting eq. (13) into eq. (11) gives:

$$1/R_s = YKS - b \quad \ldots\ldots (14)$$

As Y, K and b are all constants, eq. (14) relates effluent quality as a function of sludge age.

9.16 Oxygen consumption rate

Oxygen is utilised for the synthesis of new cell material and for maintenance of cell function (endogenous respiration). Oxygen consumption is due mainly to two groups of organisms, the heterotrophs which break down carbonaceous compounds and the nitrifiers which convert ammonia to nitrate. As no nitrification occurred in the studies reported here only the kinetics of heterotrophic oxygen consumption will be considered. The formulation given here is adapted from that presented by Marais (1973).

a) Oxygen consumption for synthesis

During synthesis of cell mass oxygen is consumed to release energy so that the organism can incorporate some of the energy in its protoplasm. The cell mass synthesised is proportional to the substrate utilised as follows:

$$\Delta X_1 = Y \Delta S_c$$

X_1 = concentration of biological mass synthesised (mg/l)

S_c = concentration of utilisable substrate, COD (mg/l).

The oxygen equivalent of protoplasm can be obtained experimentally from COD analyses or theoretically using a stoichiometric equation of

bacterial cell composition and has been estimated as approximately 1,42 mg COD/mg cell mass (Marsden and Goloi, 1974). Hence the COD locked up in the cell mass S_c' is

$$\Delta S_c' = 1{,}42\ \Delta X_1$$
$$= 1{,}42\ Y_{cv}\ \Delta S_c$$

The energy (COD) lost as heat to enable synthesis to take place is: total heat lost - energy appearing as cell mass

$$= \Delta S_c - \Delta S_c'$$
$$= \Delta S_c - 1{,}42\ Y_{cv}\ \Delta S_c$$
$$= \Delta S_c\ (1 - 1{,}42\ Y_{cv})$$

b) Oxygen consumption for endogenous respiration

Endogenous respiration has an active mass loss associated with it. This active mass loss per day is given by:

$$\Delta X = b\ X_{av}$$

However, as a fraction of this mass, about 10% remains in the effluent as a residue that is not readily biodegradable, the net mass loss is:

$$0{,}9\ b\ X_{av}$$

The COD associated with this mass loss per day is:

$$= 1{,}42\ (0{,}9\ b\ X_{av})$$

The total COD lost from the CMAS system per day can now be written down.

Mass COD removed/day = Mass COD lost to provide for synthesis + Mass COD lost to maintain cell function

$$= (S_{ci} - S_c)\ (1 - 1{,}42\ Y_{cv})\ Q + 1{,}42\ (0{,}9\ b\ X_{av})\ V \quad \ldots\ldots\ldots\ldots \quad (15)$$

Substituting X_v for X_{av} using eq. (5) into eq. (15).

Mass COD removed/day = Mass O_2 used/day =

$$(1 - 1{,}42\ Y_{cv})\ (S_{ci} - S_c)\ Q + \frac{1{,}42\ (0{,}9\ b\ X_v)\ V}{1 + 0{,}1\ b\ R_s} \quad \ldots\ldots\ldots\ldots\ldots \quad (16)$$

Now for oxygen utilised/l reactor volume/day (O_2 mg/l/day), eq. (16) is divided through by V noting V/Q = retention time (R).

$$O_2 \text{ (mg/l/day)} = \frac{(1 - 1{,}42\ Y_{cv})\ (S_{ci} - S_c)}{R} + \frac{1{,}42\ (0{,}9\ b\ X_v)}{1 + 0{,}1\ b\ R_s}$$

Dividing through by X_v then $(S_{ci} - S_c)/X_v\ R - SUR_{cv}$

$$\frac{O_2 \text{ (mg/l/day)}}{X_v} = (1 - 1{,}42\ Y_{cv})\ SUR_{cv} + \frac{1{,}42\ (0{,}9\ b)}{1 + 0{,}1\ b\ R_s} \quad \ldots\ldots\ (17)$$

Expressing eq. (17) in terms of OA and X_v:

$$\frac{O_2 \text{ (mg/l/day)}}{X_v} = (2{,}86 - 1{,}42\ Y_{ov})\ SUR_{ov} + \frac{1{,}42\ (0{,}9\ b)}{1 + 0{,}1\ b\ R_s} \quad \ldots\ (18)$$

where 2,86 is the ratio of COD to OA as determined for treated fellmongery effluent.

It will be noted that if R_s is small then $0{,}1\ b\ R_s$ is negligible so that eq. (18) now has the form:

$$\frac{O_2 \text{ (mg/l/day)}}{X_v} = C'\ SUR_{ov} + C'' \quad \ldots\ldots\ (19)$$

If O_2 (mg/l/day)/X_v is plotted against SUR_{ov} obtained from experimental data, the straight line has slope C' and the intersection on O_2 (mg/l/day)/X_v axis defines C''. At long sludge ages the true relationship is curved and passes through the origin (Fig. 9.2).

9.17 Determination of bacterial growth kinetic constants

Constants Y, K and b have to be determined for a particular effluent and its treatment system before the mathematical description of the growth kinetics can be used for design purposes. The determination of constants Y and b is simple when X_{av} is used for the data plot on a straight line (eq. 8 and Fig. 9.1). However, this method is deficient in that it does not distinguish between total volatile mass and active volatile mass. Application of this method leads to gross underestimation of b. Marais (1973) developed a method which distinguishes between the two volatile masses and directly identifies Y and b. When considering X_v as opposed to X_{av} the plots are no longer linear. Taking logarithms of SUR_{th} eq. (9):

$$\log SUR_{ov} = \log [1 + b R_s]/[(1 + 0,1 b R_s) R_s] - \log Y_{ov}$$

By plotting $1/R_s$ vs $\log [1 + b R_s]/[(1 + 0,1 b R_s) R_s]$ for a series of b values from b = 0,1 to b = 0,5 (this makes Y = 1) the shape of the respective curve is a function only of b (Fig. 9.3). The experimental values $\log 1/R_s$ vs $\log (S_{oi} - S_o)/R X_v$ are now plotted on the same axes (Fig. 9.4). The theoretical curve defining a particular b value which has the nearest shape to the experimental curve identifies the approximate b value. The value of Y_{ov} can be found by solving for Y_{ov} in eq. (10).

$$Y_{ov} = \frac{1 + b R_s}{(1 + 0,1 b R_s) R_s} \Big/ \frac{S_{oi} - S_o}{X_v R} \quad \ldots\ldots\ldots\ldots\ldots (20)$$

= theoretical value / experimental value.

This technique of identifying b by fitting the shape of the theoretical curve to the experimental points is however, insensitive. Furthermore as discussed by Van Beuningen (1974), the constants tend to act in a compensatory fashion: various combinations of Y and b give approximately the same X_v (mg/l) and O_2 (mg/l/day). Therefore it is not possible when using the technique of Marais (1973) to identify the true values from the experimental results. An approach to the solution of the problem is to determine one parameter independently of the other - either Y or b. Then, from available experimental data on sludge utilisation or oxygen consumption the corresponding value of b or Y can be determined.

9.18 Determination of b using oxygen consumption

When O_2 (mg/l/day)/X_v is plotted against SUR_{ov} the straight line has slope C' and intersection C'' (eq. 19 and Fig. 9.2). As the value at the intersection of the O_2 (mg/l/day)/X_v axis = C'' = 1,42 x 0,9 b; b may be calculated. Once the value of b has been identified, the theoretical curve corresponding to that value is superimposed upon the experimental results (Fig. 9.4). An accurate fit between the shape

of the theoretical curve and the experimental results provides confirmation that the b value has been correctly determined. The value of Y_{ov} may be computed using eq. (20) as previously indicated. A check on the value of Y_{ov} can be made using eq. (19) as the slope $C' = 2,86 - 1,42\ Y_{ov}$.

9.19 Rate constant, K

The rate constant K, can be determined theoretically by using the following relationship obtained by combining and reducing eq. (8) and eq. (14).

$$SUR_{sav} = \frac{S_i - S}{X_{av}\ R} = K\ S \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (21)$$

When SUR_{av} is plotted against S, a straight line passing through the origin is obtained, the slope defining K. However when X_v is used, the relationship is no longer linear:

$$\frac{S_i - S}{X_v\ R}\ (1 + 0,1\ b\ R_s) = K_v\ S \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (22)$$

Consequently each experimental X_v must be divided by $(1 + 0,1\ b\ R_s)$ to obtain an estimate of X_{av}. If the corrected value is plotted against S a straight line passing through the origin should be obtained. However, when COD or OA is used as the substrate parameter as opposed to BOD, a fraction of the influent COD or OA is unbiodegradable. If the experimental SUR $(1 + 0,1\ b\ R_s)$ is plotted against S_o the slope still defines K although the intercept is no longer zero but cuts the S axis. This intercept on the S axis gives an estimate of the unbiodegradable COD or OA in the influent (Fig. 9.5).

Note. The value of K is not influenced by the method of substrate determination (Marais, 1973) but is influenced by the method of sludge determination. Hence the subscript v after K is included as it denotes the method of sludge determination as the volatile suspended solids.

9.191 Design chart for sludge concentration

For preliminary design it is useful to have a graphical representation

of the effects of changes in the various operating parameters on the effluent treatment process. The equation for the graphical design model presented here is adapted from that presented by Smith (1970) and Marais (1973).

Taking logarithms of eq. (6):

$$\log X_v = \log \left[Y_{ov} \frac{(S_{oi} - S_o}{1 + b R_s} (1 + 0{,}1 b R_s) R_s \right] - \log R \ldots\ldots (23)$$

For a fixed sludge age $(S_{oi} - S_o)$ is fixed (eq. 14). Consequently, the first term on the R.H.S. of eq. (23) is a constant, C. Thus $\log X_v$ is a function only of log R.

$$\log X_v = \log C - \log R.$$

If $\log X_v$ is plotted vs log R the slope will be -1, i.e. at -45^o. The value of C is given by the term within the brackets in eq. (23) with log R = 0 i.e. R = 1. To construct a chart for $\log X_v$ vs log R, C is calculated for a series of R_s values and these are plotted on log paper at R = 1 and a -45^o line is drawn through each (Fig. 9.6).

9.192 Design chart for carbonaceous oxygen demand

The equation for the graphical design chart for carbonaceous oxygen demand as adapted from Marais (1973) is:

$$\log O_2 \text{ (mg/l/day)} = \log \left[2{,}86 (S_{oi} - S_o) - \frac{1{,}42 Y_{ov} (S_{oi} - S_o)}{1 + b R_s} (1 + 0{,}1 b R_s) \right] - \log R$$

Again the first term on the R.H.S. is a constant for a particular R_s so that the equation defines a straight line at -45^o to the axis. For each sludge age the calculation of the constant need only be performed for R = 1 and the -45^o line drawn through this value as before (Fig. 9.7).

9.2 Materials and Methods

9.21 Source of effluent

A bulk supply of concentrated mixed fellmongery effluent was obtained and stored at $4^{o}C$. Twice each day the settled effluent was diluted with tap water to give an effluent of constant strength before being added to the feed reservoir. Analysis of the feed reservoir was carried out each day together with the analysis of the CMAS reactor.

9.22 Experimental procedure

A description of the CMAS apparatus used, mode of operation and adjustment of sludge age is given in Chapter V. To determine the effect of sludge age on effluent quality, the retention time and feed concentration were kept constant at 2 days and OA 520 mg/l respectively, while the sludge age was varied from 2 to 20 days. The CMAS reactors were allowed to function for a period of 40 days (twice maximum sludge age) before steady state was assumed to have been achieved. Samples of effluent were tested regularly to note trends and indicate when a steady state prevailed. After steady state was reached duplicate samples were analysed daily for 6 days and tested graphically to determine if the values were normally distributed. The results were subjected to analysis of variance and the level of significance (student's t-test) of varying the sludge age on effluent quality was determined. No attempt was made to control the sludge concentration in the reactor. The feed rate, feed concentration and sludge age were set and the sludge concentration allowed to attain its own steady state.

9.23 Temperature

It is generally recognised that temperature has little influence on the yield constant, Y (Marais, 1973). Both K_v and b, however, are

affected by temperature and therefore in these experiments the temperature was kept constant at 20°C.

9.24 Effluent analysis techniques

These are given in Chapters IV and V. Additional analyses used in these studies were:

(a) Biochemical oxygen demand (BOD) determinations were used as an additional method of substrate estimation. The BOD was determined after 5 days incubation in the dark at 20°C using the Winkler method for determining dissolved oxygen as outlined in Ministry of Housing and Local Government (1956).

(b) The sludge volume index was calculated using the settleable solids volume and total suspended solids according to:

$$\text{Sludge volume index} = \frac{\text{Settled volume of sludge percent - 30 min}}{\text{Suspended solids percent}}$$

9.3 Results

The effects of varying the sludge age on the CMAS treatment of fellmongery effluent are shown in Table 9.1.

The values of Y_{ov} and b were determined from the plots in Figs 9.2, 9.3 and 9.4 as outlined earlier. These plots give $Y_{ov} = 1,12$ and $b = 0,1$. The value of K_v was determined from the plot in Fig. 9.5 giving $K_v = 0,017$. This plot also gave an estimate of the unbiodegradable OA in the effluent as 54 mg/l.

A comparison between the experimental and theoretical relationship of sludge age and sludge utilisation rate (eq. 10) is shown in Fig. 9.1. Using X_v the experimental relationship was curved and passed through the origin. The relationship was virtually linear for $R_s < 10$ days with the curvature only becoming pronounced at $R_s > 10$ days. Included in Fig. 9.1 is a plot of $1/R_s$ vs $1 + b\ R_s/Y\ R_s$ which has the term for endogenous

residue accumulation ($1 + 0,1\ b\ R_s$) omitted. In effect this plot assumes that $X_v = X_{av}$ rather than $X_v = X_{av} + X_e$. The two plots are parallel for sludge ages < 10 days. However, for sludge ages > 10 days the term for endogenous residue must be included for the experimental relationship to agree with the model. If a linear regression analysis on the experimental points was used to obtain a straight line with intercept on the $1/R_s$ axis giving b (eq. 8), b would be underestimated. The experimental plots follow the curved relationship very well as predicted by the theory.

9.31 Effluent quality

Effluent quality was shown to improve with increasing sludge age. When OA was used as an estimate of the substrate remaining, the percentage purification increased from 84% ($R_s = 2$) to 89% ($R_s = 20$). This improvement in the OA of the effluent was found to be significant ($t_{0,01}$) between each sludge age tested (Table 9.2). The BOD determinations were more variable than the OA and although the BOD improved with sludge age from 92,7 mg/l ($R_s = 2$) to 59,6 mg/l ($R_s = 20$) there was not a significant difference ($t_{0,05}$) between each sludge age tested (Table 9.3). There was however a significant difference in BOD from the treatment plants with sludge ages $\leqslant 5$ days and sludge ages $\geqslant 10$ days.

The experimental values and theoretical predictions of effluent quality are plotted in Fig. 9.8 and were in good agreement. In this investigation effluent quality was found to be a function of sludge age according to eq. (14).

9.32 Sludge production

The experimental values for volatile suspended solids production with increasing sludge age are compared with the theoretical predictions given by eq. (6) in Fig. 9.9 and are seen to be in good agreement.

The sludge volume index (SVI) which can serve as an indication of the compactability and settleability of the sludge was shown to vary with sludge age (Table 9.1). The CMAS reactor with sludge age 5 days had a SVI considerably lower than the other three and therefore produced the sludge with the best separation and compactability characteristics.

9.33 Oxygen consumption rate

The experimental oxygen consumption rates for each of the four reactors are plotted together with the theoretical predictions in Fig. 9.2. Experimental oxygen consumption rates appeared to follow the theoretical relationship as predicted by eq. (19), where the term for endogenous mass accumulation has been considered negligible. This is in contrast to what was found in Fig. 9.1 where the term for endogenous respiration had to be included for the experimental to agree with the theory. A possible explanation for this discrepancy is that the proportion of cellular material which accumulates as a residue is less than 10%. However the difference between the experimental observation and theoretical prediction is not significant at the 5% level (vertical bars in Fig. 9.2).

9.34 Nitrification

No nitrification was observed even at sludge ages as high as 20 days. This is indicated by the level of ammonia remaining in the settled effluent (Table 9.1).

9.4 Discussion

These results confirm the observation made in Chapter V, that if an adapted sludge is used fellmongery effluent is amenable to biological treatment in a CMAS reactor ($\pm$ 85% OA reduction) without prior lowering of the pH or the addition of catalyst to remove the sulphide. Although the sludge age has a significant influence on effluent quality, the

improvement in quality from OA 83,4 mg/l (R_s = 2 days) to OA 59,4 mg/l (R_s = 20 days) is probably not an economically significant improvement particularly as no nitrification took place even at a sludge age of 20 days. Under these circumstances the optimum sludge age would be better chosen by considering the settling properties of the sludge rather than the effluent quality.

It is interesting to compare the values for the constants Y_{ov}, K_v and b obtained in these studies with those of domestic sewage. The yield coefficient (coefficient basis COD and volatile suspended solids) at 20°C for domestic sewage has been reported to range from Y_{cv} = 0,37 to 0,67 mg sludge/mg substrate (Lawrence and McCarty, 1970; Marais, 1973). Converting Y_{ov} to Y_{cv} for fellmongery effluent = 1,12/2,86 = 0,39 mg/mg which falls within the range for domestic sewage. Substrate conversion rates (K_v) for domestic sewage vary from K_v = 0,0257 l/mg-day for heavily industrialised town like Manchester to K_v = 0,0459 for less heavily industrialised cities (Marais, 1973). A low K_v = 0,017 l/mg-day as reported here for fellmongery effluent appears consistent with the observation that industrialised domestic effluents have a lower K_v than ordinary domestic effluents (Eckenfelder, 1967). The endogenous respiration coefficient reported here (b = 0,10) is within the range reported for domestic sewage, b = 0,07 (Lawrence and McCarty, 1970) and b = 0,28 (Van Beuningen, 1974).

Sludge age has been shown to be a most important parameter in the control of nitrification. Sludge ages of 4 - 5 days appear to be sufficient to allow nitrification at 20°C although a 10 day sludge age has been recommended to ensure nitrification at 15°C (McCarty and Haug, 1971). As no nitrification occurred in these studies even with a sludge age of 20 days at 20°C there appears to be some other criterion preventing nitrification from taking place.

The design charts (Figs 9.6 and 9.7) can be useful in either the design of new plants or in obtaining the optimum operating conditions of existing plants. If the sludge age for a certain treatment process is

fixed, the volatile suspended solids concentration varies with retention time (Fig. 9.6). For any particular aeration device there is a limit to its oxygenating capacity. This places a limit on the sludge concentration which that particular aeration device can support. From the chart (Fig. 9.6) it can be seen that there is a wide range of sludge ages and concomitant retention times to give the same sludge concentration in the reactor. The optimum sludge age may be set by the requirement for a particular quality effluent or by the settling properties of the sludge. In such a case the required retention time is read off the horizontal axis. If this particular retention time is uneconomical and therefore needs to be shortened, either an aeration device of greater oxygenating capacity must be added to support the greater sludge concentration or the sludge age must be lowered.

KEY TO SYMBOLS

X = general symbol for micro-organism concentration (mg/l); subscripts av, e and io refer to active, endogenous and inert organic respectively. An additional i refers to influent.

S_i = substrate concentration in the influent (mg/l)

S = unmetabolised substrate concentration in the effluent and reactor (mg/l)

R = hydraulic retention time (days)

R_s = sludge age or solids retention time (days) defined as:

$$\frac{\text{mass of sludge in reactor}}{\text{mass wasted/day}}$$

Y = growth yield coefficient, being mass of organisms synthesised per mass substrate utilised (mg/mg)

U = specific substrate utilisation rate (day^{-1})

K = substrate conversion rate ($l/mg\text{-}day$)

K_m = maximum substrate utilisation rate (day^{-1})

K_s = half-rate constant; substrate concentration where $U = \frac{1}{2} K_m$ (mg/l)

b = micro-organism decay coefficient or endogenous respiration rate (day^{-1})

SUR = sludge utilisation rate (day^{-1}) defined as:

$$\frac{\text{mass of substrate utilised/day}}{\text{mass of sludge}}$$

Table 9.1 Effect of sludge age on the treatment of fellmongery lime-sulphide effluent in a completely mixed activated sludge (CMAS) reactor.

Sludge age (days)	2	5	10	20	Untreated influent
Retention time (days)	2	2	2	2	
Oxygen Absorbed (mg/l)	83,4	73,3	64,2	59,4	520,9
BOD_5 (mg/l)	92,7	89,9	60,4	59,6	505,7
Na_2S (mg/l)	0,78-1,56	0,78-0,56	0,78-1,56	0,78-1,56	416,6
pH	8,2	8,1	8,2	8,2	12,0
MLSS (mg/l)	548	998	1678	2809	86,6
MLVSS (mg/l)	447	865	1436	2438	55,0
Settleable Solids (ml/l)	60	47	272	438	0
Sludge Vol Index	109	47	162	156	-
Oxygen consumption rate (mg/l/day)	334	391	474	598	-
NH_3-N settled effluent (mg/l)	90	92	103	113	16,2
Bacteria (ml^{-1})	$9,09 \times 10^7$	$1,21 \times 10^8$	$2,78 \times 10^8$	$2,25 \times 10^8$	$1,03 \times 10^4$
Protozoa (ml^{-1})	$4,84 \times 10^3$	$3,34 \times 10^3$	$7,22 \times 10^3$	$1,67 \times 10^4$	0

Table 9.2 Analysis of variance of OA values of CMAS reactor under steady state conditions over 6 days

Day	1	2	3	4	5	6	Total	Mean
Sludge age 2 days	78,64 82,56	83,36 83,04	78,64 80,32	77,44 76,80	88,08 87,52	92,40 92,00	1000,80	83,40
Sludge age 5 days	79,04 77,92	73,12 72,12	68,48 69,28	67,84 68,80	71,04 71,68	78,96 80,80	880,08	73,34
Sludge age 10 days	61,68 64,56	60,80 61,60	63,76 65,76	64,32 68,00	58,88 60,80	70,88 69,28	770,32	64,19
Sludge age 20 days	60,88 58,48	60,48 59,44	57,28 56,48	58,24 56,64	61,12 59,28	66,32 58,16	712,8	59,40
Total	563,76	554,96	540,00	538,08	558,40	608,80	3364,00	
Mean	70,47	69,37	67,50	67,26	69,80	76,10		

Analysis of Variance

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Days	412,6699	5	82,5340	28,60
Sludge age	4041,1824	3	1347,0608	466,78
Interaction	317,1936	15	21,1462	7,33
Error	69,2608	24	2,8859	
Total	4840,3067	47	102,9852	

$$\text{l.s.d. (0,01) between means of sludge ages} = t_{0,01} \times \sqrt{\frac{2M.S.E.}{n}}$$

$$= 2,69 \times \sqrt{\frac{2 \times 2,8859}{12}}$$

$$= 1,39$$

Table 9.3 Analysis of variance of BOD values of CMAS reactors under steady state conditions over 5 days.

Day	1	2	3	4	5	Total	Mean
Sludge age 2 days	89,0 95,5	91,0 91,0	129,0 92,0	69,5 61,5	96,5 112,0	927,00	92,70
Sludge age 5 days	104,5 84,0	105,0 110,0	79,2 84,4	81,5 89,0	74,0 88,0	899,60	89,96
Sludge age 10 days	56,0 57,0	56,5 60,0	50,5 60,5	67,7 62,5	61,0 72,7	604,4	60,44
Sludge age 20 days	52,5 57,5	47,0 57,0	68,5 70,0	69,5 61,5	60,5 52,5	596,5	59,65
Total	596,0	617,5	634,1	562,7	617,2	3027,5	
Mean	74,50	77,19	79,26	70,34	77,15		

Analysis of Variance

Source	Sum of Squares	Degree of Freedom	Mean Square	F
Days	377,6175	4	94,4044	1,27
Sludge age	9828,1708	3	3276,0569	44,09
Interaction	3646,9005	12	303,9084	4,09
Error	1486,2350	20	74,3118	
Total	15338,9238	39	393,3057	

$$\text{l.s.d. (0,05) between means of sludge ages} = t_{0,05} \times \sqrt{\frac{2\ \text{M.S.E.}}{n}}$$

$$= 2,02 \times \sqrt{\frac{2 \times 74,3118}{10}}$$

$$= 7,79$$

Fig. 9.1 Experimental and theoretical relationship between sludge age and sludge utilization rate.

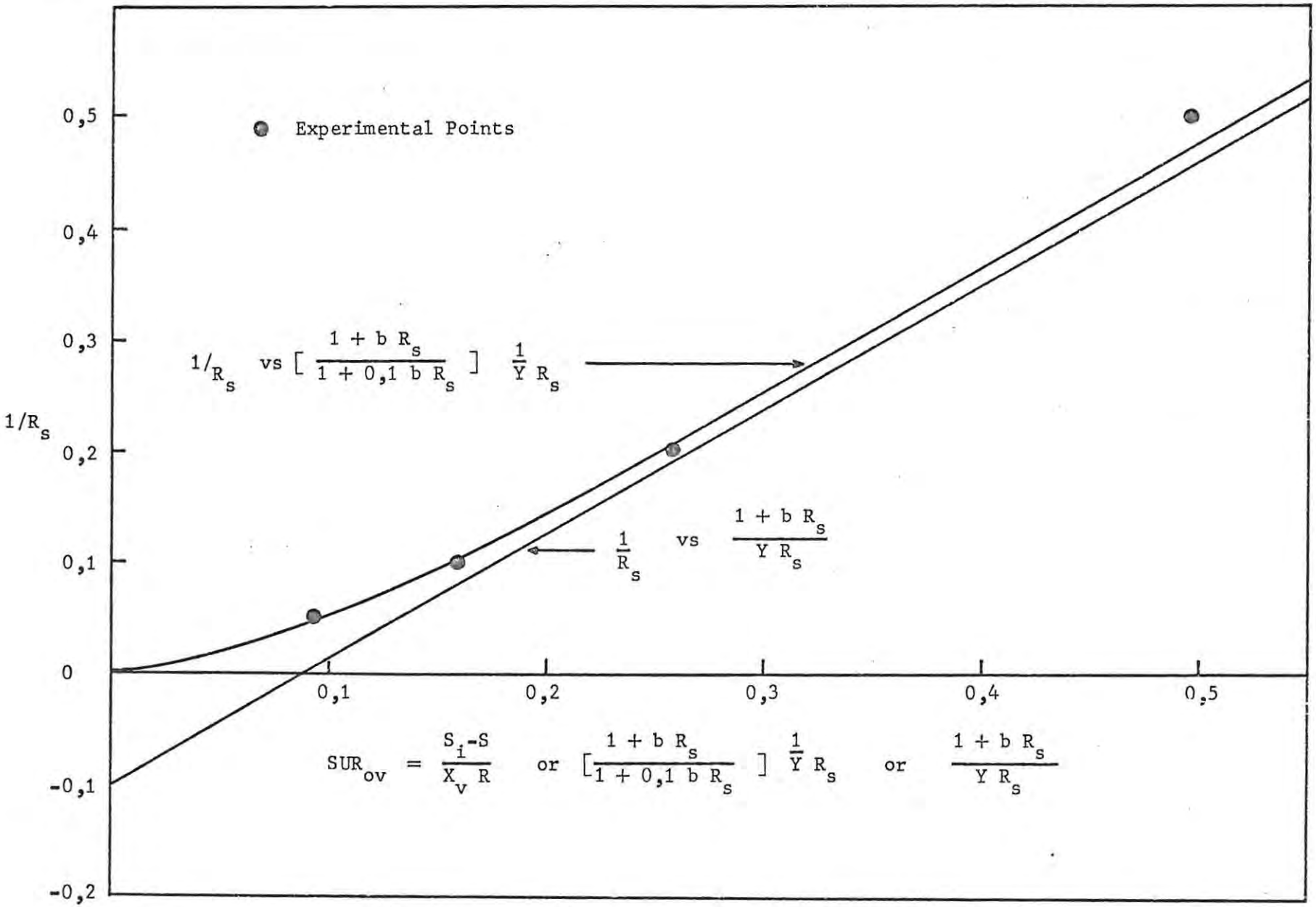


Fig. 9.2 Comparison of experimental and theoretical carbonaceous oxygen demand with [illegible]

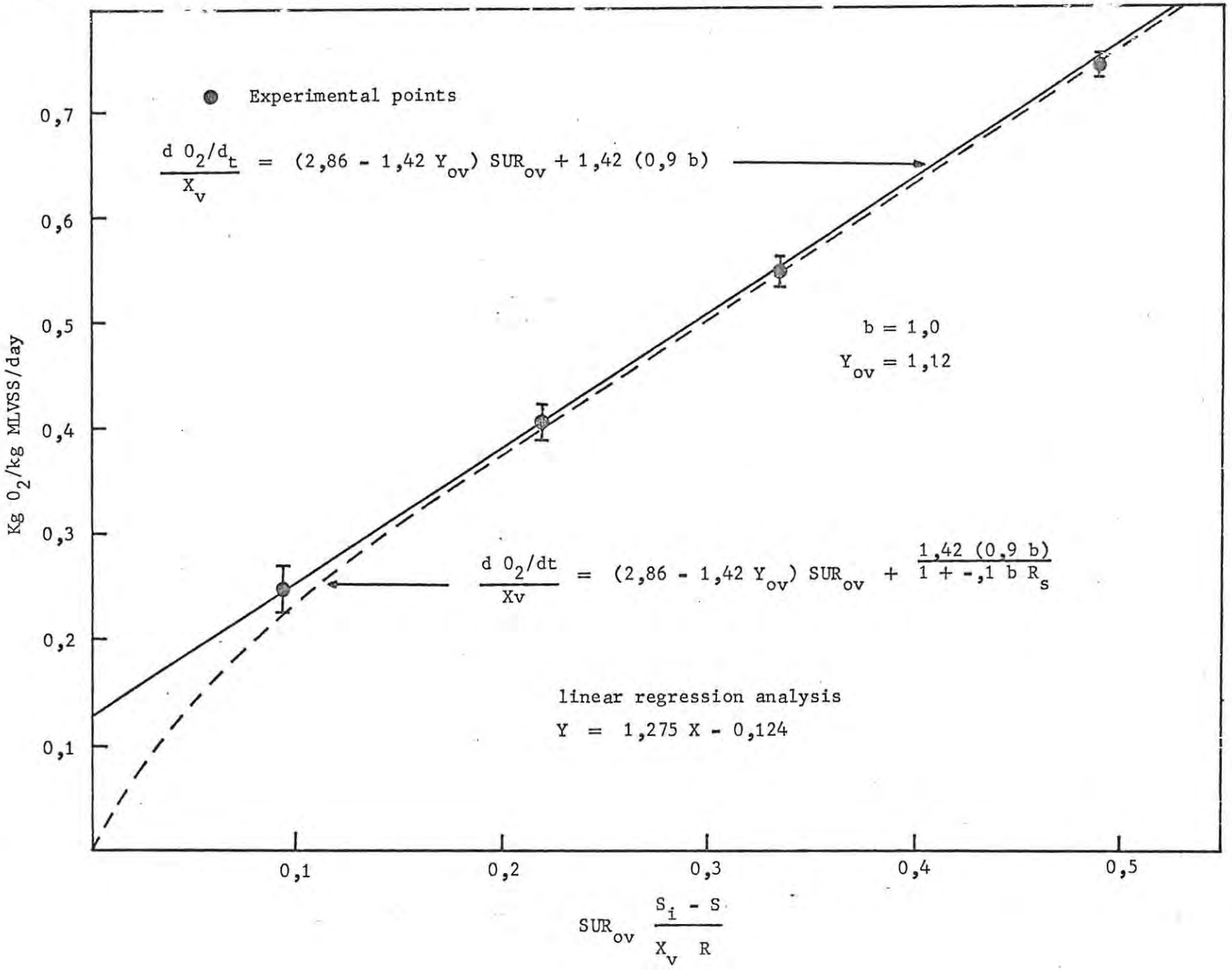


Fig. 9.3 A plot to determine the variation in shape of the curve as a function of b.

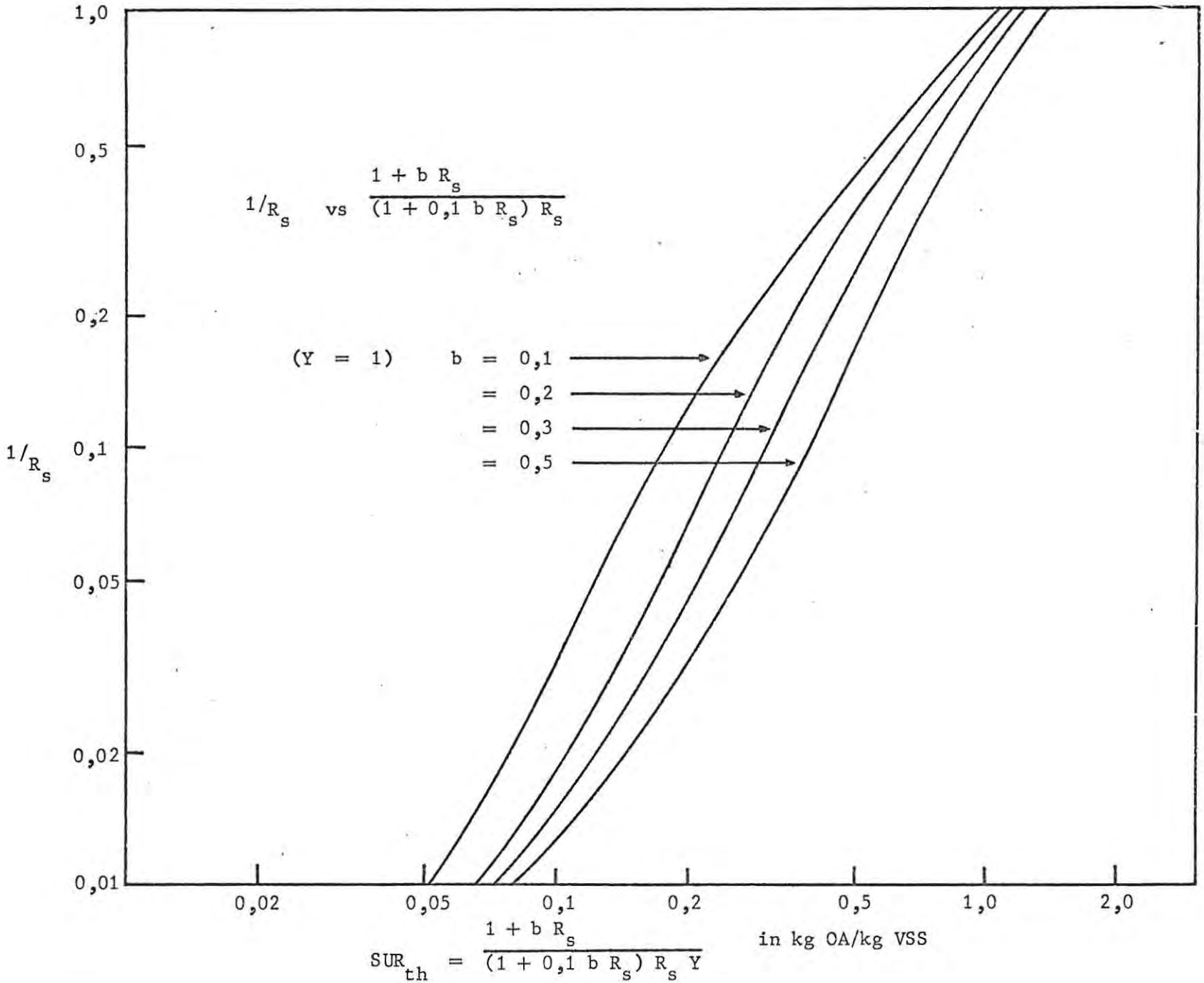


Fig. 5. The plot to determine the yield constant and endogenous respiration rate

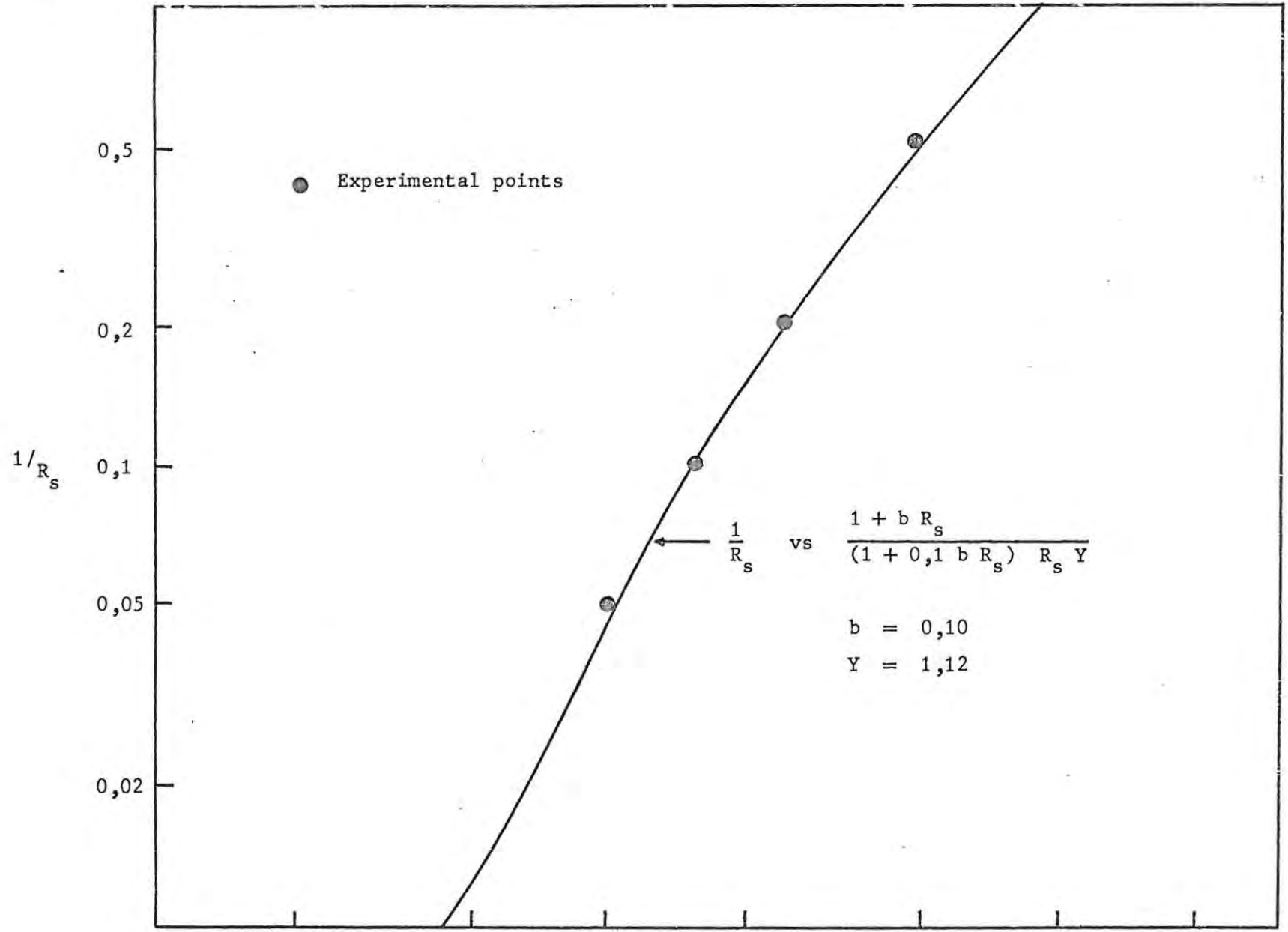


Fig. 9.5 Determination of K and unbiodegradable OA fraction in the effluent

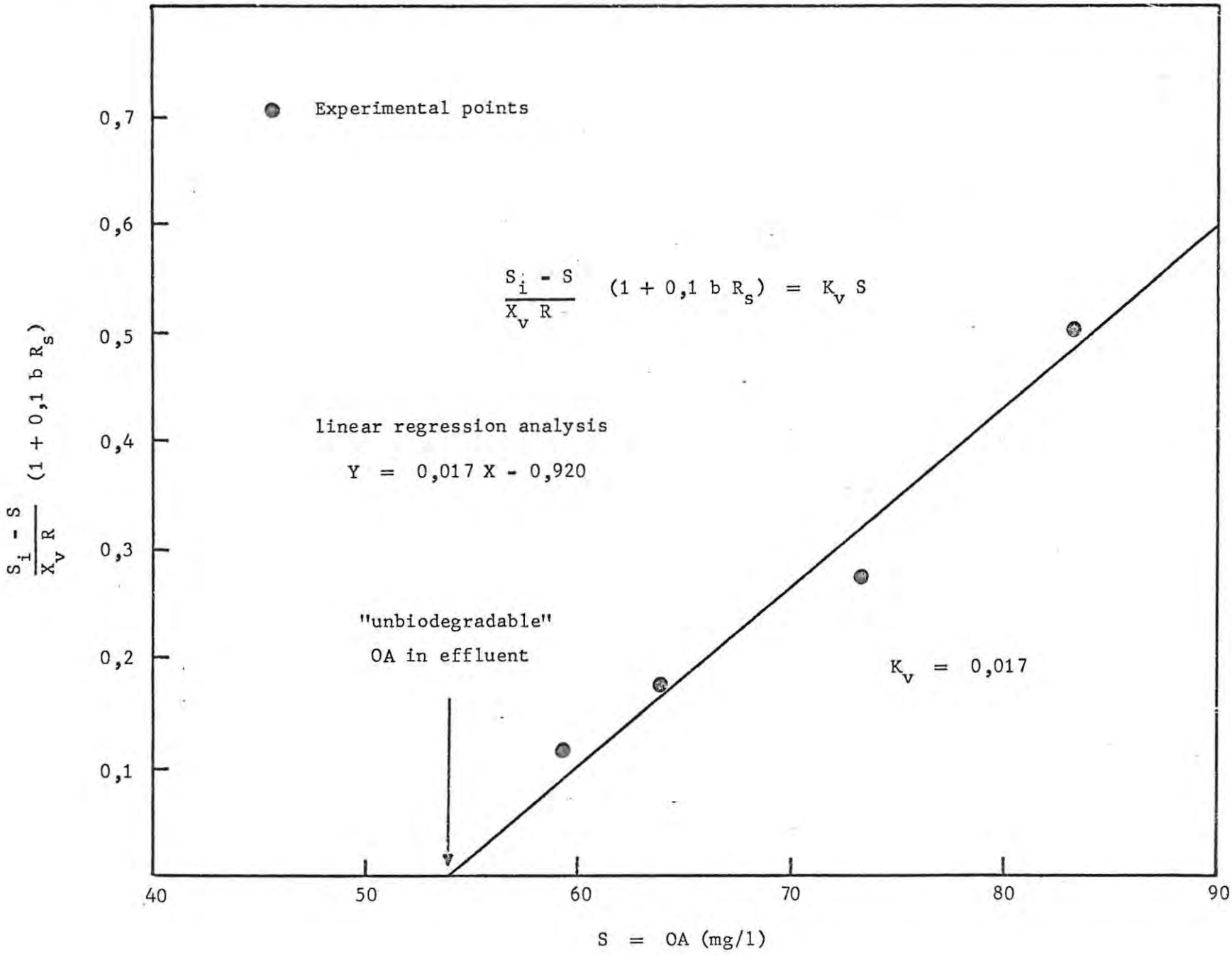


Fig. 9.6 Theoretical and experimental VSS for various retention times and sludge ages

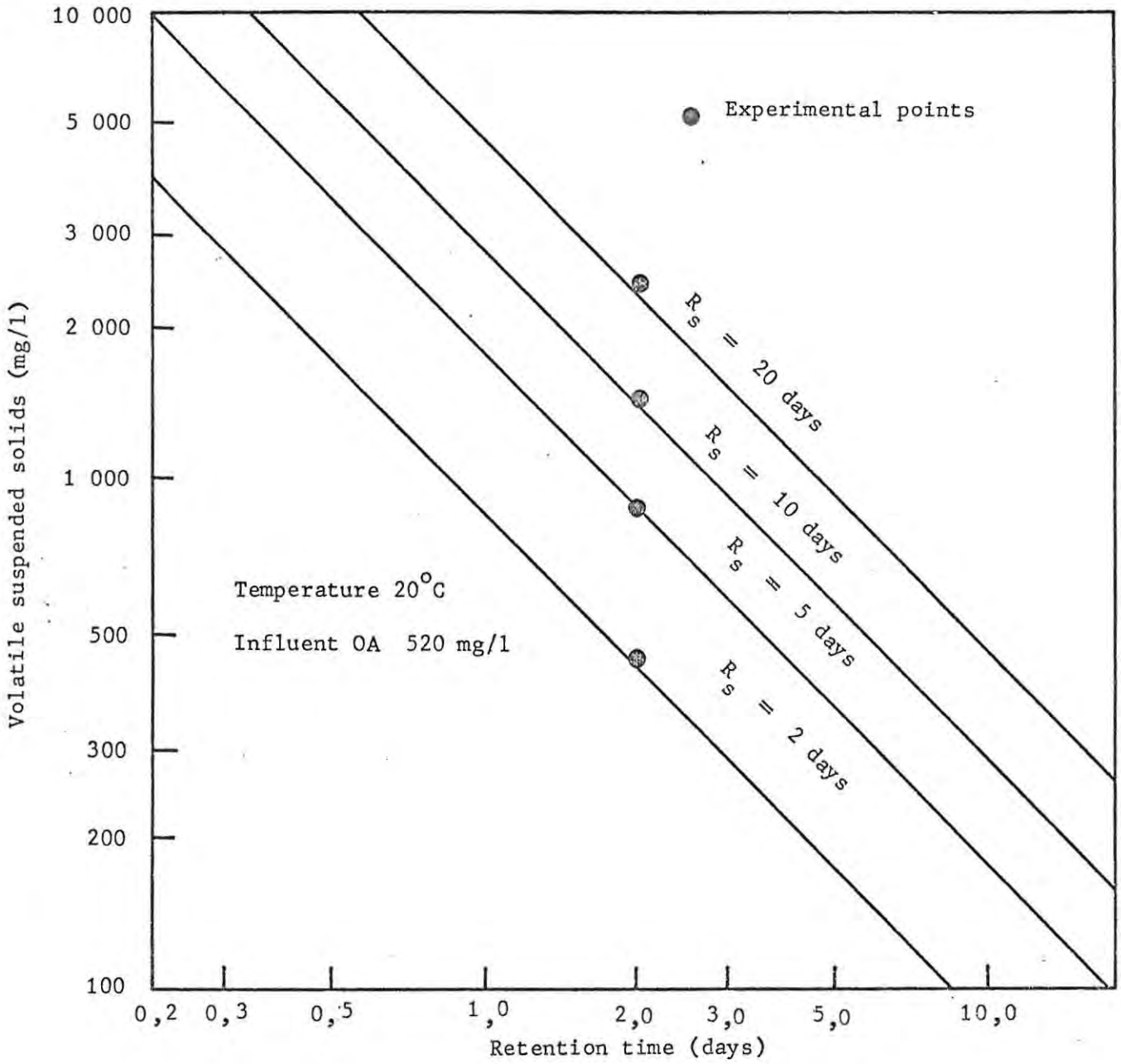


Fig. 7.7 Experimental and theoretical oxygen consumption rates for various sludge ages and retention time

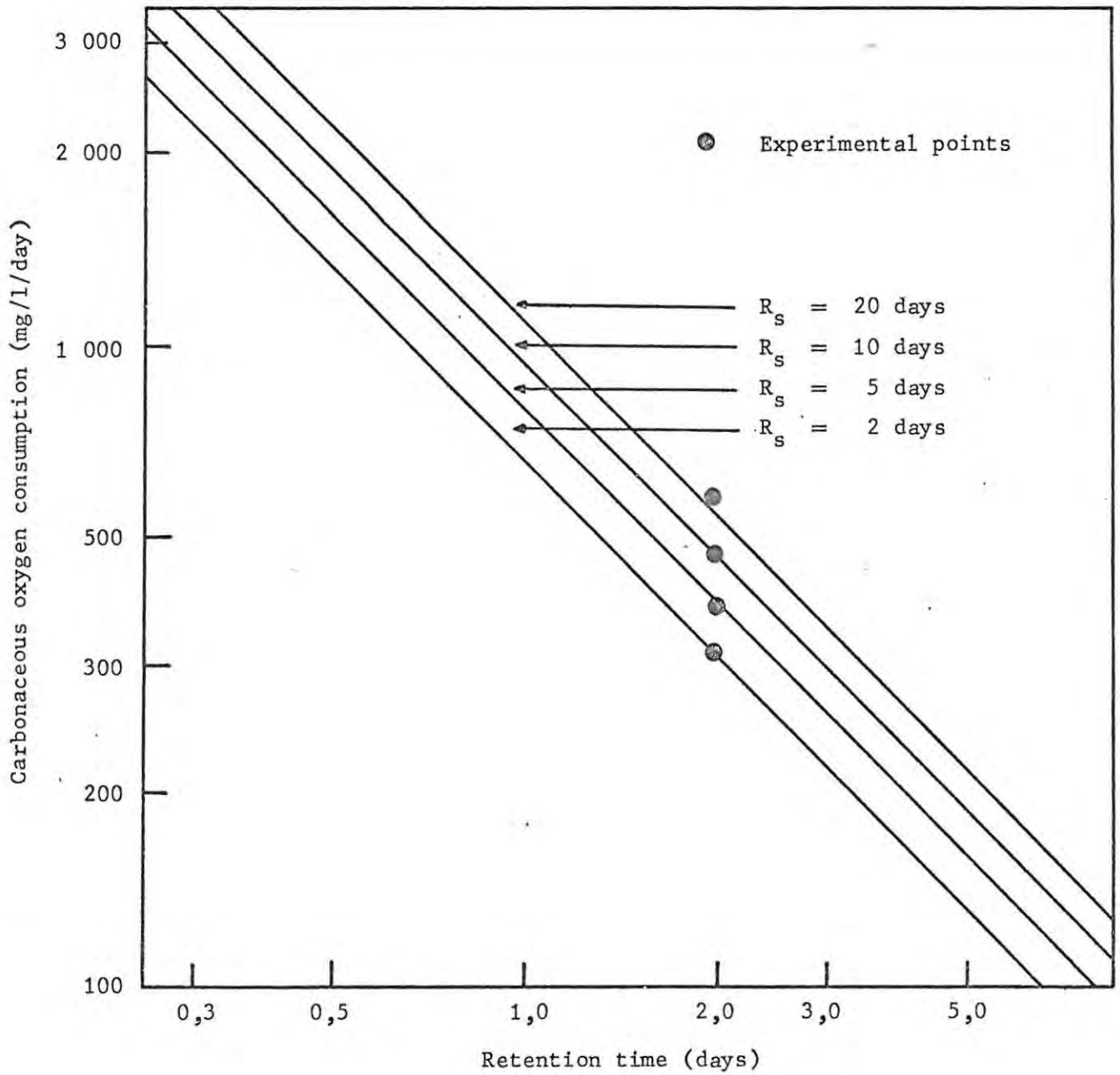


Fig. 9.8 Experimental and calculated OA remaining in effluent

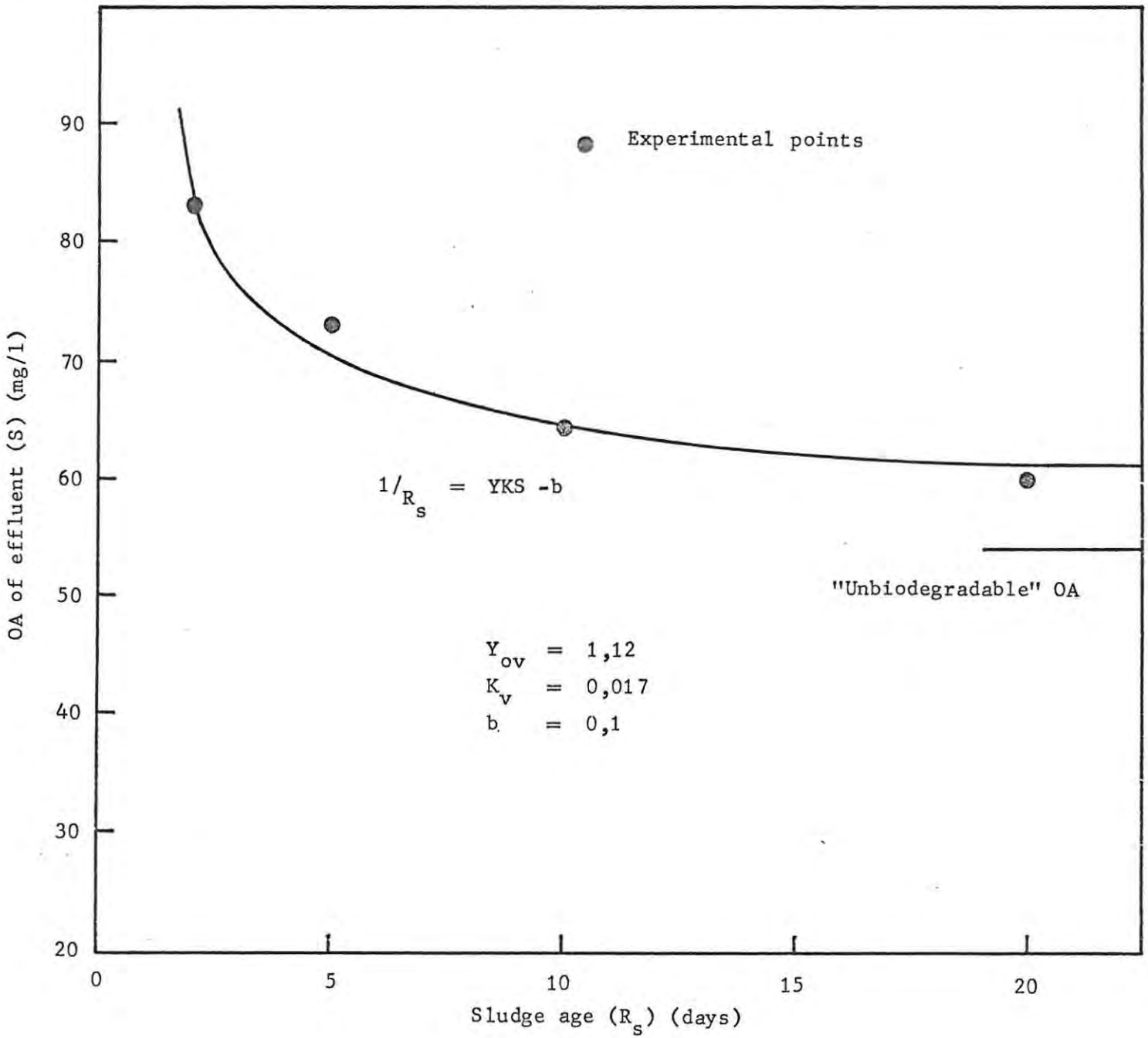
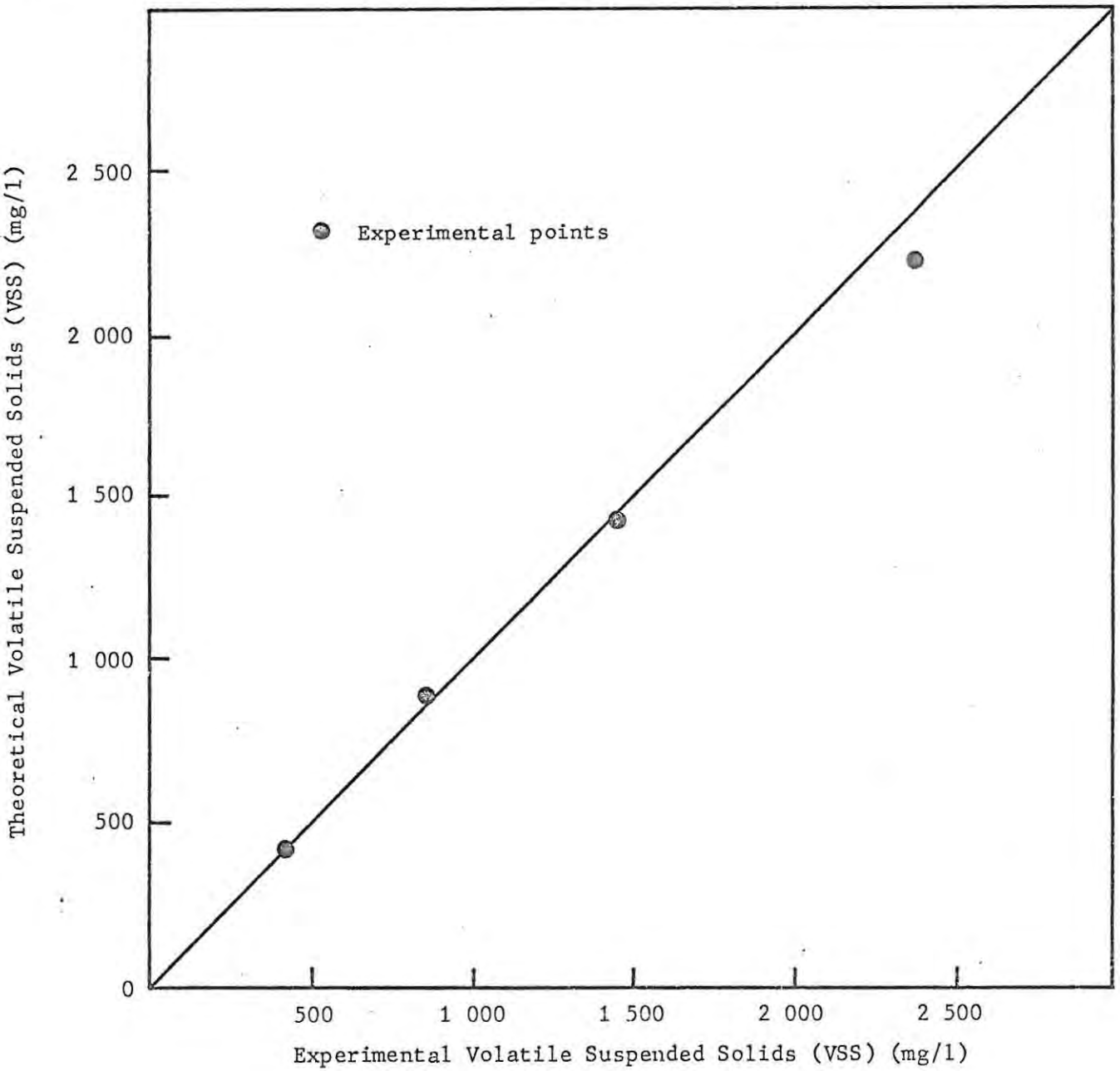


Fig. 9.9 Correlation between experimental and theoretical volatile suspended solids



CHAPTER X

MATHEMATICAL MODELLING OF FELLMONGERY WASTE WATER TREATMENT: EFFECT OF RETENTION TIME

Summary

The effects of varying the retention time in a laboratory scale CMAS reactor treating fellmongery effluent were investigated. Varying the retention time under conditions of constant sludge age, feed strength and temperature was found to have a significant effect on effluent quality. Retention times of longer than 1 day resulted in OA reductions of greater than 82%. At retention times of less than 1 day there was a rapid reduction in the efficiency of the effluent treatment process. The theoretical predictions arising from the substrate model appeared to hold under conditions of varying retention time, provided the retention time was sufficiently long for an efficient sludge to develop. However, effluent quality appeared not to be a function of the sludge age alone but also dependant on the retention time.

10.1 Introduction

In the previous chapter a mathematical model based on work by Lawrence and McCarty (1970) was shown to describe the CMAS treatment of fellmongery effluent. McKinney (1962) has also put forward a well-founded theory based on different assumptions to describe microbial growth kinetics in the CMAS process. These two theories are consistent with regard to sludge production and oxygen consumption (Marais, 1973), but differ in their prediction of effluent quality. In order to evaluate the two theories it is necessary to examine the assumptions upon which they are based.

McKinney (1962) was one of the first to produce a rational consistent kinetic theory describing the CMAS process with sludge recycling. Many of the basic relationships described by McKinney have had a strong influence on subsequent workers even though they may deviate from his original model. He postulated that under conditions of unlimited substrate supply and a small bacterial population, the rate of bacterial

growth is a function of the ability of the bacteria to utilise the substrate. The bacterial mass will increase until a critical point is reached when the substrate becomes the limiting factor. When the food to micro-organism ratio (F/M) is reduced below this critical point the food (substrate) concentration will be the factor controlling microbial growth. Therefore in an efficient CMAS effluent treatment system where the F/M ratio is low, the synthesis of microbial mass is a function only of the food remaining. Using this assumption McKinney formulated a relationship which gives effluent quality as a function of the influent substrate concentration and retention time according to:

$$S = \frac{S_i - S}{K' R} \quad \text{or} \quad \frac{S_i}{K' R + 1} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where S = unmetabolised substrate in reactor (mg/l)

S_i = substrate concentration in influent (mg/l)

R = hydraulic retention time (days)

K' = constant for a particular water at a fixed temperature.

Lawrence and McCarty (1970) developed a model to describe the CMAS treatment process as described in the previous chapter. Their model is based on Monod's description of microbial growth in continuous culture.

$$U = \frac{K_m S}{K_s + S} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where U = specific substrate utilisation rate (day^{-1})

K_m = maximum substrate utilisation rate (day^{-1})

K_s = half-rate constant, substrate concentration where $U = \frac{1}{2} K_m$ (mg/l)

S = unmetabolised substrate in the effluent (mg/l)

In the case where the food concentration is very low with respect to the food concentration allowing half the maximum rate of food utilization, ($S \ll K_s$), the bacterial growth rate is a function of both the food concentration and the active micro-organism concentration according to the relationship

$$\frac{dX}{dt} = Y K S X_{av} \quad \ldots\ldots (3)$$

where dX/dt = net growth rate of micro-organisms (mg/l/day)

X_{av} = active microbial mass already present (mg/l)

S = unmetabolised substrate (mg/l)

Y = yield coefficient (mg/mg)

K = K_m/K_s

Assuming this condition effluent quality is a function only of the sludge age according to the relationship

$$1/R_s = Y K S - b \quad \ldots\ldots (4)$$

This relationship implies that sludge age is the most important parameter in determining effluent quality and that the retention time plays no direct part in the kinetics of the process. The hydraulic retention time is required only to fix the concentration of sludge in the reactor, i.e. to act as a dilution device. The oxygenating capacity of the aeration mechanism determining the optimum concentration of sludge in the reactor. Marais (1973) showed that at a fixed sludge age (12,5 days), varying the retention time from 0,25 to 2 days had no significant effect on the effluent quality of domestic sewage treated in laboratory scale CMAS units.

In the previous chapter it was shown that effluent quality was a function of sludge age according to eq. (4). The experiments reported here were designed to determine whether effluent quality in the CMAS treatment of fellmongery effluent is independant of retention time as reported by Marais (1973) or whether effluent quality is dependant on retention time as suggested by McKinney (1962) and the relationship in eq. (1).

10.2 Materials and Methods

The source of effluent is as described in Chapter IX. Effluent analysis techniques are given in Chapters IV, V and IX while the laboratory scale CMAS apparatus and mode of operation is described in Chapter V.

10.21 Experimental procedure

In order to determine the effect of retention time on effluent quality, the sludge age (5 days), feed concentration (OA 520 mg/l) and temperature (20°C) were kept constant and only the retention time (0,5, 1, 2 and 4 days) varied between each of the four reactors. Once steady state was achieved duplicate samples were analysed daily for 6 days and tested graphically to determine if the values were normally distributed. The results were subjected to analysis of variance and where the F values were significant at the 1% level, the least significant difference (student's t-test) between the various retention times on effluent quality was determined.

10.3 Results

The effects of varying the retention time on the CMAS treatment of fellmongery effluent at constant sludge age, influent strength and temperature are shown in Table 10.1.

A retention time of 0,5 days resulted in an almost complete breakdown of the treatment process. The pH in the CMAS reactor was one pH unit above its normal level and 12% of the sulphide was still present in the reactor liquor. An efficient activated sludge had not developed as is shown by the low volatile suspended solids, oxygen consumption rate, bacterial count and absence of protozoa. The percentage OA reduction in this reactor was only 31,5%.

Retention times of 1, 2 and 4 days resulted in the lowering of the pH and removal of the sulphide to their usual levels of $\pm$ pH 8,2 and Na_2S 0,78 - 1,56 (mg/l). Efficient activated sludges developed at these longer retention times resulting in OA reductions of 82,5, 86,2 and 92,0% for retention times 1, 2 and 4 days respectively. The composition of these activated sludges varied considerably as is indicated by the varying bacterial and protozoan counts between reactors. In the CMAS reactor

Table 10.1. Effect of retention time on the purification of fellmongery lime-sulphide effluent in a completely mixed activated sludge (CMAS) reactor.

Retention time (days)	0,5	1,0	2,0	4,0	Untreated influent
Sludge age (days)	5	5	5	5	
OA (mg/l)	357,9	91,7	72,3	41,5	522,5
BOD_5 (mg/l)	317,7	134,5	99,4	38,9	540,9
Na_2S (mg/l)	53,2	0,78 - 1,56	0,78 - 1,56	0,78 - 1,56	444,2
pH	9,4	8,3	8,2	8,4	12,3
MLSS (mg/l)	1598,8	1677,0	1240,3	728,3	121,0
MLVSS (mg/l)	696,7	972,7	858,0	470,0	89,0
Settleable solids (ml/l)	44,0	68,0	63,7	76,7	0
Sludge Vol Index	27,5	40,5	50,8	105,1	-
Oxygen consumption rate (mg/l/day)	387,9	460,6	374,4	182,1	-
NH_3-N settled effluent (mg/l)	79,8	96,5	91,8	93,7	18,4
Bacteria/ml	$4,20 \times 10^7$	$2,62 \times 10^8$	$1,60 \times 10^8$	$1,28 \times 10^8$	$2,6 \times 10^3$
Protozoa/ml	$6,0 \times 10^1$	$2,18 \times 10^3$	$4,93 \times 10^3$	$8,5 \times 10^3$	0

with a retention time of 1 day the activated sludge micro-organisms were dominated by bacteria with relatively few protozoa present. Among these protozoa there was a high proportion of flagellates with only a few ciliates present. This suggests that an oxygen deficiency was occurring in this reactor inhibiting the growth of the oxygen sensitive ciliates. The two reactors with retention times 2 and 4 days had greater numbers of protozoa present and a large proportion of these were crawling and stalked ciliates. This explains the greater efficiency of OA removal that occurred in these two reactors.

10.31 Sludge production

Experimental values and theoretical predictions of the sludge concentration (volatile suspended solids) are compared in Fig. 10.1. The design curve for the relationship between sludge concentration and retention time at a constant sludge age of 5 days was constructed according to the relationship:

$$\log X_v = \log \left[Y_{ov} \frac{(S_{oi} - S_o)}{1 + b R_s} (1 + 0{,}1\ b R_s) R_s \right] - \log R$$

by the method outlined in Chapter IX. There is good correlation between the experimental and theoretical volatile suspended solids in the two reactors with retention times 2 and 4 days. However, in the reactors with retention times 0,5 and 1 day, the theoretical sludge concentration predicted no longer correlates with the experimental sludge concentration observed. This lack of correlation is explained in the case of the reactor with a 0,5 day retention time, by the failure of the treatment system to function under these conditions. In the case of the reactor with a retention time of 1 day this lack of correlation may be due to the immature nature of the activated sludge and possibly an alteration in the values of the various kinetic "constants" as will be discussed later in this chapter.

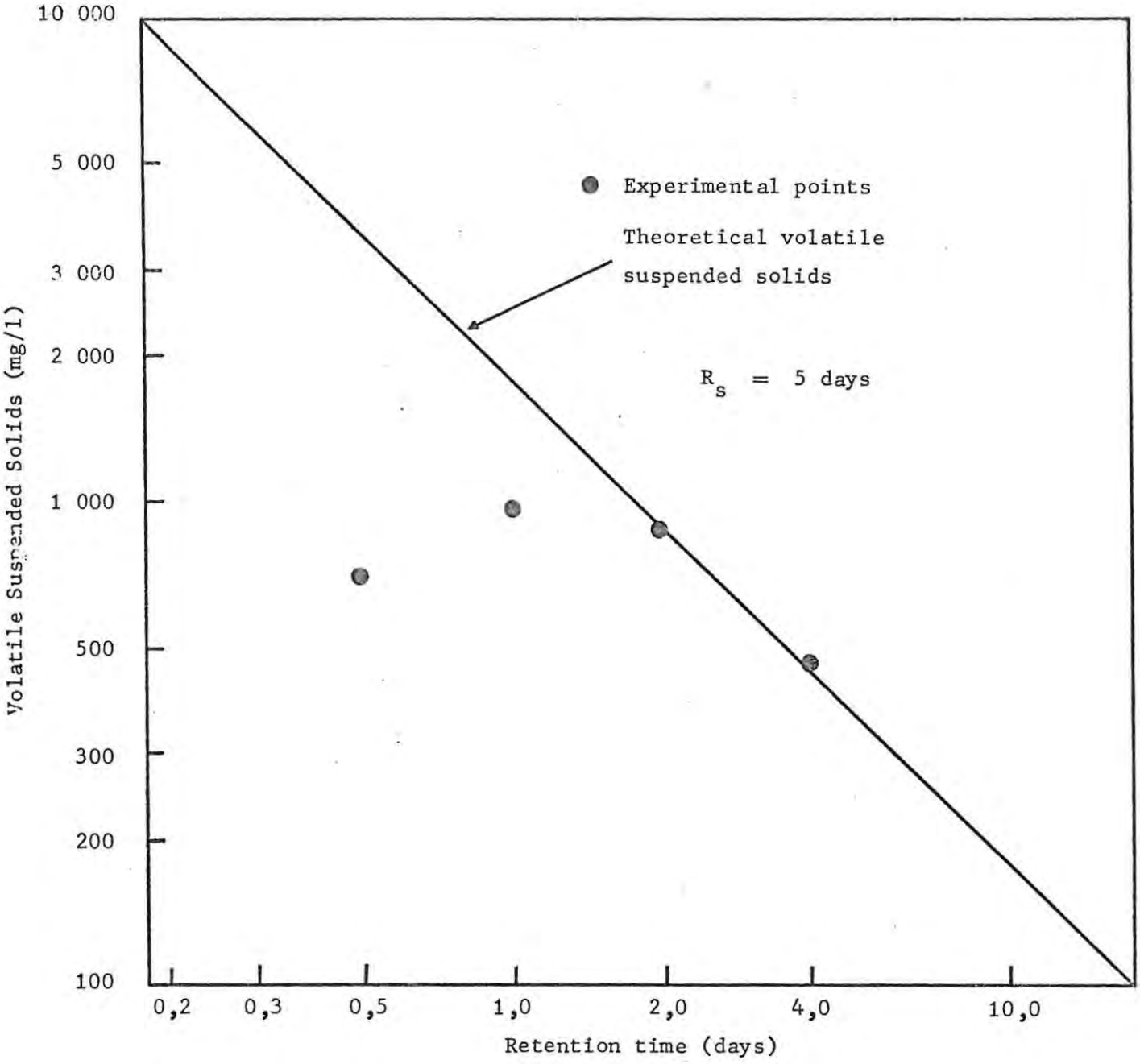


Fig. 10.1 A comparison between experimental and theoretical volatile suspended solids at various retention times.

10.32 Oxygen consumption

The theoretical predictions and experimental observations for the variation in oxygen consumption with retention time at sludge age 5 days are compared in the design chart in Fig. 10.2. The design chart was constructed according to the relationship:

$$\log O_2 \text{ (mg/l/day)} = \log \left[2{,}86 \left(S_{oi} - S_o\right) - \frac{1{,}42\, Y_{ov} \left(S_{oi} - S_o\right)}{1 + b\, R_s} \left(1 + 0{,}1\, b\, R_s\right)\right] - \log R$$

as outlined in Chapter IX. Again there is good correlation between the theoretically predicted and experimentally observed oxygen consumption rates in the cases of the two reactors with retention times 2 and 4 days. The theoretical and experimental observations do not correlate in the cases of the reactors with retention times 0,5 and 1 day. This lack of correlation is probably also due to the immaturity of the sludge in these reactors.

10.33 Effluent quality

A plot of the change in effluent quality with retention time is shown in Fig. 10.3. There was a significant improvement in effluent quality between each of the retention times tested as determined by both the OA ($t_{0,01}$) and BOD ($t_{0,05}$) values (Tables 10.2 and 10.3). These results plotted together with those of Chapter IX, show that effluent quality is not a function of the sludge age alone according to $1/R_s = Y K S - b$, but is also affected by retention time (Fig. 10.4). The assumption that S is negligible ($S \ll K_s$) in an efficient treatment process is an oversimplification.

A comparison between McKinney's formulation of the relationship between retention time and effluent quality (eq. 1) is made in Fig. 10.5. A plot of S vs $S_i - S/R$ should yield a straight line with slope $1/K'$. However, although the graph approaches a straight line at the longer

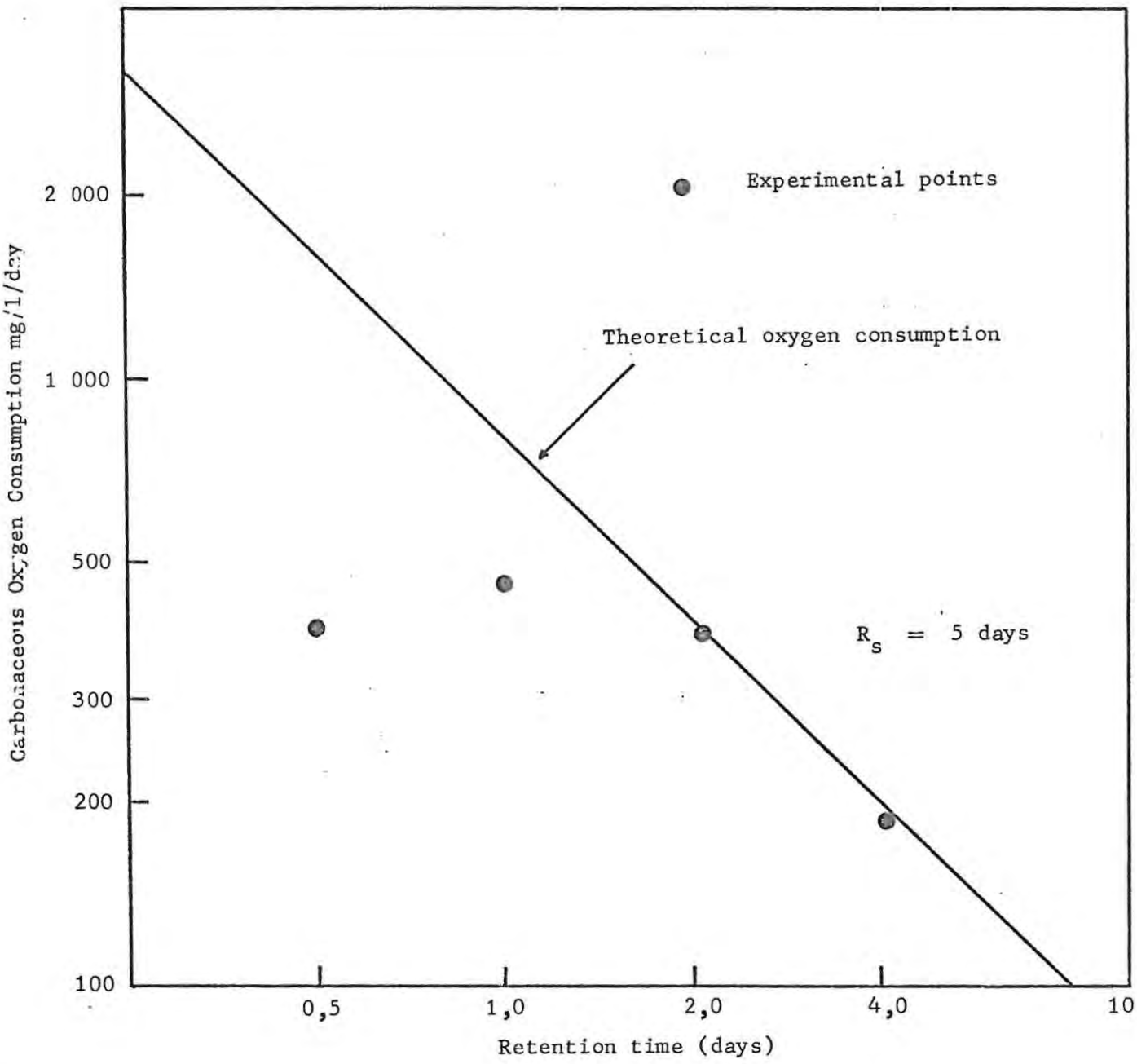


Fig. 10.2 A comparison between experimental and theoretical oxygen consumption rates at various retention times

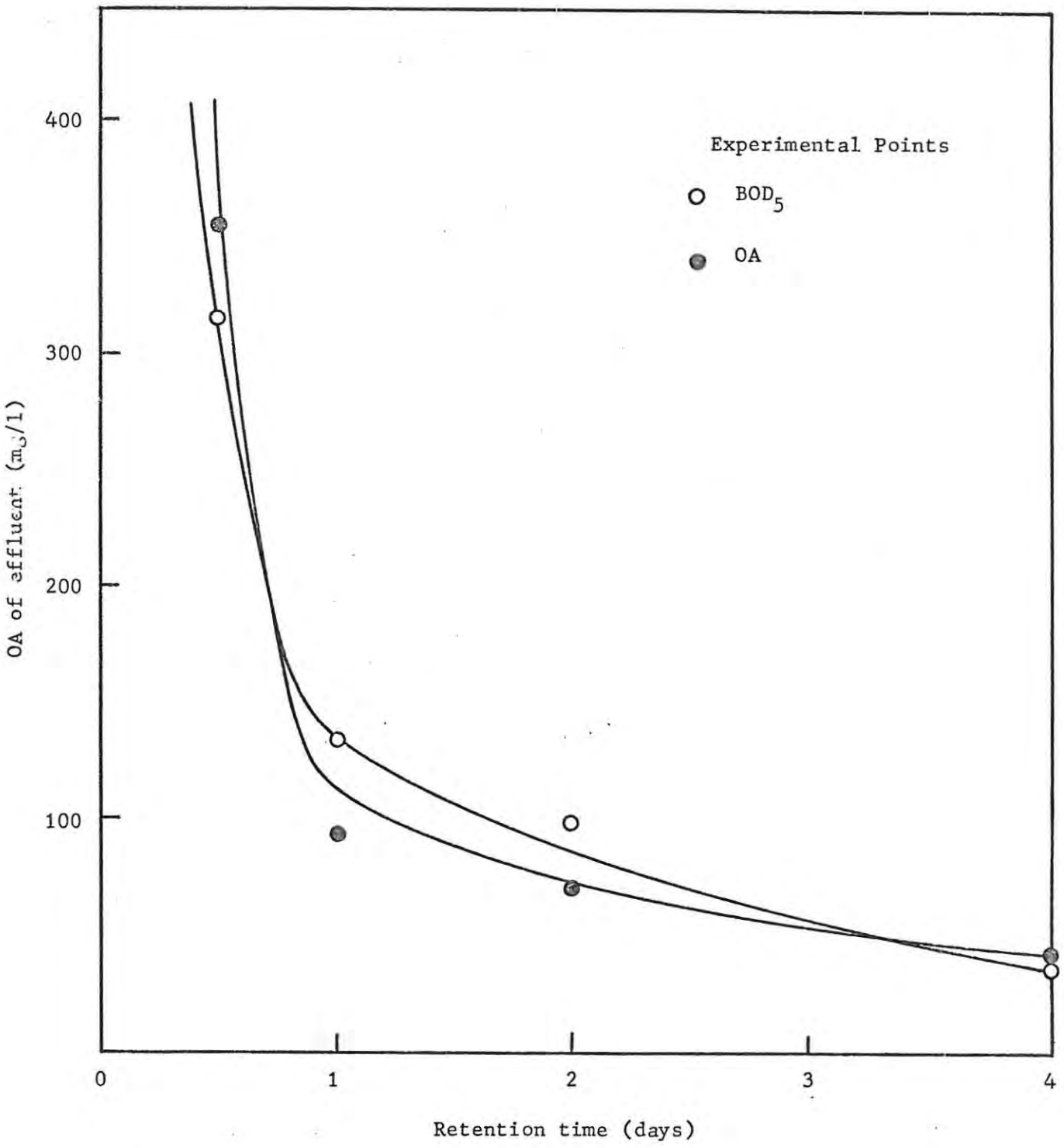


Fig. 10.3 The effect of retention time on effluent quality

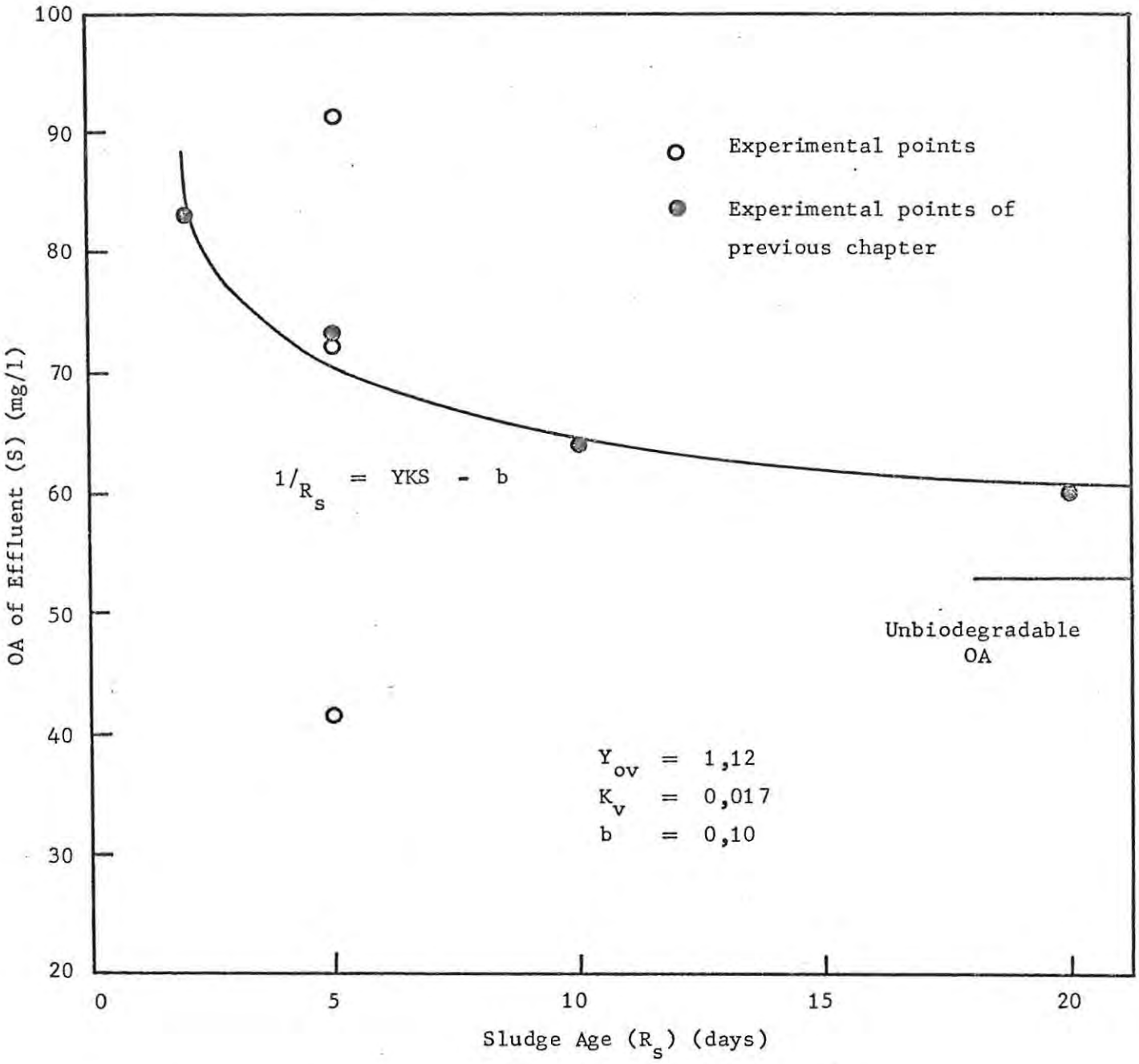


Fig. 10.4 Experimental and calculated effluent soluble OA remaining

Table 10.2. Analysis of variance of OA values of CMAS reactor under steady state conditions over 6 days.

Day	1	2	3	4	5	6	Total	Mean
Retention time 0,5 days	361,6 375,2	372,0 360,8	343,2 352,0	351,2 348,8	364,4 369,6	350,2 346,4	4295,4	357,9
Retention time 1,0 days	92,0 90,9	91,3 90,5	89,5 88,6	93,6 93,4	92,9 92,1	93,4 92,4	1100,6	91,7
Retention time 2,0 days	85,5 85,4	78,9 78,5	67,3 67,0	63,8 63,6	66,1 66,9	72,1 72,8	867,9	72,3
Retention time 4,0 days	44,2 44,1	40,8 40,8	38,3 39,8	41,0 41,8	41,2 40,9	42,8 42,3	498,0	41,5
Total	1178,9	1153,6	1085,7	1097,2	1134,1	1112,4	6761,9	
Mean	147,4	144,2	135,7	137,2	141,8	139,1		

Analysis of Variance

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Days	782,31	5	156,46	16,91
Retention time	769351,08	3	256450,36	27718,74
Interaction	1021,39	15	68,09	7,36
Error	222,05	24	9,25	
Total	771376,83	47	16412,27	

$$\text{l.s.d. (0,01) between means of retention time} = t_{0,01} \times \sqrt{\frac{2M.S.E.}{n}}$$

$$= 2,68 \times 1,24$$

$$= 3,33$$

Table 10.3. Analysis of variance of BOD values of CMAS reactor under steady state conditions over 5 days.

Day	1	2	3	4	5	Total	Mean
Retention time 0,5 days	300,8 293,6	362,3 243,5	350,9 265,4	365,4 280,2	320,6 394,4	3177,1	317,7
Retention time 1,0 days	153,8 133,2	127,3 152,0	98,0 144,4	154,0 123,2	159,9 99,4	1345,2	134,5
Retention time 2,0 days	85,3 87,8	107,7 117,5	124,5 135,5	81,4 94,6	75,4 84,3	994,0	99,4
Retention time 4,0 days	50,6 29,4	36,7 36,7	30,0 38,0	29,4 44,1	45,2 49,6	389,7	38,9
Total	1134,5	1183,7	1186,7	1172,3	1228,8	5906,0	
Mean	141,8	147,9	148,3	146,5	153,6		

Analysis of Variance

Source	Sum of Squares	Degrees of Freedom	Mean Square	F.
Days	570,30	4	142,57	0,13
Retention time	432322,05	3	144107,35	133,42
Interaction	8549,86	12	712,49	0,66
Error	21601,47	20	1080,07	
Total	463043,68	39	11872,92	

1.s.d. (0,05) between means of retention times $= t_{0,05} \times \sqrt{\frac{2M.S.E.}{n}}$

$= 2,01 \times 14,70$

$= 29,54$

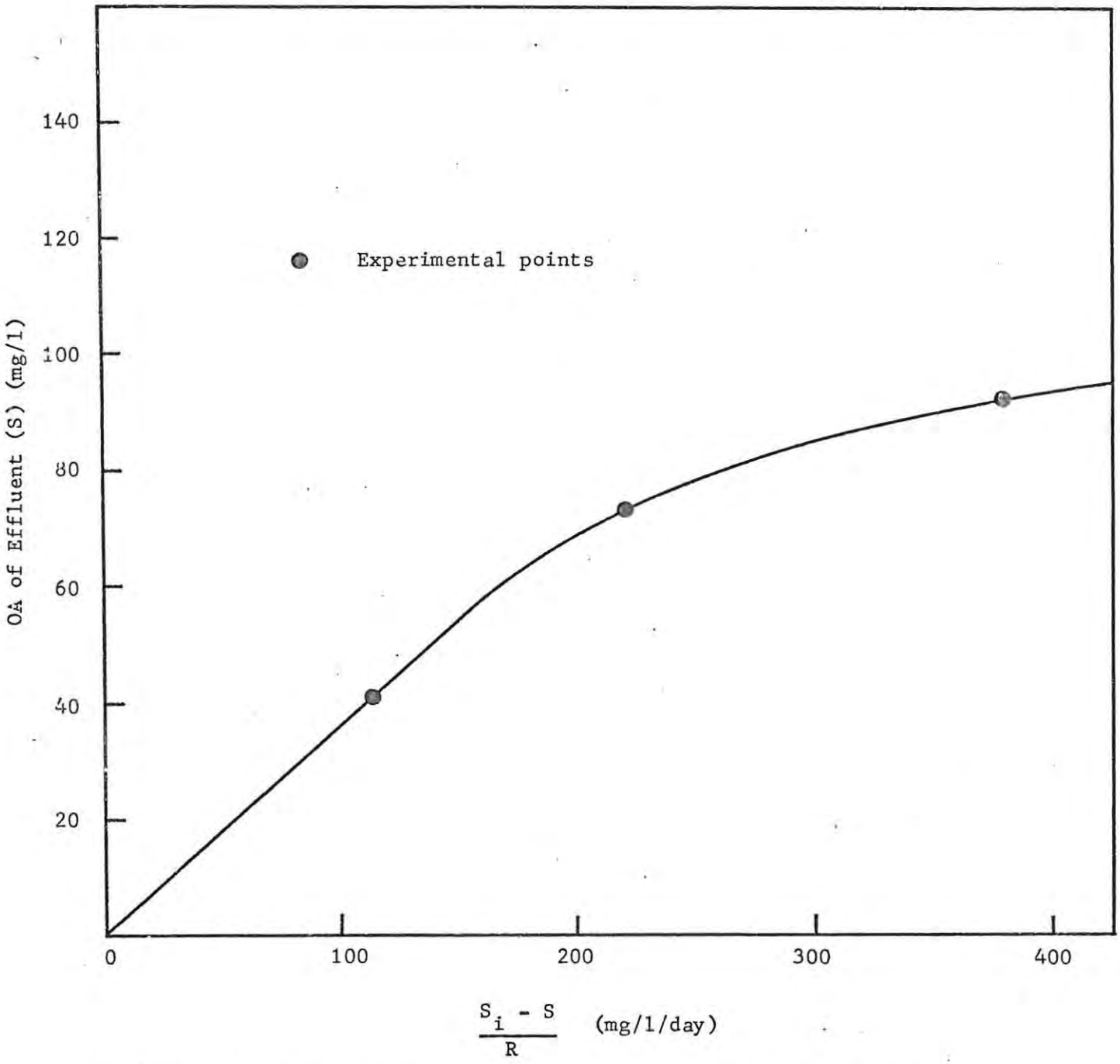


Fig. 10.5 A plot of the experimental relationship : S vs S_i - S/R

retention times, as the retention time decreases the straight line relationship no longer holds. The probable explanation of this curvature is that at short retention times the F/M ratio increases to a point where microbial growth is no longer a function of food concentration alone but of the micro-organism concentration as well.

10.4 Discussion

The mathematical model for sludge production and oxygen consumption described in Chapter IX appears to hold under conditions of varying retention time, provided the retention time is sufficiently long for an efficient sludge to develop. However, at constant influent strength, the indication from this study is that effluent quality cannot be said to be a function of either the retention time or sludge age alone. The true relationship is probably a complex interaction between the sludge age and the retention time which is more sophisticated than the mathematical model allows for.

As discussed earlier in this dissertation, current mathematical models which use pure culture kinetic equations to describe mixed culture microbial growth on a multicomponent substrate, have their limitations. The argument for the use of pure culture equations is that the many substrates in the medium give rise to a variety of enzymes which can be distributed in manifold combinations between the various micro-organisms resulting in regulatory and feedback mechanisms which can be considered to operate in a manner comparable to the way they operate in pure culture. However, there are important differences between a pure culture growing on a defined substrate and the semi-naturally occurring bacterial population growing in the aquatic environment afforded by an industrial effluent. In pure culture experiments the genetic material is constant and limited and only the phenotypical expression of the population varies with varying environmental conditions. In the heterogenous activated

sludge community a variation in environmental conditions results in a change of phenotype expression arising from selection of suitable genetic material from a large and varied pool of microbial genes. This selection will result in a shift in microbial population with possible corresponding changes in the organism to substrate yield (Y), as well as the substrate conversion (K) and endogenous respiration (b) coefficients. These kinetic "constants" can therefore only be considered stable over a limited range of environmental conditions.

Nevertheless, considerable success has been achieved in applying a mathematical model to the treatment of lime-sulphide waste waters. Various kinetic constants required for the mathematical description of the CMAS process have been calculated and theoretical predictions arising from this description shown to hold under a workable range of operating conditions, thereby establishing the design and operation of the process on a proper theoretical basis.

CHAPTER XI

GENERAL DISCUSSION

As a result of an urgent need for an effective treatment method for fellmongery and tannery effluents, most of the investigations into meeting this need in South Africa have been empirical aeration attempts. This investigation was the first fundamental study on the completely mixed activated sludge treatment of these effluents to be conducted in this country. The study involved an experimental investigation into certain aspects of the treatment process from both the ecological and kinetic points of view.

Initially methods for the removal of the high sulphide concentrations and the lowering of the high pH were investigated. It was found that either partial neutralisation of effluent with commercial grade sulphuric acid or the addition of a manganese oxidising catalyst prior to aeration were effective methods for the removal of sulphide. The use of manganese was however found to be disadvantageous as the catalytic oxidation of the sulphide could be reversed under anaerobic conditions.

The laboratory scale-continuous flow studies described in this dissertation have shown that in spite of their high pH and sulphide concentrations, fellmongery and tannery effluents are amenable to treatment by the completely mixed activated sludge process. Furthermore, that an acclimated activated sludge is able to maintain the pH in the reactor of such a system at a sufficiently low level to permit the rapid removal of sulphide from the mixed liquor without prior neutralisation or the addition of an oxidising catalyst. Although partial neutralisation may assist in the acclimation of an activated sludge to the effluent, once this has been achieved neutralisation is no longer necessary.

Optimum conditions for the enumeration of the activated sludge bacterial populations were determined and these populations investigated both enzymically and taxonomically. The enzymic investigation was conducted using two different techniques. The levels of protease, phosphatase, catalase, urease and keratinase enzymes during extended aeration of lime-sulphide mixed liquor were estimated as well as the numbers of bacteria able to produce certain degradative enzymes. This approach was found to be a potentially good method of characterising activated sludge on a quantitative basis. Partial taxonomic characterisation of the bacterial populations revealed that the activated sludge flora was dominated by an association between 2 strains of Pseudomonas and 4 strains of Acinetobacter.

In order to establish the design and operation of the completely mixed activated sludge treatment of lime-sulphide effluents on a sound theoretical basis a kinetic approach to the study of the treatment process was adopted. The efficiency of the treatment process, judged by the percentage oxygen demand removal, was found to be significantly affected by the feed concentration, sludge age and the hydraulic retention time. It was found that the maximum feed concentration that could be tolerated in the laboratory pilot plants was 1 500 mg/l (OA value). At higher feed concentrations the efficiency of the process was impaired due to the occurrence of oxygen depletion in the mixed liquor as well as the combined inhibitory effects of the high influent pH and sulphide concentrations.

These studies showed that effluent quality was not a function of either the sludge age alone as predicted by the substrate model or a function of the retention time alone as predicted by McKinney (1962), but that it was probably affected by both these operating parameters. The mathematical model described was found to give reliable predictions of both the sludge production and the carbonaceous oxygen consumption rate over a workable range of operating conditions.

No nitrification of fellmongery effluents even at long sludge ages (20 days) was observed in these studies. As there has already been a recommendation (Stanley Report) to the United States Environmental Protection Agency that tannery effluents should be treated to the extent of almost complete nitrification for discharge into surface waters by mid-1983, there is the need for an investigation into the conditions necessary to ensure nitrification of these effluents.

This study was concerned chiefly with the treatment of settled lime-sulphide effluents. However, large quantities of primary sludge in addition to excess sludge from the biological treatment process may accumulate and have to be disposed of. There is also therefore an urgent need for an investigation into an efficient method for the treatment and useful disposal of these solid wastes.

APPENDIX

LIST OF THE MORE IMPORTANT MATERIALS USED AND THEIR SUPPLIERS

Note: The use of % to describe the concentration of a solution in the text refers to w/v.

Agar: Oxoid agar No. 3. - Oxoid Ltd., London, England.

Acriflavine - British Drug Houses, Ltd., Poole, England.

Azocoll - Calbiochem, Los Angeles, California.

Bovine albumin - British Drug Houses.

Carboxymethyl cellulose - British Drug Houses.

Casein (vitamin and fat free) - British Drug Houses.

Casitone - Difco Laboratories, Detroit, Michigan.

DNA (ex herring sperm) - Koch-Light Laboratories, Colnbrook, England.

Folin - Ciocalteaus phenol reagent - E. Merck, Darmstadt, Germany.

Gelatine - British Drug Houses.

Glucose - British Drug Houses

Glycerol - Merck.

Hexadecyltrimethyl ammonium bromide - British Drug Houses.

Nesslers reagent - British Drug Houses.

Nutrient agar - Difco.

Peptone - Difco.

p-Nitrophenyl phosphate - Sigma Chemical Co., St. Louis, Missouri.

RNA (ex yeast) - British Drug Houses.

Silcolapse 5001 - I.C.I. (South Africa) Ltd.

Sodium azide - British Drug Houses.

Sodium alkylarylsulphonate - British Drug Houses.

Starch (soluble) - British Drug Houses.

Tryptone - Difco.

Tween 20 - Merck.

Yeast extract - Difco.

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