AN ACTIVATED SLUDGE BASED SYSTEM FOR THE TREATMENT OF A LEACHATE CONTAINING CHLOROPHENOLS AND PHENOXYACETATE HERBICIDES.

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biotechnology at Massey University.

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1990

ABSTRACT

A study was made on the biological treatment of a landfill leachate containing high concentrations of the phenoxy herbicides 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), along with significant quantities of *para*-chloro-*ortho*-cresol (PCOC), methanol, butan-1-ol and butan-2-ol.

A mixed, natural microbial population (consisting of Pseudomonas species) was developed from a soil inoculum. The culture was found to be capable of mineralising 2,4-D and reducing the toxicity of the leachate by 92 %. The culture was found to be stable in continuous culture (residence time = 14.5 h) for 872 days.

The optimum concentrations for degradation were found to be 5-10% leachate (217-435 mg/l phenoxies, 33-66 mg/l PCOC, 40-80 mg/l alcohols and 0.6-1.2 g/l ash) for batch work and 10-15 % leachate (435-708 mg/l phenoxies, 66-107 mg/l PCOC, 80-120 mg/l alcohols and 1.2-1.9 g/l ash) for CSTR work. Batch studies showed sequential utilisation of the substrates: alcohols, followed by PCOC followed by phenoxies.

Studies were carried out to determine the kinetics of degradation for each group of substrates. The results showed that alcohols were the most rapidly degraded (μ_{MAX} = 0.3 h^{-1}), although growth was inhibited by PCOC and phenoxies.

PCOC was inhibitory to its own degradation, with inhibition directly proportional to PCOC concentration up to 290 mg/l, above which no degradation occurred. Both alcohol and PCOC degradation were described well by a linear inhibition model.

A comparison was made between the batch determination of PCOC degradation kinetics and a relatively new method, the Modified Infinite Dilution Test (MIDT). The MIDT results showed rates 50 % higher than the batch methods, indicating there was a change in the nature of the biomass in batch studies.

The kinetics of phenoxy degradation indicated that there was no inhibition in the concentration range of interest for MCPA and 2,4-D. However, 2,4,5-T was apparently degraded by cometabolism, with PCOC the best stimulator of degradation.

An interactive three substrate model was used to describe degradation and was found to fit measured data for CSTR systems. The model was robust and could predict the single substrate (ie pure compound kinetics) on simplification, indicating the wide range of application of the model.

The model showed that the presence of the alcohols in leachate considerably accelerated the degradation of PCOC and phenoxies. Critical points for washout were significantly shifted and reversed

from those of pure compounds, indicating interactions between the substrates could not be ignored. The model provides a method for quantifying the effect of a secondary substrate on the target compounds.

Results from laboratory activated sludge experiments showed that this process was capable of degrading the alcohols, PCOC and phenoxies present in both 10 and 15 % leachate. Loading rates (1.9-3.0 kgsubstrate/m^3.d) were high in comparison to the typical loadings quoted in the literature. The three substrate model, in association with the critical point method predicted three regions of plant operation, total substrate removal, stable operation with residual substrate and no degradation, compared with the two regions of the critical point method.

The results also showed that the system could be treated as non-inhibitory for design purposes, as the Monod model gave a closer prediction of behaviour than the critical point method. However, as the composition of the leachate is expected to change, the three substrate model is required to predict the effect of these changes on an AS plant.

The sludge produced by the AS plant had low concentrations of residual organic and inorganic ions, indicating it could be treated as a non-hazardous byproduct. While AS reduced the toxicity by 71 %, the effluent toxicity could be reduced further by the use of activated carbon treatment. This produced a final effluent with an EC_{50} (48h) of 46 %.

Preliminary economic analysis showed that AS followed by activated carbon treatment was capable of treating the leachate for 42c/l, lower than the alternatives of activated carbon alone and incineration. The cost was most sensitive to leaching rate, with lower rates resulting in smaller and cheaper processes.

To conclude, it was shown that a biologically based process is capable of producing non-hazardous byproducts and is economically viable as compared to alternative treatment processes.

ACKNOWLEDGMENTS

Thanks are extended to Dr. C. Hickey, Water Quality Centre, Department of Scientific and Industrial Research, Hamilton for performing the toxicity tests and Dr. J. Lee, Biotechnology Division, Department of Scientific and Industrial Research, Palmerston North for the ICP-AES analysis of leachate and sludge.

The author wishes to thank John Alger, Bruce Collins and Wayne Mallet, the workshop staff in the Biotechnology Department. Without their abilities and efforts to maintain ageing equipment and turn ideas and sketches into working equipment, this study would have taken much longer.

Thanks are also extended to Bob Chong, Graham Manderson and Rao Bhamidimarri for their supervision of the project. This required a mixture of their skills in chemistry, microbiology and chemical engineering.

The assistance of Dr G.V. Bhaskar in the development of the computer program for solving the three substrate model and of Dr A.H.J. Patterson for advice on the economic analysis was also appreciated.

The efforts of DowElanco staff Chris Collins, Dave Catt and Colin Mercer were greatly appreciated, especially the use of an HPLC for the duration of the project and the prompt replies to requests for information and chemicals. The financial support of DowElanco was also appreciated.

The financial support of the University Grant Committee was also warmly welcomed.

The support of the Department of Soil Science, who allowed the use of equipment for TOC analysis (performed by J.Sykes) and the scintillation counting equipment is also acknowledged.

The assistance of the laboratory staff and administrators, Mike, Ann-Marie, Janice, John and Judy in obtaining obscure chemicals and equipment was also greatly appreciated.

The sense of humour of long term office-mates, Carlo Bogoni (Switzerland) and Xabi Chiura (Japan) was greatly appreciated, as both were capable of succinctly putting mishaps and bad days into their proper perspective.

Finally, my greatest appreciation is extended to my wife, Rosalie. I am deeply indebted to her, for putting up with the frequent trips to Massey, the constant bind of a bioreactor and the problem of having a husband present in body but not mind. It has all been worth it and this thesis is dedicated to her.

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Disks containing Appendices 1,7,9,11,15 and 18 can be found inside the back of this thesis. The data were stored on a write protected 5 1/4 inch, 360 K disk using the spreadsheet VP-PLANNER. The data can be read into the public domain spreadsheet ASEASYAS, along with VP-PLANNER, LOTUS 1-2-3 and QUATTRO. The remaining Appendices can be found (on microfiche) in the same envelope.

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THESIS PRESENTATION

The reader will note that many of the chapters are broken into discrete Results and Discussion sections. This was done to emphasise the logical progression of the work, rather than adhere to the conventional thesis structure.

CHAPTER 1
BACKGROUND AND INTRODUCTION

The disposal of toxic wastes to unsecured landfills has been practised in many countries. For example, the United States Environmental Protection Agency has identified 1,100 sites which require clean-up to remove hazardous chemicals. Of these sites only 13 have been renovated and withdrawn from the National Priorities List (NPL). However, the rate of clean-up of the U.S. Superfund sites is increasing (Mackerron, 1988).

In New Zealand there are very few sites which require such action. One example however, is a landfill, constructed on an elevated coastal site, which contains significant quantities of phenoxy herbicides (phenoxies) such as 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The site also contains, in lower concentrations, the chlorophenols that are associated with these herbicides, 2,4-dichlorophenol (2,4-DCP), *para*-chloro-*ortho*-cresol (PCOC) and a trace quantity of 2,4,5-trichlorophenol (2,4,5-TCP). The background to the problem and remediation steps taken so far will be described, followed by the selection of a suitable process for complete cleanup of the site.

Background.

In December 1982 chemical odours were noticed on the foreshore below and adjacent to the old clifftop dumpsites (Collier and Oldham, 1986). As the beach was used regularly for recreational purposes, there was public concern over the safety of the site, especially with respect to the future availability of the beach itself.

After a study in 1984 it was determined that the leakage was not a threat to public health and that the amount and composition of leakage had stabilised. Nonetheless, further deterioration of the site was considered possible and natural degradation of the hazardous chemical components could not be expected to occur in the site (Collier and Oldham, 1986). Containment on site was not considered feasible and it was decided to construct a new secure landfill to contain all the soil from the old site while options were reviewed as to the best and most economic strategy for completely removing the chemicals it contained (Collier and Oldham, 1986).

A secure landfill (Figure 1.1) was constructed following the guidelines of the Resource Conservation and Recovery Act from the USA. This required the facility to have;

(1) an impermeable soil liner (in this case a compacted clay bed)
(2) a synthetic liner, in this case high density polyethylene (HDPE).
(3) a secondary leakage detection system and
(4) a primary leachate collection system (Collier and Oldham, 1986).

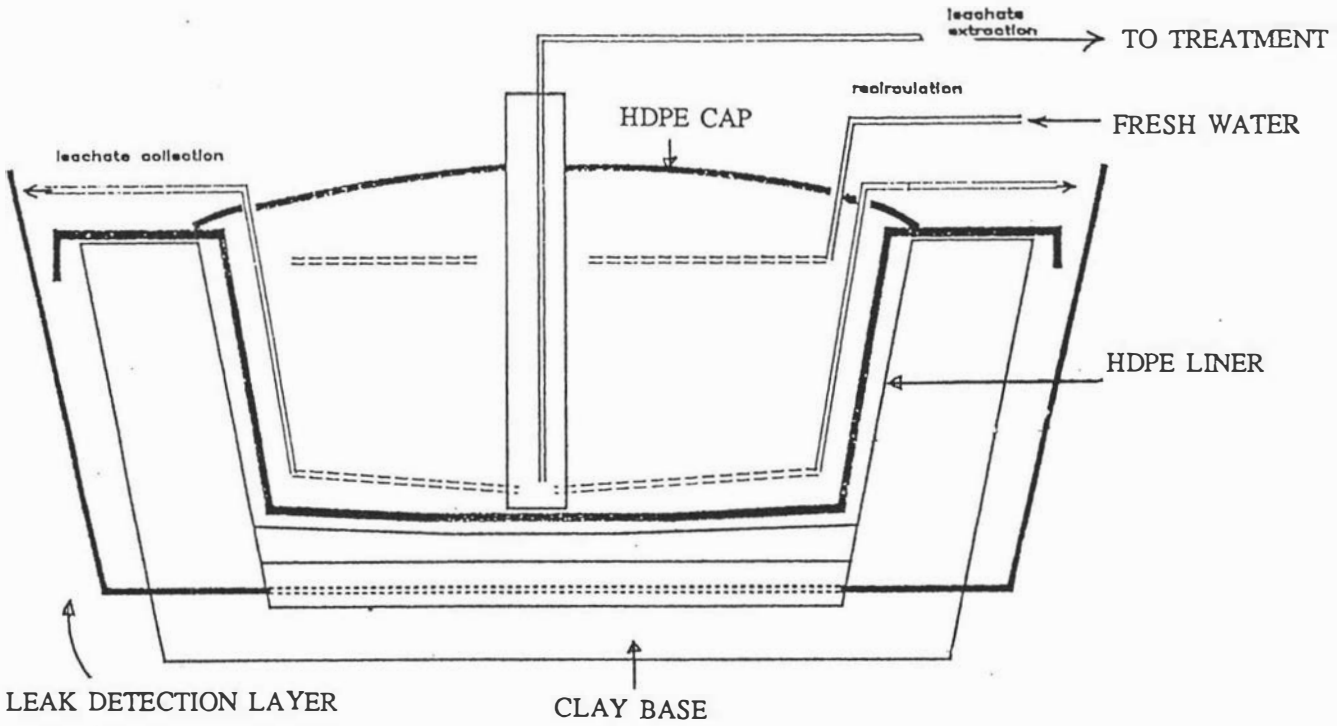


Figure 1.1: Schematic Diagram of Waireka Secure Landfill.

During the installation of the HDPE liner all joints were tested along their entire length to ensure the integrity of the site. The contents of the original dumps were transferred to the new dump, along with the surrounding contaminated soil. The dump was then capped with a HDPE cap.

The new landfill was designed to allow for the injection of water into the site and the collection of the leachate for analysis and future treatment.

Landfill Leachate Treatment.

In recent years the biological treatment of hazardous wastes has attracted increasing interest. In the context of this thesis it is useful to explore the reasons for this approach.

The options for the treatment of a leachate (Cope, 1983) are:

(1) Biological treatment,

(2) Physical and/or chemical treatment such as wet air oxidation, adsorption to activated carbon, supercritical water, and

(3) Recirculation and spray irrigation.

In this case, the herbicidal nature of the leachate prevents the use of recirculation and spray irrigation.

Of the remaining options, physical/chemical treatments require either continual overheads in the purchase or disposal of adsorbents, or high capital costs as in the case of wet air oxidation or supercritical water treatment. Biological treatment however, generates its own catalyst for the reaction by the production of new biomass and also transforms the waste into CO_2 and water. The main operating cost is providing aeration and small quantities of nutrients. Therefore if a suitable biological process is available, it may have significant cost advantages over physical/chemical methods.

Choice of a Biological Process.

There are a number of different biological processes that could be considered:

(i) attached growth reactors such as trickling filters, rotating biological contactors and fluidised bed bioreactors,

(ii) dispersed growth reactors, such as activated sludge plants and aerobic ponds,

(iii) anaerobic processes, such as anaerobic ponds and anaerobic digestion and

(iv) *in situ* treatment while still contained in the landfill.

In situ treatment of 2,4,5-T (on a laboratory scale) has been reported in the literature (Chatterjee et al. 1982), along with the use of bacterial enzyme preparations (Loos et al.,1967c). However such a process requires effective oxygenation and mixing of nutrients, which while easily achieved on a small scale or on the surface, would be very difficult in a dump as shown in Figure 1.1 (Wu et al., 1990).

Kirk and Lester (1989) showed that anaerobic digestion of chlorinated phenols and phenoxies was possible, although the residence times were long.

Hannah et al. (1986) compared the efficiency of six processes (conventional activated sludge, high rate trickling filter, primary treatment with chemical addition, filtration and aerated and facultative lagoons) for the removal of toxic pollutants, and found that none could outperform the conventional AS plant. This indicated that the activated sludge plant would probably be the best option for the degradation of the landfill leachate. It therefore seemed appropriate to study further the degradation of the leachate using activated sludge.

Activated sludge systems have been used in the past with some success. Mills (1959) used activated sludge to treat a waste containing 2,4-DCP and 2,4-D and found it to be suitable, providing sewage was added to give additional stability. Nakashio (1969) reported the use of a 300 m^3 activated sludge system for the treatment of phenolic wastes, the second such system in operation in Japan. Hashim et al. (1989) reported the use of activated sludge for the treatment of wastes containing alkylbenzenesulphonates. Not all reports have been positive, however, with Hill et al. (1986) reporting that phenoxy herbicides were not effectively removed during the activated sludge treatment of municipal wastewater.

With respect to hazardous leachates, Cope (1983) suggests the use of granular activated carbon, either prior to biological treatment (to remove the toxic organics that may upset a biological process) or after biological treatment (to remove refractory organics). The most logical approach would be to use a acclimated culture to remove the bulk of the organics (alcohols/PCOC/phenoxies) and use activated carbon columns to remove any refractory organics.

This thesis therefore describes the development and study of an activated sludge based process for the treatment of the landfill leachate, which produces byproduct streams that can be discharged into the environment. This study will consider the development of a suitable microbial culture, the determination and modelling of degradation kinetics, the design of activated sludge plants treating hazardous wastes, and the cost of such treatment systems, especially for a two stage system of activated sludge and activated carbon.

CHAPTER 2
REVIEW OF THE LITERATURE

2.1 Introduction.

For the biological treatment of a waste to be successful, there are two basic requirements; firstly that the individual components must be degradable by microorganisms, and secondly that there must be a suitable process configuration available for use. This chapter will review the current literature on the biodegradation of the alcohols, chlorophenols and phenoxies (the composition of the leachate is given in Table 2.1), along with that on activated sludge technology. As work carried out during this research was on a small scale, it was also necessary to review literature on modelling of microbial cultures, to enable the scale up of the work to be achieved.

Table 2.1 Composition of Leachate.

Component	Concentration (mg/l)
Methanol	260
Butan - 1 -ol	390
Butan - 2 -ol	330
2,4-dichlorophenoxyacetic acid (2,4-D)	1420
4-chloro-2-methylphenoxyacetic acid (MCPA)	2020
para-chloro-*ortho*-cresol (PCOC)	520
2,4-dichlorophenol (2,4-DCP)	50
2,4,5-trichlorophenoxyacetic acid (2,4,5-T)	390
2,4,5-trichlorophenol (2,4,5-TCP)	0.5
4-chloro-2-methylphenoxybutyric acid (MCPB)	4
4,5-dichloro-2-methylphenoxyacetic acid (MDCPA)	30

2.2 Biodegradation.

2.2.1 Biodegradation of the Chlorophenols and Phenoxies.

The biodegradation of 2,4-D was first reported in 1945, by workers attempting to produce a herbicide that maintained toxicity in soil for a significant period of time (Nutman et al., 1945). Since then, numerous reports have appeared on the biodegradation of 2,4-D and related compounds by a large variety of microorganisms (Table 2.2).

Before the biodegradation of the substituted phenoxyacetic acids can be fully understood, the pathways of phenol degradation in microorganisms need to be briefly covered as there are key

Table 2.2 Summary of Organisms Degrading Phenoxy Herbicides.

Organism	2,4-D	MCPA	2,4,5-T	Reference
Mycoplana sp.	*	*	*	Audus (1962)
Rhizobium meliloti	*	*		Audus (1962)
Corynebacterium sp.		*		Rogoff and Reid (1956)
Achromobacter	*		*	Audus (1962); Bell (1957)
Flavobacterium	*			Audus (1962)
Pseudomonas sp.	*		*	Tyler and Finn (1974); Pierce et al.(1982); Evans et al.(1971); Gaunt and Evans (1971a and b); Gamar and Gaunt (1971); Kim and Maier (1986)
Pseudomonas cepacia			*	Kilbane et al. (1982); Kilbane et al. (1983); Chatterjee et al.(1982); Karns et al. (1983a and b)
Pseudomonas fluorescens			*	Rosenberg and Alexander (1980a)
Alcaligenes sp.	*	*		Don and Pemberton (1981); Don et al. (1985)
Arthrobacter		*		Duxbury et al.(1970);Bollag et al.(1968a and b);Loos et al.(1967a and b) Tiedje et al. (1969); Tiedje and Alexander (1969); Sharpee et al.(1973)
Brevibacterium			*	Horvath (1971a)
Streptomyces viridochromogenes		*	*	Kearney and Kaufman (1975)
Phanerochaete chrysosporium			*	Ryan and Bumpus (1989)
Nocardia	*			Sinton et al. (1986)
Xanthanobacter	*	*		Ditzelmuller et al. (1989)

similarities and differences between the two pathways. Phenol pathways will be dealt with briefly, followed by details of 2,4-D, MCPA and 2,4,5-T metabolism.

2.2.1.1 Pathways of Degradation.

Phenol Biodegradation.

Phenol is oxidised by two pathways in bacterial cells, the β-ketoadipate pathway (also known as the *ortho* cleavage pathway) or the *meta* cleavage pathway (2,3-oxygenase pathway) (Stanier and Ornston, 1973).

In the *ortho* cleavage pathway the phenol is oxidised to catechol, followed by ring fission in the 1,2 position and further oxidation via β-ketoadipic acid to succinic acid. The *meta* pathway involves oxidation to catechol, followed by ring fission on the 2,3 position to produce a muconic semialdehyde, with further decomposition to formate, acetaldehyde and pyruvate. These two pathways are shown in Figures 2.1 and 2.2 respectively. These pathways were elucidated using almost exclusively Pseudomonas species, however variations have not been found for other organisms studied (Stanier and Ornston, 1973). The presence of two divergent pathways for the degradation of catechol into products readily utilised by cells leads to the question of pathway selection, which is beyond the scope of this work.

Chlorinated Phenoxyacetic Acid Biodegradation.

The aerobic biodegradation pathways for 2,4-D and MCPA are well defined after work carried out in the late 1960's by two groups: one led by Alexander (Tiedje and Alexander, 1969; Bollag et al., 1968a and b; Tiedje et al., 1969 and Duxbury et al. 1970) using an Arthrobacter species on 2,4-D and the second led by Evans (Evans et al., 1971; Gaunt and Evans, 1971a and b; Gamar and Gaunt, 1971) using a soil pseudomonad on MCPA.

The pathways proposed have been accepted widely. The pathway for 2,4-D involves the cleavage of the ether bond of 2,4-D to form 2,4-DCP, with the glyoxylate formed from the side chain being metabolised further to produce alanine (Tiedje and Alexander, 1969). The dichlorophenol is subsequently hydroxylated, with the aid of molecular O_2 and NADPH to the 3,5-dichlorocatechol (Bollag et al.,1968a). The chlorocatechol is then *ortho* cleaved and degraded to chloromaleylacetic acid (Tiedje et al., 1969), which is broken down to acetyl CoA and succinate, releasing free chloride ions (Duxbury et al.,1970). This pathway is shown in Figure 2.3 and was confirmed by Evans with a pseudomonad (Evans et al., 1971).

Subsequent work has made no significant changes to this pathway for organisms studied, except to allow flexibility in timing of the dechlorination reactions. Chlorine removal has been observed prior to ring cleavage in some Pseudomonas (Evans et al., 1971), Nocardia and Arthrobacter species (Sinton et al., 1986), but this does not affect the general structures of the intermediates, or the final products.

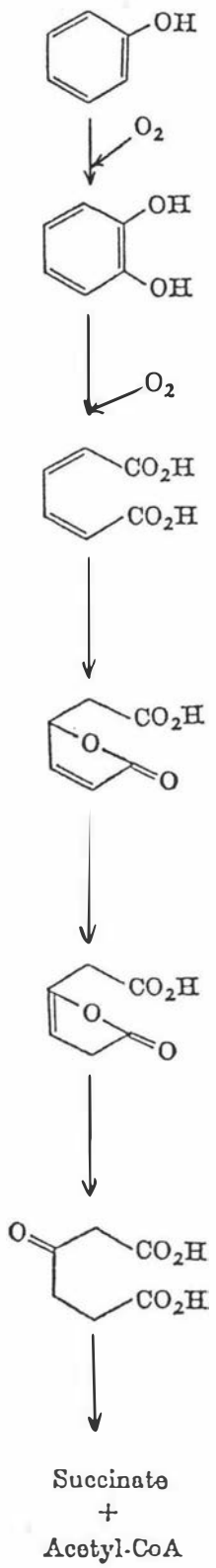


Figure 2.1: Pathway for *ortho* Cleavage of Phenol (Dagley,1971).

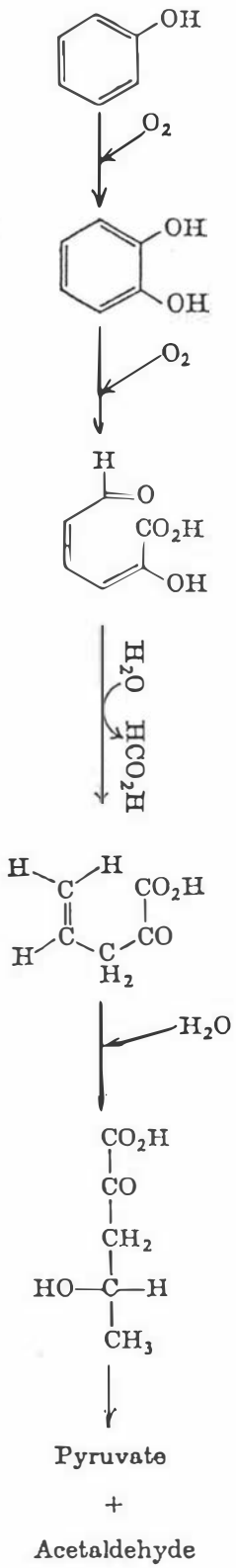


Figure 2.2: Pathway for *meta* cleavage of Phenol (Dagley,1971).

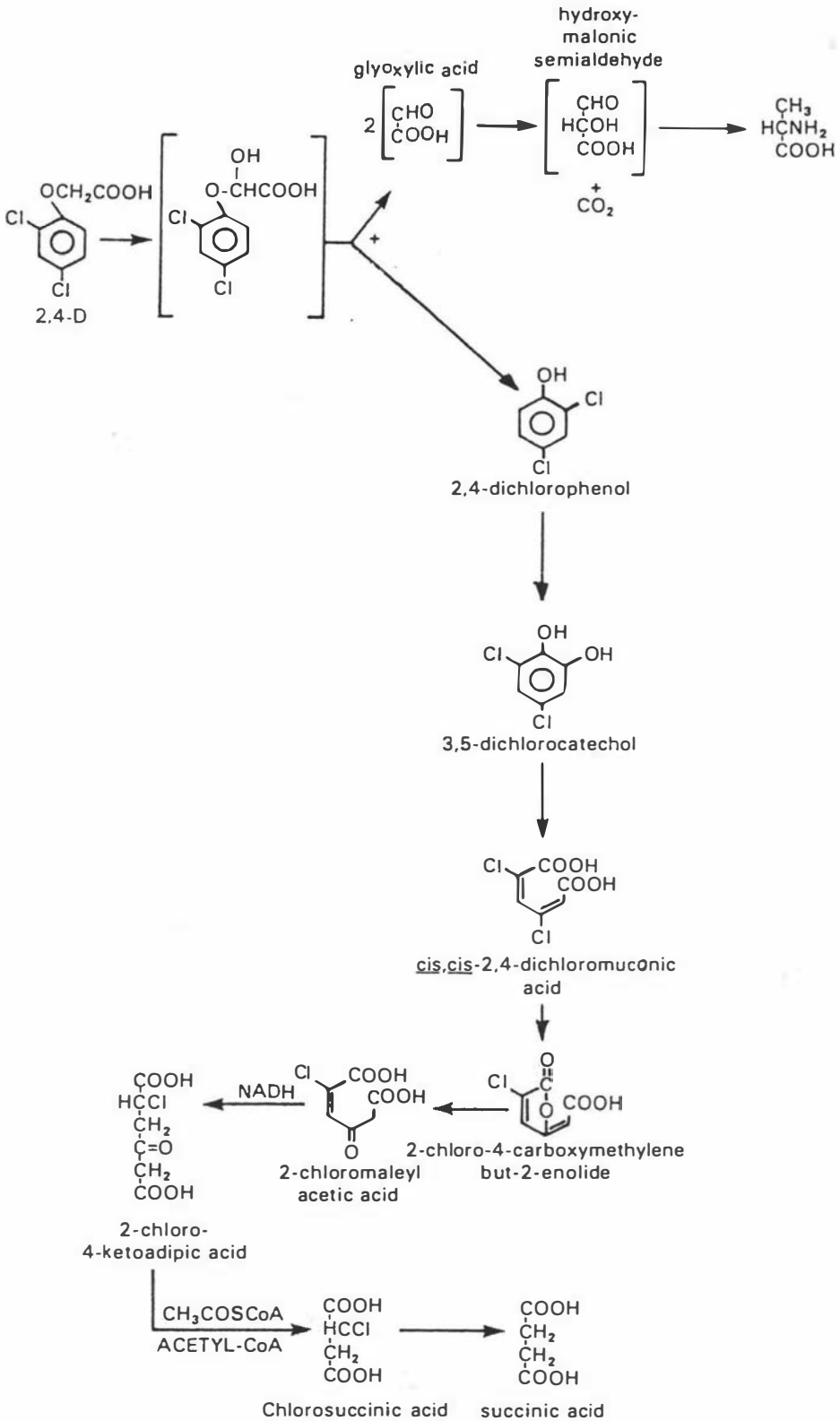


Figure 2.3: Degradation Pathway of 2,4-D by Arthrobacter.
(Rochkind et at.,1986)

The pathway for the degradation of MCPA (Figure 2.4) is similar to that for 2,4-D metabolism, except the last readily identifiable metabolite is 3-methylmaleylacetate. The fate of this compound has not been described in the literature but it is thought to be degraded by β-oxidation (Knackmuss,1984). Several organisms utilising MCPA as a sole carbon and energy source have been isolated (Gaunt and Evans,1971a; Lappin et al.,1985; Don and Pemberton, 1981) indicating that degradation to readily utilisable compounds does occur.

The pathway of 2,4,5-T metabolism is the least studied. A pathway was proposed by Rosenberg and Alexander (1980a) for the degradation of 2,4,5-T by sidechain cleavage to the trichlorophenol, followed by dehalogenation and hydroxylation to 3,5-dichlorocatechol. The chlorocatechol is then thought to be degraded via the pathway of 2,4-D metabolism to succinate and acetyl CoA (Figure 2.5).

This pathway was elucidated with a mixed culture isolated from soil, as at that time, no 2,4,5-T degrading organisms had been reported . The mode of degradation using mixed cultures was reported to be cometabolism (Horvath, 1971a;Rosenberg and Alexander, 1980b) although ^{14}C ring labelled 2,4,5-T in soil was found to be dissimilated as $^{14}CO_2$ (Rosenberg and Alexander, 1980b; McCall et al., 1981) signifying total degradation of the molecule. Horvath (1971a) reported accumulation of 3,5-dichlorocatechol with a pure culture of Brevibacterium and also noted that this compound was toxic to whole cells.

Kilbane et al. (1982) isolated the first organism capable of utilizing 2,4,5-T as a sole carbon and energy source. This organism, a Pseudomonas cepacia, was genetically modified by plasmid assisted molecular breeding and was found to be unstable, rapidly losing the degrading ability upon subculturing in the absence of 2,4,5-T (Kilbane et al., 1982).

The degradation of MCPB has been reported (MacRae, 1989),although the pathway was not stated. A similar compound, 4-(2,4-dichlorophenoxy)butyric acid has been studied in more depth (Rochkind et al.,1986). The pathway involves sidechain cleavage to the phenol and the aliphatic acid (Rochkind et al.,1986). The fate of the acid (in this case butyric acid) has been shown to be degradation by β-oxidation, and the chlorophenol is degraded by the same pathway as for the more simple phenoxies (Rochkind et al.,1986). Thus, in the case of MCPB, the expected pathway is the degradation to PCOC and butyric acid both of which should be readily degradable.

One of the phenoxy compounds present in the leachate (MDCPA) (to the author's knowledge) has not been investigated. It may be assumed that if it is degradable this compound follows a similar degradation pathway to the better known compounds.

The cofactor requirements for these pathways are not well defined. Molecular oxygen is required (Shaler and Klecka,1986) and manganese ions have been shown to be required (Evans et al.,

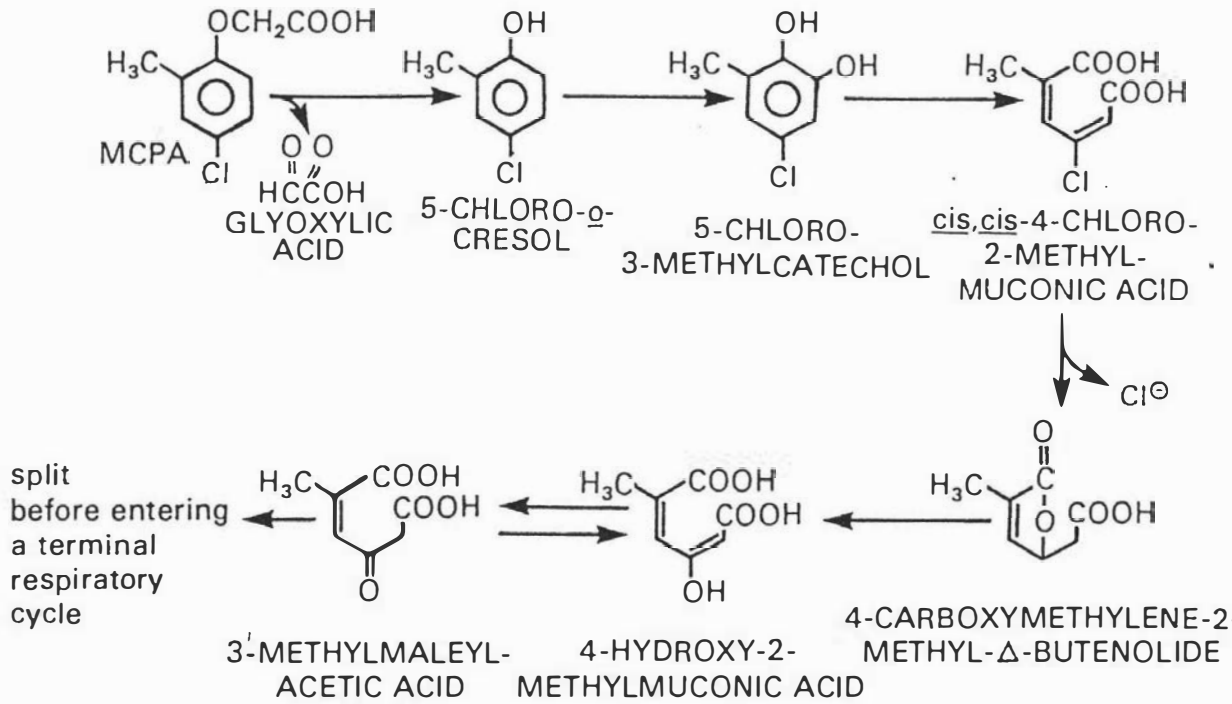


Figure 2.4: Degradation Pathway of MCPA by Pseudomonas (Loos, 1975).

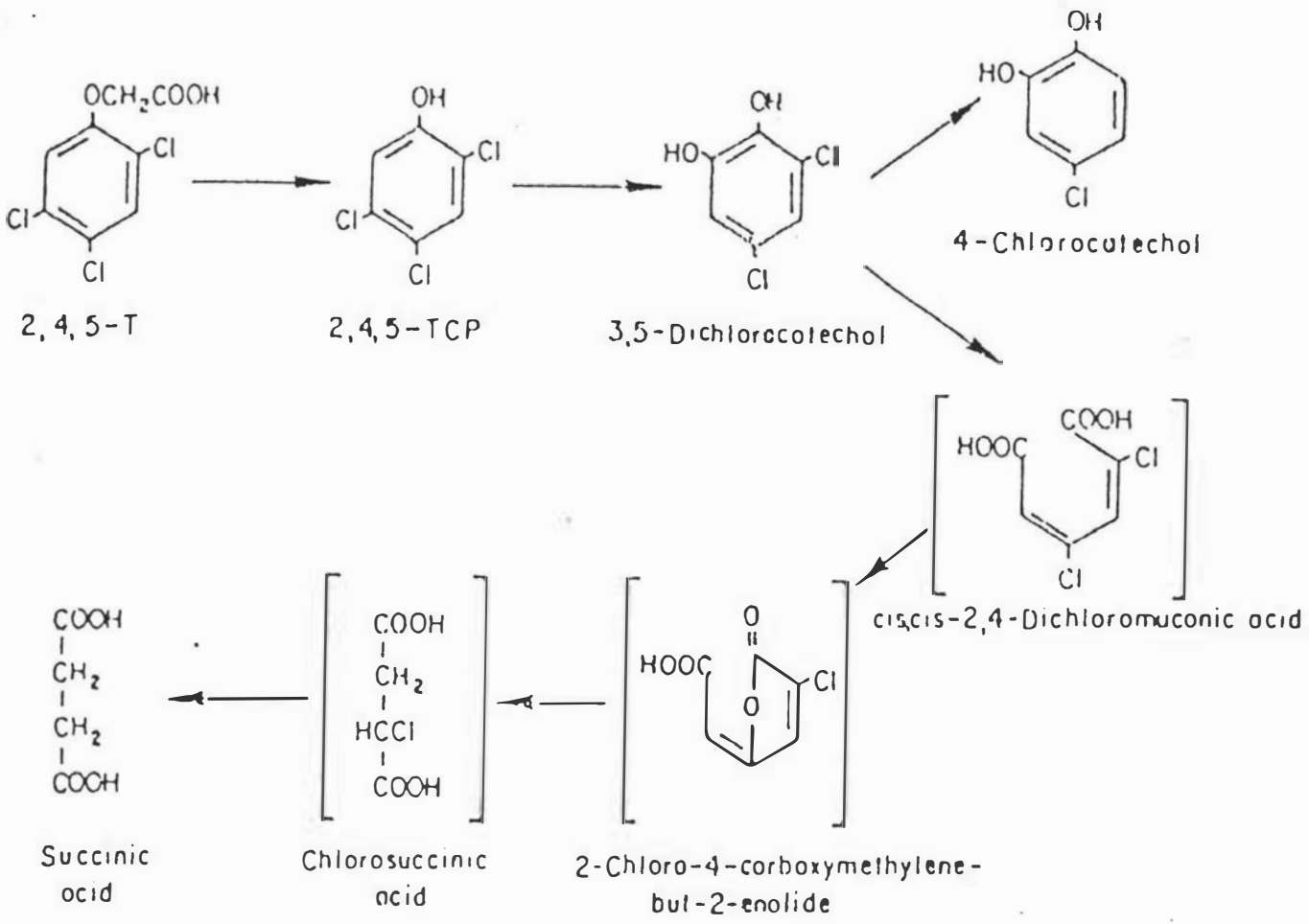


Figure 2.5: Degradation Pathway of 2,4,5-T by Pseudomonas (Rosenberg and Alexander,1980a).

1971), along with NADPH, NADH and coenzyme A (Bollag et al.,1968a;Tiedje et al.,1969;Duxbury et al., 1970). The organisms used in the studies quoted grew on salts only media, implying the organic cofactors required could be synthesised by the cells.

Chlorophenol Degradation.

The catabolic pathways for the chlorophenols of concern were covered by the phenoxy pathways, with the chlorophenols being the first intermediate in the pathways. Table 2.3 lists many biodegradable chlorophenols that have been described in the literature.

Table 2.3 Chlorophenols Reported to be Biodegradable.

Chlorophenol	Reference
2 - chloro	Loos et al. (1967a);Evans et al. (1971);Philbrook and Grady (1986)
4 - chloro	Loos et al. (1967a);Bollag et al.; 1968a; Schwein and Schmidt (1982);Spain and Nishino (1987)
2,4 - dichloro	Loos et al. (1967a);Karns et al.(1983b);Evans et. al. (1971)
2,5 - dichloro	Steiert et al. (1987); Spain and Nishino (1987)
2,4,5 - trichloro	Karns et al. (1983b); Horvath (1971a)
2,4,6 - trichloro	Karns et al. (1983b);Steiert et al. (1987)
2,3,4,6 - tetrachloro	Karns et al. (1983b);Steiert et al. (1987)
2,3,5,6 - tetrachloro	Steiert et al. (1987)
pentachloro	Karns et al. (1983b);Moos et al.(1983);Klecka and Maier(1985);Steiert et al. (1987)

2.2.1.2 Side Reactions to the Phenoxy Degradation Pathways.

Side reactions to the degradation pathway have the potential to form products that are not readily degraded by the organisms present, or that are toxic to humans and microorganisms alike. These possible side reactions need to be known, as after the leachate has been treated, the effluent must eventually be discharged to the environment. Side reactions mentioned in the literature will be discussed in relation to the leachate to be treated (Table 2.1).

Chloroanisole Formation.

The formation of chloroanisoles (Figure 2.6) from 2,4-D and 2,4,5-T has been noted by Loos et al. (1967b), Smith (1985) and McCall et al. (1981). These compounds have been found to have a high potential for bioconcentration and some authors consider they are at least as toxic as the

chlorophenols from which they are derived (Neilson et al., 1984). Allard et al. (1987) found that *o*-methylation of phenols with electron-attracting substituents might be a significant alternative to biodegradation and 2,4-D degraders have been shown to be unable to degrade 2,4-dichloroanisole (Loos et al.,1967b). In experiments using ^{14}C 2,4-D, Smith (1985) reported up to 10 % of carbon applied as 2,4-D could be extracted as 2,4-dichloroanisole. Loos et al. (1967b) isolated 2,4-dichloroanisole from cold traps on the gas outlets of fermenters degrading 2,4-D, stating that while it normally appeared in low concentrations (along with 2,4-DCP), sometimes it appeared in high concentrations and occasionally it failed to appear.

These results indicate that there is a real possibility of the production of chloroanisoles from the biological treatment of leachate and hence care should be taken to ensure the total degradation of the waste and not merely a transformation.

Hydroxylated Phenoxyacetates.

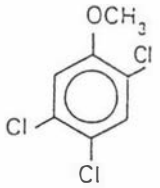
During work by the group led by Evans, the production of 2,4-dichloro-6-hydroxyphenoxyacetate (Evans et al.,1971) and 4-chloro-6-hydroxy-2-methylphenoxyacetate (Gaunt and Evans,1971a) from 2,4-D and MCPA respectively during degradation by Pseudomonas was observed. The structures of these compounds can be found in Figure 2.6. As Gaunt and Evans (1971a) found that the organism under study could not metabolise the hydroxylated phenoxyacetate further, it was postulated that it was not a major metabolite, but was formed by a side reaction.

Ring Dechlorination.

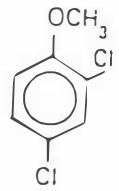
Evans et al. (1971) also found evidence of the dechlorination of 2,4-D prior to ring cleavage or sidechain removal. Elimination of the 4-chloro group of 2,4-D leads to a degradation pathway via 2-chlorophenol and 2-chloromuconic acid, which have been produced by organisms growing on 2,4-D (Evans et al.,1971). A similar pathway for MCPA via 2-methylphenoxyacetic acid and *ortho*-cresol may exist (Gaunt and Evans,1971a). 3,5-Dichlorocatechol and 4-chlorocatechol were isolated from 2,4,5-T degrading cultures, indicating ring dehalogentaion, but how this was generated from 2,4,5-TCP was not postulated (Rosenberg and Alexander, 1980a). Rosenberg and Alexander (1980a) however did not isolate any 2,4-DCP, which is one compound that would be expected to appear after a ring dehalogenation reaction.

Summary.

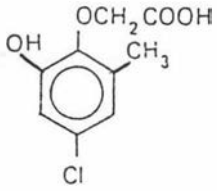
It can be seen that the degradation of the chlorinated aromatics follows the *ortho* cleavage route as opposed to *meta* cleavage. According to Knackmuss (1984), during adaptation of a Pseudomonas culture to chlorophenols, the culture initially exhibits *meta* cleavage. This, however, does not allow the productive breakdown of chlorocatechols, and the first response of the culture is to suppress *meta* cleavage activity by suicide inactivation. To enable the culture to survive, *ortho* cleavage is induced and the accumulation of toxic chlorocatechols no longer occurs (Knackmuss, 1984).



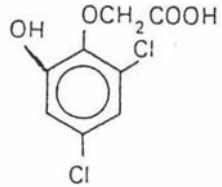
2,4,5-trichloroanisole



2,4-dichloroanisole



4-chloro-6-hydroxy-2-methylphenoxyacetic acid



2,4-dichloro-6-hydroxyphenoxyacetic acid

Figure 2.6: Side Reactions: Products (Rochkind et al..1986)

Dorn and Knackmuss (1978), using the same culture as above, described two enzymes for the *ortho* cleavage of catechols; pyrocatechase I, capable of cleaving only catechol and pyrocatechase II, which has a higher activity with chlorocatechols relative to catechol. This enzyme also appears to have a high affinity for 3-methyl-5-chlorocatechol (Kilpi et al., 1980).

As specific enzymes for the degradation of the chloro-derivatives of the breakdown products have not been described, it would be reasonable to assume subsequent enzymes have a loose specificity and hence the phenol pathway is not blocked by ring substitution. This implies the same inducers and control mechanisms apply to the pathway for chlorophenol metabolism as apply to the phenol pathway.

2.2.1.3 Microbiology and Genetics of Degradation.

As previously indicated (Table 2.2) a number of cultures capable of degrading 2,4-D and MCPA have been described. The pathways were elucidated using Pseudomonas (Evans et al.,1971) and Arthrobacter (Loos et al.,1967a;Tiedje et al.,1969) for 2,4-D and MCPA, while the culture used for 2,4,5-T (Rosenberg and Alexander,1980a) contained Pseudomonas fluorescens as the predominant organism.

Horvath (1971a) used the term cometabolism to describe the ability of a microorganism to degrade 2,4,5-T without being able to utilise the energy released for cell growth, in this case 2,4,5-T was oxidised by a Brevibacterium species to 3,5-dichlorocatechol. Cometabolism of 3,5-dichlorocatechol had previously been demonstrated in an Achromobacter species (Horvath,1970) and it was postulated that a mixed culture would degrade 2,4,5-T completely. This has since been demonstrated (Rosenberg and Alexander,1980a and b), as has the metabolism of other phenoxy herbicides such as mecoprop (2-(2-methyl-4-chlorophenoxy)propionic acid) (Lappin et al.,1985, Kilpi,1980, Kilpi et al.,1980) and silvex (2-(2,4,5-trichlorophenoxy)propionic acid) (Ou and Sikka,1977), usually with a Pseudomonas species dominant in the mixture.

Cometabolism was found to be enhanced by selecting an appropriate carbon source for growth, ie one with a structure close to the structure of the molecule to be degraded (Jacobsen and Alexander,1981). However, growth enhancement by a cocarbon source has been noted for compounds that are used as a sole carbon and energy source (Papanastasiou and Maier, 1983), implying that these two forms of metabolism cannot be distinguished apart on the basis of behaviour with a second carbon source.

The current literature indicates that the cultivation of mixed cultures allows the degradation of molecules considered recalcitrant, either by metabolism or cometabolism, giving such mixed cultures a distinct advantage over pure cultures for the degradation of phenoxy herbicides and associated chlorophenols. Recently Lu and Grady (1988) showed that a mixed culture was capable of reducing the substrate to a lower chlorophenol concentration than pure cultures derived from the mixture.

The use of defined mixed cultures for the treatment of such wastes has also been reported. Schmidt et al.(1983), having recognised that the dissimilation of chlorocatechols was the key step in the degradation of chlorophenols, used a defined mixed culture containing a pseudomonad capable of using methanol, a phenol-degrading Alcaligenes species and a chlorocatechol degrading Pseudomonas species to completely degrade a mixture of phenol, acetone, alcohols and isomeric chlorophenols. A transconjugant strain of Alcaligenes was isolated from the acclimated culture that was capable of total chlorophenol degradation, a property that none of the parent strains possessed (Schmidt et al.,1983). The experiment implied that the genes for the degradation of chlorocatechols were transmissible and hence probably plasmid borne.

It was noted that throughout the literature, the organisms most commonly associated with the degradation of phenoxies were from the family Pseudomonadaceae, and frequently Pseudomonas species.

Plasmid Involvement in Biodegradation.

Kilbane et al. (1982) bred a Pseudomonas capable of degrading 2,4,5-T as a sole carbon and energy source by the method of plasmid assisted molecular breeding. The Ps. cepacia AC1100 of Kilbane et al. (1982) harbours at least two plasmids and there is circumstantial evidence of their involvement in the 2,4,5-T degradation (Ghosal et al.,1985).

Plasmid involvement in 2,4-D metabolism was first described by Pemberton and Fisher (1977). Don and Pemberton (1981) isolated two plasmids from an Alcaligenes species and designated them pJP2, capable of degrading phenoxyacetic acid and 2,4-D and pJP4, capable of degrading 3-chlorobenzoic acid, MCPA and 2,4-D, as well as encoding resistance to merbromin and mercuric chloride. Further studies of plasmid pJP4 by Don et al.(1985) found five genes for enzymes involved in the catabolic pathways of 2,4-D and 3-chlorobenzoate. Four of the genes were found to code for 2,4-DCP hydroxylase, dichlorocatechol-1,2-dioxygenase, chloromuconate cycloisomerase and chlorodienelactone hydroxylase respectively (Don et al.,1985). Inactivation of the last three genes resulted in the prevention of the degradation of both substrates, indicating a common pathway for chlorocatechol dissimilation (Don et al.,1985). All essential steps for 2,4-D metabolism were plasmid encoded (Don et al.,1985). Considerable homology between the pJP4 fragment harbouring chlorocatechol degradation and a fragment of the plasmid DNA isolated from Ps. cepacia AC1100 has been demonstrated (Ghosal et al.,1985).

A second plasmid, pAC25, capable of dissimilating chlorocatechols has been isolated from Ps. putida grown on 3-chlorobenzoate was isolated by Chatterjee et al.(1981). Schwein and Schmidt (1982) demonstrated the transfer of chlorocatechol-degrading genes from a Pseudomonas to an Alcaligenes strain capable of growing on phenol. The resulting exconjugant strain was able to degrade all isomeric chlorophenols, which were not attacked by either parent (Schwein and Schmidt,1982). An identical isolate was later described by Schmidt et al.(1983), although the second isolate was produced in an uncontrolled manner.

This recognition that a complete set of genes for the degradation of chlorocatechols is borne on some transmissible plasmids has allowed the construction of bacterial strains capable of degrading chlorinated phenols and carboxylic acids (Ghosal et al.,1985).

2.2.1.4 Regulation of the Pathways.

Karns et al. (1983b) found the enzyme(s) responsible for degrading 2,4,5-T to 2,4,5-TCP were constitutive and that this step was rate limiting in metabolism. The reaction was not inhibited by excess 2,4,5-TCP, hence it was postulated that a high half saturation constant for either the uptake or conversion of 2,4,5-T was the only method of regulation on this reaction.

The inducer for 2,4,5-T, 2,4,5-TCP and pentachlorophenol (PCP) metabolism was postulated to be 2,4,5-TCP or a subsequent metabolite (Karns et al., 1983b). This correlates well with an *ortho* cleavage mechanism, the inducer of which is thought to be the cis,cis muconate (the first ring cleavage product), with sequential induction after the formation of β-ketoadipate (Stanier and Ornston,1973).

Karns et al.(1983b) also showed that a lack of expression of chlorophenol metabolism during growth on succinate was probably due to the exclusion of the inducer from the cytoplasm, a process resembling catabolite repression. The organism tested (Ps. cepacia AC1100) was unable to grow on either 2,4-D or PCP as a sole carbon and energy source, but 2,4,5-T grown cells could degrade both substrates, indicating induction was necessary (Ghosal et al., 1985).

The effects of other substrates on 2,4-D and 2,4,5-T metabolism are variable. Lackmann et al.(1980) found that glucose or lactose added to a culture actively using 2,4-D was not metabolised until 2,4-D metabolism was complete. These workers also found the addition of glucose at the start of biodegradation increased the overall rate of 2,4-D metabolism, probably by increasing the active biomass concentration (Lackmann et al.,1980). Reber and Kaiser (1980) postulated the formation of different central intermediates prevented either substrate from suppressing the utilization of the partner.

The regulation of MCPA degradation does not appear to be reported in the literature, but because of pathway similarities for 2,4-D and MCPA utilisation, it would be reasonable to assume similar mechanisms.

The Effect of Toxic Metabolites on Degradation.

There are two classes of metabolites that fall into this category; the chlorocatechols (Knackmuss, 1984; Horvath,1971a) and the chlorophenols (Karns et al., 1983a).

Chlorocatechols have been shown to inhibit the catechol-2,3-dioxygenase from Ps. putida by chelating iron (Klecka and Gibson,1981). Both Knackmuss (1984) and Horvath (1971a) describe the chlorocatechols as toxic although neither presents any evidence.

The chlorophenols are also toxic metabolites (Karns et al., 1983a), with high concentrations toxic to whole cells as well as inhibitory to degradation (Tyler and Finn,1974). The toxic effect of the chlorophenols is thought to be due to a decoupling mechanism, separating electron transport from oxidative phosphorylation, which prevents the cells performing energy releasing oxidation (Weinback and Garbus,1965). However recent work with yeast (Saccharomyces cerevisae) has indicated that both 2,4,5-TCP and 2,4,6-TCP attack DNA in the cell nucleus (Kleist-Welch Guerra and Lochmann, 1988).

With these toxic effects, both the chlorophenols and chlorocatechols would be expected to decrease the rate of degradation of the phenoxies.

2.2.1.5 Kinetics of Biodegradation.

Very little work was reported on the kinetics of 2,4-D biodegradation prior to 1980. The first reported attempt at degrading a waste containing 2,4-D and 2,4-DCP was in 1958 (Mills, 1959), which found that trickling filter and activated sludge treatment were both effective and cheap, provided sewage was added to the waste to provide extra nutrients and give added stability to the process.

Subsequently, further work using synthetic wastes using pure substrates has produced more data. The results of a number of studies are given in Table 2.4.

Table 2.4 Summary of Kinetic Parameters from the Literature.

Compound	μmax (h^{-1})	k_s (mg/l)	Y (g/g)	Ref	Comments
2,4-D	0.096	2.7	0.14	Shamat and Maier (1980)	20 C, 2,4-D not inhibitory up to 200 mg/l
2,4-D	0.09	0.6	0.14	Shaler and Klecka (1986)	25 C, 2,4-D not inhibitory up to 200 mg/l
2,4-D	0.14	ND	ND	Tyler and Finn (1974)	25 C, 2,4-D not inhibitory up to 2 g/l
2,4-DCP	0.12	5.1	ND	Tyler and Finn (1974)	25 C,2,4-DCP inhibitory at > 25 mg/l no growth at 100 mg/l
2,4-D	0.15	40	0.14	Papanastasiou and Maier (1982)	20 C, 2,4-D inhibitory at > 95 mg/l
Penta-chlorophenol	0.074	0.06	0.14	Klecka and Maier (1985)	20 C, PCP inhibitory at >0.4 mg/l

The kinetic parameters measured are quite consistent, except for those reported by Papanastasiou and Maier (1982). The variation observed here is probably due to the use of different cultures by the various workers.

Literature pertaining to the kinetics of degradation of MCPA or PCOC could not be found. The degradation kinetics of 2,4,5-T are also not reported, although the data of Kilbane et al.(1982), indicates a slow growth rate and inhibition at concentrations higher than 2 g/l of 2,4,5-T.

As the kinetics of the degradation of MDCPA and MCPB have not been reported, very little is known about the rates of degradation. However, the inhibitory effect appears to be correlated with the degree of substitution of the phenol ring, implying the rate of degradation of MDCPA would be low (Beltrame et al.,1988). These results also imply that the rate of metabolism of MCPB would be equivalent to that of MCPA.

The effect of a second carbon source on kinetics is such that mutual inhibition occurs when 2,4-D and glucose were present together Lackmann et al. (1980), although the overall effect on 2,4-D was masked by the larger concentrations of active biomass produced by the glucose (Papanastasiou and Maier,1982). This has a major influence on continuous systems, as the mean cell residence time required for a specific level of degradation is reduced (Papanastasiou and Maier,1982). More recently, Lindstrom and Brown (1989) stated that acceleration of a target compound removal by the addition of a secondary substrate could offer a benefit in bioremediation systems.

In the case of chlorophenols, very little work has been done. Beltrame et al.(1982) found that both phenol and glucose were equally good at accelerating 2,4-DCP degradation in a CSTR. Scrutiny of their results suggests that high rates of glucose degradation can be achieved, even at high 2,4-DCP concentrations (up to 134 mg/l). This indicates the that toxic properties of 2,4-DCP as described by Tyler and Finn (1974) appear to have no effect on the utilization of the cocarbon source. Recent work by Kleist-Welch Guerra and Lochmann (1988) however, indicated that 2,4,5-TCP and 2,4,6-TCP strongly inhibited the growth of yeast on glucose. There is clearly a considerable difference between these two findings. This situation may apply to the degradation of alcohols in leachate, so there is a need for further investigation into this interaction between substrates.

2.2.2 Aerobic Biodegradation of Alcohols.

The biodegradation of alcohols by microbial cultures is relatively well understood. This section will briefly outline the pathways and kinetics of degradation.

2.2.2.1 Degradation of Methanol.

The pathway for methanol degradation is relatively well defined, after work in the early 1970's investigated this and methane as carbon sources for single cell protein production (Harrison,1973). The pathway contains few steps, with methanol converted to formaldehyde (by methanol dehydrogenase), which is either assimilated, or converted to CO_2 by formate dehydrogenase (reducing NAD^+ to $NADH + H^+$) (Colby et al.,1979). While Pseudomonas species were the predominant organisms capable of degrading methanol, both yeast and bacteria are capable of the degradation and it is thought that the pathways are the same (Colby et al.,1979).

There was very little data available on the kinetics of methanol degradation. Luong (1987) reported the growth rate to be 0.725 h^{-1} (at 30 °C), but qualified that by stating the average was approximately 0.25 h^{-1}. Wayman and Tseng (1976) reporting the data of others, gave the maximum growth rate for Ps. methanica as 0.214 h^{-1}.

2.2.2.2 Degradation of Butan-1-ol and Butan-2-ol.

There is little published data on the degradation of the butanols. It is apparent, however, that these compounds are intermediates in the degradation of the corresponding alkanes (Doelle,1969). Two pathways, which are very similar, are suspected of being involved: monoterminal oxidation and the 2-keto pathway.

Monoterminal Oxidation.

In the degradation of hydrocarbon chains, the most common path is conversion firstly to the primary alcohol, then to the aldehyde and finally to the carboxylic acid (Doelle,1969). This is then subject to β-oxidation, and in the case of butane, produces two molecules of acetic acid which can be used in the TCA cycle (Doelle,1969). It can be seen that the butan-1-ol would enter the pathway at the first step.

2-Keto Pathway.

This alternative route, which has been demonstrated for propane, involves the conversion of the hydrocarbon to the secondary alcohol, propan-2-ol (van der Linden and Thijsse,1965). This propan-2-ol is then either converted to propan-1-ol and degraded by monoterminal oxidation to propionic acid, or oxidised to acetone then 1-hydroxypropan-2-one (van der Linden and Thijsse,1965).

The degradation pathway for butan-1-ol and butan-2-ol therefore is likely to be via the primary alcohol and monoterminal oxidation, based on information presented by Doelle (1969).

With respect to the microbiology of degradation, both bacteria (Doelle,1969; Wayman and Tseng,1976) and yeast (Tseng and Wayman,1975) have been reported as degrading butan-1-ol.

Pseudomonas and Arthrobacter. Both are also associated with chlorophenol and phenoxy degradation, (Table 2.2) along with a Mycobacterium sp. (van der Linden and Thijsse,1965).

A number of studies have been carried out using n-butanol, with maximum growth rates given of 0.34 h^{-1} (Luong,1987) and 0.31 h^{-1} (Wayman and Tseng,1976). However, the kinetics of degradation of butan-2-ol were not reported.

It can be seen that there is a considerable difference between the kinetics of alcohol degradation and phenoxy degradation, although there is considerable overlap in the organism's degradation capacities. Unfortunately, the references on alcohol degradation are old. This is a reflection of the shift from interest in SCP production based on these simple hydrocarbons to other areas of concern such as the formation of alcohols anaerobically (Clarke et al.,1988) and the metabolism of long chain alcohols and fatty acids, in the context of the degradation of surfactants (Rigby et al.,1986).

2.2.3 Summary.

In summarising the information presented in this section, the following points may be noted:

(1) The degradation of the major leachate components has been described. The pathways for alcohol, 2,4-D and 2,4,5-T metabolism are well defined, although that for MCPA is less so.

(2) Chlorocatechols and chlorophenols are the two main toxic compounds likely to be formed during biodegradation.

(3) The degradation of the phenoxies is plasmid mediated.

(4) The degradation kinetics vary with the organism studied and results appear to be contradictory. Thus although some studies found 2,4-D to be inhibitory, many found it to be non-inhibitory.

2.3 Activated Sludge.

2.3.1 Process Description.

The activated sludge process was first proposed as a wastewater treatment method in England by Ardern and Lockett (1914). This name was given to the active mass of microorganisms which were responsible for aerobically stabilising the organic components in the waste. Since then the process has become popular, and is the most widely used biochemical operation in wastewater treatment (Grady and Lim,1980).

Activated sludge systems consist of an aeration tank where an aerobic bacterial population is maintained. The waste to be treated is introduced into the aeration tank, under aerated conditions (referred to as the mixed liquor) where the waste is degraded.

The environment in the reactor is kept aerobic through the addition of air or oxygen by the use of diffused or mechanical aeration. In completely mixed systems, this also provides the agitation required. Mixed liquor is simultaneously withdrawn from the aeration tank. This is then passed to a settling tank, where the cells (which are characteristically flocculating) are separated from the treated waste. A portion of the biomass is then recycled into the aeration tank, and the remainder is wasted. This re-seeding ensures the maintenance of a consistent amount of biomass in the aeration tank. The concentration at which it is maintained depends upon the nature of the waste, the desired treatment efficiency and the kinetics of the microbial growth. Consequently the quantity of biomass used to re-seed the reactor provides the key to the overall kinetics of the waste stabilisation.

This recycling of a portion of the biomass leads to two different residence times for the reactor; the mean cell residence time (MCRT) or solids retention time (SRT), which is the average time which the sludge is retained within the system, and a second parameter, the hydraulic residence time (HRT). The MCRT is controlled by the amount of sludge that is wasted from the effluent, which is known as the wastage rate. These parameters will be discussed further in later sections.

The mixed liquor suspended solids (MLSS) not recycled must be wasted from the system to balance the growth of organisms, and hence maintain a constant MCRT. This wasted sludge requires further treatment to allow safe disposal.

2.3.1.1 Process Variations.

There are many versions of the original process, listed in Table 2.5, however they are all fundamentally similar. Each system will be discussed briefly in turn, as the process configuration has a major effect on the performance, especially with respect to inhibitory compounds.

Table 2.5: Alternative Activated Sludge Process Configurations (from Tchobanoglous,1979).

Conventional (plug flow)
Modified aeration
Step aeration
Completely mixed
Pure oxygen
Contact stabilisation
Extended aeration

Plug Flow Activated Sludge.

The first configuration described for continuous activated sludge (AS) systems was the plug flow or conventional activated sludge system. Influent waste and recycled mixed liquor are introduced at one end of a long rectangular tank and effluent is removed at the other (Figure 2.7). Air is introduced through evenly spaced air diffusers. The design is intended to minimise longitudinal mixing (Grady and Lim,1980). These activated sludge systems are usually designed with a MCRT of 3 to 15 days, with a liquid residence time of 4 to 8 hours and a MLSS of approximately 2000 mg/l for domestic sewage (Grady and Lim, 1980).

Modified Aeration Activated Sludge.

Systems in which the oxygen supply is matched to the oxygen demand are referred to as tapered aeration or modified aeration activated sludge plants (Grady and Lim, 1980). With the conventional plug flow system, the oxygen demand is greatest at the influent end of the tank, where the bulk of the substrate is consumed. The modified aeration system is designed to make more efficient use of the air supply capacity of the plant by supplying a greater proportion of the air to the inlet end of the tank, than to the effluent end where far less oxygen is required.

Step Aeration Activated Sludge.

Initiated in 1942 as a means of more effectively utilising the aeration capacity of the AS plant, the step aeration system is characterised by having the influent waste split into several portions, which are fed at different points (Figure 2.8) into the aeration basin. This design gives a more even distribution of the oxygen demand than occurs in the conventional plug flow system (Grady and Lim, 1980). The system provides considerable flexibility, and has found widespread use.

Completely Mixed Activated Sludge.

The chemical process industries, producing wastes of high strength and containing organic compounds not found in domestic sewage, introduced in the 1950's, the completely mixed activated sludge system, also referred to as the continuous stirred tank reactor (CSTR) configuration (Grady and Lim, 1980). It utilises a completely mixed approach (Figure 2.9) and was developed in an attempt to provide the microorganisms of the sludge a relatively constant environment for growth.

This alteration to the process resulted in an increase in stability, which has lead to the rapid acceptance of the process by engineers (Grady and Lim,1980). Cell and hydraulic residence times and MLSS concentrations in these systems are similar to those for plug flow, although the recycle ratio tends to be higher, as the sludges do not compact as well (Grady and Lim, 1980).

Pure Oxygen Activated Sludge.

One of the major limiting steps in activated sludge is the rate of transfer of oxygen from air bubbles to the cells. When air is used, the sensitivity of the biomass to shear (preventing the use of vigorous aeration), severely limits the rate of transfer of oxygen to the biomass. The use of pure O_2 can increase that rate of transfer by up to five times by increasing the concentration gradient, thereby

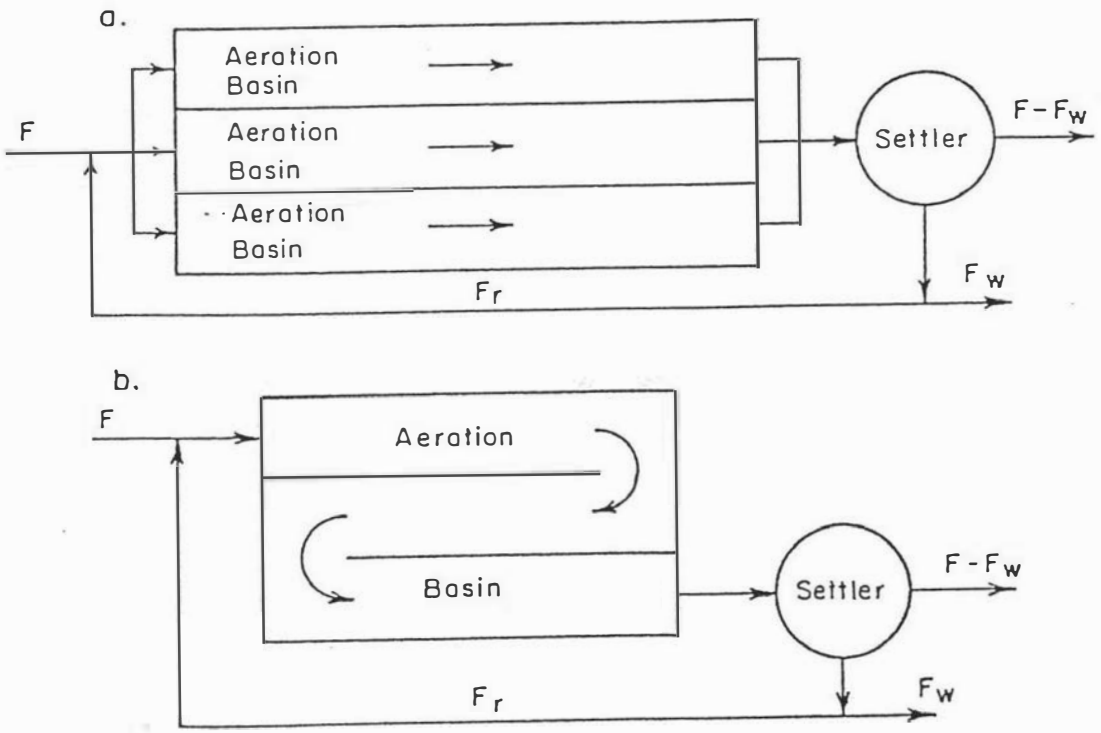


Figure 2.7: Schematic Diagram of a Conventional Activated Sludge Plant: Two Techniques Used (Grady and Lim,1980).

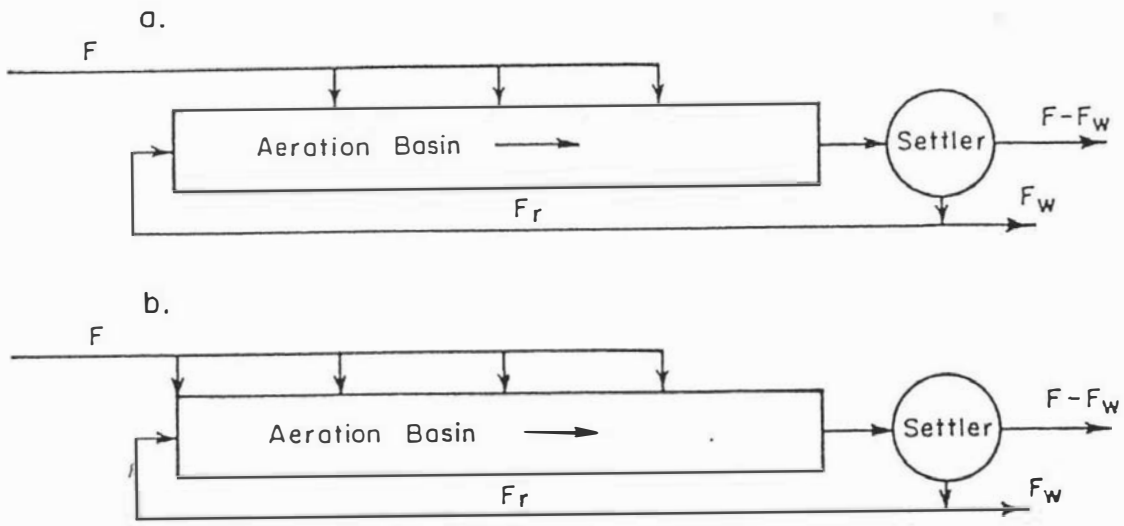


Figure 2.8: Schematic Diagram of a Step Aeration Activated Sludge Plant (a) as proposed, and (b) as commonly used.(Grady and Lim,1980).

greatly increasing the activity of biomass and hence efficiency of the system (Grady and Lim,1980).

To overcome the major problem of oxygenation costs which characterise this system, a stepwise scheme was devised so that the effluent gas from the first stage was recompressed and used in the second. A diagram of the system is shown is Figure 2.10. It has been found necessary in some instances to scrub out the CO_2 generated by the biomass to prevent large decreases in pH from occurring.

Because of the high rate of O_2 transfer, MLSS concentrations of 4000 - 8000 mg/l are possible. With the conventional MCRT of 3 - 15 days still applicable, it is possible to reduce the hydraulic residence time to 2 h for many wastes. The recycle ratio also compares well with the other methods discussed.

Contact Stabilisation Activated Sludge.

The contact stabilisation process is characterised by the rapid removal of substrate from a contact tank (Figure 2.11), by the sludge organisms, followed by stabilisation of the sludge prior to recycle. The residence time in the contact tank is typically 0.5 to 2 h (Grady and Lim,1980). The sludge is then separated from the effluent which is discharged. The sludge is returned to a stabilisation tank, where it is aerated prior to being returned to the contact tank.

The MCRT for this system is still in the 3 to 15 day bracket as used for the other systems, although the recycle ratio is usually higher, being in the 40 to 70% range (Grady and Lim,1980).

Extended Aeration Activated Sludge.

The extended aeration version of the activated sludge process was developed to reduce the large amount of sludge produced by other options (Grady and Lim,1980). Most systems employ a CSTR system, with MLSS concentrations of approximately 5000 mg/l, MCRT's of 20 to 30 days, and recycle ratios of 75 to 150 %. The extended MCRT results in a high rate of endogenous respiration, causing the degradation and removal of large quantities of the cells (Grady and Lim,1980).

A modification to this method has been proposed for the treatment of toxic wastes, hence minimising the amount of potentially hazardous sludge produced. The modified extended aeration system, as proposed by Rozich and Gaudy (1985) (Figure 2.12) includes a chemical hydrolysis step to aid in the aerobic digestion of the recycled biomass. The extra cost of the hydrolysis could by recovered by not having to pay large sums of money to use alternative sludge disposal techniques.

2.3.2 The Microbiology of Activated Sludge.

Activated sludge is the name given to the complex ecosystem of microorganisms that exists in the mixed liquor of an activated sludge plant. The mixed liquor is usually characterised by a single

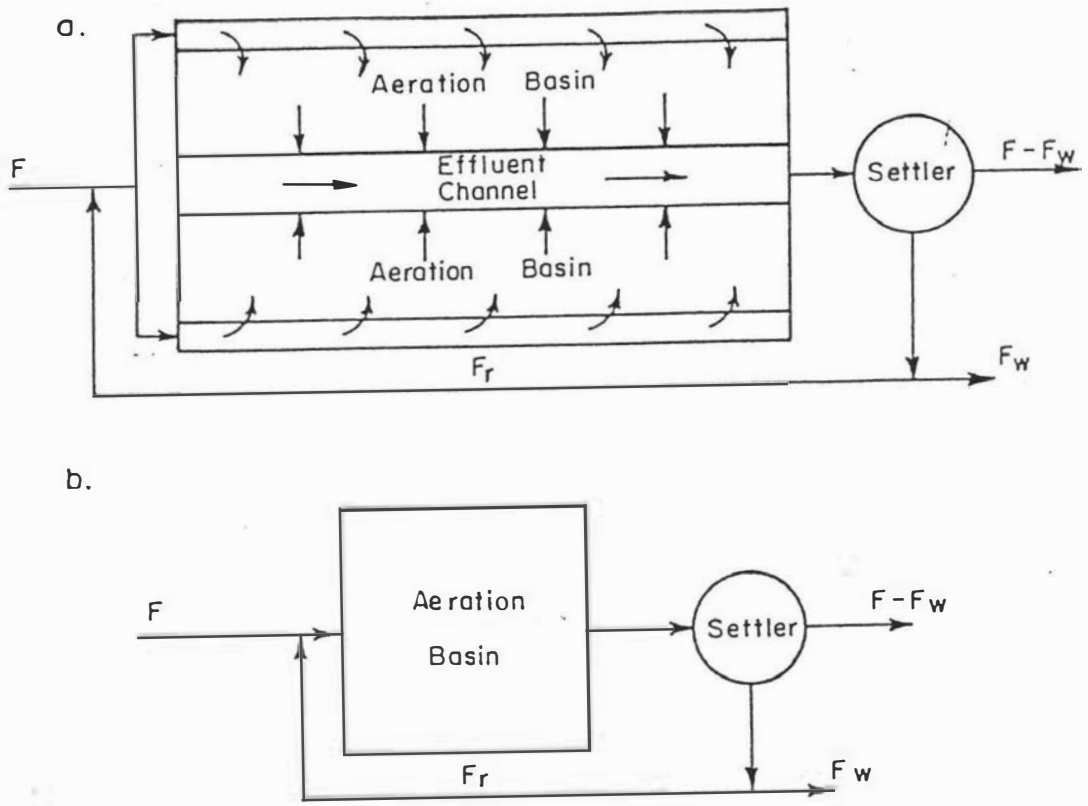


Figure 2.9: Schematic Diagram of a Completely Mixed Activated Sludge Plant (Grady and Lim,1980)
(a) Cross flow, converting plug flow to CSTR and (b) square (new construction)

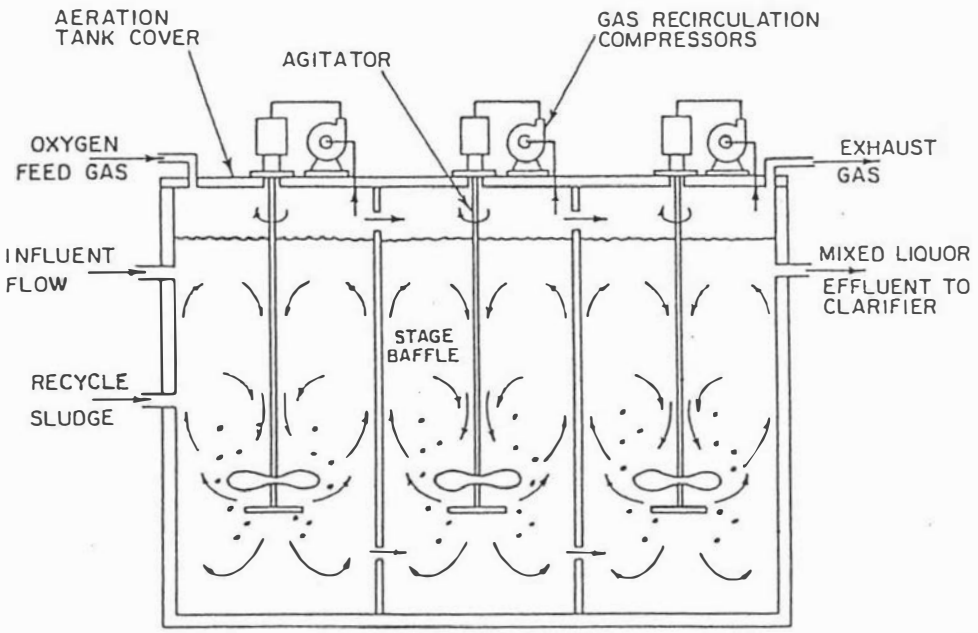


Figure 2.10: Schematic Diagram of a Pure Oxygen Sludge Plant (Grady and Lim,1980)

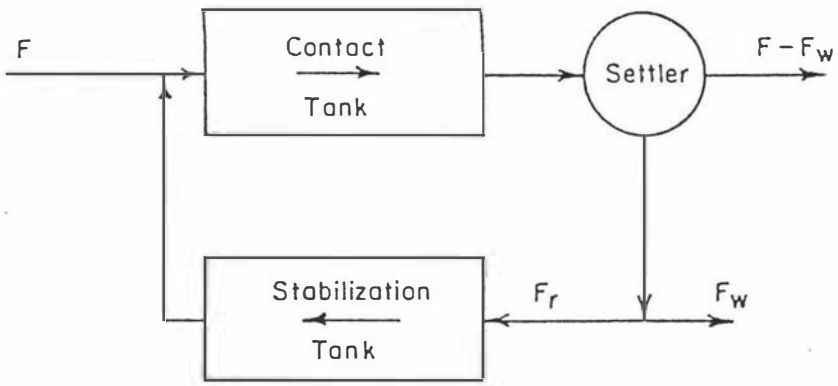


Figure 2.11: Schematic Diagram of a Contact Stabilisation Activated Sludge Plant (Grady and Lim,1980)

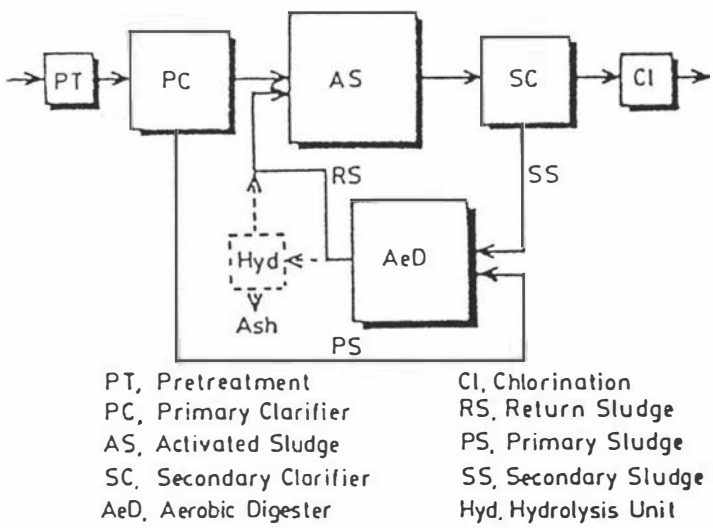


Figure 2.12: Schematic Diagram of an Extended Aeration Activated Sludge Plant as Described by Rozich and Gaudy (1985).

term, the mixed liquor volatile suspended solids (MLVSS), which leads to a drastic oversimplification of the complex microbial interactions that occur in such a treatment system. The following sections give a brief description of the organisms that are usually found in the mixed liquor.

2.3.2.1 Bacteria

The bacteria present in mixed liquor are predominantly heterotrophic bacteria, present in flocs and dispersed in the liquor (Pike,1975). The nature of the process, treating a complex waste and relying on flocculation to concentrate the sludge for recycle, places selective pressures on the community present i.e. the use of a gravity clarifier, and the presence of free swimming predators (such as rotifers) inherently selects for flocculating bacteria. Dissolved oxygen concentrations, pH, nutrient limitations and the presence of toxic compounds also has a profound effect on the organisms present (Pike,1975).

A discrepancy has been noted between the numbers of viable organisms and the total number of organisms present (Greenfield,1987), although this discrepancy appears to decrease as the growth rate and loading increase (Pike,1975). These results imply that a large proportion of bacteria present in the mixed liquor are moribund or dead, as the process is usually operated at very low growth rates.

The majority of the organisms present are usually gram-negative, such as Pseudomonas, Alcaligenes, Flavobacterium and Achromobacter (Pike,1975). Other genera present include Bacillus and Micrococcus , although the range of genera isolated depends greatly on the source and media used (Pike,1975). Flocculation is thought to be aided by the secretion of a polymeric, capsular slime (Pike,1975).

As mentioned earlier, the majority of degradation is performed by the saprophytic bacteria, and as such the can be referred to at the 'prime movers' of the substrate to be degraded. Other organisms found in the mixed liquor are generally secondary to these organisms (Greenfield,1987). However, filamentous bacteria (such as Sphaerotilus) can contribute to the bulking problem in a similar manner to fungi (Pike, 1975)

2.3.2.2 Fungi

Fungi are usually present in small numbers in activated sludge, although they are rarely dominant when the plant is running at the correct organic loading (Greenfield,1987). The presence of large numbers of filamentous fungi in a plant leads to the problem of sludge 'bulking', a problem where the specific gravity of the sludge is reduced to a point where gravity settling is very difficult (Grady and Lim,1980). This may be caused by a number of factors such as a low pH, the addition of toxic compounds, underloading or overloading (Tomlinson and Williams,1975). Geotrichum is often responsible for this problem (Tomlinson and Williams,1975).

2.3.2.3 Protozoa and Rotifers.

Protozoa

These are small, multi- or uni-cellular eukaryotic organisms that are frequently present in activated sludge (Greenfield,1987). They are motile, and generally 10 times larger than bacteria (Greenfield,1987). Ciliated organisms are more likely to be present in an established system than the flagellated species (Curds,1975). The majority are aerobic heterotrophs (Greenfield,1987), and are sensitive to low dissolved oxygen, pH variations and large amounts of carbon dioxide (Curds,1975). Their role is to add a "final polish" to the effluent by reducing the BOD_5 which microbial cells represent. They consume free swimming bacteria, and particulate organic matter, hence reducing the amount of material that will not be removed by gravity settling (Greenfield,1987). The presence of these organisms in activated sludge can be used as an indicator of the operation of the plant and effluent quality.

Rotifers

Rotifers are aerobic multicellular animals, usually possessing a foot for attachment, and two sets of rotating cilia for motility and catching food (Doohan,1975).

These organisms also prey on bacteria, both dispersed and flocculated, and degrade particulate organics (Greenfield,1987). By breaking up flocs, they provide nuclei for further floc formation, as well as clearing the effluent by removing free swimming organisms (Doohan,1975). The presence of rotifers is thought to indicate a highly efficient purification process (Greenfield,1987).

2.3.3 Process Considerations.

2.3.3.1 Toxic Compounds.

The effects of toxic compounds on the oxidative efficiency of activated sludge plants are exactly the same as would occur in any active biological process faced with an inhibitor. In the case of activated sludge, the inhibitors fall into two classes: inorganic and organic.

Inorganic Inhibitors.

This class contains boron and the heavy metals such as chromium, copper, lead, silver and arsenic. It also includes anions such as cyanides and chromates present in some industrial wastes (Tchobanoglous,1979). If such compounds are present in concentrations sufficiently high to cause inhibition, then they must be physically or chemically removed prior to biological treatment. Care must also be taken to ensure that toxic concentrations do not bioaccumulate in the sludge, and cause sludge disposal problems (Melcer,1987).

Organic Inhibitors.

At first glance, the presence of high concentrations of a toxic organic chemical would appear to preclude the use of a biological treatment system. As the waste to be considered here contains high concentrations of chlorophenols, this appears to eliminate the use of activated sludge as a treatment

option. The presence of biodegradable toxic organics does not, however, prevent the use of activated sludge, but does affect process configuration selection.

According to current thinking plug flow systems, and their various modifications cannot be used for toxic organics, because the presence of the such compounds will reduce the overall rate of removal to values much lower than can be achieved by alternative configurations. This reduction can be explained in term of inactivation of the returned sludge due to exposure to the fresh incoming waste, and increasing the volume requirements of the system (Grady and Lim,1980). For this reason plug flow systems are not used for toxic compounds.

A recent theoretical study by Santiago and Grady (1989) has suggested that plug flow systems are more stable than expected. These authors characterise the inhibitory nature of a compound by the ratio of K_I to K_s, as the β value. It is suggested that plug flow systems will perform better than a CSTR to a shock load of a mild inhibitor ($\beta = 5$), but will be more sensitive to subsequent shock loads. Based on the phenol data of Rozich and Gaudy (1984), phenol is four times more inhibitory than the mild inhibitor of Santiago and Grady (1989), with a β value of 1.05. The data of Tyler and Finn (1974) however, indicates a β value of 3.05 for 2,4-DCP, implying it is less inhibitory than phenol, although the K_I for 2,4-DCP is 35.7 mg/l c.f. 62 mg/l for phenol. As it appears that 2,4-DCP would be more inhibitory than phenol, there are grounds for questioning this approach.

A second flaw in the study of Santiago and Grady (1989) is the assumption that the inhibition is reversible. The toxic nature of the chlorophenols, and their ability to attack DNA (Kleist-Welch Guerra and Lochmann,1988) suggests such an assumption may be invalid. As it appears two of the assumptions may be violated, it would be wise to use a more conservative approach and choose the CSTR approach.

For the reasons outlined above, this thesis is concerned with the oxidation of toxic compounds and hence further discussion will be limited to CSTR approaches.

CSTR systems are the most widely used AS systems for the degradation of toxic compounds (Melcer,1987). This is because the aeration basin provides equalisation facilities in addition to the treatment (Grady and Lim,1980). The nature of CSTR systems is such that newly introduced toxic compounds are immediately dispersed throughout the aeration basin, and hence do not reach concentrations high enough to warrant concern.

Another recent development (Melcer,1987) is the introduction of the powdered activated carbon treatment process (PACT). This process involves the addition of small quantities of activated carbon to the aeration basin, which serves as a support for microbial growth, and as a matrix for organic compounds prior to their oxidation by the organisms present (Melcer,1987). Other claimed benefits include better sludge settling and greater stability to shock loads (Melcer,1987). Work has shown that this addition (at approximately 100mg/l activated carbon) does not assist the removal of readily

degraded compounds such as benzene and toluene, but does assist in the removal of the more recalcitrant molecules such as 1,2-dichlorobenzene and 1,2,4-trichlorobenzene (Melcer,1987).

2.3.3.2 Control of Mean Cell Residence Time.

The mean cell residence time (MCRT) is probably the most important control parameter in an activated sludge plant, especially one treating inhibitory compounds (Lange et al.,1989). The main factor affecting the control of the MCRT is the ease of separation of the biomass from the treated waste (Grady and Lim,1980). The most common method of achieving cell separation in activated sludge plants is gravity settling (Tchobanoglous,1979). However, effective settling can only be achieved when the organisms have flocculated into particles large enough to settle rapidly (Grady and Lim,1980).

It has been observed that flocculation only occurs between two threshold values of MCRT, a lower value of about 2 days, below which dispersed growth makes settling difficult, and an upper limit of 15 days, above which endogenous respiration causes the flocs to break up and become dispersed pin flocs (Grady and Lim,1980).

Both of these problems have the same effects: the inability to produce a good, well thickened sludge for recycle and wasting, and also to allow large amounts of the biomass to be lost in the effluent, decreasing its quality.

Despite the pin floc problem at high MCRT's, such times are often required for industrial wastewaters (Grady and Lim,1980). As a result of this, it is important to study sludge settling characteristics during laboratory scale testing of such processes.

Clarifier design plays an important role in the control of MCRT. For activated sludge use, the clarifier must perform three functions:

(1) allow the flocculation of the bacteria to occur under conditions of low shear,

(2) allow sufficient time for settling to occur under conditions that do not cause the flocs to break up,

(3) allow the thickening of the sludge to a point where the recycle ratio does not require an excessively large aeration basin (Tchobanoglous,1979).

A typical clarifier used with activated sludge plants is shown in Figure 2.13. The circular system is based on the influent mixed liquor being introduced to the centre of the clarifier, and the clarified effluent being removed at the edges via a weir system. The tank bottoms are also scraped and slope to the centre to encourage the movement of sludge to the drawoff point. Drawoff is usually by vacuum (Eckenfelder,1980).

The thickening of the sludge is also important to maintain the MCRT. If the sludge was not thickened appreciably, then the recycle ratio would be increased to the point where the recycle flow

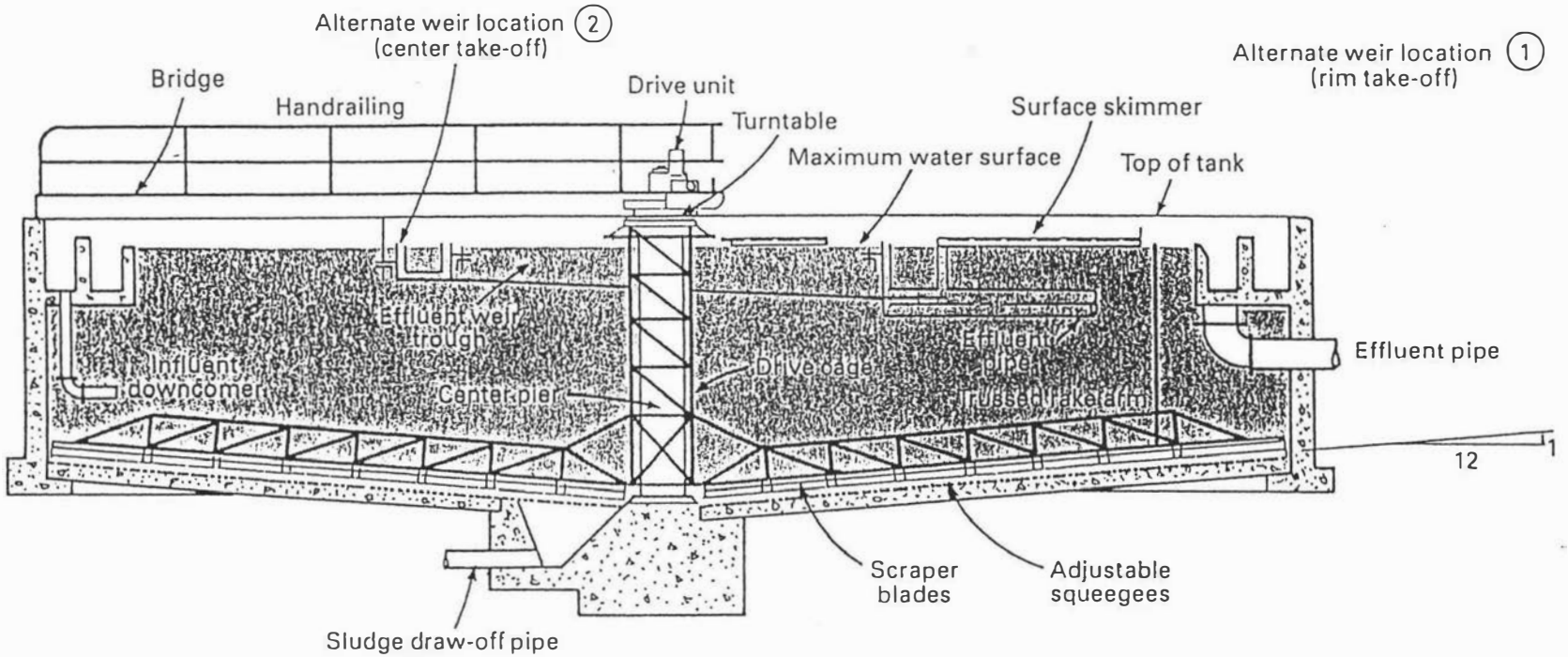


Figure 2.13: Typical Clarifier Used in Activated Sludge Systems (from Eckenfelder,1980)

from the clarifier was much greater than the waste flow ie a recycle ratio much greater than 100%. Such high recycle ratios, while not operationally impossible to maintain, are almost never used in practice.

The main operational problem which may affect the settling and thickening of the sludge (and hence the MCRT) is bulking, where the presence of filamentous bacteria and fungi produces light, 'fluffy' flocs which do not settle well, resulting in poor effluent quality (Pike,1975). As stated earlier, bulking can be caused by a number of factors, and to solve a bulking problem or any given plant will require a detailed analysis of the mixed liquor and plant loading rate (Pike,1975).

With these criteria satisfied, it is possible to run an effective AS plant. Varying the MCRT is an effective means of coping with variation in feed quality, temperature and other factors providing the variations are relatively slow (Grady and Lim,1980). Whilst automatic control of the major parameters is not difficult (Vaccari et al.,1988), process control with rapid changes in influent conditions is much more difficult than if physical or chemical treatment were used (Grady and Lim,1980).

2.3.3.3 Sludge Disposal.

Sludge disposal needs to be mentioned as wasted sludge is the largest by-product of the activated sludge process. As this thesis is not concerned principally with the disposal of sludge, a brief summary of a recent review of the topic will be provided for completeness.

Sludge for further treatment typically has a moisture content of 94 to 98 % water (Bogoni,1988), and the first step of the disposal process is to reduce the moisture to give a solids content of up to 15 %. There are three main methods of disposal of sludge:

(1) Anaerobic digestion of the sludge to produce methane and a small quantity of digested sludge which is then easily dewatered or incinerated (Bogoni,1988). This process is widely used, although the size of systems and control difficulties are a problem (Bogoni,1988).

(2) Aerobic digestion of the sludge to produce CO_2 and water by the action of aerobic bacteria to reduce the volume and organic content, which includes composting (Bogoni,1988) and

(3) Other techniques such as incineration (applicable only to small quantities due to the cost) and land and landfill disposal, both of which require the sludge to be of a low moisture content, and relatively harmless (with respect to pathogens and heavy metals) (Bogoni,1988).

Other methods are available, such as marine dumping (which has been found to contribute to heavy metal poisoning) newer, more expensive techniques such as the production of crude oil by thermal liquefaction are being investigated (Bogoni,1988).

The most widely used method for sludge stabilisation has been anaerobic digestion, although the trend today is towards aerobic digestion, which is the easier process to operate (Grady and Lim,1980). Dewatering of sludge can be achieved by filtration, centrifugation or the use of sludge drying beds (Tchobanoglous,1979).

2.3.3.4 Tertiary Treatment of the Effluent.

The term tertiary treatment can be used to describe a number of processes, such as denitrification, nitrification-denitrification, phosphate removal, removal of inorganics and removal of refractory organics (Tchobanoglous,1979). In this case, it will be narrowed down to look only at the removal of refractory organics.

The most common form of tertiary treatment for the removal of residual organics is carbon adsorption (because of its relatively low cost) followed by chemical oxidation using chlorine or ozone (Tchobanoglous,1979). This review will be confined to granular carbon adsorption as a method for tertiary treatment, as recommended by Cope (1983).

Description.

Granular activated carbon is often used in fixed bed columns as a method of contacting the carbon with the wastewater. A typical column is shown in Figure 2.14. Generally such columns are operated in a down flow mode, with provision for backwashing to prevent excessive pressure drops occurring (Tchobanoglous,1979). Columns can be operated singly, in series or in parallel.

Spent carbon usually requires regeneration to make the process economic, usually accomplished by heating the carbon in the absence of air (Vesilind et. al,1988). However this process usually results in a carbon of lower capacity, and the loss of 5 - 10 % of the carbon in transit, so there is a need for the addition of virgin replacement carbon.

Activated carbon has been used to treat effluent from plants producing phenoxy herbicides (Fox,1985). More recently synthetic resins, such as Amberlite XAD-4, are being used, as they are easier to regenerate and have a longer useful life (Catt,1990). The adsorbents may have different capacities which depend greatly on the waste in question, but the same process analysis can be applied to both.

Process Analysis.

There are four principle types of adsorption: exchange, physical, chemical and specific (Weber,1985). The extent of adsorption also relates to surface tension and solubility. These factors, along with surface area and nature of the adsorbate determine the equilibrium capacity and rate of adsorption (Weber,1985). Equilibrium is reached when the rate of adsorption is equal to the rate of desorption.

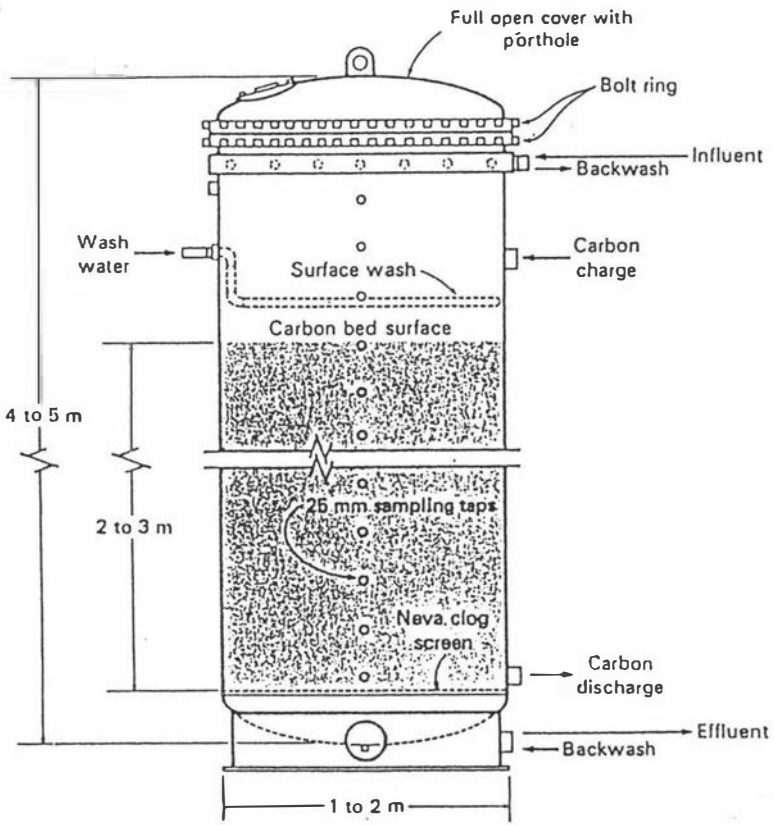


Figure 2.14: Typical Activated Carbon Adsorption Column (from Tchobacoglous, 1979)

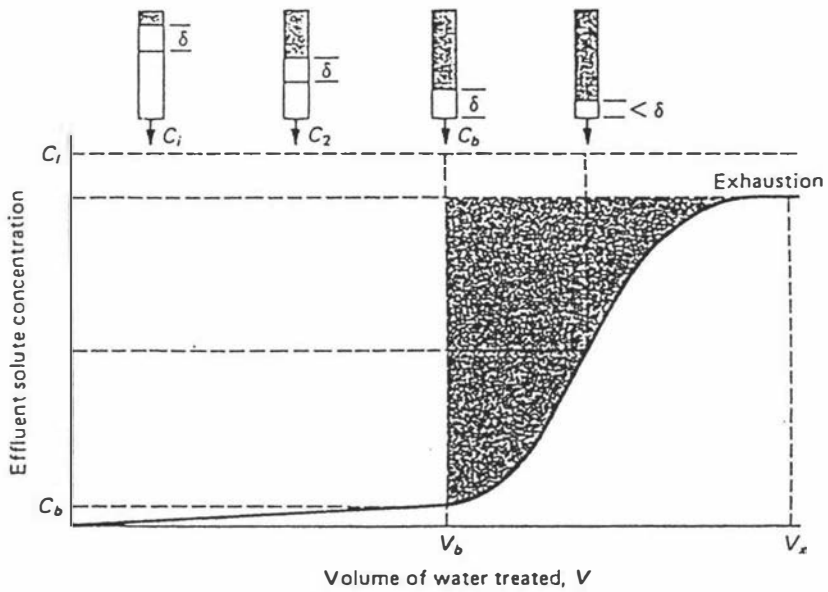


Figure 2.15: Typical Breakthrough Curve for Activated Carbon Column (from Tchobanoglous,1979) V_b = Breakthrough Volume, V_x = Exhaustion Volume, C_b = Breakthrough Concentration, C_I = Inlet Concentration, δ = Active Zone.

There are two major equilibrium equations used in wastewater treatment, the Freundlich isotherm and the Langmuir isotherm (Tchobanoglous,1979). These are given in equations (2-1) and (2-2) respectively.

$$\frac{X}{M} = kC^{1/n} \qquad (2\text{-}1)$$

$$\frac{X}{M} = \frac{abC}{1 + bC} \qquad (2\text{-}2)$$

where X/M = amount adsorbed per unit weight of adsorbent

C = equilibrium concentration of adsorbate in solution after adsorption

k,n,a,b = empirical constants

Constants can be determined by plotting X/M versus C on a log/log graph for eqn (2-1) and plotting C/(X/M) versus C for eqn (2-2).

The rate of adsorption is usually controlled by the rate of diffusion through the stationary layer surrounding the adsorbent particle (Tchobanoglous,1979).

Once the capacity of the absorbent has been determined, it is possible to determine the amount of carbon required to treat a given volume of effluent at equilibrium. Equilibrium usually exists in the top of a column (Tchobanoglous,1979), however the breakthrough phenomenon (Figure 2.15) occurs at the bottom of the bed, requiring columns to be run in series to allow the adsorbent to be equilibrated throughout the bed (Weber,1985). Optimum flowrate and bed depth need to be determined using pilot scale equipment, as these parameters can only be found using dynamic column tests (Tchobanoglous,1979). However the isotherm can be used to evaluate the economic feasibility of adsorption (Bernardin,1985).

2.4 Mathematical Description of a CSTR Activated Sludge System.

This section will describe the models available for the description of activated sludge systems, and their use. The description can be divided into two areas, (1) the microbial system: describing the manner in which the microorganisms present in the activated sludge mixed liquor behave in relation to the physical and chemical characteristics of the feedstock, and (2) the physical system: modelling the physical flow parameters of the plant, and their interaction.

2.4.1 The Microbial System.

Experimentally, it has been found that the effect of a growth limiting, non-inhibitory substrate or nutrient can be defined by the Monod equation (Pirt,1975):

$$\mu = \mu_{MAX}.S/(K_s + S)$$

where

μ = specific growth rate (h^{-1})

μ_{MAX}= maximum specific growth rate (h^{-1})

S = residual concentration of the growth limiting substrate (mg/l)

K_s = half saturation constant: the substrate concentration that gives μ equal to one-half of μ_{MAX} (mg/l)

From the value of μ it is possible to calculate the rate of growth (r_g) from the following equation:

$$r_G = \mu_{MAX}XS/(K_s + S)$$

where

X = biomass concentration (mg/l)

The rate of growth of new cells is linked to the rate of substrate removal (r_s) by the following equation:

$$r_G = -Y_{X/S}r_s$$

where

$Y_{X/S}$ = growth yield coefficient, which is the ratio of the mass of cells formed to the mass of substrate consumed. (mg/mg)

The term μ_{MAX}/Y is the maximum specific substrate removal rate (Q_{MAX} or k), and when this is substituted into previous equations the result is;

$$r_s = \frac{-Q_{MAX}XS}{(K_s + S)} \qquad (2\text{-}3)$$

The equations given all describe balanced growth, where all the cells are in the same growth phase (ie log phase). In wastewater treatment systems there are age distributions (Tchobanoglous,1979), and the effect of this variation often needs to be accounted for. The easiest manner of doing this is to assume that the loss of active cells is proportional to the total number of organisms present (Tchobanoglous,1979). This leads to the following equations:

$$r_G' = \frac{\mu_{MAX}XS}{(K_s+S)} - k_dX$$

or

$$r_G' = -Y_{X/S}r_s - k_dX$$

where

k_d = endogenous decay coefficient (h^{-1}), and

r_G' = net rate of bacterial growth (mg/l.h).

The effect of this respiration on the yield is accounted for by defining the observed yield coefficient (Y_{obs}):

$$Y_{obs} = -r_G'/r_s$$

Whenever measurements are made of the growth rate or yield coefficient, the values generated are the observed values, and not the true values that were previously defined.

These equations are the basic, widely accepted expressions used to describe the microbial population degrading a waste that does not contain any inhibitory compounds. The equations used to describe inhibited systems will be discussed in a later section.

2.4.2 The Physical System.

The physical system cannot be considered without the use of the expressions developed in the previous section. This section will look at the effect of the physical configuration, and derive the equations needed to describe the degradation of a non-inhibitory waste in an activated sludge system.

The system is shown in Figure 2.16. There are a number of assumption that need to be made before analysis can be performed:

(1) The concentration of microorganisms in the influent is negligible.
(2) Steady state conditions apply ie $dX/dt = 0$.
(3) There is no wall growth.
(4) There is perfect mixing in the system.
(5) The distribution of states, segregation and stochastic phenomena that regulate cell growth are negligible.
(6)There is no degradation of substrate in the clarifier.
(7)The only volume in the system is that of the aeration basin.

The hydraulic residence time (θ) is defined as:

$$\theta = V/F$$

where

V = aeration basin volume (m^3)
F = waste flowrate (m^3/h)

The mean cell residence time (θ_C)is described by;

$$\Theta_C = VX/(Q_W X_R + (F-Q_W)X_E)$$

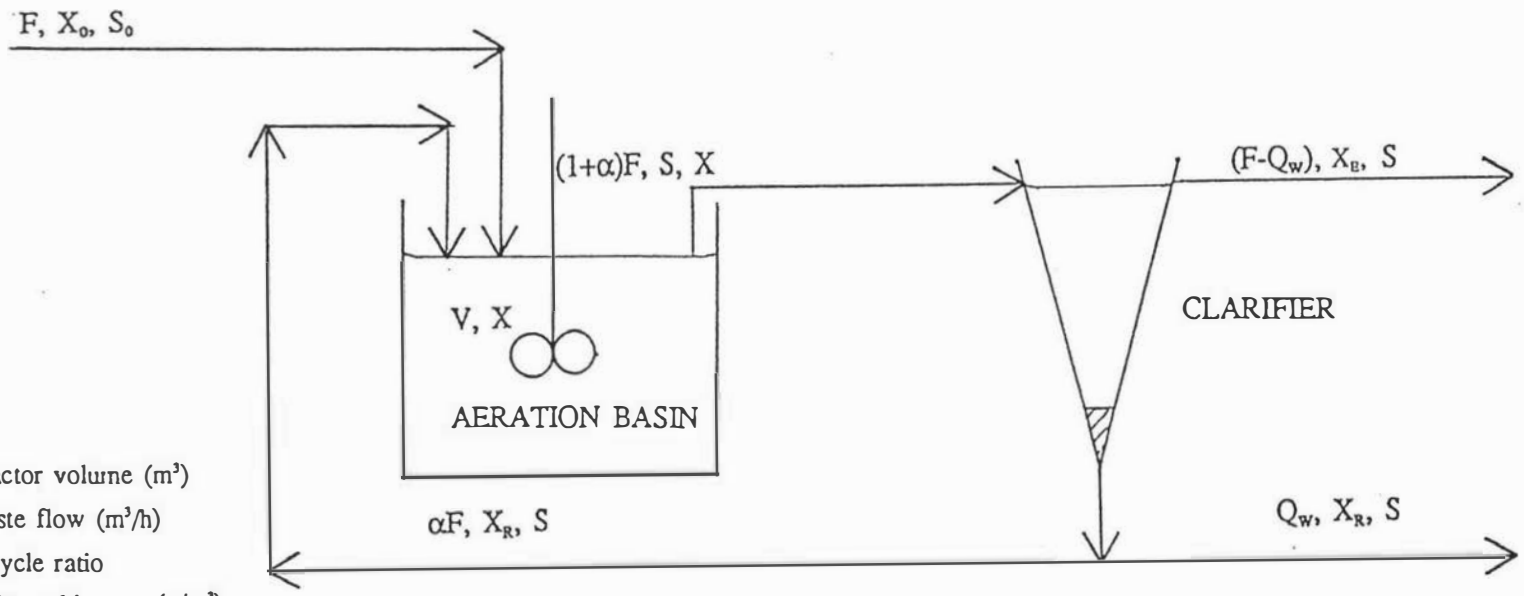
where X_R = biomass concentration in the returned sludge (mg/l)
X_E = biomass concentration in the effluent (mg/l)

assuming X_E is very small, which should be true for a plant that is operating well, this equation simplifies to;

$$\Theta_C = VX/Q_W X_R$$

A mass balance around the clarifier reveals X_R and $\propto$ are not independent. Again assuming $X_E = 0$ gives

$$X_R = \frac{(1 + \propto)FX}{Q_W + \propto F}$$



V = reactor volume (m^3)
F = waste flow (m^3/h)
α = recycle ratio
X_0 = influent biomass (g/m^3)
S_0 = influent substrate (g/m^3)
X = reactor biomass (g/m^3)
X_R = recycle biomass (g/m^3)
S = reactor substrate (g/m^3)
X_E = effluent biomass (g/m^3)
Q_w = wastage rate (m^3/h)

Figure 2.16: Typical CSTR Activated Sludge Flow Diagram.

A mass balance over the entire system can be written as:

Accumulation = inflow - outflow + net growth

Assuming no biomass in the feed and steady state conditions prevail, the following expression is true;

$$1/\theta_c = -Y_{x/s}r_s/X - k_d$$

or

$$1/\theta_c = Y_{x/s}Q - k_d$$

The form of this equation is linear, and hence it can be used to determine the values of the constants. This procedure will be discussed in the next section. The equation can, however, be rearranged to give the following expression:

$$X = \frac{\theta_c Y_{x/s}(S_o-S)}{\theta(1+\theta_c k_d)} \qquad (2\text{-}4)$$

Where S_o = initial substrate concentration (mg/l)

2.4.3 Determination of Uninhibited Kinetic Parameters of an AS Plant.

According to a number of authorities on activated sludge design, the best and most reliable method of determining the parameters required is from a series of steady state experiments at varying SRT's (Grady,1985; Busch,1971; Eckenfelder,1980). This method is incapable of producing any data on the inhibition constants for toxic substrates (Grady,1985), but does have the advantage of producing results suitable for design purposes, even if a model cannot be found to satisfactorily fit the data (Busch,1971).

To determine the four constants required (Q_{MAX} or k, k_s,k_d and $Y_{x/s}$), two separate plots of the data are required. As indicated earlier in this chapter, the microorganism mass balance produces one of the useful plots. By plotting Q versus $1/\theta_c$, the resulting plot should yield a straight line of slope $Y_{x/s}$, and with a y-intercept of $-k_d$. This plot thus gives two of the four parameters.

By altering the expression for the rate of substrate removal the following expression can be found:

$$Q_{MAX}S/(k_s + S) = (S_o - S)/\theta X$$

inverting this equation gives;

$$X\theta/(S_o-S) = (k_s/Q_{MAX})/S + 1/Q_{MAX}$$

thus by plotting $X\theta/(S_o-S)$ versus 1/S, Q_{MAX} can be found as the intercept, and k_s can be back calculated from Q_{MAX} and the slope of the line.

These two plots of data, to yield four parameters, are sufficient to describe the behaviour of uninhibited activated sludge systems.

It is possible to base the design of systems on batch data, but work with straw paper wastewater has determined that such methods tend to oversize the aeration basin, i.e. underestimate the rate at which degradation can occur (Del Borghi et al.,1978).

2.4.4 Mathematical Description of Inhibited Microbial Growth.

2.4.4.1 Models Described in the Literature.

The most common expression used for modelling the degradation of inhibitory compounds is the Haldane model, which has been used to describe the degradation of phenol (Hill and Robinson,1975; Pawlowsky and Howell,1973a and b; Gaudy and Rozich,1982), PCP (Klecka and Maier,1985), 2,4-DCP (Tyler and Finn,1974) and 2,4-D (Papanastasiou and Maier,1982). The model is in the form

$$\mu = \frac{\mu_{MAX} S}{k_s + S + S^2/k_I} \qquad (2\text{-}5)$$

Where k_I = inhibitor constant, the concentration where the growth rate is reduced to half the maximum by the substrate (mg/l).

Pawlowsky and Howell (1973a) tested five different models for describing phenol degradation and found that due to experimental scatter, there was no significant difference between them, however, these authors continued to use the Haldane model for subsequent work (Pawlowsky and Howell,1973b). The one major disadvantage of this model is that it will never predict total inhibition.

Linear inhibition kinetics have been reported for substrates such as 2,4-DCP (Tyler and Finn,1974) and alcohols such as methanol and n-butanol (Wayman and Tseng,1976). In these cases, there was a linear reduction in growth rate with increasing substrate concentration until a concentration was reached above which there was no growth.

Exponential and Teissier kinetics have also been used to describe substrate inhibition, although both suffer from being unable to predict total inhibition (Luong,1987).

Luong (1987), adapting work done by Levenspiel (1980) on product inhibition proposed a generalised model for substrate inhibition;

$$\mu = \frac{\mu_{MAX} S}{(k_s + S)} \left(1 - \frac{S}{S_M}\right)^n \qquad (2\text{-}6)$$

Where S_M = substrate concentration above which growth is completely inhibited

and n = empirical constant.

This model can predict linear inhibition curves, as well as curves similar to the Haldane equation.

Most recently, Watkin and Eckenfelder (1989) proposed the following general model, based on work with 2,4-DCP;

$$\mu = \frac{\mu_{MAX}\, S}{(K_s+S)(1+(s/k_I)^n)} \qquad (2\text{-}7)$$

Where k_I is the inhibition constant and n is an empirical constant.
This model can also predict a variety of inhibition curves, but cannot predict total inhibition.

2.4.4.2 Determination of Inhibition Constants.

As stated earlier, it is not possible to run a CSTR system under inhibitory conditions without some sort of feedback control, which is impractical for experimental use (Grady,1985). It is therefore necessary to determine the inhibition constants by another method.

There are two methods available for determining these parameters. The first, and most widely used is the batch method. In this method, large amounts of biomass are added to a prepared medium containing the substrate of study, and the decrease in substrate and increase in biomass with time are monitored.

From this data, the kinetic parameters can be determined. In the case of the Haldane model, a plot of $1/\mu$ versus S gives a y-intercept of $1/\mu_{MAX}$ and a slope of $1/(\mu_{MAX}.k_I)$. Non-linear curve fitting can also be used (D'Adamo et al.,1984). Parameters for the linear model can be determined directly from a plot of μ versus S. Determination of the constants for other models, however, require the use of nonlinear least-squares regression techniques.

The principle disadvantage of this method, especially with toxic materials, is that when the biomass is introduced to a high concentration of a toxic compound, even though it may be acclimated to it, a proportion of the biomass can die, changing the nature of the biomass, and preventing it from predicting what will happen in a CSTR (Moos et al.,1983; Philbrook and Grady,1985). Despite this major disadvantage it is the most widespread technique in use.

Recently a newer, and potentially more effective method appeared in the literature, the Modified Infinite Dilution Test (MIDT) (Philbrook and Grady,1985) or the Fed Batch Reactor method (FBR) (Watkin and Eckenfelder,1989). Both these tests work on the principle of adding substrate to a sample of biomass at a rate faster than the biomass is able to utilise. The buildup of substrate in the medium can be monitored, and from the resulting plot of substrate versus time, inhibition constants can be determined. Figure 2.17 shows typical responses of biomass to an MIDT test.

According to Philbrook and Grady (1985), this technique can also be used to determine the k_s value of a substrate by feeding the biomass at one half of the maximum rate, and measuring the residual substrate concentration.

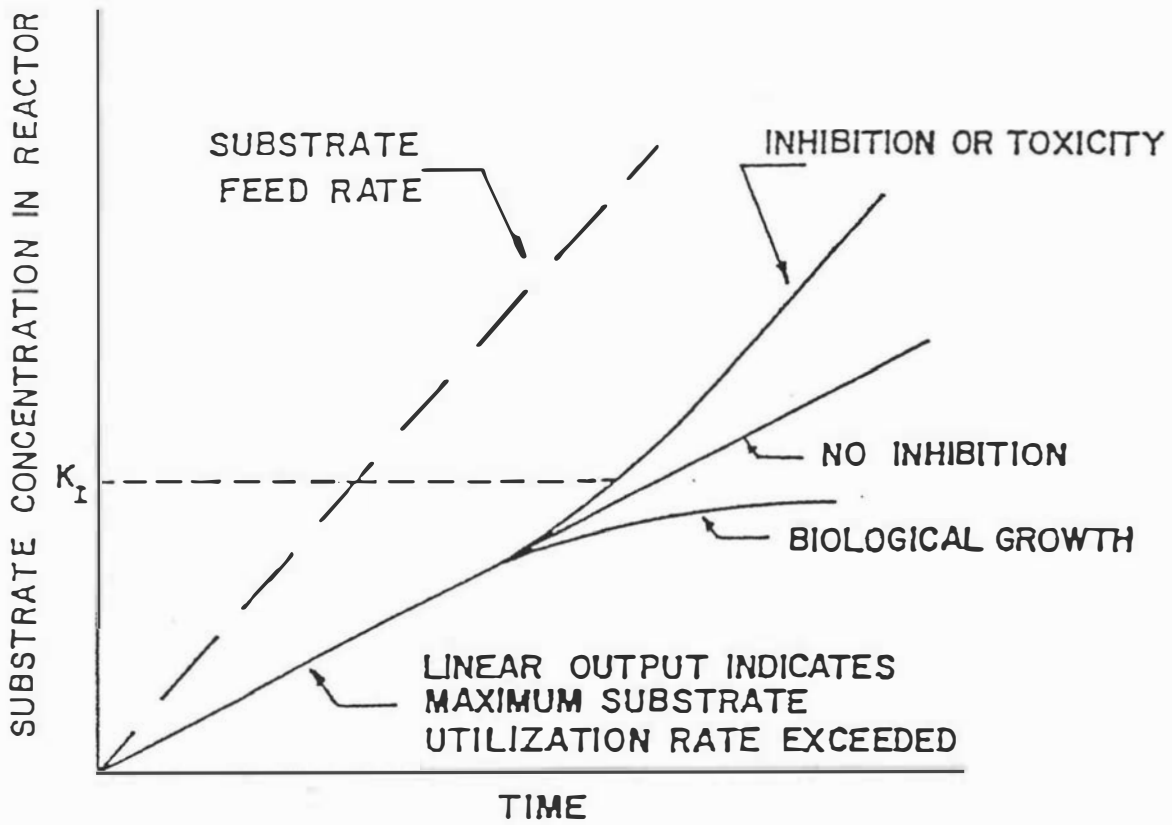


Figure 2.17: Theoretical Fed Batch Reactor Response (from Watkin and Eckenfelder,1989)

This method provides a rapid and accurate method of determining the parameters for a culture, provided the following conditions can be met:

(1)There is no significant change in the biomass nature or mass in the system.
(2)There is no significant change in the volume of the system.
(3)The substrate flowrate exceeds the rate at which the biomass can degrade it (Watkin and Eckenfelder,1989; Philbrook and Grady,1985).

Of the two methods for the determination of inhibition kinetics, the MIDT method is probably the better of the two providing the conditions can be met. If the substrate is not inhibitory, and produces large amounts of biomass (has a high $Y_{X/S}$ value) then the test may fail to produce good results, and hence batch tests will be required to determine the kinetic parameters.

2.4.4.3 Effect of a Biodegradable Inhibitor on an AS Plant.

The presence of a biodegradable inhibitor in the feed to an activated sludge system exhibits a marked effect on the performance of the plant. The effect of a small increase in the basin concentration of the inhibitor has the effect of inhibiting the biomass removing that compound, leading to a further increase in the concentration of that component. The end result of this is that at a critical point , that is dependant on the growth rate and inhibition characteristics, there is a step increase in the concentration of the inhibitory compound, causing sudden plant failure and a rapid washout of the cells in the system. This is in total contrast to a noninhibited system, where a small change would merely result in a failure to meet a discharge standard (Gaudy and Rozich,1982; Rozich and Gaudy,1984). The difference can be seen in Figure 2.18, generated from model data for the degradation of phenol (Gaudy and Rozich,1982).

Some authors prefer to use an uninhibited model for inhibitory wastes, and restrict the range of use to dilution rates where inhibition does not occur (Gaudy and Rozich,1982). This, however, implies any other user of the model must equally be aware of the limitations, without such limitations being obvious from the model.

For this reason, and for the generation of a more robust model, Gaudy and Rozich (1982) proposed the method of Critical Point Analysis for toxic waste treatment. This method relies on determining the inhibition constants for the waste to be treated (usually by batch methods), and combining these data with other information required for design of an AS plant, such as X_R and α, to give the critical dilution rate D^*, above which there will be rapid biomass washout and plant failure. The dilution rate of Rozich and Gaudy (1982) is an hydraulic rate.

Knowing the biokinetic constants, and the engineering parameters to be used, it is possible using this method to select design and operational strategies that will not cause plant failure. Gaudy et al. (1988) implies that this method can be used to determine the effect of an inhibitor on an AS

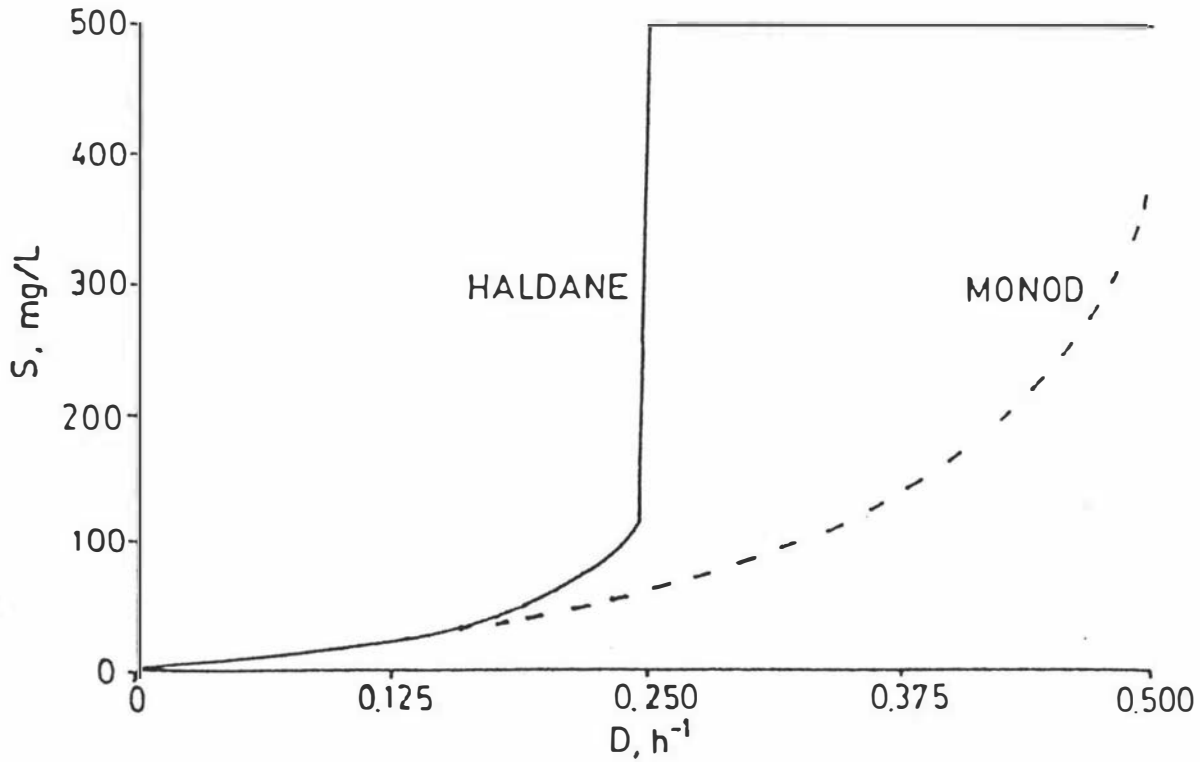


Figure 2.18: Dilute out Curves Calculated for Monod and Haldane Models for Phenol (from Gaudy et. al.,1988).
(μ_{MAX} = 0.6 h^{-1}, k_s = 75 mg/l, k_I = 150 mg/l, Y = 0.5 g/g)

plant treating domestic sewage, although the model takes no account of any substrate interactions. The model also allows the calculation of substrate concentrations at varying dilution rates up to the D^*, by the use of the equations given by Gaudy and Rozich (1982).

For this method to work it is necessary to know the inhibition constants for the inhibitory compounds in the waste. The Haldane model for inhibition was used in the case of phenol (Gaudy and Rozich,1982). As stated earlier, these constants cannot be determined by CSTR methods, and hence must be determined by other procedures (Section 2.4.4.3).

It should be noted that the work done with phenol was performed using phenol was a pure substrate. Earlier (Section 2.2.2.5) it was stated that the presence of a secondary substrate can have a major effect on the kinetics of degradation. As no account of this possibility was made in the work of Rozich and Gaudy, this is an area which deserves further investigation.

2.4.5 Multisubstrate Models.

In the previous sections, substrate, S, was confined to a general parameter, such a BOD, COD or TOC. With the increased interest in the degradation of hazardous and toxic pollutants in domestic waste plants, the idea that the waste can be treated as one uniform compound has had to be discarded (Cloonan,1984).

In the case under study, the leachate to be treated can be easily defined chemically and as the composition is likely to change during the leaching of the dumpsite, a blanket substrate term may not describe the system sufficiently. For this reason multisubstrate models must be considered.

Once models move from one substrate to multiple substrates, mathematics and verification of the model becomes complex (Cloonan,1984). Every substrate must have its own term in such a model, and each also has kinetic constants which must be estimated (Cloonan,1984). What can now be presented is a general description of two substrate systems, which can be extended to a greater number of substrates, requiring the addition of more equations and mathematical complexity. A more detailed treatise can be found in Cloonan (1984).

Dual substrate models fit into two categories: interactive, where the uptake of one substrate affects the uptake of the other, and non-interactive, which assumes the assimilation pathways for the two compounds are so dissimilar as to behave as if they were independent (Cloonan,1984).

Non-interactive models have been used by Moos et al. (1983) for pentachlorophenol in dog food extract. Non-interactive models cannot, however, be applied to the leachate system, due to the relationship between the chlorophenols and the phenoxies. As the chlorophenols are the first intermediate in the breakdown of the phenoxies (Section 2.2.2), the key assumption required for non-interactive systems is violated.

Interactive models are based on the assumption that the degradation of one substrate exerts an effect, either enhancing or depressing, the degradation of a second substrate. The simplest form of interactive model is formed by multiplying two single nutrient limiting equations (Bader,1978) to produce:

$$\mu = \mu_{MAX} S_1 S_2 / (k_{s1}+S_1)(k_2+S_2)$$

However this model implies that both are required for growth, and hence is more suitable for a substrate and nutrient combination than two substrates (Cloonan,1984).

There is, however, a more theoretically correct model, by Yoon et al.(1977). This model was based on the work of Law and Button (1977) who showed that the growth rate of a coryneform bacteria on two substrates simultaneously was the sum of the two individual growth rates. The postulated reaction sequence is;

$$X + S_1 \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} X'$$

$$X + S_2 \underset{k_{-2}}{\overset{k_2}{\Leftrightarrow}} X''$$

$$X' \overset{k_3}{\Rightarrow} 2X$$

$$X'' \overset{k_4}{\Rightarrow} 2X$$

where X' and X'' are intermediate cell states.

By following the procedure used to derive the Monod equation from first principles, the following expressions were derived;

$$\mu = \frac{\mu_{MAX,1} S_1}{(K_1 + S_1 + a_1 S_2)} + \frac{\mu_{MAX,2} S_2}{(K_2 + S_2 + a_2 S_1)}$$

where

$$K_1 = (k_{-1}+k_3)/k_1$$

$$K_2 = (k_{-2}+k_4)/k_2$$

To make the model account for enzymic interaction between substrates, and for the possibility of enhancement, the model was generalised to:

$$\mu = \frac{\mu_{MAX,1} S_1}{(K_1+S_1+a_2 S_2)} + \frac{\mu_{MAX,2} S_2}{(K_2+S_2+a_1 S_1)}$$

where a_1 and a_2 are independent parameters, similar to K_I in the Haldane model (Yoon et al.,1977). These parameters can, however, also account for enhancement if they are set at less than 1. There is no mechanistic justification for these changes to the model, other than that they generalise the model (Yoon et al.,1977). For any number of substrates, this generalises to

$$\mu = \sum_{I=1}^{n}[\mu_{M,I}S_J/(k_I + \sum_{J=1}^{n}a_{I,J}S_J)]$$

Where $a_{I,J}$ is the inhibition of the jth substrate on the utilization of the ith

If a mass balance is performed on S_1 in a CSTR at steady state it can be shown that:

$$Y_1D(S_{1,0}-S_1) = \mu_{MAX,1}S_1X/(K_1+S_1+a_2S_2)$$

where

Y_A is the yield coefficient on Substrate a (mg/mg)

D is dilution rate (h^{-1})

$S_{0,A}$ is the influent concentration of substrate a (mg/l), and

X is the total biomass concentration (mg/l) (Yoon et al.,1977).

For the equations to be strictly true, it is necessary for a_1 to be equal to $1/a_2$ (Yoon et al.,1977). However, if they are not related in this manner the equations should still predict the situation relatively well (Yoon et al.,1977).

Papanastasiou and Maier (1982 and 1983) used a similar reaction sequence to develop a model for the degradation of 2,4-D and glucose. They found that glucose degradation fitted the Monod equation, and 2,4-D was inhibitory according to the Haldane model. When the equations were developed the following expression for growth on 2,4-D was found:

$$\mu_1 = \frac{\mu_{MAX,1}S_1}{[K_1+S_1+(S_1^2/K_{11})+f_1(S_2)]}$$

and on glucose

$$\mu_2 = \frac{\mu_{MAX,2}S_2}{[K_2+S_2+f_2(S_1)]}$$

where $f_1(S_2)$ accounts for the effect of glucose on 2,4-D and $f_2(S_1)$ accounts for the effect of 2,4-D on glucose. The inhibition functions were determined from batch work, and found to be:

$$f_1(S_2) = A[1-(s_2^2/S_{2,0}^2)]$$

and

$$f_2(S_1) = a(S_1-S_1^*) \text{ where } S_1^* = S_1(t \rightarrow \infty) \leq S_1$$

Overall, $\mu = \mu_1+\mu_2$. The model was found to be good for batch work, but was never tested for a CSTR (Papanastasiou and Maier,1983). A similar approach was taken for growth on PCP and other chlorinated aromatics (Klecka and Maier,1988). These authors also found such an interactive approach to be effective.

More recently Hutchinson and Robinson (1988) studied the degradation of *p*-cresol and phenol simultaneously in batch systems. They found that for these two very similar compounds, the growth rate on a mixture of the compounds was equal to the growth rate on either of the two compounds. The authors found that the rate of utilization of each substrate was related to the fraction of the total organic substrate present (Hutchinson and Robinson,1988). This appears to be more consistent with

loose specificity on the part of the enzymes involved than a true multiple substrate system, although such a system may be applicable to modelling the degradation of the phenoxies in leachate.

To summarise, two models appear to be useful for the prediction of the removal of multiple substrates, that of Yoon et al.,(1977) and Papanastiou and Maier (1982 and 1983). These models can be expanded to account for a larger number of compounds, and hence may be useful for describing the degradation of leachate, which contains three major classes of compounds, as the composition of the leachate is expected to change with time.

2.5 Summary and Conclusions.

A number of major points were raised in the review of the literature:

(1) The key components present in the leachate were biodegradable if the correct organism, or consortia of organisms were used.

(2) Of the activated sludge configurations described, the CSTR approach would be the least affected by the inhibitory nature of the substrates.

(3) The presence of inhibitory substrates indicates the critical point method would probably be the design method of choice.

(4) The presence of PCOC and phenoxies precludes the use of a non-interactive model to describe the degradation of individual substrates.

(5) The model of Yoon et al. (1977), which can be expanded for use with three substrates may be suitable for describing leachate degradation.

To conclude, based on the above points, an activated sludge system, possibly followed by tertiary treatment could be used to dispose of the leachate. This thesis will describe a study of the degradation of leachate and determine whether this hypothesis is correct or not.

CHAPTER 3
MATERIALS AND METHODS

3.1 Introduction

This chapter describes the materials and methods common to subsequent chapters. Methods were for the analysis of chlorophenols/phenoxies by HPLC, alcohols by GLC, biomass, ash, total dissolved solids and total organic carbon by gravimetric methods.

Also listed in this chapter are the suppliers of miscellaneous chemicals and equipment and the design of the novel small scale bioreactor used for many of the experiments.

3.2 The Determination of Chlorophenol and Phenoxy Concentrations.

The chromatographic system is the method used by DowElanco (New Plymouth, New Zealand) for routine analysis of phenoxy samples. The company also supplied an instrument to be dedicated to analysis for this project. This consisted of a Waters 6000A pump (Alphatech Systems, Auckland, New Zealand), a Tracor 970A variable wavelength detector (used at 230 nm)(Advanced Electronics, Takapuna, New Zealand), a DuPont Instruments Column compartment (DuPont N.Z., Manakau, New Zealand). Two integrators were used: a Hewlett-Packard 3373B integrator with a Sekonic SS-250F chart recorder (Gough Technology, Christchurch, New Zealand) (1 mv, 150 mm/h) and a Spectraphysics SP4000 integrator (Watson Victor, Wellington, New Zealand) provided by DowElanco. Samples were centrifuged (Wifug Chemico, Sweden, 3000 xg, 2-3 min) and filtered through a 0.45 µm filter (Millex-HV,duropore 25 mm-non-sterile, or Alltech Nylon 66,25 mm-non-sterile luer lock) placed in-line with the injector. The injection volume was fixed at 100 µl.

Zorbax CN columns, usually 200 mm x 4.6 mm (8 micron packing packed by DowElanco) were used for the analysis. Occasionally 150 mm columns were used (DowElanco) without loss of resolution. One column was packed with 5 micron packing (DuPont Chromatography Products, Auckland, New Zealand), which also had good resolving power.

The mobile phase used with these columns was made by adding 450 ml of Acetonitrile-210 (Unichrom grade, Ajax Chemicals, Sydney, Australia) and 35 ml tetrahydrofuran (HiPerSolve grade,BDH,Poole, England) to 2 l of 0.1 M sodium dihydrogen orthophosphate (Analar grade,BDH,Poole, England) made up using water purified using a MilliQ Reagent Water System (Millipore Corp). The pH was adjusted to 3.2 with concentrated phosphoric acid (May and Baker,Pronalys grade, Australia). The flowrates used varied with column length and age, starting with high flows (3 ml/min) and reducing the flow to maintain run times as the columns aged (down to 1 ml/min). The column was cleaned with methanol at least once per month. A CN guard column (DowElanco) was used to extend column life. To reduce the demand for acetonitrile, the effluent from the detector was recycled into the completely mixed solvent reservoir. This allowed the same mobile phase to be used for several months.

Table 3.1 Peak Identities and Retention Times from Figure 3.1.

Peak N°	Compound	Retention Time (min)
1	2,4-D	7.3
2	MCPA	8.4
3	MDCPA	9.3
4	PCOC	10.3
5	2,4-DCP	10.9
6	2,4,5-T	12.7
7	2,4,6-TCP	17.4
8	2,4,5-TCP	23.2
9	MCPB	25.2

A typical standard is shown in Figure 3.1, with the compounds and run time listed in Table 3.1. Table 3.1 shows that the resolution of all the components in the standard was satisfactory. To reduce the complexity of the analysis and subsequent interpretation only 2,4-D, MCPA, PCOC and 2,4,5-T were used as the key compounds of interest. Other compounds present in leachate were ignored with minimal significance on the final result. If any of the compounds were not degraded, they would easily be detected by HPLC once the need to dilute samples lessened as the major compounds were degraded.

To determine the accuracy of the method, standards of known concentrations (0.5, 5, 12.5, 25, 37.5 and 50 mg/l) were injected and the peak areas recorded. Calibration curves linking peak area to concentration were drawn and found to be linear over the range tested. Quantification was performed on the basis of these standard curves. The error for all four compounds was approximately 2 % (varying from 1.4 - 2.2 %), indicating that the method was suitable for routine use.

As the phenoxy herbicides are often prepared as esters, it is necessary to hydrolyse any esters present to the free phenoxy. There was no difference between a NaOH hydrolysed sample and an untreated sample, indicating there was no significant ester content.

After major changes (i.e. lamp replacement in the detector, fresh mobile phase or a new column) a fresh standard curve was prepared.

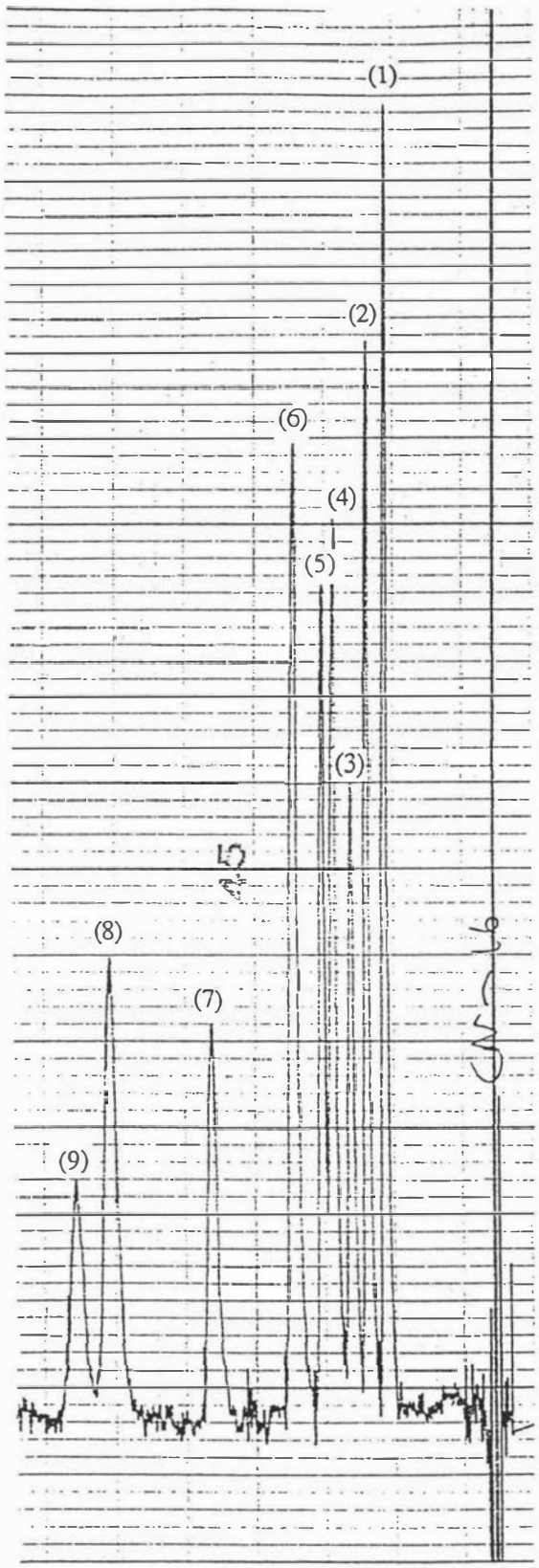


Figure 3.1: Chromatogram of Chlorophenols and Phenoxies Generated by HPLC System used for this Study. Peaks were (1) 2,4-D, (2) MCPA, (3) MDCPA, (4) PCOC, (5) 2,4-DCP, (6) 2,4,5-T, (7) 2,4,6-TCP, (8) 2,4,5-TCP, (9) MCPB.

3.3 Determination of Alcohol Concentrations.

Alcohol concentrations were determined by Gas Liquid Chromatography (GLC). A Shimadzu GC-8A chromatograph (SciMed, Wellington, N.Z.), using a 210 cm stainless steel column ((I.D. 2 mm) packed with Porapak Q (Salmond Smith Biolab, Palmerston North, N.Z.). Detection was by Flame ionisation Detector (FID), with attenuation and range of 8 and 1 respectively. FID output was recorded on a Sekonic SS-250F chart recorder (10 mv, 300 mm/h)(Gough Technology, Christchurch,N.Z.). Nitrogen (New Zealand Industrial Gases, Palmerston North, N.Z.) was run at 100 ml/min, hydrogen (N.Z.I.G.) at 12 ml/min, air at 4 ml/min, with the primary gas at 120 ml/min. The injector and detector were both held at 220 °C, while the column was run at 180 °C.

Concentrations were determined on the basis of peak height. A linear response was assumed, and the response factor was calculated daily on the basis of the standards run that day. A typical standard chromatogram is shown in Figure 3.2. Table 3.2 contains the component identity, retention times and concentrations. The injection volume in all cases was 2 µl, delivered from a 1-10 µl syringe (Hamilton, Nevada). Samples were centrifuged (Wifug, as used for HPLC samples) prior to injection.

Table 3.2 Peak Identity and Run Times for Alcohol Quantification.

Peak N°	Component	Retention Time (min)	Concentration (mg/l)
1	Methanol	1.2	39.6
2	Butan-2-ol	8.2	80.6
3	Butan-1-ol	11.8	81.0

Six replicate standards were run through the GLC, and the errors in measured concentrations calculated. The 95 % confidence intervals were found to be 5, 15 and 13 % for methanol, butan-2-ol and butan-1-ol respectively. These errors are large, because the instrument was run near the limits of component detection. It is likely any attempt to extract the alcohols from the aqueous phase to analysis by other methods would result in greater errors. As a result, the method, and the subsequent errors were accepted.

3.4 Determination of Biomass Concentration.

Various methods were used for the determination of biomass during the study and all referred to a fundamental parameter, the mixed liquor suspended solids (MLSS). As the feed medium did not contain a significant quantity of suspended material, the mixed liquor suspended solids (MLSS) was taken as being equal to the biomass in the system.

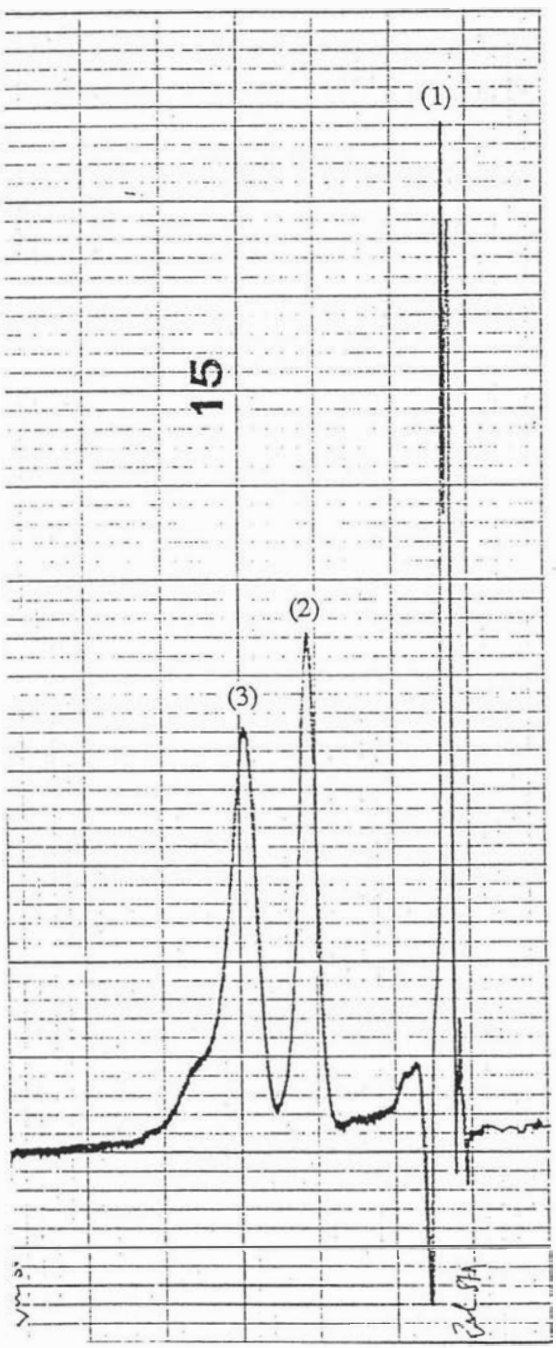


Figure 3.2 Typical Chromatogram for the Determination of Alcohol Concentrations. Peaks were (1) Methanol, (2) Butan-2-ol, (3) Butan-1-ol.

MLSS for a given sample was determined by the following method;

(1) A 0.45 μm membrane filter (Whatman, cellulose nitrate, plain white, 47 mm diameter) was labelled with a code number and pre-dried overnight at 105 °C (Watvic Oven, Cat No. 01124, Watson Victor, Wellington, N.Z.).

(2) The filter was then cooled in a desiccator in the presence of silica gel (BDH, Poole, England), and after equilibration (30 min) was weighed (Mettler AE160 digital balance, Watson Victor, Wellington, N.Z.).

(3) If the sample volume was greater than 10 ml, it was centrifuged (RC5C Sorvall centrifuge, Watson Victor, Wellington, N.Z., using a GS3 head if greater than 50 ml, otherwise with a SS34 head, at 9500 and 6700 xg respectively, for 10 min at 4 °C), and the supernatant passed through the filter under vacuum.

(4) The pellet of biomass was then suspended in a small volume of distilled water (5 - 10 ml) and the slurry was transferred quantitatively to the filter.

(5) The wet filter was then dried at 105 °C overnight, and was subsequently weighed after equilibration in a desiccator.

Good laboratory practice was used throughout. The volume for the analysis varied with the expected MLSS value. In all cases 10 mg (dry weight) were present on the filter to minimise weighing errors. The biomass was calculated using the following formula:

$$\text{MLSS (mg/l)} = \frac{\text{Nett weight in filter (mg)}}{\text{Volume of sample (ml)}} \times \frac{1000 \text{ ml}}{1 \text{ l}}$$

Other methods used in subsequent chapters all refer to MLSS on a dry weight basis.

3.5 The Determination of Ash and Total Dissolved Solids.

Ash and total dissolved solids (TDS) in the treated effluent were determined by the following method.

(1) The sample (50 - 100 ml) was filtered through a 0.45 μm filter (Whatman, cellulose nitrate, plain, 47mm) and placed into a predried (105 °C, overnight), preweighed (Mettler AE160 digital balance) silica crucible.

(2) The sample was dried at 105 °C overnight, cooled in a desiccator for 30 min and reweighed. The TDS was calculated using the following formula:

$$\text{TDS (g/l)} = \frac{\text{Dried weight(g) - tare weight(g)}}{\text{Sample Volume (ml)}} \times \frac{1000\text{ml}}{1 \text{ l}}$$

(3) The dried sample was then ashed at 600 °C for 2 h (Wild Barfield Furensco, model no MF2, Furnace Equipment Ltd, Christchurch, New Zealand), cooled in a desiccator, and reweighed. The ash was calculated using the following formula:

$$\text{Ash (g/l)} = \frac{\text{Ashed weight(g) - tare weight(g)}}{\text{Sample Volume (ml)}} \times \frac{1000\text{ml}}{1 \text{ l}}$$

3.6 Determination of Dissolved Oxygen Concentration.

The dissolved oxygen was determined using a YSI M57 dissolved oxygen meter (Watson Victor, Wellington, N.Z.), fitted with a YSI 5739 probe equipped with a 0.001" standard membrane.

3.7 Determination of Total Organic Carbon.

Total organic carbon was determined using the following method;

(1) The sample to be analyzed (approximately 100 ml) was freeze dried on a Virtis 10-020 freeze drier (Salmond Smith Biolab, Palmerston North, N.Z.).The TDS of the sample was also determined.

(2) The freeze dried solids were then analyzed for carbon using a Leco induction furnace (Med Bio Enterprises, Christchurch, N.Z.), according to the method used by Bogoni (1988). The total carbon for the sample is given by:

$$\text{Total carbon (mg/l)} = \frac{\text{TDS(g/l)} \times \text{carbon(\%)} \times 1000}{100}$$

(3) The inorganic component of the total carbon in leachate was determined by taking a sample and determining the TDS and total carbon.The pH of a further sample was then lowered to 3 with 1 M HCl (May and Baker, Pronalys grade, Australia) and nitrogen gas (N.Z.I.G.) was sparged through the liquid for approximately 4 h. This solution was then freeze dried and the total carbon and TDS were determined. The difference in carbon values was equal to inorganic the carbon.

$$\text{Total Inorganic carbon (mg/l)} = \frac{\text{TDS(g/l)} \times \text{carbon content change(\%)} \times 1000}{100}$$

This value was found to be 11 mg/l carbon per 5% leachate. This value was used throughout the study to correct for inorganic carbon.

(4) The total organic carbon is equal to the difference between the total carbon and the total inorganic carbon.

It should be noted that as the samples were freeze dried, alcohols were lost from the sample at that stage and hence were not included in the determination.

3.8 Small Scale Bioreactors.

Perspex bioreactors (Figure 3.3) were made in the Biotechnology Department. Perspex was cemented together, with rubber 'o' rings used to seal the head of the vessel, the air port and the D.O. probe port. These bioreactors were designed to allow the headspace to be reduced to a negligible volume, and a dissolved oxygen probe to be inserted. The measurement of the specific oxygen uptake rate of the culture can be performed without the need to remove a sample.

Undiluted leachate was allowed to stand in a reactor for 7 days. As no crazing, cracking or discolouration of the perspex was observed, the perspex was considered to be inert with respect to the leachate.

3.9 Miscellaneous Materials and Methods.

pH Measurement

pH was measured using an Orion Research 701A/Digital Ionalyser (Watson Victor, Wellington, N.Z.). The meter was calibrated prior to measurements at pH 4.0 and pH 7.0 using colour key buffer solutions (Laboratory reagent grade, BDH, Poole, England).

Recovery of Biomass for Inoculation

The desired volume of effluent from the parent bioreactor was collected, and then recovered by centrifugation: either using the RCSC Sorvall centrifuge (Watson Victor, Wellington, N.Z.)(9500 xg, 20°C, 10 min) or using a Clandon T52.1 centrifuge (Clandon Scientific,England) at room temperature and 2350 xg.

Chemicals.

Chemicals and materials not listed previously, and their suppliers can be found in Table 3.3

Table 3.3 Suppliers of Miscellaneous Chemicals and Equipment.

Item	Supplier
Butan-1-ol	Analar Grade, BDH, Poole, England.
Butan-2-ol	Analar Grade, BDH, Poole, England.
Methanol	Analar Grade, BDH, Poole, England.
NaOH	Analar Grade, BDH, Poole, England.
HCl	Pronalys Grade, May and Baker, Australia.
HNO_3	Analar Grade, BDH, Poole, England.
Phenolphthalein	Indicator reagent, BDH, Poole, England.
0.45 μm Filters	47mm dia, Whatman, Salmond Smith Biolab, Palmerston North, N.Z.

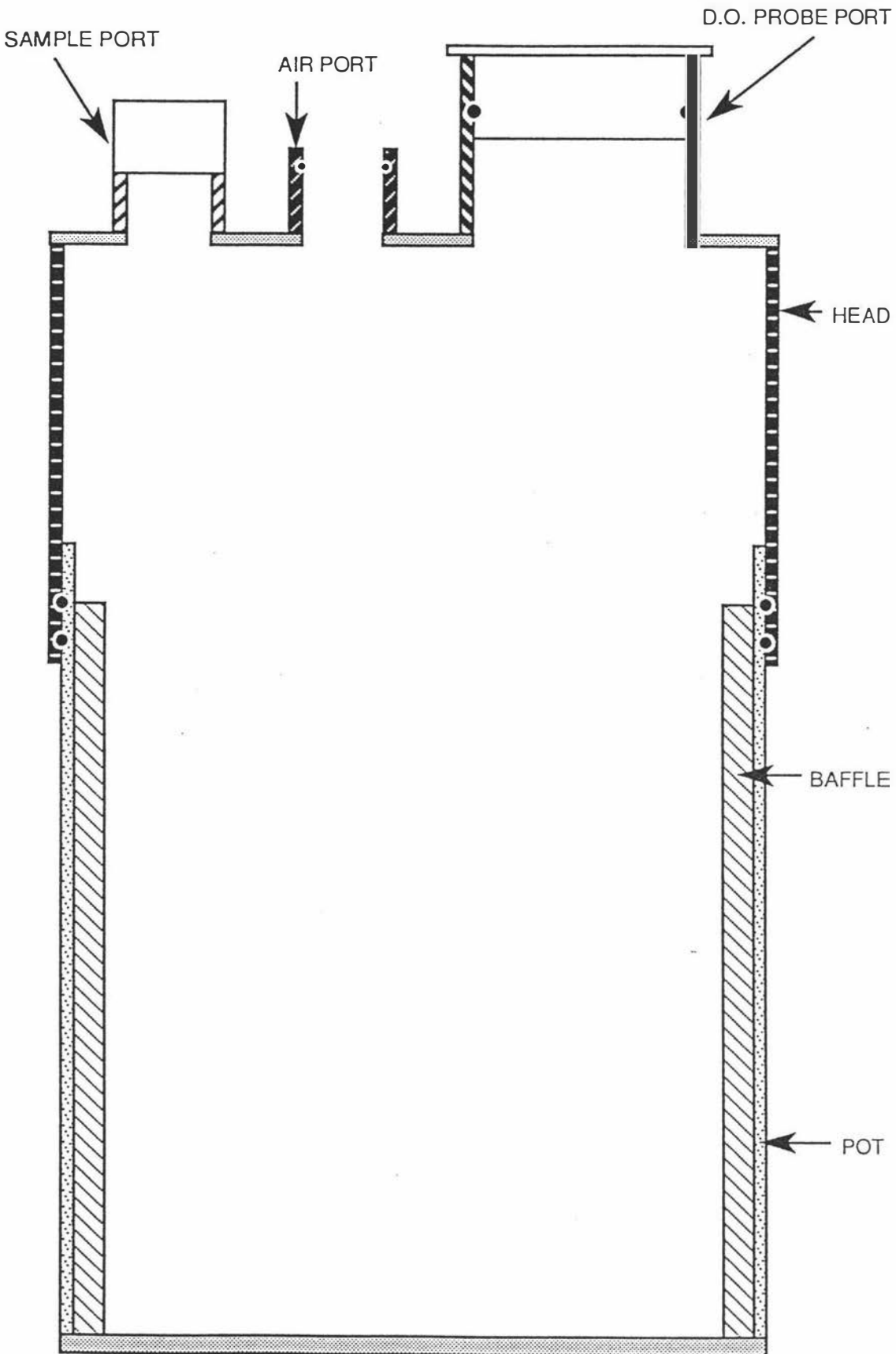


Figure 3.3 Schematic Diagram of the Perspex Bioreactor Built in by the Biotechnology Department, Massey University, Palmerston North (Scale 1:1).

CHAPTER 4
THE DEVELOPMENT AND MAINTENANCE OF A BACTERIAL CULTURE CAPABLE OF DEGRADING LEACHATE.

4.1 Introduction.

As stated in Chapter 2, the bacterial degradation of the major components in leachate has been reported. From this starting point it was necessary to develop a culture that would meet the following requirements:

(1) A capability of removing the alcohols, chlorophenols and phenoxies from leachate to give low residual concentrations,

(2) The capacity to retain the ability to degrade a wide range of substrates, even when challenged by potential contaminants in a non-sterile system, and

(3) Acceptability to authorities regulating the use of microorganisms.

This chapter will report and discuss the development, maintenance and microbiology of a culture capable of degrading the landfill leachate under study. The determination of optimum leachate concentrations and conditions will also be reported.

4.2 Initial Development.

4.2.1 Development of an Enriched Culture.

On July 30 1987, a 5 litre volume of medium (Table 4.1) was added to a 15 l glass pot on a New Brunswick Microferm laboratory bioreactor (model ME114, Watson Victor, N.Z.), along with 500 g of soil from Wairoka Research farm, New Plymouth, that in the past, had been sprayed regularly with herbicides. The mixture was stirred at 200 rpm, aerated at 1 vol/vol/min using mains air, and held at 25 °C.

Under these conditions the concentration of PCOC was reduced over 6 days, after which phenoxies were then removed. The batch was complete by day 13. The removal profile is shown in Figure 4.1. On day 15 the mixture was supplemented with leachate, increasing the PCOC/phenoxy concentrations to initial values. After a further two days, degradation was complete.

After 13 days, prior to the addition of the fresh leachate, 30 ml was withdrawn from the initial culture and added to 1.5 l of fresh medium (in a 2 l pot, using a Multigen F2000 bioreactor, Watson Victor, N.Z.). The operating conditions were maintained. After 68 h, degradation was complete. Extra leachate was added to return the concentrations to initial values, and after 96 h the majority of PCOC/phenoxies were removed.

This culture was used as seed for a third batch, under identical conditions. After 38 h degradation was complete. A substrate versus time plot is shown in Figure 4.2. After 38 h the bioreactor was run as a chemostat on the same medium until 12.5 days. Feed was provided using a

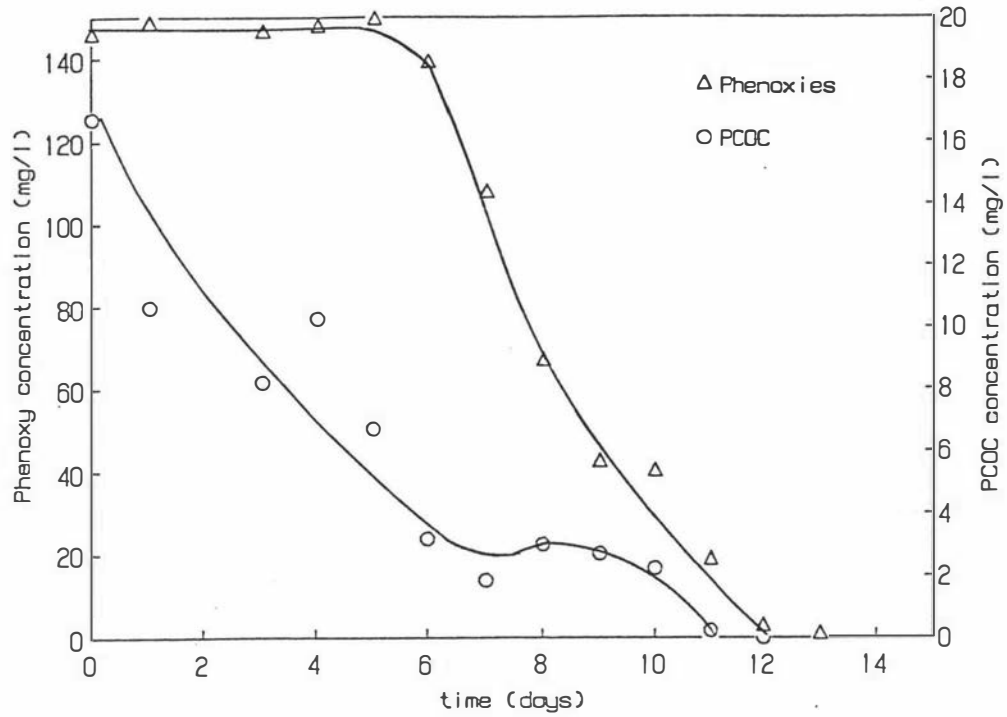


Figure 4.1: Plot of PCOC/Phenoxies versus Time for Initial Batch using Soil Inoculum.

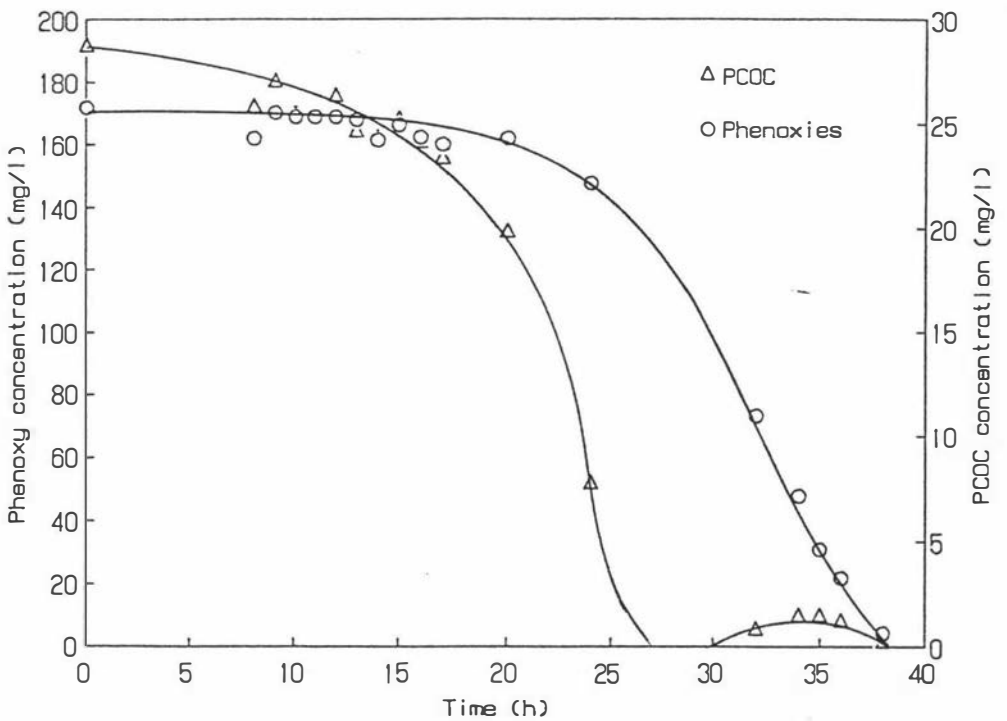


Figure 4.2: Plot of PCOC/Phenoxies versus Time for Batch Prior to the First Chemostat Run.

Table 4.1 Initial Medium Used for Leachate Degradation.

Compound	Initial Batch (5 % leachate) Concentration (mg/l)	10 % Leachate Concentration (mg/l)
Phenoxies	155	310
PCOC	24	46
Alcohols	35	80
KH_2PO_4	420	420
K_2HPO_4	375	375
$(NH_4)_2SO_4$	244	244
NaCl	30	30
$CaCl_2.2H_2O$	30	30
$MgSO_4.7H_2O$	30	30
$FeCl_2$	3	3

Masterflex 7013 pump head (C-flex tubing) and effluent removed by suction from the surface with a Masterflex 7014 pump head (also with C-flex tubing) both mounted on the same pump drive (Cole-Parmer 7546-10, 5-100 rpm peristaltic pump, Salmond Smith Biolab, Palmerston North, N.Z.). This method ensured the level in the bioreactor remained constant. The flowrate was varied between 1 ml/min (20 h residence time) and 1.5 ml/min (13.3 h residence time). The results of this can be seen in Figure 4.3. The final flowrate used was 1.3 ml/min (residence time 15.4 h), which removed 95 % of PCOC and 98.7 % of phenoxies present in the feed.

After 12.5 days the feed strength was doubled (composition given as 10 % leachate in Table 4.1) and run for a further 11.7 residence times. This system removed 99.6 % of the phenoxies and 95.6 % of PCOC. At this stage the bioreactor was stopped and used to start a larger (5.5 l) bioreactor, known as the parent bioreactor (see Section 4.3). The larger volume was chosen to enable up to 500 ml to be collected quickly (before any autolysis occurred) without disturbing the bioreactor's steady state. This culture sample was used to start other experiments.

4.2.2 Simplification of the Medium.

An attempt was made to simplify the medium. For this endeavour to be successful, it was necessary to know the composition of the leachate.

Establishing the composition of the leachate necessitated the use of a number of methods:e.g. metals, phosphorus and sulphur were determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) by the Biotechnology Division, Department of Scientific and Industrial Research (DSIR), Palmerston North, N.Z.. The chloride content was determined by titration with silver

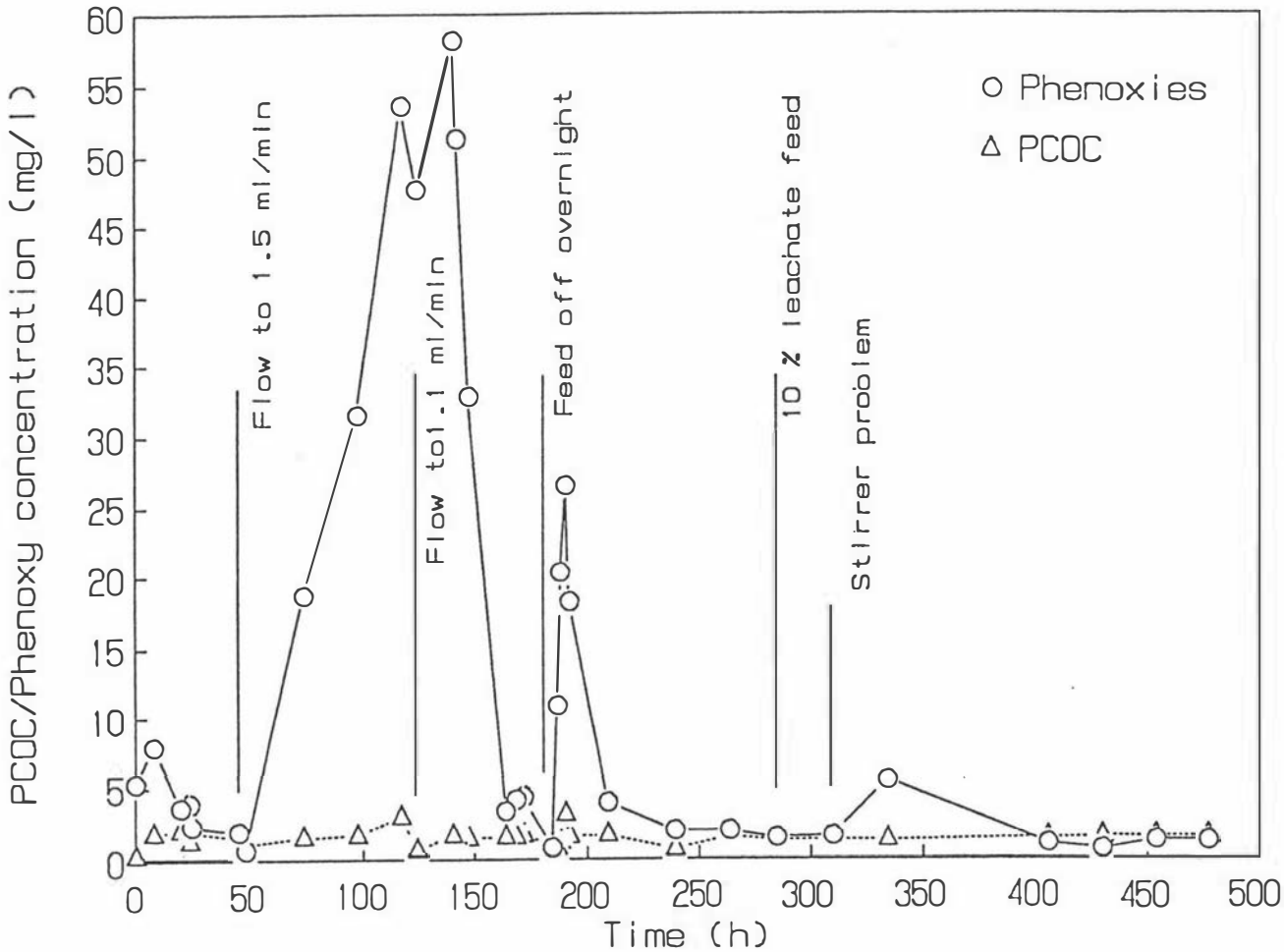


Figure 4.3:Plot of PCOC/Phenoxy Concentrations versus Time for First Chemostat Run.

nitrate solution according to Vogel (1961). Nitrogen (as NH_4^+) was determined by the Kjeldahl method (Vogel,1961). pH was measured using the method described in Section 3.9. The concentration of important metals, expressed in milligrams per litre (mg/l) present in 10% leachate and in the nutrients added to the medium are given in Table 4.2.

Table 4.2 Metal Concentrations in 10 % Leachate and Complex Medium**

Metal	10% leachate (mg/l)	Complex medium (mg/l)
Na	548	12
K	65	288
Ca	21	8
Mg	8	3
Fe	1	-*
Mn	4	-
Co	0.02	-
Cu	0.02	-
Zn	0.6	-

* - none added

** See Table 4.1 for the concentrations of PCOC/phenoxies

A comparison made of the metal concentrations in the leachate and that in the complex medium shows that all necessary cations are present in leachate and in quantities that should not be nutritionally limiting. Potassium is not required in the concentrations supplied (Pirt,1975) and therefore the removal of the supplement should not effect the medium's performance.

Concentrations of non-metal ions in the leachate and added in the complex medium are given in Table 4.3.

Table 4.3 Non Metal Ion Concentrations in 10 % Leachate and Complex Medium.

Non-metal	10% leachate (mg/l)	Complex Medium (mg/l)
N as NH_4^+	4	66.5
S as SO_4^{2-}	21.3	189
P as PO_4^{3-}	0.4	498
Cl^-	374	47.2

Table 4.3 shows that there is little ammonium, sulphate and only trace quantities of phosphate in the leachate compared to the complex medium. As there is no need for metal supplementation, but a requirement for the non-metals, a simple buffered medium containing 356 mg/l $(NH_4)H_2PO_4$, 210 mg/l $(NH_4)_2HPO_4$ and 244 mg/l $(NH_4)_2SO_4$ (all Analar grade, BDH, Poole) was formulated. In 10 % leachate, this simple supplement provided all elements of the complex medium and a small amount of buffer capacity (required because of HCl production from 2,4-D degradation (Loos,1975)) at a pH of approximately 6.5.

To verify that the simple buffered medium was suitable, two trials were undertaken, one in a batch system and a second using chemostats.

Batch trials were conducted using 50 ml of 5% leachate in simple buffered medium in 500 ml Erlenmeyer flasks. Duplicate flasks were inoculated with 5 ml of culture from the continuous bioreactor running on complex medium (Table 4.1) and incubated at 25 °C (shaken at 200 rpm) in an Environ-shaker (3597-1234&10, Lab-line Instruments,Illinois). These flasks were subcultured twice, achieving a 1000 fold dilution of any nutrients carried over. The degradation rates in these flasks and in controls consisting of 5% leachate in complex medium were compared.

Continuous bioreactor trials were performed using 10 % leachate in complex medium and in simple buffered medium. The results for batch and chemostat trials are given in Table 4.4.

Table 4.4 Substrate Removal in Under Batch and Chemostat Conditions in Simple and Complex Media.

	Complex Medium		Simple Medium	
	Chemostat	Batch	Chemostat	Batch
% removal PCOC*	95.6	87	98.4	91
% removal Phenoxies	99.6	97	98.8	96

* Removals are an average of the effluent concentrations over three days at steady state (residence time = 15 h)

These results demonstrate clearly that the simple buffered medium was suitable for the degradation of leachate. The medium is less complex than the initial medium and therefore cheaper, particularly for a large scale system. It was decided to use this simple buffered medium for subsequent work with the leachate.

4.3 Establishment of the Parent Bioreactor

A parent bioreactor was established to provide a large source of organisms for further experimentation. It also provided information about the stability of the culture over a long period of time.

This bioreactor was established on September 8, 1987 as a batch, using 5.5 l of medium (Table 4.5) in the same apparatus used for the initial batch (Section 4.2.1). After running as a batch for 23 hours, medium of the same composition was pumped at 6.4 ml/min (residence time 14.5 h, D = 0.069 h^{-1}). A schematic diagram of the equipment is shown in Figure 4.4, and its photograph in Figure 4.5.

Table 4.5 Simple Buffered Medium using 10 % Leachate.

Compound	Concentration (mg/l)
Phenoxies	316
PCOC	53
Alcohols	80
$(NH_4)H_2PO_4$	356
$(NH_4)_2HPO_4$	210
$(NH_4)_2SO_4$	244

This bioreactor was operated throughout the entire project,i.e. 872 days or an equivalent 1443 reactor volumes. During operation, the feed reservoir was replaced with a clean vessel filled with fresh medium every 1-2 days. The composition of the leachate obtained from DowElanco was found to vary slightly, batch to batch. The composition of the leachate, and the dilutions used can be found in Table 4.6.

Table 4.6 Composition of the Leachate Batches Obtained from DowElanco.

Component	Batch							
	I	II	III	IV	V	VI	VII	VIII
Phenoxies (g/l)	3.16	4.07	4.49	4.05	4.68	4.58	4.35	3.44
PCOC (g/l)	0.53	0.63	0.69	0.59	0.60	0.67	0.66	0.60
Alcohols (g/l)	0.8	0.84	0.88	0.78	0.78	0.82	0.80	0.66
Concentration Used (%)	10	10	10	10	10	10	10	12.5

Wall growth was scraped off using magnetic fleas inside the bioreactor, carried by a horseshoe magnet moved manually over the outside of the glass bioreactor. Tubing between the reservoir and pump was also changed every 1-2 days. When not in use, the feed reservoirs and tubing were soaked in Pyroneg (Diversey N.Z. Ltd) to prevent contamination of the fresh feed. A cotton wool bung was used to prevent airborne contamination of the feed. Throughout the course of the study, no evidence of degradation of the feed in the reservoir was found. No other precautions were take to prevent

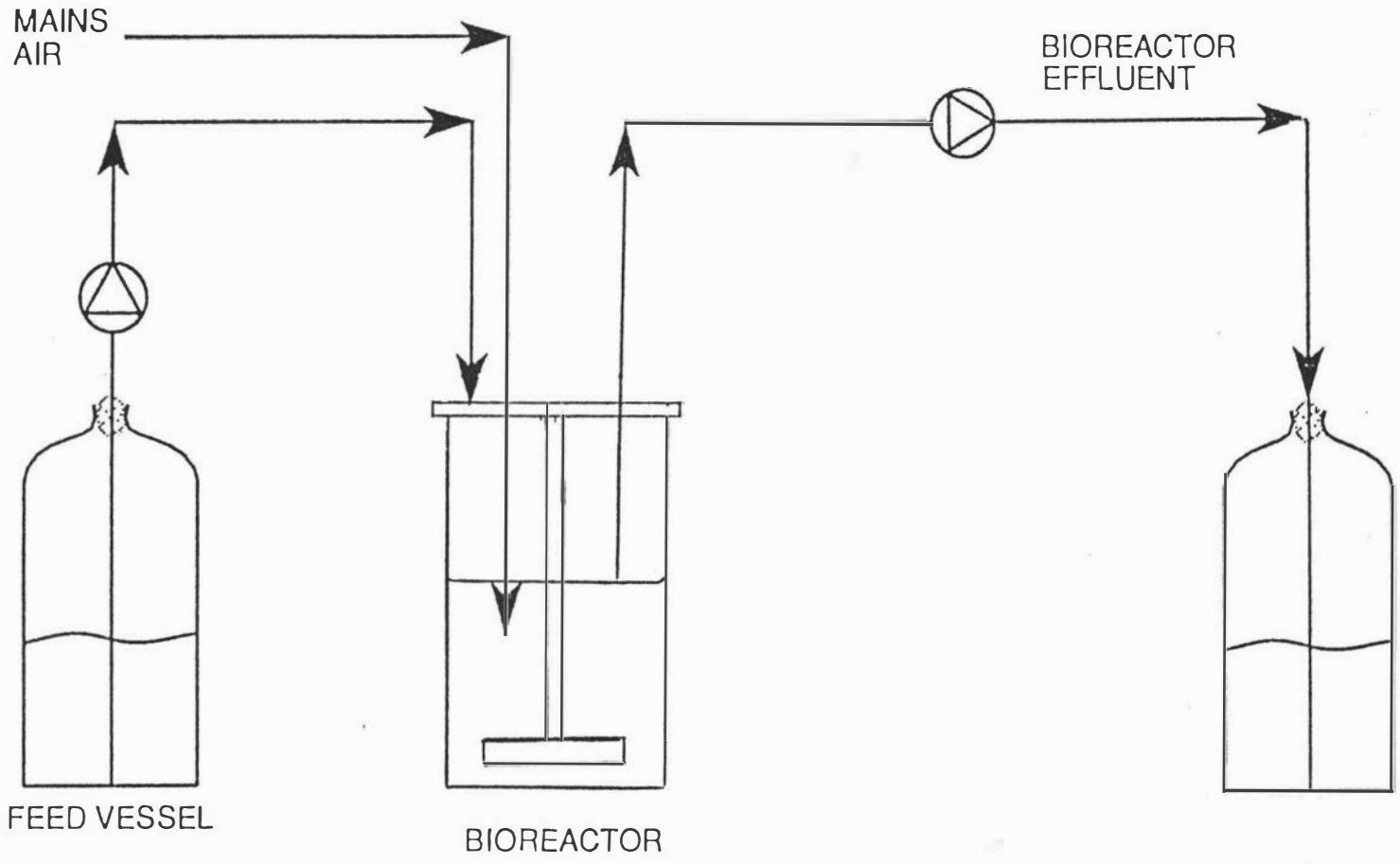


Figure 4.4: Schematic Diagram of the Parent Bioreactor.

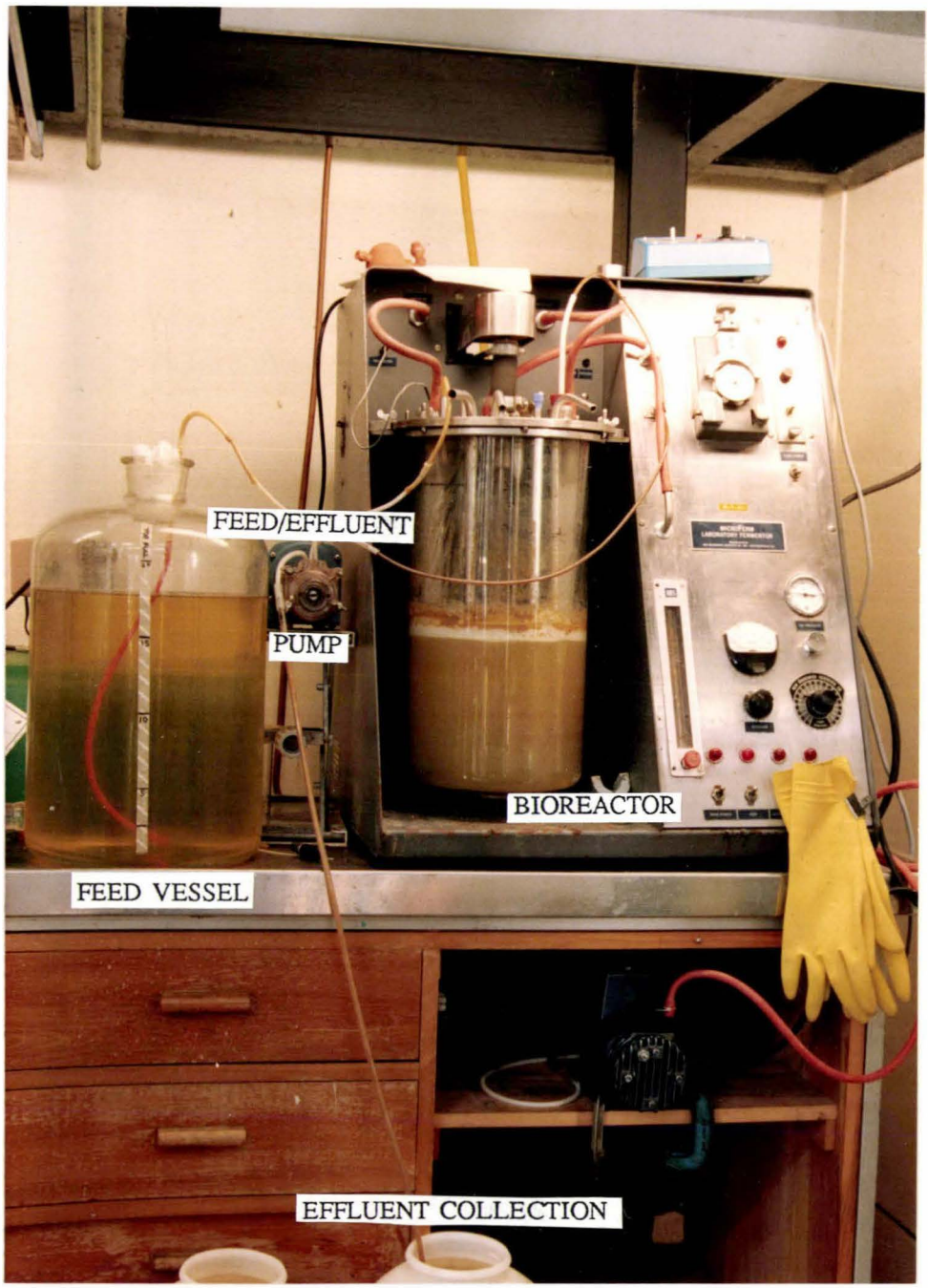


Figure 4.5: Photograph of Parent Bioreactor Experimental Equipment.

contamination of the culture.

Figures 4.6 and 4.7 show effluent concentrations of PCOC and phenoxies respectively. During the course of the study several 'spikes' can be seen in the substrate concentrations. These spikes were due to operational problems, such as pump or stirrer failures. It should be noted here that the biomass produced by this bioreactor was slightly brown in colour and capable of rapid flocculation and settling under quiescent conditions. This is a very desirable characteristic for a culture to be used for activated sludge. The parent bioreactor was used not only as a source of inocula for all other experiments but also to maintain the consortia of organisms developed.

4.4 Proof of Total Degradation.

If the culture is to be useful on a large scale, then it must be able to metabolise PCOC/phenoxies to benign products such as CO_2 and H_2O, rather than transformation to equally undesirable products. Incomplete degradation has been reported as occurring in some environments (Allard et al.,1987; Loos et al.,1967b) and it is essential that total degradation be verified.

According to Glaser (1988), two methods are suitable:

(1) Toxicity testing of the feed and effluent to show that a reduction in toxicity has occurred, and

(2) performing a radioactive tracer study to verify the production of radioactive CO_2.

4.4.1 Toxicity Testing.

Samples of medium to the parent bioreactor and collected effluent (2 l), centrifuged (RC5C Sorvall centrifuge (9500 xg,10min 20°C)) to remove any suspended solids, were tested for acute toxicity using Daphnia magna and Microtox® systems. The results, expressed as equivalent concentration where 50% of organisms survive after the desired time period (EC_{50}), 24 h for D.magna and 15 min for the Microtox method. The results are shown in Table 4.7.

Table 4.7 Toxicity of Feed to and Effluent from the Parent Bioreactor.

Test Organism	Feed EC_{50} % V/V	Effluent EC_{50} % V/V	Reduction %
D.magna	2.4	19.9	88
95 % confidence	(0.99 - 3.1)	(13.4 - 29.0)	
Microtox	1.4	26.9	95
95 % confidence	(1.1 - 1.8)	(20.5 - 35.2)	

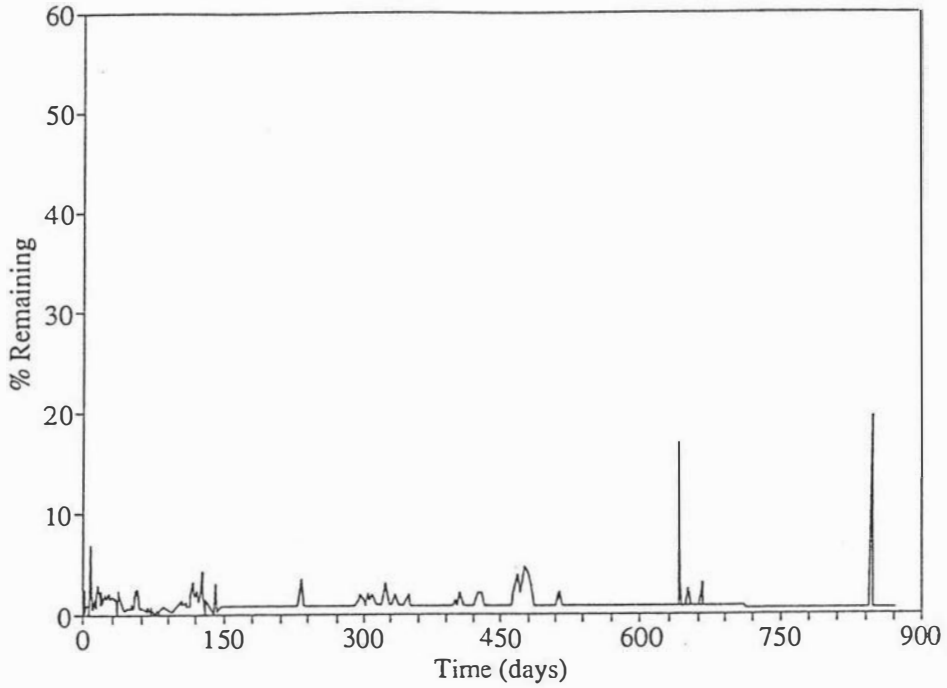


Figure 4.6: Plot of Residual PCOC versus Time for Parent Bioreactor.

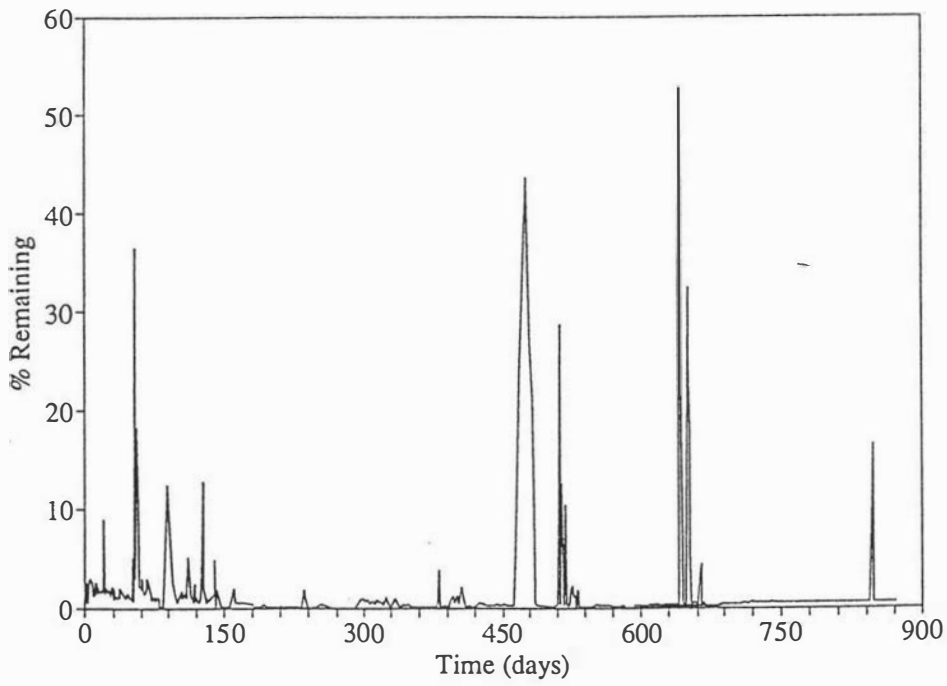


Figure 4.7: Plot of Residual Phenoxy Concentration versus Time for the Parent Bioreactor.

These results indicate a 92 % reduction in toxicity and represents a substantial removal of toxicity, indicating a toxic metabolite is not present (Glaser,1988).

4.4.2 Radioactive Tracer Study.

4.4.2.1 Experimental Procedure.

A sample of 100 μCi of 2,4-Dichloro[ring-u-^{14}C]phenoxyacetic acid was obtained from Amersham (UK). This was dissolved in 10 ml methanol (Analar,BDH,Poole) and stored at -20 °C until needed. When required, 1 ml was removed, made alkaline by adding 2 drops of 1 M NaOH (Analar,BDH, Poole), placed in a vacuum flask and held under vacuum until all methanol had been removed. This was then washed quantitatively into 500 ml of the medium in Table 4.5. A 5 ml sample was taken and stored at -20 °C until counting. The medium was then inoculated with 81 mg (dry weight) of biomass from the parent bioreactor. The experimental equipment is shown in Figure 4.8. The culture was grown in a perspex bioreactor (Section 3.8). Metabolic CO_2 was collected in 1 M NaOH solutions through which all air leaving the bioreactor was passed. Inlet air was also passed through a scrubber to remove traces of CO_2 that would otherwise be introduced to the system from ambient air. Air was supplied at 300 ml/min via a fish tank aerator (Second Nature Whisper 400, Pets International). Mixing was provided by an external magnetic stirrer (Heidolph MH2002, Watson Victor, N.Z.) and a magnetic flea. The temperature was maintained at approximately 25 °C and the batch ran for 30 h.

After the batch was complete the effluent caustic scrubbing solutions were pooled and neutralised with 5 M HCl using phenolphthalein as an indicator (to prevent chemiluminescence from interfering with the counting procedure). The sample was held in an ice/water bath to minimise losses of CO_2. The last traces of colour from the indicator were removed by adding 2 drops of 2% acetic acid to prevent any interference with the counting procedure. The volume was then made up to 250 ml with MilliQ water.

The biomass was recovered by centrifugation and collected on 0.45 μm filters . The biomass was then washed with isotonic saline and dried over P_2O_5. After drying the samples were incinerated in a Leco induction furnace (Model 785-000) and the gases were collected in 1 M NaOH. After neutralisation, as above, the volume was made up to 500 ml.

The spent effluent along with the washings from the biomass were pooled and made up to a final volume of 550 ml with MilliQ water.

A 1 ml of each sample was added to 9 ml of scintillation cocktail (Scintran, BDH, Poole) and counted in a Beckman LS5801 series scintillation counter (Alphatech Systems, Takapuna, N.Z.). HPLC analysis was performed on the CO_2 fraction and spent broth from the bioreactor. Safety procedures used were based on recommendations made by Faires and Boswell (1981).

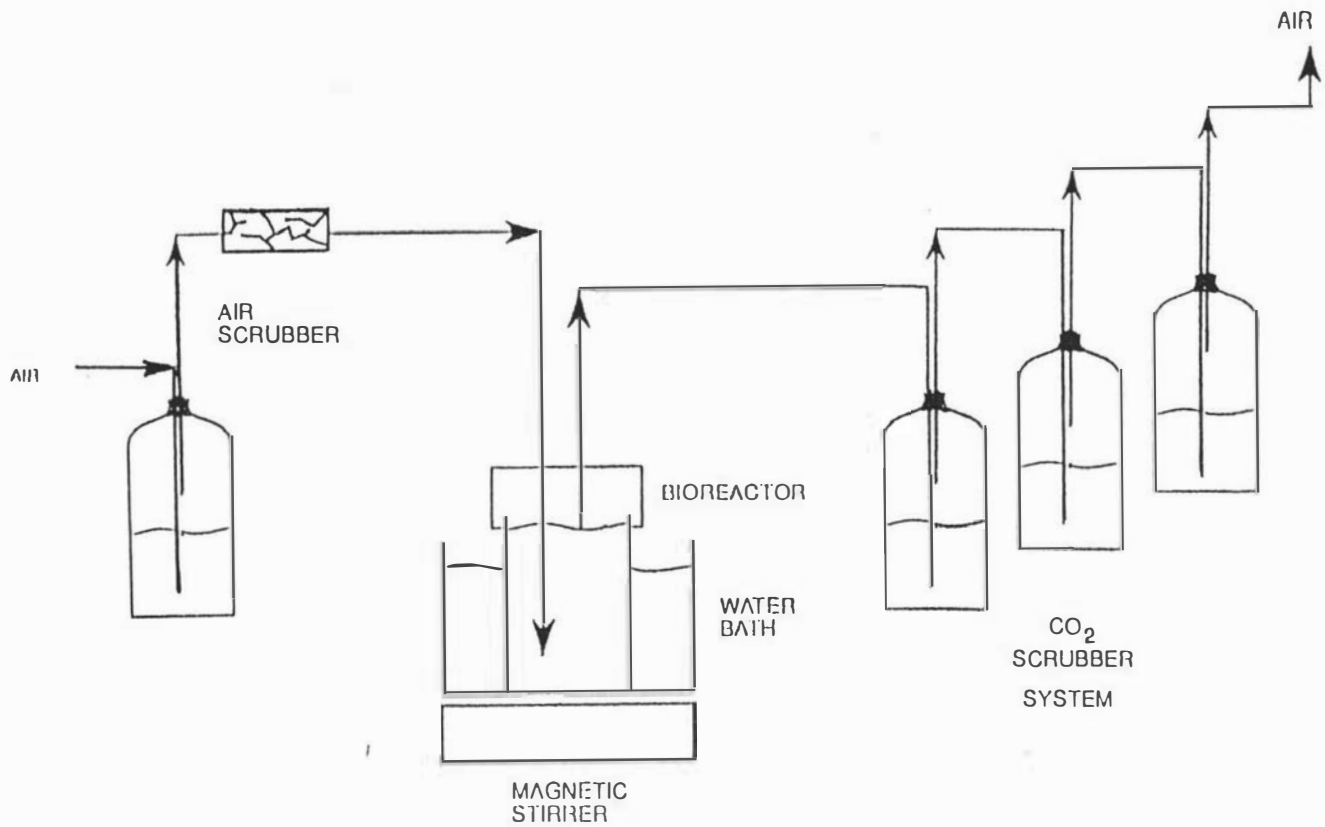


Figure 4.8: Schematic Diagram of Experimental Apparatus for Radioactive Tracer Study.

4.4.2.2 Results.

Results of scintillation counting can be found in Table 4.8.

Table 4.8 Scintillation Counts from Radioactive Tracer Study.

Fraction	Volume (ml)	Counts/min	TOTAL cpm	% initial
Initial	490	103058	5.05×10^7	100
Effluent	550	6332	3.48×10^6	6.9
CO_2	250	134600	3.37×10^7	66.7
Biomass	500	6538	3.27×10^6	6.5
TOTAL RECOVERED			4.05×10^7	80.1

The reason for the low biomass count was that during the process of incinerating the biomass, a hose connecting the furnace to the collection train disconnected, venting the CO_2 into the fumehood instead of the caustic solution. HPLC analysis of the effluent and CO_2 fractions showed no 2,4-D or 2,4-DCP were present in the samples.

From these results, applying the rule of Grady (1985), mineralisation of the phenoxies is occurring.

4.4.3 Discussion.

The toxicity test results and the radioactive tracer study results show there is mineralisation occurring. The most toxic compounds present in the leachate are the chlorophenols (Verschueren,1983). If these compounds were not being mineralised, but transformed to another compound such as the related chloroanisole, it would be expected the toxicity would not be greatly reduced, if any reduction occurred at all.

The tracer study results indicate that 2,4-D is mineralised. Chlorophenols are also degraded, because they are the first intermediate in the breakdown pathway of the phenoxies (Section 2.2.1.1).

Moos et al. (1983) reported the results of 3 separate studies using pentachlorophenol which showed that 67, 68 and 50 % of ring carbon could be detected as CO_2. These compare well with the results of this study. Studies have been carried out with labelled 2,4-D (McCall et al., 1981; Smith, 1985) and 2,4,5-T (Rosenberg and Alexander 1980a and b; McCall et al., 1981) but could not be compared with these results as the experiments were conducted in soil and soil suspensions.

Thus the culture developed is potentially suitable for use in a biological treatment system for leachate treatment, because the key components are mineralised.

4.5 Determination of Suitable Operating Conditions.

4.5.1 The Effect of Leachate Concentration.

Maximum throughput of leachate in a biological treatment system is possible if the optimum operating conditions, including feed concentration are known. From Chapter 2, a CSTR system, which maintains low residual substrates should be able to treat a higher concentration of leachate than batch systems.

4.5.1.1. Batch Conditions.

Experimental Procedure.

The media used were based on the simple buffered medium (Section 4.2.2). The substrate concentration was varied between 5% and 100% (5,10,20,30,50 and 100%) leachate. A precipitate formed in all media at concentrations greater than 30 % leachate. Alcohols were analyzed by GLC (Section 3.3) and PCOC/phenoxies were analyzed by HPLC (Section 3.2). Experiments were carried out in the same bioreactor used for the initial chemostat work (1.2 l working volume) under the same conditions. The inoculum (75 mg/l dry weight) was prepared by centrifugation from the parent bioreactor. The cultures were monitored for 20 h.

Results and Discussion.

No degradation was recorded in any batch containing more than 20% leachate. Results of the remaining batches (5,10 and 20% leachate) are summarised in Table 4.9

Table 4.9 Summary of Batch Degradation of Leachate.

Feed Concentration (% leachate)	5	10	20
	Removal in 20 h (mg/l)*		
Phenoxies	190 (100)	60 (20)	12 (1.6)
PCOC	24 (100)	16 (27)	20 (11)
Alcohols	31 (100)	62 (100)	50 (41)
TOTAL REMOVED	243	138	82

* ( ) number in brackets indicates % removal

Experiments using a 10 % leachate batch (inoculum 90 mg/l dry weight) showed that (Figure 4.9) there was sequential utilisation of the leachate components. Initially the alcohols were degraded, then PCOC and finally the phenoxies.

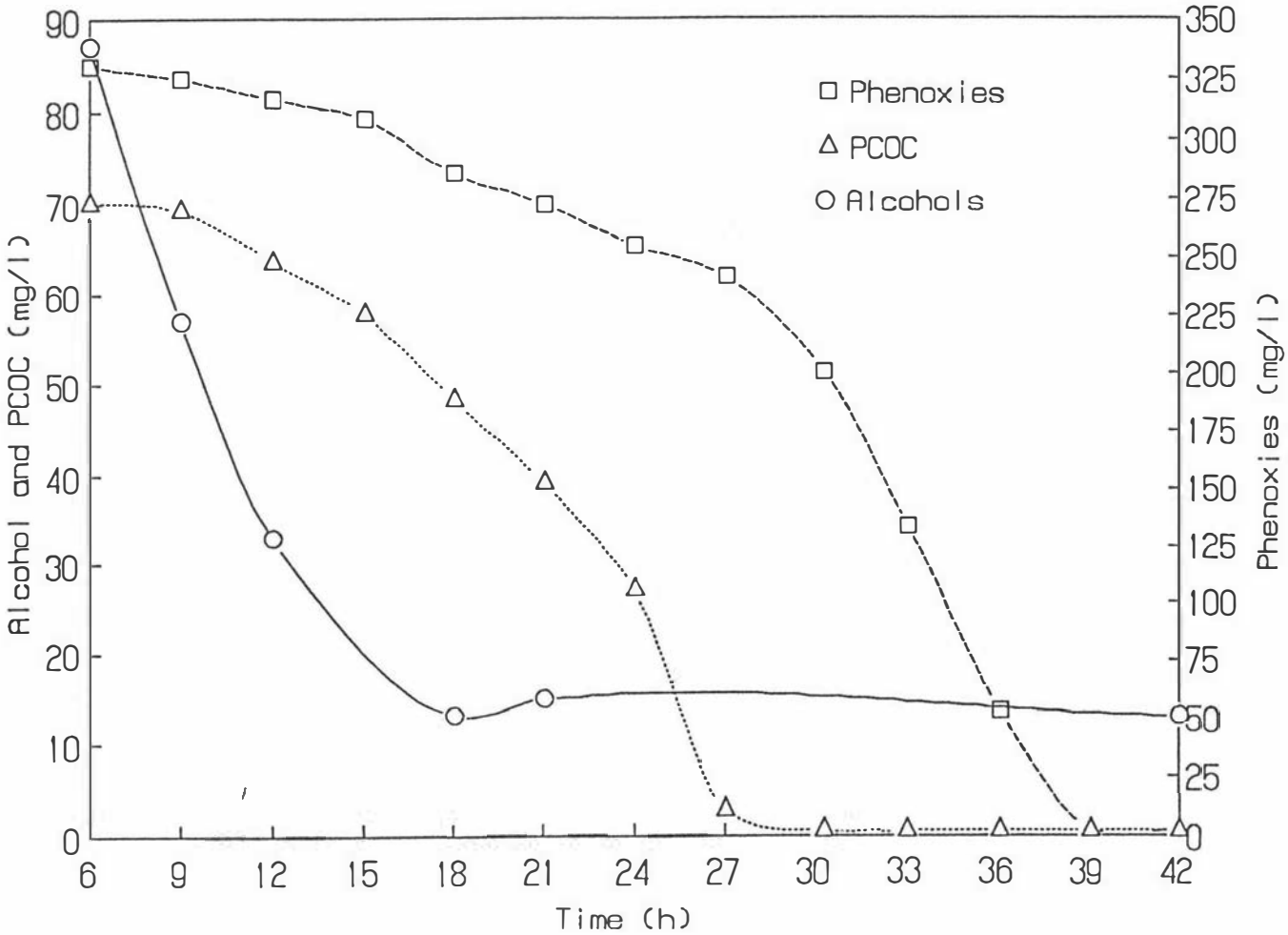


Figure 4.9: Plot of Substrate versus Time for the Degradation of 10 % Leachate.

As stated earlier the chlorophenols are the first breakdown product of phenoxy degradation (Section 2.2.1.1). Therefore it is expected that PCOC will be removed prior to the degradation of significant quantities of phenoxy, as phenoxy degradation would be inhibited at the sidechain cleavage step by product inhibition (Conn and Stumpf,1976).

Alcohols were the first compounds to be degraded (Figure 4.9), possibly as they are simpler to degrade. Catabolite repression (Lindstrom and Brown,1989) would probably prevent the degradation of PCOC while significant quantities of alcohols were present.

However, the removal data listed in Table 4.9 indicated that there was substrate inhibition occurring. As the leachate concentration increased, the total substrate degraded decreased, to the point where in 20 % leachate not all alcohols were degraded in the 20 h period. Higher concentrations showed no degradation at all during the 20 h, indicating total inhibition. Therefore it was only possible to use leachate concentrations of 20% or lower.

4.5.1.2 CSTR Conditions.

Batch experiments indicated that there was substrate inhibition, however, such effects may not occur under CSTR conditions because the residual substrate concentrations are very low. There may also be other factors which influence the degradation rates. It was therefore necessary to determine the maximum leachate concentration that could be tolerated in a CSTR system.

Experimental Procedure.

The medium used for these experiments was the simple buffered medium (Section 4.2.2). Four different leachate concentrations were used, 5,10,15 and 20 %. The composition is given in Table 4.10. Biomass was measured directly as MLSS (Section 3.4). pH was measured directly using the Orion Research pH meter (Section 3.9).

The cultures were grown in a perspex bioreactor (Section 3.8), with a working volume of approximately 900 ml, in the same manner as described earlier (Section 4.2.1). Effluent was passed from the bioreactor pump into a measuring cylinder to measure the volume passing through the system. Working volume was measured after the run was completed.

Temperature was controlled to 25 °C by placing the bioreactors in a water bath, with stirring provided by stirrer bars driven by magnetic stirrers (Chiltern, MM21, Salmond Smith Biolab, Palmerston North, N.Z.) placed under the water bath. Wall growth was removed daily by scraping the stirrer bar over the inside surface of the bioreactor. The tubing to the bioreactor was cleaned and fresh feed was made daily.

Samples were removed directly from the mixed liquor and either analyzed immediately, or stored frozen (-18 °C) until analysis. The absorbance of the culture at 600 nm (Cecil CE202 UV

Visible Spectrophotometer, Wiltons Scientific, Wellington, N.Z.) was recorded daily and while not used for biomass determination was used to determine when the culture was at steady state. Daily substrate concentrations (PCOC/phenoxies) were measured by HPLC (Section 3.2).

Each run was started either by collecting effluent from the parent bioreactor, placing this (including biomass) into the bioreactor and starting the feed at the required concentration and flowrate, or, if a run had just finished, collecting 900 ml of effluent from the parent bioreactor, recovering by centrifugation and adding the biomass to the existing reactor and restarting the feed at the new (higher) concentration. If the new concentration was lower, the feed was changed to the lower concentration.

Results and Discussion.

The results are summarised in Table 4.10 and the data illustrated in Figures 4.10 - 4.13. The raw data from these experiments can be found in Appendix 1 (spreadsheet APPEND1.WKS).

Table 4.10 Summary of the Effect of Initial Concentration on CSTR Performance.

Feed Concentration (% leachate)	5	10	15	20
Initial Concentrations (mg/l)				
Phenoxies	218	435	708	944
PCOC	33	66	107	142
Alcohols	41	82	123	164
Residual Concentrations (mg/l)				
Phenoxies	1.0	1.7	2.6	3.9
PCOC	ND*	ND	ND	ND
Alcohols	ND	ND	ND	ND
Biomass (mg/l)	212	538	800	1338
Dissolved Oxygen (mg/l)	3.7+	3.4+	4.6	4.3
Retention Time (h)	15.0	15.0	14.4	14.8
Ash (g/l)	0.6	1.2	1.9	2.9
TDS (g/l)	1.3	1.8	2.5	3.4
TOC (mg/l)	40	70	162	198
pH drop	0.11	0.28	0.30	0.38

* ND Not Detected

+ Hole noted in Gas distribution tube: O_2 transfer reduced.
Experiments were run for a minimum of 16 residence times.

During the course of the runs, HPLC analysis showed that there were a small number of minor components which were not degraded by the culture. They were eluted early in the chromatogram; at 82 and 87% of the run time for 2,4-D and the peak height indicated they were present at approximately

50 mg/l in the undiluted leachate. These compounds were detected in the leachate, but only by injecting an undiluted sample of the feed. Chromatograms for analysis of effluent from 10% and 15 % leachate media, along with a chromatogram of 15 % feed are shown in Figure 4.14. The identity of these compounds is unknown, and as a CN system operates in a mixture of separating modes (Millipore, 1985) it is not possible to predict their nature.

From these results it was concluded that, firstly, 20 % leachate (944 mg/l phenoxy, 142 mg/l PCOC, 164 mg/l alcohols and 2.9 g/l ash) was the maximum substrate concentration at which a bioreactor could be operated. At higher concentrations it was observed that initially phenoxies were degraded, while the concentration of PCOC increased. Subsequently the phenoxy concentration increased, causing failure of the reactor. This observation is contrary to earlier observations (Section 4.5.1.1) that phenoxies were not degraded in the presence of high concentrations of PCOC. The reason or mechanism of this effect was not investigated further.

The second observation was related to the speed of recovery after stressing the bioreactor. While the 20 % leachate reactor ran well, both the 10 and 15 % systems were stressed by mechanical failures and the time taken for the system to recover varied considerably. The 15 % leachate bioreactor required 9.9 residence times to recover after an increase in substrate to 50 % of the feed concentration (S_o), where as the 10 % leachate bioreactor required only 3.4 after an increase to 43 % of S_o.

Extrapolation of the data indicates that any failure in the bioreactor running on 20 % leachate would result in even longer recovery times.

4.5.1.3 Choice of Leachate Concentration for Experimentation.

As noted previously, there is a maximum leachate concentration above which inhibition of degradation occurs. The best operating concentration for a CSTR system would be in the range of 10 - 15 % leachate (435-708 mg/l phenoxies, 66-107 mg/l PCOC, 80-120 mg/l alcohols and 1.2-1.9 g/l ash). For such ranges, bioreactors should operate well at steady state, and will also recover from any operational upsets, as was shown in the previous section.

For batch experiments, 5 - 10 % leachate appears most suitable (Section 4.5.1.1). This minimised inhibition and allowed rapid experimentation.

4.5.2 Effect of pH on Leachate Degradation.

Throughout previous experiments the pH of the leachate was left unaltered. However, it was important to determine if the medium's pH would produce any increase in degradation rates.

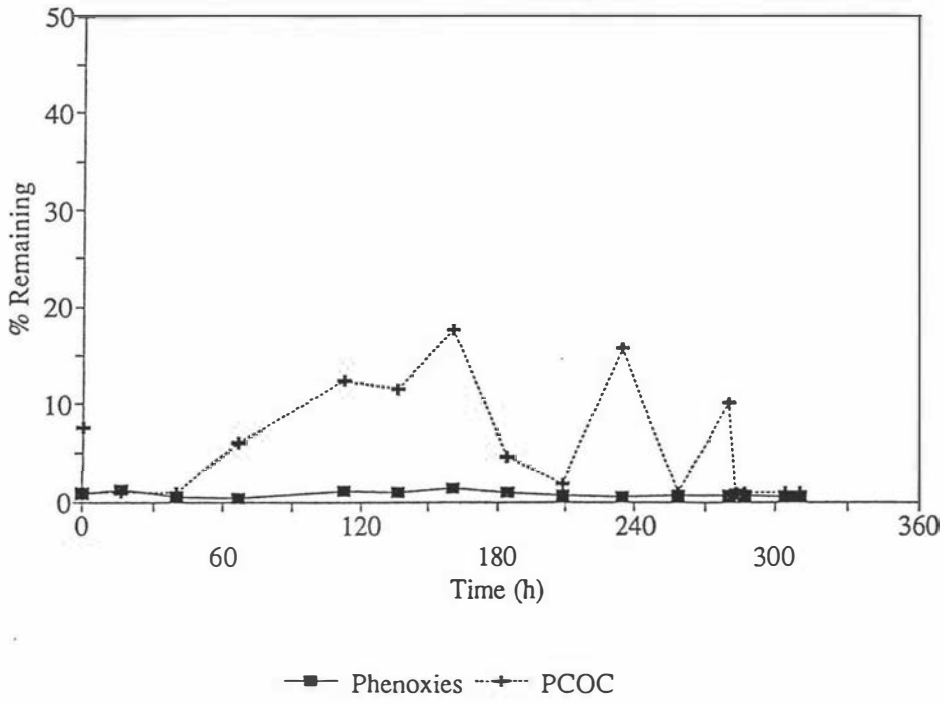


Figure 4.10: Plot of Residual PCOC and Phenoxy versus Time for Chemostat Run on 5 % Leachate.

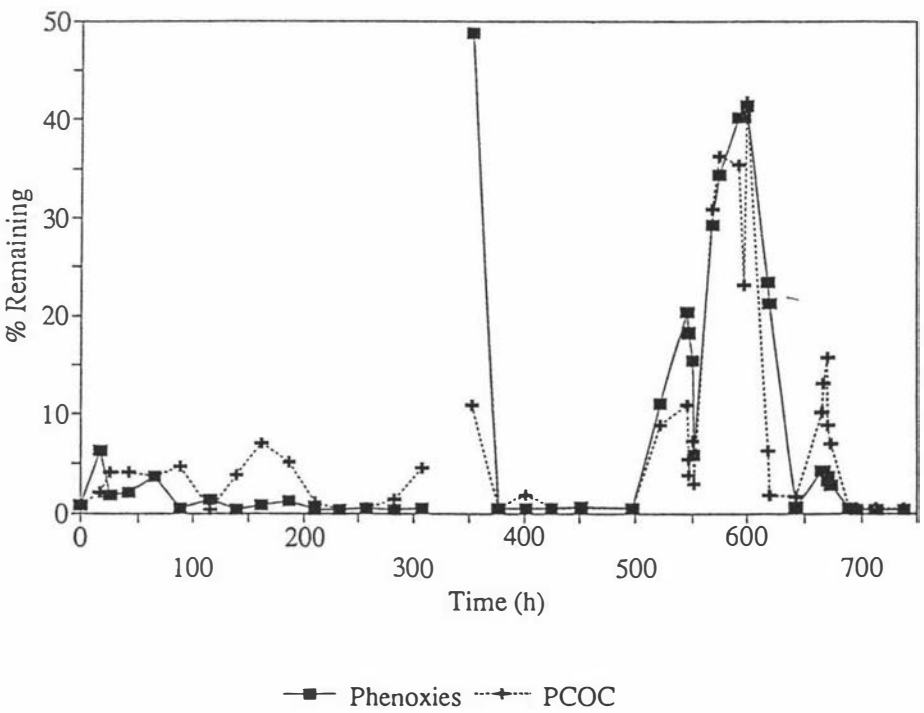


Figure 4.11: Plot of Residual PCOC and Phenoxies versus Time for Chemostat Run on 10 % Leachate.

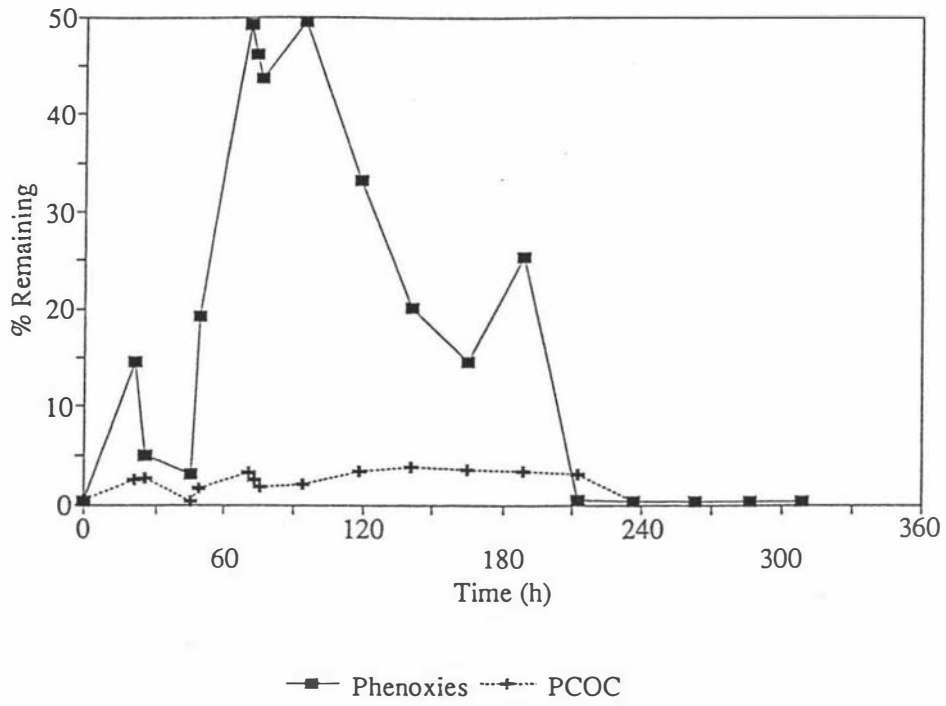


Figure 4.12: Plot of Residual PCOC and Phenoxies versus Time for Chemostat Run on 15 % Leachate.

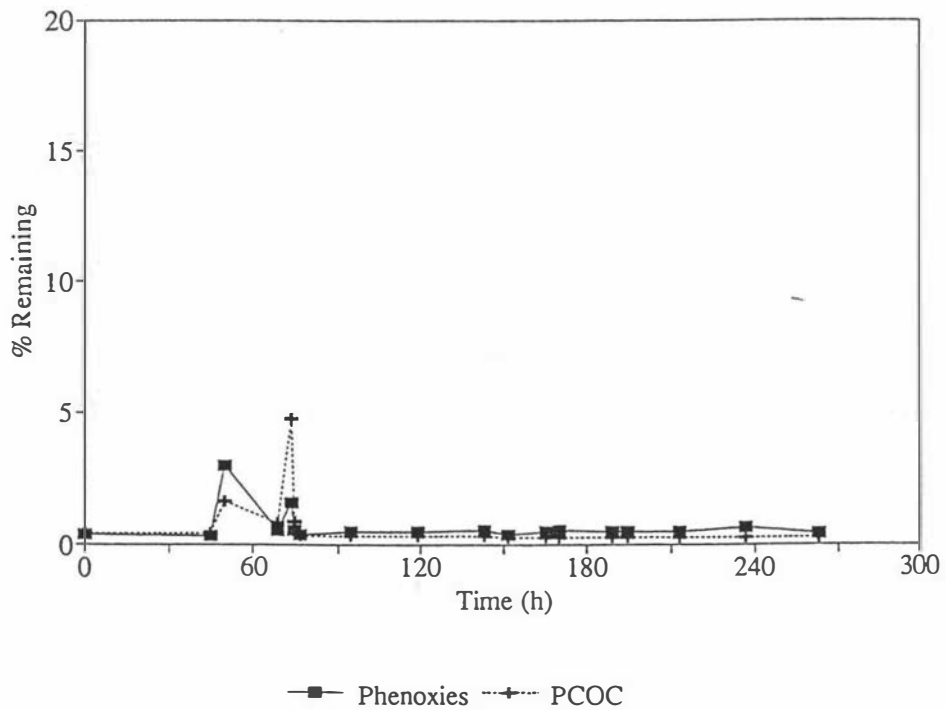


Figure 4.13: Plot of PCOC and Phenoxies Versus Time for Chemostat Run on 20 % Leachate.

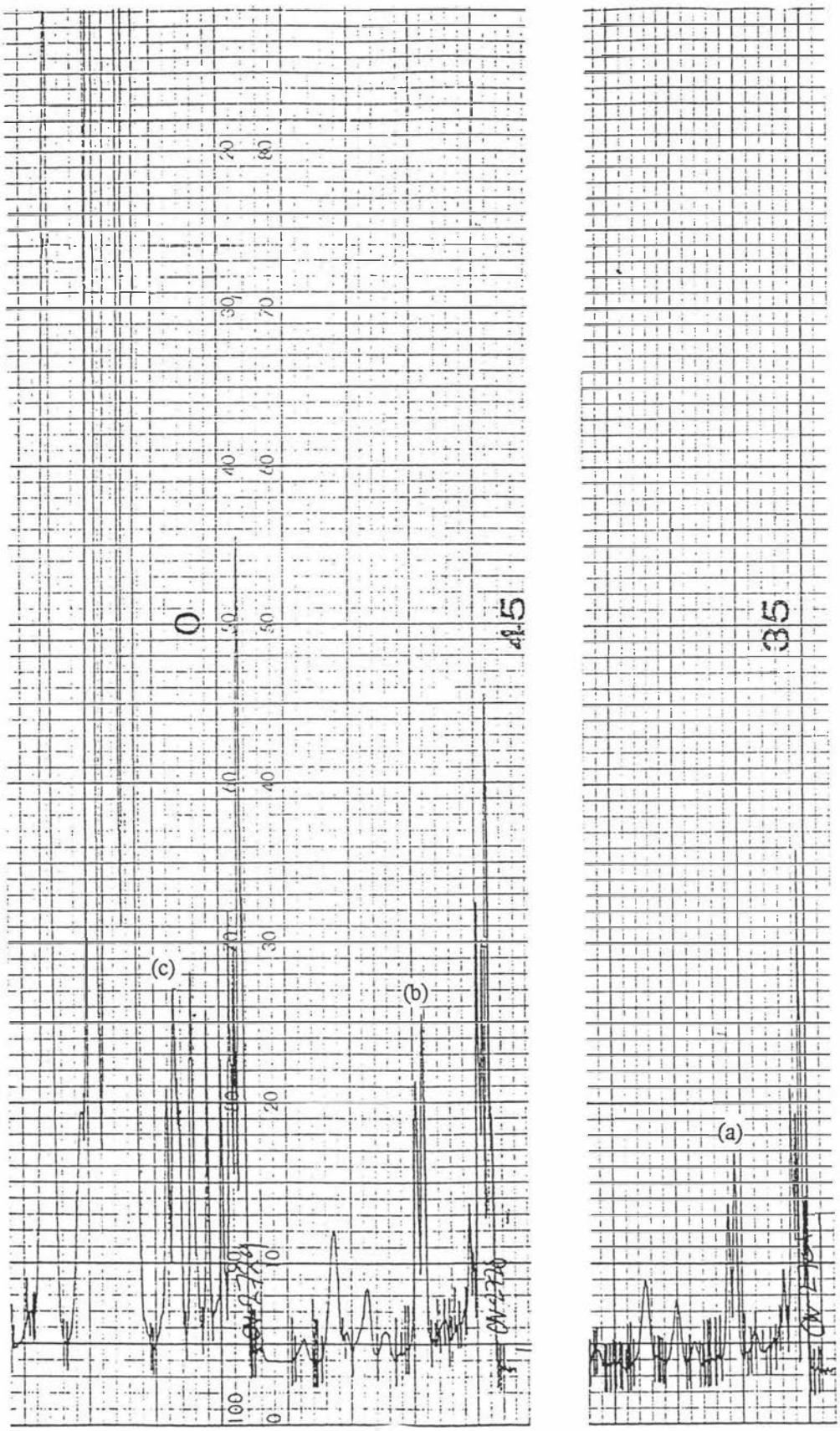


Figure 4.14: Chromatograms Generated Using CN-HPLC System:(a) 10 % Leachate ex Parent Bioreactor;(b) 15 % Leachate ex Bioreactor;(c) 15 % Feed, Undiluted.

4.5.2.1 Experimental Procedure.

Aliquots (100 ml) of 5% leachate in simple buffered medium (250 ml Erlenmeyer flasks) were adjusted to the desired pH, inoculated to 24 mg/l with biomass from the parent bioreactor and incubated at 25 °C in an Orbit Water Bath Shaker (Cat No 3536, Lab-line Instruments Inc, Illinois) at 150 rpm. Samples were analyzed by HPLC (Section 3.2) to determine the PCOC/phenoxy concentrations. The time to complete degradation was determined from time course data over 16-26 h. The experiments were run in two groups, each containing a batch at pH 6.65. The ratio of the batch time at each test pH to the standard pH of 6.65 was determined, giving a relative rate of leachate degradation at each pH.

4.5.2.2 Results and Discussion.

The raw data from this experiment can be found in Appendix 2. Only the final results are shown here, in Figure 4.15. It is shown that the fastest leachate degradation occurred between pH 6.1 and pH 6.65. Below pH 6.1 the degradation rate fell rapidly, and above pH 6.65 the degradation rate decreased slowly. Two observations were drawn. Firstly that no pH adjustment of leachate medium was required, unless the pH was to fall below pH 6. This is lower than was observed when degrading 20% leachate (Table 4.10), therefore no pH adjustment should be required, an obvious advantage in a large scale situation. Secondly, these results compare favourably to those of Tyler and Finn (1974) using 2,4-D as the substrate (Figure 4.16). Their results for 2,4-DCP were quite different to results described here. This may be due to phenoxies being the major components in leachate, with their properties influencing the overall behaviour of the leachate.

4.5.3 Effect of Recycled Effluent on Degradation.

Recycle of the treated effluent through the landfill would reduce the water volume required to treat the site. Such a system would lead to a buildup of ash and recalcitrant organics in the system, which may be detrimental to the culture, thus it was necessary to determine the effect of the residual solids on leachate degradation.

4.5.3.1 Experimental Procedure.

The methods used for this section were basically the same as Section 4.5.2. Non-degradable solids were prepared by taking 5 l of parent bioreactor effluent and concentrating to 200 ml by distillation. The remaining liquor contained 40.6 g/l total solids and 26.3 g/l ash. The difference (14.3 g/l) was considered to be organic. This solution was used to prepare a series of media in 5 % leachate (Experiment 1, Table 4.11). Leachate (5 %) was also amended with NaCl (Experiment 2, Table 4.11). This was done to determine whether any observed effect was due to the presence of organics in the recycle, or due to osmotic pressure from a high ash concentration. Phenoxy concentrations were determined by HPLC (Section 3.2), and the final rate of removal was measured for each batch. These

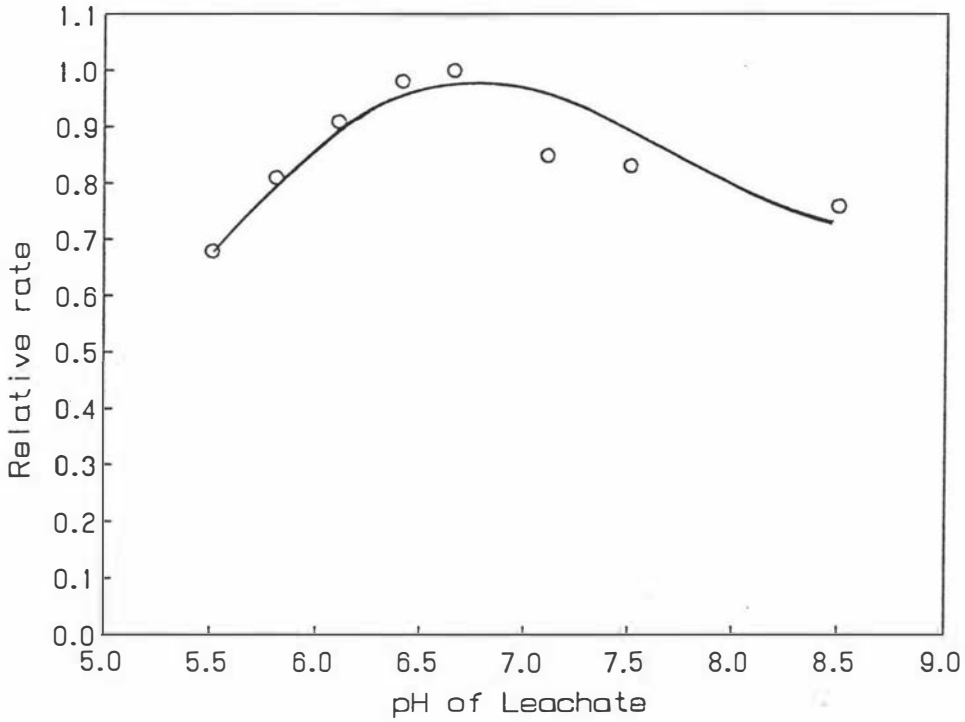


Figure 4.15: Plot of Relative Degradation Rate versus Leachate pH.

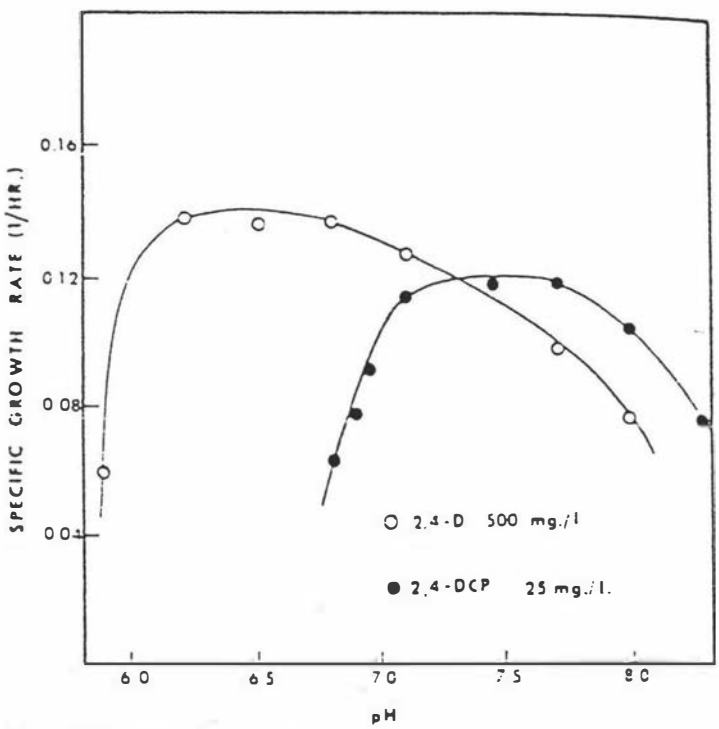


Figure 4.16: Plot of Specific Growth Rate of a Pseudomonad versus Growth pH using 2,4-D and 2,4-DCP (Reproduced from Tyler and Finn,1974).

rates were then standardised using 5 % leachate as a relative rate of 1.0.

4.5.3.2 Results and Discussion.

The results of this experiment can be found in Table 4.11.

Table 4.11 The Effect of Dissolved Solids and NaCl on Leachate Degradation Rates.

Rate (mg/l.h)	Rel rate	TDS (g/l)	Ash (g/l)	NaCl* (g/l)
		Expt 1 Recycled Solids		
22.8•	1.00	1.3	0.6	0.5
21.7	0.95	5.3	3.2	2.7
22.5	0.99	9.2	5.6	4.8
18.6	0.82	13.3	8.3	7.1
11.5	0.50	16.9	10.5	8.9
		Expt 2 NaCl amended		
20.9•	1.00	1.3	0.6	0.5
21.0	1.00	4.3	3.6	3.5
12.8	0.61	8.8	8.1	8.0
6.4	0.31	10.4	9.7	9.6
5.3	0.25	16.2	15.5	15.4

* Based on 85 % of cations in leachate are Na^+ (section 4.2.2)

All experiments performed at pH 6.65

• Reference runs

The results for Experiment 1 indicate that there was an inhibitory effect on phenoxy degradation by the recycled solids. This effect could be caused by two factors, the presence of inhibitory organics in the recycled solids, or changes in osmotic pressure exerted by the high ash concentration. The second experiment was designed to differentiate between these two effects. It can be seen that the NaCl amended leachate also had an inhibitory effect on phenoxy degradation.

A plot of relative rate of phenoxy degradation versus NaCl concentration is shown in Figure 4.17. This graph indicates coincidence between the inhibition due to recycled solids and NaCl, hence it was concluded that the NaCl present in the leachate was probably the major cause of inhibition at higher recycled solids concentrations. As the degradation rate started to decrease at NaCl concentrations greater than 4.8 g/l, it would be advisable to ensure that NaCl concentrations did not rise above this level when recycling through the dumpsite.

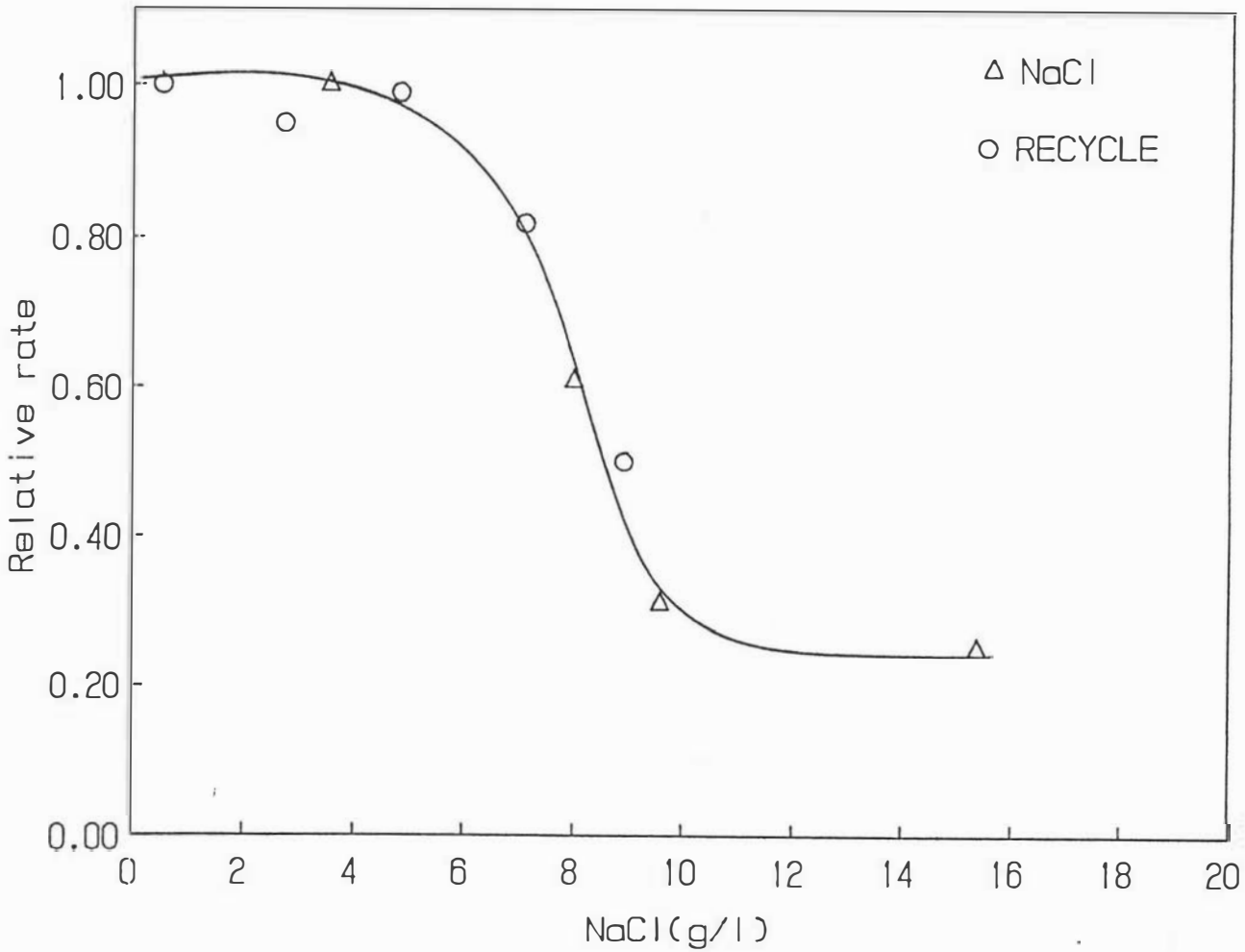


Figure 4.17: Plot of Relative Degradation Rate versus NaCl Concentration in the Growth Medium.

4.5.4 Summary.

The work described in this section has made three major points.
(1) The chosen concentration for continuous bioreactor work was 10 - 15 % leachate (435-708 mg/l phenoxies, 66-107 mg/l PCOC, 80-120 mg/l alcohols and 1.2-1.9 g/l ash), resulting from a trade off between increased degradation rates at higher concentrations and increased recovery rates from reactor upsets at lower concentrations. The chosen concentration for batch work was found to be 5 - 10 % leachate (217-435 mg/l phenoxies, 33-66 mg/l PCOC, 40-80 mg/l alcohols and 0.6-1.2 g/l ash).

(2) The native pH of the leachate was found to be best for the degradation of leachate, with no pH adjustment required when running at up to 20 % leachate.

(3) If recycling of the treated waste through the dump is to be used, the NaCl concentration should not be allowed to rise above 4.8 g/l. The residual organics appear to have no effect on degradation at the levels tested.

These results indicate that the diluted leachate, when incorporated into a simple buffered medium, was amenable to biological treatment.

4.6 Microbiology.

In previous sections, the process of removing the PCOC/phenoxies from the leachate has been described as biodegradation, although no evidence was presented to suggest that microbial agents were involved. This section of work will present evidence for biodegradation further data on the microbial population.

4.6.1 Proof of Microbial Degradation.

It was important to prove that the removal of alcohols, PCOC and phenoxies from the leachate was microbial and not caused by any other mechanism, such as air stripping, photodegradation or adsorption. This was to show microorganisms were involved and ensure that routine determinations of PCOC/phenoxy and alcohol concentrations truly indicated the extent of biodegradation.

4.6.1.1 Experimental Procedure.

Two perspex bioreactors (Section 3.8) containing 1 l of 10% leachate in simple buffered medium (filter sterilised, 0.45 μm filters) were prepared. Conditions were maintained as previously described in Section 4.5.1.2 (but run as a batch). One bioreactor was inoculated with 50 mg/l of active biomass from the parent bioreactor, while the second was inoculated with 50 mg/l of previously

autoclaved (121 °C for 15 min) parent bioreactor biomass. The alcohol and PCOC/phenoxy concentrations were monitored for 42-46 h using methods previously described (Sections 3.2,3.3).

4.6.1.2 Results and Discussion.

The alcohol and PCOC/phenoxy concentrations during the batch for both sterile and viable inocula are shown in Figure 4.18. These results indicate the only removal mechanism for PCOC/phenoxies was biodegradation. Alcohol removal was observed in both systems, but was removed much faster in the viable bioreactor (15 h) than in the non-viable (46 h). The increase in rate indicates the viable biomass contributes to the removal of the alcohols. The loss of alcohols in the non-viable reactor could have been due to one of two factors: either air stripping of the volatile alcohols or contamination of the medium with microorganisms introduced in the air or during sampling. The former is considered more likely than the latter. Air stripping would be expected to be less in a CSTR system, as the concentrations of these compounds is very low, reducing the driving force for mass transfer (Moos et al., 1983).

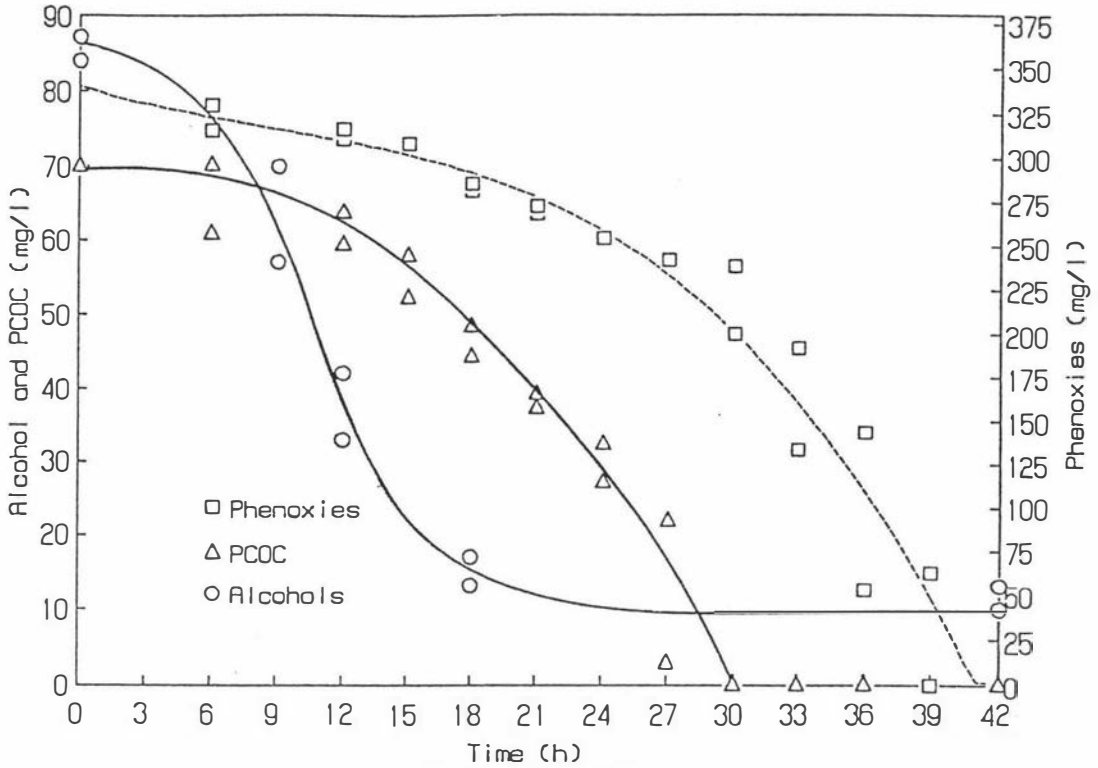
Other removal mechanisms (such as photodegradation) were probably not operating in this system either, as any such removal would have resulted in a significant concentration change of either PCOC or phenoxies in the control. These did not occur.

It was concluded that biodegradation was the major, if not sole, removal mechanism of alcohols, PCOC and phenoxies in a CSTR system. As a CSTR system would be used on a large scale, biodegradation would be expected to be the dominant removal mechanism.

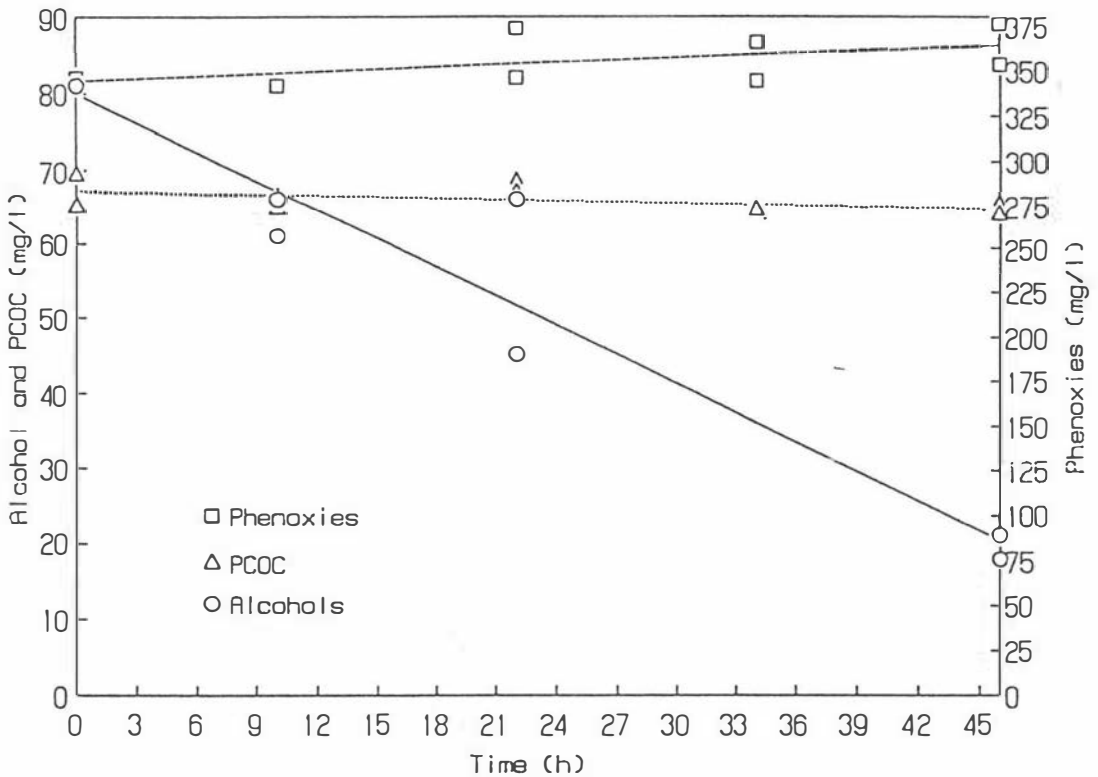
4.6.2 Isolation and Identification of Microorganisms in the Mixed Culture.

Two studies were carried out on the microorganisms degrading leachate, the first, using a sample taken at the end of the alcohol degradation phase in 10 % leachate after 7 months of operation and directly from the parent bioreactor after 26 months. The aim of these studies was to obtain information on the organisms present in the biomass to compare with the literature and to determine whether there was a significant change in the population over the 18 months between the two studies. Details of the isolation and identification of organisms can be found in Appendix 3.

Data presented in Appendix 3 indicates Ps. aeruginosa and Ps. fluorescens were present in both sets of isolates. Other Pseudomonas species were also noted in the second study. As stated earlier (Section 2.2.1.3), Pseudomonas species are frequently associated with the degradation of phenoxy herbicides and their associated chlorophenols, with just under 50 % of the references to specific organisms studied referring directly to these organisms (Table 2.2). In several cases, the species is unknown (e.g. Gaunt and Evans, 1971a, b; Schwein and Schmidt, 1982; Spain and Nishino, 1987); presumably as there was difficulty in placing the organism into any particular species. The detection of Pseudomonas species in the mixed culture degrading leachate was therefore not unexpected.



(a) Viable Inoculum.



(b) Autoclaved Inoculum.

Figure 4.18: Plots of Substrate Concentration vs Time for (a) viable and (b) autoclaved inocula in 10 % Leachate.

The presence of higher organisms, such as the rotifer Philodina was an indication that the culture was ecologically diverse. Rotiferia are considered to be an indication of a high degree of substrate removal (Doohan, 1975). These organisms were not deliberately introduced into the parent bioreactor, but probably became established after an opportunistic contact. Bdelloids (such as Philodina) secrete a protective cyst under adverse conditions and can withstand long periods of desiccation (Doohan,1975), so it is not surprising that this organism was present.

The detection of Enterobacteriaceae in the second study was also not unexpected. While there are no reports of these organisms degrading PCOC or phenoxies (Section 2.2.1.3), in a system where no sterile precautions were taken, the presence of secondary organisms was likely. The presence of such secondary organisms is quite common in waste treatment systems (Greenfield,1987).

The presence of an amorphous matrix, holding the cells together in flocs, was noted in both studies. The presence of flocculant biomass is an important criterion for an activated sludge plant, as such a system relies on gravity settling and thickening of the biomass prior to recycle (Grady and Lim,1980). In this case it can be seen that the requirement of flocculation was met by the culture. The residence time in the parent bioreactor was 14.5 h. This is significantly shorter than the 2 - 3 days quoted by Grady and Lim (1980) as necessary for effective flocculation to occur.

The two studies, separated by 18 months of operation in a non sterile manner, indicated that the stable culture consisted of gram-negative rods of varying lengths, held together by an amorphous matrix. The same Pseudomonas species were present in both studies and it was concluded that no gross changes in the microbial population had occurred over the period of operations. The nature of the biomass in both studies was comparable to that expected for an activated sludge plant.

As stated earlier (Section 2.2.1.3), the degradation of PCOC/phenoxies is plasmid mediated. Work done in this department has shown the parent bioreactor culture contains a number of plasmids. The plasmids were c.a. 15.3 megadaltons (23 K base pairs) in size (Chiura et al.,1990), compared to the 50-70 megadaltons reported elsewhere (Chatterjee et al., 1981; Don and Pemberton, 1981), and could be transferred to E.coli DH5∝, allowing the E.coli to grow on 2,4-D (Chiura et al.,1990). These results indicated that degradation was plasmid mediated in this culture, in agreement with the findings of other workers using different cultures.

4.7 Culture Maintenance.

Chiu et al. (1972b) stated that as activated sludge is a continuous enrichment culture of microorganisms and that unless all conditions are kept constant, the composition of the microbial population will fluctuate. This has also been borne out by other workers, using both experimental results (Chiu et al., 1972a, Yoon et al., 1977; Gottschal and Thingstad, 1982) and theoretical models (Yoshida et al.,1979). Therefore the parent bioreactor was operated under constant conditions.

At no time did the culture lose the ability to degrade leachate. It also recovered relatively rapidly after upsets caused by mechanical failures. Reinoculation was never required. The longest period of upset operation (22 days) occurred over days 464 - 486 (Christmas - New Year 1988-89), when two parameters changed simultaneously (flow increased by 25 % and temperature decreased by 20 %). After correction (day 480) the return to steady state required 6 days.

Thus the parent bioreactor was a suitable method of maintaining an active culture for experimental use. However, for security reasons, samples of the culture were freeze dried, according to the method of Kirsop and Snell (1984). Such cultures were found to be viable, and capable of degrading 5 % leachate in 48 h, after a lag of 48 h. The viability was checked immediately after drying and again after storage at room temperature for 1 year. In each case the culture retained its activity.

4.8 General Discussion.

This chapter has described the development of a natural population capable of degrading the alcohols, PCOC and phenoxies present in the leachate. Methods of culture development abound in the literature, but there is one common thread through most: the repeated batch enrichment, often followed by isolation of a single organism responsible for the degradation. This method has been used with success including many recent workers (Ditzelmuller et al.,1989; Kilpi et al.,1980; Ou and Sikka, 1977; Lappin et al.,1985). However some (Ou and Sikka,1977; Lappin et al.,1985) have found that while an enrichment culture was capable of degradation, the pure isolates were not. Rosenberg and Alexander (1980a, b) showed that the degradation of 2,4,5-T was mediated by a mixed culture and that pure isolates were not capable of performing the degradation. A number of other reports also show that pure isolates from mixed cultures were able to perform only part of the degradation, but not complete mineralisation (Horvath and Alexander, 1970; Horvath, 1971a, b).

These reports lead to the conclusion that such an approach may not produce the best culture as the basic method selects for organisms capable of degrading a single compound, when all nutrients are present in excess (Slater and Lovatt, 1984). The method also selects the organisms with the highest growth rate under conditions of excess of all nutrients (r-strategist) which may not be able to compete with organisms with lower maximum growth rates, but with very low saturation constants (K-strategists), under conditions of substrate limitation (Slater and Lovatt, 1984). Such conditions of substrate limitation occurs in CSTR systems, as was proposed for use in Section 2.3.3.1.

Slater and Lovatt (1984) proposed a method of culture enrichment which allows the isolation of highly stable associations of microorganisms. They recommended the use of chemostats, as unnecessary organisms are rapidly washed out. The system is also limited by a nutrient and hence selects for a k-strategist.

There is one drawback of the chemostat approach, particularly when related to inhibitory compounds as in this study. As shown in Section 2.4.4.2 if the dilution rate in a system is above the critical dilution rate (described by Rozich et al.,1983), or the initial concentration of the substrate is too high, the chemostat will be operating in an unstable region and therefore may fail to degrade the compound. It is therefore necessary to have a culture that can degrade the compounds of interest, however slowly, prior to the use of the chemostat for enrichment. In the case of this study, three preliminary batches were run, the first taking 13 days, and the second 96 h, prior to the establishment of a chemostat. The chemostat was established after a 38 h batch, once the inhibitory compounds had been degraded, allowing the system to operate in a stable region.

The source of inoculum for the first batch was important. In an approach similar to Kilpi et al. (1980) the first batch was seeded with soil that had been repeatedly exposed to the compounds studied, ensuring organisms were present in the inoculum capable of degrading phenoxies.

The presence of secondary, more easily metabolised substrates may also enhance the degradation, especially in a CSTR system. Under growth limiting carbon concentrations Lindstrom and Brown (1989) state that a secondary carbon source adds to the biomass present, but does not induce catabolite repression. This effect stabilises the population in the presence of high and inhibitory concentrations of the target compound (Lindstrom and Brown,1989). This effect could be occurring in the system under study, with the alcohols acting as the secondary substrate, and the PCOC/phenoxies as the target compounds.

The parent bioreactor was established 39 days after the initial batch was started. While few authors give total times for enrichment, Lappin et al., 1985, quoted 4 months as the time required to produce an enriched culture degrading Mecoprop (a related herbicide). The method employed in this study was significantly faster than previously reported. Culture viability was shown to be the only factor involved in the removal of PCOC and phenoxies, and a major factor in the removal of alcohols in batches. Air stripping was considered to be negligible in CSTR systems, due to the low driving force for mass transfer (Moos et al., 1983). Biodegradation was the only removal mechanism.

The mixed population enriched and maintained in the parent bioreactor was shown to consist of Pseudomonas species along with other organisms, and to be relatively stable over the period of the study. The culture produced many of the characteristics of an activated sludge population.

The culture was also capable of producing an effluent with concentrations of PCOC/phenoxy consistently at or below the limits of detection (approximately 0.5 mg/l per compound).

As any large scale system will probably use this culture, it would be hoped that the culture could perform well on a large scale. The sponsoring company (DowElanco) has conducted batch trials on a 50 m^3 scale using inocula from the parent bioreactor. They have found the culture can rapidly degrade the PCOC/phenoxies, and still retains the ability to flocculate under quiescent conditions (Catt,

1990). This is an indication the culture should be suitable for a large scale activated sludge system. The culture was found to completely mineralise 2,4-D, according to the rule proposed by Grady (1985). The reduction in toxicity of the leachate supported this conclusion.

The optimum concentrations for degradation were found to be 5-10 % leachate (217-435 mg/l phenoxies, 33-66 mg/l PCOC, 40-80 mg/l alcohols and 0.6-1.2 g/l ash) for batch work and 10 - 15 % leachate (435-708 mg/l phenoxies, 66-107 mg/l PCOC, 80-120 mg/l alcohols and 1.2-1.9 g/l ash) for CSTR work. The concentrations of chlorophenols in these media are well above those reported as inhibitory to Pseudomonas species by Tyler and Finn (1974). No pH control was required, with the native pH of the leachate suitable for growth. A simple medium was developed to ensure sufficient nitrogen, phosphate and sulphate were present in the feed.

The order of substrate uptake in a batch was interesting (Figure 4.9). Initially the alcohols were used, followed by PCOC and finally phenoxies were degraded. In CSTR experiments that were carbon limited, there was simultaneous uptake of all three groups of substrates. It therefore appeared that there were interactions occurring between the three groups of substrates.

The presence of these interactions indicated the leachate may not be able to be treated as one substrate, as is the norm for waste treatment systems (Grady and Lim,1980). It was therefore necessary to study the kinetics of degradation of the individual groups of compounds separately and together in leachate to determine the need for a multisubstrate approach. This work will be the subject of the next chapter.

4.9 Conclusions.

This chapter has shown that the leachate under study could be degraded by a mixed microbial population developed for the purpose. A simple buffered medium was developed that allowed rapid degradation of the key components present in the leachate. No pH adjustment was required. The population developed was capable of mineralising the leachate and was stable in continuous culture (residence time 14.5 h, D = 0.069 h^{-1}) over 872 days. The characteristics of the culture indicated its suitability for activated sludge.

The optimum concentrations for degradation were found to be 5-10 % leachate (217-435 mg/l phenoxies, 33-66 mg/l PCOC, 40-80 mg/l alcohols and 0.6-1.2 g/l ash) for batch work and 10 - 15 % leachate (435-708 mg/l phenoxies, 66-107 mg/l PCOC, 80-120 mg/l alcohols and 1.2-1.9 g/l ash) for CSTR work.

Batch studies showed sequential utilisation of the substrates, indicating there may be a need to use complex multisubstrate models to describe degradation, rather than the more usual single substrate models frequently applied to waste treatment systems.

CHAPTER 5
THE KINETICS OF PURE COMPOUND DEGRADATION.

5.1 Introduction.

An important part of the design and economic analysis of a potential cleanup method is the determination of the kinetics of the waste degradation. Variation in composition often occurs with landfill leachates (Cope, 1983), indicating the need to be able to predict degradation kinetics under a wide variety of leachate compositions.

A mathematical description of leachate degradation will be complex if it is to account for a number of interacting phenomena such as the effect of each substrate on overall removal rates, and any interactions between substrates. It was shown earlier (Chapter 4) that classification of the substrates into three classes, alcohols, PCOC and phenoxies was useful for describing batch degradation. Using this taxonomy, a three substrate model, similar to that of Yoon et al. (1977) could be developed. The basic model of Yoon et al. (1977) has already been applied successfully to describe chlorinated phenol degradation in the presence of second substrates such as glucose (Papanastasiou and Maier,1982) and other chlorinated phenols (Klecka and Maier,1988).

Usually, such models rely on the determination of the degradation kinetics of each compound alone, followed by the determination of interaction parameters based on batch experiments (Yoon et al., 1977).

This chapter describes the determination of degradation kinetics of the pure compounds, the data from which will be used later in a three substrate model. As common assumption of constant k_s and $Y_{x/s}$ values under all conditions was made.

5.2 Initial Batches.

Preliminary experiments were carried out using leachate as the medium to determine initial estimates of the kinetic parameters such as the specific growth rate, yield coefficient and half saturation constant on each substrate. This was done to allow better design of the subsequent experiments.

5.2.1 Experimental Procedure.

Duplicate batches were run using 10 % leachate in simple buffered medium (Section 4.2.2). The experiments were carried out at 25°C in the perspex bioreactors (Section 3.8). All conditions were the same as described in Section 4.6.1, except both inocula were viable, and the medium was not sterilised. Biomass was determined by either the COD or Biuret method as described in Appendix 4.

5.2.2 Results.

The results of these experiments are shown in Figures 5.1 and 5.2. It can be seen from these data that alcohols were being removed first (with the exception of part of the secondary alcohols), followed by PCOC and finally phenoxies. From the biomass data it was possible to estimate the growth rate and yield coefficient for the alcohols and phenoxies. These are given in Table 5.1. Figures 5.1 and 5.2 also show there was very little biomass produced from the PCOC degradation, with an apparent lag in growth until all was degraded. After PCOC degradation was complete, phenoxy degradation was rapid.

Table 5.1 Initial Estimates of μ_{MAX} and Yield Coefficients of Alcohols and Phenoxies in Leachate.

	$Y_{X/S,ALC}$ (mg/mg)	$\mu_{MAX,ALC}$ (h^{-1})	$Y_{X/S,OXY}$ (mg/mg)	$\mu_{MAX,OXY}$ (h^{-1})
Average	1.32	0.20	0.37	0.048
(diff ±)	0.08	0.01	0.01	0.004

5.2.3 Discussion.

Considerable differences were apparent between the kinetics of degradation of the different classes of compounds. There was also considerable variation between the kinetic values of phenoxy degradation and that reported in the literature (see Table 2.4) with the measured growth rate being less than half the value reported previously, and the yield coefficient was greater than twice that reported previously.

Therefore it was considered necessary to study the degradation of each component in isolation. It was decided to study alcohol degradation first, followed by PCOC degradation with the last compounds to be studied being the phenoxies.

5.3 Alcohol Degradation.

5.3.1 The Kinetics of Alcohol Degradation.

The maximum growth rate of the culture on alcohols was measured and it was determined whether there was any growth inhibition by alcohols. An estimate of the half saturation constant (k_s) was also calculated so that a useful substrate degradation model could be developed.

5.3.1.1 Experimental Procedure.

Experiments were conducted in two parts; initially to determine whether substrate inhibition would be a factor at high concentrations, and secondly to follow a time course of the degradation.

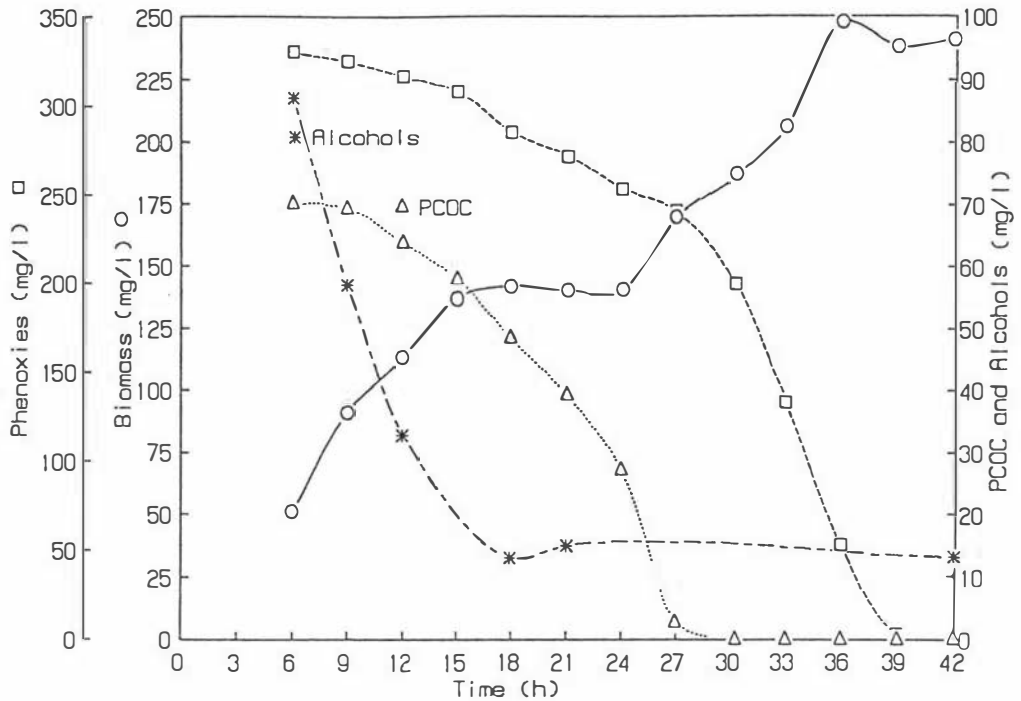


Figure 5.1: Plot of Substrate and Biomass versus Time for the Degradation of 10 % Leachate: Batch I.

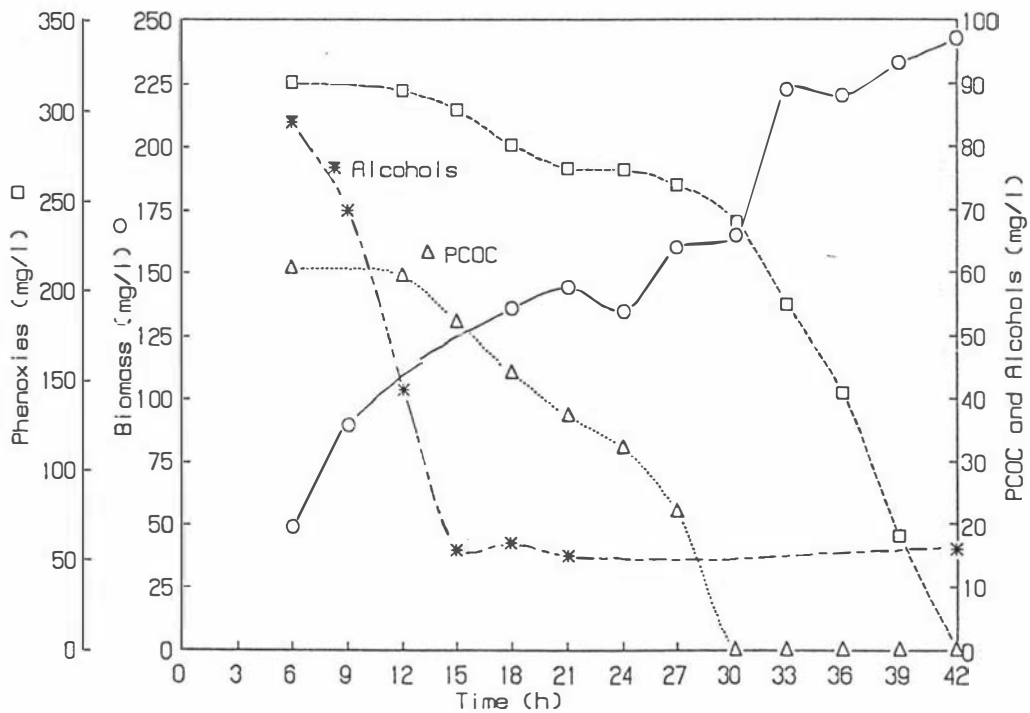


Figure 5.2: Plot of Substrate and Biomass versus Time for the Degradation of 10 % Leachate: Batch II.

Inhibition studies were conducted using Basal Ash Medium (BAM) (Appendix 5) amended with 78, 195, 390, 585 and 780 mg/l alcohols (butan-1-ol, butan-2-ol and methanol all in the same proportions as found in leachate), and inoculated with culture stored at 4 °C. The growth rate was determined in the same manner as used in Appendix 5. All other conditions (volumes etc) were the same as those described in Appendix 5.

Observations on degradation were conducted using duplicate flasks containing 390 mg/l alcohols in BAM. The same inocula and conditions were employed as per inhibition studies (above) except that the volume of media used was increased to 200 ml to ensure sample withdrawal would have a minimal effect on the degradation. Throughout the course of the batch 2 ml samples were taken for alcohol analysis by GLC (Section 3.3).

5.3.1.2 Results.

Inhibition Experiments.

The growth rates were determined in the same manner as previously described (Appendix 5) The measured growth rates can be found in Table 5.2. A plot of $1/\mu$ versus 1/S can be found in Figure 5.3. This plot indicates that there was no apparent substrate inhibition.

Table 5.2 Specific Growth Rates at Five Different Alcohol Concentrations.

Concentration (mg/l)	Growth rate (h^{-1})
78	0.34
195	0.28
390	0.35
585	0.28
780	0.29
Average	0.31 (Std Deviation = 0.03)

Slope of line in Figure 5.3 not significantly different from 0

There was no apparent pattern in the measured growth rates. This variation in measured growth rates made it impractical to perform a direct plot (Wharton and Eisenthal,1981) to determine $k_{s,ALC}$.

Alcohol Utilisation.

Figure 5.4 illustrates alcohol utilisation by the culture. The specific growth rate was found to be 0.26 ± 0.01 h^{-1}, and the yield coefficients for the culture on alcohols was 1.2 and 1.1 mg biomass/mg alcohol for the duplicate batch experiments.

Figure 5.4 also shows that the secondary alcohols were not totally removed, with 78 % still present after 43 h. The primary alcohols were exhausted by approximately 35 h. This will be addressed

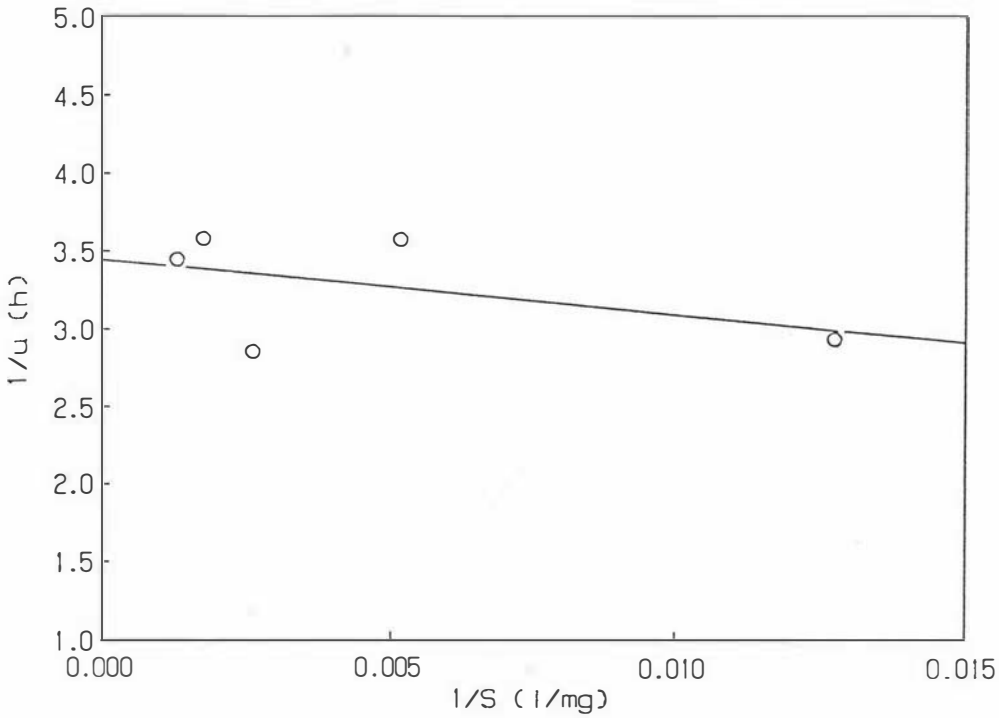


Figure 5.3: Plot of 1/μ versus 1/S to Determine Whether Alcohols are Inhibitory.

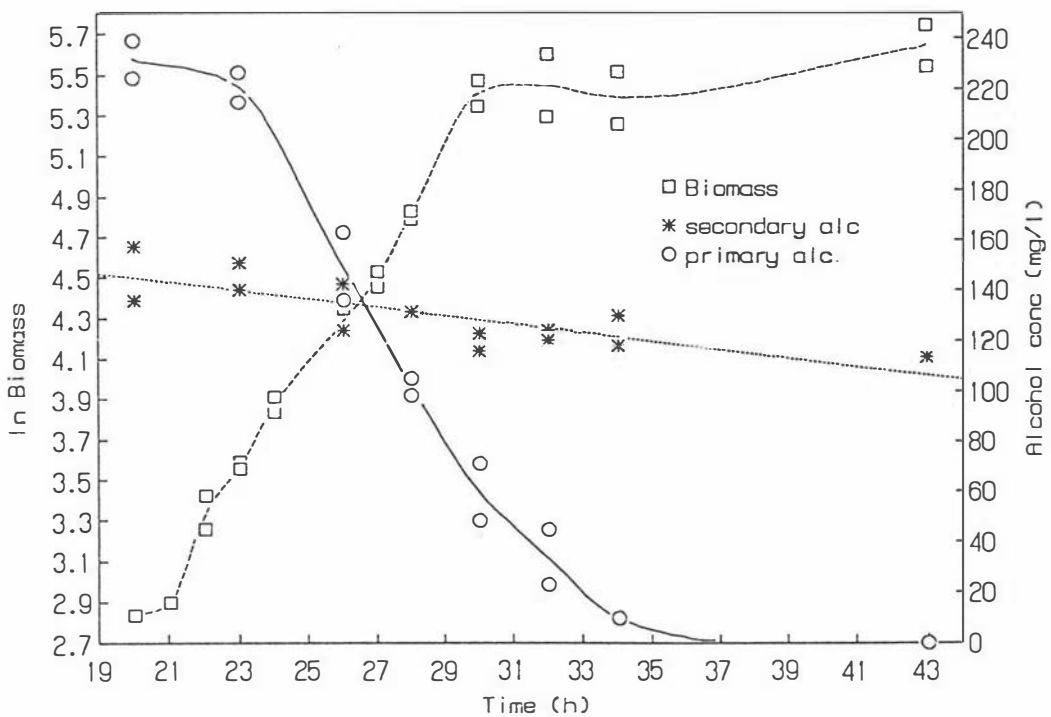


Figure 5.4: Plot of Alcohol and Biomass Concentrations versus Time for the Batch Degradation of Alcohols in BAM.

further in the next section.

5.3.1.3 Discussion.

The average maximum specific growth rate (observed) for the culture degrading alcohols was found to be 0.31 h^{-1}, with no substrate inhibition observed up to 780 mg/l alcohols. The specific growth rate measured in separate experiments was found to be approximately one standard deviation lower, which was an acceptable difference. The average maximum compared well with an expected value (0.31 h^{-1}) determined using BAM (Appendix 5). These values for the maximum specific growth rate agree well with data obtained by other workers using butan-1-ol, namely 0.34 and 0.31 h^{-1} (Luong, 1987, Wayman and Tseng, 1976).As the incubation temperature was not given in these references, it was not possible to make precise comparisons.

The maximum specific growth rate reported for methanol (Luong,1987) was, however, variable, with the highest being to be 0.725 h^{-1} (30 °C), but the average was reported to be approximately 0.25 h^{-1} (temperature unknown). The measured values, therefore are of a similar order of magnitude to those reported in the literature, although again, temperature differences may be a factor.

That secondary alcohols were not completely removed in the batch runs was noted also in previous experiments (Figures 5.1, 5.2 and 4.9). It was also noted that their degradation stopped after the primary alcohols were exhausted. The reason for this was not elucidated although cometabolism of the secondary alcohols in the presence of primary alcohols seems likely. As the metabolism of all alcohols seems to be closely linked and because the residual alcohol concentration in leachate based batches was also small (13-15 mg/l) it was assumed that degradation could approximated by considering all alcohols as one chemical group. CSTR experiments (Section 4.5.1.2) showed that residual concentrations of secondary alcohols were frequently below the GLC detection limit, indicating that any error that may be introduced in batch modelling by this assumption would be negligible in CSTR systems.

Four estimates of the yield coefficient of growth on alcohols, gave an average of 1.2 mg/mg (std deviation 0.1). This value was regarded as an initial estimate, as further determinations of the yield coefficient can be made based on data yet to be described.

The determination of $k_{s,ALC}$ using a direct plot (Wharton and Eisenthal,1981) posed problems as it was unsuitable. A second method available determining k_s is computer fitting of the substrate versus time data to the model (Ong,1983; Robinson and Characklis,1984). This, however, can have the effect of producing unrealistic values, such as those of Luong (1987), who quotes the k_s value for Ps. methanicia growing on methanol of 0.00478 mg/l. Such a concentration is one that could not be easily measured, nor would it have any real significance because the model would be very close to zero order kinetics.

As it was not possible to determine the value of $k_{s,ALC}$, it was necessary to obtain a reasonable estimate of its value. Table 5.3 gives some literature values.

Table 5.3 Literature Valued of k_s on Butan-1-ol and Methanol for Some Bacteria.

Organism	k_s (mg/l)	Substrate	Reference
Arthrobacter	113	Butan-1-ol	Luong (1987)
Arthrobacter	8.8	Butan-1-ol	Wayman and Tseng (1976)
Ps. methanica	0.005	Methanol	Luong (1987)
Ps. extorquens	0.6	Methanol	Harrison (1973)
Ps. species	1.6	Methanol	Harrison (1973)

As the data indicated the $k_{s,ALC}$ value was less than 78 mg/l, the first value of Luong (1987) was ignored. Luong's second value was, as mentioned earlier, an unreliable value, so that too was ignored. That left three values, differing by approximately a factor of ten. A weighted average was made, depending on the proportion of the alcohol present in the media. This gave an average $k_{s,ALC}$ of 5 mg/l. This value appeared to conform with the observed behaviour of the culture, and hence will be used for further work.

This work has determined the maximum specific growth rate of the culture degrading the alcohols present in leachate, and an estimate of the k_s has been obtained.

5.3.2 Determination of the Effect of PCOC on Alcohol Degradation.

It is necessary to determine whether PCOC has any effect on the kinetics of degradation of alcohols, so that interactions between substrates can be determined. Two approaches were taken to measuring the effect of PCOC on the degradation kinetics of alcohols: firstly experiments were conducted using 10 % leachate with extra PCOC added to increase the concentration and secondly using alcohols and the appropriate concentration of PCOC in BAM to determine the growth rate on alcohols at different PCOC concentrations.

5.3.2.1 Experimental Procedure.

Amended Leachate Experiments.

The experimental procedure used to ensure the effect of PCOC on growth rate was the same as that described in Section 5.2.1 except that the following changes were made. The PCOC concentration in each experimental medium was made up to the desired concentration by the addition of PCOC dissolved in the manner described in Appendix 5. The initial alcohol concentration was also

increased to 320 mg/l. The final phenoxy concentration was not altered from 370 mg/l. The pH was adjusted to 6.65 prior to inoculation.

BAM Experiments.

The experimental procedure for the BAM medium experiments was based on that used previously in Appendix 5. The final alcohol concentration was made up to 390 mg/l as before, and the PCOC concentrations used were 0 (triplicate), 80 mg/l (triplicate), 130 mg/l and 190 mg/l. Biomass was determined by absorbance at 600 nm using the standard curve described in Appendix 5.

5.3.2.2 Results.

Amended Leachate Experiments.

Experiments were carried out at PCOC concentrations between 60 mg/l (the normal concentration in 10% leachate) and 250 mg/l. The growth rate of the culture was determined as described previously (Appendix 5). These values at corresponding PCOC concentrations can be seen in Figure 5.5.

A linear decrease in specific growth rate with increasing substrate PCOC concentration was noted. PCOC was not acting as a substrate in these experiments, but as an inhibitor. The equation of the best fit straight line was found to be

$$\mu_{APP} = 0.26 - 9.77 \times 10^{-4} \times [PCOC]$$

where μ_{APP} is the apparent growth rate and
[PCOC] is the PCOC concentration in mg/l.

R^2 was 96.36 %. The standard deviation of the slope was 8.25×10^{-5}, or 8.4 %.
The 95 % confidence interval (CI) for the intercept was calculated by the method given in Mendenhall and Ott (1980) and found to be (0.25 - 0.27 h^{-1}).

The yield coefficient for alcohols was determined for four of the batches and the average, with previous data included, was found to be 1.3 mg/mg (SD = 0.2 mg/mg, n = 8).

BAM Experiments.

The specific growth rate of the culture was determined for each flask using the method described in Appendix 5. The results, in the form of a plot of specific growth rate versus PCOC concentration are shown in Figure 5.6. Again, the data were best fitted with a straight line:

$$\mu_{APP} = 0.30 - 1.03 \times 10^{-3} \times [PCOC]$$

R^2 was 96.9 %. The standard deviation of the slope was 7.03×10^{-5}, or 6.8 %. The 95 % CI for the intercept was found to be (0.29 - 0.31 h^{-1})

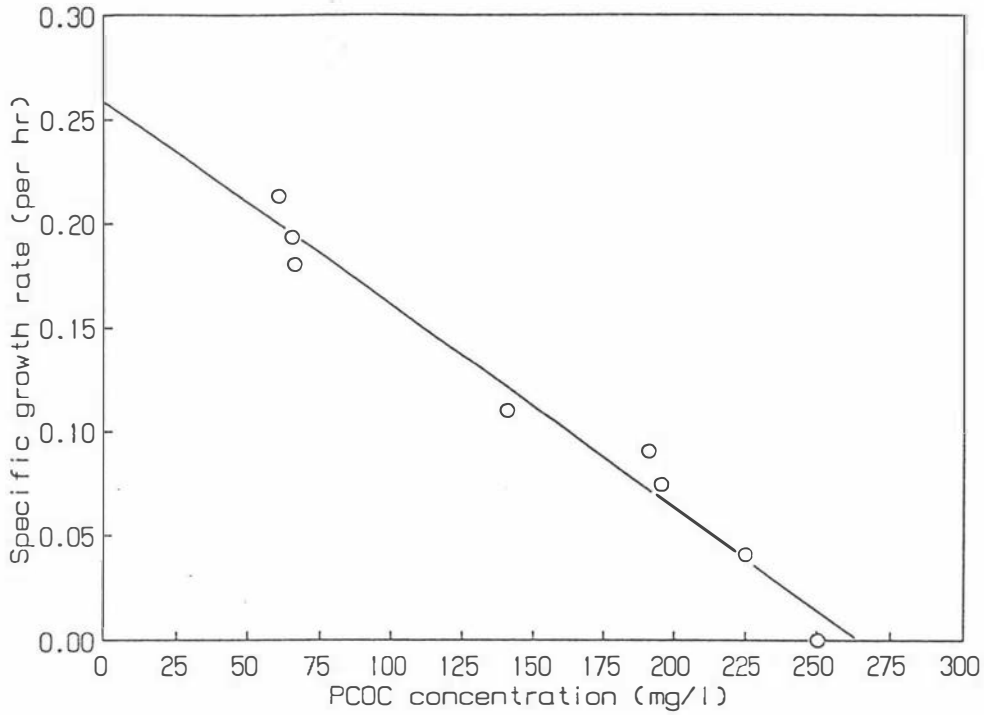


Figure 5.5: Plot of Specific Growth Rate on Alcohols versus PCOC (inhibitor) Concentration for the Culture Grown in Amended Leachate.

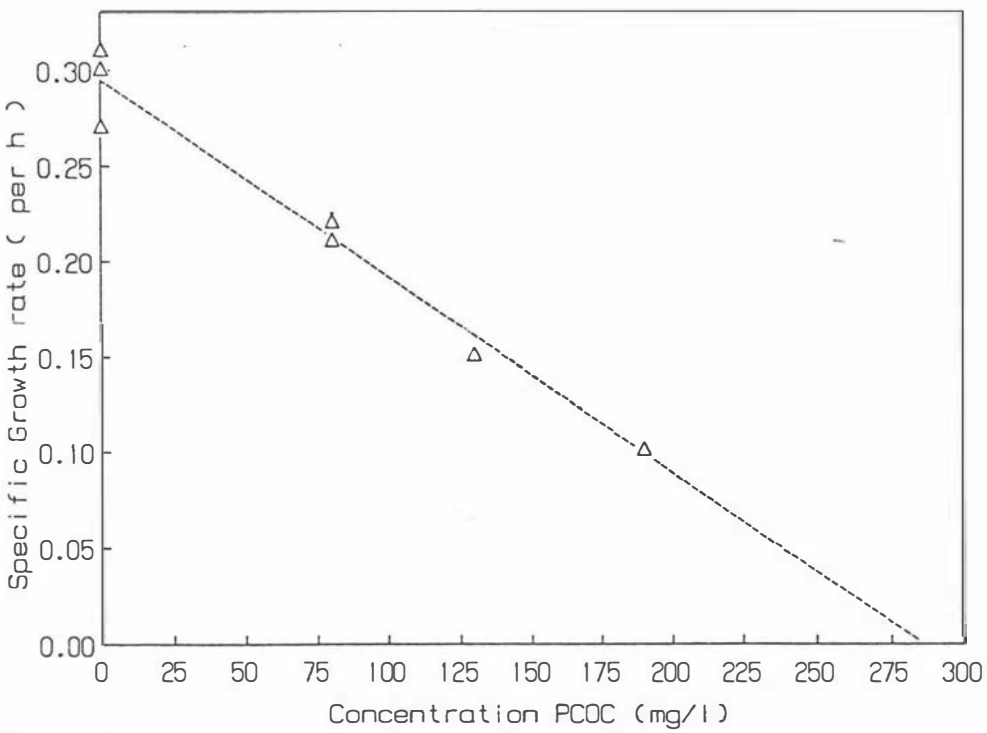


Figure 5.6: Plot of Specific Growth Rate on Alcohols Versus PCOC (inhibitor) Concentration for Culture Grown in BAM.

5.3.2.3 Discussion.

Amended Leachate Experiments.

Considering the yield coefficient data first, it can be seen that the average yield coefficient on alcohols was 1.3 mg/mg. The standard deviation was found to be 14 %. No data could be found in the literature on the yield coefficient of bacteria on butan-1-ol. The average yield coefficient on methanol was 0.50 g/g for bacteria and 0.36 g/g for yeast, according to Atkinson and Mavituna (1983). Tseng and Wayman (1975) however, found with three yeast (C. utilis, C. lipolytica, and S. cerevisiae) having an average yield of 1.32 mg/mg on ethanol and 1.07 mg/mg on butan-1-ol. These values agree well with the values reported in this study.

The inhibition of alcohol degradation by PCOC in leachate was, as mentioned earlier, found to be linear with increasing PCOC concentration. Such linear inhibition curves have been encountered before (Tseng and Wayman, 1975) using yeast and bacteria growing on simple alcohols, acids and esters, and also with 2,4-DCP degradation using bacteria (Tyler and Finn, 1974), where above a certain "threshold" concentration of substrate there was a linear reduction in growth rate with any further increase in concentration. The modelling of these results will be discussed in Section 5.3.4.

Kleist-Welch Guerra and Lochmann (1988) found that 2,4,5- and 2,4,6 -TCP are harmful to the nuclear DNA and reduced the DNA synthesis rate in yeast cells. This fact, along with the observation that the two metabolic pathways are quite dissimilar (Chapter 2), leads to the conclusion that the effect of PCOC on the degradation of alcohols found in this study could have been due to a toxic effect of the chlorophenol on the whole cell.

It has been observed in another instance that 2,4-DCP, however has no effect on the degradation rate of phenol or glucose by activated sludge, even at concentrations as high as 134 mg/l (Beltrame et al., 1982), despite evidence that this concentration of 2,4-DCP would be highly inhibitory (Tyler and Finn, 1974). These results appear to be at odds with the results reported here and elsewhere. This discrepancy would appear to imply either the culture of Beltrame et al. (1982) was very different to the cultures used elsewhere, in that it has an extremely high tolerance for 2,4-DCP. Work by Watkin and Eckenfelder (1989) showed that while 2,4-DCP was not inhibitory to its own degradation at concentrations up to 150 mg/l, the degradation of glucose was inhibited by relatively low concentrations of 2,4-DCP (K_I = 7 mg/l).

Results of other workers indicate there is considerable variation in the effects of the chlorinated phenols on degradation kinetics. It was therefore concluded that the kinetics are culture dependant and any data taken from the literature must be considered in relation to the culture used.

BAM Experiments.

It can be seen that the slopes of the lines describing the effect of PCOC in alcohol degradation in BAM and in leachate are very similar (10.3×10^{-4} and 9.8×10^{-4} l/h.mg) and are within 1 standard deviation of each other. There was no significant difference between the two slopes.

The measured intercepts, however, were significantly different at the 95 % level. It should be noted that the 95 % CI for BAM includes the measured μ_{MAX} on alcohols for this medium.

As mentioned earlier, linear inhibition curves have previously been reported. These are characterised by a maximum concentration, above which there is no growth. Extrapolating the data, it was found that in the case of PCOC in BAM, this occurred at a PCOC concentration of 291 mg/l (95% CI ± 18 mg/l). In leachate medium, total inhibition occurred at 266 mg/l (95% CI ± 21 mg/l).

There was one major difference between the media used that could have contributed to the difference in intercepts, the presence of the phenoxies in the leachate medium. If the phenoxies were inhibitory, then this would have the effect of lowering the observed growth rate. As the concentration of the phenoxies was constant, this difference would also be expected to be constant, hence the similarity in the slopes. Therefore it was necessary to investigate the effect of phenoxies on the degradation of alcohols.

It can be seen from this work that PCOC has a significant inhibitory effect on the degradation of alcohols present in leachate.

5.3.3 Determination of the Effect of Phenoxies on Alcohol Degradation.

The aim of this section of work was to quantify the effect of the phenoxies on alcohol degradation. It is also necessary to determine whether the different phenoxies (2,4-D, MCPA and 2,4,5-T) have the same effect on degradation kinetics. Work with mixtures of phenoxies will be described first, followed by work with the individual compounds.

5.3.3.1 Experimental Procedure.

Mixtures of Phenoxies.

The experimental procedure used was that as described in Section 5.3.2.1 except for one change. Instead of PCOC being added to the BAM along with the alcohols, a stock solution of phenoxies was added to give the necessary range of phenoxy concentrations. The stock solution was prepared by dissolving 350 mg 2,4-D, 500 mg MCPA and 95 mg 2,4,5-T in water and 1 M NaOH as previously described, and making up to 250 ml with MilliQ water. Each experiment (15, 25 and 35 ml stock per 100 ml medium) was performed in triplicate. Samples were retained for HPLC analysis using the CN system.

Pure Phenoxies.

The method used for the pure phenoxy experiments was the same as above, except that stock solutions of each phenoxy were made up individually. 2,4,5-T was difficult to dissolve under the conditions employed, but solution was achieved after vigorous mixing for 2-3 hours. The concentration of each phenoxy was made up to 800 mg/l in each flask. Duplicates batches were performed for each individual phenoxy.

5.3.3.2 Results.

Mixtures of Phenoxies.

The results are shown in Figure 5.7 where a linear relationship between specific growth rate and measured phenoxy concentration was given by.

$\mu_{APP} = 0.30 - 1.45 \times 10^{-4} \times [oxy]$

where [oxy] was the concentration of phenoxies in mg/l.

R^2 was 96.5 %. The standard deviation of the slope was 9×10^{-6}, or 6.2 %. The maximum concentration of phenoxies prior to total inhibition was found to be 2040 mg/l (95 % CI ± 80 mg/l).

Pure Phenoxies.

The measured growth rate and phenoxy concentration are given in Table 5.4.

Table 5.4 Measured Specific Growth Rates Using Alcohol in the Presence of Individual Phenoxies.

Sp. Growth rate (h^{-1})		Phenoxy	Concn (mg/l)	Pred * (h^{-1})
	0.19	2,4,5-T	841	
	0.22	2,4,5-T	750	
Avg	0.205		796	0.19±0.025
	0.21	2,4-D	712	
	0.22	2,4-D	829	
Avg	0.215		771	0.19±0.025
	0.20	MCPA	804	
	0.20	MCPA	760	
Avg	0.20		782	0.19±0.025

TOTAL 0.21 h^{-1} SD = 0.01 h^{-1} 782 mg/l SD = 50 mg/l

* indicates ± 95 % CI

It can be seen that in all cases the average measured growth rate on the pure phenoxies fell within the confidence interval around the value predicted based on the equation developed from mixture data. Therefore there was no reason to suggest that there was any difference in the ability between 2,4-D, MCPA and 2,4,5-T to inhibit alcohol degradation.

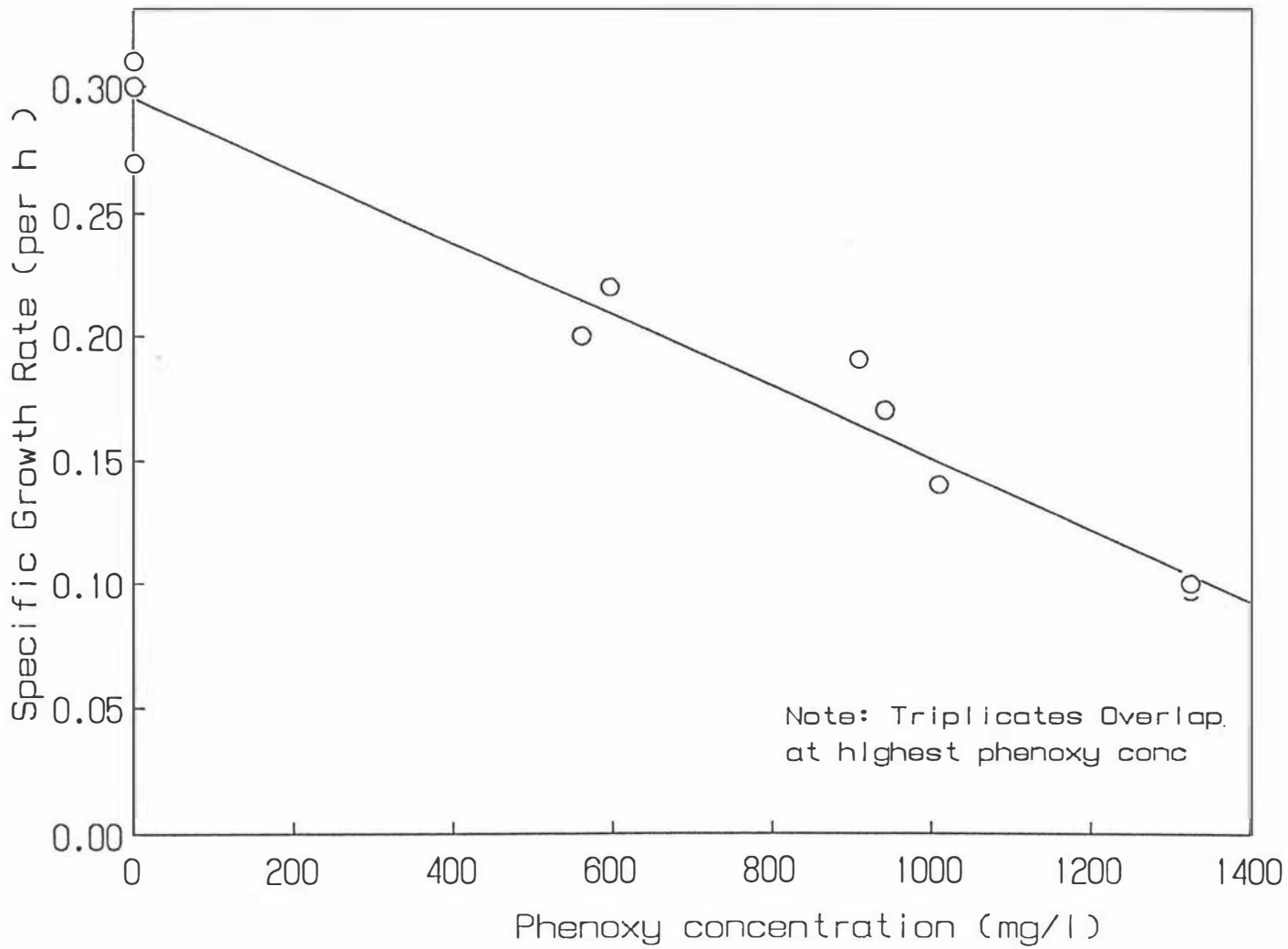


Figure 5.7: Plot of Specific Growth Rate on Alcohols versus the Inhibitor (Phenoxy) Concentration (Mixture of Phenoxies).

5.3.3.3 Discussion.

The section above described the linear inhibition of alcohol degradation by phenoxies, a pattern also observed with PCOC. Phenoxies were, however, seven times less inhibitory than PCOC (based on the slopes of the inhibition curves). This was not unexpected, as the chlorophenols are considered to be more toxic than the phenoxies (Mottet, 1985)

Differences in intercept between the BAM/PCOC medium and the amended leachate medium may be due to the phenoxies present in the leachate medium (Section 5.3.2.3). With a phenoxy concentration of 380 mg/l, the growth rate would be expected to be $0.25 \pm 0.025\ h^{-1}$. This would be the expected value of the intercept of the leachate experiments, if the remaining inhibition was caused by the phenoxies. The measured intercept was actually $0.26 \pm 0.02\ h^{-1}$ (Section 5.3.2.2). The confidence intervals overlap, indicating the inhibition was probably caused by the phenoxies.

This overlap also indicated that there was probably no interaction between the PCOC and phenoxies with respect to alcohol degradation. This should allow the separate effects, quantified in this section, to be combined into one model and still give an accurate prediction of growth rates.

5.3.4 Modelling of Alcohol Degradation.

5.3.4.1 Development of a Mathematical Model.

Kinetic descriptions of the degradation of alcohols which account for the influence of other compounds on the measured parameters will enable the development of a three substrate model. In the present study the model sought was one capable of describing the inhibition of degradation by PCOC and phenoxies.

Linear inhibition patterns have been studied previously using yeast (Tseng and Wayman, 1975) and bacteria (Tyler and Finn, 1974), and reported that above a certain "threshold" concentration of substrate there was a linear reduction in growth rate with any further increase in concentration. The result of this work was a postulated model where

$\mu = \mu_{MAX}.S/(k_s+S)$ when $S < S_T$ (S_T = threshold concentration) and

$\mu = \mu_{MAX}.S/(k_s+S) - i(S-S_T)$ when $S > S_T$.

The major disadvantage of this approach is that the model is discontinuous, making it more difficult to incorporate into an integrated model for a number of substrates.

Recently Luong (1987) proposed a generalised model which could replace one described by Haldane and was capable of being fitted to any curve shape. The model is

$\mu = \mu_{MAX}.S/(k_s+S) \times (1 - S/S_M)^n$

where

S_M is the substrate concentration where growth is totally inhibited, and

n is an empirical constant.

When n approaches 1 the model produces a line very similar to the model of Tseng and Wayman (1975). If n is greater than 1, then the model approximates the Haldane model.

This model was found to fit the data of Tseng and Wayman and others very well (Luong, 1987). However, the major problem with this model is the difficulty in fitting the parameters to the data using simultaneous nonlinear least-squares regression techniques, which are sensitive to both the initial guess for the parameters and can easily give results with no physical meaning (Luong, 1987).

As the models of Tseng and Wayman (1975) and Luong (1987) both appear to have significant disadvantages, a new model was developed.

Assuming that ash is not limiting (i.e. greater than 5-6 times the half saturation constant of 75 mg/l), and that PCOC and phenoxies have a toxic effect on the biomass (i.e. reduce the amount of viable cells) a mass balance can be written for the biomass.

Apparent Growth rate = maximum rate - inhibition
(mg/h) (mg/h) (mg/h)

The inhibition was a reduction on growth rate caused by the interference of chlorophenols on DNA synthesis (Kleist-Welch Guerra and Lochmann, 1988). 2,4,6-T has also been reported as causing damage to DNA in Bacillus subtilis (Kitchin and Brown, 1988), therefore, it was assumed the phenoxies interfered in a similar manner. Earlier data (Section 5.3.3.3) indicated that this was proportional to the concentration of the toxic compound.

$Inhibition = V.K_{PCOC}.[PCOC].X + V.K_{OXY}.[oxy].X$

where

V = system volume (l)

X = biomass concentration (mg/l)

[PCOC] = PCOC concentration (mg/l)

[oxy] = phenoxy concentration (mg/l)

K_{PCOC} = rate reduction due to PCOC ($l/mg_{PCOC}.h$)

K_{OXY} = rate reduction due to phenoxies ($l/mg_{OXY}.h$)

The actual growth rate was the growth model developed earlier (Sections 5.3.2 and 5.3.3);

$Growth\ rate = \mu_{MAX,ALC}.S.V.X/(k_{S,ALC}+S)$

where

$\mu_{MAX,ALC}$ = maximum specific growth rate (alc) (h^{-1})

$k_{S,ALC}$ = half saturation constant (mg/l)

S = substrate concentration (alc) (mg/l)

Converting into a mathematical equation yields;

$$V.dX/dt=(\mu_{MAX}.X.V-(K_{PCOC}.[PCOC]+K_{OXY}.[oxy]).X.V).S/(k_{S,ALC}+S)$$

eliminating V, taking X common on the left hand side and transposing to the right hand side gives

$$1/X.dX/dt = (\mu_{MAX}-(K_{PCOC}.[PCOC]+K_{OXY}.[oxy])).S/(k_{S,ALC}+S)$$

as 1/X.dX/dt is equal to μ, the final model was

$$\mu = (\mu_{MAX}-(K_{PCOC}.[PCOC]+K_{OXY}.[oxy])).S/(k_{S,ALC}+S) \quad (4\text{-}1)$$

The only values required to use this model are K_{PCOC} and K_{OXY}. These can easily be determined by from the data for alcohol degradation in the presence of PCOC and phenoxies in BAM. Under these conditions, when $S >> k_s$, and only one of the inhibitors was present, i.e. PCOC alone, equation (1) simplifies to;

$$\mu = \mu_{MAX} - K_{PCOC}.[PCOC] \quad (4\text{-}2)$$

or for phenoxies alone;

$$\mu = \mu_{MAX} - K_{OXY}.[oxy] \quad (4\text{-}3)$$

Equations (4-2) and (4-3) are the equations of straight lines, with Y-intercepts of μ_{MAX} and slopes of K_{PCOC} and K_{OXY} respectively. These plots and determinations have already been made in Sections 5.3.2.2 and 5.3.3.2 respectively.

This model has advantages over the other models discussed; it is a continuous function (if negative at high inhibitor concentrations, indicating cell death), in contrast to the model of Tseng and Wayman (1975), and the constants are very easy to determine, compared with the mathematical complexities of the model of Luong (1987).

5.3.4.2 Verification of the Model.

Verification of the model requires data not used in the determination of the model parameters. To this end a 15 % leachate batch was performed, according to the method used in Section 5.2.1. The results are shown in Figure 5.8. The experimental points are shown and the solid curves are those predicted by the model. The predicted data was generated using the parameters in Table 5.5 using the interactive simulation package ISIS run on an IBM XT clone. The program used is given in Appendix 6.

A chi squared test was performed (Mendenhall and Ott, 1980) on the biomass and substrate concentrations up to 20 h. The test statistic was found to be 6.83, less than the critical value of 14.06 (7 degrees of freedom, $\propto$ = 0.05), indicating there was no significant lack of fit.

Table 5.5 Parameters Used to Generate Figure 5.8.

Parameter	Value	units#
μ_{MAX}	0.30	h^{-1}
$k_{S,ALC}$	5	mg/l
$Y_{X/S,ALC}$	1.3	mg/mg
K_{PCOC}	$1.0x10^{-3}$	l/mg.h
K_{OXY}	$1.45x10^{-4}$	l/mg.h
S	125	mg/l
[PCOC]	100	mg/l
[oxy]	550	mg/l

units were converted to min^{-1} basis for model to avoid rounding problems.

5.3.4.3 Discussion.

As shown in Figure 5.8 the fit of the model was good up to 20 h, when alcohol degradation stopped, leaving only butan-2-ol present at 24 mg/l. There was no significant lack of fit up to this point. The model, however predicted total removal of these alcohols at longer times. As mentioned in Section 5.3.1.3, this discrepancy was expected in batch systems, but should not be apparent in CSTR systems.

One assumption not previously described was that PCOC and phenoxies were not removed. In the case of this batch, 3 % of the original PCOC and none of the phenoxies were removed during alcohol degradation. This assumption was therefore acceptable.

The model developed in this section was found to be suitable for describing the kinetics of alcohol degradation in batches, both in BAM and leachate. An inability to predict the residual butan-2-ol concentration should not be a problem when applied to CSTR systems.

5.3.5 Overview of Alcohol Degradation.

Work in this section has shown that PCOC and phenoxies both inhibit the degradation of alcohols, and the inhibition was first order with respect to inhibitor concentration. The work also showed there was no interaction between PCOC and the phenoxies with respect to the extent of inhibition.

The model developed was found to fit experimental data well, with the exception that the model was unable to predict the residual concentration of butan-2-ol. The data presented earlier indicated butan-2-ol degradation stopped when the primary alcohols were exhausted. This may have been due to the culture only being able to co-metabolise the secondary alcohols. If this were the case, a CSTR system would probably not have a large residual butan-2-ol concentration, as the system would be

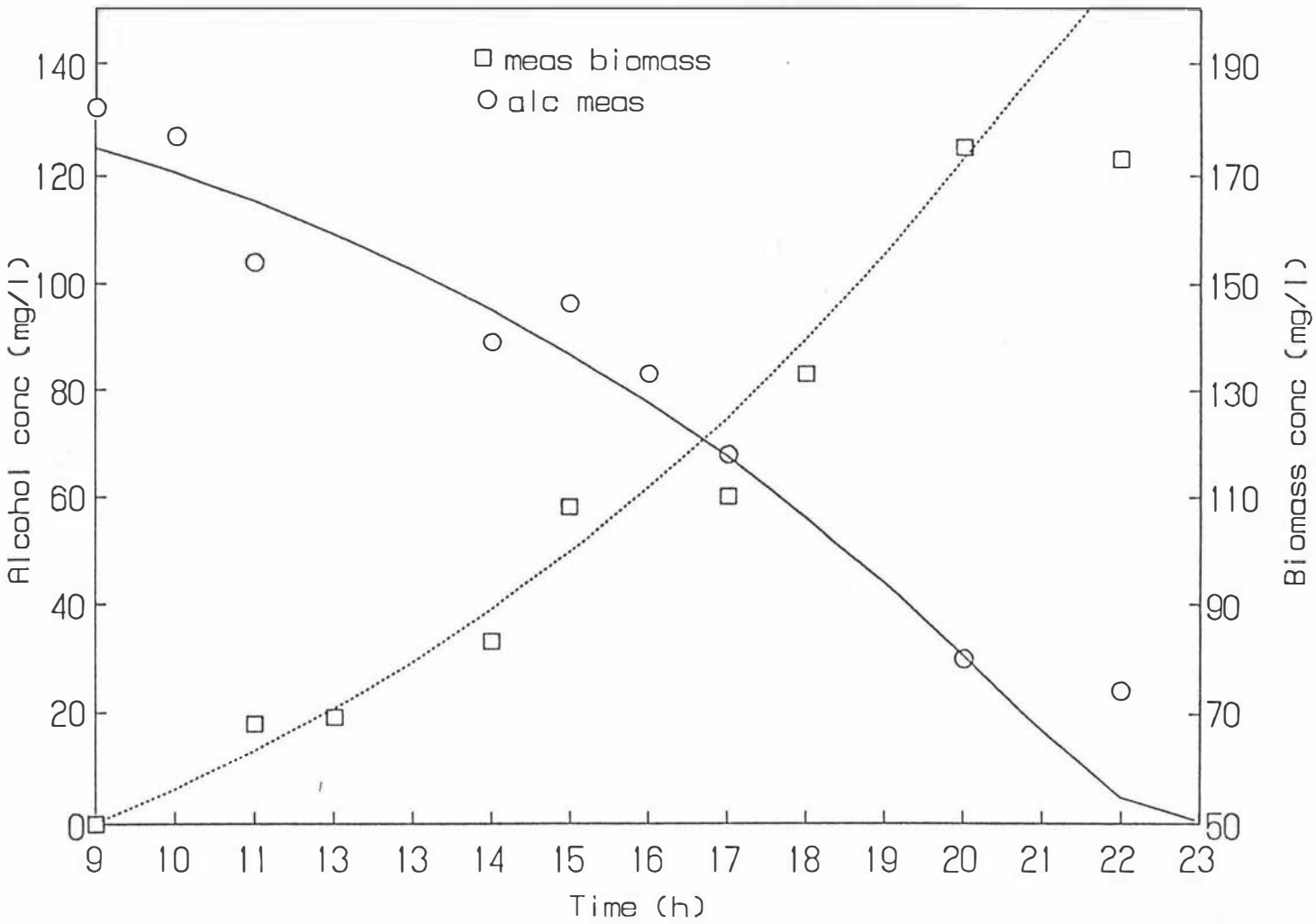


Figure 5.8: Plot of Measured and Predicted (Appendix 6) Alcohol and Biomass Concentrations versus Time for the Degradation of Alcohols in 15 % Leachate.

carbon limited, with fresh easily degraded primary alcohols aiding the degradation of the secondary alcohols. This remains to be proven.

The model developed has advantages over those of Luong (1987) and Tseng and Wayman (1975) two potential models described in the literature, as it was simple to determine the necessary parameters, and it was a continuous function. This allows it to be used in a number of situations.

This model will be used in subsequent chapters for the quantitative description of the alcohol degradation in leachate.

5.4 PCOC Degradation.

The second group of compounds to be degraded in leachate were the chlorophenols, of which PCOC was the major compound present. In this section, the kinetics of PCOC degradation will be described, along with the evaluation of a relatively new method of determination, the Modified Infinite Dilution Test (MIDT).

As stated earlier, the determination of kinetics can be performed in a number of ways; batch methods, where a change in the nature of the biomass used can occur, CSTR methods, where no information on inhibition can be elucidated, and the newest method, the MIDT method, which reportedly allows the evaluation of the maximum rate of degradation and inhibition kinetics in a very short time (Philbrook and Grady, 1985).

This section describes work performed to determine the effect of PCOC on its own degradation using batch, CSTR and MIDT methods for the measurement of kinetic parameters.

As the accurate assessment of small changes in biomass concentration was very difficult at the concentrations used, it was decided to measure the initial rates of substrate removal. Once these rates had been determined, they could then be converted to growth rates by the use of the yield coefficient on PCOC (determined from CSTR experiments).

5.4.1 Batch Determination of Degradation Kinetics.

5.4.1.1 Experimental Procedure.

The experiments were conducted using BAM, with PCOC added to the desired concentration. Perspex bioreactors (Section 3.8) were used, with a working volume of 900 ml. The temperature was maintained at 25 °C using an external water bath and mixing was provided by magnetic stirrer. Air was provided at 1 l/min (non-sterile). No sterile precautions were taken as the substrate was considered inhibitory. Each concentration was run in duplicate.

After inoculation, samples were taken and analyzed every 15-30 min to determine the PCOC concentration. When at least 10 mg/l of PCOC had been degraded a 100 ml sample of the medium was taken to determine the biomass concentration by direct measurement (Section 3.4).

5.4.1.2 Results.

An example of the results obtained is shown in Figure 5.9. The slope of the best fit line was determined using the statistical package MUTAB. The specific substrate removal rate Q_{PCOC} (mg_{PCOC}/ $mg_{BIOMASS}$.h) was determined by dividing the rate of removal by the measured biomass. The remainder of the raw data can be found in the VP-planner worksheet APPEND7.WKS (Appendix 7). The data are summarised in Figure 5.10, where measured Q_{PCOC} was plotted against the average PCOC concentration during the experiment.

It can be seen in Figure 5.10 that the data again appear to fit a straight line. The best fit line was found to be;

$$Q_{PCOC} = 0.080 - 4.16x10^{-4}[PCOC]$$

where all symbols are previously defined. R^2 was 96.5 % and the standard deviations around the intercept and slope were 4 % and 6 % respectively. The 95 % CI of the slope was found to be 4.04 - $4.36x10^{-4}$ l/mg/h. These data indicated total inhibition at 192 mg/l (95% CI = 9).

5.4.1.3 Discussion.

It can be seen that there was considerable similarity between Figures 5.10 and 5.6. Both were linear inhibition curves, with total inhibition occurring around the region of 200 - 300 mg/l PCOC.

The data of Tyler and Finn (1974) showed that linear inhibition curves were found with 2,4-DCP, and that total inhibition of growth occurred at concentrations higher than 100 mg/l. In contrast Beltrame et al. (1982) found that 2,4-DCP was not inhibitory at concentrations up to 134 mg/l and Watkin and Eckenfelder (1989) found no inhibition at concentrations up to 150 mg/l on its own degradation, but it did inhibit glucose degradation (K_I = 7 mg/l 2,4-DCP). These results contradict the work of Tyler and Finn (1974), and may be due to culture variations.

5.4.2 Effect of PCOC on Cell Viability.

Moos et al. (1983) state that batch experiments may be effected by the nature of the substrate, and hence the experiments may be considered unreliable. This section of work was conducted to determine whether PCOC had a major effect on the viability of the organisms, and hence determine whether there was basis for concern over the batch results.

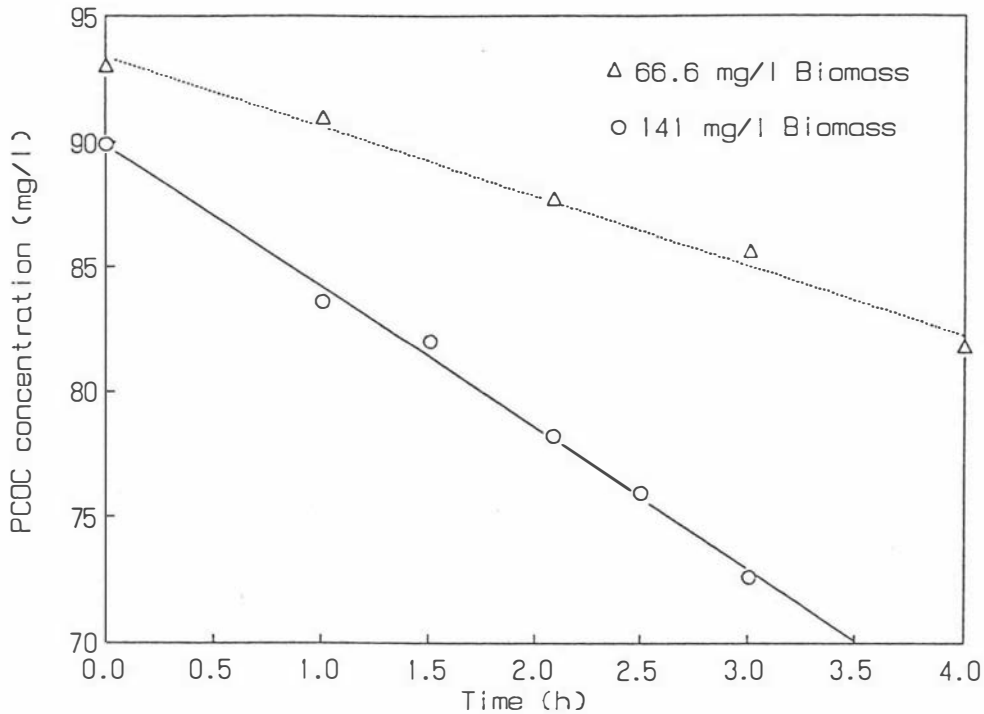


Figure 5.9: Example of Batch Data for the Determination of PCOC Degradation Kinetics.

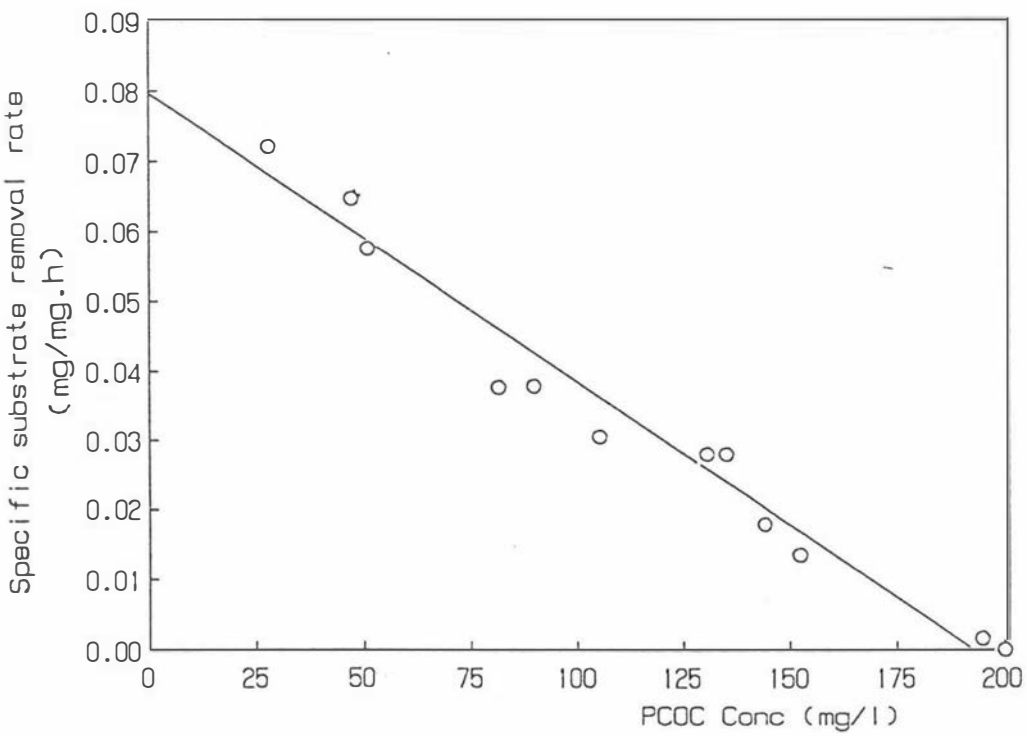


Figure 5.10: Summary of Results for the Batch Determination of PCOC Degradation Kinetics.

5.4.2.1 Experimental Procedure.

Two media were used for this experiment, BAM with 300 mg/l of PCOC and BAM with no carbon source (10 ml). Both the test and control were sterilised by filtration (0.45 µm filter, Whatman) and inoculated with culture from the parent bioreactor and 1 ml samples were taken at times 0, 3, 6 and 9 hours. Each sample was rapidly filtered on 0.22 µm, sterilised cellulose nitrate filters (Whatman), and the biomass was then washed with sterile isotonic saline at pH 6.65 to remove any residual PCOC that may interfere with the determination of the viable cell count. The filter papers were then aseptically placed in 10 ml of sterile peptone water (Gibco, cat no. M38100) containing sterile glass beads. This mixture was agitated for 30 seconds to remove the bacteria from the filter. The bacteria were then counted using the standard plate count method (APHA,1981).

5.4.2.2 Results.

Results, expressed as colony forming units (CFU) per ml were plotted against time and can be found in Figure 5.11. These results indicate a rapid reduction in the viability of the culture after exposure to PCOC compared to the control.

5.4.2.3 Discussion.

As the chlorophenols are known to interfere with DNA synthesis (Kleist-Welch Guerra and Lochmann,1988), it was not unexpected that there was a reduction in the viable count of the culture after exposure to 300 mg/l PCOC. These results were consistent with earlier results (Section 5.3.2.2) indicating that PCOC reduced the growth rate of the culture on alcohols.

The main implication of these results was that the batch method used in Section 5.4.1, which was similar to the method used for the determination of loss of viability, may also be experiencing a loss of viability due to the toxic effect of PCOC. This would effectively reduce the measured specific removal rate of PCOC to a value lower than the true removal rate of the culture. Inoculum size was an important factor affecting the measured kinetics of pentachlorophenol (PCP) degradation in batches (Moos et al.,1983), with small inocula showing inhibition at much lower concentrations than larger. Existing published data were therefore consistent with the data presented in this section.

It was therefore considered necessary to determine the kinetics by a second method, in order to determine whether the batch results were a valid measure of the culture performance.

5.4.3 MIDT Determination of PCOC Degradation Kinetics.

The MIDT method (Philbrook and Grady, 1985) and the FBR test (Watkin and Eckenfelder, 1989) are relatively new techniques for the determination of the kinetics of degradation of some compounds. As the two techniques are very similar, they are both referred to as the MIDT technique

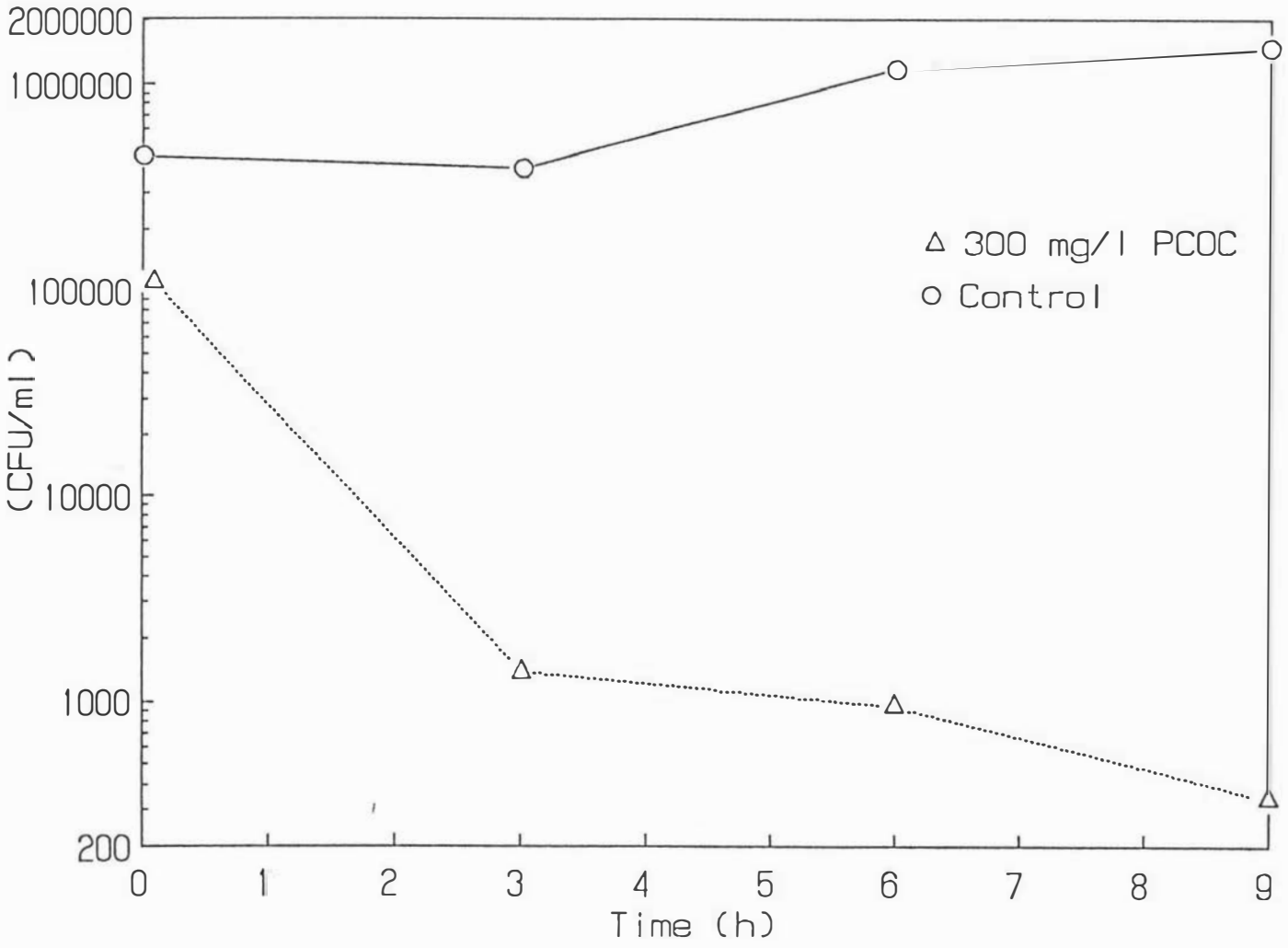


Figure 5.11: Plot of Viable Cell Count versus Time of Exposure to PCOC Showing the Death of Cells Due to PCOC.

for the purpose of this thesis. This section of work described the determination of PCOC degradation kinetics by the MIDT method.

5.4.3.1 Experimental Procedure.

One major modification was made to BAM for use in these experiments. As the method requires a very high strength feed, the pH was required to be pH 10 to ensure the PCOC remained in solution. Therefore it was necessary to increase the buffer capacity of BAM to prevent pH changes occurring. This was achieved by adding Na_2HPO_4 and NaH_2PO_4 at 2.1 and 3.56 g/l respectively. This medium had a Na^+ concentration of 3.4 g/l, well below the 5 g/l limit for inhibition shown in Figure 4.17. The medium (600 ml) was placed in a standard perspex bioreactor, air was sparged through at 300 - 500 ml/min, and the temperature was maintained at 25 °C by means of an external water bath.

The feed provided was prepared by dissolving 100 mg of PCOC in 1M NaOH as before, adjusting the pH to 10 and making up to 50 ml. The feed was prepared the same day as the experiments were conducted. The feed was then placed in a 50 ml measuring cylinder, to allow the measurement of the volume remaining at any time. The inoculum used for the experiments was prepared as described in Section 3.9.

Once inoculated, two 50 ml aliquots of the medium were withdrawn for a biomass assay. The biomass was determined directly as MLSS using the method described in Section 3.4.

Feed was pumped into the reactor using a Buchler multi-staltic pump (model #2-6200, Wilton Scientific, Wellington, N.Z.), at approximately 9 ml/h. The flow was determined exactly by measuring the initial and final volumes of feed to the experiment. The pump was fitted with Masterflex J-6419-13 Viton^R pump tubing (Extech Equipment, Wantima, Victoria, Australia) as only Viton^R tubing was chemically resistant to the PCOC solution at pH 10. The experimental equipment is shown in Figure 5.12.

Samples were taken from the reactor every 20 or 30 minutes. The sample volume was adjusted to ensure 9 ml of the medium was removed every hour. Volume changes in the reactor during the course of the experiment were then negligible. To prevent the loss of biomass from the system, samples were centrifuged (Wifug, 3000 xg, 3 min), the supernatant used for HPLC analysis and the pellet of biomass was returned to the reactor. At the completion of the experiments, two further 50 ml aliquots of the medium were taken for MLSS determination.

One control run was performed using the identical procedure, except no biomass was added and no MLSS determinations were made.

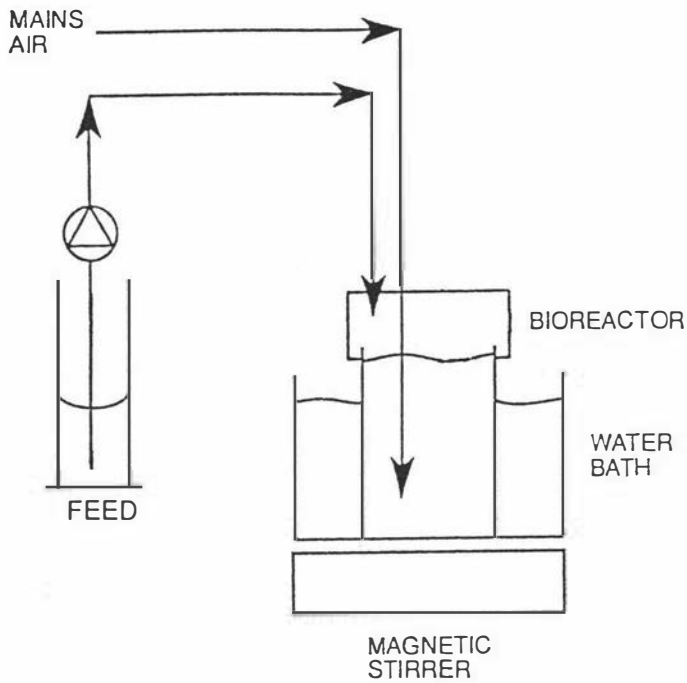


Figure 5.12: Experimental Apparatus Used for the Modified Infinite Dilution Test.

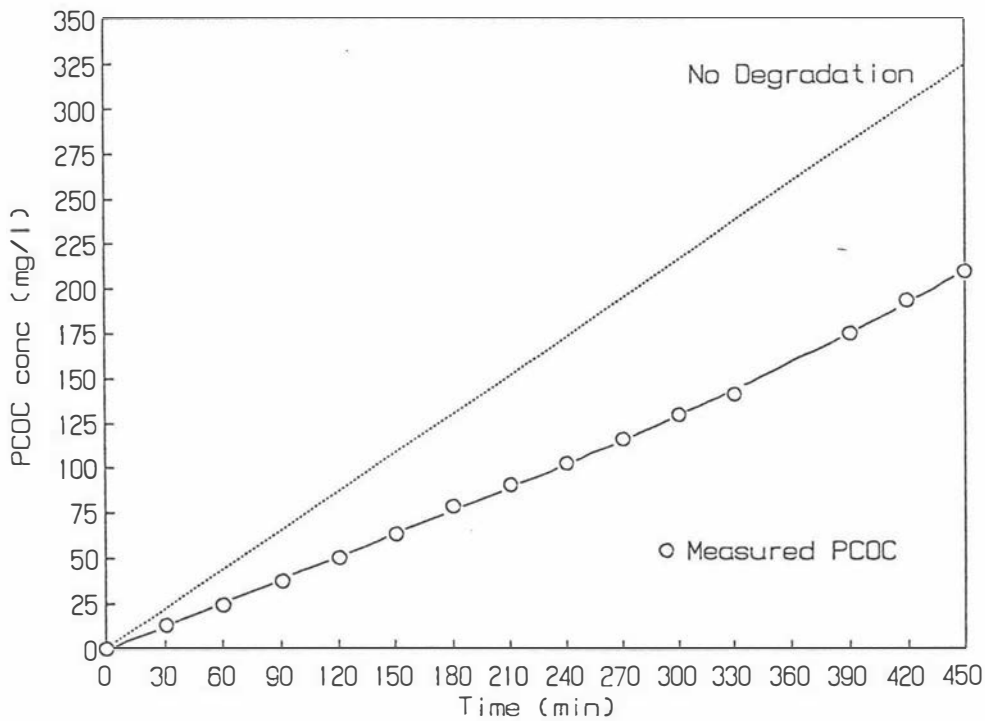


Figure 5.13: Time Course Data for a Typical MIDT Experiment. The difference between the no degradation and the measured PCOC lines was the PCOC degraded.

5.4.3.2 Results.

The results obtained from one of the four experimental runs using PCOC will be shown as an example. Figure 5.13 shows the measured substrate versus time data for this run. Other important data are given in Table 5.6.

$Q_{PCOC,MAX}$ was determined from this data using the following equation;

$$Q_{PCOC,MAX} = \frac{\text{(Feed rate - initial buildup rate)}}{\text{(Biomass present)}}$$

Table 5.6 Data generated by MIDT.

Parameter	Value	Units
Flow	9.8	ml/h
Feed Concn	2208	mg/l
Buildup rate	24.4	mg/l.h
Initial MLSS	154	mg/l
Final MLSS	172	mg/l

In this case $Q_{PCOC,MAX}$ was found to be 0.122 mg/mg.h. Once $Q_{PCOC,MAX}$ had been quantified, then inhibition parameters could be determined.

Mathematics of Inhibition Parameter Determination.

If the volume of the system under study is constant, then the following equations are generated from a substrate balance.

accumulation = input - output - conversion

or in mathematical terms

$$V.dS/dt = F.S_F - F.S - Q.X.V$$

where F is equal to the flowrate of feed (and the flow withdrawn) (l/h)

or $$dS/dt = F/V.S_F - F/V.S - Q.X \qquad (5\text{-}4)$$

Watkin and Eckenfelder (1989) suggested that if S is much greater than k_s, as it should be during these experiments, then

$$Q = Q_{MAX}/(1 + (S/K_I)^n)$$

Where K_I is an inhibitor constant (mg/l) and

n is a constant describing the shape of the inhibition curve.

As the biomass concentration can change a small, but significant amount, to account for this dX/dt can be set to the slope of the line joining the initial and final biomass values (m).

This results in two differential equations;

$$dS/dt = F/V.S_F - F/V.S - Q_{MAX}.X/(1 + (S/K_I)^n) \quad (5\text{-}5)$$

$$dX/dt = m \quad (5\text{-}6)$$

which, if integrated and solved simultaneously, would describe the variation of S with time as has been measured. By the use of BMDP85, a non-linear regression package which includes an integrator, it was possible to determine K_I and n from the measured substrate versus time data.

As the batch data indicated a linear inhibition curve may also be appropriate, this was also fitted to the data, using the following model as a basis;

$$Q_{PCOC} = (Q_{PCOC,MAX} - K_{OH_1}S).S/(k_{S,PCOC}+S) \quad (5\text{-}7)$$

where

K_{OH_1} = growth rate reduction due to PCOC ($l/mg_{BIOMASS}.h$)

$k_{S,PCOC}$ = the half-saturation constant for PCOC (mg/l)

When the same assumptions applied to Watkin and Echenfelder's model were applied to equation 5-7, the resulting equation was equation 5-8. Equation 5-8 was then substituted for 4-5.

$$dS/dt = F/V.S_F - F/V.S - (Q_{MAX} - K_{OH_1}S).X \quad (5\text{-}8)$$

The package then solved for both Q_{MAX} and K_{OH_1} as this would use all the data to determine Q_{MAX} instead of just the first hour.

Both these sets of equations were solved using BMDP85. The programs used can be found in Appendix 8 and the results can be found in Table 5.7.

Figure 5.14 shows the resulting models expressed as a plot of Q_{PCOC} versus substrate concentration. It can be seen that the model of Watkin and Eckenfelder (1989) follows very closely the linear model. The linear model was chosen for further experiments, as the model was simpler, fitted the data better, and was similar to that used for the alcohol degradation section.

The results of other MIDT runs using PCOC are given in Table 5.8.

Averaging the $Q_{PCOC,MAX}$ values, and the K_{OH_1} values for the longer runs gave the following final equation;

$$Q_{PCOC} = 0.123 - 4.35\times10^{-4}.[PCOC]$$

Table 5.7 Curve Fitting Data for Selected Models.

Model	Watkin and Eckenfelder	Linear Model
$Q_{MAX,PCOC}$ (mg/mg.h)	0.123	0.126
K_I (mg/l)	153.8	NA
n	1.72	NA
K_{OH_1} (l/mg.h)	NA	$4.15x10^{-4}$
SSresid*	20.6	17.7
Total inhibition (mg/l)	*NA	304

* SSresid is the sum of the residuals squared. The smaller of the two was the better fit.

* The concentration of PCOC which would give total inhibition of degradation. As the model of Watkin and Eckenfelder (1989) cannot predict total inhibition, there was no value.

NA the data was not evaluated in this model.

Table 5.8 MIDT Results using PCOC as the Substrate.

Run	$Q_{MAX,PCOC}$	K_{OH_1}	SS resid	Total Inhib
1	0.126	$4.15x10^{-4}$*	17.7	304 mg/l
2	0.119	$3.4x10^{-4}$	11.6	350 mg/l
3	0.125	$5.0x10^{-4}$	5.5	250 mg/l
4	0.123	$4.54x10^{-4}$*	18.7	271 mg/l

* High PCOC concentrations reached so K_{OH} more accurate.

The standard deviation about the slope and intercept were 6% and 2.5% respectively. As mentioned in the experimental procedure, a control was performed with no biomass added, to ensure the biomass was responsible for PCOC removal. The measured buildup rate was 37.75 mg/l.h, whereas the measured feed rate was 38.09 mg/l.h. As the difference was less than 1 % it was clear that biomass was responsible for the removal of PCOC from solution.

5.4.3.3 Discussion.

The inhibition of batch cultures was found to be linear and similar to the inhibition of alcohol degradation by PCOC. Using the MIDT method, linear inhibition curves were again found and fitted, both using the linear model developed earlier and a more general model (Watkin and Eckenfelder, 1989). As Figure 5.14 shows, the general model forms an approximation of the linear model, but does not fit the data as well. Therefore the linear model was used for the subsequent runs.

The observation that the general model was found to fit a straight line indicated that the use of linear models in this and earlier Sections (5.3.2.2 and 5.3.3.2) was justified. These linear models

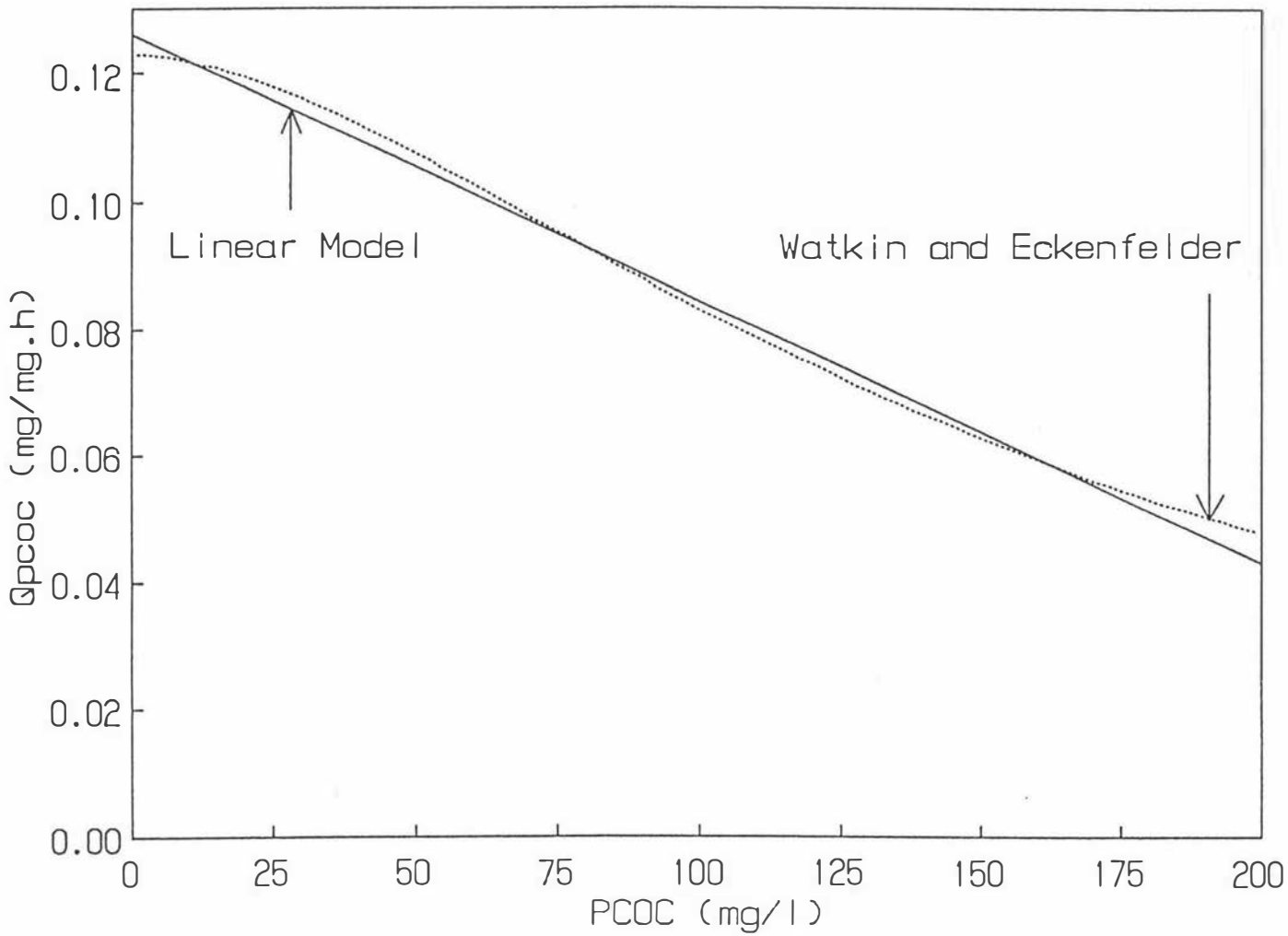


Figure 5.14: Comparison of the Linear Model Developed and the Model of Watkin and Eckenfelder (1989). Note that the curve of Watkin and Eckenfelder approximates a straight line.

predict total inhibition (which the general model of Watkin and Eckenfelder (1989) fails to do), and which indeed occurs, but they are simpler to use to quantify the inhibition parameters.

The speed of the MIDT procedure was found to be a significant advantage. It was possible to determine the entire inhibition curve in one run, so population dynamics in the parent bioreactor were not a factor. Population dynamics over longer periods can be measured using the MIDT method, by repeatedly determining inhibition curves over a period of time, as was done by Philbrook and Grady (1985). These authors measured the standard deviation of Q_{MAX} at 5 % over a period of 5 days and their value compares well with the standard deviation of 2.5 %, measured for PCOC in these present studies over a period of 12 days.

The MIDT method is essentially a CSTR system that is confronted with a shock load of substrate which cannot be totally degraded by the biomass present. This similarity makes the MIDT method excellent for determining unsteady state kinetics for CSTR systems, as proposed by Watkin and Eckenfelder (1989).

It can be seen on comparing the batch and MIDT results, that $Q_{PCOC,MAX}$ determined by the MIDT method was 50 % higher than that measured in batch experiments (0.080 mg/mg.h c.f. 0.123 mg/mg.h). The average slope from the MIDT experiments (K_{OH_1}) however falls within the 95 % CI of the data from the batch experiments (4.04 - 4.36×10^{-4} $l/mg_{BIOMASS}.h$). This indicates there was no significant difference between the K_{OH_1} values measured for the two systems.

Therefore there was a significant change in the culture during batch experiments, as was observed by Moos et al. (1983), and discussed by other workers (Philbrook and Grady, 1985; Grady, 1985).

This phenomenon could be explained as being the effect of the reduction in cell viability due to PCOC, as was observed in Section 5.4.2.3. If a proportion of the cells was killed by the PCOC, the $Q_{PCOC,MAX}$ value would be expected to be reduced, as dead cells would still be measured as MLSS. This bias would remain throughout the experiment, reducing the value of Q_{PCOC} by the same proportion. This would account for the measured slopes being equal.

Batch experiments were found to be unsuitable for the determination of the kinetics of PCOC, whereas the MIDT method appeared to be much better. Batch methods have been used by other workers to determine the kinetics of inhibitory compounds; i.e. PCP (Klecka and Maier, 1985), phenol (Rozich et al., 1985) and 2,4-DCP (Tyler and Finn, 1974). It would therefore be expected that these authors results would be conservative.

It would appear from the data presented here that the MIDT method has significant advantages over batch methods, as

(1) the MIDT method was significantly faster for the determination of kinetics,

(2) the MIDT method gave higher values for $Q_{PCOC,MAX}$, and its similarity to CSTR systems would appear to make these results more realistic than batch experiments.

(3) Population dynamics are minimised throughout the determination of the inhibition curve, making the determinations potentially more accurate.

Therefore, it was concluded that the MIDT method was superior to the batch method for the determination of PCOC degradation kinetics.

Concerning the PCOC concentrations at which total inhibition occurs, the MIDT method yields a value of 283 mg/l, which was within the confidence interval for total inhibition of alcohol degradation by PCOC (291 ± 18 mg/l). It was concluded that PCOC cannot be tolerated at concentrations of greater than 290 mg/l because there was no significant difference between the two intercepts.

The same mechanism was possibly responsible for the inhibition in both sets of experiments, as the effects of PCOC were almost identical on alcohol and PCOC degradation; i.e. linear inhibition down to no growth at 290 mg/l PCOC.

Before the kinetics of PCOC degradation can be compared with literature values, it is necessary to determine the yield coefficient ($Y_{X/S,PCOC}$) and half-saturation constant ($k_{S,PCOC}$) of the culture on PCOC. This will be discussed in the next section of work.

5.4.4 CSTR Work with PCOC as the Sole Source of Carbon and Energy.

A number of authors (Chiu et al., 1972a and b: Philbrook and Grady, 1985; Yoon et al., 1977) have reported that mixed populations may change their composition and kinetic characteristics at different dilution rates and feed compositions. Furthermore, batch experiments had been shown to be unsuitable for the determination of kinetics of PCOC (see above). Because there was no other satisfactory method for the determination of $Y_{X/S,PCOC}$ it was therefore decided to use the CSTR method for the determination of $Y_{X/S,PCOC}$ and $k_{S,PCOC}$.

5.4.4.1 Experimental Procedure.

The experimental procedure used for this section of work was very similar to that described in Section 4.5.1.2. BAM was used, with PCOC added to the desired concentration. Ash, TDS and TOC were not measured on the effluent from the bioreactor. In these experiments pH adjustment was found to be necessary. This was performed manually.

5.4.4.2 Results.

Four runs were performed at different dilution rates. The results are given in Table 5.9 and the raw data can be found in the file APPEND7.WKS in Appendix 7. Substrate versus time data are

not given here, as throughout all four runs the PCOC concentration was consistently at or near the limits of detection of the HPLC.

Table 5.9 Measured CSTR Parameters with PCOC as the Substrate.

Run	D (h^{-1})	Q_{PCOC} (mg/mg.h)	MLSS (mg/l)	$Y_{X/S,PCOC}$ (mg/mg)	Feed (mg/l)	[PCOC] (mg/l)	$k_{s,PCOC}$* (mg/l)
1	0.0127	0.047	86	0.27	319	0.22	0.36
2	0.016	0.067	62	0.24	260	0.20	0.16
3	0.019	0.077	90	0.25	358	0.10	0.06
4#	0.021	0.084	ND	ND	ND	0.10	0.04

* $k_{s,PCOC}$ determined by equation of Philbrook and Grady (1985)

MLSS not determined: Q_{PCOC} given on basis of average $Y_{X/S,PCOC}$

Each run lasted approximately 300 h, i.e. between changing the flowrate and sampling for steady state. Thus at least 3 residence times passed during each run. The MLSS was not determined on run 4 because of feed problems prior to sampling.

The pH was found to fall approximately 0.3 units over the 24 h period between adjustments. The amount of NaOH used for pH adjustment was not measured.

A Gram stain (as described in Appendix 3) was performed on the culture during run 3. This showed the presence of gram-negative rods in an amorphous matrix, along with the presence of the rotifer Philodina (Appendix 3).

The average yield coefficient ($Y_{X/S,PCOC}$) was found to be 0.25 (SD = 0.02) mg biomass/mg PCOC. The average half-saturation constant ($k_{s,PCOC}$) was found to be 0.15 (SD = 0.15) mg/l.

5.4.4.3 Discussion.

The measured yield coefficient for PCOC has not to the authors knowledge been reported elsewhere. The value was found to be approximately 80 % higher than the values reported for PCP of 0.14 mg/mg (Klecka and Maier, 1985), and within the range of values reported for a variety of chlorobenzoates (0.14 - 0.25 mg/mg) (Shamat and Maier, 1980).

By comparison, the yield coefficient on similar, unchlorinated compounds as reported is much higher,i.e. 0.70 mg/mg for phenol and *p*-cresol (Hutchinson and Robinson, 1988) and 1.02 mg/mg phenol (Rozich and Gaudy, 1984).

The k_s value for PCOC has also not been reported previously. Reported values for similar compounds can be found in Table 5.10 where it can be seen that they span two orders of magnitude and that the values currently measured for PCOC fall into the middle of the range. The assumption used in Section 5.4.3.2 that k_s was small compared to the measured PCOC concentrations was therefore valid.

Table 5.10 Literature Values of k_s for Some Chlorinated Compounds.

Compound	k_s (mg/l)	Reference
2,4-DCP	5.1	Tyler and Finn (1974)
PCP	0.06	Klecka and Maier (1985)
2-chlorophenol	0.030	Philbrook and Grady (1985)
m-chlorobenzoate	2.0	Shamat and Maier (1980)
o-chlorobenzoate	2.4	Shamat and Maier (1980)
p-chlorobenzoate	1.1	Shamat and Maier (1980)

It should be noted that the highest specific substrate removal rate measured was 0.084 mg/mg.h, higher that the intercept measured for batch experiments (0.080 mg/mg.h). This was further evidence that there had been a significant culture change during the batch experiments. The Gram stain of the culture revealed very similar characteristics to the parent culture. This indicated no gross changes had occurred within the culture during the course of the experiments. Detailed work to determine whether any minor changes had occurred was considered inappropriate.

5.4.5 Overview.

From the data presented above, it was possible to determine the maximum specific growth rate and inhibition curve for growth of the culture on PCOC. As shown in Chapter 2

$$\mu_{MAX} = Y_{X/S}.Q_{MAX}$$

therefore the Q_{PCOC} data can be reexpressed as;

$$\mu_{PCOC} = (\mu_{MAX,PCOC} - K_{OII}.[PCOC]).[PCOC]/(k_{s,PCOC} + [PCOC]) \qquad (5\text{-}9)$$

where $\mu_{MAX,PCOC} = 0.031\ h^{-1}$

$K_{OII} = 1.09 \times 10^{-4}$ l/mg PCOC.h

$k_{s,PCOC} = 0.15$ mg/l

A plot of μ versus [PCOC] is shown in Figure 5.15. This graph shows linear inhibition of growth by PCOC at concentrations greater than approximately 5 mg/l. This compares well with the data using 2,4-DCP (Tyler and Finn, 1974) with respect to curve shape, but the values of μ_{MAX} and total inhibition are completely different. Table 5.11 gives literature values for μ_{MAX} for related compounds.

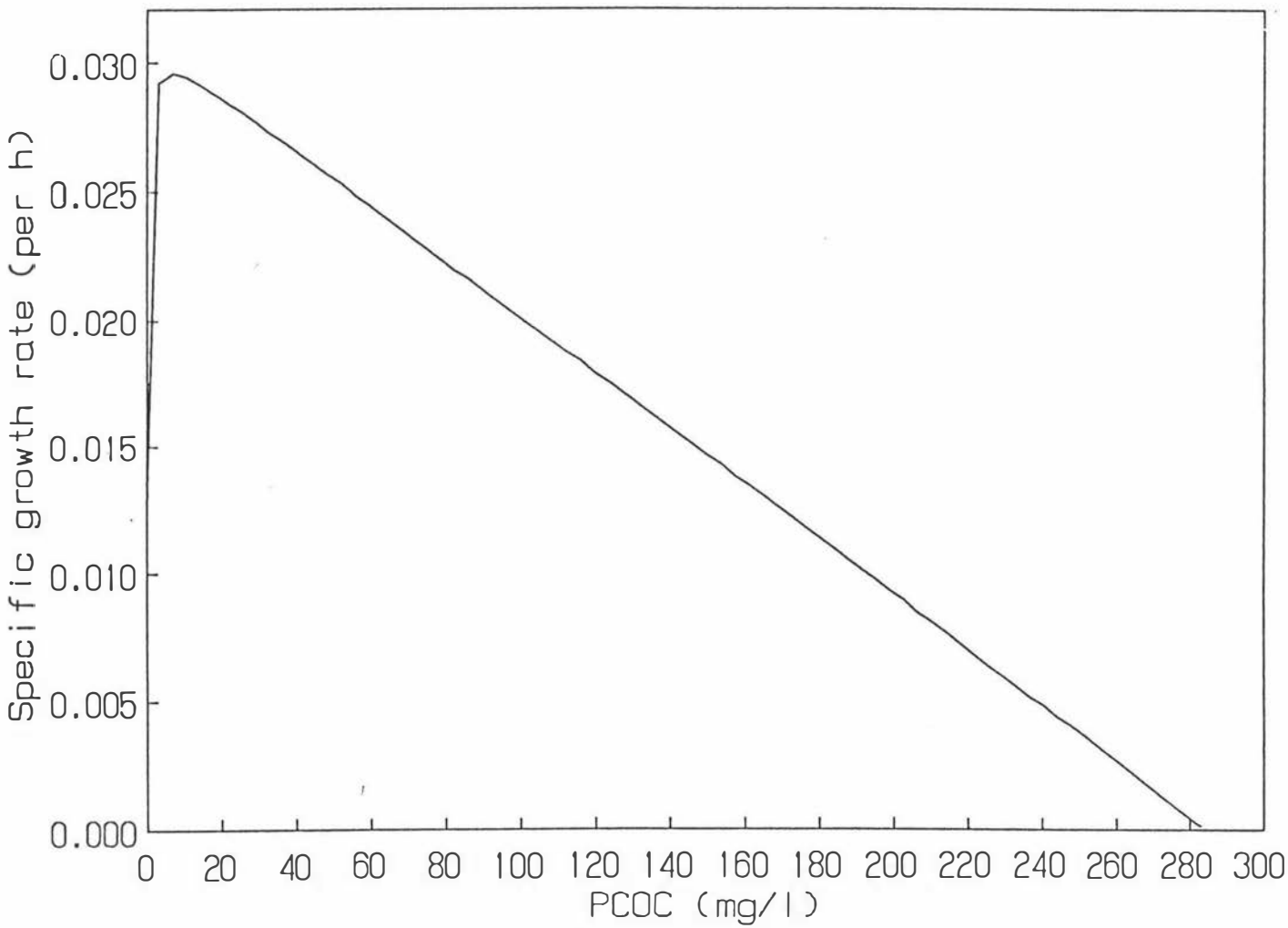


Figure 5.15: Plot of Specific Growth Rate on PCOC versus PCOC Concentration Predicted by the Linear Model.

It can be seen that the measured maximum growth rate on PCOC was within the range of those measured by Shamat and Maier (1980) for the mono-chlorinated benzoates. Of the compounds listed in Table 5.11, these chlorobenzoates were the closest in structure and composition to the PCOC tested, as they all contained 7 carbon atoms and 1 chlorine. These authors also used a mixed bacterial population derived from an activated sludge plant. Their experiments were carried out at 20°C, and this 5°C difference would result in a 10% difference in measured growth rates (Tchobanoglous,1979). However, after accounting for this fact the measured growth rate for PCOC was within the range reported (0.028 - 0.055 h^{-1}).

Table 5.11 Literature Values of μ_{MAX} for Some Chlorinated Compounds.

Compound	μ_{MAX} (h^{-1})	Reference
2,4-DCP	0.140	Tyler and Finn (1974)*
PCP	0.074	Klecka and Maier (1985)
m-chlorobenzoate	0.025	Shamat and Maier (1980)
o-chlorobenzoate	0.042	Shamat and Maier (1980)
p-chlorobenzoate	0.050	Shamat and Maier (1980)

* Only workers to use a pure culture at 25°C. Remainder used mixed cultures at 20 °C.

On comparing $\mu_{MAX,PCOC}$ with $\mu_{MAX,ALC}$ it can be seen that the culture grows ten times faster on the alcohols than on PCOC, although PCOC had the same effect on both substrates; the growth rate was reduced in a linear fashion to give no growth above approximately 290 mg/l PCOC. The yield of biomass using PCOC was found to be 20% of the yield using alcohols. It was therefore obvious that alcohols were a superior growth substrate than PCOC.

5.5 Phenoxy Degradation.

The third class of compounds present in the leachate was the phenoxies. There were three major phenoxies present in the leachate under study, MCPA (≈ 56 % of the total phenoxies), 2,4-D (≈ 35 %) and 2,4,5-T (≈ 9 %). In this section the kinetics of degradation of each phenoxy will be described, and where possible the degradation kinetics of mixtures of phenoxies will be modelled. The same techniques will be used in for these studies as were used for the PCOC degradation work.

5.5.1 MIDT Experiments.

5.5.1.1 Experimental Procedure.

The experimental procedure used for the determination of phenoxy degradation kinetics was very similar to the method used for PCOC (Section 5.4.3.1). The concentrations of the feed solutions were different; 2,4-D 3500 mg/l, MCPA 4000 and 4500 mg/l and 2,4,5-T 2500 mg/l. In all other aspects the method was unchanged.

5.5.1.2 Results.

The results of one of the MIDT experiment performed using 2,4-D can be found in Figure 5.16. It can be seen that there was considerable curvature of the substrate versus time graph after 100 minutes of feeding. Therefore only the first 80 minutes were used for the determination of Q_{MAX}. As a similar effect was noted with MCPA, only the first 80 minutes were used. The results for 2,4-D and MCPA are summarised in Table 5.12.

Table 5.12 MIDT Results with 2,4-D and MCPA as Substrates.

Compound	Feed rate (mg/l.h)	Buildup rate (mg/l.h)	Q_{MAX} (mg/mg.h)	Avg Concn (mg/l)
2,4-D	53.4	9.6	0.219	5.2
2,4-D	62.6	21.5	0.218	18.5
AVERAGE			0.219	
MCPA	115.2	85.2	0.160	42.5
MCPA	86.2	56.4	0.155	28.3
MCPA	92.8	57.0	0.160	28.4
AVERAGE			0.158	

MIDT experiments were performed using 2,4,5-T. Typical results are shown in Figure 5.17. The feed rate was 23.9 mg/l.h and the measured buildup rate was 23.6 mg/l.h. It was therefore concluded that there was no degradation of 2,4,5-T throughout the course of the experiment, even after the feed had been stopped.

5.5.1.3 Discussion.

It can be seen from Figure 5.16 that there was significant curvature of the substrate versus time profile. This was probably caused be either a large increase in biomass in the system, or that initially the substrate concentration was less than k_s for the phenoxy, and as the concentration increased, the rate of substrate removal also increased. This was considered unlikely, as the graph continued to curve down, indicating the rate of removal was increasing, while the substrate level was decreasing. If the curvature was due to a k_s effect, an equilibrium would be reached at a constant level. Such curvature was also experienced by Philbrook and Grady (1985), who attributed the effect to growth of biomass.

This curvature prevented the use of the MIDT method for determining whether any inhibition was occurring during degradation. Therefore it would be necessary to use batch techniques at higher substrate concentrations.

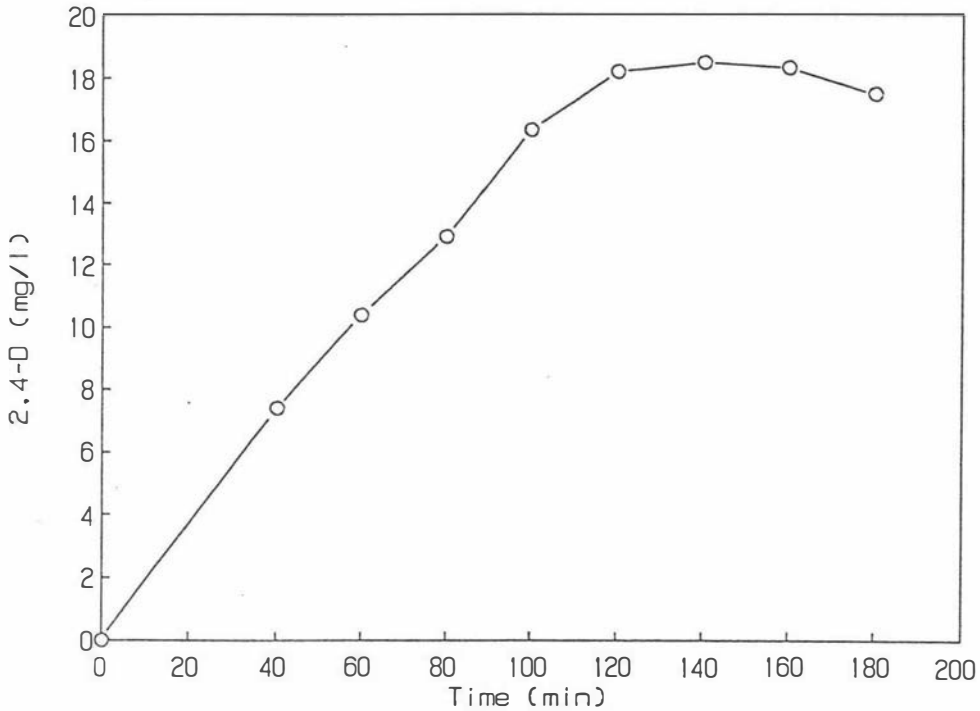


Figure 5.16: Typical Plot of Substrate Concentration versus Time for MIDT using 2,4-D. Only the first 60 - 80minutes were used for calculations.

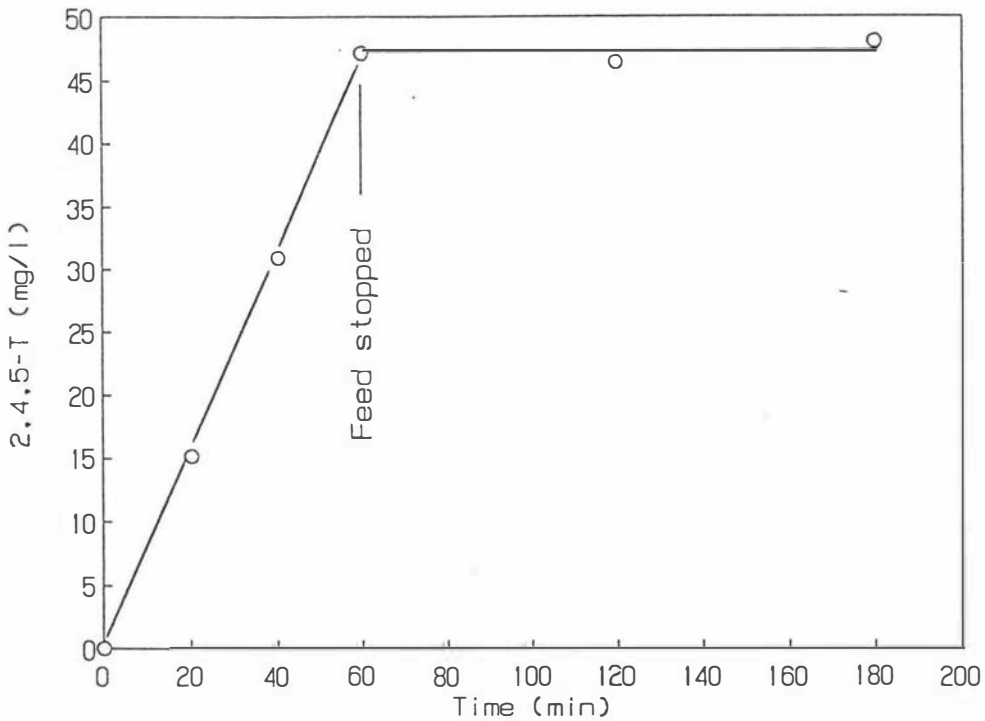


Figure 5.17: Plot of MIDT Result using 2,4,5-T as the Substrate. The buildup rate was equal to the feed rate, indicating no degradation was occurring. After the feed was stopped, there was also no degradation.

The measured values for Q_{MAX} on each substrate were quite different. Even after converting the degradation rates to molar removal rates ($9.7x10^{-4}$ and $8.0x10^{-4}$ mM/mg.h for 2,4-D and MCPA respectively), there was still a difference of 20 %. The molar rate of PCOC degradation, calculated from Section 5.4.3.2, was found to be $8.6x10^{-4}$ mM/mg.h, a difference of 7.5 % between PCOC and MCPA degradation. This similarity would be expected, as the two compounds are degraded via the same pathway. The difference between 2,4-D and MCPA may indicate the involvement of different enzymes in degradation or steric hindrance due to the methyl group of MCPA. It is also possible that different organisms in the mixed culture were responsible for the degradation of the different compounds.

5.5.2 Batch Experiments Using 2,4-D and MCPA.

5.5.2.1 Experimental Procedure.

The experimental procedure used was very similar to that used previously for PCOC (Section 5.4.1.1). Samples were taken every 30 - 60 min and the substrate concentrations determined by HPLC (Section 3.2). Two 50 ml biomass samples were taken after 1.5 h and the degradation was followed until at least 10 % of the substrate was removed.

5.5.2.2 Results.

As an example of the results obtained, Figure 5.18 shows duplicate results for a typical batch run using MCPA at 520 mg/l as the substrate. The substrate removal rates were found to be 26.8 and 27.7 mg/l.h (R^2 99.4 % and 98.2 % respectively). With measured MLSS values of 170 and 174 mg/l, the measured values for Q_{MCPA} were both 0.16 mg/mg.h. Other results for MCPA and 2,4-D are given in Table 5.13.

Batch experiments allow the removal of MCPA at the same rate as that obtained in MIDT experiments (0.16 mg/mg.h), even at concentrations as high as 930 mg/l. 2,4-D on the other hand appears to be inhibitory at concentrations greater than 500 mg/l. A plot of 1/Q versus S was performed to determine whether Haldane kinetics applied. The plot is shown in Figure 5.19. From the best fit line, Q_{MAX} was found to be 0.22 mg/mg.h and K_I was 2475 mg/l (R^2 = 80.3%).

5.5.2.3 Discussion.

There was no difference between the Q values determined by the MIDT method and those determined by batch experiments. Therefore for these less inhibitory substrates, both the batch and MIDT methods may be used for determining the kinetics of degradation.

Table 5.13 Measured Q for 2,4-D and MCPA using Batch Methods.

Compound	Concn (mg/l)	Deg rate (mg/l.h)	MLSS (mg/l)	Q (mg/mg.h)
2,4-D	493	14.6	82	0.18
2,4-D	496	14.8	77	0.19
2,4-D	661	16.2	94	0.17
2,4-D	658	15.7	93	0.17
2,4-D	326	20.8	95	0.22
2,4-D	322	19.7	91	0.22
2,4-D	88	15.7	78	0.20
2,4-D	77	16.6	80	0.21
MCPA	467	26.8	170	0.16
MCPA	463	27.7	174	0.16
MCPA	930	56.1	322	0.17
MCPA	918	53.4	335	0.16
MCPA	96	14.4	84	0.17

5.5.3 CSTR Experiments.

CSTR experiments were carried out to determine k_s and $Y_{x/s}$ on the phenoxies. The reason for this approach was the same as that previously outlined for PCOC in Section 5.4.4.

5.5.3.1 Experimental Procedure.

The procedure used was almost identical to that used for PCOC (Section 5.4.3.1), with two exceptions; the amount of NaOH required for pH adjustment was recorded when 2,4-D was the substrate. The feed concentration was 500 mg/l of the test substrate.

5.5.3.2 Results.

The raw data for these experiments can be found in the file APPEND9.WKS in Appendix 9. Only the important data will be presented here.

The yield coefficients on 2,4-D and MCPA ($Y_{x/s,2,4\text{-}D}$ and $Y_{x/s,MCPA}$) were found to be 0.24 and 0.29 mg/mg respectively. Only one experiment was used to determine these yields, and the standard deviation about these values was considered to be the same as for PCOC, as the experimental procedure and culture were the same.

In both cases the residual concentrations of substrate measured in the medium was 0.1 mg/l. Using the formula of Philbrook and Grady (1985), $k_{s,2,4\text{-}D}$ and $k_{s,MCPA}$ were found to be 0.01 mg/l (from

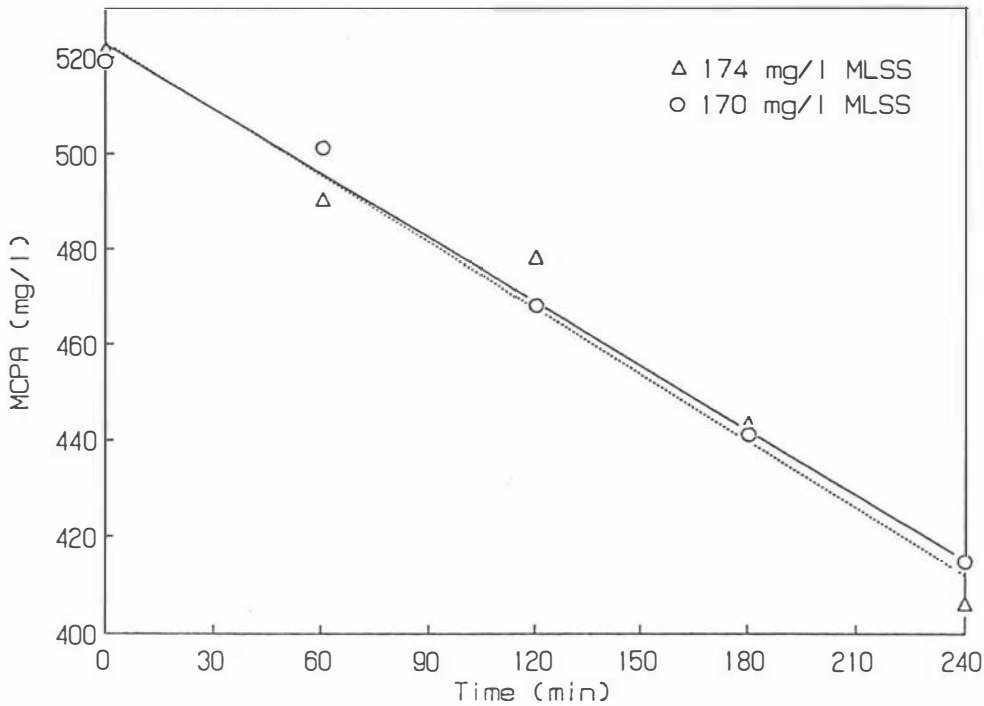


Figure 5.18: Typical Batch Data for the Degradation of MCPA (Duplicate Experiments).

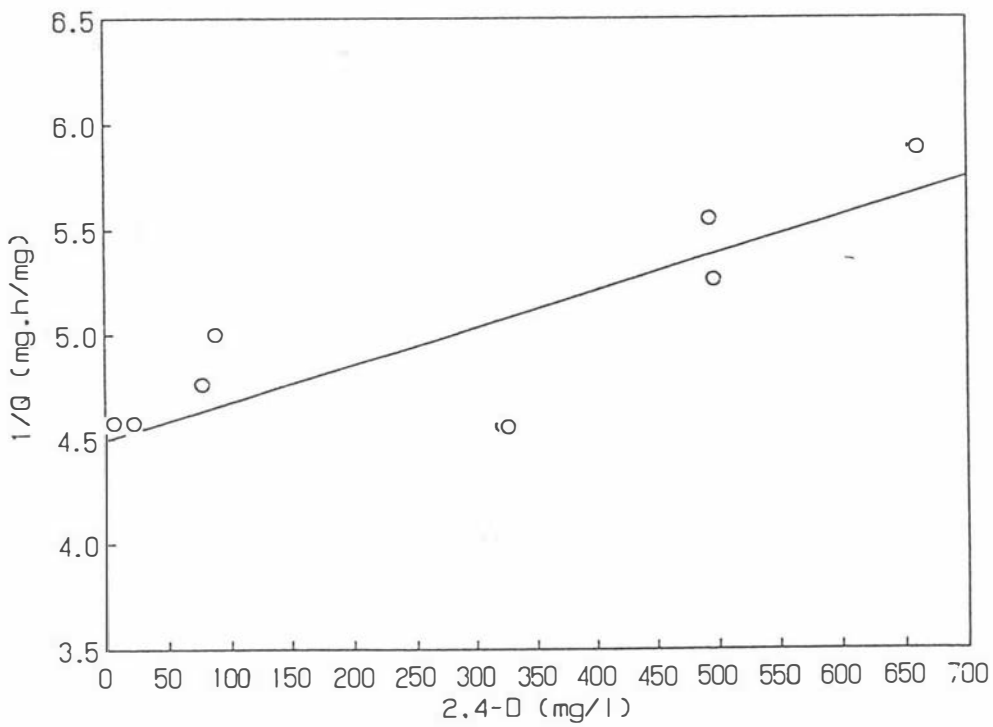


Figure 5.19: Plot of 1/Q versus S to Determine the Kinetic Constants for Haldane Model on 2,4-D.

measured Q values of 0.188 and 0.152 for 2,4-D and MCPA respectively). The NaOH usage rate was found to be 0.085 mM/h when 2,4-D was the substrate. The rate of 2,4-D utilisation was 0.089 mM/h.

5.5.3.3 Discussion.

It can be seen that NaOH was required almost mole for mole with 2,4-D degradation (difference 5 %). This was consistent with the data of Loos (1975) who reported the production of 1 mole of HCl per mole of 2,4-D degraded. Loos (1975) also reported a much lower acid production using MCPA, considered to be due to the production of organic acids and not HCl.

The incorporation of the k_s and yield coefficients into the previously collected data for the phenoxies gave the following equations to describe growth.

$$\mu_{MCPA} = 0.046.[MCPA]/(0.01 + [MCPA]) \quad (5\text{-}10)$$

and

$$\mu_{2,4\text{-}D} = 0.052.[2,4\text{-}D]/(0.01+[2,4\text{-}D]+[2,4\text{-}D]^2/2500) \quad (5\text{-}11)$$

These curves, along with the measured data are plotted in Figures 5.20 and 5.21 respectively.

The average maximum specific growth rate was found to be 0.048 h^{-1}. The highest leachate concentration suitable was 20 % (phenoxy concentration = 944 mg/l), which contained 330 mg/l 2,4-D. This maximum concentration of 2,4-D was in the range where there was no substrate inhibition, and hence a simplifying assumption; that 2,4-D followed the Monod equation, could be made. Using the average measured growth rate, the following general expression was derived;

$$\mu_{OXY} = \mu_{MAX,OXY}.[oxy]/(k_{s,OXY} + [oxy]) \quad (5\text{-}12)$$

where

μ_{OXY} = growth rate on phenoxies (h^{-1})
$\mu_{MAX,OXY}$ = maximum growth rate on phenoxies (0.048 h^{-1})
[oxy] = phenoxy concentration (mg/l)
$k_{s,OXY}$ = half-saturation constant for phenoxies (0.01 mg/l)

The maximum specific growth rate on phenoxies was considerably lower than those reported in the literature (0.09 - 0.15 h^{-1}, Table 2.4). This could be due to differences between the parent culture and those used by other workers. The measured yield coefficients (0.24 and 0.29 mg/mg) were considerably higher than literature values (0.14-0.15 mg/mg), although similar to that determined earlier for PCOC. Again the differences were probably due to culture differences. The observation that MCPA has a higher yield coefficient than 2,4-D could be related to the fact that MCPA has an extra carbon atom in the molecule. The low k_s values were consistent with selecting a k-strategist culture, as described in Section 4.8, an advantage in CSTR systems according to Slater and Lovatt (1984).

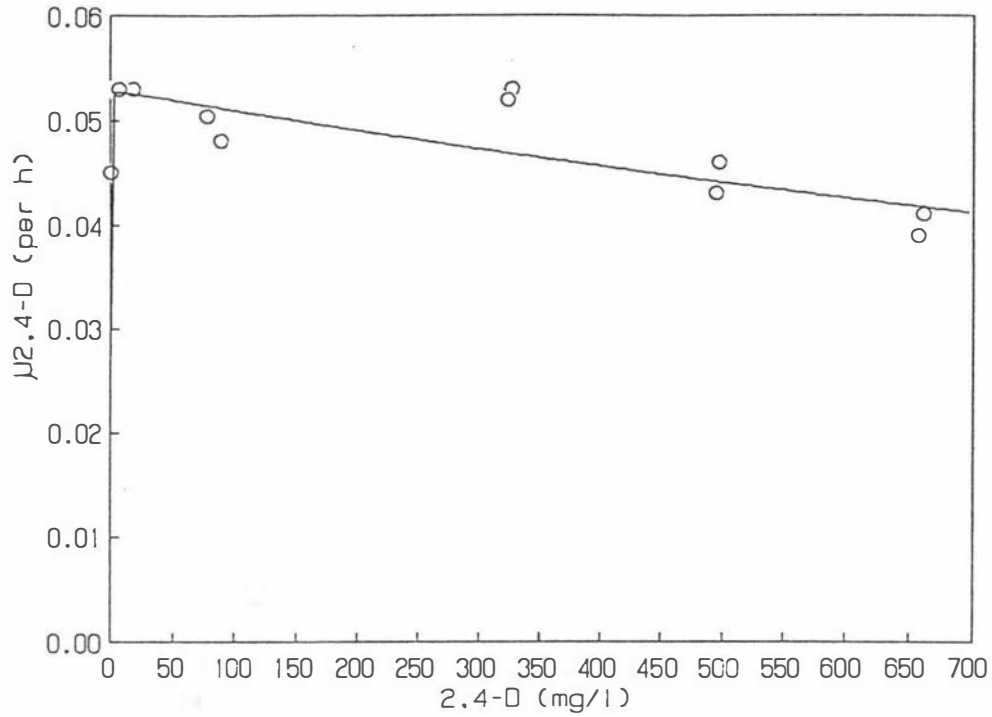


Figure 5.20: Plot of Specific Growth Rate on 2,4-D versus 2,4-D Concentration Predicted By the Haldane Model, along with Measured Points.

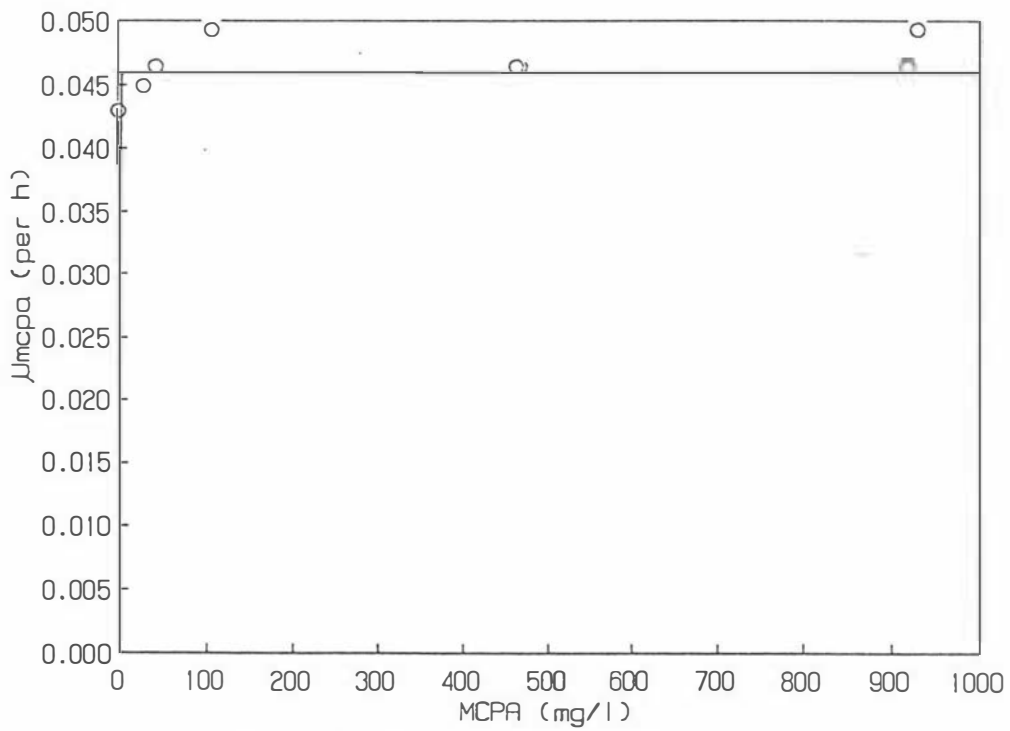


Figure 5.21: Plot of Specific Growth Rate on MCPA versus MCPA Concentration Predicted By the Monod Model, along with Measured Points.

With respect to inhibition by 2,4-D, the literature contains a number of conflicting results, varying from inhibitory at > 95 mg/l (Papanastasiou and Maier, 1982) to not inhibitory at up to 2 g/l (Tyler and Finn, 1974). This culture was found to be inhibited slightly at concentrations greater than 500 mg/l. This was well within the reported range.

5.5.4 Degradation of Mixtures of Phenoxies.

5.5.4.1 Experimental Procedure.

Experiments were carried out using 100 ml of BAM, amended to the desired concentrations of phenoxies. The media were placed in 250 ml Erlenmeyer flasks and incubated at 150 rpm and 25 °C in a orbital water bath. In all other respects the method was as used previously in Section 5.4.1.1.

5.5.4.2 Results.

Mixtures of 2,4-D/MCPA.

Duplicate experiments were conducted using two different 2,4-D/MCPA ratios; firstly 100 mg/l of each and secondly, 105 mg/l MCPA and 67 mg/l 2,4-D (the same proportion as found in leachate).

The resulting substrate versus time profiles can be found in Figures 5.22 - 5.25 where points represent measured concentrations and the lines representing the predicted values based on the model of Hutchinson and Robinson (1988) produced using ISIS. Details of the model, and the ISIS program that generated the predictions can be found in Appendix 10. It can be seen that the model satisfactorily predicted the experimental data.

2,4,5-T Degradation.

As shown earlier, 2,4,5-T was not degraded as a pure compound. It was therefore necessary to find out which compounds stimulated degradation. Media containing 2,4,5-T and each test compound (≈50 mg/l) were monitored for 22 h. Figure 5.26 shows a plot of 2,4,5-T concentration versus time for each experiment. The number in brackets following the compound in the key is the percentage of that compound degraded in the 22 h. A 50 mg/l 2,4-D control was totally degraded in 6 hours.

It can be seen that there was substantial inhibition of phenoxy degradation by 2,4,5-T, with little accompanying 2,4,5-T degradation. PCOC however was not affected, and 65 % of 2,4,5-T was removed. There was no degradation of 2,4,5-T alone. This indicated that 2,4,5-T was apparently removed by cometabolism.

A further experiment was conducted to determine whether 2,4,5-T, in the proportion present in leachate, had a significant effect on total phenoxy degradation. A mixture of 400 mg/l MCPA and 50 mg/l 2,4,5-T was inoculated (141 mg/l) and incubated, along with a control containing 200 mg/l of MCPA. The specific degradation rates were measured, and found to be 0.17 mg/mg.h for MCPA alone, and 0.14 and 0.02 mg/mg.h for MCPA and 2,4,5-T respectively in the mixture. Therefore the two total

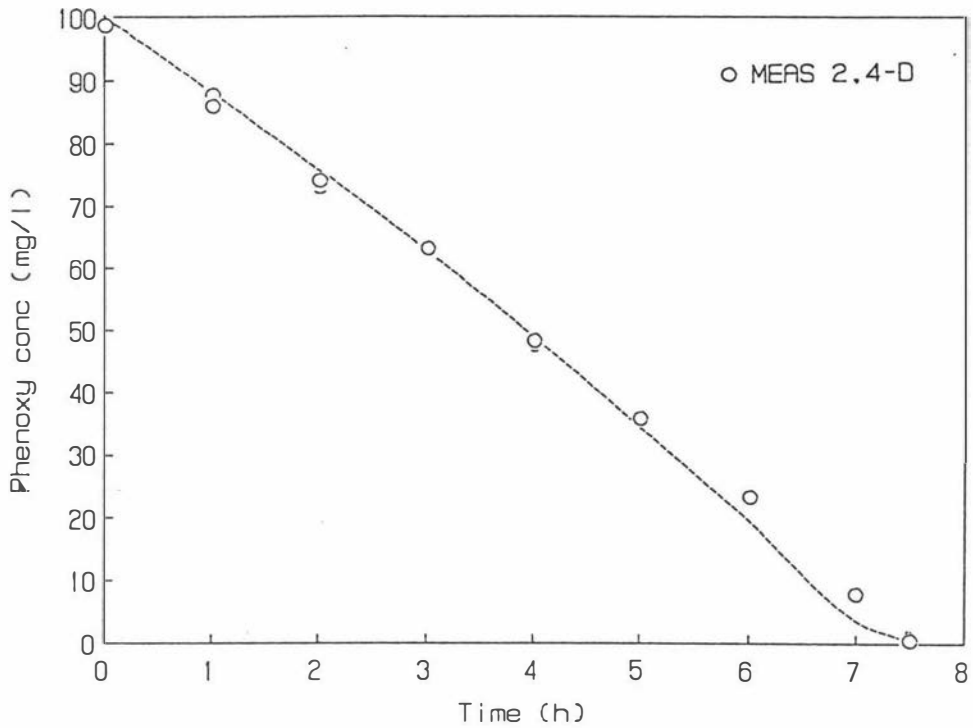


Figure 5.22: Comparison of Predicted and Measured 2,4-D Concentrations for a 1:1 Mixture of MCPA and 2,4-D.

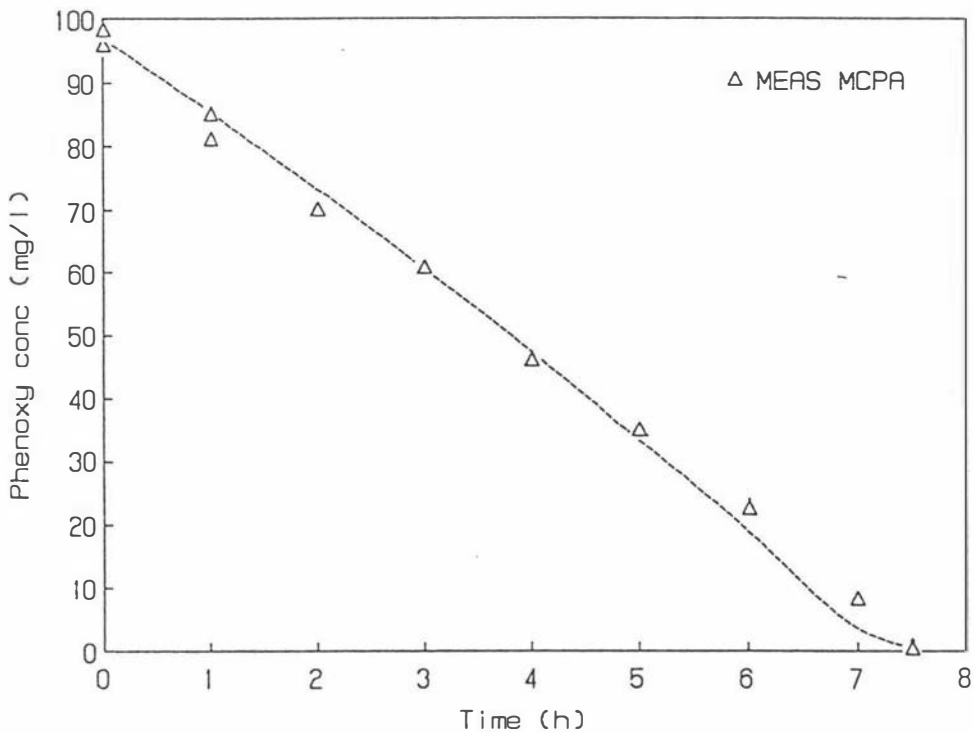


Figure 5.23: Comparison of Predicted and Measured MCPA Concentrations for a 1:1 Mixture of MCPA and 2,4-D.

substrate removal rates were equal.

5.5.4.3 Discussion.

The model of Hutchinson and Robinson (1988) was found to fit the degradation of mixtures of 2,4-D and MCPA. A weighted average of the yield coefficient was used to drive the model, rather than the individual yields as suggested by Hutchinson and Robinson (1988). This was because the average yield was found to give a better fit than the individual yields.

Figure 5.26 shows that PCOC was the most effective compound which stimulated 2,4,5-T degradation. There also appeared to be no inhibition of PCOC degradation by 2,4,5-T. Phenoxy degradation on the other hand was inhibited strongly by 2,4,5-T. This implies that 2,4,5-T was affecting phenoxy degradation at the first metabolic step (the cleavage of the sidechain to produce the associated chlorophenol). While further work is necessary determine details of the mechanism, it would appear that there may be some monooxygenase inactivation by 2,4,5-T.

The total measured rate of substrate removal with a mixture of MCPA and 2,4,5-T was found to be 0.16 mg/mg.h. comparable to the average rate for previous experiments and for the control (0.17 mg/mg.h). Therefore the effect of 2,4,5-T on the degradation of other phenoxies, in the proportions present in leachate was considered to be negligible.

These results indicate that the model derived from Hutchinson and Robinson (1988) was a good predictor of phenoxy degradation. The main driving equation was

$$\mu_{OXY} = 0.048.[oxy]/(0.01 + [oxy]) \qquad (5\text{-}12)$$

This equation will be taken as the best available model for phenoxy degradation. The phenoxy yield coefficient ($Y_{X/S,OXY}$) for further work will be the weighted average of the values for 2,4-D and MCPA in leachate (0.27 mg/mg).

5.6 Conclusions.

The results of these experiments can be summarised by three equations:

$$\mu_{ALC} = (\mu_{MAX,ALC} - (K_{PCOC}.[PCOC] + K_{OXY}.[oxy])).S/(k_{S,ALC} + S) \qquad (5\text{-}1)$$

$$\mu_{PCOC} = (\mu_{MAX,PCOC} - K_{OH}.[PCOC]).[PCOC]/(k_{S,PCOC} + [PCOC]) \qquad (5\text{-}9)$$

$$\mu_{OXY} = \mu_{MAX,OXY}.[oxy]/(k_{S,OXY} + [oxy]) \qquad (5\text{-}12)$$

The parameters required are given in Table 5.14

It can be seen that the degradation kinetics of the three classes of compounds vary by a factor of 10.

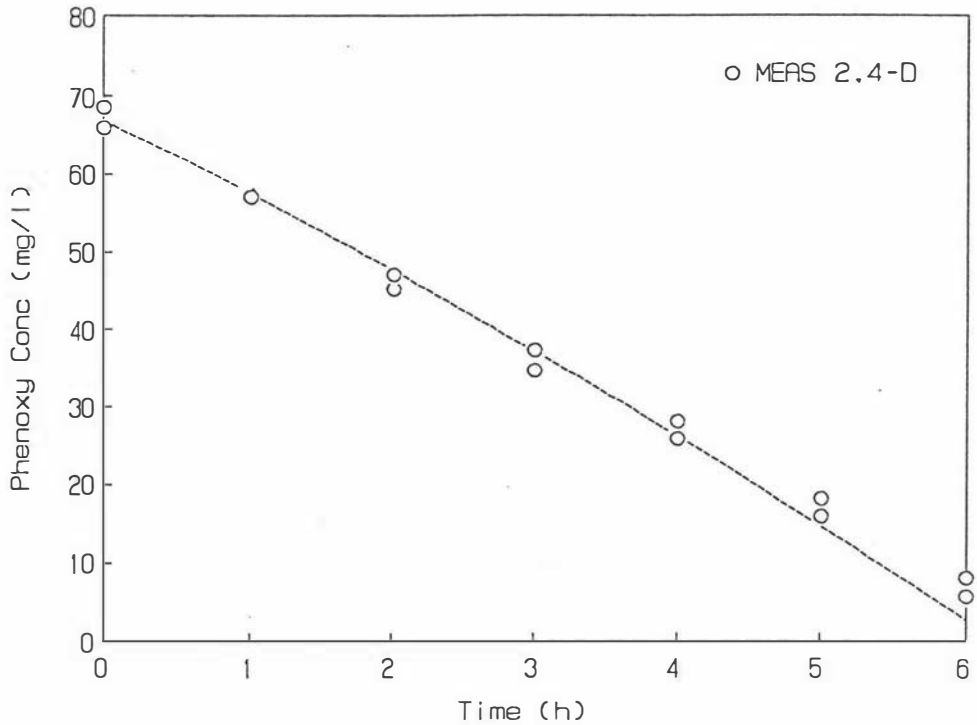


Figure 5.24: Comparison of Predicted and Measured 2,4-D Concentrations for a 1.6:1 Mixture of MCPA and 2,4-D.

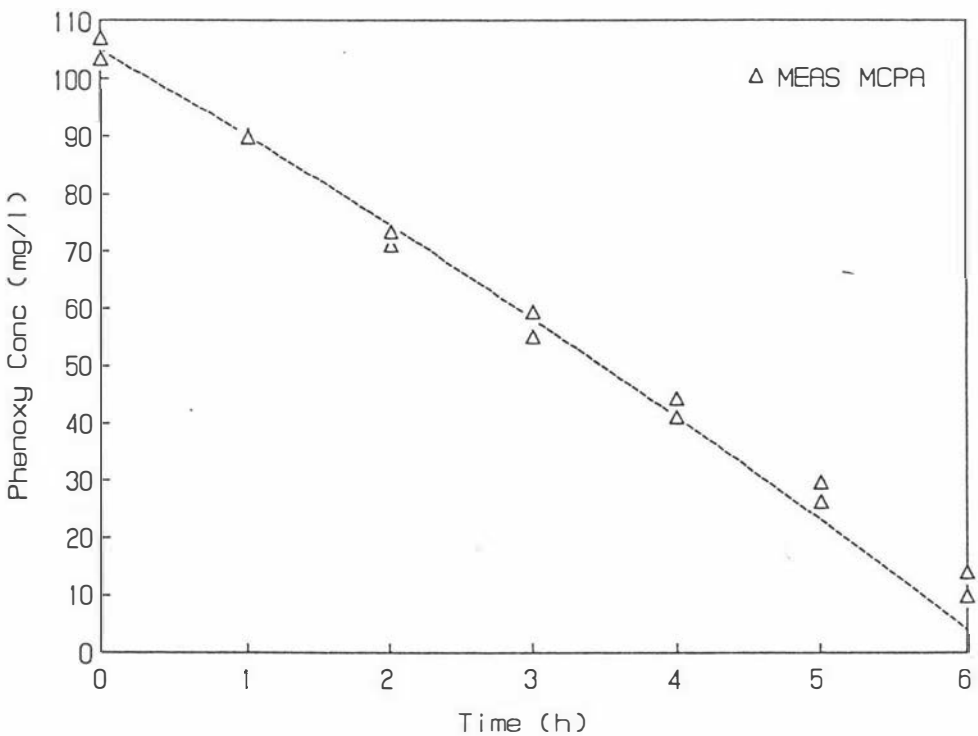


Figure 5.25: Comparison of Predicted and Measured MCPA Concentrations for a 1.6:1 Mixture of MCPA and 2,4-D.

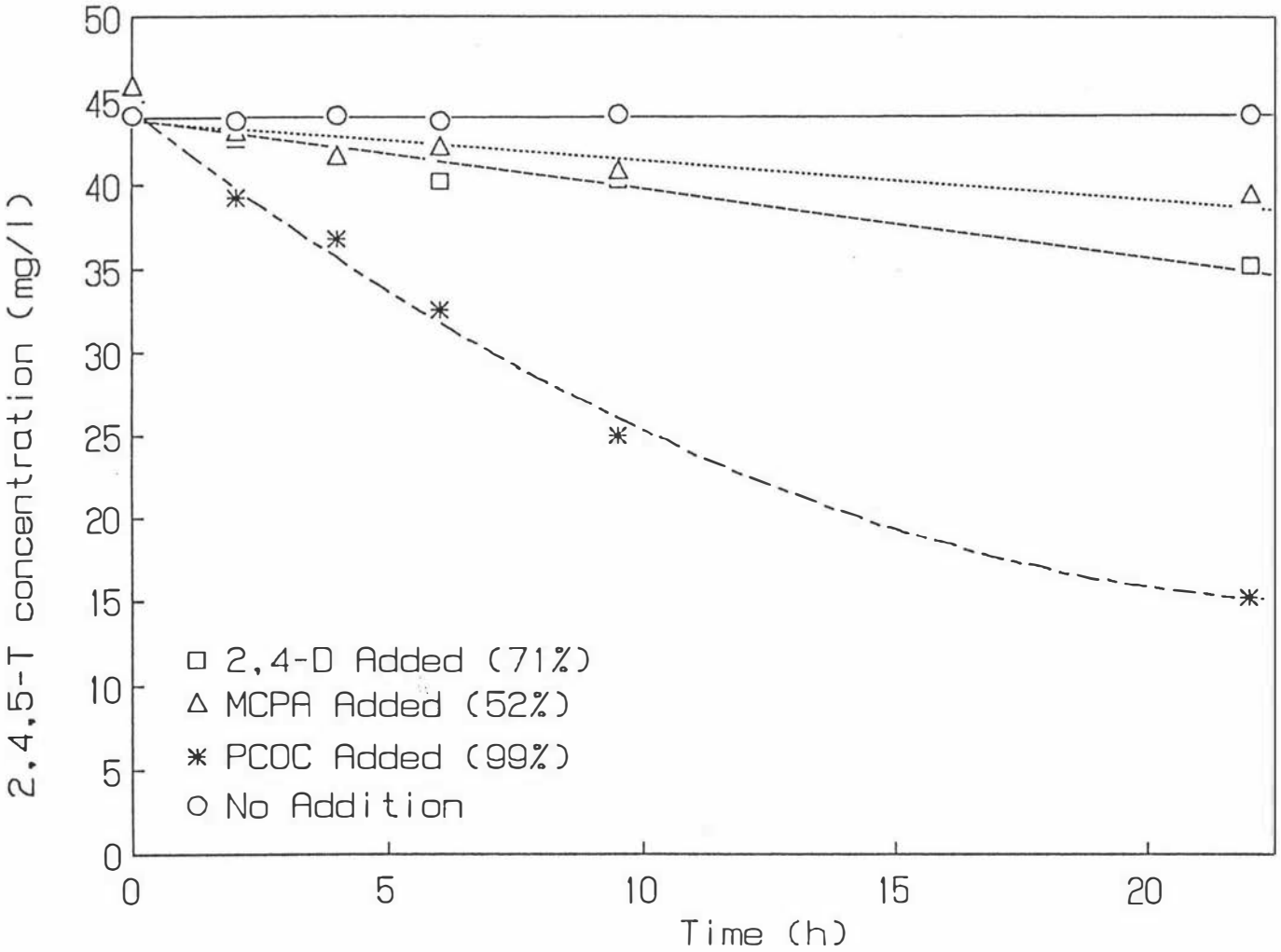


Figure 5.26: The Effect of Phenoxies and PCOC on the Degradation of 2,4,5-T. The numbers in brackets indicate the percentage of the second substrate degraded in the 22 h experiment.

Table 5.14 Summary of Parameters Describing Pure Compound Degradation.

	alc	PCOC	oxy	Units
μ_{MAX}	0.30	0.031	0.048	h^{-1}
k_S	5	0.15	0.01	mg/l
$Y_{X/S}$	1.3	0.25	0.27	mg/mg
K_{PCOC}	$1.0x10^{-3}$			$l/mg_{PCOC}.h$
K_{OXY}	$1.45x10^{-4}$			$l/mg_{OXY}.h$
K_{OH}		$1.09x10^{-3}$		$l/mg_{BIOMASS}.h$

The linear model was found to fit well the data for alcohol and PCOC degradation. No degradation of the substrates could occur at PCOC concentrations greater than ≈ 290 mg/l.

The conventional batch methods for the determination of the kinetics of PCOC degradation were found to give results 50 % lower than the newer MIDT method. The MIDT method was found to be a rapid and reliable procedure for the determination of the kinetics of PCOC and the phenoxies.

2,4,5-T was found to be degraded, apparently by cometabolism, with PCOC the best compound tested for stimulating degradation.

The resulting models were in a form that could be used in a three substrate model.

CHAPTER 6
THE REMOVAL KINETICS OF MIXED SUBSTRATES IN LEACHATE.

6.1 Introduction.

The critical dilution rate method of Rozich and Gaudy (1984) has become the design method of choice, as studies have shown PCOC to be inhibitory. It was necessary, however to determine whether the assumptions made using this method were valid for the leachate system.

This chapter describes the effects of varying SRT on leachate degradation.Data generated are used to determine activated sludge plant design parameters and the interaction coefficients required for a three substrate model describing leachate degradation. These results are then compared to those expected using the method of Rozich and Gaudy (1984).

6.2 Experimental Procedure.

The experimental procedure for these experiments was the same as that previously in Section 4.5.1.2. All experiments were carried out using 10 % leachate. In addition, the sludge volume index (SVI) was determined at each dilution rate, by the method described in Tchobanoglous (1979), and microscopic examination of the culture was also performed at each dilution rate. Fourteen different dilution rates were tested, with the parent bioreactor used as the data point for 14.5 h. Wall growth was removed daily in early runs, and twice daily in later runs.All experiments were conducted without cell recycle.

6.3 Results.

6.3.1 Overview.

All measurements made during the course of these experiments can be found in Appendix 11, a VP-PLANNER spreadsheet named APPEND11.WKS. Steady state was deemed to be achieved when approximately three days of operation had shown no variation in residual substrate concentrations or A_{600}. A summary of the results can be found in Table 6.1.

Substrate concentration versus time was plotted for each experiment, and the resulting graphs are given in Figure 6.1 (a-m). The data for 14.5 h SRT (parent bioreactor) has been presented previously (Figures 4.6 and 4.7). Figures 6.1(a)-(e) represent total substrate removal during the bioreactor runs. Spikes in the substrate levels were usually due to operational upsets. The length of operation varied from 260 to 700 h. Figure 6.1(f) shows the results of a reactor (SRT 9.9 h) that appeared to be cycling (period ≈ 3 days). This reactor was monitored for 5 cycles, with the average measured values considered steady state.

For runs shown in Figures 6.1 (g)-(m), complete substrate removal was not observed, with phenoxy removal incomplete. At the highest dilution rates, both PCOC and phenoxies were found to

Table 6.1 Summary of Results: Varying Dilution Rates on Leachate Degradation.

SRT (h)	Figure 6.1	D (h^{-1})	No of RT*	Δ pH	Biomass (mg/l)	SVI (g/ml)	Alc (%)#	PCOC (%)	Phenoxy (%)	TOC (mg/l)	Higher orgs&
20.3	(a)	0.049	12.7	0.15	503	182	14	0.4	0.1	44	Y
19.1	(b)	0.052	39.0	0.12	323	201	1	0.5	0.1	59	Y
17.2	(c)	0.058	41.2	0.29	333	289	2	0.4	0.26	55	Y
14.6	NA	0.068	1001	0.24	305	147	1	0.5	0.4	61	Y
12.9	(d)	0.078	25.3	0.35	268	ND	6	3.5	0.8	73	ND
11.4	(e)	0.088	30.9	0.36	368	175	7.8	0.8	0.5	71	ND
9.9	(f)	0.101	54.9	0.29	447	439	15	2.3	6.3	70	ND
9.6	(g)	0.104	83.5	0.08	343	2914	2.4	0.8	34.6	131	Y
8.3	(h)	0.120	90.6	0.10	234	4325	1	1.6	43.8	184	N
6.2	(i)	0.161	179.8	0.26	159	6289	1	3.8	61.6	182	Y
6.0	(j)	0.166	26.8	ND	155	6452	1	3.7	63.3	ND	N
5.2	(k)	0.192	30.3	0.17	140	7149	1	45.1	82.2	302	N
5.0	(l)	0.200	154.4	0.09	151	6623	1	43.2	94.4	276	N
4.8	(m)	0.208	35.1	0.05	137	7300	5.6	63.7	98.1	302	N

ND: Not determined

* Number of Retention times the bioreactor was run at the desired dilution rate.

(%) was the percentage of the initial substrate remaining in the bioreactor.

Where no alcohol was detected, 1 % remaining was recorded to indicate there was error in the determination.

& the presence of higher organisms such as rotifers and ciliates in the culture.

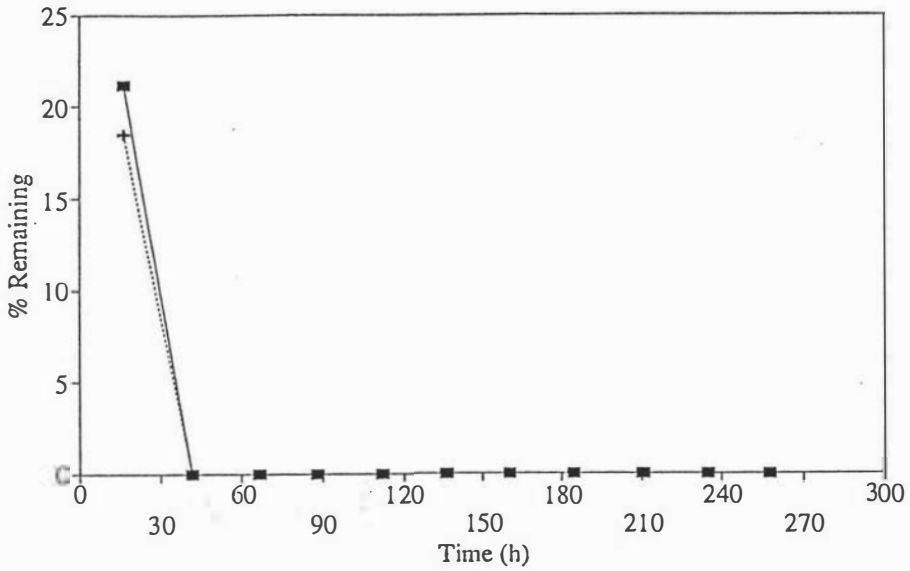


Figure 6.1(a):

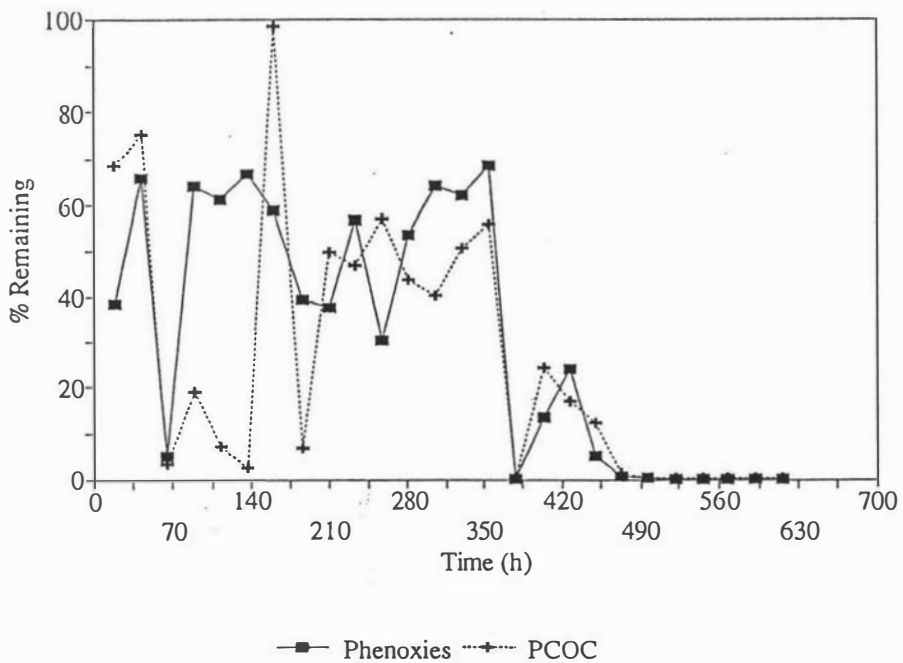


Figure 6.1 (b)

Figure 6.1: Plots of Residual PCOC and Phenoxy Concentration versus Time for Various SRT's. (a) 20.3 h, (b) 19.1 h.

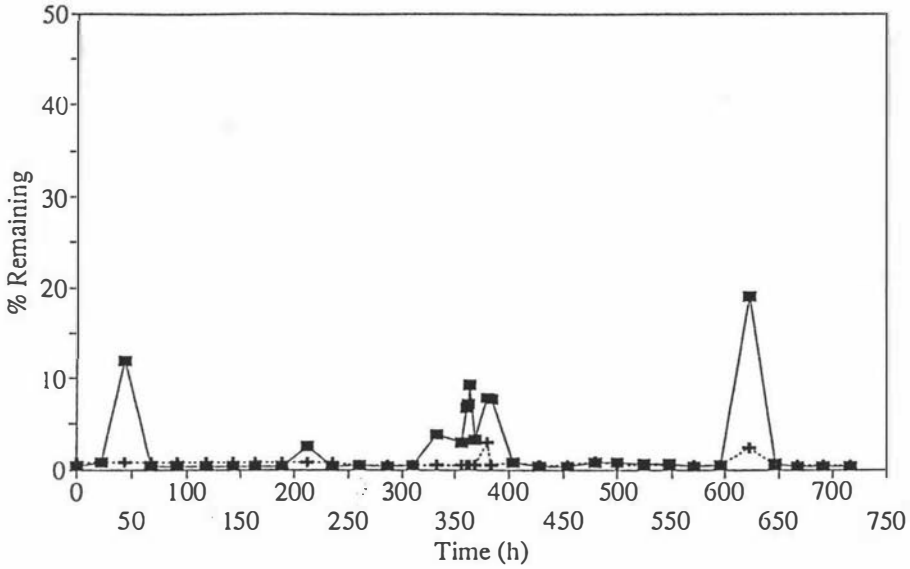


Figure 6.1 (c)

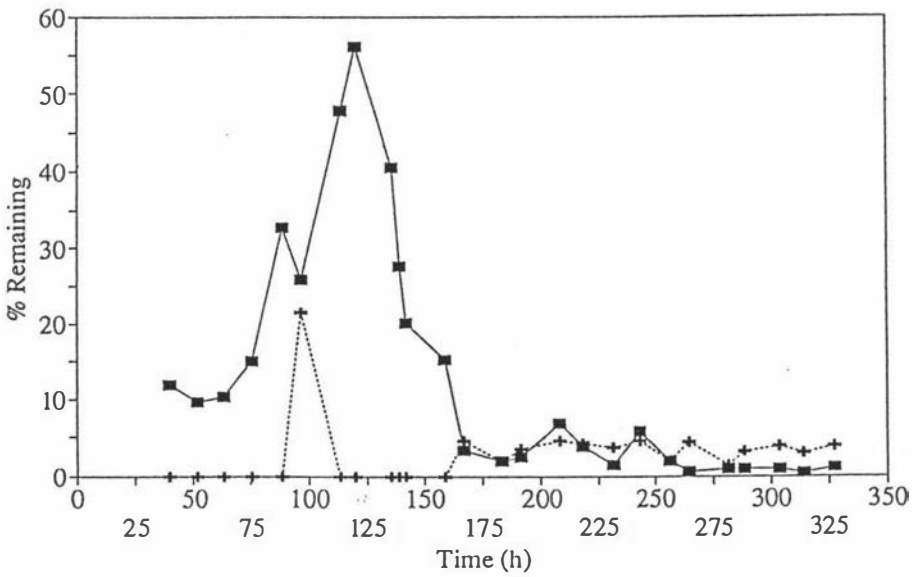


Figure 6.1 (d)

Figure 6.1: Plots of Residual PCOC and Phenoxy Concentration versus Time for Various SRT's. (c) 17.2 h, (d) 12.9 h.

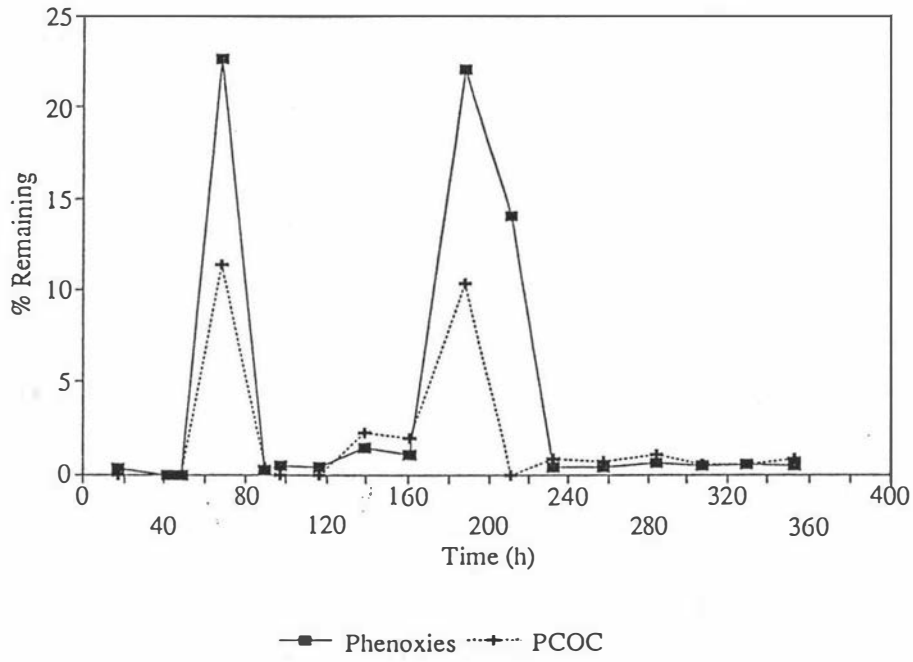


Figure 6.1 (e)

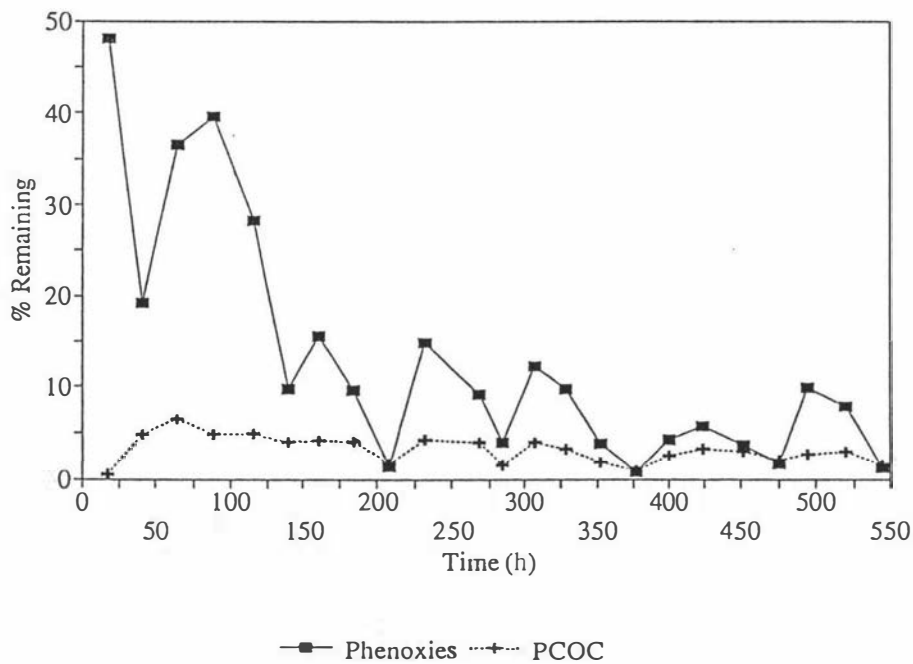


Figure 6.1 (f)

Figure 6.1: Plots of Residual PCOC and Phenoxy Concentration versus Time for Various SRT's. (e) 11.4 h, (f) 9.9 h.

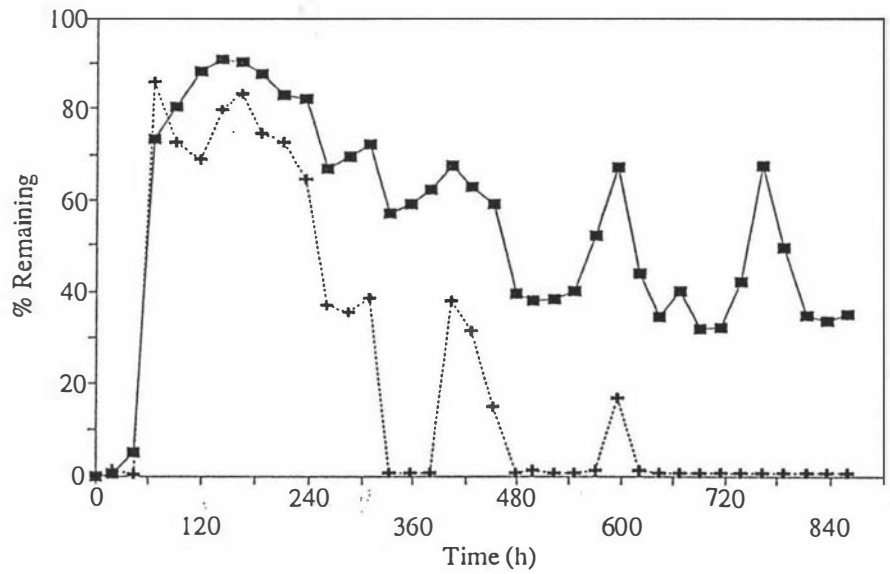


Figure 6.1 (g)

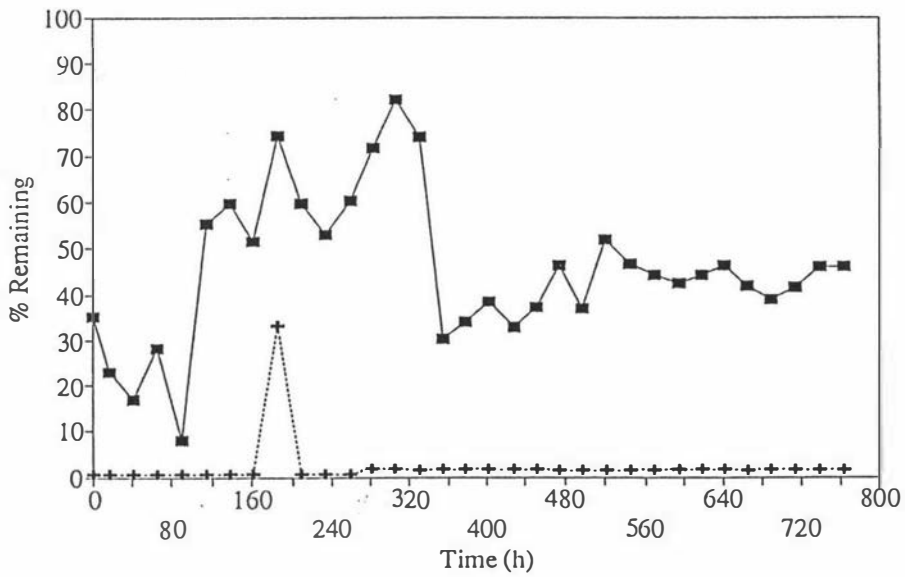


Figure 6.1 (h)

Figure 6.1: Plots of Residual PCOC and Phenoxy Concentration versus Time for Various SRT's. (g) 9.6 h, (h) 8.3 h.

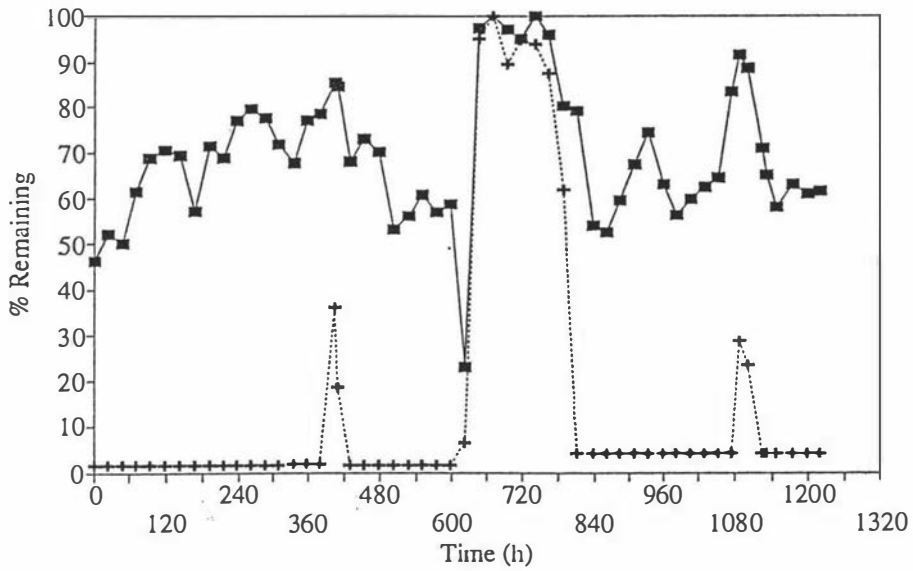


Figure 6.1 (i)

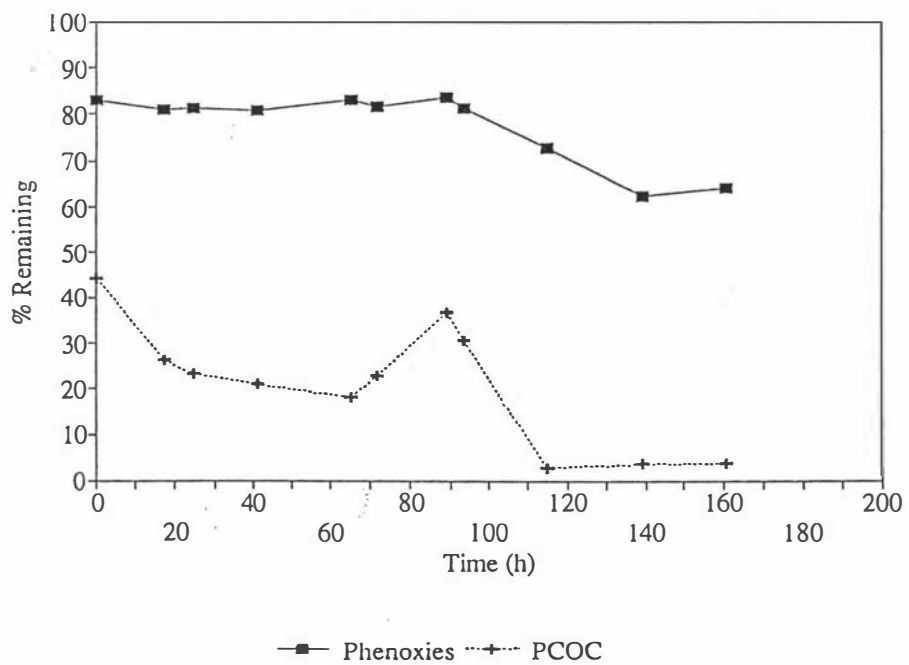


Figure 6.1 (j)

Figure 6.1: Plots of Residual PCOC and Phenoxy Concentration versus Time for Various SRT's. (i) 6.2 h, (j) 6.0 h.

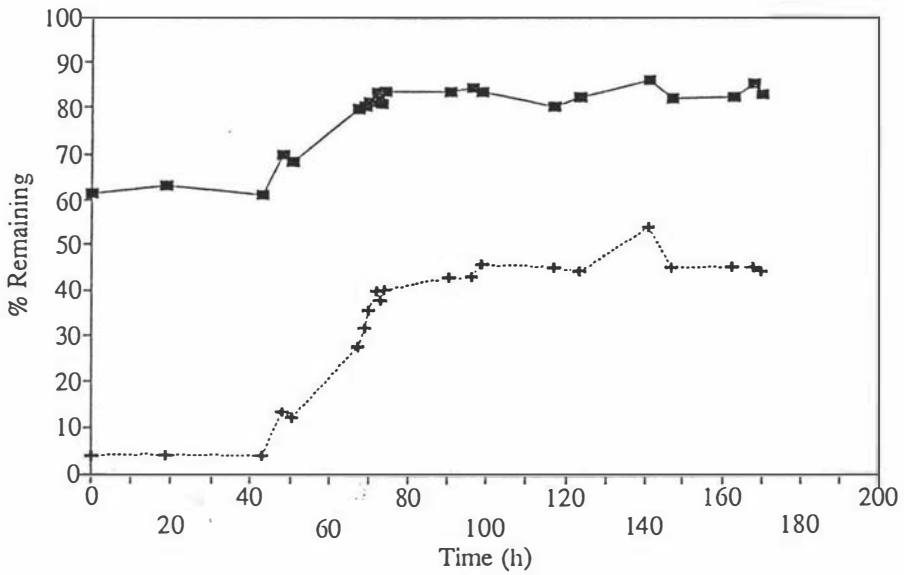


Figure 6.1 (k)

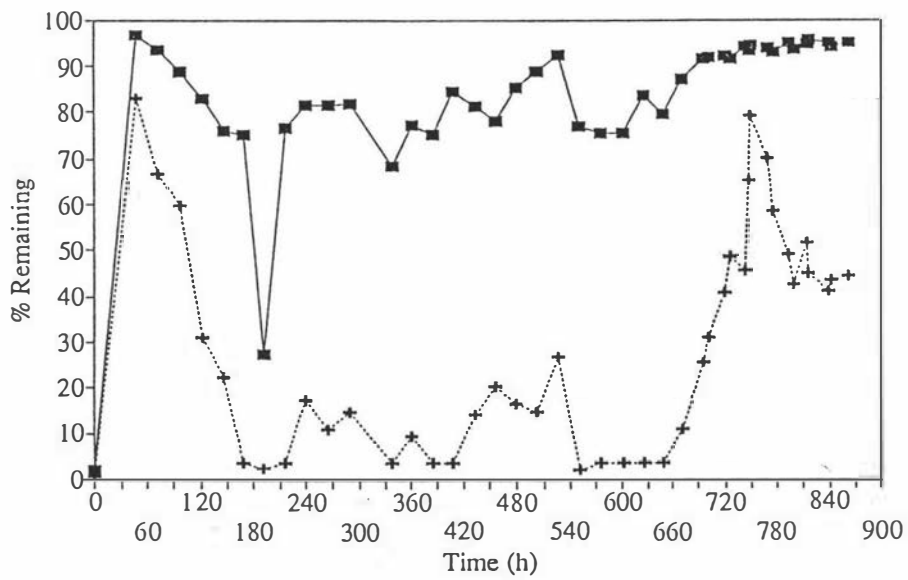


Figure 6.1 (l)

Figure 6.1: Plots of Residual PCOC and Phenoxy Concentration versus Time for Various SRT's. (k) 5.2 h, (l) 5.0 h.

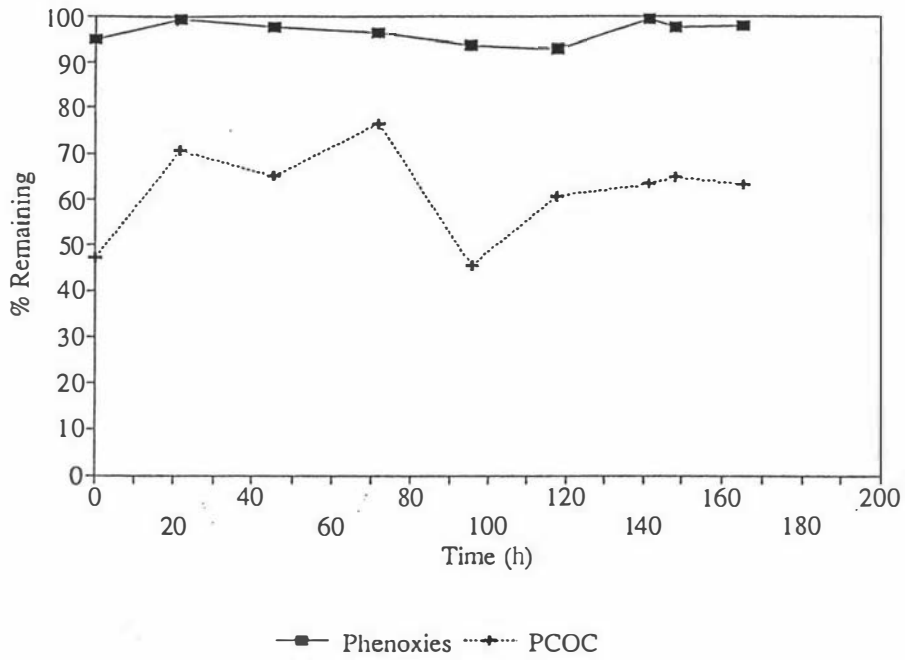


Figure 6.1 (m)

Figure 6.1: Plots of Residual PCOC and Phenoxy Concentration versus Time for Various SRT's. (m) 4.8 h

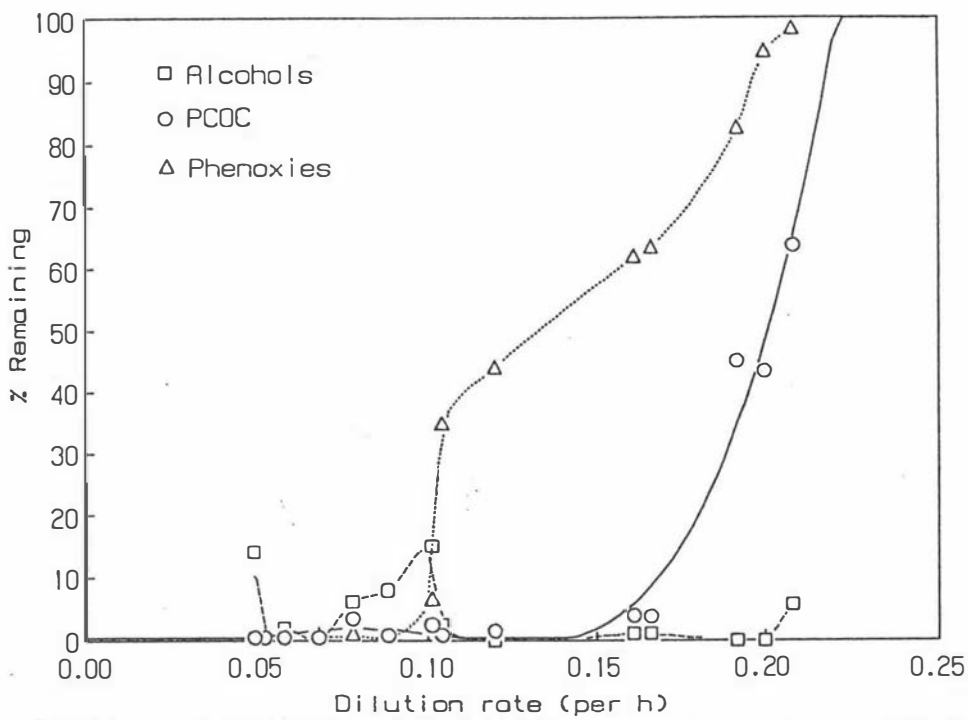


Figure 6.2: Plot of Residual PCOC, Alcohol and Phenoxy Concentrations versus Dilution Rate.

be present in the effluent, although alcohol degradation was still essentially complete. The steady state criteria were relaxed from 3 days to approximately 3-10 residence times in the later work, as the retention times did not warrant the use of such long periods of time.

As the alcohol concentrations were measured only at steady state, these data do not appear on the graphs. The residual alcohol determinations were accurate to ± 10 mg/l, sufficient for the purposes of the data analysis. Figure 6.2 is a plot of residual substrate versus dilution rate. It can be seen that there were two distinct portions on the graph, one at low dilution rates where practically all of the substrate was removed, and the second where there was significant residual PCOC and/or phenoxies. The results for the two section were analyzed differently, with the range where total degradation occurred used to determine activated sludge parameters and the total pool of results was used to determine the interaction parameters for the three substrate model.

6.3.2 Determination of Activated Sludge Parameters.

Chapter 2 (Section 2.4.3) described the graphs plotted to determine the uninhibited kinetics of substrate removal for an activated sludge plant. The experiments used for this purpose were the seven runs, with SRT values between 20.3 and 9.9 h (inclusive).

Table 6.1 shows considerable variation (80%) in the biomass values measured for these runs, even though the feed composition was constant. This difference was probably due to two factors: wall growth, and biomass thrown above the liquid level in the bioreactor that was reintroduced during wall scraping. It was found that transferring the mixed liquor from one bioreactor to a second clean vessel had no effect on the substrate removal, but did reduce the measured A_{600} by up to 40 %. Also comparing the data at SRT of 20.3 and 19.1 h, a difference of 50 % was noted in the biomass data, with no difference in substrate removal. These results indicated the extra biomass measured had a negligible effect on the degradation. It was therefore decided to substitute an average biomass value from the parent bioreactor for errant values. The parent bioreactor data are given in Table 6.2

Table 6.2 Determination of the 95% Confidence Interval on Parent Bioreactor Biomass.

Measured Values 278, 313, 322, 280, 316, 305, 305 (mg/l)
Average = 303 mg /l SD = 17 mg/l
95 % CI = 2.447xSD = 42 mg/l therefore 96 % CI 261 mg/l < biomass < 345 mg/l
Any measured biomass outside these limits was replaced with the mean of 303 mg/l

For the determination of 1/S, the PCOC and phenoxy concentrations only were used (designated 1/s*). This was because the alcohol analysis was unreliable at low concentration and would have an undue effect on the determinations. This approach has been taken previously by Moos et al. (1983)

for determining the degradation kinetics of mixtures of PCP and soluble COD. In other situations where the residual substrate concentration was required, the total substrate (alcohols + PCOC + phenoxies = S) was used. After applying these corrections, Table 6.3 was generated.

Table 6.3 Data for the Determination of Activated Sludge Parameters.

SRT (h)	$1/\theta_C$ (h^{-1})	X #	S_0 #	S #	S_0-S #	Q_B (mg/mg.h)	$1/Q_B$ (mg.h/mg)	$1/S^*$ #
20.3	0.049	303	613	12	601	0.098	10.2	1.25
19.1	0.052	323	577	1	576	0.093	10.8	1.22
17.2	0.058	333	618	3	615	0.107	9.3	0.67
14.6	0.069	305	581	2	579	0.130	7.7	0.47
12.9	0.078	268	559	13	546	0.158	6.3	0.18
11.4	0.088	303	546	9	537	0.155	6.5	0.37
9.9	0.101	303	541	39.1	502	0.167	5.99	0.03

units mg/l

The resulting plots are shown in Figures 6.3 and 6.4 respectively. Straight lines were fitted using MUTAB, (R^2 91.9% and 92.4% respectively) and the slope and intercept of each line was determined. The data, along with the estimated values of the parameters can be found in Table 6.4.

These are the four parameters required for the design and operation of an activated sludge plant. The data were also analyzed in the same manner using TOC as a measure of substrate. The results indicated k_d was comparable at 0.003 h^{-1}, with $Y_{LEACH,TOC}$ = 1.3 mg/mgTOC, $Q_{MAX,TOC}$ = 0.10 mg/mg.h and $k_{s,TOC}$ = 46 mg/l.

Table 6.1 also gives information on the SVI and pH changes measured at different dilution rates. These data are also plotted in Figures 6.5 and 6.6 respectively. A plot of TOC versus dilution rate is shown in Figure 6.7. It can be seen that the SVI increased rapidly as the dilution rate increased above 0.1 h^{-1}, indicating the culture was producing dispersed growth rather than the flocculant growth observed at lower dilution rates. The measured pH reduction was found to be maximum at retention times of approximately 11.4-12.9 h, with less reduction occurring at higher and lower retention times.

The residual TOC suggests there were some residual organic compounds that were not degraded. As the feed was 10 % leachate, these recalcitrant compounds were present in the undiluted leachate at concentrations of approximately 440 mg/l TOC. The theoretical TOC of the PCOC/phenoxies that were degraded was 250 mg/l in this medium. As the feed TOC was 288 mg/l, the residual TOC was expected to be ≈ 38 mg/l. This was close to the measured 44 mg/l.

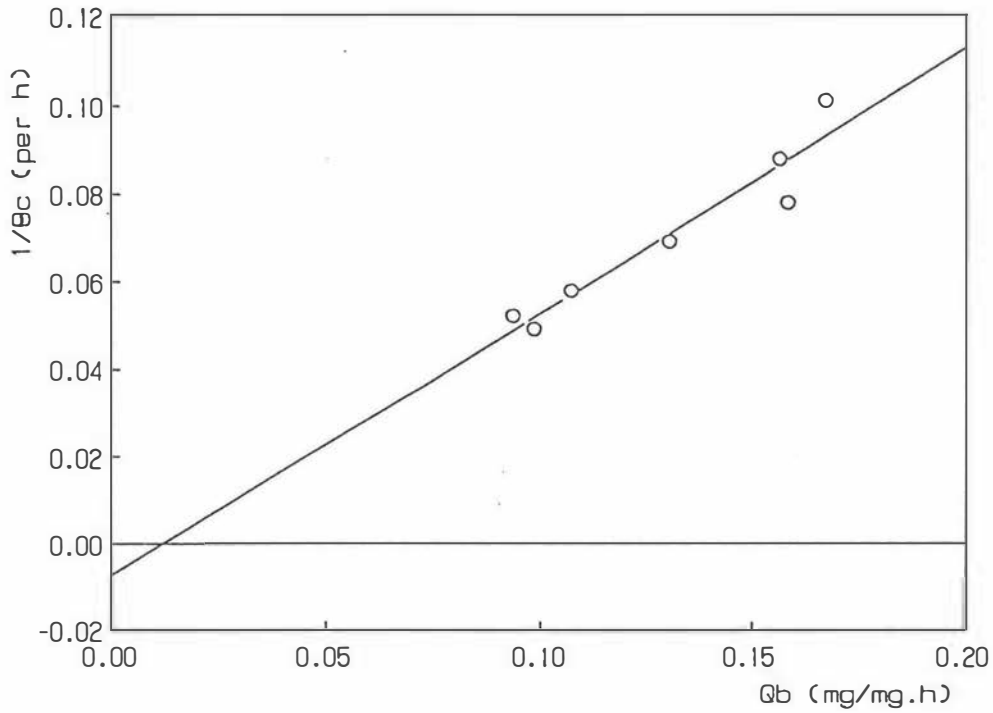


Figure 6.3: Plot of $1/\theta_c$ versus Q for the Determination of k_d and Y.

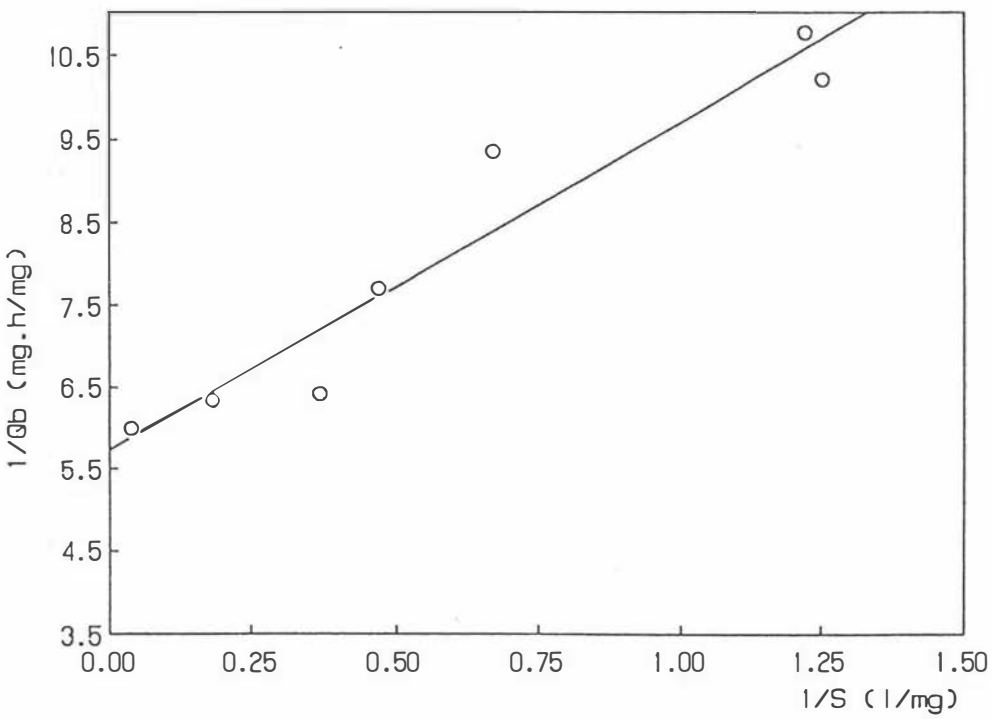


Figure 6.4: Plot of 1/Q versus $1/S^*$ for the Determination of Q_{MAX} and k_s.

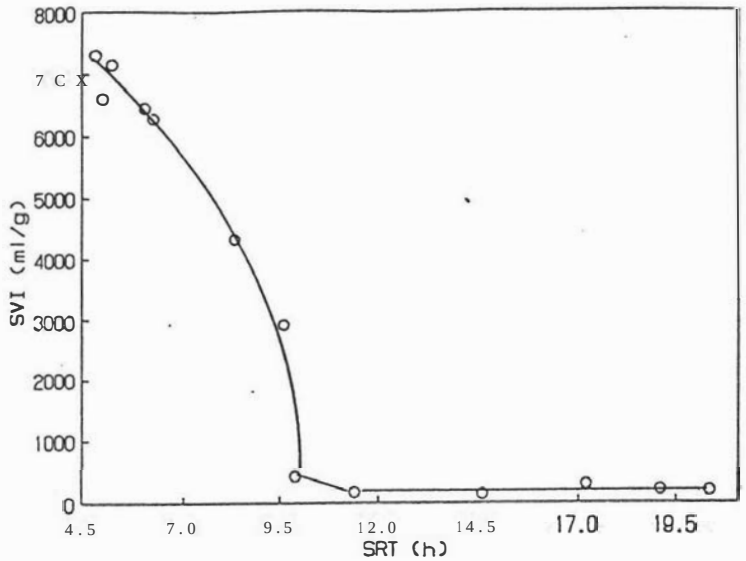


Figure 6.5: Plot of SVI versus SRT Indicating the Loss of Flocculating Ability at SRT's Less than 10 h.

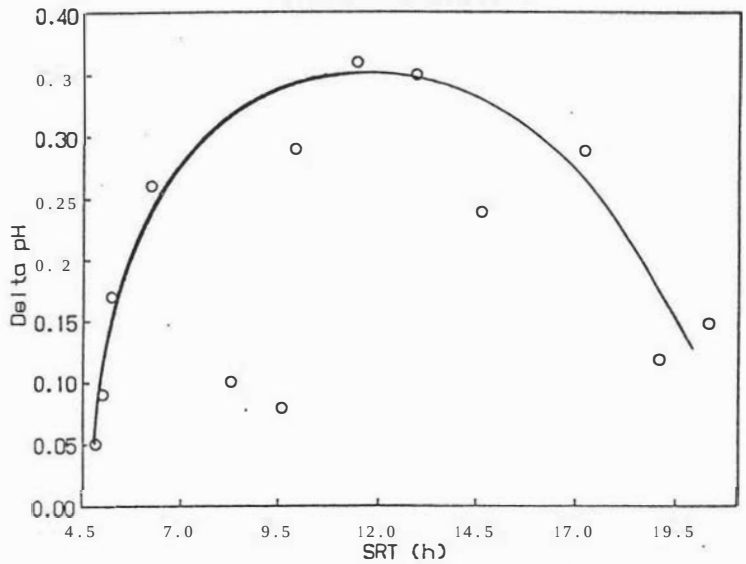


Figure 6.6: Plot of pH Fall During Degradation versus SRT.

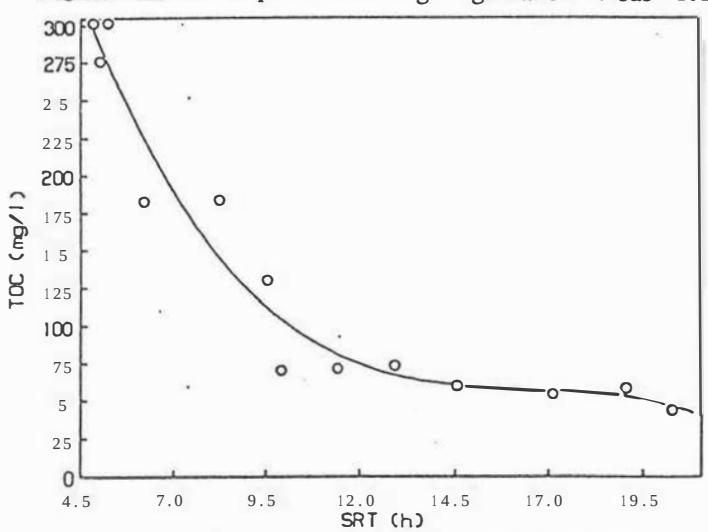


Figure 6.7: Plot of Residual TOC versus SRT.

Table 6.4 Measured Activated Sludge Design Parameters.

From Figure 6.3	Slope = 0.603		(SD = 0.08)
	intercept = -0.007		(SD = 0.011)
therefore	Yield (Y_{LEACH})	= 0.60 mg/mg	(SD = 13 %)
and	Death rate (k_d)	= 0.007 h^{-1}	(SD = 140%)
From Figure 6.4	Slope = 3.962		(SD = 0.5)
	intercept = 5.74		(SD = 0.4)
Therefore	Maximum rate (Q_{MAX})	= 0.174 mg/mg.h	(SD = 7 %)
and	(k_s)	= 0.7 mg/l	(SD = 14 %)

It can be seen in Figure 6.2 that there was no one dilution rate where the bioreactors suddenly and totally failed, as would be expected based on the critical point method for design. There were however, two critical points, where firstly phenoxies and secondly PCOC started to wash out. These occurred at dilution rates of 0.101 and 0.17 h^{-1} respectively. No critical point was observed for alcohols within the range of dilution rates tested.

6.3.4 Development of a Three-Substrate Model to Describe Leachate Degradation.

In Chapter 2, interactive 2 substrate models from the literature were described, which have been applied to mixtures containing 2,4-D (Papanastasiou and Maier, 1982) and mixtures of chlorophenols (Klecka and Maier, 1988). These models were based on the work of Yoon et al. (1977), who gave a general formula to allow expansion to any number of substrates, where the total growth rate was equal to the sum of the individual growth rates. Applying this to leachate, and using the equations developed in the previous chapter for the pure compounds, gave the following equation;

$$\mu = \frac{(\mu_{MAX,ALC}-(K_{PCOC}.[PCOC]+K_{OXY}.[oxy])).[alc]}{(k_{S,ALC}+[alc])} + \frac{(\mu_{MAX,PCOC}-K_{OH}.[PCOC]).[PCOC]}{(k_{S,PCOC}+[PCOC]+k_1[alc]+k_2[oxy])} + \frac{\mu_{MAX,OXY}.[oxy]}{(K_{S,OXY}+[oxy]+k_3[alc]+k_4[PCOC])} \quad (6\text{-}1)$$

Where k_1 is the coefficient for the effect of alcohols on PCOC degradation,
k_2 is the coefficient for the effect of phenoxies on PCOC degradation,
k_3 is the coefficient for the effect of alcohols on phenoxy degradation, and
k_4 is the coefficient for the effect of PCOC on phenoxy degradation.

All other symbols were as defined previously. There was no need for coefficients to define the effect of PCOC and phenoxies on alcohol degradation as this was already determined in the batch experiments and included in the growth model for alcohol degradation.

At steady state in a continuous culture;

$$X = Y_1(S_{10}-S_1) + Y_2(S_{20}-S_2) + Y_3(S_{30}-S_3) \qquad (6\text{-}2)$$

Where S_{10}, S_{20} and S_{30} are the initial concentrations of alcohol, PCOC and phenoxy respectively, and S_1, S_2 and S_3 are the residual concentrations. These subscripts will be used henceforth to simplify the equations, and will be used with all the parameters.

The residual concentrations at steady state are those that satisfy the following equations simultaneously at a given dilution rate D (h^{-1}):

$$Y_1.D(S_{10}-S_1) - \frac{(\mu_{MAX,1}-(K_{PCOC}.S_2+K_{OXY}.S_3)).S_1.(Y_1(S_{10}-S_1)+Y_2(S_{20}-S_2)+Y_3(S_{30}-S_3))}{(k_{S,1}+S_1)} = 0 \qquad (6\text{-}3)$$

$$Y_2.D(S_{20}-S_2) - \frac{(\mu_{MAX,2}-K_{OH}.S_2).S_2.(Y_1(S_{10}-S_1)+Y_2(S_{20}-S_2)+Y_3(S_{30}-S_3))}{(k_{S,2}+S_2+k_1.S_1+k_2.S_3)} = 0 \qquad (6\text{-}4)$$

$$Y_3.D(S_{30}-S_3) - \frac{\mu_{MAX,3}.S_3.(Y_1(S_{10}-S_1)+Y_2(S_{20}-S_2)+Y_3(S_{30}-S_3))}{(K_{S,3}+S_3+k_3.S_1+k_4.S_2)} = 0 \qquad (6\text{-}5)$$

Before the model can be used it was necessary to evaluate k_1 - k_4. As the data collected for leachate at different dilution rates included all the measured substrate concentrations, and the kinetic constants had previously been determined, these values were substituted into equations (6-4) and (6-5). Each dilution rate gave two linear simultaneous equations, one in terms of k_1 and k_2 and the second in terms of k_3 and k_4. Solving pairs of the equations for any two dilution rates will produce estimates of the coefficients. The data in Table 6.5 were determined using the dilution rates of 0.052, 0.068 and 0.104 - 0.208 h^{-1},.

Table 6.5. Data for the Determination of Interaction Parameters.

D (h^{-1})	S_1 (mg/l)	S_2 (mg/l)	S_3 (mg/l)	A	B
0.052	1	0.3	0.3	2.122	0.248
0.068	1	0.3	1.7	1.514	0.712
0.104	2	0.5	150.5	1.130	26.64
0.120	1	1.05	190.5	1.875	22.96
0.161	1	2.5	267.9	2.270	21.89
0.166	1	2.4	275.3	1.970	23.21
0.192	1	29.8	357.6	33.16	209.6
0.200	1	28.5	410.6	21.68	1364.9
0.208	4	42.0	426.7	52.99	4274.8

Where $S_1.k_1 + S_3.k_2 = A$ and $S_1.k_3 + S_2.k_4 = B$

With respect to k_1 and k_2, regression was used instead of solving equations, dividing the equation in Table 6.5 by S_3, gave the equation $A/S_3 = k_2 + k_1.S_1/S_3$, the equation of a straight line of slope k_1 and intercept k_2. Analysis by MUTAB indicated an excellent fit (R^2 = 99.7%) with k_1 = 2.1 (SD = 0.05) and k_2 = -0.009 (SD = 0.05). As it was not possible to have a negative value for the coefficient, and as k_2 was within 1 standard deviation of zero, k_2 was assigned the value of 0.

With k_3 and k_4 the same approach was not possible, as the equations were very sensitive to changes in S_1 and S_2. To overcome this, equations for dilution rates of 0.161, 0.166 and 0.192 h^{-1} were each solved simultaneously with the equations for D = 0.052 and 0.068 h^{-1} to determine k_4. Six estimates of k_4 were obtained, with a mean of 9.3 (SD = 1.8). Backsubstitution into these equations gave negative values for k_3, probably due to the fact that all of the equations had low alcohol concentrations, again making the equations very sensitive.

To determine k_3 it was necessary to vary its value in a program to solve equations (6-3) - (6-5) simultaneously, and find the value which minimised the sum of squared residuals about the phenoxy concentration. The program to perform this (written in Fortran 77l) can be found in Appendix 12. The program used the three equations, along with the partial derivatives of each equation, and uses an iterative subroutine to determine the values of S_1, S_2 and S_3 which satisfy all equations simultaneously. Estimates of k_3 were input into the program, and the phenoxy concentrations were calculated. The residuals were then calculated manually, and the squared residuals plotted against the estimate in Figure 6.8. It can be seen that there was a minimum at k_3 = 5.5, and the minimum was relatively flat, indicating the model was relatively insensitive to error in k_3. To check there was no error in the program or mathematical manipulation of the equations, the solution values of S_1, S_2 and S_3 were backsubstituted into equations (6-3), (6-4) and (6-5). At D = 0.1 h^{-1}, the solutions were found to be 0.023, 0.047 and 0.001 respectively. These values were sufficiently close to zero to indicate the program and manipulation were correct.

A plot of the measured and predicted substrate concentration versus dilution rate can be found in Figure 6.9. It can be seen that there was good agreement between the predicted and measured values. One way analysis of variance on the residuals was carried out. The runs with SRT's of 20.3 and 19.1, 6.2 and 6.0 and 5.2 and 5.0 h were used as replicates. The results of the analysis are given in Table 6.6. These results indicate that there was no significant lack of fit at the 95 % level, and hence the model was a good predictor of the bioreactor performance.

To test the effect of error in the interaction coefficients on the performance of the model, the program was run with each k value varied by ± 1 standard deviation from the best fit value. The difference between the new prediction and old was then calculated and expressed as a percentage change. The results of this exercise are given in Table 6.7. For k_3, where no lower value could be used, only the higher value was calculated.

Table 6.6 Summary of Analysis of Variance on Three Substrate Model.

Component	MSS Factor	MSS Error	F
Alcohol	136.1	19.97	6.82
PCOC	15.40	13.57	1.13
Phenoxy	755.7	248.3	3.04
Degrees of Freedom	9	3	

$F_{9,3,0.05}$ = 8.81: As calculated F values were less than $F_{9,3,0.05}$ there was no significant lack of fit.

Table 6.7 Determination of the Sensitivity of Predictions to Variations in Interaction Coefficients.

	k_1	k_2	k_3	k_4
Value	2.1	0	5.5	9.3
Standard Deviation	0.05	0.05	0.5	1.8
% change in k	2.4	-	9.1	19.3
% change in Predictions	3.2	0.1	0.3	1.4

It can be seen in Table 6.7 that the model was relatively insensitive to variations in k_3 and k_4, with large changes in the coefficients producing a change in the predictions approximately ten times smaller. However, k_1 produced changes in predictions larger than the change in coefficient. These results indicate the model was sensitive to the values of k_1 and k_2, but relatively insensitive to the values of k_3 and k_4.

The model was also used to predict the degradation of leachate in a batch system. An ISIS program (Appendix 13) to model the batch shown in Figure 4.1 was written. The results are shown in Figure 6.10. It can be seen that the model predicted total degradation in 22 h, compared with the measured time of 39 h. This difference will be discussed in Section 6.4.

The model was also used to predict the biomass concentrations expected in the bioreactors at different SRT's. These data, along with measured data, can be found in Figure 6.11. It can be seen that while there was good agreement for short retention times, at times greater than 6.2 h there was a significant difference. This difference will also be discussed in Section 6.4.

6.4 Discussion.

6.4.1 Substrate Removal.

It was noted in Figure 6.1 (f) that the residual substrate concentrations were oscillating, with a period of ≈ 3 days. Oscillatory behaviour has been noted in some fermentations, notably the acetone butanol ethanol fermentation using Clostridium acetobutylicum (Clarke et al., 1988) and in the ethanol fermentation using Saccharomyces cerevisiae (Porro et al., 1988). In both cases the effect was

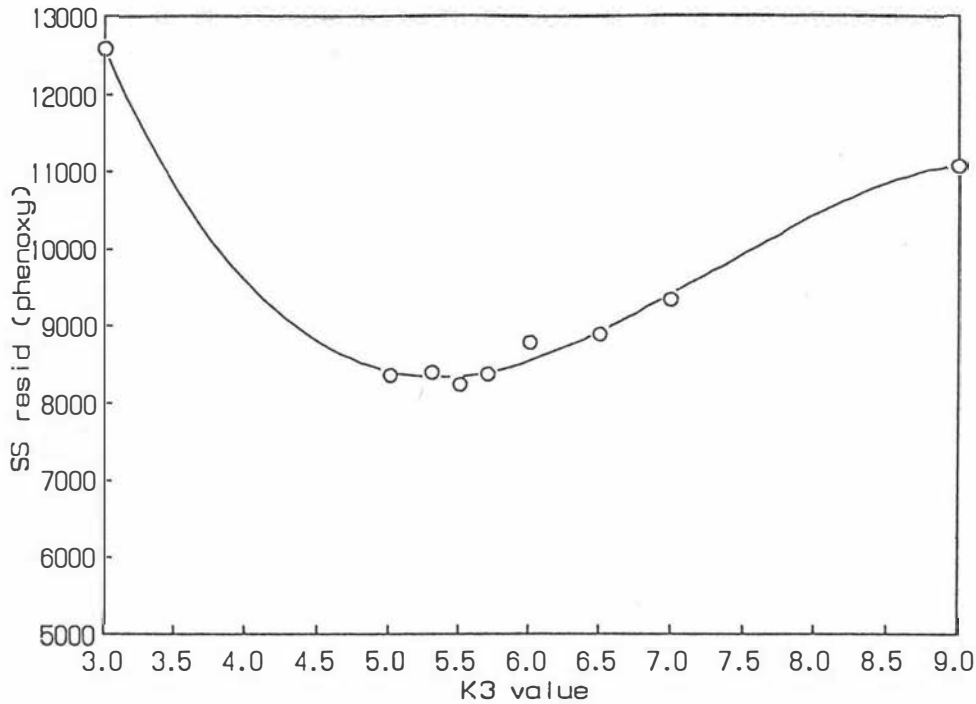


Figure 6.8: Plot of Residual Sum of Squares for the Phenoxy Concentration Versus k_3, Indicating a Minimum at $k_3 = 5.5$

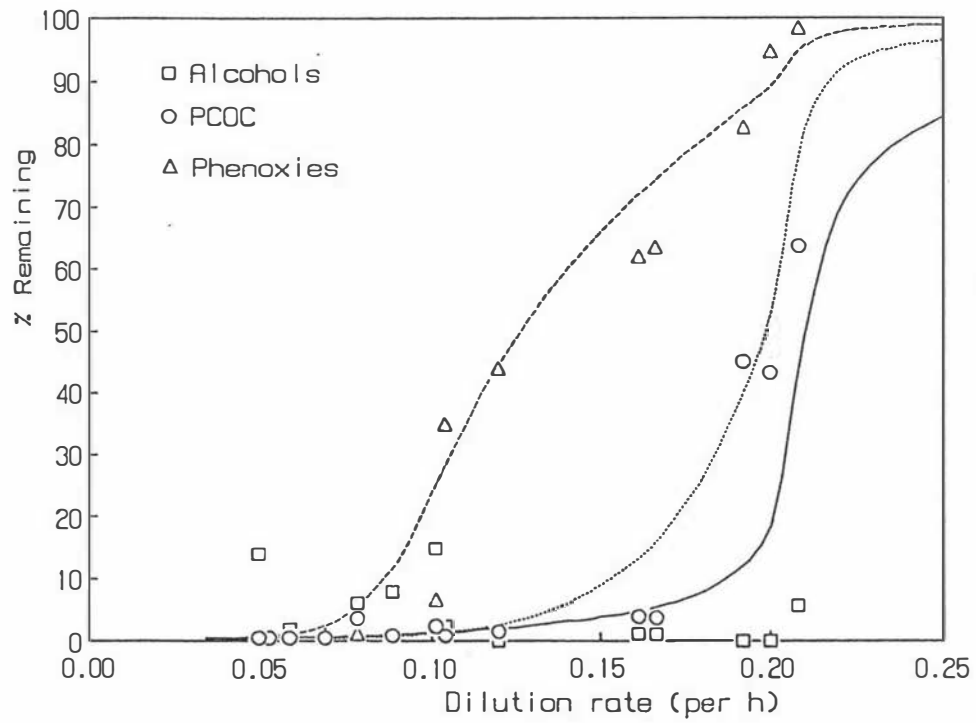


Figure 6.9 Plot of Measured and Predicted Substrate Concentrations versus Dilution Rate for the Three Substrate Model.

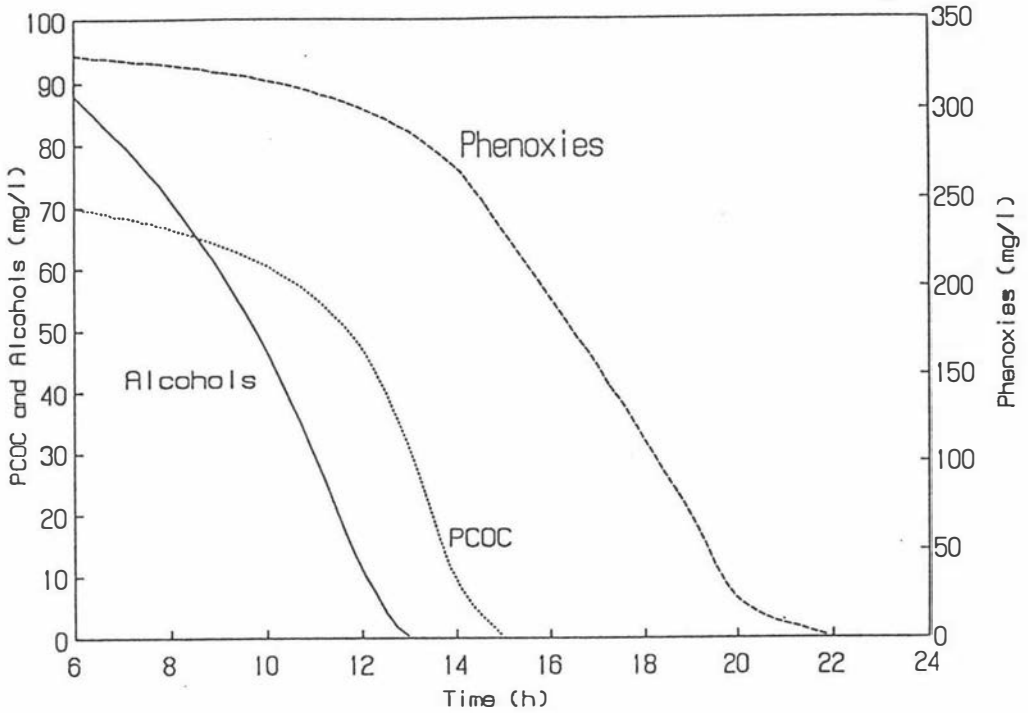


Figure 6.10: Batch Substrate Removal Profile Predicted Based on the Three Substrate Model.

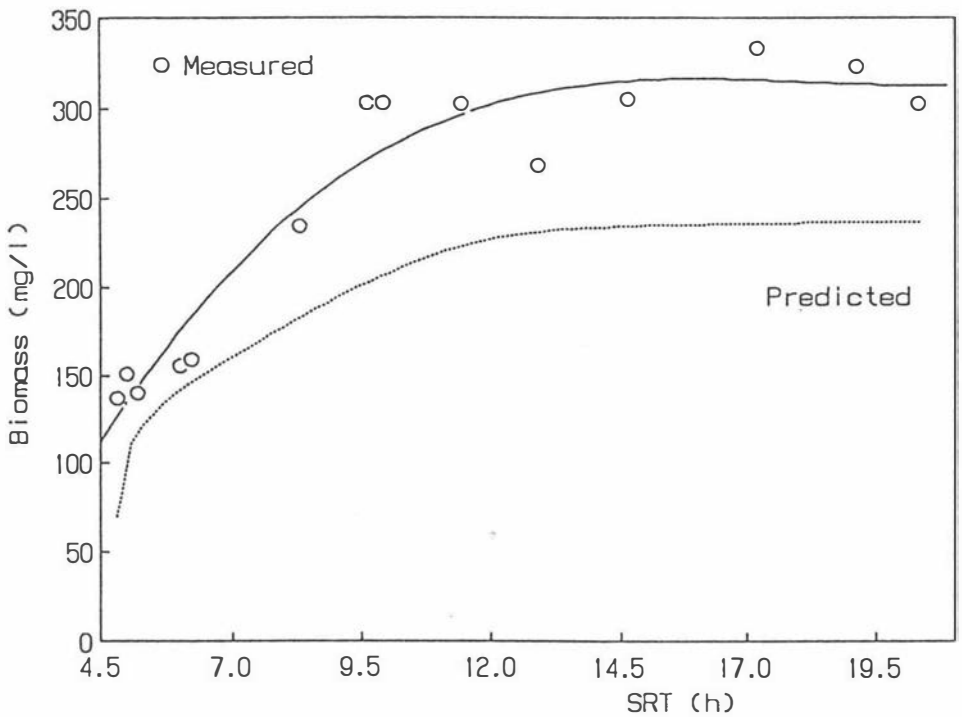


Figure 6.11: Plot of MLSS Concentrations (Measured and Predicted by the Three Substrate Model) versus Time.

considered to be due to a shift in metabolism, from growth on the initial carbon source to growth on a secondary source, acids in the case of Clostridium acetobutylicum (Clarke et al., 1988) and ethanol in the case of Saccharomyces cerevisiae (Porro et al., 1988). A similar effect may be occurring in this case with the two classes of substrates, the alcohols and the aromatic compounds. Further investigation would be required to confirm this, using a more accurate method of alcohol analysis to determine whether their concentrations were also cycling.

The residual TOC (Figure 6.7) was found to level out at higher SRT's and appeared to approach a value the TOC could not be reduced below by this culture. Based on the initial TOC and the theoretical TOC of the compounds degraded this was calculated to be 38 mg/l. The nature of the compounds contributing to this residual TOC was not studied, however two possibilities were apparent: firstly the unknown peaks detected in leachate that had been biologically treated could contribute a portion of that, and secondly there was the possibility that humic and fulvic acids had been leached from the soil and were present in the leachate.

It was noted that there was a very rapid change in the SVI as the dilution rate was increased above 0.101 h^{-1}. This dispersed growth occurred at dilution rates where total substrate removal was not occurring. As flocculation is often associated with conditions of low substrate concentration, when the cells present are in the decline and endogenous growth phases (Pike, 1975), it would appear that the increase in dispersed growth could be attributed to the higher substrate concentrations resulting from incomplete degradation.

It was observed that phenoxies were the first group of compounds to washout, despite the growth rate on these compounds (0.048 h^{-1}) being higher than the growth rate on PCOC (0.031 h^{-1}). This indicates that there is a significant interaction between these two classes of compounds. This interaction was not unexpected, as the chlorophenol is the first breakdown product of the phenoxy (Section 2.4.5). It should be noted that for most runs there was almost total removal of alcohols. This is consistent with the assumption made earlier in Section 5.3.5 with respect to modelling alcohol degradation. The corrections applied to biomass data will be discussed later (Section 7.2.4)

6.4.2 The Three Substrate Model.

The use of an interactive three substrate model for describing biological growth has never been previously reported in the literature to the authors knowledge. This model will be useful, as a means of predicting the effect of changes in leachate composition on an activated sludge plant.

There were a number of assumptions made during the development of the model. The major assumption was that all the biomass was capable of degrading any of the substrates, i.e. the biomass behaved like a pure culture. There was no evidence from the fitting of the model which indicated this assumption was violated. It was also assumed that the interaction parameters were independent. This assumption was made by Yoon et al. (1977) in order to generalise the model. If this assumption was

not made, the expression $k_2 = 1/k_4$ must hold (based on the postulated enzyme sequence). In this case $k_2 \neq 1/k_4$, so this assumption was necessary for the model to be useful. The last major assumption made was that the growth models for the pure compounds were valid. This has already been discussed (Chapter 5), and assumptions made there must be considered in the application of the model, especially relating to the proportion of 2,4,5-T in the total phenoxies, and the 2,4-D concentration where the substrate becomes inhibitory.

As Table 6.6 showed, there was no significant lack of fit at the 95 % confidence interval. The analysis of variance required replicates, which were described earlier. These experiments were originally designed as replicates, and as such were set up independently. Although the dilution rates were slightly different, the runs were still considered replicates for the statistical analysis.

The interaction parameters required for the model were relatively easy to estimate. This was because experiments were performed at dilution rates where substrate removal ranged from total degradation to only one substrate being removed. This allowed the use of simultaneous equations to determine three of the four parameters. The fourth (k_3) could not be estimated in this manner as there was no data at dilution rates where no alcohol was removed. However, the fitting procedure used was capable of determining the parameter easily.

According to Yoon et al. (1977), an interaction parameter greater than 1 indicates inhibition of degradation of one substrate by a second, and a parameter less than 1 indicates enhancement. The results for this model indicate the first part of the statement was true, but a coefficient less than 1 does not indicate enhancement, but rather a lack of inhibition[1]. The "enhancement" described by Yoon et al. (1977) was not due to the interaction between substrates, but the higher growth rate and biomass achieved with two substrates being degraded simultaneously. From the measured values of k it can be seen that alcohols inhibit the degradation of both PCOC and phenoxies (k_1 and k_3), and PCOC inhibits the degradation of phenoxies (k_4). However, phenoxies have no effect on the degradation of PCOC ($k_2 = 0$). These observations agree with those reported earlier (Chapter 4), with respect to unsteady state behaviour.

Figure 6.10 showed the results of predicting the behaviour of a batch using the model. While the sequential utilisation of substrates was observed, the batch time was 40% lower than measured. This difference does not detract from the applications of the fitted model. Earlier results (Chapter 5) indicated that there were changes in the biomass in batch studies due to the toxic effect of PCOC. A second possible reason for this is related to the involvement of plasmids in the degradation of the phenoxies. It was recently reported (Greenfield et al.,1990) that a cured strain of bacteria can grow at rates 4 times higher than the strain containing the plasmid. In a batch situation, organisms lacking the degradative ability may grow rapidly, producing a large bulk of biomass unable to degrade the

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[1] As k_1, k_2, k_3 and $k_4 \rightarrow 0$ the individual terms in equation (6-1) become equal to the equations given in Chapter 5.

aromatic compounds in leachate, resulting in a lower than predicted rate of degradation. This effect would probably not occur under CSTR conditions, as there is never an excess of the substrates at steady state.

Fitting the interaction parameters to the CSTR data has one major advantage over fitting to batch data, as CSTR data will account for some of the variations that may occur at different dilution rates. Therefore finding the model predicted faster degradation than occurred in a batch was consistent with the results reported by both Chiu et al. (1972b) and Del Borghi et al. (1978).

These results indicate fitting the three substrate model to the CSTR results will produce a model that is capable of predicting the performance of a CSTR system, as will be used in an activated sludge plant. It was therefore the best model to use.

As shown in Figure 6.11, there was a significant difference between the measured biomass and predicted biomass at SRT's greater than 6.2 h. Examining Table 6.1 indicated that of the 5 runs at 6.2 h or less, only one showed the presence of higher organisms such as rotifers and ciliates. In contrast, all but one of the runs examined at longer residence times (6) contained higher organisms. These longer retention times also produced flocculant bacteria, along with the associated extracellular polysaccharides. The presence of these secondary organisms and metabolites, which were not predicted by the model, at the longer residence times could have been responsible for the difference. If this were so, approximately 22 % (w/w) of the biomass at an SRT of 14.5 h would be secondary organisms or products.

A second version of the program to solve the equations was prepared, which would allow the input of any initial substrate concentrations (providing the assumptions mentioned previously apply) and the concentration of each substrate at different dilution rates were calculated. This program is given in Appendix 14. This version of the model was used for the remainder of this discussion.

It was found (data not shown) that when only one substrate was entered into the program, the resulting curve was as predicted using the pure compound data. While this may appear trivial, the results serve two functions. Firstly they indicate the program entered was correct. Secondly, and more importantly, the major advantage of the model of Yoon et al. (1977) over the model of Bader (1978) is revealed. The model of Bader (based on the multiplication of a series of Monod equations) predicts no growth, unless all substrates are present. This major flaw in the Bader model was not present in the three substrate model used here.

Figure 6.12 shows the effect of varying the initial concentration of alcohols on the dilute out curve for phenoxies. Phenoxies were chosen, as these compounds will always be the first to wash out. Figure 6.12 shows that with no alcohols present, there would be very rapid washout, at a dilution rate of $\approx 0.065\ h^{-1}$. However, with increasing alcohol concentration, both the initial point of washout, and the sensitivity of substrate concentration to dilution rate are altered, with washout occurring at higher

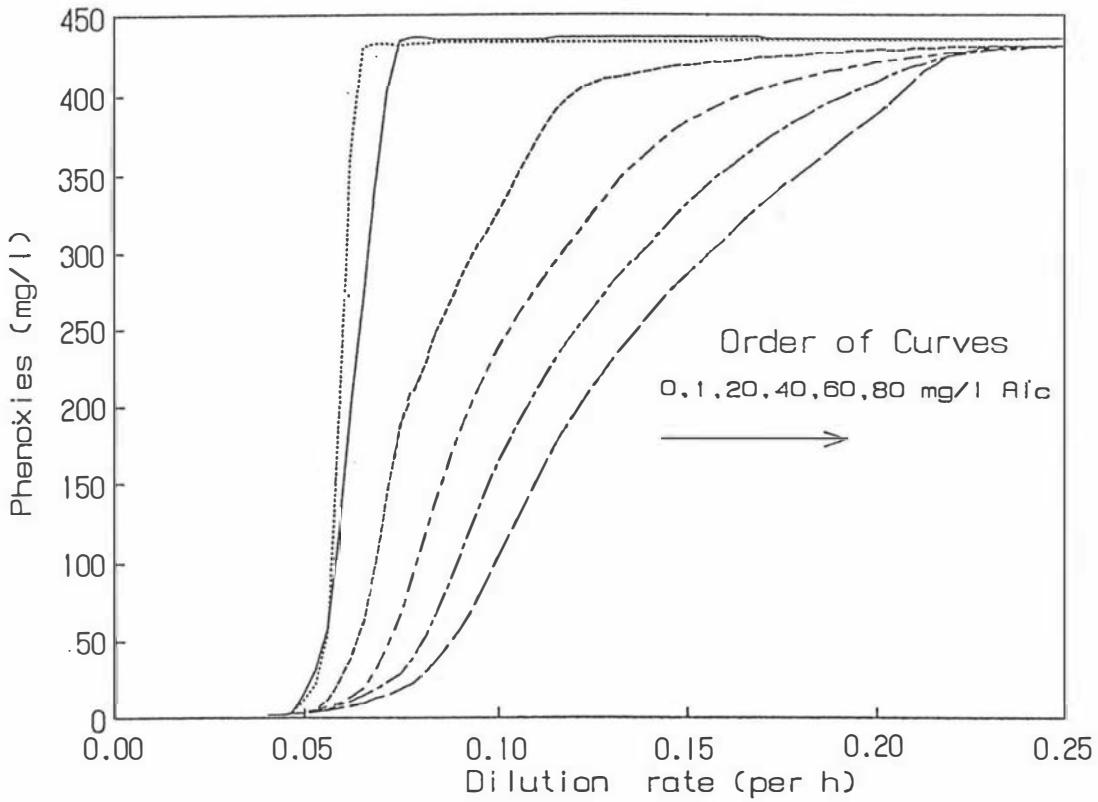


Figure 6.12: The Effect of Alcohols on the Dilute Out Curve for Phenoxies.

It can be seen that increasing the alcohol concentration increases the dilution rate where washout occurs and reduces the sensitivity of substrate concentration to dilution rate.

dilution rates and substrate levels changing less rapidly with dilution rate.

The effect of a readily degraded substrate on the degradation kinetics of a more recalcitrant molecule has been discussed in the literature in the past, but is currently under more intensive scrutiny. Mills (1959) reported the addition of domestic sewerage to a mixture of 2,4-D and 2,4-DCP stabilised the biological processes used for treatment. Batch studies have shown that the simultaneous addition of conventional metabolites does not enhance the degradation of 2,4-D and 3,5-dichlorobenzoate (Kim and Maier, 1986) and nor does glucose enhance 2,4-D degradation (Lackmann et al., 1981). However Lackmann et al. (1981) did note that the addition of glucose prior the addition of 2,4-D did enhance degradation. Results were taken to indicate the extra biomass generated by the glucose increased the rate of 2,4-D degradation. Dynamic analysis of batch data has indicated that a second substrate will dramatically enhance the degradation of 2,4-D in a CSTR, however no experiments were conducted to confirm this (Papanastasiou and Maier, 1983). More recently support for this postulate has come from Lindstrom and Brown (1989), who stated that the reason for the failure of earlier batch work to show enhancement was catabolic repression. The results reported in this thesis indicate there was considerable enhancement of degradation by the presence of mg/l quantities of alcohols and that it was possible to quantify the effect of the secondary substrate on the target compound in a CSTR system.

This difference was best seen by studying the critical points. These are summarised in Table 6.8. This table indicates that there was a large difference between the degradation kinetics of the pure compounds in isolation and in mixtures. The difference was most obvious in the SRT required for degradation. It can be seen therefore that the substrate interactions in the leachate contribute a great deal to the kinetics, and the alcohols in particular have the major effect. Unless the alcohols were present, the model indicates the parent bioreactor (SRT 14.5 h) would have washed out rapidly, and not been able to run for the 1443 residence times that were observed.

As there was a significant change in the critical points from the pure compounds, and also a region where there were high residual substrate concentrations without washout, there was a significant difference between suitable operating conditions predicted by the critical point method, and the actual required conditions as measured in this case. By replacing the Haldane function in the critical point method with the three substrate model, a more accurate predictor of activated sludge plant performance could be obtained. This will be discussed further in the next chapter.

It can be seen that the presence of the alcohols significantly improves the performance of the culture with respect to the degradation of the hazardous compounds present in the leachate. This improvement was entirely consistent with literature postulates. This work, however, provides a method for quantifying and predicting this effect by the use of an interactive three substrate model.

Table 6.8 Predicted and Measured Critical Points for 10 % Leachate.

Compound	Predicted		Measured	
	μ (h^{-1})	SRT (h)	μ (h^{-1})	SRT (h)
Pure Compounds				
PCOC	0.03	33.3	NA*	
Phenoxy	0.048	20.8	NA*	
Alcohol	0.22	4.5	NA*	
2 Substrate Mixtures				
PCOC +				
Phenoxy	0.06	16.7	ND#	
3 Substrate Mixture				
PCOC	0.15	6.7	0.17	5.9
Phenoxy	0.08	12.5	0.101	9.9
Alcohol	0.2	5.0	> 0.208	< 4.8

NA* Not Appropriate: The measured data were used to generate the predicted values

ND# Not Determined: No CSTR experiments were performed with this mixture.

6.5 Conclusions.

An interactive three substrate model was developed and found to fit measured data for CSTR systems. The model was capable of predicting the pure compound data when only one substrate was present, indicating the wide range of application of the model.

The model showed that the presence of the alcohols in the leachate considerably accelerated the degradation of the PCOC/phenoxies in leachate. The critical points for washout were significantly shifted from those of the pure compounds, indicating interactions between the substrates could not be ignored. The model provides a method for quantifying the effect of a secondary substrate on the target compounds.

It is necessary to use the three substrate model in the critical point method to determine suitable operating conditions for an activated sludge plant.

CHAPTER 7
THE DEGRADATION OF LEACHATE BY ACTIVATED SLUDGE: PROCESS KINETICS AND EFFLUENT QUALITY.

7.1 Introduction.

Activated sludge (AS) is the most widely used biological waste treatment system (Grady and Lim,1980). Described here is work that uses a laboratory AS plant to demonstrate the degradation of leachate. Results will be presented showing the successful operation of a laboratory scale AS plant, and the quality of the effluent and wasted sludge produced. Tertiary treatment of the effluent will also be investigated, aimed at producing non-toxic streams suitable for environmental release. This chapter will also show the usefulness of the three substrate model in design, based on the critical point method.

7.2 Laboratory Activated Sludge Experiments.

7.2.1 Development of a Cell Recycle System.

The essential requirements for a cell recycle system are that the biomass is concentrated (to minimise the recycle ratio) and the biomass spends a short time in the system to ensure there is no endogenous metabolism of the sludge, either aerobically or anaerobically. The conventional laboratory scale AS plant consists of a partially partitioned aeration tank (Eckenfelder et al. ,1972), where the MLSS overflows into the partitioned section, the biomass settles and the clear supernatant is removed. The settled biomass then returns to the MLSS by passing under the partition. Biomass is wasted from the aeration tank. With this system it is not possible to accurately determine X_R or the recycle ratio, but the biomass spends very little time in anoxic conditions, and is hence still active. Some systems employ an external clarifier, with periodic return of biomass to the aeration basin. The main problem with this is the timing of the return of sludge. If the sludge is left too long in the clarifier, it may become anaerobic, and if too short a time, the concentration will be low, and a large recycle ratio would be required.

In an effort to overcome these problems for this project, a novel cell recycle system was developed. An external clarifier (an Imhoff cone) was employed, with recycle controlled by an optic system, which detected the buildup of biomass in the bottom of the clarifier. When the biomass built up past the trigger point, the control system started two pumps simultaneously: one to recycle settled sludge into the bioreactor, and the second to pump sludge to waste. The flowrates of the pumps could be controlled independently. A schematic diagram of the system can be found in Figure 7.1.

The control system consisted of a light dependent resistor (LDR) on one side of the cone and a light source on the other. When the biomass built up to a level covering the LDR, the resistance increased above that of the reference resistance. When this occurred, a threshold comparator then started the pumps until the original conditions were restored. The major potential faults in the system were if the light source failed and if the biomass became attached to the cone over the LDR. To prevent

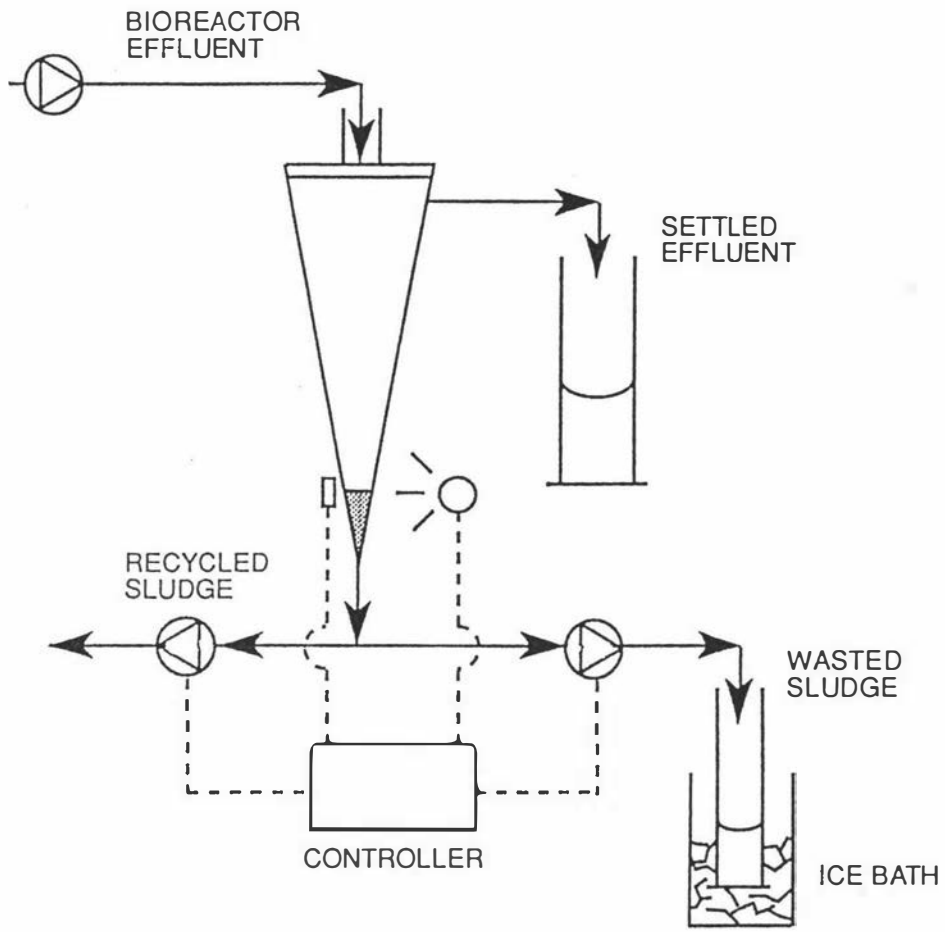


Figure 7.1: Schematic Diagram of the Novel Cell Recycle System.

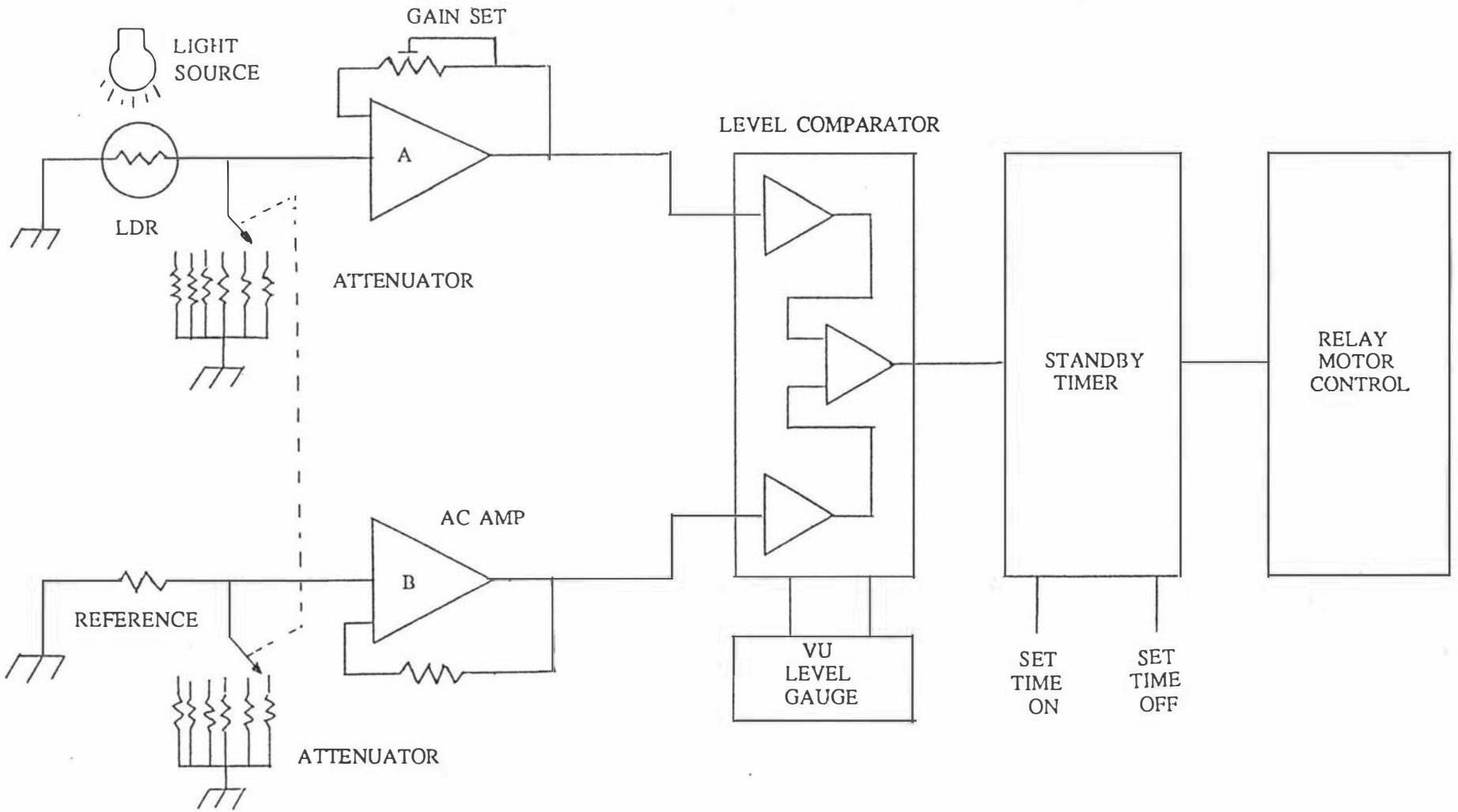


Figure 7.2: Block Diagram of the Cell Recycle Controller.

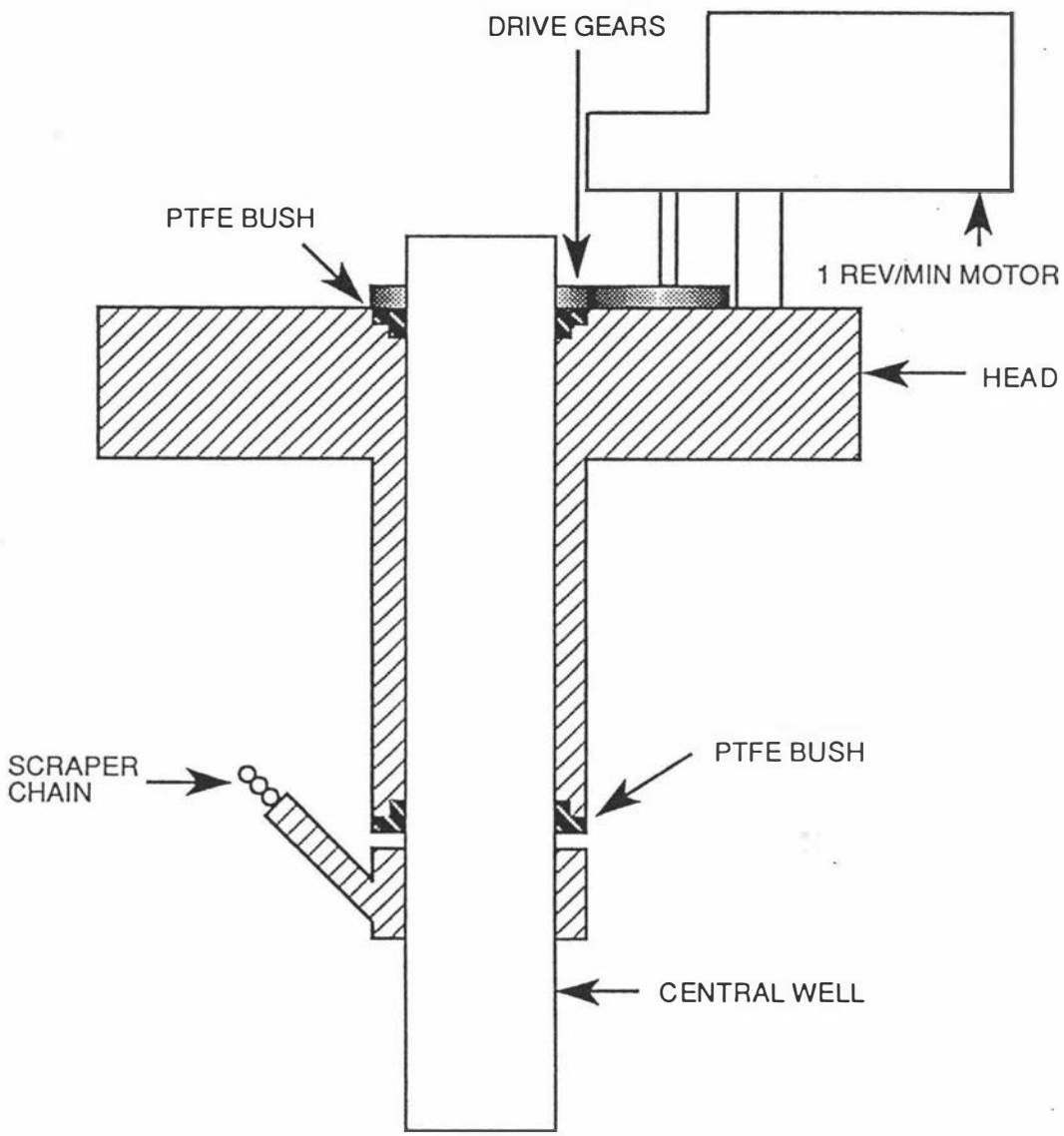


Figure 7.3: Schematic Diagram of the Scraper System for the Clarifier.

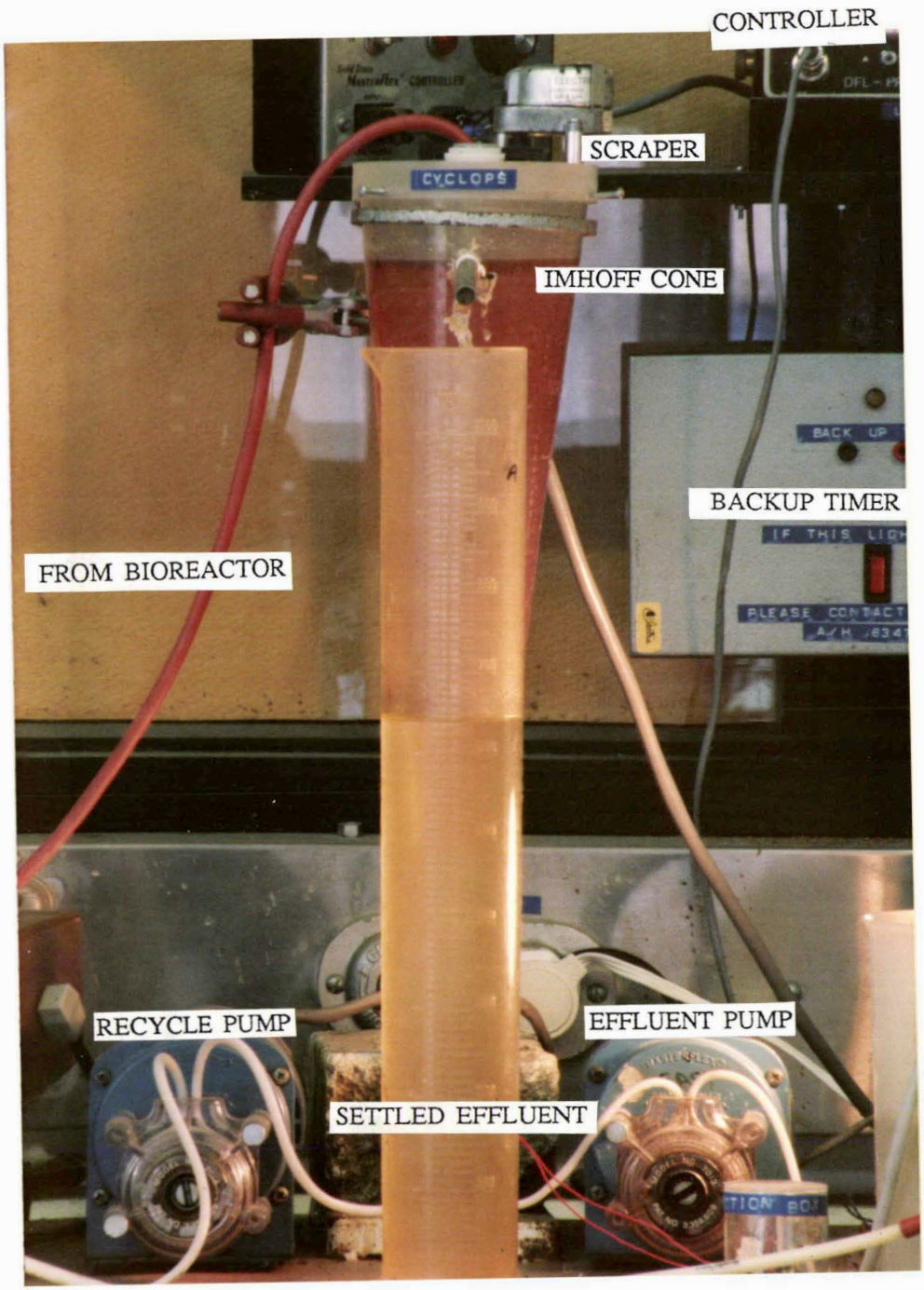


Figure 7.4: Photograph of Cell Recycle System.

the second fault from occurring, a backup timing system was used. If the pumps were activated for more than 30 seconds, the timer would turn off the pumps, and operate on a cycle of 30 seconds pumping every 45 min. If the LDR cleared, the backup timer would be stopped and the system would run as normal. If there was a light source failure and the resistance on the LDR was very high, the comparator detected a fault and no action was taken. This would cause a build up of biomass in the clarifier, therefore a second LDR was added, directly adjacent the light source. If there was a significant increase in the resistance of this LDR, the backup timing system was again activated. This system is shown in block diagram form in Figure 7.2.

The major operational problem encountered was attachment of the flocs to the walls of the settling cone. To overcome this a motorised scraper was developed. The feed to the clarifier was introduced down a central well. The central well was driven by a small electric motor to rotate at 1 rev/min. Attached to this central well was a length of chrome plated bathroom plug chain. As the well rotated, the chain scraped the walls of the clarifier. To prevent biomass from building up on the chain, small ridges were attached to the inside of the cone in two places. As the chain fell back onto the cone from the ridge, the chain was shaken and the biomass freed. A schematic diagram of this can be found in Figure 7.3 and a photograph of the entire recycle system is shown in Figure 7.4.

It was envisaged that this system would have a relatively short residence time for the sludge, and hence there would be little change in the activity of the biomass. The system also allows the wastage of concentrated sludge from the underflow, and also relatively easy control of the recycle ratio by altering the relative pump flows.

To confirm the biomass was still active the specific oxygen uptake rate (Jorgensen,1984) was determined using biomass from the bioreactor and the underflow from the clarifier. The biomass was placed in a 250 ml BOD bottle (stirred by magnetic stirrer, 100 rpm), filled with a well aerated solution of 2 % leachate, and the dissolved oxygen concentration was measured over time. The biomass was determined using A_{600} (standard curve given in Section 7.3.2). The results can be found in Table 7.1.

It can be seen that there was no reduction in the specific oxygen uptake rate of the sludge. The calculated residence time of the sludge in the bioreactor was 1.7 h, and the volume of sludge in the cone was ≈ 20 ml. It can therefore be seen that the clarifier system developed was suitable for use in a laboratory scale AS plant.

7.2.2 Experimental Procedure.

The experimental procedures can be broken into three sections: (1) the operation of a laboratory AS plant, (2) the determination of sludge quality and (3) the determination of the effectiveness of tertiary treatment. Each will be described in turn.

Table 7.1 Comparison of Specific Oxygen Uptake Rates of MLSS and Clarifier Underflow.

Source	Uptake Rate (mg/l.min)	Biomass (mg/l)	Sp. uptake rate (mg/mg.min)
Underflow	1.393	260	5.4×10^{-3}
Underflow	1.592	299	5.3×10^{-3}
Average			5.4×10^{-3}
MLSS	0.525	99	5.3×10^{-3}
MLSS	0.523	93	5.6×10^{-3}
Average			5.5×10^{-3}

(1) The Operation of a Laboratory AS Plant.

The experimental procedure for this section of work was very similar to that used previously (Section 4.5.1.2). The effluent from the bioreactor was passed from the pump into the top of the clarifier (described in Section 7.2.1). The clarified effluent was collected and the daily volume used to check the flowrate. The wasted sludge was collected in a 100 or 250 ml measuring cylinder (depending on the production rate), which was placed in an ice bath (replenished twice daily) to prevent autolysis. All substrate samples were taken from the bioreactor to ensure the measured degradation was occurring in the bioreactor, and not the clarifier.

Bioreactors were run using 10 % (runs 1 and 2) and 15 % (run 3) leachate. The wall growth was removed two or three times daily. As the biomass was found to give reproducible absorbance values when the bioreactors were near steady state, A_{600} was used to determine the biomass. A plot of A_{600} versus biomass can be found in Figure 7.5.

Samples of the mixed liquor were frequently taken and the MLSS determined by the method described in Section 3.4 to check the standard curve. Samples of the clarified effluent, mixed liquor and underflow were taken daily and the MLSS determined by A_{600}. Steady state was achieved when the residual PCOC/Phenoxy and biomass concentrations, along with the SRT and HRT had been constant for at least 3 SRT's.

The specific oxygen uptake rate was measured (in duplicate) in each activated sludge run by the following method:

(1) the dissolved oxygen was increased to a high value (18 - 20 mg/l) using pure oxygen (N.Z.I.G.).

(2) the headspace of the bioreactor was then reduced to a minimum, all the ports were sealed, and the reduction in dissolved oxygen concentration with time was measured (Section 3.6). During the experiments the feed was continued to provide substrate, and the volume and oxygen added by this was considered to be negligible. Experiments

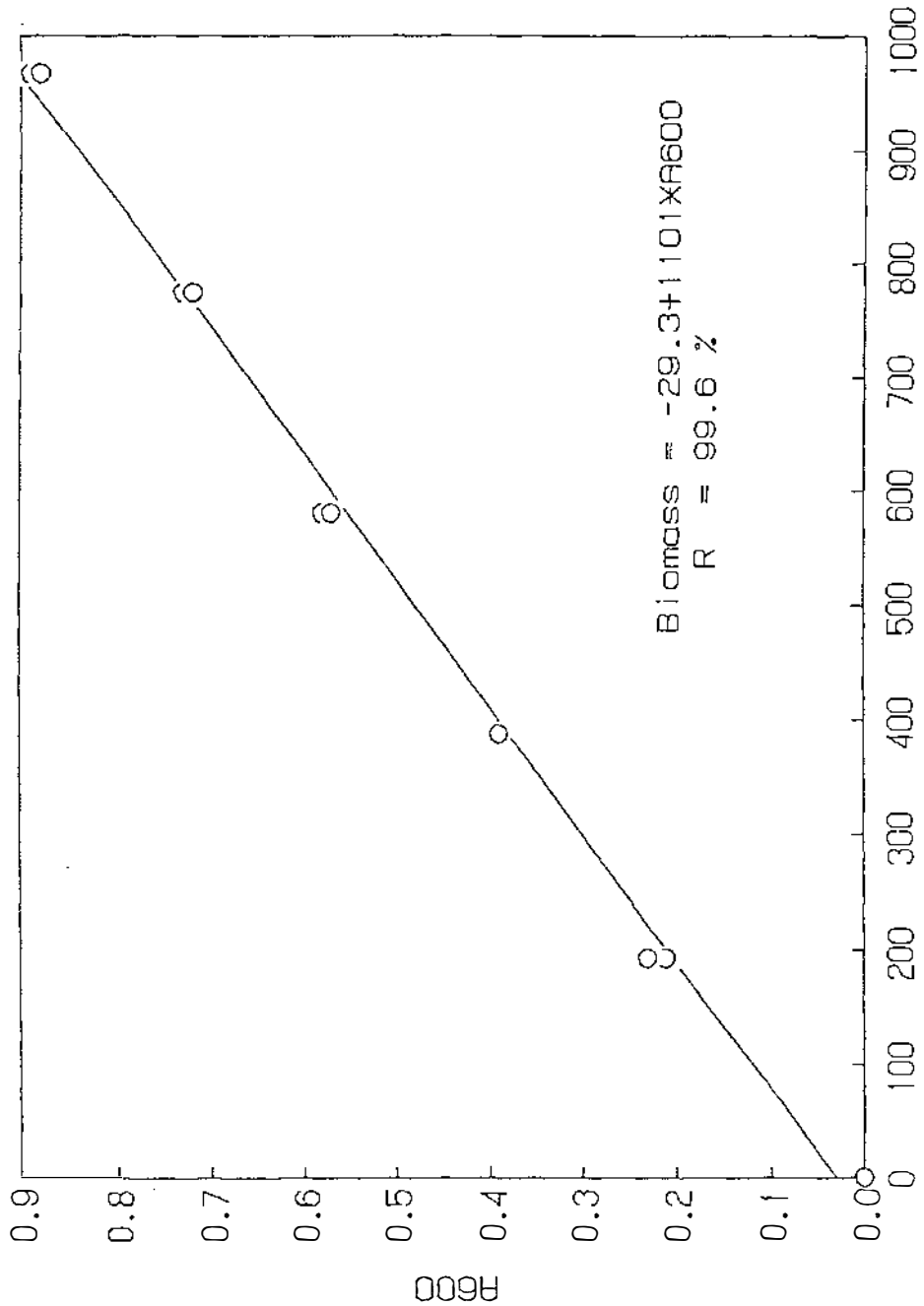


Figure 7.5: Standard Curve for the Determination of MLSS from A_{600}.

lasted approximately 5 minutes.

(3) After the experiment was completed, a sample of MLSS was taken to determine the biomass, and the bioreactor contents were then used for the determination of SVI and/ or settling rate.

Samples for TOC determination (Section 3.7) were taken at steady state and stored in the freeze dried state (with desiccant) prior to analysis. Pooled samples (approximately 500 ml per day) were taken for toxicity tests, and for tertiary treatment experiments. These samples were stored at 4 °C until required. Sludge samples for further analysis were stored at -18 °C until analysis could be performed.

In later experiments, a subsurface drawoff, similar to that described by Cloonan (1984), for mixed liquor from the bioreactor was used, to reduce problems encountered with the biomass concentration in the effluent line. In the last AS experiment, antifoam (Dow Corning Antifoam AF emulsion, Food Grade, 1.5 mg/ml) was added at 4 ml/h (using a Cole Palmer 754-01, 1 rpm pump, fitted with a 7013 Masterflex head) to reduce foam concentration of the biomass.

The full experimental apparatus is shown in schematic in Figure 7.6. A photograph is shown in Figure 7.7.

The first AS plant was started by taking 2 l of effluent from the parent bioreactor and allowing the biomass to settle for 1 h. The top 1 l was then used to fill the clarifier, and the sediment was placed in a perspex bioreactor. The feed was then started at the desired rate with the apparatus set up as in Figure 7.6. Subsequent runs were performed by altering the conditions on this bioreactor. During the transition from Run 1 to Run 2, a novel approach was taken. Rather than increase the flowrate in one step, and allow the biomass to build up, subjecting the AS plant to a major shock, the wastage from the plant was halted 36 h prior to the flowrate change. This allowed the biomass to build up prior to the change in loading rate. When the flowrate was changed, there was no detectible change in substrate concentration, indicating the preemptive biomass buildup was suitable for preparing for changes in conditions. The bioreactor body was changed every 400 - 500 hours to help minimise wall growth.

Microscopic examination of the sludge was carried out frequently during each run. Gram stains (Appendix 3) were carried out, along with direct observation using methods of Eikelboom and van Buijsen (1981).

(2) Determination of Sludge Quality.

For the determination of sludge quality, three analyses were carried out: TOC, determination of intracellular organics and the determination of the quantity of inorganics present. TOC as analyzed as before (Section 3.7). Other analyses are listed below.

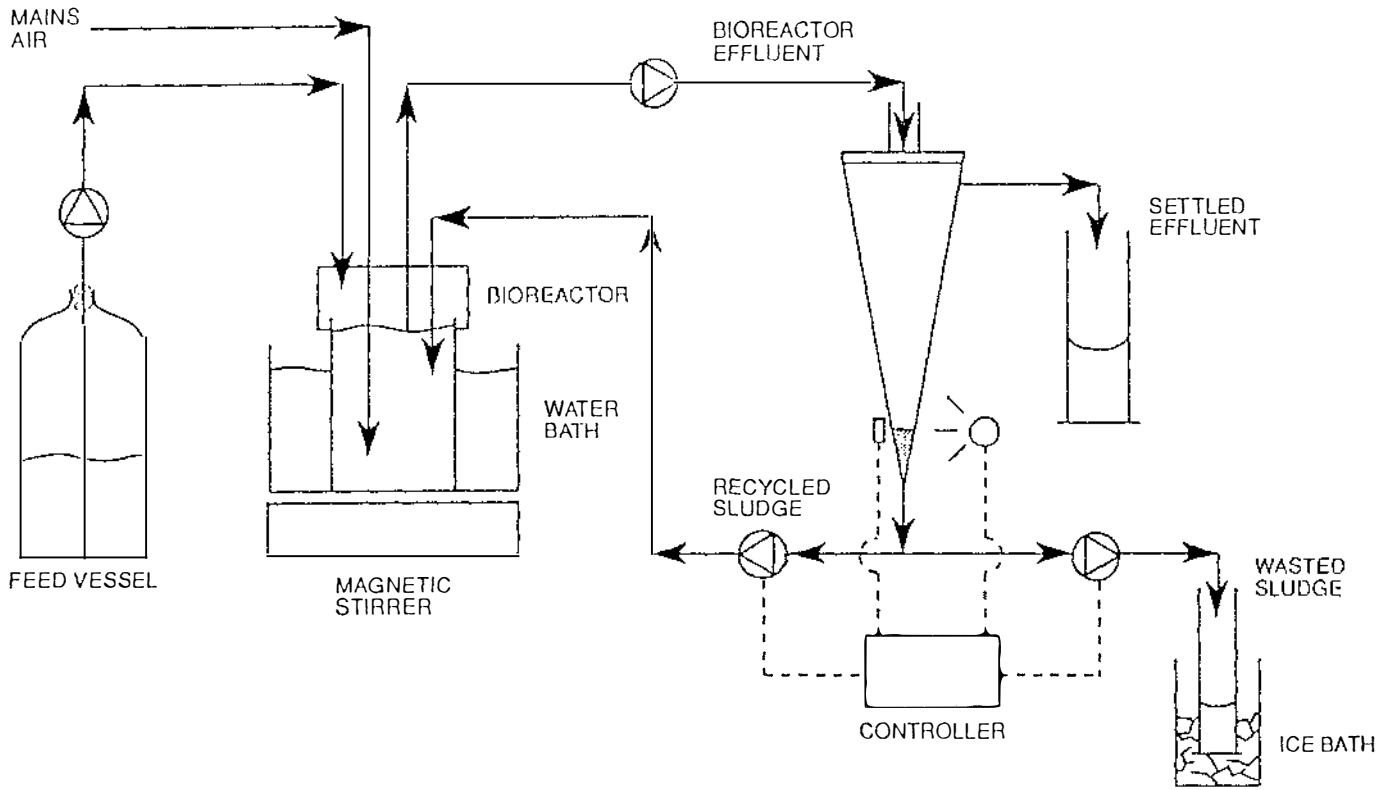


Figure 7.6: Schematic Diagram of the Laboratory Activated Sludge Plant.

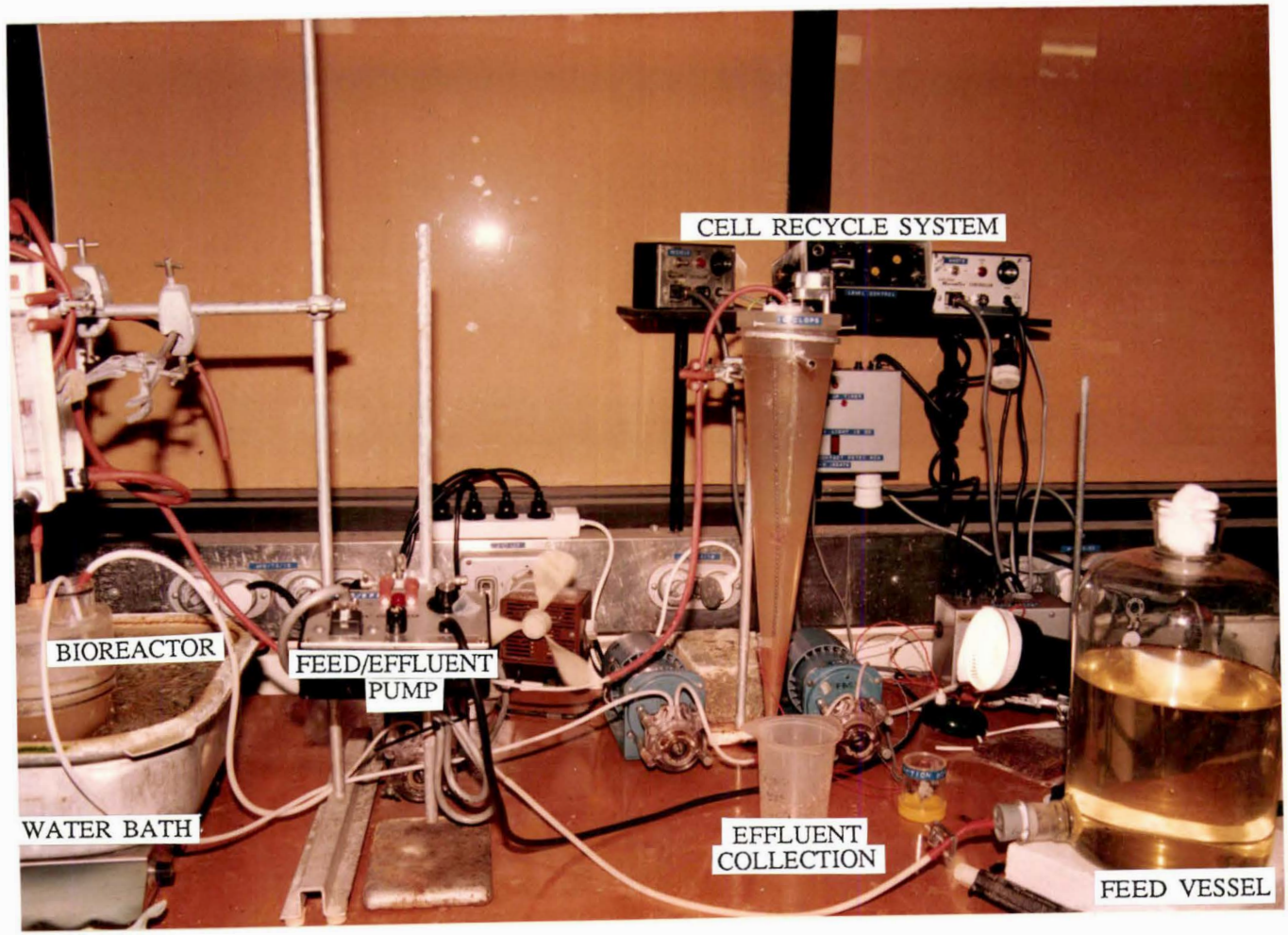


Figure 7.7: Photograph of the Laboratory Activated Sludge Plant.

Intracellular Organics.

A 50 ml sample of the sludge was homogenised (Braun 853 034 homogeniser, Selby Wilton, Wellington, N.Z.) for 30 sec. The sludge was then centrifuged (27,000 xg, 10 min) to remove the cell debris and homogeniser beads. The supernatant was quantitatively transferred to a freeze drying flask and freeze dried. The solids were then extracted with 5 ml of methanol and the final volume made up to 20 ml with MilliQ water. This sample was analyzed by HPLC (Section 3.2) for PCOC and phenoxies.

Metal Analysis.

A 50 ml sample was evaporated to dryness over a steam bath. Concentrated HNO_3 (20 ml) was added, the mixture evaporated to dryness and cooked until fuming. The residue was made up to 50 ml with 1 M HCl and submitted for ICP-AES analysis.

(3) Tertiary Treatment.

Determination of Adsorption Isotherm.

Various weights of Calgon F400 activated carbon (DowElanco, New Plymouth, New Zealand) were dispensed into 250 ml Erlenmeyer flasks, along with 100 ml of the test solution. This was then agitated at 150-200 rpm in an orbital water bath (25 °C). After 24 h, aliquots were taken, centrifuged (Wifug, 3000 xg, 2 min) and filtered through 0.45 µm filter (Whatman). The absorbance of the solution was then measured at 284 nm in a Philips PU8625 UV/VIS spectrophotometer (Philips N.Z. Ltd, Auckland, N.Z.). A sample of MilliQ water (A_{284} = 0) was also incubated at 25 °C with a replicate of the largest amount of carbon added during the experiment. The solute concentration was considered the be the difference between the measured A_{284} of the sample and the A_{284} contributed by the amount of carbon added to the flask. In the case of the effluent from the AS plant, the sample was not prefiltered to remove residual biomass, as in a real situation it would be desirable to avoid this practice. However, if particulates interfered with GAC column performance, then normal practice is to incorporate a sand filter prior to the AC system.

Preparation of Samples for Toxicity Testing.

Samples of 10% and 15 % leachate that had been treated by AS were then treated with the appropriate amount of AC to reduce the residual A_{284} to ≈ 0.05 AU (near the limits of accuracy of the spectrophotometer). The same method was used to treat undiluted leachate to the same level. A total of 2 l of each sample were placed in 5 l Erlenmeyer flasks and shaken at 200 rpm (25 °C) for 24 h. The carbon was then removed by filtering firstly through Whatman no 1 filter paper, and secondly through a 0.45 µm filter. The samples were then stored at 4 °C prior to submission for toxicity testing. Two assays were performed, an acute test using D. magna at 25 °C, and an acute test using alga (Selenastrum capricornutum) at 24 °C in standard dilution water without EDTA. Ten cladocerans were used for each assay. The TOC of each sample was determined by the method used previously (Section 3.7).

After activated carbon treatment of the leachate, the pH was found to be 9.8. This was adjusted to 7.0 prior to testing.

7.2.3 Results.

(1) The Operation of a Laboratory AS Plant.

Activated sludge plants were run at three different sets of conditions, with a total operating time of 3717 h. The results of these experiments can be found in the file APPEND15.WKS in Appendix 15. Table 7.2 contains a summary of the important information collected during these experiments. Plots of PCOC/phenoxy versus time for each experiment can be found in Figures 7.8 (a), (b) and (c). Alcohol concentrations were not included on these graphs, as they were not measured on all samples, only those at steady state at the end of each run. In all cases, residual alcohol concentrations were below the limits of detection.

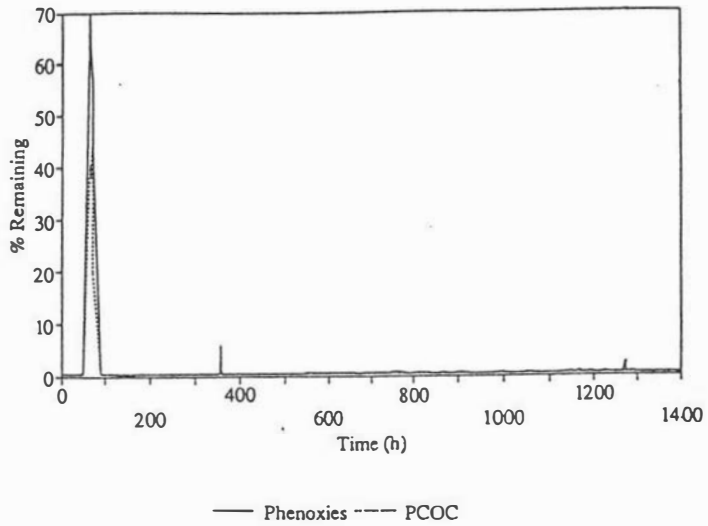
The prominent peak in Figure 7.8 (a) was caused by an air failure. A greater number of problems were encountered in run 3 than in other runs (Figure 7.8 (c)), with a number of incidents occurring. However, for the majority of the time the AS plants were running, total removal of the substrates was observed.

Plots of θ_c versus time for each run can be found in Figures 7.9 (a),(b) and (c) where it can be seen that the SRT was relatively well controlled by the clarifier system.

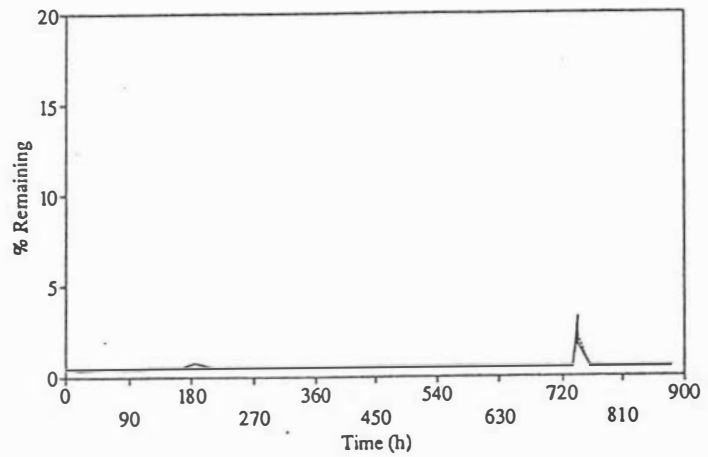
Adding the Q and θ_c data for these runs to the data obtained in the previous chapter, yields the plot shown in Figure 7.10 (R^2 = 96.7 %). It can be seen that the AS plant data were consistent with the data determined by varying SRT alone. The extra data altered the measured coefficients slightly to k_d = 0.014 h^{-1} (SD = 0.005) and Y = 0.65 mg/mg (SD = 0.04). These new values were within 1 standard deviation of those determined in Section 6.3.2.

Microscopic examination of a wet mount of the sludge revealed the presence of the rotifer Philodina in all preparations. These organisms were present in large numbers, along with large spherical objects, which appeared to be rotifer eggs. The presence of a large number of flocs was also noted. The Gram stain of the cultures revealed the presence of tight flocs, held together by an amorphous matrix. The culture was found to be gram-negative, with mainly short rods present in the flocs. There were very few free living organisms. A study on the population present in the AS plant (Run 2) showed that the organisms present in the AS plant were the same as those present in the parent bioreactor (Appendix 16).

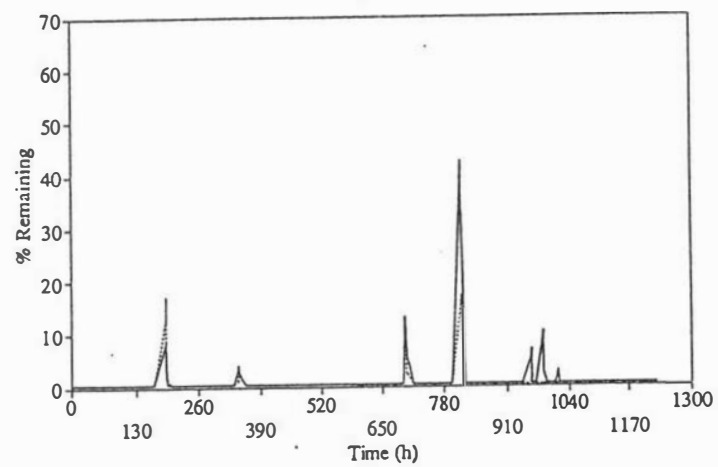
The average oxygen uptake rate measured for the activated sludge plants was 1.65×10^{-3} mg/mg.min. This value was considerably lower than the value observed during the measurement of the performance of the clarifier (Section 7.3.1). The average dissolved oxygen was found to be 5.5 mg/l.



(a) Run 1

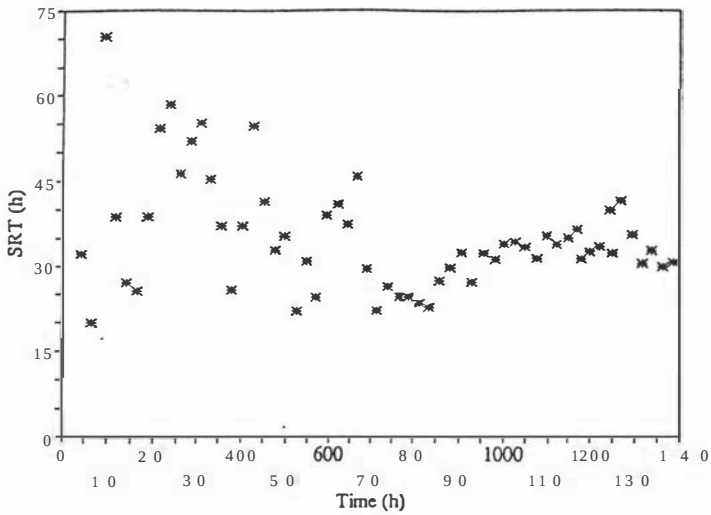


(b) Run 2.

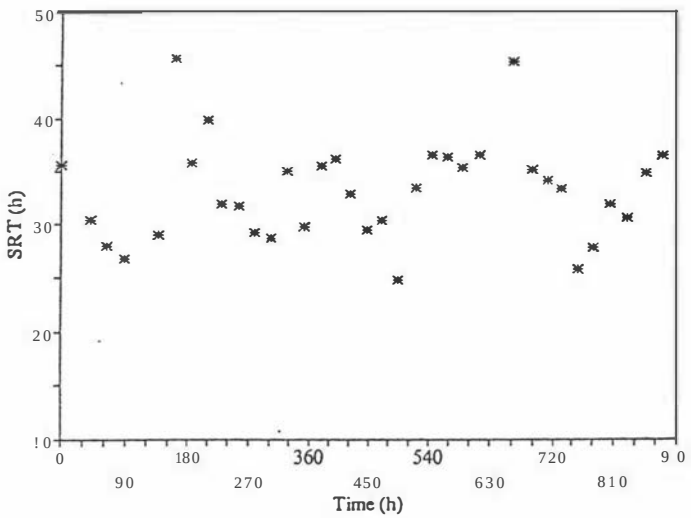


(c) Run 3.

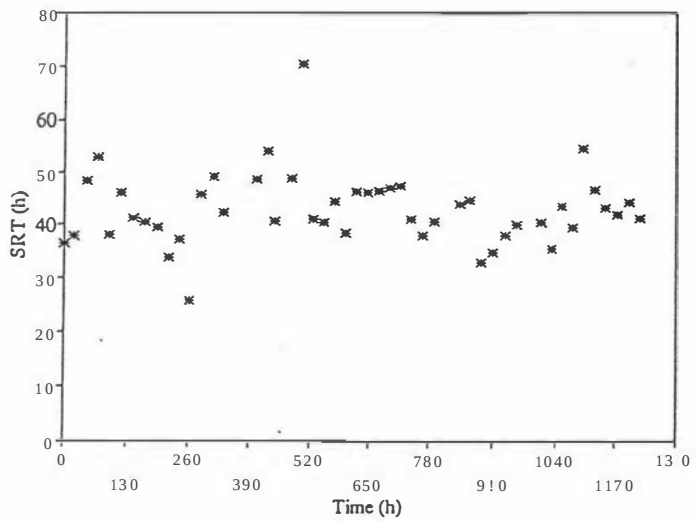
Figure 7.8: Plots of Residual PCOC and Phenoxies vs Time for the Three Activated Sludge Runs.



(a) Run 1.



(b) Run 2.



(c) Run 3.

Figure 7.9: Plots of θ_c vs Time for the Three Activated Sludge Runs.

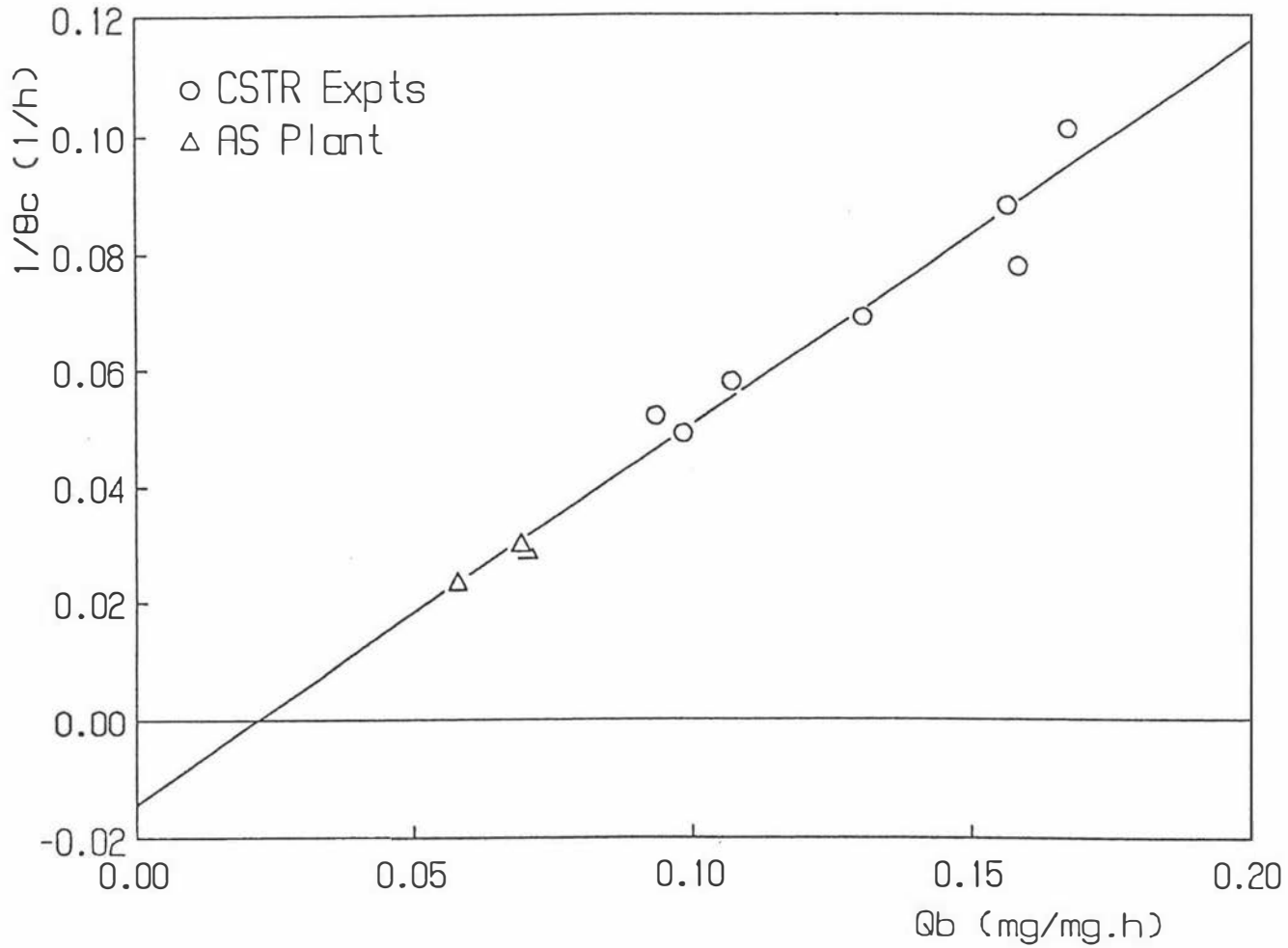
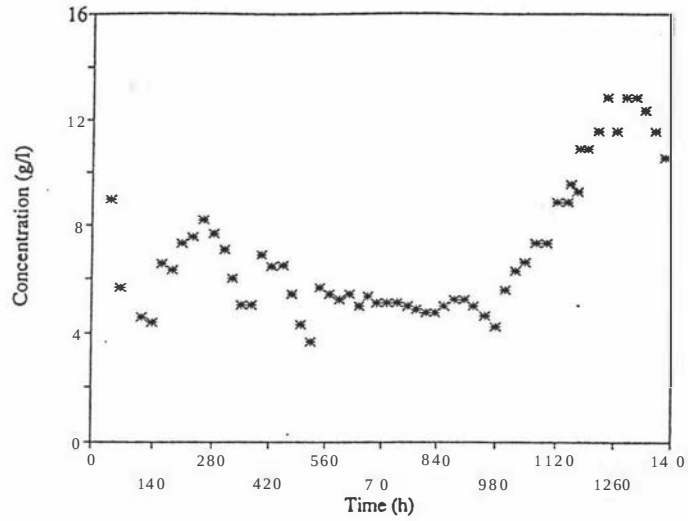
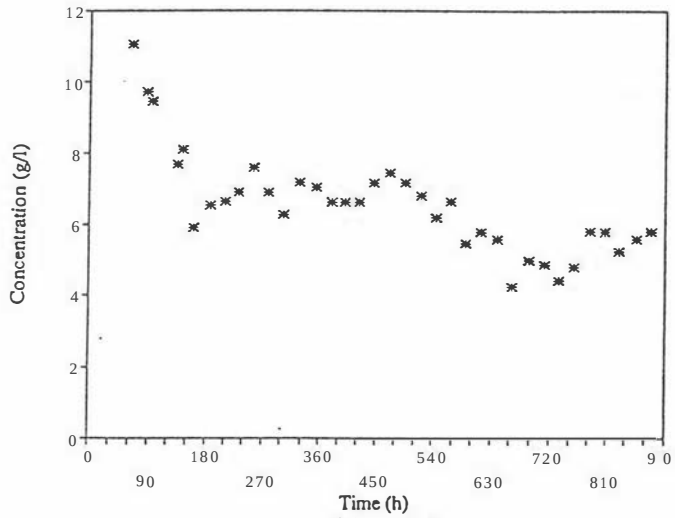


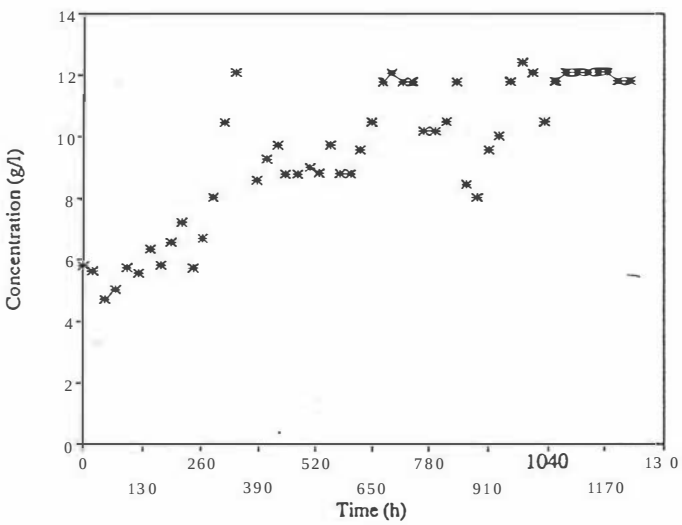
Figure 7.10: Plot of $1/\theta_c$ vs Q Using Both AS Runs and Data from Chapter 6.



(a) Run 1.



(b) Run 2.



(c) Run 3.

Figure 7.11: Plot of Recycle Concentration vs Time for the Three Activated Sludge Runs.

Table 7.2 Summary of Principal Results from Activated Sludge Experiments.

Parameter	Run 1 Value	SD	Run 2 Value	SD	Run 3 Value	SD
Reactor Volume (ml)	860		860		800	
Flowrate (ml/h)	118.6		177.0		115.6	
HRT (h)	7.25	0.14	4.9	0.04	6.92	0.06
SRT(h)	33.7	3	34.9	4	43.6	2.2
MLSS (mg/l)	1158	80	1701	76	2162	44
PCOC (% remaining)	0.5		0.5		0.3	
Phenoxy (% remaining)	0.5		0.6		0.5	
Alcohol (% remaining)	0		0		0	
Q (mg/mg.h)	0.0691		0.0702		0.0578	
TOC (mg/l)	56	3	46	2	87	4
SVI (ml/g)	81		187		104	
pH drop	0.26		0.24		0.54	
O_2 uptake rate (mg/mg.min x 10^3)	1.40	0.15	2.05	0.06	1.49	
Dissolved Oxygen (mg/l)	4.5	0.5	6.6	0.4	5.3	
Sludge Wastage Rate (mg/h)	30.1	3.1	43.0	5.9	39.9	1.3
TDS (g/l)	1.9		1.7		2.5	
Ash (g/l)	1.3		1.2		1.8	
X_R (g/l)	11.4		5.5		11.9	
X_E (mg/l)	24		68		33	
Recycle ratio	5.0 %		8.1 %		10.4 %	
Clarifier residence time (h)	2.4		1.25		1.66	

Figures 7.11 (a),(b) and (c) demonstrate the variation of X_R with time during the experiments. It can be seen that all experiments showed some variation of this parameter. It was noted that after an increase in substrate concentration, X_R fell. This was corrected by the addition of a small quantity of biomass from the parent bioreactor.

During Run 3 it was noted that the wastage rate was decreasing spontaneously. Comparison of the MLSS in the mixed liquor and in the feed to the clarifier indicated that the draw off system was not taking a representative sample from the mixed liquor, but taking disproportionately more liquid than suspended solid. To overcome this problem, a subsurface draw-off (Section 7.3.2) was used. This corrected the problem. It was also noted during this run that a significant proportion of the biomass was being concentrated in the foam. To overcome this, antifoam was used in this run alone.

The results of toxicity tests, using D. magna and the alga Selenastrum capricornutum are presented in Table 7.3.

Table 7.3 Toxicity Test Data for Activated Sludge Plant Effluents.

Test	Run 2 Feed (%)	Run 2 Effluent (%)	Run 2 Toxicity Red^n (%)	Run 3 Feed (%)	Run 3 Effluent (%)	Run 3 Toxicity Red^n (%)
D. magna *EC_{50} 24 h,25 °C	2.4	9.7	76	1.6	3.2	50
Alga EC_{50} 96 h,24 °C	ND	ND		0.6	4.2	86

*EC_{50} = Equivalent Concentration where 50 % of the test organisms die within the period of the test.

There was a significant reduction in toxicity achieved by the activated sludge system.
Pooled samples of the sludge and effluent from runs 2 and 3 were retained (as described in Section 7.3.1) for further study.

The loading rates for the three runs were 1.92, 2.86 and 3.02 kg(substrate)/m^3.d. The theoretical oxygen demand, assuming all carbon was oxidised completely to CO_2 was calculate to be 656 mg/l for 10 % leachate. The ratio of TOD to feed concentration was therefore 1.13. The loading ranged from 2.2 - 3.4 kg(TOD)/m^3.d, which compares well with the range for CSTR systems as described by Tchobanoglous (1979) of 1.2 - 3.0 kg(BOD_u)/m^3.d for domestic wastes.

(2) Determination of Sludge Quality.

The results are summarised in Table 7.4. Also included in Table 7.4 is the approximate elementary composition of bacterial cells. It can be seen that the composition of the sludge was very similar to that given by Greenfield (1987). With respect to trace metals, only two, zinc and manganese were present in concentrations greater than 1 mg/kg(dry wt) (part per million).

HPLC analysis showed the concentrations of the target compounds were below the limits of detection, based on the initial sample size.

(3) Tertiary Treatment.

Determination of Adsorption Isotherm.

The results of a plot of C/(X/M) versus C for leachate and AS treated effluent can be found in Figures 7.12 and 7.13 respectively. This plot determined the parameters for fitting the Langmuir isotherm, the parameters of which are given in Table 7.5.The data were also fitted to the Freundlich isotherm, but the data for leachate did not fit as well (R^2 = 89.6 %), and the fit on effluent was comparable (R^2 = 97.3 %). The Langmuir isotherm was used throughout for consistency.

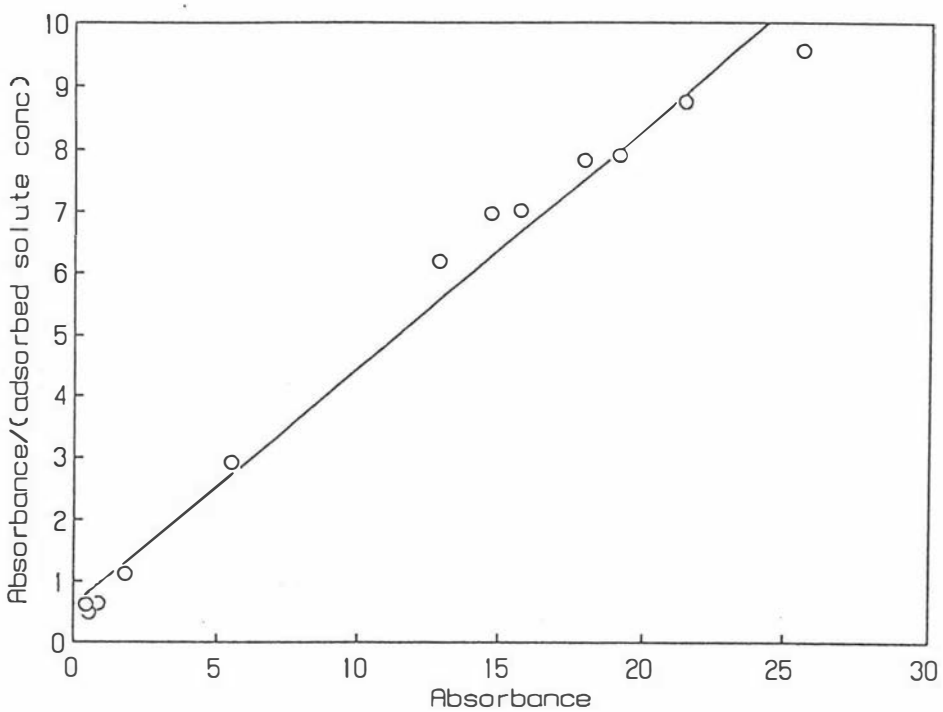


Figure 7.12: Plot to Determine the Langmuir Isotherm Parameters for the Treatment of Leachate with Activated Carbon.

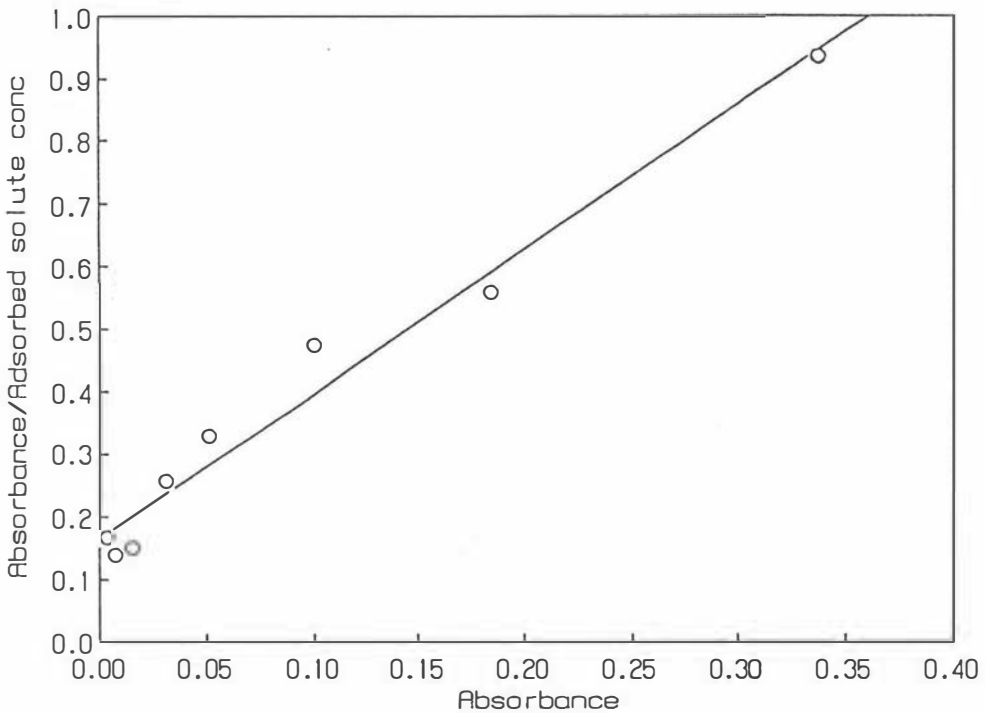


Figure 7.13: Plot to Determine the Langmuir Isotherm Parameters for the Treatment of Effluent with Activated Carbon.

Table 7.4 Summary of the Results of Sludge Analysis.

Analysis	Concentration	Units (dry wt)	Typical Sludge Values (Greenfield, 1987)	Limit for Land appl[@]
Macronutrients				
TOC	40.1	%	50 %	
Phosphorus	3.1	%	3 %	
Sulphur	1.2	%	1 %	
Potassium	1.6	%	1 %	
Calcium	0.6	%	0.5 %	
Magnesium	0.3	%	0.5 %	
Iron	0.4	%	0.2 %	
Sodium	4.9	%	-	
All others	0.4	%	0.8 %	
Trace Elements				
Mn	1.5	mg/kg		
Cu	0.05	mg/kg		500
Al	0.2	mg/kg		
Co	0.01	mg/kg		100
Cr	0.01	mg/kg		500
Mo	0.001	mg/kg		
Ni	0.01	mg/kg		50
Pb	ND			500
Si	0.15	mg/kg		
Sn	ND			
Sr	0.06	mg/kg		
Zn	1.3	mg/kg		2000
Organics				
PCOC	< 16	mg/kg		
MCPA	< 16	mg/kg		
2,4-D	< 16	mg/kg		
2,4,5-T	< 16	mg/kg		

[@] Lowest of Limits for Western Europe as described in Kirk, 1987

ND None Detected

Based on these isotherm data it was determined that the amounts of carbon required to reduce the A_{284} of leachate, AS treated 15 % and 10 % leachate to a nominal value of 0.05 AU were 421 g/l, 2.13 g/l and 1.5 g/l respectively. These quantities were used for the treatment of pooled samples for toxicity testing.

Table 7.5 Langmuir Isotherm Parameters for AC Treatment of Leachate and AS Effluent.

Neat Leachate

intercept = 0.605 therefore maximum capacity (b) = 1/0.605 = 1.65 (AU/g)

Slope = 0.386 therefore constant (a) = 1/(0.386x1.65) = 1.57 (1/AU)

R^2 = 98.4 %

AS Effluent

intercept = 0.155 therefore maximum capacity (b) = 1/0.155 = 6.45 (AU/g)

Slope = 2.364 therefore constant (a) = 1/(2.364x6.45) = 0.066 (1/AU)

R^2 = 96.9 %

Where $\frac{X}{M} = \frac{a.b.C}{1 + a.C}$

Toxicity Testing Results.

The results of the toxicity tests carried out by the DSIR are given in Table 7.6 , along with A_{284} and TOC data. It can be seen that the use of activated carbon as a tertiary treatment step produces an effluent that has a relatively low toxicity.

Table 7.6 Summary of Toxicity Test Results.

Sample	A_{284}	TOC (mg/l)	Red^n (%)	EC_{50}* (24h)	Red^n (%)	EC_{50}# (96h)	EC_{50}* (48h)
15 % Feed	6.31	378	-	1.6	-	0.6	ND
15 % + AS	0.441	87	77	3.2	50	4.2	1.5
15% + AS + AC	0.073	22	94.2	>100	> 98.4	ND	48
10 % Feed	4.03	280	-	2.4	-	ND	ND
10 % + AS	0.321	46	84	9.8	76	0.6	ND
10% + AS + AC	0.064	19	93.2	> 100	> 97.6	3.1	44
Leachate	33.6	2800	-	0.24@	-	ND	ND
AC alone	0.053	17	99.4	57	99.6	6.7	20

* Test organism Cladoceran D. magna

Test organism Alga, Selenastratum capricornutum

@ calculated from the values for 10% and 15% leachate

ND Not Determined

- cannot be calculated

Confidence intervals, although calculated, were not given because the statistical nature of the test gives very wide limits when few organisms die, and tight limits when the majority of the organisms die (Lankford et al., 1988).

It can be seen in Table 7.6 that the acute toxicity to D. magna after AC treatment of both 10% and 15 % was excellent, with fewer than 50 % of the test organisms dying in 24 h in undiluted effluent. This represents a major improvement in effluent quality by activated carbon treatment. By comparison, the AC treated leachate was found to be more toxic, despite the lower A_{284}. This residual toxicity was probably due to the presence of a higher concentration of inorganic compounds in this waste stream than others.

7.2.4 Discussion.

(1) The Operation of a Laboratory AS Plant.

Determination of design parameters for this AS system was a mixture of conventional uninhibited AS plant parameters, and the use of the three substrate model. The three substrate model can be used to determine the substrate concentrations at the dilution rate the system is operating ($1/\theta_c$), however it has been shown to be inaccurate in the prediction of biomass, probably due to the inability to account for secondary organisms. Parameters from conventional AS parameter plots, however, can readily determine the biomass at a given θ_c (from the yield and k_d), but were less able to determine the residual substrate concentrations. The logical approach was therefore to use the three substrate model to predict substrate concentrations based on θ_c, and the AS parameters of Y and k_d to predict biomass concentrations from these substrate levels and θ_c.

The value of Y (0.65 mg/mg) could not be compared with literature values, due to the lack of work using similar wastes reported in the literature. The death rate, however, could be compared as this is less sensitive to waste characteristics. Table 7.7 shows literature values for k_d using wastes containing similar compounds. It can be seen that the measured k_d for this system was well within the range reported for similar systems.

In Section 6.3.2, it was assumed that the corrections made to the biomass concentrations were acceptable. The AS plant experiments were at higher SRT's and as such were at extremes of the plot. Statistically, these points are very influential on the measured parameters. These data, however, did not significantly change the measured parameters, indicating that the decision to substitute the errant biomass values for a corrected value was valid. Again, as total alcohol removal was observed, the assumption of Section 5.3.5 was found to be valid.

Run 3 indicated that an AS plant can run easily on 15 % leachate. During this run, leachate was obtained that had 20 % less PCOC/phenoxy/TDS than the previous batches. To overcome this the concentration was increased from 15 to 18.75 % leachate (this will still be designated 15 % leachate for convenience). No changes were observed in the bioreactor.

Table 7.7 Literature Values of k_d for Waste Treatment Systems on a Variety of Substrates.

Substrate	k_d (h^{-1})	Reference
Phenol	0.021	Gaudy and Rozich (1982)
Phenol	0.008	Beltrame et. al. (1980)
Phenol	0.006	Kim et. al. (1981)
Phenol	0.0195	Rozich and Gaudy (1984)
Methanol	0.003	Kim et. al. (1981)
2,4-D + Phenol	0.014	Beltrame et. al. (1982)
PCP + SCOD	0.005	Moos et. al. (1983)
PCP + Glucose+ Cellobiose	0.023	Edgehill and Finn (1983)
AVERAGE	0.012	Standard Deviation = 0.007

The SRT's used in these experiments ranged from 33.7 - 43.6 h. These retention times were short in relation to the values quoted in Table 7.8.

Table 7.8 Literature Values of SRT for the degradation of Similar Compounds.

Substrate	SRT range (h)	Flocs*	Reference
PCP + SCOD	76.8 - 439.2	only at 439 h	Moos et. al. (1983)
PCP + Sugars	148.8	Y	Edghill and Finn (1983)
2,4-DCP + Phenol	42 - 257	Y	Beltrame et. al. (1982)
2,4-DCP + Glucose	20 - 109	Y	Beltrame et. al. (1982)
PCP + Sewage	240 - 480	Y	Melcer and Bedford (1988)

* Flocs = the presence of biomass flocs either stated or implied.

Apart from the work of Beltrame et al. (1982) flocculation occurred only at high SRT's, as stated by Grady and Lim (1980). The work described in this chapter was performed at the lower SRT's of the range in Table 7.8.

From the oxygen uptake rates measured in the AS plants and measured during the study on the clarifier, it was noted that the clarifier values were 3.3 times higher. There were two possible reasons for this difference: the first that the substrate was limiting in the AS experiments, while there was no limitation in the case of the clarifier experiments. The second possible reason is that the specific oxygen uptake rate of the sludge is higher when alcohols are used as the substrate. In either case, it would be necessary to ensure the air supply system of a large scale system can provide sufficient air so that O_2 is not limiting during operation when substrate is not limiting, as may occur when an AS

plant is recovering from a shock load. The D.O. was above the 2 mg/l concentration required for good settling (Surucu and Cetin,1990)

The measured recycle ratios for the three AS plants were considered to be low. A mass balance on the biomass around the clarifier for each run can be found in Table 7.9. These data indicate there was a major difference between the measured recycle ratio and the value expected by mass balance. This may have been partially due to the method of measuring the recycle ratio, which relied on measuring the relative flowrates of the recycle and waste pumps. As the recycle pump was a high speed pump (6-600 rpm) and the wastage pump a low speed (1-100 rpm), the recycle pump continued to pump (due to inertia of the heads) for a longer period after the controller stopped both pumps. As pumping was characterised by frequent (every 2 - 4 min) pumping of small volumes, significantly more sludge could be pumped than occurred when testing the relative flowrates continuously.

The second factor that may have contributed to the difference was the effluent drawoff in the bioreactor, which, as mentioned earlier, was found to select for the liquid at the interface. This resulted in an internal recycle of biomass that was not measured in pump flows. This was noted in run 3 and corrected, but was probably occurring in run 2, based on the difference between the mass balance and measured values. The calculated value for run 2 was high, although this value was the only one in the range for CSTR systems using domestic wastes (25 - 50 %, Tchobanoglous, 1979). It is likely that the first effect caused the differences in runs 1 and 3. As neither of these problems would be expected in a larger scale system, recycle ratios determined by mass balance will be used for the rest of the work.

Table 7.9 Mass Balance Around Clarifier for the Activated Sludge Runs.

Parameter	Run 1	Run 2	Run 3
Biomass (mg/l)	1158	1701	2162
Flowrate (ml/h)	118.6	177.0	115.6
Clarifier loading (mg/h)	137.3 + 137.3α	301.1 + 301.1α	249.9 + 249.9α
Wastage rate (mg/h)	30.1	43.0	39.9
Underflow concentration (mg/l)	11400	5502	11900
Therefore recycle rate (mg/h)	1352α	973.9α	1376α
Expected recycle ratio (%)	8.8	38.4	18.7
Measured recycle ratio (%)	5.0	8.1	10.4

(2) Sludge Quality.

The HPLC analysis of the intracellular PCOC/phenoxy concentrations indicated that the degradation was complete, and that there was no bioaccumulation of these compounds. No analyses were carried out for dioxins. The main reason for this was the fact that no dioxins were detected in the leachate (detection limit ng/kg or parts per trillion, Hannah, 1989), and hence it was considered that

there should be none in the biomass. For large scale work, it would be advisable to attempt quantification.

The macronutrients listed in Table 7.4 were comparable to the data of Greenfield (1987). The only difference was that Greenfield did not give a value for sodium, which was found to be 4.9 % of the total dry weight. This sodium may not have been accumulated in the biomass, but could have been carried over from the treated leachate in which the 12 g/l sludge was suspended. Undiluted leachate contained 40 mg/l of Mn, so a portion was probably accumulated by the biomass. The zinc concentration in the leachate was much lower (6 mg/l), indicating it was probably not the source. However, the metal concentrations measured in this sludge were at least 100 times lower than the limits applied in Western Europe (Kirk,1987).

Overall, the analyses indicate that the sludge produced by this AS plant was suitable for disposal into the environment, as there were no significant quantities of hazardous metals or organics found in the biomass, and hence the sludge can be handled in the same manner as domestic sludge.

(3) Tertiary Treatment.

The Langmuir isotherm used for this study was initially developed based on the mechanism of adsorption of gases to a solid. (Weber,1985). Even though all the assumptions associated with the derivation of the model may not be valid, the fact that this isotherm gives the best description of the adsorption is sufficient justification for its use (Weber,1985).

Kuhn et al. (1989a) determined the EC_{50} and EC_0 values for a 48 h daphnid test using a number of chlorophenols. A plot of EC_0 versus EC_{50} can be found in Figure 7.14. The best fit line showed $EC_0 = EC_{50} \times 0.68 - 0.29$ ($R^2 = 95.5$ %). Using this correlation, the EC_0 for each final effluent can be found in Table 7.10. Kuhn et al. (1989b) showed that for chlorophenols, the NOEC (no observed effect concentration) using a 21 day test was an average of 13 times lower than the EC_{50} in a 24 h test. These values for the effluent are also given in Table 7.10.

Table 7.10 Predictions of EC_0 and NOEC based on Measured Data for AC Treated Effluents.

Sample	EC_{50} (48 h)	EC_0 (48 h)	EC_{50} (24 h)	NOEC (%)
15 % AS + AC	48 %	32 %	> 100 %	> 7.7 %
10 % AS + AC	44 %	29 %	> 100 %	> 7.7 %
AC Alone	20 %	13 %	57 %	4.4 %

Note: % is the percentage of the original solution that has the EC effect.

These results compare well with the data of Neiheisel et al. (1988), who reported a survey of 6 AS plants treating domestic sewage, and found the NOEC's to range from 1 - 30 % (ave 19.2 %, SD = 14.2 %).

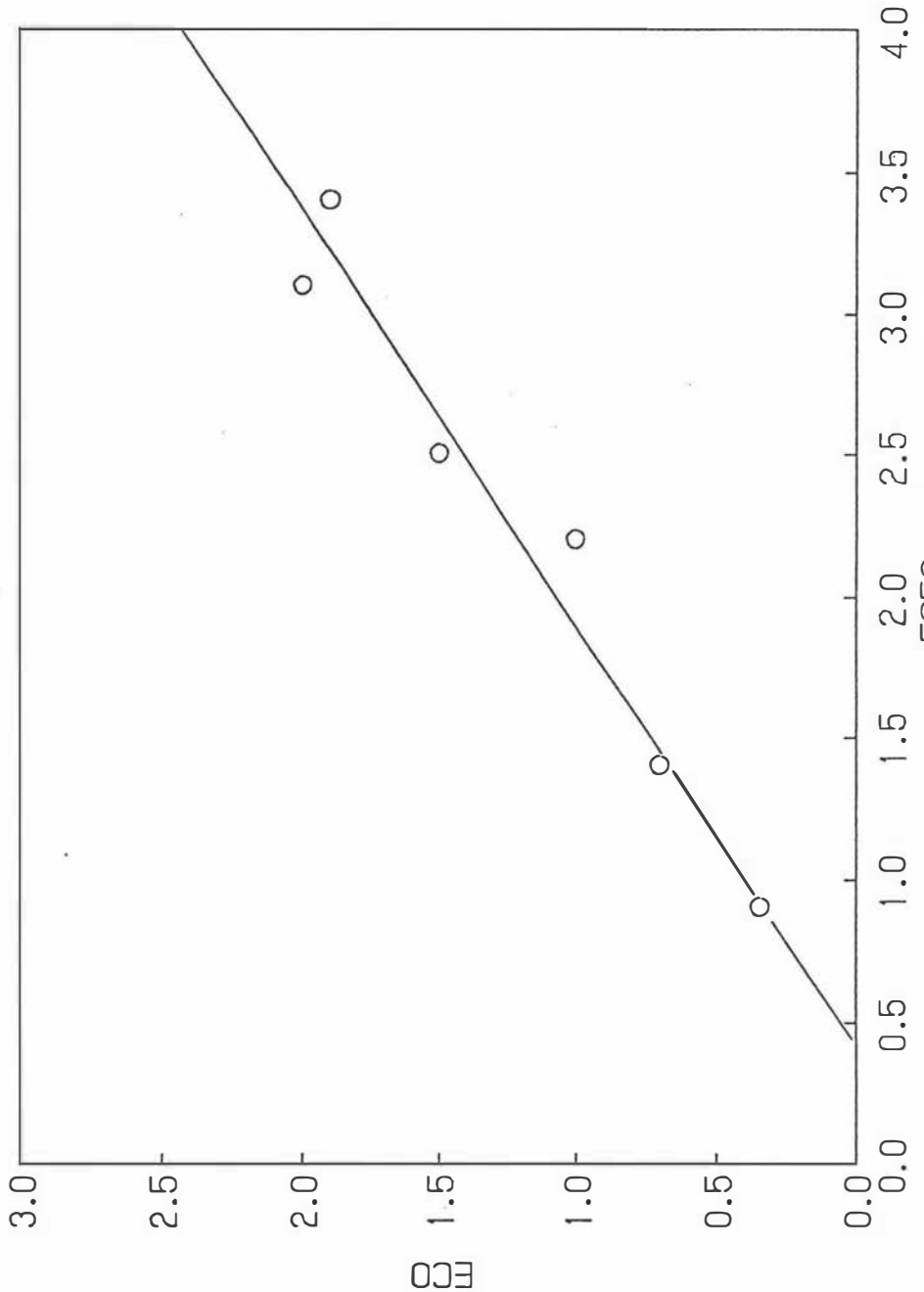


Figure 7.14: Plot of EC_0 vs EC_{50} for the Data of Kuhn (1989a).

It can be seen that diluting the AC treated effluent by a factor of 13 with a receiving water will result in there being no observable effect on the test organisms, providing the residual compounds behave like the chlorophenols. While further tests would be required to determine if the correlation used was valid, these results indicate that the treated leachate was similar in toxicity to effluent from a domestic sewage AS plant. The nature of the toxicity, however, may be different.

No measurement of the ammonium or phosphate content of the treated effluent was made during the course of the project. It is likely that these compounds are present in excess in the effluent, as large quantities were added in the feed. A mass balance for these compounds over run 3 is shown in Table 7.11.

Table 7.11 Mass Balance on Nutrients around Run Three.

Stream	NH_3-NH_4^+	PO_4^{-3}
Nutrients (mg/l)	178	450
At 115.6 ml/h load (mg/h)	20.6	52
Biomass produced (mg/h)	39.9	39.9
Nutrient used* (mg/h)	6.8	3.5
Unused Nutrient (mg/h)	14.4	48.4
Concentration (mg/l)	125	419
Typical AS (Neiheisel et. al., 1988) (mg/l)	4.5	3.5

* Based on biomass composition of Greenfield (1987). Assumes no nitrification/denitrification or other removal

It can be seen that there was probably a vast excess of nitrogen and phosphate in the treated effluent. This would mean that removal would be necessary prior to discharge to a surface water to prevent oxygen depletion and stimulation of algal growth (Tchobanoglous, 1979). To overcome this, it could be possible to reduce the nutrient dosing to a lower level, although this would require further experimentation to determine the minimum requirement. Topp and Hanson (1990) reported the degradation of PCP at NH_4^+ and PO_4^{-3} concentrations of 23 and 12.1 mg/l respectively, indicating large reductions could be made. In a full scale system, adequate (but not grossly excessive) nutrients would be added without the necessary buffer capacity. It would then be necessary to practice pH control to maintain suitable operating conditions. Tertiary treatment using land treatment is also possible (Anon, 1990), however such a system involves the removal of nutrients that need never have been initially added to the system.

As mentioned earlier in this discussion, the best approach for modelling the degradation of leachate was a combination of the three substrate model and conventional activated sludge parameters. By using this to replace the Haldane model in the critical point method, a model could be developed that would predict the critical points for this leachate system.

7.3 The Three Substrate Model in the Critical Point Method.

7.3.1 Development of Computer Program.

At this point in the thesis it should be pointed out that there has been recent criticism of the approach taken by Rozich and Gaudy (1982). This is due to a fundamental assumption made by these authors that the recycle ratio and recycled solids concentration were independent variables. This was valid for the system they were considering, with a holding tank that allowed sludge to be recycled back at a constant concentration. However this assumption is not valid for an activated sludge system containing an normal settler, where the two parameters are linked by a mass balance around the clarifier.

The net result of this assumption is to increase the emphasis on Θ as the primary control variable, and not Θ_c as is more usual. Therefore in this thesis the critical points will be expressed in terms of Θ_c, along the lines of Bertucco et at. (1990).

Critical points can also be expressed in terms of other operating parameters such as Θ and biomass concentration or X_R and $\propto$. The critical point is related to Θ and X by the following equation:

$$X = \frac{Y(S_o - S).\Theta_c}{(1 + k_d.\Theta_c).\Theta} \qquad (7\text{-}1)$$

A similar expression was developed by mass balance over Figure 2.16 that allows the critical point to be expressed in terms of $\propto$ and X_R at fixed substrate and Θ values;

$$\frac{1}{\Theta_c} = \frac{1}{\Theta}.\left(1 + \propto - \propto\frac{X_R}{X}\right) \qquad (7\text{-}2)$$

Unlike the model of Rozich and Gaudy (1984), the three substrate model has no point of sudden failure, but rather a gradual buildup of substrate. Therefore it was necessary to define two critical points, the first when a significant amount of residual substrates (phenoxies) starts to appear (the breakthrough critical point) and the second when there is total failure of the plant, defined by the substrate concentrations being very close to the concentrations in the feed. A computer program was thus written which determined the critical points based on the substrate concentration (using the same procedures as the program PURE). The program determines the critical points for leachate concentrations between 5 and 20 % leachate. The program also determines the critical point based on the PCOC data. The program, known as CRIT can be found in Appendix 17. To check the correctness of the program, the critical points were determined for a CSTR using the program PURE, and compared with those given by CRIT. The comparison is made in Table 7.12.

Table 7.12 Comparison of Critical Points Predicted using PURE and CRIT.

Leachate Concentration Washout	20 mg/l oxy	CRIT* Washout	PURE# 20 mg/l oxy
5 %	13.0	4.26	13.3 - 12.5 4.36 - 4.25
10 %	13.2	4.72	13.3 - 12.5 4.54 - 4.76
15 %	13.2	5.46	13.3 - 12.5 5.55 - 5.40
20 %	13.3	5.88	13.3 - 12.5 5.88 - 5.75

* The values given are the residence times predicted with $\alpha = 0$; i.e as a CSTR.
# The values given are residence times ($1/D^*$) where the critical substrate concentration, or washout occurred. As a smaller step was used for CRIT, the values of PURE will bracket the CRIT values.

It can be seen that the two programs do coincide, and hence CRIT was suitable for further use. It should be noted the biomass predicted by the model was based on a yield coefficient determined when there was total degradation in an AS plant and not from the yields of individual substrates. As the growth rate is increased, however, washout of all three substrates does not occur simultaneously. This will lead to a error in the predicted biomass compared with the true biomass. This difference will only affect the predicted total washout point, and not the important breakthrough critical point. Therefore this error was accepted.

7.3.2 Discussion.

A plot of θ_c versus S_0 can be found in Figure 7.15. There are three regions on the graph, above the lines; where all substrate is removed, between the lines, where there is only partial substrate removal, although the plant does not fail, and below the lines, where there is no significant degradation. This is different to the results of Rozich and Gaudy (1984) who found only two regions, total removal, and total failure. The results of using the approach of Rozich and Gaudy (1984) and breakthrough using the three substrate model are given in Figure 7.16.

It can be seen that the approach of Rozich and Gaudy (1984), which takes no account of interactions between the substrates, could not accurately determine the aeration basin volume required. In a number of publications (Rozich and Gaudy, 1984; Gaudy and Rozich, 1982; and Gaudy et al., 1988), these authors have expounded the use of this method for the design of activated sludge plants treating inhibitory wastes. The method, however, only applies accurately to activated sludge systems treating the pure compounds, a situation that would be extremely rare. The presence of other, more easily degraded compounds, will effectively alter the kinetics, and move the observed critical points (as was seen in Chapter 6).

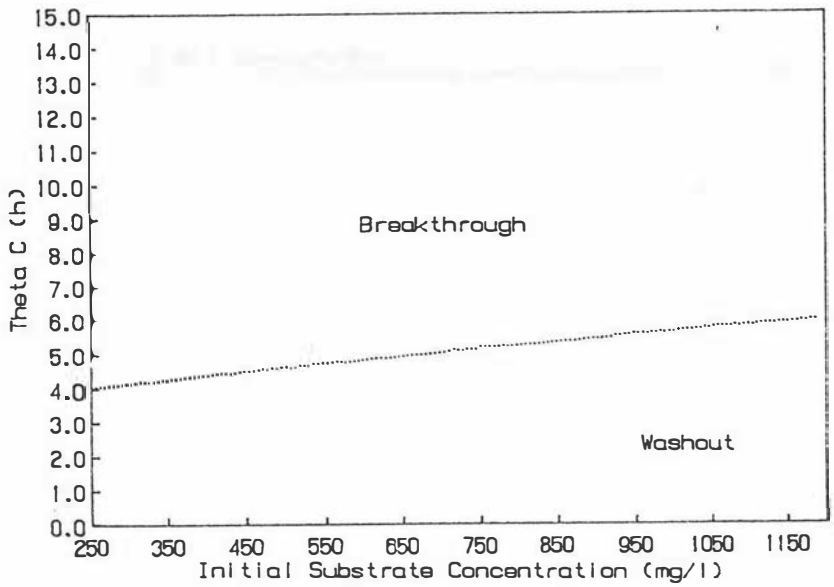


Figure 7.15: Plot of Critical Points vs S_0 for Run 1 based on the 3 Substrate Model.

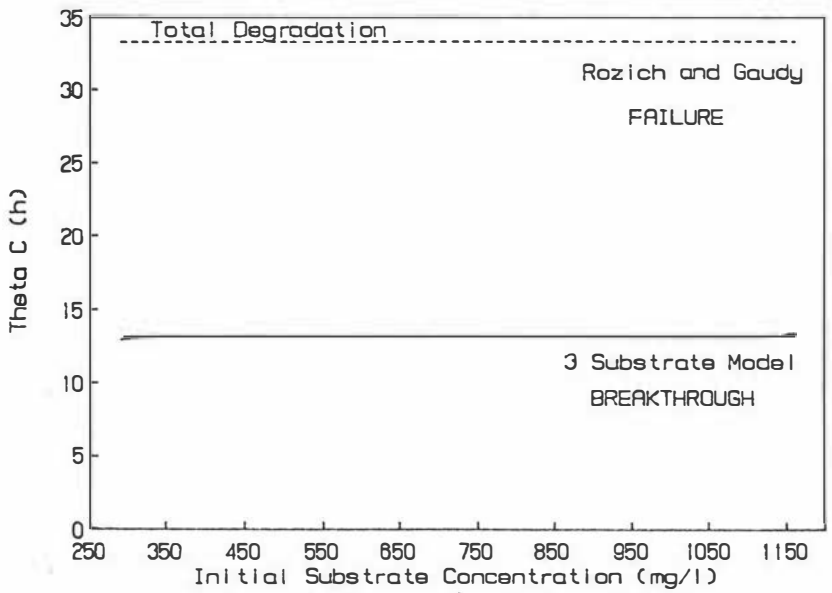


Figure 7.16: Plot of Critical Points Based on Rozich and Gaudy (1984) and Breakthrough (3 Substrate Model) for Run 1.

Gaudy and Rozich (1982) state that there are two schools of though on the biological treatment of toxic wastes, where one group believe the characteristics of the waste are controlled entirely by the toxic compound, and plants should be designed with respect to the toxic compound only, and the second group believing non-inhibitory models should be used. Results presented in this thesis indicate that both groups are partially correct. A plot of total substrate versus SRT can be found in Figure 7.17 for the method of Gaudy and Rozich, Monod kinetics and the three substrate model. The presence of other substrates enhances the degradation of the toxic compounds at most dilution rates, increasing the range where non-inhibitory kinetics can be used. In this case uninhibited AS plant kinetics were determined (Chapter 6) at dilution rates where the pure compound data indicated that there should be no degradation. However at higher dilution rates, the inhibitory characteristics of PCOC were controlling, with rapid failure predicted to occur at high dilution rates (Table 7.12). Therefore it is necessary to consider interactions between the substrates to prevent overdesign of an AS plant. In this particular case, where the leachate composition may suffer considerable variation (Cope,1983), the 3 substrate model should be able to predict changes in the AS plant behaviour, whereas the Monod model can not.

As shown in Table 6.8 there were large differences between the pure substrate and mixture critical points when looking at CSTR systems. This difference (a factor of 3 between washout based on PCOC degradation and that measured by phenoxy breakthrough) would cause a reactor design to be oversized. The same growth rates in a cell recycle system, however, would probably not produce such dramatic differences in reactor volume.

These data indicate the three substrate model can be incorporated easily into the critical point method. The resulting model predicts three operating regions. The effect of varying engineering parameters on the effectiveness of an AS plant can easily be seen. To this end, operating diagrams were constructed for an activated sludge plant. Figure 7.18 shows safe operating biomass concentrations at the design hydraulic retention time and substrate concentration. By entering the figure at the design HRT, a safe operating biomass concentration is any concentration above the operating line for the feed substrate concentration. Biomass concentrations above these lines correspond to SRT's greater than the critical point. Operating points for the three AS plant runs were also plotted on this graph. It can be seen that all three were in the safe operating region.

Figure 7.19 is a more specific operating diagram showing the relationship between alpha and X_R at a variety of initial substrate concentrations and a fixed HRT of 7.25 h (as measured in Run 1). A different nest of curves is required for each different HRT. By plotting design underflow concentration and recycle ratio on this operating diagram it is possible to determine whether the proposed operational regime is in a stable region. If the plotted point is above the operating curve for the feed substrate concentration, then the Θ_c is in the safe region. These safe operating regions were very large. Whether

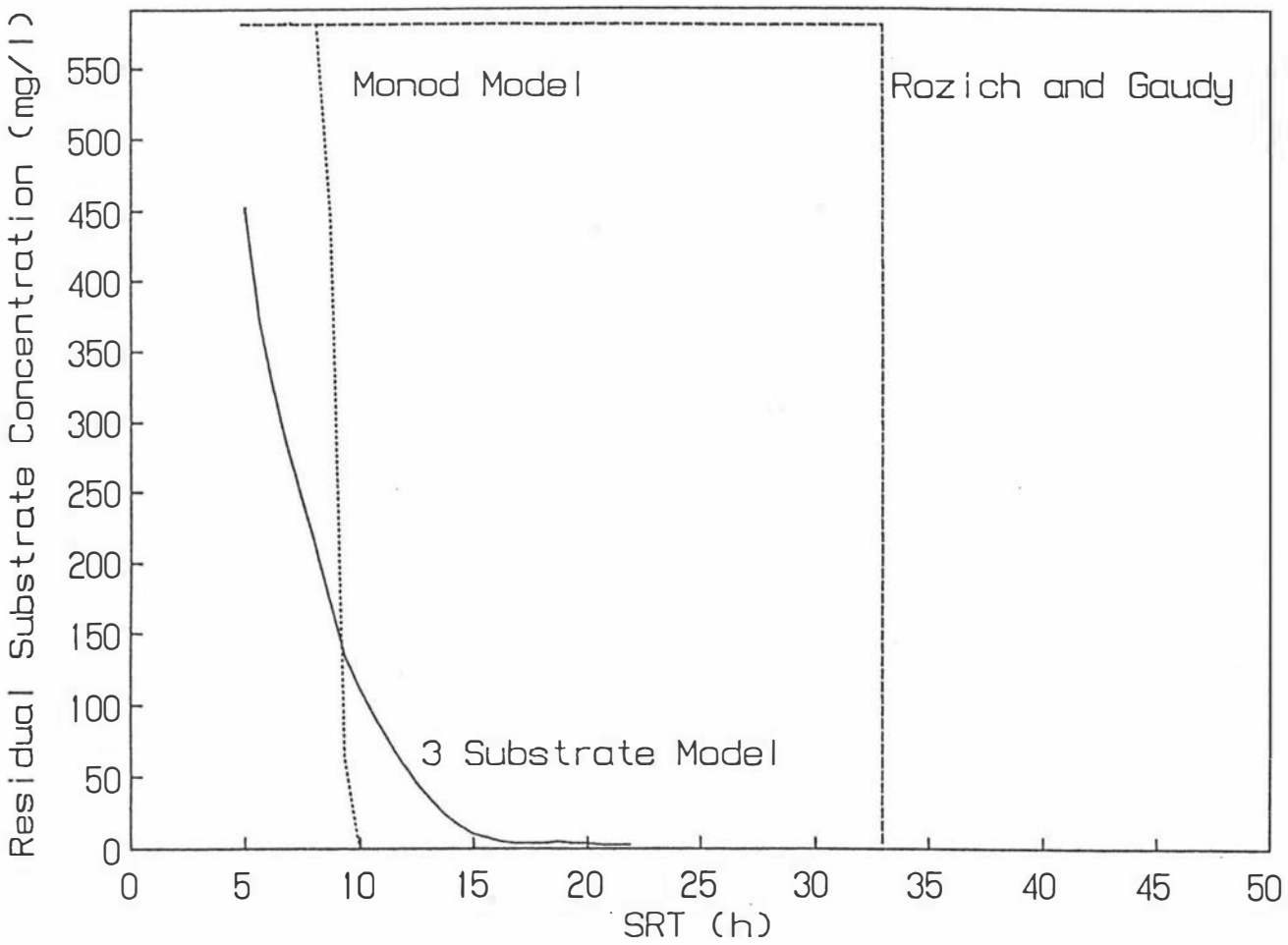


Figure 7.17: Plots of Total Residual Substrate vs SRT for Monod Kinetics, the Method of Rozich and Gaudy and the Three substrate Model.

or not operation close to these critical points can be achieved will depend on how well the culture continues to settle at shorter SRT's. Table 6.1 showed that good settling was achieved at low SRT's in laboratory experiments, although this may not occur in larger systems. If good settling does not occur on the large scale, then the empirical rules will need to be used (Grady and Lim, 1980).

For runs 1 and 2, the operating point was very close to the critical point as predicted by the Rozich and Gaudy method, implying the system would be expected to be potentially unstable. Figures 7.8(a) and (b) however, indicate the system was stable, adding further weight to the idea that the model of Rozich and Gaudy (1984) was not a suitable predictor of AS plant behaviour.

It can be seen from this work that the three substrate model can successfully be used for the design of an AS plant. The design method proposed here is a mixture of the methods used by the two schools of thought involved in the design of biological systems treating toxic chemicals.

7.4 Overview.

Results presented in this chapter indicate that the biological treatment of the landfill leachate can be achieved by an activated sludge plant. The effluent toxicity can be greatly reduced by treating the AS plant overflow with activated carbon.

Such two stage processes have been reported in the literature as being used on small scale systems for the degradation of hazardous compounds; i.e. Skladany (1989) reported the use of a submerged fixed film reactor, followed by AC treatment as a method used to treat up to 1.14 m^3/h of a leachate containing 119 mg/l of isomers of toluic acid.

The AC column step, as well as constantly removing the residual TOC at steady state also has an important part to play when there are variations in effluent quality, as may occur with any biological process. In the case of the system under study in this thesis, such a tertiary treatment system would provide a means of containing disturbances within the system, i.e. no effluent with a high toxicity would be produced, as the AC column would remove the compounds of interest.

Although the kinetics were not measured, adsorption is expected to be rapid. Paprowicz (1990) tested a number of different activated carbons and found the average time constant for the process at 1 mg/l phenol was 6.7 min.

It may also be possible to reduce the amount of carbon required for this final step by encouraging the growth of microorganisms on the carbon granules. Hutchinson and Robinson (1988)

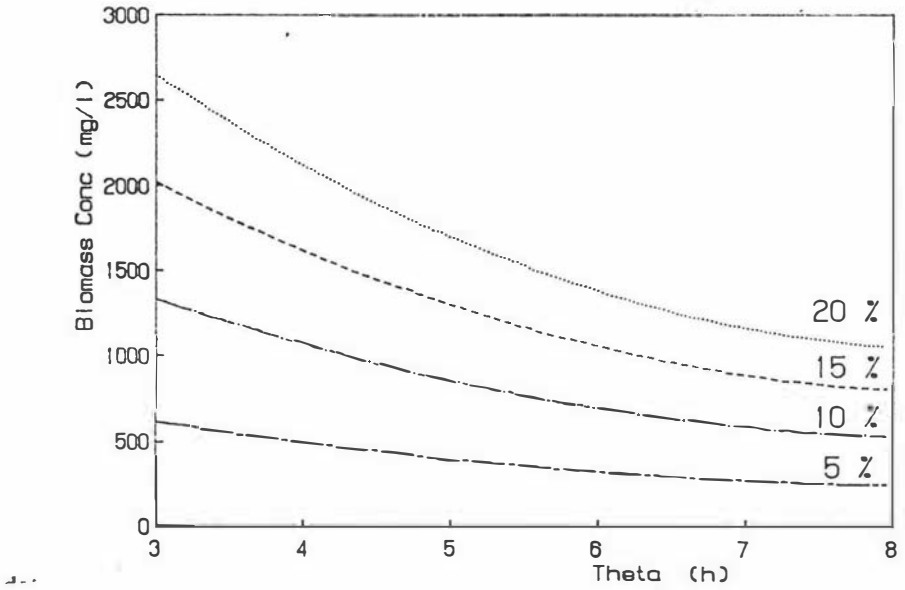


Figure 7.18: Operating Curves for an AS Plant Based on Θ and X.
The series of curves follow lines of leachate concentration. By entering the graph at the design HRT and substrate concentration, the biomass corresponding to the critical detention time for breakthrough can be determined.

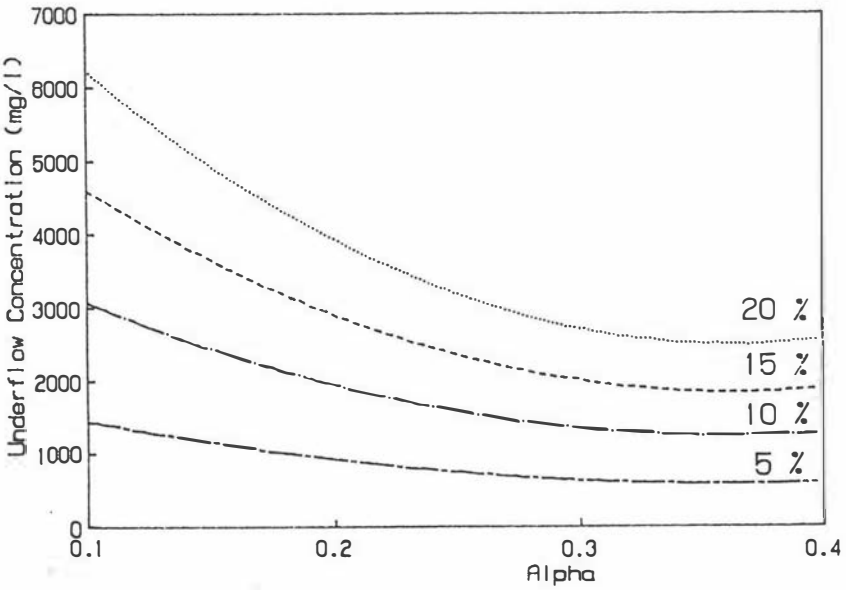


Figure 7.19: Operating Curves for an AS Plant Based on ∝ and X_R.
The series of curves follow lines of constant substrate concentration. By entering the graph at the design underflow concentration and recycle ratio, if the point is above the curve for the feed concentration, then the SRT is greater than the critical value. Valid for HRT = 7.25 h.

showed microbial regeneration of carbon was possible, and more recently Speitel et al. (1989) and Ying et al. (1990) showed the biodegradation of trace concentrations of chlorinated compounds occurred in granular activated carbon columns.

As air stripping was not considered a problem, due to the low substrate concentrations present in the bioreactor (Section 4.6.1.2), the quality of the effluent air was not studied

This two step process, where the biological system removed almost all of the target compounds, and a total of 80 % of the TOC, with the physical adsorption system removing the majority of the remaining TOC, and reducing the toxicity of the effluent. This process used very little carbon (2.13 g/l of 15 % leachate) compared with AC alone (412 g/l leachate). This indicates the process may be significantly cheaper than AC alone. Therefore there is a need to perform an economic analysis to determine the relative cost of three options; (1) AS alone which produces an effluent that will require safe disposal, (2) AS + AC, which produces a good quality effluent by the use of two processes in series, and (3) AC alone, which, as there is no need for nutrients and aeration , may be significantly cheaper than any other option. To perform an analysis, it is necessary to have a preliminary plant design. This preliminary design and economic analysis will be the subject of the next chapter in this thesis.

7.5 Conclusions.

Results presented in this chapter indicate that the AS plants were capable of removing the alcohols, PCOC and phenoxies from both 10 % and 15 % leachate. The loading rates 1.9 - 3.0 kgsubstrate/m^3.d (2.2 - 3.4 kg TOD/m^3.d) was high in comparison to the typical loadings quoted in the literature. The three substrate model, in association with the critical point method predicted three regions of plant operation, total substrate removal, stable operation with residual substrate and no degradation, compared with the two regions of the critical point method.

The sludge produced by the AS plant had low concentrations of residual organics and inorganic ions, indicating it could probably be treated as a non-hazardous waste, and disposed of in the same manner as domestic sludge. While the effluent from the AS plant had a reduced toxicity (average 71 %), the toxicity could be reduced further by the use of activated carbon treatment. This produced an effluent with an EC_{50} (48 h) of 46 %. This residual toxicity was comparable to that of AS treated domestic sewage. The ammonium and phosphate concentrations, however, will probably be too high for discharge to surface water, and hence medium changes will probably be required.

The results presented in this chapter suggest the need for economic analysis to be carried out on the proposed treatment system, and this will be the subject of the next chapter.

CHAPTER 8
DESIGN AND ECONOMIC ANALYSIS OF OPTIONS FOR TREATING LEACHATE.

8.1 Introduction.

The previous chapter showed that biological treatment of the leachate using AS was feasible, and that the use of a polishing AC step produced an effluent with a low residual toxicity. It is therefore necessary to determine whether this two step process has any cost advantage over activated carbon treatment, or AS treatment followed by an alternative effluent disposal method (AS alone).

In order to compare the costs of the three options, it is necessary to design and cost three equal service plants. The results of this will be presented in this chapter, along with data on the sensitivity of costs to such factors as leachate strength and leaching rate.

As there were no data available on the rate of leaching from the dump, or on expected leachate composition, it was necessary to make two assumptions;

(1) The leaching rate will be set at 15.6 kg substrate/day, and will be constant throughout the life of the plant, and that

(2) the leachate concentration will be 5.8 g/l (total alc/PCOC/phenoxies), as it has been for the majority of the project. This too will be assumed to be constant for the life of the plant.

It will not be possible to test these assumptions until the dump is actually leached, however, the use of consistent data for all three options will ensure comparisons between options are valid.

8.2 Design of AS and AC plants.

8.2.1 Flow Sheet Development.

According to Ulrich (1984), the development of a flowsheet, and the accompanying mass balance, is the basic requirement for plant design and economic analysis. At this stage, a number of process choices can be made as to equipment selection and configuration. These choices are based on information presented in Tchobanoglous (1979) and Ulrich (1984). Subsequent redesign may alter the choices made in this section.

A flowsheet for the two step process is shown in Figure 8.1. The equipment is labelled with a letter followed by a three digit number, according to the method of Ulrich (1984). Each flow is also numbered. Some pieces of equipment are numbered twice, with the second number referring to the same piece of equipment if the system were an AC treatment system alone. The AS system consists of all the equipment with a number between 100 and 200, the polishing system between 200 and 300 and the AC alone between 300 and 400.

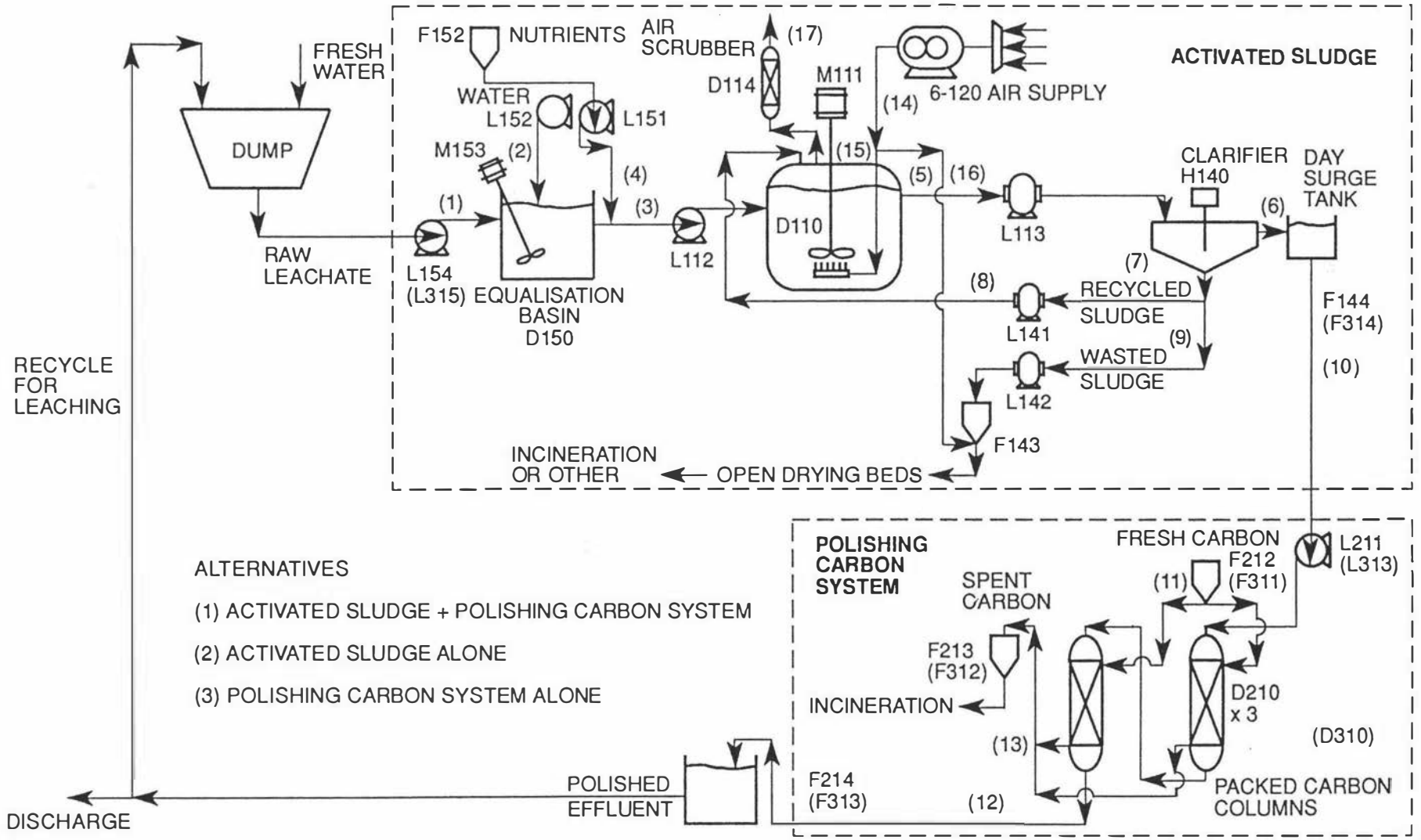


Figure 8.1: Process Flow Diagram for the Proposed Treatment of Leachate.

A number of assumptions were made at this stage. Pumps required to deliver a precise flow (L151, L152) were gear pumps, those handling biomass or likely to contain suspended solids were Mono® pumps (L113, L141, L142 and L315). All others were selected as centrifugal pumps, due to their low cost and robustness at reasonable efficiencies. Carbon columns (D114, D210 and D310) were considered to be vertical process vessels constructed of stainless steel (grade selected to resist corrosion likely on an exposed cliff-top). The air supply (G120) was provided by a package compressor system, to avoid the need to design the ancillary equipment at this early design stage. Mixing in the aeration basin (M111) would be provided by a radial turbine, as frequently used for bioreactors (Ulrich, 1984). Equalisation was considered necessary (Arbuckle and Kennedy,1989) and would occur in a concrete basin (D150) mixed with a propeller turbine (M153) (Tchobanoglous, 1979).

The aeration basin (D110), sludge storage tank (F143), nutrient makeup tank (F152) and surge tanks (F144, F214, F314 and F316) were stainless steel or concrete horizontal process vessels. Carbon storage hoppers (F311, F312, F212, F213) were considered storage bins according to Ulrich (1984). The clarifier (H140) was considered to be a typical clarifier/thickener used in waste treatment.

Air will be supplied to the tank holding the settled sludge to prevent the bulk from becoming anaerobic (creating a potential odour problem) and to maintain live biomass, in case the sludge is required in the plant. Sludge drying beds were chosen as the best method of disposal, as they are cheap, require little land area, and as the sludge appears to be non-hazardous, provides a final product suitable for land disposal or incineration (Tchobanoglous, 1979).

Carbon columns would be placed countercurrent in series. Three columns will be required, with two in operation at any given time. This operating mode will ensure the effluent quality can be maintained.

8.2.2 Mass Balance over Processes.

Mass balances were performed for each option. The calculations were performed using VP-planner, to allow easy updating with changing conditions. The calculations can be found in the spreadsheet APPEND18.WKS (Appendix 18). This spreadsheet also contains information on equipment design and economic analysis. A block diagram of the various sections in the program can be found in Appendix 19. It was assumed there was perfect separation in all solid/liquid separations to simplify the calculations. The results for the assumed conditions can be found in Table 8.1.

These data form the basis of a preliminary design and costing of the three options. It can be seen that the plants will be treating relatively low flowrates (no greater than 1 m^3/h) under the assumed conditions, resulting in a small plant in terms of domestic wastewater treatment plants (Tchobanoglous, 1979).

Table 8.1: Mass Balance Summary for Three Options for Leachate Treatment.

Stream	No	Flowrate (l/h)	Substrate (g/l)	Biomass (mg/l)	Residual (AU)	O_2 (kg/h)	Carbon (kg/h)
AS + AC Plant							
Leachate	1	112	5.8	-	3.4	-	-
Water	2	635	-	-	-	-	-
Plant Feed	3	747	0.87	-	0.51	-	-
Nutrients	4	3.3	-	-	-	-	-
AS Eff	5	939	-	2426	0.51	-	-
Sett Eff	6	729	-	-	0.51	-	-
Underflow	7	209	-	12000	0.51	-	-
Recycle	8	190	-	12000	0.51	-	-
Wastage	9	20.6	-	12000	0.51	-	-
Effluent	10	729	-	-	0.51	-	-
Polish AC	11	-	-	-	-	-	1.82
Pol Eff	12	729	-	-	0.05	-	-
Used AC	13	-	-	-	-	-	1.82
Comp Air	14	171*	-	-	-	4.28	-
Air to AS	15	157*	-	-	-	3.93	-
Sludge Air	16	14*	-	-	-	0.35	-
Scrub Air	17	157*	-	-	-	3.54	-

* units m^3/h

For AS Plant alone

Streams 10,11,12 and 13 are not included in the mass balance.

For AC alone

Streams 2 - 10 and 14 - 17 are not required

Leachate	1	112	5.8	-	3.4	-	-
Polish AC	11	-	-	-	-	-	59.3
Pol Eff	12	112	-	-	0.05	-	-
Used AC	13	-	-	-	-	-	59.3

8.2.3 Plant Design at the Selected Conditions.

Detailed plant design can be found in the VP-planner spreadsheet APPEND18.WKS (Appendix 18). The method of design and sizing of the major pieces of equipment will be described in this section, along with any assumptions made.

Based on the leachate concentration and desired loading rate, the volume, flowrate and HRT can be calculated by simple mass balance. A design SRT (in this case 48 h) was then used to determine the MLSS concentration in the aeration basin based on data presented in Section 7.2.3 (equation 7-1). Choosing a settled solids concentration (X_R) of 12 g/l (approximately that measured in runs 1 and 3) the recycle ratio was calculated using equation (7-2). With these data, it can be confirmed that operation is in a safe region using Figure 7.18. Providing the plotted point fell in the safe region, the design was considered satisfactory.

Design of a clarifier was performed using the method given by Tchobanoglous (1979) for design based on a single batch curve, measured during run 3 (Figure 8.2). The resulting proposed clarifier had loading rates of 2.7 kg/m².h and 22.4 m³/m².d, which compared well with typical data (Tchobanoglous, 1979).

From the data above, and other data presented earlier in the thesis (Chapters 6 and 7), it was possible to calculate nutrient requirements, oxygen requirements and to size a carbon column to scrub effluent air.

All vessels, both process and storage were designed to allow three days uninterrupted operation without the need to provide fresh supplies of nutrients, empty sludge storage tanks, or replace spent carbon columns. To this end, F152, F143, F212, F213, F312, F311 were sized to hold three days requirement (plus an additional safety factor), and columns D310, D210 and D114 were sized to operate for 3, 9 and 9 days respectively, to maintain the residence times required for treatment.

Surge tanks, such as F144 and F214 were sized as day tanks (residence time 8 h) according to Ulrich (1984). Pumps were sized according to the flowrate and a nominal pressure drop of 100 kPa, unless the application indicated a higher pressure drop may be appropriate. Mixers were sized according to the volume to be mixed by the method described in Tchobanoglous (1979). Sludge disposal beds were sized using the lowest loading rate quoted by Tchobanoglous (1979).

Safety factors, ranging from 1.1 to 2.5 were used to ensure the design would be suitable. A summary of the design results can be found in Table 8.2. This table will be discussed further in a subsequent section.

These design calculations were performed in the same spreadsheet as the mass balance calculations, and subsequent economic analysis calculations. This was done to develop a spreadsheet that could perform the design calculations under a variety of conditions.

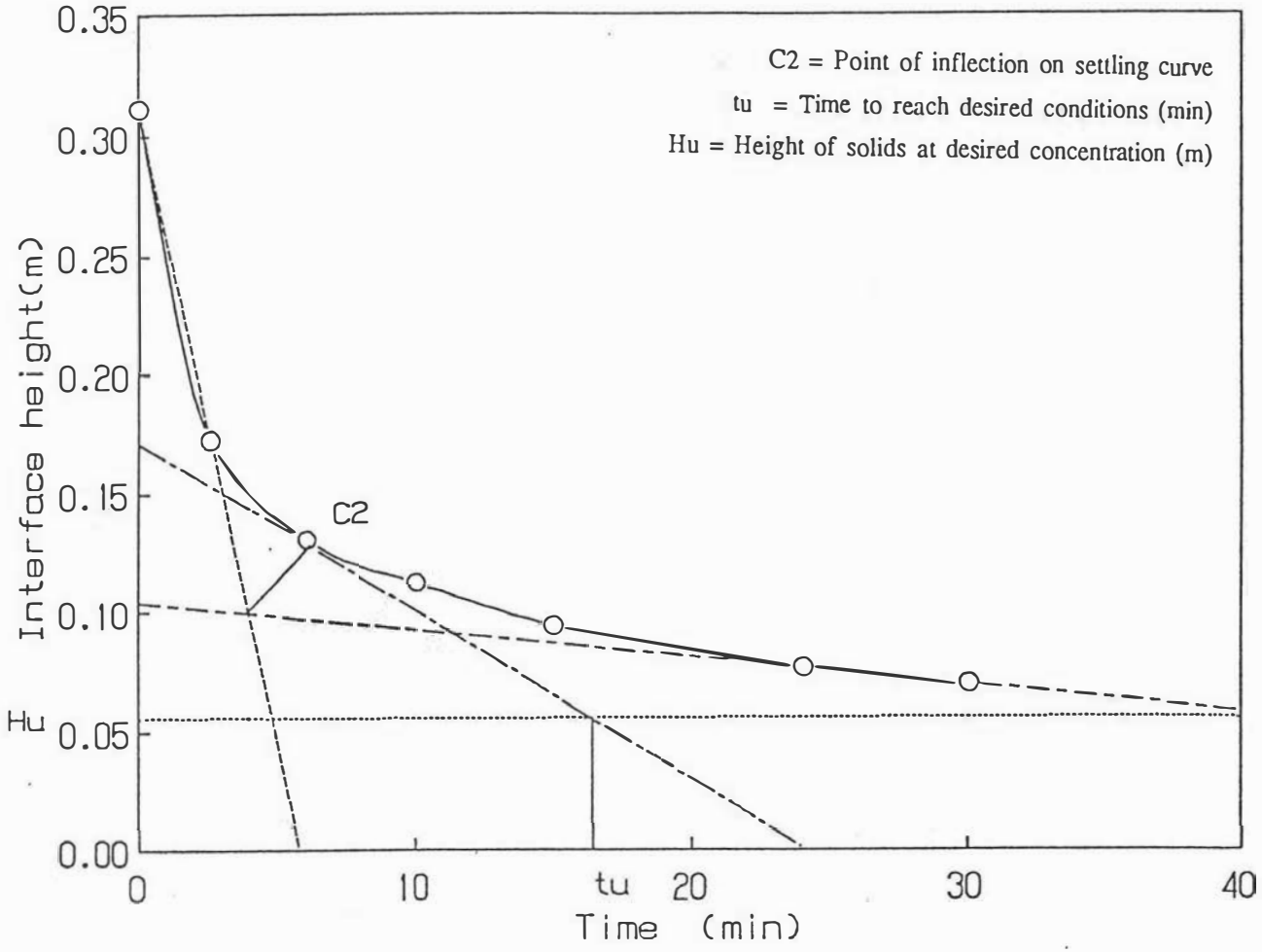


Figure 8.2: Construction for the Graphical Design of a Clarifier from Batch Settling Data (Run 3)

8.3 Cost Estimation for Leachate Treatment.

8.3.1 Capital Costs of Alternatives.

The capital costs for each item of equipment were estimated from data presented in Ulrich (1984). This data allowed the calculation of the total installed cost (including instrumentation) of each piece of equipment required, based on the flowrate, dimensions, power consumption and the materials of construction. The resulting costs were in $US (mid-1982). This method was chosen as the simplest method of estimating the costs and allowing comparisons between the options. Errors in costing by this method can be up to 30 % (Ulrich, 1984) which was considered sufficiently accurate.

Costing was performed under the conditions described for the mass balance and equipment design. To increase the utility of the spreadsheet containing the data, the 6/10 rule was used to scale the costs to other conditions. The indices varied from 0.5 to 0.8 and were taken from Table 5-1 of Ulrich (1984). Table 8.2 contains the cost of each item of equipment, along with the conditions and index used in the analysis.

The capital costs for each item required as an option were summed to give an installed cost. contingency and grass roots factors 0.18 and 0.3 respectively were then included to give the total capital cost in $US (1982). This was then escalated to 1989 prices using the chemical engineering (CE) index, and converted to $ NZ using the exchange rate of 59 c/dollar. The results can be seen in Table 8.2. The total cost for AS, AS and AC and AC alone were found to be $565,000, $740,000 and $318,000 respectively. It can be seen therefore that the capital cost for an AS and AC plant were the highest.

8.3.2 Operating Costs of Alternatives.

Again the detailed calculations can be found in the spreadsheet APPEND18.WKS. The costs of electricity, activated carbon and incineration were obtained from DowElanco (Catt, 1990 and Mercer, 1990). Factors for the estimation of indirect costs such as maintenance and insurance from capital costs were obtained from Ulrich (1984). Labour cost was calculated assuming the plant was manned for 1 half day per 3 days, due to DowElanco's desire to use highly automated systems. Administrative costs/overheads were assumed to be equal to the labour costs. The operating costs were calculated on a daily basis, and annualized assuming continuous operation for 365 days/year.

In the case of AS alone, there is a need to dispose of the AS plant effluent for further treatment, as its toxicity probably prevents direct discharge into the environment. The method of choice of DowElanco is transport from the site for disposal into the local city trade waste system (Mercer,1990). The cost of this transport is $0.12/l (Catt,1990).

A summary of the estimated operating costs can be found in Table 8.3. It can be seen that the operating costs of an AC system would be very high, almost $5 million per year, compared to the AS

Number	Equipment	Size	Safety Factor	Type	Cost	Power	Index
Activated Sludge							
D110	Aeration basin	6.47 m³	1.2	Steel/concrete	12,000	-	0.66
M111	Stirrer	1.8 kW	1.2	Radial turbine	11,000	1.8	0.5
G120	Air compressor	216 m³/h	1.2	Package system	60,835	7.6	0.7
L112	Feed pump	897 l/h	1.2	Centrifugal	6,748	0.1	0.5
L113	Effluent pump	1124 l/h	1.2	Mono[R]	9,484	0.1	0.7
D114	Air scrubber	216 m³/h	1.2	Steel column	2,024	-	0.6
H140	Clarifier/thickener	4.23 m²	2.5	Steel/concrete vessel	27,079	0.2	0.64
L141	Sludge recycle pump	897 l/h	1.2	Mono[R]	8,097	0.1	0.7
L142	Sludge waste pump	37 l/h	1.2	Mono[R]	4,504	0.1	0.7
F143	Sludge storage tank	3112 l	1.4	Steel/concrete vessel	7,000	-	0.52
F144	Surge tank	8218 l	1.1	Steel/concrete vessel	15,000	-	0.52
D150	Equalisation tank	10.8 m³	12 h feed	Concrete hole in ground	8,370	-	0.8
L151	Nutrient pump	4 l/h	1.2	Gear pump	4,499	0.1	0.7
F152	Nutrient tank	360 l	1.2	Steel tank	500	-	0.8
M153	Equalisation mixer	0.1 kW	1.2	Propeller	6,098	0.1	0.5
L154	Leachate pump	168 l/h	1.5	Centrifugal	4,007	0.1	0.5
L155	Dilution water pump	953 l/h	1.5	Gear pump	8,370	0.1	0.7
				$US 1982	195,769	10.2 kW	
				$US 1989+fact	333,677 =	$NZ 1989	565,555
Activated Carbon Polishing							
D210	Activated carbon columns	0.24 m³	1.2	Packed bed steel column	35,604	-	0.6
L211	Feed pump to column	0.1 kW	1.5	Centrifugal pump	8,214	0.1	0.5
F212	Fresh carbon hopper	236 l	1.2	Hopper	999	-	0.8
F213	Spent carbon hopper	236 l	1.2	Hopper	999	-	0.8
F214	Polished effluent day tank	6394 l	1.1	Steel/concrete tank	14,668	-	0.8
				$US 1982	60,485	0.1 kW	
				$US 1989+fact	103,093 =	$NZ 1989	174,733
Activated Carbon Alone							
D310	Activated carbon columns	539 l	1.0	Packed bed steel column	47,779	-	0.6
F311	Fresh carbon storage hopper	3.0 m³	1.4	Steel hopper	15,868	-	0.8
F312	Spent carbon storage hopper	3.0 m³	1.4	Steel hopper	15,868	-	0.8
L313	Carbon column pump	135 l/h	1.2	Centrifugal pump	4,809	0.1	0.5
F314	Surge tank (leachate)	1345 l	1.25	Concrete tank	9,967	-	0.8
L315	Leachate pump	135 l/h	1.2	Mono[R]	5,738	0.1	0.7
F316	Effluent day tank	1345 l	1.25	Concrete tank	9,967	-	0.8
				$US 1982	109,997	0.2 kW	

based system ($ 866,000 for trade waste disposal and $240,000 for AC treatment). The capital cost of the AC plant is, however, considerably less than the AS plant. Therefore there is a need to perform a discounted cash flow to truly compare the three options.

Table 8.3 Summary of Estimated Costs at Nominal Flows.

Item	AS alone ($/day)	AS + AC ($/day)	AC alone ($/day)
Direct Costs			
Nutrients	22.22	22.22	-
Stripping carbon	69.26	69.26	-
Effluent Carbon	-	19.98	11,385
Power	35.60	35.60	0.70
Incineration	12.99	80.15	2134
Labour	13.70	13.70	13.70
Disposal	2161	-	-
Indirect Costs			
Maintenance	46.48	60.85	26.12
Insurance	7.75	10.14	4.35
Administration	13.70	13.70	13.70
TOTAL ($/day)	2373	664	13,578
TOTAL PER ANNUM ($/annum)	866,000	242,000	4,956,000
CAPITAL COSTS ($ NZ)	565,000	740,000	318,000

8.3.3 Discounted Cash Flow Analysis of the Three Options.

In conventional economic analysis, the aim is to determine which option is capable of generating the greatest profit. In this case, where there is no final product to sell, the question becomes which option will perform the task at the least cost.

8.3.3.1 Estimation of Total Costs.

In order to compare the options the total cost of the project was determined using discounted cash flow techniques, and the results for each option expressed in two ways; as a present worth and as a cost per litre of undiluted leachate (based on the equivalent uniform annual cost (EUAC)).

It was necessary to assume an interest rate (15 % p.a. was applied) and a corporate tax rate (33 %,Dept. Inland Revenue, 1990). Plant life was calculated from the leaching rate and the known content

of the dump. Depreciation of the capital (straight line over 10 years or plant life, whichever is least, with a salvage value of zero) results in a tax credit, which was subtracted from the operating costs. These calculations were performed in the spreadsheet APPEND18.WKS, and the results at the nominated conditions are summarised in Table 8.4.

Table 8.4 Summary of Discounted Cash Flow at Nominated Conditions.

Cost Basis	Activated Sludge Alone	AS and AC	AC Alone
Cost/l leachate	$ 1.02	$ 0.42	$ 5.12
Total Cost (present worth)	$ 3,344,000	$ 1,390,000	$16,860,000

It can be seen that activated carbon treatment will be over ten times as expensive as the two step process to produce comparable effluents. AC would also be over 5 times more expensive than AS followed by dumping. Therefore on a cost basis, activated carbon treatment alone for the leachate should be discounted.

It can be seen that the AS + AC option is the cheapest of the methods examined for the disposal of the leachate. However, it should be noted that the bulk of the cost for AS alone is from the cost of transport to the trade waste sewer. Without this cost, AS alone is cheapest at $0.22 /l.

8.3.3.2 Effect of Variation From Nominated Conditions on Economics.

The nominated conditions fixed the leaching rate and leachate concentration, which subsequently defined the plant life and AS plant size. By altering these two key parameters in the spreadsheet APPEND18.WKS, it was possible to see the effect these had on the economics of the plant. Firstly the effect of leachate concentration will be investigated, followed by the effect of leaching rate.

Variation of Leachate Concentration.

The nominal leachate concentration was 5.8 g/l, the concentration noted during the bulk of the work, but the last batch of leachate had a reduced concentration of 4.6 g/l. It was therefore assumed that 5.8 g/l was the highest concentration that would be encountered, and that 15 % leachate (feed concentration, 870 mg/l, Chapter 7) would be the lowest encountered. The spreadsheet was recalculated using values in this range, and the total cost (present worth) was noted. The present worth was plotted against the concentration, and can be found in Figure 8.3. This indicates the leachate concentration has no effect on the cost of treatment.

The effect of the feed concentration to the AS plant was also investigated using a similar technique, and the plot of present worth versus feed concentration can be found in Figure 8.4. This indicates the higher the feed concentration the cheaper the overall cost: at a constant organic loading rate.

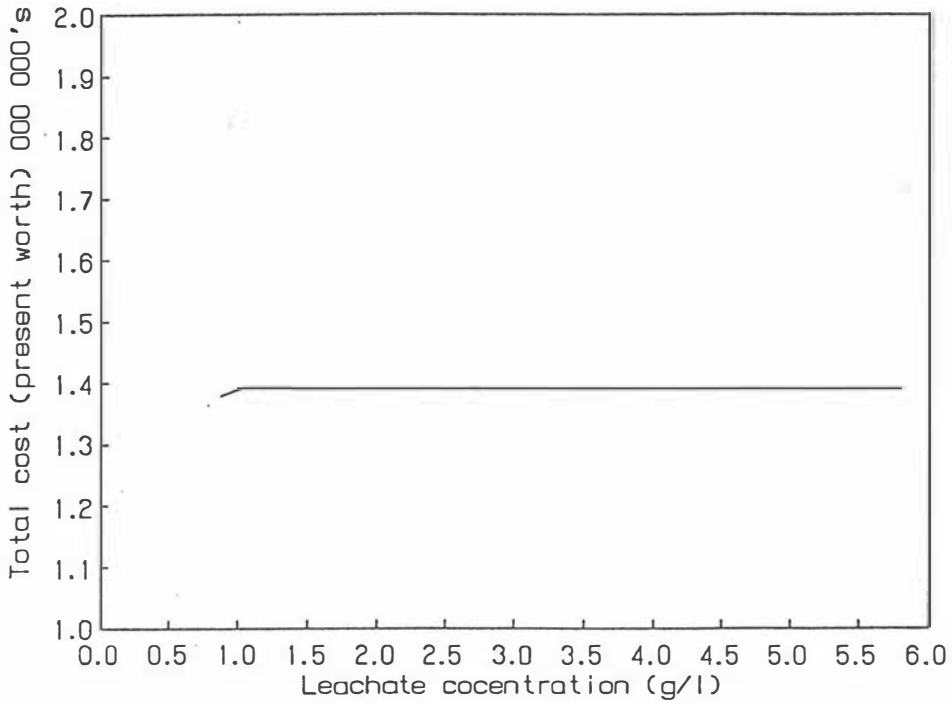


Figure 8.3: Plot of Present Worth versus Leachate Concentration for AS+AC Treatment.

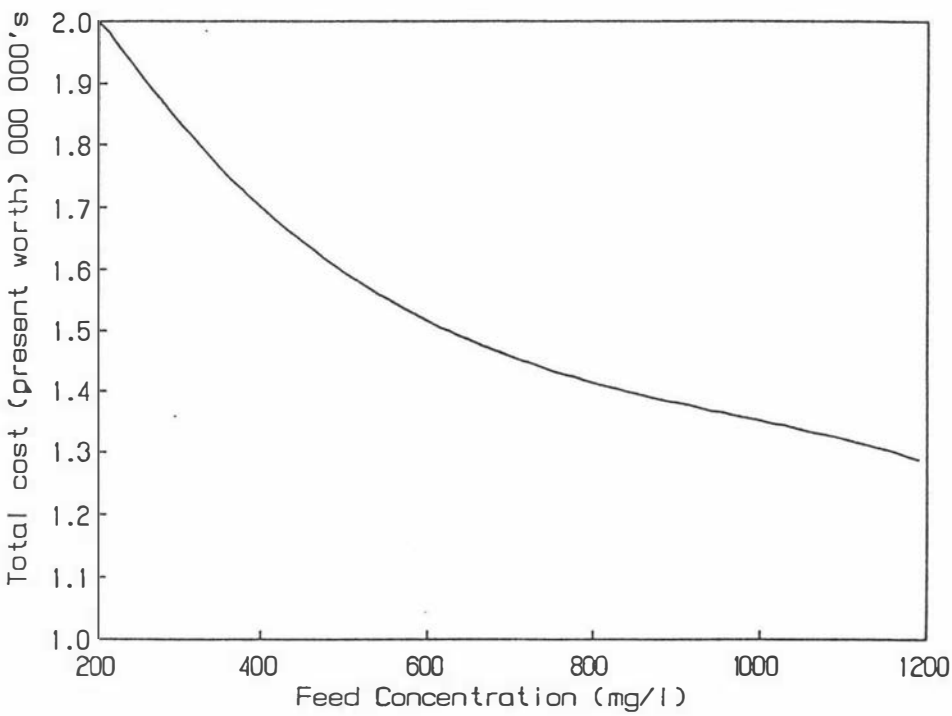


Figure 8.4: Plot of Present Worth versus Feed Concentration for AS+AC Treatment.

Variation of Leaching Rate.

The second key parameter to be investigated is the rate of leaching from the dump. Currently there is no information available on the expected rates, the cost was determined at a number of different leaching rates. The present worth was plotted against the leaching rate, and can be found in Figure 8.5. Figure 8.6 is a plot of present worth versus the time required to leach the site, assuming a constant leaching rate.

It can be seen that the leaching rate has a major effect on the cost of the system. Very rapid leaching results in the requirement of a large plant, and the provision of services and nutrients over a short period of time. Lower rates of leaching, however, require a smaller plant, with lower capital costs, but still require the same total nutrients and services, but over a longer period of time. The net result of this, and the time value of money, is that the longer the clean up time, the less the total cost. There will, of course, be an upper limit placed on the time allowed by regulatory authorities, however this study showed that costs decreased for plant lives of up to 20 years.

Other Possible Variables.

With respect to economics, it is also be possible to vary the AS plant loading rate, interest rate, power and carbon costs and determine the effect of these parameters on the overall cost. This has not been done. For the loading rate, the effect is obvious, i.e. higher loading rates give lower capital costs. Other parameters can be tested by interested parties by simply varying the spreadsheet and recalculating the total costs.

8.4 Comparison With Other Costs.

Comparing this cost to others is difficult, due to the variations which may be encountered, but work has indicated the cost for incineration of the leachate would be in the order of $2/l (Catt, 1990), ≈ 5 times higher than the cost of AS + AC treatment. The largest error in the analysis conducted in this thesis would be in the estimation of capital costs, which were based on US prices (1982) and escalated forward to $NZ 1990. Ulrich (1984) estimates these errors to be 20 to 30 %. As the cost data was not New Zealand based, equipment was oversized to ensure the capital costs were an overestimate. Therefore the estimated cost can be treated as being at the upper end of the errors. An error of 40 % in capital cost would lead to a 14 % difference in the final cost per litre. It could be seen, however, the AS+AC was less than one quarter cost of incineration. It would be advisable, to obtain better, New Zealand based, capital cost estimations before a final commitment to a particular process is made.

Information in the literature about the cost of these processes is scarce. Glynn et al. (1987) quotes $0.35 US/US gallon or $NZ 0.16/l as the cost for mobile biological treatment of contaminated groundwater and leachate, which was similar to the cost calculated for AS without the disposal costs. Skladany (1989), however, gives the cost as $0.006 US/US gallon ($NZ 0.003/l) for a bioreactor treating toluic acid. The author, unfortunately, gives no indication whether this figure includes the capital cost, and also ignores some basic costs such as nutrients and activated carbon used in a

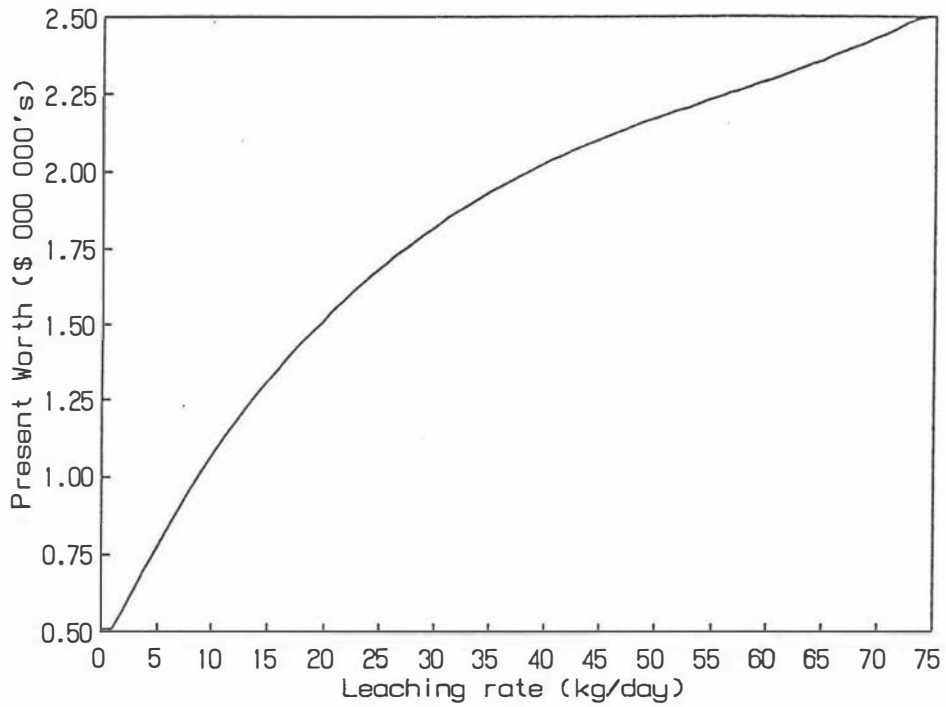


Figure 8.5: Plot of Present Worth versus Leaching Rate for AS+AC Treatment.

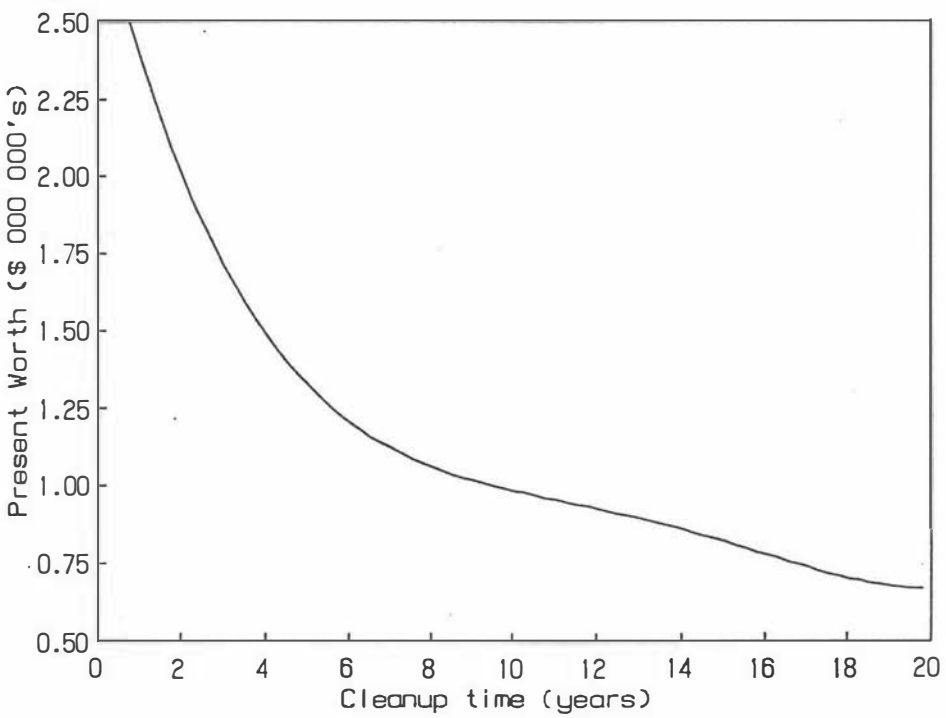


Figure 8.6: Plot of Present Worth versus Cleanup Time for AS+AC Treatment.

polishing step. Skladany's value, therefore, was considered to be inappropriate.

The use of a spreadsheet for this economic analysis was invaluable. The ability to alter inputs such as leaching rates allows the determination of sensitivity to parameters, greatly easing the work. It should be noted, however, that like all systems, the program is limited by the accuracy of the input data.

8.5 Conclusions.

It has been shown that activated sludge (followed by trade waste sewage disposal) and AS + AC processes are cheaper than AC alone for this leachate by factors of 5 and 10 respectively. The costs were determined at the nominal leaching rate of 15.6 kg/day and concentration of 5.8 g/l. Activated sludge and AS+AC cost $1.02 and $0.422 per litre of undiluted leachate, both of which were less than the cost of incineration ($2/l).

The cost was most sensitive to the leaching rate, with lower rates resulting in smaller and cheaper designs. The cost was insensitive to the leachate concentration. It was concluded that AS + AC was the cheapest method of totally treating the landfill leachate evaluated.

CHAPTER 9
GENERAL DISCUSSION AND CONCLUSIONS

It was stated that this work was based on the thesis that a biologically based process was capable of treating a hazardous waste to produce environmentally acceptable products at a competitive cost.

For biological treatment to be successful, it is necessary to use a microbial population enriched and acclimated to the stream to be treated. Work described earlier (Chapter 4) indicated that the mixed, unmodified, microbial population developed during this study was relatively stable, capable of mineralising the key components in the leachate to be treated and able to reduce the toxicity of the waste by 70 %.

An unmodified mixed culture has three major advantages over pure cultures:
(i) mixed cultures can achieve lower effluent concentrations than pure cultures derived from the same mixture (Lu and Grady, 1988).
(ii) Mixed populations can attack complex compounds, an ability that pure cultures may lack (Rosenberg and Alexander, 1980b).
(iii) Genetically modified organism technology currently faces difficult regulatory paths, which has, so far, resulted in few organisms being released (ABC,1989).

In terms of these points, the population developed during this study possesses the advantages of (i) and (ii), without the disadvantage of (iii). The population was found to be stable over an extended period (872 days) and could also be used in an activated sludge plant due to its flocculation properties.

These advantages, together with the freedom to operate successfully in a non-sterile environment make the culture excellent for use in waste treatment applications.

The MIDT method employed for the determination of the kinetics of degradation of PCOC and the phenoxies possesses many advantages over the more common batch methods. In the case of PCOC it was demonstrated clearly that batch techniques produce results that do not represent the true characteristics of the biomass. The MIDT method, however, appears to measure rapidly the true characteristics of the biomass, giving substrate removal rates 50 % higher than measured by batch experiments. The MIDT method worked well for the determination of inhibition kinetics where inhibition occurs at low substrate concentrations, but is probably not as good for situations where the yield of biomass is high. This makes the method especially suitable for the determination of the kinetics of degradation of hazardous chemicals.

Linear inhibition curves were noted during this study, and a continuous, simple model was developed. Such curves can be found in the literature. However, many authors attempt to fit the

Haldane model to these data, even though it is apparently unsuitable (i.e. Gaudy et al, 1988, fitted the Haldane curve to straight line data with *ortho*-chlorophenol). Linear models have one major advantage over the Haldane model, namely, that they can predict a concentration above which no growth is possible. This point (referred to as the total inhibition concentration by Tseng and Wayman, 1975) does not exist according to the Haldane approach. The concept of total inhibition is very useful when quantifying the effects of toxic substrates.

This study has shown that interactions between the substrates had a major effect on their degradation kinetics: ie as pure compounds, PCOC and phenoxies would wash out at residence times of 33 and 20.8 hours respectively. Substrate washout was observed at 5.9 and 9.9 hours respectively, not only at shorter residence times, but in a different order. It was found that a three substrate model was able to model this behaviour. The use of a three substrate, interactive model to describe leachate degradation has not been previously reported. This model allowed interactions between the substrates to be considered, while retaining the ability to predict pure substrate data. This is an improvement on the model of Bader (1978), which predicts no growth if one or more of the substrates is absent. The model is relatively simple, a simple summation of Monod models for each substrate, with an interaction term for the effect of each substrate on every other. In this case, the linear inhibition models were used in place of the Monod model, with good results. It was shown that there was no significant lack of fit between the measured and predicted data, indicating the model was suitable for further use.

When this model was incorporated into a model for an AS plant, as Rozich and Gaudy (1984) did with the Haldane model, it was found that there were three potential operating regions, as opposed to two suggested by Rozich and Gaudy (1984). It was also noted that the critical point was at a dilution rate three times faster than would otherwise be expected. Thus, ignoring such interactions can lead to overcautious design.

As stated earlier (Section 7.2.4.1) there are currently two views concerning the design of systems treating toxic wastes: one which holds that the waste can be regarded as non-inhibitory (for design purposes) and the other which maintains that the toxic compound itself should control the entire design process. The present work clearly demonstrates that both views are partially correct. It was shown that there was a wide range of dilution rates across which there was total substrate removal, and the system would therefore behave as an uninhibited one. This range covered the normal operating conditions. It was also shown that the inhibitory nature of the substrates does have a controlling effect at high dilution rates.

In the case of the present study, the effect of an easily metabolised substrate, the alcohols, on the degradation of the more hazardous PCOC/phenoxies was successfully modelled using the three substrate model. There is currently considerable interest in accelerating the rate of degradation of priority pollutants using a secondary substrate (Lindstrom and Brown, 1989), and this three substrate model offers a method of quantifying the interactions between the substrates.

Interactive models could also be applied to modelling the behaviour of priority pollutants in domestic sewage treatment plants. Non-interactive approaches have been taken in the past by Moos et al. (1983) and more recently by Melcer and Bedford (1988). The use of an interactive model as was used in this thesis may provide more information and perhaps betters design of such treatment facilities. Interactive models may also be useful for modelling the dynamic oscillations mentioned in Chapter 6, caused by the switching of the culture from one substrate to a second. This is an area which requires further work.

The cell recycle system used for activated sludge experiments in this thesis was novel. It was capable of recycling a concentrated biomass to the aeration basin, with a short residence time in the clarifier, with no apparent loss of activity. If the problems with the determination of the recycle ratio were overcome, then the optical system could be widely applied. Such a system is flexible, as there is no need to readjust timers to ensure the correct recycle ratio and it has proved reliable over 3000 hours of operation.

The loading rates used in the experiments were high in comparison to the typical values quoted in the literature for domestic sewage. This indicated that the activated sludge system used was efficient.

Analysis of the sludge produced during this project indicated that there was no bioaccumulation of the substrates, or of the metal ions present. The wasted sludge was therefore considered safe for disposal to the environment.

It was also shown that the effluent from an AS plant could easily be treated with activated carbon to yield an effluent of comparable toxicity to AS treated domestic sewage. The concentrations of residual nutrients however, were high, and further work is necessary to determine the minimum nutrient requirement for the process.

These results indicated that biologically based systems are capable of producing non-hazardous products from hazardous wastes. There is currently also opposition to alternative techniques such as incineration and sea disposal (Fry, 1988). According to Vesilind et al. (1988) biodegradation of hazardous wastes is a science currently in the research and development stages and is receiving more funding and attention in the U.S. (Crawford, 1989). The results of this study show that biological treatment can effectively be used to degrade hazardous landfill leachates.

Preliminary economic analysis of the proposed process has shown that the two stage process of AS followed by AC can treat the leachate for a cost of ≈ $ 0.42/l. This is cheaper than incineration ($ 2/l) and over ten times less expensive than AC alone. These results indicated that bioremediation in this situation is likely to be the least expensive method of site cleanup. There is, however, room for further optimisation of the process (along the lines of Tyteca et al., 1977), when more information on leaching rates from the dumpsite become available. This leaching rate data could be obtained from laboratory scale experiments, which may also be able to predict changes in leachate composition. This

information, in conjunction with the three substrate model could allow close control of the activated sludge system.

Many processes described in the literature remain at the laboratory scale. This study, however, has a direct field application. Currently, large scale batch work is underway at the premises of the sponsoring company, to treat the excess of leachate present in the dump. Using a 50 m^3 (working volume) bioreactor, the treatment of approximately 100,000 l of leachate has been achieved. As a batch process was used (due to equipment availability), the degradation rates were not high, however, the treatment of this quantity of leachate demonstrates the feasibility of this process on a large scale. Hsu et al. (1984) showed small scale systems were good predictors of the behaviour of large scale waste treatment systems, which indicates the AS system should also work well on a large scale.

Currently, both in New Zealand and overseas, there is considerable interest in cleaning up the environment and maintaining the biosphere in a sustainable condition. This is shown by the large number of articles in both the multidisciplinary technical literature (such as Science and Current Contents) and the mass media. To reduce pollution, it is necessary to provide cheap, effective methods of treating hazardous wastes, to give the technical ability to meet standards and balance the increased penalties proposed in new legislation to be enacted here in the near future. The use of a "Best Practicable Option" system for setting standards (Ministry for the Environment, 1988) means that the cheaper and more effective the method, the more likely it is to be used. Therefore the biologically based process developed in this thesis, which provides a means of cheaply treating a hazardous waste, and producing non-hazardous byproducts which should be suitable for disposal to the environment, should be acceptable to regulatory authorities. New Zealand significantly lags other countries with respect to environmental regulation. Proposed changes recommended the establishment of an Environmental Protection Agency in New Zealand, some 20 years after Japan, and 17 years after the US and some Australian states established such organisations. However legislators have not take up this proposal.

It is concluded that a biologically based process is capable of producing non-hazardous byproducts and is economically viable as compared to alternative treatment processes.

ABBREVIATIONS AND NOMENCLATURE.

2,4-D	2,4-dichlorophenoxyacetic acid
MCPA	2-methyl-4-chlorophenoxyacetic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,4-DCP	2,4-dichlorophenol
PCOC	*para*-chloro-*ortho*-cresol
2,4,5-TCP	2,4,5-trichlorophenol
MDCPA	2-methyl-2,5-dichlorophenoxyacetic acid
MCPB	2-methyl-4-chlorophenoxybutyric acid
PCP	Pentachlorophenol
A_{600} and A_{284}	Absorbance at the specified wavelength
ABC	Association of Biotechnology Companies
AC	Activated Carbon
AS	Activated Sludge
BAM	Basal Ash Medium
BOD	Biological Oxygen Demand (mg/l)
CI	Confidence Interval
CN	Cyanopropyl HPLC packing
COD	Chemical Oxygen Demand (mg/l)
CSTR	Continuous Stirred Tank Reactor
D	Dilution rate (h^{-1})
D^*	Critical dilution rate (h^{-1})
DO	Dissolved Oxygen concentration (mg/l)
EC	Effective Concentration (%)
EUAC	Equivalent Uniform Annual Cost
F	Flowrate (m^3/h)
F_R	Recycle rate (m^3/h)
F_W	Wastage rate (m^3/h)
FBR	Fed Batch Reactor
x g	Times Gravity
GLC	Gas Liquid Chromatography
HPLC	High Performance Liquid Chromatography
HRT, θ	Hydraulic Residence Time (h)
ICP-AEC	Inductively Coupled Plasma - Atomic Emission Spectroscopy
k_d	Death rate (h^{-1})
K_I	Haldane inhibition constant (mg/l)
k_S	Monod half saturation constant (mg/l)
K_{OXY},k_{OH}	Inhibition constants for phenoxies and PCOC respectively ($l/mg_{SUBSTRATE}.h$) (See Addendum 3)
k_1, k_2, k_3, k_4	Interaction terms in 3 substrate model ($l/mg_{SUBSTRATE}$)
LDR	Light Dependent Resistor

m	Rate of biomass increase (mg/h)
MCRT	Mean Cell Residence Time (h)
MIDT	Modified Infinite Dilution Test
MLSS	Mixed Liquor Suspended Solids (mg/l)
MLVSS	Mixed Liquor Volatile Suspended Solids (mg/l)
n	Empirical constant
NOEC	No Observable Effect Concentration
PACT	Powdered Activated Carbon Treatment
Q	Specific substrate removal rate (mg/mg.h)
S	Substrate concentration (mg/l)
S_I	Threshold inhibition substrate concentration (mg/l)
S_M	Maximum substrate concentration prior to total inhibition (mg/l)
S_1, S_2, S_3	Concentrations of alcohol, PCOC and phenoxies in 3 substrate model (mg/l)
SRT, θ_c	Solids Retention Time (h)
SVI	Sludge Volume Index (ml/g)
TDS	Total Dissolved Solids (g/l)
TOC	Total Organic Carbon (mg/l)
TOD	Theoretical Oxygen Demand (mg/l)
USEPA	United States Environmental Protection Agency.
V	Reactor volume (m^3)
X	Biomass Concentration (mg/l)
X_R	Recycle sludge concentration (mg/l)
Y, $Y_{X/S}$	Yield coefficient (mg/mg)
α	Recycle ratio
β	Ratio of K_I to k_s (Santiago and Grady, 1989)
μ	Specific growth rate (h^{-1})
[ ]	Concentration (mg/l)
Subscripts.	
ALC	Refers to Alcohols
OXY	Refers to Phenoxies
OH,PCOC	Refers to PCOC
MAX	Maximum
APP	Apparent

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ADDENDUM

Addendum 1: The Program CRIT.

Due to very late changes to the thesis, the program CRIT on microfiche is incorrect. The correct version can be found below.

```
      Program Criticalpoints
      implicit real *8 (a-h,o-z)
      dimension s(5), g(5,5), r(5),crit(3,30)
      write(*,*)'     CRITICAL POINT DETERMINATION'
      write(*,*)'     ============================'
      write(*,*)'                                 '
      Write(*,*)'This  program  takes engineering parameters'
      write(*,*)'for an AS plant and determines the critical'
      write(*,*)'SRT (theta C) for the biomass to be'
      write(*,*)'retained in the activated sludge system for good op-'
      write(*,*)'eration, based on a 3 substrate,interactive'
      write(*,*)'model. Please respond to the questions.'
      write(*,*)
      write(*,*)'input Leachate concs, Alc, PCOC, Phenoxy  '
      read(5,*)alc,PCOC,pheno
      write(*,*)'input phenoxy threshold '
      read(5,*)scrit
      k1 = 2.1
      k2 = 0
      k3 = 5.5
      k4 = 9.3
      smax = alc+pcoc+pheno
      do 100 f = 5,20,1
      ct = 0
      c2 = 0
      s(1) = 1
      s(2) = 1
      s(3) = 1
      s10 = alc*f/100
      s20 = pcoc*f/100
      s30 = pheno*f/100
      sin = s10+s20+s30
      Xmax = 1.3*s10+.25*s20+.27*s30
50    do 1000 d=0.001,0.3,.001
30    do 10 i=1,3
        do 10 j=1,4
        g(i,j) = 0.
10    continue
11    g(1,1) = ((1.3*s10-6.5)*d-.3*xmax)+(1.0e-3*xmax+.075)*s(2)
     1  +(1.4e-4*xmax+.081)*s(3)-2.6e-3*s(1)*
     1    s(2)-3.6e-4*s(1)*s(3)-2.5e-4*s(2)*s(2)
     1    -3.8e-5*s(3)*s(3)-3.5e-4*s(2)*s(3)+s(1)*(0.78-2.6*d)
      g(1,2) = (1.0e-3*xmax+.075)*s(1)-1.3e-3*s(1)*s(1)
     1  -5.0e-4*s(1)*s(2)-3.5e-4*s(1)*s(3)
      g(1,3) = (1.4e-4*xmax+.081)*s(1)-1.8e-4*s(1)*s(1)
     1  -7.8e-5*s(1)*s(3)- 3.5e-4*s(1)*s(2)
```

```
      g(2,1) = .25*k1*s20*d+(.0403-k1*.25*d)*s(2)-1.42e-4*s(2)*s(2)
      g(2,2) = ((.25*s20-.0375)*d-.031*xmax)+(0.0403-k1*.25*d)*s(1)
     1  +(8.37e-3-k2*.25*d)*s(3)+2*(1.09e-4*xmax+7.75e-3-0.25*d)*s(2)
     1    -2.83e-4*s(1)*s(2)-5.88e-5*s(2)*s(3)-8.19e-5*s(2)*s(2)
      g(2,3) = k2*.25*s20*d+(8.37e-3-k2*.25*d)*s(2)-2.94e-5*s(2)**2
      g(3,1) = k3*.27*s30*d+(0.0624-k3*.27*d)*s(3)
      g(3,2) = k4*.27*s30*d+(0.012-k4*.27*d)*s(3)
      g(3,3) = ((.27*s30-2.7e-3)*d-.048*xmax)+(0.0625-k3*.27*d)
     1    *s(1)+(0.012-k4*.27*d)*s(2)+2*(.0129-.27*d)*s(3)
        g(1,4) = 6.5*s10*d+((1.3*s10-6.5)*d-.3*xmax)*s(1)
     1    +(1.0e-3*xmax+.075)*s(1)*s(2)+(1.4e-4*xmax+.081)*s(1)*s(3)
     1    -1.3e-3*s(1)*s(1)*s(2)-1.8e-4*s(1)*s(1)*s(3)+
     1  (.39-1.3*d)*s(1)*s(1)-2.5e-4*s(1)*s(2)*s(2)-
     1  3.8e-5*s(1)*s(3)*s(3)-3.5e-4*s(1)*s(2)*s(3)
      g(2,4) =.0375*s20*d+k1*.25*s20*d*s(1)+((.25*s20-.0375)*d
     1   -.031*xmax)*s(2)+k2*.25*s20*d*s(3)+(.0403-k1*.25*d)
     1    *s(1)*s(2)+(8.37e-3-k2*.25*d)*s(2)*s(3)+(1.09e-4*xmax
     1    +7.75e-3-0.25*d)*s(2)*s(2)-1.417e-4*s(1)*s(2)*s(2)-2.94e-5
     1   *s(3)*s(2)*s(2) -2.73e-5*s(2)**3
     g(3,4) = 2.7e-3*s30*d +((.27*s30-2.7e-3)*d-.048*xmax)*s(3)
     1    +.27*s30*d*k3*s(1)+.27*s30*k4*d*s(2)+(.0625-.27*k3*d)
     1   *s(1)*s(3)+(.012-.27*k4*d)*s(2)*s(3)+(.0129-.27*d)*s(3)*s(3)

            call gausl(5,5,3,1,g)

      r(1) = s(1)-g(1,4)
      r(2) = s(2)-g(2,4)
      r(3) = s(3)-g(3,4)
      er1 = sqrt(abs((r(1)**2-s(1)**2)/r(1)*r(1)))
      er2 = sqrt(abs((r(2)**2-s(2)**2)/r(2)*r(2)))
      er3 = sqrt(abs((r(3)**2-s(3)**2)/r(3)*r(3)))
      er = er1+er2+er3
      if (er .le. 1.e-4) go to 40
      s(1) = r(1)
      s(2) = r(2)
      s(3) = r(3)
      if (s(3) .le. -1.e-1) go to 35
      if (s(2) .le. -1.e-1 .and. s10 .ge. 1) go to 36
      go to 30
35    s(3) = s30
      go to 11
36    s(2) = s20
      s(3) = s30
      go to 11
40    sout = s(1)+s(2)+s(3)
      if (s(3) .ge. scrit .and. ct .le. 0) crit(1,f)= 1/dold
      if (s(3) .ge. scrit) ct = ct+1
      dec = sout/sin
      if (dec .ge. 0.90 .and. c2 .le. 0) crit(2,f)=1/dold
      if (dec .ge.  0.90) c2 = c2+1
      if ( d .le. 3.1e-2 .and. d .ge. 3e-2) crit(3,f) = 1/d
45    format(4(f10.5,1x))
      dold = d
1000  continue
100   continue
      write(*,*)
      write(*,*)'Breakthru     Washout     Gaudys     Feed conc'
      write(*,*)'  (hr)          (hr)       (hr)       (mg/l)'
```

```
      write(*,*)'=========================================='
      do 12 i = 5,20,1
      write(6,31)crit(1,i),crit(2,i),crit(3,i),i*smax/100
12    continue
      stop
31    format(4(f7.2,6x))
      end
```

Addendum 2

The following reference became available after submission of the thesis, however the information it contained has a significant effect on the interpretation of the modelling. It was therefore included subsequently.

Bertucco, A., Volpe, P., Klei, H.E., Anderson, T.F. and Sundstrom, D.W.,'The stability of activated sludge reactors with substrate inhibition kinetics and solids recycle.'Water Res.,24, 1990, pp 19-176.

Addendum 3

K_{OH1} refers to the effect of PCOC on PCOC degradation in terms of substrate removal.

K_{OH} refers to the effect of PCOC on PCOC degradation in terms of growth rate.