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THE OCCURRENCE OF CHROMATIACEAE
IN WASTE TREATMENT LAGOONS AND THEIR
UTILISATION TO TREAT FELLMONGERY EFFLUENT

A thesis presented in partial fulfilment of
the requirements for the degree of Doctor
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THE OCCURRENCE OF CHROMATIACEAE IN
LAGOON SYSTEMS AND THEIR UTILISATION TO
TREAT FELLMONGERY EFFLUENT

ABSTRACT

A study of the occurrence of Chromatiaceae in waste treatment lagoons was made. To determine the important factors leading to their dominance, an investigation of the effect of various environmental parameters on the growth of a Chromatium species was made.

Chromatium minutissimum was isolated and identified from an anaerobic lagoon treating meatworks effluent. An experimental design was used to screen the effects of temperature, pH, sulphide and acetate concentrations and light intensity on the batch growth of this bacterium in pure culture. Empirical models were developed which described the maximum population and the exponential growth rate as a function of these variables. Comparison of these models with lagoon data indicated that they provided a conservative estimate of the exponential growth rate and maximum population under lagoon conditions and that, under the range of environmental conditions expected in New Zealand, the hydraulic retention time is of major importance in limiting the development of this phototrophic bacterium in lagoons. The developed models may possibly be used to characterise the growth of other Chromatiaceae.

To study the growth of the Chromatiaceae in mixed culture various lagoon samples were incubated in daylight. A succession from anaerobic non-phototrophic bacteria to phototrophic bacteria to algae was observed in these batch cultures. Thus, in addition to low hydraulic retention times preventing the growth of the Chromatiaceae, competition from the algae precludes their dominance at longer retention times.

Seven lagoon systems in which the Chromatiaceae were known to occur were then investigated. The lagoons studied ranged from facultative to anaerobic. The wastes treated varied from domestic sewage to strong industrial and agricultural effluents. A succession from non-phototrophic anaerobes to Chromatiaceae to algae was observed in many instances and a three stage succession theory was formulated.

This theory was used to explain the occurrence of the Chromatiaceae in all the lagoon systems studied and it may be used to design lagoons in which the dominance of the Chromatiaceae is favoured or prevented.

The study of the lagoon systems indicated the potential of the Chromatiaceae for treating effluents containing reduced sulphur compounds. In N.Z., fellmongery effluent is the most important sulphide-bearing effluent. Experiments were therefore performed to develop criteria for the design of anaerobic lagoons using the Chromatiaceae to treat fellmongery effluent. Experiments were conducted to determine the effects of temperature and sulphide concentration on the performance of .088 m³ laboratory lagoons, in which Thiocapsa roseopersicina was dominant, treating a synthetic fellmongery effluent. Temperatures from 10°C to 25°C and influent sulphide concentrations of 200 mg/l to 1,500 mg/l were studied. Good treatment was obtained under a wide range of conditions although inhibition of growth occurred at influent sulphide concentrations of approximately 900 mg/l. Concentrated fellmongery effluents may therefore be treated by these lagoons. COD removals varied from 66.1% - 87.1% and sulphide removals from 89.5% - 98.4%.

Design equations which described the performance of the laboratory lagoons were developed. To confirm the accuracy of these equations, pilot scale experiments were conducted on a 5.74 m³ lagoon system treating actual fellmongery effluent. A good degree of treatment was again achieved and the laboratory-developed equations provided a good estimate of the pilot-scale effluent over the range of conditions studied. Suitable criteria have therefore been developed for the design of anaerobic lagoons using the Chromatiaceae to treat fellmongery effluent.

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CHAPTER 1

PRELUDE

1 PRELUDE

The occurrence of the Chromatiaceae, the purple sulphur bacteria, in lagoon systems has frequently been observed. However, there is little information describing the reasons for their prevalence or outlining possible methods for their control or utilisation. This thesis seeks to rectify this situation.

The Biotechnology department first became involved with the Chromatiaceae when a local meat works was accused of discharging blood directly to an adjacent river. The works' management protested that the red colouration of the effluent was due to some organism which had become the dominant microflora of their anaerobic lagoons, changing the supernatant liquor from its usual mud-brown colour to a red-pink. Preliminary work demonstrated that the Chromatiaceae were the organisms causing the colour.

Subsequent research indicated that the occurrence of these bacteria was quite widespread. They were observed in lagoons treating a variety of effluents under widely varying climatic conditions. However, no general theory explaining their occurrence had been developed. Much of the research contained in this thesis was performed to solve this problem.

To gain insight into the factors favouring the growth of the Chromatiaceae, a Chromatium species was isolated and subjected to an experiment to determine the effects on it of several environmental parameters believed to be important in such lagoons. This work is described in Chapter Three.

The results of this experiment and the observation of the Chromatiaceae in actual lagoon systems led to the development of a general theory to account for the survival and growth of the bacteria in waste treatment lagoons. As their prevalence is often unintended and, in some instances, undesirable due to the colour they impart to

the effluent, this theory provides for the control of the appearance of these organisms by indicating the important parameters which should be manipulated. Chapter Four outlines the development of this theory.

However, it was believed that the ability of the Chromatiaceae to oxidise sulphur compounds under anaerobic conditions could be exploited in the treatment of certain effluents. In New Zealand, this is most applicable to the fellmongery industry in which sheep pelts are treated producing a noxious, sulphide-bearing effluent. The research described in Chapters Five and Six was performed to elucidate this area of waste management.

CHAPTER 2

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 INTRODUCTION

This literature review seeks initially to outline the problems caused by the discharge of effluents containing sulphur compounds and to demonstrate the necessity for adequate treatment prior to their discharge.

The suitability of the conventional biological treatment techniques for treating such effluents will then be assessed. Lagoon treatment using the Chromatiaceae will be shown to be potentially feasible. In this thesis, a lagoon is defined as any natural or artificial body of still water used specifically as a waste treatment process. A pond is a natural, small body of still water not used intentionally for any waste treatment purposes.

To study the possible effects of the Chromatiaceae in lagoons their sulphur and carbon metabolisms and their ecology will be reviewed.

The potential for applying these bacteria to the treatment of sulphur-bearing industrial wastes in New Zealand will be assessed. It will be shown that fellmongery effluent is the most important sulphur-bearing effluent in New Zealand and this effluent and its current treatment methods will be outlined.

This review therefore delineates the problem of sulphur-bearing wastes, outlines the characteristics of the Chromatiaceae and provides a survey of current fellmongery treatment technology with which to compare the potential of the photosynthetic bacterial lagoon.

2.2 THE TOXICITY OF SULPHUR COMPOUNDS

2.2.1 Introduction:

The quality of receiving waters may be detrimentally affected in various ways. For example, if an excessive organic discharge occurs, aerobic micro-organisms will utilize oxygen at a rate greater than can be replenished by surface re-aeration. Anaerobiosis

results. Also, inorganic compounds can chemically react with oxygen resulting in an additional immediate oxygen demand. Further, the discharge of toxic chemicals may result in the destruction of aquatic life.

Effluents containing reduced sulphur compounds may cause oxygen depletion in all three modes described above. It is essential that these effluents be adequately treated prior to discharge.

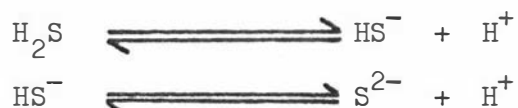
2.2.2 Toxicity of Sulphur Compounds to Multicellular Organisms:

This section is concerned with the effect of sulphur compounds on organisms present in receiving waters. Sulphate-sulphur accumulates in nature because of its stability to oxidation and its concentration in natural waters varies from 10 - 10,000 mg/l SO_4^{2-} . Sea water contains 800 - 900 mg/l SO_4^{2-} (Lambert 1972). For health reasons and to maintain a palatable taste, the Committee on Water Quality Criteria, United States Environmental Protection Agency (Rooney 1973), recommends that sulphate in public water supplies does not exceed 250 mg/l and the W.H.O. recommends a maximum level of 500 mg/l (Appleyard and Shaw 1974).

In water, the sulphite ion is a transitory intermediate species which is readily oxidized to sulphate (Lambert 1972). Sulphite is less toxic than sulphide with a TL_m of 203 mg/l in 100 hours being reported for Daphnia magna (Dowden and Bennett 1965). The median tolerance limit TL_m , or TL_{50} , represents the concentration which kills 50% of the test organism within a specified time span, usually 96 hours. In Switzerland, public water must not contain more than 1 mg/l sulphite (IULCS Effluent Commission 1972). The toxic effect of sulphite-containing effluents has also been investigated. For example, the threshold mortality for salmon is 500-600 mg/l of sulphite waste liquor (Neale 1958).

Thiosulphate also exerts a toxic effect although such data is relatively sparse. Daphnia magna showed a TL_m of 805 mg/l in 100 hours in the presence of $\text{Na}_2\text{S}_2\text{O}_3$ (Dowden and Bennett 1965).

Sulphides, especially hydrogen sulphide (H_2S) are very toxic. Hydrogen sulphide is soluble in water to the extent of 4,000 mg/l at atmospheric pressure and 20°C (Rooney 1973). Upon solution it dissociates according to the following equations :



The relative proportions of the three sulphur moieties vary with pH as shown in Fig. 2.1. Sulphide solutions are more toxic in acid conditions as the concentration of H_2S is then increased (Hynes 1963).

For human beings H_2S is a particularly insidious poison. It is perceptible in air at a concentration of .002 mg/l as an odour of rotten eggs (Klein 1959, Stecher 1968) but higher levels may not be detected as the sense of smell is then impaired (Stecher 1968). Toxic threshold concentrations for humans are in the range 10-14 mg/l (Christensen 1973, Sax 1968). Exposure to 800 - 1,000 mg/l may be fatal in 30 minutes and high concentrations are instantly fatal. Hydrogen sulphide poisoning may occur more rapidly than poisoning from exposure to a similar concentration of hydrogen cyanide.

Much of the available data on the toxicity of sulphide to aquatic organisms is inadequate. Many studies used extremely short exposure periods and failed to give adequate account to the effect of environmental factors such as temperature, pH, dissolved oxygen and dissolved salts (Hynes 1963). Consequently, whereas early data suggested that 0.3 - 4.0 mg/l H_2S allowed fish to survive more recent studies have demonstrated sulphide toxicity at lower concentrations (Oseid and Smith 1974, Rooney 1973, Smith and Oseid 1970). Recommended total sulphide levels are .002 mg/l in fresh water and 0.005 mg/l in salt water (Rooney 1973).

2.2.3 Toxicity of Sulphur Compounds to Micro-organisms in Biological Treatment Systems:

2.2.3.1 Introduction:

Many compounds exert a toxic effect on biological treatment processes and inhibition may be partial or complete depending upon

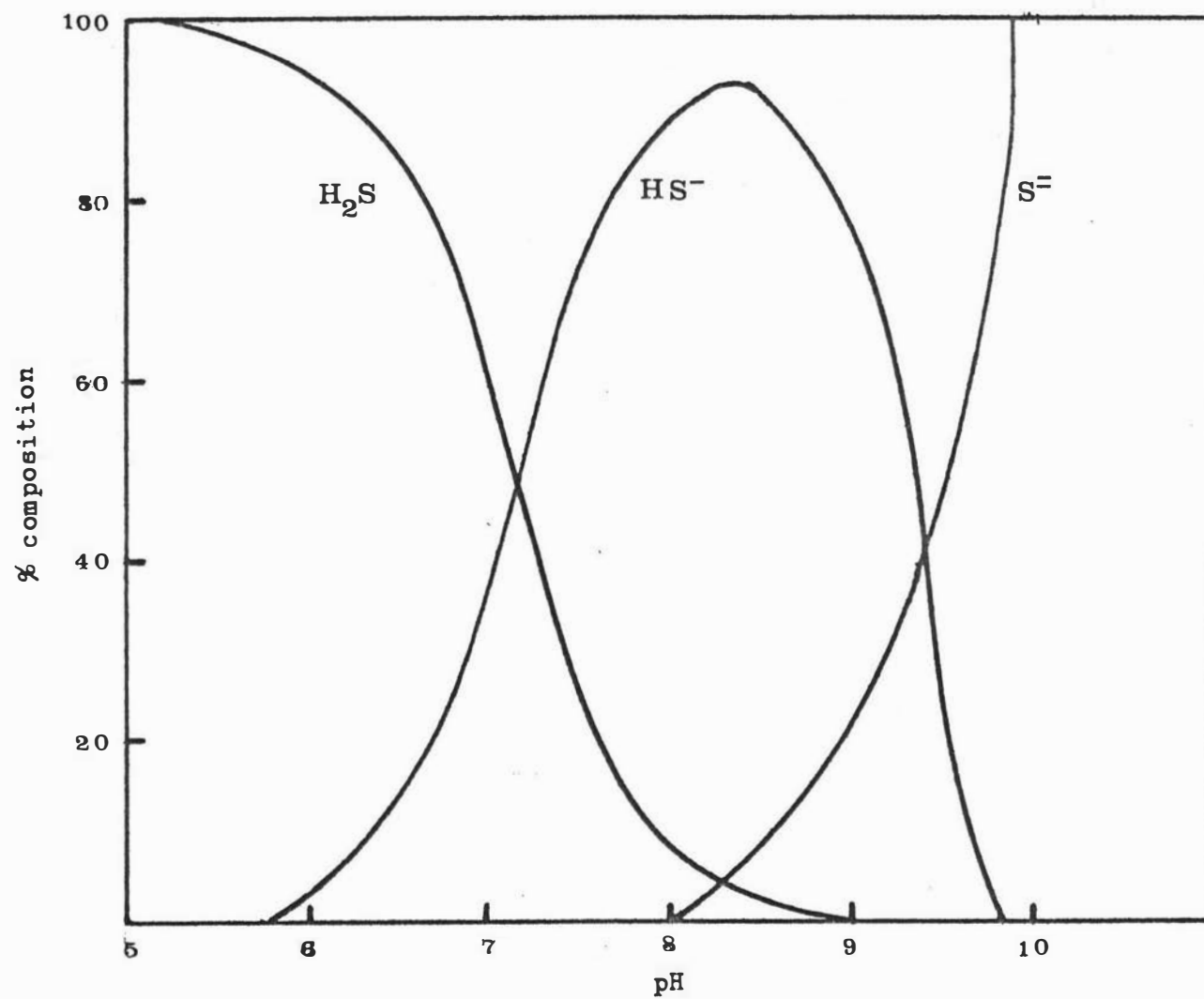


Figure 2.1: Effect of pH on Hydrogen Sulphide-Sulphide Equilibrium
(10⁻³ M Solution) (Sawyer and McCarty 1967)

the substance and its concentration. The toxic concentration is influenced by factors such as the temperature, the nature of the organism and the availability of food (Ingols 1955).

Some organisms may acclimatize to certain levels of toxic substances. This may result from a neutralisation of the toxic material by microbial metabolism or the growth of selected organisms which may or may not be able to metabolise the toxic substance (Eckenfelder and O'Connor 1961).

2.2.3.2 Activated Sludge:

Activated sludge systems are capable of operating effectively at influent sulphide concentrations in the vicinity of 20 mg/l (Aulenbach and Heukelekian 1955a, Bailey 1971, Leafe et al 1972, Ludvik et al 1973). Data on the effect of higher concentrations are difficult to interpret due to the various operating and influent characteristics of the systems used. Concentrations of 50 - 250 mg/l sulphide may be tolerated for brief periods (Bailey 1971, Ludvik et al 1973) but extended loading results in floc dispersion (Aulenbach and Heukelekian 1955a). Under such conditions system recovery is slow and pretreatment for sulphide is advised to protect the unit (Leafe et al 1972). Batch loading does not have so pronounced an effect as continuous (Coe 1952). However toleration of higher influent sulphide levels of 300 - 444 mg/l S^{2-} has been reported (Rawlings et al 1975c, Villa 1969).

2.2.3.3 Biofiltration:

The biological filter is more resistant to toxic wastes than the activated sludge system and it also recuperates more rapidly from shock loadings (Gurnham 1955). For this reason it is usually preferred for the treatment of strong industrial wastes and many authors report successful treatment of sulphide-bearing effluents by this system.

A lab-scale system treating synthetic fellmongery effluent gave complete sulphide removals for influents containing 60-150 mg/l S^{2-} (Hill 1972). With screened fellmongery effluent, the

sulphide removal rate was proportional to the recycle ratio and sulphide concentrations greater than 100 mg/l resulted in decreased COD removals (Ryder 1973). Average removals of 47% COD and 40% BOD were obtained on a pilot plant biofilter treating fellmongery effluent averaging 80 mg/l S^{2-} (Hicks and Martin 1973).

A laboratory-scale biofilter loaded at $9.6 \times 10^{-2} \text{ m}^3/\text{m}^2 \cdot \text{d}$ with neutral tannery effluent withstood up to 440 mg/l S^{2-} (Green 1960). Shock loads were irregularly endured. Doubling the hydraulic retention time reduced the tolerance to below 90 mg/l.

Biofiltration of a 40% sewage/60% tannery effluent mixture containing 82 mg/l S^{2-} resulted in a 90% BOD reduction and complete sulphide removal (Rosenthal 1957).

Domestic sewage containing 180 mg/l S^{2-} was successfully treated on full-scale filters (Bailey 1969). The effluent averaged 2 mg/l S^{2-} .

2.2.3.4 Aerobic Lagoon Systems:

Because of their high oxygen demand, industrial sulphide wastes are seldom treated in aerobic or aerated lagoons.

Sulphides and the proliferation of sulphate-reducing bacteria are commonly associated with failing aerobic lagoons (Brockett 1975, Kendler and Donagi 1971). Concentrations of 7 mg/l S^{2-} are toxic to some algae (Espino and Gloya 1967).

2.2.3.5 Anaerobic Processes:

Under anaerobic conditions, oxidized sulphur compounds become reduced to sulphide which may result in digester failure. Sulphide selectively inhibits the Methanobacteriaceae (Aulenbach and Heukelekian 1955b, Rudolfs and Amberg 1952). Gas production ceases and volatile acids accumulate. The concentration at which inhibition occurs varies from 100 mg/l to greater than 200 mg/l S^{2-} (Aulenbach and Heukelekian 1955b, Lawrence et al 1966, Orford 1958, Rudolfs and Amberg 1952) with the sensitivity to sulphide increasing with decreasing pH (Bannink and Muller 1951). Such inhibition

may be restrained by adding heavy metals to control the level of soluble sulphides and form insoluble metal/sulphide precipitates (Lawrence and McCarty 1965, Lawrence et al 1966).

With open anaerobic systems an important problem is that of odour. In the U.S.A., 73% of the lagoons had odour problems (Porges and Mackenthun 1963) with sulphides being identified as one of the most important constituents of such odours (Dague 1972, Hovious et al 1973).

2.2.4 Conclusions:

Reduced sulphur compounds cause severe problems when discharged into a receiving water. It is therefore imperative that effluents containing these compounds are adequately treated prior to discharge.

Aerobic biological treatment systems have been shown to be susceptible to sulphide toxicity, with the biofilter being more resistant to upset than the activated sludge process.

Anaerobic digestion is also inhibited by sulphide.

Thus, influents containing high concentrations of sulphur compounds are difficult to treat using the conventional biological treatment processes. However, some phototrophic bacteria have been periodically observed in lagoon systems and as these micro-organisms may withstand high sulphide concentrations (Lopanitsyna and Novozhilova 1969, Yakoleva and Novozhilova 1969) and provide effective deodorisation (May and Stahl 1967, Meredith and Pohland 1970) they should be able to provide a suitable biological treatment system for reduced sulphur compounds. In view of this potential, the role of these bacteria in the sulphur cycle will be studied and then the growth and physiology of these organisms will be elaborated.

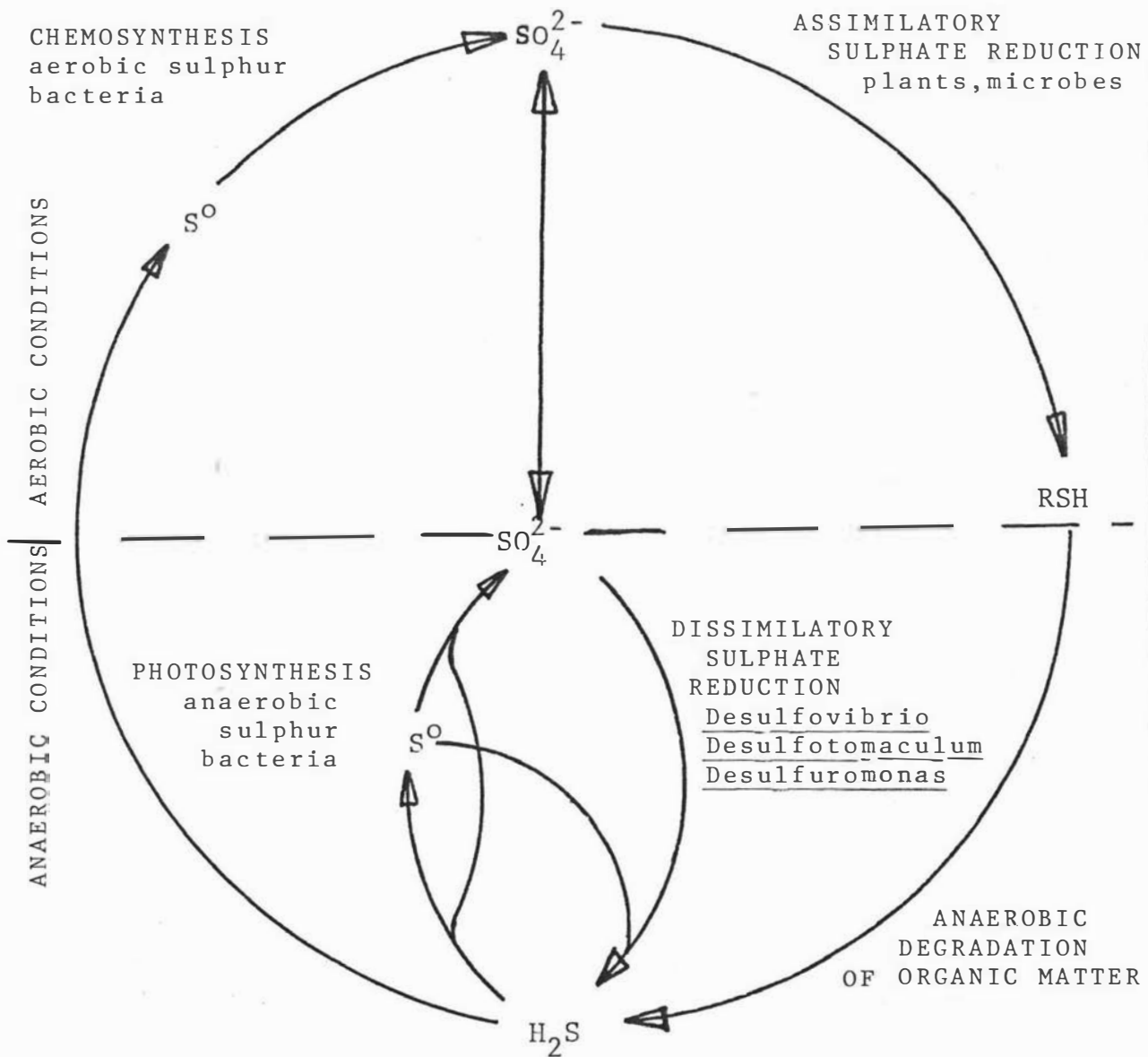
2.3 MICROBIAL TRANSFORMATIONS OF SULPHUR COMPOUNDS

2.3.1 Introduction:

The major pathways in the transformation of sulphur compounds in nature are outlined in Fig. 2.2. Due to its importance in anaerobic lagoons emphasis will be placed upon the anaerobic sub-cycle involving the phototrophic bacteria and dissimilatory sulphate reducers, such as Desulfovibrio. This sub-cycle has been termed a sulphuretum (Baas-Becking 1925).

Figure 2.2: THE SULPHUR CYCLE

(Adapted from Pfennig 1975, Pfennig and Biebl 1976)



While the aerobic portion of the cycle is unimportant in the above context, it is of significance in some aerobic treatment systems. The thiobacilli are often the most important organisms under these conditions (Kreye et al 1973 and 1974, Ryder 1973) and their characteristics have been documented (Trudinger 1967, Vishniac 1974).

2.3.2 Anaerobic Oxidation of Sulphur Compounds

The phototrophic bacteria, the Rhodospirillales, are aquatic organisms inhabiting marine and freshwater environments. Unlike other phototrophs, their photosynthesis only occurs under anaerobic conditions and without oxygen evolution (Pfennig 1967). The gram negative cells cover a wide morphological range and possess bacteriochlorophyll and carotenoid pigments (Pfennig and Truper 1974). Their photosynthetic metabolism is dependent upon the presence of oxidisable external electron donors such as reduced sulphur compounds, molecular hydrogen and organic compounds. (Kondrat'eva 1965). Some genera are therefore able to oxidise reduced sulphur compounds under anaerobic conditions.

The classification of the phototrophic bacteria is shown in Fig. 2.3.

2.3.2.1 Rhodospirillineae

The Rhodospirillineae (purple phototrophic bacteria) contain bacteriochlorophyll a or b as the main bacteriochlorophyll. Various carotenoids are also present. The photopigments are located in internal membrane systems continuous with the cytoplasmic membrane (Pfennig and Truper 1974).

2.3.2.2 Rhodospirilliaceae

The phototrophic development of the Rhodospirillaceae (purple non-sulphur bacteria) is dependent upon simple organic compounds which are photo-assimilated or serve as electron donors for CO₂ assimilation (Pfennig and Truper 1974).

Fig. 2.3 Classification of The Phototrophic Bacteria (Truper 1976)

Order :

RHODOSPIRILLALES
(phototrophic bacteria)

Sub-Order

RHODOSPIRILLINEAE
(purple)

CHLOROBIIINEAE
(green)

Family

RHODOSPIRILLACEAE
(purple non-sulphur)

CHROMATIACEAE
(purple sulphur)

CHLOROBIIACEAE
(green sulphur)

CHLOROFLEXACEAE
(green flexiform)

Type Genera:

RHODOSPIRILLUM

CHROMATIUM

CHLOROBIIUM

CHLOROFLEXUS

Type Species:

R. rubrum

C. okenii

C. limicola

C. aurantiacus

These bacteria are generally microaerophilic but many species may grow at full atmospheric tension in either the light or the dark. Under aerobic conditions photopigment production is inhibited and an aerobic respiration functions (Elsden 1962, Elsden and Omerod 1956). Under anaerobic conditions, CO_2 is fixed via the reductive pentose phosphate cycle and an anaerobic light-dependent tricarboxylic acid cycle functions (Pfennig 1967).

Hydrogen donors and carbon sources used by the Rhodospirillaceae have been listed (Pfennig and Truper 1973).

Three genera and twelve species of this family are recognized (Pfennig and Truper 1974). Although earlier research indicated that no species were able to utilize sulphide, more recent work has contradicted this. Rhodopseudomonas palustris (Hansen and van Germerden 1972) and Rps. sulphidophila (Hansen and Veldkamp 1973) are able to oxidise sulphide to sulphate without the accumulation of intracellular sulphur. Rps. sphaeroides, Rps. capsulata and Rhodospirillum rubrum are capable of oxidising sulphide only to sulphur which is deposited extracellularly (Hansen and van Germerden 1972). Under conditions of competition for sulphide these latter organisms do not compete successfully with other phototrophic bacteria (Pfennig 1975).

As all species are unable to utilise elemental sulphur, this criterion may be used to differentiate between the Rhodospirillaceae and the Chromatiaceae (Hansen and van Germerden 1972, Pfennig and Truper 1974).

2.3.2.3 Chromatiaceae

Ten genera and twenty-six species of the Chromatiaceae (purple sulphur bacteria) are recognized (Pfennig and Truper 1974). Not all have been isolated in pure culture. Most species are strictly anaerobic and obligately phototrophic. All are capable of photolithotrophic CO_2 fixation in the presence of sulphide. Under these conditions sulphur accumulates inside or outside the cells with sulphate being the final oxidation production. Further details are presented in Section 2.4.1.

All species photoassimilate a number of simple organic substrates of which acetate and pyruvate are the most widely used. All forms are therefore potentially mixotrophic (Pfennig and Truper 1974).

2.3.2.4 Chlorobiineae

The Chlorobiineae (green phototrophic bacteria) contain bacteriochlorophylls c, d or e as the major bacteriochlorophyll components and various carotenoids. The photopigments are located in the chlorobium vesicles which underlie, and are attached to, the cytoplasmic membrane (Pfennig and Truper 1974, Truper 1976).

2.3.2.5 Chlorobiaceae

Five genera and nine species of the Chlorobiaceae (green sulphur bacteria) have been recognized (Pfennig and Truper 1974). These organisms are strictly anaerobic and obligately phototrophic. All species are capable of photolithotrophic CO₂ assimilation in the presence of sulphide. Under these conditions elemental sulphur accumulates outside the cells and is further oxidised to sulphate.

A number of simple organic compounds are photoassimilated. The Chlorobiaceae are therefore potential mixotrophs.

2.3.2.6 Chloroflexaceae

The Chloroflexaceae (green flexiform bacteria) contain bacteriochlorophylls c, d or e besides bacteriochlorophyll a. The cells exhibit a gliding motility, the cell walls are flexible and growth is filamentous (Truper 1976).

One genus and one species, Chloroflexus aurantiacus, is recognized. Pending pure culture studies, the genus Chloronema and its two species C. giganteum and C. spiroideum have not been included in this classification.

Although C. aurantiacus prefers photo-organotrophic growth conditions and exhibits a capacity to grow aerobically in the dark, these properties have not been elevated to the family level (Truper 1976).

2.3.3 Anaerobic Reduction of Sulphur Compounds

2.3.3.1 Desulfovibrio:

Five species of the genus Desulfovibrio are recognized (Postgate 1974). These bacteria are strictly anaerobic chemo-organotrophs which obtain their energy from anaerobic respiration reducing various sulphur compounds to sulphide. Lactate, pyruvate and usually malate are oxidised to acetate and CO_2 .

These bacteria can tolerate high sulphide concentrations (Miller 1950) and are important in anaerobic treatment processes. 5×10^4 cells/ml of D. desulphuricans have been reported for sludge from an anaerobic digester (Toerien et al 1968). These numbers increased if the level of sulphur compounds in the waste rose. Tests of sulphur compounds reduced by Desulfovibrio have been reported in the literature (Baas-Becking 1925, Postgate 1959). Sulphur is reduced non-enzymically only under limited conditions (Postgate 1959).

Ecologically Desulfovibrio are more abundant and more active in sediments than in overlying waters (Le Gall and Postgate 1973).

The effect of the sulphide produced by these organisms in anaerobic processes has been described in Section 2.2.3.5.

2.3.3.2 Desulfotomaculum:

One genus and three species of Desulfotomaculum are recognised (Campbell 1974). Members of this genus may be differentiated from the Desulfovibrio by their ability to sporulate and their peritrichous flagella. The biochemistry of the two genera is very similar.

2.3.3.3 Desulfuromonas:

One genus and one species of Desulfuromonas are recognized (Pfennig and Biebl 1976). These bacteria may be differentiated from Desulfovibrio and Desulfotomaculum by their ability to use elemental sulphur as an electron acceptor for its anaerobic respiration. Acetate is completely oxidised to CO_2 .

The ability of these organisms to form syntrophic cultures with Chlorobiaceae is ecologically important.

Thus the phototrophic bacteria and the sulphate reducers occupy similar environments and possess complementary sulphur metabolisms, allowing the completion of the sulphur cycle under anaerobic conditions.

As the oxidation of sulphur compounds under anaerobic conditions is of more importance to this research, the metabolism of sulphur and carbon compounds by the phototrophic bacteria will now be outlined.

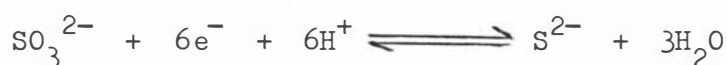
2.4 TRANSFORMATIONS OF SULPHUR AND CARBON COMPOUNDS BY PHOTOTROPHIC BACTERIA

2.4.1 Metabolism of Sulphur Compounds:

Two forms of sulphur metabolism may be distinguished in the phototrophic bacteria. Quantitatively, dissimilatory utilisation, in which the sulphur compounds provide electrons for the functioning of the photosynthetic electron chain, is more important. Assimilatory sulphur metabolism supplies the sulphur necessary for the synthesis of cell material.

All Chromatiaceae and Chlorobiaceae (Pfennig and Truper 1974) and several Rhodospirillaceae (Hansen and Van Germerden 1972, Hansen and Veldkamp 1973, Hashwa 1975, Rolls and Lindstrom 1967) exhibit a dissimilatory sulphur metabolism. All Rhodospirillaceae and some Chromatiaceae are capable of assimilatory sulphate reduction while the Chlorobiaceae lack this ability (Pfennig and Truper 1974, Truper 1975).

The present state of knowledge of the sulphur metabolism of the phototrophic bacteria is outlined in Fig. 2.4. Important enzymes in the dissimilatory metabolism of sulphur identified in Chromatiaceae are given in Table 2.1. From this Table, a lack of metabolic coupling between sulphide, or sulphur, and sulphite is evident. The presence of a sulphur-oxidizing enzyme has never been demonstrated in the Chromatiaceae (Truper 1975). However, heterotrophically and autotrophically grown cells of Chromatium vinosum have been shown to contain sulphite reductase (E.C. 1.8.1.2) (Peck et al 1974). The enzyme catalyses the following reaction :



Although Peck et al (1974) imply its use in assimilatory metabolism the occurrence of this enzyme in autotrophically grown C. vinosum and Chlorobium limicola, which is incapable of assimilatory reduction (Pfennig and Truper 1974), suggests a role for this enzyme in dissimilatory sulphur metabolism.

The occurrence of sulphur as an oxidation intermediate is therefore unlikely. The formation of intracellular sulphur is more probably a storage side reaction which can be used as a sink for electrons under anaerobic conditions in the dark (Van Gernerden 1968b). (See Section 2.4.2.2.). Such a postulate is reinforced by the following evidence :

- (i) Those Rhodospirillaceae capable of oxidising sulphide form either elemental sulphur or sulphate (Hansen and Van Gernerden 1972, Hansen and Veldkamp 1973).
- (ii) Fractionation of sulphur isotopes by a Chromatium spp. led Kaplan and Rittenberg (1964) to conclude that elemental sulphur was not an intermediate in the oxidation of sulphide to sulphate.

As this evidence is at present indirect, two alternative pathways for the oxidation of sulphide to sulphate have been included in Fig. 2.4.

(Reproduced with permission from Truper 1975)

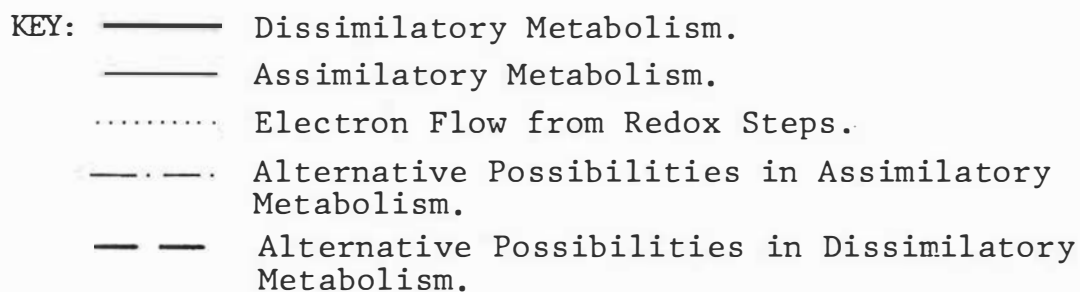


TABLE 2.1

Enzymes of Dissimilatory Sulphur Metabolism in the Chromatiaceae
(After Truper 1975).

Reaction Number	Enzyme	Reaction Catalysed	Reference
1	Sulphide oxidizing	$S^{2-} \rightarrow S^0 + 2e^-$	4,8
2	Thiosulphate reductase	$S_2O_3^{2-} + 2e^- \rightarrow S^{2-} + SO_3^{2-}$	1,2,6
3	APS reductase	$SO_3^{2-} + AMP \rightarrow APS + 2e^-$	3,5,7
4	ADP sulphurylase	$APS + Pi \rightarrow ADP + SO_4^{2-}$	3

Reference key :

- 1 Hashwa and Pfennig (1972)
- 2 Smith and Lascelles (1966)
- 3 Thiele (1968b)
- 4 Truper (1964)
- 5 Truper and Peck (1970)
- 6 Truper and Pfennig (1966)
- 7 Truper and Rogers (1971)
- 8 Truper and Schlegel (1964)

The reaction numbers refer to Figure 2.4.

The Chromatiaceae vary in their ability to perform assimilatory sulphate reduction. Only those organisms possessing this characteristic are capable of utilising organic compounds in the absence of reduced sulphur compounds (Pfennig and Truper 1974). This property will therefore be of importance in determining the bacteria present in an environment devoid of sulphide.

The fact that dissimilatory sulphur metabolism is common to all Chromatiaceae but only a limited number of species are capable of assimilatory utilisation suggests that, at some point, different enzymes are involved in the two pathways.

Sulphur compounds play an important role in selecting the phototrophic bacteria most suited to an environment. This will be considered in Section 2.5.2.1. Prior to this, the associated transformations of carbon compounds by these bacteria will be reviewed.

2.4.2 Metabolism of carbon compounds

Phototrophic bacteria are capable of obtaining the carbon necessary for cell growth from diverse substrates such as CO_2 and simple organic compounds (Doelle 1969, Kondrat'eva 1965, Weissner 1970). In anaerobic lagoons, CO_2 and volatile acids (Holm and Vennes 1970) are present in significant quantities, with acetate being the most important intermediate for methanogenesis (Jeris and McCarty 1965, Smith and Mah 1966).

The transformation of carbon compounds by the phototrophic bacteria is important in determining how the organic component of an effluent is stabilised in lagoons in which these bacteria are dominant. In lagoons, dispersed growth occurs and the organisms present are discharged with the effluent. The cells will therefore exert a BOD on the receiving water and ultimate stabilisation of the organic component of the effluent only occurs through discharge of gaseous products from the lagoon.

Such a situation may be compared to the activated sludge and biofiltration systems where the majority of the cells are removed from the effluent prior to discharge and subsequently stabilised, often by an anaerobic process.

It is noteworthy that the cell yield from the anaerobic processes is less than from aerobic processes (Eckenfelder 1970) and therefore less biomass will be present in the effluent from an anaerobic process than from a comparable aerobic process.

Although all phototrophic bacteria possess an active reductive pentose phosphate cycle (Pfennig 1967), further pathways of carbon metabolism are more diverse. The Rhodospirillaceae, typified by R. rubrum, possess a complete tricarboxylic acid (TCA) cycle (Truper 1964) which allows the nearly quantitative conversion of organic compounds to CO_2 and H_2 (Gest et al 1962). Chlorobium thiosulphatophilum possesses a carbon reduction cycle which is effectively a reversal of the TCA cycle (Evans et al 1966) while the Chromatiaceae tested lack a TCA cycle but possess a glyoxylate cycle (Truper 1964, Fuller 1969).

Also, the metabolic fate of CO_2 and organic compounds is dependent upon the stage of growth of the bacterium, its carbon requirements and its environment (Fuller 1969, Weissner 1970). Thus, in the presence of nitrogen compounds, an actively growing cell diverts most of its carbon into amino acid synthesis and minimises the transformation of organic compounds into storage products. The presence of organic compounds can lead to the partial inhibition of CO_2 fixation pathways.

While generalisations concerning metabolic pathways are difficult to make and it is possible that alternative pathways exist in organisms yet to be investigated, the present knowledge on the metabolism of CO_2 and organic compounds by the Chromatiaceae can be summarised.

2.4.3. Carbon Metabolism of the Chromatiaceae:

2.4.3.1 Carbon Dioxide Fixation:

Being photosynthetic organisms, carbon dioxide fixation is one of the fundamental reactions of the Chromatiaceae.

The investigation of Chromatium enzymes and the tracing of labelled intermediates has demonstrated a functional reductive pentose phosphate cycle in these bacteria (Fuller and Anderson 1957, Fuller et al 1961, Smillie et al 1962). The enzyme catalysing the CO_2 fixation reaction in this path, ribulose diphosphate (RuDP) carboxylase (E.C. 4.1.1.39), is an important metabolic control point. In the Chromatiaceae, the activity of this enzyme is greatest in autotrophically grown cells (Asami and Akazawa 1975a, Fuller et al 1961, Hurlbert and Lascelles 1963). Photo-organotrophic conditions lead to a rapid decrease in RuDP carboxylase concentration (Table 2.2). A change to mixotrophic conditions partially restores the activity of this enzyme (Hurlbert and Lascelles 1963).

Although in the Rhodospirillaceae RuDP carboxylase is inhibited under aerobic conditions and its specific activity is inversely proportional to the light intensity (Lascelles 1960), in at least one species of the Chromatiaceae RuDP carboxylase is not inhibited by oxygen (Kondrat'eva et al 1976a).

TABLE 2.2

Partial Inhibition of Ribulose Diphosphate Carboxylase by Organic Compounds.

(RuDP carboxylase activity as a % of autotrophic level)

Organism		<u>Chromatium vinosum</u> Str.D			<u>Thiocapsa</u> *
Substrate	Reference	Hurlbert and Lascelles (1963)	Fuller et al (1961)	Asami and Akazawa (1975a)	Hurlbert and Lascelles (1963)
acetate		41	42	31	45
malate		37	11	36	77
fumarate		46	-	-	68
succinate		86	-	-	41
pyruvate		25	-	-	60
propionate		65	-	-	100
lactate		39	-	-	55

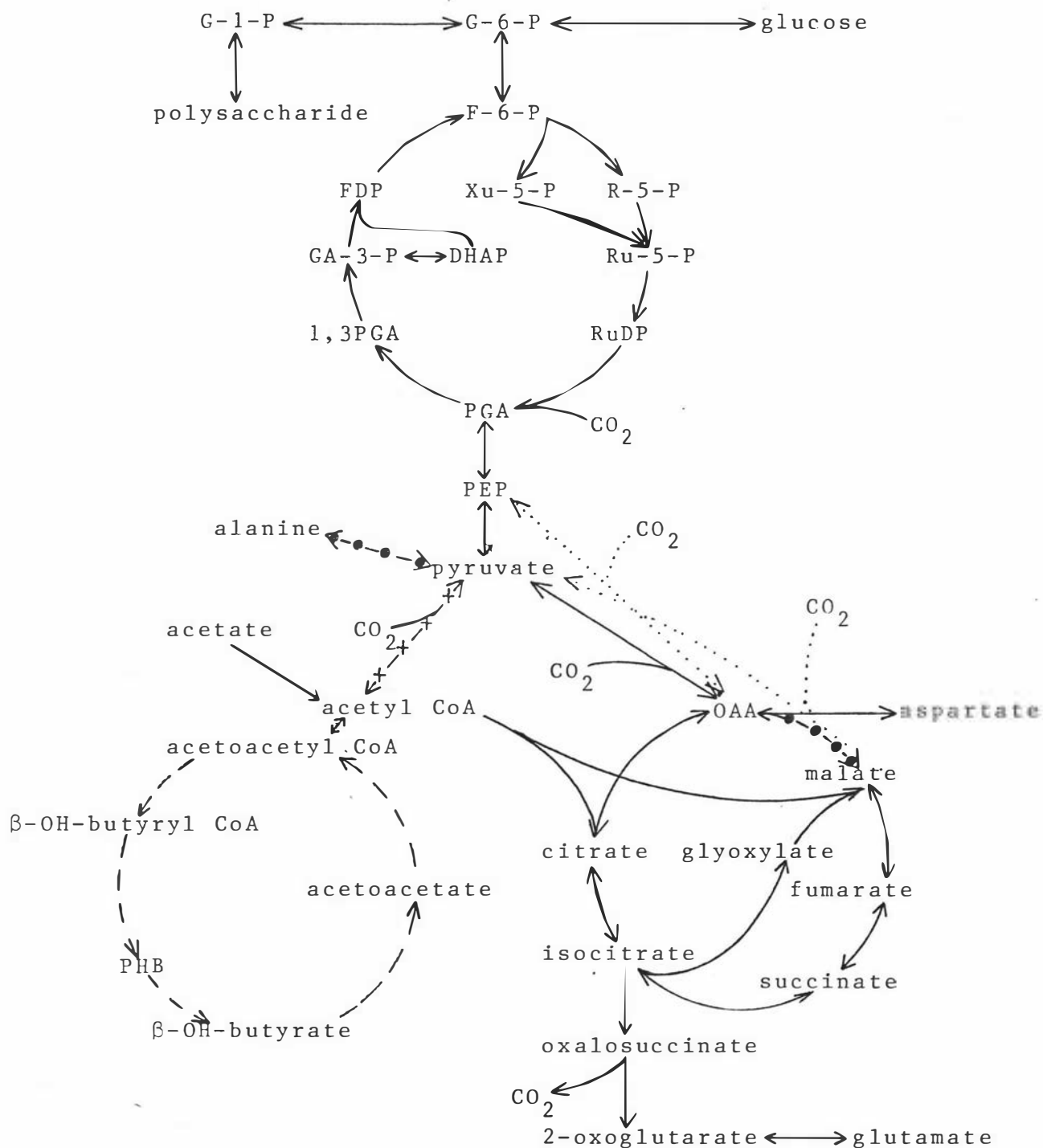
*This organism was identified as Thiopedia by Hurlbert and Lascelles (1963) but was subsequently re-identified as Thiocapsa (Pfennig 1967).

Under photoautotrophic conditions, the reductive pentose phosphate cycle operates as the main carbon assimilation pathway.

If simple organic compounds are present in the environment, they are preferentially metabolised. Such control has probably evolved to minimise the expenditure of ATP and NADH_2 . Thus, the RuDP carboxylase of bacteria in anaerobic lagoons will be under partial inhibition resulting in less CO_2 fixation via the pentose phosphate cycle.

Possible pathways for the linking of autotrophic and heterotrophic metabolism in Chromatium spp. are shown in Fig. 2.5.

Figure 2.5: Pathways of Autotrophic and Heterotrophic Carbon Metabolism in Chromatium spp.



KEY: -●-●- Truper(1964) for *C. okenii*

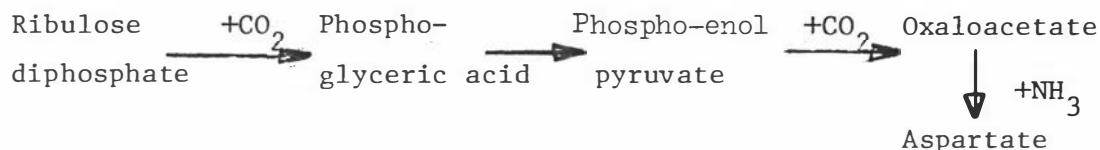
..... Fuller et al (1961) for *Chromatium* D

———— Common to both species

-x-x- Pyruvate phosphoroclastic reaction(Bennett and Fuller 1964,Bennett et al 1964,Buchanan and Arnon 1965,Buchanan and Bachofen 1964)

---- PHB cycle(Stanier et al 1971,Truper 1964)

Other CO_2 fixation reactions also occur. Kinetic isotope analysis of $^{14}\text{CO}_2$ fixation in growing cells of Chromatium vinosum strain D (hereafter called Chromatium D) shows aspartate as an early metabolic product (Fuller 1969). A primary carboxylation occurs in the reductive pentose phosphate cycle and a second carboxylation of phospho-enol pyruvate (PEP) occurs as shown below (Fuller 1969):



In Chromatium okenii two possible secondary carboxylations exist, via either PEP carboxykinase (E.C. 4.1.1.32) or pyruvate carboxylase (E.C. 6.4.1.1.), (Truper 1964).

Also, particulate fractions of Chromatium spp. have been shown to catalyse the synthesis of pyruvate from acetate and CO_2 (Buchanan et al 1964, Evans and Buchanan 1965). The significance of this reaction will be considered in the section on the metabolism of organic compounds.

The pattern of CO_2 incorporation has also been studied. Chromatium D grown in a hydrogen atmosphere incorporate CO_2 into the following compounds (Arnon et al 1963).

metabolic intermediates (mainly alanine)	36 - 57%
proteins	31 - 43%
polysaccharides and nucleic acids	16 - 21%
poly- β -hydroxybutyrate (PHB)	0.6 - 0.8%

The major storage product formed during autotrophic growth is therefore a polysaccharide which has been identified as a glycogen/amylopectin polymer (Hara et al 1973). Glycogen synthase has also been isolated (Hara and Akazawa 1974).

2.4.3.2 Metabolism of Organic Compounds

The ability of the Chromatiaceae to grow on simple organic substrates in the absence of reduced sulphur compounds was first demonstrated by Van Niel (1931). Subsequently Muller (1932)

confirmed these results and indicated that the organic substrates were virtually completely converted to cell material and CO_2 . These observations were explained by the postulate that compounds which were more oxidised than cell material evolved CO_2 during assimilation. Such compounds included acetate, lactate, malate and succinate. Compounds more reduced than cell material, such as butyrate lead to the uptake of CO_2 .

Also Gaffron (1933, 1935) interpreted his study of the organic metabolism of the Rhodospirillaceae as the direct light dependent conversion of organic carbon into cell constituents.

Despite this evidence, it was believed until the late 1950's that organic compounds served solely as hydrogen donors for photosynthesis (Foster 1951). This was a direct result of Van Niel's unitary theory of photosynthesis (Van Niel 1935, 1936, 1941). Stanier et al (1959) finally dispelled such thoughts by demonstrating that direct photoassimilation occurred in Rhodospirillum rubrum.

Comprehensive lists of organic substrates utilised by the Chromatiaceae have been published (Holm and Vennes 1970, Hurlbert and Lascelles 1963, Pfennig and Truper 1971, Thiele 1968a). Possible pathways for the metabolism of some substrates will be considered to demonstrate how the Chromatiaceae may stabilize organic matter.

Whereas the Rhodospirillaceae possess a complete TCA cycle (Elsden and Ormerod 1956, Stopanni et al 1955) which also functions under anaerobic conditions in the light (Gest et al 1962), the Chromatiaceae tested lack 2-oxoglutarate dehydrogenase (E.C. 1.2.4.2) (Fuller et al 1961, Losada et al 1960, Truper 1964). They are therefore unable to generate CO_2 and reduced pyridine nucleotides via this cycle. In this family, the glyoxylate cycle (Truper 1964), or a modified glyoxylate cycle (Fuller et al 1961, Losada et al 1960), is used to generate the intermediates essential for amino acid synthesis (Fig. 2.5). Chromatium D lacks malate dehydrogenase (E.C. 1.1.1.37) which is necessary for the glyoxylate cycle (Fuller et al 1961, Fuller and Kornberg 1961, Sisler and Fuller 1959). In this bacterium, malate is converted to oxalacetate via pyruvate using malate dehydrogenase (decarboxylating, NADP) (E.C. 1.1.1.40)

and pyruvate carboxylase (E.C. 6.4.1.1.) (Fuller et al 1961). However, a full complement of glyoxylate cycle enzymes is present in Chromatium okenii (Truper 1964).

The glyoxylate cycle is important in transforming and maintaining a pool of essential metabolic intermediates. The purpose is therefore assimilatory and it does not directly evolve CO_2 . Limited information is available on the importance of this cycle in the Chromatiaceae and further enzymatic analysis is required to prove its significance in genera other than Chromatium.

To demonstrate the interactions of the pathways shown in Fig. 2.5 and to study possible CO_2 - evolving reactions, the metabolism of acetate and pyruvate will be studied. These are the only two organic compounds utilised by all species of Chromatiaceae (Foster 1951).

2.4.3.3 Metabolism of Acetate

Acetate may be metabolised in the absence of CO_2 (Losada et al 1960) but its assimilation is light dependent (Losada et al 1960, Muller 1932).

The first stage of acetate metabolism is its activation by acetyl CoA synthetase (E.C. 6.2.1.1.). Further pathways are dependent upon the cell's environment and the stage of growth. In the presence of nitrogen compounds, actively growing cells incorporate most of the acetate carbon into amino acids, especially glutamate (Losada et al 1960, Weissner 1970). The glyoxylate cycle, which requires a supply of acetyl CoA, ensures a ready supply of the necessary intermediates (Fig. 2.5).

In resting cells, much of the acetate carbon appears in poly- β -hydroxybutyrate (PHB). This transformation requires both ATP and NADH (Stanier et al 1971, Weissner 1970). As Chromatium spp. lack the TCA cycle they are unable to generate NADH in this manner and therefore PHB is only synthesised in the presence of external hydrogen donors (Arnon et al 1963, Buchanan and Bachofen 1969, Buchanan et al 1964, Weaver et al 1965). Thus cells of Chromatium D grown in a

hydrogen atmosphere incorporated 74% of the labelled acetate into PHB and only 19% into protein. In an argon atmosphere only 7% was incorporated into PHB and 64% into protein (Eisenberg 1955).

The Rhodospirillineae are one of the few groups of bacteria capable of synthesising the two organic reserve materials, PHB and polysaccharide (Stanier et al 1971). Also the Chromatiaceae studied possess a full complement of Embden - Meyerhoff - Parnas pathway enzymes (Fig. 2.5.). Exogenous substrates such as acetate may therefore be converted to either storage product (Weissner 1970) and one organic reserve material may be converted to the other. This is particularly important for energy generation in darkness.

With Chromatium spp. an average of 0.17 moles of CO_2 are evolved per mole of acetate consumed (Muller 1932). That is approximately one mole of CO_2 produced per six moles of acetate metabolised although the figure could be expected to vary with the growth and the environment of the cell. The metabolic pathways outlined in Fig. 2.5 indicate the alternative routes available for acetate assimilation. Some involve the fixation of CO_2 such as the conversion of acetate to pyruvate and some involve the direct assimilation of acetate as in the formation of aspartate via the glyoxylate cycle. Others involve the evolution of CO_2 as indicated by the formation of glutamate via isocitrate and 2-oxoglutarate.

A study of these pathways only demonstrates the physiological feasibility of such reactions. It fails to indicate the balanced operation of the pathways under different environmental conditions. This is what will determine whether the cell evolves or assimilates CO_2 .

2.4.3.4 Metabolism of Pyruvate

As with acetate, several alternative pathways are available for the metabolism of pyruvate depending upon the organism, the environment and the phase of growth (Weissner 1970).

The ability of Chromatium D to convert pyruvate into PEP using PEP synthetase has been demonstrated (Buchanan and Evans 1966). In

R. rubrum, PEP may be used to form organic acids as precursors to amino acids (Evans et al 1966) or, under conditions favouring the accumulation of storage compounds, it may be used in the synthesis of polysaccharides (Stanier et al 1959). Similar reactions could be expected for the Chromatiaceae.

Under autotrophic conditions, a supply of acetyl CoA must be generated to allow the glyoxylate cycle to function (Truper 1964). Essential aminoacids may then be formed. The decarboxylation of pyruvate via the phosphoroclastic reaction allows the generation of acetyl CoA under such conditions (Bennett et al 1964, Bennett and Fuller 1964, Buchanan and Arnon 1965, Buchanan et al 1964). In this reaction either acetyl phosphate or acetyl CoA may be formed (Racker 1965) with the latter compound being in the probable product Chromatium (Buchanan et al 1964). The reaction, which is shown below, is catalysed by pyruvate synthetase (Evans and Buchanan 1965).



The importance of this reaction in the metabolism of organic compounds is shown in Fig. 2.5 and reinforced by the following observations :

(i). The incorporation of acetate carbon into cell polysaccharide requires CO_2 . The incorporation of acetate into PHB does not (Stanier et al 1959).

(ii) The conversion of stored PHB into polysaccharides and nitrogenous constituents requires CO_2 (Stanier et al 1959).

(iii) Labelling experiments with R. rubrum grown on acetate indicated that the addition of CO_2 to acetate to yield pyruvate was an important step in amino acid formation (Cutinelli et al 1951a, b).

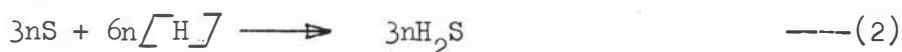
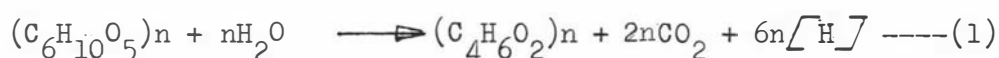
(iv) Such a reaction explains the ability of Chromatium to convert stored carbohydrate to PHB and CO_2 under anaerobic conditions in the dark (Van Germerden 1968b). This transformation is important as it allows the cell to generate energy by substrate phosphorylation under those conditions where photosynthetic energy is not available.

This will be considered in the following section .

2.4.3.5 Anaerobic Dark Metabolism

The existence of a maintenance metabolism under dark/anaerobic conditions was first examined by Gaffron (1933, 1935) who concluded that an oxidation of a reserve material coupled to a reduction of sulphate to sulphide occurred. Subsequent research (Roelofsen 1935, Van Niel 1936) failed to demonstrate such a sulphate reduction and Van Niel (1936) indicated that sulphur was the precursor of sulphide. This observation has been confirmed for Chromatium D (Hendley 1955), C. okenii (Truper and Schlegel 1964) and Thiocapsa roseopersicina (Truper and Pfennig 1966). In addition to sulphide, Chromatium D formed varying proportions of acetate and CO_2 under these conditions (Hendley 1955). Also, C. okenii grown photo-heterotrophically on acetate and containing PHB granules, produced less sulphide under dark anaerobic conditions than cells grown photoautotrophically on sulphide (Truper and Schlegel 1964).

To explain these observations it has been postulated that under anaerobic conditions in the dark, elemental sulphur acts as an electron acceptor in the conversion of storage polysaccharide to PHB and CO_2 as shown by the following equations (Van Germerden 1968b):



The degradation of polysaccharide via the Embden-Meyerhoff-Parnas pathway to PHB via acetyl CoA (see Fig 2.5) yields 2 moles of ATP per mole of glycosyl residue degraded. This energy would then be available to maintain the cell structure under conditions unsuitable for photosynthesis.

With Chromatium strain 6412 used to verify this hypothesis no acetate production was detected (Van Germerden 1968b). However, two moles of CO_2 were produced per mole of glycosyl residue degraded, demonstrating that some organic matter may be ultimately stabilised by this means.

Subsequently, it has been reported that Ectothiorhodospira shaposhnikovii (Kondrat'eva 1974) and Thiocapsa roseopersicina strain BBS (Bogarov 1974, Krasil'nikova et al 1975) are capable of growing, rather than maintaining themselves, under anaerobic conditions in the dark. With T. roseopersicina strain BBS significant growth only occurred with glucose as a substrate (Krasil'nikova et al 1975). Small quantities of pyruvate accumulated in the medium. This strain of T. roseopersicina lacks the enzymes required for the phosphoroclastic cleavage of pyruvate (Krasil'nikova and Kondrat'eva 1974) and is therefore incapable of converting pyruvate to acetyl CoA by this path.

Thus, although Chromatium strain 6412 does not produce organic compounds under anaerobic conditions in the dark, other genera may do so. The implications of such a metabolism for a lagoon system are that whereas CO_2 evolution may be considered beneficial, the production of sulphide and organic acids are detrimental as they will result in an increased BOD and COD.

2.4.3.6 Aerobic Metabolism

The effluent from an anaerobic photosynthetic lagoon is highly coloured and, under most circumstances, will require further treatment. Algal lagoons are the most practical method of decolourisation and the effect of oxygen on the metabolism of the Chromatiaceae is therefore, worthy of further consideration.

The Chromatiaceae were originally believed to be strict anaerobes, requiring both light and absence of oxygen for growth (Van Niel 1931, 1936). Some species have been shown to be negatively aerotactic (Kondrat'eva 1965), and this plays an important role in determining the distribution of these bacteria in nature (see Section 2.5.2.3).

Chromatium vinosum strains (Asami and Akazawa 1975a, Breuker 1964, Hurlbert 1967, Smith and Lascelles 1966) oxidise reduced sulphur compounds and fix CO_2 under aerobic conditions. However, oxygen partially inhibits the oxidation of intracellular sulphur and the

metabolism of pyruvate by Chromatium D (Hurlbert 1967). Oxygen also affected the pattern of acetate assimilation in this bacterium. Under aerobic conditions PHB was the major product whereas in an anaerobic environment the acetate was more evenly distributed throughout the cell fractions.

Although, in the presence of oxygen, Chromatium D can maintain its viability, motility and ability to metabolise both organic and inorganic compounds, it is unable to grow at a significant rate (Gibson 1967, Hurlbert 1967). No definite physiological explanation has been formulated to account for this inability.

Under aerobic conditions in the light, autotrophically grown cells of Chromatium D exhibit a prototype of photorespiration (Asami and Akazawa 1974). Glycolic acid is excreted. The phenomenon is similar to the Warburg effect (Warburg 1920) demonstrated by green plants and algae under a high partial pressure of oxygen and a low CO₂ partial pressure (Tolbert et al 1967, Jackson and Volk 1970). No glycolate is produced under anaerobic conditions (Asami and Akazawa 1975a). The path of glycolate fermentation has yet to be fully elucidated, although intermediates of the reductive pentose phosphate cycle are believed to be involved (Asami and Akazawa 1975b, Lorimer et al 1976).

Although the aerobic metabolism of Chromatium D is typical of most Chromatiaceae, some species exhibit growth under aerobic conditions (Kondrat'eva et al 1976a). Thiocapsa roseopersicina (Kondrat'eva et al 1975, Kondrat'eva et al 1976b, Petushkova and Ivanovskii 1976, Pfennig and Truper 1974) and Amoebobacter roseus (Gorlenko 1974) are capable of chemoautotrophic growth with sulphide, sulphur or thiosulphate as substrates. Ectothiorhodospira shaposhnikovii is capable of growth on the above sulphur compounds if simple organic acids (e.g. acetate, pyruvate) are present (Kondrat'eva et al 1976b).

Of these organisms, T. roseopersicina has received the most study. Under these growth conditions the reduced sulphur compounds act as electron donors for aerobic respiration (Kondrat'eva et al 1976b, Petushkova and Ivanovskii 1976) generating sufficient energy

for growth. Carbon dioxide assimilation proceeds primarily via the reductive pentose phosphate cycle (Kondrat'eva 1975, 1976a). T. roseopersicina therefore exhibits an anaerobic/light phototrophic metabolism and an aerobic chemoautotrophic metabolism. Although the latter conditions are less favourable for growth (Kondrat'eva et al 1976a), such a metabolism may be important for the survival of these bacteria in the absence of light and utilisable organic compounds. It also implies these bacteria would be capable of growth in an aerobic lagoon provided suitable reduced sulphur compounds were present.

2.4.4 Conclusions:

It is important to determine the fate of the sulphur and carbon compounds in any waste treatment process.

It has been shown that all Chromatiaceae are capable of dissimilatory sulphide oxidation, with sulphate being the final oxidation product. The metabolic reactions for such pathways have yet to be fully elucidated.

The carbon metabolism is more complex. As photosynthetic organisms, the Chromatiaceae may be expected to increase the BOD of the effluent due to CO_2 fixation. Carbon dioxide is photo-assimilated primarily through the reductive pentose phosphate cycle in which RuDP carboxylase is a key enzyme. However, RuDP carboxylase is partially inhibited by low molecular weight organic compounds such as those found in anaerobic lagoons. Under these conditions, CO_2 fixation via this cycle will be impaired and the Chromatiaceae will exhibit a mixotrophic metabolism.

Organic matter may then be stabilised by various CO_2 -evolving reactions. However the Chromatiaceae do not possess a complete complement of TCA cycle enzymes and cannot perform the decarboxylation reactions associated with this cycle. The glyoxylate cycle, which does not involve any decarboxylations, is the major means of generating essential metabolic intermediates.

However, CO_2 may be evolved by other reactions. The most important of these is the phosphoroclastic cleavage of pyruvate to yield acetyl CoA. This is a key reaction in the conversion of storage polysaccharide to PHB under anaerobic conditions in the dark with 2 moles of CO_2 being produced per mole of glycosyl residue degraded. Thus, alternation of light and dark plays an important role in the stabilisation of organic compounds by the Chromatiaceae under anaerobic conditions.

2.5 THE ECOLOGY OF THE PHOTOTROPHIC BACTERIA.

2.5.1 Introduction:

Two groups of organisms may be considered to be masters of the anaerobic environment. Both operate at the end of a series of anaerobic respiratory and fermentative activities and effectively recycle metabolic end-products which would otherwise accumulate (Pfennig 1967).

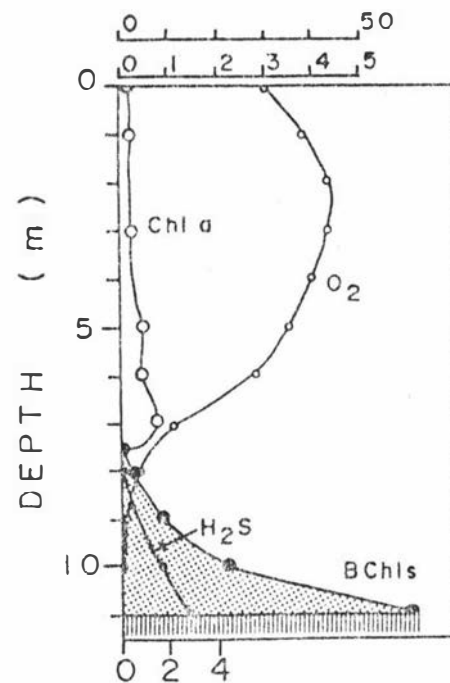
Firstly, the Methanobacteriaceae use CO_2 as a hydrogen acceptor for their metabolism of highly reduced compounds (Bryant 1974).

Secondly, the phototrophic bacteria metabolise reduced sulphur compounds and simple organic compounds. Because these bacteria obtain much of their energy under anaerobic conditions in the light by photophosphorylation, they function more efficiently than the Methanobacteriaceae.

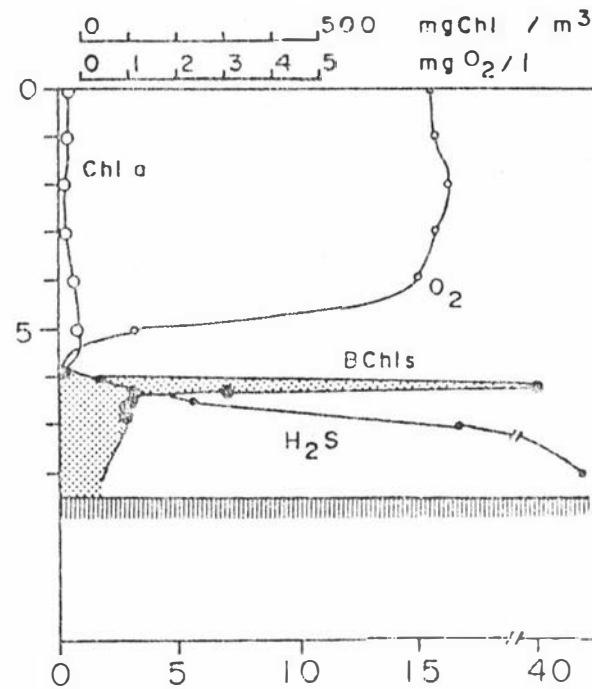
The natural ecological niches of the phototrophic bacteria are defined by their major metabolic requirements. Therefore, they thrive in anaerobic environments exposed to the light, with typical habitats being stratified lakes, stagnant ponds and waste treatment lagoons. In any given niche, many environmental factors are important in selecting for the various families, genera and species of the Rhodospirillales. By examining the physiological requirements

Figure 2.6: The Two Patterns of Vertical Distribution of Phototrophic Sulphur Bacteria in Lakes (Reproduced from Takahashi and Ichimura 1970).

(a) Pattern I



(b) Pattern II



HYDROGEN SULFIDE (mg S / l)

of the bacterial types occurring in their different locations an understanding of these environmental parameters may be obtained and a knowledge of the occurrence, and the role, of Chromatiaceae in lagoon systems may be gained.

2.5.2 Phototrophic Bacteria in Lakes:

Large numbers of phototrophic bacteria frequently inhabit the chemocline of lakes. They form stable populations in meromictic lakes (Pfennig 1967) but normally only occur in holomictic lakes during the summer stratification (Takahashi and Ichimura 1970).

The patterns of vertical distribution may be differentiated (Fig. 2.6) (Takahashi and Ichimura 1970).

Pattern I is characteristic of holomictic lakes and shows an increase in bacterial numbers with depth early in the summer stagnation. Collins (1960) has outlined the microbial events occurring during this period. They are as follows.

After stratification, the activity of the facultatively aerobic organisms in the mud reduces the oxygen tension allowing the sulphate reducing bacteria to become active. The resulting sulphide diffuses into the overlying water creating an anaerobic zone suitable for the growth of many anaerobic phototrophic bacteria.

As the stagnation proceeds, the zone of anaerobiosis, and the associated phototrophic bacteria, extend further into the overlying water until a distribution approximating that of pattern II reveals itself.

In the autumn, the lake's contents become thoroughly mixed and these microbial zones are dispersed.

Pattern II is characteristic of meromictic lakes and the predominant biomass is restricted to a narrow layer between the oxidative and reductive zones. Because of the stability of these zones in such lakes, pattern II has been more commonly recorded

(Caldwell and Tiedje 1975, Culver and Brunskill 1969, Genovese 1963, Hutchinson 1957, Ruttner 1963).

Caldwell and Tiedje's (1975b) detailed study of two meromictic lakes revealed two distinct communities in the upper layers of the hypolimnion. The layer nearest the surface was turbid, red and contained Chromatiaceae while the layer immediately below contained Chlorobiaceae. Microstratification of various genera occurred within each of these communities (Table 2.3). Populations of Rhodospirillaceae have been occasionally reported as overlying the purple and green sulphur bacteria (Collins 1960, Pfennig 1967).

Significant seasonal fluctuations are observed in the numbers of phototrophic bacteria in such platelets. Maximum populations occur in the late summer (Culver and Brunskill 1969, Takahashi and Ichimura 1970).

As the light intensity and the concentrations of sulphide, oxygen and organic compounds vary significantly across the chemocline, it is apparent that the selection of various genera of phototrophic bacteria at different levels within a lake is governed by these four, and possibly other, variables. These variables will now be considered in detail.

2.5.2.1 The Effect of Sulphide:

The sulphide tolerance of the phototrophic bacteria varies widely. The Chlorobiaceae tolerate the highest sulphide concentrations of 128 - 256 mg/l while the Chromatiaceae withstand concentrations of 26 - 128 mg/l and the Rhodospirillaceae 13 - 64 mg/l (Pfennig 1975). Higher tolerances have been reported with growth of Chromatium spp. observed at 700 - 900 mg/l (Yakoleva and Novozhilova 1969, Lopanitsyna and Novozhilova 1969).

The observed stratification of these families of bacteria in lakes may be explained partly by their varying tolerance to sulphide.

TABLE 2.3

Microstratification of Phototrophic Bacteria in Two Meromictic Lakes in Michigan, U.S.A.
(Caldwell and Tiedje 1975 b).

-	Burke Lake			Wintergreen Lake		
Family	Depth(m)	Organism	% of Population	Depth(m)	Organism	% of Population
<u>Chromatiaceae</u>	8.5	<u>Thiospirillum</u>	50	2.6-3.5	<u>Thiopedia</u>	90%
	8.6	<u>Chromatium</u>	4	3.3-3.5	<u>Thiocystis</u>	4%
	8.7-9.0	<u>Thiocystis</u>	40			
<u>Chlorobiaceae</u>	9.0-9.1	<u>Pelodictyon</u>	50*	3.3-5.5	<u>Prosthecochloris</u>	80%
	9.0-9.5	<u>Chlorochromatium</u>	35*		<u>Clathrochloris</u>	8%

* estimated from the authors' data.

The sulphide concentration is also capable of selecting different genera and species within a family. In a continuous mixed culture of Chromatium and Thiocystis, Chromatium exhibited the faster growth rate at low sulphide concentrations while Thiocystis predominated numerically at higher concentrations (Van Germerden and Jannasch 1971, Veldkamp and Jannasch 1972).

Those organisms containing gas vacuoles appear to have a selective advantage at low sulphide concentrations (Pfennig 1975). Examples among the Chromatiaceae are Lamprocystis, Thiodictyon and Thiopedia.

Also various Rhodospirillaceae, notably Rhodopseudomonas sulphidophila and Rps. capsulata, are capable of growing at sulphide concentrations tolerated by the Chromatiaceae.

2.5.2.2 The Effect of Light Intensity:

The light intensity is important in selecting the genera which predominate in any niche. The motile phototrophic bacteria exhibit a positive phototaxis. Kondrat'eva (1965) provides a review of this subject. This characteristic allows these bacteria to grow in the maximum light intensity within the restrictions imposed by other parameters.

The in vivo absorption of the various bacteriochlorophylls in the near infra-red is also ecologically significant as photosynthetic eucaryotes do not absorb significantly at these wavelengths (Stanier and Cohen-Bazire 1957). Phototrophic bacteria may therefore grow under layers of algae (Naguib 1959) or aquatic plants such as Lemna (Kaiser 1966).

However, near infra-red light is absorbed very strongly in pure water while blue and blue-green light (450-550 nm) penetrates to the greatest depths (Wetzel 1975, Jerlov 1951). Consequently, in deep, clear environments such as clear lakes where plates of phototrophic bacteria have been found at depths of up to 50m (Ruttner 1963), the photosynthetic role of the carotenoids becomes important.

These pigments selectively absorb in the blue and blue-green regions of the spectrum (Stanier and Cohen-Bazire 1957) and their photosynthetic efficiency varies from 98% to 30-40% depending upon the bacteria (Goedheer 1959). Thus, carotenoid-rich species often inhabit regions of lakes (Pfennig 1967, Truper and Genovese 1968).

In water containing dissolved and particulate organic matter, the light absorption pattern changes markedly. Although the absorption by particulate matter is virtually independent of wavelength, dissolved organic matter absorbs selectively at wavelengths less than 600 nm (Wetzel 1975). Carotenoid-rich organisms possess few advantages under such situations and would therefore not be expected to be dominant in waste treatment systems.

The rate of oxidation of sulphide is light dependent up to a critical intensity (Truper and Schlegel 1964). Beyond this intensity light saturation occurs and substrate removal is strictly dependent upon the density of the bacterial suspension. The value at which light saturation occurs is dependent upon the organism and its environment (Truper and Schlegel 1964, Lippert and Pfennig 1969, Shaposhnikov et al 1961, Takahashi and Ichimura 1970). For Chromatium grown in artificial culture a value of 2,000 lux is commonly reported (Takahashi and Ichimura 1970, Truper and Schlegel 1964).

Also the bacteriochlorophyll content of the cells and their photosynthetic efficiency is inversely proportional to the light intensity. (Fuller et al 1963, Takahashi et al 1972). This appears to be a natural response to allow growth under poorly illuminated conditions.

Various light intensities, in conjunction with other variables, have been used to favour the growth of different Chromatiaceae by selective enrichment techniques (Van Niel 1971).

The duration of the photoperiod is also important in selecting the dominant organism. With intermittent light/dark illumination,

Chromatium vinosum SMG 185 and Chromatium weissei SMG 171 showed a balanced co-existence when grown in continuous culture. The steady state abundance of C. vinosum was positively correlated with the length of the light period and that of C. weissei to the length of the dark period (Van Germerden 1974).

2.5.2.3 The Effect of Oxygen:

The dissolved oxygen concentration plays an important role in determining the phototrophic bacteria present in any niche.

Many Rhodospirillaceae exhibit an aerobic metabolism (Pfennig and Truper 1974) while, of the Chromatiaceae only Thiocapsa roseopersicina (Pfennig and Truper 1974, Kondrat'eva et al 1975, Kondrat'eva et al 1976a) and Amoebobacter roseus (Gorlenko 1974) are capable of growth in the presence of oxygen. The Chlorobiaceae are strict anaerobes (Pfennig and Truper 1974).

Under poorly illuminated conditions, the motile Rhodospirillaceae exhibit a positive aerotaxis. This response becomes negative under high light intensities (Clayton 1958). Such action ensures that these organisms maintain themselves within the optimum environment available for growth.

The strictly anaerobic phototrophic bacteria only exhibit a negative aerotaxis (Kondrat'eva 1965).

2.5.2.4 The Effect of Organic Compounds:

The effect of organic compounds in selecting organisms is more difficult to ascertain than the three previous parameters.

Numerous organic compounds are utilised by the phototrophic bacteria and a variety of metabolic pathways are available for transformation of these molecules. These topics have been discussed in Section 2.4.3.2. However, these data have been obtained from pure culture studies in synthetic medium and there is a dearth of information on the selective role of organic compounds in a natural environment.

2.5.3 Phototrophic Bacteria in Ponds:

The phototrophic bacteria are widespread in stagnant ponds. In a survey of 37 ponds and canals, Kaiser (1966) demonstrated Chlorobiaceae, Chromatiaceae and Rhodospirillaceae were present in virtually every pond sampled even if there was no visible evidence of their presence. Blooms of these bacteria only occurred in the more polluted environments and, under these situations, the Chromatiaceae and the Chlorobiaceae predominated. Growth of the Chlorobiaceae was favoured by slightly acid conditions, while the Chromatiaceae preferred slightly alkaline conditions.

Chromatiaceae have also been shown to occur under a cover of Lemna in a 20 cm deep pond (Schlegel and Pfennig 1961). Although these bacteria are present throughout the year they regularly bloom from April to July. A succession of species has been noted, with the Chromatium okenii initially being dominant. As the sulphide concentration is reduced this organism is replaced by Thiospirillum jenense.

Large populations of Thiopedia have been found covering the mud surface of shallow marl ponds (Hirsch 1969).

The role of the phototrophic bacteria in ponds is similar to that in lakes, recycling fermentation end-products and reduced sulphur compounds.

2.5.4 Phototrophic Bacteria in Waste Treatment Lagoons.

2.5.4.1 Introduction:

The presence of phototrophic bacteria in facultative or anaerobic waste treatment lagoons has quite frequently been observed as the red colour produced is quite striking. However, few detailed studies have been made of this phenomenon.

Table 2.4 outlines some of these studies and indicates that the Chromatiaceae are the most common family of phototrophic bacteria occurring in such lagoons.

2.5.4.2 Common Genera of the Chromatiaceae in Lagoons:

Chromatium and Thiocapsa are the most common genera of the Chromatiaceae observed in waste treatment lagoons.

Table 2.4 implies that Thiopedia is also a common genus. It is believed that in some circumstances Thiocapsa has been misidentified as Thiopedia. The primary reason for this error is the description of Thiopedia given by the definitive texts of the time, Breed et al (1957) and Skerman (1967). Insufficient emphasis was placed on the presence of the readily observable gas vacuoles in the cells of Thiopedia. The more recent classification of Pfennig and Truper (1974) has clarified the differences between Thiocapsa and Thiopedia.

The probability of such a mis-identification is reinforced by studying the photo-micrographs of Green (1966) and May and Stahl (1967). No gas vacuoles are apparent and, on morphological grounds, the organism is probably Thiocapsa.

However, Cooper's (1963) photo-micrograph of bacteria from a lagoon treating rendering plant wastes does tend to confirm his identification of Thiopedia.

Sletten and Singer (1971) reported observing Rhodotheca in three lagoons treating pig, poultry and feed lot wastes respectively. The organism was described as being unicellular, spherical, occurring singly, in pairs or in short chains. It was non-motile. Breed et al (1957) and Skerman (1967) state that Rhodotheca contain gas vacuoles. Pfennig and Truper (1974) do not recognise Rhodotheca as a genus, classifying such organisms as Amoebobacter which also contains gas vacuoles.

It is therefore most likely that the bacteria described by Sletten and Singer belong to the genus Thiocapsa.

TABLE 2.4

Summary of Data on the Observation of Chromatiaceae in Lagoons.

Example No.	Location	BOD loading (kg/ha.d)	Effluent Treated	Lagoon Dimensions		Hydraulic Retention Time (days)	BOD Removal (%)	Dominant genus **	Permanent Bloom	Reference
				Area(ha)	Depth(m)					
1	Melbourne, Aust.	1850	Sewage	3.76	1.35	3.8	81	<u>Chromatium</u>	No	8
2	California, U.S.A.	644	Poultry Waste	0.30	1.70	infinity	-	<u>Thiocystis</u>	Yes	3
3	Melbourne, Aust.	334	Example 1 effluent	4.00	1.57	4.7	54	<u>Chromatium</u>	No	8
4	North Dakota, U.S.A.	270*	Sewage and Potato Wastes	28.3	1-1.5	-	-	<u>Chromatium</u> <u>Thiocapsa</u>	No	4,6
5	California, U.S.A.	255	Rendering	0.23	2.1	160	88	<u>Thiopedia</u>	No	3
6	Moerewa, N.Z.	170	Fellmongery	2.2	1.74	100	75	<u>Thiocapsa</u>	Yes	1
7	California, U.S.A.	60	Petroleum	58.3	1.4	9-45	39	<u>Chromatium</u>	No	2,3
8	Washington State, U.S.A.	-	Rat Wastes	0.025	0.70	-	-	<u>Thiopedia</u> <u>Chromatium</u>	No	5,7,9

* The potato season lasts from September to June and the wastes produced result in the loading quoted. During the rest of the year the loading averages 17 kg BOD/ha.d.

** The generic names are those used by the original authors.

Reference Key : 1. Cooper et al (1975)
2. Cooper (1963)
3. Cooper et al (1965)

4. Fillipi and Vennes (1971)
5. Green (1966)
6. Holm and Vennes (1970)

7. May and Stahl (1967)
8. Parker (1962)
9. Stahl and May (1967)

The confusion of such classification during the 1960's is typified by Pfennig's (1967) reidentification of Hurlbert and Lascelles (1963) Thiopedia as Thiocapsa.

2.5.4.3 The Occurrence of Chromatiaceae in Lagoons:

The Chromatiaceae become the dominant population in a wide variety of wastes treated by anaerobic lagooning. Some of these effluents possess no obvious properties which select for these organisms. Under these circumstances, physical and environmental parameters must favour their growth. Some such treatment systems will now be considered.

In Washington State, U.S.A., a lagoon system treating rat wastes was studied (Green 1966, May and Stahl 1967, Stahl and May 1967). The second of three lagoons contained a seasonal bloom of "Thiopedia". The rapid growth of this bacteria was associated with increasing lagoon temperature and the maximum number of 10^9 cells/ml was obtained at the end of July and the beginning of August. A slight decrease in BOD was observed with the onset of the bloom (Green 1966).

Neel (1963) identified Chromatiaceae in a lagoon system treating milk wastes. Chromatium and Thiospirillum were dominant during the spring while Thiopedia and many unidentified rods and spirilla were prevalent in the autumn.

In Melbourne, Australia, Chromatium spp. were identified in heavily loaded lagoons treating domestic sewage (Parker 1962). During the summer months they were the dominant population in the first two lagoons in a series of eight. Populations of 7×10^3 cells/ml and 7.1×10^6 cells/ml were observed in the first and second lagoons respectively. Concomitant BOD removals were 81% and 54%

Certain effluents do, however, appear to be selective for the growth of Chromatiaceae. For example, the anaerobic lagoon treatment of effluents containing a significant level of sulphates can result in these organisms contributing substantially to the lagoon population.

Espino and Gloyna (1967) studied sulphide production in a pilot scale lagoon and demonstrated that the lagoon's sulphide concentration was directly proportional to the influent sulphate concentration. The presence of reduced sulphur compounds in the influent could be expected to affect the model developed. Chromatium spp. predominated in this lagoon.

A series of studies (Hovious et al 1973) indicated that a 0.7m deep lagoon loaded at 14-16 kg COD/ha.d treating petrochemical wastes containing a high concentration of acetic acid could tolerate as much as 4,000 mg/l SO_4^{2-} without inhibitory concentrations of sulphide being produced. Such lagoons contained well developed populations of Chromatium and possessed a significant profile of sulphides ranging from 10 mg/l at the surface to 150 mg/l at the sludge interface.

Samples were incubated in these lagoons under ambient, diurnal light and temperature conditions. Near the surface the phototrophic bacteria were active with significant removals of COD, volatile acids and sulphide occurring. Bottles incubated at a depth of 0.915 m showed no significant difference between light and dark samples. Light transmission was presumed to be insufficient for photosynthetic activity at this depth.

Cooper and co-workers (1963, 1965) reported on a petroleum refinery lagoon which originally contained Euglena as the dominant organism and possessed a liquor D.O. of 6-10 mg/l. The addition of a waste stream containing sulphonates rapidly affected the flora of the lagoon. It shortly turned bright pink and a microscopic examination revealed the presence of Chromatium spp. The average BOD loading in this lagoon was 5.8 g BOD/m².d.

Over a period of 23 weeks the number of Chromatiaceae ranged from 1×10^4 cells/ml to 1.5×10^7 cells/ml.

A Chromatiaceae count in excess of 10^6 cells/ml resulted in the lagoon appearing pink. An algae count greater than 10^4 cells/ml and a Chromatiaceae count less than 10^6 cells/ml resulted in the lagoon appearing green. The former conditions were associated with BOD loadings higher than average and the latter with below average loadings.

Cooper et al (1975) describe a lagoon loaded at an average of $17.0 \text{ g BOD/m}^2 \cdot \text{d}$ treating settled fellmongery effluent. The lagoon influent contained approximately 95 mg/l sulphide and a pH of 12.5. When the lagoon was first commissioned, in the off-season of 1972, it was lightly loaded and colonised by Chlamydomonas and a diatom similar to Esterionella.

Once loaded with fellmongery effluent the lagoon turned red with patches of green algae and, after two months, was a brilliant red. Little odour was evident at this time and the dominant organism was identified as Thiocapsa roseopersicina at a level of 3.4×10^7 cells/ml.

Chromatiaceae also occur in the anaerobic zones of facultative lagoons (Green et al 1963) and their appearance as the dominant population in such lagoons has been associated with seasonal fluctuations in the algal population and lagoon overloading (Brockett 1975).

Thus, the Chromatiaceae occur in lagoons treating a variety of wastes. In some situations they form a stable dominant population and, in others, their pre-eminence is of a more transient nature. In this thesis (Chapter 4) a theory will be developed to account for the occurrence of these bacteria in different lagoon systems.

2.5.4.4 The Effect of Chromatiaceae on Effluent Quality:

As outlined in Section 2.4.2.2., the Chromatiaceae are capable of metabolising low molecular weight organic acids. This is essentially a direct assimilation of organic carbon into cell material. This characteristic associated with their ability to fix CO_2 indicates that the growth of these organisms could be detrimental to the quality of the lagoon's effluent.

Green (1966) has stated that although the Chromatiaceae are significant in reducing odours, their presence is detrimental to the treatment capability of lagoons. These conclusions were based primarily on data obtained from the growth of pure cultures of Chromatium and "Thiopedia" in a synthetic waste medium. However, in the lagoon situation, no increase in BOD was evident during the period of the bloom of "Thiopedia".

Neel (1963) noted a decrease in BOD and coliform removals when the Chromatiaceae were dominant in a lagoon treating milk wastes. While the purple sulphur bacteria were prevalent the BOD removal was 75% and the coliform removal was 96%. When algae predominated, the removals were 92% and 99.99% respectively.

Other authors have noted a decrease in BOD during blooms of Chromatiaceae. Vennes and co-workers (Fillipi and Vennes 1971, Holm 1969, Holm and Vennes 1970, Olsen et al 1968) observed a rapid decrease in BOD from 700-1000 mg/l to 100 mg/l while Thiocapsa and Chromatium were prevalent in a lagoon treating sewage and potato processing effluent.

Cooper and co-workers (1963, 1965) indicated that a lagoon treating petroleum refinery wastes operated more efficiently when Chromatiaceae rather than algae, were the dominant population. When the lagoon was pink the average BOD removal was 48.7% and when green it was 26.5%.

The interpretation of the role of the Chromatiaceae in such lagoon systems is difficult due to the presence of mixed populations. It is likely that other organisms, notably the Methanobacteriaceae, stabilise significant amounts of organic material. The effect of the Chromatiaceae is therefore somewhat difficult to establish.

2.6 TREATMENT OF INDUSTRIAL EFFLUENTS CONTAINING REDUCED SULPHUR COMPOUNDS.

Section 2.2.3 outlined some limitations of the traditional aerobic biological treatment processes in treating reduced sulphur effluents and subsequent sections (2.3.2, 2.4.1) indicated the ability of the Chromatiaceae to oxidise sulphur compounds under anaerobic conditions. To investigate the applicability of a treatment system utilizing these organisms, industrial sources of reduced sulphur effluents and their treatment methods will be briefly considered.

Reduced sulphur compounds may be an important and noxious constituent of effluents from the following industries :

- (i) Chemical pulping of wood (Klein 1959, Wilson 1960)
- (ii) Petroleum refineries (Beychok 1967, Weston et al 1961)
- (iii) Gas treatment plants (Hodge 1961, Moyes and Wilkinson 1974)
- (iv) Fellmongeries and Beamhouses (Cooper et al 1975).
- (v) Sulphur dyeing of textiles (Akhmedov and Garibov 1966, Beach and Beach 1956, Salmon 1976)
- (v) Viscose rayon manufacture (Koziorowski and Kucharski 1972)
- (vii) Synthetic phenol manufacture (Wilson 1960)

Due to the problems associated with the discharge of sulphur bearing effluents (Section 2.2.2), adequate treatment methods are essential. As the effluent characteristics vary from one industry to another, each industry has developed treatment systems to suit its particular requirements. However, the literature indicates a predominance of physical/chemical systems. This is primarily due to the effect of high sulphide concentrations on biological treatment systems (Section 2.2.3).

In New Zealand, the most important of these industries are the chemical pulping of wood and fellmongery and beamhouse processing.

However, in modern pulp mills sulphur compounds are not a significant component of the effluent. In the Kraft process, recovery of the chemicals from the spent cooking liquor is an economic necessity (Gurnham et al 1976) while in the sulphite processes chemical recovery is practised to allow effluent discharge requirements to be met (Canty et al 1973, Scott and Willard 1973). The removal of troublesome compounds, such as sulphides, from the effluent allows conventional biological processes to be used, with aerated lagoons and activated sludge systems being the most favoured (Johnson 1976).

Fellmongeries and beamhouses do discharge substantial quantities of sulphides and as few New Zealand processors possess adequate waste treatment systems (Mason 1974), the potential for the photosynthetic bacterial lagoon treatment of these effluents will be investigated. The following section seeks to describe the processes carried out in a fellmongery and a beamhouse, to consider the nature of the effluents produced and to present briefly the available technology for treating such wastewaters.

2.7 TREATMENT OF FELLMONGERY AND BEAMHOUSE EFFLUENTS.

2.7.1 Depilatory Processes:

Three stages may be discerned in the processing of animal skins. Initially a depilatory process removes the hair or wool. The skins are then conditioned in preparation for the final stage which is tanning.

Depilation and conditioning of lamb and sheepskins are performed in a fellmongery, cattle hides in a beamhouse. In N.Z., fellmongeries are usually attached to an export meat works and pelts are often exported in a semi-processed state for subsequent tanning. This differs from overseas practice in which fellmongeries are often an integral part of a tannery.

The 38 fellmongeries in N.Z., produce 28-35 million pelts annually (Mason 1974, New Zealand Official Yearbook 1975) ranking this country the third largest producer of pelts (Aloy et al 1976). The 9 beamhouses process approximately 200,000 cattle hides per year (Mason 1976, New Zealand Official Yearbook 1975).

In a fellmongery, depilation is commonly accomplished by applying a lime/sulphide paint to the flesh side of the washed skin. During overnight storage, the chemicals diffuse through the skin and the sulphide attacks the disulphide bond of the cystinyl residues of keratin. These bonds are weakest in the incompletely keratinised wool roots and their cleavage allows the recovery of the valuable wool. This is removed manually the following day.

Lime supplies the alkalinity necessary for this nucleophilic attack by sulphide which occurs only at a pH above 12 (Oldfield 1970). It also provides the viscosity necessary for the paint to adhere to the skin.

The depilated pelts are then subjected to a series of conditioning operations. During liming, overnight agitation in a lime/sulphide solution removes any remaining wool, plumps the collagen and opens the fibres to allow good penetration of the tanning chemicals. Deliming follows in which the pelts are agitated in a weak acid solution, usually $(\text{NH}_4)_2\text{SO}_4$, NH_4Cl or H_3BO_3 . Excess lime is removed from the pelts reducing their pH to a value between 8 and 9. Pancreatic enzymes are used in the bating stage to open the fibre structure more fully and cleanse the grain of detritus loosened in previous processes. Finally, pickling with NaCl and H_2SO_4 preserves the pelts during transportation.

Beamhouses are usually associated with tanneries. They receive hides covered with salt and other preservatives (Oldfield 1970). Soaking removes these chemicals and any attached dirt and blood. Hair recovery is uneconomic and is not practised. Depilation is accomplished in the liming stage in one of two ways. The hair burning process uses a high sulphide concentration to totally solubilise the hair. A lower sulphide concentration is used in the hair saving process which partially degrades the keratin. Hair can then be removed from the effluent by screening, thereby reducing the BOD load. Subsequent processes are similar to those practised in fellmongeries.

2.7.2 Effluent Characteristics:

Effluents from beamhouses have been more frequently characterised than those from fellmongeries. The available data has been reduced to a common form to illustrate the extent to which each process contributes to the total effluent (Tables 2.5.1 - 2.5.4). It is apparent that the liming effluent dominates the characteristics of the total effluent.

Soaking produces a relatively large volume of moderately strong, neutral effluent. It contains blood, soluble proteins, naphthalene, NaCl and Na_2CO_3 (Anonymous 1969). The liming process yields a relatively large volume of a strong alkaline waste. This effluent contains sulphide, bisulphide, Ca^{2+} , dissolved albumin, mucoids,

TABLE 2.5

Composition of Fellmongery and Beamhouse Effluents:-Process Contributions as Percentages of Total Load:2.5.1 Volume

Reference Process	Volume discharged ($\frac{V}{W} \%$)						
	1	2	3	4	5 *		
					A	B1	B1
soaking	43	35	47	37	47	52	46
unhairing	54	36	35	42	26	24	32
deliming/bating	3	14.5	11.5	14	18	16	15
pickling	-	14.5	6.5	7	9	8	7

2.5.2 BOD, COD

Reference Process	BOD ($\frac{W}{W} \%$)									COD ($\frac{W}{W} \%$)	
	1	2	3	4	5 *				6	7	
					A1	A2	B1	B2		1	2
soaking	20	26	20	14	30	26	28	29	19	37	20
unhairing	76	63	54	78	54	60	56	68	68	50	73
deliming/bating	4	4	13	8	17	14	16	12	6	8	4.5
pickling	-	7	4	-	-	-	-	-	7	5	2.5

*A = older technology (as defined by USDI 1968)

B = prevalent and newer technology (as defined by USDI 1968)

1 = hair saving

2 = hair burning

TABLE 2.5 (cont'd)

Composition of Fellmongery and Beamhouse Effluents:-Process Contributions as Percentages of Total Load:2.5.3 Total Solids

Reference Process	w/w %							
	1	2	3	5 *				6
				A1	A2	B1	B2	
soaking	30	22	37	50	40	50	34	16
unhairing	69	38	45	39	50	40	59	46
deliming/bating	1	3	5	7	6	6	4	5
pickling	-	37	13	4	4	4	3	33

2.5.4 pH

Reference Process	1	2	8	6
soaking	7.45	6.1	7.2	6.2
unhairing	-	11.6	11.1-12.1	11-12
deliming/bating	6.7	8.2	8.8	8
pickling	-	2.4	-	2.5

* A = older technology (as defined by USDI 1968)

B = prevalent and newer technology (as defined by USDI 1968)

1 = hair saving

2 = hair burning

Reference key:

<u>Reference No.</u>	<u>Reference</u>
1	Riffenberg and Allison (1941)
2	Sutherland (1947)
3	Masselli et al (1958)
4	Eckenfelder (1970)
5	U.S.D.I. (1968)
6	Del Pezzo and Simoncini (1958)
7	Van Vlimmeren and Van Meer (1974)
8	Tomlinson et al (1969)

mucopolysaccharides, keratin, dissolved and emulsified fats and insoluble organic and inorganic compounds (Cooper et al 1974). Deliming and bating produce a moderate volume of a slightly alkaline, medium strength waste. This effluent contains Na_2SO_3 , ammoniacal salts and mineral or organic acids (Anonymous 1969). Pickling produces a small volume of a dilute, acidic effluent. In some beamhouses no pickle liquor is discharged. If produced, it will contain NaCl and mineral or organic acids.

Table 2.6 compares some typical fellmongery effluents. However, process modifications will alter the effluent characteristics. Some workers (Folachier 1975, Miller 1970, Money and Adminis 1974) have shown that lime liquors may be recycled up to twenty times with no adverse effects on the pelts or hides. A reduced volume of concentrated effluent results.

Due to the effluent problems created by the lime/sulphide process, alternative depilatory techniques have been investigated (Frendrup 1974). In New Zealand, it is unlikely that these alternative methods will be used widely in the near future (Mason 1972, Vivian 1972).

2.7.3 Treatment Technology:

Methods of treating fellmongery and beamhouse effluents are reviewed in a chronological order to demonstrate the significant technological developments. It will be shown that although research indicates a trend towards biological treatment methods, physical/chemical treatment systems currently predominate.

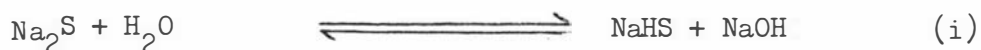
2.7.3.1 Precipitation with Metal Salts:

Certain metal salts may be used to precipitate sulphide from solution. Traditionally, ferrous sulphate is used. The pH is reduced from 11 - 12 to 8 - 9 mainly through the removal of alkalinity due to the sulphide-hydrosulphide equilibrium (Oger and Lassere 1969, Scholz 1957).

TABLE 2.6

Composition of Total Fellingmongery Effluent

Parameter (mg/l except pH)	Reference	Sawyer 1965	Cooper et al 1975	Cooper 1973	BLMRA 1975	Ryder 1972
pH		11.7-12.1	12.6	12.0-12.3	12.0-12.5	9.5-12.4
BOD		640-1,670	2,290	1,224	2,000	1,100-2,400
COD		-	6,311	6,109-12,202	3,750	760-5,920
Total Solids		-	14,360	7,853-18,100	-	-
Suspended Solids		400-1,660	3,800	1,560-1,930	1,700	7,360-15,097
Settleable Solids		-	56	12-23	-	539-1,457
Grease		120-340	384	196-254	350	-
Total Nitrogen		-	-	243-420	-	40
Sulphide		-	97	52-93	150	10-150
Chloride		-	105	1,675-1,833	2,500	3,000



Some alkalinity due to lime is also removed by atmospheric carbonation.

The major disadvantage of this method is that the precipitated FeS is colloidal, flocculating only within a narrow pH range (Scholz 1959). Lack of control results in the black FeS imparting a residual colour to the effluent. The resulting sludge is bulky and difficult to dewater (Oger and Lassere 1969).

These problems were partially overcome by adding ferrous bicarbonate to the effluent from the ferrous sulphate process. This allowed the control of the pH between 6.0 and 8.5. Below pH 8.2 many effluent proteins become insoluble due to iso-electric effects, giving an increased removal of organic compounds. These precipitated proteins adsorb the FeS and, after sedimentation, a clear effluent is produced. However, very large volumes of a sludge which is difficult to dewater are produced. This method is used successfully in the treatment of 5000 m³/day of chrome tanning effluent (Scholz 1963a).

A further development was the oxidative FeSO₄ process. Sufficient FeSO₄ is added to achieve a neutral solution. The effluent is then passed to an aeration unit where part of the Fe²⁺ is oxidised to Fe³⁺. This results in the oxidation of sulphide to elemental sulphur which is adsorbed onto the surface of the air bubbles. These rise to the surface where the sulphur is removed. Another oxidation product, Fe(OH)₃, also aids in the flocculation process, reducing the volume of the sludge and making it easier to dewater (Scholz 1959, 1967, Wolff 1970). Scholz (1959) describes a plant using this process to treat 11,000 m³/day of tannery effluent. 4,200 kg of FeSO₄·7H₂O are consumed daily and 250 m³/day of sludge are produced. On drying, this sludge is reduced to 13 m³.

The economics of the ferrous salt processes are becoming less favourable as FeSO_4 , once available as a cheap by-product of the steel industry, is becoming increasingly expensive (Scholz 1959, Wolff 1970).

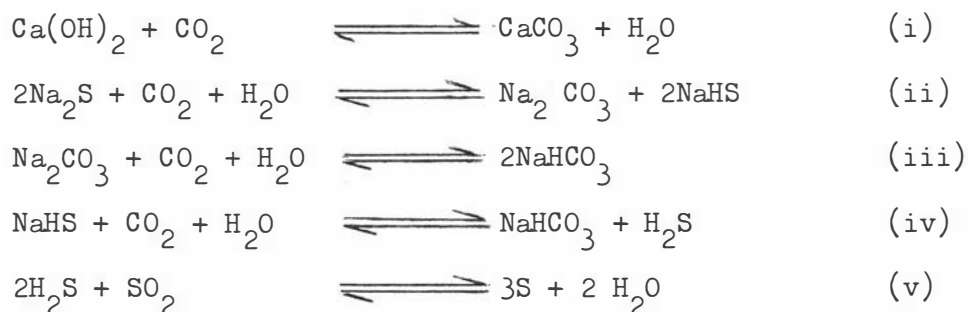
$\text{Al}_2(\text{SO}_4)_3$ may be used as a flocculant but only with prior sulphide removal since its hydrolysis results in the evolution of H_2S (Oger and Lassere 1969). Munteanu and Weiner (1963) describe a plant using such a process to treat a mineral tanning effluent. This method proved expensive and produced a poor sludge.

2.7.3.2 Chemical Oxidation:

Various agents may be used to oxidise the sulphide present in lime liquors. Air is most commonly used, superceding SO_2 available in flue gas. Although laboratory trials appear promising (Zehender 1970), no commercial applications of chlorination have been reported.

2.7.3.2.1 Sulphur dioxide:

When flue gas is bubbled through lime liquors the following series of reactions occur (Scholz 1967).



Reaction (i) occurs almost instantaneously removing the alkalinity due to lime by precipitating CaCO_3 . Reaction (ii), in combination with reaction (iv), removes the alkalinity due to sulphide. This neutralisation results in the evolution of H_2S which is oxidized by SO_2 (reaction (v)). The resulting liquor has a high concentration of Na_2CO_3 and NaHCO_3 which exercises a strong buffering action which is useful for neutralising other tannery effluents.

This process was introduced in the early 1960's to minimise the sludge handling problems associated with the FeSO_4 processes (Scholz 1963b). The characteristics of one plant reported are given in Table 2.7 (Agapoff 1969). Similar results are reported by other authors (Corning and Robinson 1972, Degermann 1970, Scholz 1963b). Prior to discharge, biological treatment (Agapoff 1969) or a substantial dilution with other waste streams (Degermann 1969) is necessary.

As no chemicals need be added, this process has a relatively low operating cost provided corrosion can be minimised. This may be achieved by use of non-corrosive coatings on all metal surfaces (Degermann 1970) but even so the life of such reactors is 6 - 8 years. Also, the capital cost of a flue gas treatment plant is high (Agapoff 1969).

Because of corrosion, foaming and odour problems, a French tannery has recently abandoned its flue gas plant in favour of a catalytic oxidation system followed by biological treatment (Vulliermet and Aloy 1974).

TABLE 2.7

The effect of Flue Gas Treatment on Lime Liquors (Agapoff 1969).

Parameter (mg/l except pH)	Before Treatment	After Treatment
pH	11.5-12.5	7.5-8.0
Sulphide	1,000-1,100	300-400
Suspended Solids	23,000	18,400
Settleable Solids	5,400	7,700
COD	13,200	9,500
BOD	2,760	2,330
Nitrogen	709	578

2.7.3.2.2 Oxygen:

Aeration is the most common method of introducing oxygen to the effluent. Alternative processes include the addition of ozone (Eye and Clement 1972) and hydrogen peroxide (Aloy et al 1976, Raleigh 1975). Although successful both are limited by the high cost of operation.

The uncatalysed oxidation of sulphide solutions by air is slow and exhibits a complex reaction mechanism, the stoichiometry of which varies with temperature (Murray 1959), pH (Chen and Morris 1972) and initial sulphide (Bowers et al 1966) and oxygen concentrations (Chen and Morris 1972). To explain these observations Chen and Morris (1972) postulated an autocatalytic reaction based upon polysulphide under slightly acid or alkaline conditions. Other work supports these observations (Bowers et al 1966, Cooper 1974, Murray 1959, Tsuchiya and Johnson 1972). Initially sulphide reacts with oxygen to form polysulphides. A subsequent chain reaction involves the reaction of polysulphides to form sulphate via thiosulphate and sulphate (Chen and Morris 1972).

As the oxidation of sulphide solutions by air is so slow, catalysts are added to render the process a viable waste treatment method. The first practical application of the system was in 1933 when nickel and copper salts were used in conjunction with pH adjustment and aeration to reduce the level of soluble sulphides in the bleed water from sulphur wells (Schwab and Butterworth 1933). Subsequently the British Leather Manufacturers Research Association applied this principle to the treatment of beamhouse and fellmongery effluents (Bailey and Humphreys 1966a, 1966b, 1967, Pepper 1966). Development in South Africa (Berg et al 1967), France and Holland (Van Vlimmeren et al 1967, 1969) followed rapidly until 1973 when a satisfactory stage of development was reported. At this time 49 plants were operating in Europe with capacities of 4 - 1,200 m³/day (Bailey and Van Meer 1974).

Of the catalysts investigated, most belong to the fourth period transition elements. Their activity in beamhouse effluent

has been ranked in decreasing order :

Mn^{2+} , Co^{2+} , Ni^{2+} , Fe^{2+} , Cu^{2+} (Bailey and Humphreys 1966a, 1967).

This order varies slightly with pH and catalyst concentrations in aqueous sulphide solutions (Chen and Morris 1972, Krebs 1929).

Manganese salts also have the advantage of low toxicity and have gained wide acceptance as the preferred catalyst. The relative performance of $MnSO_4$, $KMnO_4$, $MnCl_2$ and MnO_4 have been investigated (Eye and Clement 1972) with $KMnO_4$ being the most effective catalyst. However, $MnSO_4$ is often used because of its lower cost (Corning 1976, Eye and Clement 1972, Rawlings et al 1975b).

Only a small proportion (4 - 11%) sulphide is catalytically oxidised to sulphate (Bailey and Humphreys 1966a, 1967). Partially oxidised intermediates are produced with thiosulphate the most commonly reported (Bailey and Humphreys 1966a, 1967). Elemental sulphur is not usually a significant product although its presence has been recorded (Bailey and Van Meer 1974, Zehender 1970).

Catalytic oxidation using diffused aeration systems generally proved unsatisfactory (Bailey and Humphreys 1966b, 1967, Berg et al 1967, Eye and Clement 1972, Van Vlimmeren and Van Meer 1974). In alkaline solutions, Mn^{2+} is principally present as the insoluble hydroxide which may be adsorbed onto either hair particles or air bubbles and carried to the surface. It is then retained in the surface wool mat, effectively lowering the concentration of catalyst in solution (Berg et al 1967, Van Vlimmeren and Van Meer 1974). Mechanical aeration systems have been used successfully as they minimise this effect (IULCS Effluent Commission 1973). Systems investigated include paddle aerators (Berg et al 1967, Van Vlimmeren et al 1969), surface aerators (Aloy 1974, Bailey 1970, Bailey and Robinson 1969, Vulliermet and Aloy 1974), modified beamhouse drums (Van Vlimmeren et al 1969, fixed spiral aerators (Corning 1976), and jet pumps (Corning 1976). In Europe, surface aerators are the most popular system (Corning 1976).

Catalytic oxidation during the latter stages of liming has also been studied (Berg et al 1967, Van Vlimmeren et al 1969). Although offering significant capital savings in effluent treatment

equipment, this practice is impractical as oxidation rates are lower than those achieved by post-liming oxidation (Berg et al 1967). This leads to extended liming times and adversely affects the availability of processing equipment. (IULCS Effluent Commission 1972).

Table 2.8 presents operating conditions and oxidation rates of catalytic oxidation systems calculated from published data. There is a great variation in rates and the multiplicity of systems, catalysts, effluents and operating parameters makes a detailed analysis difficult.

The prevalence of full-scale systems at the bottom of the Table and laboratory-scale systems at the top indicates that aeration may be limiting in the former case. Agitation and aeration is likely to be more efficient in laboratory systems and hence higher oxidation rates will result. It is possible that the importance of these parameters is being ignored in the scale-up of aeration systems.

A wide range of catalyst concentrations is also used. The $\text{Mn}^{2+}/\text{S}^{2-}$ ratio allows the most meaningful comparison of catalysts and a ratio of 0.10 has been shown to give the most economic catalyst use (Eye and Clement 1972). Situations with a high $\text{Mn}^{2+}/\text{S}^{2-}$ ratio and a low oxidation rate indicate that aeration is limiting. This is most clearly demonstrated by the data of Thorstensen (1976). Although an $\text{Mn}^{2+}/\text{S}^{2-}$ ratio of approximately 0.50 is used the oxidation rate is only $55 \text{ mgS}^{2-}/\text{l.hr.}$

2.7.3.3 Acidification Processes:

The acidification of lime liquors results in the evolution of hydrogen sulphide gas (Arnould 1969):

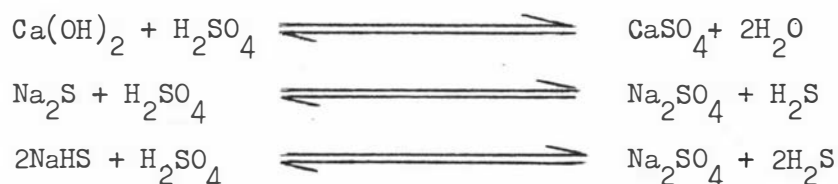


TABLE 2.8

Summary of Operating Parameters and Oxidation Rates for the Treatment of Fellmongery and Beamhouse Effluents by Catalytic Oxidation Systems.

Catalyst		Aeration System	Scale	$[S^{2-}]_i$ (mg/l)	Oxidation Rate (mgS ²⁻ /l.hr)	Reference
Type	Concn. (mg/l)					
KMnO ₄	476-572	D.A.	L	4,400-5,650	3,500-7,300	6
KMnO ₄	100	S.A.	L	2,000	1,440	4
MnCl ₂	100	D.A.	L	2,600	420-2,170	2
MnSO ₄	70	P.A.	P	1,900	1,200	3
MnSO ₄	100	S.A.	L	2,000	980	4
MnCl ₂	50-200	D.A.	P	2,590-3,010	420-1,040	2
MnCl ₂	100-200	D.A.	P	1,040-2,430	180-670	2
Mn ²⁺	44	S.A.	L	2,000	500	9
MnCl ₂	38-200	D.A.	P	560-805	100-330	2
MnSO ₄	20-400	P.A.	P	1,500-3,000	80-300	10
MnSO ₄	28-350	-	L	2,060-3,500	19-270	5
-	-	D.A.	F	1,300	200	1
CoCl ₂	40	S.A.	F	583	80	1
MnSO ₄	500	D.A.	F	233	55	8
MnSO ₄	25-100	D.A.	L	870	15-20	7
MnSO ₄	50	D.A.	F	120-160	12-15	1

A zero - order oxidation rate is assumed for the above table.

Key : D.A. = diffused aeration

P.A. = paddle aeration

S.A. = surface aeration

L = laboratory scale

P = pilot scale

F = full scale

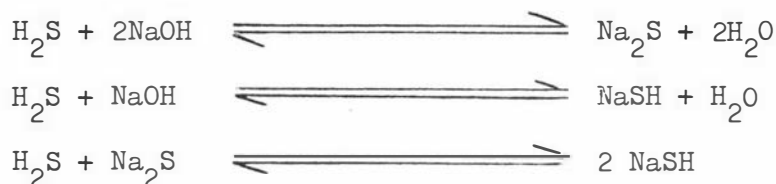
$[S^{2-}]_i$ = initial sulphide concentration

Reference Key:

1. Aloy (1974)
2. Bailey and Humphreys (1967)
3. Berg et al (1967)
4. Corning (1976)
5. De (1973)

6. Eye and Clement (1972)
7. Rawlings et al (1975b)
8. Thorstensen (1976)
9. Van Meer (1973)
10. Van Vlimmeren et al (1969)

This can then be recovered by passage through an alkaline solution to yield a mixture of HS^- and S^{2-} .



Acidification, although slower than catalytic oxidation, results in a greater reduction in Permanganate Value due to the volatilisation of H_2S (Rawlings et al 1975a). Catalytic oxidation forms sulphur oxyanions which still possess an oxygen demand.

Only lime liquors are treated by this process (Arnould 1969, Grosjean 1970). After sedimentation to remove excess lime, they pass to the reaction vessel where the retention time is 1 - 2 hours and the pH is reduced to 5.0. Recycled air is passed through the liquor to strip out the H_2S . The gas stream carries the sulphide to an absorption column where it is stripped into a sodium hydroxide solution. The resulting solution may be used for liming. Sulphide recoveries of 50 - 97% are reported (Arnould 1964, Grosjean 1970, Harenberg and Heidemann 1974).

Acidification may also result in the precipitation of proteins. Pretreatment or recovery of the sulphides is necessary. Initially this technique was conceived solely as a waste treatment process and the acidification of the lime liquor to pH 3.5 resulted in a 90% COD reduction (Van Meer 1973). Subsequent workers (Happich et al 1974, Shivas 1976) purified the precipitates aiming to market this product as a feedstuff. Such a process has not proven economic (Shivas 1976).

2.7.4 Biological Treatment Processes:

The high pH and sulphide concentration of fellmongery effluents were responsible for the belief that biological treatment of such wastes was impractical (Scholz 1963b), and resulted in the subsequent development of the numerous physical/chemical systems outlined.

More recent work has indicated the potential of various biological treatment processes.

2.7.4.1 Activated Sludge:

Although activated sludge systems are difficult to control and are sensitive to the shock loads characteristic of fellmongery discharges, their application to these effluents has evolved from the catalytic oxidation process.

Acclimated biomass increased the oxidation rate of sulphide in the presence of manganese (Hill 1972, McDougall et al 1972, Ryder 1973) and in a neutralisation system (Shuttleworth et al 1974), a reduction in permanganate value (P.V.) greater than could be accounted for solely by the oxidation of sulphide was observed. As counts of 2.9×10^9 cells/ml were indicated and the pH in the aeration unit was approximately 7.0, some bacterial oxidation of organic compounds could be postulated. The importance of micro-organisms in the oxidation of fellmongery effluent was confirmed by the aeration of a chemically sterilised and an unsterilised effluent over a period of four days (Rawlings et al 1975b). The unsterilised effluent showed a 75% decrease in P.V. while a 27% reduction was noted for the sterilised effluent.

The activated sludge treatment of fellmongery effluent without pretreatment has only recently been investigated. Using laboratory scale equipment, the effect of varying the dilution of the effluent at a retention time of two days and a sludge age of five days was studied (Rawlings and Wood 1976). Provided the P.V. of the influent was maintained below 1500 mg/l the system operated effectively giving P.V. reductions of greater than 72%, complete sulphide removal and pH reduction from 12.3 to 8.3. The influent sulphide concentration was 884 mg/l at a P.V. of 1500 mg/l

Further work (Every et al 1976) investigated the effect of varying sludge ages and retention times using a dilute fellmongery effluent (BOD 500 - 540 mg/l, P.V. 520 mg/l). A sludge age of

five days and a one day retention time gave 80% reductions in BOD and P.V. The sulphide concentration was reduced from 440 mg/l to 1.0 mg/l. Varying the sludge age from two to twenty days at a liquor retention time of two days did not affect the process significantly.

The effect of lower retention times has also been investigated (Cooper et al 1976). Using a lime liquor diluted 20:1, a laboratory scale system was operated for a few days at a retention time of ten hours, a mixed liquor suspended solids concentration of 3,000 - 3,900 mg/l and a sludge age of 0.74 - 0.96 days. The results obtained are presented in Table 2.9.

Laboratory-scale activated sludge systems are therefore capable of treating relatively dilute fellmongery effluent. Research with larger scale equipment is required to investigate suitable operating conditions and the long-term stability of such systems more closely.

The microbiology of the process, in which the thiobacilli could be expected to play a dominant role, also requires elucidation.

TABLE 2.9
Activated Sludge Treatment of Diluted Lime Liquor (Cooper et al 1976).

Parameter (mg/l)	Influent	Effluent	% Removal
BOD	1584	289	85
Suspended Solids	1228	71	94
Total Nitrogen	228	89	61
Organic Nitrogen	195	36	82
Sulphide	175	14	92
COD	2219	370	83

2.7.4.2 Biofiltration

Much of the work published on this topic has been performed in N.Z. Table 2.10 records the data from a pilot-scale Flocor tower operated by a meat works (Halliburton 1972, Hicks and Martin 1973).

Batch operation of a pilot-scale stone-packed filter has also been investigated (Ryder 1973). COD loadings of $0.94 - 1.57 \text{ kg/m}^3 \cdot \text{d}$ were applied using an eight-hour day and a five day week. The recycle ratio (RR) was varied from 5:1 to 34.5:1. The results obtained are shown in Table 2.11. Continuous loading of $2.23 \text{ kg COD/m}^3 \cdot \text{d}$ at 21°C and a recycle ratio of 12.2:1 gave an average COD removal of 53% and an average sulphide removal of 90.3%. The effluent pH averaged 8.20. Maximum COD removals occurred when the influent sulphide concentration was in the range 90-110 mg/l (Melcer and Ryder 1975, Ryder 1973).

TABLE 2.10

Performance of a Pilot Scale Flocor Biofilter Treating Fellmongery Effluent (Halliburton 1972, Hicks and Martin 1973).

Parameter (mg/l except pH)	Influent		Effluent		Removal (%)
	Range	Average	Range	Average	
pH	-	-	8.4-11.3	9.0	-
BOD	1,100-2,400	1,571	600-1,700	943	40.0
COD	760-8,640	3,208	680-3,750	1,766	45.0
S ²⁻	40-150	80	0-10	3.7	95.4

The operating conditions were :

organic loading : $4.5 \text{ kg BOD/m}^3 \cdot \text{d}$
hydraulic loading : $43 \text{ m}^3/\text{m}^3 \cdot \text{d}$
recycle ratio : 8:1

TABLE 2.11

Performance of a Batch-Loaded Stone-Packed Biofilter Receiving Screened Fellmongery Effluent (Ryder 1973).

Parameter	Influent range (mg/l except pH)	Recycle Ratio				
		5:1	9.3 :1	14.5:1	27.7:1	34.5:1
COD	2349-3919	53.3	64.5	57.3	67.7	62.7
S ²⁻	66-95	90.5	93.8	96.9	97.1	100
pH	9.85-11.45	8.44	8.42	8.40	8.63	8.36

The figures in Table 2.11 are % removal except for pH

A study of the microbiology of the filter showed that Thiobacillus thioparus was the major organism responsible for sulphide oxidation. Chromatiaceae were also isolated from the inside of a clear plastic recirculation line (Ryder 1973).

A high-rate pilot-scale Floccor tower has also been used to upgrade pretreated fellmongery effluent (Bailey et al 1972). The plant was initially operated for a year at a 3:2 recycle ratio and a hydraulic loading of $4.0 \text{ m}^3/\text{m}^3 \cdot \text{d}$ with the influent averaging 673 mg/l BOD and a pH of 7.4. The BOD removal averaged 37% at an applied loading of $2.84 \text{ kg BOD}/\text{m}^3 \cdot \text{d}$. The recycle ratio was then reduced to 1:1 altering the hydraulic load to $6.0 \text{ m}^3/\text{m}^3 \cdot \text{d}$, 43% of the applied BOD was removed at a loading of $4.10 \text{ kg BOD}/\text{m}^3 \cdot \text{d}$. The increased performance at higher loadings was due to plant modifications resulting in increased ventilation.

2.7.4.3 Lagoons:

There is little information available on the lagoon treatment of fellmongery and beamhouse effluents. An anaerobic lagoon receiving settled fellmongery effluent, in which Thiocapsa roseopersicina was dominant has been described (Cooper et al 1975). Operating at an average hydraulic retention time of 100 days, the sedimentation tank/lagoon system achieved a remarkable degree of treatment:

91% COD removal, 89% BOD removal, 99% sulphide removal, 62% removal of total solids and a pH reduction from 12.5 to 7.5. Populations of 3.4×10^7 cells/ml of Thiocapsa were recorded.

Also the occurrence of Chromatiaceae in some aerated lagoons treating tannery effluent has been observed (Aloy et al 1976).

This lack of information of the lagoon treatment of fellmongery effluent stimulated much of the research described in this thesis.

2.7.5 Conclusions:

Limitations exist with the physical/chemical treatment methods, their major function being the removal of sulphide and reduction of alkalinity prior to biological treatment or sewer discharge. All of these plants require quite large capital expenditure and the catalytic oxidation, acidification and metal salt precipitation processes have significant operating costs, primarily due to chemical addition. Acidification partially defrays this expense by sulphide recovery and, possibly in the future, protein recovery. In many instances, major operational problems also exist.

Biological treatment systems require further research but indicate potential. Activated sludge systems give adequate treatment of dilute fellmongery effluents but the long term stability and ease of operation of this process has yet to be confirmed. Bio-filtration has been performed successfully on fellmongery effluents containing less than 110 mg/l sulphide. However, lagoons containing Chromatiaceae also give adequate treatment with the advantages of cheapness and ease of operation. Further investigation of this process is therefore warranted.

2.8 GENERAL CONCLUSIONS FROM LITERATURE SURVEY:

This literature survey has indicated three aspects of the occurrence of the Chromatiaceae in waste treatment lagoons which require further elucidation.

Firstly, the effects of various environmental parameters on the growth of these bacteria must be determined. The importance

of these parameters may be assessed by using an experimental design to screen the variables. Such an investigation is presented in Chapter 3.

Further, a detailed study of the occurrence of these bacteria in lagoon systems is required. The genera and species which develop in such lagoons may thus be determined and a general theory for their occurrence formulated. Such a study is presented in Chapter 4.

Finally, the ability of the Chromatiaceae to oxidise reduced sulphur compounds under anaerobic conditions is worthy of exploitation. In New Zealand, anaerobic photosynthetic lagoons are most applicable to the treatment of fellmongery effluent and experiments performed to determine design data for such lagoons are presented in Chapters 5 and 6.

CHAPTER 3

STUDIES ON THE GROWTH OF CHROMATIUM MINUTISSIMUM IN BATCH CULTURE

3. STUDIES ON THE GROWTH OF CHROMATIUM MINUTISSIMUM IN BATCH CULTURE

3.1 Introduction:

A survey of the relevant literature (Chapter 2) has indicated a dearth of information on the effects and interactions of environmental parameters on the growth of those Chromatiaceae which occur in waste treatment lagoons. Therefore a species of Chromatiaceae was isolated from a lagoon in which they were known to occur, and subsequently identified. Assuming this bacterium was typical of those occurring in such lagoons, a screening experiment was performed to determine the environmental factors important for their growth and any interaction between these parameters.

This study utilised a Chromatium species, one of the two important genera of Chromatiaceae occurring in the lagoons. Further experiments must be conducted to determine if the results obtained are applicable to the other genus Thiocapsa.

The effects of temperature, pH, sulphide and acetate concentrations ^{and light intensity} were the environmental factors considered to be worthy of investigation. Chromatiaceae isolated from anaerobic lagoons had a temperature optimum of 25-30°C (Holm and Vennes 1970, Meredith and Pohland 1970). Little investigation of the growth rate has been made at the lower temperatures likely to prevail in New Zealand during the cooler months.

The optimum pH for Chromatiaceae lies in the range 6.5 to 8.0 (Holm and Vennes 1970, Meredith and Pohland 1970, Pfennig and Truper 1974). In view of the intended use of these bacteria to treat alkaline fellmongery effluents, the investigation of their growth under more alkaline conditions was warranted. Acid conditions were considered less important as below pH 6.0 substantial volatilisation of H₂S occurs (Figure 2.1).

The sulphide tolerance of the Chromatiaceae has already been considered (Section 2.5.2.1). In this experiment, high concentrations were investigated to assess the feasibility of using this bacterium to treat concentrated fellmongery effluent which may contain in excess of 1,000 mg/l sulphide. However, to determine growth rates at concentrations typical of lagoons, low levels were also studied.

As acetate is metabolised by all Chromatiaceae (Section 2.4.3.3) and is an important metabolic intermediate under anaerobic conditions (McCarty 1964), the effect of a wide range of concentrations of this compound on growth rates was studied.

The effect of light intensity on the growth of the phototrophic bacteria has already been outlined (Section 2.5.2.2). A range of light intensities from very low to above saturation were investigated. Emphasis was placed on studying the lower range of light intensities occurring in waste treatment lagoons as it is these that will limit the photosynthetic ability of bacteria in such situations. Above saturation, little alteration in the rate of photosynthesis should occur.

The levels of the five independent variables selected for the experimental design are presented in Table 3.1. (Page 69).

3.2 Isolation and Identification of Chromatium minutissimum from an Anaerobic Lagoon:

On March 13, 1974, the supernatant liquor of an anaerobic lagoon treating screened and settled meatworks effluent was sampled. At this time there was no visible evidence of the presence of the Chromatiaceae in the lagoons.

Initial enrichment cultures of the Chromatiaceae were developed using Winogradsky columns as described by Van Niel (1971). These columns were incubated at 30°C in a light intensity of 1,000 lux and at 15°C in a lower light intensity of 250 lux. The former

conditions are selective for Thiocapsa, Thiocystis, Amoebobacter and the small species of Chromatium while the latter favour the growth of the large Chromatium species such as C. okenii and C. weissei (Van Niel 1971).

After three weeks there was visible growth in the 30°C enrichment culture. Microscopic examination showed the dominant microorganism was a small ($1 - 1.5\mu \times 2 - 3\mu$), motile rod containing intracellular sulphur granules. The sulphur granules were identified by their solubility in ethanol (Van Gernerden 1968a).

No growth occurred in the 15°C enrichment culture.

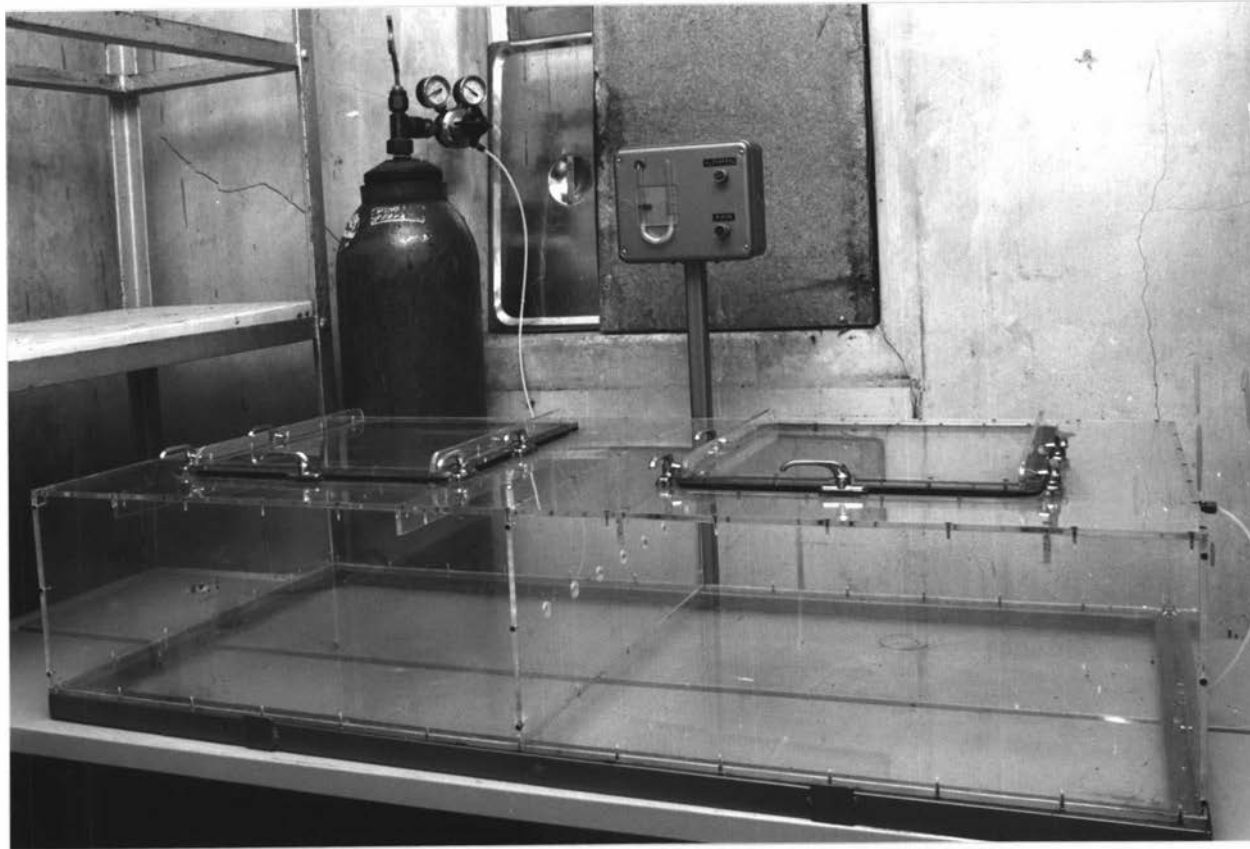
Further enrichment of the small rods in the 30°C culture was achieved by four serial transfers through an aqueous medium described by Pfennig and Lippert (1966). This medium will be referred to as Pfennig's medium. No cyanocobalamine was added to the enrichment cultures of this medium and they were incubated at 30°C and in a light intensity of 1,000 lux.

Final isolation of the bacterium was achieved by four serial passages through plate cultures of Pfennig's medium containing 3% agar. These plates were incubated at 30°C and approximately 1,000 lux in a specially constructed anaerobic incubator (Figure 3.1).

Microscopic examination indicated the colonies were of a small rod morphologically similar to the dominant organism in the first enrichment culture.

A single colony was then transferred to the aqueous Pfennig's medium in a 100 ml screw top bottle. This was incubated at 30°C and 1,000 lux and, after a fortnight, microscopic examination indicated the small rod as the only morphological type present. The absence of growth on nutrient agar incubated both aerobically and anaerobically at 30°C and in sulphate reducer API broth (BBL 11678) incubated at 30°C indicated that no contaminating bacteria were present.

Figure 3.1: Photograph of the Anaerobic Perspex Incubator.



The Figure shows the incubator, the N_2/CO_2 cylinder and the gas flow control box consisting of a manometer, a gas flow valve (upper) and a purge valve (lower).

The following observations led to the identification of the bacterium as Chromatium minutissimum as described by Pfennig and Truper (1974).

- (i) Washed suspensions of the bacterium showed the absorption maxima in the near infra-red region characteristic of living cells of the red phototrophic bacteria (Figure 3.2.) Two maxima were observed, one at 805 nm and the other at approximately 870 nm. These maxima are typical of bacteriochlorophyll a which is the major bacteriochlorophyll of Chromatium minutissimum (Pfennig and Truper 1974).
- (ii) When growing on sulphide, sulphur was deposited intracellularly. Aged cultures were devoid of such sulphur deposits indicating its subsequent oxidation. This sulphur metabolism is characteristic of the Chromatiaceae other than Ectothiorhodospira (Pfennig 1975).
- (iii) Being a motile rod devoid of gas vacuoles and depositing sulphur intracellularly the bacterium may be identified as a member of the genus Chromatium (Pfennig and Truper 1974).
- (iv) The growth of the bacterium in Pfennig's medium containing $0.1\frac{V}{\%}$ acetic acid but with sodium sulphate substituted for sodium sulphide indicated the presence of assimilatory sulphate reduction (Thiele 1968b). This, allied with the small cell dimensions ($1 - 1.5\mu \times 2 - 3\mu$) resulted in a species identification of Chromatium minutissimum.

3.3 THE EFFECT OF TEMPERATURE, pH, SULPHIDE AND ACETATE CONCENTRATIONS AND LIGHT INTENSITY ON THE GROWTH OF CHROMATIUM MINUTISSIMUM

3.3.1 The Experimental Design:

Data was collected using a half-replicate 2^5 central composite design. The levels of the five variables studied were selected to cover the range of conditions expected in lagoons in New Zealand. The values chosen are presented in Table 3.1.

An orthogonal experimental design requires variables to be investigated at equal increments. Therefore to study the desired range of sulphide and acetate concentrations from 1 to 10,000 mg/l logarithmic intervals were necessary in each case. Similarly, the light intensity was investigated at half-logarithm intervals from 31.6 lux to 3,160 lux.

Those variables which are used in a logarithmic form to provide equal increments between coded levels are preceded by the letter q. This indicates a positive logarithm of a concentration distinguishing this from the letter p symbolising the negative logarithm of a concentration, as in pH.

TABLE 3.1

Definition and Levels of Independent Variables Selected to Test the Growth Response of *Chromatium minutissimum*.

Independent Variable	Coded Symbol	Coded Level				
		-2	-1	0	1	2
Temperature ($^{\circ}\text{C}$)	X_1	10	15	20	25	30
pH	X_2	10	9	8	7	6
Sulphide concentration (mg/l)		1	10	100	1,000	10,000
Log sulphide concentration (=qS)	X_3	0	1	2	3	4
Acetate concentration (mg/l)		1	10	100	1,000	10,000
Log acetate concentration (=qA)	X_4	0	1	2	3	4
Light intensity (lux)		31.6	100	316	1,000	3,160
Log light intensity (=qL)	X_5	1.5	2.0	2.5	3.0	3.5

Central composite designs are composed of three sets of points (John 1971): (i) The cube points at $X_i = \pm 1$. Five independent variables were considered and a design consisting of a half-replicate 2^5 was

chosen giving sixteen cube points ($n_c = 16$).

(ii) The star, or axial, points (n_a) located two per axis a distance $\pm \alpha$ from the origin. With five independent variables, five axes are required and therefore $\alpha = 10$. To achieve rotatability $\alpha^4 = n_c$, thus a rotatable half-replicate 2^5 central composite design has $\alpha = 2$.

(iii) The centre points (n_o) located at the origin. To achieve orthogonality,

$$n_o + n_a = \frac{4 \alpha^2 (n_c + \alpha^2)}{n_c}$$

$$\therefore n_o = 10$$

To achieve rotatability and orthonogonality, this design required 36 trials as shown in Table 3.2 (Pages 75 and 76).

The design used offers the benefits of economy, orthogonality, and near rotatability while estimating the second order effects (Davies 1978). Higher effects cannot be estimated due to confounding. An empirical mathematical equation of the following form can be fitted to the data :

$$Y = \beta_0 + \beta_1 X_1 + \beta_{1j} X_{1j} + \beta_{1i} X_{1i}^2 \quad (3.1)$$

where : Y is the dependent variable (i.e. the measured growth response)

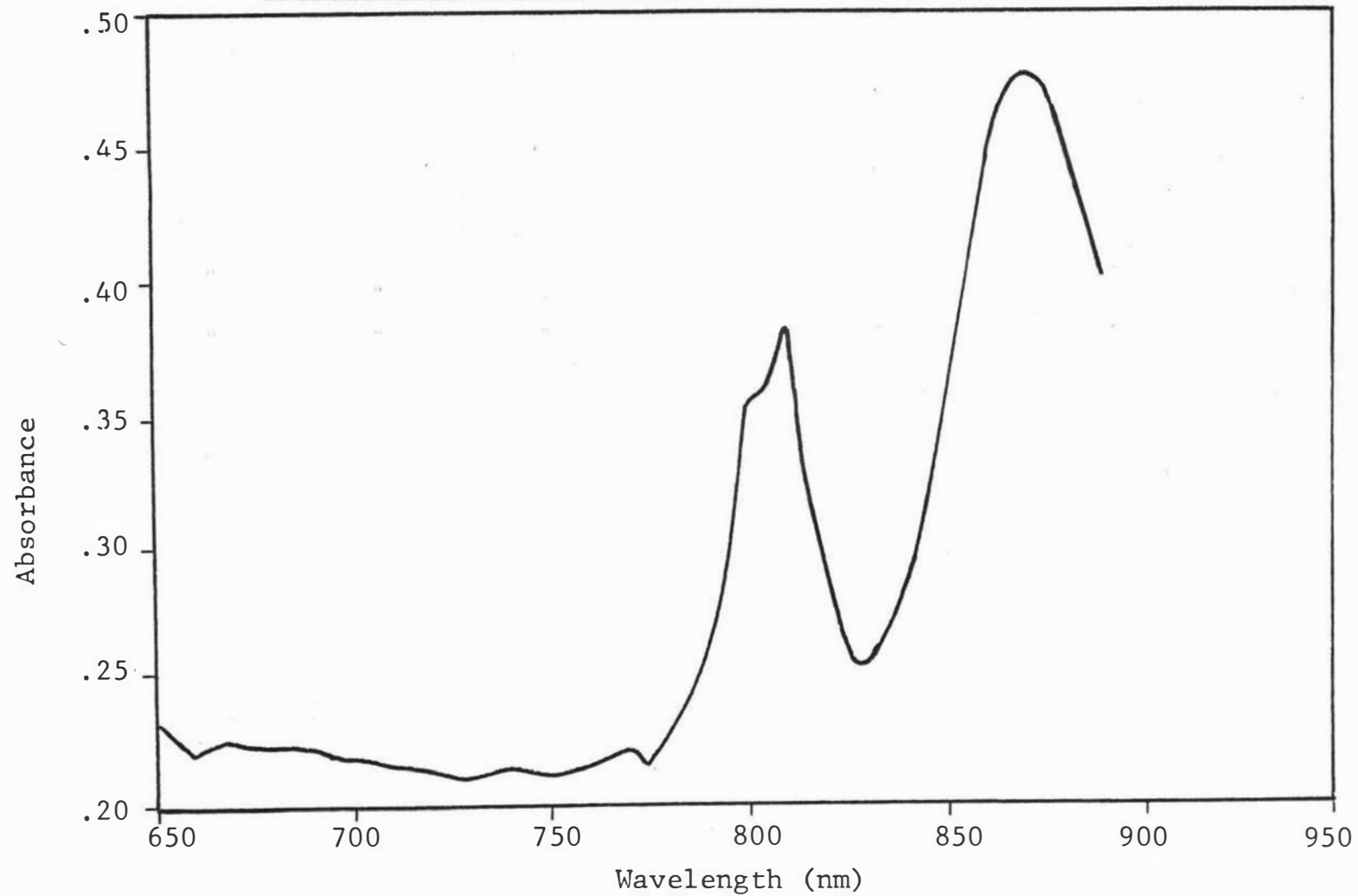
β_0 is a constant

β_1 is a coefficient describing the linear response of the independent variable X_1 .

β_{1j} is a coefficient describing the interaction of the two independent variables X_1 and X_j .

β_{1i} is a coefficient describing the quadratic response of the independent variable X_1 .

Figure 3.2 : The Effect of Wavelength on the Absorbance of Washed Cells
of *Chromatium minutissimum*



By fitting such a model to the observed data the response of the bacterium, within the field of the independent variables considered, may be described.

3.3.2 Experimental Procedure:

Chromatium minutissimum was grown in Pfennig's medium and the cells were harvested during the exponential growth phase. After washing with $\frac{1}{4}$ strength Ringer's solution they were resuspended to give approximately 10^7 cells/ml. This culture was then inoculated in 2.5 ml aliquots into each of the 36 x 250 ml glass stoppered bottles containing Pfennig's medium amended as required by the experimental design.

Prior to inoculation, Analar grade acetic acid (99.7% minimum purity, BDH catalogue number 10001) was aseptically added in quantities calculated to provide the necessary acetate concentrations. The sulphide concentration was incrementally increased to the desired value by aseptically adding a sterile solution of sodium sulphide. The sulphide concentrations were determined using a sulphide specific ion electrode as described by Papp (1971). Samples were then aseptically withdrawn and the pH measured using a Metrohm E 350B pH meter. If necessary, the culture pH was adjusted to that required by the experimental design using 5M NaOH or 5M HCl.

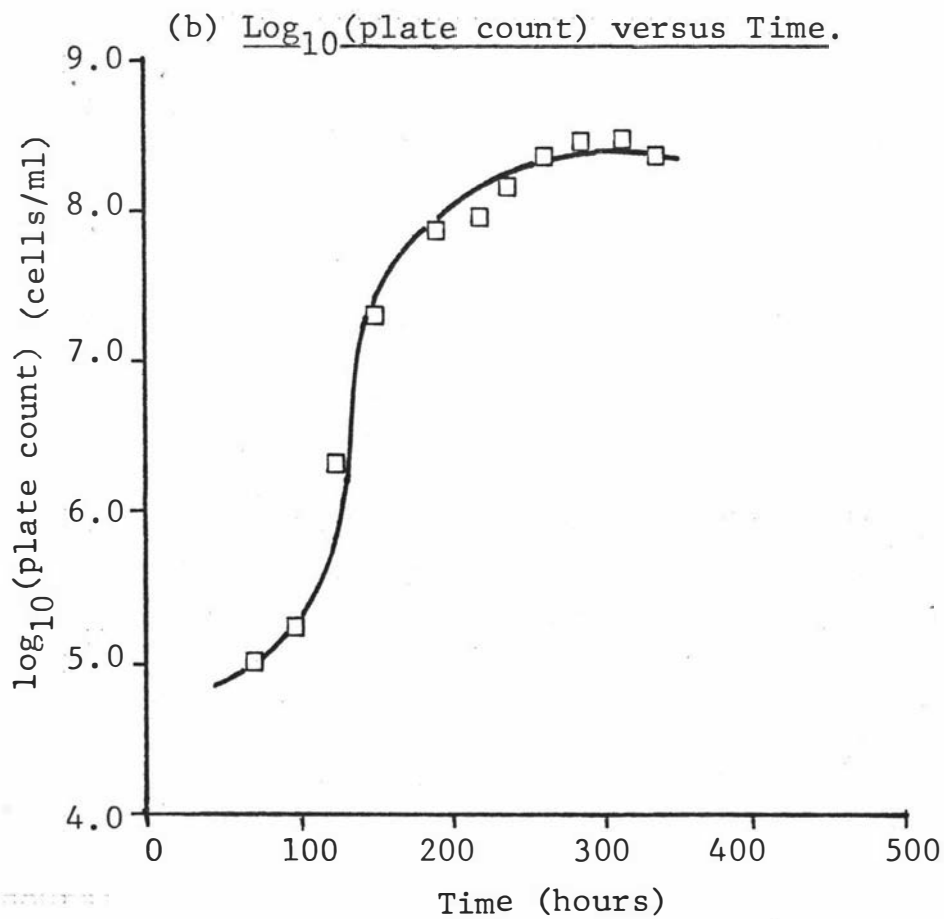
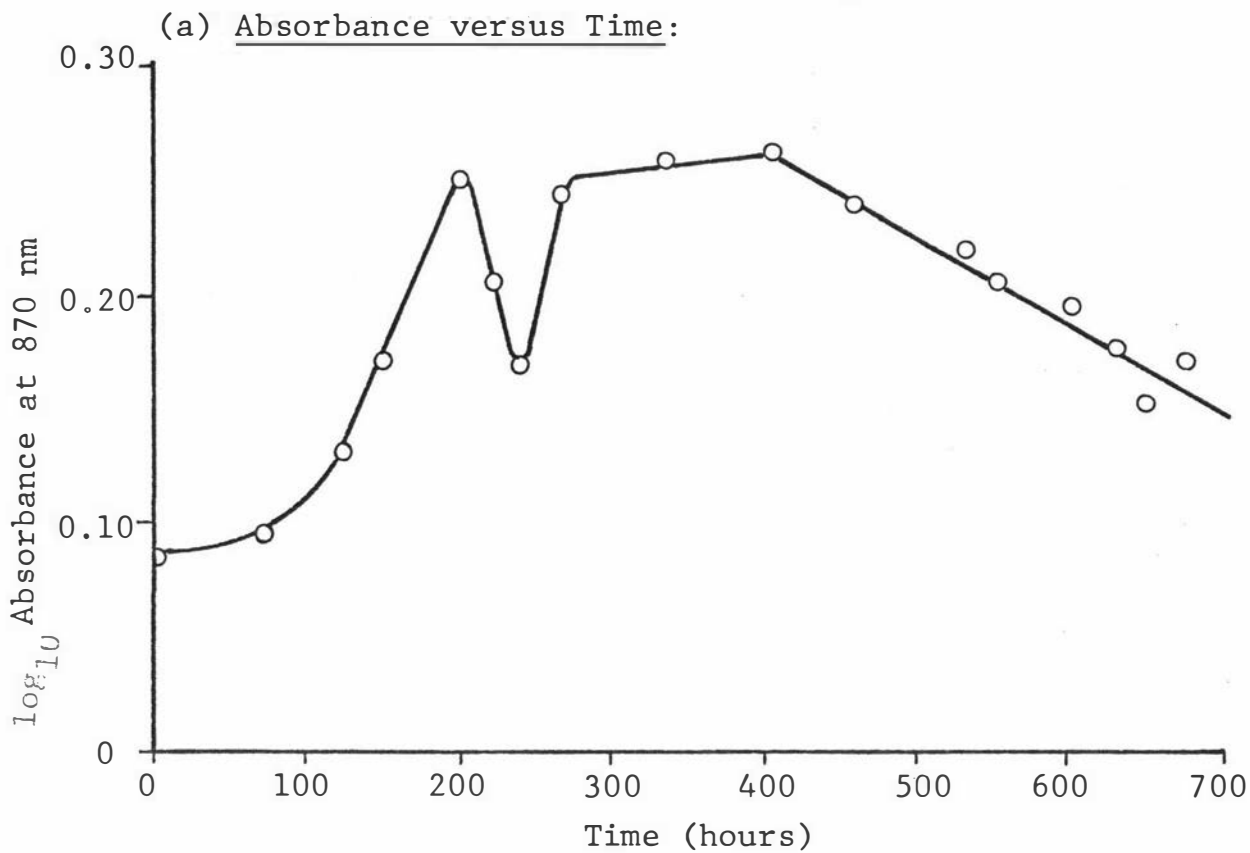
The inoculated bottles were placed in rooms or incubators controlled at the desired temperature. The required light intensities were obtained by placing the bottles at various distances from a 100 W incandescent bulb. The incident light intensity at the surface of the bottles was measured using a Sangamo Weston model S.511 photometer.

3.3.3 Measurement of Growth:

For a unicellular population, two types of parameters may be used to measure growth (Stanier et al 1971):

- (i) cell numbers
- (ii) cell mass.

Figure 3.3 : Growth Curves for Trial 7.



The viable count is the most sensitive technique for measuring bacterial numbers (Stanier et al 1971). Viable cells may be determined by performing plate counts. Although time consuming, for batch cultures this technique has the benefit of requiring only small sample volume. For this reason and due to its wide acceptance as a technique for quantifying bacterial growth, the plate count was the major method used to measure the growth of C. minutissimum.

The cell mass may be assessed by determining the dry weight of cell material in a known volume of culture medium. Such a technique is seldom used for unicellular bacteria due to the insensitivity of the analysis. Correlating the optical density to the mass of a cell suspension is the preferred technique. However, such an analysis is only adequately sensitive for cultures containing more than 10^7 cells/ml (Stanier et al 1971).

With the Chromatiaceae, optical density measurements have been shown to be an inadequate measurement of growth as the deposition of intracellular sulphur affects the optical density more than does the mass of cells (van Gernerden 1968a). However, the growth of Chromatium has been studied by measuring the absorbance of the cells' bacteriochlorophyll (van Gernerden 1968a). Such a measurement should be limited to situations where the light intensity is above saturation as below this value the cells adjust their bacteriochlorophyll concentration in inverse proportion to the light intensity (Pfennig 1967). The bacteriochlorophyll content of Chromatium cells has also been shown to vary with the initial sulphide concentration (van Gernerden 1968a) and with the presence or absence of organic compounds (Fuller et al 1963).

Despite these disadvantages, the in vivo measurement of bacteriochlorophyll offered potential as a rapid field measurement technique.

The culture absorbance at 870 nm, the wavelength which exhibited the strongest absorbance (Fig. 3.2), was therefore studied to assess whether this could be used as a valid growth parameter.

Thus, three dependent, or response, parameters were studied:

(i) The exponential growth rate calculated from the change in culture absorbance at 870 nm with time. This parameter will be referred to by the abbreviation μ_a .

(ii) The maximum count observed on plates of Pfennig's medium containing 3% agar incubated in the light.

(iii) The exponential growth rate calculated from the plate counts described in (ii) above. This parameter will be referred to by the abbreviated μ_p .

Absorbances were read on an Hitachi model 101 spectrophotometer. To minimise light scattering a filter paper impregnated with paraffin oil was sandwiched between two sheets of opal glass and placed immediately behind the sample in the spectrophotometer (Shibata et al 1954).

The plate counts were obtained by incubating the inoculated petri plates for fourteen days in the perspex incubator at 30°C. A gas consisting of 90% N₂ and 10% CO₂ was used to sparge the incubator and beakers containing a solution of pyrogallol (1^w/v%) and sodium bicarbonate (3^w/v%) (Willis 1969) were placed in the incubator to ensure that anaerobic conditions prevailed.

3.3.4 Analysis of Results:

The growth data was read into Massey University's Burroughs B6700 computer and analysed using the MINITAB language.

Growth curves were obtained for each trial by plotting log₁₀ absorbance and log₁₀ plate count versus time. Typical curves are presented in Figure 3.3 for trial 7. Visual inspection of these curves indicated the phases of lag, exponential and stationary growth. The maximum count was obtained from the graph and recorded as a base 10 logarithm. The exponential growth rate was obtained by linear regression of the points in the exponential growth phase.

These analyses resulted in the calculation of the three dependent variables and their values for each trial are presented in Table 3.2

As anticipated, a wide range of growth rates and maximum counts were observed. The maximum counts ranged from 4.06 to 8.81 (Table 3.2). The μ_{up} values ranged from 0.016×10^{-3} to $1.12 \times 10^{-3} \text{ hr}^{-1}$ for the same trials suggesting some correlation between μ_{up} and the maximum count. A correlation analysis (Table 3.3) confirmed this surmise. The two factors are significantly correlated at the 1% level. Insignificant correlation was observed between μ_{ua} and other factors (Fisher and Yates 1963).

TABLE 3.3

Correlation Coefficients for the Dependent Variables

	μ_{ua}	μ_{up}
μ_{up}	.136	-
maximum count	.100	.528

If the absorbance at 870 nm was a good estimate of the cell mass, μ_{ua} and μ_{up} should have been significantly correlated. This is due to nearly all the cells formed during the exponential growth phase being viable and of a constant size and, thus, the cell mass and cell numbers increase in parallel (Stanier et al 1971).

Also, the measurement of growth by the absorbance at 870 nm revealed some unusual effects in some cultures. A typical example is presented in Figure 3.3(a) where a substantial decrease in absorbance was observed at the onset of the stationary phase. No decrease in cell numbers was apparent during this period (Fig. 3.3 (b)). No reference to this phenomenon has been found in the literature and the reasons for such an effect are worthy of further study.

TABLE 3.2

The Experimental Design Used and the Responses of *Chromatium minutissimum* to the Independent Variables

Independent Variables						Trial Number	Dependent Variables		
Coded Symbol	X1	X2	X3	X4	X5		$\text{hr}^{-1} \times 10^{-2}$ ^{ua}	$\text{hr}^{-1} \times 10^{-3}$ ^{up}	Maximum count (log ₁₀)
Description	Temperature	pH	qS	qA	qL				
	1	1	1	1	1	12	.695	.440	6.47
	-1	1	1	1	-1	14	1.88	.525	5.75
	1	-1	1	1	-1	11	.310	.091	4.23
	1	1	-1	1	-1	24	1.40	1.12	8.81
	1	1	1	-1	-1	15	.712	.966	5.86
	-1	-1	1	1	1	16	.242	.392	4.16
	-1	1	-1	1	1	10	1.31	.711	8.20
	-1	1	1	-1	1	2	.719	.223	7.50
	1	-1	-1	1	1	3	1.91	.662	8.45
	1	-1	1	-1	1	18	0.21	0.16	4.06
	1	1	-1	-1	1	6	0.45	.152	7.90
	-1	-1	-1	1	-1	21	.692	.332	8.30
	-1	-1	1	-1	-1	22	.018	.766	5.83
	-1	1	-1	-1	-1	8	.725	.026	6.65
	1	-1	-1	-1	-1	23	.241	.265	7.39
	-1	-1	-1	-1	1	1	.010	.752	6.84
	0	0	0	0	0	4	.846	.691	7.48

Continued

TABLE 3.2 Continued

Independent Variables						Trial Number	Dependent Variables		
Coded Symbol	X1	X2	X3	X4	X5		μ_a (hr ⁻¹ x 10 ⁻²)	μ_p (hr ⁻¹ x 10 ⁻³)	Maximum count (log ₁₀)
Description	Temperature	pH	qS	qA	qL				
	0	0	0	0	0	13	.242	.603	8.02
	0	0	0	0	0	5	.658	.494	7.85
	0	0	0	0	0	17	.367	.752	8.16
	0	0	0	0	0	7	.928	1.05	8.30
	0	0	0	0	0	19	.581	.787	8.07
	0	0	0	0	0	9	.503	.738	7.85
	0	0	0	0	0	20	.683	.744	7.86
	2	0	0	0	0	27	3.39	.766	7.86
	-2	0	0	0	0	30	.968	.704	7.20
	0	2	0	0	0	31	3.59	.450	6.40
	0	-2	0	0	0	34	1.33	.086	4.69
	0	0	2	0	0	35	.751	.110	4.09
	0	0	-2	0	0	36	.542	.137	6.70
	0	0	0	2	0	32	1.58	.407	5.65
	0	0	0	-2	0	29	2.01	.143	6.70
	0	0	0	0	2	33	.746	.362	8.30
	0	0	0	0	-2	25	.534	.464	7.15
	0	0	0	0	0	28	.520	.299	6.48
	0	0	0	0	0	26	.479	.508	7.80

The absorbance at 870 nm was therefore a poor parameter with which to study the growth of Chromatium minutissimum and no further reference to this variable will be made.

3.3.5 Development of Growth Models:

An empirical model in the form of equation 3.1 was then fitted to each of the dependent variables, μ and maximum count, by performing multiple regression analyses using the B6700 computer and the MINITAB language. This involved multiple regression of the coded linear, quadratic and first order interaction terms of the independent variables to the dependent variables. Thus the five main effects (X_1 to X_5), the ten interaction terms (X_1X_2 to X_4X_5) and the five quadratic terms (X_1X_1 to X_5X_5) were regressed with μ and the maximum count respectively.

For each regression, the computer print out contained a regression coefficient for each term, the standard deviation of regression coefficient and the Student's t statistic. Coefficients which had a t statistic significant at the 0.05 level were considered to fit the data adequately and therefore contributed significantly to any model developed. In this manner, coefficients with t statistics not significant at the 0.05 level were discarded and only those with significant t statistics contributed to the final, 'parsimonious' model.

The parsimonious models developed for μ and the maximum count are presented in Tables A.1.1. and A.1.2. (Appendix 1).

The adequacy of fit of the models to the observed data was assessed using the F statistic (John 1971). In this instance;

$$F_{n_1, n_2} = \frac{\text{mean sum of squares due to lack of fit.}}{\text{mean sum of squares due to pure error.}}$$

$$= \frac{MS_{\text{lof}}}{MS_{\text{pe}}}$$

The MS_{pe} may be calculated by performing a one way analysis of variance on the replicate centre point results (Trials 4, 5, 7, 9, 13, 17, 19, 20, 26, 28). The MS_{lof} may then be obtained by difference from the residual mean sum of squares. For example, with the $\nearrow$ up model (Table A.1, Appendix 1.)

$$\begin{aligned}
 &MS_{pe} = .0416 \\
 \text{and} \quad &\text{residual mean sum of squares} = .0688 \\
 \therefore \quad &MS_{lof} = .0688 - .0416 \\
 &= .0272 \\
 n_2 &= \text{degrees of freedom of replicates} \\
 &= 10 - 1 \\
 &= 9 \\
 n_1 &= \text{residual degrees of freedom} - n_2 \\
 &= 32 - 9 \\
 &= 23 \\
 \therefore \quad &F_{23,9} = \frac{.0272}{.0416} \\
 &= .654
 \end{aligned}$$

As this value is less than $F_{23,9,0.05} = 2.91$ there is no significant lack of fit at the 0.05 level.

Similar calculations for the maximum count model also indicated no significant lack of fit.

However, the correlation coefficients (r^2) were 25.8% and 84.1% for the $\nearrow$ up and maximum count models respectively. This means that the $\nearrow$ up model, for example, explained 25.8% of the observed variation in the data points and was therefore inadequate. Thus a means of improving the fit of the $\nearrow$ up model was sought and, while the maximum count model gave an adequate description of the data, a search for a better data fit was made.

Thus, the growth rate and maximum count data were manipulated prior to regression. Two manipulations, the natural logarithm and the inverse of each datum point were investigated.

Manipulation of the $\sqrt{\text{up}}$ data resulted in a substantial improvement in the model. The inverse $\sqrt{\text{up}}$ model yielded a correlation coefficient of 85.7% (Table A.3) compared to 25.8% for the $\sqrt{\text{up}}$ model (Table A.1). However, there was a significant lack of fit at the 0.05 level. The $\ln \sqrt{\text{up}}$ model was a further improvement with an r^2 value of 86.0% and no significant lack of fit (Table 3.4).

Data manipulation also resulted in an improvement in fit of the model to the maximum count results. The inverse model resulted in an r^2 value of 91.9% and had no significant lack of fit (Table 3.5). The logarithm model had a correlation coefficient of 88.1% and showed no significant lack of fit (Table A.4). Therefore, both maximum count models adequately described the observed data but because of its higher r^2 value, the inverse model was preferred.

Residual plots for the two selected models are presented in Figures 3.4 and 3.5. The random distribution of points indicates the absence of any bias or time trends.

Further, the selected models can be manipulated to yield an equation giving the predicted response using uncoded independent variables (e.g. temperature in $^{\circ}\text{C}$). Such a decoded model is much more practical for general use.

The decoding was performed as outlined by Belz (1973) using Table XXIII of Fisher and Yates (1963).

The decoded models are presented in Tables 3.6 and 3.7.

3.3.6 Interpretation of Growth Models:

Interpretation of the developed models is difficult due to their complexity. The relatively large number of independent variables studied and the number of significant interactions and quadratic effects in each model makes a simple analysis impossible.

TABLE 3.4

Parsimonious Model and Regression Statistics for $\ln \uparrow$ upParsimonious model:

$$\begin{aligned}
 \ln Y = & - .457 + .225X_2 + .360X_4 + .575X_1X_2 \\
 & - .370X_1X_3 - .388X_1X_5 + .460X_2X_3 + .263X_2X_4 \\
 & - .266X_3X_4 - .443X_3X_5 + .253X_4X_5 \\
 & - .257X_2X_2 - .375X_3X_3 - .206X_4X_4
 \end{aligned}$$

Correlation Coefficient:

$$r^2 \text{ (adjusted for degrees of freedom)} = 86.0\%$$

Analysis of Variance Summary:

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Sum of Squares
regression	13	32.0	2.46
residual	22	3.07	.140
total	35	35.07	

Test for Goodness of Fit:

$$MS_{pe} = .118$$

$$\therefore MS_{lof} = .022$$

$$\frac{MS_{lof}}{MS_{pe}} = .186$$

As $F_{13, 9, 0.05} = 3.04$ there is no significant lack of fit at the 0.05 level.

TABLE 3.5

Parsimonious Model and Regression Statistics for the Inverse of the Maximum Count

Parsimonious model:

$$\begin{aligned} \underline{1} &= .129 - .0160X_2 + .0285X_3 + .00838X_1X_3 \\ Y &- .00696X_1X_4 - .0159X_2X_3 - .00997X_2X_5 \\ &+ .0105X_3X_4 + .0123X_2X_2 + .0153X_3X_3 \\ &+ .00691X_4X_4 \end{aligned}$$

Correlation Coefficient:

$$r^2 \text{ (adjusted for degrees of freedom)} = 91.9\%$$

Analysis of Variance Summary:

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Sum of Squares
regression	10	.0489	.00489
residual	25	.0030	.00012
total	35	.0519	

Test for Goodness of Fit:

$$\begin{aligned} MS_{pe} &= .0000924 \\ \therefore MS_{lof} &= .0000276 \\ \frac{MS_{lof}}{MS_{pe}} &= .299 \end{aligned}$$

As $F_{16,9,0.05} = 2.98$ there is no significant lack of fit at the 0.05 level.

Figure 3.4: Plot of Residual versus Y for $\ln \mu_p$ Model.

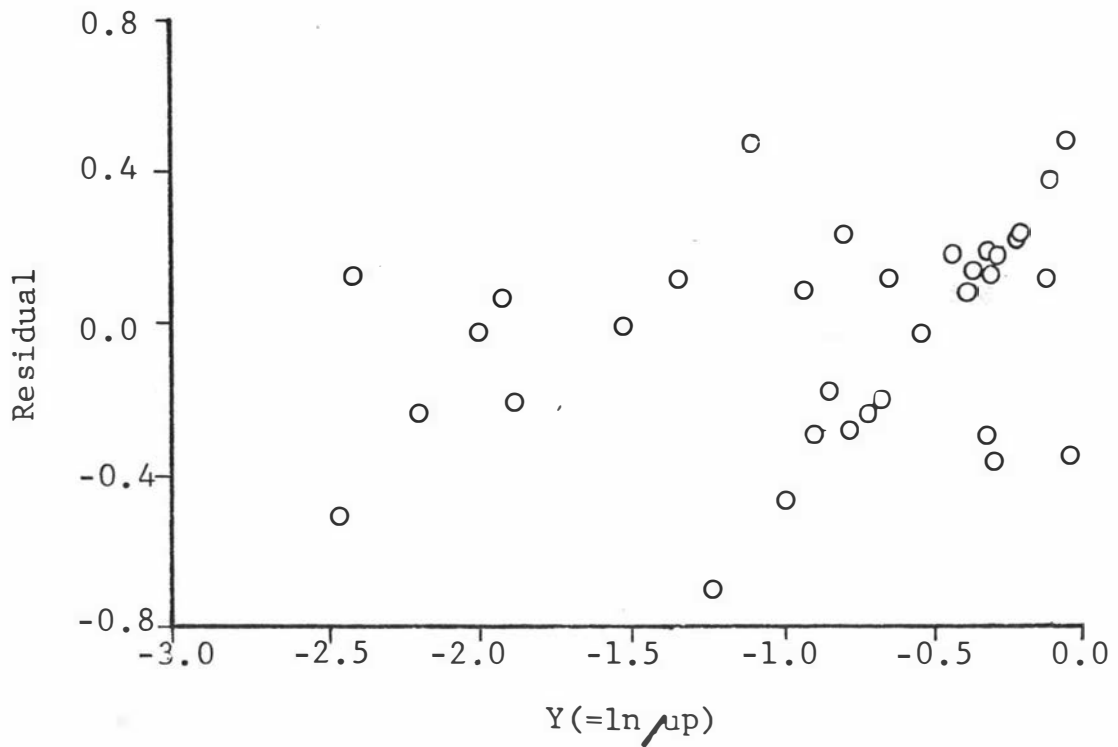


Figure 3.5: Plot of Residual versus Y for Inverse Maximum Count Model.

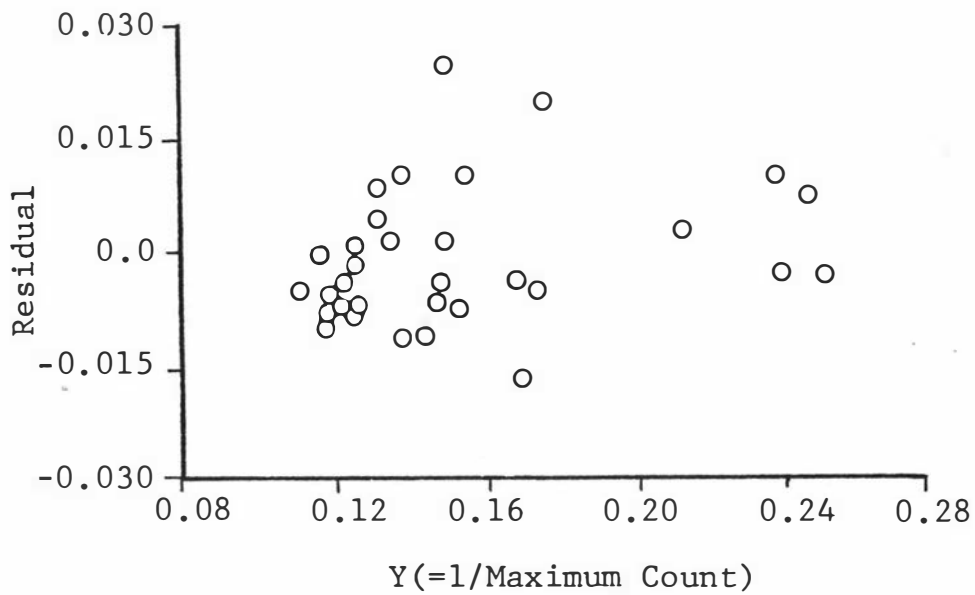


TABLE 3.6Decoded ln_{up} Model

$$\begin{aligned}
 \ln Y = & -1.243 - .024T + .0788\text{pH} + .127\text{qS} \\
 & - .0353\text{qA} + .242\text{qL} + .00719 T.\text{pH} \\
 & - .00462 T.\text{qS} - .0097 T.\text{qL} + .0287 \\
 & \text{pH}.\text{qS} + .0164 \text{pH}.\text{qA} - .0166 \text{qS}.\text{qA} \\
 & - .0554 \text{qS}.\text{qL} + .0316 \text{qA}.\text{qL} \\
 & - .0161 \text{pH}^2 - .0234 \text{qS}^2 - .0129 \text{qA}^2
 \end{aligned}$$

TABLE 3.7Decoded Inverse Maximum Count Model

$$\begin{aligned}
 1/Y = & .164 - .000035T - .0112 \text{pH} + .00785\text{qS} \\
 & - .0013\text{qA} + .00997\text{qL} + .000105 T.\text{qS} \\
 & - .000087 T.\text{qA} - .000994 \text{pH}.\text{qS} \\
 & - .00125 \text{pH}.\text{qL} + .000656 \text{qS}.\text{qA} + .000769 \\
 & \text{pH}^2 + .000956 \text{qS}^2 + .000432 \text{qA}^2
 \end{aligned}$$

Key to Tables 3.6 and 3.7:

- T : Temperature °C
 pH : pH
 qS : log (sulphide concentration in mg/l)
 qA : log (acetate concentration in mg/l)
 qL : log (light intensity in lux).

This section seeks to interpret the models as simply as possible to indicate the important effects of the independent variables. This is achieved by considering the isolated effects of the variables and their interactions. However, these techniques result in only a partial representation of the models and an accurate prediction of the growth rate and the maximum count can only be obtained by inserting the correct levels of the independent variables into the equations presented in Tables 3.6 and 3.7.

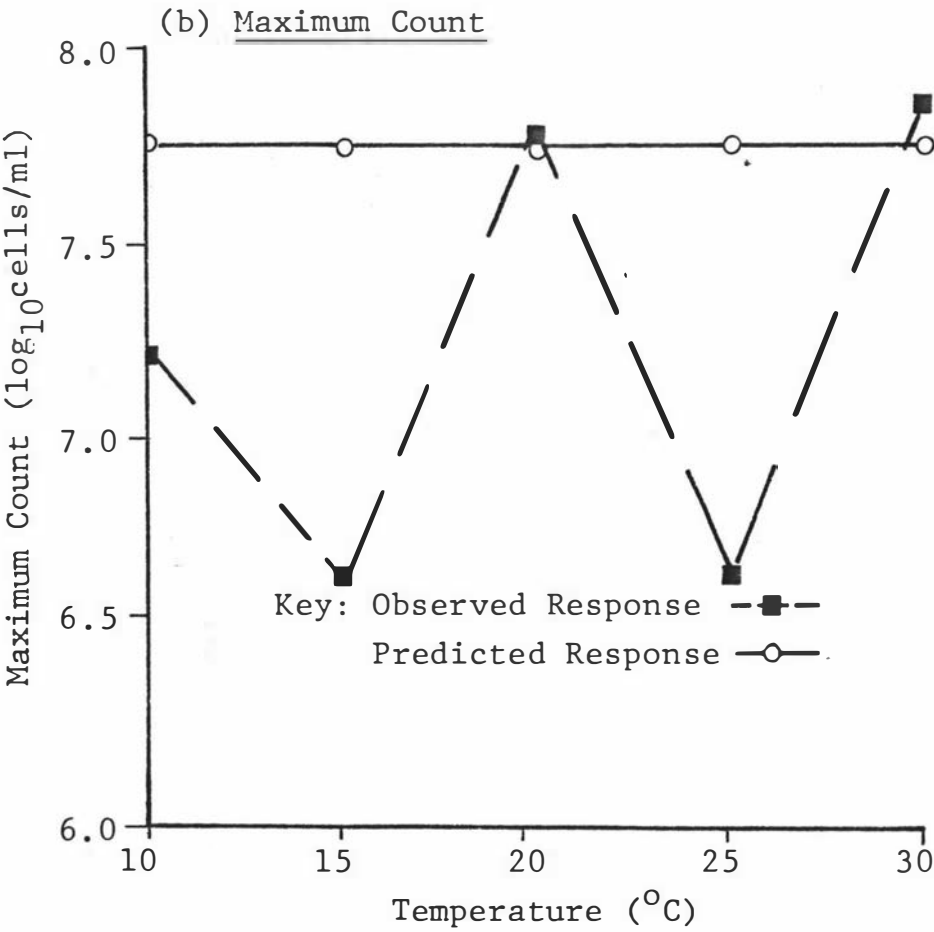
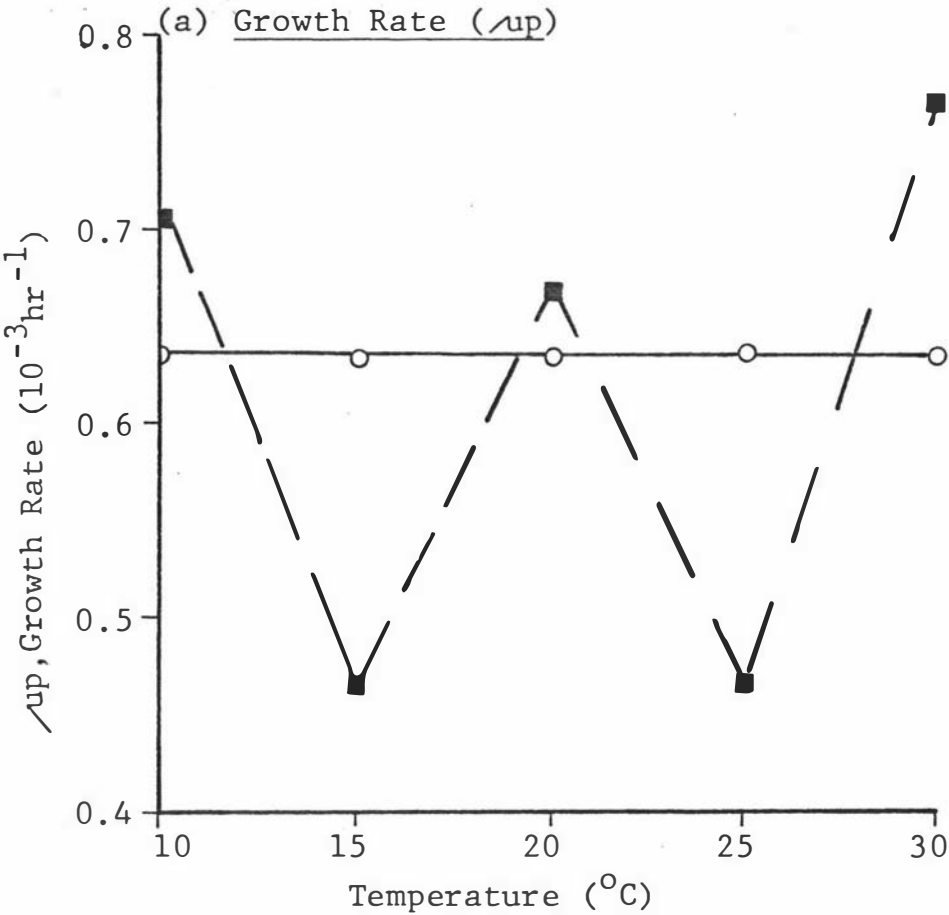
3.3.6.1 Consideration of the Isolated Effects of the Variables:

The fit of the selected models to the observed data and the nature of the responses was investigated by plotting the isolated effects of the independent variables on the dependent variables (Richert 1972) (Figures 3.6-3.10). This was achieved by only considering the effect of each dependent variable on the independent variables. An example calculation for the derivation of these effects and the tabulated data for each model are presented in Appendix 2. Figures 3.5 to 3.9 are a graphical representation of these data. Such plots include only a fraction of the field covered by the models and therefore exclude much information. They do represent μ_p and the maximum population under the conditions considered in the graph but it is important to realise that they do not represent the optimum growth conditions and that varying the levels of the other independent variables will greatly affect the observed response. Plots of the isolated effects are most useful in visualising the adequacy of fit of the model to the observed data.

From Tables 3.4 and 3.5 it is apparent that there are no main or quadratic effects for temperature in either of the developed models. Thus, the predicted μ_p and maximum count were constant across the range of temperatures studied (Figure 3.6.). This was not as expected.

It is usual for the growth rate to follow Arrhenius' law for much of the temperature range over which growth is observed (Stanier et al 1971) and such curves with optima in the range of 25-30°C have been observed for Chromatium (Meredith and Pohland 1970, Takahashi and Ichimura 1970). However the latter authors also isolated a

Figure 3.6: The Isolated Effect of Temperature on the
Dependent Variables.



Chromatium spp. which showed a relatively uniform rate of photosynthesis from 8°C to 30°C. The observed isolated effect of temperature in this study shows a similar response. However, a study of the interaction terms will demonstrate the importance of other independent variables such as light intensity, pH and sulphide concentration in determining the effect of temperature on μ_{up} . A direct comparison of results is therefore not possible as the previous authors have not specified their incubation conditions.

Figure 3.7 presents the isolated effect of pH on the μ_{up} and the maximum growth rate. Quite good agreement is obtained between the observed and predicted μ_{up} data. However, the model is unable to account for the sharp change in shape of the observed data line at pH 8.0. This results in the observed maximum occurring at pH 8.0 and the predicted maximum μ_{up} at pH 8.44. Also, this explains the relatively poor agreement between observed and predicted data points at pH's 9 and 10. Meredith and Pohland (1970) obtained the maximum growth rate of a Chromatium spp. at pH 7.5 but they observed little variation in growth rate between pH 7.0 and 8.0. The maximum growth rate of C. minutissimum occurs between pH 6.5 - 7.6 (Pfennig and Truper 1974) although growth of the Chromatiaceae has been observed between pH 5.5 (Kaplan 1956) and 11.0 (Jannasch 1957). However, variation in other parameters will affect the pH at which the highest growth rate occurs. The effect of various temperatures sulphide and acetate concentrations on the optimum pH for growth will be demonstrated shortly.

Good agreement is also obtained between the observed and predicted maximum count data, although the model has some difficulty in following the sharp inflexion in the observed data curve. This results in a disparity between the observed and predicted values at pH 9 and 10 and between the observed and predicted optimum pH. The highest maximum count was observed at pH 8.0 and the model predicts an optimum at pH 8.65. A previous study observed the highest maximum counts of Chromatium vinosum at pH 7.8 (Holm and Vennes 1970).

Figure 3.7: The Isolated Effect of pH on the Dependent Variables.

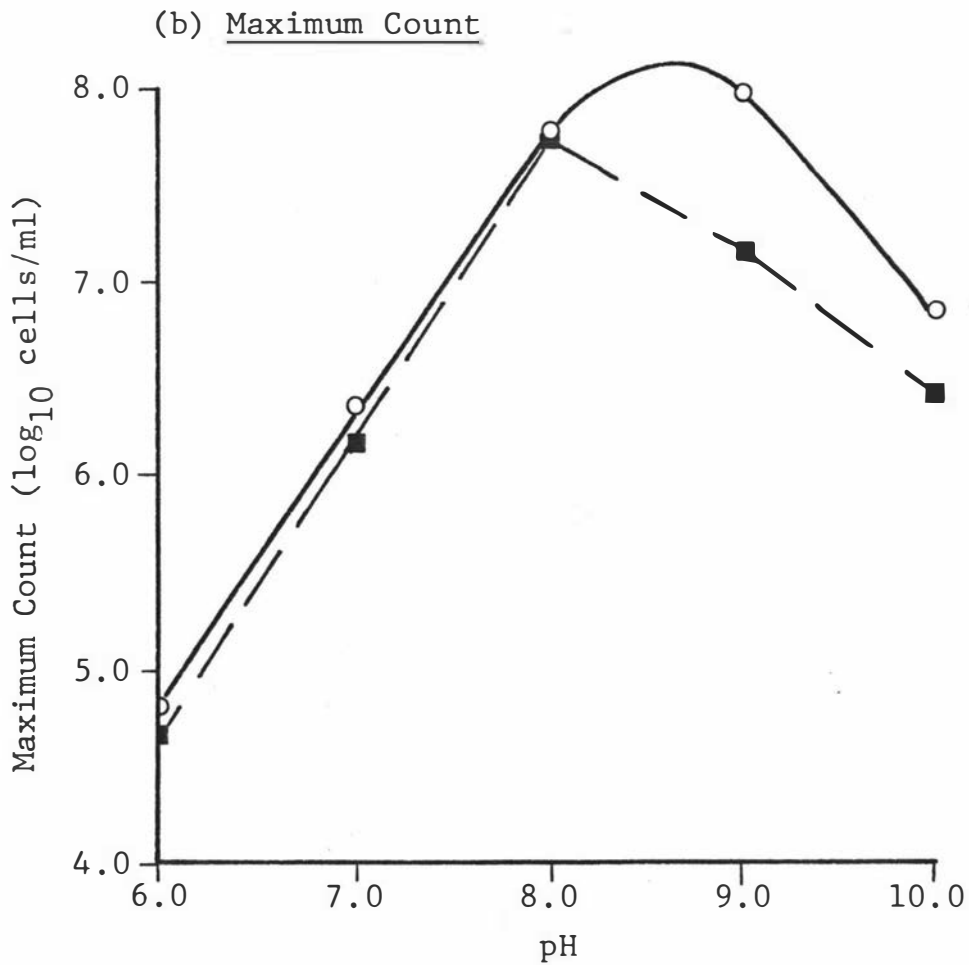
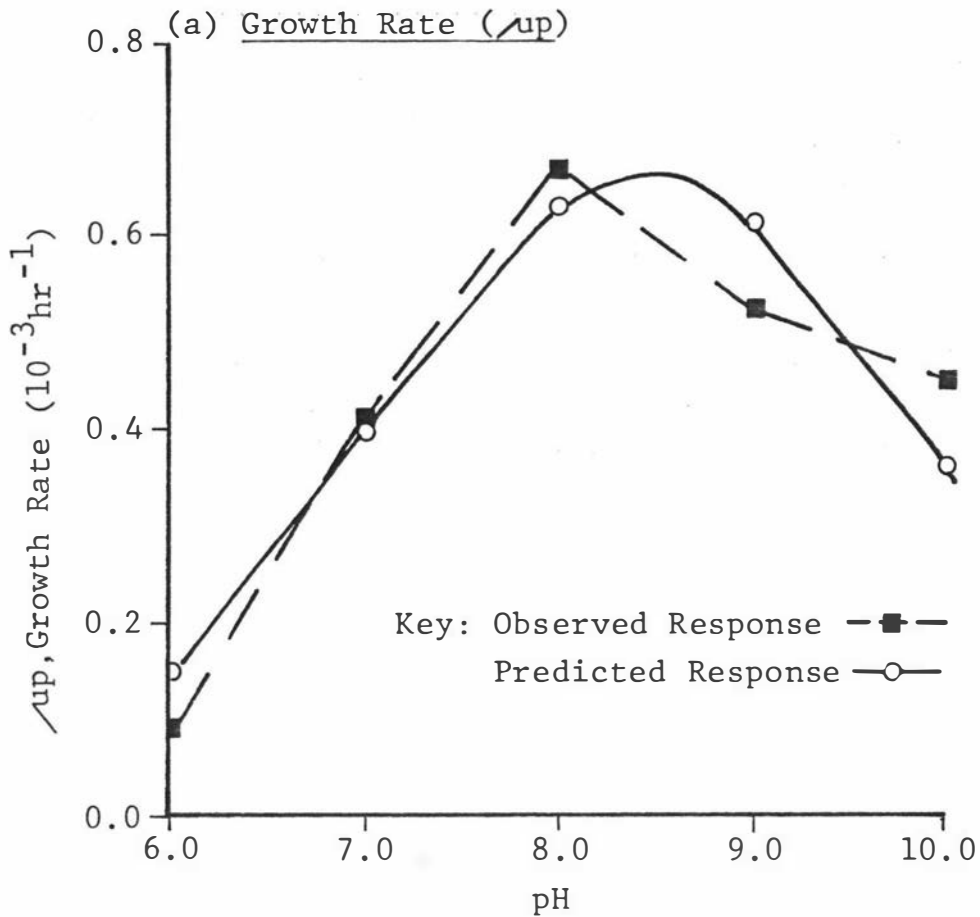


Figure 3.8 : The Isolated Effect of Sulphide on the
Dependent Variables.
 (a) Growth Rate (μ_p)

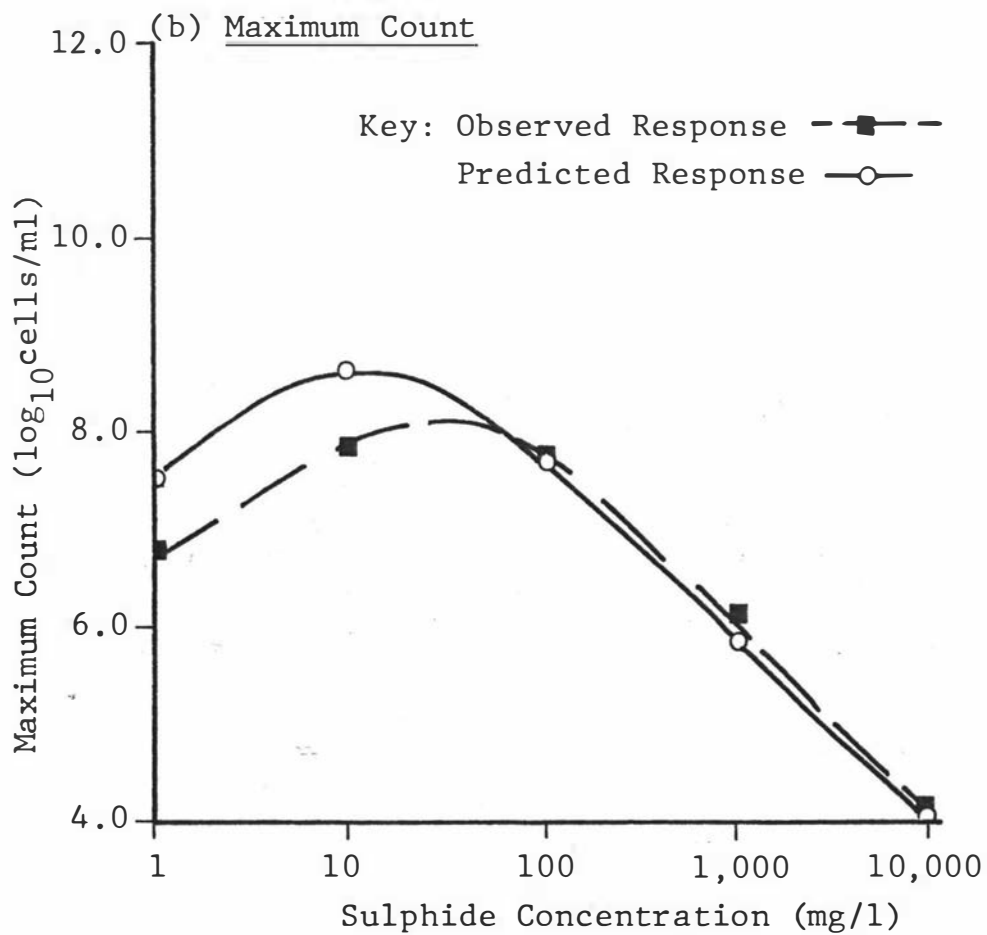
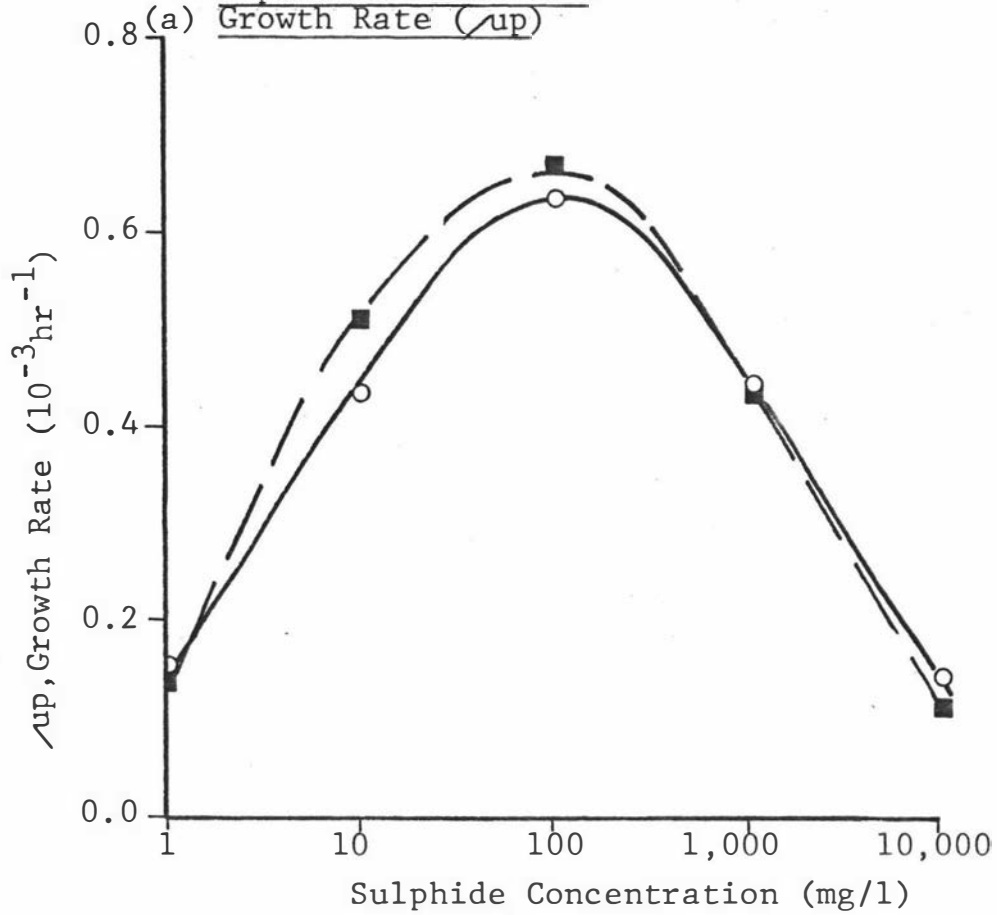


Figure 3.9: The Isolated Effect of Acetate on the Dependent Variables.

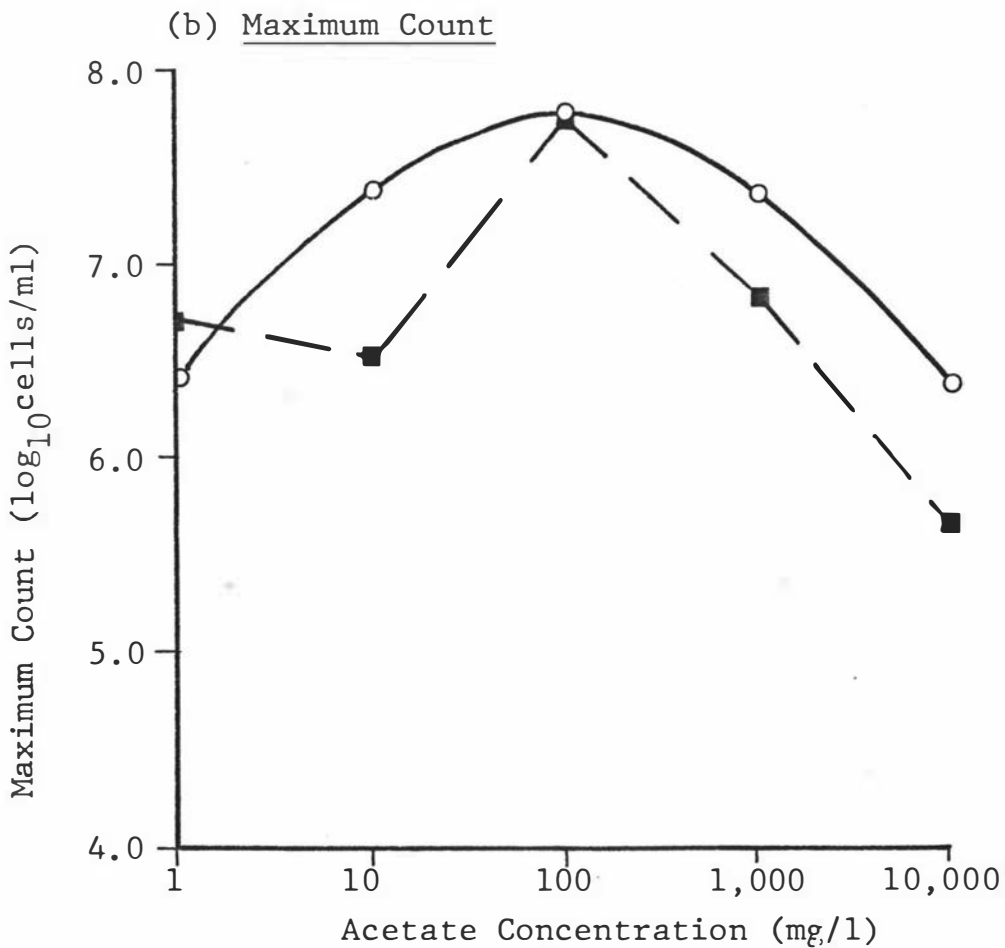
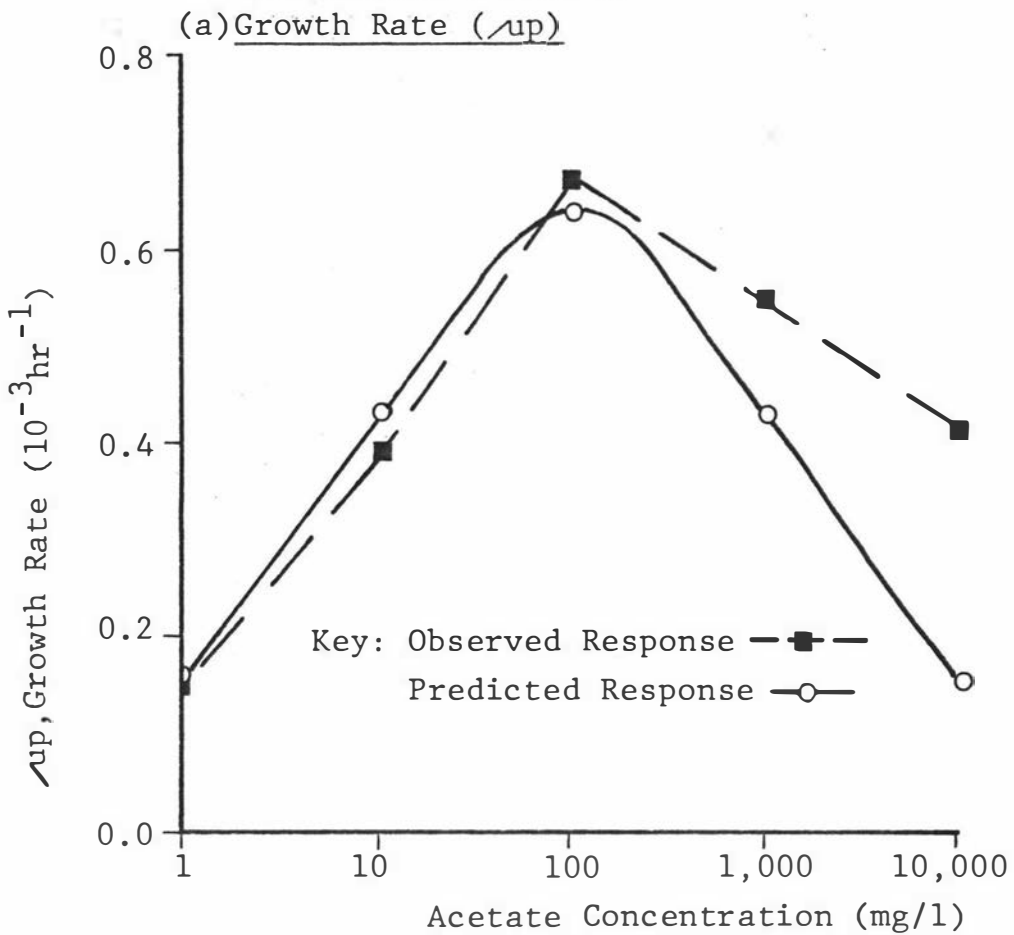
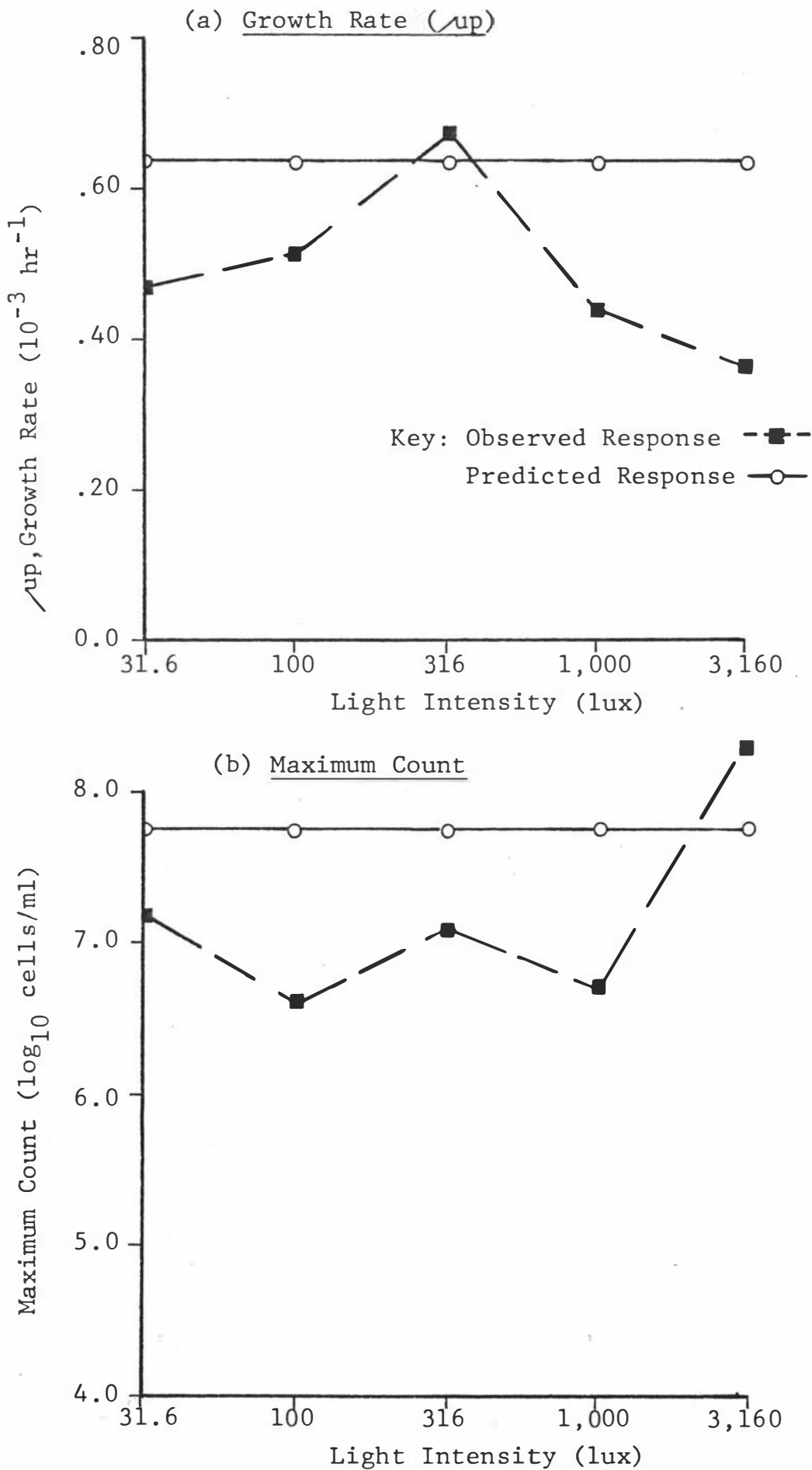


Figure 3.10: The Isolated Effect of Light Intensity on the Dependent Variables.



The isolated effect of sulphide on μ_{up} and the maximum count is presented in Figure 3.8. There is good agreement between the observed and predicted μ_{up} data. In both instances the maximum growth rate occurs at 100 mg/l. This is within the range of 26 - 128 mg/l sulphide tolerated by the Chromatiaceae (Pfennig 1975).

The model again has difficulty in following the curvature of the observed maximum population data at low sulphide concentrations. This results in discrepancies between the observed and predicted maximum counts at 1.0 and 10 mg/l sulphide. Also the observed data indicate that the highest maximum count is obtained at 32 mg/l sulphide while the model predicts an optimum at 12 mg/l sulphide. The observed data is in reasonable agreement with that of 48-56 mg/l sulphide found for Chromatium vinosum (Holm and Vernes 1970) although these values will be affected by the levels of the other independent variables.

The model also fits the acetate data adequately (Figure 3.9). The model and the observed data agree in predicting that both the maximum μ_{up} and the highest maximum count occur at 100 mg/l acetate. Lower concentrations stimulate, and higher concentrations inhibit, growth. Unfortunately there is no published data with which to compare these results.

As observed for the temperature, there are no main nor quadratic effects for the light intensity in either model (Tables 3.4 and 3.5.). The predicted μ_{up} and maximum count are therefore independent of the light intensity (Figure 3.10). A study of the literature indicates that the growth rate should be proportional to the light intensity, up to the saturation intensity (Takahashi and Ichimura 1970, Truper and Schlegel 1964) with saturation occurring at different intensities depending upon the conditions of growth and the bacterial species (see Section 2.5.2.2). For Chromatium species, saturation is most frequently observed at 2,000 lux and therefore an increase in growth rate up to this light intensity could have been anticipated. This was not observed and the maximum growth rate occurred in the vicinity of 316 lux (Figure 3.10(a)).

The highest maximum count was observed at a light intensity of 3,160 lux (Figure 3.10(b)). However, this point is the result of only one observation and could be an outlier as the other data points indicate a maximum count independent of the light intensity. Unfortunately, no maximum count data have been published in the literature with which to compare the results of this study.

The major findings from a study of the isolated effects of the independent variables may be summarised as follows:

(i) No significant effects of temperature or light intensity were observed.

(ii) Adequate fit of the models to the pH data was observed. Best growth occurred at pH's in the vicinity of 8.0.

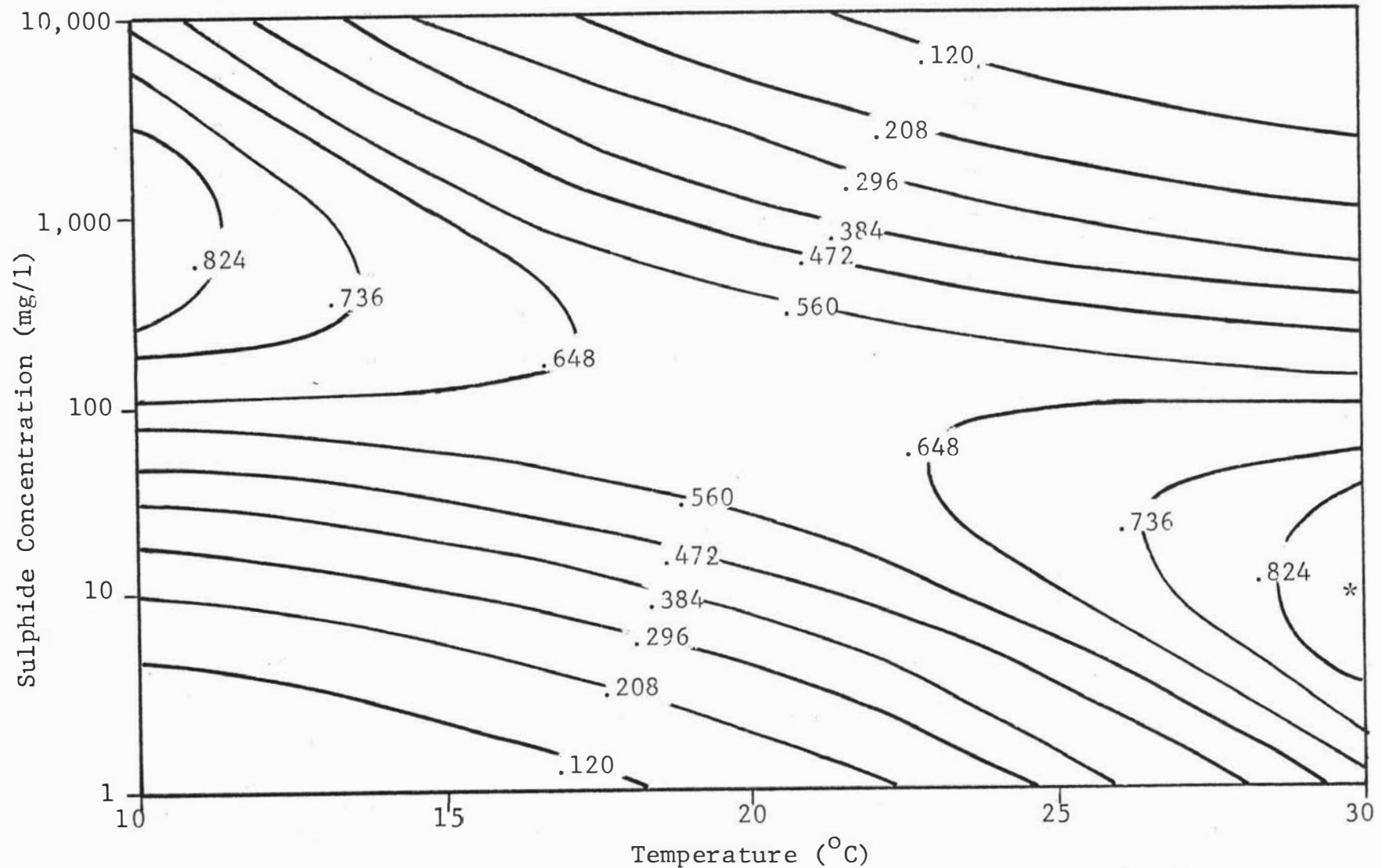
(iii) The models fitted the observed sulphide data well. Low sulphide concentrations stimulated growth while high concentrations proved inhibitory. The maximum growth rate was observed at 100 mg/l sulphide and the highest maximum count occurred at 32 mg/l sulphide.

(iv) Adequate fit of the model to the acetate data was observed with low concentrations proving stimulatory and high concentrations inhibitory. Best growth was obtained at 100 mg/l acetate.

(v) The above are the results of considering only the isolated effects and do not account for the effects of any interactions between the independent variables. Such interactions will affect the predicted optimum growth conditions and to demonstrate the effects of the interactions, some will now be considered.

Figure 3.11: The Isolated Temperature-Sulphide Interaction of the $\ln_{up}$ Model

The Maximum Growth Rate is $.912 \times 10^{-3} \text{ hr}^{-1}$ at 30°C and 10 mg/l S^{2-} .



The figures in the body of the diagram are $\ln_{up}$ in units of 10^{-3} hr^{-1} .

3.3.6.2 Consideration of the Isolated Interaction of Two Variables:

Further information on the developed models may be obtained by studying the significant interactions between the independent variables. It will be demonstrated that a consideration of these interactions is essential and that they substantially affect the highest μ and maximum counts observed in the study of the isolated effects.

The effects of the interactions of the independent variables on the response variable may be obtained by studying response surface contour plots of the fitted models using a computer programme developed by Dr. S.H. Richert (Personal Communication 1976).

However, there are eight interaction terms in the $\ln \mu$ model and five in the inverse maximum count model and to consider these interactions would require 1625 contour plots. As it is impractical to include these in this thesis, only 4 of the 13 isolated interactions observed in the two models are presented. To study these, the independent variables not involved in the interactions were set at the centre point. For example, consider the X_1X_2 interaction of the $\ln \mu$ model (Table 3.4). By setting X_3 , X_4 and X_5 equal to zero, the model becomes:

$$\ln Y = - .457 + .225X_2 + .575X_1X_2 - .257X_2X_2$$

or $Y = \exp(-.457 + .225X_2 + .575X_1X_2 - .257X_2X_2)$

and a contour plot of Y with X_1 on the abscissa and X_2 on the ordinate can be obtained. Such plots are presented in Figures 3.11-3.14.

Firstly, the $\ln \mu$ model will be considered by studying some contour plots. It must be remembered that such plots are only a partial representation of the data as, in Fig. 3.11 for example, the pH, the light intensity and acetate concentrations have all been set at their centre point values to remove any effect they may have on μ . At other levels of these variables, a different temperature - sulphide contour plot would be obtained.

Figure 3.11 demonstrates some interesting effects. At all temperatures, low sulphide concentrations stimulate the growth rate while high concentrations depress it. However, the sulphide concentration at which the maximum growth rate is predicted, decreases with increasing temperature. Thus at 30°C the highest growth rate is observed at 10 mg/l sulphide while at 10°C it is observed at 1,000 mg/l sulphide. It is interesting that the predicted μ_p is the same at these two points while at 20°C and 100 mg/l sulphide it is only two thirds of the maximum value. Also, temperature stimulates the growth rate at sulphide concentrations less than 100 mg/l but inhibits it at higher concentrations. No evidence to corroborate this latter fact could be found in the literature.

This information again emphasises the importance of considering the effects of any interactions when interpreting the models, as the isolated effect of sulphide (Figure 3.7) indicated that the maximum growth rate occurs at 100 mg/l sulphide. However, the effect of temperature significantly modifies this response.

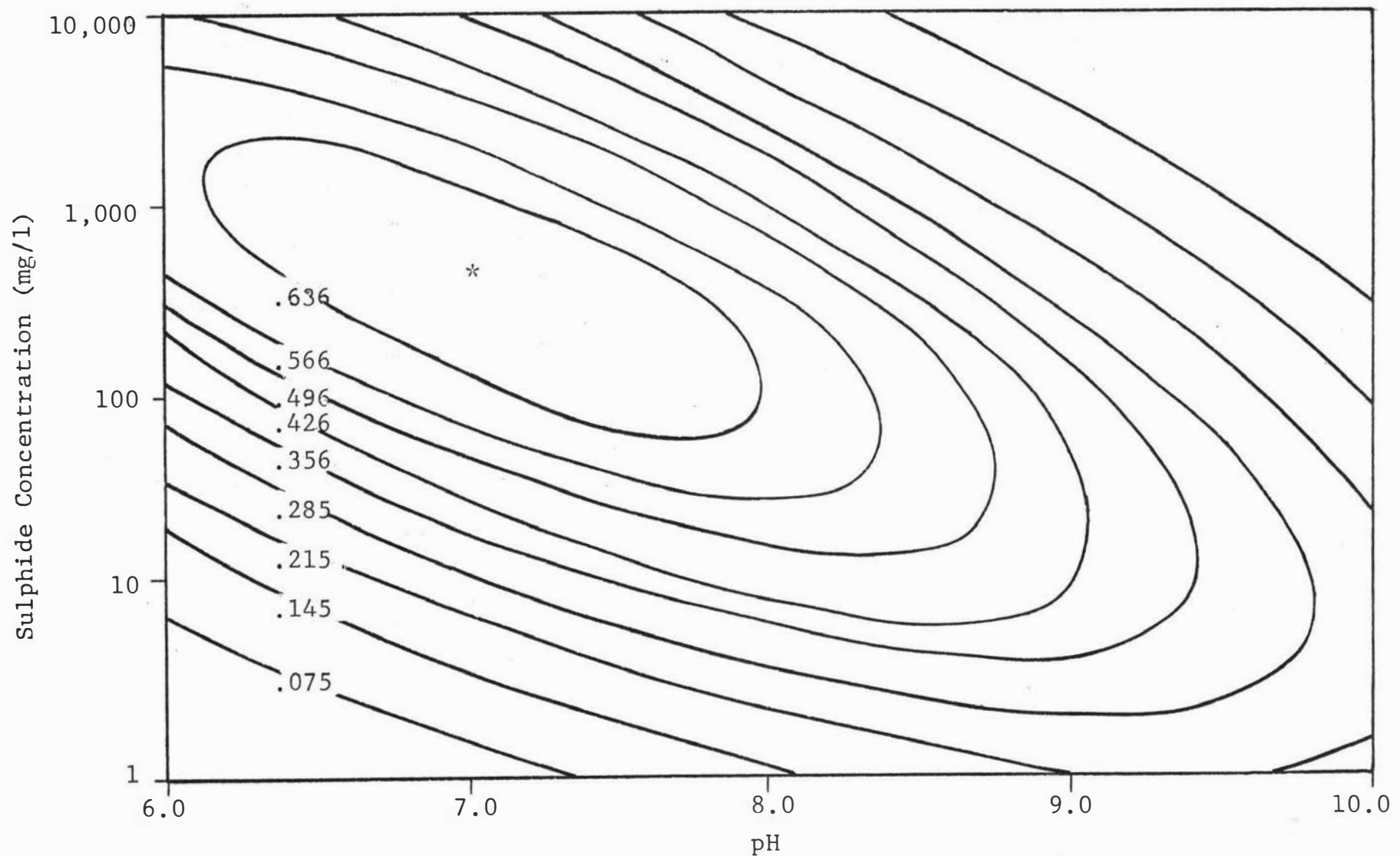
The pH - sulphide isolated interaction (Fig. 3.12) demonstrates that at 20°C, 316 lux and 100 mg/l acetate, the maximum growth rate is predicted to occur at pH 7.0 and 400 mg/l sulphide. This information reveals a significantly different optimum to that indicated by a consideration of only the isolated effects of pH and sulphide (Figure 3.6 and 3.7). This indicates the difficulty in interpreting the results when only a part of the data is considered.

However, this interaction confirms that low concentrations of sulphide stimulate growth while high concentrations retard it.

However, the sulphide concentration at which the maximum growth rate is observed decreases with increasing pH. Thus, at pH 10.0, the maximum predicted μ_p occurs at 5.8 mg/l sulphide while at pH 6.0 it is at 1,060 mg/l sulphide. This example also demonstrates that the pH at which the maximum μ_p occurs decreases with increasing sulphide concentration.

Figure 3.12: The Isolated pH-Sulphide Interaction of the $\ln_{up}$ Model

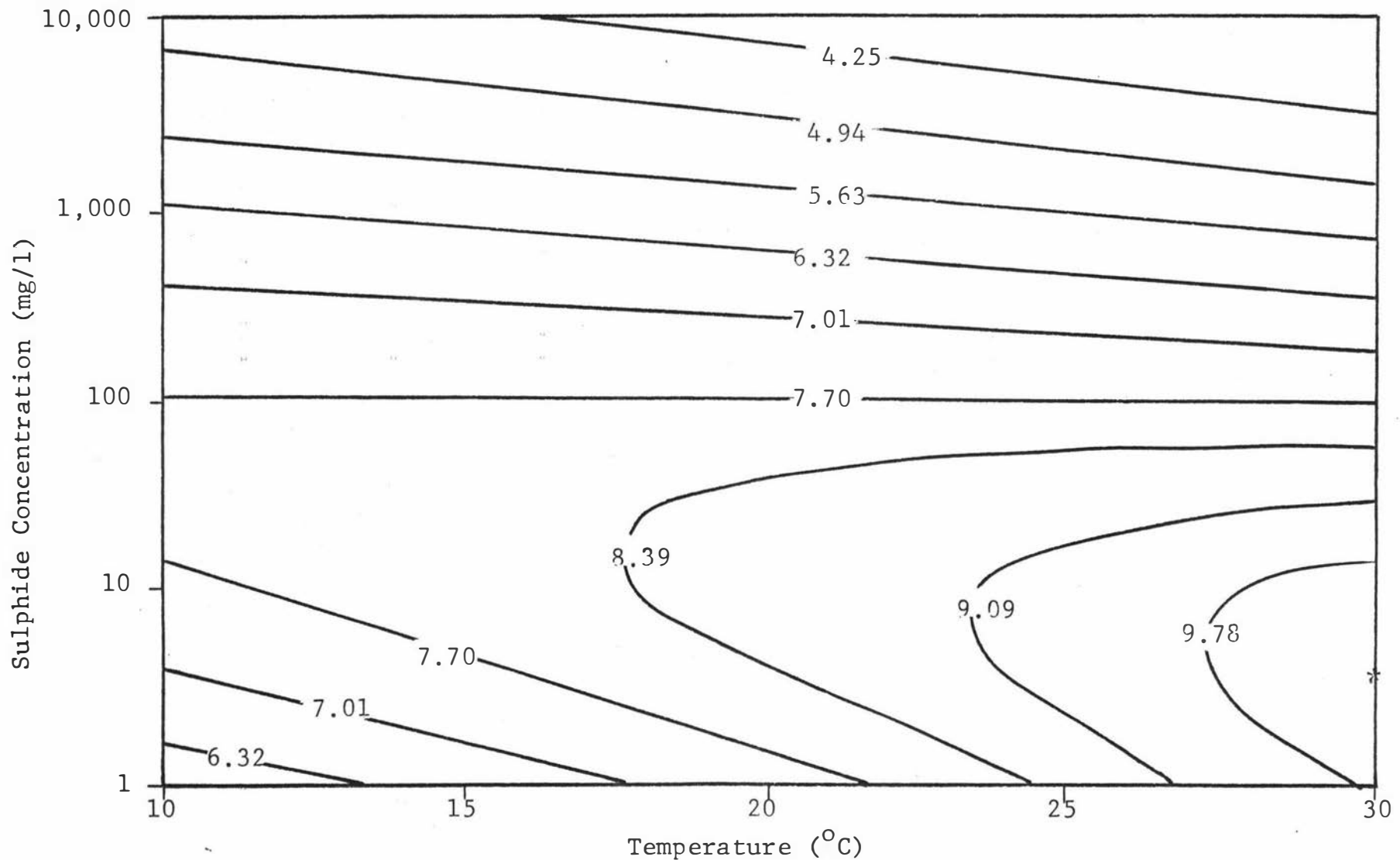
The Maximum Growth Rate is $.706 \times 10^{-3} \text{ hr}^{-1}$ at pH 7 and 400 mg/l S^{2-}



The figures in the body of the diagram are $\ln_{up}$ in units of 10^{-3} hr^{-1}

Figure 3.13: The Isolated Temperature-Sulphide Interaction of the Maximum Count Model

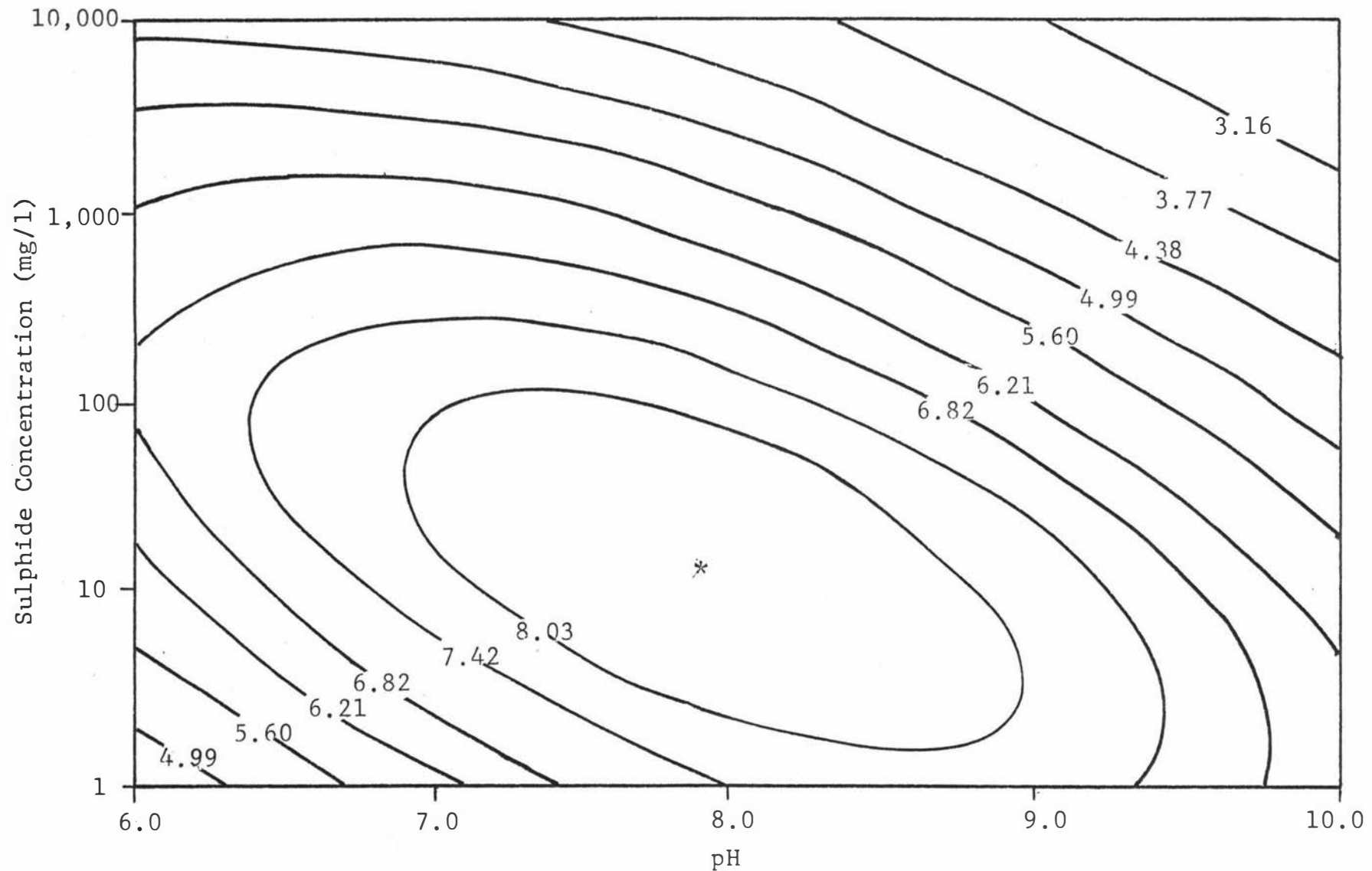
The Highest Maximum Count is 10.5 ($\log_{10}$ cells/ml) at 30°C and 3.2 mg/l S^{2-} .



The figures in the body of the diagram are the Maximum Count in $\log_{10}$ cells/ml.

Figure 3.14: The Isolated pH-Sulphide Interaction of the Maximum Count Model

The Highest Maximum Count is 8.64($\log_{10}$ cells/ml) at pH 8.0 and 12.5 mg/l S^{2-} .



The figures in the body of the diagram are the Maximum Count in $\log_{10}$ cells/ml.

Secondly, the maximum count model will be considered by studying some of the significant interactions.

The temperature - sulphide isolated interaction (Figure 3.13) indicates that at low sulphide concentrations (less than 100 mg/l) increasing the temperature results in increased maximum counts. The predicted maximum count is 3.20×10^9 cells/ml at 30°C and 3.2 mg/l sulphide. Also, the sulphide concentration at which the highest maximum count is observed increases with decreasing temperature.

Above 100 mg/l sulphide, temperature has little effect on the maximum count, which is reduced by the inhibitory effect of high sulphide concentrations.

The isolated pH - sulphide interaction shows a similar shape for both the inverse maximum count (Figure 3.14) and the $\ln \mu_{up}$ models (Figure 3.12). However, the highest maximum count is observed at a lower sulphide concentration (12.5 mg/l compared to 400 mg/l) and a higher pH (8.00 compared to 7.06). Thus the maximum count is more readily reduced by high sulphide concentrations than is the growth rate.

3.3.6.3 Consideration of the Combined Effects of More Than Two Variables:

The isolated interactions considered in Figures 3.11 to 3.14 only present the effects of two independent variables on a growth parameter while the other three variables are held at their centre points. This partial representation of the data is necessary due to the inability to visually represent a five dimensional space. However, the independent variables not considered may have an important effect on the interaction. An example of this is presented in Table 3.8 where the sulphide - acetate interaction is considered at various pH levels. Decreasing the pH increases the predicted maximum μ_{up} while increasing both the sulphide and acetate concentrations at which this maximum μ_{up} occurs. The magnitude of these shifts is substantial and so while the isolated interactions are useful as they indicate the general trends caused by the

TABLE 3.8

Summary of the Effect of pH on the Sulphide-Acetate Interaction of the $\ln \mu$ Model

pH	Maximum μ predicted at		Predicted maximum μ ($\times 10^{-3} \text{h}^{-1}$)
	sulphide (mg/l)	acetate (mg/l)	
6	400	6,000	1.02
7	103	2,500	.985
8	25	1,030	.776
9	10	650	.501
10	4	320	.264

independent variables, a truly quantitative prediction of the growth rate and maximum population can only be obtained by inserting the correct levels of the independent variables into the equations presented in Tables 3.6 and 3.7.

3.4 Application of the Models to Waste Treatment Lagoons:

The aim of this experiment was to provide an insight into conditions causing the growth of Chromatium minutissimum in waste treatment lagoons and, to this end, models predicting the growth rate and maximum count have been developed. To make use of these equations, the expected levels of the independent variables in typical lagoon systems must be determined.

Lagoon temperatures may range from 5°C to 30°C depending upon the latitude and the season.

Lagoon pH's vary widely but lagoons in which the Chromatiaceae are dominant do not exhibit the large diurnal pH fluctuations characteristic of facultative lagoons. The pH of Chromatiaceae lagoons usually falls in the range 7.1 - 9.1 (Chapter 4).

The sulphide concentration in such lagoons also varies widely and is dependent upon the characteristics of the effluent being treated. Concentrations of 0 mg/l to 62.4 mg/l sulphide have been observed (Chapter 4).

A wide range of acetate concentrations has been observed in anaerobic systems with values of 30 - 1,900 mg/l having been reported (Hindin and Dunstan 1960). However the acetate concentration most frequently falls within the range 30 - 300 mg/l (Eckenfelder 1970, Hindin and Dunstan 1960). To a certain extent the acetate concentration will be dependent upon the sulphide concentration, with soluble sulphide concentrations of 200 mg/l inhibiting methanogenesis and resulting in increased concentrations of volatile acids.

In the vicinity of Palmerston North, N.Z., the daily average light intensity varies from 11,000 lux in July to 43,000 lux in January (De Lisle 1966, Kimball 1924). However, in lagoons, the amount of light transmitted decreases rapidly with depth and typical extinction coefficients which have been observed in lagoons are presented in Table 3.9. These coefficients indicate that a light intensity of 31.6 lux may be obtained at a depth of .418 m in the winter and .814 m in the summer. So the range of light intensities investigated in this study may be expected to occur in the upper

TABLE 3.9

Extinction Coefficients Observed in Five Waste Treatment Lagoons in North and South Dakota (Bartsch and Allum 1957).

season	range of coefficient	average coefficient
spring	9.9 - 46.0	21.4
summer	5.9 - 10.8	8.80
autumn	10.8 - 22.9	17.1

regions of a typical lagoon.

The extreme values of the independent variables which would be anticipated for a New Zealand lagoon, and which fell within the range

of the experimental design, were substituted into the developed models. There were 2^5 , or 32, combinations of the independent variables and these, along with the calculated growth rates and maximum counts, are tabulated in Appendix 3. The range of dependent variables predicted and the conditions under which they occur are presented in Table 3.10.

TABLE 3.10

Range of the Dependent Variables Predicted Under Extreme Lagoon Conditions.

Dependent Variable		Independent Variables				
		Temp. (°C)	pH	sulphide (mg/l)	acetate (mg/l)	light (lux)
lowest μ (hr ⁻¹ x 10 ⁻³)	.362	10	9.1	1	30	31.6
highest μ (hr ⁻¹ x 10 ⁻³)	.840	30	9.1	62.4	1,900	31.6
lowest maximum count (cells/ml)	2.72×10^7	10	7.1	62.4	1,900	3,160
highest maximum count (cells/ml)	9.10×10^8	30	9.1	1	1,900	3,160

There is therefore nearly a hundred-fold variation in the predicted maximum counts and approximately a 2.5-fold variation in the predicted growth rate.

The growth rate calculations indicated that washout of C. minutissimum would occur at retention times between 50 days and 115 days. These values are higher than might be expected under field conditions (Chapter 4) and may be explained by the following:

(i) the organism was grown in pure culture. Changing from pure culture to mixed culture may substantially alter the growth characteristics of a bacterium with the nature of the change being

dependent upon the relationship between the organisms (Pirt 1975). As syntrophic growth has been demonstrated for some of the phototrophic bacteria (Biebl and Pfennig 1978) more rapid growth in mixed culture than in pure culture may be anticipated.

(ii) the use of a synthetic culture medium. There will be a substantial difference between the media in which this experiment was conducted and the lagoon environment. Such a difference may significantly alter the growth characteristics of the bacterium.

(iii) only one species of the Chromatiaceae, C. minutissimum, was studied. Although isolated from an anerobic lagoon, it has not been frequently observed in such situations (Section 2.5.4.2 and Chapter 4) and further research is required to determine whether its growth characteristics are similar to those bacteria frequently inhabiting such environments.

Thus, the points covered in (i) and (ii) above are likely to result in increased growth rates and, as indicated by point (iii), other Chromatiaceae may have the genetic capability for faster growth than C. minutissimum.

The models developed are likely to result in a conservative prediction of the growth rate and populations of Chromatiaceae observed in waste treatment lagoons.

The maximum count calculations (Appendix 3) indicate that, under the range of environmental conditions expected for lagoons in New Zealand, substantial populations of C. minutissimum would occur at retention times above washout. As red lagoons have been observed at populations as low as 2×10^6 cells/ml, a highly coloured effluent would be produced. The calculations also indicate that manipulation of the environmental parameters would not provide control over the occurrence of this bacterium. Only by reducing the hydraulic retention time below the washout point will its dominance be prevented.

3.5 Conclusions:

Two models which adequately described the growth of C. minutissimum in pure culture in synthetic medium were developed. These models yield a conservative prediction of the growth rate and maximum count of this bacterium in a waste treatment lagoon.

The models indicated that high populations of these bacteria would develop in lagoons under the range of environmental conditions likely to prevail in N.Z. and that their prevalence could only be prevented by reducing the hydraulic retention time below that at which washout would occur.

However, this is a simplistic explanation of what will occur in a waste treatment lagoon as these experiments were performed on pure cultures and any competition by other organisms for the niche occupied by the phototrophic bacteria is ignored. It will be shown in Chapter 4 that at relatively long retention times and low organic loading rates green algae successfully compete for the upper reaches of the lagoon. This generally restricts the dominance of Chromatiaceae to a relatively narrow range of retention times and loading rates and at the retention times required for the dominance of C. minutissimum it is probable that the green algae would be the dominant lagoon microflora.

CHAPTER 4

OBSERVATIONS ON THE OCCURRENCE OF THE
CHROMATIACEAE IN WASTE TREATMENT LAGOONS

4. OBSERVATIONS ON THE OCCURRENCE OF THE CHROMATIACEAE IN WASTE TREATMENT LAGOONS

4.1 INTRODUCTION:

The occurrence of Chromatiaceae in lagoons treating a variety of wastes has been noted (Section 2.5.4.). In some situations, they formed a permanent dominant population while in others, their prevalence was of a more transient nature. This chapter presents and discusses observations of the Chromatiaceae in a wide variety of waste treatment lagoons.

A succession from anaerobic heterotrophs to phototrophic bacteria to algae has been demonstrated during the incubation of an organic waste in the light (Kobayashi 1972). To confirm these studies, the succession of microorganisms in the batch culture of samples removed from lagoons treating piggery and meatworks wastes was investigated.

A number of lagoon systems were then studied to investigate the typical Chromatiaceae present and to elucidate the reasons for their occurrence. To ensure a thorough investigation of the conditions under which these bacteria became prevalent, as many lagoons as possible in which they were known to occur were observed. Lagoons ranging from facultative to anaerobic were investigated and the effluents treated varied from domestic sewage to strong industrial and agricultural effluents. As many of these lagoons were geographically remote from Palmerston North, it was impractical for the author to perform all the necessary analyses and to assess the seasonal fluctuation in the lagoons' population. He was therefore dependent upon the regular analyses by the people involved in the monitoring of the lagoons and he is most appreciative of their assistance.

The author was only closely involved with the monitoring of a meat works lagoon system in the vicinity of Palmerston North. Brief visits to four of the other lagoons allowed some chemical and microbial analyses to be carried out. The author only performed microbial investigations on the two South African lagoon systems.

These studies showed that the three stage succession observed in batch cultures may be extrapolated to full scale lagoons and that the organic loading rate exerted on a lagoon is an important parameter in determining the predominance of the Chromatiaceae.

4.2 METHODS OF STUDYING LAGOONS

4.2.1 Organic Loading Rates on Lagoons:

As the organic loading rate is an important parameter when considering the occurrence of Chromatiaceae in lagoons, it is essential to establish typical loadings for facultative and anaerobic lagoons. However, such loadings vary with climatic conditions, effluent composition and other factors (Oswald 1968) and only serve as a base from which the loadings of the lagoons subsequently studied may be assessed.

Organic loadings on facultative lagoons are usually expressed on a surface area basis ($\text{g BOD/m}^2 \cdot \text{d}$) due to the dependence of the algal oxygen contribution upon sunlight (Aguirre and Gloyna 1970). Generalised BOD loadings for such lagoons treating domestic sewage are presented in Table 4.1. The effect of the environmental conditions can readily be discerned. In the U.S.A., BOD loadings of $1\text{--}10 \text{ g BOD/m}^2 \cdot \text{d}$ are common (McKinney et al 1971, Canter and Englande 1970, Aguirre and Gloyna 1970) while the Auckland Regional Authority operates the largest lagoon system in New Zealand at loadings of $8.4\text{--}16.8 \text{ g BOD/m}^2 \cdot \text{d}$ (Brockett 1975, Collum 1972). The recommended loading for N.Z. is $8.4 \text{ g BOD/m}^2 \cdot \text{d}$ (Ministry of Works and Development 1974).

For anaerobic lagoons, algal oxygenation is unimportant and BOD loadings are usually expressed on a volumetric basis

(g BOD/m³.d). Loadings of 190-320 g BOD/m³.d are considered common in the U.S.A. (McKinney et al 1971, White 1970) while the Israeli Ministry of Health quotes 125 g BOD/m³.d (Shuval 1966).

This variation in units makes a comparison between loading rates on facultative and anaerobic lagoons difficult. However, the minimum surface loading rate necessary to maintain anaerobic conditions does indicate the region in which the transition from facultative to anaerobic conditions occurs. This loading varies with environmental conditions with values quoted for California of 9.0 g BOD/m².d in the winter and 35.0 g BOD/m².d in the summer (Oswald 1968).

TABLE 4.1

Generalised Surface BOD Loadings for Facultative Lagoons
Under Various Environmental Conditions (Gloyna 1971)

Surface Loading (g BOD/m ² .d)	Environmental Conditions
Less than 1.0	Frigid zones, with seasonal ice cover, uniformly low water temperatures and variable cloud cover.
1.0 - 5.0	Cold seasonal climate, with seasonal ice cover and temperate summer temperatures for short season.
5.0 - 15.0	Temperate to semi-tropical, occasional ice cover, no prolonged cloud cover.
15.0 - 35.0	Tropical, uniformly distributed sunshine and temperature, and no seasonal cloud cover.

In Section 4.6, information on two South African lagoon systems treating tannery effluent is presented. In South Africa, the BOD is not used as a parameter for determining the organic loading (Funke 1977) and the oxygen absorbed (OA) as measured by the 4 hour permanganate value (Standard Methods for the Examination of Water

and Wastewater 1975) is the preferred technique. It is therefore difficult to compare the loadings of the South African and New Zealand lagoons.

4.2.2 Lagoon Retention Time:

In this chapter, the hydraulic retention time of a lagoon is defined as the lagoon volume divided by the daily hydraulic flow. Although this definition has limitations due to the various flow regimes observed in different lagoons, it is useful as a basic parameter for comparing lagoon systems.

4.2.3 Analytical Techniques:

Chemical, biochemical and physical parameters were measured in accordance with Standard Methods for the Examination of Water and Wastewater (1975). Sulphides were determined by the titrimetric method.

Chromatiaceae were enumerated by direct counts using a Hawksley improved Neubauer counting chamber (Holm and Vennes 1970). Enrichment cultures of these bacteria were prepared according to van Niel (1971). Identification of genera and species followed Pfennig and Truper (1974). Refractile cell inclusions which could be removed by centrifugation at 5,000 rpm for 10 minutes or applying pressure to the coverslip were considered to be gas vacuoles (Caldwell and Tiedje 1975a, Irgens 1977). Refractile cell inclusions which were eluted with ethanol were considered to be sulphur granules (Van Gernerden 1968a). Polyhydroxybutyrate (PHB) was identified by staining with sudan black B (0.02% in 96% ethanol) for 20 minutes and then washing for 1 minute with 96% ethanol (Schlegel et al 1970). PHB then appeared as dark cell inclusions.

Total anaerobic counts were obtained by performing three dilutions in the expected range. Each of these was then plated

in duplicate on Oxoid's Tryptone Glucose Extract Agar (Tasman Vaccine Laboratory, 35 Whakatiki Street, Upper Hutt, N.Z.) and incubated under anaerobic conditions at 30°C for 48 hours (Holm 1969).

Algae were identified according to Prescott (1970) and were enumerated by direct microscopic counts as described for the Chromatiaceae.

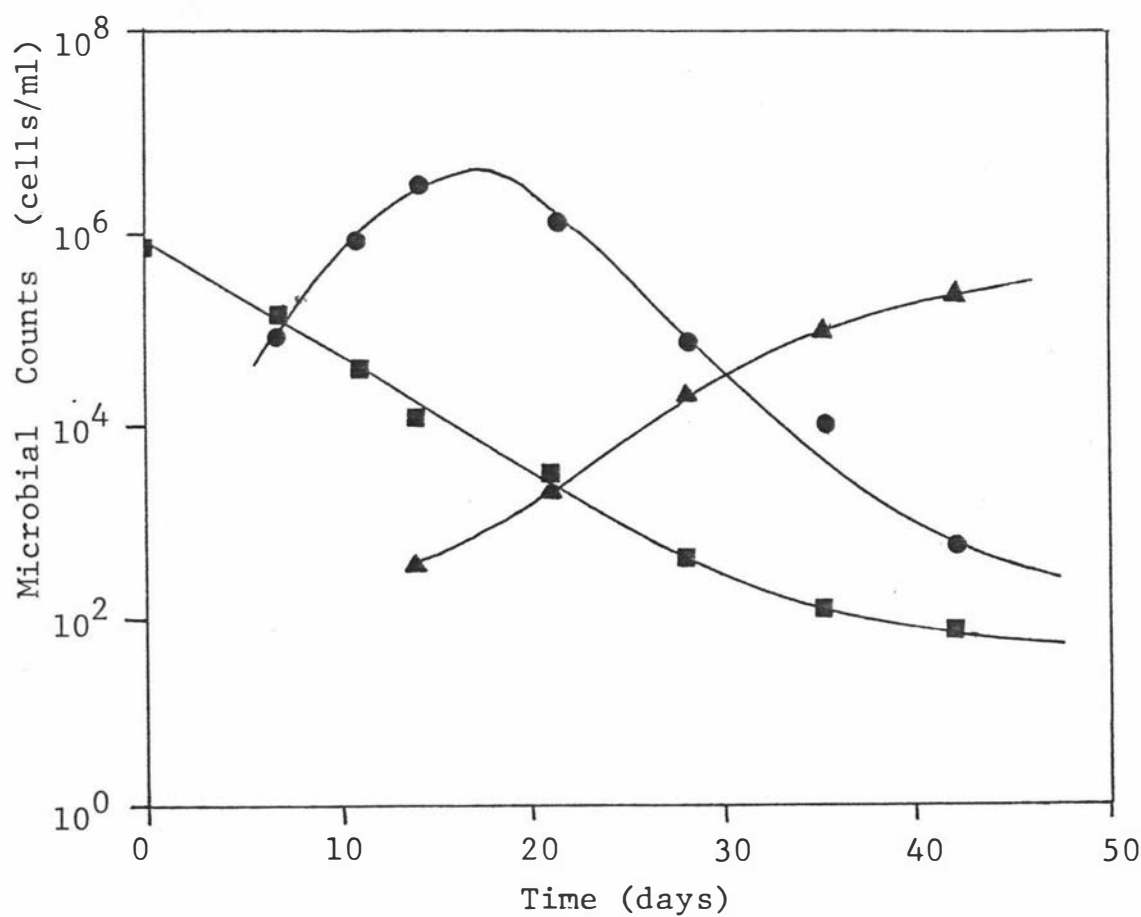
Photomicrographs were taken on either an Olympus Photomax (Olympus Optical Co. Ltd., 3-7 Kanda-Ogawamachi, Chiyodaku, Tokyo, Japan) or a Leitz Ortholux 2 (Ernst Leitz, GMBH D6330 Wetzlar, Germany) microscope.

4.3 BATCH CULTURE OF LAGOON LIQUORS:

To confirm prior studies of the succession of micro-organisms in wastes exposed to the light (Kobayashi 1972, Kobayashi and Nakanishi (1970)), various lagoon samples were incubated in sealed 250 ml glass bottles and exposed to natural diurnal fluctuations in light and temperature.

Samples were taken from two lagoon systems. The first was from an anaerobic lagoon treating meat works effluent (see Section 4.4). Each bottle was filled with 225 mls of lagoon supernatant liquor mixed with 25 mls of bottom sludge and the variations in bacterial and algal populations were observed with time (Figure 4.1). At the time of sampling, the lagoon was brown and there was no visible evidence of the presence of the Chromatiaceae in the lagoon. After eight days incubation, the liquor in the bottle appeared pink corresponding to a Chromatiaceae count of approximately 10^5 cells/ml. Thiocapsa roseopersicina was the dominant organism at this stage and remained so until the fourteenth day of incubation. Subsequently, Chromatium spp. became dominant. Cell counts of the Chromatiaceae observed are presented in Table 4.2. The predominant alga observed was Chlamydomonas. Although Euglena spp. were also present, they did not contribute significantly to the cell count.

Figure 4.1: Sample from an Anaerobic Lagoon
Treating Meatworks Effluent.
(Section 4.4)



KEY: Total Anaerobic Counts —■—
 Chromatiaceae —●—
 Algae —▲—

TABLE 4.2

Cell Counts of the Chromatiaceae Observed During the Incubation of Meatworks Lagoon Liguor.

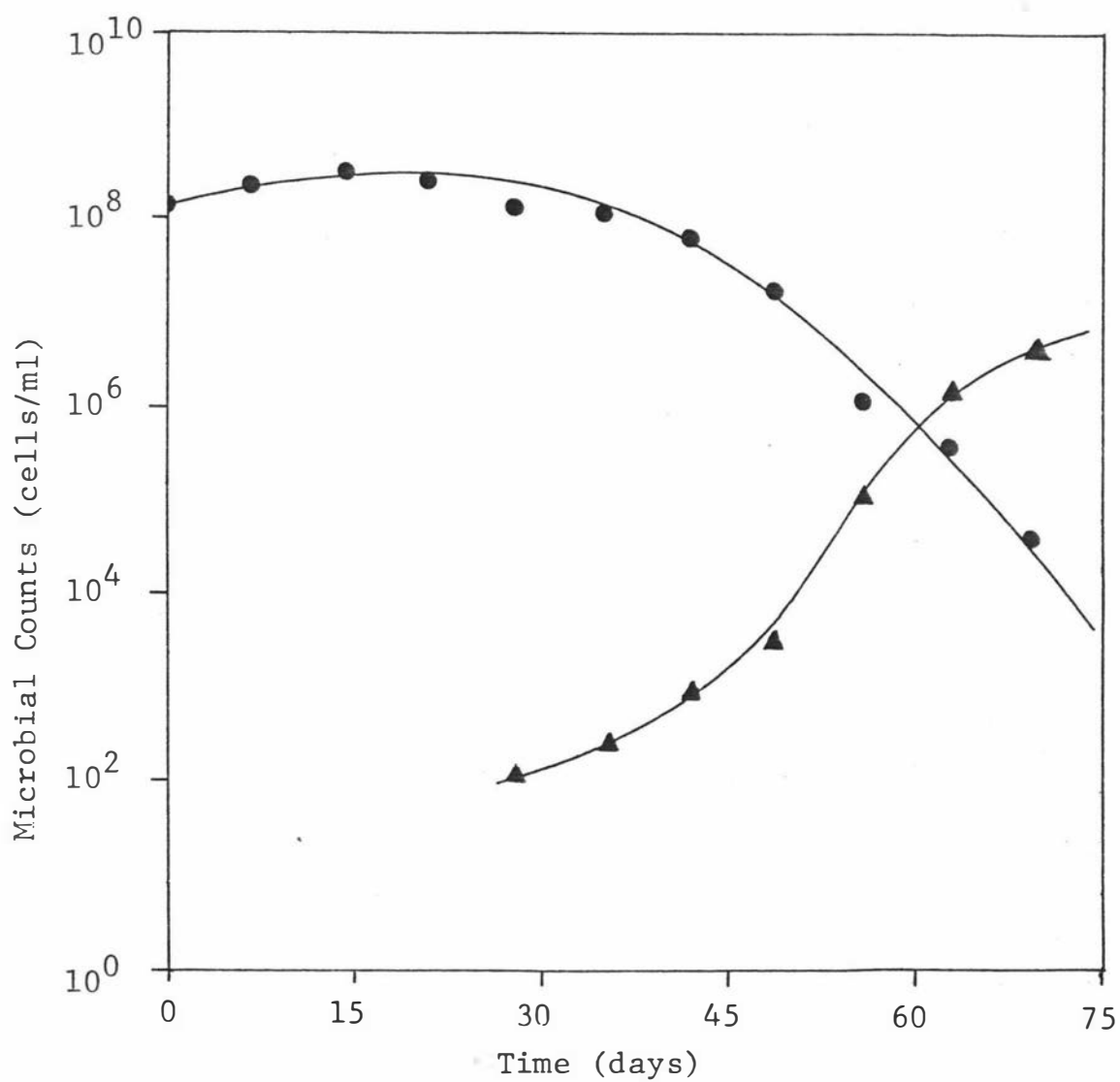
Incubation Time (days)	<u>Thiocapsa</u> spp. (cells/ml)	<u>Chromatium</u> spp. (cells/ml)
7	7.80×10^4	-
11	7.98×10^5	5.07×10^4
14	2.91×10^6	2.63×10^5
21	3.41×10^5	6.62×10^5
28	6.44×10^4	6.14×10^5
35	-	1.00×10^4
42	-	3.80×10^3

This sample therefore demonstrated a succession from the anaerobic bacteria initially prevalent to the algae which finally predominated. The Chromatiaceae occupied a niche located temporarily between these two populations.

A similar succession was also observed in samples taken from a lagoon system treating piggery effluent.

Two samples from different lagoons were incubated (see Section 4.5.1). Sample A (Fig. 4.2) was taken from the first of a series of three lagoons. This lagoon was anaerobic, pink and contained a relatively high initial population of 1.53×10^8 cells/ml of Thiocapsa roseopersicina. No other Chromatiaceae contributed significantly to the cell count. In this instance no succession of Chromatiaceae was observed. Thiocapsa counts peaked after 14 days incubation and subsequently declined very slowly. The high initial level of suitable substrates is the likely reason for the slow decrease in numbers (c.f. Fig's 4.1, 4.3). Similarly, algal growth was very slow and only after approximately two months did they become dominant. Again, Chlamydomonas was the dominant genus.

Figure 4.2: Sample from Lagoon 1 of Piggery Lagoon
System A (Section 4.5.1)



KEY: Chromatiaceae —●—
 Algae —▲—

Sample B (Fig. 4.3) was taken from the third and final lagoon of this system. In this instance a far more rapid transition from Chromatiaceae dominance to algal dominance is apparent. Thiocapsa roseopersicina was the dominant Chromatiaceae observed while Euglena and Chlorella were the dominant genera of algae.

In this example, the Chromatiaceae were dominant in both lagoons sampled and therefore no transition from anaerobic heterotrophs to the phototrophic bacteria was apparent. A succession from Chromatiaceae to algae was observed in each sample but the rate of this succession varied greatly. This observation may be explained by considering the sampling locations. Sample A was removed from lagoon 1 of the three lagoon system.

High levels of organic compounds, and presumably sulphur compounds, were present in this lagoon and the Chromatiaceae were only prevalent due to the lagoon's relatively long retention time (Section 4.5.1). The high substrate levels therefore resulted in an extended phase of Chromatiaceae dominance and the algae only predominated when the phototrophic bacteria provided conditions suitable for their growth.

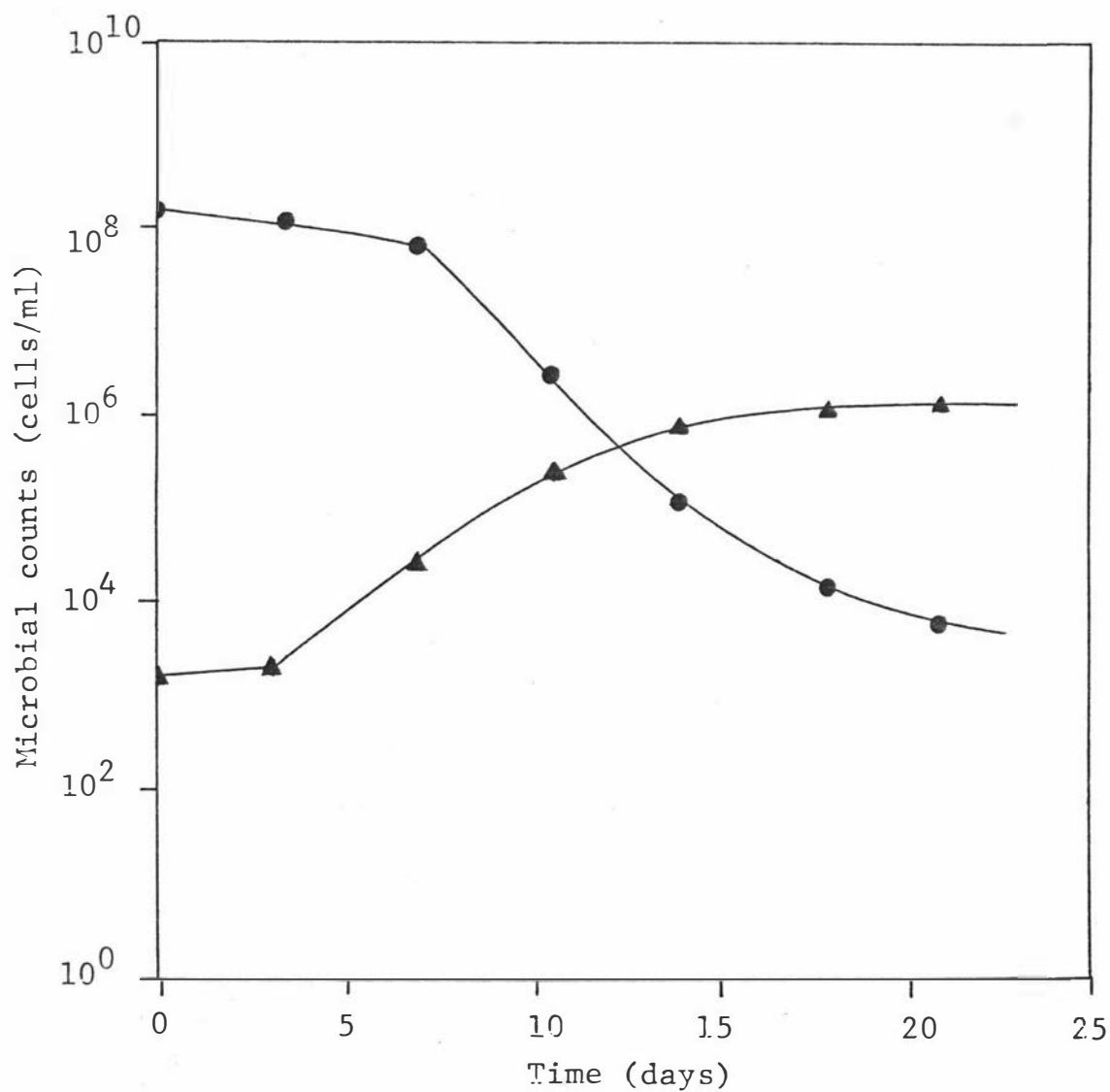
Sample B was taken from the final lagoon and subsequently the levels of organic and sulphur compounds were much lower. The Chromatiaceae were therefore nearly at the end of their period of dominance and a rapid transition to an algal phase was observed.

These results confirm the succession from anaerobic heterotrophs to phototrophic bacteria to algae. Such a succession may therefore be anticipated during the degradation of any waste exposed to the light if adequate retention time and suitable substrate concentrations are available.

There are problems in extrapolating these results to full scale lagoons due to :-

(i) The closed nature of the laboratory system. This prevents any diffusion of oxygen into, and carbon dioxide out of, the system.

Figure 4.3: Sample from Lagoon 3 of Piggery Lagoon
System A (Section 4.5.1)



KEY: Chromatiaceae ●
Algae ▲

Obviously, this will affect the microflora which develops.

(ii) The much higher surface area to volume ratio of the bottle system than a lagoon. This will allow better penetration of sunlight and will therefore greatly affect the growth rate of both the phototrophic bacteria and the algae. It may also influence which microorganisms are favoured.

Having ascertained that a three stage succession took place in bottle cultures, a series of lagoons in which the Chromatiaceae had been observed were studied to investigate whether such a succession theory could be applied to the lagoon situation. If so, it would lead to a greater understanding of the occurrence of Chromatiaceae in lagoon systems.

4.4 CHROMATIACEAE IN A LAGOON SYSTEM TREATING MEATWORKS EFFLUENT

4.4.1 System Description:

A meatworks capable of slaughtering approximately 10,000 sheep and lambs daily treats its effluent by primary sedimentation followed by anaerobic lagoons. Prior to sedimentation, gross solids are removed by bar screens.

The settled effluent is passed to three anaerobic lagoons operated in parallel whose physical and operational characteristics are described in Table 4.3

As the volume of effluent produced is dependent upon the number of animals slaughtered, the hydraulic retention time in the lagoon is variable. Under normal operating conditions it is approximately 18 days (Fig. 4.4.).

Intermittent sludge recycling is practised to increase the solids retention time.

TABLE 4.3

Characteristics of Meatworks Lagoon System.

Parameter	Value
Number of Lagoons	3
Mode of Operation	Parallel
Total Area (m ²)	17,000
Average Depth (m)	5
Volume (m ³)	85,000
Average Volumetric BOD load (g/m ³ .d)	83
Average Surface BOD load (g/m ² .d)	415

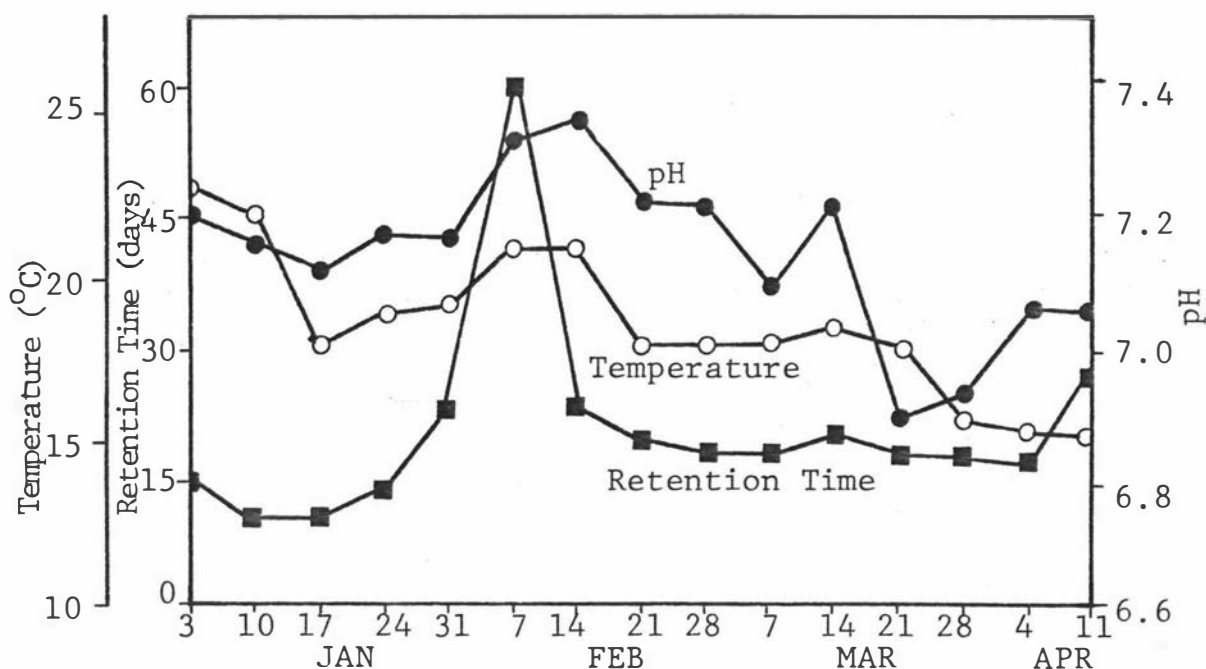
4.4.2 Observation of Meatworks Lagoon System:

With the exception of the bacterial counts, the COD and the sulphide data, the observations presented in this section have been calculated from information kindly provided by the management of the meatworks.

The observations presented in this section are restricted to two periods during, and immediately subsequent to, the dominance of the Chromatiaceae in this lagoon system. Firstly, the period from 3rd January to 11th April, 1974, is considered. Subsequently, data from 13th February to 20th March, 1975, are presented. The data points are the average of a weeks analyses.

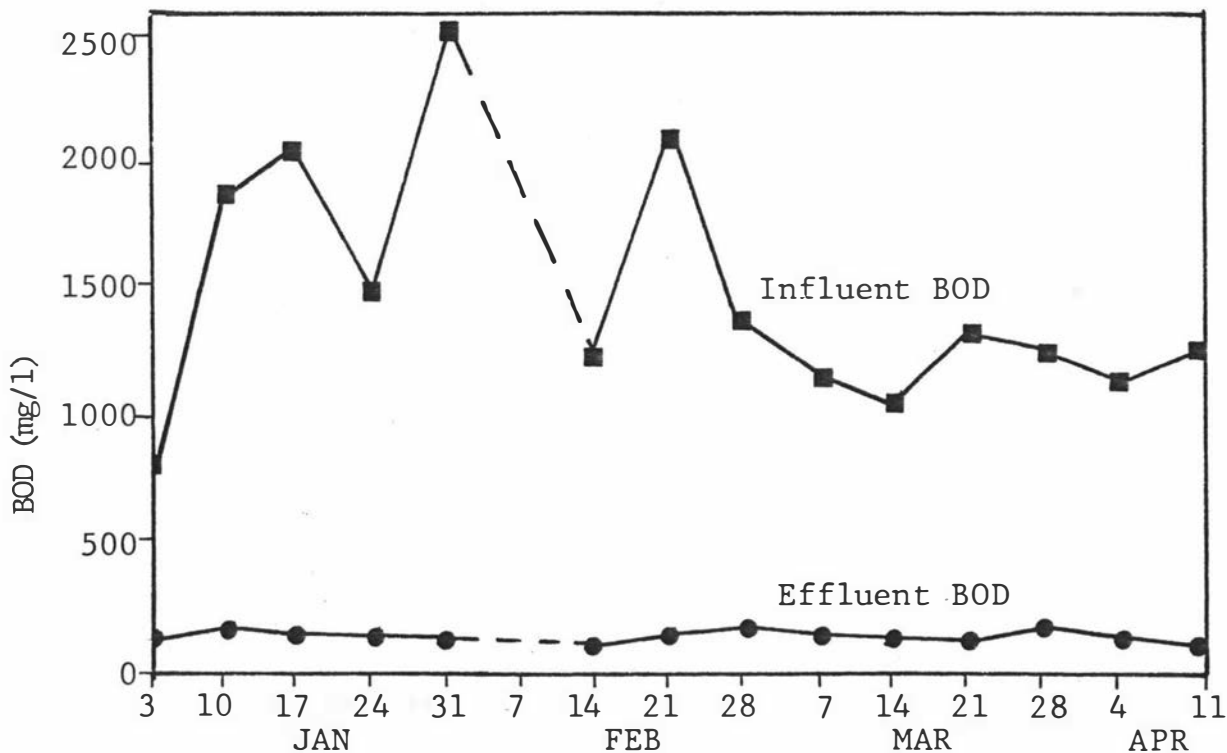
During the 1973 Christmas vacation, no effluent was produced for a week, resulting in an increased retention time. When processing resumed, all three lagoons were a distinct red colour due to the presence of Chromatiaceae. However, these bacteria did not form a stable dominant population and by March 21, 1974, the lagoons had resumed their usual mud brown colour. The results of the variation in retention time, temperature, pH, and BOD in the lagoons in early 1974 are presented in Figures 4.4. and 4.5. The increased retention time during the week of February 7, 1974, was due to industrial action which halted processing.

Figure 4.4: Meatworks Lagoon Environmental Parameters(1974)



Each datum point represents the average of a weeks analyses for all three lagoons.

Figure 4.5: Meatworks Lagoon BOD Data(1974)



Each datum point represents the average of a weeks analyses for all three lagoons.

During the following year, further observations were made. From February 5, 1975, Lagoon 3 did not receive any effluent for a month and rapidly turned red. The remaining two lagoons were then treating the entire effluent and remained their normal mud brown colour. The results obtained during this period are presented in Figures 4.6 and 4.7. In addition, the variation in COD and sulphide concentrations are presented in Figures 4.8. and 4.9.

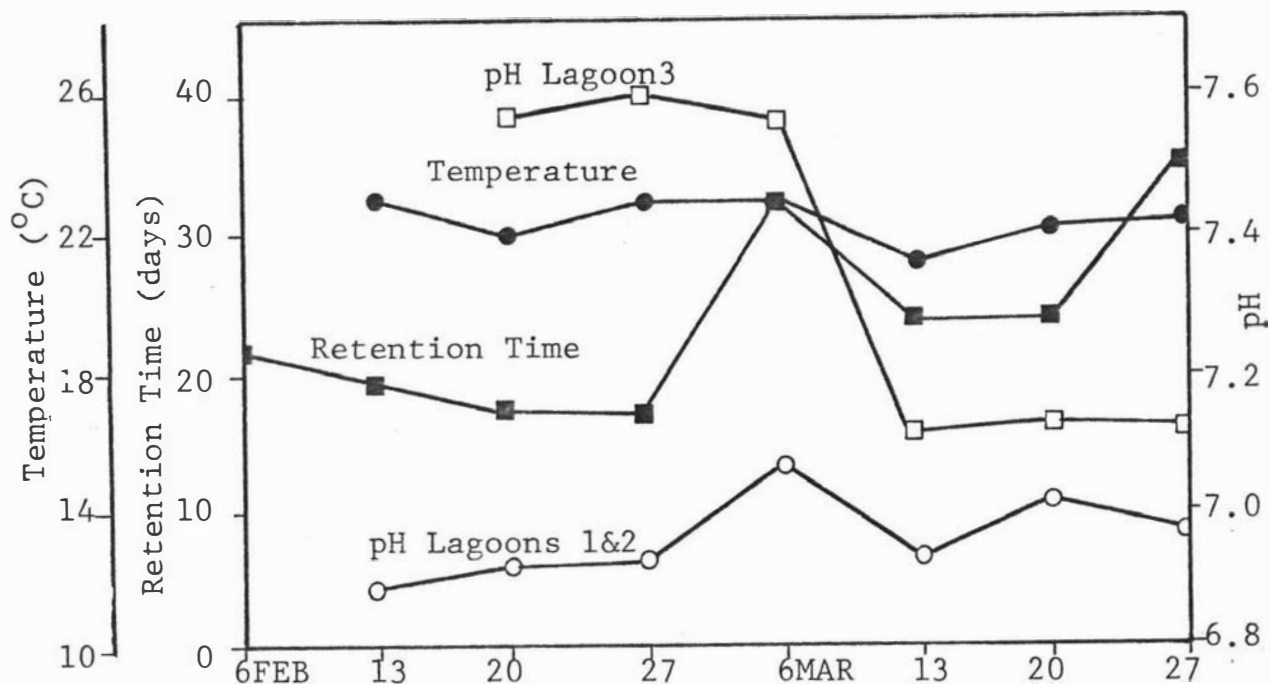
Microscopic examination of the liquor from Lagoon 3 demonstrated that Thiocapsa was initially the dominant genus at 10^7 cells/ml with Chromatium spp. also present at 10^4 cells/ml. As the retention time increased, a succession of Chromatiaceae was observed. After two weeks, Chromatium spp. were as numerous as Thiocapsa at 10^6 cells/ml. On March 6, 1975, when the lagoon recommenced receiving effluent, the liquid contained 5×10^6 cells/ml of Chromatium spp. and 10^5 cells/ml of Thiocapsa. At that stage, bacteria resembling Thiopedia were appearing. Once loading resumed, the numbers of Chromatiaceae rapidly decreased with 10^2 and 10^3 cells/ml Chromatium spp. and Thiocapsa being recorded on March 13. No Thiopedia were observed.

Photomicrographs of the bacteria observed are presented in Fig. 4.10. No intracellular sulphur deposits are visible. This is to be expected in environments where sulphide is absent. On culturing on Pfennig's medium containing sulphide (Van Niel 1971), sulphur granules were observed in the cells. The refractile bodies apparent within the cells of the Chromatium spp. were sudanophilic implying they were PHB (Schlegel et al 1970).

4.4.3 Discussion:

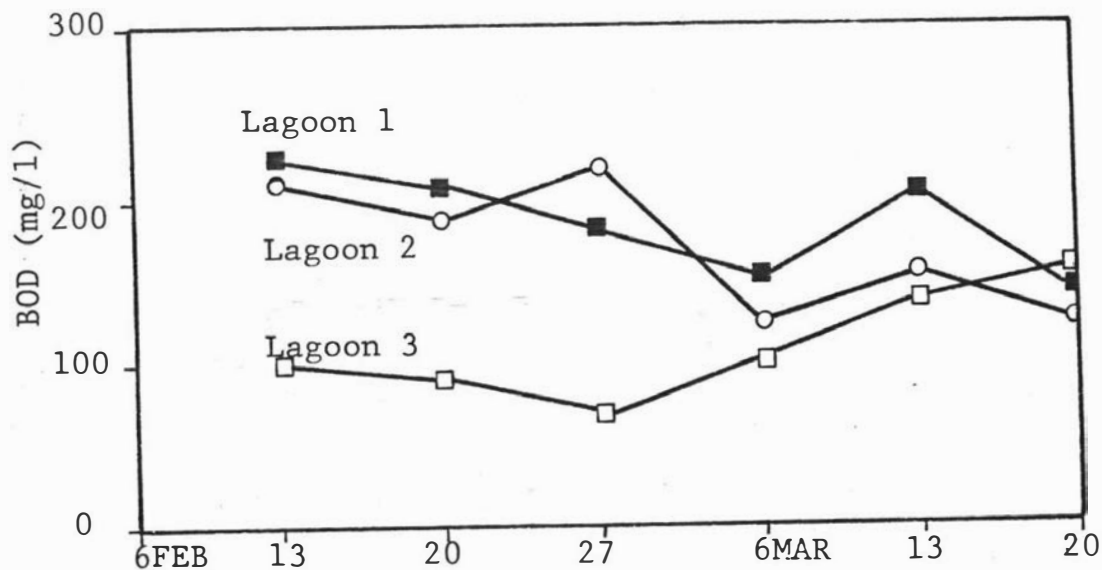
The 1974 results indicate that the lagoon performance was satisfactory while the Chromatiaceae were the dominant population (Fig's. 4.4. and 4.5). No significant variation occurred in the effluent BOD levels while the pH showed a slight increase. Only the colour of the effluent caused discharge problems.

Figure 4.6: Meatworks Lagoon Environmental Parameters (1975)



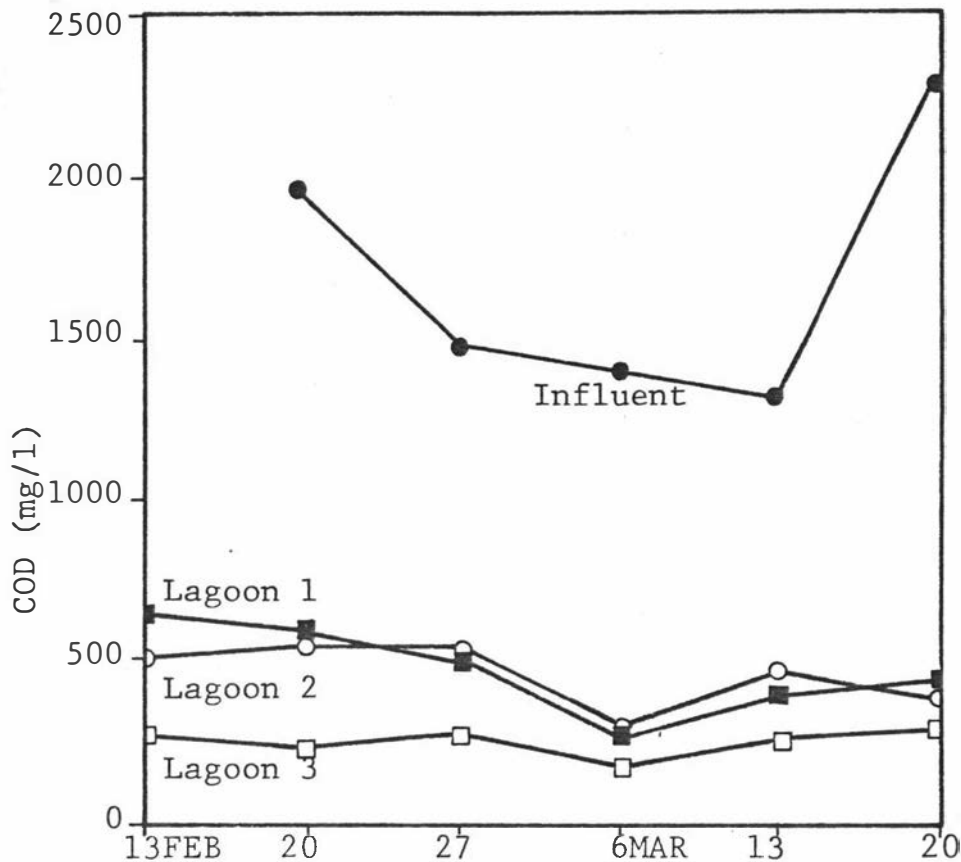
Each datum point represents the average of one weeks analyses.

Figure 4.7: Meatworks Lagoon BOD Data (1975)



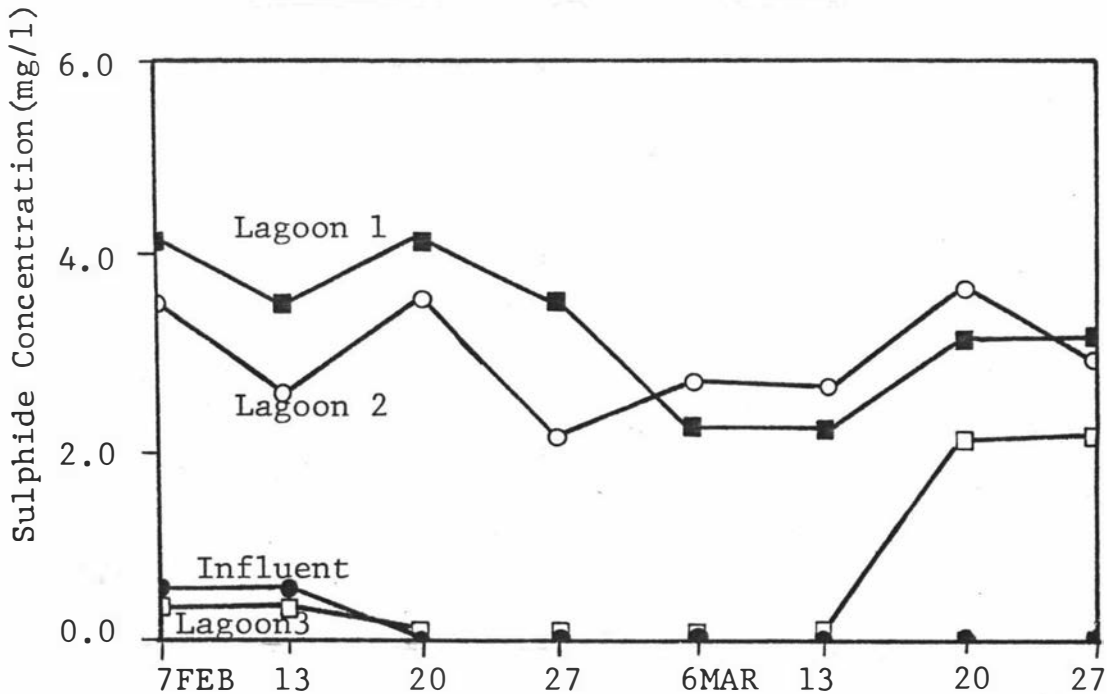
Each datum point represents the average of one weeks analyses.

Figure 4.8: Meatworks Lagoon COD Data (1975)



Each datum point represents the average of one weeks analyses.

Figure 4.9: Meatworks Lagoon Sulphide Data (1975)



Each datum point represents the average of one weeks analyses.

Thus, of the parameters measured in 1974, only the pH appears indicative of the numbers of Chromatiaceae present. Any pH change is dependent upon the buffering capacity of the medium and the production or consumption of acid or alkaline substances (Lijklema 1969). In fresh waters (Wetzel 1975) and effluents (Lijklema 1969) the $\text{CO}_2\text{-HCO}_3^-\text{-H}_2\text{CO}_3$ system is the major buffering mechanism. The pH is therefore very dependent upon the CO_2 concentration and the photosynthetic fixation of CO_2 can result in significant increases in pH (Gloyne 1968, Weber and Stumm 1963).

Conversely, the oxidation of sulphide leads to a decrease in pH (Weber and Stumm 1963).

These two observations must therefore be balanced when considering the effect of the growth of the Chromatiaceae on the lagoon pH. In this lagoon system, an increase of 0.3 to 0.5 pH units was associated with the growth of these bacteria (Fig's 4.5 and 4.7). The effect of CO_2 fixation therefore predominates over any sulphide oxidation effects. This is to be expected as the low sulphide concentrations observed in these lagoons during periods when the Chromatiaceae were not dominant would minimise the pH variation from this source.

The growth of the Chromatiaceae may therefore be approximately followed by studying the pH. In the first week of January 1974, the pH was high implying the Chromatiaceae had proliferated during the Christmas-New Year period when the retention time was long (Fig. 4.4.). As loading was resumed, a decrease in pH occurred implying decreasing photosynthetic activity. During the week beginning February 7, industrial action resulted in an increased retention time and an associated increase in pH. After this period, the hydraulic retention time decreased and remained in the vicinity of 18 days. A gradual decrease in pH to 6.9 was associated with this period.

Similar pH effects were observed in 1975 (Fig. 4.6). In this instance, lagoons 1 and 2 effectively provide control data. During the period when the Chromatiaceae were dominant, lagoon 3

had a pH significantly higher than lagoons 1 and 2. Once it commenced receiving influent, a rapid decrease in pH and numbers of Chromatiaceae was observed.

As in 1974, no adverse effects on the lagoon characteristics were noted, although the successive years are not strictly comparable as lagoon 3 was not loaded for much of the 1975 observation period. However, when the Chromatiaceae predominated, the BOD and COD of lagoon 3 were lower than those of lagoon 1 and 2 (Fig's 4.7 and 4.8).

No sulphide could be detected in lagoon 3 when the Chromatiaceae were prevalent (Fig. 4.9). Once loading was resumed, the sulphide concentration reached 2 mg/l within one week indicating a rapid transition back to normal lagoon conditions. Data for lagoons 1 and 2 indicate a sulphide concentration in the range 2 to 5 mg/l. As the influent contains negligible sulphide, sulphate reduction and protein degradation must account for this level.

Although Thiopedia was observed in the succession of phototrophic bacteria in lagoons treating meatworks effluent (Fig. 4.10(c)), no cells of Thiopedia were evident in a bottle culture of the same effluent. As it is known that this genus of the Chromatiaceae is favoured by low light intensities (Pfennig and Truper 1974), the higher light intensities throughout the bottle culture may have precluded the growth of these bacteria.

4.5 CHROMATIACEAE IN LAGOONS TREATING PIGGERY EFFLUENT

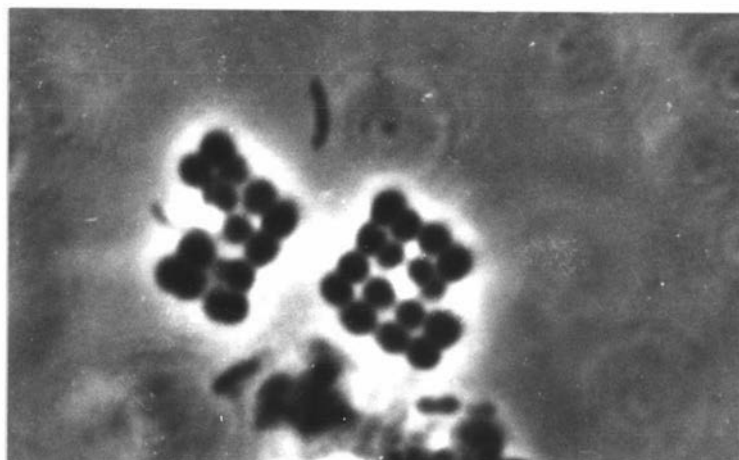
4.5.1 Piggery Lagoon System A:

4.5.1.1 System Description:

A piggery treats the effluent from approximately 2,500 pigs by passage through three lagoons in series. The physical and operational characteristics of these lagoons are summarised in Table 4.4.

Figure 4.10: Phototrophic Bacteria Observed in the Meatworks
Lagoon (— = 2.0 μ for all photomicrographs).

(a) Thiocapsa



(b) Chromatium



(c) Thiopedia

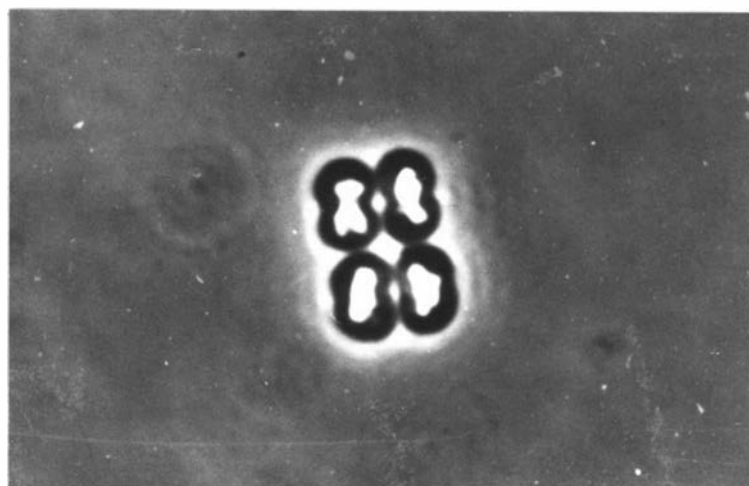


TABLE 4.4

Physical and Operational Characteristics of Piggery
Lagoon System A.

Parameter	Lagoon 1	Lagoon 2	Lagoon 3
Area, (m^2)	6,070	3,240	1,620
Depth, (m)	2.10	0.91	0.91
Volume, (m^3)	12,750	2,950	1,475
Retention Time, (d)	165	38	19
Volumetric BOD Load, ($g/m^3 d$)	11.2	4.6 ^A /7.7 ^B	6.2 ^A /7.8 ^B
Surface BOD Load, ($g/m^2 d$)	23.5	4.2 ^A /7.0 ^B	5.4 ^A /6.7 ^B
BOD Influent, (mg/l)	1,850	175 ^A /293 ^B	119 ^A /149 ^B
BOD Effluent, (mg/l)	175 ^A /293 ^B	119 ^A /149 ^B	114 ^A /141 ^B
BOD Removal, %	90.5 ^A /84.2 ^B	32 ^A /49 ^B	4.2 ^A /5.4 ^B
<u>Thiocapsa</u> , Cells/ml	1.50-3.82 x 10 ⁸	3.00 x 10 ⁸	2.54 x 10 ⁸

^A Average 1975 data.

^B Average 1971-72 data.

Hydraulic loading = 77.2 m^3/d

Total hydraulic retention time = 222 d

Total BOD removal = 93%

Thiocapsa counts taken on April 14, 1976.

Short circuiting was apparent in all three lagoons but was most significant in lagoons 2 and 3 due to poor inlet and outlet location.

4.5.1.2 Observations of Piggery Lagoon System A:

All observations, other than the cell count data were calculated from information kindly provided by the N.Z. Pork Industry Council.

The variations in lagoon parameters observed from April, 1971 to March, 1972, and from 12th August to 21st October, 1975, are presented in Figures 4.11-4.16.

When this lagoon system was observed by the author on March 14, 1976, all three lagoons were distinctly red. A microscopic examination indicated that Thiocapsa roseopersicina was the dominant organism present. The cell counts observed are recorded in Table 4.4. Algae were only present in lagoon 3 at a level of 1.3×10^3 cells/ml.

Vigorous gassing, indicative of methanogenesis, was observed only in lagoon 1.

As few counts of the Chromatiaceae were made, the lagoon colour was used to indicate the major type of microflora present. Green was presumed to be indicative of algal dominance, red of the dominance of the Chromatiaceae and brown as either an intermediate algal/Chromatiaceae stage or a stage in which the anaerobic and facultatively anaerobic heterotrophs predominated.

Table 4.4 demonstrates that there is only a slight variation in volumetric BOD loading throughout the three lagoons. The load of $11.2 \text{ g BOD/m}^3 \cdot \text{d}$ is very low for an anaerobic lagoon but the surface load of $23.5 \text{ g BOD/m}^2 \cdot \text{d}$ is probably sufficient to maintain anaerobic conditions (Section 4.2.1). However, on a volumetric basis, this lagoon is underloaded by approximately a power of ten. This results in a wastage of land as the effluent from a lightly loaded anaerobic

lagoon is not greatly different from that of a heavily loaded anaerobic lagoon (Loehr 1967).

The surface BOD loading on lagoons 2 and 3 should be low enough to allow the development of a stable algal population (Table 4.4). This has not occurred and, from the 1976 observations, the Chromatiaceae were gradually infiltrating these lagoons. A possible explanation of this phenomenon is presented in Section 4.9.

4.5.1.3 Lagoon 1 of Piggery Lagoon System A:

The data presented in Fig's 4.11 to 4.16 allow a detailed analysis of the seasonal changes to be made.

The red colour of lagoon 1 during 1971-72 implied that the Chromatiaceae were dominant in this lagoon throughout the year, with greatest numbers occurring in January and February (Fig. 4.11). However, in 1975, this lagoon was brown during the August-September period indicating the dominance of the anaerobic heterotrophs at the onset of spring (Fig. 4.12). Towards the end of October, the Chromatiaceae once again became dominant. In both instances, increasing red colouration was associated with increasing temperature. Also, temporary increases in pH accompanying the red colour could have been due to either reduced volatile acid concentrations or decreased levels of dissolved CO_2 .

Suspended solids showed little variation throughout the year (Fig. 4.11). It is evident that the Chromatiaceae only slightly modified the behaviour expected of an anaerobic lagoon.

During the winter when the temperature was low the lagoon acted primarily as a sedimentation basin, removing mainly the suspended solids. Methanogenesis was negligible as below 15°C very little methane is produced by anaerobic lagoons (Oswald 1968). Soluble organic material therefore remained in suspension resulting in an effluent with a relatively high BOD. With increasing temperatures, active methanogenesis resumed resulting in the upflow of masses of

Figure 4.11: Lagoon 1 of Piggery Lagoon System A (1971-72)

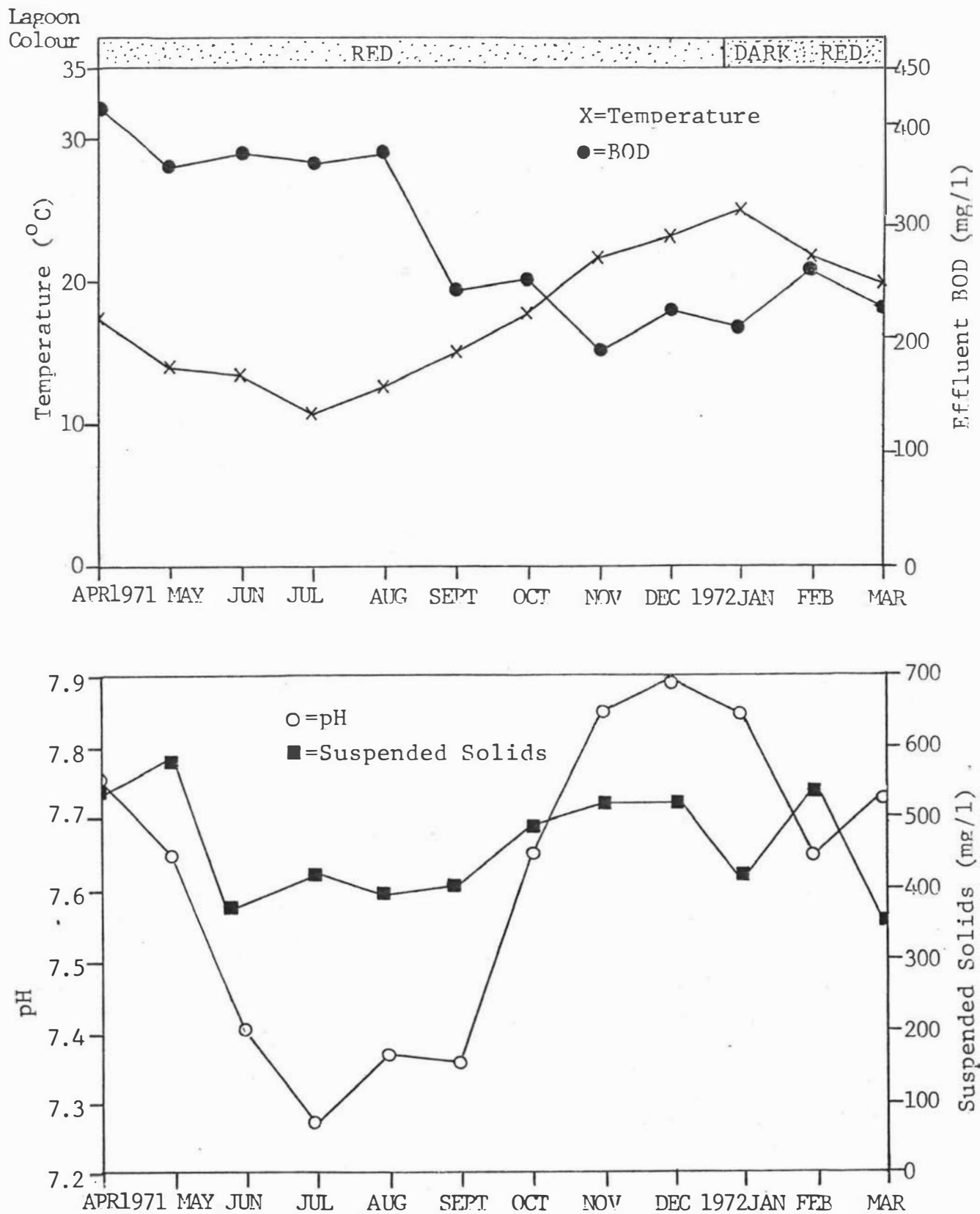
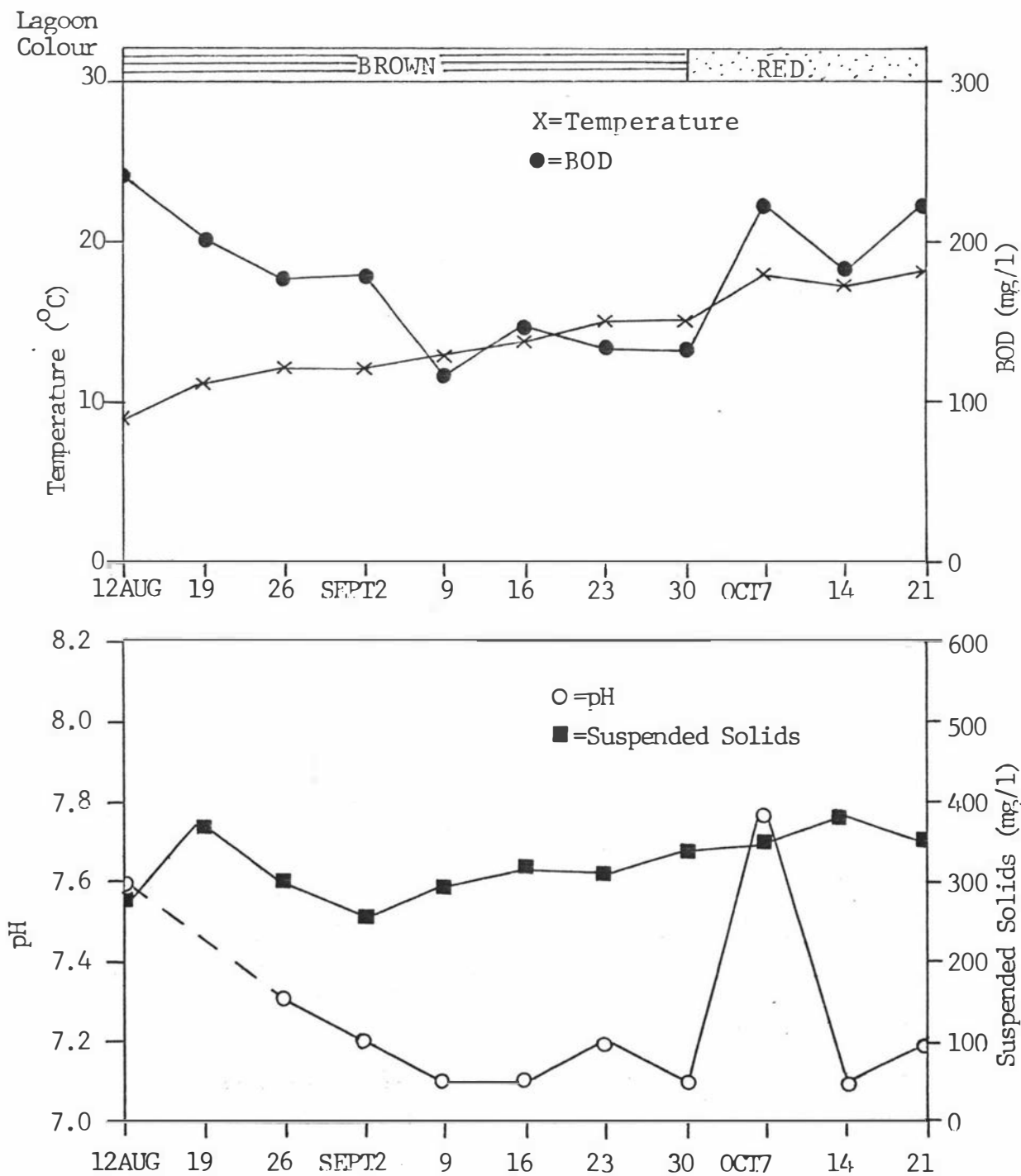


Figure 4.12: Lagoon 1 of Piggery Lagoon System A (1975)



gas-impregnated sludge. This sludge was capable of adsorbing dissolved organic matter and rendering it insoluble (Oswald 1960). When the sludge settled these compounds were carried into the zone of active digestion. Counteracting this effect was the partial resolution of sludge fermentation end-products (Marais 1970).

The decreasing BOD of the liquor with increasing temperature indicated that the former effect was quantitatively more important (Fig. 4.11).

Thus from July to November 1971, the BOD decreased from 370 mg/l to 185 mg/l. From January to March, a slight increase to 225-265 mg/l was associated with the darker red colour of the lagoon.

During 1975, an effluent BOD minimum of 107 mg/l was achieved on September 9. Subsequently an increase from 130 mg/l to 180-220 mg/l was associated with the red colouration of the lagoon.

However, average BOD removals of 84.2% during 1971-72 and 90.5% during 1975 indicated that this lagoon was operating effectively (Table 4.4).

4.5.1.4 Lagoon 2 of Piggery Lagoon System A:

In 1971-72, the red colour in lagoon 2 became apparent towards the end of June (Fig. 4.13). At this stage the lagoon temperature was approaching its winter minimum. This red colour predominated during the period of increasing lagoon temperature until October. During this phase of dominance by the Chromatiaceae, the BOD decreased from 155 to 100 mg/l. Much of this organic removal may be attributable to the Methanobacteriaceae. During this period the pH was reasonably high and relatively static, as were the suspended solids.

Figure 4.13: Lagoon 2 of Piggery Lagoon System A (1971-72)

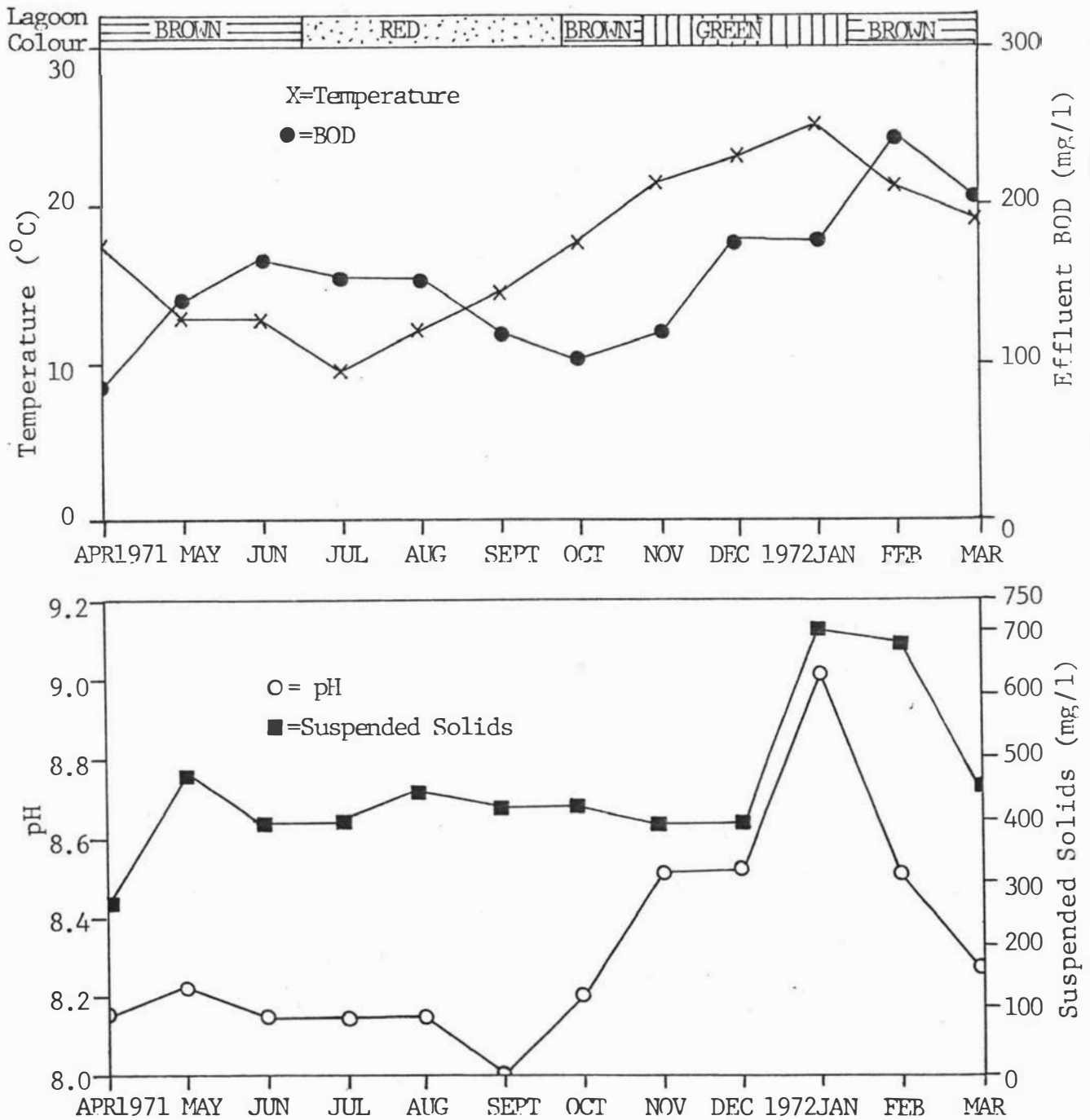
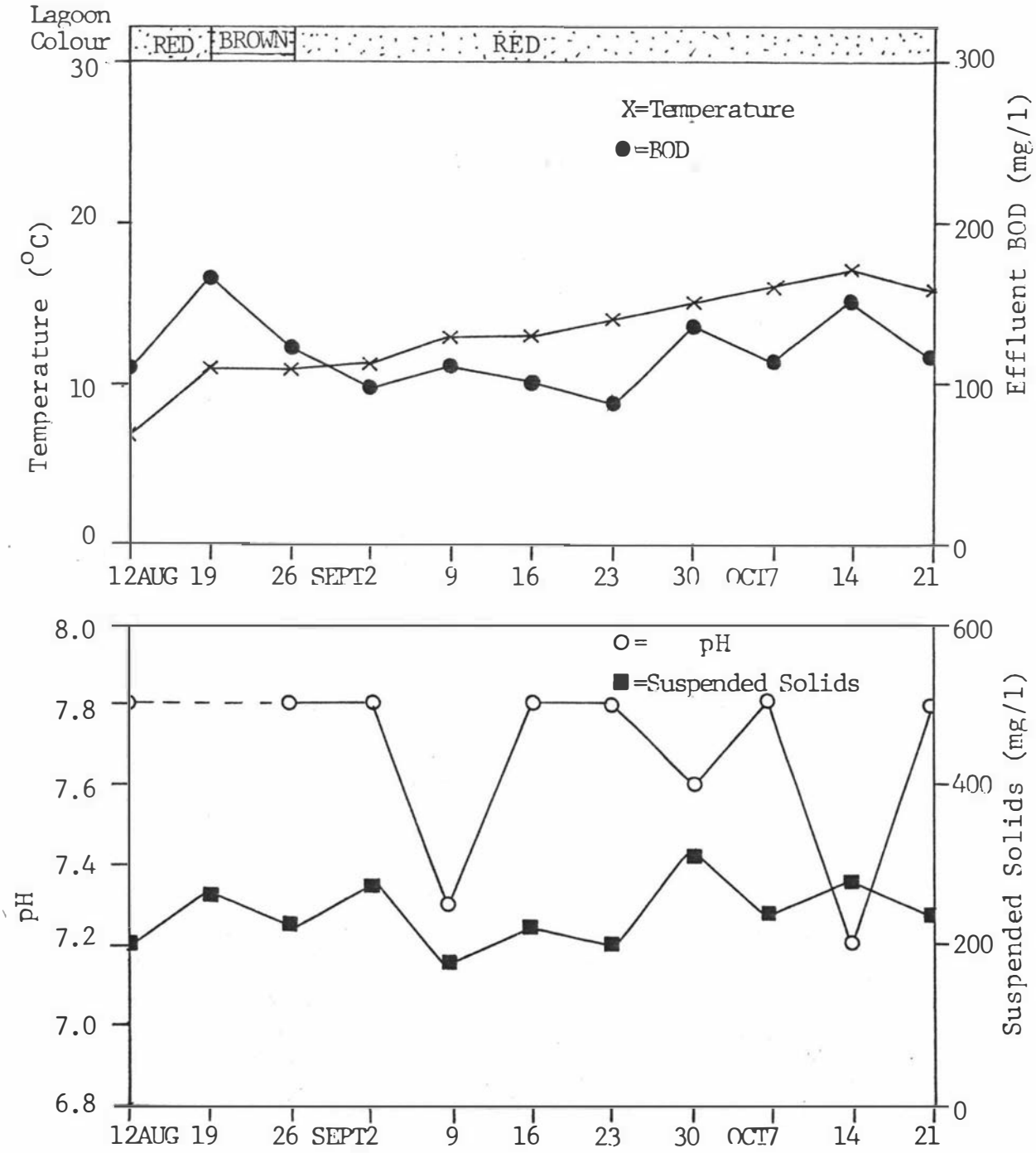


Figure 4.14: Lagoon 2 of Piggery Lagoon System A (1975).



During October the red colour of the lagoon altered to brown indicating increasing algal numbers. By the beginning of October, the lagoon appeared green, implying that algae were now the dominant microflora. Increasing levels of suspended solids, pH and BOD were associated with this phase of algal dominance. Suspended solids increased from 400 to 700 mg/l, the pH from 8.2 to 9.0 and the BOD from 118 mg/l to 200 mg/l.

By mid-January this phase of algal dominance had passed and the brown colour indicative of a combined algae/Chromatiaceae regime predominated. During this phase, all parameters measured decreased.

The 1975 data (Fig. 4.14) does not allow many conclusions to be drawn due to the limited time over which observations were made. The results tend to confirm the 1971-72 conclusions. From August to October, the Chromatiaceae were dominant. A slight decrease in BOD was observed during this period. Only minor fluctuations occurred in pH and suspended solids but both were significantly lower than the 1971-72 values.

This lagoon was much less efficient than lagoon 1 with BOD removals of 49% and 32% being achieved in 1971-72 and 1975 respectively (Table 4.4).

4.5.1.5 Lagoon 3 of Piggery Lagoon System A:

During 1971-72 lagoon 3 was only briefly classified as red in late July. However from May to mid-July and from August to September a brown colour, indicative of the presence of significant numbers of both algae and Chromatiaceae, predominated. Apart from an initial increase from 60 to 120 mg/l there was little change in the BOD during this period. Suspended solids were also reasonably constant. However, towards the end of the August to November 'brown' period, a significant increase in pH occurred. This was probably due to the increasing numbers of algae and the intensification of photosynthetic CO₂ fixation.

Figure 4.15: Lagoon 3 of Piggery Lagoon System A(1971-72)

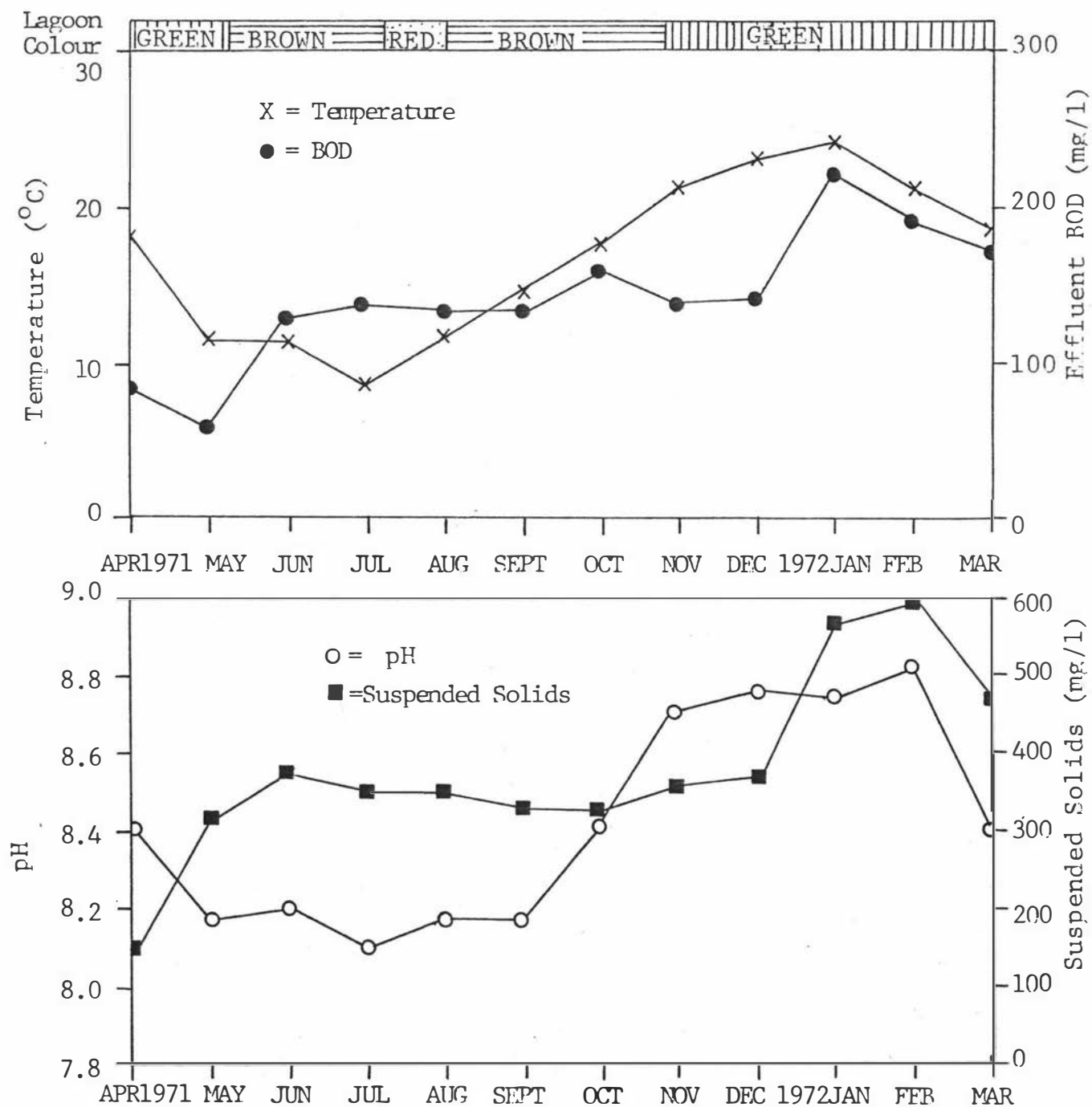
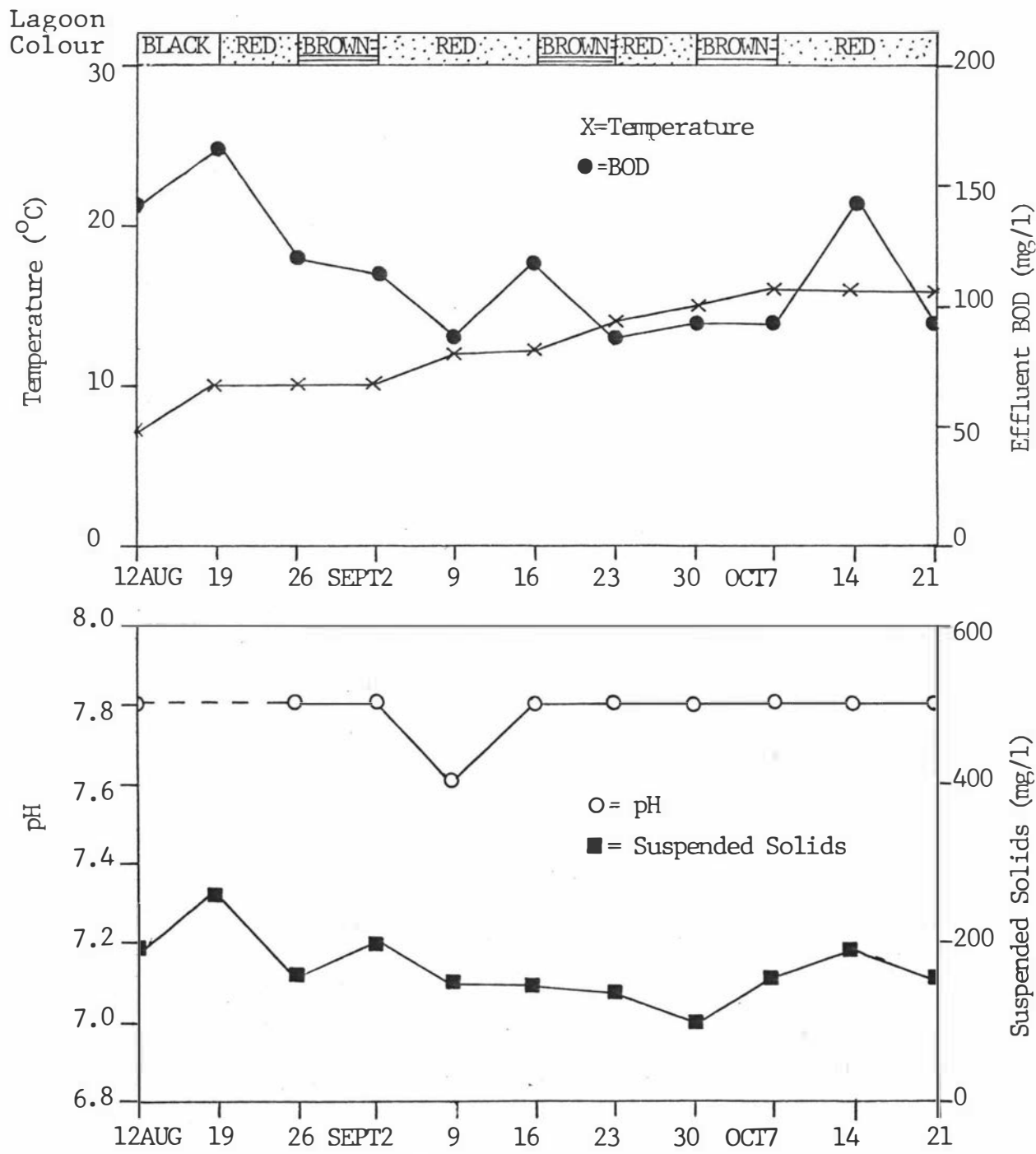


Figure 4.16: Lagoon 3 of Piggery Lagoon System A (1975).



With increasing temperatures during spring, this brown phase passed into a green phase during November. This algal domination persisted until May. The parameters measured indicate the maximum algal population occurred during January and February. Increases in BOD, pH and suspended solids were associated with this maximum population (Fig. 4.15).

From August to October 1975, a slightly different pattern was apparent (Fig. 4.16). The lagoon tended to oscillate between red and brown indicating that the Chromatiaceae were dominant for a longer period than in 1971-72 but that the dominance was somewhat tenuous. Also, the BOD varied between 90 and 145 mg/l compared to 140-160 mg/l for 1971-72. As with the data for lagoon 2, the pH and suspended solids were significantly lower than their 1971-72 values.

Average BOD removals for this lagoon were very poor, being 5.4% in 1971-72 and 4.2% in 1975.

When this lagoon system was observed on March 14, 1976, all three lagoons were a distinct red colour indicating an extended dominance of the Chromatiaceae.

4.5.2 Piggery Lagoon System B:

4.5.2.1 System Description:

This system treats the effluent from approximately 3,500 pigs by using four lagoons in series. The physical and operational characteristics of this lagoon system are presented in Table 4.5. This system was observed in March, 1976.

4.5.2.2 Observations of Piggery Lagoon System B:

With the exception of the cell count data, the observations in this section were calculated from information provided by the management of the pig farm.

TABLE 4.5

Physical and Operation Characteristics of Piggery Lagoon System B.

Parameter	Lagoon 1	Lagoon 2	Lagoon 3	Lagoon 4
Area, (m^2)	200	2,020	2,020	2,048
Depth, (m)	3	1.84	2.15	4.3
Volume, (m^3)	600	3,720	4,350	8,820
Hydraulic retention time, (d)	8.6	53	62	126
Volumetric BOD load, ($g/m^3 d$)	1,520	122	21	2
Surface BOD load, ($g/m^2 .d$)	4,450	225	45	8
BOD Influent, mg/l	12,000-14,000	6,000-7,000	1,200-1,400	200-250
BOD Effluent, mg/l	6,000-7,000	1,200-1,400	200-250	100-110
BOD Removal, %	50	80	83	50
<u>Thiocapsa</u> , cells/ml	0	8.2×10^6	4.3×10^5	9.1×10^3
<u>Chlorella</u> , cells/ml	0	8.5×10^2	8.7×10^3	2.1×10^5

Hydraulic loading = 70 Cu M/D

Total Hydraulic Retention Time = 250 D

Total BOD Removal = 99.2%

Vigorous gassing, implying active methanogenesis, was observed only in lagoons 1 and 2.

Lagoon 1 was black-brown in colour and a microscopic examination of the liquor revealed a large number of rods and cocci. Spores and spore-containing cells were also present. No algae or Chromatiaceae were evident. However, incubation of a sludge sample in the light resulted in the growth of a red film on the glass surface nearest to the light source. A microscopic examination indicated that Thiocapsa and Chromatium spp. were the major organisms present.

The second lagoon appeared red-pink and Thiocapsa roseopersicina were present at 8.2×10^6 cells/ml. Chromatium spp. were present at 7.4×10^2 cells/ml and a green algae, identified as Chlorella spp. occurred at 8.5×10^2 cells/ml. Free spores were also present.

Lagoon 3 appeared red-brown and counts of 4.3×10^5 cells/ml were recorded for Thiocapsa roseopersicina, 1.2×10^3 cells/ml for Chromatium spp. and 8.7×10^3 cells/ml for Chlorella spp.

Lagoon 4 appeared green-brown and contained 9.1×10^3 cells/ml of Thiocapsa roseopersicina and 2.1×10^5 cells/ml of Chlorella spp. No Chromatium spp. were evident, and Chlorogonium was the next most numerous algal genus with a count of 3.4×10^3 cells/ml being recorded.

At one stage, a red colour indicative of the dominance of the Chromatiaceae had permeated the final three lagoons. The addition of nitrate to lagoons 3 and 4 resulted in a return to aerobic conditions. Algae subsequently became dominant in these lagoons.

4.5.2.3 Discussion:

This lagoon system operated very efficiently with a total BOD removal of 99.2% being achieved. Both the surface and volumetric BOD loads on successive lagoons decreased steadily. This indicated a decreasing availability of organic compounds for bacterial metabolism and favoured the gradual dominance of the algae.

The volumetric BOD load on the first lagoon was approximately five times that common for anaerobic lagoons in the U.S.A. (Section 4.2.1). However, this lagoon has proved stable and has shown no tendency toward the acid conditions indicative of a failing anaerobic system. As this lagoon has a 50% BOD removal, it serves admirably as a roughing process. The hydraulic retention time of 8.6 days is too short to allow the presence of the Chromatiaceae in the liquor. However small numbers of these organisms were present in the sludge as demonstrated by the incubation of a sludge sample in the light.

Lagoon 2 operated at a volumetric BOD loading of $122 \text{ g/m}^3 \cdot \text{d}$ which is somewhat low for an anaerobic lagoon. However, the surface loading of $225 \text{ g/m}^2 \cdot \text{d}$ was high enough to ensure that algae did not flourish. Therefore this lagoon, which had a relatively long retention time of 53 days, provided a suitable environment for the growth of the Chromatiaceae. A good BOD removal of 80% was achieved by this lagoon.

Lagoon 3 operated at a volumetric loading of $21 \text{ g BOD/m}^3 \cdot \text{d}$. This is very low for an anaerobic system and probably explains why no active gassing was apparent in this lagoon. The surface loading of $45 \text{ g BOD/m}^2 \cdot \text{d}$ was however sufficient to maintain anaerobic conditions and as a continuous inoculum of Chromatiaceae was provided by lagoon 2, these bacteria dominated this lagoon. However, the lighter loading allowed a greater number of algae to maintain themselves. As with lagoon 2, a good BOD removal efficiency was achieved by this lagoon.

Lagoon 4 operated at a surface loading of $7.7 \text{ g BOD/m}^2 \cdot \text{d}$ which is a reasonable loading for a facultative lagoon in N.Z. The algae therefore flourished and became the dominant microorganism. The number of Chromatiaceae in this lagoon was reduced but still measurable. The effluent from lagoon 3 provided a continuous inoculum of these bacteria and any seasonal decrease in algal activity led to temporary dominance of the Chromatiaceae.

A comparison between the two piggery lagoon systems will be made in Section 4.9.

4.6 CHROMATIACEAE IN LAGOONS TREATING TANNERY EFFLUENT.

4.6.1 Tannery Lagoon System A:

4.6.1.1 System Description:

After pretreating its total effluent by aeration, a South African tannery treats it by serial passage through nine evaporation lagoons. The mode of operation of these lagoons is shown in Fig. 4.17 and the physical and operational characteristics are presented in Table 4.6.

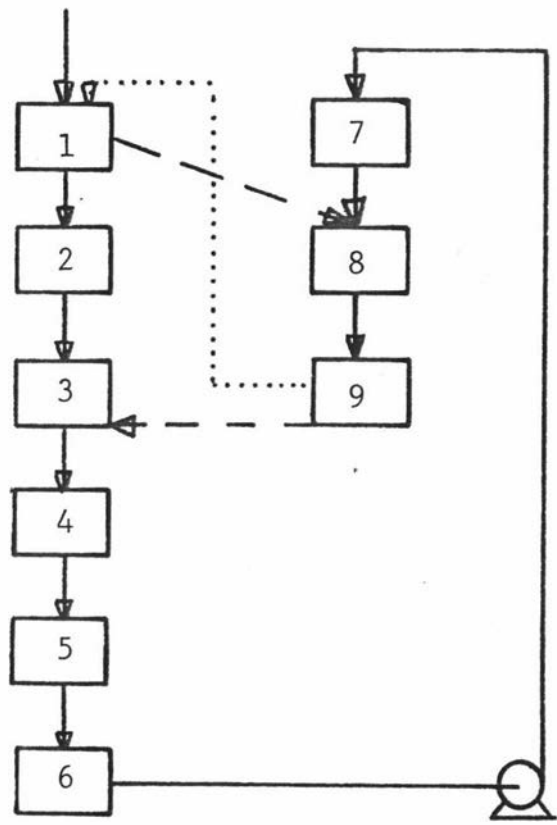
4.6.1.2 Observations of Tannery Lagoon System A:

The observations in this section have been obtained from information kindly received from Mr. J.W. Funke, National Institute for Water Research, South Africa, and has been used with the permission of the tannery's management.

The results of a sampling survey performed on 18 February, 1977, is shown in Table 4.7. Unfortunately no BOD data are available for this lagoon system as the BOD is not used in South Africa as a parameter for determining the organic loading (Funke 1977). Thus the volumetric and surface BOD loads cannot be calculated. However, some indication of these figures might be obtained by calculating the OA (oxygen absorbed) loadings. The OA has been shown to approximate the BOD for a South African fellmongery effluent (Rawlings and Woods 1977) and for a tannery effluent a ratio of approximately 1.3 to 1.0 BOD:OA is suggested for the untreated effluent and 1.0 to 1.0 for the treated effluent (Rawlings 1977).

A succession of Chromatiaceae was observed in the samples analysed on 10th May, 1977. Unfortunately, a delay of 57 days was incurred in transporting the samples from South Africa to New Zealand. The lack of exposure to light for this period may have altered the

Figure 4.17: Mode of Operation of Tannery Lagoon System A.



Key: — — Recycle path used to extend the retention time under unfavourable conditions.

..... Overflow path.

TABLE 4.6

Physical and Operational Characteristics of Tannery Lagoon System A.

Parameter	Lagoon 1	Lagoon 2	Lagoon 3	Lagoon 4	Lagoon 5	Lagoon 6
Area (m ²)	3,000	3,000	3,000	3,000	3,000	3,000
Depth (m)	2.5	2.5	2.5	2.5	2.5	2.5
Volume (m ³)	7,500	7,500	7,500	7,500	7,500	7,500
Nominal Hydraulic Retention Time (days)	13.75	13.75	13.75	13.75	13.75	13.75
Volumetric OA Load* (g oA/m ³ .d)	50.9	26.2	11.3	10.5	10.5	9.1
Surface OA Load* (g oA/m ² .d)	127	65.4	28.2	26.3	26.3	22.7
<u>Chromatiaceae</u> (cells/ml)	1×10^6 (3.54×10^7) ^a	7.12×10^6	9.08×10^6	7.75×10^6	2.92×10^6	9.63×10^5
<u>Spirulina</u> (cells/ml)	-	-	-	-	-	(3.3×10^5) ^a
Colour at 28.1.77	Black	Pink	Pink	Pink	Pink	Green
Colour at 18.2.77	Pink	Pink	Pink	Pink	Pink	Green
Colour at 10.5.77	Pink	Pink	Pink	Pink	Pink	Pink

* Based on unfiltered OA results obtained on 18.2.77 (See Table 4.7)

^a Microbial counts taken on 18.2.77. Other microbial counts taken on 10.5.77.

Daily Hydraulic Loading = $545 \text{ m}^3/\text{d}$.

TABLE 4.7

Results of a Sampling Survey Performed on Tannery Lagoon System A on 18.2.77 (mg/l except pH)

Parameter	Influent	Lagoon 1	Lagoon 2	Lagoon 3	Lagoon 4	Lagoon 5	Lagoon 6
pH	7.3	7.9	8.3	8.4	8.5	8.7	8.8
4 hr OA unfiltered	700	360	155	145	145	125	105
4 hr OA filtered	-	185	58	56	68	60	65
COD	4,900	-	-	-	-	1,400	1,000
Chloride	2,200	-	-	-	-	-	-
Grease and oil	1,345	86	-	-	-	68	20
Sulphide	45	52	2.4	3.2	2.4	1.3	0.3
Sulphate	1,650	835	742	703	670	763	873
Total Solids	13,500	8,450	10,560	10,150	10,910	11,400	12,830
Dissolved Oxygen	-	0.2	0.2	0.4	-	0.4	12.0

composition of the microbial populations. The cell counts of each genus observed are presented in Table 4.8.

4.6.1.3 Discussion:

This lagoon system has been designed as evaporation ponds and is therefore not intended to produce an effluent. Thus, care is needed in interpreting the nominal hydraulic retention times presented in Table 4.6 as, under ideal conditions, the system operates at an infinite retention time. However, this situation is entirely dependent upon the weather. Under unfavourable climatic conditions, a recycle system transfers the effluent from lagoon 9 to lagoon 1. Also, lagoon 1 supernatant may be transferred to either lagoon 2 or lagoon 8 (Fig. 4.17). This makes interpretation of the loading figures difficult.

Consideration of the analyses presented in Table 4.7 does allow some conclusions to be drawn. A steady, and significant, increase in pH through the first six lagoons may be presumed to be indicative of increasing photosynthetic activity. Similarly the total solids give some indication of the cell numbers. In the first lagoon, a decrease in suspended solids was observed. This was anticipated as the increased retention time allowed sedimentation of suspended particles and bioflocculation (Lackey and Smith 1955, Oswald 1960), further reducing the suspended solids concentration. Also, near the time of sampling, lagoon 1 appeared black indicating low numbers of Chromatiaceae were present. Unfortunately, no counts of Chromatiaceae were made at the time of sampling.

Subsequent increases in suspended solids levels may be assumed to indicate increasing numbers of Chromatiaceae as all three lagoons appeared pink (Table 4.6). The large increase in suspended solids in lagoon 6 may be presumed to be associated with the presence of algae as shown by the green appearance of this lagoon (Table 4.6).

The dissolved oxygen (DO) data reinforces the above assumptions as lagoons 1 to 5 had a negligible D.O. while lagoon 6 had a D.O. of 12 mg/l.

TABLE 4.8

Cell Counts of Chromatiaceae genera observed in Tannery Lagoon System A samples on 10 May, 1977 (Cells/ml).

	<u>Amoebobacter</u> spp.	<u>Thiocapsa</u> spp.	<u>Chromatium</u> spp.
Lagoon 1	8.23×10^5	1.81×10^5	-
Lagoon 2	3.69×10^6	3.32×10^6	1.10×10^5
Lagoon 3	3.27×10^6	4.32×10^6	1.49×10^6
Lagoon 4	8.50×10^5	3.88×10^6	3.02×10^6
Lagoon 5	-	1.06×10^6	1.86×10^6
Lagoon 6	-	2.10×10^5	2.42×10^6

The sulphide analyses lead to the same conclusions. The influent sulphide concentration of 45 mg/l increased slightly in the first, black lagoon. This may be assumed to be primarily due to dissimilatory sulphate reduction as a decrease in sulphate concentration from 1;650 mg/l to 835 mg/l was observed. However only 7 mg/l of this 815 mg/l SO_4^{2-} reduced appeared as sulphide. Presumably, the difference is accounted for by other sulphur moieties not analysed, by sedimentation of sulphur and by volatilisation of sulphides and associated compounds.

On passing to lagoon 2, the effluent sulphide was reduced from 52 mg/l to 2.4 mg/l indicating the activity of the Chromatiaceae. This activity was maintained in lagoons 3 to 5. The aerobic nature of lagoon 6 allowed a further reduction of sulphide to 0.3 mg/l. This lagoon system achieved a 99.3% reduction in sulphide concentration. Removals of other parameters achieved are 79.6% COD, 85.0% 4 hour OA unfiltered and 98.5% grease and oil.

4.6.2 Tannery Lagoon System B

4.6.2.1 System Description:

A South African tannery treats its effluent by passage through a series of four lagoons designed to serve as evaporation ponds with zero discharge. The physical and operational characteristics of these lagoons during March, 1976, are presented in Table 4.9. At this time, the first lagoon was bypassed to allow the removal of accumulated sludge. Thus lagoon 2 was effectively the first in a series of three lagoons.

4.6.2.2 Observations of Tannery Lagoon System B:

The observations presented in this section have been obtained from data kindly forwarded by Dr. D.E. Rawlings, Leather Industry Research Institute, South Africa and is used with the permission of the tannery's management.

TABLE 4.9

Physical and Operational Characteristics of Tannery Lagoon System B (March 1976).

Parameter :	Lagoon 1	Lagoon 2	Lagoon 3	Lagoon 4
Area (m ²)	9,110	9,110	13,200	6,070
Average depth (m)*	1.75	1.75	1.75	1.75
Volume (m ³)	15,900	15,900	23,100	10,600
Nominal Hydraulic Retention Time (days)	56.2	56.2	81.6	37.5
Volumetric OA load (g OA/m ³ .d)	-	-	1.92	0.764
Surface OA load (g OA/m ² .d)	-	-	2.85	1.34

Daily Hydraulic Loading = 283 m³/d.

Total Nominal Hydraulic Retention Time = 231.5 days.

*The average depth is used as the lagoon depth varies across the lagoon from 0.525 m at the shallow side to 3.05 m at the deep side.

The results of analyses performed during March, 1976, are displayed in Table 4.10. Unfortunately, no O.A. loadings can be calculated for the first two lagoons. Lagoon 1 was receiving no effluent and no data is available on the influent to lagoon 2.

Subsequently, the factory has improved its water management and reduced its discharge from 283 m³/d to 159 m³/d. This has affected the appearance of the lagoons as shown by the observations made on 28 January, 1977 (Table 4.11). No chemical analyses were made at this time.

4.6.2.3 Discussion:

The effect of the lagoon retention time on the microorganisms dominant in a lagoon is clearly demonstrated by this system. When 283 m³/d of effluent were being treated the first lagoon was grey, the second and third were pink and the fourth was green. A reduction in the volume treated to 159 m³/d resulted in the first two lagoons appearing pink and the third and fourth lagoons green. Under these conditions Amoebobacter spp. were the dominant Chromatiaceae in lagoons 1 and 2. Thiocapsa and Chromatium spp. were present at less than 10³ cells/ml.

The interpretation of data from the samples analysed during March, 1976, is difficult as the lagoons were operating in an atypical fashion at this stage. The results for lagoon 1 therefore lack relevance and those for lagoon 2 may be expected to be higher than usual. Even so, good removals of sulphide and O.A. were achieved by the lagoons in which the Chromatiaceae were dominant. The overall removals of the system were also good with reductions of 80.4% in O.A. and 96.0% in sulphide being achieved through lagoons 2 to 4.

TABLE 4.10

Results of a Sampling Survey Performed on Tannery Lagoon System B during March 1976.

Parameter	Lagoon 1	Lagoon 2	Lagoon 3	Lagoon 4
4 hr OA (mg/l)	45.6	156.8	28.6	25.2
Na ₂ S (mg/l)	3.2	62.4	2.5	1.5
pH	8.7	9.0	9.1	9.0
Colour	grey	milky pink	pink	green

TABLE 4.11

Tannery Lagoon System B Parameters Observed on 28 January, 1977.

Parameter	Lagoon 1	Lagoon 2	Lagoon 3	Lagoon 4
Nominal Hydraulic Retention time (days)	100	100	145	66.7
Colour	milky pink	pink	green	green
<u>Amoebobacter</u> spp. (cells/ml)	3.45×10^7	1.50×10^7	-	-

Daily Hydraulic Loading = $159 \text{ m}^3/\text{d}$

Total Nominal Hydraulic Retention Time = 412 days.

4.7 CHROMATIACEAE IN A LAGOON SYSTEM TREATING FELLMONGERY EFFLUENT.

4.7.1 System Description:

This lagoon treats the settled fellmongery effluent produced by a North Island meat works. Primary treatment of the fellmongery effluent is performed by a 332 m³ rectangular sedimentation tank which provides a retention time of 1.8 hours at the maximum flow rate of 591 m³/d (Cooper et al 1975). The tank possesses top and bottom scrapers to remove floating grease and wool and the settled solids.

The sedimentation tank effluent is pumped to the lagoon via a small holding pond. The physical characteristics of this lagoon are presented in Table 4.12.

4.7.2 Observations of the Fellmongery Lagoon:

The observations in this section have been obtained by the Meat Works concerned and the author is indebted to the management for providing access to published and unpublished information.

The operational characteristics of this lagoon are presented in Table 4.13. The data for the effluent column has been calculated from ten samples taken over a period of sixteen months. The data for the influent column is from one sample.

The lagoon was commissioned in April 1972 and, as it was filled during the off-season, effluent was not produced until mid-August of that year. At that stage, the lagoon was odourless and contained freshwater diatoms and algae. By mid-December, shortly after the commencement of the fellmongery season, the lagoon was red with isolated pockets of green algae. There was a strong sulphide and mercaptan smell in the lagoon vicinity at this stage (Cooper et al 1975). By mid-February 1973, the lagoon was entirely red and there was no noticeable odour. Since this time the Chromatiaceae have formed the predominant population in this lagoon with Thiocapsa roseopersicina being the dominant organism at concentrations between 4.0×10^6 - 8.0×10^7 cells/ml. Chromatium spp. were also present in lesser numbers.

TABLE 4.12Physical Characteristics of Fellmongery Lagoon (Cooper et al 1975)

Parameter	Value
Area, (m^2)	21,700
Depth, (m)	1.74
Volume, (m^3)	37,600
Hydraulic Retention Time, (d)	100
Volumetric BOD Loading, ($\text{g}/\text{m}^3 \cdot \text{d}$)	9.9
Surface BOD Loading, ($\text{g}/\text{m}^2 \cdot \text{d}$)	17.2

TABLE 4.13Operational Characteristics of Fellmongery Lagoon (Cooper et al 1975)

Parameter	Influent	Effluent	% Removal
BOD, mg/l	990	13-227	77.1-98.7
COD, mg/l	3,435	279-1,182	65.6-91.9
pH	12.5	7.5	-
Sulphide, mg/l	95	0-35	63.2-100
Solids, mg/l			
Total	10,860	3,440-5,480	49.5-68.3
Mineral	8,580	1,936-4,540	47.1-77.4
Volatile	2,280	309-2,708	-18.8-86.4
Suspended	400	34-832	-108-91.5

At one stage, the supply of fellmongery effluent was stopped for a short period of two months and although Thiocapsa did not disappear, algae became dominant with Chlorella, Euglena, Chlamydomonas and Scenedesmus being most numerous (Cooper et al 1975).

4.7.3 Discussion:

The degree of treatment achieved by this lagoon was very good (Table 4.13). High reductions in BOD, COD, pH and sulphides were achieved. The performance with respect to solids concentration was more variable with increases occurring in two instances. It is noteworthy that anaerobic conditions were maintained at the relatively low organic load of $9.9 \text{ g BOD/m}^3 \cdot \text{d}$. The surface BOD loading of $17.2 \text{ g BOD/m}^2 \cdot \text{d}$ was only slightly above that expected to maintain aerobic conditions in a facultative lagoon treating domestic sewage in a temperate to semi-tropical climate (Table 4.1). The temperature of this lagoon varied from 10°C to 30°C during the year. Thus, the nature of the effluent must play an important role in maintaining anaerobic conditions.

Also, the sulphide component of the fellmongery effluent will be selective for the Chromatiaceae.

4.8 CHROMATIACEAE IN A LAGOON SYSTEM TREATING DOMESTIC SEWAGE

4.8.1 System Description:

The Auckland Regional Authority (A.R.A.) uses facultative lagoons for the treatment of the 2 million population equivalent of domestic sewage and industrial waste generated by the City of Auckland. Four lagoons of approximately equal area are operated with 1.5 to 3.5 volumes of lagoon effluent recycled per volume of settled sewage influent (Shelef et al 1977). The varying recycle is due to fluctuations in sewage flow.

The characteristics of this lagoon system are described in Table 4.14.

TABLE 4.14

Characteristics of Domestic Sewage Lagoons (from Brockett 1975, Richardson 1974, Shelef et al 1977).

Parameter	Value
No. of Lagoons	4
Mode of Operation	Parallel
Recycle Ratio (Recycled Effluent: Settled Sewage)	1.5:1-3.5:1
Total Area, (m^2)	5.2×10^6
Average Depth, (m)	1.35
Volume, (m^3)	7.02×10^6
Volumetric BOD Load, ($g/m^3 \cdot d$)	6.2-9.1
Surface BOD Load, ($g/m^2 \cdot d$)	8.4-12.3

4.8.2 Observations of a Domestic Sewage Lagoon System:

This lagoon system functioned well and, until autumn, 1973, a satisfactory effluent was always produced (Brockett 1975). Total phytoplankton numbers decreased during autumn and this phenomenon was limiting the organic load which could be applied (Rowe 1975a). In March, 1973, this autumn pause, in association with increased loading due to the growth of industry and population, led to the failure of the lagoon system. In all four lagoons, algae lost their dominance and a phototrophic bacterium identified as Thiopedia, became dominant. However, the description of this bacteria more closely resembles Thiocapsa. The lagoons appeared pink at this stage. Brockett (1975) details the observations made during this period.

Photosynthetic bacteria and sulphide-producing bacteria have been isolated from the sludge/liquor interface by both Brockett (1975) and this author. Employing enrichment culture techniques using Pfennig's medium (VanNiel 1971) a mixed culture of Chromatium spp. and Thiocapsa roseopersicina was obtained.

4.8.3 Discussion:

These results indicate the importance of the Chromatiaceae in a failing facultative lagoon.

Fluctuations in the phytoplankton populations of sewage lagoons have been observed quite frequently (Dust and Shindala 1970, Rashke 1970, Shelef et al 1977). In a study of the A.R.A. lagoons, Rowe (1975b) detected three seasonal phases. In the summer, a stable diverse phytoplankton population was observed, while in the winter a stable but less diverse population was present. The changeovers between these two major seasons were characterised by a less stable, short-lived population component.

During these transition periods the lagoon system was most susceptible to overloading. Any increase in the organic load resulted in bacterial activity and if the algae were unable to provide sufficient oxygen to meet this demand anaerobic conditions eventually prevailed.

With the demise of the algae and the onset of anaerobic conditions, the sulphate reducing bacteria flourished and sulphide became distributed throughout the lagoons. This provided a suitable medium for the growth of the Chromatiaceae which moved upwards from the sludge interface into the liquor due to their positive phototactic response. The presence of these bacteria in samples of the sludge/liquor interface demonstrated that the sediment served as a source of inoculum for these microorganisms.

Once the organic load was decreased, or conditions more suitable for the growth of the algae prevailed, the liquor gradually returned to an aerobic state.

Similar events have been shown to occur when a holomictic lake becomes stratified (Collins 1960).

4.9 THEORY OF THE OCCURRENCE OF CHROMATIACEAE IN WASTE TREATMENT LAGOONS

The results presented indicate that the occurrence of the Chromatiaceae in waste treatment lagoons may be described by a three phase succession theory. At short retention times, or in heavily loaded lagoons, anaerobic heterotrophs predominate. At longer retention times, or in lightly loaded lagoons the algae are dominant. The Chromatiaceae occupy a niche intermediate to these extremes and therefore predominate in the following situations:

- (i) underloaded anaerobic lagoons.
- (ii) overloaded facultative lagoons.
- (iii) lagoons treating selective effluents.

A theory for the occurrence of Chromatiaceae in waste treatment lagoons will be developed by using the lagoons already studied

(Sections 4.4-4.8) to demonstrate each of the above situations.

4.9.1 Underloaded Anaerobic Lagoons:

In an anaerobic lagoon, the hydraulic retention time is of major importance in determining the dominance of the Chromatiaceae. The lagoon liquor is likely to have all the substrates required for the growth of these bacteria with low molecular weight organic compounds being present. The limiting criterion therefore becomes the dilution rate, equivalent to the inverse of the hydraulic retention time. If the dilution rate is greater than the growth rate of the Chromatiaceae under these conditions, these bacteria will be unable to maintain themselves in the liquor. However, in anaerobic lagoons, the solids retention time is much greater than the hydraulic retention time and, as shown by lagoon 1 of piggery lagoon system B, the Chromatiaceae are able to maintain themselves in the sludge zone. As the light intensity reaching this area is negligible (Bartsch and Allum 1957), these bacteria must exhibit an anaerobic dark metabolism. A maintenance metabolism has been postulated for Chromatium spp. exposed to such conditions for short periods (Van Gernerden 1968b). Alternating light and dark periods would be necessary to maintain the integrity of the cell. However, more recently, species capable of growth under anaerobic conditions in the dark have been reported (Bogorov 1974, Krasilnikova 1976, Krasilnikova et al 1975, Kondrat'eva et al 1975).

Thus, certain species of the Chromatiaceae are capable of slow growth under the conditions which might be expected in the sludge zone of an anaerobic lagoon. If sufficient hydraulic retention time is provided in the lagoon liquor and environmental conditions are favourable, the lagoon liquor will provide a good medium for the growth of the Chromatiaceae and they could be expected to become the dominant microflora. Those phototrophs present in the sludge would serve as an inoculum for growth in the overlying liquor. Examples of such an occurrence are provided in the meatworks lagoon (Section 4.4) and in lagoon 1 of piggery lagoon system A (Section 4.5.1).

The performance of these lagoons appeared satisfactory during the period of Chromatiaceae dominance, with only the colour of the effluent causing concern. Thus, for the meatworks lagoon system 1, no significant deterioration in the effluent quality was observed during 1974 (Fig's 4.4-4.5) or 1975 (Fig's 4.6-4.9). However, in 1975, the BOD of lagoon 1 of piggery lagoon system A increased with the onset of Chromatiaceae dominance. Whether this is part of the annual lagoon cycle or is directly attributable to the dominance of the Chromatiaceae is difficult to ascertain.

The three stage succession theory may therefore be used to design anaerobic lagoons in which the Chromatiaceae may or may not predominate. As there is little improvement or deterioration in the effluent quality, the major benefit would be a reduction in the sulphide odour which is very often important. In the U.S.A. 80% of anaerobic lagoons treating industrial wastes have reported problems with odours (Porges 1963) and the intentional growth of Chromatiaceae to minimise the objectionable sulphide odours has been advocated (Cooper 1963). It should be noted that the Chromatiaceae produce a characteristic odour of their own. However, this odour is generally considered to present less of a problem.

If an anaerobic lagoon in which the Chromatiaceae predominate discharges directly to a receiving water the red colour which the bacterial cells impart to the effluent is likely to create problems. However, it is improbable that such an anaerobic lagoon would be capable of treating an effluent to a standard suitable for direct discharge. Thus a further treatment stage to upgrade the effluent would most likely be necessary and, in this instance, the colour would be inconsequential as it is removed by secondary processes such as aerated or algal lagoons (Birch 1977).

The major disadvantage of using a lagoon in which the Chromatiaceae are dominant is the increased land required. The major factor favouring the dominance of these phototrophic bacteria is the hydraulic retention time and, depending upon the effluent to be treated and the climatic conditions, significantly larger lagoons may be needed.

4.9.2 Overloaded Facultative Lagoons:

In an efficiently-operating facultative lagoon, the organisms participating in this three-stage succession are spatially distributed. The Chromatiaceae occupy a zone located between the algae in the liquor and the methanogenic and sulphate-reducing bacteria in the sludge. Although the motile Chromatiaceae attempt to move upwards due to their positive phototactic response, the strictly anaerobic purple sulphur bacteria exhibit a negative aerotactic response which maintains them in the anaerobic zone (Kondrat'eva 1965). Due to the low light intensity reaching such zones, these bacteria must exhibit an anaerobic dark metabolism. Thus, under normal operating conditions, few Chromatiaceae are present and they are restricted to the upper layers of the sludge zone.

If a facultative lagoon becomes anaerobic, the sulphide-reducing bacteria flourish and sulphide becomes distributed throughout the lagoon. With the onset of anaerobic conditions, the Chromatiaceae are free to move toward the surface due to their positive phototactic response. The presence of sulphide and low molecular weight organic compounds makes the liquor a suitable medium for their growth.

The period for which the Chromatiaceae are dominant is primarily dependent upon the organic loading. If the lagoon is overloaded throughout the year, the Chromatiaceae will form a stable dominant population and the situation is similar to that in a lightly loaded anaerobic lagoon. If the lagoon is a heavily loaded facultative system and the failure is due to the seasonal fluctuation in the activity of the algae, the dominance of the Chromatiaceae will be of a more transient nature.

The domestic sewage lagoon (Section 4.8) is an example of the latter situation. Over a period of years, the organic loading on the lagoons has gradually increased. During the summer of 1972-1973, the algal activity was sufficient to maintain aerobic conditions, but during the autumn, the activity of the algae decreased (Brockett 1975, Rowe 1975 a & b) and was unable to meet the oxygen demand of

the bacteria. Anaerobic conditions and the dominance of the Chromatiaceae ensued. When the seasonal conditions favoured the growth of the algae, they became dominant again. In order to reduce the loading on the lagoons to allow algal dominance throughout the year, a large biofiltration plant is being installed.

4.9.3 Lagoons Treating Selective Effluents:

The fellmongery lagoon (Section 4.7) is an example of the use of Chromatiaceae in treating selective effluent. Fellmongery effluent is an alkaline waste containing significant amounts of sulphide (Section 2.7.2), which is toxic to some algae at a concentration of 7 mg/l (Espino and Gloyna 1967). Concentrations of 200 mg/l will inhibit the Methanobacteriaceae (Lawrence et al 1966). Therefore, the lagoon treatment of this effluent favours the dominance of the Chromatiaceae and these bacteria successfully treat a noxious industrial effluent (Table 4.12) (Cooper et al 1975).

The three-stage succession theory is still applicable to selective wastes. Although high sulphide concentrations may limit the activity of the primary anaerobes, the Chromatiaceae are capable of lowering the sulphide concentration to levels suitable for the growth of algae. Therefore, algae may eventually become the dominant population in such an effluent, as demonstrated by the fellmongery lagoon when it was not loaded for two months. However, selective wastes do allow an extended phase of Chromatiaceae dominance.

This extended dominance is also observed in the lagoon systems treating tannery effluent (Section 4.6). The sulphide content of the tannery effluents undoubtedly contributed significantly to the observed dominance of the Chromatiaceae.

4.10 APPLICATION OF THE SUCCESSION THEORY TO LAGOON DESIGN

Careful lagoon design is required to prevent the Chromatiaceae from dominating any following algal lagoons. A study of piggery lagoons A and B (Section 4.5) is useful in determining suitable design techniques for preventing this phenomenon and for demonstrating the usefulness of the succession theory.

Both lagoon systems treat piggery effluent and operate at similar total retention times so similar removal efficiencies would be expected. In fact, whereas piggery lagoon system B achieves a 99.2% BOD removal (Table 4.5) lagoon system A only removes 93% of the BOD (Table 4.4). Lagoon system B demonstrates the three stage succession theory predicted with anaerobic heterotrophs being dominant in lagoon 1, Chromatiaceae in lagoons 2 and 3 and algae in lagoon 4. It is noteworthy that the volumetric and surface BOD loadings decrease significantly through successive lagoons (Table 4.5). This indicates the decreasing availability of organic compounds throughout the lagoons which undoubtedly favours the ready succession of the different microflora and eventually leads to a stable algal phase. Good BOD removal efficiencies are observed in all lagoons.

By comparison lagoon system A demonstrates what may occur with poor design of lagoon hydraulics. Over a period of five years, the Chromatiaceae have infiltrated the facultative lagoons and have eventually become dominant throughout.

Lagoon 1 is a lightly loaded anerobic lagoon with a long retention time which provides suitable conditions for the growth of the Chromatiaceae, which are the dominant population throughout the year. Lagoon 1, therefore, provides an inoculum of Chromatiaceae for the second lagoon. Theoretically, at the surface loading of $4.2 - 7.0 \text{ g BOD/m}^2 \cdot \text{d}$, this lagoon should be aerobic. However, significant short circuiting occurred in this lagoon. Therefore, the organic load is not evenly distributed throughout the lagoon, resulting in localized overloading and reduced hydraulic retention times. The effect of this overloading is to favour the dominance of Chromatiaceae which are present in large numbers in the effluent from the first lagoon. This inoculum also overcomes any washout of these bacteria due to lower retention times.

A similar phenomenon occurs in lagoon 3, where there is again short circuiting due to poor inlet and outlet locations.

It is noteworthy that the organic loading varies only slightly throughout the three lagoons of Lagoon System A. This indicates that relatively constant amounts of organic matter are available for bacterial metabolism. Algae would be favoured by a lagoon system in which the loadings decrease significantly in successive lagoons. It is, therefore, apparent that short circuiting and under-sized facultative lagoons have led to the eventual dominance of the Chromatiaceae in this system.

4.11 CONCLUSIONS

A theory based upon a three stage succession of microbial populations has been shown to be useful in describing the occurrence of Chromatiaceae in waste treatment lagoons. By manipulating the organic loading and the hydraulic retention time, lagoon systems may be designed to either allow or avoid the dominance of Chromatiaceae. By exercising such control, the potential of these organisms in minimising odours from anaerobic lagoons may be utilised.

This succession theory also provides an explanation for the dominance of the Chromatiaceae in overloaded facultative lagoons.

The observations made on lagoon systems treating sulphide-bearing wastes, such as fellmongery and tannery effluents, indicate that the Chromatiaceae constitute a stable, dominant population and provide a good degree of treatment.

These bacteria may therefore be beneficially exploited in the treatment of these noxious wastes which are difficult to treat using conventional technology. The subsequent chapters therefore assess the ability of the Chromatiaceae to treat fellmongery effluent and develop criteria for the design of such lagoons.

CHAPTER 5

LABORATORY EVALUATION OF DESIGN PARAMETERS
FOR ANAEROBIC LAGOONS USING THE CHROMATIACEAE
TO TREAT FELLMONGERY EFFLUENT.

5. EVALUATION OF DESIGN PARAMETERS FOR ANAEROBIC LAGOONS USING THE CHROMATIACEAE TO TREAT FELLMONGERY EFFLUENT.

5.1 INTRODUCTION:

Lagoons have become widely accepted as an alternative form of waste treatment. However, the use of facultative lagoons to treat wastewaters containing significant concentrations of sulphide can lead to severe operational problems with sulphide concentrations as low as 4 mg/l inhibiting some algae (Aguirre and Gloyna 1970).

The Chromatiaceae are able to grow at quite high sulphide concentrations (Chapter 3) and provide a good degree of treatment for sulphide-bearing effluents (Chapter 4). Lagoons may therefore be built to intentionally utilise the Chromatiaceae to treat wastes such as fellmongery and tannery effluents. However, little information is available on the design of such lagoons. The studies outlined in this chapter were implemented to generate design data for fellmongery effluent lagoons and to assess the effects of temperature and sulphide concentration on the effluent treatment capability of these bacteria.

5.2 LABORATORY PLANT DESIGN

5.2.1 Equipment:

Two identical 88 l model lagoons were constructed in 6 mm perspex sheeting after Eckenfelder and Ford (1970) and Eckenfelder et al (1972)(Fig. 5.1). The sides were covered with blackened cardboard to prevent lateral light penetration. Four 100 w tungsten bulbs were suspended above the lagoon at a height which resulted in a light intensity at the surface of 5,000 lux. The light intensity was measured with a Sangamo Weston Model S511 Photometer.

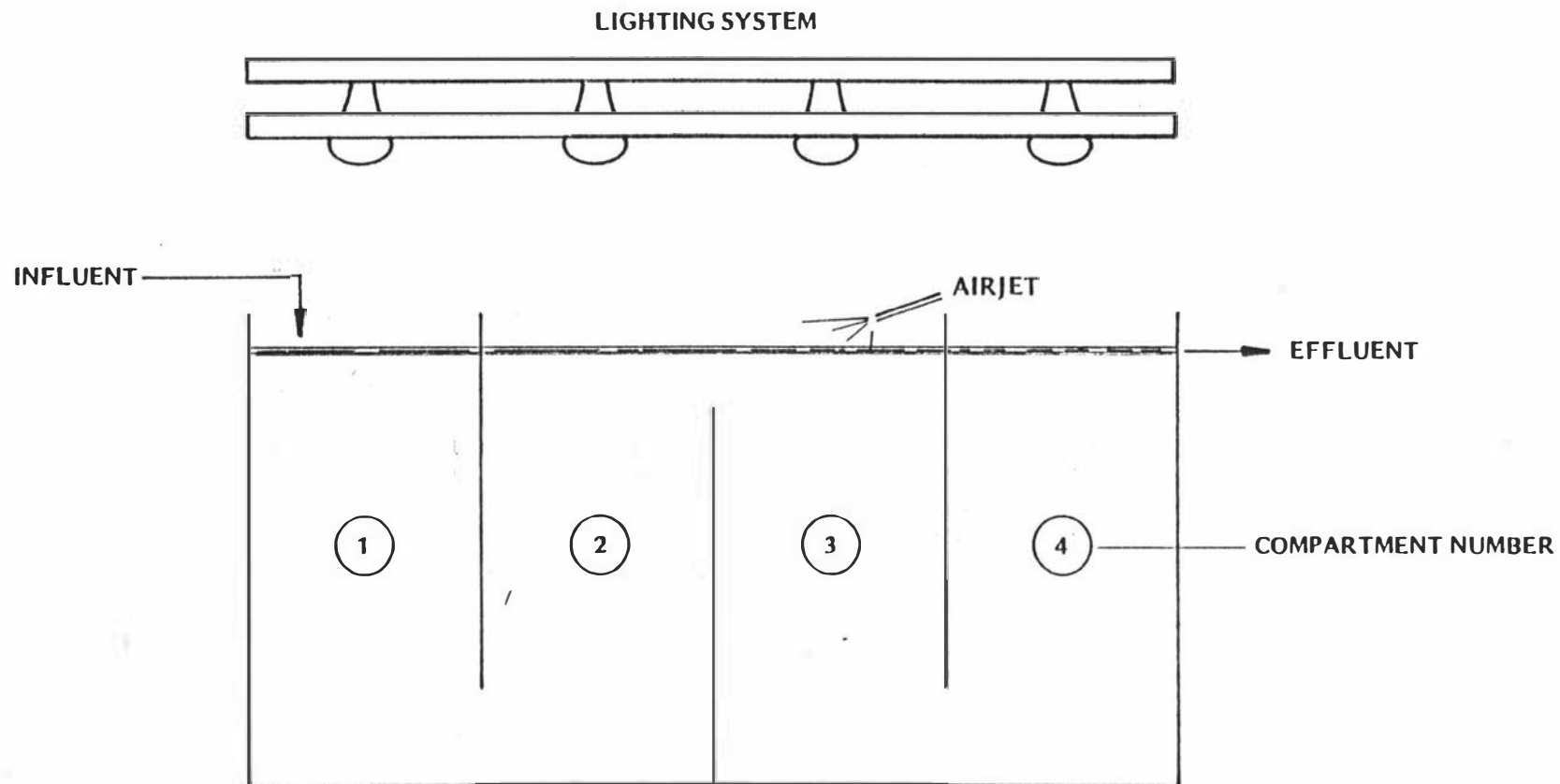


Figure 5.1: SCHEMATIC DIAGRAM OF MODEL LAGOON
(AFTER ECKENFELDER AND FORD, 1970)

A time switch provided control for the lights and pumps both of which were operated for 12 hours daily. To compensate for evaporative losses, a separate pump supplied sterile distilled water (Fig. 5.2). A fan was located to blow air across the surface of the lagoon in an attempt to simulate the effect of wind on a full scale lagoon.

Each lagoon and its ancillary equipment was placed in a separate temperature controlled room. This allowed the study of the effect of temperature on the lagoon performance.

5.2.2 Synthetic Fellmongery Effluent:

The effect of influent sulphide concentration also merited investigation. As the variable operating conditions in a fellmongery result in varying sulphide concentrations in the effluent, a synthetic effluent had to be formulated. Analysis of BOD, COD, ammoniacal nitrogen and total nitrogen and comparison with published data on the composition of N.Z. fellmongery effluents (Ryder 1972, Cooper 1973, Mason 1974) yielded a synthetic effluent of the composition outlined in Table 5.2 .

5.3 EXPERIMENTAL PROCEDURE

5.3.1 Experimental Design:

The effects of temperature and sulphide on the effluent quality were investigated at the levels indicated in Table 5.2. The range of temperatures was selected to cover the extreme environmental conditions expected in N.Z. during that period of the year that fellmongery effluent is produced. High sulphide concentrations were studied to assess the feasibility of treating concentrated fellmongery effluents. If effluents containing in the vicinity of 1,000 mg/l sulphide could be adequately treated, the segregation of the lime liquors from the relatively innocuous wash waters would result in a smaller volume of more concentrated effluent allowing smaller, cheaper fellmongery lagoons to be constructed. Lower sulphide concentrations, in the vicinity of those currently produced by fellmongeries, were also investigated.

Figure 5.2: Schematic Diagram of Lagoon and Ancillary Equipment.

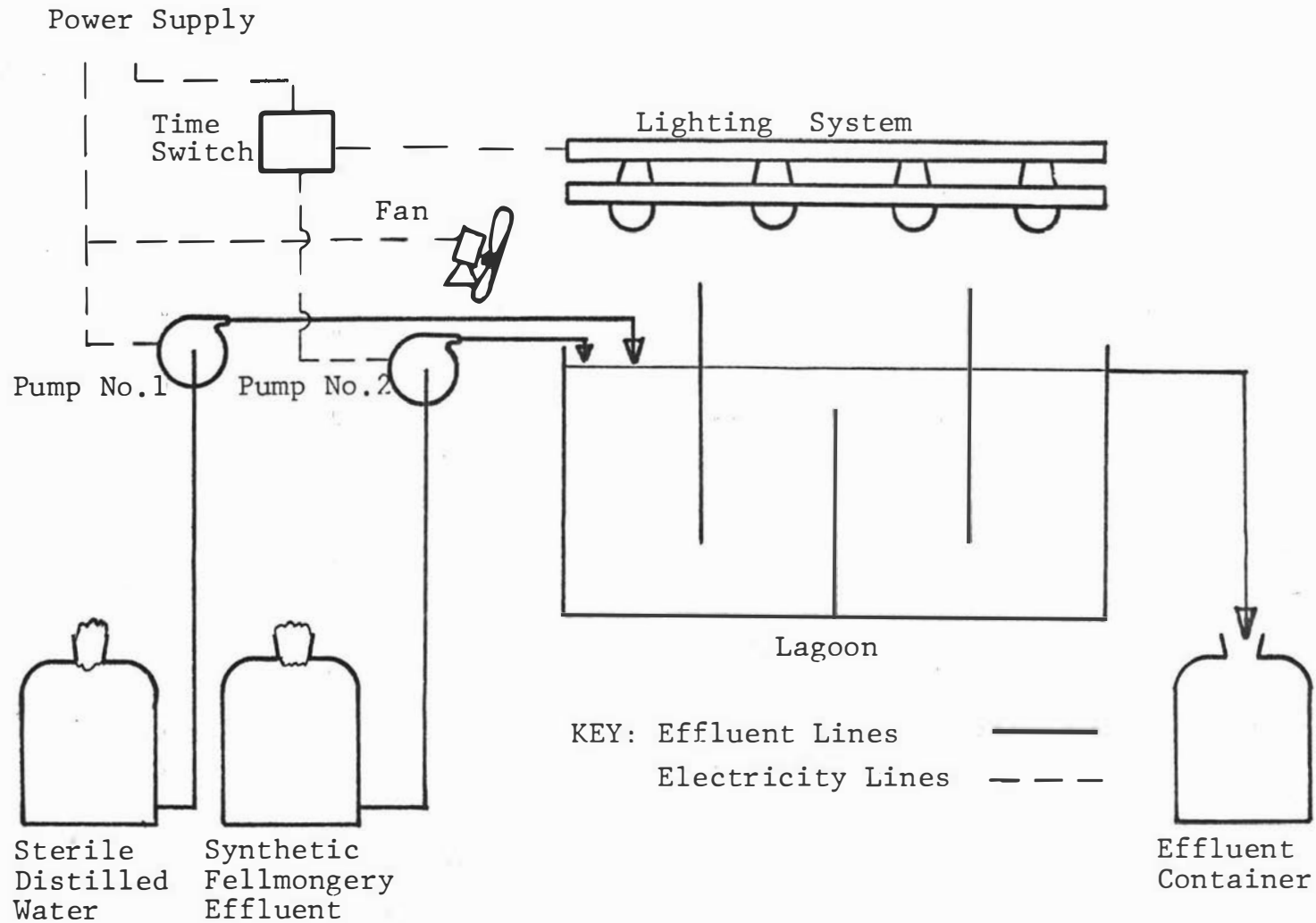


TABLE 5.1Composition of Synthetic Fellingmongery Effluent.

Component	Concentration (mg/l)
$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$	200, 850, 1,500 (as sulphide)
Bacteriological Peptone	2,500
$\text{Ca}(\text{OH})_2$	1,000
NH_4Cl	400
NaCl	1,000

TABLE 5.2Experimental Conditions Investigated.

Experiment No.	Temperature °C	Sulphide Concentration (mg/l)
1	25	200
2	25	1,500
3	10	200
4	10	1,500
5	17.5	850
6	17.5	850

5.3.2 Experimental Control:

To provide an inoculum of Methanobacteriaceae and obligate and facultative anaerobes, 10 l of sludge obtained from an anaerobic lagoon treating meatworks effluent was placed in each lagoon. Active gassing of the sludge was observed at the time of inoculation.

A diverse inoculum of Chromatiaceae was sought to allow the natural selection of the population best suited to the lagoon conditions. Thus, 15 l of a mixture of cultures of Chromatium D, Chromatium minutissimum, Thiocapsa roseopersicina and other unidentified phototrophic bacteria grown in Pfennig's medium were placed in each lagoon.

Ten litres of synthetic fellmongery effluent (Table 5.1) with an initial sulphide concentration of 20 mg/l and containing no calcium hydroxide was then added daily to the lagoon for the next six days. These low levels of sulphide and alkalinity were selected to prevent inhibition of growth. As the bacterial population in the lagoons developed the concentrations of lime and sulphide were incrementally raised over a period of four months to 200 mg/l and 1,000 mg/l respectively.

Under the temperature and sulphide conditions defined by the experimental design the lagoons were operated at a specified retention time until steady state was achieved. This was defined as less than a 5% variation in the effluent COD over a week. Generally, 1.5 to 2.0 retention times were required to achieve steady state. As retention times of up to 120 days were investigated in these experiments, extensive periods were required for the system to achieve equilibrium. A method which would maximise the results obtained from each run was therefore required. This was achieved by taking samples from each of the four compartments created by the baffles (Fig. 5.1). Thus, at each steady state, samples from four different retention times were analysed.

5.3.3 Analytical Methods:

Five parameters were used to determine the effectiveness of the lagoon as a treatment system. The biochemical oxygen demand (BOD_5) was measured manometrically using a Hach Model 2173 BOD apparatus. The chemical oxygen demand (COD) was measured in accordance with Standard Methods for the Analysis of Water and Wastewater (1975). Sulphides were determined using an Orion Model 94-16 sulphide specific ion electrode following the technique described by Papp (1971). The pH was measured in situ using an E.I.L. Model 7030 pH meter. The phototrophic bacteria were identified in accordance with Pfennig and Truper (1974) and were enumerated by direct counts using a Hawksley improved Neubauer counting chamber (Holm and Vennes 1970). The Chromatiaceae were readily identified visually due to their intracellular sulphur inclusions. Occasional measurements of thiosulphate, sulphite and polysulphides were performed as described by Papp (1971).

A problem was encountered because the initially high values of alkalinity and sulphide prevented successful BOD_5 measurement. The BOD_5 therefore could not be obtained for the influent nor for samples at short retention times. To correct this deficiency, organic removal rates were also calculated by correcting the COD value for the sulphide concentration. This resulted in the corrected COD, or COD_c , where

$$\text{COD}_c = \text{COD} - 2S$$

where $\text{COD} = \text{COD concentration mg/l}$
 $S = \text{sulphide concentration mg/l}.$

A factor of two was used as the stoichiometry of sulphide oxidation indicates that 2 moles of oxygen were required per mole of sulphide :



5.3.4 Methods of Data Analysis:

Data were analysed using the completely mixed model of Eckenfelder and Ford (1970) and Marais and Shaw (1961):

$$\frac{S_e}{S_o} = \frac{1}{1 + kt} \quad (5.1)$$

where :

S_e = effluent parameter (mg/l except pH)

S_o = influent parameter (mg/l except pH)

k = first order removal coefficient (day^{-1})

t = retention time (days)

The first order removal coefficient was determined by plotting $S_o - S_e/t$ versus S_e . A regression of the points yielded a line of slope k .

However, such a model has the constraint that at an infinite retention time, $S_e = 0$. Thus, the regression line must pass through the origin of the graph.

Although this model is correct for parameters such as the BOD, it is inadequate for the description of parameters such as the COD or pH. Such parameters are characterised by a positive intercept on the abscissa of the $S_o - S_e/t$ versus S_e graph. To describe such parameters the model must be amended to:

$$\frac{S_o - S_e}{t} = k (S_e - S_r) \quad (5.2)$$

where :

S_r = residual concentration (mg/l) and is defined as the intercept on the abscissa of the $S_o - S_e/t$ versus S_e graph.

To predict the effluent quality with this model both k and S_r must be known. The effect of temperature on these terms must be assessed. This was done using the technique of Marais (1970) and

Eckenfelder and Ford (1970) where:

$$k_2 = k_1 \theta^{(T_2 - T_1)} \quad (5.3)$$

and
$$S_{r2} = S_{r1} \theta^{(T_2 - T_1)} \quad (5.4)$$

where : k_2 = removal rate coefficient (day^{-1}) at T_2 ($^{\circ}\text{C}$)
 k_1 = removal rate coefficient (day^{-1}) at T_1 ($^{\circ}\text{C}$)
 θ = temperature coefficient ($^{\circ}\text{C}^{-1}$)
 S_{r2} = residual (mg/l) at T_2 ($^{\circ}\text{C}$)
 S_{r1} = residual (mg/l) at T_1 ($^{\circ}\text{C}$)

5.4 RESULTS

To demonstrate the effect of retention time on effluent quality, the results obtained in each trial are presented as chronological plots (Figures 5.3-5.8).

The data for each of the trials from which these plots were made are presented in Appendix 4. The values in the tables are the average of five samples analysed during a week of steady state operation.

The blank values in the BOD columns of these tables result from an inability to measure the BOD at high concentrations of sulphide and alkalinity.

A summary of each experiment is presented in Table 5.4 where the influent quality is compared with the final effluent quality.

Thiocapsa roseopersicina was the dominant micro-organism in the lagoons under the range of conditions studied.

5.5 CONSIDERATION OF CHRONOLOGICAL PLOTS

The chronological plots (Figures 5.3-5.8) followed the pattern expected for continuous culture systems (Aiba et al 1973, Pirt 1975). At long retention times, or low dilution rates, bacterial numbers

Figure 5.3: Chronological Plot for Run 1 (25°C, 200mg/l S^{2-})

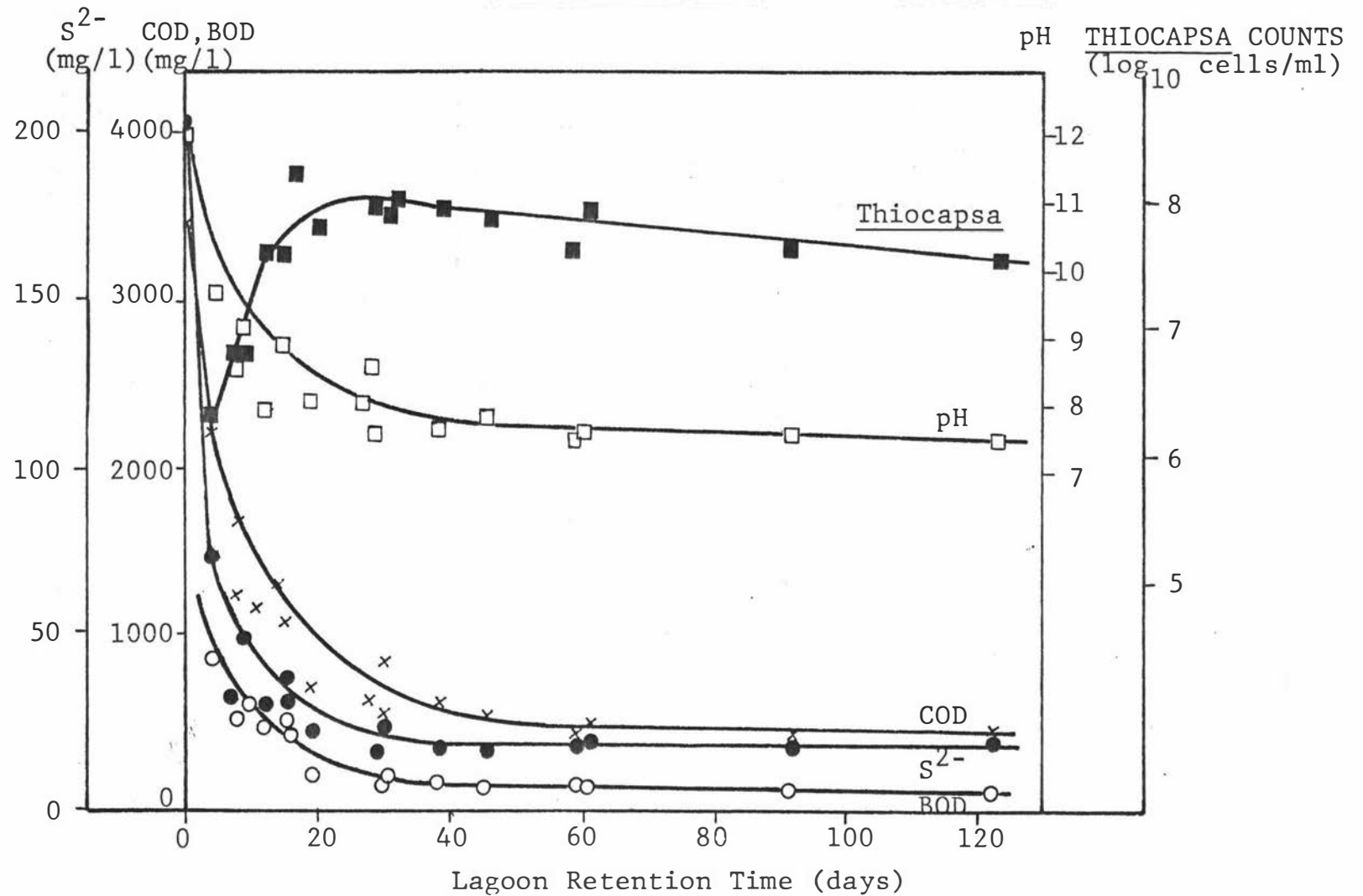


Figure 5.4: Chronological Plot for Run 2 (25°C, 1500 mg/l S²⁻)

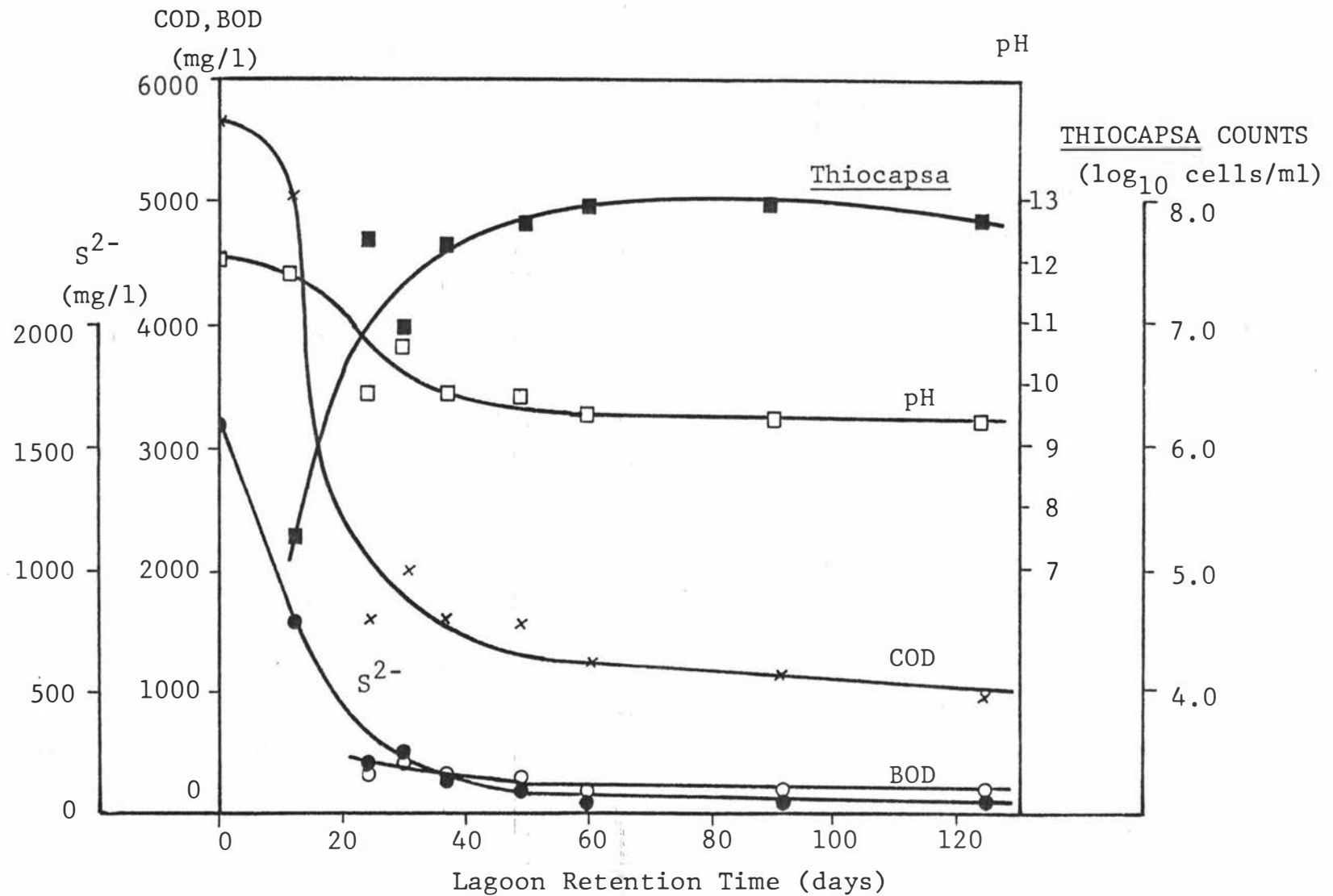


Figure 5.5: Chronological Plot for Run 3 (10°C , 200 mg/l S^{2-})

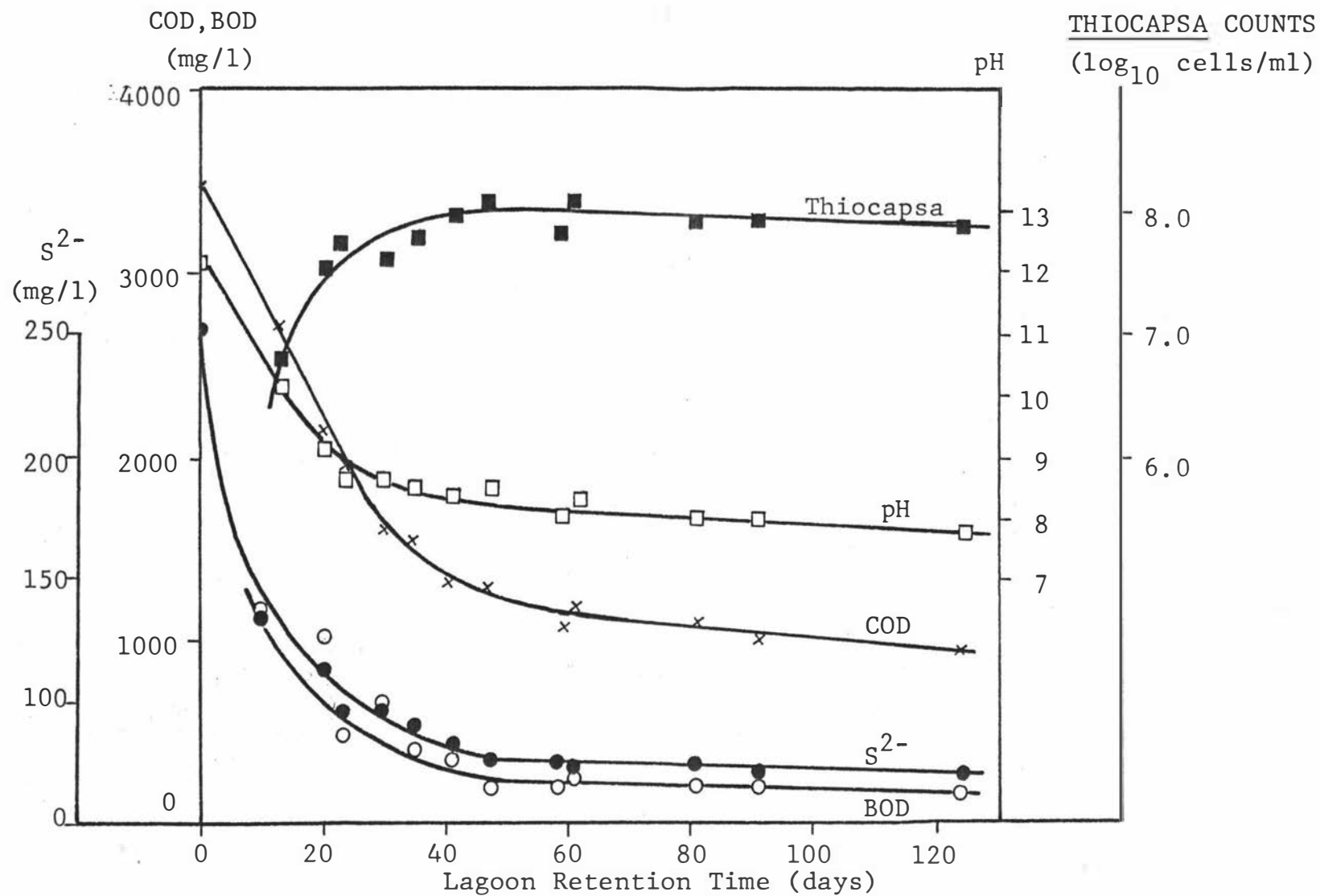


Figure 5.6: Chronological Plot for Run 4 (10°C , 1500 mg/l S^{2-})

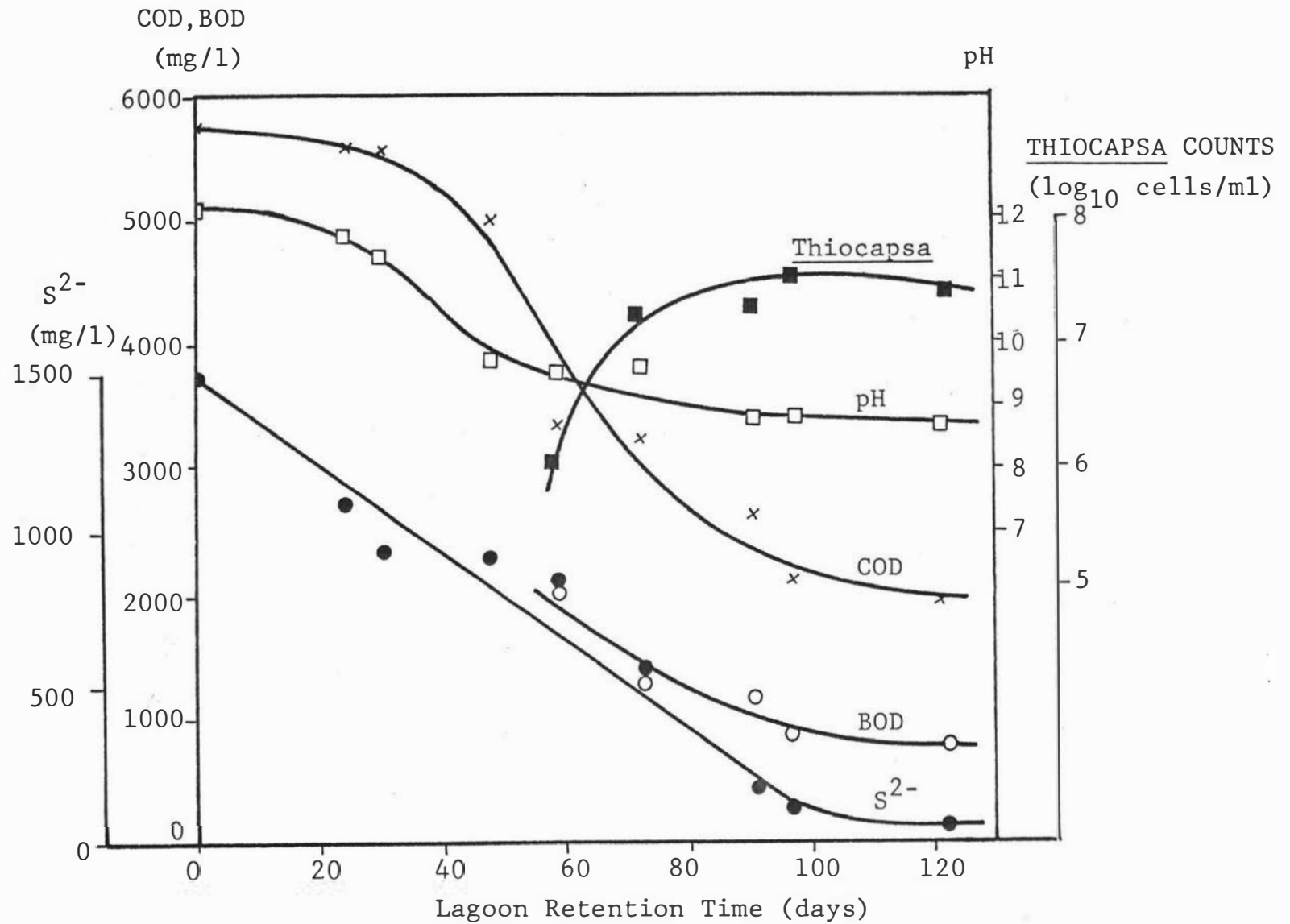


Figure 5.7: Chronological Plot for Run 5 (17.5°C, 850 mg/l S²⁻)

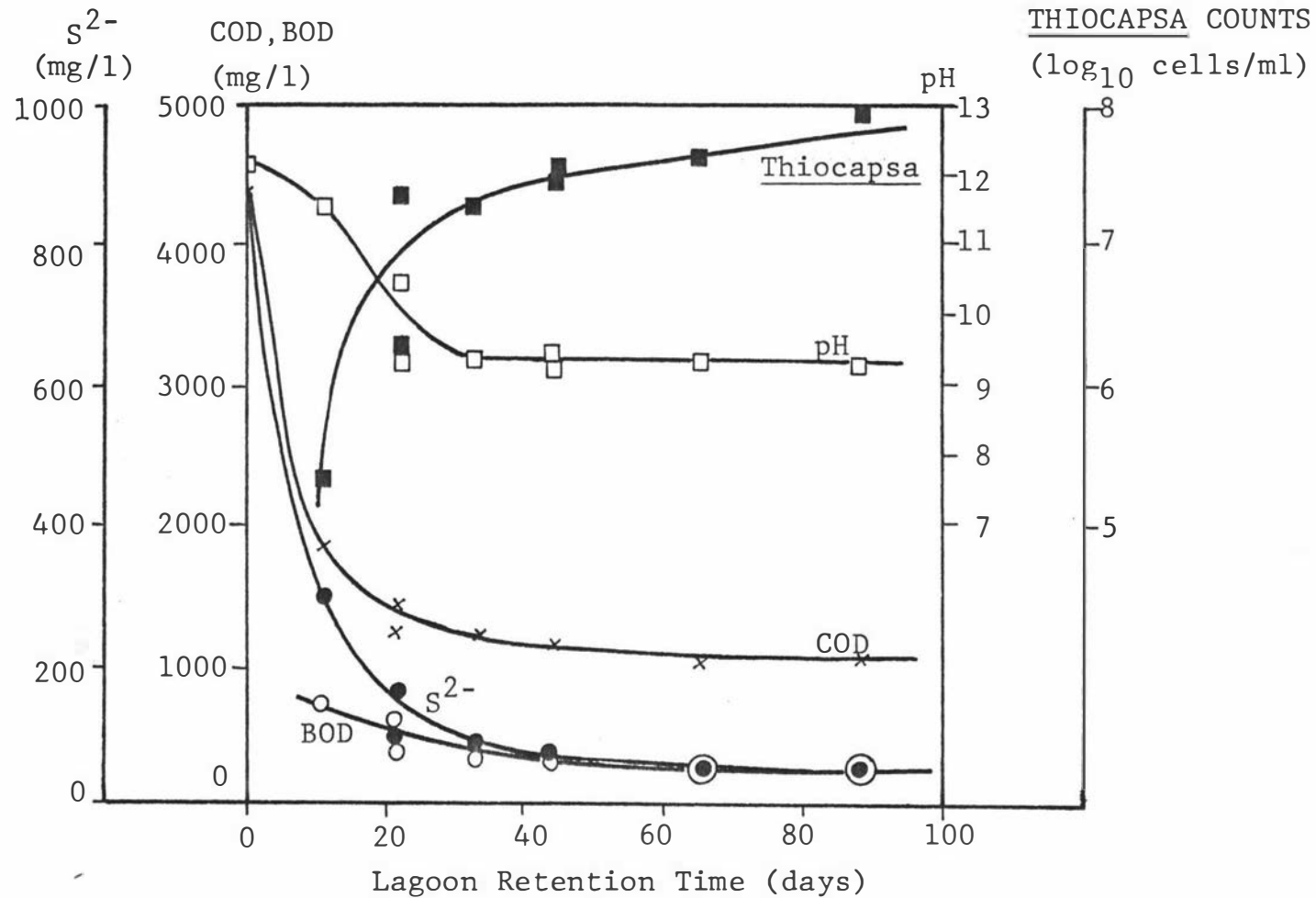
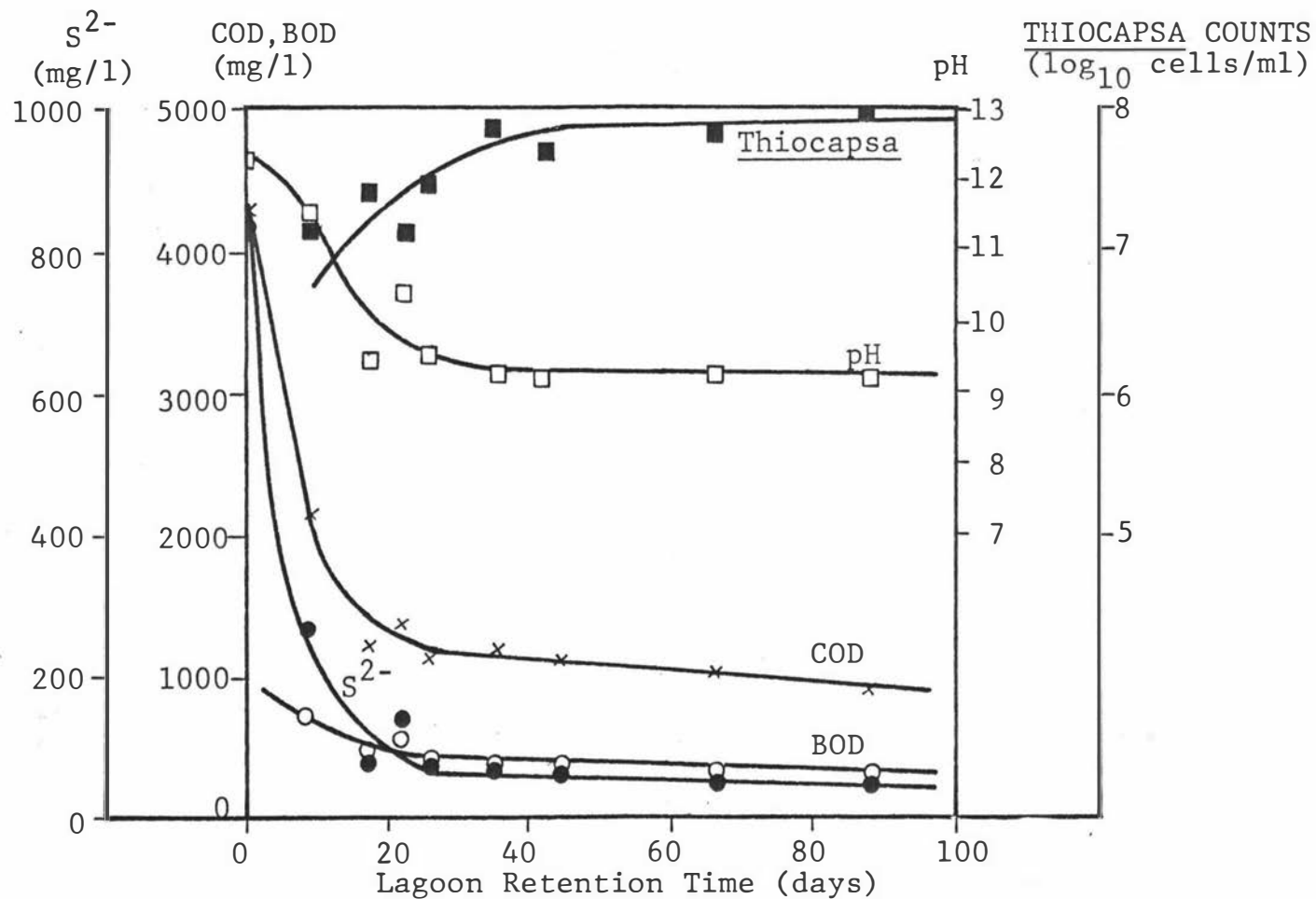


Figure 5.8: Chronological Plot for Run 6 (17.5°C, 850 mg/l S^{2-})



were high and substrate concentrations were low. Little variation in these parameters was observed until a critical retention time occurred. Below this retention time the bacteria could no longer grow at a sufficient rate to maintain their numbers and a decrease in population was observed. Increasing substrate concentrations were associated with this period. The retention time at which the bacterial population approaches zero has been defined as 'washout' (Aiba et al 1973).

Due to the problems of measuring low populations of phototrophic bacteria, an attempt was made to approximate the point at which washout occurred in each trial by determining the retention time at which 50% of the influent COD was removed. This information is presented in Table 5.3.

Under the most advantageous conditions (Run 1 : 25°C and 200 mg/lS²⁻) the lowest retention time of 8.0 days for a 50% COD reduction was observed. Little variation in this retention time was observed under more moderate conditions (Runs 5 and 6 : 17.5°C, 850 mg/lS²⁻). However under more severe conditions the performance of the lagoons deteriorated with a retention time of 75.0 days

TABLE 5.3

The Retention Time at Which a 50% COD Removal Occurs as a Function of the Experimental Conditions.

Run No.	Experimental Conditions		Retention Time for 50% COD removal (days)
	Temperature (°C)	S ⁻ (mg/l)	
1	25	200	8.0
2	25	1,500	17.5
3	10	200	30.0
4	10	1,500	75.0
5	17.5	850	8.5
6	17.5	850	9.0

required to achieve a 50% reduction in COD under the most extreme conditions (Run 4 : 10°C , $1,500 \text{ mg/l S}^{2-}$). Thus, the influent sulphide concentration and the lagoon temperature substantially affect the retention time required to produce an adequate effluent.

The effluent quality at the longest retention time for each trial is presented in Table 5.4. The percent removals of COD and sulphide indicate that these lagoons operate efficiently under a wide range of temperatures and influent sulphide concentrations. This is confirmed by a study of Figure 5.9 which indicates the trends observed in the treatment efficiencies of the lagoons. The COD removal efficiency is highest at low sulphide concentrations and high temperatures and decreases with increasing sulphide concentration and decreasing temperature. This closely parallels the effects of sulphide and temperature on the growth of Chromatium minutissimum (Chapter 3). A similar trend is observed with the BOD and pH results (Figure 5.9 (b) and (d)). However, the highest percentage removals of sulphide occur at the high sulphide concentrations (Figure 5.9 (c)). This might be expected as increasing concentrations of sulphide will increase the driving force for its removal.

Therefore the best effluent quality is achieved at the highest temperature and the lowest concentration studied (Run 1: 25°C , 200 mg/l sulphide) and the observed values were:

COD 440 mg/l
BOD 95 mg/l
Sulphide 20 mg/l
pH 7.4

The poorest effluent quality was observed at the lowest temperature and highest sulphide concentration studied (Run 4 : 10°C , $1,500 \text{ mg/l S}^{2-}$) and the observed values were:

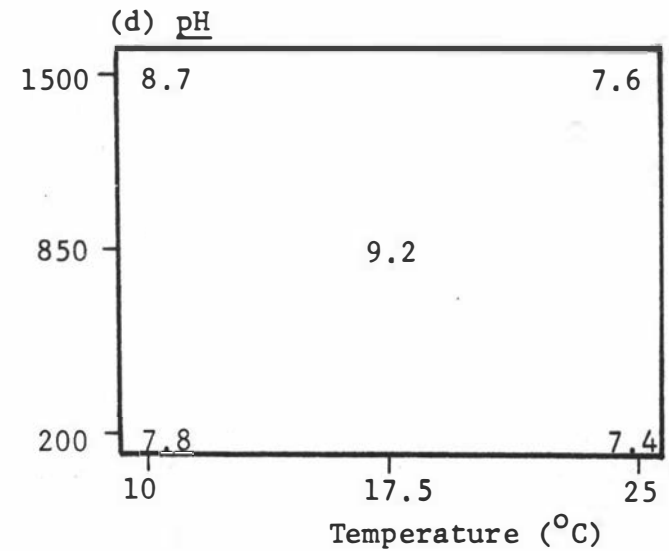
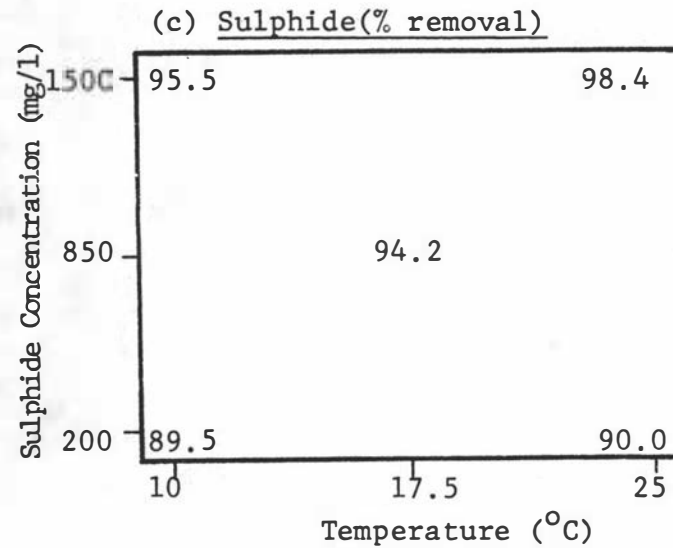
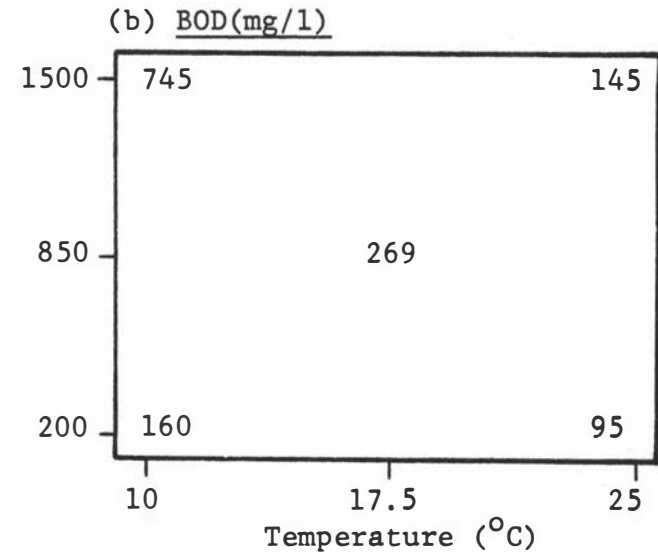
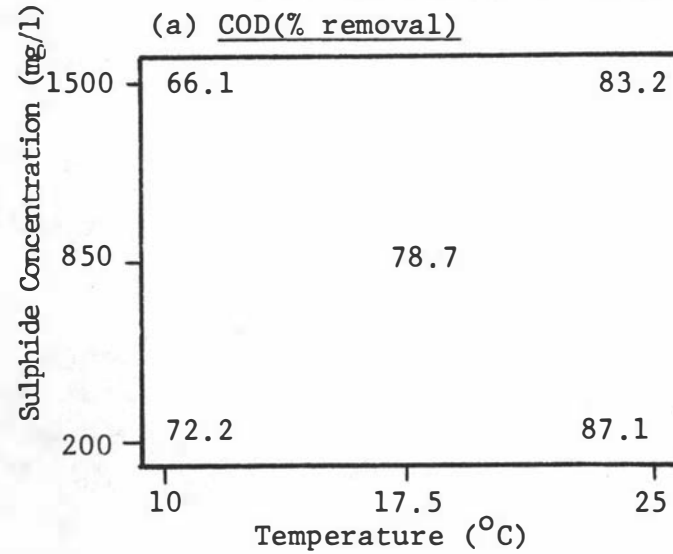
COD 1,952 mg/l
BOD 745 mg/l
Sulphide 67 mg/l
pH 8.7

TABLE 5.4

Effluent Quality at the Longest Retention Time for Each Trial.

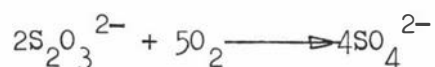
Run No.	Influent Characteristics					Final Detention Time (days)	Effluent Characteristics				Percent Removals	
	COD	COD _C	S ⁼	pH	Temp. (°C)		COD	BOD	S ⁼	pH	COD	S ⁼
	(mg/l)	(mg/l)	(mg/l)				(mg/l)	(mg/l)	(mg/l)			
1	3,424	3,024	200	11.8	25	122	440	95	20	7.4	87.1	90.0
2	5,694	3,072	1,536	12.1	25	122	956	145	24	7.6	83.2	98.4
3	3,476	3,076	200	11.8	10	122	966	160	21	7.8	72.2	89.5
4	5,764	2,786	1,489	12.2	10	122	1,952	745	67	8.7	66.1	95.5
5	4,357	2,641	858	12.3	17.5	88	975	251	53	9.2	77.6	93.8
6	4,295	2,611	842	12.3	17.5	88	869	288	45	9.2	79.8	94.6

Figure 5.9: Plots Demonstrating the Effects of Temperature and Influent Sulphide Concentration on the Treatment Efficiency of the Lagoons.



5.6 CALCULATION OF REMOVAL RATE COEFFICIENTS

To quantify the rates of removal of the various effluent parameters, regression curves of $S_o - S_e/t$ versus S_e were plotted (Figures 5.10-5.13). Unfortunately, the problems with BOD measurement mentioned in Section 5.3.3 precluded the use of BOD as a regression parameter. In no case was it possible to obtain influent BOD values and thus, such a regression analysis was impractical. Therefore the corrected COD, COD_c , was used as an estimate of the organic content as described in Section 5.3.3. Although the COD_c does not take account of the sulphide content of the wastewater, it will measure any other reduced inorganic compounds. Occasional analyses for thiosulphate, sulphide and polysulphides failed to detect any of these compounds in the lagoons or their effluent. In some instances thiosulphate was detected in the influent but never at levels greater than 5% of the sulphide concentration. Thiosulphate is oxidised according to:



Thus one mole of thiosulphate reacts with 2.5 moles of oxygen. During its oxidation, sulphide reacts with 2.0 moles of oxygen (Section 5.3). Due to the similarity of the stoichiometry and the low thiosulphate concentrations observed, it was assumed that thiosulphate made a negligible contribution to the inorganic COD of the influent when compared to sulphide. Therefore subtracting the COD due to sulphide from the total COD is equivalent to removing the effect due to inorganic sulphur compounds.

No compensation for the effect of inorganic nitrogen compounds has been made. This prevents the COD_c from being solely an organic COD. However, published data indicate only a small variation between influent and effluent ammoniacal nitrogen concentrations. Cooper et al (1975) reported an influent concentration of 22.2 mg/l and an effluent concentration of 17.6 mg/l. This small variation in ammoniacal nitrogen concentrations therefore allows the COD_c to provide a useful indication of the rate of removal of organic compounds.

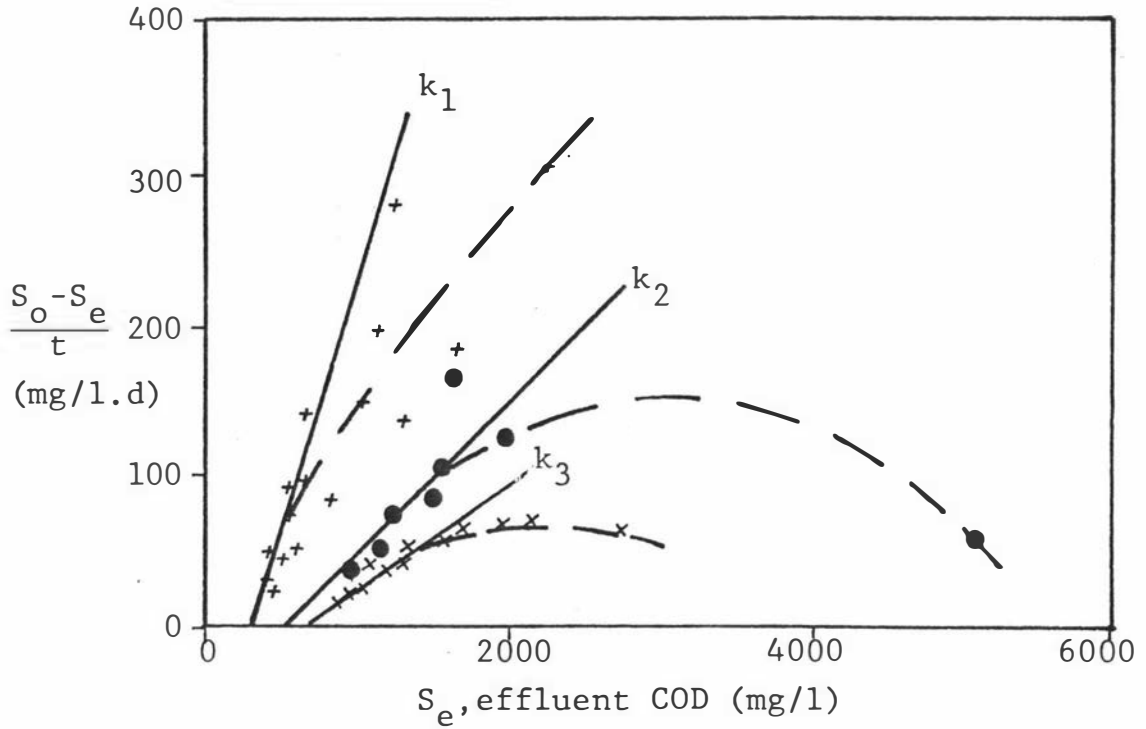
A study of the regression curves (Figures 5.10-5.13) yields some interesting information. In nearly all cases, reduction of the removal rate coefficients is observed at high Se values (i.e. short retention times). This effect may be explained by the instability of the lagoon under these conditions. A large, stable microbial population is unable to develop due to the short retention time, and the adverse growth conditions arising from the high pH and the high sulphide concentration. As the retention time increases, the pH and the sulphide concentration decreases slowly, even in the absence of the Chromatiaceae (Fig. 5.6). This non-microbiological decrease in pH is probably caused by atmospheric carbonation removing the alkalinity due to lime (see Section 2.7.3.2.1) while the decrease in the sulphide concentration is probably due to oxidation by air.

Thus, at short retention times, k values are low due to their dependence upon chemical oxidation and their value is probably limited by the rate of diffusion of CO_2 and O_2 into the lagoon. As the pH and sulphide concentrations are reduced, the conditions in the lagoon become suitable for the growth of the Chromatiaceae and a slow increase in k values is observed until a stable population of phototrophic bacteria is established. This postulate is confirmed by a study of the removal rate graphs (Figures 5.10-5.13) and explains why the regression curves accurately model the low Se values. This is the region in which the highest removal rates are observed and under these conditions a stable bacterial population has developed. It is therefore the best region in which to operate the lagoon.

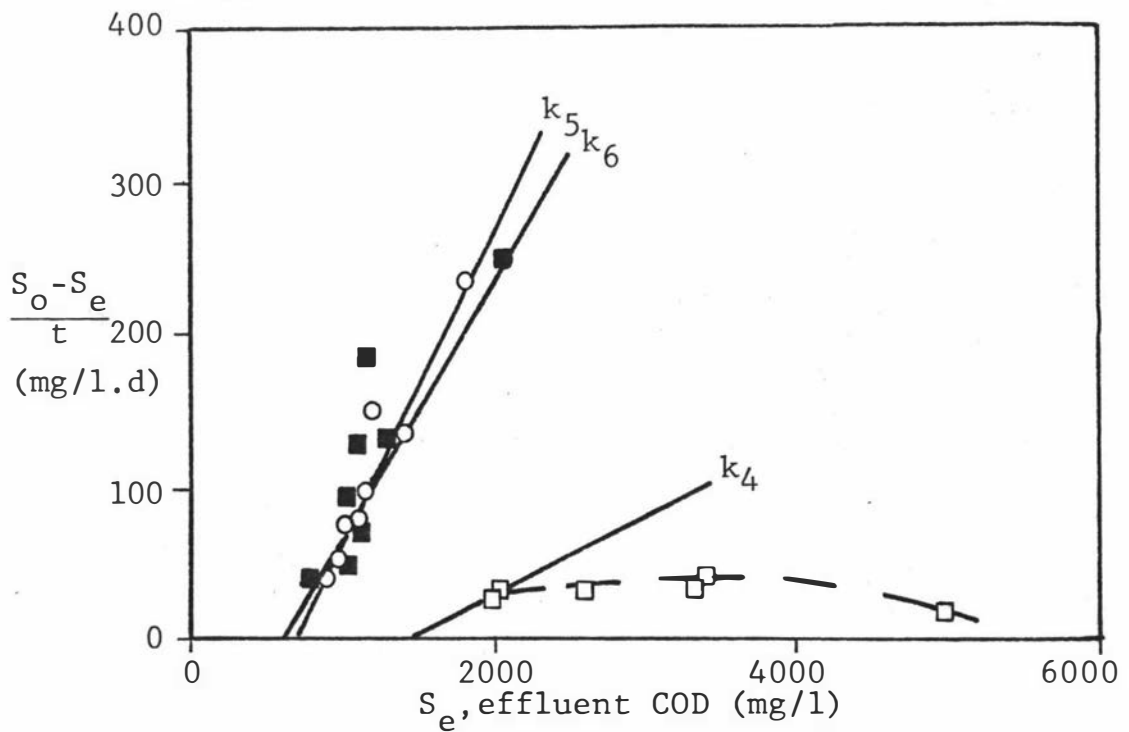
Some scatter of points about the regression lines may be observed. This is largely due to the use of the completely mixed model to analyse the data. This assumes that the lagoons are completely mixed whereas in fact their flow regime is likely to fall between the extremes of plug and completely mixed flow.

Figure 5.10: Plots to Determine the COD Removal
Rate Coefficients

(a) Runs 1, 2 and 3



(b) Runs 4, 5 and 6



5.6.1 COD Removal Rate Coefficients:

No uniformity as to the point at which the removal rate coefficients become inhibited was observed. The COD values at which the COD removal rate coefficient becomes reduced varies from 800 mg/l for run 1 (25°C, 200 mg/l S²⁻) to 2,000 mg/l for run 4 (10°C, 1,489 mg/l S²⁻). This variation is not unexpected due to the wide range of experimental conditions studied. Obviously, the activity of the bacteria will be severely curtailed even at high retention times under the extreme conditions prevailing in run 4. Also, the validity of the k value obtained in run 4 is questionable due to the regression including only two points. To determine k more accurately, the system would have to be studied at longer retention times.

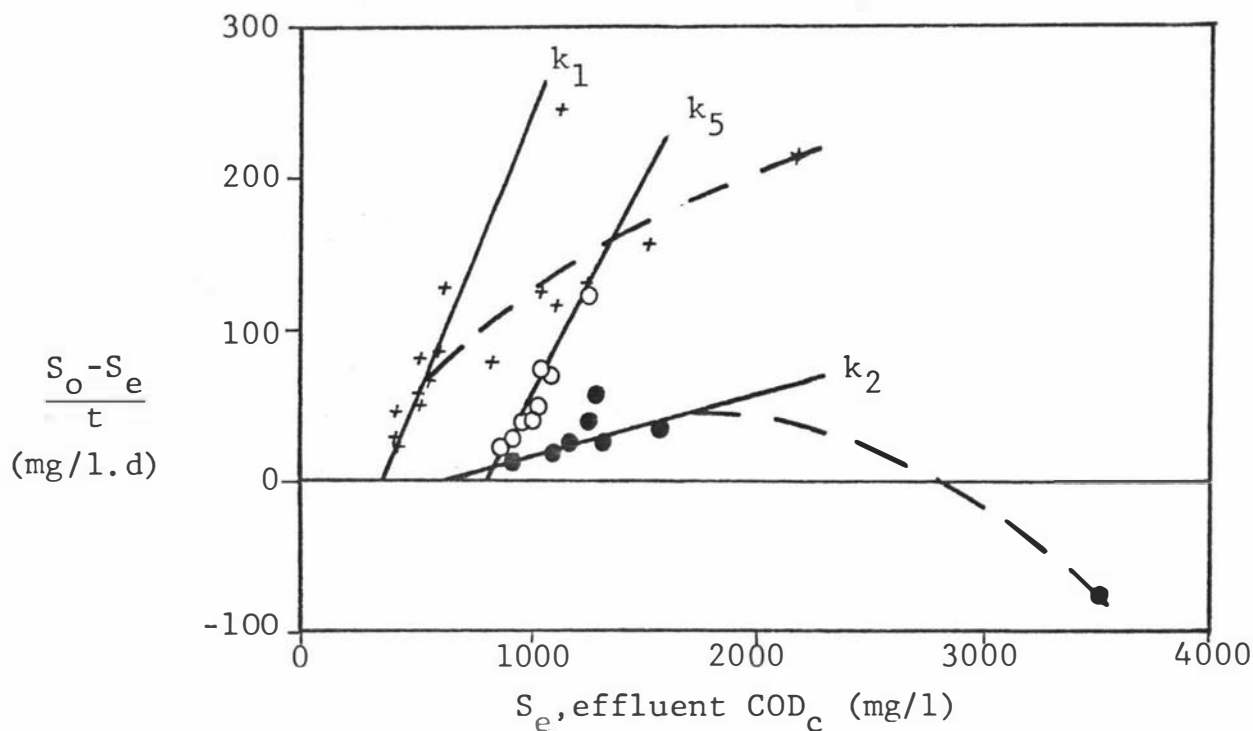
5.6.2 COD_c Removal Rate Coefficients:

The corrected COD regression analyses (Figure 5.11) show similar results to the COD data. However, two unexpected results may be observed. Firstly, run 4 shows a negative removal rate coefficient. Although this could be interpreted as an increase in the organic content of the effluent due to photosynthesis, it is more probably caused by the inhibitory effects of the adverse lagoon conditions on the removal rate coefficient. It is likely that a study of longer retention times would yield a positive k value.

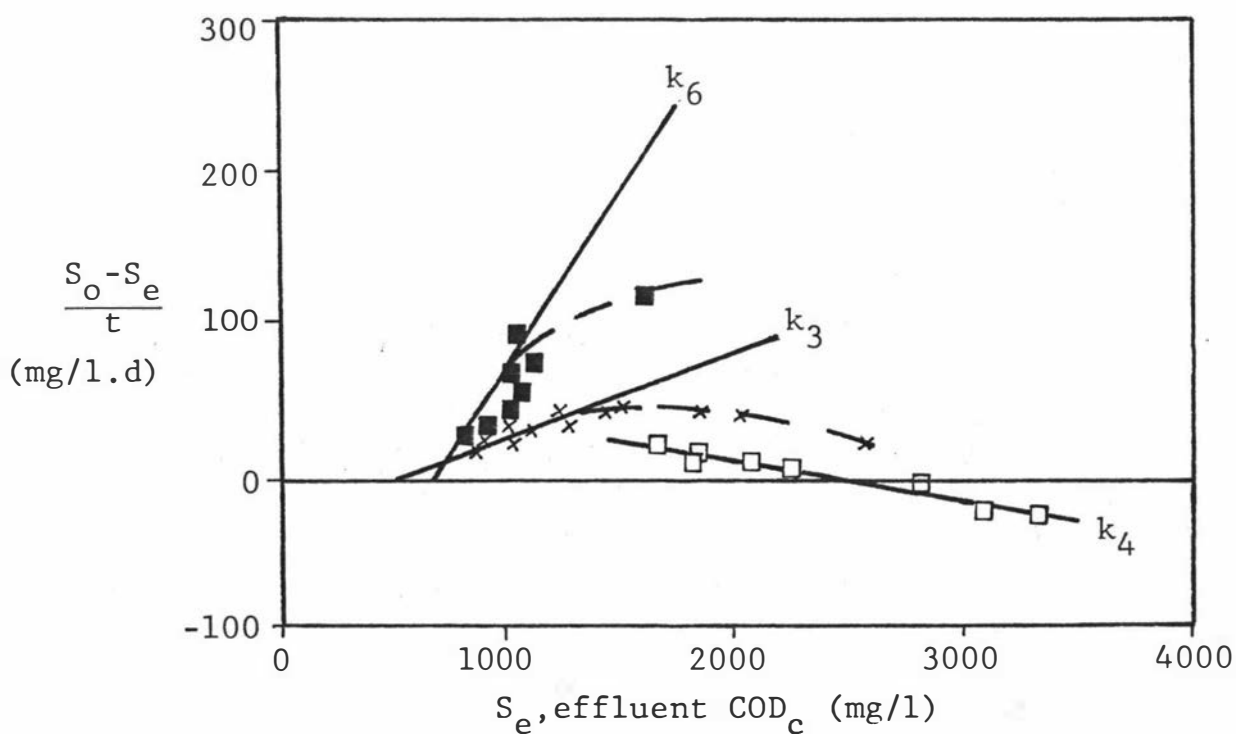
The second unexpected observation is the negative So-Se/t values. All the negative values occurred at short retention times on the experiments using a high influent sulphide concentration (Runs 2 and 4). These results imply that an increase in the organic content of the effluent occurs at short retention times. However, all other results indicate that this is improbable indicating some inconsistencies between the sulphide and COD analyses at high sulphide concentrations.

Figure 5.11: Plots to Determine the COD_c Removal Rate Coefficients

(a) Runs 1, 2 and 5



(b) Runs 3, 4 and 6



5.6.3 Sulphide Removal Rate Coefficient:

The plots to determine the sulphide removal rate coefficient (Figure 5.12) demonstrate the inhibitory effect of high sulphide concentrations. Again, run 4 yields a spurious result due to being studied for an insufficiently long retention time as indicated by the positive intercept on the ordinate. Similarly, the pH removal rate coefficient is inhibited at high pH values (Figure 5.13).

5.6.4 Summary:

The removal rate coefficients, the residuals and the correlation coefficients for each run are summarised in Table 5.5. In all experiments, sulphides were removed more rapidly than the COD. The rate coefficients for sulphide removal ranged from $.878 \text{ day}^{-1}$ to $.698 \text{ day}^{-1}$ while the corresponding range for COD was from $.348 \text{ day}^{-1}$ to $.0591 \text{ day}^{-1}$. For all four parameters studied the highest removal rates were observed in run 1 (25°C , 200 mg/l S^{2-}) and the lowest rates were observed in run 4 (10°C , $1,489 \text{ mg/l S}^{2-}$).

A study of the residual concentrations indicates that they do not fit such a neat pattern. Although the lowest residual occurs in run 1 and the highest in run 4 for both the COD and the COD_c , the highest S_r for sulphide is observed in Run 5, and the lowest in run 4. However, the negative value observed in run 4 is questionable due to the regression being based on only two points. The S_r values for pH range from 7.0 for run 4 to 9.06 for run 6.

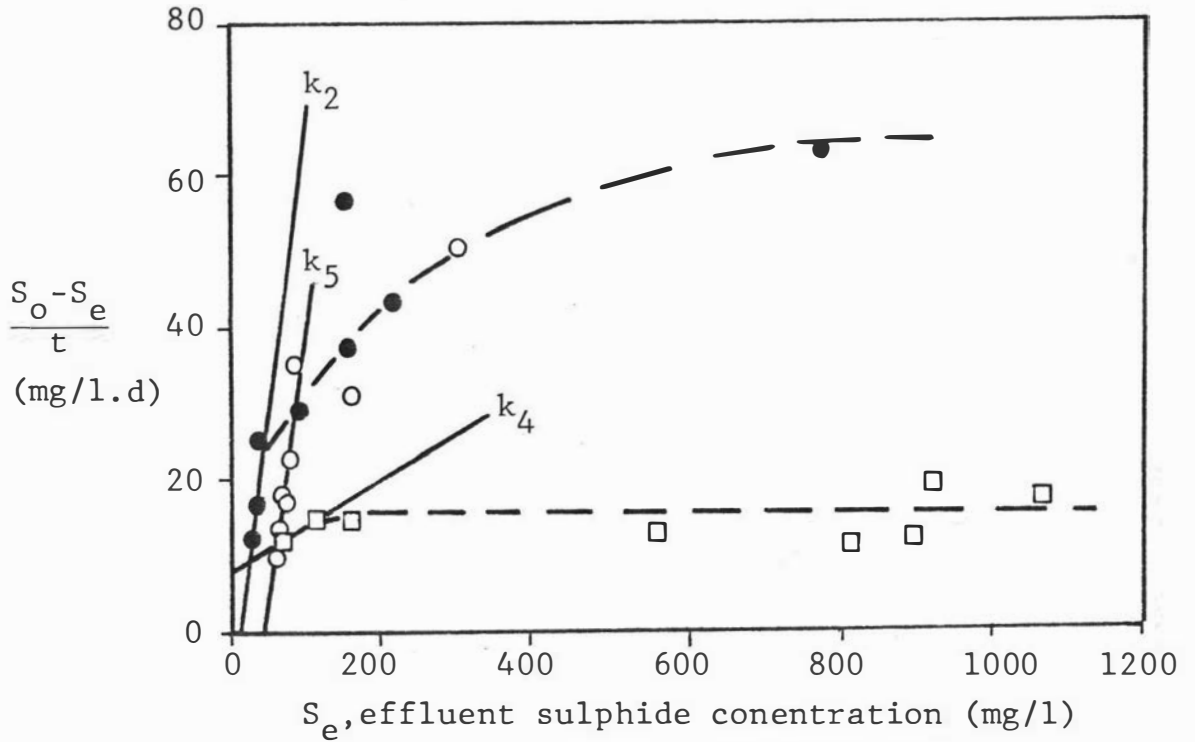
5.7 COMPARISON OF DEVELOPED MODELS WITH OBSERVED DATA

As the regression analyses indicate some lack of fit at high S_e values Figure 5.14 was plotted to demonstrate the adequacy of fit of the COD model to the raw COD data.

In all cases, good fit is observed at high retention times. The models for runs 5 and 6 fit all the data well. However, in other experiments the models provide an optimistic prediction of

Figure 5.12: Plots to Determine the Sulphide Removal Rate Coefficients

(a) Runs 2, 4 and 5



(b) Runs 1, 3 and 6

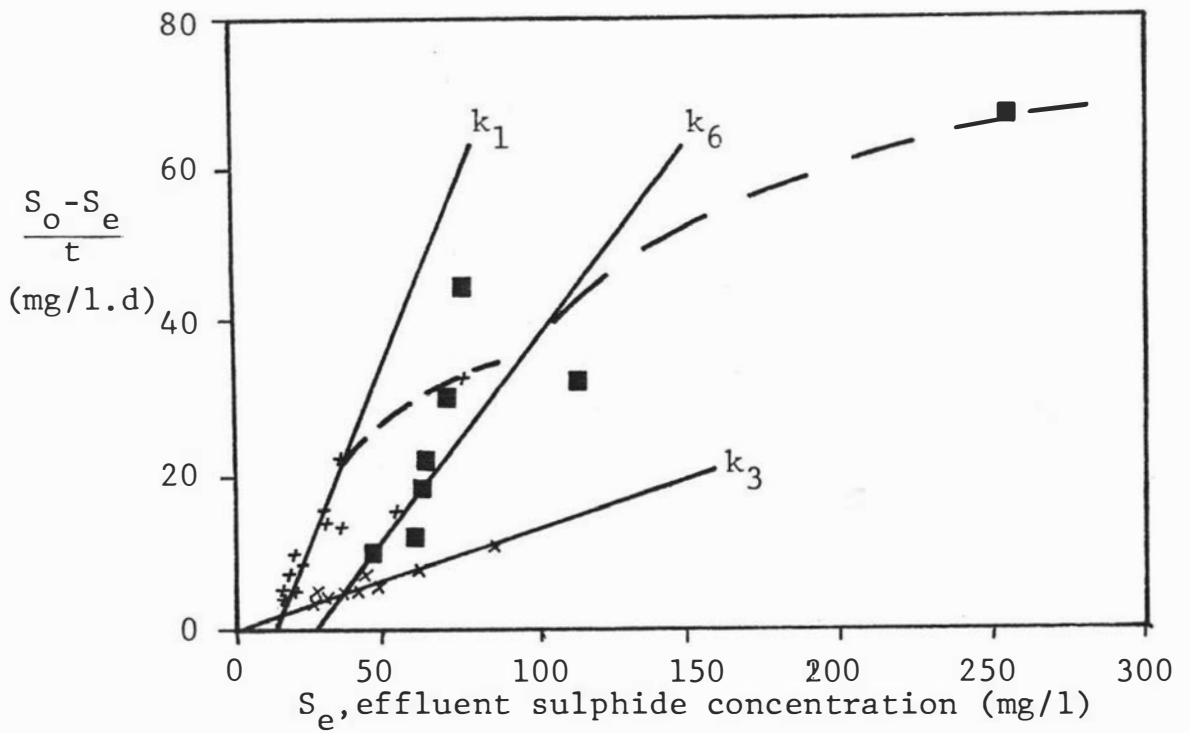
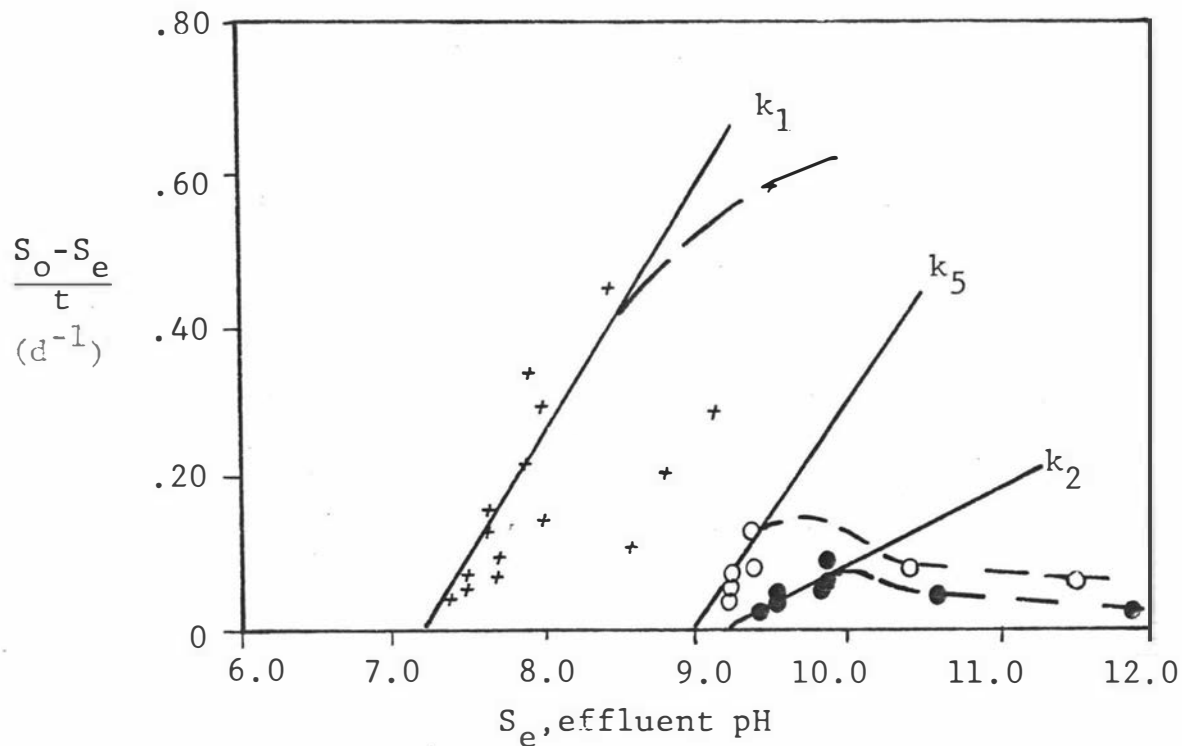


Figure 5.13: Plots to Determine the pH Removal Rate Coefficients

(a) Runs 1,2 and 5



(b) Runs 3,4 and 6

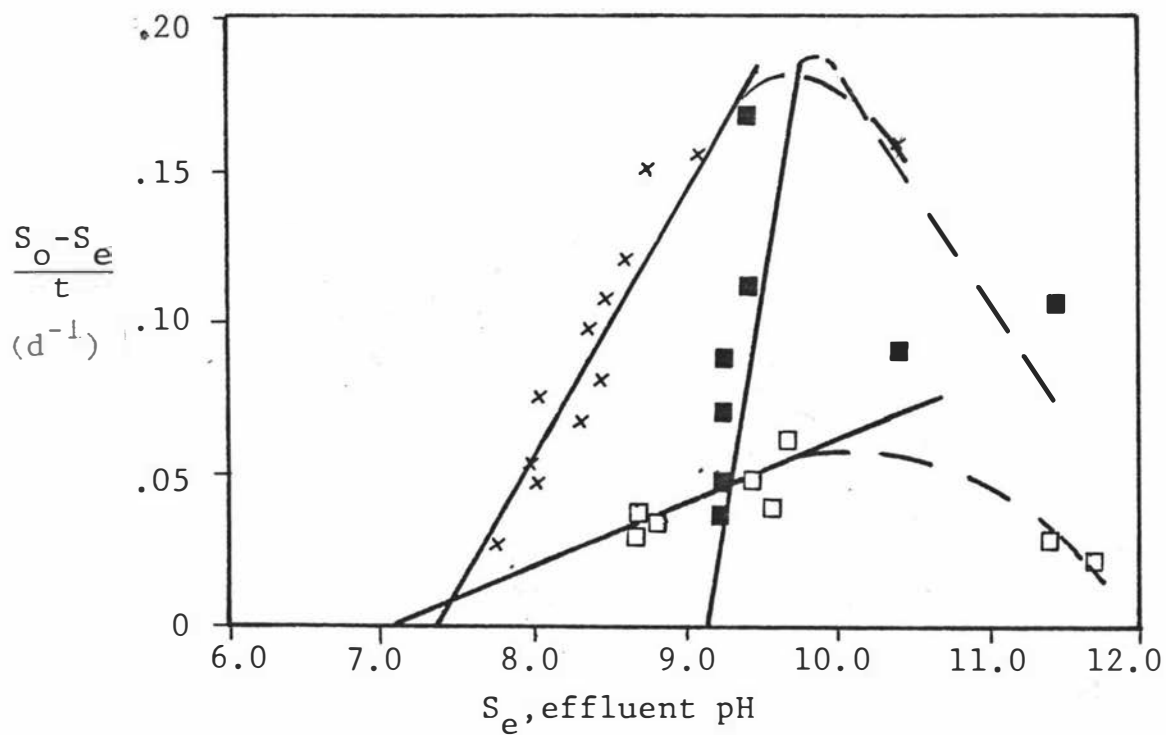


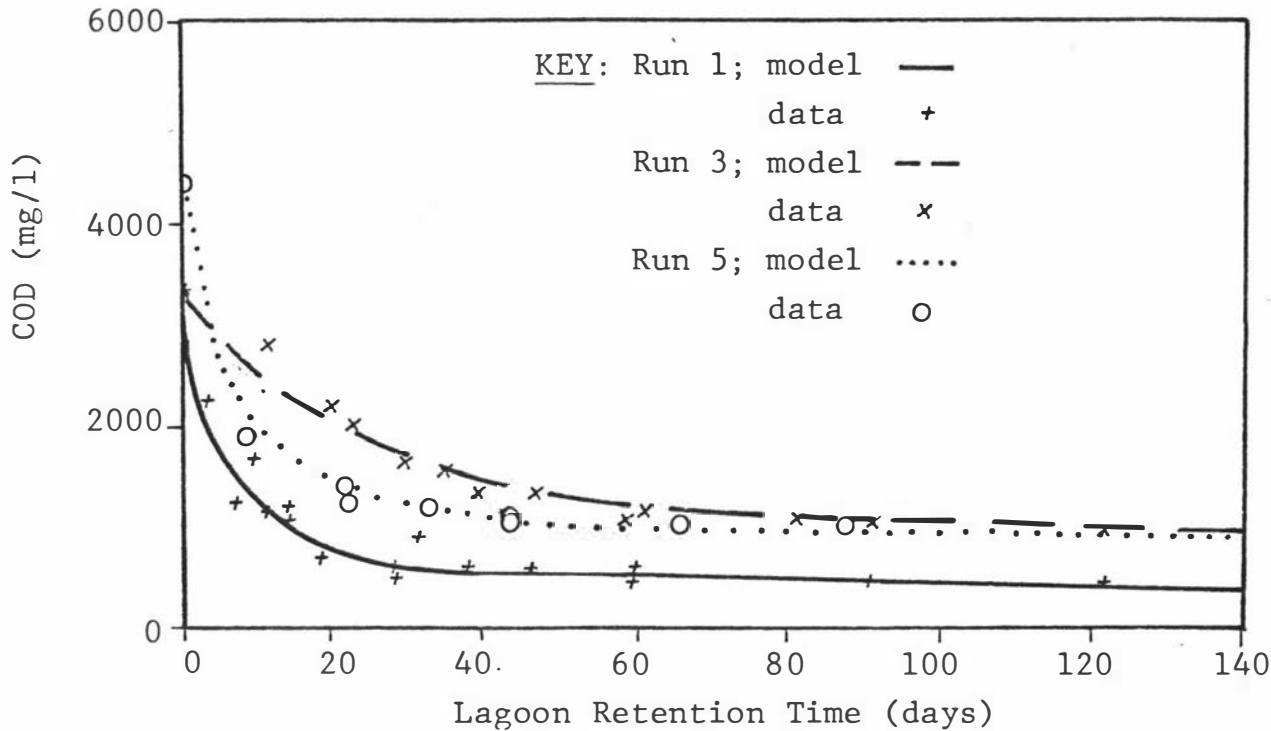
TABLE 5.5

Summary of the Removal Rate Coefficients, the Residuals and the Correlation Coefficients
Obtained for Each Run.

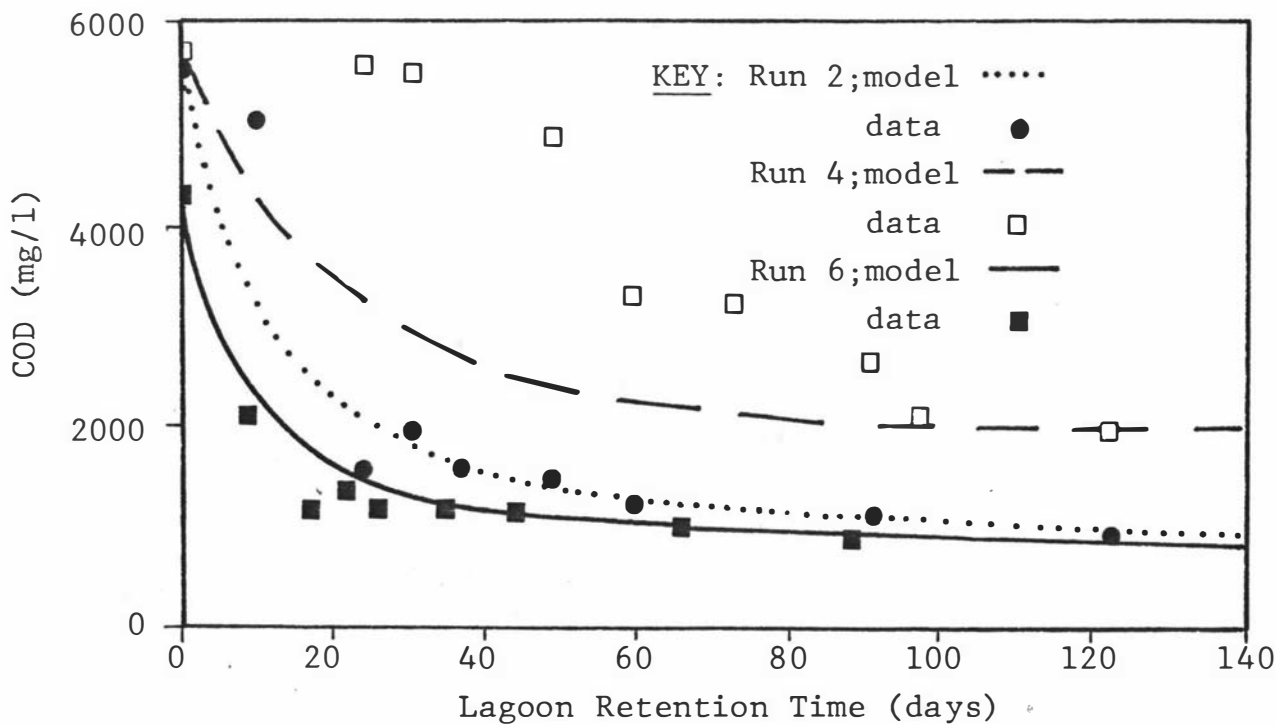
Run No.	1	2	3	4	5	6	
Temperature (^o C)	25	25	10	10	17.5	17.5	
Sulphide concentration (mg/l)	200	1,536	200	1,489	858	842	
Parameter							
COD	k (day ⁻¹)	.348	.103	.0751	.0591	.208	.178
	S _r (mg/l)	327	516	642	1,425	718	677
	r ²	.811	.585	.847	1.00	.918	.988
COD _c	k (day ⁻¹)	.331	.0374	.0526	-.0216	.272	.223
	S _r (mg/l)	318	523	578	2,428	803	680
	r ²	.845	.726	.803	.936	.934	.484
Sulphide	k (day ⁻¹)	.878	.669	.122	.8698	.711	.526
	S _r (mg/l)	14.4	4.57	1.67	-100	40.5	28.5
	r ²	.830	.486	.923	1.00	.750	.552
pH	k (day ⁻¹)	.327	.102	.0820	.0190	.291	.286
	S _r (mg/l)	7.30	9.14	7.39	7.00	8.96	9.06
	r ²	.5670	.700	.863	.883	.488	.314

Figure 5.14: Plots Showing the Fit of the COD Models
to the COD Data

(a) Runs 1,3 and 5



(b) Runs 2,4 and 6



the effluent quality at short retention times. In runs 1, 2 and 3 the inaccuracy of the model is not very substantial. However, in run 4 a severe lack of fit is apparent. This is due to the lag period observed in this COD data. Up to 40 days retention time only a small reduction in COD occurs (Figure 5.6) and an inverted sigmoid curve would be needed to fit the data. An exponential decline in the COD is predicted by the completely mixed model and any system in which the COD removal is initially inhibited will be inadequately described by this model. Thus, care must be taken when applying this model to any effluent that is potentially inhibitory. In these studies, severe inhibition was only observed in run 4 (10°C , $1,489 \text{ mg/l S}^{2-}$). Minor inhibition was observed in run 2 (25°C , $1,536 \text{ mg/l S}^{2-}$) (Figure 5.4).

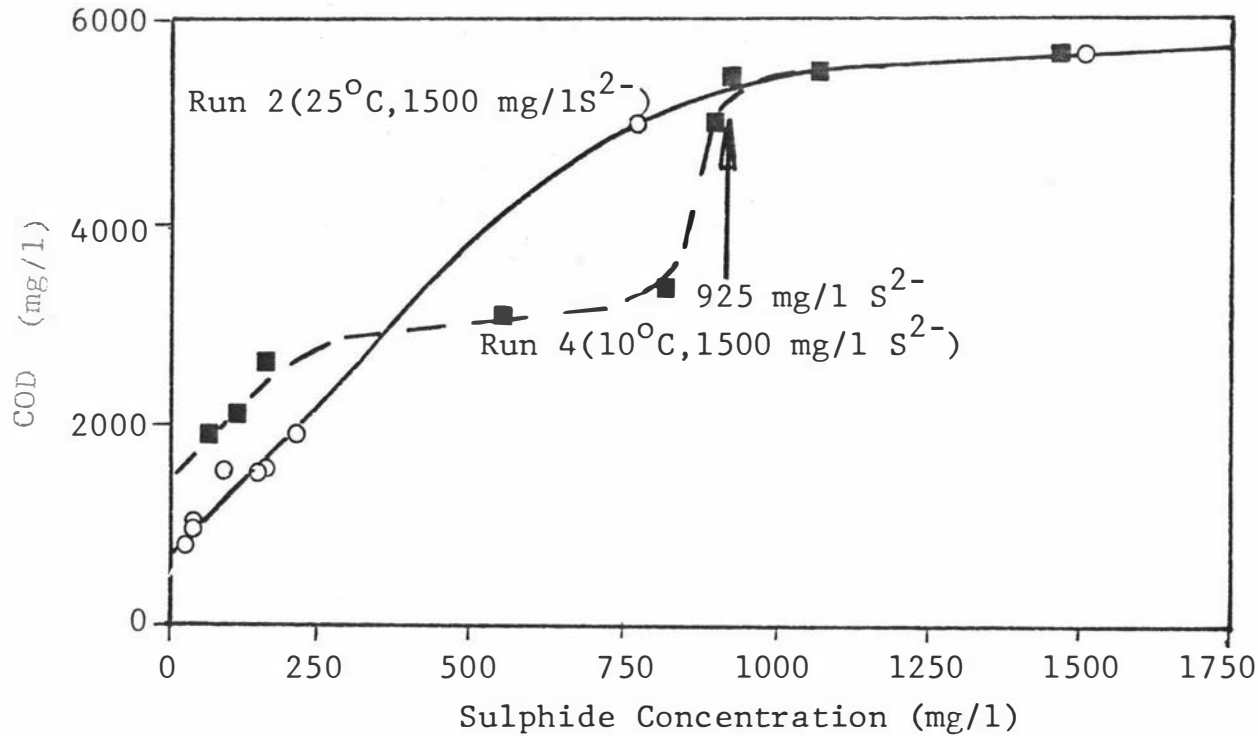
Thus in both instances where a high sulphide influent was applied to the lagoons some inhibition was observed. As there was only a minor variation in pH from one run to another, this implies that the high sulphide concentration caused the inhibition. To assess this posulate, Figure 5.15 was plotted. In both cases, a negligible COD removal occurs above 925 mg/l sulphide (Figure 5.15 (a)). Below this concentration substantial COD removals occur, although the pattern of removal differs in each experiment. High sulphide concentrations also proved inhibitory to Thiocapsa roseopersicina with negligible populations being observed above $1,000 \text{ mg/l}$ sulphide (Figure 5.15(b)). Again, temperature affects the manner in which sulphide inhibition occurs. At 10°C (run 4), no decrease in the population of T. roseopersicina was observed up to approximately 400 mg/l sulphide. At 25°C (run 2), the maximum population occurs at 40 mg/l sulphide.

These data compare favourably with the results obtained for Chromatium minutissimum in Chapter 3.

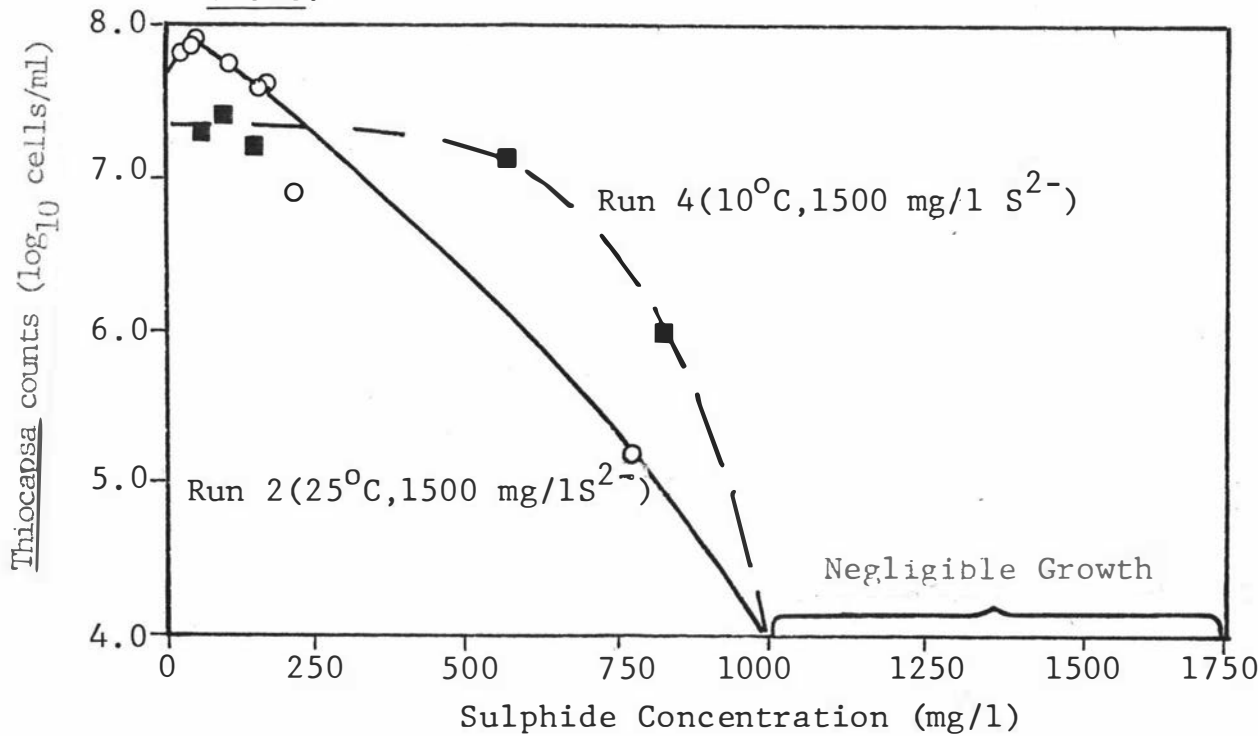
One may therefore conclude that lagoons containing Thiocapsa roseopersicina can adequately treat fellmongery effluent containing up to 900 mg/l sulphide.

Figure 5.15: The Effect of Sulphide on Runs 2 and 4

(a) The Effect of Sulphide Concentration on COD



(b) The Effect of Sulphide Concentration on the Thiocapsa Count.



5.8 EFFECT OF TEMPERATURE ON REMOVAL RATES AND RESIDUAL CONCENTRATIONS.

As expected, the removal rate coefficients are stimulated by temperature (Figure 5.16). This effect may be described by equation 5.3. A study of Figure 5.16 indicates that the removal rate coefficient data may be split into two groups:

- (i) those runs at an influent sulphide concentration below 900 mg/l
- (ii) those runs at an influent sulphide concentration above 900 mg/l.

It is presumed that inhibition occurs at 900 mg/l S^{2-} as at some point between 850 mg/l S^{2-} and 1,500 mg/l S^{2-} a reduction in the effect of temperature on k occurs. As previously discussed, other evidence indicates that such inhibition is likely to occur in the vicinity of 900 mg/l S^{2-} .

This grouping of data can be made as the k values at 850 mg/l S^{2-} fall close to, or above, the line described by the 200 mg/l S^{2-} temperature coefficient. The 200 mg/l S^{2-} data therefore either:

- (a) adequately describes the 850 mg/l S^{2-} k value
- (b) slightly underestimates the k value at 850 mg/l S^{2-} resulting in a conservative prediction of the effluent quality under these conditions.

Therefore, the 200 mg/l S^{2-} data in Table 5.6 may be used to predict the removal rate coefficients at sulphide concentrations up to 900 mg/l.

At sulphide concentrations in excess of this value the 1,500 mg/l S^{2-} data presented in Table 5.6 must be used.

The 200 mg/l S^{2-} removal rate coefficients exhibit temperature coefficients in the range 1.10-1.14 (Table 5.6) which is slightly

Figure 5.16: Plots Demonstrating the Effect of Temperature
on the Removal Rate Coefficients

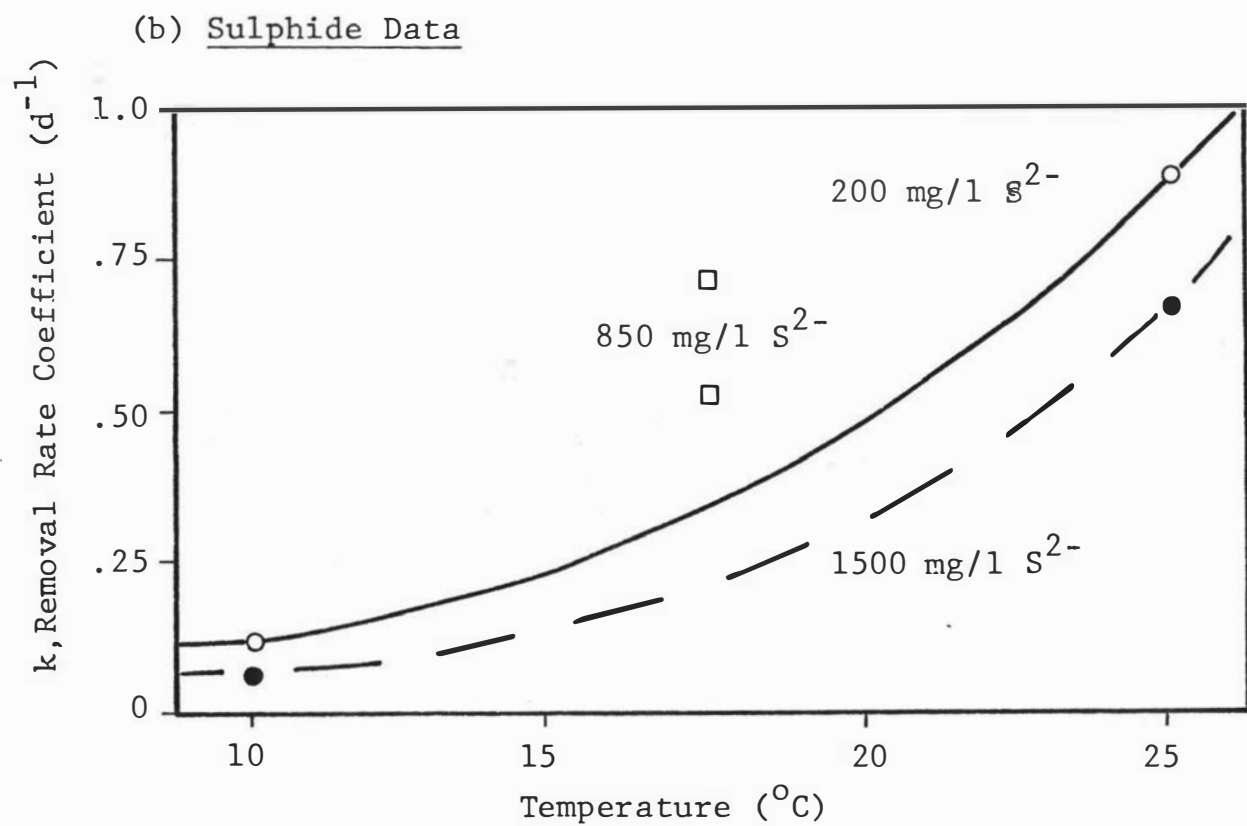
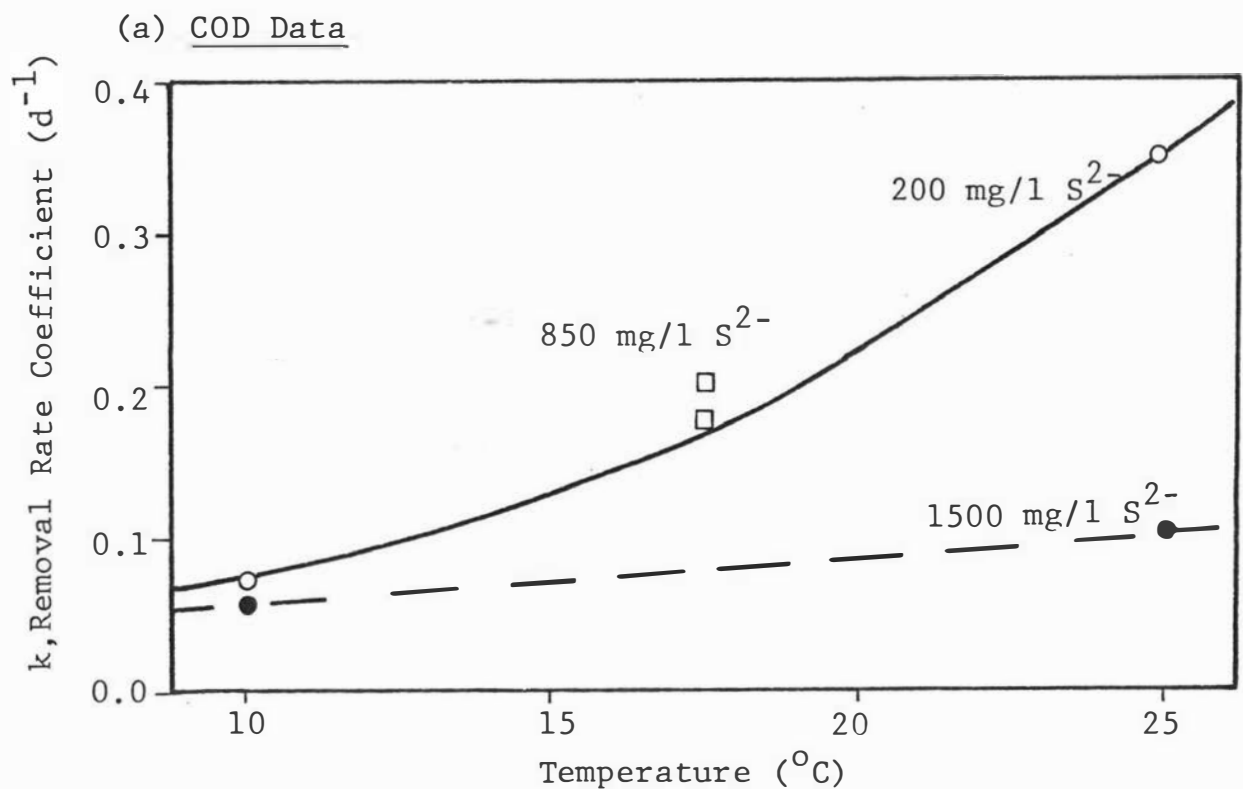


Figure 5.16(continued): Plots Demonstrating the Effect of Temperature on the Removal Rate Coefficients

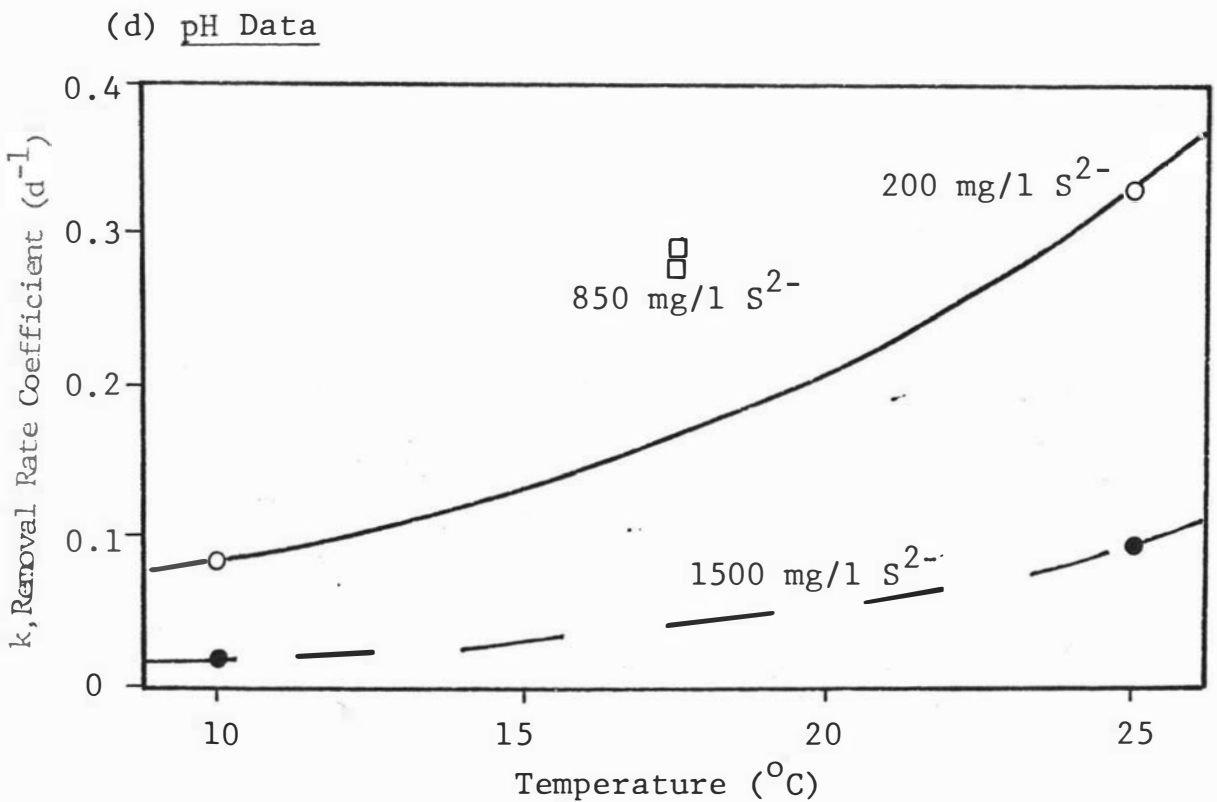
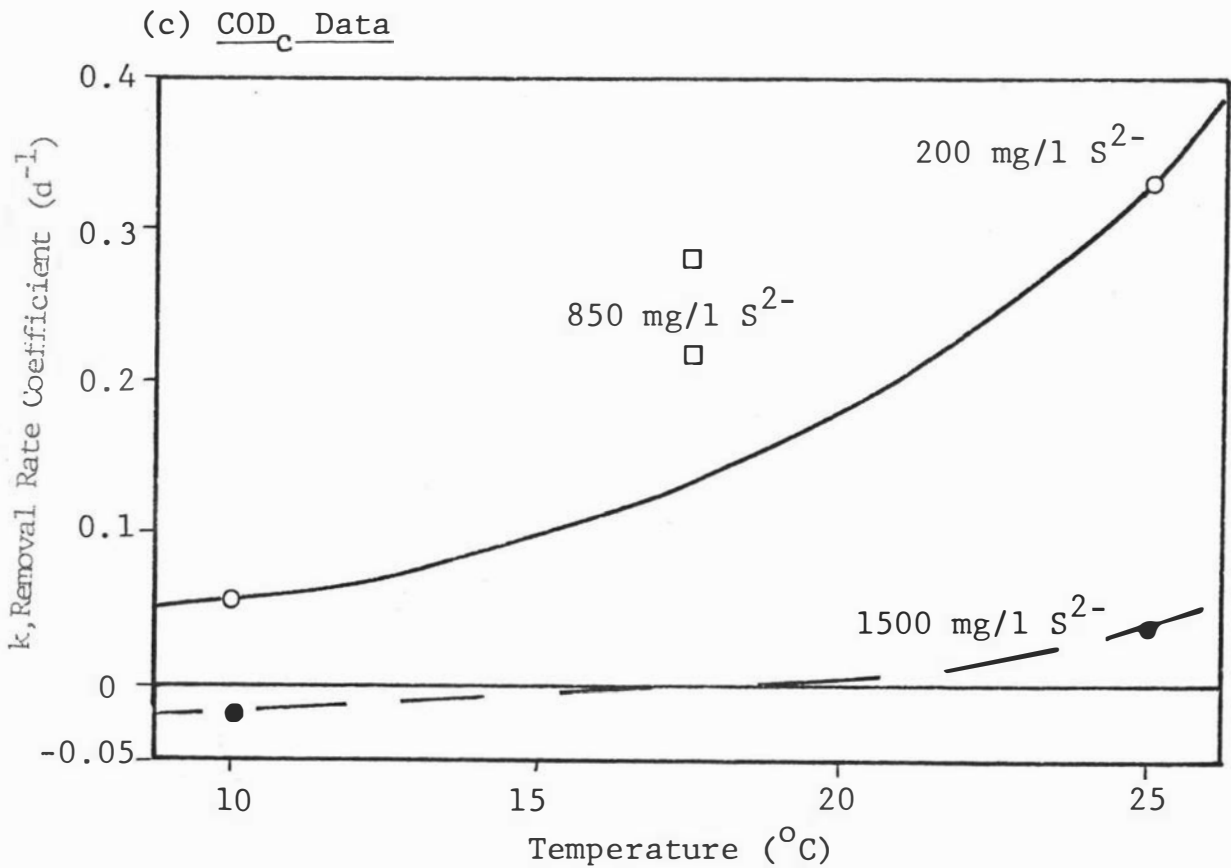


TABLE 5.6

Temperature Coefficients for the Removal Rate Coefficients and k_{10} Values.

parameter	sulphide concentration			
	200 mg/l		1,500 mg/l	
	k_{10} (d^{-1})	θ	k_{10} (d^{-1})	θ
COD	.0751	1.11	.0591	1.04
COD _c	.0526	1.13	-.0216	1.09
Sulphide	.122	1.14	.0698	1.16
pH	.0820	1.10	.0190	1.12

k_{10} = removal rate coefficient at 10°C

θ = temperature coefficient

Comment: If influent sulphide concentration is less than 900 mg/l use 200 mg/l sulphide data.

If influent sulphide concentration is above 900 mg/l use 1,500 mg/l data.

TABLE 5.7

Temperature Coefficients for the Residuals and S_{r10} Values.

Parameter	sulphide concentration			
	200 mg/l		1,500 mg/l	
	S_{r10} (mg/l)	θ	S_{r10} (mg/l)	θ
COD	642	.956	1425	935
COD _c	578	.961	2428	903
Sulphide	1.67	1.15	0.50	1.16
pH	7.39	.999	7.00	1.02

S_{r10} = residual at 10°C

θ = temperature coefficient

higher than those observed for other lagoon systems (Aguirre and Gloyna 1970, Eckenfelder and Ford 1970, Gloyna 1971, Thirumurthi 1974). These values indicate that temperature stimulates the rate of removal of all parameters to nearly the same extent.

Increasing the influent sulphide concentration to 1,500 mg/l results in decreased temperature coefficients for COD and COD_c and increases those of sulphide and pH (Table 5.6). This implies that increasing temperatures and sulphide concentrations interact positively to stimulate the rate of removal of sulphide and pH but interact negatively to inhibit the removal of organic compounds.

The data in Table 5.6 therefore allows the prediction of the removal rate coefficient if the influent sulphide concentration and the lagoon temperature are known.

As no consistent temperature trends are apparent in the residual concentration data, it is more difficult to categorise than the removal rate coefficient data. For both the COD and COD_c , increasing the temperature decreases the residual. However, the 850 mg/l S^{2-} residuals fall between the 200 mg/l S^{2-} and 1,500 mg/l S^{2-} lines (Figure 5.17(a) and (c)). Thus, neither line adequately describes the 850 mg/l S^{2-} data and interpolation of the results is required.

Similarly, neither the 200 mg/l or 1,500 mg/l S^{2-} data can be used to model the 850 mg/l S^{2-} data on the sulphide and pH graphs (Figure 5.17 (b) and (d)).

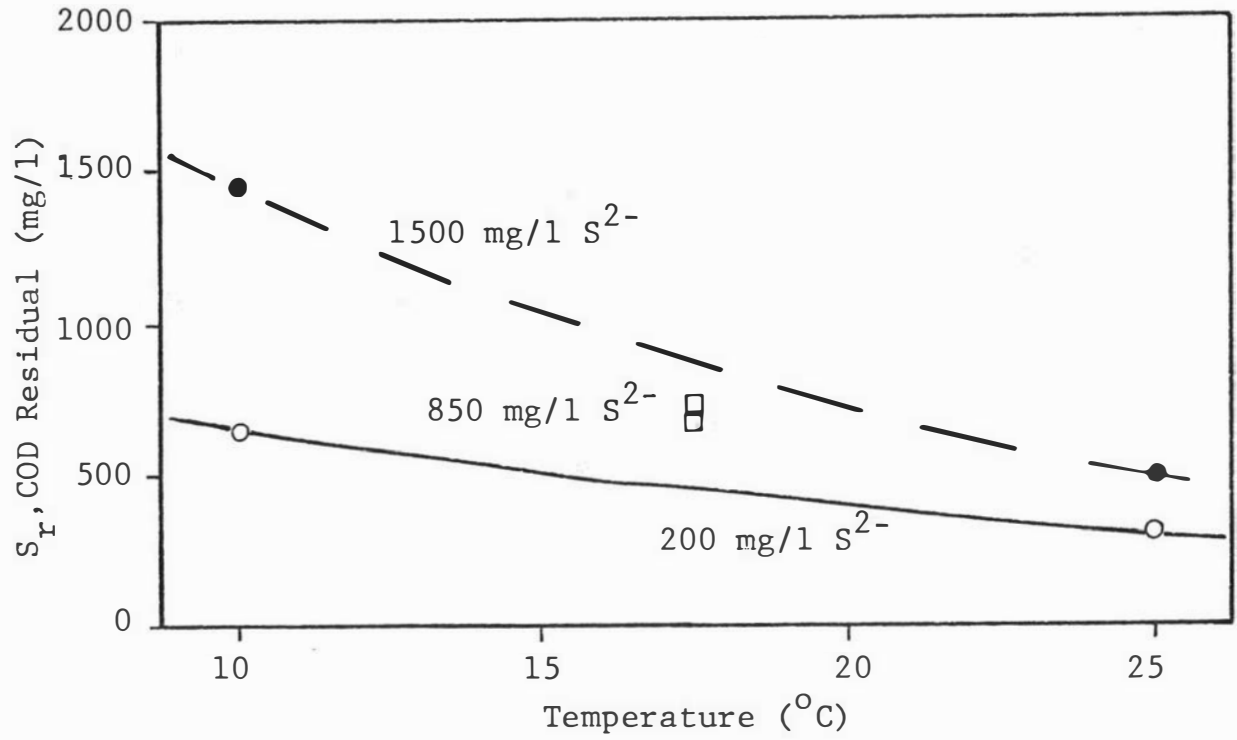
It is therefore difficult to accurately predict the effect of temperature on S_r and the value to be used in the design equation (equation 5.2) must be either estimated from the data presented in this study or experimentally determined in each particular instance.

5.9 APPLICATION OF THE DEVELOPED MODELS TO FELLMONGERY LAGOON DESIGN

The information presented in this chapter allows the design of anaerobic lagoons using the Chromatiaceae to treat fellmongery effluent. Rearranging equation 5.2 allows the retention time required

Figure 5.17: Plots Demonstrating the Effect of Temperature on the Residual Concentration

(a) COD Data



(b) Sulphide Data

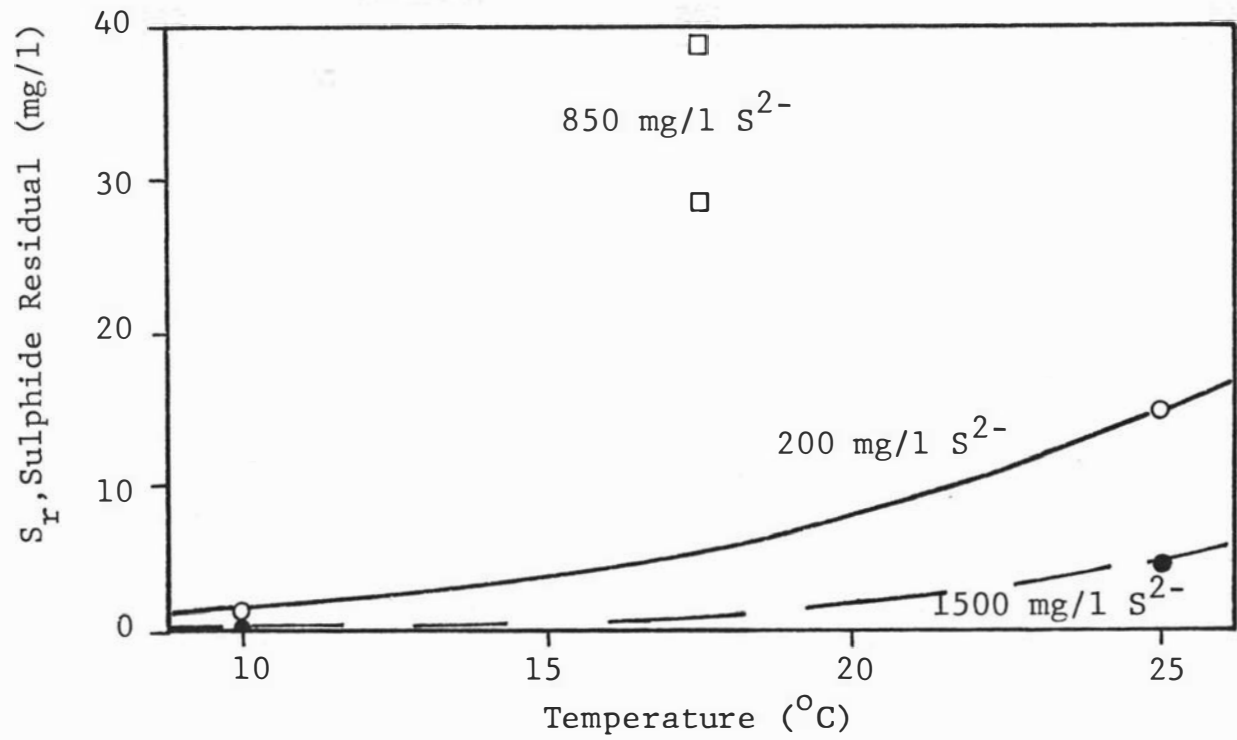
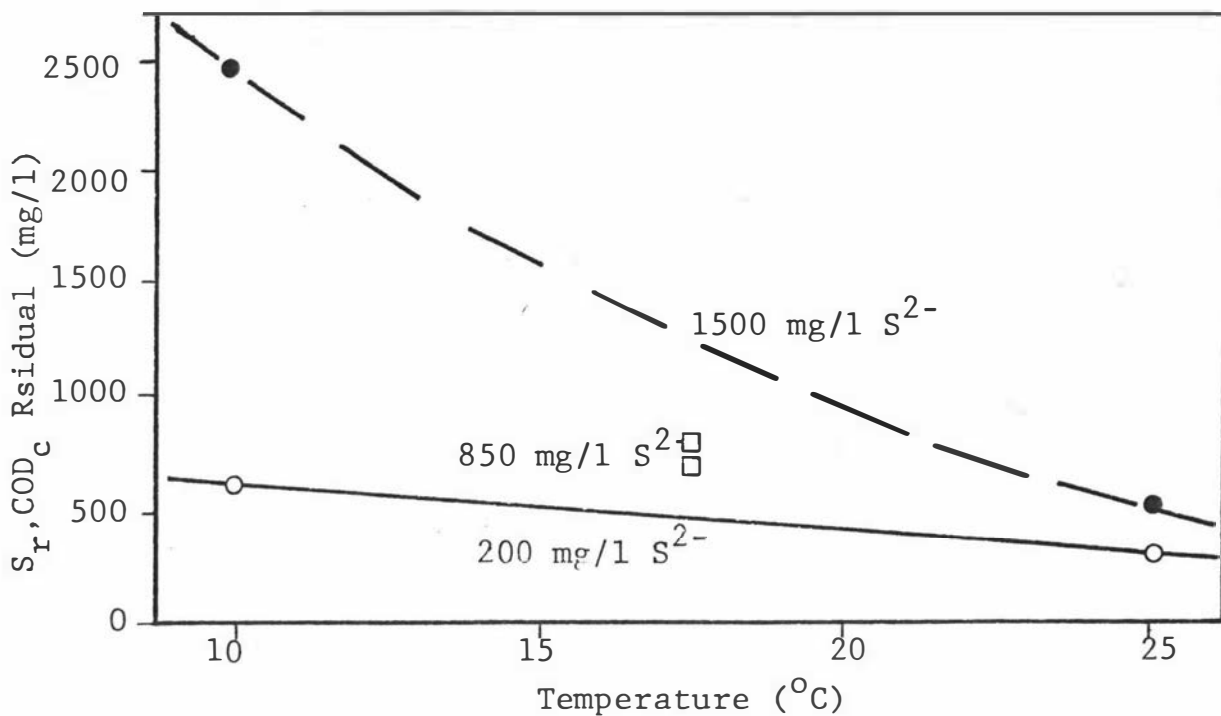
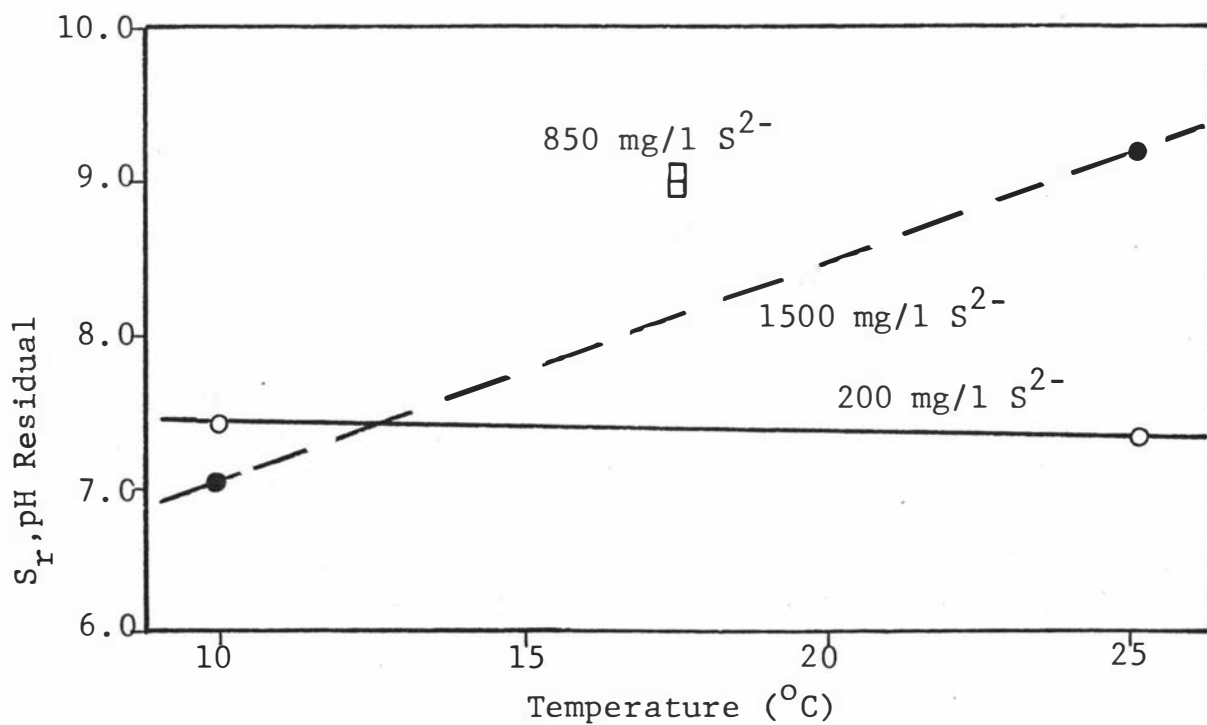


Figure 5.17(continued): Plots Demonstrating the Effect of Temperature on the Residual Concentration.

(c) COD_c Data



(d) pH Data



to achieve the desired effluent quality to be calculated:

$$t = \frac{S_o - S_e}{k(S_e - S_r)} \quad (5.5)$$

k may be calculated from the data presented in Table 5.12 using equation 5.3 (see P.142) provided the influent sulphide concentration and the lagoon temperature are known. The usual design temperature used is the average water temperature of the coldest month in which the lagoons will be operating (Gloyna 1971). This is when the lowest quality effluent will be produced.

S_r may be estimated from the data presented in Table 5.13 using equation 5.4 (see P.142).

Best treatment is achieved if the influent sulphide concentration is below 900 mg/l. Above this figure the growth of Thiocapsa roseo-persicina is inhibited. As good treatment was obtained at sulphide concentrations close to the inhibitory value it would be most economic to treat more concentrated fellmongery effluents (e.g. 850 mg/l S^{2-}) of a smaller volume than is current practice. To achieve this, the concentrated lime liquor must be prevented from being contaminated by the more dilute wash waters.

If discharge requirements are more stringent than the effluent quality observed in this study, the anaerobic photosynthetic lagoon may be regarded as the first stage of a series of two or three lagoons. Their detention times may be designed to utilise the rapid reduction of pH, sulphide and COD in a primary lagoon, allow a microbial transformation to an algal species in a secondary lagoon (Chapter 4) and permit further aerobic treatment in a second algal lagoon if required.

In New Zealand, many fellmongeries are attached to export meat works and the maximum production of fellmongery effluent occurs during the summer months when ambient temperatures are relatively high. Advantage may be taken of this situation by carefully analysing the lagoon loading as a function of temperature. This will enable the minimum lagoon volume to be computed, thereby reducing the area of land required.

Climatic conditions and land availability are the important variables restricting the applicability of fellmongery lagoons. While recognising these limitations, the data generated in this study have indicated the potential of these lagoons under a wide range of conditions and provide a scientific basis for the design of such lagoons.

5.10 CONCLUSIONS

1. Laboratory anaerobic lagoons utilising the Chromatiaceae effectively treat fellmongery effluent. Sulphide removals of up to 98% and COD removals of up to 87% have been observed.
2. Such lagoons will reduce the pH from 11.8 - 12.3 to 7.4 - 9.2 depending upon influent conditions.
3. Removal of organic compounds was inhibited at sulphide concentrations greater than 900 mg/l.
4. The dominant organism in the lagoon under a wide range of conditions was Thiocapsa roseopersicina whose growth was inhibited at sulphide concentrations greater than 900 mg/l.
5. A model for the design of these lagoons was developed using Tables 5.6 and 5.7 and equations 5.3 and 5.4.

CHAPTER 6

PILOT-SCALE EVALUATION OF DESIGN PARAMETERS FOR
ANAEROBIC LAGOONS USING THE CHROMATIACEAE TO TREAT
FELLMONGERY EFFLUENT.

6. PILOT-SCALE EVALUATION OF DESIGN PARAMETERS FOR ANAEROBIC LAGOONS USING THE CHROMATIACEAE TO TREAT FELLMONGERY EFFLUENT.

6.1 INTRODUCTION:

Design criteria necessary for the construction of anaerobic photosynthetic lagoons to treat fellmongery effluent were developed in Chapter 5. Due to the small size of the lagoons studied and the use of synthetic effluent, an investigation of a larger lagoon treating actual fellmongery effluent was necessary to verify the design data obtained.

With the agreement of a local meatworks, a 4.50 m³ pilot plant was installed adjacent to their fellmongery and the fellmongery effluent was treated at ambient temperature under synthetic lighting conditions.

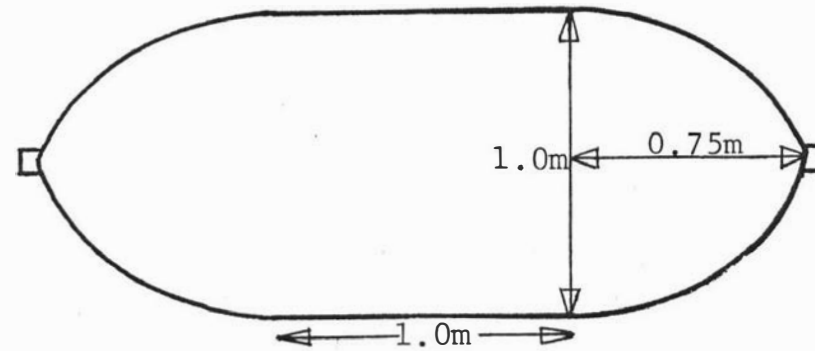
6.2 DESIGN OF THE LAGOON SYSTEM

Two concrete septic tanks each with a nominal capacity of 2.25 m³ (Cement Products, Palmerston North, N.Z.) were coupled in series and installed adjacent to the fellmongery and pelt processing area. Measurement indicated that the actual volume of each tank was 2.37 m³. The tank dimensions are detailed in Figure 6.1. A schematic diagram of the plant is presented in Figure 6.2.

The effluent from the fellmongery department travelled by gravity to a 1 mm brushed screen and thence into a sump prior to further primary treatment. The effluent from this sump was passed to the first of the pilot lagoons through 19 mm i.d. steam tubing (Skellerup (N.Z.) Ltd.) using an 'M' series Mono pump (Dalhoff and King N.Z. Ltd.). This pump was adapted to fit a D.C. motor and was fitted with speed control (Barlow Electronics, Palmerston North, N.Z.). This provided control of the influent volume and allowed a range of retention times to be studied. The pump was controlled by a Venner time switch to operate for ten hours daily.

Figure 6.1: Dimensions of the Pilot-scale Lagoon

(a) Plan:



(b) Elevation:

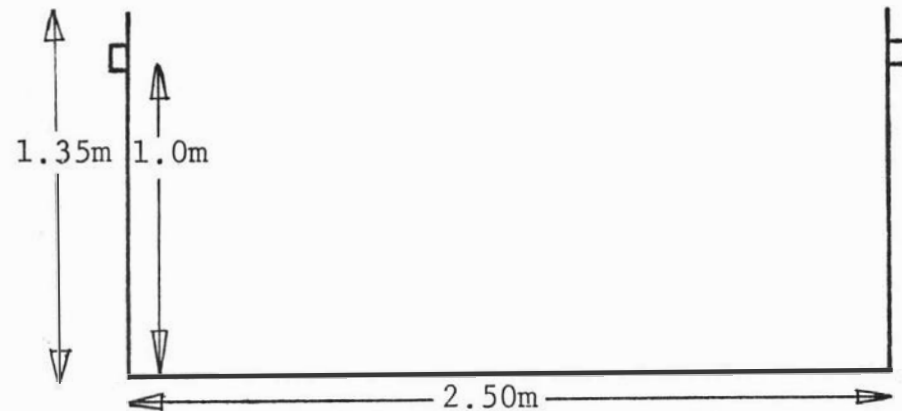
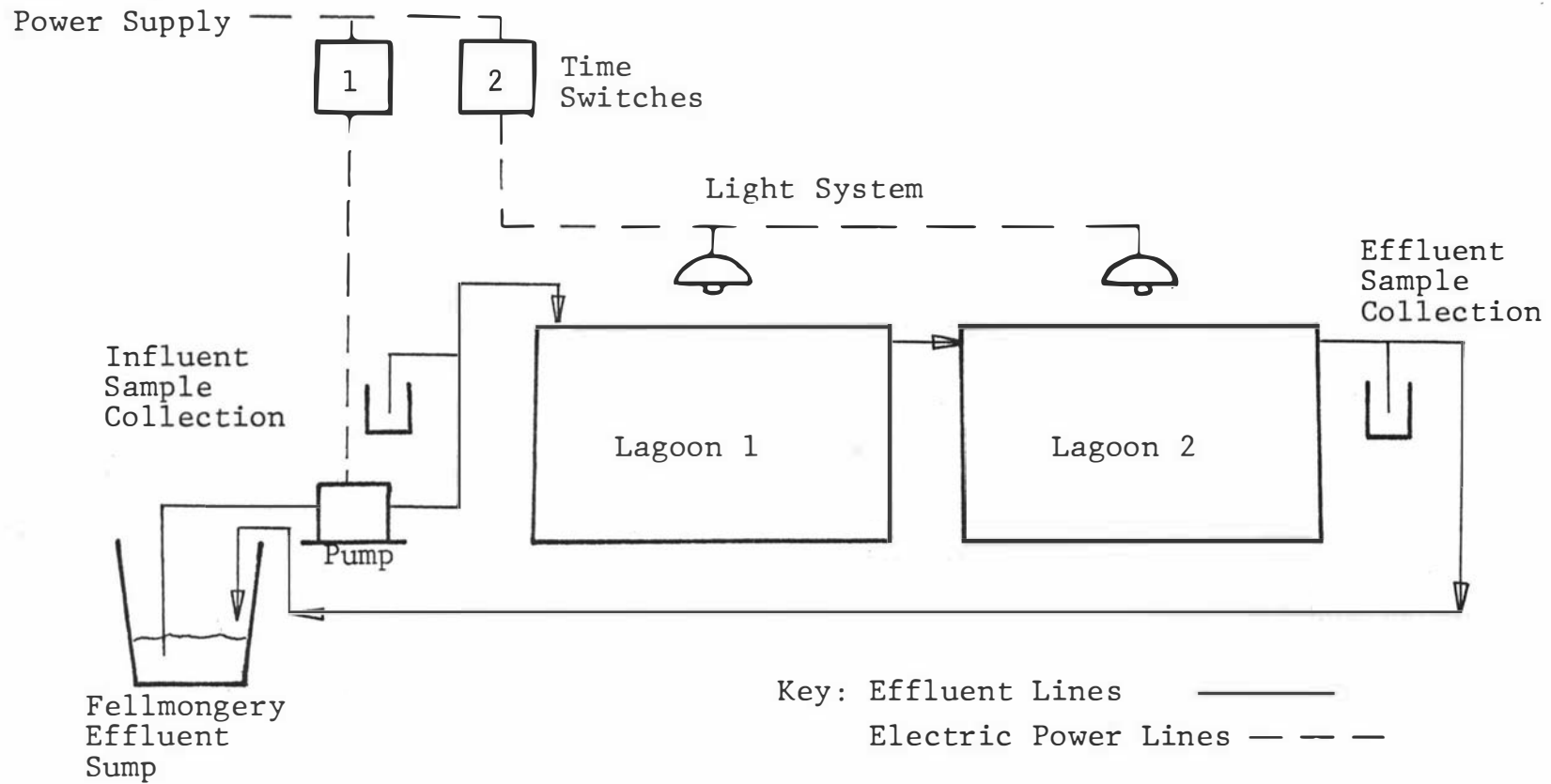


Figure 6.2: Schematic Diagram of the Pilot-scale Lagoons.



The first lagoon was joined to the second using 150 mm i.d. spiral reinforced PVC ducting (Skellerup N.Z. Ltd.). The effluent from the second lagoon was returned to the effluent drain below the point of intake using 19 mm i.d. steam hosing.

A 1 kW and six 200 W incandescent bulbs were suspended above each lagoon to provide a surface illumination of 5,000 lux as measured by a Sangamo Weston Model S511 Photometer. The lights were operated on a 12 hour light/dark cycle controlled by a Venner time switch.

6.3 OPERATION OF THE LAGOON SYSTEM

Each lagoon was inoculated with 225 l of effluent from a meatworks anaerobic lagoon. Microscopic examination of this effluent yielded a Chromatiaceae count of 7×10^5 cells/ml. Ten litres of laboratory lagoon effluent containing Thiocapsa roseopersicina was also added to each lagoon. The lagoons were then gradually filled with fellmongery effluent. For the first week 70 l were added daily. For the second week 100 l/day were added and then 130 l/day were added until the system was full.

Samples were continuously withdrawn from the influent and effluent lines via T-junctions inserted into the tubing (Figure 6.2). Samples were collected in 10 l containers. Analyses were performed within five hours of sample collection and the analytical techniques used were as described in Section 5.3.3.

The lagoons were operated at a specified retention time until steady state, as defined by a 10% variation in the COD, was achieved. This retention time was then maintained for a further week and sample analysis was performed daily. The values reported are therefore the average of five steady state analyses.

Thiocapsa roseopersicina was the dominant phototrophic bacterium observed in the lagoons under all the conditions investigated. Fellmongery practice led to the production of an effluent containing only 90-100 mg/l sulphide.

6.4 DEGREE OF TREATMENT ACHIEVED AT VARIOUS RETENTION TIMES

Five different retention times were evaluated using the pilot scale lagoons. The results obtained are presented in Table 6.1

At a retention time of 90 days, an 85.9% COD removal and an 84.9% removal of sulphide was observed. These results compare favourably with other studies. Ryder (1977) obtained an 80.1% COD removal and an 83.9% sulphide removal at a retention time of 68 days. The values reported were 853 mg/l COD and 13 mg/l sulphide. Cooper et al (1975) observed an 83.3% COD removal and a 98.7% sulphide removal with figures of 572 mg/l and 1.2 mg/l being reported at a retention time of 100 days.

6.5 DETERMINATION OF REMOVAL RATE COEFFICIENTS

Plots of the regression analyses performed to determine the removal rate coefficients and residual concentrations are presented in Figure 6.3 and the results obtained from these analyses are summarised in Table 6.2.

The regression analyses of the pilot scale data yielded better fit than did the laboratory data (Figure 6.3). Correlation coefficients of .892 to .989 were obtained (Table 6.2). This may be due to the flow regime in the larger lagoons being closer to completely mixed rather than plug flow.

6.6 COMPARISON OF LABORATORY AND PILOT PLANT RESULTS

The removal rate coefficients and residual concentrations calculated using the data obtained from the laboratory scale lagoons (Tables 5.6 and 5.7) are also presented in Table 6.2 and an analysis of the difference between the laboratory and pilot scale k and S_r values is presented in Table 6.3.

TABLE 6.1

Results of the Pilot Scale Lagoon Treatment of Actual Fellmongery Effluent.

Retention Time (Days)	Temperature (°C)	Influent Variables				Effluent Variables					
		COD (mg/l)	COD _c (mg/l)	Sulphide (mg/l)	pH	COD (mg/l)	COD _c (mg/l)	Sulphide (mg/l)	pH	BOD (mg/l)	<u>Thiocapsa</u> counts (cells/ml x 10 ⁷)
15	16.8	4,462	4,256	103	12.2	1,503	1,454	24.6	8.6	284	4.13
30	16.0	4,345	4,161	92	12.0	1,065	1,024	20.7	8.2	219	7.26
50	15.6	4,578	4,376	101	12.3	762	731	15.6	8.0	177	8.54
70	17.6	4,867	4,673	97	11.9	697	669	14.2	8.0	179	9.10
90	17.0	4,269	4,097	86	12.2	601	575	13.0	7.9	167	8.79

TABLE 6.2

Comparison of the Results of the Pilot Scale Regression Analyses with the Predictions from the Laboratory Scale Model.

Parameter	Results of Pilot Scale Regression Analyses			Predictions from Laboratory Scale Model	
	k (day ⁻¹)	S _r (mg/l)	Correlation Coefficient (r ²)	k (day ⁻¹)	S _r (mg/l)
COD	.167	347	.988	.150	477
COD _c	.162	323	.989	.118	444
Sulphide	.341	11.0	.892	.290	4.20
pH	.278	7.74	.981	.154	7.34

TABLE 6.3

Analysis of the Difference Between the Observed and Predicted Removal Rate Coefficients and Residual Concentrations.

Parameter	% difference in k	% difference in S _r
COD	10.2	22.4
COD _c	27.2	27.2
Sulphide	15.0	61.2
pH	44.6	5.17

Figure 6.3: Regression Plots to Determine the Removal Rate Coefficients and Residual Concentrations for Pilot-scale Lagoons.

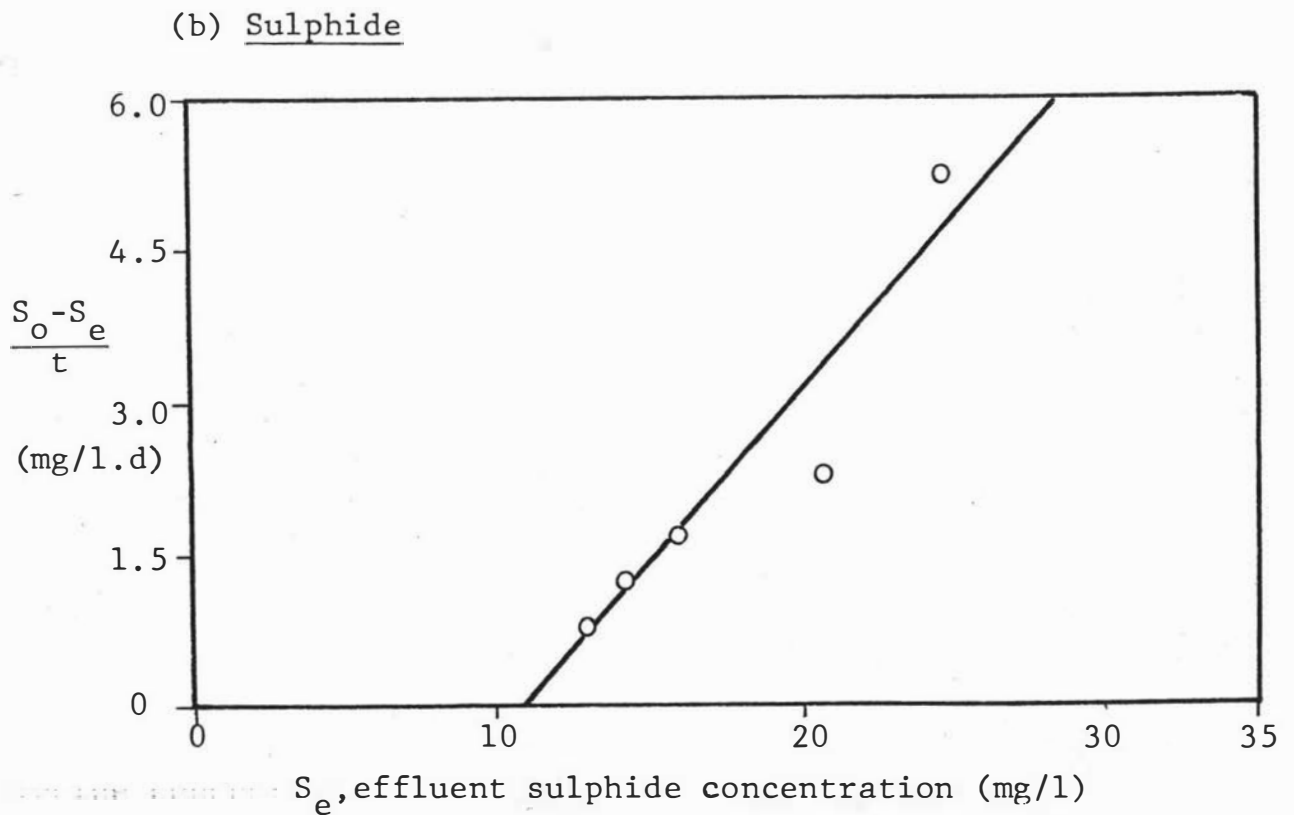
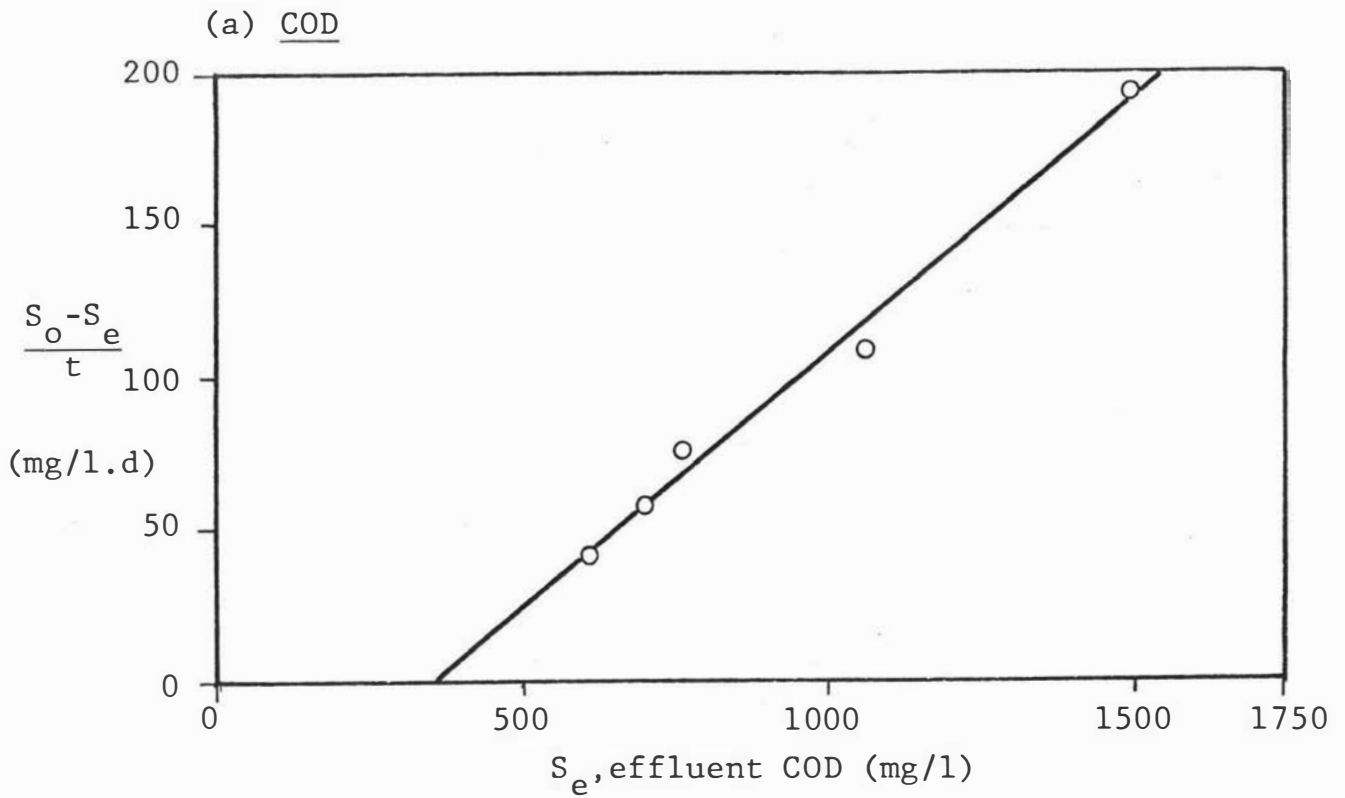
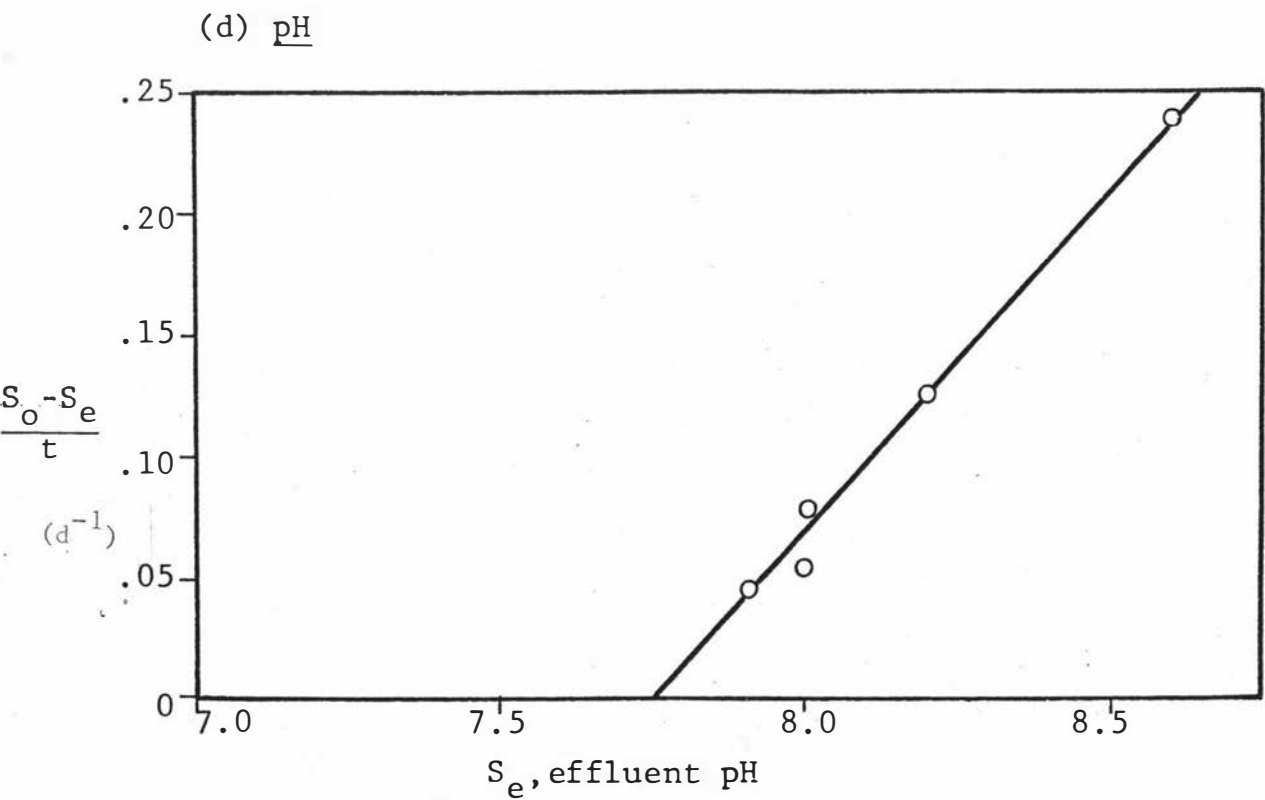
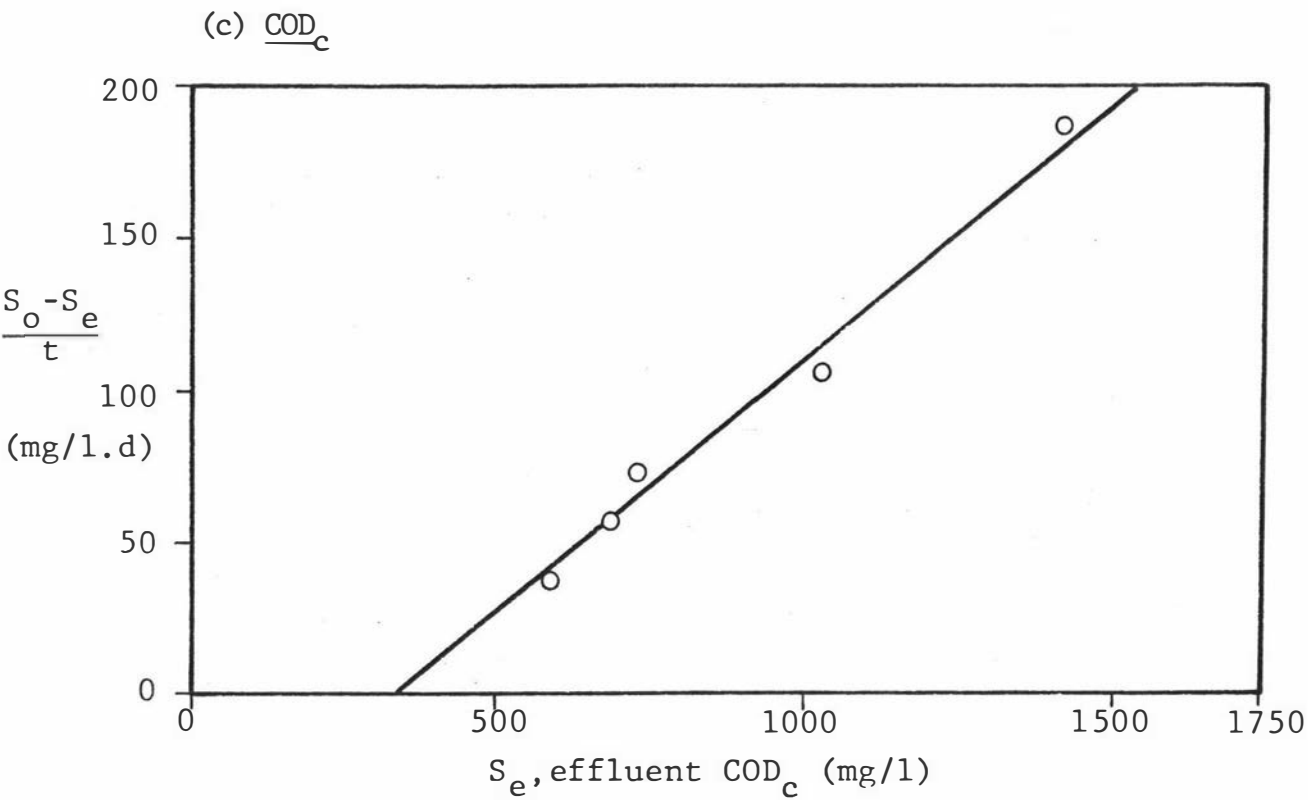


Figure 6.3(cont.):Regression Plots to Determine the Removal Rate Coefficients and Residual Concentrations for Pilot-scale Lagoons.



The k and S_r values predicted from the laboratory scale data show a reasonable agreement with the observed removal rate coefficients and residuals (Table 6.2). For the COD and COD_C, both k and S_r are adequately predicted with similar accuracy in the estimation of each variable being observed. For the sulphide concentration, k is estimated much more accurately than S_r . This is due to the poor estimation of the sulphide residual concentration by the model, with slightly low S_r values being predicted. However, for the pH, the residual concentration is more accurately estimated than the removal rate. This may be due to an excessive concentration of lime in the synthetic fellmongery effluent used in the laboratory studies. Subsequently, the higher pH's observed in the laboratory influent may have inhibited microbial CO₂ production thereby reducing the rate at which the pH fell.

An analysis of the difference between the observed and predicted values indicates a 10.2% - 44.6% difference in k and a 5.17% - 61.2% variation in S_r . Although these variations appear large, a number of factors contribute to these differences.

Firstly, the pilot lagoons treated an actual fellmongery effluent as compared to a synthetic effluent for the laboratory-scale lagoons. The organic compounds present in each effluent could be expected to be different and therefore different removal rate coefficients might be observed. Also, the different substrates may have resulted in a change in the microbial population present in the lagoons. This could also be expected to alter the removal rate coefficients. However, as only the Chromatiaceae were enumerated the extent of such a change cannot be ascertained. No substantial difference between the populations of T. roseopersicina in the laboratory and pilot-scale lagoons was observed.

Secondly, the influent fed to the pilot lagoons was of a fluctuating quality whereas the laboratory lagoons were loaded with a uniform influent.

In addition, the pilot lagoons were exposed to diurnal and seasonal fluctuations in temperature while the laboratory lagoons were studied in a constant temperature environment.

Both the pilot and laboratory lagoons were studied under incandescent lighting. This was due to the pilot lagoons being located under a large overhang. The effect of diurnal and seasonal fluctuations in the light intensity on the performance of these lagoons could therefore not be assessed.

Further, the average influent sulphide concentration of 96 mg/l was substantially less than the lowest concentration studied in the laboratory lagoons.

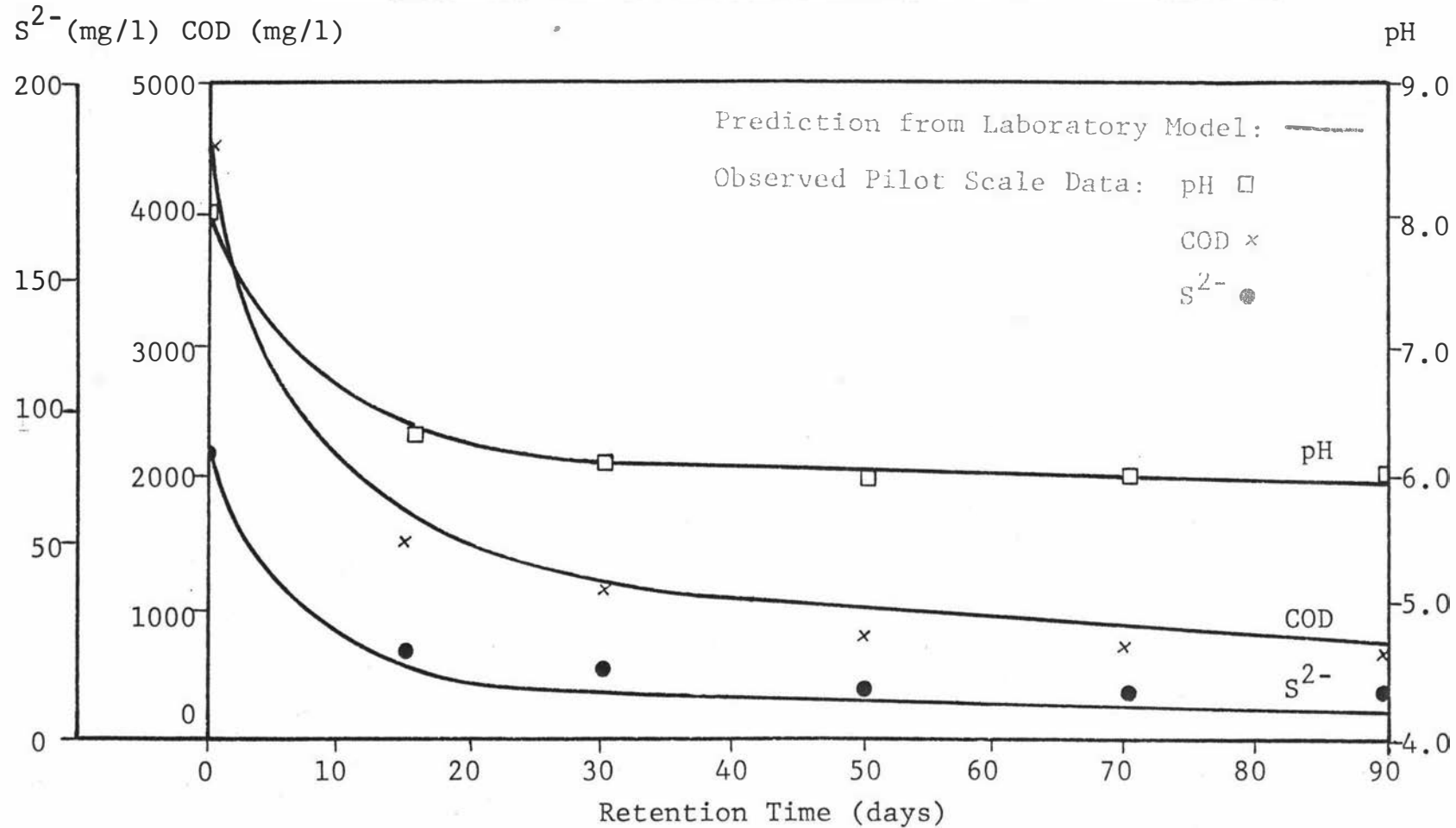
The above factors will have contributed to the differences observed between the laboratory and pilot-scale results. However, most of these variations will be encountered in full scale lagoons and therefore the models developed from the laboratory data must still be capable of providing adequate predictions of the effluent quality under such conditions.

Figure 6.4 indicates the adequacy of the laboratory models in predicting the quality of the effluent from the pilot lagoons. The predicted COD line is located just above the observed COD data due to the predicted k and S_r values being less than the observed values (Tables 6.2 and 6.3). A conservative prediction of the effluent COD results.

The sulphide and pH lines fall slightly below the observed pilot-scale results. As all the predicted k values are less than those observed in the pilot lagoon (Tables 6.2 and 6.3), these results are due to the predicted S_r values for sulphide and pH being less than the observed values. This results in a slightly optimistic prediction of these parameters.

The models developed from the laboratory study may therefore be used to predict the effluent quality from lagoons using the Chromatiaceae to treat fellmongery effluent.

Figure 6.4: Plot to Compare the Effluent Quality Predicted from the Laboratory Scale Lagoons with that Observed in the Pilot Scale Lagoons.



6.7 CONCLUSIONS ON THE DESIGN OF FELLMONGERY EFFLUENT LAGOONS.

1. These pilot-scale studies indicate that lagoons in which the Chromatiaceae are the dominant population can provide a high degree of treatment for fellmongery effluent.

2. The models developed from laboratory scale studies provide an adequate prediction of the effluent quality from such lagoons.

CHAPTER 7

GENERAL CONCLUSIONS

7 GENERAL CONCLUSIONS

The important environmental parameters causing the dominance of the Chromatiaceae in lagoon systems were investigated by studying the batch growth of Chromatium minutissimum isolated from an anaerobic lagoon. Empirical models describing the growth of this bacterium were developed and it is believed that the trends observed in the models provide useful information on the growth of other Chromatiaceae likely to occur in lagoon systems. However, such conjecture is dangerous and confirmation should be sought by performing experiments of the type described in this thesis on other important Chromatiaceae such as Thiocapsa roseopersicina.

Although all the environmental factors studied affected growth in axenic batch cultures, comparison with lagoon data indicated that none of the environmental parameters investigated would prevent the dominance of the Chromatiaceae in New Zealand lagoons. The hydraulic retention time is the major factor limiting the growth of these bacteria in lagoons. At dilution rates above the maximum growth rate, wash-out of cells will occur and the bacteria will be unable to maintain their presence in the lagoon liquor. However, the longer solids retention time may facilitate the growth of some bacteria in the sludge zone.

The batch incubation of lagoon samples indicated that at long retention times, the Chromatiaceae reduced the concentrations of organic and sulphur compounds to levels which allowed competition from, and eventual dominance of, algae.

A threestage succession theory from non-photosynthetic anaerobes to Chromatiaceae to algae was formulated and a study of seven lagoon systems demonstrated its applicability. The Chromatiaceae were observed to predominate at retention times intermediate to those of typical anaerobic and facultative lagoon flora. However, the breadth of retention times at which they were dominant was highly variable depending upon the effluent treated and the climatic conditions. This theory may be used to design lagoons favouring or preventing the dominance of the Chromatiaceae.

The study of the full scale lagoon systems also demonstrated that effective effluent treatment was achieved while the Chromatiaceae were the dominant population. No substantial increases in effluent BOD were observed. Whether this indicates a minimal amount of photosynthetic CO_2 fixation or a balance between CO_2 fixation and evolution cannot be deduced from these studies. A literature survey of the carbon metabolism of the Chromatiaceae demonstrated some mechanisms for CO_2 evolution and indicated that low molecular weight organic compounds inhibited RuDP carboxylase. However, in the mixed cultures studied, the contributions of the non-photosynthetic bacteria in stabilizing organic material as CO_2 and CH_4 must be taken into account. Experiments using labelled carbon compounds and carbon balances in pure and mixed culture systems would assist in indicating the mechanisms by which organic matter is stabilized in such lagoons.

The major problem observed in lagoons in which the Chromatiaceae were dominant was the colour of the effluent. While green, algae-laden effluents are currently acceptable to N.Z. water pollution control authorities, red effluents in which the Chromatiaceae predominate are not. This largely due to the aesthetic problems associated with red effluents. Research on the effects of such Chromatiaceae lagoon effluents on receiving waters is required to indicate whether the discrimination against these effluents is justified.

If such research indicates the Chromatiaceae have a severely detrimental effect on receiving waters, the technology is available to facilitate their conversion to algal cells.

The full scale lagoons also demonstrated the usefulness of these bacteria in treating effluents containing reduced sulphur compounds. A survey of the relevant literature indicated that such effluents are difficult to treat using conventional technology. Fellmongery effluent was identified as the most important sulphide-bearing effluent in N.Z. Laboratory scale lagoons were therefore studied to develop criteria for the design of lagoons to treat this effluent. A good degree of treatment was obtained under a wide range of operating conditions.

Effluents containing up to 900 mg/l sulphide were treated without inhibition. Concentrated fellmongery effluents may therefore be treated. Current fellmongery practice is to discharge wash water with the lime and other liquors produced. An effluent containing from 50-200 mg/l sulphide results. Segregation of the wash waters and the stronger liquors to produce a stream containing approximately 800 mg/l sulphide would greatly reduce the size of the lagoon needed while affording an 11% safety factor against inhibition.

Design equations were therefore developed for two conditions. One for effluents containing less than 900 mg/l sulphide and the other for inhibitory effluents containing more than 900 mg/l sulphide.

To confirm the laboratory design data, pilot-scale studies were performed on an actual fellmongery effluent. The results indicated that the laboratory data adequately described the pilot-scale lagoon effluent and could therefore be used to design such lagoons.

In N.Z., fellmongering is a seasonal industry, closely linked to the killing of lambs, sheep and cattle. The maximum effluent flows therefore occur during the spring, summer and autumn when the environmental conditions favour a high degree of treatment. In N.Z., the climatic conditions are unlikely to limit the applicability of these lagoons. The availability of land will therefore be the major factor influencing the adoption of this waste treatment technique.

ABBREVIATIONS AND NOMENCLATURE

ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
APS	Adenosine-5'-phosphosulphate
ATP	Adenosine triphosphate
ELMRA	British Leather Manufacturers Research Association
BOD	Biochemical Oxygen Demand (mg/l)
CoA	Coenzyme A
DHAP	Dihydroxyacetone phosphate
DO	Dissolved Oxygen (mg/l)
EPA	United States Environmental Protection Agency
FDP	Fructose 1,6 Diphosphate
F-6-P	Fructose-6-phosphate
G-1-P	Glucose-1-phosphate
G-6-P	Glucose-6-phosphate
Ga-3-P	Glyceraldehyde-3-phosphate
IULCS	International Union of Leather Chemists Societies
k	Reaction rate coefficient (days ⁻¹)
MS _{lof}	Mean sum of squares due to lack of fit
MS _{pe}	Mean sum of squares due to pure error
n _a	Number of axial points in experimental design
n _c	Number of cube points in experimental design
n _o	Number of centre points in experimental design
NAD	Nicotinamide adenine nucleotide
NADH ₂	Reduced nicotinamide adenine nucleotide
OA	Oxygen absorbed
OAA	Oxaloacetic acid
P _i	Inorganic phosphate
PAP	Adenosine-3',5'-diphosphate
PAPS	3'-phosphadenosine-5'-phosphosulphate
PEP	Phosphoenol pyruvate
PGA	Phosphoglyceric acid
1,3 PGA	1,3 Diphosphoglyceric acid
PHB	Poly-β-hydroxybutyric acid
P.V.	Permanganate value

ABBREVIATIONS AND NOMENCLATURE (cont.)

R-5-P	Ribose-5-phosphate
Ru-5-P	Ribulose-5-phosphate
RuDP	Ribulose diphosphate
S _e	Effluent parameter (mg/l except pH)
S _o	Influent parameter (mg/l except pH)
S _r	Residual concentration (mg/l except pH)
t	Retention time (days)
TCA	Tricarboxylic acid cycle
TL _m	Median tolerance limit
USDI	United States Department of the Interior
WHO	World Health Organisation
X	Independent variable in experimental design
Xu-5-P	Xylulose-5-phosphate
Y	Dependent variable in experimental design
α	Distance of axial points from origin in experimental design
β	Coefficient in the experimental design model
θ	Temperature coefficient ($^{\circ}\text{C}^{-1}$)
μ_a	Exponential growth rate measured by the absorbance (hr^{-1})
μ_p	Exponential growth rate measured by plate counts (hr^{-1})

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APPENDIX 1 : PARSIMONIOUS MODELS DEVELOPED PRIOR TO THE FINAL
up AND MAXIMUM COUNT MODELS.

Table A.1.1: Parsimonious Model and Regression Statistics for the
growth rate calculated from plate counts (up).

Parsimonious model:

$$Y = .572 + .150X_1X_2 - .134X_3X_4 - .111X_3X_3$$

Correlation Coefficient:

$$r^2 \text{ (adjusted for degrees of freedom) } = 25.8\%$$

Analysis of Variance Summary:

Source of Variation	Degrees of Freedom	Sum of Squares	Mean sum of Squares
regression	3	1.0414	0.3471
residual	32	2.2013	0.0688
total	35	3.2428	

Test for goodness of fit:

$$MS_{pe} = .0416$$

$$\therefore MS_{lof} = .0272$$

$$\frac{MS_{lof}}{MS_{pe}} = .654$$

As $F_{23, 9, 0.05} = 2.91$, there is no significant lack of fit at the 0.05 level.

Table A.1.2: Parsimonious Model and Regression Statistics for the Maximum Count.

Parsimonious model:

$$Y = 7.79 + .471X_2 - .996X_3 - .324X_1X_3 + .420X_2X_3 \\ + .327X_2X_5 - .476X_3X_4 - .476X_2X_2 - .514X_3X_3 \\ - .319X_4X_4$$

Correlation Coefficient:

$$r^2 \text{ (adjusted for degrees of freedom)} = 84.1\%$$

Analysis of Variance Summary:

Source of Variation	Degrees of Freedom	Sum of Squares	Mean sum of Squares
regression	9	57.9	6.44
residual	26	7.78	.299
total	35	65.7	

Test of goodness of fit:

$$MS_{pe} = .261$$

$$\therefore MS_{lof} = .038$$

$$\frac{MS_{lof}}{MS_{pe}} = .146$$

As $F_{17, 9, 0.05} = 2.97$ there is no significant lack of fit at the 0.05 level.

Table A.1.3: Parsimonious Model and Regression Statistics for $1/\mu$.

Parsimonious Model:

$$\begin{aligned} \frac{1}{Y} = & 3.02 - 2.03X_2 - 4.33X_4 - 6.63X_1X_2 \\ & + 6.13X_1X_3 + 5.69X_1X_5 - 6.58X_2X_4 \\ & - 4.77X_2X_5 + 5.74X_3X_5 - 2.46X_4X_5 \\ & + 1.90X_2X_2 + 2.22X_3X_3 \end{aligned}$$

Correlation Coefficient:

$$r^2 \text{ (adjusted for degrees of freedom)} = 85.7\%$$

Analysis of Variance Summary:

Source of Variation	Degress of Freedom	Sum of Squares	Mean sum of Squares
regression	11	4,328	393.5
residual	24	469	19.6
total	35	4,797	

Test for goodness of fit:

$$MS_{pe} = .393$$

$$\therefore MS_{lof} = 19.2$$

$$\frac{MS_{lof}}{MS_{pe}} = 48.8$$

As $F_{15, 9, 0.05} = 3.00$ there is significant lack of fit at the 0.05 level.

Table A.1.4: Parsimonious Model and Regression Statistics for the Natural Logarithm of the Maximum Count.

Parsimonious Model:

$$\begin{aligned} \ln Y = & 2.05 + .0857X_2 - .165X_3 \\ & - .0508X_1X_3 + .0812X_2X_3 + .0564X_2X_5 \\ & - .0693X_3X_5 - .0757X_2X_2 - .0871X_3X_3 \\ & - .0467X_4X_4 \end{aligned}$$

Correlation Coefficient:

$$r^2 \text{ (adjusted for degrees of freedom)} = 88.1\%$$

Analysis of Variance Summary:

Source of Variation	Degrees of Freedom	Sum of Squares	Mean sum of Squares
regression	9	1.604	.178
residual	26	.156	.00599
total	35	1.760	

Test for goodness of fit:

$$MS_{pe} = .00489$$

$$\therefore MS_{lof} = .00110$$

$$\frac{MS_{lof}}{MS_{pe}} = .225$$

As $F_{17, 9, 0.05} = 2.97$ there is no significant lack of fit at the 0.05 level.

APPENDIX 2 : EXAMPLE CALCULATION OF THE DATA USED TO COMPARE THE PREDICTED AND OBSERVED RESPONSES OF μ_{up} TO THE ISOLATED EFFECT OF pH.

1. Predicted Response

From Table 3.4, the parsimonious model that best describes the μ_{up} data is:

$$\begin{aligned} \ln Y = & - .457 + .225X_2 + .360X_4 + .575X_1X_2 \\ & - .370X_1X_3 - .388X_1X_5 + .460X_2X_3 + .263X_2X_4 \\ & - .266X_3X_4 - .443X_3X_5 + .253X_4X_5 \\ & - .257X_2X_2 - .375X_3X_3 - .206X_4X_4 \end{aligned} \quad (A-1)$$

The isolated effect of pH is:

$$\ln Y = - .457 + .225X_2 - .257X_2X_2 \quad (A-2)$$

By substituting coded pH values into equation A-2 $\ln Y$ and thence Y may be calculated as in the following table:

X_2	-2	-1	0	1	2
$\ln Y$	-1.93	-.939	-.457	-.489	-1.03
Y	.144	.391	.633	.613	.355

Y is the predicted μ_{up} ($\text{hr}^{-1} \times 10^{-3}$) and these results are plotted in Figure 3.7(a).

It is also possible to calculate maxima and minima of the predicted models. Differentiation of equation A-2 yields:

$$\frac{d(\ln Y)}{dX_2} = .225 - .514X_2 \quad (A.3)$$

At a minimum or a maximum, $\frac{d(\ln Y)}{dX_2} = 0$

$$\begin{aligned} X_2 &= \frac{.225}{.514} \\ &= .438 \end{aligned}$$

Substitution of this value into equation A-2 yields the growth rate at this pH value:

$$\begin{aligned} \ln Y &= - .457 + (.225 \times .438) - (.257 \times .438^2) \\ &= - .408 \\ Y &= .665 \end{aligned}$$

This assists substantially in obtaining the correct shape of the predicted response curve.

2. Observed Response:

The observed response of μ to pH is obtained by averaging the μ values observed at each pH value, as outlined in the following table. The values in the table are obtained from Table 3.2.

Coded pH	-2		-1		0		1		2	
	Trial No.	μ	Trial No.	μ	Trial No.	μ	Trial No.	μ	Trial No.	μ
Observed μ Values	34	.086	1	.752	4	.691	2	.223	31	.450
			3	.662	5	.494	6	.152		
			11	.091	7	1.05	8	.026		
			16	.395	9	.738	10	.711		
			18	.016	13	.603	12	.440		
			21	.332	17	.752	14	.525		
			22	.766	19	.787	15	.966		
			23	.256	20	.744	24	1.12		
					26	.508				
					28	.299				
Sum of μ		.086		3.289		6.666		4.163		.450
Average μ		.086		.411		.667		.500		.450

Similar calculations for the other variables allowed Figures 3.6 to 3.10 to be plotted.

APPENDIX 3 : THE PREDICTED μ_{up} AND MAXIMUM COUNT AT VARIOUS EXTREME LAGOON CONDITIONS.

Independent Variables					Dependent Variables	
Temperature ($^{\circ}\text{C}$)	pH	Sulphide Concentration (mg/l)	Acetate Concentration (mg/l)	Light Intensity (lux)	μ_{up} ($\text{hr}^{-1} \times 10^{-3}$)	Maximum Count (cells/ml)
10	7.1	1	30	31.6	.429	1.51×10^8
30	7.1	1	30	31.6	.551	2.53×10^8
10	9.1	1	30	31.6	.362	1.83×10^8
30	9.1	1	30	31.6	.619	3.10×10^8
10	7.1	62.4	30	31.6	.547	4.66×10^7
30	7.1	62.4	30	31.6	.595	4.36×10^7
10	9.1	62.4	30	31.6	.511	9.16×10^7
30	9.1	62.4	30	31.6	.741	8.52×10^7
10	7.1	1	1,900	31.6	.485	1.56×10^8
30	7.1	1	1,900	31.6	.622	4.42×10^8
10	9.1	1	1,900	31.6	.433	1.89×10^8
30	9.1	1	1,900	31.6	.741	5.48×10^8
10	7.1	62.4	1,900	31.6	.585	3.61×10^7
30	7.1	62.4	1,900	31.6	.637	5.14×10^7
10	9.1	62.4	1,900	31.6	.580	6.95×10^7
30	9.1	62.4	1,900	31.6	.840	1.02×10^8
10	7.1	1	30	3,160	.630	1.08×10^8
30	7.1	1	30	3,160	.549	1.78×10^8
10	9.1	1	30	3,160	.530	2.87×10^8
30	9.1	1	30	3,160	.616	5.00×10^8
10	7.1	62.4	30	3,160	.618	3.48×10^7
30	7.1	62.4	30	3,160	.489	3.26×10^7
10	9.1	62.4	30	3,160	.618	1.39×10^8
30	9.1	62.4	30	3,160	.608	1.29×10^8
10	7.1	1	1,900	3,160	.797	1.12×10^8
30	7.1	1	1,900	3,160	.694	3.06×10^8
10	9.1	1	1,900	3,160	.712	2.97×10^8
30	9.1	1	1,900	3,160	.827	9.10×10^8
10	7.1	62.4	1,900	3,160	.793	2.72×10^7
30	7.1	62.4	1,900	3,160	.586	3.83×10^7
10	9.1	62.4	1,900	3,160	.786	1.04×10^8
30	9.1	62.4	1,900	3,160	.773	1.56×10^8

APPENDIX 4 : RAW DATA FROM THE SIX EXPERIMENTS PERFORMED ON THE
LABORATORY FELLMONGERY LAGOONS.

Table A.4.1: Effluent Characteristics for Run No. 1 (25°C, 200 mg/l sulphide).

Retention Time (days)	COD (mg/l)	S ²⁻ (mg/l)	COD _c (mg/l)	BOD (mg/l)	pH	Thiocapsa counts (cells/ml x 10 ⁷)
0	3,424	200	3,024	-	11.8	-
3.90	2,249	75	2,174	864	9.5-	0.07
7.80	1,223	35	1,153	518	8.4-	0.20
9.68	1,635	51	1,533	660	9.1-	0.19
11.7	1,177	30	1,117	471	7.9	1.08
15.2	1,314	38	1,238	420	8.8-	1.05
15.6	1,089	30	1,029	365	8.0	4.75
19.1	685	21	643	180	7.9	1.89
28.7	631	18	595	157	8.0	2.48
29.6	576	19	538	135	7.6	2.25
30.5	858	25	808	225	8.6-	2.77
38.2	588	19	550	126	7.6	2.63
45.7	559	19	521	122	7.7	1.98
59.1	440	19	402	110	7.5	1.10
60.8	524	19	486	110	7.7	2.54
91.5	427	18	391	90	7.5	1.23
122.0	440	20	400	95	7.4	1.01

Table A.4.2: Effluent Characteristics for Run No. 2 (25°C, 1,500 mg/l sulphide)

Retention Time (days)	COD (mg/l)	S ²⁻ (mg/l)	COD _c (mg/l)	BOD (mg/l)	pH	Thiocapsa counts (cells/ml)
0	5,694	1,536	2,622	-	12.1	-
12.2	5,031	774	3,483	-	11.85	1.90 x 10 ⁵
24.4	1,598	151	1,296	312	9.86	4.86 x 10 ⁷
30.5	1,973	216	1,541	400	10.6	8.46 x 10 ⁶
36.6	1,564	149	1,266	286	9.85	4.39 x 10 ⁷
48.7	1,497	93.0	1,311	279	9.80	6.27 x 10 ⁷
59.1	1,229	35.2	1,159	157	9.50	7.91 x 10 ⁷
91.5	1,165	36.0	1,093	145	9.50	8.34 x 10 ⁷
122	956	23.6	909	145	9.40	7.62 x 10 ⁷

Table A.4.3: Effluent Characteristics for Run No. 3 (10°C, 200 mg/l sulphide).

Retention Time (days)	COD (mg/l)	S ²⁻ (mg/l)	COD _d (mg/l)	BOD (mg/l)	pH	Thiocapsa count (cells/ml x 10 ⁷)
0	3,476	200	2,786	-	12.2	-
11.7	2,752	84	2,584	1,146	10.36	0.433
20.3	2,157	61.2	2,035	1,005	9.10	3.28
23.5	1,951	43.6	1,864	462	8.72	5.09
30.5	1,605	46.7	1,512	670	8.60	3.43
35.2	1,540	40.5	1,459	414	8.48	5.35
40.7	1,319	32.7	1,253	328	8.35	8.87
47.0	1,310	25.0	1,260	218	8.45	10.3
59.1	1,072	25.2	1,022	215	7.97	6.12
61.0	1,143	23.1	1,097	275	8.32	11.3
81.3	1,082	24.2	1,034	209	8.04	8.18
91.5	1,003	21.1	961	205	7.97	7.40
122	966	21.0	924	160	7.76	6.82

Table A.4.4: Effluent Characteristics for Run 4 (10°C, 1,500 mg/l sulphide).

Retention Time (days)	COD (mg/l)	S ²⁻ (mg/l)	COD _c (mg/l)	BOD (mg/l)	pH	Thiocapsa count (cells/ml)
0	5,764	1,489	2,786	-	12.2	-
24.5	5,564	1,074	3,416	-	11.7	-
30.5	5,534	916	3,702	-	11.4	-
48.7	4,948	903	3,142	-	9.7	-
59.1	3,317	824	1,669	1,982	9.5	1.07 x 10 ⁶
73.2	3,218	559	2,100	1,276	9.6	1.62 x 10 ⁷
91.5	2,585	160	2,265	1,138	8.7	1.70 x 10 ⁷
97.3	2,067	103	1,861	810	8.8	3.14 x 10 ⁷
122	1,952	67.2	1,818	745	8.7	2.46 x 10 ⁷

Table A.4.5: Effluent Characteristics for Run 5 (17.5°C, 850 mg/l sulphide).

Retention Time (days)	COD (mg/l)	S ²⁻ (mg/l)	COD _c (mg/l)	BOD (mg/l)	pH	Thiocapsa count (cells/ml)
0	4,357	858	2,641	—	12.2	—
11.0	1,841	302	1,237	683	11.5	2.00 x 10 ⁵
22.0	1,410	160	1,090	495	10.4	1.90 x 10 ⁶
22.0	1,204	80	1,044	346	9.35	2.01 x 10 ⁷
33.0	1,204	81	1,042	301	9.35	1.80 x 10 ⁷
44.0	1,100	71	958	291	9.40	3.72 x 10 ⁷
44.0	1,062	61	940	262	9.25	5.74 x 10 ⁷
66.0	993	60	873	282	9.25	3.73 x 10 ⁷
88.0	975	53	869	251	9.25	9.81 x 10 ⁷

Table A.4.6: Effluent Characteristics for Run 6 (17.5°C, 850 mg/l sulphide).

Retention Time (days)	COD (mg/l)	S ²⁻ (mg/l)	COD _c (mg/l)	BOD (mg/l)	pH	Thiocapsa count (cells/ml)
0	4,295	842	2,611	—	12.3	—
8.72	2,106	255	1,596	708	11.4	1.50 x 10 ⁷
17.4	1,152	73.6	1,004	442	9.40	2.33 x 10 ⁷
22.0	1,352	114	1,124	525	10.4	1.00 x 10 ⁷
26.1	1,126	68.8	988	390	9.45	2.71 x 10 ⁷
34.8	1,170	62.4	1,045	353	9.30	6.92 x 10 ⁷
44.0	1,115	61.7	992	342	9.22	4.39 x 10 ⁷
66.0	1,020	60.8	898	305	9.25	6.06 x 10 ⁷
88.0	869	44.8	779	288	9.25	1.02 x 10 ⁸