THE USE OF NATURALLY GENERATED VOLATILE FATTY ACIDS FOR PESTICIDE REMOVAL DURING THE DENITRIFICATION PROCESS

A THESIS SUBMITTED IN FULFILMENT

OF THE

DEGREE OF DOCTOR OF PHILOSOPHY IN CIVIL ENGINEERING

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THE UNIVERSITY OF CANTERBURY

NEW ZEALAND

DECEMBER 2006

Acknowledgements

The work carried out for this research would not have been successful without contributions from a number of people to whom I would like to express my appreciation. A big thank-you to my supervisor, Dr David Wareham, for your encouragement and vision. I appreciate the good work you do with research supervision. Your comments and ideas helped me a lot through the experimental part of the work and thesis writing. I appreciate the quick turn-around times when reviewing my work. We have also had many fun times and laughter together. I am also impressed with your outlook on life, especially the balance between work and play. It has been an encouragement to me.

I would like to thank my co-supervisor Dr Mark Milke for his help in fulfilling that important role.

Special thanks also to the technical staff in the Department of Civil Engineering. Dave Macpherson helped me a lot with the construction of the experimental reactors and was always available to solve any problems which might occur during my lab work. Richard Newton was helpful with the SBR software programme and fixing any electrical problems. Additionally, I would like to thank Dr Joseph Holman who continuously carried sewage to my experiments throughout the research and contributed lots of ideas and technical support to my lab work.

Thank you to the Department of Chemical & Process Engineering for allowing access to the HPLC. I would like to give my thanks to Trevor Berry and Tony Allen for their technical support.

The financial support offered by the University of Canterbury via a doctoral scholarship is gratefully acknowledged.

Thank you to my fellow students especially Peter Brown for taking time to listen to me and providing help on my thesis editing.

Finally, thanks to my family who have made significant contribution to support my study. I am indebted to my husband Bo for his support, encouragement and understanding, and a very special love to my daughter Nicole for bringing joy and comfort.

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Abstract

The effect of naturally produced volatile fatty acids (VFAs) on the removal of 2, 4-D from a wastewater during the denitrification process was studied in this thesis.

The VFAs were generated from an anaerobic digester using soya flour solution as a synthetic feed. The digester was operated at an SRT and HRT of 10 days. The pH (4.8 ± 0.2) and temperature (32 ± 3 °C) of the digester were not controlled. A mean VFA concentration of 3153 ± 801 mg/L was achieved with acid speciation results of acetic (51.4 %), propionic (27.5 %), n-butyric (19.6 %) and iso-valeric (1.4 %). The specific VFA production rate was 0.014 mg VFA/mg VSS/day. The extent of the digestion process converting the substrate from a particulate to soluble form was evaluated as the specific TOC solubilization rate (0.007 mg TOC/mg VSS/day), soluble COD production rate (0.022 mg SCOD/mg VSS/day) and percent VSS reduction (14 %). The low solubilization rate is possibly due to high feed solids (3.4%) which led to a heavily overloaded bioreactor. It also suggests that the particulate substrate was not entirely amenable to solubilization.

The acclimation of 2, 4-D degrading bacteria was developed successfully in an SBR fed with sewage and 2, 4-D (30-100 mg/L) as carbon and energy sources. A mean MLSS of 3653 ± 547 mg/L and an SRT of 20 ± 9 days were observed during the research period. The settleability of the SBR sludge was excellent evidenced by a low sludge volume index (SVI) of 101 ± 50 mL/g and less than 5 mg/L of effluent suspended solids. The specific 2, 4-D degradation rate was 0.046 ± 0.018 mg/mg MLSS/day. However, the removal of 2, 4-D during 60 minutes of non-aerated phase was negligible while more than 90 % of the 2, 4-D was removed within 240 minutes of the aerated phase. The successful degradation of 2, 4-D is related to the length of the acclimation period, as the acclimation period increased, the specific biodegradation rate increased. A biosorption study using ultrasound pre-treatment of the SBR acclimated biomass suggested that less than 10 % of the removal of 2, 4-D was due to biosorption, while more than 90 % removal of the 2, 4-D was likely due to biodegradation.

Denitrification batch tests (using SBR-acclimated biomass) demonstrated that the addition of a digester effluent rich in naturally-produced VFAs increased both the

specific denitrification rate and the 2, 4-D degradation efficiency, as compared to that using 2, 4-D as a sole carbon source. In particular, the specific denitrification rates increased from 0.0119 ± 0.0039 to 0.0192 ± 0.0079 to 0.024 ± 0.003 g NO_3-N/g VSS per day, when using 2, 4-D alone, 2, 4-D plus natural VFAs and natural VFAs alone as carbon sources. The percent 2, 4-D removal increased from 28.33 ± 11.88 using 2, 4-D alone as a carbon source to 54.17 ± 21.89 using 2, 4-D plus natural VFAs as carbon sources. The specific 2, 4-D degradation rate and 2, 4-D removal efficiency of unacclimated biomass were 2.0 to 2.5 times less than those of the acclimated biomass. Natural VFAs and synthetic VFAs were found to be identical in denitrification batch tests in terms of their use as a carbon source. The mean specific denitrification and VFA-C consumption rates as well as the mean specific 2, 4-D degradation rate derived from experiments using natural VFAs and 2, 4-D as carbon sources were close to the valuess from experiments using synthetic VFAs and 2, 4-D as carbon sources.

Further exploration of 2, 4-D degradation behaviour with pulsed additions of NO_3-N did not find further significant 2, 4-D removal, although almost all of NO_3-N was used by the end of the experimental run due to endogenous carbon sources used for cell maintenance and growth. However, the higher the concentration of biomass used in the denitrification batch system, the larger the amount of 2, 4-D degraded and the faster the VFA-C and NO_3-N were consumed.

Further research with respect to optimisation of the acid-phase anaerobic digestion process (e.g. to adjust SRT and HRT or to lower the solid content of synthetic feed) would improve the specific VFA production rate and the solubilization rate. More research on the SBR could be carried out to investigate its maximum 2, 4-D removal capability as well as the removal of other structurally related herbicides. Attempts could be made to stimulate the growth of denitrifiers in the SBR (e.g. to add certain amounts of NO_3-N according to proper C: N ratios or to increase the length of non-aerated time). More microbiological studies of 2, 4-D degrading bacteria may also be helpful to understand the combined SBR/denitrification and 2, 4-D degradation process. More theoretical aspects of modelling kinetics could be developed to apply the combined process in-situ at 2, 4-D contaminated sites.

Chapter 1: Introduction

As a consequence of human activities, many toxic compounds are released to the environment from industry. These include compounds such as polychlorinated biphenyls (PCBs), polychlorodioxins, and trinitrotoluene (TNT) all of which are classified as xenobiotics because their chemical structure is alien to the biosphere. In addition, many natural compounds (e.g. fossil fuel hydrocarbons and heavy metals) have been mobilized to a bioavailable form that is toxic to organisms. Major sources of these pollutants include: (i) chemical (e.g. pharmaceutical) industries that produce a wide array of xenobiotics; (ii) pulp and paper industries that produced chlorinated organics; (iii) mining activities that release heavy metals; (iv) fossil fuels which cause acid rain as well as the greenhouse effect; and (v) intensive agricultural practices which release massive amounts of fertilizers and pesticides (Dua et al., 2002; Rieger et al., 2002).

The aim of this research is to study the pesticide biodegradation capability under denitrification conditions. The literature review therefore will discuss firstly, general concepts behind pesticide degradation using microbial means, secondly, the microbiology and use of organic materials in the denitrification reaction with a particular focus on the carbon to nitrogen ratio, thirdly, the acid-phase digestion process to generate volatile fatty acids (i.e. one of the co-substrates) and fourthly, sequencing batch reactor technology. The systems used in this research are an anaerobic digester, a sequencing batch reactor and a series of batch reactors to conduct denitrification tests.

Chapter 2: Literature Review

2.1 Pesticide poisoning and microbial degradation

2.1.1 Introduction

Pesticides are a broad group of heterogeneous chemicals that have a significant public health benefit by increasing food production productivity and decreasing food-borne and vector-borne diseases. However, depending on the agent and the exposure, they may pose health risks (Weiss et al., 2004). Pesticides can be classified according to their use (insecticides, fungicides, herbicides, raticides etc.) or by their chemical family (organochlorates, organophosphates, carbamates, pyrethroids, bipyridilium compounds, inorganic salts etc.) (Ferrer, 2003). All of them are biocides, which normally imply a high toxicity for humans; thus they have been a cause for concern since the mid-20th century due to the widespread and indiscriminate use of these products. Some organochlorine compounds (such as DDT) were first used in massive fumigations to fight malaria and these have had to be banned because of their capacity for bioaccumulation and environmental persistence. However, increased awareness of the harmful effects of pesticides has led to continuous research into various strategies that may be employed to clean up the environment. It is now realized for example that microbial metabolism provides a safer and less expensive alternative to physicochemical methods of degrading pesticides (Pandey and Jain, 2002). Microorganisms are highly adaptable to changes in the environment and consequently may evolve the genes that specify the degradation of target compounds (Ogawa et al., 2003). There is a great deal of variability however in pesticide toxicity among microbial species as sometimes pesticides may reduce certain microorganism populations while at other times pesticides stimulate the growth of some species (Iyaniwura, 1991).

2.1.2 Environmental factors affecting pesticide toxicity

A large number of environmental factors influence the toxicity of pesticides to microorganisms (Sanchez et al., 2004). For example, pH, temperature, and the level of other organic compounds present will affect the toxicity of pesticides. Picton and Farenhorst (2004) found that herbicide sorption generally increased with increasing soil

organic content, but the extent of 2, 4-D sorption per unit organic carbon varied among the soils due to differences in soil pH, clay content and/or organic matter quality. Natural processes of detoxification of compounds carried out by organisms are also important factors affecting toxicity. Some compounds are less easily absorbed and persistent in the body in their original form (e.g. the insecticide DDT) than when they have been metabolised (e.g. to DDE) by organisms (Kiely, 1997). Thus in an attempt to detoxify compounds, the organism may produce an end product which is in fact more toxic.

Some environmental parameters not only affect the metabolism of organisms themselves but also influence the bioavailability of pesticides to organisms (Kiely, 1997). Chemicals that are released into the atmosphere, water or soil are often transformed from one form to another, e.g. elements may be transformed from an inorganic state to an organic state or vice versa. These chemical changes may be brought about by oxidation, methylation or other chemical processes in the soil, water or air and often result in an increase in toxicity of the compound. For example, mercury (Hg), a compound released in large concentrations from crematoria and formerly a fungicide widely used in agriculture, in its inorganic form, is virtually unavailable to biological systems and hence has no toxicity. However, when the mercury is transformed into methylated mercury by bacteria and fungi in soil and water, it becomes extremely toxic to biological systems (Kiely, 1997).

2.1.3 Microbial degradation strategies

Microorganisms can be classified by cell carbon source, electron donor or acceptor, as well as end products and these are summarized in Table 2.1-1 (Metcalf and Eddy, 2003).

Table 2.1-1 Classification of microorganisms by electron donor, electron acceptor, source of cell carbon, and end products. Adapted from (Metcalf and Eddy, 2003)

Type of Bacteria	Common Reaction Name	Carbon Source	Electron Donor	Electron Acceptor	End Products
Aerobic Heterotrophic	Aerobic Oxidation	$C_xH_yO_z$	$C_xH_yO_z$	O_2	CO_2, H_2O
Aerobic Autotrophic	Nitrification	CO_2	NH_3^-, NO_2^-	O_2	NO_2^-, NO_3^-
	Iron Oxidation	CO_2	Fe^{2+}	O_2	Fe^{3+}
	Sulfur Oxidation	CO_2	H_2S, S^0, $S_2O_3^{2-}$	O_2	SO_4^{2-}
Facultative Heterotrophic	Denitrification (Anoxic Reaction)	$C_xH_yO_z$	$C_xH_yO_z$	NO_2^-, NO_3^-	N_2, CO_2, H_2O
Anaerobic Heterotrophic	Acid Fermentation	$C_xH_yO_z$	$C_xH_yO_z$	$C_xH_yO_z$	VFAs
	Iron Reduction	$C_xH_yO_z$	$C_xH_yO_z$	Fe^{3+}	Fe^{2+}, CO_2, H_2O
	Sulfate Reduction	$C_xH_yO_z$	$C_xH_yO_z$	SO_4^{2-}	H_2S, CO_2, H_2O
	Methanogenesis	$C_xH_yO_z$	VFAs	CO_2	CH_4

Note: $C_xH_yO_z$ -organic compounds

Oxygen is the most common final electron acceptor for microbial respiration, and aerobic processes provide the highest amount of energy available to cells (Fig. 2.1-1 (Field et al., 1995)). In chemotrophic reactions, a portion of the substrate is oxidized to obtain energy and another part is assimilated into cell mass. In aerobic respiration, oxygen not only is the electron acceptor but also participates in activation of the substrate via oxygenation reactions (Diaz, 2004). However, many polluted environments are often anoxic, e.g. aquifers, aquatic sediments and submerged soils. In such environments, biodegradation is carried out by either strict anaerobes or facultative microorganisms using alternative electron acceptors, such as nitrate (denitrifying organisms), sulfate (sulfate reducers), Fe (III) (ferric-ion reducers), CO_2 (methanogens), or other acceptors (chlorate, Mn, Cr, etc.) (Fig. 2.1-1) (Widdel and Rabus, 2001; Gibson and Harwood, 2002; Lovley, 2003). In terms of energy, using nitrate and Fe (III) as terminal electron acceptors is almost as efficient as using oxygen, however, sulfate reducers and methanogenic conditions generate comparatively much less energy (Fig. 2.1-1) (Field et al., 1995).

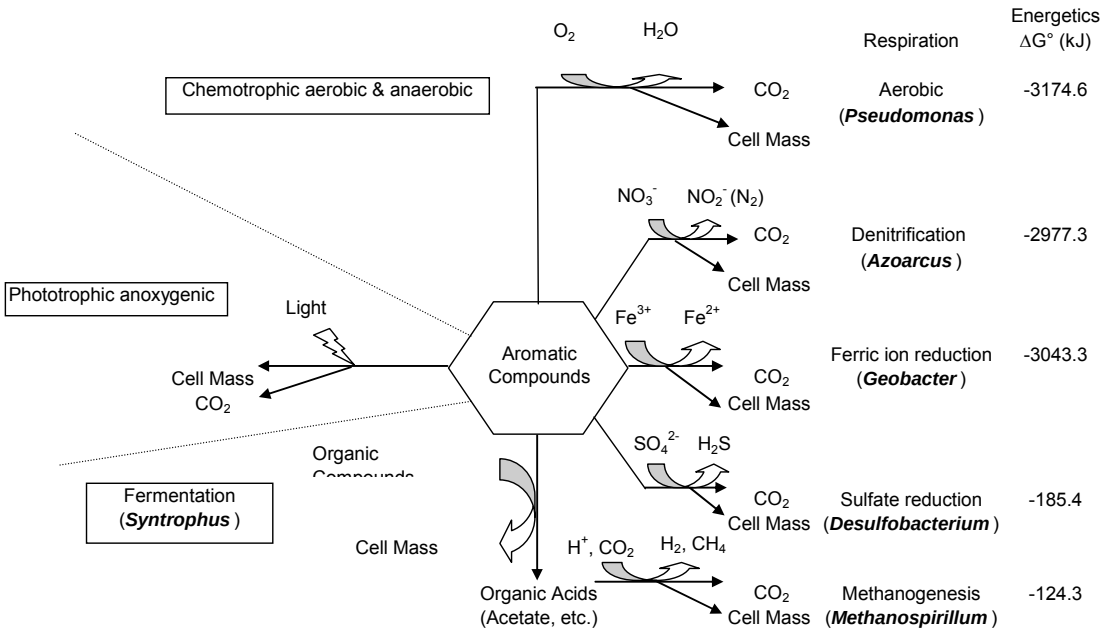


Figure 2.1-1 Microbial Utilization of Aromatic Compounds. Bacterial genera representative of each type of metabolism are shown in parentheses. Adapted from (Widdel and Rabus, 2001).

Most organisms are endowed with detoxification abilities, i.e. mineralization, transformation and/or immobilization. The abundance of microorganisms, together with their great ability for horizontal gene transfer and their high growth rates, allows them to evolve quickly and to adapt to environmentally changing conditions, even to extreme environments that do not allow proliferation of other living organisms (Diaz, 2004). The great gene diversity of microorganisms accounts for their great metabolic versatility (De Lorenzo, 2001; Lovley, 2003). By expressing different catabolic (biodegradative) pathways, microorganisms can use a wide array of compounds as sole carbon and energy sources (Harayama and Timmis, 1992). For example, many aromatic compounds which are widely used in pesticide formulations have become major environmental pollutants due to the thermodynamic stability of the benzene ring which increases their persistence in the environment. Aromatic compounds however are the second most abundant class of organic compounds in nature (next to carbohydrates) and therefore serve as an important growth substrate for many organisms. Almost thirty years ago it was found that aromatic compounds can be metabolized by bacteria even under anoxic conditions and that the aromatic ring becomes reduced (Evans, 1977; Evans and Fuchs, 1988). A wide phylogenetic diversity of microorganisms has been

observed to use such compounds ranging from a single bacteria strain to a syntrophic bacterial consortium (Dagley, 1986; Harayama and Timmis, 1992; Field et al., 1995; Andserdon and Lovley, 2000; Gibson and Harwood, 2002).

Adequate acclimation has been the key issue in achieving the degradation of some recalcitrant compounds (Spain and Van Veld, 1983). The acclimation duration, when mixed cultures are exposed to new or unusual chemicals, ranges from a few hours to several weeks and/or months and depends on the quantity and quality of microorganisms, as well as on the conditions under which acclimation is carried out (Moreno and Buitron, 2004a). Generally, where induced enzymes are involved, such an induction occurs over relatively short periods (minutes to days), whereas more fundamental changes, such as the modification of the structure of enzymes or the emergence of mutants usually take much longer (weeks to years) (Buitron and Capdeville, 1995). It has been noted that the induction process for biodegrading toxic compounds is favored by the dynamic conditions of operation that prevail in discontinuous systems (Ellis et al., 1996) such as Sequencing Batch Reactors (SBR). The periodic operation of an SBR imposes selective pressures that can enhance the growth of microbial populations able to degrade problematic compounds (Irvine and Ketchum, 1989; Wanner, 1992). However, it has been observed that the degree of adaptation varies depending on the acclimation procedure. Watson (1993) found differences with two procedures of acclimation and suggested that the acclimation in a single flask was more effective for the biodegradation of six chemical compounds, than the acclimation by the enrichment or multiple transfer procedure. The acclimation performed by Moreno and Buitron (2004b) found that a variable time (fixing a removal efficiency) strategy produced a microbial community with higher specific activity on the 4-chlorophenol degradation compared with those obtained for the fixed time (fixing the reaction time independent of the removal efficiency) strategy.

In general, it is recommended that activated sludge be used as a suitable inoculum to treat toxic compounds due to its microbial diversity (Spain and Van Veld, 1983; Watson, 1993). Semple et al. (2001) pointed out that sludge is a source of microorganisms such as bacteria, actinomyces and fungi, capable of degrading pollutants to harmless compounds like CO_2 and H_2O. They can also transform compounds into other, less toxic ones and/or retain them in the organic matrix and

therefore reduce their bioavailability. However, the diversity of the microbial population may vary depending on the type and composition of wastewater treated and this may explain the differences observed with respect to the degree of degradation of some toxic compounds (Jianmin et al., 1993).

2.1.4 Pesticide biodegradation potential

The aerobic biodegradability of pesticides has been studied extensively over the last two decades (Shaler and Klecka, 1986; Oleszkiewicz et al., 1991; Basu and Oleszkiewicz, 1995; Mangat and Elefsiniotis, 1999; Yoong et al., 2000; Chiavola et al., 2004; Moreno and Buitron, 2004b). However, due to their high energy costs and abundant sludge production, aerobic processes are not considered to be practicable. Pesticide biodegradation under anaerobic conditions such as in groundwater, sediments, landfills, sludge digesters and anaerobic bioreactors has therefore gained increasing attention. Anaerobic degradation may occur in environments that are not entirely oxygen free but where the oxygen supply is limited and hard to control. This is true for anoxic polluted aquifers that are characterized by little oxygen access and a variety of pollutants that are sufficient to consume not only the available oxygen but also reduce other electron acceptors present (especially Fe (III) oxides and sulphate) (Schink, 2002). Some aromatic hydrocarbons such as benzene, toluene, xylene and ethylbenzene compounds are now known to be mineralized under anaerobic conditions under a variety of electron accepting conditions ranging from methanogenic, sulphate-reducing, iron-reducing, manganese-reducing and denitrification (Frazer et al., 1995; Kazumi et al., 1997; Langenhoff et al., 1997; Heider et al., 1999). Anaerobic biodegradation of benzene is relatively slow and laboratory enrichment cultures required almost two years to mineralise 225 μM of benzene under methanogenic conditions (Kazumi et al., 1997). By comparison, anaerobic toluene degradation is more rapid with doubling times as little as 6 hr observed for denitrifying bacteria (Heider et al., 1999). The utilization of chlorobenzoates was also found to be the fastest under denitrifying conditions as compared with other reducing conditions such as iron-reducing, sulfate-reducing and methanogenic conditions (Kazumi et al., 1995). However, one disadvantage of anaerobic bacteria is their slow growth and the corresponding problem of long detention times, though this can be overcome by advanced reactor concepts such as fixed and fluidised-bed reactors, or UASB technology (Schink, 2002).

Under anaerobic conditions, the enzymatic reactions common to many pesticides include dechlorination, hydrolysis, nitro reduction, and dealkylation (Willams, 1977). Figure 2.1-2 describes the anaerobic reactions of three structurally-distinct pesticides, 2, 4-dichlorophenoxyacetic acid (2, 4-D), parathion (o,o-diethyl-o-p-nitropheno phosphorothioate) and atrazine.

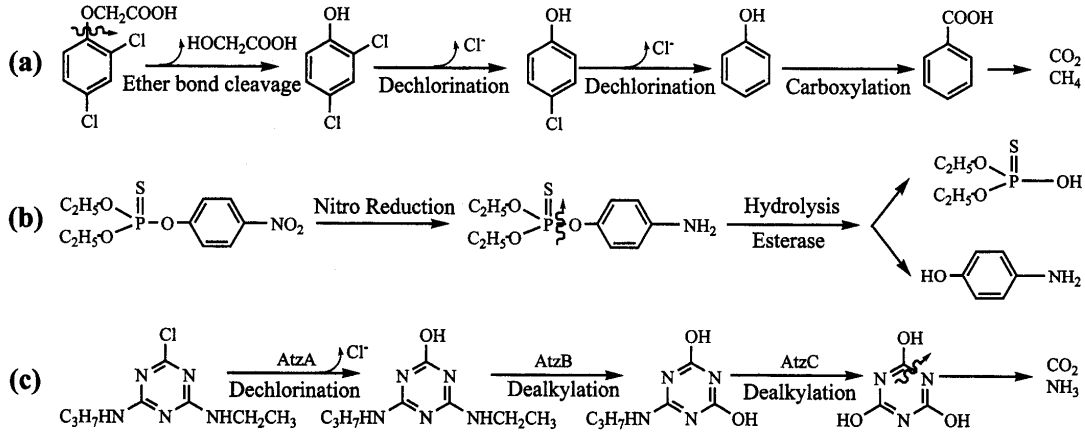


Figure 2.1-2 Anaerobic pathways for the biodegradation of three selected pesticides

a) **2,4-dichlorophenoxyacetic acid (2,4-D),** b) **parathion (o,o-diethyl-o-p-nitropheno phosphorothioate), and** c) **atrazine. Adapted from (Zhang and Bennett, 2005)**

Reductive dechlorination is common to all halogenated pesticides (Fig.2.1-2 a, c), including aliphatic (fumigants), cyclic aliphatic (lindane), aromatic (DDT; PCP), phenoxyalkanotes (2, 4-D), aniline-based (alachlor), and cyclodiene (aldrin) (Cookson, 1995). While lightly halogenated pesticides are more biodegradable under aerobic conditions, it is commonly believed that highly halogenated pesticides often biodegrade more rapidly under anaerobic conditions (Van Eekert and Schraa, 2001; Zhang and Bennett, 2005). Hydrolysis of phosphate esters, catalyzed by esterase, is an important mechanism for organophosphate pesticides (Fig.2.1-2 b). The degradation of the nitrogen-containing pesticide atrazine shown in Fig. 2.1-2 c (partially aerobic processes) involves hydrolytic dechlorination, dealkylation, and the cleavage of C-N in the cyclic ring, yielding ultimate mineralization to CO_2 and NH_3 (Zhang and Bennett,

2005). Van Eekert and Schraa (2001) point out that the microbial degradation mechanism of chlorinated compounds depends on the nature of the redox conditions and the presence and activity of the (co)-enzymes required for transformation. In addition, methanogenic and acetogenic bacteria are also able to reduce chlorinated compounds via a cometabolic process which does not yield energy; that is, another substrate providing a carbon and energy source is required to support growth of cometabolically dechlorinating bacteria.

2.1.5 2, 4-D

2, 4-D is a chlorinated phenoxy compound that functions as a systemic herbicide and is used to control many types of broadleaf weeds. It may be found in emulsion form, in aqueous solutions (salts), and as a dry compound. It bears the Signal Word DANGER-POISON because 2, 4-D has produced serious eye and skin irritation among agricultural workers (Kamrin, 1997). There are many forms or derivatives of 2, 4-D including esters, amines and salts. Its molecular structure is shown in Figure 2.1-3.

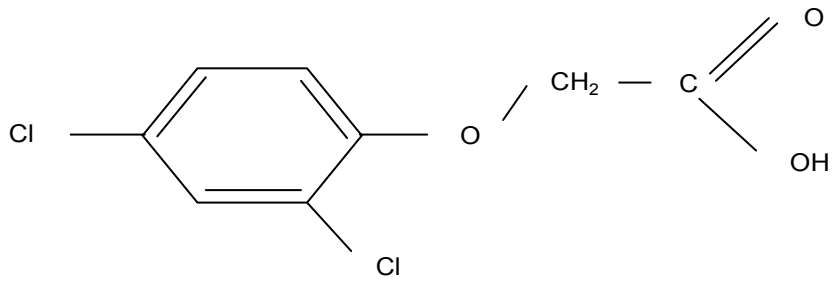


Figure 2.1-3 Molecular structure of 2, 4-D

The main physical-chemical properties of 2, 4-D are presented in Table 2.1-2 (Kamrin, 1997).

Table 2.1-2 Physical-chemical properties of 2, 4-D

Chemical Formula	$C_8H_6Cl_2O_3$
Molecular Weight	221.04 g/mol
Appearance	White to yellow powder, phenolic odour
Melting point	134-140 °C
Solubility in water	900 mg/L @ 25 °C
Boiling point	160 °C
Half life in soil	10 to 50 days
Half life in water	1 week to several weeks under oxygenated conditions

2.1.6 Biosorption of pesticides

Assessment of a microorganism's ability to utilise a target compound usually starts with liquid culture experiments where the compound is added to the medium either as a sole carbon source or in the presence of a growth supporting substrate. In these types of experiments, often a decrease in the concentration of the target compound is not completely attributed to 'degradation'. A reduction in the concentration of the target compound may occur due to surface binding of the compound to the biomass. The special surface properties of bacteria, yeasts, fungi and algae enable them to adsorb different kinds of pollutants from solutions. "Biosorption" is the term used to indicate a number of metabolism-independent processes (physical and chemical adsorption, electrostatic interaction, ion exchange, complexation, chelation, and microprecipitation) taking place essentially in the cell wall rather than oxidation through anaerobic or aerobic metabolism (biodegradation) (Aksu, 2005). Although using fungal, yeast and bacterial biomass to remove organic pollutants in a wastewater is still in the research stage, microbial adsorption is regarded as a promising alternative to replace or supplement present treatment processes for the removal of very high concentrations of dyes and very low concentrations of phenolics and pesticides from the wastewater (Benoit et al., 1998; Hong et al., 2000; Aksu, 2005).

2.2 Biological denitrification

2.2.1 Introduction

Rising nitrogen (N) pollution is indicated by the deterioration of water quality in aquifers, the eutrophication of receiving water bodies and the rise in N-related health problems, all of which have triggered extensive treatment of N-rich effluents. Excessive application of fertilizers and intensive animal rearing are regarded as the major sources of N-pollution (Choi et al., 2004). The common method of treating N-pollution is nitrification followed by denitrification (Bilanovic et al., 1999). Nitrification converts ammonia to nitrite and then to nitrate and while in many cases, conversion of ammonia to nitrate provides adequate treatment, it is often essential to remove nitrates by encouraging denitrification. Biological denitrification represents an important part of the biogeochemical cycle of the element nitrogen and constitutes the predominant pathway of the reductive dissimilation of nitrate in the environment. Nitrate is transformed via four enzymatic reactions stepwise to nitrite (NO_2^-), nitric oxide (NO), and nitrous oxide (N_2O), to finally yield dinitrogen gas (N_2) (Einsle and Kroneck, 2004).

2.2.2 Microbiological and biochemical characteristics

Denitrification is widespread in nature. Denitrifiers are common among the Gram-negative *Proteobacteria*, such as *Pseudomonas*, *Alcaligenes*, *Paracoccus*, and *Thiobacillu*s. Some Gram-positive bacteria, including *Bacillus*, can denitrify and even a few halophilic Archaea, such as *Halobacterium*, are able to denitrify. All the denitrifiers are facultative aerobes, which means that they shift to NO_3^- or NO_2^- respiration when O_2 becomes limiting (Rittmann and McCarty, 2001). Because of their great metabolic diversity, denitrifiers are commonly found in soils, sediments, surface waters, groundwaters, and wastewater treatment plants.

The oxygen concentration controls whether or not facultative aerobes respire with nitrates. Research has found that denitrification can occur when D.O. concentration is well above zero (Rittmann and Langeland, 1985; deSilva, 1997). However, very low concentrations of the electron donor or too high concentrations of D.O. can lead to

accumulation of denitrification intermediates: NO_2^-, NO_2, and N_2O (Rittmann and McCarty, 2001). The latter two are greenhouse gases whose release should be avoided.

Like other biological reactions, temperature and pH affect the rate of denitrification. Temperature generally exerts a stronger effect below 15 ºC than above it (Rittmann and McCarty, 2001; Carrera et al., 2003), which is consistent with Elefsiniotis and Li (2006), who explored the effect of temperature on denitrification using volatile fatty acids and found that a temperature change from 10 to 20 °C exerted a larger effect on both the specific denitrification and carbon consumption rates than a temperature increase from 20 to 30 °C. In general, for any given temperature, the highest rates occur within the pH range of 7.0 and 7.5 and pH values outside the optimal range of 7 to 8 can lead to accumulation of intermediates (Glass and Silverstein, 1998). Denitrification will produce alkalinity, so the pH will increase if high concentrations of NO_3^- are to be removed (USEPA, 1993).

Although heterotrophic denitrifiers exhibit an affinity for a wide range of organic substrates, a few simple substrates have been intensively studied primarily because many systems have high NO_3-N levels but little or no BOD (Rittmann and McCarty, 2001). Thus, early research addressed exogenous electron donors (carbon sources) where simple compounds purchased in bulk quantity were evaluated e.g. methanol, acetate, glucose, ethanol, and a few others (Akunna et al., 1993; Constantin and Fick, 1997; Thalasso et al., 1997; Bickers and van Oostrom, 2000). Because methanol (CH_3OH) was relatively inexpensive, it gained widespread use and a very large database on methanol has been developed (Akunna et al., 1993; Thalasso et al., 1997; Gomez et al., 2000). However, use of exogenous carbon sources can represent a significant cost, and alternative inexpensive but effective carbon sources would be preferable, especially since the cost of methanol has risen in recent years.

2.2.3 Variety of organic materials used for denitrification

Different criteria have been used to choose a specific external carbon source for denitrification. First, as shown in Table 2.2-1, Elefsiniotis et al. (2004) have indicated that denitrification rates are strongly dependent on the type of organic carbon source.

Table 2.2-1 Summary of denitrification rates achieved with various organic carbon substrates. Adapted from (Elefsiniotis et al., 2004)

Organic C source	Denitrification rate	Reference
Methanol	0.289 g NO_3-N/g VSS per day	Xu (1996)
Methanol	2.0 g NO_3-N/m^2 per day	Aspergren et al. (1998)
Methanol	1.9 kg NO_3-N/m^3 per day	Rajapakse and Scutt (1999)
Acetate	2.1 kg NO_2-N/m^3 per day	Rahmani et al.(1995)
Acetate	0.603 g NO_3-N/g VSS per day	Xu (1996)
Acetate	0.091 g NO_3-N/g COD_{tot} per day	Moser-Engeler et al. (1998)
Acetate	0.016 g NO*x*-N/g VSS per day	Li (2001)
Propionate	0.041 g NO_3-N/g COD_{tot} per day	Moser-Engeler et al. (1998)
Propionate	0.008 g NO*x*-N/g VSS per day	Li (2001)
Propionate	0.362 g NO_3-N/g VSS per day	Xu (1996)
Acetate and propionate	0.014 g NO*x*-N/g VSS per day	Li (2001)
Butyrate	0.519 g NO_3-N/g VSS per day	Xu (1996)
Valerate	0.487 g NO_3-N/g VSS per day	Xu (1996)
Mixed VFA	0.36 g NO_3-N/g SS per day	Fass et al.(1994)
Mixed VFA	0.754 g NO_3-N/g VSS per day	Xu (1996)
Effluent VFA	0.57 g NO_3-N/g VS per day	Æsøy and Ødegaard (1994)
Effluent VFA	0.22 g NO*x*-N/g MLSS per day	Hatziconstantinou et al.(1996)
Effluent VFA	0.144 g NO_3-N/g COD_{tot} per day	Moser-Engeler et al. (1998)
Effluent VFA	0.28 g NO_3-N/g VSS per day	Pavan et al. (1998)
Effluent VFA	0.054 g NO_3-N/g VSS per day	Llabres et al.(1999)

Note: The rates included are not directly comparable due to many different reporting units, reactor configurations, scales, temperatures and waste types (i.e. real versus synthetic sewage).

Published references, however, sometimes give conflicting results. For example, some authors suggest that acetic acid achieves greater rates than glucose, methanol or ethanol (Constantin and Fick, 1997), while other authors show denitrification rates with acetic acid similar to those achieved with methanol (Nyberg et al., 1992). Several references also indicate that ethanol has a higher rate than methanol (Christensson et al., 1994; Andersson et al., 1998), while an earlier study indicated that the rate with methanol was greater than that with ethanol (Henze, 1991).

No matter what kind of chemical is used, it constitutes a cost to the operation which must be considered. It is also necessary to consider the availability of the external carbon source. For example, an alternative to pure chemical compounds is to use a by-product as the carbon source, for example, municipal and agricultural effluents which are often rich in readily biodegradable volatile fatty acids (VFAs). Oleszkiewicz et al. (1991) found that denitrification in anoxic reactors was strongly dependent on the presence of easily available carbon. In addition, the effluent from the anaerobic fermentation of municipal solid waste may contain 25,000 mg VFAs/L (Sans et al., 1995) which on a carbon basis, is roughly equivalent to 35,000 mg/L of CH_3OH. The use of this effluent as a carbon source could help decrease denitrification operational expenses (Bilanovic et al., 1999). Lee et al. (2002) used anaerobically fermented leachate of food waste (AFLFW) in a biological nutrient removal (BNR) process and found that the addition of AFLFW as an external carbon source increased the removal efficiency of total nitrogen and phosphorus from 60% and 2% to 77% and 67%, respectively. The concentration of AFLFW was calculated on the basis of the theoretically required amount (2.86 kg COD/kg NO_3-N). Chen et al. (2004) also found that using swine waste as an electron donor for denitrification in a SBR could achieve nitrogen removal efficiencies of 95%. When an organic waste is used as an external carbon source, its characteristics are important for determining the denitrification rate. For example, the supernatant from biologically hydrolysed sludge has led to a denitrification rate as high as that obtained with acetic acid (Kristensen and Jorgensen, 1990). In addition, thermally or chemically hydrolysed sludge showed a denitrification rate approximately half the acetic acid rate (Kristensen and Jorgensen, 1990).

2.2.4 Effect of the C/N ratio on the denitrification process

The effect of influent C/N (carbon to nitrate-nitrogen) ratios on denitrification has been investigated by many authors in the last three decades (Narkis et al., 1979; Skrinde and Bhagat, 1982; Her and Huang, 1995; Carrera et al., 2004). For example, Elefsiniotis and Li (2006) indicated that a C: N ratio of 2:1 was sufficient for complete denitrification using acetic and propionic acid as carbon sources. Theoretically, 2.86 g of COD is needed for complete removal of 1 g NO_3-N. Due to synthesis of denitrifying bacteria, more carbon is needed in practice for a complete denitrification (Dangcong et al., 2004). Carrera et al. (2004) reported that the influent chemical oxygen demand to nitrogen (COD/N) ratio directly influences the competition for growth among many different microbial populations. In their study, a COD/N ratio of 7.1 g COD/ g N was required to achieve total denitrification.

Her and Huang (1995) indicated that the denitrification efficiency and the C/N ratio would be significantly influenced by both the chemical structure and the molecular weight of the carbon source used. In their study, the minimum C/N ratio required for near complete denitrification (92-99%) using an aromatic carbon source (benzoic acid) ranged from 3.0 to 3.6. These experimental values were significantly higher than those of using other non-aromatic sources (such as methanol, acetic acid and glucose which ranged from 0.9 to 2.0). It is suggested that aromatic compounds are more difficult to degrade than non-aromatic compounds because of their structure (e.g. the 6 carbon compound benzene vs. glucose). In addition, not all aromatic compounds may be able to be degraded fully. For non-aromatic carbon sources, the minimum C/N ratio required for near complete denitrification (97-100%) increased with an increase in molecular weight. If insufficient carbon was supplied for denitrification, accumulation of intermediate nitrite species could be observed. Their research also suggested that the actual C/N ratio for complete denitrification is larger than the theoretical value based on the stoichiometry, usually by up to 20 to 30% because some of the carbon is used in new biomass formation.

In recent years, some researchers have considered that the C/N ratio could be one of the specific parameters influencing nitrous oxide (N_2O) production during biological nitrogen removal. Kishida et al. (2004) investigated the effect of the C/N ratio on N_2O

emission from a swine wastewater treatment process and found that the total N_2O emission with a BOD/TN ratio of 2.6 was 10 times greater than that with a BOD/TN ratio of 4.5. In the latter case, both successful nitrogen removal and N_2O emission control were attained simultaneously. It was observed by Itokawa et al. (2001) that 20-30% of influent nitrogen was emitted as N_2O in bioreactors with influent COD/N ratios less than 3.5. A tracer study showed that this N_2O originated from denitrification in the anoxic phase. However, the N_2O reduction capacity of the denitrifiers was always larger than the NO_3-N or NO_2-N reduction capacity. It was suggested that a high N_2O emission rate under low COD/N ratio operations was mainly due to endogenous denitrification using NO_2-N in the latter part of the anoxic phase. The NO_2-N build-up was attributed to the difference between the NO_3-N and NO_2-N reduction capacities which was the feature observed only in low COD/N ratio operations.

2.2.5 The potential of denitrifiers to degrade toxic compounds

While various bacterial species have the ability to degrade synthetic chemical compounds, denitrifying microorganisms, those which biologically reduce nitrate, are of particular interest for bioremediation applications for two important reasons. First, denitrifiers are widely distributed in soils, sediments and wastewater treatment plants. Second, increasing nitrate-pollution problems required novel and cost-saving strategies to enhance nitrate removal. There is a limitation to the traditional denitrification process with commonly used external carbon sources because of the cost. The work of others has demonstrated that denitrifiers may utilize a variety of toxic compounds as a sole carbon source. For example, Rockne and Strand (2001) have shown that highly-enriched denitrifying cultures can mineralise bicyclic and polycyclic aromatic hydrocarbons. Mineralization was nitrate dependent and the cultures produced N_2O, a denitrification product when supplied with PAHs as the sole carbon and energy source. Reyes-Avila et al. (2004) also indicated that denitrification was a feasible process for the simultaneous removal of carbon, nitrogen and sulphur from effluents of the petroleum industry. Aslan and Turkman (2005) observed a high removal efficiency of nitrate with the pesticides, trifluralin, fenitrothion and endosulfan ($\alpha + \beta$) in a biological denitrification continuous reactor packed with wheat straw as carbon source and which simultaneously provided a platform to support microorganism growth. Sherwood et al. (1998) also proved that a broad denitrifying consortium was capable of degrading

chlorinated solvents. In view of this research, it appears that an integrated biological denitrification and toxic pollutant degradation approach has the potential to be an effective tool for environmental clean-up of pesticides.

2.3 Anaerobic digestion

2.3.1 Introduction

Anaerobic treatment technologies are used throughout the world for the effective treatment of municipal wastewaters and sludges as well as for the treatment of a wide variety of industrial wastewaters. Anaerobic technologies are particularly attractive to industrial and municipal officials in developing countries, since the energy required for operating the process is minimal compared to the energy required for aerobic processes. The anaerobic digestion process is a naturally occurring decomposition process which is not a recent development. At least 100 years ago in Vesoul, France, Louis H. Mouras designed and constructed fermenters to treat sludges obtained from domestic wastewater. Abbe Moigno, in 1881, reported that anaerobic digestion of domestic wastes was completed in about eighteen days (Malina and Pohland, 1992). During this process a variety of microorganisms convert organic material into biogas, leaving solid and liquid residues. The degraded organic material is a complex mixture of primarily carbohydrates, proteins and lipids. The biogas is primarily methane (CH_4) and carbon dioxide (CO_2) but it may contain trace amounts of nitrogen (N_2), ammonia (NH_3) and hydrogen sulphide (H_2S), depending on the degraded organic material. The overall process can be simplified to:

$$\text{Organic matter} + H_2O \xrightarrow{\text{anaerobes}} CH_4 + CO_2 + \text{New biomass} + NH_3 + H_2S + \text{heat}$$

2.3.2 Major biochemical processes of anaerobic digestion

At the simplest level, the complete process of anaerobic digestion can be divided into three sequential stages which involve specific types of microorganisms and biochemical reactions (Fig. 2.3-1).

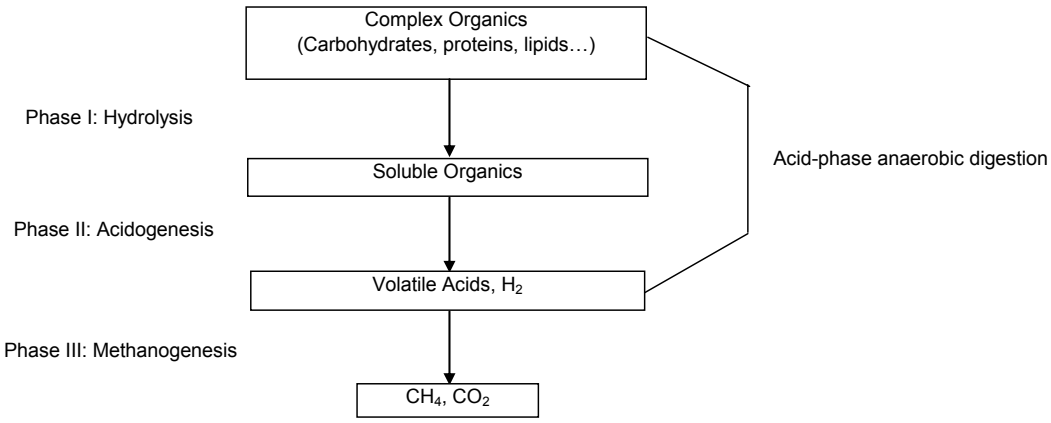


Figure 2.3-1 Three phases of anaerobic digestion

First, complex insoluble organic polymers are solubilised and broken down into organic monomers by extracellular enzymes produced by hydrolytic microorganisms. Proteins, carbohydrates (such as starch and cellulose) and fats are broken down into amino acids, simple sugars and long chain fatty acids, respectively. The main bacterial species of hydrolytic fermentative organisms belong to the genera of *Clostridium*, *Eubacterium* and *Peptococcus* (Dewison, 1998).

Secondly, soluble organic compounds produced in the hydrolysis stage are fermented by acid-forming bacteria (acidogens) to volatile fatty acids, carbon dioxide, and some hydrogen gas as well as other simple organic compounds. Some bacterial species of acidogens are from the family of *Streptococcaceae* and *Enterobacteriaceae* and belong to the genera of *Bacteriods*, *Clostridium*, *Butyrivibrio*, *Eubacterium* and *Lactobacillus* (Banerjee et al., 1998). The primary volatile fatty acids produced are acetic, propionic, and butyric acids. However, other volatile acids frequently are found in smaller quantities and these include: formic, valeric, isovaleric, and caproic acids.

Volatile fatty acids become the substrate in the third stage for methane-forming bacteria (methanogens) that convert the acids to methane and carbon dioxide. The principal organic compound that is utilized by methane-producing bacteria is acetic acid, although almost all volatile acids can be used by specific species of methane-producing bacteria. The most predominant bacterial species of methanogens belong to the genera of *Methanobacterium*, *Methanobacillus*, *Methanococcus* and *Methanosarcina* (Tchobanoglous et al., 1993).

The mechanism of anaerobic digestion of sludge is sequential in nature; however, acid fermentation and methane fermentation take place simultaneously and synchronously in a well-buffered, actively-digesting system. Effective anaerobic digestion requires the maintenance of a balance among the rates of acid production and conversion of volatile acids to methane. Therefore, it is essential that the environment in the anaerobic digestion tank be maintained at conditions optimum for both acidogenic and methanogenic growth. However, in reality, conditions that favour acidogens, such as a short HRT and low pH, are inhibitory to methanogens which are strict anaerobes and extremely sensitive to changes in temperature and pH (Ince, 1998). This problem has led researchers to develop a two phase digester, which physically separates the acidogenesis phase from the methanogenesis phase and allows a more heterogeneous and stable population of bacteria to be selected and enriched in each digester (Ince, 1998). The system efficiency and stability are enhanced by providing the optimal conditions for both acidogens and methanogens (Chyi and Dague, 1994). Overall organic matter degradation rates can thus be increased by separately optimising conditions for each bacterial group (Yu and Fang, 2003).

2.3.3 Acid-phase anaerobic digestion

Much of the research on anaerobic digestion has been focused on the rate-limiting, methanogenic phase as it is the energy yielding phase. However, in wastewater treatment applications, the soluble organic products of acidogenic activity can be used as an energy and carbon source for microorganisms carrying out other processes, such as biological phosphorus or nitrogen removal (Elefsiniotis and Oldham, 1994). It has been observed that the presence of VFAs in the influent to a biological nutrient removal (BNR) facility can significantly improve the phosphorus or nitrogen removal capacity of the process (Danesh and Oleszkiewicz, 1997; Christensson et al., 1998; Elefsiniotis et al., 2004). The VFA concentration in the wastewater entering a BNR plant can be increased by the addition of pre-formed VFAs such as sodium acetate or by controlled primary sludge digestion with return of the digested material to the main bioreactor (Bouzas et al., 2002). For this purpose, acid-phase anaerobic digestion has received increasing attention in wastewater treatment research.

Acid-phase anaerobic digestion products are greatly influenced by parameters such as retention time, pH, temperature, wastewater characteristics, reactor configuration, trace minerals and oxidation-reduction potential.

2.3.3.1 pH

Since pH affects the growth rate of micro-organisms, pH changes may cause significant shifts in the relative numbers of different species in a heterogeneous population such as is present in an acidogenic reactor (Horiuchi et al., 1999). Many aspects of microbial metabolism are greatly influenced by pH variations over the range within which the microorganisms can grow. These aspects include utilisation of carbon and energy sources, efficiency of substrate degradation, synthesis of proteins and various types of storage material, and release of metabolic products from cells. Moreover, pH variations can affect cell morphology and structure and, therefore, flocculation and adhesion phenomena (Gottschalk, 1986). A substantial number of studies have been carried out on the effect of pH on acidogenesis of wastewaters and these have found that the optimal pH range for acidogenesis varies with waste composition. For example, Yu and Fang (2003) investigated the influence of pH on the acidification of a synthetic gelatin-based wastewater and found that the gelatin degradation efficiency substantially increased with pH, from 60.0% at pH 4.0 to 97.5% at pH 7.0. In addition, the degree of acidification increased from 32.0% at a pH 4.0 to 71.6% at pH 6.5, but dropped to 66.8% when the pH increased to 7.0. The optimum pH for the overall acidogenic activity was found to be 6.0. Ince (1998) observed an optimal pH of 5.7-5.8 for dairy wastewater while Chyi and Dague (1994) observed an optimal pH of 5.6 for a synthetic cellulose wastewater. Finally, an optimum pH range of 5.0-5.5 for a synthetic carbohydrate-rich wastewater has been found for glucose (Zoetemeyer et al., 1982a), lactose (Kissalita et al., 1987) and sucrose (Zoetemeyer et al., 1982b).

2.3.3.2 Temperature

It has been reported that there are two efficient temperature regions for the anaerobic degradation process: a mesophilic range with an optimum temperature of around 35-37°C and a thermophilic range with an optimum temperature of around 55-60°C (Zoetemeyer et al., 1982a). In between these two temperature ranges, e.g. 45-50°C, both the degradation efficiency and rate decrease sharply. In most cases, methanogenesis is

the rate-limiting step for the overall degradation process, thus the anaerobic reactor should be operated around 37°C or 55°C to ensure methanogens grow at their optimum temperatures. However, acidogens are not as sensitive to temperature changes as methanogens. As demonstrated by Yu and Fang (2003), temperature had little influence on gelatin degradation and the degree of acidification. Interestingly, Bouzas et al (2002) reported higher VFA yields with an increase in temperature from 20°C to 30°C, mainly due to an improvement in the hydrolysis of particulate organic matter. Lipids and proteins were found to be more soluble as the temperature increased from 14°C to 30°C, making them more readily biodegradable (Ruel et al., 2002). Like pH, the optimum temperature range also varies slightly with waste composition (Banerjee et al., 1998).

2.3.3.3 Solids retention time (SRT)

The SRT affects the mix of organisms which eventually predominate in a system because it tends to select organisms in accordance with their generation times. The physiology, environmental requirements, and growth kinetics of the acidogenic and methanogenic groups of microbes may differ greatly from each other. It has been observed that the maximum specific growth rate of acid-producing bacteria can be up to one order of magnitude higher than that of methane-producing organisms (Elefsiniotis and Oldham, 1994). This suggests that it is possible to maximize net VFA production in an acid-phase digester by operating the system at an SRT below some critical value. The critical SRT can range from several hours to several days, depending on the wastewater source (Elefsiniotis and Oldham, 1994). Banister and Pretorious (1998) observed that acid fermentation proceeded rapidly at retention times of less than 6 days for primary sludge, with reduced VFA yields at 10 days. This work agrees with observations found by Skalsky and Diagger (1995) and Bouzas et al. (2002) when degrading primary sludge.

As mentioned, the predominant VFA species that usually form during anaerobic digestion are acetic, propionic and butyric acids (Yu and Fang, 2003); however, the SRT value can affect VFA speciation. For example, Elefsiniotis and Oldham (1994) observed that SRTs of more than 10 days encouraged the generation of isobutyric acid and the three isomers of valeric acid. These acids are associated with the fermentation

of proteins and are not normally generated under conditions of elevated partial pressures of hydrogen. Their work suggests that different metabolic pathways for VFA production may predominate at different SRT values.

2.3.3.4 Hydraulic retention time (HRT)

The HRT controls the contact time between bacteria and their feed sources, affecting the amount and type of substrate being used by the bacteria (Elefsiniotis and Oldham, 1994). However, it is difficult to assess the role of HRT independently of SRT, as SRT is usually similar to HRT in most research published, since non-recycle systems are often used. The optimum HRT value, like SRT, will depend on the particular type of wastewater being degraded.

The effect of HRT on VFA and COD production has also been investigated by researchers able to vary both HRT and SRT independently. For example, Elefsiniotis and Oldham (1994) reported an increase in VFA and COD production as the HRT increased from 6 to 12 hours, but beyond that there was a reduction mainly because of the onset of methanogenesis. Banerjee et al. (1998) observed a profound effect of HRT on the net VFA production with an increase in HRT from 18 to 30 hours, increasing the VFA production by 37% in a mixed wastewater and 14% in a primary sludge. Chyi and Dague (1994) reported that an HRT of 48 hours was required for acidogenesis of a synthetic carbohydrate-rich wastewater and observed little effect on acidogenesis when the HRT was increased incrementally up to 72 hours.

2.3.3.5 Organic loading rate

Ince (1998) showed that there were significant increases in effluent VFA with increases in the organic loading rate from 10 to 20 kg COD/m^3/day. Chyi and Dague (1994) also reported proportional increases in effluent soluble COD and VFA with increases in influent particulate COD. However, there is likely to be a maximum loading rate beyond which VFA production does not increase significantly, due to enzyme saturation (with high substrate levels) or enzyme inhibition (at high VFA levels).

2.3.3.6 Solids concentration

Anaerobic digestion is well known as a treatment process for high-strength wastes such as sludges and manures that contain elevated levels of suspended solids. When the majority of the organic material is insoluble, lengthy digestion periods are required to allow for the relatively slow biological processes of hydrolysis and solubilization of insoluble materials. Once solubilized, the dissolved organics can undergo further conversion to volatile organic acids and methane fairly rapidly. Banister and Pretorius (1998) investigated ways of optimising the performance of primary sludge acidogenic fermentation systems and suggested that it would be advantageous to limit influent solids concentration of the primary sludge to approximately 0.5 to 2% TS. Skalsky and Diagger (1995) also reported higher VFA yields at solids concentrations of less than 1% (m/v) compared to 2.6% (m/v) solids at 2 days of retention time. This may have been linked to better mixing or a reduction in inhibitory substance effects in the dilute reactor.

2.3.3.7 Presence of toxic compounds

Industrial wastewaters very often contain substances that have been related to toxic responses and, in some cases, to process failure in anaerobic bioreactors. The characteristic of the toxicity will depend on the nature of the toxic substance, its concentration, and the degree to which the process has become acclimated to it (Parkin and Owen, 1986). However, it should not be assumed that because a substance is known to be toxic to anaerobic bacteria, wastewaters containing that substance cannot be successfully treated. As with most microorganisms, anaerobic bacteria can develop a tolerance to a wide variety of inhibitors. With proper process design and adequate bacterial acclimation, some organic inhibitors can be biodegraded, and susceptibility to toxicants can often be reversed (Malina and Pohland, 1992).

2.3.3.8 Mixing

It is a common research method to continuously mix a digester contents with the aim of improving the contact between degrading bacteria and their substrates. Ruel et al. (2002) observed low VFA yields below stirring speeds of 300 rpm supporting field observations that mixed, heated digesters have a faster fermentation rate than unmixed,

unheated digesters. However, with the presence of some inhibitory substances, mixing may possibly disrupt the microenvironment where fermentation is taking place, as suggested by Banister and Pretorius (1998) who found higher VFA yields in an unmixed digester than in a mixed digester that was degrading the same type of wastewater.

2.4 Operation of sequencing batch reactor (SBR)

SBRs have experienced a rebirth in use since the early 1970s for the biological treatment of wastewater (Irvine and Davis, 1971). Owing to the nature of the process, and advances in process control technology, there is renewed interest in the SBR for the treatment of inhibitory industrial wastewaters. The batch discharge of treated effluent allows close monitoring of the wastewater and further treatment, if necessary, to meet discharge licence conditions. It is also uniquely suited for the selection and enrichment of desired microbial populations because of the ease with which a diverse array of operating strategies and selective pressures can be implemented (Buitron et al., 2005). That is, SBR reactors are very versatile and reactions and aeration times can be varied during a cycle period. The general operating steps in an SBR are shown in Figure 2.4-1 and include Fill, React, Settle, and Decant (Irvine and Ketchum, 1989).

The convenience of an SBR operation stems from the time-oriented nature of the process (Irvine et al., 1997). For example, organism selection can be controlled by manipulating physical variables such as the length of the Fill period, the oxygen tension in the reactor (anaerobic to aerobic), and the mixing pattern. During the React period, selective pressures can be applied by controlling the length of time the organisms are subjected to starvation conditions. After treatment, the microbes are allowed to separate by sedimentation during the period called Settle. The treated effluent is subsequently withdrawn from the reactor during an additional and distinct Decant period (Irvine et al., 1997).

The benefits of SBRs have been demonstrated for nutrient removal, the control of filamentous bacteria and the removal of specific organic compounds present in industrial wastewaters (Buitron et al., 2005). In particular, the operational flexibility of the SBR in terms of selection pressures led to the eventual formation of microbial granules with excellent settling characteristics and high specific tert-butyl alcohol

(TBA) biodegradation rates (Zhuang et al., 2005). The mechanism of granulation is mostly unknown, although it may be related to adaptation to inhibitory compounds and severe environments (Hu et al., 2005). This adaptation towards granule formation broadens the benefits of using the SBR to target the biodegradation of toxic or recalcitrant chemicals (Zhuang et al., 2005). The enforcement of controlled, short-term, unsteady-state conditions may favor the induction of enzymes required for degrading biorefractory compounds.

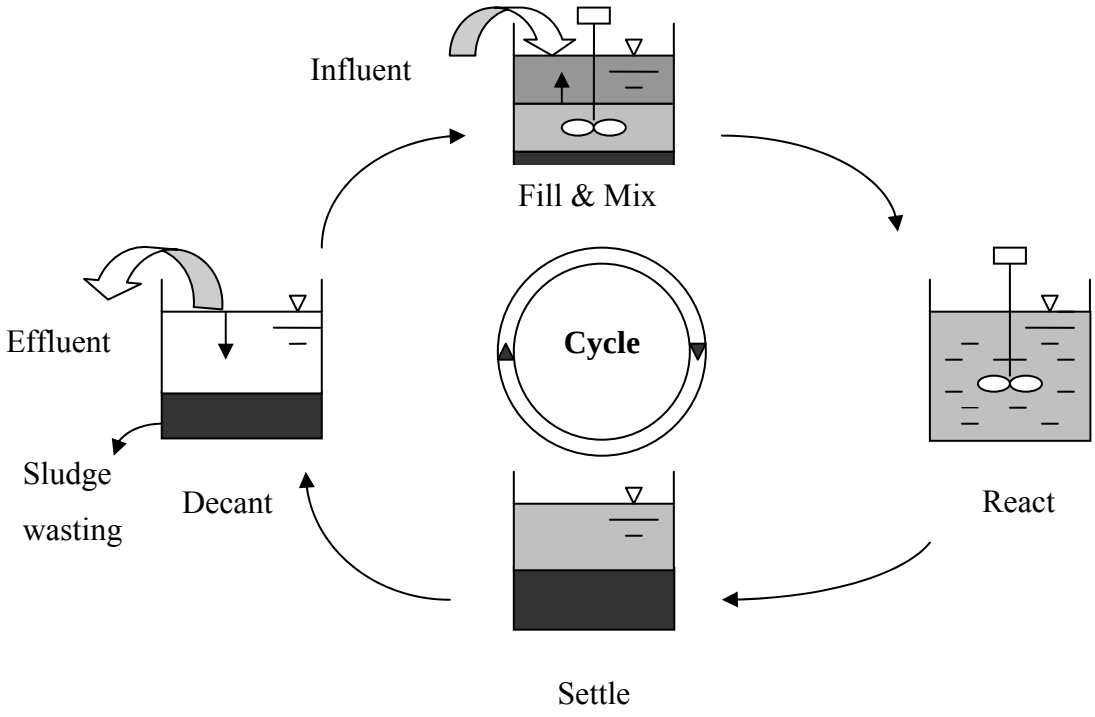


Figure 2.4-1 Schematic of a typical SBR process cycle, adapted from (Irvine et al.,1997)

Chapter 3: Research Objectives

Biological processes for the decontamination of wastewaters are often more complex when several organic compounds with different biodegradability rates are present. However, the interaction among such compounds can sometimes enhance or suppress the biodegradation rate of potentially toxic and biorefractory compounds (Chiavola et al., 2004). Many chemicals, some of which are persistent, are degraded co-metabolically by microorganisms unable to use them as a source of energy or essential nutrients. Such microorganisms need one substrate to support growth while another substrate, the cometabolite, is converted to another product. This phenomenon is linked to the concept of cometabolic transformation kinetics which are difficult to interpret and depend upon many factors (Chiavola et al., 2004).

In the literature, the major studies on pesticide treatment conducted so far have focused on the biodegradation behaviour of a single compound, either aerobically or anaerobically. Although more recent reports have demonstrated pesticide degradation with readily biodegradable co-substrates, there is limited information available with respect to using naturally produced volatile fatty acids as the co-substrate.

The aim of this research is to investigate the pesticide biodegradation capability of the herbicide 2, 4-D when naturally-produced VFAs are used as a co-substrate under denitrification conditions. The research will provide some comparisons on aspects such as denitrification rates, VFA production and consumption rates as well as pesticide biodegradation rates. In particular, this research aims to comment on the effect of the VFA as co-substrate on denitrification and pesticide removal efficiency and to investigate the feasibility for combined treatment of wastewaters contaminated by pesticides and nitrates.

To achieve these goals, the experimental work had the following specific objectives:

1. Develop a semi-continuous fermenter-type batch system (i.e. an acid-phase anaerobic digester) that can produce a stable concentration of volatile fatty acids;

2. Assess the performance of the acid-phase anaerobic digester via the VFA production rate;

3. Evaluate the acclimation of biomass to the presence of the herbicide (2, 4-D) under anoxic-aerobic conditions using sequencing batch reactor technology;

4. Explore the 2, 4-D removal efficiency in the SBR and compare effects of feeding real sewage versus synthetic wastewater;

5. Investigate the nitrate-nitrogen reduction, VFA carbon utilization and 2, 4-D removal profiles during denitrification;

6. Discuss the effect of using naturally-produced VFAs as co-substrates for 2, 4-D removal;

7. For completeness sake, the effect of denitrification and 2, 4-D removal efficiency between naturally-produced VFAs and synthetic VFAs will be studied; and

8. Compare the 2, 4-D biodegradation efficiency between the acclimated biomass from SBR and unacclimated biomass from a local WWTP.

Chapter 4: Experimental Set-up, Operation and Testing Regime

4.1 Acid-phase anaerobic digester

As mentioned in the Introduction, there are 3 systems involved in this research. The first system was an anaerobic digester which was used to generate volatile fatty acids for use in the third system (the denitrification batch tests).

4.1.1 Digester configuration

To produce a stable concentration of volatile fatty acids, a semi-continuous batch digester was designed (Figure 4.1-1). The digester consisted of a 30 L stainless steel cylinder with a sealed base and a removable lid containing a liquid volume of 20 L. While the digester was operating, the system needed mechanisms to mix and sample the digester contents as well as to vent gases from the digester head space. Thus, the purposes of the main components are as outlined below:

Airtight Seal: A grease and rubber seal was used to generate an airtight seal, allowing the system to operate anaerobically;

Pump: An external pump was used to continuously mix the digester contents. Mixed liquor was removed from the bottom sampling port and returned through the top port to the digester. The pump was calibrated to have a flow rate of approximately 8 L/min;

Sampling outlet: The digester had a bottom port to allow the contents to be sampled and which could also be used to drain the digester;

Gas venting outlet: To prevent gases building up in the headspace, a vent from the top port released gases into water to maintain anaerobic conditions; and

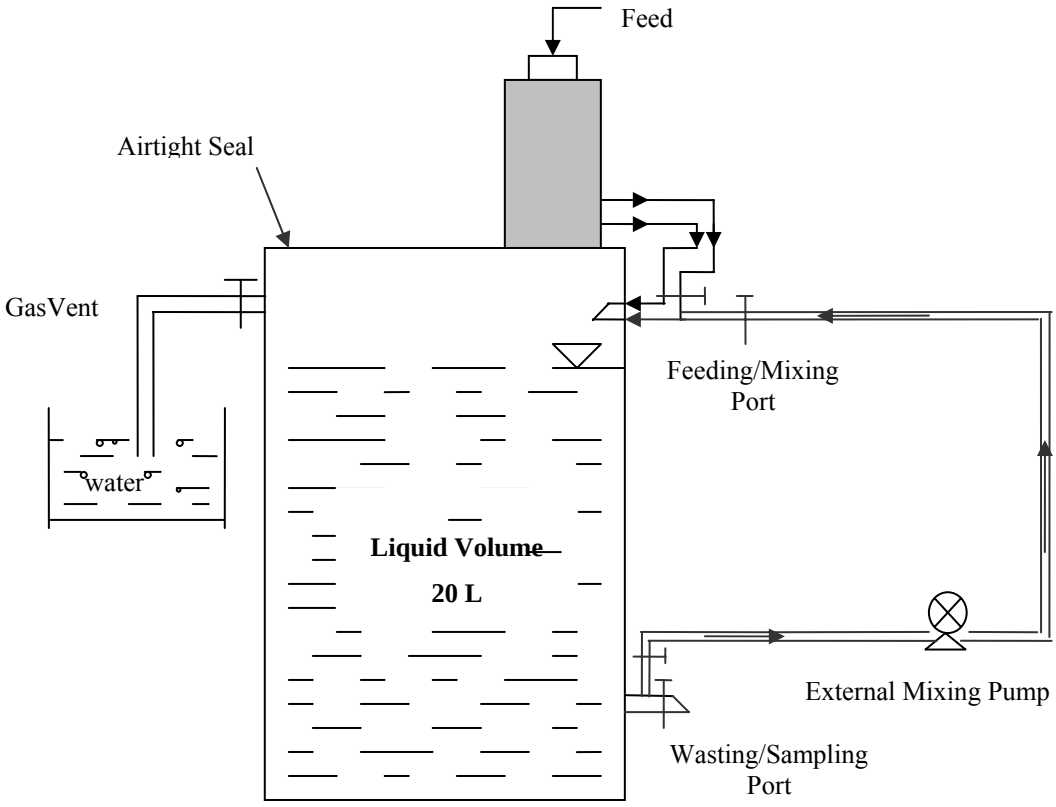


Figure 4.1-1 Schematic of Acid-phase Anaerobic Digester

Feeding/Wasting mechanism: A feeding and wasting system based on a pressure head differential was developed. The "feed" solution was added to the digester through the top port, forcing digester contents to be "wasted" out through the bottom port.

A photo of the anaerobic digester is shown in Figure 4.1-2.

Figure 4.1-2 Photo of Anaerobic Digester

4.1.2 Reactor inoculation, start-up and operation

To provide the initial biomass, the digester was seeded with 10 L of digested sludge obtained from the Christchurch Wastewater Treatment Plant located at Bromley, Christchurch, New Zealand. The pump was then switched on to circulate the biomass. A soya flour solution was chosen as a synthetic feed, thus full-fat, enzyme-active soya flour was purchased from Weston Milling Co. Ltd, Christchurch, New Zealand. The biochemical composition of soya flour was assumed to contain approximately 46% protein, 38% carbohydrate and 7% lipid, as reported in a nutrient database published electronically by the U.S. National Agricultural Library (2003). This composition falls within the ranges reported by Metcalf and Eddy (2003) for untreated wastewater. Experimentally, 40 g/L of soya flour solution was found to have a COD of approximately 50,000 mg/L. After the initial 10 L of digested sludge was added, ten litres of 40 g/L of soya flour solution was added and the digester was sealed. The flow through the pump was closely monitored for the first 4 to 6 hours and valve blockages were removed manually by removing the valve, sealing the pipe with a rubber bung, clearing the blockage, removing the bung and reconnecting the valve.

Each day, 2 L of the soya flour solution was added through the top of the digester while at the same time 2 L of digester contents was wasted through the wasting outlet at the bottom of the digester. The external mixing pump was stopped prior to feeding and wasting, and then restarted immediately afterwards. The 2 L daily feeding and wasting regime of the 20 L liquid volume gave the digester an SRT and HRT of 10 days. The digester was sampled daily for the first few months, and then every 2-3 days until the end of the study. Samples taken from the wasted digester contents were normally analysed for pH, temperature, total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), total organic carbon (TOC) and volatile fatty acids (VFAs).

4.1.3 Sampling and analysis

Sample pH was measured by an electronic pH meter, calibrated at a standard pH of 7.0. Temperature was measured by a standard mercury thermometer adhering to the outside wall of the digester reflecting ambient conditions. Solids concentrations and COD were determined according to Standard Methods (A.P.H.A et al., 1998). TOC analysis was performed on a Teledyne Tekmar Apollo 9000 Combustion TOC analyser and was quantified by comparing the data to that of known standards from calibration curves (Appendix A).

The concentration of VFAs including acetic, propionic, n-butyric and iso-valeric acids were determined on a Hewlett-Packard Gas Chromatography unit equipped with a HP 19091N-133 column (HP INNOWax Polyethylene Glycol 30 m × 250 μm × 0.25 μm) and a flame ionization detector (FID). Samples were centrifuged at 4,400 rcf (relative centrifugal force) for 10 minutes. The supernatant from the centrifugation tube was filtered through 0.45 μm nitrocellulose filter paper (Millipore) under a vacuum, and then acidified with 10% v-v sulphuric acid diluted in deionized water to a final pH value below 2. Samples of 2 ml were transferred to a gas chromatography vial and stored at 4°C until analyzed. The injection volume for analysis was 1.0 μL and the inlet temperature was 280°C. The initial temperature in the oven was 120°C, which was maintained constant for 1 minute and then increased up to 250°C at a rate of 10°C per minute. Once this temperature was reached, the temperature was held for 2 minutes, then decreased to 120°C and held for 0.5 minute. The FID temperature was 300°C. The

nitrogen gas flow was 2.1 mL/min from 0 min to 16.0 minutes and 0.2 mL/min from 16.0 minutes to 16.5 minutes. The pressure was 180.6 kPa. Each acid present was identified by comparing the travel time with that of known standards. The amount of each acid was quantified by comparing the area under the peak of the chromatograph to that of known standards from calibration curves (Appendix A).

4.2 Sequencing batch reactor (SBR)

The second system was an SBR and its purpose was to generate an acclimated biomass able to degrade the pesticide 2, 4-D.

4.2.1 SBR configuration

The SBR was constructed from a 2 mm-thick stainless steel cylinder with an internal diameter of 300 mm and a total volume of 30 L. The operating liquid volume was 22 L. The SBR was designed with five ports for feeding, decanting, sample collection, air supply and mixing (Figure 4.2-1). A photo of the SBR is shown in Figure 4.2-2.

Aeration was provided by compressed air sourced from the laboratory reticulated air supply. The upstream side of the aeration system started with a regulator to reduce the mains air pressure from 80 PSI to 40 PSI allowing for pressures within the range specified by the manufacturers of the variable rate solenoids and air flow meters. The regulator was followed by a Burket 0-10 Bar 230 Volt on/off solenoid and then a Burket variable rate solenoid (Burket 0-9 L/min, 0-20mA, 0-2.8 Bar Model 6021). The purpose of the first on/off solenoid was to remove air pressure from the variable rate solenoid when the air supply was off. The air inflow rate was controlled by a Cole Parmer air flow meter at approximately 6 L/min at a gauge pressure of 25 PSI. A porous air diffuser stone was mounted in the base of the SBR to provide fine bubbles during aerobic periods; the dissolved oxygen (DO) level in the mixed liquor was maintained at a value greater than 2 mg/L.

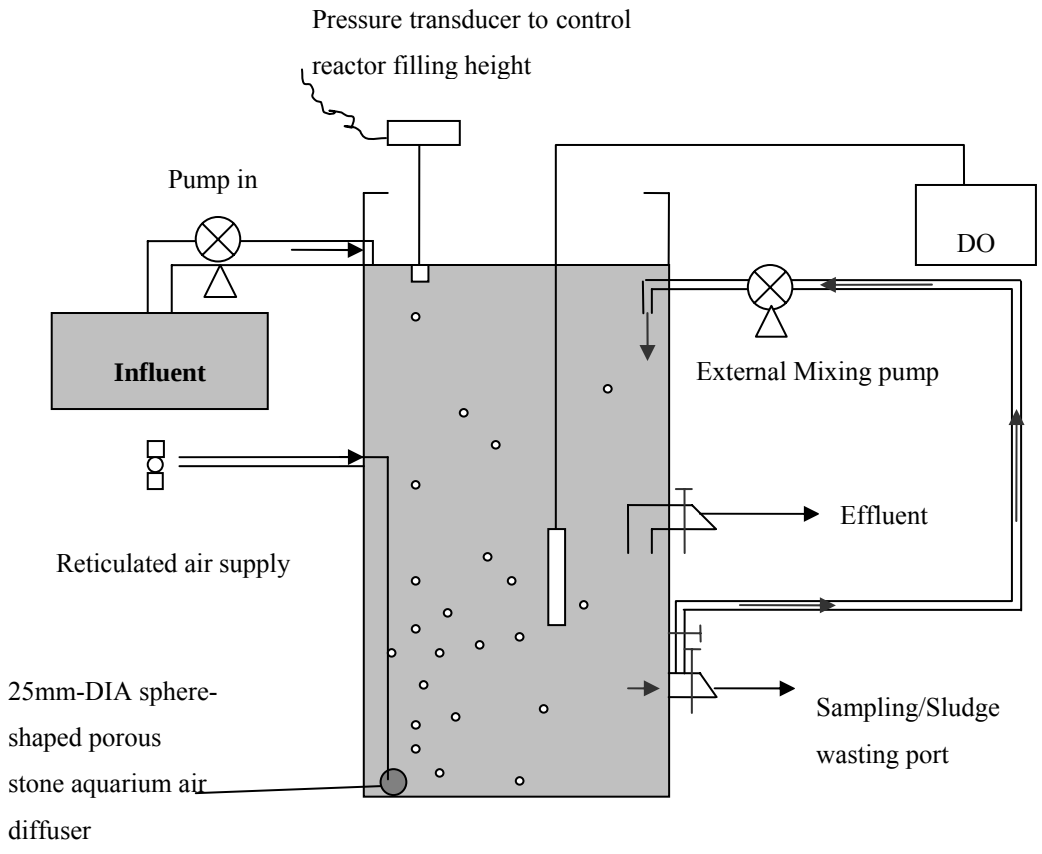


Figure 4.2-1 Schematic of Sequencing Batch Reactor

During anoxic periods, the air flow was eliminated and an external pump was used for mixing. Filling, mixing, anoxic or aerobic reactions, settling and decanting functions were controlled through a programmable controller. The system was operated under room temperatures that ranged from 20 to 22°C. At the end of the Settle phase, 11 L of effluent was decanted while the same volume of influent from a container stored in the refrigerator was added at the beginning of each cycle. A simple, open-shut, solenoid valve (230 volt) was used for control of clarified effluent decanting. The solenoid was normally shut but would open upon supply of 230 volts from the process control system. The influent was delivered through a feed supply system as described in section 4.2.4.

Figure 4.2-2 Photo of Sequencing Batch Reactor

4.2.2 SBR seed

To provide the initial biomass, activated sludge from the Lyttleton sewage treatment plant, Christchurch, New Zealand, was obtained by collecting 20 L of mixed liquor from the oxidation ditch system.

The contents were allowed to settle for 30 minutes before the supernatant was decanted to provide approximately 10 L of concentrated seed activated sludge (~5000 mg VSS/L). Once seeded, the SBR was filled to the 22 L mark with a fresh soya flour solution of 0.6 g/L (equal to 750 mg/L of COD). To acclimate and grow the mixed liquor, the SBR was initially operated with a default value of 150 minutes of aeration at a DO of 4.0 mg/L.

4.2.3 SBR feed

As mentioned, the SBR was initially fed with 0.6 g/L of soya flour solution; however, little COD was removed using soya flour as a synthetic feed; thus, five months later, the soya flour feed solution was replaced with fresh municipal wastewater obtained from the inflow region of the primary sedimentation tanks at the main Christchurch wastewater treatment plant located at Bromley. Figure 4.2-3 shows a schematic of the Christchurch wastewater treatment plant with the location of the feed collection point. The wastewater at this point had been through bar screens to remove large objects such

as rags and sticks as well as being through an aerated grit chamber sequence to remove most of the inert coarse grit.

No pesticide was added during the start-up stage in order to allow the biomass to adapt to the change from soya flour to raw wastewater feed. After 24 days, the SBR was fed with fresh wastewater containing 2, 4-D, a pesticide representative of a variety of chlorinated aromatic compounds. Because the solubility of 2, 4-D is low in water at neutral pH and room temperatures, 2, 4-D was first dissolved in 0.05N NaOH. To avoid potential detrimental effects on the biomass growth, the initial 2, 4-D concentration was 30 mg/L applied to the feed as this concentration would allow time for the biomass to develop/produce the appropriate enzymes to degrade and remove the pesticide from the wastewater. After 60-70% of 2, 4-D degradation was observed in the SBR, the 2, 4-D feed concentration was increased to 50 mg/L, then finally to 100 mg/L. Due to a dilution ratio of 2 (SBR total volume 22 L to influent feed volume 11 L), the 2, 4-D concentration in the SBR was initially 15 mg/L, then it was increased to 25 mg/L and finally it became 50 mg/L.

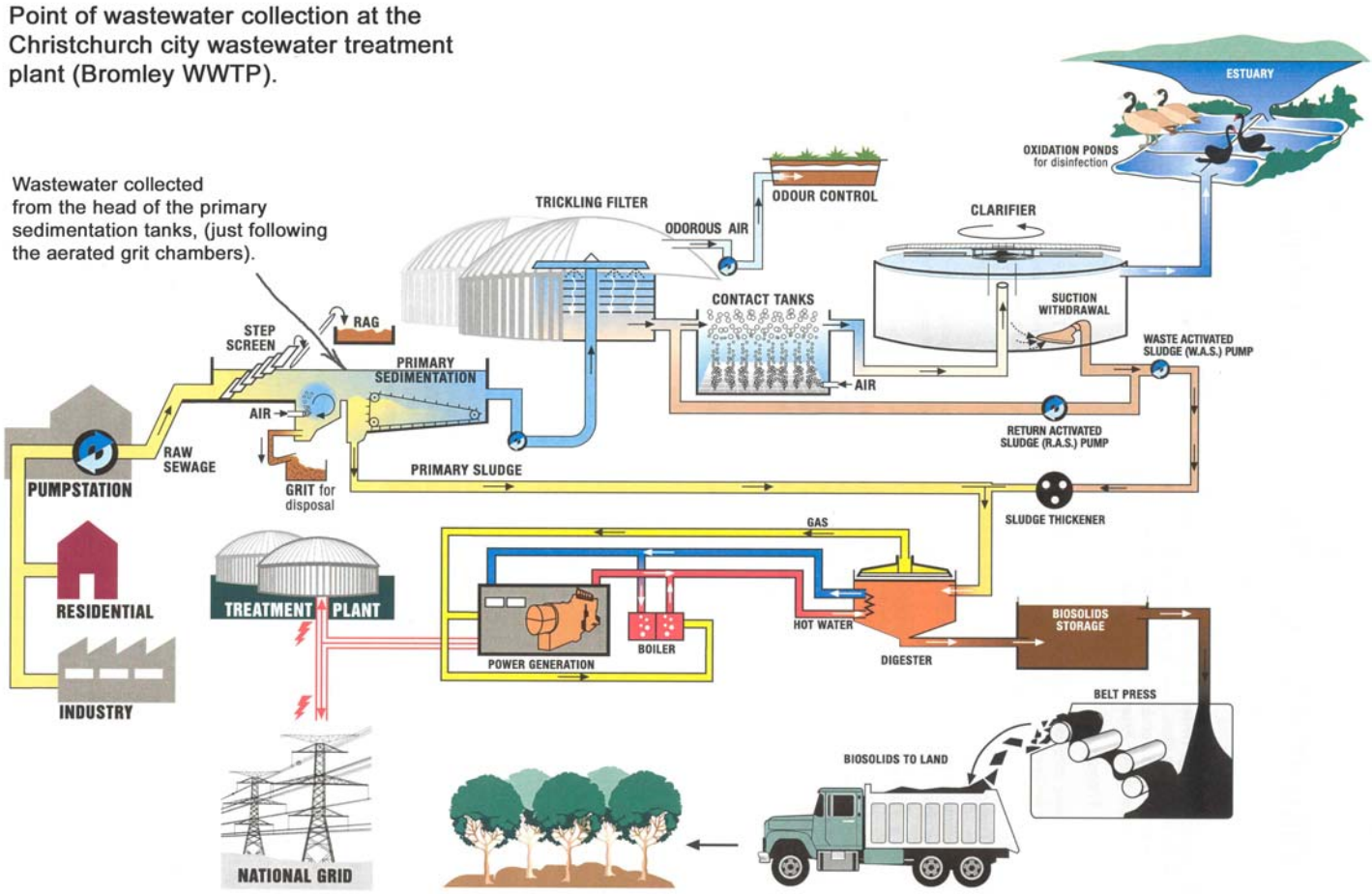


Figure 4.2-3 Schematic of the Christchurch wastewater treatment plant with the location of experimental feed collection indicated (Adapted from Christchurch City Council public information brochure and Holman, 2004)

4.2.4 Feed supply system

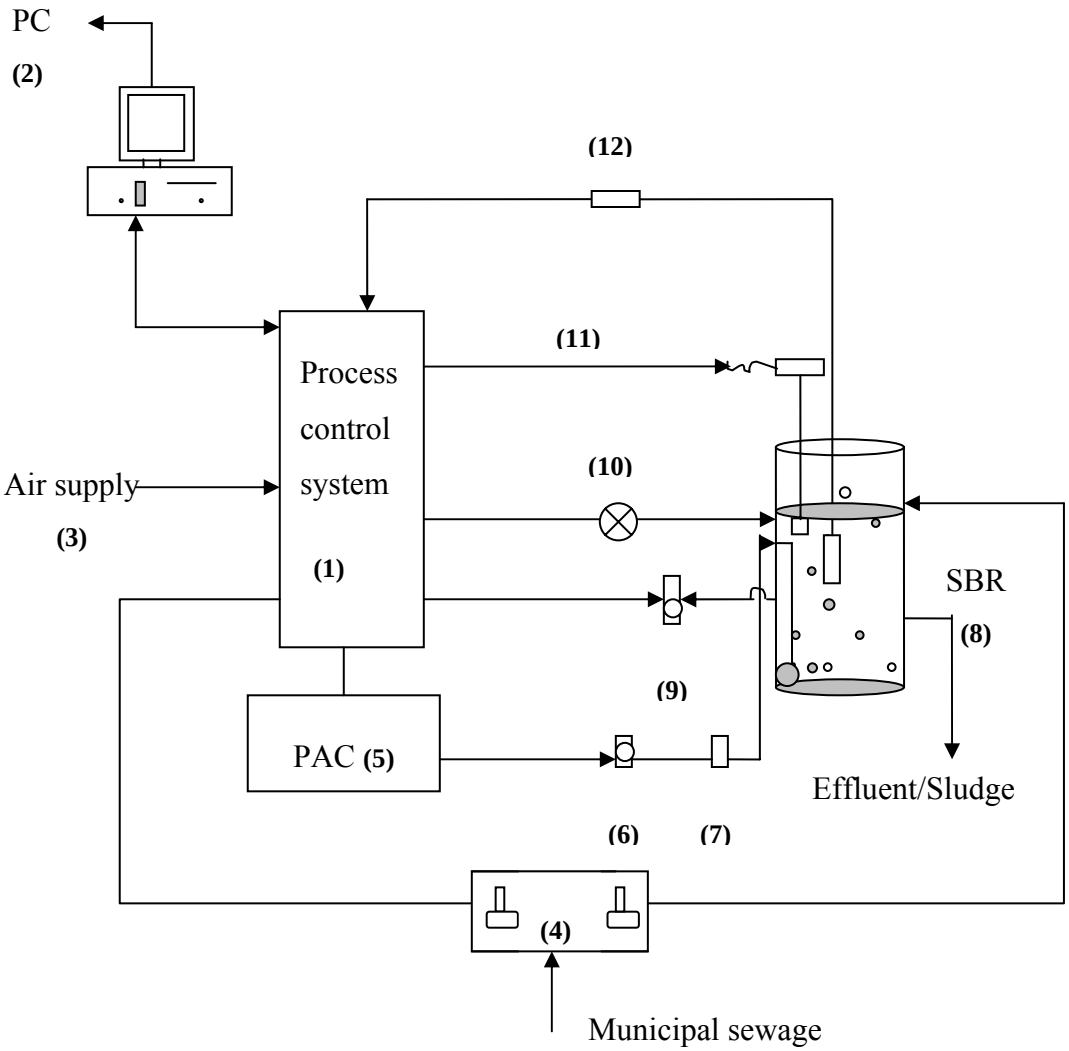
To store the raw feed wastewater and limit degradation, a 500 L chest freezer was purchased and fitted with an external temperature controller built in-house at the University of Canterbury. The temperature controller required an input of a desired temperature set point, so that the freezer turned on/off depending upon the actual temperature as measured by a submersed thermostat. The set point temperature was 5°C so that there was no risk of forming ice on the sides of the freezer and possibly damaging the pumps.

The freezer contained one submersible feed pump and one submersible mixing pump. The mixing pump was connected to the SBR waste solenoid which switched it on for 5 minutes during the SBR Decant period to ensure the wastewater composition was as consistent as possible before the Feed period. The mixing pump was located so that mixing could be undertaken without the pump acting as an aerator (thus reducing biological degradation). To prevent the growth of bacteria in the freezer it was emptied and washed thoroughly before each fresh batch of wastewater was added.

4.2.5 Process control system

Automated control of the SBR was necessary to allow the experimental setup to run continuously when no operator was present. A microprocessor based control system as illustrated in Figure 4.2-4 was developed in house at the University of Canterbury. The process control system was composed of two key elements: a central control box and a personal computer.

The central control box was microprocessor based and contained 16 analogue inputs, 16 digital inputs, and 16 digital outputs. This allowed complete and independent control of all the required SBR operations. Included within the system was an independent “watch dog” microprocessor whose purpose was to maintain control of the SBR on a limited basis in the event of a computer malfunction. The central control box checked the status of the personal computer every six seconds. In the event of identifying a computer problem, the “watch dog” microprocessor was programmed to act as a stand-alone unit



KEY:

(1) Process controller, microprocessor control, controlled and monitored on AMD 1 GHz Athlon PC running Windows XP. Box incorporates 16 analogue inputs, 16 digital inputs, and 16 digital outputs.

(2) On-line real time data reports for the analysis of treatment process

(3) Regulated compressed air supply

(4) 500L capacity cold wastewater storage with submersible feed pumps

(5) Proportional air valve controller

(6) Air solenoid

(7) Air flow meter

(8) 22L capacity sequencing batch reactor

(9) Waste solenoid

(10) Mixer

(11) Fill control pressure transducer

(12) DO meter

Figure 4.2-4 Process Control System

operating on a predetermined timer basis until being manually overridden by an operator. This provided additional operational security to reduce the possibility of a "biological" upset, if the computer had problems during an unattended period.

The personal computer ran a software program developed with the Lab View package from National Instruments. The software allowed the computer to control and monitor the process using either an onboard A/D card or via one of the computer communication ports. Other researchers who have used Lab View for similar types of application include Yu et al. (1998), Andreottola et al. (2001) and Holman (2004).

4.2.6 Sampling and analysis

The influent wastewater was obtained once or twice a week depending on requirements. The composition while in cold storage was checked on a regular basis. The key parameters analysed included pH, total COD, soluble COD, NO_3-N, NO_2-N and NH_3-N according to Standard Methods (A.P.H.A et al., 1998).

The SBR samples were collected every day to determine mixed liquor suspended solids (MLSS) and sludge volume index (SVI) (A.P.H.A et al., 1998). To maintain the reactor within +/- 10% of the desired operational MLSS of 4000 mg/L, the amount and frequency of sludge wasting was determined daily depending upon the relative growth rates of the bacteria. The desired operational MLSS of 4000 mg/L was chosen following classification of the wastewater as medium-high strength. Typical design parameters for sequencing batch reactors detailed in Metcalf and Eddy (2003) give an operational MLSS range of 2000-5000 mg/L dependent upon factors such as the COD concentration. The influent wastewater total COD while in cold storage was typically between 500-700 mg/L, corresponding to a medium-high strength wastewater.

Numerous track studies were carried out in order to explore the performance of the SBR system with respect to 2, 4-D biodegradation. Samples were collected during the whole reaction phase from feeding to decanting and filtered through 0.45µm nitrocellulose filter paper (Millipore) under a vacuum. The parameters analysed during track studies include TOC, TC, TN and SCOD, NO_3-N, NH_3-N as well as 2, 4-D. TOC, TC and TN were measured in soluble form by a Teledyne Tekmar Apollo 9000 Combustion TOC analyser with an additional TN module. The concentration of 2, 4-D

was determined by high performance liquid chromatography (HPLC) equipped with HP 1100 series system and HP ChemStation software. Filtered 10 µl samples were injected into the HPLC system at 230 nm wavelength. An All Tech Adsorbosphere C18 5 Micron column (250 × 4.6 mm × 1/4") was eluted with a mixture of acetonitrile (HPLC grade) and orthophosphoric acid (0.1%) at a flow rate of 1 mL/min. The areas, retention times and UV spectra of the sample peaks were compared with those of the known standards (Appendix A). The eluent gradient is described in Table 4.2-1.

Table 4.2-1 HPLC eluent gradient time table

Time (min)	B = Deionized Water + 0.1% H_3PO_4	A = Acetonitrile + 0.1% H_3PO_4
0	50%	50%
4.5	5%	95%
6.5	5%	95%
8	50%	50%
10	50%	50%

In order to explore the possible biosorption of 2, 4-D during the SBR operation, an ultrasonic converter (The Virtis Company, INC., U.S.A.) was used to break the bacterial cells at a frequency of 20 kHz. Samples (50 ml) were placed in a 150 ml beaker with the ultrasonic probe positioned 2 cm above the bottom of the beaker. The sonication time was 5 min to release 2, 4-D from the solids. Then the sample solution was centrifuged at 4,400 rpm for 10 min and filtered through 0.45 µm nitrocellulose filter paper (Millipore) under a vacuum and made ready for HPLC measurement. The result was compared with the sample without ultrasonic treatment to see whether there was a difference between 2, 4-D concentrations.

4.2.7 Operating strategy

The operating strategy, and particularly the length of the cycle, was initially adjusted by monitoring the amount of degradation of the sewage with a particular focus on the amount of 2, 4-D degradation. As mentioned, soya flour showed little COD removal but as soon as the SBR was switched over to using sewage as a feed, greater than 80 %

COD removal was obtained. However, most importantly, the amount of 2, 4-D degradation effectively went from zero removal up to 30 % removal. As the length of the operating cycle was increased from 3.17 hours to 5.67 hours, up to 90 % of 2, 4-D was removed. The final operating strategy showing the length of each phase of the cycle is shown in Table 4.2-2.

Table 4.2-2 SBR Operating Strategy

	Parameter
Maximum reactor liquid volume, L	22
Feed volume per cycle, L	11
Cycles per day	4.23
Hydraulic retention time, hours	11.34
Time for : Fill and Mix, minutes	5
Non-Aerated Time, minutes	60
Aerated Time, minutes	240
Settle, minutes	30
Decant, minutes	5
Total cycle time, hours	5.67

4.3 Denitrification batch tests

Once VFAs (from the digester) and a 2, 4-D acclimated biomass from the SBR existed, denitrification batch tests (the third system) were started.

4.3.1 Preparation of seed

Wasted sludge from the above SBR system was used as seed for a series of denitrification experiments. Denitrifying bacteria were expected to exist in the sludge, thus, sludge processing was required in order to minimize the initial SCOD. That is, residual SCOD would render the initial C: N ratio meaningless, since it would mask the

consumption of the external carbon (i.e. the VFAs in the anaerobic digester effluent and/or 2, 4-D) (Elefsiniotis et al., 2004).

SCOD removal was a multi-step centrifuge, decant and wash process. Sludge was allowed to sit for 7-10 days during which it was assumed that any easily degradable organic carbon would be fermented into VFAs. The sludge was then poured into 35 ml tubes and centrifuged for 10 minutes at 4,400 rcf. The supernatant was decanted without loss of solids and a small amount of tap water was added to the tubes which were then vigorously agitated to break up the centrifuged solids. More tap water was added to restore the 35 ml volume, and the process was repeated twice more. This treatment has been proved to be sufficient to ensure the removal of most SCOD in the sludge (Elefsiniotis et al., 2004).

4.3.2 Feed sources

Three-liter completely-mixed batch reactors were used for the denitrification experiments and these were fed using fermented and settled acid-phase anaerobic digester effluent (to provide the source of VFAs) and/or 2, 4-D as the carbon sources (Figure 4.3-1). To explore the effect of VFAs on 2, 4-D removal and denitrification efficiency, reactor A was fed with both digester effluent and 2, 4-D, reactor B was fed with digester effluent only and reactor C was fed with 2, 4-D only as carbon source. Nitrate-nitrogen (NO_3-N) was added in the form of sodium nitrate in a range of C: N ratios (i.e. 1-3). To quantify any potential abiotic losses of 2, 4-D, VFAs and NO_3-N, three additional batch reactors were operated as control reactors (with no addition of biomass) and fed with 2, 4-D only, digester effluent only and NO_3-N only, respectively. Each test condition was carried out in duplicate to make sure of consistent results.

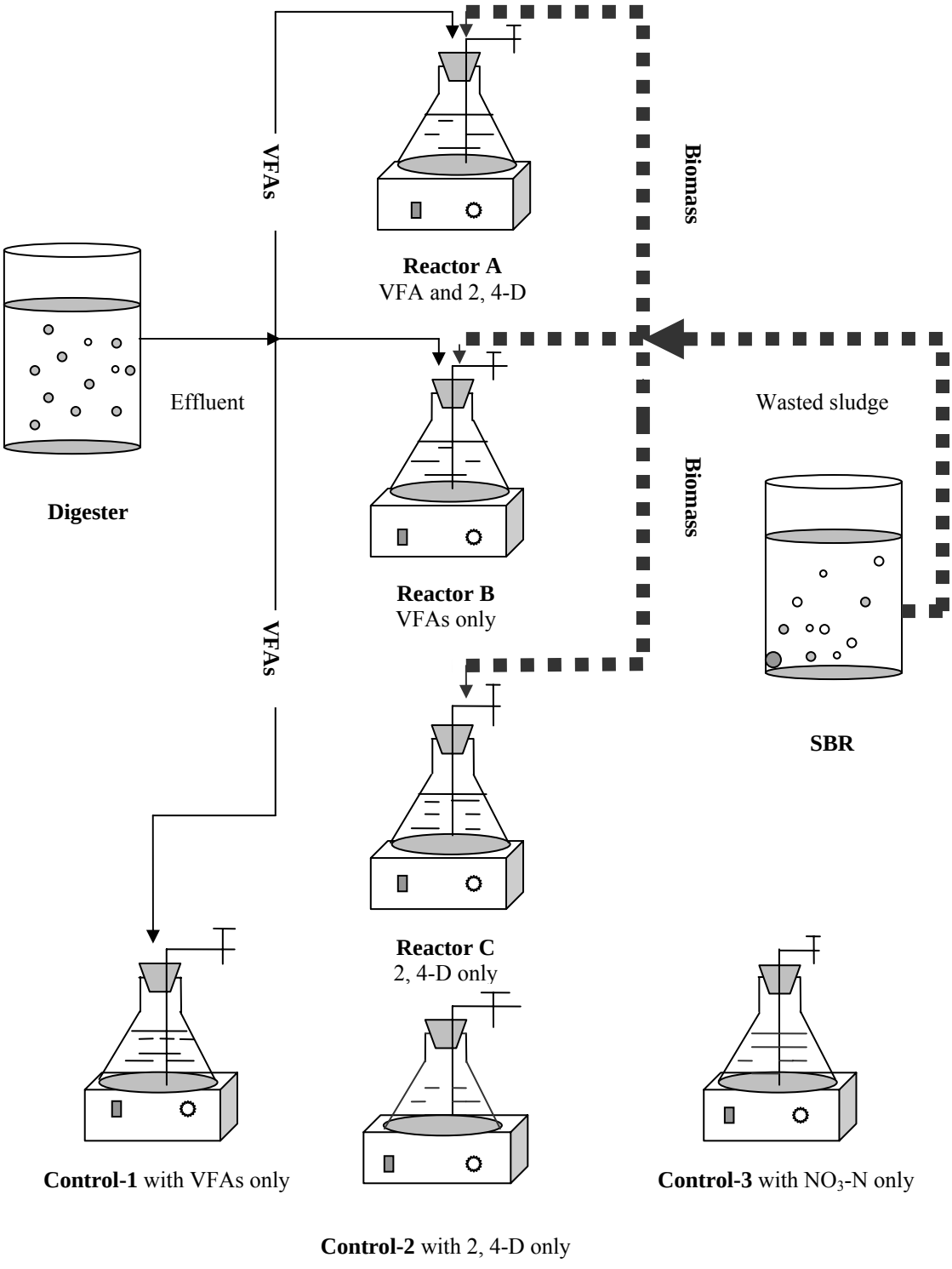


Figure 4.3-1 Schematic of Denitrification Batch Reactor Set-up

A photo of the denitrification batch reactors is shown in Figure 4.3-2.

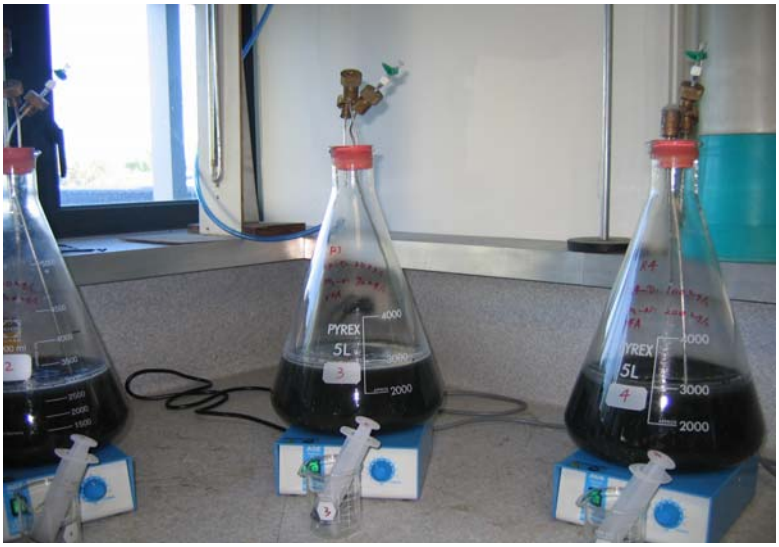


Figure 4.3-2 Photo of Denitrification Batch Reactors

4.3.3 Sampling schedule and analytical methods

The denitrification batch test runs were operated at 21 ± 2°C. Samples were taken on the first day at 0, 4, 8 and 12 hours. On the second to third day, 24 - 32 hours and 48 - 56 hours, the reactors were sampled every 4 h. Finally, after 72 hours, samples were drawn twice every day until one week had elapsed. Samples were analysed for VFAs (acetic, propionic, n-butyric and iso-valeric acids), 2, 4-D, NO_x-N, pH, alkalinity, SCOD, NH_3-N, TOC and VSS. Analytical tests were conducted according to Standard Methods (A.P.H.A, 1998), except in the case of VFAs, 2, 4-D and TOC which have been described previously.

4.3.4 Degradation behaviour with unacclimated biomass

In order to compare the 2, 4-D biodegradation efficiency between acclimated biomass from the SBR and unacclimated biomass from a local wastewater treatment plant, activated sludge was collected from the Christchurch WWTP, New Zealand. The sludge was processed as per the procedure mentioned above and carbon sources and nitrate-nitrogen were also added as above. Denitrification batch tests were carried out in the same manner as the acclimated biomass.

4.4 Experimental difficulties & problem solutions

Over and above the initial attempt to use soya flour as a feed source to the SBR (which the biomass did not readily degrade within a period of 5 months), start-up difficulties were encountered with the operation of the GC, TOC and HPLC machines. About 3 months elapsed before these machines were working properly and able to give consistent and reasonable results. Other than these difficulties, normal operational problems (e.g. pump failures, valve blockages etc.) were encountered and fixed on a routine basis.

Chapter 5: Experimental Results and Discussion: Acid-phase Anaerobic Digester Performance and VFA Production

5.1 Overall aims

The primary aim of operating an anaerobic digester was to produce a stable level of VFAs using soya flour as a synthetic wastewater feed. The secondary aim was to report on the performance of the digester (i.e. organic carbon solubilization and VFA production rates) using different performance indices.

5.2 Characteristics of the digester seed and feed

Sieved digested sludge which provided the initial seed in the digester was found to have a total solids concentration of 20,700 mg/L and a volatile solids concentration of 17,000 mg/L. The total COD value was approximately 66,000 mg/L.

As mentioned, a soya flour solution (40 g/L) provided the feed to the anaerobic digester and its characteristics are shown in Table 5.2-1.

Table 5.2-1 Characteristics of the anaerobic digester feed soya flour solution (40 g/L)

	pH	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)	Total COD (mg/L)	Soluble COD (mg/L)	TOC (mg/L)	VFAs (mg/L) as acetic acid
Mean	6.7	34245	32267	24200	23722	54068	10473	3815	348
±σ	0.1	1317	1095	421	410	4108	1573	174	65

5.3 Digester performance

5.3.1 Variation in digester temperature

The temperature of the digester was not controlled during the operating period but was 32 ± 3 °C which lies in the mesophilic range (30 to 38 °C) that most municipal and domestic digesters operate. Figure 5.3-1 presents the digester temperature variation throughout the research.

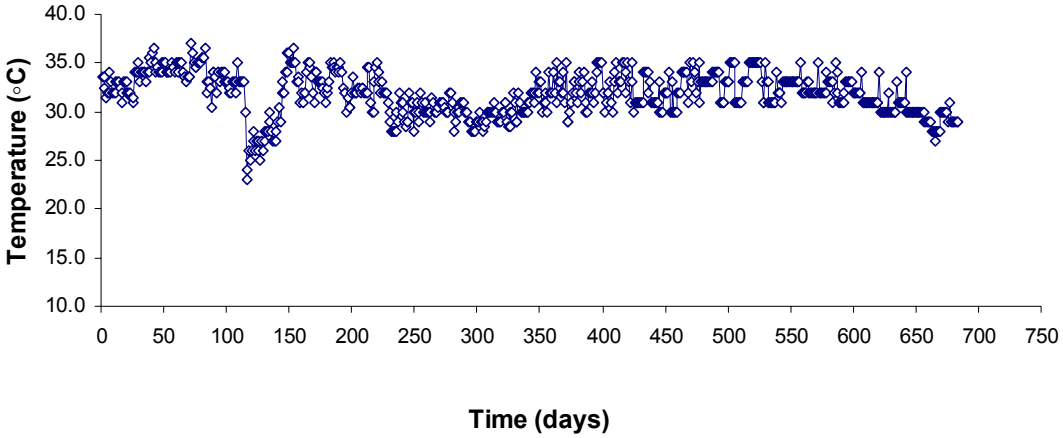


Figure 5.3-1 Profile of temperature of anaerobic digester

Since acidogens are not as sensitive to temperature changes as methanogens (Yu and Fang, 2003), this study did not focus on finding the optimum temperature with respect to the process performance. Both diurnal and seasonal temperature changes were deemed insignificant with respect to the degree of acidification and VFA formation.

5.3.2 Digester pH

The pH of samples taken from the digester over the research period are shown in Figure 5.3-2.

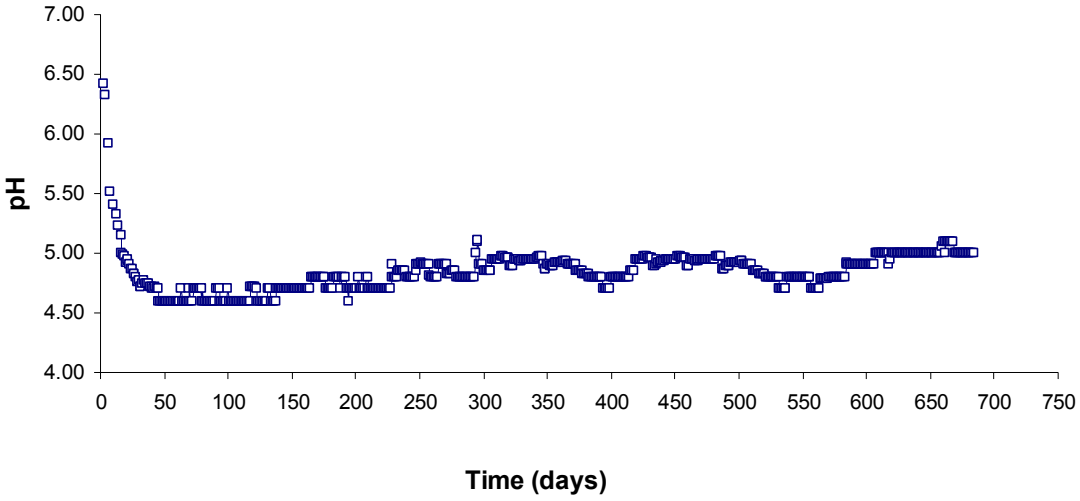


Figure 5.3-2 Profile of pH of anaerobic digester

The pH in the digester decreased from approximately 6.5 to 4.8 within the first 30 days followed by it being relatively constant (between 4.7 and 5.0) during the rest of research period. Since the mean influent pH was 6.7, the pH indicates that stable acidogenesis occurred, although the mean pH (4.98 ± 0.2) is lower than some studies that report optimal values of 5.5 to 6.0 (Yu and Fang, 2003).

5.3.3 Digester solids concentration

The volatile suspended solids concentration inside the digester initially dropped from 17,000 mg/L to 15,000 mg/L in the first 15 days before increasing to a mean VSS concentration of 20,441mg/L for the rest of the research period. The profile of VSS is shown in Figure 5.3-3 and the relatively stable value suggests that a healthy population of bacteria was established and maintained in the digester (i.e. steady state conditions were achieved).

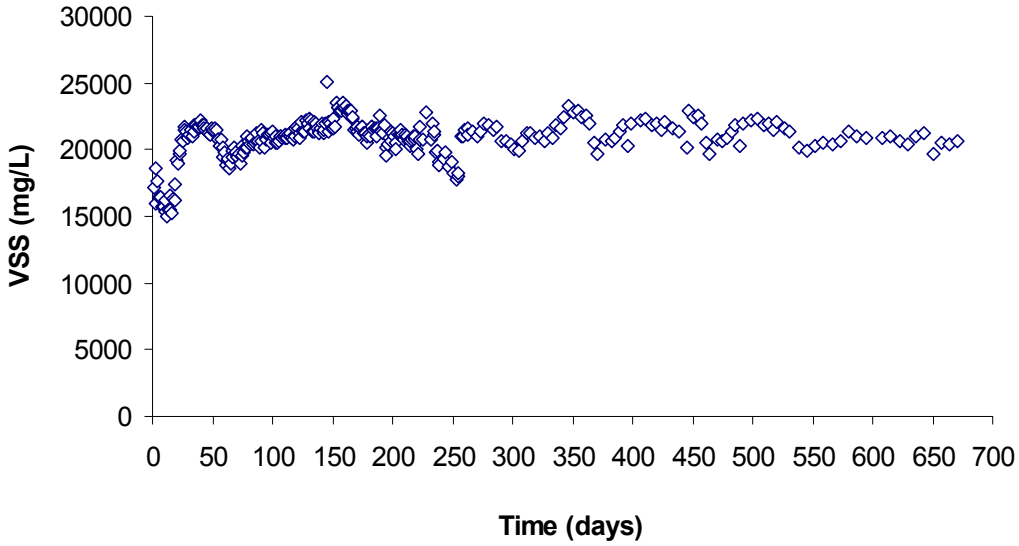


Figure 5.3-3 Profile of total solids of anaerobic digester

5.3.4 Digester VFA concentration and speciation

The total concentration of VFAs (expressed as acetic acid) as well as the speciation of acids (acetic, propionic, n-butyric and iso-valeric acid) in the digester are presented in Figure 5.3-4.

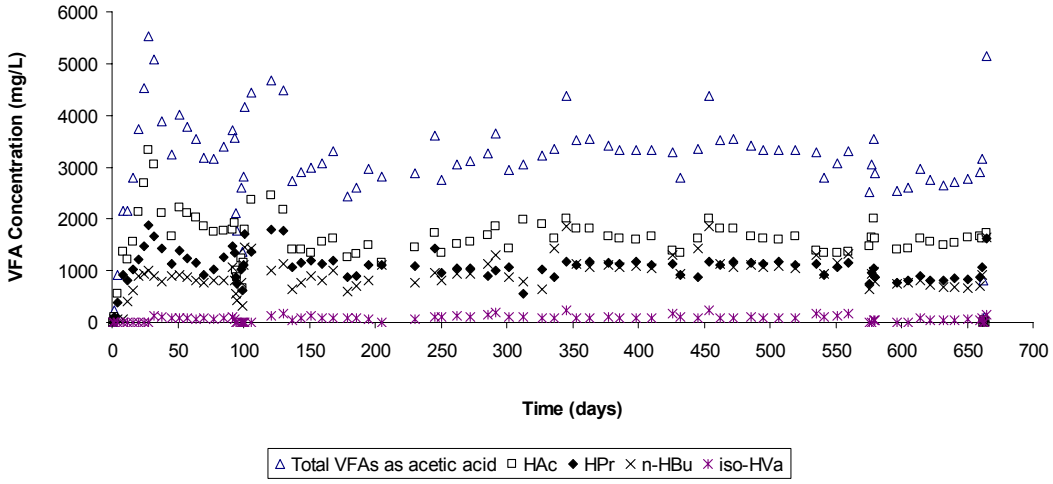


Figure 5.3-4 Profile of VFA concentration

Total VFA levels fluctuated in the first 4 months between 2100 and 5500 mg/L then became relatively stable ranging from 2500 to 3000 mg/L during the rest of research period. Levels of acetic and propionic acid predominated in the anaerobic digester throughout the research period; however levels of n-butyric acid also rose substantially after two weeks. Levels of iso-valeric acid remained constantly low throughout the period of research. The digester appears to have reached a stable state with respect to VFAs concentration and speciation after 4 months, with mean concentrations of 1621 mg/L (51.4%) acetic, 1071 mg/L (27.5%) propionic, 909 mg/L (19.6%) n-butyric and 75 mg/L (1.4%) iso-valeric acid (Figure 5.3-5).

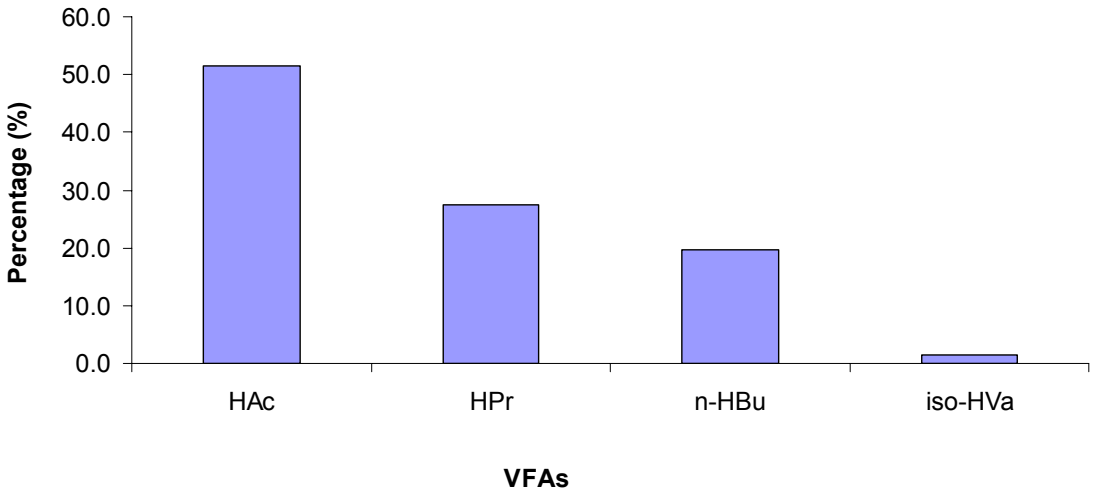


Figure 5.3-5 Percentage VFAs speciation

As mentioned, VFA speciation results indicate that acetic and propionic acids were the most prevalent VFA generated throughout the research. The proportions of VFA species produced were similar to those found by Elefsiniotis and Oldham (1994), who observed levels of approximately 48% acetic acid, 30% propionic acid and 10% butyric acid when digesting primary sludge in an acidogenic anaerobic digester at an SRT of 10 days. The distribution of acetic and propionic acids is also relatively consistent with other researchers where acetic acid ranged from 33% to 73% and propionic acid from 28% to 44% (Skalsky and Diagger, 1995; Moser-Engeler et al., 1998; Mavinic et al., 2000; Bouzas et al., 2002). The n-butyric acid amount is relatively larger than other research listed in Table 5.3-1, and this may be due to the different wastewaters studied.

Table 5.3-1 Typical VFA composition distributions for fermentation effluents, adapted from (Rossle and Pretorius, 2001)

Acetic acid (%)	Propionic acid (%)	Butyric acid (%)	Valeric acid (%)	Reference
38	36	16	10	(Pitman et al., 1992)
43	41	8	-	(Randall et al., 1997)
70	25	5	-	(Carlsson et al., 1996)
56	30	7	0	(Rabinowitz et al., 1997)
71	24	3	3	
61	27	7	-	(Munch, 1998)
55	45	0	0	(Munch, 1998)
49	33	13	6	(Munch, 1998)
63	25	12	-	(Munch, 1998)
				(Rodriguez et al., 1998)
MEAN				
56	32	8	2	-
51.4	27.5	19.6	1.4	In this study (2006)

5.3.5 Particulate organic carbon solubilization

Particulate organic matter first undergoes hydrolysis before being taken up by microorganisms. Since 70-80 % of the substrate in the feed is in the particulate form (as indicated by the VSS/VS ratio), solubilization of organic matter is a major step in acidogenic digestion. The rate of hydrolysis is a function of pH, temperature, type of substrate, nature of the biomass, size of particles, and remaining concentration of biodegradable matter (Elefsiniotis et al., 1996).

The amount of particulate organic carbon solubilization in the digester can be estimated from certain non-specific chemical parameters such as filtered TOC, soluble COD or VSS destruction. For example, if the net filtered TOC concentration (effluent TOC minus influent TOC) increases in the digestion system, it is the result of substrate

conversion from particulate to soluble form. The specific solubilization rate of TOC can be expressed as the net amount of filtered TOC generated per day per unit mass of VSS in the digester (mg TOC/mg VSS/day) (Table 5.1-3). Similarly, the degree of COD solubilization can be expressed as the net soluble COD concentration (effluent soluble COD minus influent soluble COD) or specific COD solubilization rate (mg soluble COD/mg VSS/day) (Table 5.3-2). Since the amount of gas generated in the process was minimal (section 5.3.7), gas production was not taken into account in the calculation of the TOC or COD solubilization rate. Finally, the extent of organic carbon solubilization can be also viewed from the perspective of the reduction in VSS, which will provide additional evidence whether the particulate substrate in the feed was amenable to solubilization or not.

Table 5.3-2 Anaerobic digester particulate organic carbon solubilization

HRT (day)	Net filtered TOC (mg/L)	Specific TOC solubilization rate (mg TOC/mg VSS/day)	Net soluble COD (mg/L)	Specific COD solubilization rate (mg soluble COD/mg VSS/day)	VSS reduction (%)
10	1431	0.007	4517	0.022	14

The extent of organic carbon solubilization in terms of the specific TOC solubilization rate and VSS reduction rate were relatively lower than previous research using primary sludge as feed (Elefsiniotis et al., 1996), which obtained a maximum specific TOC solubilization rate of 0.07 mg TOC/mg VSS/day and a maximum VSS reduction rate of 72 %. Similarly, the specific soluble COD production rate was found to be lower than the value of 0.124 mg SCOD/mg VSS/day reported when using mixtures of starch-rich industrial wastewater with municipal wastewater as feed to an anaerobic digester (Elefsiniotis et al., 2005). Because anaerobic digestion is biologically mediated and depends upon the growth of microorganisms, complete volatile solids destruction does not occur, with 40 to 65 % being typical (Vesilind, 2003). Lower percentage destruction occurs with solids containing significant concentrations of materials that are difficult to degrade (Vesilind, 2003). That is, high-percentage destruction is achieved when the digestion of primary sludge contains easily degraded materials such as simple carbohydrates, complex carbohydrates (cellulose), proteins, and lipids (grease) (Vesilind, 2003). The relatively low solubilization rate in this study suggests that the

particulate substrate in question (i.e. soya flour) was not entirely amenable to solubilization. However, the feed concentration chosen (TS = 34,250 mg/L or 3.42 %) (Table 5.2-1) is well above the 0.5 % to 2.0 % TS range (Section 2.3.3.6) because the initial attempt was to match the COD load to values typical of primary sludge. Therefore, the low percent VSS reduction can possibly be attributed to solids overloading of the digester. Nonetheless, some solubilization of particulate matter was occurring and while not optimum, the digester was producing a source of naturally generated VFAs as detailed in the next section. It must also be remembered that the purpose of this research was not per se to optimise VFA production, but merely to use the digester as a VFA-generator (Section 5.1).

5.3.6 Anaerobic digester VFA production rate

The net values for VFA production were obtained by subtracting the corresponding influent concentration (Table 5.2-1) from that in the reactor (Figure 5.3-4) and expressing them as acetic acid for comparison purposes. The VFA specific production rate was expressed as the net amount of VFAs produced per day per unit amount of VSS in the digester (mg VFA as acetic acid/mg VSS/day). The results are listed in Table 5.3-3.

To estimate the fraction of soluble COD in the form of VFAs, VFA concentrations were converted to COD equivalents by using conversion factors: 1.067 for acetic acid, 1.514 for propionic acid, 1.818 for butyric acid, and 2.039 for isovaleric acid (Rossle and Pretorius, 2001).

Table 5.3-3 Anaerobic digester VFA production rate

Net VFAs (mg/L)	Net VFAs (mg COD/L)	VFA-COD/ Soluble COD (%)	Specific VFA production rate (mg VFA as acetic acid/mg VSS/day)
2805	4785	32	0.014

Though net VFAs production (2805 mg/L) is substantial, VFA production (4785 mg COD/L) accounted for only 32 % of soluble COD in the anaerobic digester. The specific VFA production rate (0.014 mg VFA/mg VSS/day) is again lower than data found in the literature (Elefsiniotis et al., 2005) which obtained a proportion of VFA in

the soluble COD from 70% to 90 % and a specific VFA production rate of 0.07 mg VFA/mg VSS/day. Since it is evident that SCOD contributing compounds in the digester were not only in the form of VFAs, it means that other compounds were contributing to SCOD and these may have included unused soluble substrate, products of cell lysis and extracellular intermediate metabolites. It is also possible that the less complex components of the soya flour feed may have limited the growth of a dynamic population of VFA producers.

5.3.7 Gas production

The acid-phase step of anaerobic digestion is generally characterized by very low gas generation, mostly in the form of CO_2, N_2, and H_2, which are the by-products of many pathways followed for substrate metabolism (Elefsiniotis and Oldham, 1994). Ideally, the methane (CH_4) content in the digester should be negligible; however, in practice, varying amounts of CH_4 have been detected in acid-phase digesters. This may be due to either incomplete separation of the two phases which results in the coexistence of heterotrophic methane producers and/or the presence of certain fast-growing autotrophic methanogenic microorganisms such as *Methanobacterium* (Novaes, 1986).

In this study, the digester gas was analyzed by a Landfill Gas Analyser (Geotechnical Instruments, UK) and the gas composition averaged 10% CH_4, 90% CO_2 and extremely low level of N_2 and H_2 (less than 1%). These results are similar to those previously reported for acidogenic-phase digestion (Elefsiniotis and Oldham, 1994) and suggests that the low pH value (4.7-5.0) encountered during this study resulted in little gas production, indicating successful suppression of methanogenesis (Kayhanian and Tchobanoglous, 1992).

Chapter 6: Experimental Results and Discussion: Acclimation and Biodegradation of 2, 4-D in the SBR

6.1 Introduction

The following section provides a summary of the operational results for the SBR. The MLSS, SRT and SVI results are presented as well as the carbon, nitrogen and dissolved oxygen profiles during the acclimation phase of the SBR system. The biodegradation of 2, 4-D in the SBR system was also studied and the results are presented in the following section. The operational objective was to maintain a relatively stable population of 2, 4-D degrading bacteria (either aerobic, anaerobic or anoxic) in the system in order to develop their biodegradation capability in the subsequent denitrification batch reactor tests (Chapter 7).

6.2 Reactor start-up and Influent characteristics

The composition of influent wastewater is illustrated in Table 6.2-1 which shows the statistics from tests taken over the duration of the work.

Table 6.2-1 Influent wastewater characteristics

	pH	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)	TCOD (mg/L)	SCOD (mg/L)	NH_3-N (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	TOC (mg/L)	TC (mg/L)	TN (mg/L)
Maximum	7.42	753.50	739.40	269.00	271.00	735.00	370.00	28.10	6.10	0.00	82.00	105.00	40.00
Minimum	7.15	477.00	426.00	164.00	161.00	615.00	184.00	23.60	2.10	0.00	48.00	87.00	30.00
Mean	7.28	625.39	585.04	207.89	205.21	679.78	303.78	25.70	3.36	0.00	63.22	91.56	34.22
±σ	0.08	110.13	114.25	35.79	37.10	44.86	66.06	1.65	1.45	0.00	11.12	5.48	3.15

The influent BOD_5 was also tested by a HACH BODTrak (HACH, USA) (Figure 6.2-1), however, a 5-day period was generally considered too long to wait for results in most instances. It can however provide evidence with respect to differentiating between biologically oxidizable and biologically inert organic matter in the influent. Generally, a BOD_5 concentration of domestic wastewater is around 50% of the unfiltered COD concentration (total COD) or around 0.4-0.8 BOD_5/COD (Metcalf and Eddy, 2003). It can be seen that this sewage had a BOD_5/COD ratio of approximately 400/680 ≈ 0.6 so it was a fairly typical influent sewage.

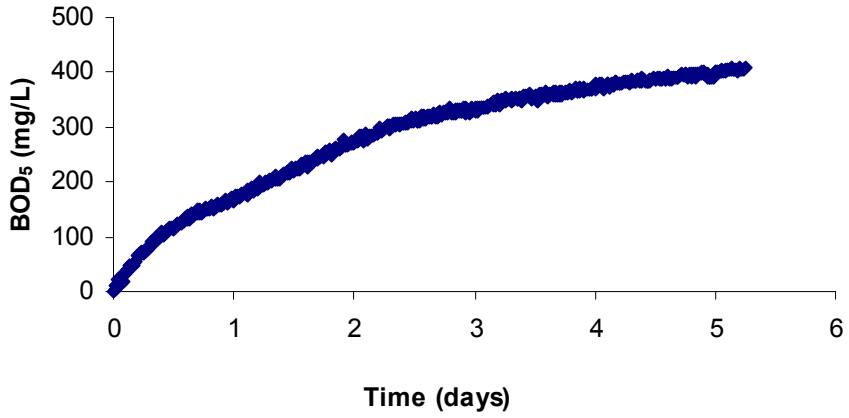


Figure 6.2-1 Influent wastewater BOD_5 profile

The wastewater was obtained once or twice a week depending on requirements. Checks to monitor the wastewater composition while in cold storage were carried out on a regular basis. An example set of tests are presented in Table 6.2-2 and these results indicate how the wastewater changed over a seven day period.

Table 6.2-2 Wastewater degradation while in cold storage

	pH	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)	TCOD (mg/L)	SCOD (mg/L)	NH_3-N (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)
Day 1	7.34	753.52	739.44	210.00	218.89	735.00	370.00	23.60	6.10	0
Day 2	7.61	650.00	527.63	205.49	204.40	680.00	347.00	28.80	5.90	0
Day 3	7.26	684.00	572.00	200.00	201.08	596.00	300.00	27.30	6.30	0
Day 4	7.55	668.83	520.78	217.02	218.09	569.00	249.00	26.50	6.80	0
Day 5	7.63	604.00	457.33	189.47	190.53	533.00	220.00	30.70	6.30	0
Day 6	6.78	571.05	406.58	204.26	202.13	516.00	206.00	31.10	5.40	0
Day 7	6.87	556.58	398.68	209.38	201.04	498.00	190.00	31.40	5.70	0

The wastewater pH was typically between 6.8-7.4, while the COD declined steadily. The ammonia nitrogen increased slightly as organic nitrogen was converted to ammonia according to data from Table 6.2-1. The large decline in total COD appeared to be caused by the fall in soluble COD (Figure 6.2-2). The total solids and volatile solids also changed significantly during the 7 days of storage (Figure 6.2-3) which was probably due to bacteria activity, although the wastewater was stored at 5°C. Therefore the management strategy was changed such that the wastewater was collected every 3 to 4 days to minimize the loss of COD and solids.

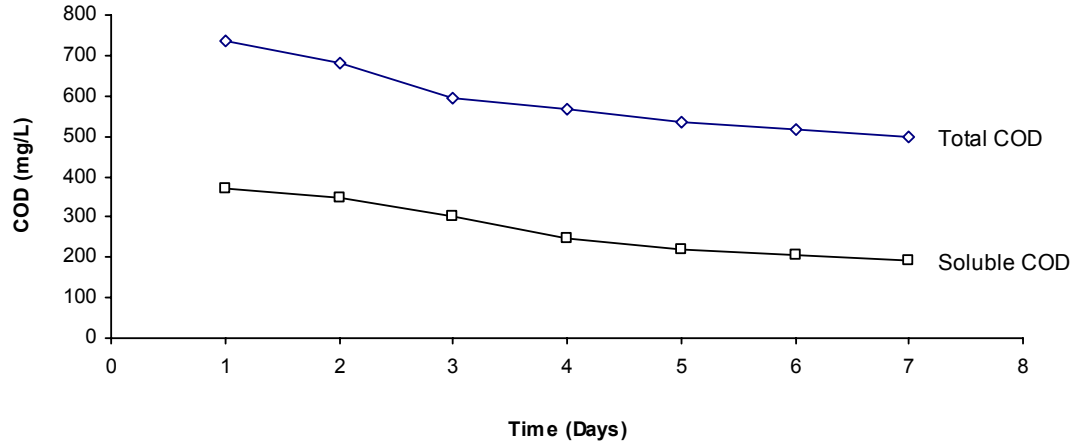


Figure 6.2-2 Organic carbon degradation during wastewater storage period

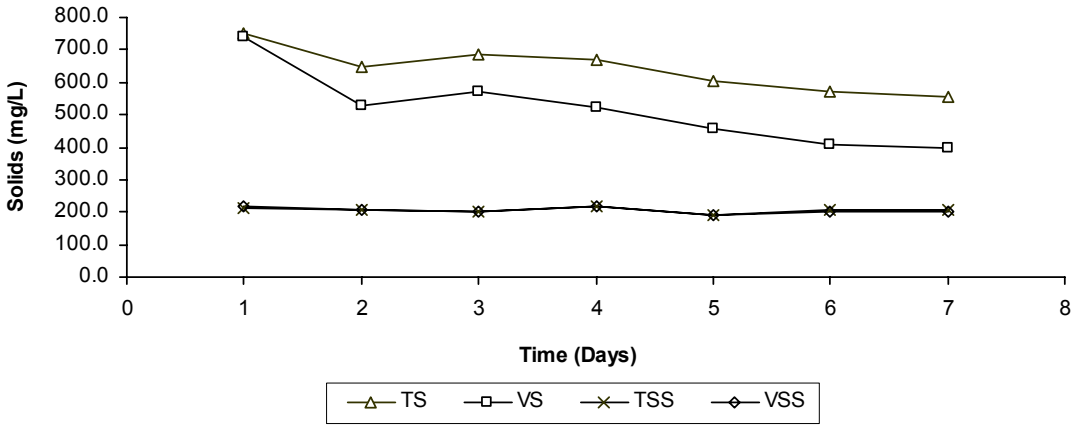


Figure 6.2-3 Solids change during wastewater storage period

6.3 Nitrogen, carbon and dissolved oxygen profiles in the SBR without 2, 4-D in feed

Before adding pesticide, the SBR was fed only with sewage to allow the biomass to increase, to minimize any problems due to potential toxic effects, and improve the stability of the SBR system. Figure 6.3-1 illustrates a typical nitrogen profile including NH_3-N, NO_3-N and total nitrogen (in soluble form and included organic N, ammonia N and NO_x-N) in the SBR with no addition of 2, 4-D to the feed.

The first 60 minutes of the cycle were anoxic (Table 4.2-2).The NO_3-N and the NH_3-N showed negligible change. The following 240 minutes were aerobic (Table 4.2-2) and the NH_3-N decreased quickly due to it being oxidized to the NO_3-N, which was observed to increase correspondingly. Once the NH_3-N was completely oxidized (after 190 minutes), the NO_3-N returned to a stable level. At the end of the cycle, there was a difference between the measured TN and the sum of the NH_3-N and NO_3-N which was due to soluble organic N released by lysis of bacteria, likely due to over-aerating conditions (Figure 6.3-3).

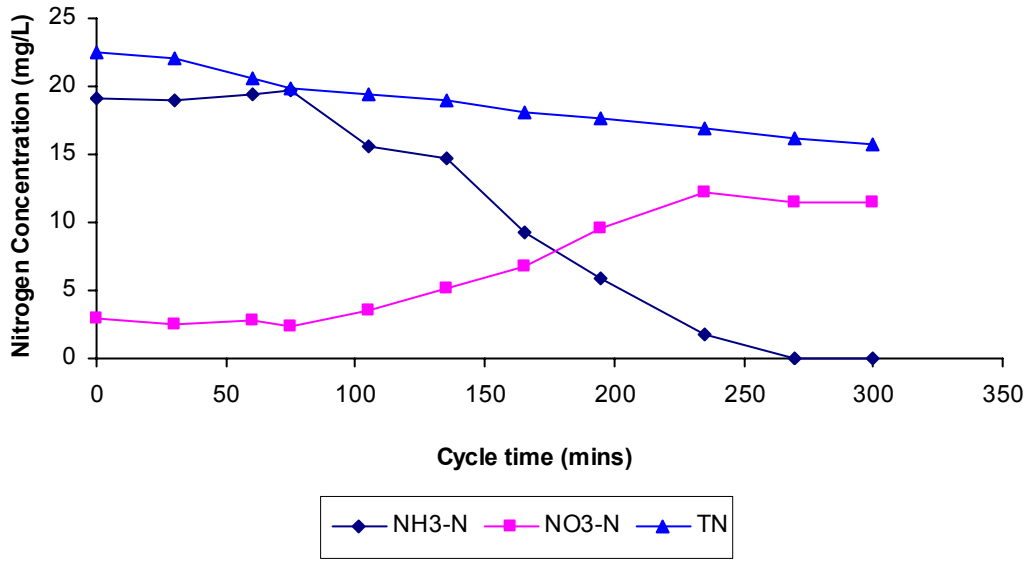


Figure 6.3-1 Nitrogen variation in SBR without adding 2, 4-D in feed

The carbon profile including the SCOD, TOC and TC in the SBR is presented in Figure 6.3-2. The SCOD dropped about 10% under anoxic conditions while 60% was removed at the end of aerobic period. The TOC and TC removals showed a similar trend in the anoxic environment with both of them recording less than 10% removal. At the end of the aerobic period, the TOC removal reached 63% while the TC removal reached 46% implies that the organic carbon was being consumed at a faster rate than the inorganic carbon.

The DO profile in Figure 6.3-3 gives an indication of the pattern of food utilization during the aerobic period. There is negligible oxygen (< 0.5 mg/L) in the SBR during

the anoxic period. During the aerobic period, the DO was consumed due to NH_3-N oxidation, however, with the decrease in NH_3-N (Figure 6.3-1) the DO became surplus and started to increase. After NH_3-N oxidation was complete (about 250 minutes later), the DO stayed at the maximum level until the end of the cycle.

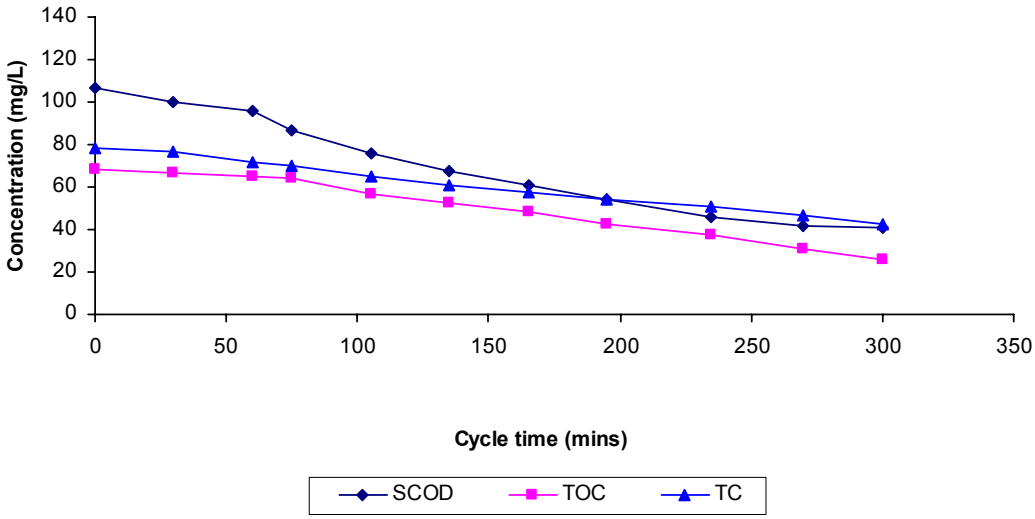


Figure 6.3-2 Carbon profile in SBR with no addition of 2, 4-D in feed

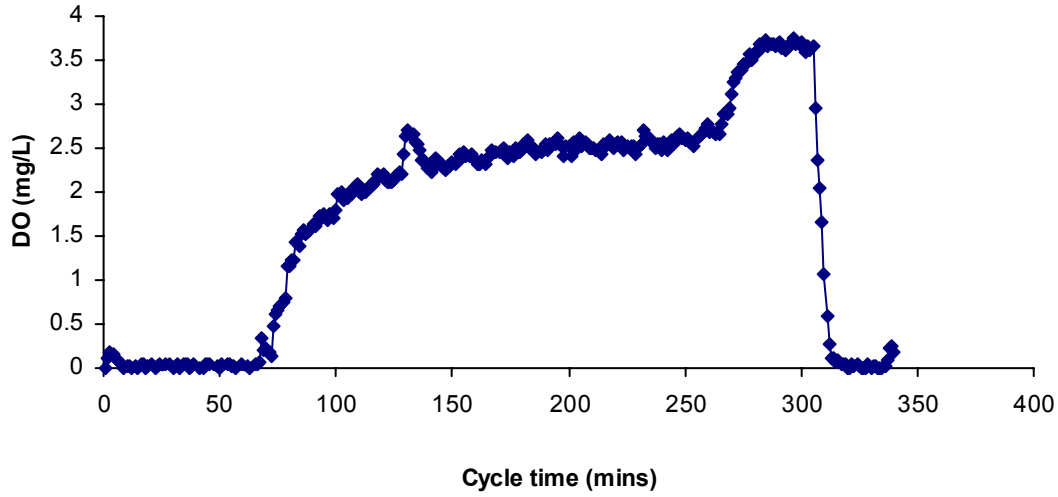


Figure 6.3-3 DO profile in SBR with no addition of 2, 4-D in feed

6.4 SBR operation and control parameters

A targeted biomass concentration of 4000 mg/L (expressed as MLSS) was selected which is within the typical range of aerobic activated sludge reactors (Metcalf and Eddy, 2003). Figure 6.4-1 shows the MLSS over the duration of the SBR operation. The mean MLSS was 3653 ± 547 mg/L. The mean SRT was 20 ± 9 days.

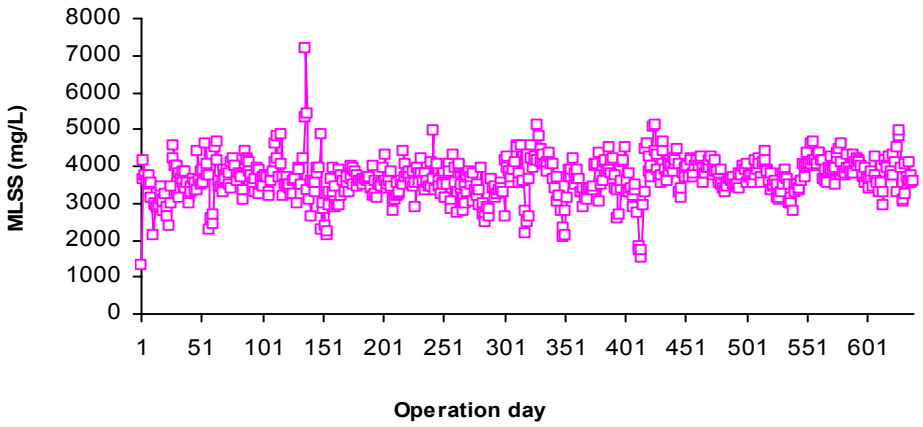


Figure 6.4-1 SBR mixed liquor suspended solids concentration

The settleability of the sludge fluctuated throughout the entire period of operation with the sludge volume index (SVI) averaging 101 ± 50 mL/g resulting in low effluent suspended solids content (typically less than 5 mg/L). Figure 6.4-2 shows the SVI data. The SVI excursions were a result of operational problems (eg. power failures, foaming etc.), however over 600 days of the research, the impact was thought to be minimal.

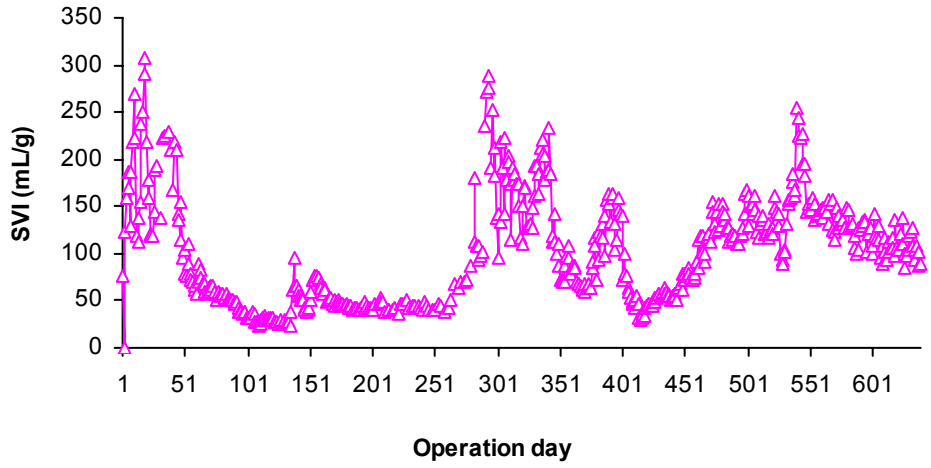


Figure 6.4-2 SBR sludge volume index

6.5 Biomass acclimation to 2, 4-D in the SBR

6.5.1 Storage of 2, 4-D

Some internal checks on the wastewater containing 2, 4-D were carried out to monitor the possibility of 2, 4-D degradation in the freezer. The results (Figure 6.5-1) indicate that there was only 6-15% variation during the 7 days of storage. This was probably due to analytical error as the possibility of biodegradation in the freezer was considered to be negligible.

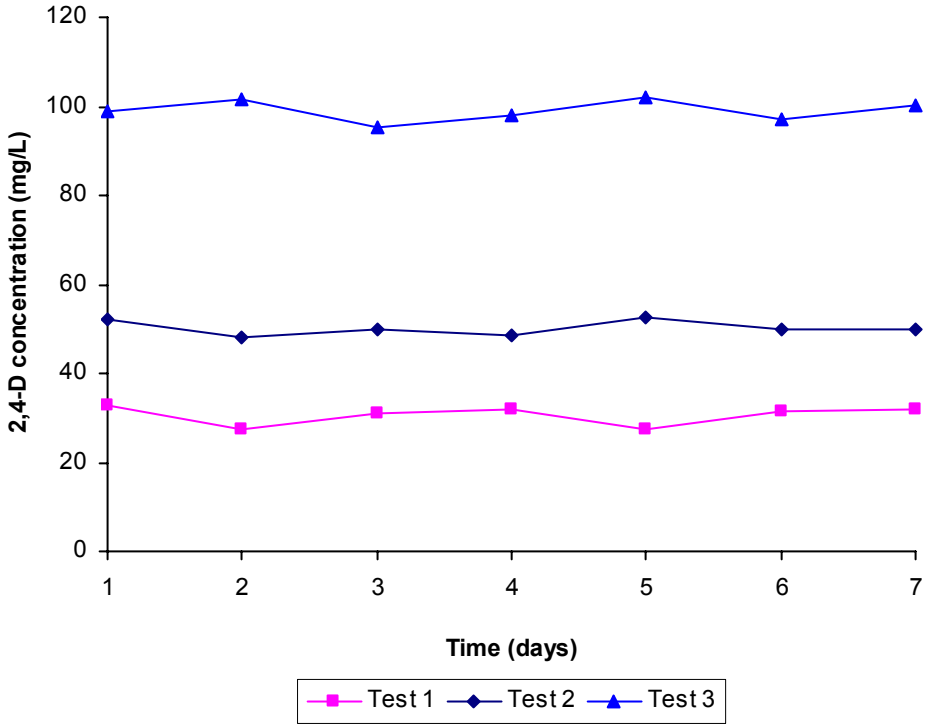


Figure 6.5-1 Variation of 2, 4-D concentration during cold storage with wastewater

6.5.2 2, 4-D removal in the SBR

After exposure to 30 mg/L of 2, 4-D for approximately 2 weeks, the SBR exhibited the first signs of biodegradation with an increasing trend until 90 % removal was accomplished approximately 30 days later (Figure 6.5-2).

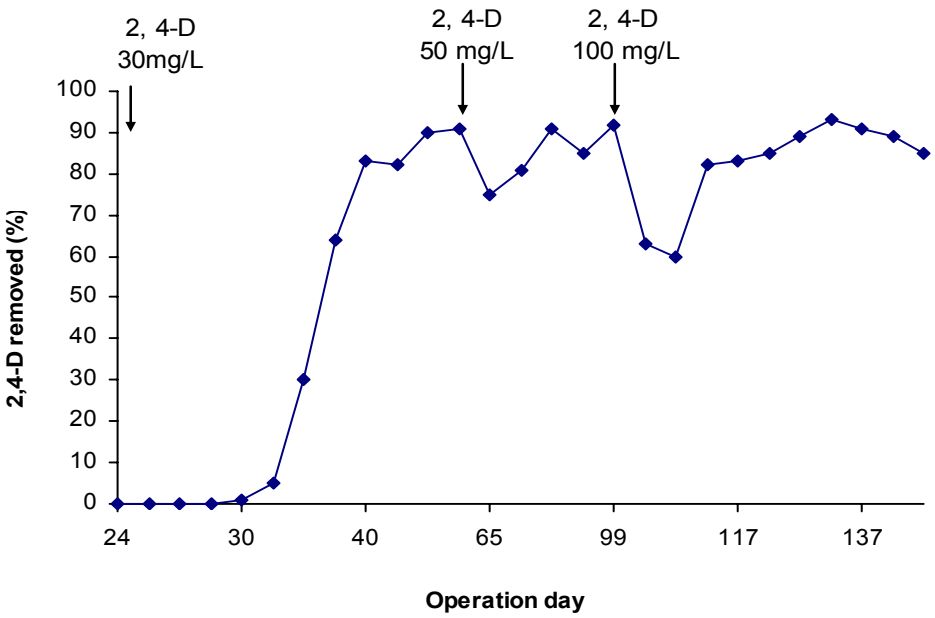


Figure 6.5-2 Removal of 2, 4-D in the SBR

Subsequent to this, when the 2, 4-D concentration was raised from 30 mg/L to 50 mg/L on day 66, a slight decrease in the 2, 4-D degradation efficiency was observed (it dropped from 91 % to 75 %). About one week later, the biomass had adapted to the new 2, 4-D concentration and the efficiency gradually increased to around 80 %, reaching approximately 90 % two weeks after the new concentration (50 mg/L) was added. The second increase of 2, 4-D (from 50 mg/L to 100 mg/L) however had a greater effect on the SBR system, which led to a large drop in MLSS from nearly 4000 mg/L to around 2600 mg/L and an accompanying drop in 2, 4-D degradation efficiency down to 63 %. This lasted for nearly 10 days and less sludge was wasted during this period until the MLSS slowly increased. Thirty-six days later, 93 % of 2, 4-D removal was achieved.

The success of this strategy emphasizes the importance of an acclimation period. The non-biodegradable nature of 2, 4-D at short exposures to microbial cultures, can be attributed to the lack of appropriate enzyme systems for its metabolism as a substrate. This obstacle is overcome by an acclimation phase where development of the necessary enzymatic structure takes place, presumably by induction or depression of the existing enzyme production or by random genetic mutation which confers the new enzyme production capability (Lackmann et al., 1980). As the acclimation period proceeded, it was found that the affinity of the consortia to biodegrade the toxic compound increased (Moreno-Andrade and Buitron, 2004). The need for biomass acclimation to 2, 4-D has been frequently reported in the literature. Orhon et al. (1989) showed that in order to degrade 100 to 400 mg/L of 2, 4-D, an acclimation period of 35 to 45 days was needed. However, an elevated concentration of the toxic compound may cause inhibition problems. For example, Moreno-Andrade and Buitron (2003) observed that inhibition is not only a function of the initial substrate concentration, but also of the initial biomass concentration. In general, a low biomass concentration will produce a greater inhibition. For this reason, the acclimation of microorganisms is preferably done at a lower initial substrate to microorganism ratio. Greer et al. (1990) reported that the starting bacterial population density and the initial 2, 4-D concentration were linearly related to the lag time observed prior to the start of 2, 4-D degradation. The effect of initial 2, 4-D concentration on the acclimation period has been explored by many other researchers (Ou et al., 1978; Davidson et al., 1980; Parker and Doxtader, 1982). Mangat and Elefsiniotis (1999) proved that the presence of an initial 40 mg/L of 2, 4-D in their SBR system resulted in reduced supplemental substrate removals which indicated the inhibitory effect of 2, 4-D; however, once the microorganisms are acclimated, the increase of 2, 4-D concentration to 100 mg/L did not result in any noticeable inhibitory effects on supplemental substrate degradation, and the treatability limit of the system was reached at a feed concentration of 300 mg/L 2, 4-D.

6.5.3 Track studies on 2, 4-D removal in the SBR

Several track studies were carried out during the operating cycle particularly at each point of 2, 4-D increase in order to observe the 2, 4-D removal pattern (Appendix C). Samples of the mixed liquor were collected at regular intervals during the non-aerated and aerated phases, filtered and analyzed on the HPLC for 2, 4-D concentration. The

decant of both the previous cycle and the tested cycle was analyzed for each track study. Figure 6.5-3 illustrates the 2, 4-D profile with time in the SBR.

Track studies 1, 2 and 3 were carried out during the period of 30 mg/L of 2, 4-D in the feed. Track studies 4, 5 and 6 were carried out after the 2, 4-D concentration was increased to 50 mg/L while track studies 7, 8, 9 and 10 were performed when the 2, 4-D concentration was raised to 100 mg/L. The removal of 2, 4-D during the 60 minutes of non-aerated period was found to be negligible in all the track studies while most of the 2, 4-D was removed within 240 minutes of aerated conditions. The removal efficiency ranged from 60 to 96 % according to the different acclimation times and the variable 2, 4-D concentration in the feed. The specific consumption rate of 2, 4-D was calculated for each track study and the results are listed in Table 6.5-1.

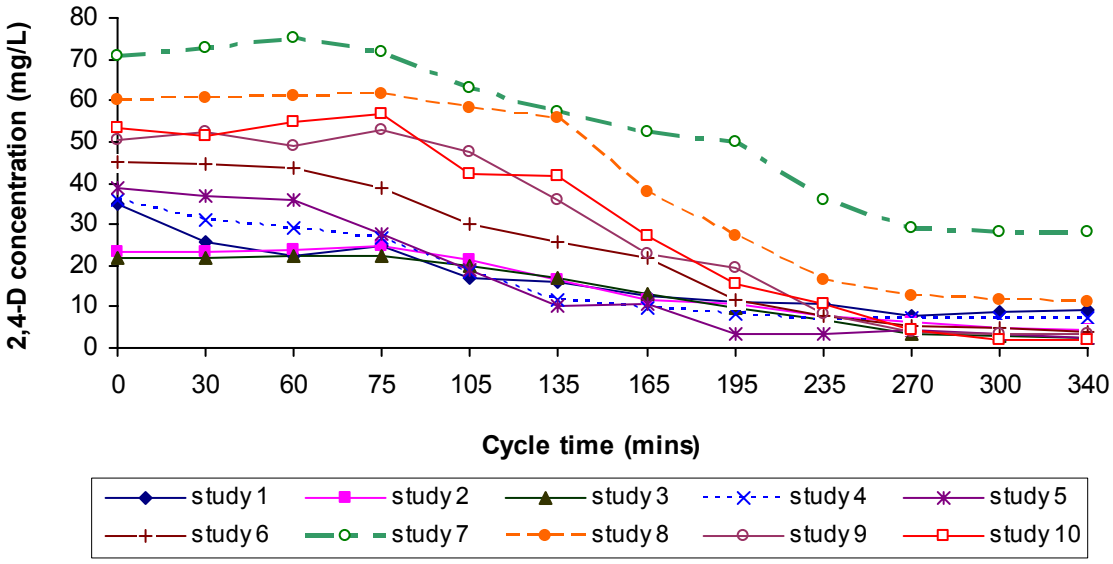


Figure 6.5-3 Track studies on 2, 4-D removal in the SBR

The results depicted in Figure 6.5-3 reveal that the SBR proved to be a suitable technology for the degradation of toxic and biorefractory compounds, such as the herbicide studied. However, the biodegradation efficiency of 2, 4-D in the SBR system is influenced by the availability of dissolved oxygen. The effects of oxygen limitation on microbial activities have been noted by Shaler and Klecka (1986) who found that dissolved oxygen concentrations below 1 mg/L may be rate limiting for the biodegradation of chlorinated aromatic compounds such as 2, 4-D. This is because they

have a requirement for molecular oxygen as a co-substrate for the initial cleavage of the ether side chain, and both the hydroxylation and subsequent fission of the aromatic nucleus. Celis (2005) similarly reported that under aerobic conditions, complete 2, 4-D removal was achieved at a feed concentration up to 500 mg/L, however, under anaerobic conditions, only partial consumption (about 40%) of 2, 4-D was observed at a feed concentration of 300 mg/L. Microbial degradation of 2, 4-D was also reported to be more rapid under aerobic conditions (half-life 1.8 to 3.1 days) than under anaerobic conditions (half-life 69 to 135 days) by Montgomery (1997). Thus further investigations of 2, 4-D degradation under anaerobic conditions is deemed to be necessary if sufficient time and enough nutrients to allow for the growth of anaerobes (such as denitrifying organisms).

Table 6.5-1 Specific 2, 4-D degradation rate in the SBR

2,4-D concentration in feed (mg/L)	Track #	MLSS (mg/L)	Specific 2,4-D degradation rate (mg/mg MLSS-d)
30	1	3500	0.031
30	2	3057	0.027
30	3	3882	0.021
50	4	3381	0.036
50	5	3039	0.050
50	6	4200	0.042
100	7	4134	0.044
100	8	2600	0.080
100	9	3100	0.064
100	10	3598	0.060
Maximum			0.080
Minimum			0.021
Mean			0.046
±σ			0.018

The average value of the specific 2, 4-D degradation rate is linked to initial feed 2, 4-D concentrations in Table 6.5-1. The average specific 2, 4-D degradation rate is 0.026 mg/mg VSS/day when the initial 2, 4-D concentration was 30 mg/L; the average specific 2, 4-D degradation rate increased to 0.043 mg/mg VSS/day when the initial 2, 4-D concentration was 50 mg/L; the average specific 2, 4-D degradation rate increased again to 0.062 mg/mg VSS/day when the initial 2, 4-D concentration increased to 100 mg/L. As can be seen, the specific 2, 4-D degradation rates increased gradually with an increase in concentration up to 100 mg/L suggesting that the system had not reached its

full biodegradation potential which confirms the ability of the biomass to degrade the herbicide. Similar studies of 2, 4-D in an aerated SBR have revealed that following acclimation, complete 2, 4-D biodegradation was accomplished (at feed concentrations up to 500 mg/L) with a specific 2, 4-D utilization rate of 0.041 mg/mg VSS/day (Celis, 2005). Mangat and Elefsiniotis (1999) achieved 2, 4-D specific removal values ranging from 0.035 to 1.62 mg/mg MLVSS/day (the high rate of 1.62 was associated with a low concentration, specialised-biomass degrading 2, 4-D only) as a function of HRT in an aerobic SBR system. An average of specific 2, 4-D degradation rate (0.046 mg/mg MLSS per day) from this study is slightly lower than other reported research.

6.5.4 Carbon and nitrogen consumption during acclimation to 2, 4-D

The carbon (TOC, TC and SCOD) and nitrogen (TN, NH_3-N and NO_3-N) were also tracked to explore the consumption behaviour of carbon and nitrogen forms in the feed containing 2, 4-D. Representative plots of the above parameters are depicted in Figures 6.5-4 and 6.5-5. There was no evident influence on the removal pattern as compared with carbon and nitrogen consumption profiles plotted without the addition of the 2, 4-D to the feed (Figures 6.3-1 and 6.3-2).

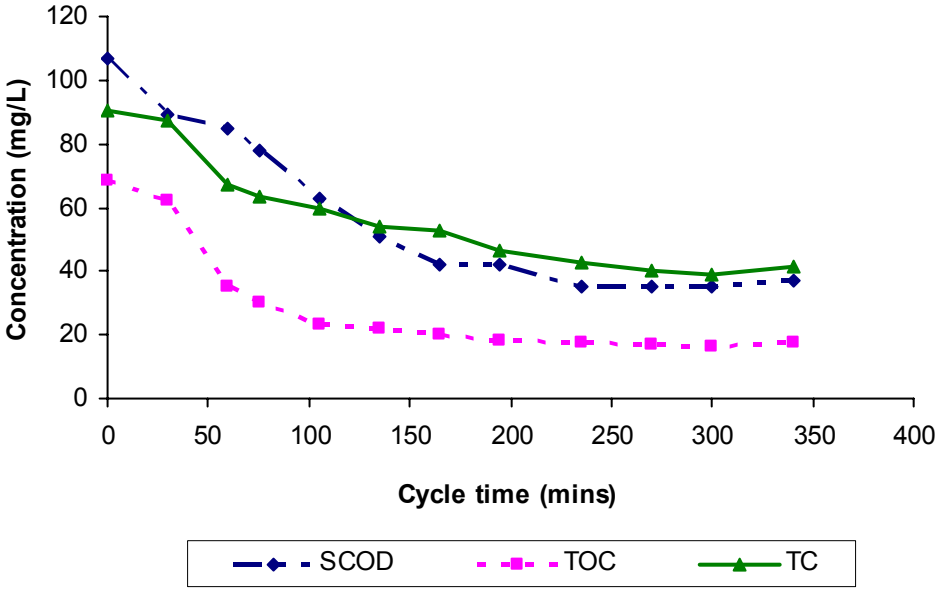


Figure 6.5-4 Carbon consumption in the SBR during acclimation to 2, 4-D

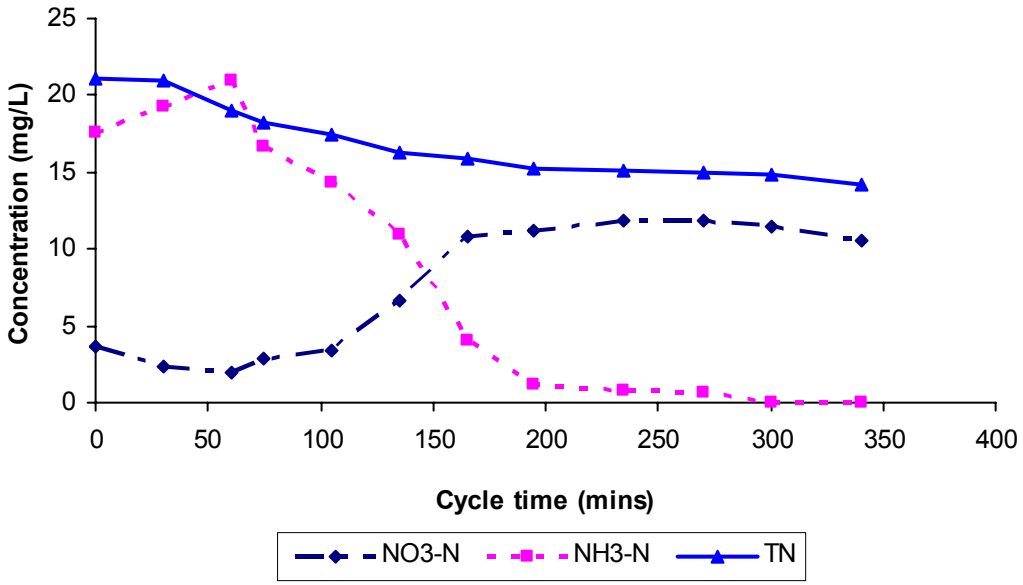


Figure 6.5-5 Nitrogen consumption in the SBR during acclimation to 2, 4-D

6.5.5 Alkalinity and DO profile during acclimation to 2, 4-D

Figure 6.5-4 for both TC and TOC indicates a decreasing trend. To investigate the inorganic carbon change during the SBR cycle, alkalinity tests were performed on a few track studies and a representative plot is shown in Figure 6.5-6. During the non-aerated phase, the alkalinity increased by about 20 % probably due to denitrification and removal of some organic products. Then after the aerated phase started, the alkalinity began to decrease gradually due to nitrification until 50-60 % of the initial value was reached at the end of the cycle. The DO utilization pattern (Figure 6.5-7) seemed to have little difference with Figure 6.3-3 which had no 2, 4-D in the feed. It implies that the concentration of 2, 4-D (30 to 100 mg/L) used in this study would not have detrimental effect on the bioactivity of microorganisms in the SBR.

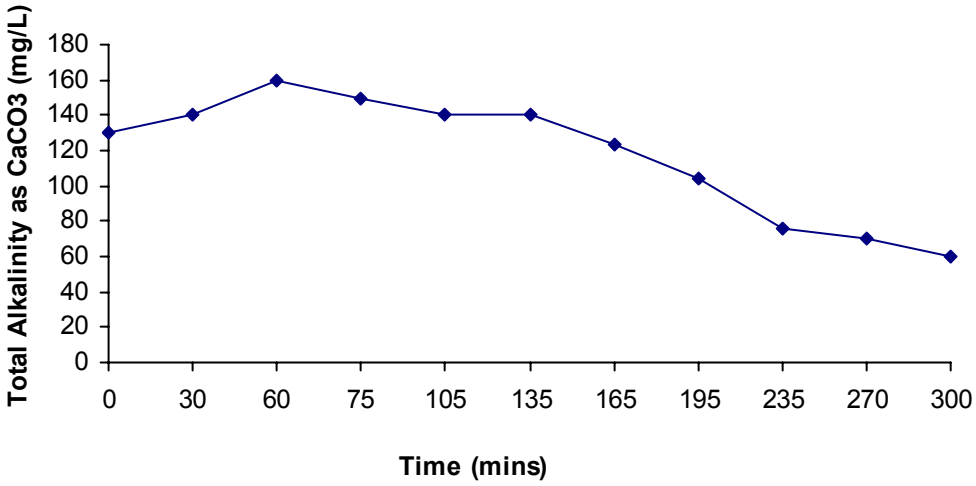


Figure 6.5-6 Alkalinity change in the SBR

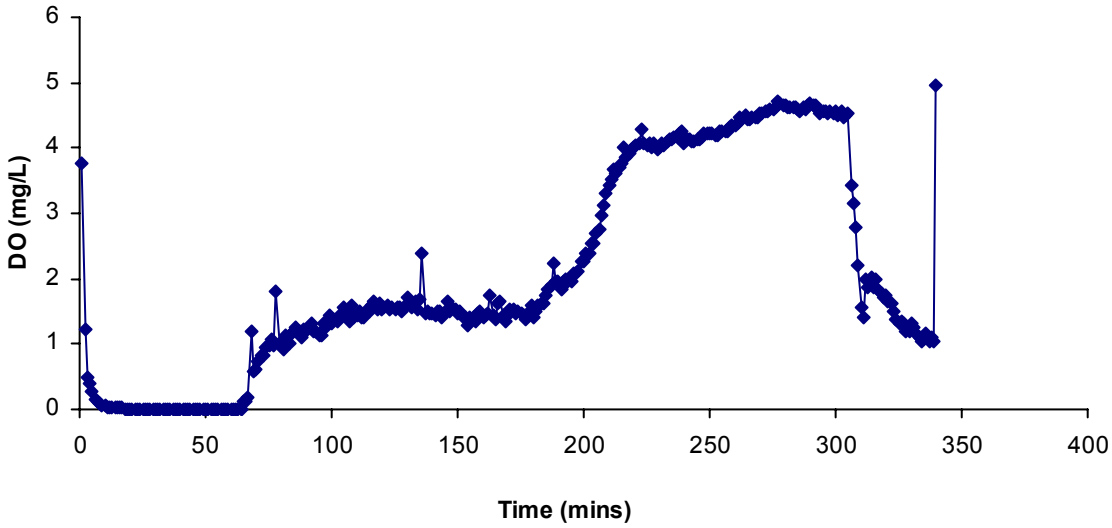


Figure 6.5-7 DO profile in the SBR during acclimation to 2, 4-D

6.6 Biosorption test

Both living and dead biomass could potentially act as biosorbents due to the passive uptake of pollutants from aqueous solutions. The disappearance of pollutants could therefore be attributed to either "biodegradation" or "biosorption" or both. In order not to overestimate the degradative potential of the microorganisms, an ultrasonic converter

was used to break up the bacterial cells and release compounds binding to the biomass surface. The result was compared with the sample without ultrasonic treatment and the difference yielded an indication of the relative partitioning of biodegradation and biosorption (Appendix C). Figure 6.6-1 illustrates some representative results during a track study of the SBR. These results show that the average increase in 2, 4-D concentration after cell destruction was 7 %; however, the general trend of 2, 4-D concentration after ultrasound treatment was consistent with the 2, 4-D concentration trend without ultrasound treatment. It could be estimated that less than 10 % of the overall uptake of 2, 4-D in the SBR was due to biosorption and more than 90 % removal of the 2, 4-D was therefore likely due to effective biodegradation.

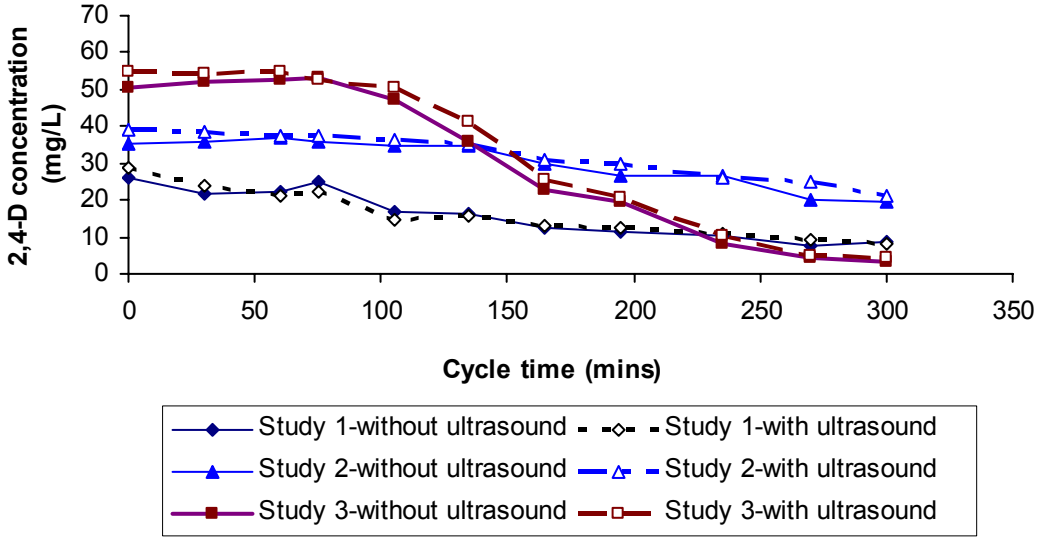


Figure 6.6-1 Biosorption track studies in the SBR

Chapter 7: Experimental Results and Discussion: Biodegradation of 2, 4-D in Denitrification Batch Reactors

7.1 Introduction

The following sections discuss the 2, 4-D biodegradation capacity of microorganisms under denitrifying conditions. Figure 7.1-1 provides a good overall map to the parts of this chapter, since the relevant section numbers are shown below (or beside) each box. The bulk of the denitrification studies were carried out by using acclimated biomass that had been collected as waste at the end of a SBR cycle and which had been stored in a container for a few days until a sufficient amount of biomass had been accumulated (approximately 1500 to 2000 mg VSS/L). Filtered effluent from the anaerobic digester (rich in VFAs) and/or 2, 4-D provided the carbon source for the microorganisms during the denitrification batch tests, while sodium nitrate solutions were made up to target various C: N ratios. All tests were conducted in 5 L glass batch reactors with an actual liquid volume of 3 L at an ambient temperature of 21-25 $^{\circ}$C. The reactors were stirred continuously at a rate deemed sufficient by visual inspection to ensure solids were suspended at all times. The effect of naturally-produced VFAs as a co-substrate on 2, 4-D removal efficiency was compared to those using 2, 4-D as sole carbon source. Denitrification studies were also carried out using synthetic VFAs plus 2, 4-D as carbon sources to investigate any differences between natural and synthetic VFAs. Finally, to compare the 2, 4-D removal capability between acclimated and unacclimated biomass, the activated sludge from a local wastewater treatment plant was collected and tested under similar conditions. All specific rates in this study have been calculated for the total length of the run (i.e. including the acclimation period) to obtain an overall assessment of the ability of the biomass to perform under the specific conditions investigated. Statistical studies were carried out to compare results from different batch tests and these are included in Appendix B.

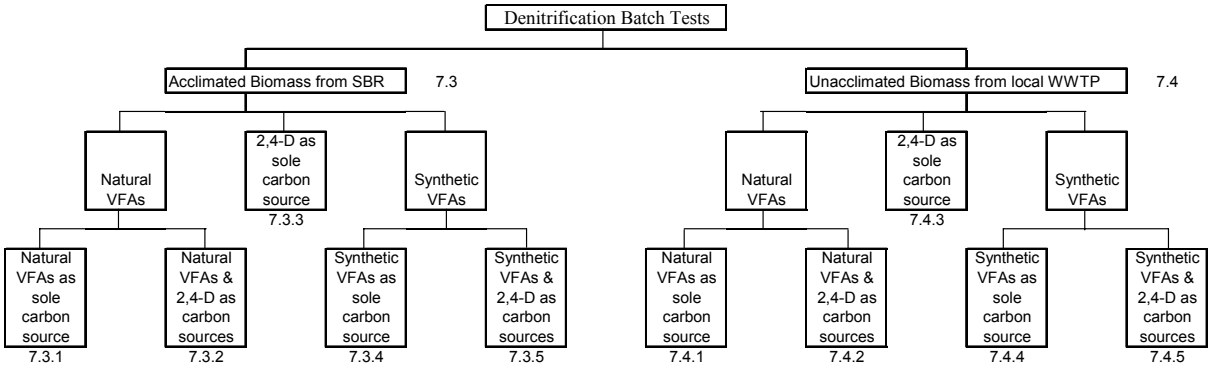


Figure 7.1-1 Experimental map of denitrification batch tests

7.2 Abiotic losses

Three batch reactors were operated as controls, with no addition of biomass to quantify abiotic losses of 2, 4-D, VFA-C and nitrate-nitrogen. The first control experiment was conducted with the addition of 100 mg/L of 2, 4-D, the second was conducted with the addition of 400 mg/L of VFA-C and the third was with the addition of nitrate-nitrogen at a concentration of 100 mg/L. Results showed that 2, 4-D, VFA-C and NO_3-N concentrations all remained essentially the same (less than 5% variation) during 5 days of operation indicating that abiotic losses of 2, 4-D, VFA-C and NO_3-N were negligible. It should be noted that the main mechanism of 2, 4-D, VFA-C and NO_3-N disappearance is biodegradation, since volatilisation, photodegradation and adsorption on biomass appear to be insignificant (Mangat and Elefsiniotis, 1999; Li, 2001).

7.3 Denitrification batch tests using SBR acclimated biomass

7.3.1 Natural VFAs as a sole carbon source (acclimated biomass)

To observe the nitrogen and VFA-C utilization patterns in the absence of any potential toxic effects associated with 2, 4-D, naturally-produced VFAs were used as a sole carbon source during baseline denitrification tests. The denitrification stoichiometric equations including cell synthesis for the three main volatile fatty acids (acetic, propionic, and butyric) are written below (Elefsiniotis et al., 2004):

Acetic acid:

$$\frac{133}{140}NO_3^- + \frac{1}{4}CH_3COO^- + \frac{23}{140}H_2CO_3 \rightarrow \frac{1}{28}C_5H_7NO_2 + \frac{1}{10}N_2 + \frac{6}{35}H_2O + \frac{34}{70}HCO_3^- \quad (7.1)$$

Propionic acid:

$$\frac{133}{140}NO_3^- + \frac{1}{7}CH_3CH_2COO^- + \frac{9}{70}H_2CO_3 \rightarrow \frac{1}{28}C_5H_7NO_2 + \frac{1}{10}N_2 + \frac{6}{35}H_2O + \frac{53}{140}HCO_3^- \quad (7.2)$$

Butyric acid:

$$\frac{133}{140}NO_3^- + \frac{1}{10}CH_3CH_2CH_2COO^- + \frac{4}{35}H_2CO_3 \rightarrow \frac{1}{28}C_5H_7NO_2 + \frac{1}{10}N_2 + \frac{6}{35}H_2O + \frac{47}{140}HCO_3^- \quad (7.3)$$

The theoretical VFA-C consumption for complete denitrification (including the requirements for the growth of biomass) is 1.82 mg of HAc-C per mg of NO_3-N, 1.56 mg of HPr-C per mg of NO_3-N, and 1.45 mg of HBu-C per mg of NO_3-N. The actual C:N ratio should be larger than the theoretical value, usually by up to 20 to 30% (Her and Huang, 1995), that is about 2.0. The anaerobic digester effluent in this study (Chapter 5) typically contained a mixture of 648 mg HAc-C/L, 521 mg HPr-C/L and 496 mg HBu-C/L, that is, a total of 1665 mg VFA-C/L.

In this study, a total of 10 tests were carried out and anaerobic digester effluent was added in various volumes to give influent VFA-C concentrations of approximately 60 to 800 mg/L. The combinations of anaerobic digester effluent and sodium nitrate solution (60 to 200 mg NO_3-N/L) generated C: N ratios ranging from 0.5 to 5.0. Tap water was added to standardize the liquid volume to 3 L. The specific denitrification rates (k_N) were expressed in mg of NO_3-N removed per mg of reactor VSS per day and the specific carbon consumption rates (k_C) were expressed in mg of VFA-C consumed

per mg of reactor VSS per day. The mean VSS concentration in the batch reactors was used as a representative value for calculation purposes.

In general, denitrification proceeded successfully in all reactors which were evidenced from pH recovery and nitrate-nitrogen disappearance as well as VFA-C consumption profiles. No nitrite production was observed. Some typical nitrogen removal profiles as a function of time are depicted in Figure 7.3-1. There is a relatively short lag period occurring within the first 6 hours probably due to acclimation of the denitrifying microorganisms to the substrate. After 6-9 hours, the nitrate-nitrogen concentration dramatically decreased, disappearing completely within 48-82 hours (depending on the initial NO_3-N concentration). This clearly indicates that denitrification is feasible using naturally produced VFAs as a sole carbon source.

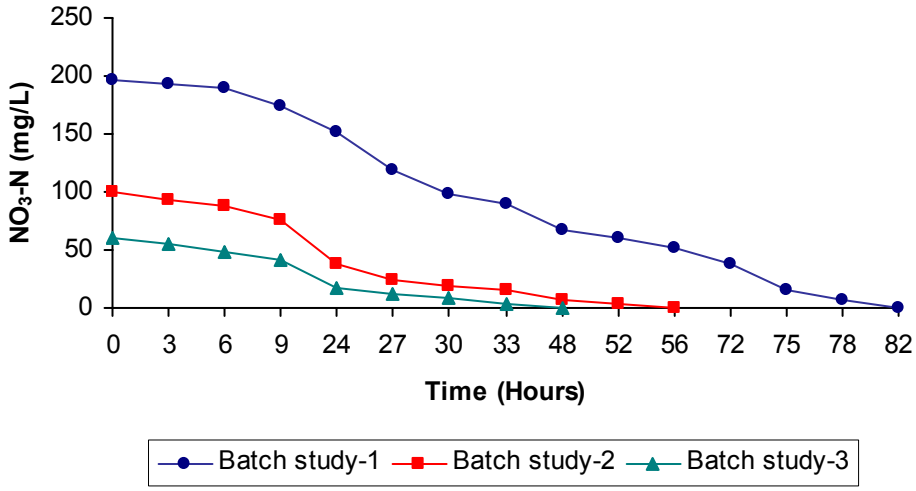


Figure 7.3-1 Nitrate nitrogen removal profile using natural VFAs as a sole carbon source and SBR acclimated biomass

In order to ascertain how well the naturally produced VFAs were used in the denitrification process, the specific denitrification and VFA-C consumption rates were calculated and these are shown in Table 7.3-1.

Table 7.3-1 Denitrification batch test results using natural VFAs as a sole carbon source and SBR acclimated biomass

Reactor	Initial [NO_3-N] (mg/L)	Initial [VFA-C] (mg/L)	C:N ratio	k_N (g NO_3-N/g VSS per day)	k_C (g VFA-C/g VSS per day)
B-1	98.4	68	0.69	0.024	0.023
B-2	100	73	0.73	0.024	0.024
B-3	76	106	1.39	0.021	0.031
B-4	79.7	118	1.48	0.022	0.033
B-5	100.2	245	2.45	0.028	0.051
B-6	60	159	2.65	0.019	0.043
B-7	196.7	805.6	4.10	0.027	0.068
B-8	200.1	799.4	4.00	0.028	0.066
B-9	95.6	428	4.48	0.022	0.062
B-10	96.8	184.6	1.91	0.024	0.055
Average ±σ				0.024 0.003	0.046 0.017

When compared to most of the specific denitrification rates reported in the literature (ranging from 0.014 to 0.754 g NO_x-N/gVSS per day (Table 2.2-1)), results from this study (0.024 ± 0.003 g NO_3-N/gVSS per day) appear to be at the low end of the spectrum. However, some of the rates in Table 2.2-1 are associated with continuous, flow-through systems which have generally higher rates than those obtained from batch systems. This is due to the non-steady state nature of batch systems and acclimation of bacteria to particular carbon sources in continuous flow through systems (Elefsiniotis et al., 2004). Despite being low, the denitrification rates of this study are comparable to a few other studies (Li, 2001; Elefsiniotis et al., 2004).

A representative plot of the VFA consumption pattern is shown in Figure 7.3-2 indicating a preferential order of utilization of VFA species. The trend indicates that acetic acid is the preferred VFA species for denitrification, an observation that is consistent with other researchers (Xu, 1996; Li, 2001; Elefsiniotis et al., 2004). Propionic acid appears to be the second most preferred VFA species while butyric and valeric acids are consumed at nearly the same time.

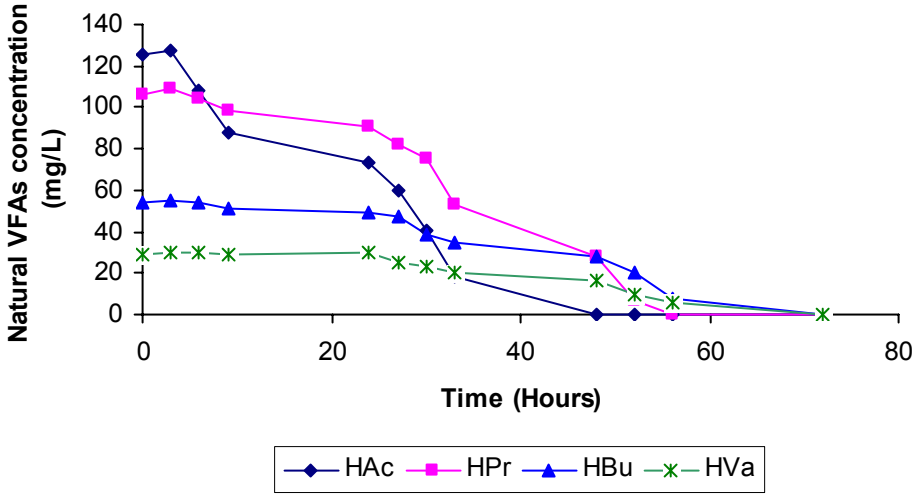


Figure 7.3-2 Natural VFA consumption pattern using SBR acclimated biomass

The initial C: N ratio plays a critical role with respect to the specific VFA carbon consumption rates. From Table 7.3-1, the specific VFA carbon consumption rates can be classified into two distinct sets based on C: N ratios which are greater than two and lower than two (Table 7.3-2).

Table 7.3-2 Comparison of specific VFA-C consumption rates (k_c) based on C: N ratios

Reactor	C:N ratio<2	k_C (g VFA-C/g VSS per day)	Reactor	C:N ratio>2	k_C (g VFA-C/g VSS per day)
B-1	0.69	0.023	B-5	2.45	0.051
B-2	0.73	0.024	B-6	2.65	0.043
B-3	1.39	0.031	B-7	4.10	0.068
B-4	1.48	0.033	B-8	4.00	0.066
B-10	1.91	0.055	B-9	4.48	0.062
Average ±σ	1.24 0.52	0.0332 0.0129	Average ±σ	3.53 0.92	0.0580 0.0107

A summary of the results (Table 7.3-2) indicates that the specific carbon consumption rate is significantly affected by the limitation of external carbon, dropping from an average value of 0.058 g VFA-C/g VSS per day at a C: N ratio above two to 0.033 g VFA-C/g VSS per day at C: N ratio below two. The rates from these two sets prove statistically different from each other at the 95 % confidence level (Appendix B). Results from this study also suggest that denitrification was not inhibited at limited

carbon conditions which are consistent with Li (2001), even at C: N ratios lower than one. This strengthens the possibility that endogenous carbon was exploited as a carbon source by the bacterial population in the presence of inadequate amounts of external VFA carbon.

7.3.2 Natural VFAs and 2, 4-D as carbon sources (acclimated biomass)

The denitrification batch reactors were filled with prepared SBR sludge seed and various concentrations of nitrate-nitrogen (50 to 300 mg/L). Anaerobic digester effluent was added in various volumes to give influent VFA-C concentrations of approximately 40 to 400 mg/L. Finally, 2, 4-D was added in concentrations ranging from 30 to 100 mg/L which was similar to the concentrations initially used in the SBR. These combinations of carbon and nitrogen resulted in C: N ratios ranging from 0.5 to 5.0. Tap water was added to standardize the liquid volume to 3 L and a total of 18 tests were carried out.

Experimental results in Table 7.3-3 show that there was an average of 54 % of the 2, 4-D removed through cometabolism with naturally produced VFAs. A representative plot of VFA consumption, NO_3-N consumption and 2, 4-D degradation is shown in Figure7.3-3. There is a large fluctuation in percent of 2, 4-D removal ranging from 12 to 85 %. The high removals (82-85 %) were at consistently low initial 2, 4-D concentrations of 31-49 mg/L, while the lowest removal (12%) was linked to the highest initial 2, 4-D concentration (122.3 mg/L).

Table 7.3-3 Denitrification batch test results using natural VFAs and 2, 4-D as carbon sources with SBR acclimated biomass

Reactor	Initial [NO_3-N] (mg/L)	Initial [VFA-C] (mg/L)	Initial 2,4-D (mg/L)	Initial C:N ratio	k_N (g NO_3-N/g VSS per day)	k_C (g VFA-C/g VSS per day)	$k_{2,4\text{-}D}$ (g 2,4-D/g VSS per day)	2,4-D removal (%)
A-1	100	220	58.6	2.45	0.025	0.1571	0.0097	68
A-2	100	46	62.4	0.73	0.021	0.051	0.0095	65
A-3	100	413	122.3	4.66	0.023	0.1632	0.0088	12
A-4	80	235.9	42	3.17	0.012	0.0951	0.0031	34
A-5	60	106.9	43.5	2.10	0.012	0.0849	0.0045	67
A-6	300	201	46.6	0.74	0.036	0.1388	0.0047	83
A-7	200	206	45.8	1.13	0.024	0.1285	0.0047	85
A-8	100	202.5	49	2.24	0.026	0.0694	0.0047	82
A-9	300	209	108.6	0.85	0.035	0.1384	0.0059	45
A-10	200	198.5	99.7	1.21	0.023	0.1384	0.0058	48
A-11	100	202	104	2.47	0.015	0.1368	0.0063	51
A-12	200	233.1	41	1.25	0.017	0.1409	0.0035	41
A-13	100	230.2	40	2.48	0.013	0.1393	0.006	24
A-14	70	235	40	3.61	0.012	0.1425	0.0028	50
A-15	200	245.9	112	1.47	0.018	0.1345	0.0067	64
A-16	100	238	104	2.83	0.012	0.1325	0.0038	28
A-17	70	257.1	106	4.33	0.011	0.136	0.0043	45
A-18	50	66.9	31	1.61	0.011	0.062	0.0042	83
Average ±σ					0.0192 0.0079	0.1216 0.0335	0.0055 0.0021	54.17 21.89

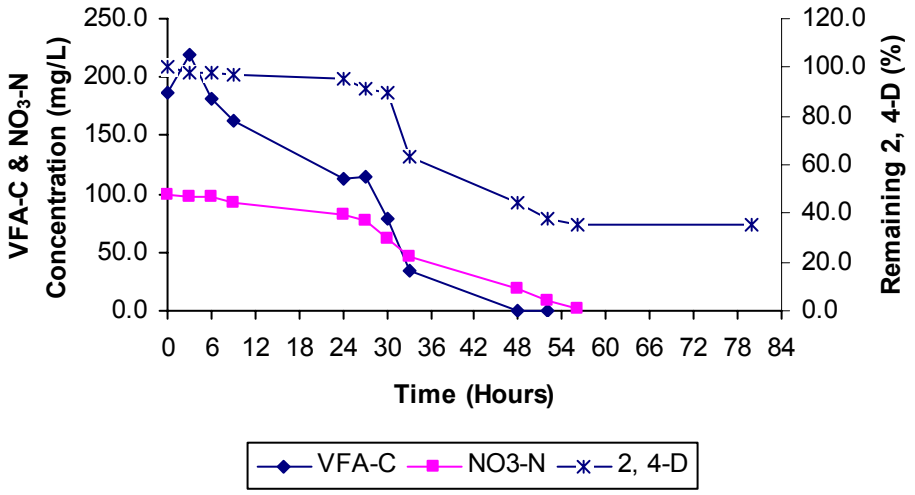


Figure 7.3-3 Natural VFA-C consumption, NO_3-N degradation and 2, 4-D removal using SBR acclimated biomass

According to Table 7.3-3, the average specific denitrification rate (0.0192 g NO_3-N/g VSS per day) using dual-substrates was slightly lower than the rate using natural VFAs as a sole carbon source (0.024 g NO_3-N/g VSS per day), however, the specific VFA-C

consumption rate (0.1216 g VFA-C/g VSS per day) using dual-substrates was much higher than the rate using natural VFAs as a sole carbon source (0.046 VFA-C/g VSS per day). These rates proved statistically different from each other at the 95 % confidence level (Appendix B). These findings suggest that the addition of a non-growth substrate (2, 4-D) below a concentration of 100 mg/L may inhibit the nitrate reduction process while at the same time not appearing to inhibit the VFA-C consumption pattern. This seems to be a puzzling result and can only be due to non-denitrifying activity; however, more in depth microbial studies would be needed to confirm or reject this hypothesis.

Figure 7.3-3 also indicates the relatively long period (30 hours) needed for biomass to acclimatize to 2, 4-D despite being exposed in the SBR to similar 2, 4-D conditions. This is probably because the SBR experienced aerobic conditions and, at high concentrations of 2, 4-D, a sufficient enrichment time is required in denitrification cultures to achieve the selection of the appropriate catabolic enzymes. Significant degradation of 2, 4-D occurred only after supplemental substrate (natural VFAs) had been significantly consumed (about 50 %). This process indicates a (not surprisingly) sequential substrate utilization pattern with simpler (non-chlorinated) organic compounds degraded first, followed by chlorinated organic compounds degradation (Mangat and Elefsiniotis, 1999). This phenomenon is known as “diauxic growth” due to catabolite repression (i.e. the repression of enzymes that degrade a less rapidly metabolised energy source in the presence of a more rapidly metabolised one) (Mangat and Elefsiniotis, 1999). Bacterial proliferation and enzyme induction then enhanced the capability of microorganisms to use 2, 4-D as a growth substrate, since 2, 4-D degradation continued even after VFAs had reached very low levels. Such a degradation pattern is in agreement with that observed by Chiavola et al. (2004) who used non-chlorinated organic compound (phenol) to biodegrade a chlorinated organic compound (3-chlorophenol) through cometabolism.

Compared with aerobic degradation of 2, 4-D in the SBR (Chapter 5), the average specific 2, 4-D degradation rate in the denitrification batch reactor is approximately 10 times lower. This is probably due to the different aerobic and anoxic/anaerobic biodegradation pathways. Nonetheless, these results show that 2, 4-D can be degraded under nitrate-reducing conditions and suggest that anoxic/anaerobic treatment may

provide a useful option for remediation of 2, 4-D contaminated sediments where aerobic methods may face many technical problems (i.e. buoyancy of air or low solubility of oxygen in water).

As Table 7.3-3 indicates, there is considerable variation in rate data. This is expected in non-steady-state batch reactor systems. Elefsiniotis et al. (2004) stated that in steady state systems, such as continuous flow systems, any influence associated with the initial operating conditions is minimized (i.e. the so-called "memory" that bacterial seed have of the initial conditions becomes muted as new generations of bacteria are produced). The lack of the smoothing-out effect in non-steady-state systems such as batch reactors makes it difficult to ensure exact replication of initial conditions.

The relationships between the specific VFA-C consumption rate and initially applied VFA-C concentrations are expressed graphically in Figure 7.3-4.

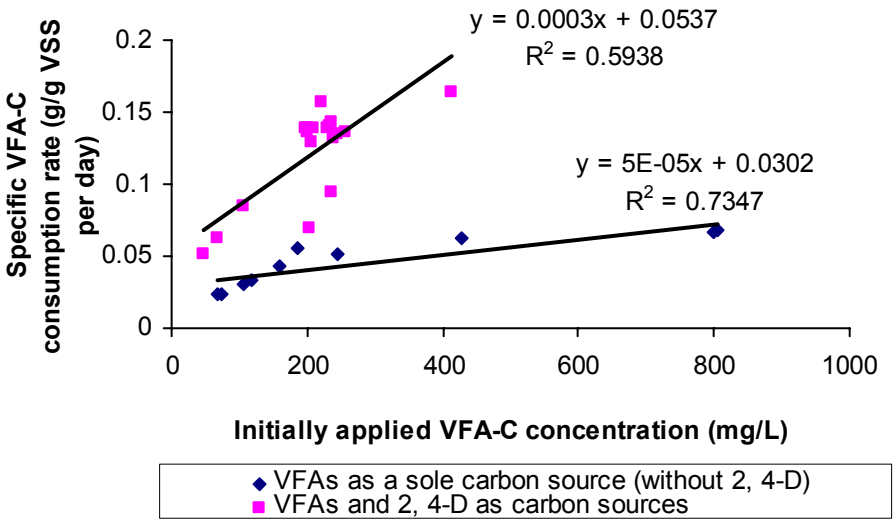


Figure 7.3-4 Relationships between the specific VFA-C consumption rate and initially applied VFA-C concentrations in the denitrification batch systems with and without 2, 4-D

As seen from this figure, for both conditions with and without 2, 4-D existing in the denitrification batch system, the specific VFA-C consumption rate presented a positive linear relationship against initially applied VFA-C concentrations with correlation coefficients (R^2) equal to 0.5938 (with 2, 4-D) and 0.7347 (without 2, 4-D), respectively.

According to the Monod model, the substrate-limited conditions are expressed as:

$$\frac{dS}{dtX} = \frac{kS}{K_m + S} \tag{7.4}$$

Where: $\frac{dS}{dt}$ = the consumption rate of the growth substrate (VFA-C);

X= the biomass concentration;

S= the growth substrate concentration;

$\frac{dS}{dtX}$ = the specific consumption rate of the growth substrate;

k= the maximum specific consumption rate of the growth substrate; and

K_m= the half-saturation constant of the growth substrate.

When S is much smaller than K_m, the specific consumption rate of the growth substrate is linearly proportional to S, which coincides with the regression result without 2, 4-D as shown in Figure 7.3-4. However, for a **competitive inhibition model** that relates the consumption of a growth substrate to the consumption of a non-growth substrate in cometabolism, the Alvarez-Cohen model is expressed as:

$$\frac{dS}{dtX} = \frac{kS}{K_m(1 + IK_I) + S} \tag{7.5}$$

Where: I= the non-growth substrate concentration (2, 4-D); and

K_I= the half-saturation constant of the non-growth substrate.

If I is much smaller than K_I, equation (7.5) will be similar to equation (7.4). Hence, the specific VFA-C consumption rate still presents linearly against initially applied VFA-C concentration for the system with 2, 4-D as shown in Figure 7.3-4. According to Figure 7.3-4, the slope of the line (k/ K_m) is equal to about 5×10^{-5} for the batch tests without adding 2, 4-D. This number is approximately 10 times lower than the batch tests with 2, 4-D applied (3×10^{-4}). Therefore, it indicates that the addition of an appropriate

concentration of 2, 4-D (below 100 mg/L) may not inhibit VFA-C consumption during the denitrification process.

7.3.3 2, 4-D as a sole carbon source (acclimated biomass)

When 2, 4-D was tested as a sole carbon source in the denitrification batch tests, the theoretical stoichiometry of 2, 4-D removal and nitrate transformation was calculated assuming complete oxidation of 2, 4-D to CO_2 coupled to denitrification, as in the following reaction equation:

$$C_8H_6Cl_2O_3 + 6NO_3^- \rightarrow 8CO_2 + 2Cl^- + 3N_2 + H_2O + 4OH^- \qquad (7.6)$$

which gives a theoretical nitrate/2, 4-D stoichiometry of 6.0 mol nitrate per mol of 2, 4-D, which equals C: N ratio of 1.14: 1.0. The actual C: N ratio should be larger than the theoretical value due to the inclusion of cell synthesis or biomass growth. In this study, the initial 2, 4-D concentration was selected to be between 30 and 100 mg/L while the initial NO_3-N concentration was from 20 to 100 mg/L. These combinations of carbon and nitrogen resulted in C: N ratios ranging from 0.1 to 3.5. The denitrification rate and 2, 4-D biodegradation efficiencies are summarized in Table 7.3-4 while representative plots of NO_3-N and 2, 4-D degradation are shown in Figure 7.3-5.

In general, denitrification proceeded successfully in each reactor which was evidenced by pH recovery and nitrate-nitrogen disappearance. No nitrite production was detected. The removal of 2, 4-D was observed to be a low efficiency process compared with the pervious batch tests using VFAs and 2, 4-D as co-substrates. The relationship between C: N ratios and 2, 4-D removal efficiency is plotted in Figure 7.3-6.

Table 7.3-4 Denitrification batch test results using 2, 4-D as a sole carbon source with SBR acclimated biomass

Reactor	Initial [NO_3-N] (mg/L)	Initial 2,4-D (mg/L)	Initial C:N ratio	k_N (g NO_3-N/g VSS per day)	$k_{2,4\text{-}D}$ (g 2,4-D/g VSS per day)	2,4-D removal (%)
C-1	100	40	0.17	0.008	0.0016	42
C-2	80	35	0.19	0.007	0.0017	48
C-3	20	71	1.54	0.01	0.0010	29
C-4	18	66	1.59	0.009	0.0008	27
C-5	16	112	3.04	0.008	0.0023	12
C-6	15	110	3.19	0.008	0.0022	14
C-7	100	32	0.14	0.014	0.0018	43
C-8	12.6	62.4	2.15	0.016	0.0041	36
C-9	14	108.8	3.38	0.02	0.0031	18
C-10	10	35.3	1.53	0.016	0.0023	43
C-11	46	67.2	0.63	0.013	0.0014	30
C-12	59	30	0.22	0.017	0.0008	32
C-13	63	50	0.34	0.014	0.0008	39
C-14	14	70	2.17	0.009	0.0006	26
C-15	21	100	2.07	0.011	0.0012	15
C-16	50	62.2	0.54	0.016	0.0009	29
C-17	21	102	2.11	0.009	0.0012	14
C-18	20	105	2.28	0.01	0.0011	13
Average				0.0119	0.0016	28.33
±σ				0.0039	0.0009	11.88

Table 7.3-4 shows that the low percent removals of 2, 4-D (12-18 %) are directly linked to high initial concentrations (> 100 mg/L) of 2, 4-D. The average specific 2, 4-D degradation rate (0.0016 g 2, 4-D/g VSS per day) and specific denitrification rate (0.0119 g NO_3-N/g VSS per day) in Table 7.3-4 are much lower than the average rates in Table 7.3-3 using both VFA and 2, 4-D as co-substrates. These rates prove statistically different from each other at the 95 % confidence level (Appendix B). The 2, 4-D removal efficiency is also low averaging only 28%. The average specific 2, 4-D degradation rate is about 30 times lower than the one in the SBR. The reasons are because it is acclimated to anoxic conditions (i.e. in the denitrification reactors) compared to primarily aerobic conditions (SBR). However, according to Figure 7.3-5, the NO_3-N concentration dropped significantly in the first three days and completely disappeared at the end of the test. This suggests that the bioactivity of the bacteria is not completely inhibited by 2, 4-D up to 100 mg/L but endogenous carbon sources rather than 2, 4-D are exploited as energy sources for cell maintenance.

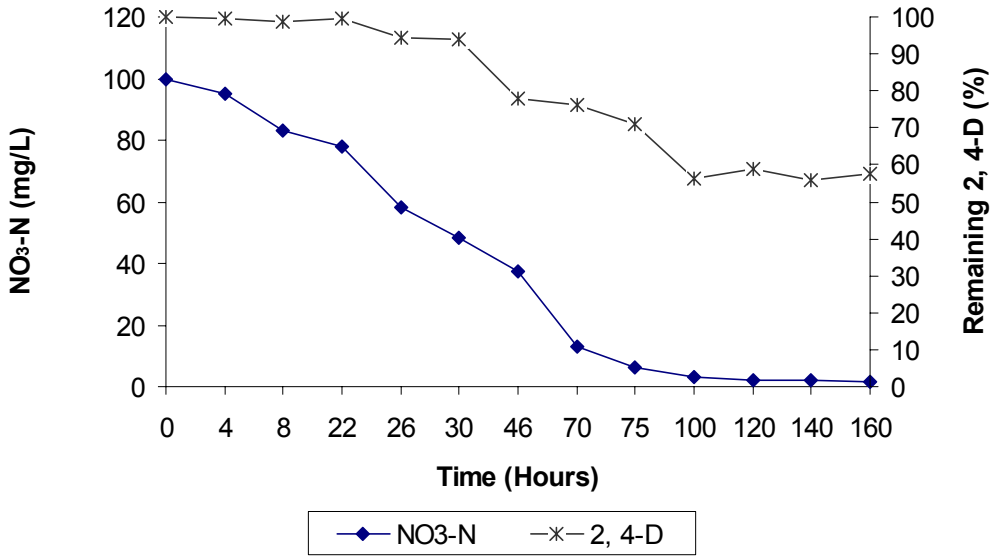


Figure 7.3-5 Plot of NO_3-N and 2, 4-D degradation using 2, 4-D as a sole carbon source with SBR acclimated biomass

Figure 7.3-5 indicates that the NO_3-N decreased significantly in 70 hours, completely disappearing by the end of the test. In contrast, the 2, 4-D concentration did not drop significantly in the first 30 hours although the biomass had in theory been acclimated in the SBR with similar 2, 4-D concentrations. This is because it was not really an acclimation to anoxic conditions, but rather an acclimation to aerobic conditions for degradation. That is, the bacteria were acclimated to 2, 4-D (the electron donor) but they were not acclimated in terms of the electron acceptor. It was thought however that sufficient enrichment time during a specific denitrification test would eventually allow expression of the enzymes to remove 2, 4-D. Figure 7.3-5 indicated that 42 % of the 2, 4-D was removed, from 30 hours onwards to the end of the test.

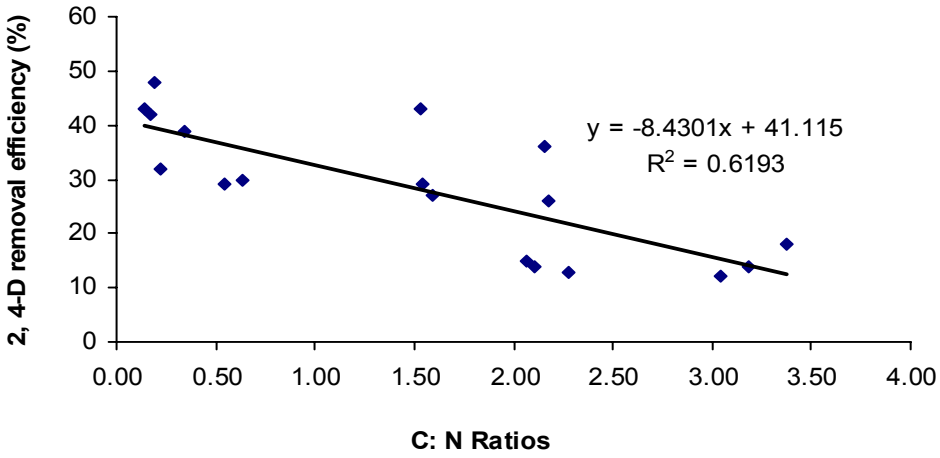


Figure 7.3-6 Relationship between C: N ratios and 2, 4-D removal efficiencies using 2, 4-D as a sole carbon source and SBR acclimated biomass

As seen from Figure 7.3-6, a negative linear relationship between C: N ratios and 2, 4-D removal efficiencies was observed with R^2 equal to 0.6193. The removal efficiency of 2, 4-D decreased from 48 % to 12 % when the values of C: N ratio increased from 0.1 to 3.5. Apparently, high level of 2, 4-D concentrations or relatively low NO_3-N concentrations resulted in low percent of 2, 4-D degradation during the denitrification process. However, despite the low efficiencies recorded, this set of experiments suggests that 2, 4-D could be utilized as a sole carbon source for acclimated biomass under nitrate-reducing conditions, but with the need of better control of the initial 2, 4-D concentrations due to its toxicity.

7.3.4 Synthetic VFAs as a sole carbon source (acclimated biomass)

These sets of experiments were carried out to observe the utilization pattern of synthetic VFAs in the denitrification reaction, and to rule out any unlikely preference by denitrifying bacteria for natural over synthetic VFAs or vice versa. Synthetic VFAs included pure acetic acid or a mixture of acetic acid (50 %), propionic acid (30 %) and butyric acid (20 %) which is similar to the natural VFAs speciation in the digester effluent (Chapter 5). A total of ten reactors were used with five reactors containing acetic acid and five reactors containing a mixture of acetic, propionic and butyric acid

to give VFA-C concentrations of approximately 40 to 220 mg/L. Sodium nitrate was added to each reactor to give various concentrations of NO_3-N (30 to 200 mg/L) resulting in approximately 1.0 to 3.5 C: N ratios. Results of the denitrification rate and VFA-C consumption rates are summarized in Table 7.3-5 while the preferential VFA consumption pattern is plotted in Figure 7.3-7.

Similar to the process using natural VFAs as a sole carbon source, the reactions using synthetic VFAs as a sole carbon source proceeded successfully in all reactors as observed by pH recovery, dramatic nitrate-nitrogen decrease and complete VFA consumption within 48-72 hours (depending on the initial concentration).

Table 7.3-5 Denitrification batch test results using synthetic VFAs as a sole carbon source with SBR acclimated biomass

Reactor	Initial [NO_3-N] (mg/L)	VFA type	Initial [VFA-C] (mg/L)	C:N ratio	k_N (g NO_3-N/g VSS per day)	k_C (g VFA-C/g VSS per day)
D-1	100	Acetic	200	2.00	0.023	0.046
D-2	70	Acetic	125	1.79	0.019	0.04
D-3	50	Acetic	63	1.26	0.016	0.029
D-4	35	Acetic	42	1.20	0.019	0.025
D-5	20	Acetic	55	2.75	0.017	0.027
D-6	200	Mixture #	220	1.10	0.043	0.047
D-7	100	Mixture #	220	2.20	0.024	0.049
D-8	95	Mixture #	142	1.49	0.023	0.042
D-9	67	Mixture #	220	3.28	0.017	0.049
D-10	42	Mixture #	105	2.50	0.015	0.036
Average					0.022	0.039
±σ					0.008	0.009

#: Mixture of Acetic, Propionic and Butyric acids

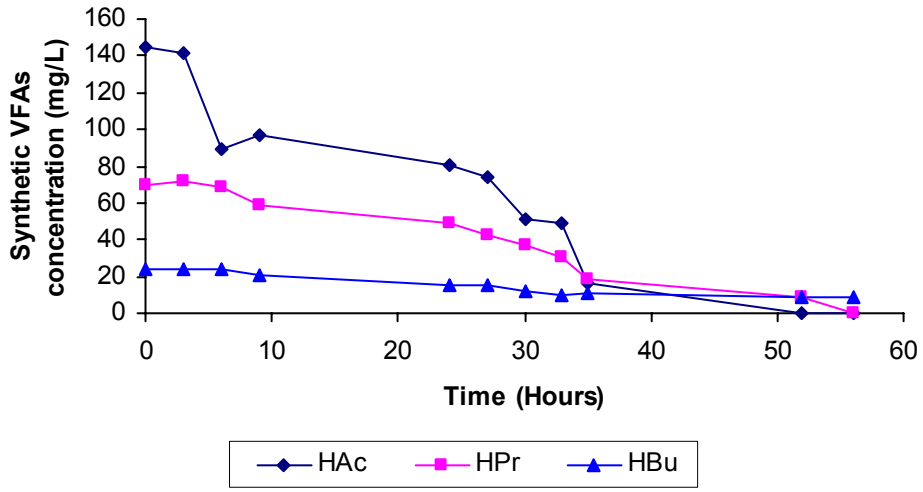


Figure 7.3-7 Synthetic VFA consumption pattern using SBR acclimated biomass

The average specific denitrification rate (0.022 g NO_3-N/g VSS per day) and specific VFA-C consumption rate (0.039 g VFA-C/g VSS per day) for the synthetic VFAs are close to the values obtained using natural VFAs (0.024 g NO_3-N/g VSS per day and 0.046 g VFA-C/g VSS per day). Statistical analysis (Appendix B) also indicates that there is no significant difference between synthetic and natural VFAs with respect to the specific denitrification and the VFA-C consumption rates at the 95 % confidence

level. The data therefore imply that both synthetic and natural VFAs provide an excellent carbon source for denitrification and that denitrifying bacteria do not differentiate with respect to the origin of VFAs. This is supported by Elefsiniotis and Wareham (2006) who found that denitrifying bacteria do not prefer naturally-generated over synthetic carbon types or vice versa. Regarding the effect of C: N ratios on synthetic VFA carbon utilization, similarly to the natural VFAs, the specific VFA-C consumption rate dropped from 0.0414 (VFA-C: NO_3-N > 2) to 0.0322 (VFA-C: NO_3-N < 2) g VFA-C/g VSS per day. It implies that the specific carbon consumption rate is affected by the limitation of external carbon. However, within the experimental set using synthetic VFAs as a sole carbon source, both the k_N value and k_C value for the mixture (0.0244 g NO_3-N/g VSS per day and 0.0446 g VFA-C/g VSS per day) are higher than the pure acetic value (0.0188 g NO_3-N/g VSS per day and 0.0334 g VFA-C/g VSS per day), indicating a preferential hierarchy. It is not clear whether the hierarchy is a result of a concentration effect or a result of denitrifying bacteria invoking certain specific metabolic pathways.

7.3.5 Synthetic VFAs and 2, 4-D as carbon sources (acclimated biomass)

To compare the effect of natural and synthetic VFAs on 2, 4-D biodegradation, synthetic VFAs including pure acetic acid and a mixture of acetic acid (50 %), propionic acid (30 %) and butyric acid (20%) were utilized in the denitrification batch tests. The VFAs were randomly mixed by different volumes of VFA type. The concentration of 2, 4-D in the denitrification batch test ranging from 30 to around 120 mg/L and a total of 18 runs were carried out. Nitrate-nitrogen concentrations ranged from 30 to 200 mg/L. The combinations of carbon and nitrogen resulted in C: N ratios ranging between 1.0 and 2.7 (with only one exception of 4.28). The specific denitrification, VFA-C consumption and 2, 4-D biodegradation rates are shown in Table 7.3-6 while a representative plot of VFA consumption, NO_3-N and 2, 4-D degradation pattern is shown in Figure 7.3-8.

Table 7.3-6 Denitrification batch test results using synthetic VFAs and 2, 4-D as carbon sources and SBR acclimated biomass

Reactor	Initial $[NO_3\text{-}N]$ (mg/L)	VFA type	Initial [VFA-C] (mg/L)	Initial 2,4-D (mg/L)	Initial C:N ratio	k_N (g NO_3-N/g VSS per day)	k_C (g VFA-C/g VSS per day)	$k_{2,4\text{-}D}$ (g 2,4-D/g VSS per day)	2,4-D removal (%)
E-1	102	Acetic	200	40.9	2.13	0.017	0.102	0.0027	61
E-2	96	Acetic	150	33.3	1.71	0.016	0.088	0.0038	66
E-3	121	Mixture	220	52.5	2.01	0.021	0.126	0.0044	48
E-4	58	Mixture	220	65.1	4.28	0.012	0.126	0.0045	32
E-5	63	Acetic	128	60.7	2.45	0.012	0.123	0.0038	33
E-6	100	Acetic	110	82.4	1.46	0.017	0.094	0.0059	41
E-7	78	Mixture	85	81	1.54	0.015	0.077	0.0069	42
E-8	66	Mixture	87	49.9	1.65	0.013	0.051	0.0053	52
E-9	59	Acetic	95	46.7	1.95	0.013	0.072	0.0061	56
E-10	77	Acetic	141	106.6	2.43	0.016	0.125	0.0045	24
E-11	48	Mixture	79	110	2.64	0.012	0.058	0.007	25
E-12	54	Mixture	70	54.3	1.73	0.014	0.031	0.0103	69
E-13	200	Acetic	162	49.2	0.92	0.036	0.081	0.0068	77
E-14	175	Mixture	158	122.1	1.21	0.034	0.086	0.0071	30
E-15	50	Acetic	73	104	2.36	0.015	0.062	0.0068	28
E-16	48	Mixture	78	62.2	2.19	0.011	0.048	0.0102	68
E-17	30	Acetic	35	44	1.80	0.011	0.041	0.0064	54
E-18	32	Mixture	62	37.4	2.45	0.012	0.065	0.0046	47
Average ±σ						0.017 0.007	0.081 0.030	0.0060 0.0020	47.39 16.59

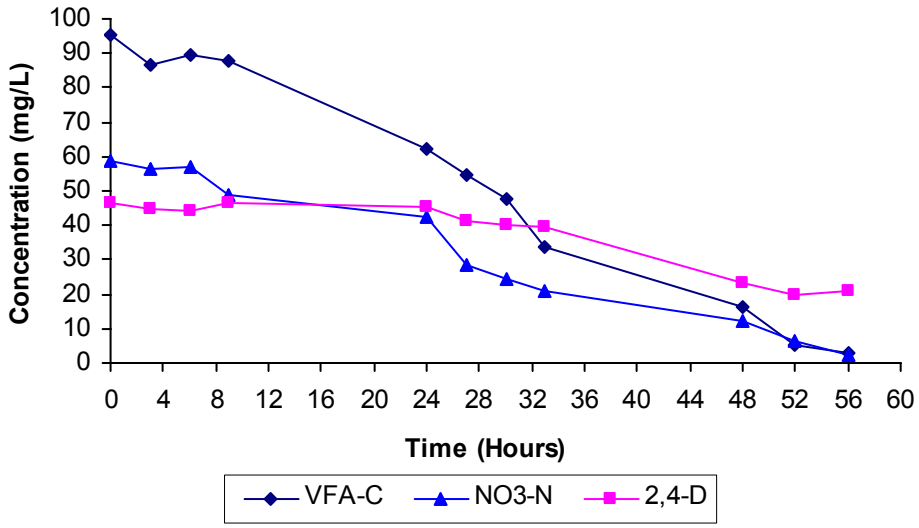


Figure 7.3-8 Synthetic VFA-C consumption, NO_3-N degradation and 2, 4-D removal using SBR acclimated biomass

Table 7.3-6 reveals that the average specific denitrification rate (0.017 g NO_3-N/g VSS per day), specific VFA-C consumption rate (0.081 g VFA-C/g VSS per day) and specific 2, 4-D degradation rate (0.0060 g 2, 4-D/g VSS per day) for synthetic VFAs

are all close to the values obtained using natural VFAs which had rates of 0.0192 g NO_3-N/g VSS per day, 0.1216 g VFA-C/g VSS per day and 0.0055 g 2, 4-D/g VSS per day. Statistical analysis (Appendix B) also proves that there is no significant difference between synthetic and natural VFAs with respect to the specific denitrification and the VFA-C consumption rates at the 95 % confidence level. The 2, 4-D removal efficiencies of synthetic and natural VFAs are similar, with 47.39 % and 54.17 %, respectively. However, compared with 2, 4-D as a sole carbon source (Appendix B), using synthetic VFAs plus 2, 4-D as carbon sources yielded higher specific denitrification and 2, 4-D degradation rates. Conversely, compared with synthetic VFA as a sole carbon source (Appendix B), using synthetic VFA plus 2, 4-D as carbon sources gave relatively lower specific denitrification rates but higher VFA-C consumption rates. This all suggests that synthetic VFAs are similar to natural VFAs, as far as the bacteria are concerned and therefore VFAs in general are a readily biodegradable substrate which supports the growth of bacteria and maintains bioactivity. Additionally, 2, 4-D is a non-growth substrate which inhibits the denitrification process.

However, within the experimental set using synthetic VFAs plus 2, 4-D as carbon sources, both the k_N and k_C value for the mixture (0.016 g NO_3-N/g VSS per day and 0.074 g VFA-C/g VSS per day) were lower than pure acetic value (0.017 g NO_3-N/g VSS per day and 0.087 g VFA-C/g VSS per day). This finding is opposite to the experimental set using synthetic VFAs as a sole carbon source. Therefore, the addition of 2, 4-D changed the preferential hierarchy between pure acetic acid and the mixture of acetic, propionic and butyric acids. However, the $k_{2,\,4\text{-}D}$ value for the mixture (0.0067 g 2, 4-D/g VSS per day) was higher than the pure acetic acid value (0.0052 g 2, 4-D/g VSS per day) suggesting that the mixture of acids favored bacterial metabolic pathways for the degradation of 2, 4-D.

Similar to Figure 7.3-4 showing the relationship between the specific VFA-C consumption rates and initially applied VFA-C concentrations in the denitrification batch system with and without 2, 4-D, Figure 7.3-9 using synthetic VFAs with or without 2, 4-D as carbon sources also indicates that the specific VFA-C consumption rate has a positive linear relationship against initially applied VFA-C concentrations with correlation coefficients (R^2) equal to 0.6700 (with 2, 4-D) and 0.9651 (without 2,

4-D), respectively. Thus, the Monod model still fits in the situation with synthetic VFA consumption.

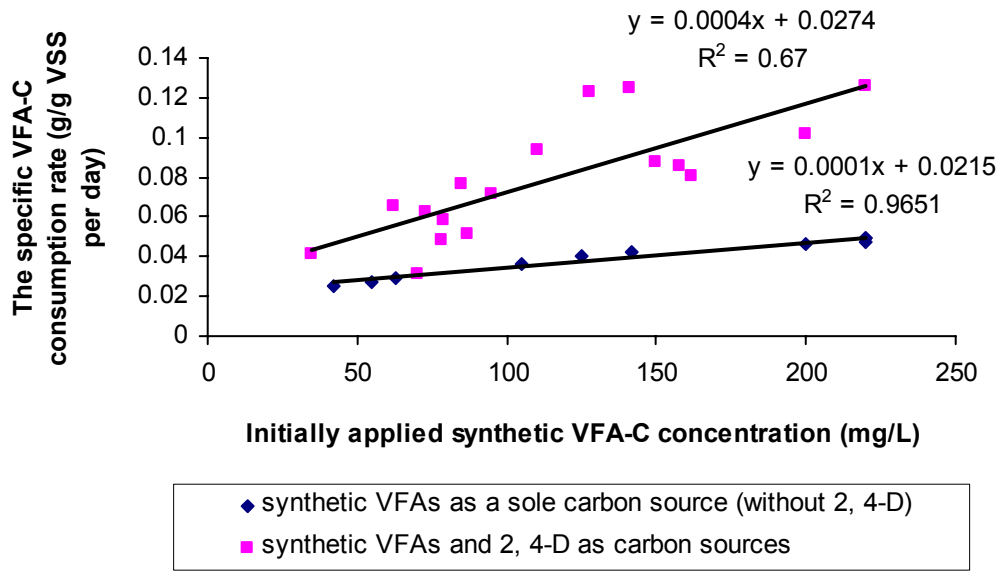


Figure 7.3-9 Relationships between the specific synthetic VFA-C consumption rate and initially applied synthetic VFA-C concentrations in the denitrification batch systems with and without 2, 4-D

According to Figure 7.3-9 and Monod model, the slope of the line (k/ K_m) is equal to about 1×10^{-4} for the batch tests without adding 2, 4-D. This number is 4 times lower than the batch tests with 2, 4-D applied (4×10^{-4}). Therefore, like natural VFAs, the addition of an appropriate concentration of 2, 4-D (30 to 100 mg/L) also promotes synthetic VFA-C consumption during the denitrification process.

7.4 Denitrification batch tests using unacclimated biomass from local WWTP

Denitrification batch tests in this section were carried out using unacclimated biomass from the Christchurch wastewater treatment plant in the same manner as the acclimated biomass from the SBR. The carbon sources used were natural VFAs alone, 2, 4-D alone, the combination of natural VFAs and 2, 4-D, synthetic VFAs alone and the combination of synthetic VFAs and 2, 4-D. There were six runs included in each test. Each reactor was seeded with unacclimated biomass of 1500 to 2000 mg MLVSS/L.

Carbon to nitrogen ratios were from 0.5 to 5.0. Statistical analyses comparing results to acclimated biomass results were performed and are shown in Appendix B.

7.4.1 Natural VFAs as a sole carbon source (unacclimated biomass)

Complete denitrification was observed using natural VFAs as a sole carbon source and unacclimated biomass as evidenced by pH recovery, NO_3-N disappearance and VFA-C consumption. There was no NO_2-N detected during the test. The specific denitrification and VFA-C consumption rates are listed in Table 7.4-1.

Table 7.4-1 Denitrification batch test results using natural VFAs as a sole carbon source and unacclimated biomass from local WWTP

Reactor	Initial [NO_3-N] (mg/L)	Initial [VFA-C] (mg/L)	C:N ratio	k_N (g NO_3-N/g VSS per day)	k_C (g VFA-C/g VSS per day)
F-1	30	77	2.57	0.017	0.032
F-2	35	99	2.83	0.018	0.034
F-3	80	110	1.38	0.022	0.041
F-4	120	128	1.07	0.025	0.044
F-5	200	263	1.32	0.028	0.052
F-6	50	159	3.18	0.019	0.049
Average				0.022	0.042
±σ				0.004	0.008

Compared with acclimated biomass using natural VFA as a sole carbon source, both k_N and k_C values are close to those values. Statistical analysis (Appendix B) also indicates that there was no significant difference at the 95 % confidence level between the two sets of data. This suggests that natural VFAs are easily degraded by biomass no matter whether it is acclimated or unacclimated to VFAs. Representative plots of the VFA-C and NO_3-N consumption patterns are shown in Figure 7.4-1. It was observed that the VFA-C kept decreasing even after the NO_3-N was eliminated. This is probably due to the diverse mixed culture in municipal activated sludge and substantial activity of non-denitrifying heterotrophic microorganisms under anaerobic conditions.

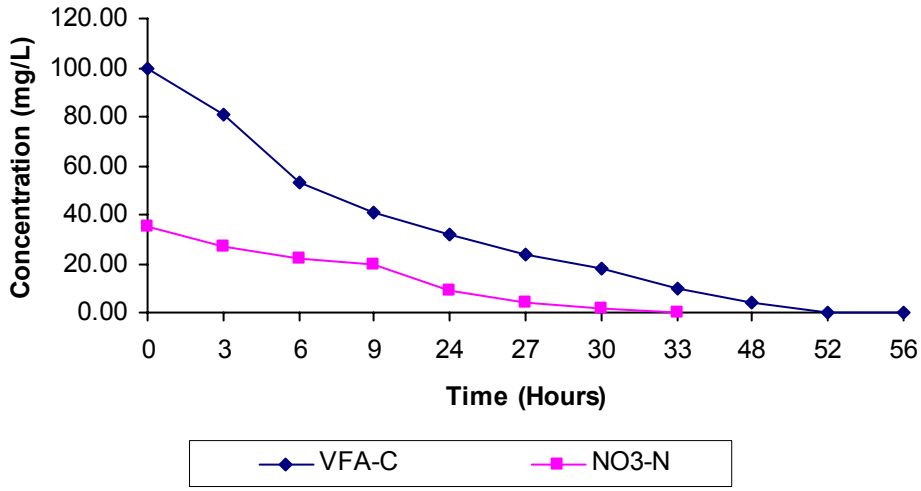


Figure 7.4-1 Natural VFA-C and NO_3-N consumption pattern using natural VFAs as a sole carbon source and unacclimated biomass from local WWTP

7.4.2 Natural VFAs and 2, 4-D as carbon sources (unacclimated biomass)

Natural VFAs and 2, 4-D were used as carbon sources by unacclimated biomass in order to compare the specific denitrification, VFA-C consumption and 2, 4-D degradation rates with the acclimated biomass. Table 7.4-2 shows the calculated rates.

Table 7.4-2 Denitrification batch test results using natural VFAs and 2, 4-D as carbon sources and unacclimated biomass from local WWTP

Reactor	Initial [NO_3-N] (mg/L)	Initial [VFA-C] (mg/L)	Initial 2,4-D (mg/L)	Initial C:N ratio	k_N (g NO_3-N/g VSS per day)	k_C (g VFA-C/g VSS per day)	$k_{2,4\text{-}D}$ (g 2,4-D/g VSS per day)	2,4-D removal (%)
G-1	100	212	56	2.36	0.019	0.0987	0.0017	19
G-2	60	46	61	1.21	0.012	0.0531	0.0015	15
G-3	100	385	100	4.28	0.021	0.1238	0.0009	10
G-4	80	232	40	3.12	0.015	0.1004	0.0031	23
G-5	60	96	43.5	1.91	0.013	0.0849	0.0029	25
G-6	154	201	46.7	1.44	0.023	0.0867	0.0028	22
Average ±σ					0.0172 0.0045	0.0913 0.0233	0.0022 0.0009	19.00 5.62

Statistical analysis (Appendix B) indicates that there was no difference for specific denitrification and VFA-C consumption rate between acclimated and unacclimated biomass. However, the acclimated biomass had a specific 2, 4-D biodegradation rate

and removal efficiency about 2.5 times higher than the unacclimated biomass. This finding supports the thesis that the acclimation process promotes the capability of biomass to utilize a toxic substance (i.e. 2, 4-D) as an organic carbon source, most probably through enzyme induction. In addition, it provides evidence that herbicide-degrading populations can be enhanced through an acclimation phase. A representative plot of VFA-C, 2, 4-D and NO_3-N consumption pattern is shown in Figure 7.4-2.

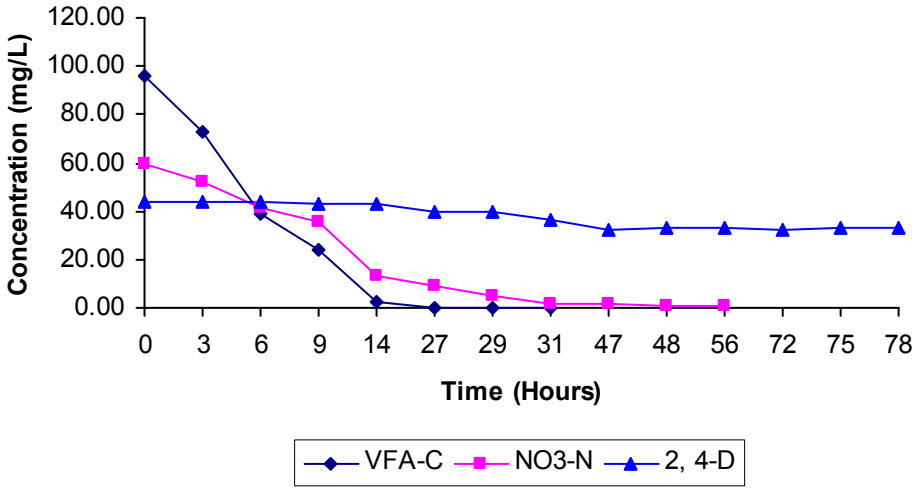


Figure 7.4-2 Natural VFA-C, 2, 4-D and NO_3-N consumption pattern using natural VFAs and 2, 4-D as carbon sources and unacclimated biomass from local WWTP

7.4.3 2, 4-D as a sole carbon source (unacclimated biomass)

It was demonstrated that 2, 4-D could be utilized as a sole carbon source during the denitrification process by SBR acclimated biomass. Therefore, this set of experiment used unacclimated biomass to compare the effect of acclimation on both denitrification and 2, 4-D biodegradation efficiency. Results are shown in Table 7.4-3. A representative plot of 2, 4-D and NO_3-N consumption pattern is shown in Figure 7.4-3.

Table 7.4-3 Denitrification batch test results using 2, 4-D as a sole carbon source with unacclimated biomass

Reactor	Initial [NO_3 N] (mg/L)	Initial 2,4-D (mg/L)	Initial C:N ratio	k_N (g NO_3-N/g VSS per day)	$k_{2,4\text{-}D}$ (g 2,4-D/g VSS per day)	2,4-D removal (%)
H-1	15	30	0.87	0.008	0.0008	12
H-2	12	33	1.19	0.009	0.0007	14
H-3	10	61	2.65	0.01	0.0010	8
H-4	12	66	2.39	0.009	0.0010	13
H-5	20	102	2.22	0.01	0.0009	9
H-6	50	98	0.85	0.012	0.0009	7
Average ±σ				0.0097 0.0014	0.0009 0.0001	10.50 2.88

Statistical analysis (Appendix B) indicates that the specific denitrification rates are close to each other however the acclimated biomass had about twice the specific 2, 4-D degradation rate and removal efficiency than the unacclimated biomass. Again, an acclimation phase is shown to be efficient with respect to herbicide biodegradation.

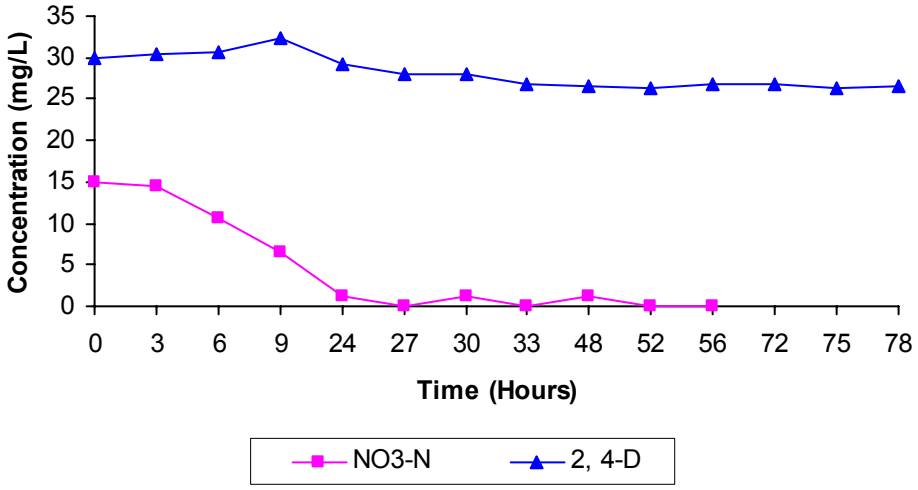


Figure 7.4-3 NO_3-N and 2, 4-D consumption pattern using 2, 4-D as a sole carbon source and unacclimated biomass from local WWTP

7.4.4 Synthetic VFAs as a sole carbon source (unacclimated biomass)

This set of experiments investigated the utilization pattern of synthetic VFAs in the denitrification reaction using unacclimated biomass. Results are shown in Table 7.4-4. A representative plot of VFA-C and NO_3-N consumption pattern is shown in Figure 7.4-4.

Table 7.4-4 Denitrification batch test results using synthetic VFAs as a sole carbon source with unacclimated biomass

Reactor	Initial [NO_3-N] (mg/L)	VFA type	Initial [VFA-C] (mg/L)	C:N ratio	k_N (g NO_3-N/g VSS per day)	k_C (g VFA-C/g VSS per day)
I-1	30	Acetic	51	1.70	0.015	0.03
I-2	66	Acetic	62	0.94	0.021	0.033
I-3	50	Acetic	110	2.20	0.016	0.041
I-4	35	Mixture #	82	2.34	0.016	0.036
I-5	44	Mixture #	93	2.11	0.018	0.037
I-6	150	Mixture #	163	1.09	0.033	0.046
Average					0.020	0.037
±σ					0.007	0.006

Statistical analysis (Appendix B) implies that, as with the acclimated biomass, unacclimated bacteria do not differentiate with respect to origin of VFAs. Thus, both natural and synthetic VFAs provide an excellent carbon source for denitrification.

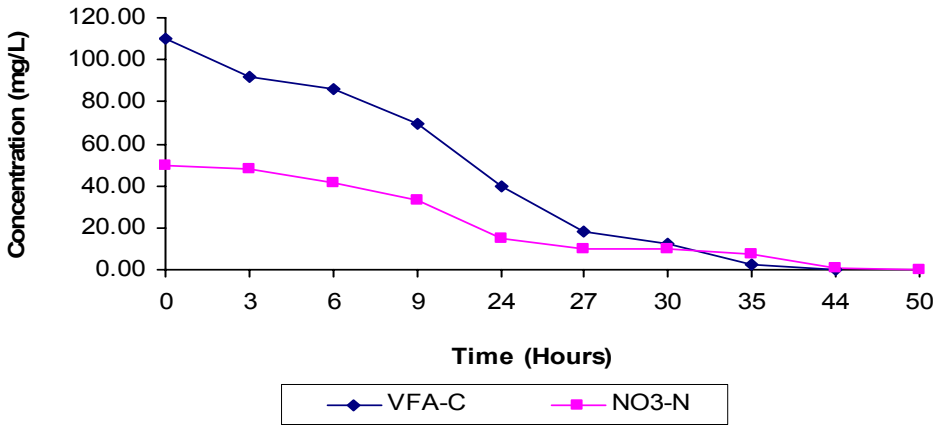


Figure 7.4-4 Synthetic VFA-C and NO_3-N consumption pattern using synthetic VFAs as a sole carbon source and unacclimated biomass from local WWTP

7.4.5 Synthetic VFAs and 2, 4-D as carbon sources (unacclimated biomass)

Synthetic VFAs and 2, 4-D were used as carbon sources for unacclimated biomass in the denitrification reaction. Results are shown in Table 7.4-5. A representative plot of VFA-C, 2, 4-D and NO_3-N consumption pattern is shown in Figure 7.4-5.

Table 7.4-5 Denitrification batch test results using synthetic VFAs and 2, 4-D as carbon sources with unacclimated biomass

Reactor	Initial [NO_3-N] (mg/L)	VFA type	Initial [VFA-C] (mg/L)	Initial 2,4-D (mg/L)	Initial C:N ratio	k_N (g NO_3-N/g VSS per day)	k_C (g VFA-C/g VSS per day)	$k_{2,4\text{-}D}$ (g 2,4-D/g VSS per day)	2,4-D removal (%)
J-1	32	Acetic	63	41	2.53	0.015	0.09	0.0022	21
J-2	46	Acetic	55	34	1.52	0.016	0.088	0.0026	22
J-3	51	Acetic	61	55	1.66	0.017	0.092	0.0014	18
J-4	62	Mixture #	153	60	2.89	0.019	0.115	0.0015	15
J-5	63	Mixture #	128	71	2.52	0.019	0.119	0.0018	11
J-6	100	Mixture #	147	96	1.89	0.021	0.124	0.0019	9
Average						0.018	0.105	0.0019	16.00
±σ						0.002	0.016	0.0004	5.29

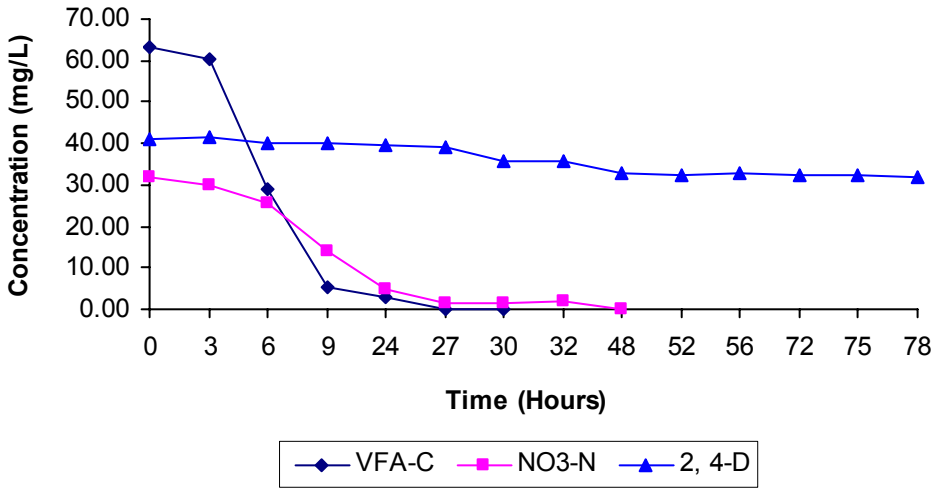


Figure 7.4-5 Synthetic VFA-C, 2, 4-D and NO_3-N consumption pattern using synthetic VFAs and 2, 4-D as carbon sources and unacclimated biomass from local WWTP

Statistical analysis (Appendix B) shows that acclimated biomass has nearly three times the specific 2, 4-D degradation rate and removal efficiency as the unacclimated

biomass. There was no significant difference with respect to the specific denitrification rate at the 95 % confidence level, however, interestingly, the unacclimated biomass was slightly higher with respect to the specific VFA-C consumption rate than the acclimated biomass. It is unclear whether this difference is caused by experimental error or is a real result with a specific metabolic pathway/explanation.

Chapter 8: Further Exploration of 2, 4-D Biodegradation

8.1 Introduction

The results from the denitrification batch tests (Chapter 7) demonstrate that the addition of a readily biodegradable carbon source adequately promotes NO_3-N reduction and 2, 4-D degradation under denitrifying conditions. Some literature indicates that continued addition of NO_3-N will increase the percent biodegradation of certain aromatic and/or phenolic compounds under denitrifying conditions (Rockne and Strand, 2001; Kesseru et al., 2005), however no literature is evident concerning whether this same effect will happen with respect to herbicide removal. That is, some denitrification results from the biodegradation of aromatic compounds report that the continued presence of NO_3-N promotes anoxic metabolism (Crawford et al., 1998; Haggblom and Young, 1999); however, in contrast, other results do not show a stoichiometric balance between nitrate and aromatic compound removal (Mihelcic and Luthy, 1988a, 1988b). Although in this research, 10 to 300 mg/L of NO_3-N was completely consumed regardless of the initial C: N ratio, no information has been obtained with respect to NO_3-N dependence on 2, 4-D degradation. Therefore a detailed investigation of the sustained biodegradation of 2, 4-D via a pulse addition of NO_3-N may promote a better understanding of the connection between the denitrifying process and the oxidative degradation of the herbicide.

In addition to this, Moreno-Andrade and Buitron (2003) observed that inhibition is not only a function of the initial substrate concentration but it is also a function of the initial biomass concentration (i.e. a high biomass concentration will show a lower inhibition). For example, Yang et al. (1999) demonstrated that an increase in biomass concentration of nitrifying bacteria would not only decrease the lag time for NH_3 oxidation in a nitrifying culture applied with TCE, but it also would increase the oxidation efficiency of TCE up to 80 %. For this reason, a set of experiments was conducted in order to determine the 2, 4-D degradation behaviour under increased biomass concentrations.

8.2 Pulse addition of NO_3-N to test 2, 4-D degradation efficiency

To determine whether 2, 4-D degradation is NO_3-N dependent, an experiment was performed consisting of four runs. The amount of biomass (collected from the SBR) during each run was approximately 2000 mg/L (expressed as MLVSS). The first run acted as a control using 2, 4-D as a sole carbon source with only an initial addition of NO_3-N (around 50 mg/L) until the end of the run. The second run was conducted under the same conditions as the first run, however, after the initial NO_3-N was completely consumed, another 50 mg/L of NO_3-N was injected into the reactor every 48 hours in three lots. The third and fourth runs were performed using both VFA and 2, 4-D as carbon sources but with the same protocol as the first two runs. The results are shown in Figure 8.2-1 and Figure 8.2-2.

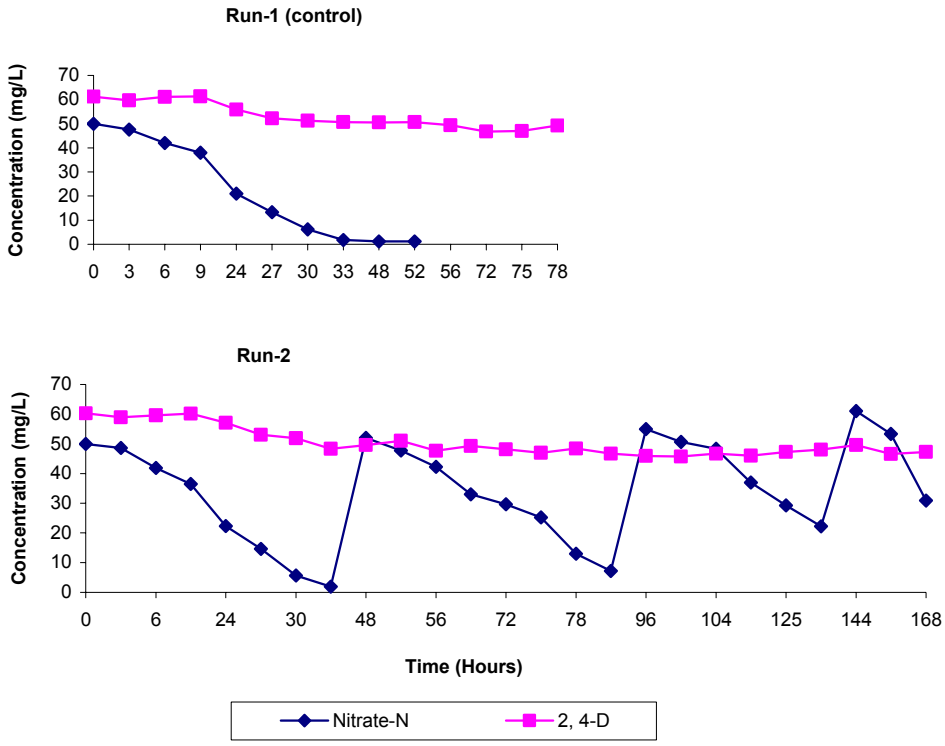


Figure 8.2-1 Pulse addition of NO_3-N using 2, 4-D as a sole carbon source

The above figure indicates that after the NO_3-N was consumed completely (50 hours), around 18 % of the 2, 4-D was removed for both runs. The control had approximately

20 % of 2, 4-D removal at the end of the run while run-2 had 22 % of 2, 4-D removal after three repeats of pulse addition of NO_3-N. No significant total change in 2, 4-D removal efficiency was therefore found after pulse injections of NO_3-N. Interestingly, almost all of the NO_3-N was finally taken up by the biomass implying that endogenous carbon rather than 2, 4-D had been utilized as a carbon source by the denitrifying bacteria. This means that the 2, 4-D removal capability was not increased by the pulsed addition of NO_3-N; however, the bioactivity of the bacteria was also not completely inhibited by the toxicity of the 2, 4-D due to the evidence of NO_3-N consumption.

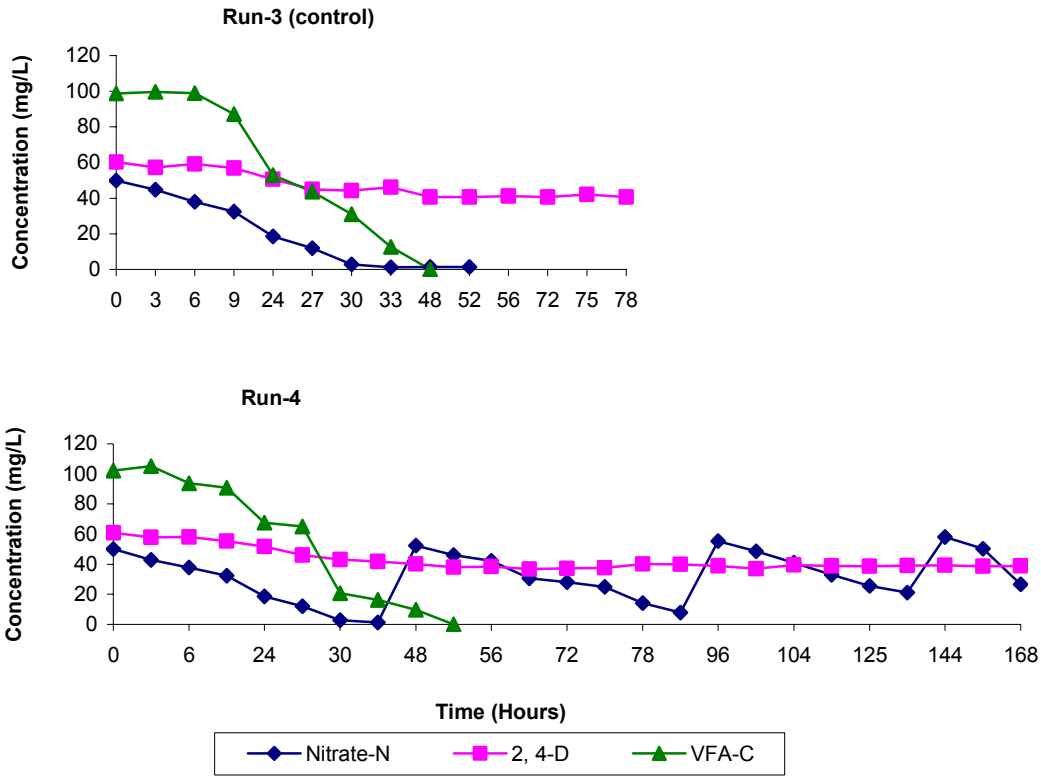


Figure 8.2-2 Pulse addition of NO_3-N using natural VFA and 2, 4-D as carbon sources

Figure 8.2-2 indicates that both VFA-C and NO_3-N were completely consumed in 50 hours, while 32 % of the 2, 4-D was removed in both of the two runs. The control (Run-3) had no further 2, 4-D removal after the VFA-C and NO_3-N disappeared. Run-4 had 36 % final removal of 2, 4-D after three repeats of pulsed addition of NO_3-N. Similar to

run-2, most of the NO_3-N was removed by the biomass regardless of the 2, 4-D biodegradation. These results suggest that the process of 2, 4-D biodegradation and nitrate reduction were not completely coupled and other factors such as growth-supporting substrates, biomass characteristics or specific metabolic pathways may influence the target compound biodegradation capability.

8.3 The effect of biomass concentration on 2, 4-D biodegradation efficiency

In this experiment, runs were operated under identical concentrations of VFA-C (120 mg/L), NO_3-N (70 mg/L) and 2, 4-D (60 mg/L) but using different biomass concentrations (1000, 2500, 4100 and 6300 mg MLVSS /L, respectively). The purpose of this set of test runs was to study the effect of biomass concentrations on the biodegradation efficiencies of VFA-C, 2, 4-D and NO_3-N. The experimental results are shown in Figures 8.3-1, 8.3-2 and 8.3-3.

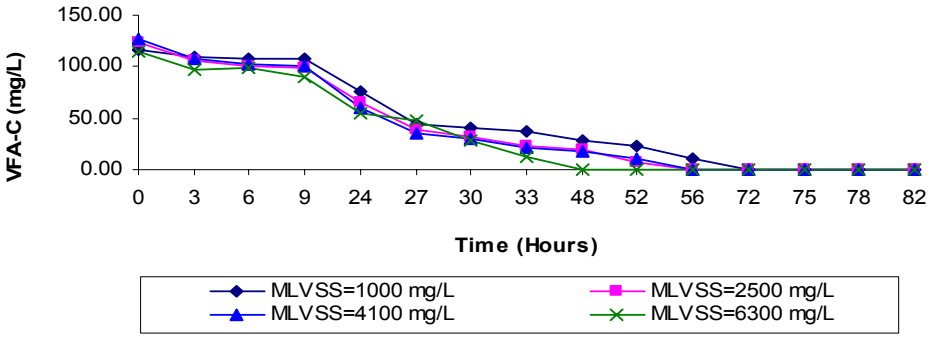


Figure 8.3-1 Natural VFA-C consumption pattern in the denitrification batch tests using various biomass concentrations

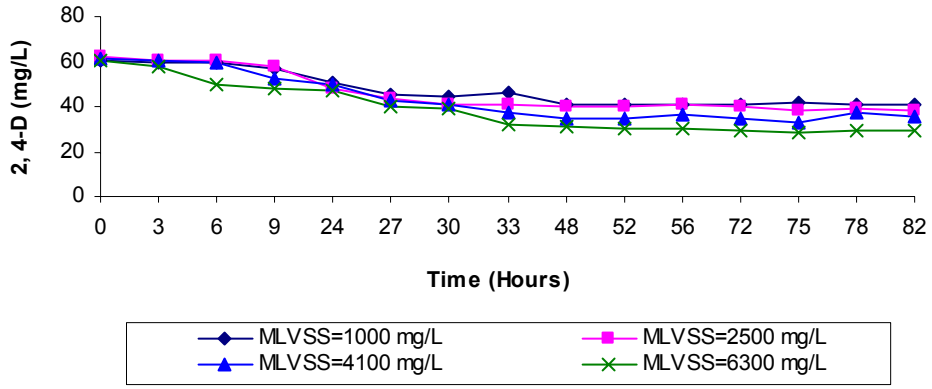


Figure 8.3-2 Biodegradation pattern of 2, 4-D in the denitrification batch tests using various biomass concentrations

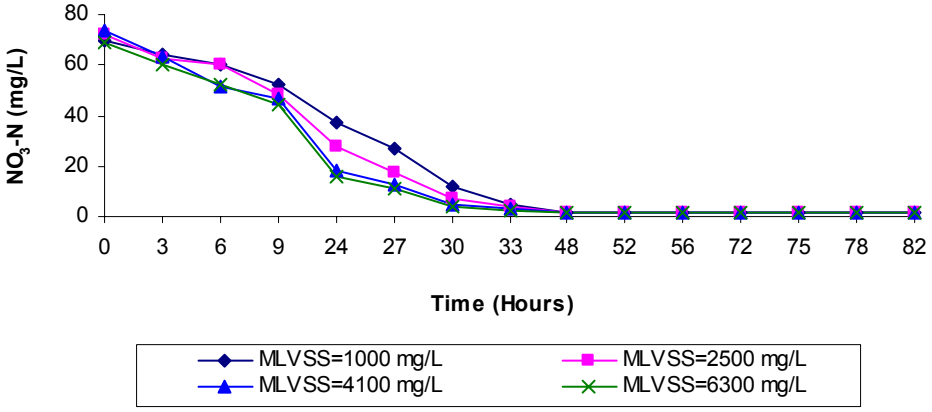


Figure 8.3-3 NO_3-N consumption pattern in the denitrification batch tests using various biomass concentrations

Figure 8.3-2 suggests that the higher the concentration of biomass in the batch reactor system, the larger the amount of 2, 4-D biodegraded. The reason might simply be that the amount of 2, 4-D biodegradation enzymes generated by specific microorganisms increases with increased biomass concentration. In addition, according to Figures 8.3-1 and 8.3-3, the larger the concentration of biomass in the system, the faster the VFA-C and NO_3-N are consumed implying on the surface that the denitrification process can be accelerated with an increase in biomass concentration. In contrast, however, Table 8.3-1 shows that the specific degradation rates of 2, 4-D, NO_3-N and VFA-C actually decreased with an increase in biomass concentration.

Table 8.3-1 Specific degradation rates with the increase of biomass concentration

MLVSS (mg/L)	k_N (g NO_3-N/g VSS per day)	k_C (g VFA-C/g VSS per day)	$k_{2,4\text{-}D}$ (g 2, 4-D/g VSS per day)
1000	0.029	0.046	0.0056
2500	0.014	0.021	0.0028
4100	0.013	0.014	0.0018
6300	0.008	0.012	0.0015

The trend in Table 8.3-1 implies that the effect on the rate seems to directly correlate to MLVSS values, arrived at either by dilution or concentration. This trend is surprising since one would most probably expect the specific rates to be the same, as long as there is an equal amount of bacteria around with the ability to express the enzymes necessary for degradation. The trend does suggest that there might be a lower limit to the rate, as the biomass concentration increases.

Chapter 9: Conclusions and Recommendations

9.1 Conclusions

A number of conclusions can be drawn from this research and these are summarized as following:

1. The anaerobic digestion process performed successfully and produced a stable supply of VFAs using soya flour as a synthetic wastewater feed. A favourable environment for acidogenic digestion was established in the anaerobic digester and methanogenesis was operationally suppressed (i.e. the low pH ranging from 4.7 to 5.0 resulted in little gas production). VFA concentrations up to 3153 ± 801 mg/L could be achieved in the anaerobic digester while the VFA species were acetic (51.4%), propionic (27.5%), butyric (19.6%), and iso-valeric (1.4%).

2. The digestion process converted the substrate from particulate to soluble form which was expressed as the specific TOC solubilization rate (0.007 mg TOC/mg VSS/day), soluble COD production rate (0.022 mg SCOD/mg VSS/day), and percent VSS reduction (14 %). However, the magnitudes of these rates are relatively lower than previous research using primary sludge as feed. Accordingly, the specific VFA production rate (0.014 mg VFA/mg VSS/day) and the fraction of soluble COD in the form of VFAs (32%) are lower than some data found in the literature. This difference is probably due to the soya flour wastewater characteristics.

3. An SBR proved to be a suitable technology for the biodegradation of 2, 4-D from 30 to 100 mg/L. The SBR used real sewage as feed and maintained a mean MLSS of 3653 ± 547 mg/L and a SRT of 20 ± 9 days. An elevated concentration of 2, 4-D had little effect on the biomass activity in terms of carbon and nitrogen removal patterns (as well as dissolved oxygen uptake profiles), however, a relatively longer acclimation period was necessary to achieve more than 90% of 2, 4-D degradation during the second increase from 50 to 100 mg/L (as compared to the first increase from 30 to 50 mg/L).

4. SBR track studies demonstrated that the removal of 2, 4-D during the first 60 minutes (i.e. the non-aerated phase) was negligible while most of the 2, 4-D was

removed within 240 minutes of the aerated phase. In general, a specific 2, 4-D degradation rate of 0.046 ± 0.018 mg/mg MLSS per day was achieved in the SBR which was comparable to some literature data. In particular, successful degradation of 2, 4-D in an SBR is related to the length of the acclimation period, and as the acclimation period increased, the specific biodegradation rate of 2, 4-D increased.

5. Ultrasound treatment of the biomass from the SBR during track studies demonstrated that less than 10 % of the overall uptake of 2, 4-D was due to biosorption while more than 90 % removal of the 2, 4-D was likely due to biodegradation. However, literature studies have found that the biosorption capacity could vary depending on pre-treatment methods as well as some environmental factors (such as pH, initial pollutant and microorganism types (live or dead, bacteria or fungus etc.) and concentrations).

6. Denitrification batch tests using SBR-acclimated biomass demonstrated that the addition of a digester effluent rich in naturally-produced VFAs promoted the specific denitrification rate as well as the 2, 4-D biodegradation efficiency as compared to that using 2, 4-D as a sole carbon source. As summarized in Table 9.1-1, the specific denitrification rate increased from 0.0119 ± 0.0039 to 0.0192 ± 0.0079 g NO_3-N/g VSS per day while the percent 2, 4-D removal increased from 28.33 ± 11.88 to 54.17 ± 21.89 when adding natural VFAs as a co-substrate. However, when natural VFAs were used as a sole carbon source, the specific denitrification rate increased to 0.024 ± 0.003 g NO_3-N/g VSS per day which is probably due to the lack of 2, 4-D toxicity. These results indicate that naturally-produced VFAs can be added to the denitrification process when both denitrification and pesticide degradation are objectives.

7. Denitrification batch tests using unacclimated biomass from a local WWTP demonstrated that the specific 2, 4-D biodegradation rate and removal efficiency using unacclimated biomass were 2.0 to 2.5 times less than those of the acclimated biomass. That is, the acclimation of microorganisms to appropriate concentrations of 2, 4-D in the SBR was shown to develop the capability of bacteria to biodegrade 2, 4-D in the following denitrification process. Despite the probable fact that microorganisms capable of 2, 4-D biodegradation were already present in the unacclimated biomass, it is suspected that very low numbers of such microorganisms possessing the entire set of

enzymes were present and these were not able to transform the toxic pollutant "productively". The acclimation phase therefore allows those microorganisms capable of 2, 4-D degradation to proliferate under a favourable environment. It is noted that the experimental data (Table 9.1-1) indicate that the specific denitrification and VFA-C consumption rates were not affected by the biomass acclimation process.

Table 9.1-1 Summary of denitrification batch tests

	Acclimated Biomass				Unacclimated Biomass			
Carbon Source	K_N (g NO_3-N/g VSS per day)	K_C (g VFA-C/g VSS per day)	$K_{2,4\text{-}D}$ (g 2, 4-D/g VSS per day)	2, 4-D removal efficiency (%)	K_N (g NO_3-N/g VSS per day)	K_C (g VFA-C/g VSS per day)	$K_{2,4\text{-}D}$ (g 2, 4-D/g VSS per day)	2, 4-D removal efficiency (%)
Natural VFAs	0.024 ± 0.003	0.046 ± 0.017	-	-	0.022 ± 0.004	0.042 ± 0.008	-	-
Natural VFAs + 2, 4-D	0.0192 ± 0.0079	0.1216 ± 0.0335	0.0055 ± 0.0021	54.17 ± 21.89	0.0172 ± 0.0045	0.0913 ± 0.0233	0.0022 ± 0.0009	19 ± 5.62
2, 4-D	0.0119 ± 0.0039	-	0.0016 ± 0.0009	28.33 ± 11.88	0.0097 ± 0.0014	-	0.0009 ± 0.0001	10.5 ± 2.88
Synthetic VFAs	0.022 ± 0.008	0.039 ± 0.009	-	-	0.02 ± 0.007	0.037 ± 0.006	-	-
Synthetic VFAs + 2, 4-D	0.017 ± 0.007	0.081 ± 0.03	0.006 ± 0.002	47.39 ± 16.59	0.018 ± 0.002	0.105 ± 0.016	0.0019 ± 0.0004	16 ± 5.29

8. In the main and not surprisingly, natural VFAs and synthetic VFAs were found to be identical in denitrification batch tests in terms of their use as a carbon source. The mean specific denitrification and VFA-C consumption rates derived from experiments using natural VFAs as carbon source were reasonably close to the values obtained from experiments using synthetic VFAs as carbon source. The mean specific 2, 4-D degradation rate derived from experiments using natural VFAs and 2, 4-D as carbon sources was also close to the value from experiments using synthetic VFAs and 2, 4-D as carbon sources.

9. An additional exploration of 2, 4-D degradation behaviour found that pulsed additions of NO_3-N to the denitrification batch tests did not yield further significant 2, 4-D degradation, although almost all of NO_3-N added to the test was used up by the end of the experimental run. Therefore, 2, 4-D removal capability was not completely NO_3-N dependent and there exists a possibility of some other particular environmental factors which may contribute to 2, 4-D biodegradation.

10. It appears that biomass concentration plays an important role in the denitrification process. The higher the concentration of biomass that was applied to the denitrification batch system, the larger the amount of 2, 4-D degraded and the faster the

VFA-C and NO_3-N are consumed. The results from this work suggest an approach for herbicide-contaminated site remediation could include "microbial richness" which is called bioaugmentation technology under optimal local conditions.

9.2 Recommendations

Based on the results obtained from this research, the following recommendations are suggested:

1. Operational and environmental parameters of the anaerobic digester (such as HRT/SRT, pH and temperature) could be adjusted in order to improve the particulate organic carbon solubilization rate and obtain a higher proportion of VFAs in the soluble COD form. In addition, primary sludge could be used as a digester feed to compare the specific VFA production rate with the synthetic feed. Variations of SRT etc. are needed to observe the effect on the specific TOC/soluble COD solubilization rate and the percent VSS reduction and to find the optimal condition for the specific VFA production rate.

2. More research could be carried out on the SBR with respect to 2, 4-D biodegradation efficiency during non-aerated periods. Certain amounts of NO_3-N could be added to the feed to stimulate the growth of denitrifiers and to look at the possibility of increased 2, 4-D biodegradation capability during extended non-aerated periods. Additionally, the critical dissolved oxygen concentration during the aerated phase could be investigated to find the level at which 2, 4-D biodegradation efficiency will be affected.

3. The 2, 4-D concentration in the SBR could be progressively increased to find out the maximum concentration of 2, 4-D to be removed and to evaluate the overall SBR performance under the influence of increasing concentrations of 2, 4-D.

4. One could investigate the biodegradation capability of some other herbicides in an SBR, such as 2, 4, 5-T, isoproturon, Mecoprop [2-(2-methyl-4-chlorophenoxy) propionic acid] or MCPA (2-methyl-4-chlorophenoxy acetic acid). All these are structurally related to 2, 4-D and can also be used extensively to control broad-leaved weeds competing with cereal crops.

5. Biosorption did not contribute to high percentage of 2, 4-D removal in this study as tested by ultrasonic pre-treatment of the biomass. However, the biosorption capability might vary depending upon the species of microorganisms (ex. bacteria, yeast or fungi) present in the wastewater; the type of pollutant and initial concentration; the pH; and the biomass concentration and pre-treatment method. In order not to overestimate the "degradative potential" of microorganisms and to precisely assess an organism's ability to utilise a target compound, some other pre-treatment methods such as alkali or acid treatments, heat treatment etc. are suggested to further investigate 2, 4-D biosorption efficiency.

6. One could explore the extent to which VFAs enhance the degradation of 2, 4-D to see whether the efficiency of biological 2, 4-D removal is affected by different ratios of VFA to 2, 4-D.

7. One could compare pure acetic acid with a mixture of acetic, propionic and butyric acids plus 2, 4-D as carbon sources in denitrification system to see the effect on 2, 4-D removal and denitrification efficiency.

8. One could isolate 2, 4-D degrading microorganisms from the denitrification system and identify their morphological and biochemical characteristics (i.e. reaction to Gram stain, morphology, motility, ability to grow under the aerobic/anaerobic etc.).

9. One could try and detect the intermediate product of 2, 4-D degradation (i.e. 2, 4-DCP) and investigate the accumulation of the 2, 4-D degradation product as that may reduce the 2, 4-D degradation rate or efficiency.

10. One could develop more theoretical aspects of the combined SBR/denitrification process by modelling the kinetics of simultaneous 2, 4-D degradation and nitrate reduction to apply the combined process in-situ at 2, 4-D contaminated sites.

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Appendix A

A1. Calibration curves for four major VFAs using Gas Chromatography (GC)

The following retention times were observed for the four major VFA species:

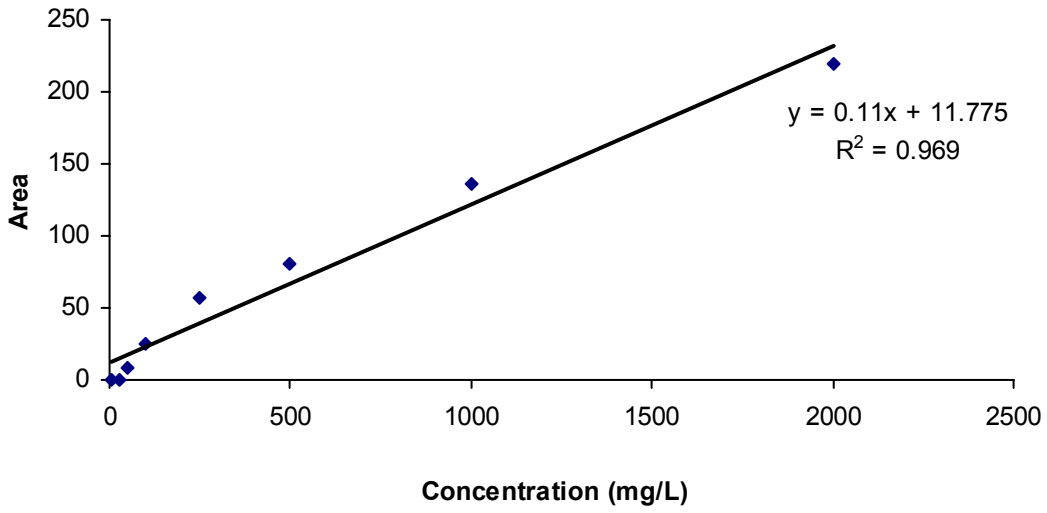
Acetic acid: 2.5 minutes

Propionic acid: 3.0 minutes

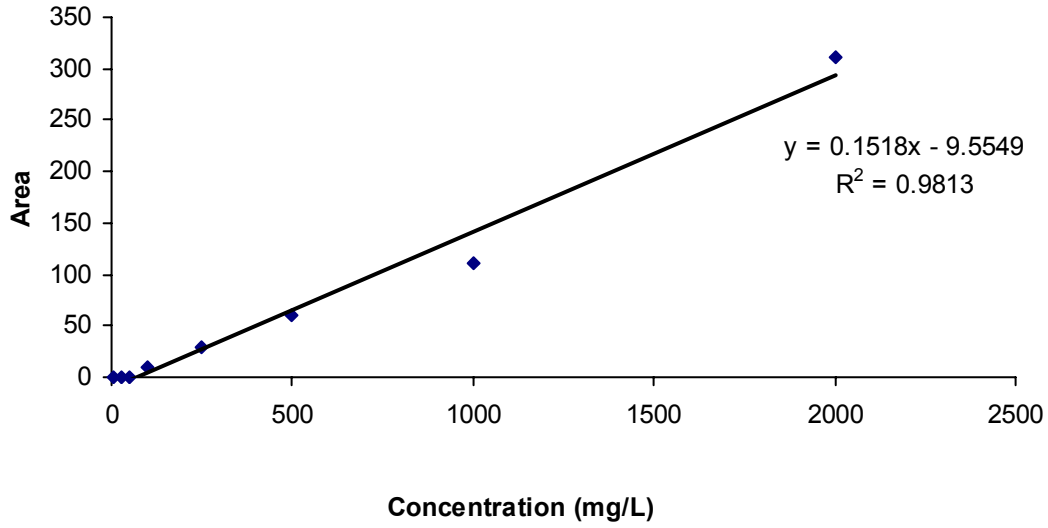
n-Butyric acid: 3.7 minutes

iso-Valeric acid: 4.0 minutes

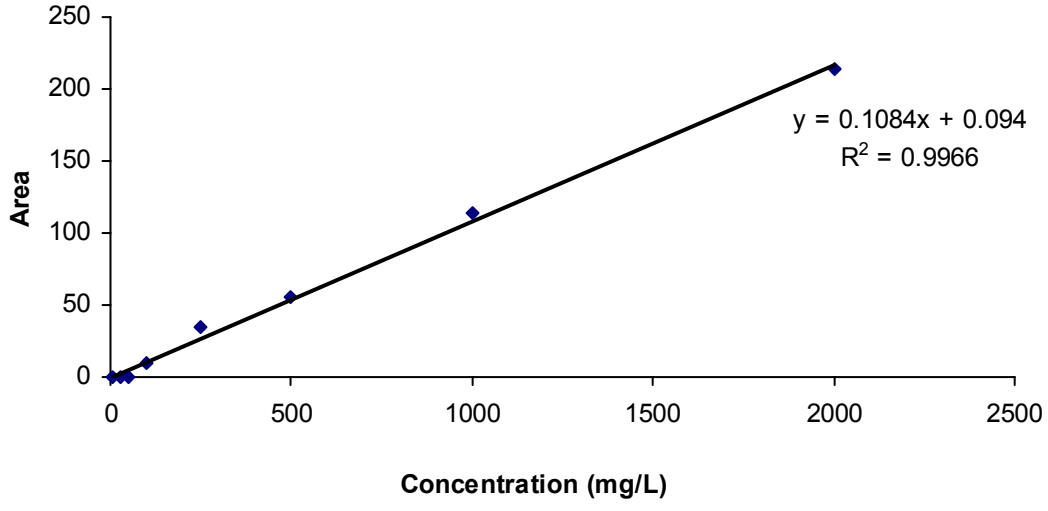
Known concentrations of acetic, propionic, n-butyric and iso-valeric acid were analysed separately by gas chromatography, calibration curves were constructed as shown below:



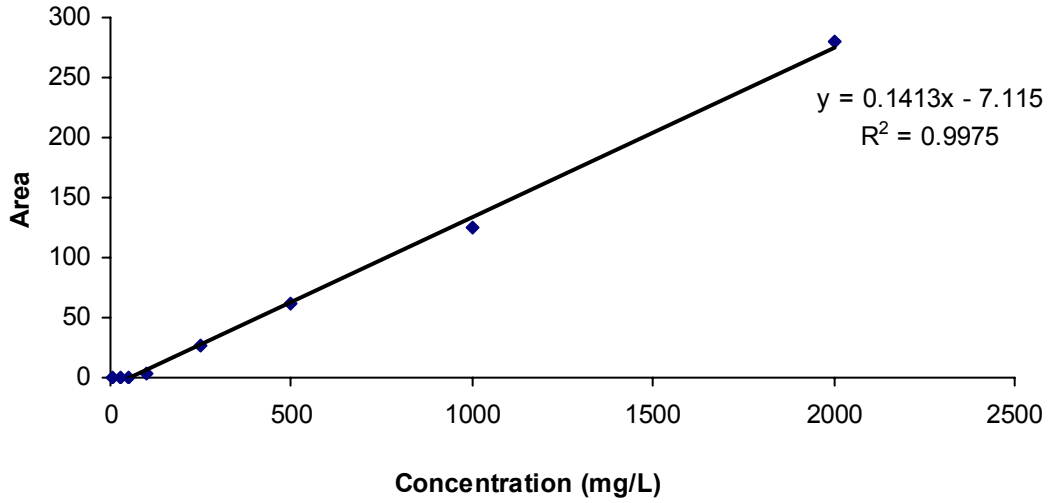
Acetic Acid Standards



Propionic Acid Standards



n-Butyric Acid Standards



iso-Valeric Acid Standards

Determining the Total VFA Concentration

The concentrations of propionic, butyric and valeric acid were converted into equivalent concentrations of acetic acid as shown below:

Propionic acid as acetic acid (mg/L) = propionic acid (mg/L) × 60/74

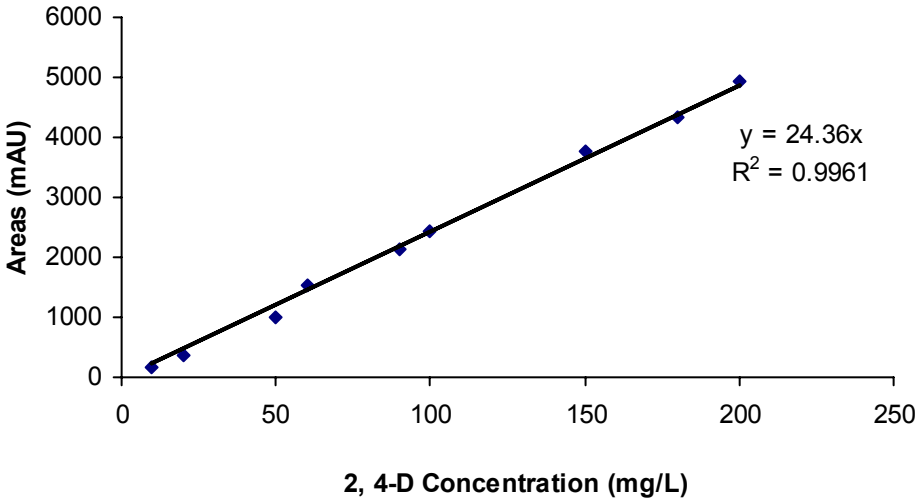
Butyric acid as acetic acid (mg/L) = butyric acid (mg/L) × 60/88

Valeric acid as acetic acid (mg/L) = valeric acid (mg/L) × 60/ 102

Where 60, 74, 88 and 102 are the molecular weights of acetic, propionic, butyric and valeric acid, respectively. The equivalent concentrations of propionic, butyric and valeric acid as acetic acid were added to the acetic concentration to obtain an approximate value for total VFA concentration.

A2. Calibration curves for 2, 4-D using High Performance Liquid Chromatography (HPLC)

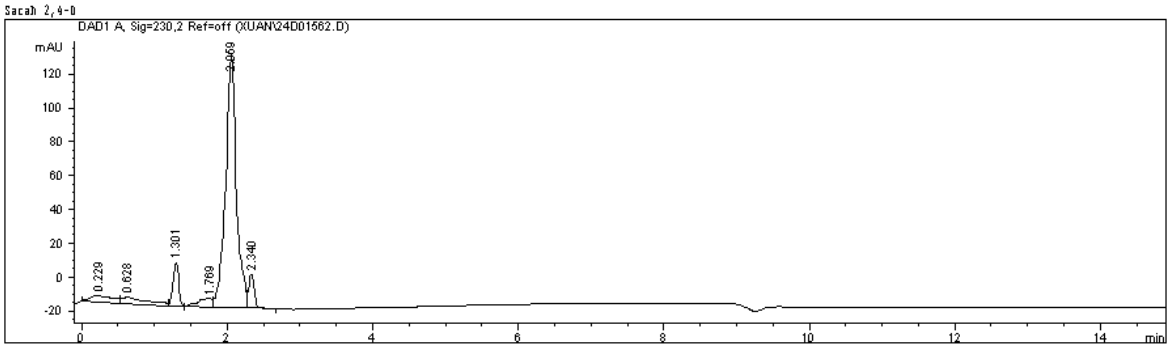
Known concentrations of 2, 4-D were analysed and a retention time of 2.0 minutes was observed.



Pure 2, 4-D Standards

An example of a chromatogram for 2, 4-D is shown below:

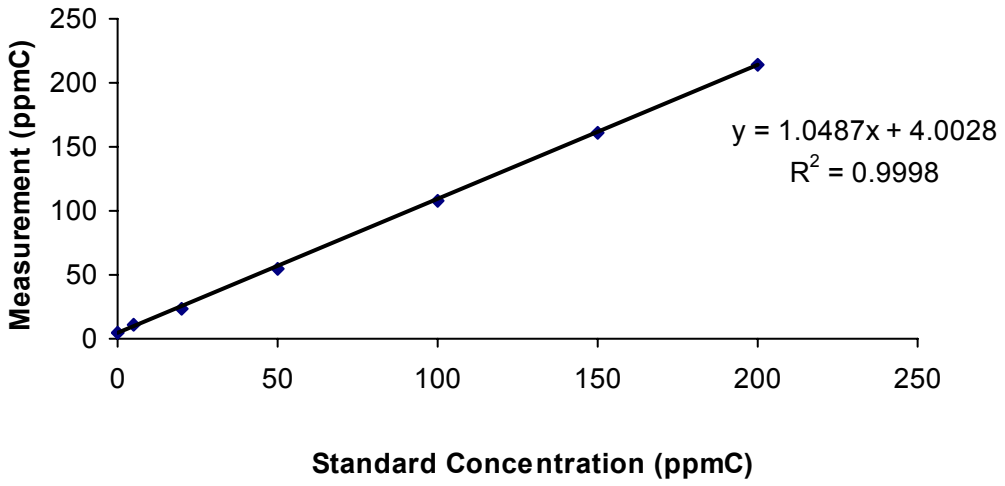
```
================================================================
Injection Date  : 27/09/2005 13:23:09 p.m.       Seq. Line :   2
Sample Name     : std-60mg/L                      Location : Vial 2
Acq. Operator   : Sacah                              Inj :   1
                                                 Inj Volume : 10 µl
Sequence File   : C:\HPCHEM\1\SEQUENCE\XUAN\XUAN.S
Method          : C:\HPCHEM\1\METHODS\XUAN\XUAN.M
Last changed    : 27/09/2005 13:21:07 p.m. by Sacah
                  (modified after loading)
Sacah 2,4-D
```




```
================================================================
                      Area Percent Report
================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000


Signal 1: DAD1 A, Sig=230,2 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   |min|        |min|    |mAU*s|     |mAU|       %
----|-------|----|-------|----------|----------|--------|
   1   0.229 BV    0.2585   74.32449    3.57509   3.6838
   2   0.628 VV    0.3262   88.05140    3.49140   4.3641
   3   1.301 VP    0.0858  135.54889   25.59514   6.7182
   4   1.769 VV    0.1793   69.90342    4.99451   3.4646
   5   2.059 VV    0.1466 1535.42053  151.01704  76.1003
   6   2.340 VP    0.0901  114.37976   20.19748   5.6690

Totals :                   2017.62849  208.87067

 Results obtained with enhanced integrator!
================================================================
                        *** End of Report ***
```

A3. Calibration curve for Total Organic Carbon (TOC) using TOC Combustion Analyser (Apollo 9000)

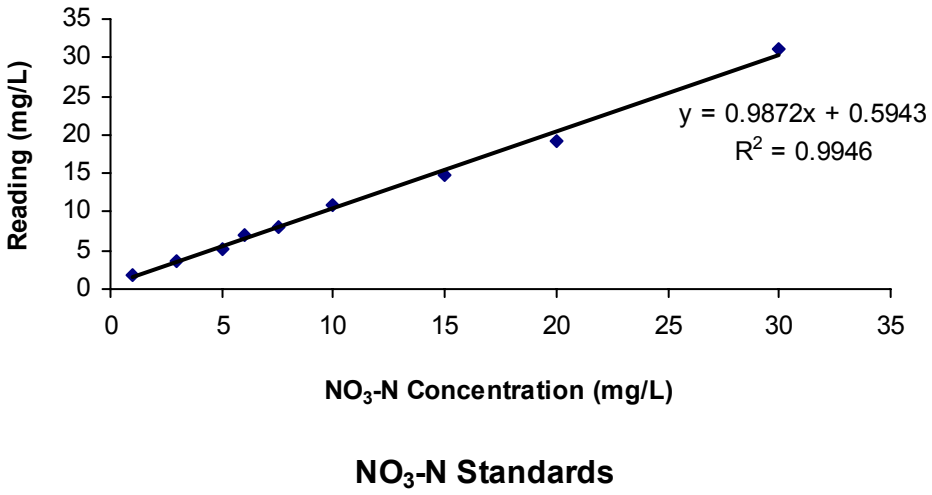
Potassium Hydrogen Phthalate (KHP) (2125 mg/L) (equals to 1000 ppm C) stock solution was used to prepare known concentrations (5-200 mg TOC/L) of standard solution, and calibration curves were constructed as shown below:



TOC Calibration Curve

A4. Calibration curve for NO_3-N using spectrophotometer

NO_3-N was measured using the cadmium reduction method (0 to 30.0 mg/L) by spectrophotometer (HACH), and calibration curves were constructed as shown below:



NO_3-N Standards

Appendix B

This section gives results from t-Tests comparing specific denitrification rates, specific VFA-C consumption rates, specific 2, 4-D degradation rates and 2, 4-D removal efficiencies as well as carbon to nitrate-nitrogen ratios with different carbon sources using SBR acclimated biomass and unacclimated biomass from the local wastewater treatment plant.

B1 Results of t-Test comparing natural VFAs as a sole carbon source & natural VFAs and 2, 4-D as carbon sources using SBR acclimated biomass

Run	Specific denitrification rate (g/g VSS per day)		Specific VFA-C consumption rate (g/g VSS per day)	
	Mean	Stdev	Mean	Stdev
A (Natural VFAs+2,4-D)	0.0192	0.0079	0.105	0.0455
B (Natural VFAs)	0.024	0.003	0.046	0.017
t_{cal}	1.85		3.93	
t_{tab}	1.706		1.706	
Significant Difference at the 95% Confidence Interval?	YES		YES	

B2 Results of t-Test comparing 2, 4-D as a sole carbon source & natural VFAs and 2, 4-D as carbon sources using SBR acclimated biomass

Run	Specific denitrification rate (g/g VSS per day)		Specific 2,4-D degradation rate (g/g VSS per day)		2,4-D removal efficiency (%)	
	Mean	Stdev	Mean	Stdev	Mean	Stdev
A (Natural VFAs+2,4-D)	0.0192	0.0079	0.0055	0.0021	54.17	21.89
C (2,4-D only)	0.0119	0.0039	0.0016	0.0009	28.33	11.88
t_{cal}	3.52		7.24		4.4	
t_{tab}	1.697		1.697		1.697	
Significant Difference at the 95% Confidence Interval?	YES		YES		YES	

B3 Results of t-Test comparing natural VFAs as a sole carbon source & synthetic VFAs as a sole carbon source using SBR acclimated biomass

Run	Specific denitrification rate (g/g VSS per day)		Specific VFA-C consumption rate (g/g VSS per day)	
	Mean	Stdev	Mean	Stdev
D (Synthetic VFAs)	0.022	0.008	0.039	0.009
B (Natural VFAs)	0.024	0.003	0.046	0.017
t_{cal}	0.74		1.15	
t_{tab}	1.734		1.734	
Significant Difference at the 95% Confidence Interval?	NO		NO	

B4 Results of t-Test comparing 2, 4-D as a sole carbon source & synthetic VFAs and 2, 4-D as carbon sources using SBR acclimated biomass

Run	Specific denitrification rate (g/g VSS per day)		Specific 2,4-D degradation rate (g/g		2,4-D removal efficiency (%)	
	Mean	Stdev	Mean	Stdev	Mean	Stdev
E (Synthetic VFAs+2,4-D)	0.017	0.007	0.006	0.002	47.39	16.59
C (2,4-D only)	0.0119	0.0039	0.0016	0.0009	28.33	11.88
t_{cal}	2.7		8.51		3.96	
t_{tab}	1.697		1.697		1.697	
Significant Difference at the 95% Confidence Interval?	YES		YES		YES	

B5 Results of t-Test comparing natural VFAs and 2, 4-D as carbon sources & synthetic VFAs and 2, 4-D as carbon sources using SBR acclimated biomass

Run	Specific denitrification rate (g/g VSS per day)		Specific VFA-C consumption rate (g/g VSS per day)		Specific 2,4-D degradation rate (g/g VSS per day)		2,4-D removal efficiency (%)	
	Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev
A (Natural VFAs+2,4-D)	0.0192	0.0079	0.105	0.0455	0.0055	0.0021	54.17	21.89
E (Synthetic VFAs+2,4-D)	0.017	0.007	0.081	0.03	0.006	0.002	47.39	16.59
t_{cal}	0.88		1.67		0.73		1.048	
t_{tab}	1.697		1.697		1.697		1.697	
Significant Difference at the 95% Confidence Interval?	NO		NO		NO		NO	

B6 Results of t-Test comparing synthetic VFAs as a sole carbon source & synthetic VFAs and 2, 4-D as carbon sources using SBR acclimated biomass

Run	Specific denitrification rate (g/g VSS per day)		Specific VFA-C consumption rate (g/g	
	Mean	Stdev	Mean	Stdev
E (Synthetic VFAs+2,4-D)	0.017	0.007	0.081	0.03
D (Synthetic VFAs)	0.022	0.008	0.039	0.009
t_{cal}	1.72		2.95	
t_{tab}	1.706		1.706	
Significant Difference at the 95% Confidence Interval?	YES		YES	

B7 Results of t-Tests comparing C: N < 2 & C: N > 2 within each run using SBR acclimated biomass

Parameters	Runs		A (Natural VFA + 2, 4-D)	B (Natural VFA)	C (2, 4-D)	D (Synthetic VFA)	E (Synthetic VFA + 2, 4-D)
Specific denitrification rate (g/g VSS per day)	C:N ≤ 2	Mean	0.0212	0.023	0.0124	0.0238	0.0188
		Stdev	0.0086	0.0014	0.0036	0.0098	0.0094
	C:N >2	Mean	0.0161	0.0248	0.0114	0.0183	0.0142
		Stdev	0.0061	0.0041	0.0043	0.0039	0.0033
	Significant Difference at the 95% Confidence Interval?		NO	NO	NO	NO	NO
Specific VFA-C consumption rate (g/g VSS per day)	C:N ≤ 2	Mean	0.1041	0.0332	–	0.0322	0.069
		Stdev	0.0462	0.0129	–	0.0009	0.0225
	C:N >2	Mean	0.1057	0.058	–	0.0414	0.0928
		Stdev	0.0473	0.0107	–	0.0008	0.0339
	Significant Difference at the 95% Confidence Interval?		NO	YES	–	YES	NO
Specific 2, 4-D degradation rate (g/g VSS per day)	C:N ≤ 2	Mean	0.0056	–	0.0013	–	0.0065
		Stdev	0.0019	–	0.0005	–	0.0017
	C:N >2	Mean	0.0054	–	0.002	–	0.0054
		Stdev	0.0023	–	0.0012	–	0.0023
	Significant Difference at the 95% Confidence Interval?		NO	–	NO	–	NO
2, 4-D removal efficiency (%)	C:N ≤ 2	Mean	64.25	–	36.2	–	54.11
		Stdev	18.16	–	7.58	–	14.98
	C:N >2	Mean	46.1	–	18.5	–	40.67
		Stdev	22.04	–	8.35	–	16.09
	Significant Difference at the 95% Confidence Interval?		YES	–	YES	–	YES

B8 Results of t-Test comparing acclimated biomass (B) and unacclimated biomass (F) using natural VFA as a sole carbon source

Run	Specific denitrification rate (g/g VSS per day)		Specific VFA-C consumption rate (g/g VSS per day)	
	Mean	Stdev	Mean	Stdev
B (Natural VFAs)-Acclimated	0.024	0.003	0.046	0.017
F (Natural VFAs)-Unacclimated	0.022	0.004	0.042	0.008
t_{cal}	1.14		0.54	
t_{tab}	1.761		1.761	
Significant Difference at the 95% Confidence Interval?	NO		NO	

B9 Results of t-Test comparing acclimated biomass (A) and unacclimated biomass (G) using natural VFA and 2, 4-D as carbon sources

Run	Specific denitrification rate (g/g VSS per day)		Specific VFA-C consumption rate (g/g VSS per day)		Specific 2,4-D degradation rate (g/g VSS per day)		2,4-D removal efficiency (%)	
	Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev
A (Natural VFAs+2,4-D)-Acclimated	0.0192	0.0079	0.105	0.0455	0.0055	0.0021	54.17	21.89
G (Natural VFAs+2,4-D)-Unacclimated	0.0172	0.0045	0.0913	0.0233	0.0022	0.0009	19	5.62
t_{cal}	0.59		0.7		3.71		3.8	
t_{tab}	1.717		1.717		1.717		1.717	
Significant Difference at the 95% Confidence Interval?	NO		NO		YES		YES	

B10 Results of t-Test comparing acclimated biomass (C) and unacclimated biomass (H) using 2, 4-D as a sole carbon source

Run	Specific denitrification rate (g/g VSS per day)		Specific 2,4-D degradation rate (g/g VSS per day)		2,4-D removal efficiency (%)	
	Mean	Stdev	Mean	Stdev	Mean	Stdev
C (2,4-D only)-Acclimated	0.0119	0.0039	0.0016	0.0009	28.33	11.88
H (2,4-D only)-Unacclimated	0.0097	0.0014	0.0009	0.0001	10.5	2.88
t_{cal}	1.38		1.89		3.5	
t_{tab}	1.717		1.717		1.717	
Significant Difference at the 95% Confidence Interval?	NO		YES		YES	

B11 Results of t-Test comparing acclimated biomass (D) and unacclimated biomass (I) using synthetic VFA as a sole carbon source

Run	Specific denitrification rate (g/g VSS per day)		Specific VFA-C consumption rate (g/g VSS per day)	
	Mean	Stdev	Mean	Stdev
D (Synthetic VFAs)-Acclimated	0.022	0.008	0.039	0.009
I (Synthetic VFAs)-Unacclimated	0.02	0.007	0.037	0.006
t_{cal}	0.51		0.68	
t_{tab}	1.761		1.761	
Significant Difference at the 95% Confidence Interval?	NO		NO	

B12 Results of t-Test comparing acclimated biomass (E) and unacclimated biomass (J) using synthetic VFA and 2, 4-D as carbon sources

Run	Specific denitrification rate (g/g VSS per day)		Specific VFA-C consumption rate (g/g VSS per day)		Specific 2,4-D degradation rate (g/g VSS per day)		2,4-D removal efficiency (%)	
	Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev
E (Synthetic VFAs+2,4-D)-Acclimated	0.017	0.007	0.081	0.03	0.006	0.002	47.39	16.59
J (Synthetic VFAs+2,4-D)-Unacclimated	0.018	0.002	0.105	0.016	0.0019	0.0004	16	5.29
t_{cal}	1.21		1.86		2.82		4.48	
t_{tab}	1.717		1.717		1.717		1.717	
Significant Difference at the 95% Confidence Interval?	NO		YES		YES		YES	

Appendix C

C1 Track Studies of 2, 4-D Degradation in SBR

Time (min)	Track -1 (23/08/05)	Track-2 (26/08/05)	Track-3 (15/09/05)	Track-4 (20/09/05)	Track-5 (06/10/05)	Track-6 (15/10/05)	Track-7 (29/10/05)	Track-8 (12/11/05)	Track-9 (20/11/05)	Track-10 (21/03/06)
0	35	23.4	22	36	38.6	45.2	71	60.2	50.6	53.2
30	25.5	23.1	21.9	31.1	36.9	44.7	72.8	60.6	52.3	51.5
60	22.5	23.9	22.1	29	36.1	43.6	75	61	49	55.0
75	24.7	24.5	22.5	26.8	27.7	38.6	71.9	61.5	53	56.7
105	17	21.1	20.1	18.5	18.9	30	63	58	47.3	42.0
135	16.1	16.3	17.2	11.8	10.0	25.7	57.4	56	36.0	41.6
165	12.6	11.5	12.9	9.9	10.5	21.8	52.5	37.7	22.7	27.2
195	11.3	10.7	9.7	8.4	3.4	11.8	49.7	27.1	19.5	15.3
235	10.5	7.8	6.8	6.7	3.2	7.7	36	16.3	8.2	10.9
270	7.8	6.2	3.4	7.1	4.2	5.3	29.1	12.6	4.1	4.6
300	8.7	4.7	2.8	7.3	3.3	4.9	28	11.7	3.5	2.1
340	9	4.2	2.3	7.5	2.5	3.7	28.3	11.2	3.4	2

C2 Biosorption Track Studies in the SBR

31/08/2005	Time (min)	2,4-D (mg/L)	2,4-D (mg/L) (ultrosound treatment)	8/09/2005	Time (min)	2,4-D (mg/L)	2,4-D (mg/L) (ultrosound treatment)	20/11/2005	Time (min)	2,4-D (mg/L)	2,4-D (mg/L) (ultrosound treatment)
	Decant	17.2			**Decant**	9			**Decant**	3.2	
Anoxic	0	35.3	38.9	Anoxic	0	26	29	Anoxic	0	50.6	55
	30	35.6	38.3		30	21.5	23.7		30	52.3	54.4
	60	36.8	37.2		60	22.5	20.9		60	52.5	55
Aerobic	75	36	37.7	Aerobic	75	24.7	22	Aerobic	75	53	52.7
	105	35	36.4		105	17	14.7		105	47.3	50.5
	135	34.6	35.1		135	16.3	15.6		135	36.0	41.1
	165	29.8	31.2		165	12.6	13.1		165	22.7	25.6
	195	26.8	30		195	11.2	12.7		195	19.5	20.7
	235	26.5	25.9		235	10.2	11.1		235	8.2	10.3
	270	20.3	24.7		270	7.8	9.2		270	4.1	4.9
	300	19.5	21		300	8.6	8.1		300	3.5	4.2
	340	13.9			340	9.3			340	3.4	3.4