KINETIC STUDIES ON READILY BIODEGRADABLE SUBSTRATES BY THE ANAEROBIC DIGESTION PROCESS

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ABSTRACT FACULTY OF ENGINEERING AND APPLIED SCIENCES DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING

Doctor of Philosophy KINETIC STUDIES ON READILY BIODEGRADABLE SUBSTRATES BY THE ANAEROBIC DIGESTION PROCESS by Ghufran Redzwan

The aim of the study was to evaluate the possibility of using a high rate anaerobic process to convert the soluble hydrolysis and acidification products from a first phase solid substrate anaerobic digester treating the organic fraction of municipal solid waste (OFMSW). To achieve this a comparative evaluation of the kinetic methods for predicting the effluent (S_e) soluble substrate concentration was undertaken. The methodology was developed by conducting anaerobic treatability studies on a readily degradable soluble wastewater, this was then extended to studies using a solids free leachate derived from the hydrolysis and acidification of OFMSW, and finally to a mixed soluble/suspended solids phase leachate produced by a high rate hydraulic flush bioreactor treating OFMSW.

Using the readily degradable wastewater two sets of experiments were undertaken to assess the kinetics of both a batch operation and fed-batch operation. These treatability studies were carried out at a laboratory scale in stirred tank reactors. Batch operation using this wastewater was inherently unstable and it was demonstrated that the problems were due to both a nitrogen deficiency in the wastewater and a lack of natural buffering capacity. Stable operation could be maintained by supplementing the buffering capacity by daily addition of NaHCO₃ and NH₃HCO₃ at 250-400 mg.l⁻¹.d⁻¹ and 10 mg.l⁻¹.d⁻¹ respectively to the batch system, this also provided sufficient nitrogen to maintain a healthy bacterial population. The kinetics of the batch reaction were best described using the equation $S_e = S_o^{kt}$ ($S_o = influent$; t=time), the constant k was equal to $0.02h^{-1}$ under normal operating conditions. For batch operation, an estimate of the maximum gas production (G_m) could be made using the specific function $G = G_m^{k/t}$ (G = gas production). Statistically, this gave a better estimate of G_m than other known methods, in addition the method developed was more straightforward.

For the fed-batch reactor treating the readily degradable wastewater a Michaelis-Menten kinetic approach was adopted and the reaction was proven to be of a first order, except at high loadings (>1.3kg.m³.d⁻¹). The effluent soluble substrate could be predicted with confidence using the equation $S_e = S_o$ / [1 + k.HRT] (HRT = hydraulic retention time). An organic loading rate (OLR) of 1.4 kg.m³.d⁻¹ could be treated at a removal efficiency (E_r) of 92%. For a fed-batch operation, the constant k in the first order model when applied to the readily biodegradable wastewater was equal to 1.25d⁻¹. The coefficients r_m and K_m for the Michaelis-Menten kinetic equation were equal to 1700 mg.l⁻¹.d⁻¹ and 310 mg.l⁻¹

For the treatment of both the solids free and the mixed leachate, no buffer or nutrient additions were required for successful treatment in both fed-batch and continuous-feeding experiments. The fed-batch reactor showed that the system could treat solid-free leachate up to an organic loading rate (OLR) of 1.7kg.m³.d⁻¹, and for mixed leachate up to 2.0kg.m³.d⁻¹ without sacrificing the E_r for COD. The average COD E_r was 90% for solid-free leachate, and 93% for mixed leachate. The reaction kinetics of the mixed leachate was adequately described using the first order rate equation of Michaelis-Menten. For a solid free leachate a better estimation of the effluent substrate concentration could be obtained using an equation incorporating a term for E_r , $S_e = [100 S_0 - E_r S_0] / [100]$. For a fed-batch operation, the constant k in the first order model was equal to 2.11d⁻¹, which signified that the leachate was more easily degraded than the readily degradable wastewater used earlier. The coefficients r_m and K_m for the Michaelis-Menten kinetic equation were equal to 5000 mg.l⁻¹.d⁻¹ and 1300 mg.l⁻¹ for mixed leachate. The estimate of S_e derived from the kinetic expression could then be applied to determine CH₄ production, but with a statistically less acceptable accuracy.

By using an continuous feed anaerobic filter (AF) mixed leachate treatment could be further improved to give 90% COD E_r at OLR's up to 2.3 kg.m³.d⁻¹.The kinetics of the reaction could best be described by use of the Monod equation which was found to give a reliable prediction of the final effluent substrate concentration.

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LIST OF ABBREVIATIONS

μ	: specific growth rate
µm,	: maximum specific growth rate
AF	: anaerobic filter
BOD	: biochemical oxygen demand
CaCO ₃	: calcium carbonate
CH₄	: methane gas
CO_2	: carbon dioxide
COD	: chemical oxygen demand
CSTR	: continuous stirred tank reactor
FV	: feed volume
G	: CH ₄ production
G_{m}	: maximum CH ₄ production
HFR	: hydraulic flushing rate
HRT	: hydraulic retention time
<i>K</i>	: Michaelis-Menten constant
K_{s}	: half-saturation for substrate concentration at one-half of $\mu_{\rm m}$
MCRT	: mean cell retention time
NaHCO ₃	: sodium bicarbonate
NH ₄ HCO ₃	: ammonium bicarbonate
OFMSW	: organic fraction municipal solid waste
OLR	: organic loading rate
0	: volumetric flow rate
\tilde{O}_{a}	: volumetric flow rate of effluent
\tilde{O}_{α}	: volumetric flow rate of influent
\widetilde{R}^{2}	: coefficient of determination
RBCOD	: readily biodegradable chemical oxygen demand
r_G	: biomass growth rate
r_s	: substrate utilisation rate
r_m	: maximum substrate utilization rate
S	: substrate concentration
Se	: effluent
So	: influent
SRT	: solid retention time
TS	: total solid
t	: time
UASB	: upflow anaerobic sludge blanket
V	: working volume
v/w	: volume per weight
VFA	: volatile fatty acid
VS	: volatile solid
w/w	: weight per weight
WAS	: waste activated sludge
Χ	: biomass concentration
Y	: biomass yield coefficient

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CHAPTER I INTRODUCTION

1.1 Introduction

Methods for the treatment of wastewater can be categorised into three groups, known as physical, chemical and biological treatment methods (Metcalf and Eddy, 1991). All of these methods are used in existing wastewater treatment plants. Currently, attention is being given to biological processes since their potential is not being fully exploited. To explain briefly, biological processes work by converting most of the remaining soluble and colloidal organic matter, nitrogen and phosphorus in the effluent, into settleable microbial solids. These solids are then separated before the treated wastewater is conveyed to a receiving body. With careful process selection, many types of wastewater, eg. from agricultural and industrial activities, can be efficiently treated by biological processing.

Several different units of operation come under the heading of biological processes, which are distinguished according to their biochemical reactions. One of these processes is known as anaerobic digestion, as it can take place without a supply of oxygen. Like any biological process, anaerobic digestion also can be modelled kinetically. Kinetic studies during the treatment process will assist the evaluation of the selected reactor design by providing information on the efficiency of the process based on the required sizing and design of the operation. Information from kinetic studies can be used to derive mathematical models that can quantitatively describe the mechanisms which regulate the events occurring during treatment. Manipulation of the kinetic model at laboratory scale can also be used to investigate the effect of alternative operational strategies such as phase-separation or operation as a hybrid-type reactor, in order to improve treatment efficiency whilst reducing operating costs.

Each biological process has its own unique features. In theory, anaerobic processes have many advantages compared to other processes. They offer a high degree of waste stabilisation, low production of excess biological sludge, low nutrient requirements, no O_2 requirement, and production of CH_4 which is a useful by-product. Nevertheless, application of anaerobic treatment methods is still limited as there is a lack

of understanding about the whole process. In practice, anaerobic processes are considered as rather complex, difficult to control and susceptible to treatment failures.

Application of biological processes is a complex task, as they work through a variety of biochemical reactions. In the case of anaerobic processes, the reaction has four main phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. In conventional anaerobic processes, the reaction phases are sequential but they occur simultaneously in the reactor, and this may lead to failure during the treatment process if the system suffers from phase-imbalance. Optimum operating conditions can be derived for each phase, but the process must operate within the optimum conditions for the most restrictive of the phases, which is generally accepted to be methanogenesis.

1.2 Aims and objectives

The overall aim of the project was to evaluate to the possibility of using a high rate anaerobic digestion process to convert the soluble hydrolysed and acidified products from a first phase solids substrate reactor treating the organic fraction of municipal solid waste (OFMSW). The research had a number of objectives as part of this overall aim, these were as follow:

- to set up and run a first phase flushing anaerobic reactor to produce leachate for second phase treatment.
- to carry out treatability studies of this leachate using fed batch and continuous feeding reactors
- to set up an run batch, fed-batch, and continuous-feeding of anaerobic reactors which are capable of treating readily biodegradable substrates.
- to evaluate the stability of the digestion system when treating a readily biodegradable wastewater that could be prepared with a high degree of consistency.
- to consider the process induced environmental factors such as pH, alkalinity and washout that can influence the process.

- to develop a methodology that would allow a simple and reproducible monitoring system to be employed from which data could be derived for the kinetic evaluation.
- to develop design guidelines for a second phase anaerobic reactor to treat leachate from the OFMSW.
- to review the kinetic models that have been used to describe the process of anaerobic digestion.
- to apply these models to base line data derived from the study of a readily biodegradable wastewater.
- to identify the strengths, weaknesses and applicability of these kinetic models to the prediction of gas production and substrate removal.
- to determine which models would be most applicable to the feeding regime employed.
- to determine the gas production potential of the leachate in a high rate reactor
- to determine the type of high rate anaerobic reactor most suited to the treatment of this type of leachate.

CHAPTER II LITERATURE REVIEW

PART A: ANAEROBIC DIGESTION PROCESSES FOR WASTEWATER TREATMENT

2.1 The role of microorganisms in anaerobic digestion

Microorganisms play the main role in the secondary treatment of wastewater. Literally, microorganisms feed on the remaining organic substances in the wastewater and convert it to tissues and various gaseous. The mixture of cells and waste residuals from the process is known as waste activated sludge (WAS) and can be removed by gravitational settlement. Similarly, anaerobic digestion is a process of decomposing the carbonaceous organic compound in the absence of molecular oxygen. The carbonaceous compound is used as the electron receptor during the oxidation, and forms CO₂ and CH₄ (McCarty, 1964). The formation of CH₄ is known as the methanisation process, which is a unique feature of anaerobic digestion. Figure 2.1 illustrates the biochemical activities of anaerobic microorganisms during stabilisation of organic matter. Several reaction phases occur during decomposition (McCarty, 1964). First, complex substrates like carbohydrate are reduced and solubilised to simpler substrates such as disaccharides or monosaccharide by a hydrolysis reaction. This process is then followed by the second phase of acidogenesis, where the end product of hydrolysis is metabolised to aliphatic compounds such as medium chain length volatile fatty acids (VFA). Thirdly, the medium chain length VFAs are degraded and converted to acetic acid in what is known as the acetogenesis phase. Fourthly, the most vital process for anaerobic digestion is the methanogenesis phase where methane is formed via several biochemical metabolic pathways from methanogenic substrates.

The anaerobic digestion process was first introduced for the stabilisation of WAS. This may explain why the application of anaerobic digestion is frequently associated with sludge digestion, resulting in a perception that anaerobic treatment is mainly for destruction of suspended solids (McCarty, 1964). There are two methods of WAS stabilisation by anaerobic digestion processes. The first method is known as standard-rate anaerobic digestion process and is a conventional method of treatment. WAS is digested in a reactor without any heating or mixing. The second method of



Figure 2.1 Schematic diagram of biochemical phases in anaerobic digestion process (Holland *et al.*, 1987 - see Metcalf and Eddy, 1991)

treatment is known as high-rate anaerobic digestion, in which sufficient heat is supplied for optimal biochemical reactions to occur (Figure 2.2a). The contents of the reactor are mixed and circulated in order to provide intimate contact between raw and digesting sludge, to discourage scum layer formation and grit settlement, to maintain a uniform temperature and to facilitate the release of gases from sludge in the lower region (IWPC, 1979).

The innovation of high-rate digestion made anaerobic digestion feasible for treatment with a higher organic loading in the wastewater. It has been found that 'washout' phenomena occur during the treatment of wastewater with a high content of biodegradable substrate that requires a very short hydraulic retention time (HRT). Essential microorganisms are washed-out because their regeneration rate is slower than the amount of biomass that is removed in the waste flow. The two-stage process or contact process was subsequently developed to overcome the wash-out problem. Biomass from the waste flow is collected in a settling chamber and reintroduced to the high-rate digestion reactor (Figure 2.2b). Solids in the effluent (digested wastewater) are separated by a clarifier or vacuum flotation unit, and the supernatant is discharged as the final effluent. Untreated wastewater is mixed with recycled sludge solids and pumped into the reactor as influent, to be anaerobically digested in a reactor sealed off from the entry of air. Settled anaerobic sludge is then recycled to seed the next influent. This method of treatment has been proven successful for the stabilisation of meatpacking and other high-strength soluble wastes (Metcalf and Eddy, 1991).

Figure 2.2 illustrates typical reactor configurations that are currently used for secondary treatment. Such reactors have been developed based on the concept of sustaining the biomass, or in other words as high solid retention time (SRT) anaerobic digestion reactors. The biomass in the reactor can be sustained by two methods of growth, known as the suspended-growth and attached-growth techniques. As in the anaerobic contact process, microorganisms in a suspended-growth reactor grow within the reactor itself and move with the wastewater throughout the process. The final effluent is separated by sedimentation and the biomass is retained for use in the treatment process once again. Continuous stirred tank reactors (CSTR) and upflow anaerobic sludge-blanket (UASB) reactors are typical designs for suspended-growth reactor.

In an attached-growth reactor, the microorganisms are provided with a growing surface. They remain attached to the surface and the wastewater flows over the surface. Collectively, the attached microorganisms are known as a biofilm. Excess microorganisms are 'sloughed off' the surface and are separated from the effluent in the final clarifier. The two most common anaerobic attached-growth reactors are the anaerobic filter and the expanded-bed reactor.

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Figure 2.2 Typical reactor configurations used in anaerobic wastewater treatment (Speece, 1983 - see Metcalf and Eddy, 1991)

2.2 Phase separation in the anaerobic digestion process

Application of anaerobic digestion as a wastewater treatment process still involves difficulties. In particular, the instability of this process makes control difficult and thereby diminishes its performance (Cohen *et al.*, 1979). The lack of stability is often due to the imbalances that occur between the different groups of microorganisms that perform the sequential phases of the process during the decomposition of complex organic matter. Two major groups of microorganism are involved throughout the process, non-methanogens and methanogens. Both groups differ greatly with respect to their physiology, nutritional requirements, growth and metabolic characteristics, and also in the degree of sensitivity to environmental stresses (Pohland and Ghosh, 1971). The hydrolysis-acidogenesis phase is performed by the non-methanogenic group, while the acetogenesis-methanogenesis phase is carried out by the methanogenic group.

The idea of separating the two groups was introduced to overcome the inherent problem in one-phase anaerobic digestion. The technique is known as two-phase separation. Most anaerobic reactor designs provided for coexistence of the two groups within the same physical and chemical environment (Pohland and Ghosh, 1971). Such practices have often been considered as leading to unstable performance as the system is susceptible to changes during operation that are liable to upset the balance between the two groups. The interacting stages of fermentation become uncoupled and system performance deteriorates. To maintain a favourable environment for groups, volatile fatty acid (VFA) production and conversion rates must be balanced. At shorter retention times, VFA production may exceeded utilisation which can lead to reactor failure (Ghosh, 1991).

Two-phase separation is an attempt to take advantage of the diphasic conditions during anaerobic digestion by keeping them physically separated. This will eventually provide the optimum environmental conditions to stimulate the hydrolysis-acidification and acetogenesis-methanogenesis phases in two different reactors. (Pohland and Ghosh, 1971). The environment in the non-methanogenic reactor can be controlled to promote the growth and proliferation of the acidogens whereas the methanogenic reactor receives the products from the non-methanogenic process and can be designed in such a way as to provide an optimum environment for the methanogens.

Pohland and Ghosh (1971) emphasised that the successful application of the twophase concept depends upon the feasibility of process separation and of maintaining dominant cultures of the acid and methane formers in two separate reactors. Phase separation in anaerobic digestion can be set-up by several methods: chemical inhibition (Pohland and Mancy, 1969), kinetic control (Pohland and Ghosh, 1971) and dialysis (Borchardt, 1969). Inhibition of methanogens in the acid reactor requires the addition of chloroform or carbon tetrachloride, limited oxygenation or adjustment of the redox potential. Separation by kinetic control is more attractive as there are difficulties with dialysis membranes and uncertainties associated with the determination of inhibitor concentration (Pohland and Ghosh, 1971). By adjusting the retention time, the growth of the selected group can be controlled. For example, the acid former reactor can be constructed to have a short retention time.

Two-phase separation should not be confused with two-stage digestion, which has been introduced in the anaerobic contact process (Pohland and Ghosh, 1971). The term 'phase' should be used for the process when different reactions occur in different reactors, and 'stage' should be applied for the same process and reaction occurring in two consecutive reactors (Fongastitkul *et al.*, 1994)

2.2.1 Potential benefits of phase-separation

Numerous potential benefits have been suggested for two-phase separation in comparison to conventional one-phase digestion. Optimisation is more favourable as the rate of substrate turnover can be increased which may allow a reduction in total reactor volume (Cohen *et al.*, 1979), higher organic loading rates for both groups are possible (Pohland and Ghosh, 1971), and the specific activity of the methanogens can be

improved and will eventually lead to an increase in methane production (Bull *et al.*, 1984).

Process instability can be caused by an imbalance of conditions between the two groups during the initial acidification and the final conversion of VFA to methane. Better process stability can be achieved by phase-separation, when the optimum operating conditions for both groups can be swiftly controlled (Cohen *et al.*, 1980). Phase-separation also provides a faster recovery following a shock loading into the reactor, and a better quality of effluent with lower suspended solids and total chemical oxygen demand (COD) for the effluent (Bull *et al.*, 1984).

2.2.2 Evaluation of phase-separation in the anaerobic digestion process

Several studies have been conducted to evaluate the efficiency of phaseseparation in anaerobic digestion. Table 2.1 shows a few studies on phase-separation in anaerobic digestion for waste treatment. Most of the studies concentrated on two-phase separation, but Kubler and Schertler (1994) developed three-phase separation (hydrolysis, acidification and methanisation) for the treatment of the organic fraction of municipal solid waste (OFMSW), which mainly consists of both soluble and solid organic matter. The waste was first pre-treated by magnetic separator and waste-pulper to isolate ferrous material as ferrous scrap. Meanwhile metals, minerals, or plastics were removed as rake and heavy fractions. The pulp then was stored in a buffer tank where spontaneous acidification started to occur. Dissolved material in the tank was later separated from the solids and immediately fed to the anaerobic reactor for methanisation. The remaining solids were then placed in a CSTR for hydrolysis, which is also carried out anaerobically. Part of the content of the hydrolysis reactor was recycled through the solid-liquid separation. Acidified products were removed together with the liquid phase and pumped into methanogenic reactor.

Type of phase- separation	Type of waste	Reference	
Three-phase	OFMSW	Kubler and Schertler, 1994	
Two-phase	OFMSW	Mtz-Viturtia et al., 1995	
Two-phase	WAS	Ghosh, 1987, 1991	
Two-phase	WAS (synthetic) - primary	Fongastitkul <i>et al.</i> , 1994	
	- secondary		
Two-phase	WAS	Bhattacharya et al., 1996	
Two-phase	Synthetic wastewater - glucose (1%)	Cohen <i>et al.</i> , 1980, 1982	
Two-phase	Synthetic wastewater - complex medium	Bull et al., 1984	
Two-phase	Synthetic wastewater - baby milk - skim milk	Jeyaseelan and Matsuo, 1995	

Table 2.1 List of comparison studies between phase-separation and one-phase anaerobic digestion

The three-phase anaerobic digestion showed promising results as it achieved a up to 84% degradation of volatile solids in pilot-scale study (Kubler and Schertz, 1994). Separating the hydrolysis phase may have enhanced the treatment process as it has been realised that the hydrolysis phase is rate-limiting in anaerobic digestion of cellulose (Noike *et al.*, 1985). However Mtz-Viturtia *et al.* (1995) observed that two-phase separation is not suitable for the treatment of MSW as the process efficiency is lower than has been observed in one-phase anaerobic digestion.

Two-phase separation studies have also been conducted on anaerobic digestion of WAS which mainly contained volatile solids (VS). Ghosh (1987) observed that 58% of the sludge VS was potentially anaerobically biodegradable under the baseline of mesophilic conditions. When compared to the one-phase system, the two-phase CSTR system performed better than the one-phase under all temperature conditions (mesophilic and thermophilic). In the same study, the VFA production rate was observed to be higher than the VFA conversion rate at lower hydraulic retention times and higher loading rates in one-phase digestion system. Thus, reliable operation can be expected only at high

HRTs, where the rates of VFA production and conversion are balanced. Subsequently, Ghosh (1991) demonstrated in a pilot-scale anaerobic digestion that the two-phase system was able to give higher treatment efficiency compared to the one-phase system. The reactor that was specifically designed for phase separation system exhibited an unusually high VS reduction of 73%.

Fongastitkul et al. (1994) also demonstrated the feasibility of two-phase separation using the UASB process on both synthetic primary and secondary sludge at laboratory scale. Waste substrates were simulated according to the sludge composition. Most primary sludge consists primarily of organic substances while secondary sludge usually contains organic and other nutrient substances such as cellular tissues, nitrogen and phosphorus. A careful combination of both sludges could potentially result in maximum removal efficiency, methane yield and operation stability. Contradicting this finding, Bhattacharya et al. (1996) observed a relatively small increase in VS reduction for a two-phase system compared to a one-phase system. The study used three sources of sludge, primary, WAS, and combination of both in the ratio of 1:1. The difference between the studies is in the method of VS measurement. Bhattacharya et al. (1996) used direct measurement of VS while Ghosh (1991) used VS reduction based on gas yield, which was measured by gas production over VS reduction and compared to theoretical gas yield. If direct measurement of VS is used as the major parameter, two-phase separation may not be worthwhile for operation considering the additional cost at full scale.

For more dilute wastewater with a high concentration of hydrolysable organic substrates, Cohen *et al.* (1980) reported that the specific sludge loading of the methanogenic phase for a two-phase system was over three times higher than the onephase system. Specific sludge loading was measured by the amount of COD turnover per biomass per day. There was no direct explanation for this feature. It might be the result of the different eco-physical interactions occurring in the two systems. These differences might involve: (1) the nature of metabolic pathways and the energy content of keyintermediates, resulting in different turnover rates, (2) maximum specific interspecies transfer rates of intermediary metabolites, (3) excretion of stimulatory or inhibitory substances by different acid-forming phases. The physical separation between acidogenic and methanogenic microorganisms in a two-phase system might lead to important alterations with respect to the composition of microorganism populations as well as to intermediary degradation routes. On theoretical grounds, it was expected that a one-phase digestion of simple substrate would comprise mainly interspecies transfer of acetate, bicarbonate and hydrogen, with the splitting of acetate and carbon dioxide reduction being the terminal steps.

A subsequent study by Cohen *et al.* (1982) characterised the two-phase system as essentially more stable for easily hydrolysable carbohydrates when compared to a onephase system. It was found that shock-loading of the system caused by an increase of feed supply in a one-phase system led to an accumulation of overflow products of the acidogenic phase followed by an inhibition of, or ineffective removal by the methanogenic phase, even if methanogenesis was not inhibited by low pH and/or high VFA or salt concentration.

A similar study by Bull *et al.* (1984) for simulated high-strength wastewater treatment showed that the phase-separation system consistently gave a better quality effluent in which the effluent contained fewer suspended solids and a lower total COD, and the methane yield improved. The improvement in effluent suspended solids content from the phase separation system is probably due to the greater sludge build-up in the separate-phase fluidised bed recycle system. This study also showed that the two-phase system was inherently more stable and recovered more rapidly after a shock loading. Suspended solids in the effluent remained stable or decreased in the phase-separation system after the cessation of shock loading, in contrast to the one-phase system where the solids tended to increase and were much more unstable. The stability of the reactors during the shock loading was measured by the ratio of alkalinity:VFA. It was observed that the ratio might be up to 60% higher in the separated-phase system than in the one-phase system. For more complex substrates in wastewater treatment, Jeyaseelan and Matsuo (1995) observed that when lipid substrates were included together with

carbohydrates, the two-phase anaerobic digestion had higher digestion efficiencies compared to the corresponding one-phase digestion. Similar findings were also reported earlier by Ghosh (1987) who monitored lipid degradation in anaerobic digestion of WAS.

2.2.3 Phase-separation operation for the disposal of organic fraction of municipal solid waste (OFMSW)

The normal procedure for the disposal of OFMSW is by landfilling. It has been realised, however, that the leachate produced by landfilling such waste can be hazardous if it enters the groundwater or any other sources of natural water. Cameron and Koch (1980) conducted toxicity assessment tests on leachate using bioassay techniques using rainbow trout. The OFMSW leachate from a natural landfill could be lethal as it reaches a concentration of 4.9 to 7.0% (v/v). The leachate from a lysimeter proved to be even more lethal, even though it only reached a concentration of 0.35% (v/v).

OFMSW leachate is produced by a simple process in which organic compounds are decomposed by a microbial chain, in conditions where there is sufficient moisture and a lack of oxygen. The leachate that is produced may contain a large amount of biodegradable organic material, and can therefore be treated anaerobically. Defining the characteristics of the landfill leachate is not an easy task as it depends on the surrounding moisture content, the amount of surface water and also the age of landfill. Leachate from domestic wastes have been found to be significantly more toxic compare to those of pure industrial wastes (Bernard *et al.*, 1996). The most toxic leachate were found in landfill receiving hazardous industrial wastes mixed with domestic wastes. Apart from defining toxicity, other leachate characteristics are difficult to categorise because the variability depends on the moisture content in the surrounding area and also on the age of the landfill. Leachate from a stabilised landfill are not suitable for biological treatment because a substantial part of the biodegradable organic matter has already been removed (Boyle and Ham, 1974).

If integrated phase-separation is introduced for treatment of OFMSW itself, this offers another disposal method that could potentially divert a large proportion of refuse away from landfill. Sans et al. (1995) explored three possibilities for integrated plants that could be operated for the treatment of OFMSW. First, OFMSW could be used as the substrate for anaerobic digestion. The sludge produced would then be composed at a later stage for final stabilisation. This process is somewhat more like a two-stage operation, however, rather than two-phase. Second, OFMSW could be used as the substrate for the first stage of operation (acidogenic) of the two-phase process. A composting process would again be used for final treatment of the sludge produced. Third, OFMSW could be used to produce valuable products such as VFAs by the anaerobic digestion process. Thus the OFMSW could be 'pre-treated' in a fermentating acidogenic process with the aim of obtaining the maximum concentration of VFA in the liquid phase. The sludge produced in the acidogenic phase could go through a pressing process for further extraction of the liquid phase. The pressed sludge could then go directly to a composting step or it would be possible to recover the organic fraction in the residuals. The effluent or the leachate from the acidogenic phase of the second and third methods would be further treated in a second phase where biogas would be obtained.

Leachate produced in the first stage of two-phase operation of OFMSW treatment would also be useful for biological nutrient removal (BNR) in a wastewater treatment plant. Llabres *et al.* (1999) used the organic fraction of the hydrolysed and acidified product of OFMSW fermentation for enhanced biological phosphate removal (EBPR). This is a biological alternative to chemical phosphate precipitation. The leachate, also known as readily biodegradable COD (RBCOD), was added when the internal RBCOD in the system was insufficient and could adversely affect the phosphate removal process. Such integrated municipal waste management systems should be introduced as both treatment methods can share materials and equipment and yields can improve due to positive synergies. Within this context, the OFMSW fraction separately collected at source could be treated in a wastewater treatment plant. The solid phase from the first stage of the operation could undergo a second-methanogenic phase together with the excess sludge or could be used as a soil conditioner in agriculture after dewatering.

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Part B: REVIEW OF KINETIC MODELS USED TO DESCRIBE ANAEROBIC DIGESTION PROCESSES

2.3 Kinetic studies for wastewater treatment

The kinetics of anaerobic digestion depend on the rate at which microorganisms degrade the organic substrate. Organic substrate concentrations can essentially be measured either with two analytical methods, an oxygen-demand parameter or by direct carbon-content measurement. Another way of measuring the degree of organic matter degradation in anaerobic digestion is by measurement of CH₄ gas production. The combination of both analytical measurement and CH₄ production will give the removal efficiency by calculation of the amount of degraded organic matter that has been converted to CH₄.

Modelling the kinetics of the anaerobic digestion process for wastewater treatment is not straightforward, as simplifying assumptions must be made in order to fit the mathematical descriptions to observed effects. It is essential that all parameters are clearly defined to ensure that models are not applied inappropriately. The general assumption that has been made for modelling the kinetics of anaerobic digestion is that a mixed population of biomass is regarded as one unit of population, and is distributed continuously through the reactor (Horan, 1990). The complexity of interactions in an anaerobic digestion system is well known; nevertheless the more simplified models may help to give a general insight to the phenomena investigated (Vavilin and Lokshina, 1996).

2.3.1 Development of kinetic model for the anaerobic digestion process

Most of the kinetic models for wastewater treatment are based on the massbalance of the system, which is considered as important as other fundamental factors like environmental conditions and operating regime. Mass-balance provides a convenient way of defining what occurs within treatment facilities as a function of time (Metcalf and Eddy, 1991). Mass-balance serves as an important tool in analysing flow systems in any natural or engineered system such as water and wastewater treatment, air pollution control, and stream pollution analysis (Ray, 1995). It is defined as an organised statement of the inputs and outputs in an arbitrary but definable system. The net rate of substrate accumulation within the system is equal to the rate of substrate input to the system minus the rate of mass output from the system and minus the net rate of substrate utilisation within the system.

In simpler format:

[Net substrate mass] = mass of [substrate_{in} - substrate_{out} - substrate_{utilisation}]

In equation form at steady state:

$$V. \,\delta S/\delta t = \Sigma \left(S_o \, Q_o \right)_{\text{in}} - \Sigma \left(S_e \, Q_e \right)_{\text{out}} - r_S \, V; \text{ and } Q_o = Q_e$$
$$= \Sigma \, Q. \left(S_{in} - S_{out} \right) - r_S \, V \qquad (\text{Equation 2.1})$$

 $\partial S/\partial t =$ net organic substrate concentration in the reactor measured at *t*-time [mass/volume.time]

V =	volume of reactor	[volume]
$S_o =$	concentration of substrate entering the system	[mass/volume]
$Q_o =$	volumetric flow rate of the substrate entering the system	n [volume/time]
$S_e =$	concentration of substrate leaving the system	[mass/volume]
$Q_e =$	volumetric flow rate of the substrate leaving the system	[volume/time]
$r_S =$	substrate utilisation rate	[mass/volume.time]
	$r_{S} \cdot V = \left[\Sigma \left(S_{o} \ Q_{o} \right)_{\text{in}} - \Sigma \left(S_{e} \ Q_{e} \right)_{\text{out}} \right] $ (Equ	ation2.2)
	$r_{S} = Q \left[\Sigma (S_{o} - S_{e}) \right] $ (Equ	ation2.3)
	V	

For a system running in steady-state conditions without substrate accumulation, the time-dependent term in Equation 2.1 goes to zero. At this point, formulation of mass
balances can be reassigned to the design procedure. The required sizing can be estimated according to the flow-rate (Q), concentration of influent (S_o) and effluent (S_e), and the organic substrate utilisation rate (r_s). The concentration of effluent that can be discharged (S_e) is governed by the maximum allowable effluent according to environmental standard regulations, with known values of V, Q and r_s .

In laboratory-scale, pilot-scale and research studies the application of a kinetic model based on r_s can be regulated by simplification of Equation2.2, which when influent and effluent flow are at the same rate gives Equation 2.3. In this equation, the parameter r_s is equal to the difference between the mass input rate and the mass output rate. Mass-balance appears to be a satisfactory method for general design and has been applied for over two decades. Application of a kinetic model derived from Equation 2.3 is also valuable in improving the process design since it allows comparison of the different versions of the process with respect to process stability. The incorporation of improved control systems in the design would also improve process stability and remove the need for oversizing (Andrews and Graef, 1971).

There are several mathematical approaches to calculating the parameter r_S in the anaerobic digestion process. Most of the kinetic models developed use the basic equation for biological kinetic growth involving growth rate (r_G), substrate utilisation rate (r_S), biomass yield coefficient (Y) to represent the relationship between essential substrate concentration and growth rate.

2.3.2 Michaelis-Menten kinetic relationship

The Michaelis-Menten kinetic relationship was introduced in the area of enzymology, which involves catalytic reactions between a specific enzyme, substrate and product. Segel (1976) gives the general equation of the Michaelis-Menten relationship for a biochemical reaction as Equation 2.4. It describes the formation of a substrateenzyme complex, followed by the breaking down of the complex, resulting in an end product and free enzyme. The enzyme remains unchanged and is capable of further catalytic reaction. Ramalho (1977) described the microbial population as acting as the catalyst with the organic substances as the substrate.

$$[S] + [E] < \stackrel{k_1}{==} > [E-S] < \stackrel{k_2}{==} > [E] + [P]$$
 (Equation 2.4)
 $\underset{k_{-1}}{k_{-1}}$ (Equation 2.4)

[S] = substrate
[E] = enzyme
[E-S] = enzyme-substrate complex
[P] = product
[k] = reaction rate constant

Parameter r_s can be obtained from this equation with the assumption that the breaking down of enzyme-substrate complex is irreversible, yielding Equation 2.5.

$$[S] + [E] < \stackrel{k_1}{==} > [E-S] \stackrel{k_2}{==} > [E] + [P]$$
 (Equation 2.5)
 $\stackrel{k_1}{=} > [E] + [P]$

Hence from Equation 2.5, r_s can be equated to the rate of formation of [P], giving Equation 2.6.

$$r_S = k_2 \ [E-S] \qquad (Equation 2.6)$$

The net change of concentration of the [E-S] complex is expressed as a differential in Equation 2.7.

To show the total concentration of enzymes in the reacting system, Equation 2.8 uses $[E_t]$ to represent the free enzyme and enzyme-substrate complex. Rearrangement of Equation 2.8 as Equation 2.9 shows the calculation of free enzyme in the system.

$$[E_t] = [E] + [E-S]$$
 (Equation 2.8)
 $[E] = [E_t] - [E-S]$ (Equation 2.9)

Replacing Equation 2.7 with Equation 2.9 for steady-state conditions gives Equation 2.10 and Equation 2.11.

 $k_1 [S][E_t] - k_1 [S][E-S] = k_1 [E-S] + k_2 [E-S]$ (Equation 2.10)

$$\frac{[E_{t}][S]}{[E-S]} = \frac{k_{-1} + k_{2} + k_{1} [S]}{k_{1}}$$
 (Equation 2.11)

 $[(k_1 + k_2) / k_1]$ represents the Michaelis-Menten constant which can be written as K_m . It is also known as the saturated substrate constant. Incorporation of K_m into Equation 2.11 gives Equation 2.12. In steady state conditions, $[S] = S_e$ and replacing Equation 2.6 into Equation 2.12 gives Equation 2.13.

$$[E-S] = \frac{[E_t][S]}{K_m + [S]}$$
(Equation 2.12)
$$r_S = \frac{k_2 [E_t] S_e}{K_m + S_e}$$
(Equation 2.13)

 K_m = Michaelis-Menten constant

At high substrate concentrations, S_e is very much higher than K_m , thus K_m in Equation 2.13 can be neglected under such conditions to give Equation 2.14 which is equivalent to r_m .

 $r_{S} = k_{2} [E_{t}] = r_{m}$ (Equation 2.14)

At low substrate concentrations, S_e is very much lower than K_m , thus S_e in the denominator of Equation 2.13 can be neglected and gives Equation 2.15.

$$r_m = \frac{r_m \cdot S_e}{K_m}$$
 (Equation 2.15)

Since r_m and K_m are both constant for a specific wastewater, Equation 2.15 can be rewritten as Equation 2.16 where $[k = r_m / K_m]$.

 $r_S = k.S_e \qquad (Equation 2.16)$

The Michaelis-Menten kinetic relationship for wastewater treatment can be summarised with reference to the substrate concentration as follows:

- at high substrate concentration, zero order kinetic relationship: Equation 2.14
- at intermediate substrate concentration, between zero and first order kinetic relationship: Equation 2.15
- at low substrate concentration, first order kinetic relationship: Equation 2.16 where
 - r_s = substrate utilisation rate (mass.volume⁻¹.time⁻¹)
 - r_m = maximum substrate utilisation rate (mass.volume⁻¹.time⁻¹)

 S_e = effluent strength (mass.volume⁻¹)

 K_m = Michaelis-Menten constant (mass.volume⁻¹)

2.3.3 First Order Model

The First Order Model is adapted from the first order Michaelis-Menten kinetic relationship. It considers the microorganisms as a 'catalyst' and represents an overall mass transfer kinetic model for a 'catalysed' reaction (Mata-Alvarez and Cecchi, 1990). It is based on the assumption that the rate of reaction depends only on the concentration of the organic matter (*S*) and is proportional to its concentration. Although this model is not sophisticated, it can provide a useful kinetic constant that can be applied when dealing with complex systems. Equation 2.16 describes the First Order Model, which is rewritten as Equation 2.17.

 $r_{S} = \delta S / \delta t = k.S$ (Equation 2.17) $r_{S} = \text{substrate utilisation rate (mass.volume^{-1}.time^{-1})}$ $k = \text{constant (time^{-1})}$

S =substrate concentration

When Equation 2.1 is applied to a batch operation, it will be the same as Equation 2.17 because the coefficient Q is equal to zero when there is no flow rate. Integration of Equation 2.17 between the limits of S equal to S_o and $S = S_t$, and t = 0 and t = t, will give Equation 2.18 which can be used to determine the constant k.

$$\int \delta S / S = \int k. \, \delta t$$

$$\ln (S_t / S_o) = k.t \qquad (Equation 2.18)$$

However the constant k cannot be calculated directly from Equation 2.18, but has to be fitted through a set of data points of $[S_e/S_o]$ at time-t. For a continuous operation under steady state conditions and assuming that the reaction is a first order kinetic relationship, the First Order Model for this operation is given in Equation 2.3 where $[\delta S/\delta t]$ is equal to zero. It is also can be rewritten as Equation 2.19 when the flow rate for influent and effluent are the same.

$$r_{S} = \frac{S_{o} - S_{e}}{\text{HRT}}$$
(Equation 2.19)

HRT = hydraulic retention time

2.3.4 Monod Model

The Monod Model considers the growth rate of the microorganisms and the substrate utilisation rate are limited by the amount of substrate present, as represented by Equation 2.20.

$$\mu = \frac{\mu_m \cdot S_e}{K_s + S_e}$$
 (Equation 2.20)

 μ = specific growth rate

 μ_m = maximum specific growth rate

 S_e = concentration of growth limiting substrate

K_s = half-velocity constant, substrate concentration at one-half of μ_{max}

The equation is similar to Michaelis-Menten kinetic relationship except this model stresses the growth rate instead of the substrate utilisation rate. The relationship between both parameters can be represented by the biomass yield coefficient, which was introduced by Lawrence and McCarthy (1969) for its application in wastewater treatment. The mathematical expression for biomass yield is shown in Equation 2.21.

 $Y = r_G / r_S$ (Equation 2.21)

Y = biomass yield coefficient

 r_G = biomass growth rate

 r_S = substrate utilisation rate

The biomass growth rate can be determined by Equation 2.22.

$$r_G = \mu \cdot X$$
 (Equation 2.22)
X = biomass concentration

Equation 2.20 can be expressed in terms of r_s by replacement with a combination of Equation 2.21 and Equation 2.22, giving gives Equation 2.23. μ_m / Y can be represented by coefficient k and gives Equation 2.24.

$$r_{S} = \frac{\mu_{m} \cdot S_{e} \cdot X}{Y \cdot [K_{s} + S_{e}]}$$
(Equation 2.23)
$$r_{S} = \frac{k \cdot S_{e} \cdot X}{[K_{s} + S_{e}]}$$
(Equation 2.24)

Coefficient k is also known as the maximum substrate utilisation rate per unit of biomass. Meanwhile the significance in waste treatment terms of a low value of coefficient K_s is that the system can be operated at 'short' SRT and correspondingly 'high' rates of specific substrate utilisation without sacrificing effluent quality and treatment efficiency. Vavilin and Lokshina (1996) used another approach with the Monod Model for a batch operation. Based on Equation 2.23, in the particular case of high initial variable X with low changes in biomass, and substrate concentration very much higher than K_s , the substrate kinetics reduce to a zero order reaction which is written in Equation 2.25. Integration of the equation gives Equation 2.26

$r_{S} = \partial S / \partial t = - \left[\mu_{m} \cdot X_{o} \right] / \left[Y \right]$	(Equation 2.25)
$S = S_o - \mu_m X_o \cdot t / Y$	(Equation 2.26)

From Equation 2.26 is obtained

$$\mu_m X_o / Y = S_o - [S / t]$$
 (Equation 2.27)

In the particular case of a low initial biomass concentration and substrate concentration very much higher than K_s , the substrate kinetics are written as

 $r_{S} = \delta S / \delta t = [\mu_{m} \cdot X_{o} \cdot e^{\mu m \cdot t}] / [Y]$ (Equation 2.28)

From 2.28 the substrate concentration is

 $S = S_o - X_o [e^{\mu m \cdot t}] / [Y] - 1$ (Equation 2.29)

2.3.5 Inhibition Model

The Monod Model has been observed to be stable except for the wash-out residence time. However, it does not take account of inhibition that may exist during the process. The use of an inhibition model for wastewater treatment is considered important to enable the prediction of process failure due to a high concentration of volatile fatty acids (VFAs) at residence times exceeding the wash-out residence time. It has also been used to study inhibition by other substrates eg. phenols, thiocyanates, nitrates and ammonia (Andrews, 1968). The Haldane Model is one of the commonly used inhibition models. It was first introduced to study the inhibition of enzymes by high substrate concentration. The equation for the Haldane Model is given in Equation 2.30. For low

values of substrate concentration or high values of K_i (less inhibitory substrate), the Haldane Model will reduce to the Monod Model.

$$\mu = \frac{\mu_m}{1 + [K_s / S] + [S / K_i]}$$
(Equation 2.30)

 μ = specific growth rate

- μ_m = maximum specific growth rate in the absence of inhibition
- K_s = half-velocity constant
- S = substrate concentration
- K_i = inhibition constant

Andrews (1968) defined K_s as numerically equal to the lowest concentration of substrate at which μ is equal to one-half of μ_m in the absence of inhibition, while K_i is numerically equal to the highest substrate concentration at which μ is equal to one-half of μ_m in the presence of inhibition. In a typical continuous culture operated at near steady state conditions, substrate concentrations are low and the term $[S/K_i]$ is therefore much less than the term $[K_s/S]$ even for low values of K_i .

The Haldane Model can be modified to reflect un-ionised acid as the limiting substrate, with the coefficient of K_s and K_i expressing the concentration of un-ionised VFA. Consideration of the un-ionised acids as inhibiting agents resolves the conflict which exists in the literature as to whether inhibition is caused by high VFA concentration or low pH (Andrews and Graef, 1971). The following equations show the modification of Equation 2.30. For pH values above 6, the total un-ionised substrate can be measured by Equation 2.31 and modification produces with Equation 2.32

HS =
$$[H^+][S^-]/K_a$$
 (Equation 2.31)

 K_a = ionisation constant, i.e. 10^{-4.5} for acetic acid at 38 °C

$$\mu = \frac{\mu_m}{1 + [K_s / \text{HS}] + [\text{HS} / K_i]}$$
HS = unionised substrate concentration
H⁺ = hydrogen ion concentration

 S^- = ionised substrate concentration

Therefore, at a fixed pH and with a known total substrate concentration, the ionised substrate concentration can be calculated from the equilibrium relationship in Equation 2.31. In fact, the Haldane Model can be modified to monitor other inhibitory factors beside substrates, such as alkalinity, pH, gas flow rate and gas composition. Several other models have also been introduced as inhibition models.

(Equation 2.32)

Andrews (1968) also noted that even when coefficients K_s and K_i are well separated, there will be a considerable reduction in the μ_m attainable when compared with the case without inhibition. Setting the first derivative of the Haldane Model in Equation 2.30 equal to zero gives μ_m ' which is shown in Equation 2.33.

$$\mu_m' = \frac{\mu_m}{1 + 2 (K_s / K_i)^{0.5}}$$
 (Equation 2.33)

 μ_m' = maximum specific growth rate attainable in the presence of inhibition

Extensive modification of the Haldane Model leads to Equation 2.34 and 2.35, by incorporating the model into the mass-balances for biomass and substrate, respectively.

$$\frac{\delta X}{\delta t} = \frac{X_o - X}{MCRT} + \frac{\mu_m}{1 + [K_s / S] + [S/K_i]} \cdot X \quad (Equation 2.34)$$

MCRT = mean cell retention time

$$\frac{\delta S}{\delta t} = \frac{S_o - S}{HRT} + \frac{\mu_m}{1 + [K_s / S] + [S/K_i]} \cdot \frac{X}{Y}$$
(Equation 2.35)

In steady state conditions, $\delta X/\delta t$ and $\delta S/\delta t$ equal zero, which gives Equation 2.36 and Equation 2.37 for Equation 2.34 and Equation 2.35, respectively. $S = K_i \cdot [\mu_m \cdot MCRT - 1] + [K_i^2 \cdot (\mu_m \cdot MCRT - 1)^2 - 4K_s \cdot K_i]^2$

 $X = Y \cdot [S_o - S] / HRT$

(Equation 2.36) (Equation 2.37)

WATERIALS AND METHODS CHAPTER III

3.0 General introduction

A series of experiments was conducted with two types of substrate in order to study the kinetics of anaerobic digestion in wastewater treatment works. Most of the materials and methods used in this study, such as the experimental set-up and chemical analysis, were adapted from established methods. All data were taken in triplicate using three reactors, except for the last experiment, which was carried out with a single reactor. The measurements taken were also repeated for each set of experimental work.

3.1 Reactor set-up

Two types of reactor design were used in this work to represent the two typical configurations for suspended-growth and attached-growth reactors. Both designs were developed to sustain the biomass population, or to provide a high solids retention time (SRT) because of the low biomass yield for anaerobic microorganisms. All reactors were set up at mesophilic temperature. Reactors for anaerobic process have to be sealed from the entry of atmospheric air. Thus, all reactors were tested for air-tightness before starting up the experiments.

3.1.1 Continuous stirred tank reactor

The continuous stirred tank reactor or CSTR is a typical design for suspended growth reactors. A 6-litre airtight glass vessel with motorised stirrer, substrate inlet and outlet and biogas outlet, placed in a water bath at a temperature of 35 $^{\circ}$ C was used as a CSTR for this study.

Figure 3.1 shows the set up for CSTR operation. Biogas from the reactors was collected in water displacement gasometers. Calculations of the generated biogas were made according to Equation 3.1.



Figure 3.1 CSTR set up for batch and fed-batch operation

Biogas production = $\eta \cdot r^2 \cdot h$ (Equation 3.1) $\eta \cdot r^2$ = surface area of the measurement flask h = height of water displacement level

2.5 l of digested sludge from Southern Water Ltd, Millbrook, Southampton were placed in each reactor as the inoculum. Nitrogen gas was purged into each reactor for 4-5 minutes to remove any oxygen from the headspace. Production of CH₄ from the newly-seeded sludge was monitored for 24 hours prior to any substrate addition to assure the viability of the inoculum as anaerobic biomass. An acclimatisation stage with the experimental substrate took place immediately after the viability of the inoculum was confirmed. At this stage, CH₄ production was analysed with a gas analyser (GA94A, Geotechnical Instrument (UK), Ltd.).



Figure 3.2 AF reactor set up for continuous feeding operation

3.1.2 Anaerobic filter reactor

The anaerobic filter or AF was set-up in an airtight 3.5 l polypropylene column. Figure 3.2 shows the set-up for the AF operation. Heat for the reactor was supplied by a thermocyclic system. Copper lining was placed around the column as the heating coil, while hot water at 55 °C from a boiler was circulated through the coil to heat the reactor up to 35 °C when placed in surroundings at room temperature.

Various sizes of plastic ring pulls (8, 15, 20 mm) with inert surfaces were packed into the column to entrap and attach the biomass. These acted as the filter media for the substrate flowing through the column. Biomass acclimatised to OFMSW leachate in the previous CSTR was allowed to settle at full depth, then supernatant was removed by a

siphon process. 2.5 l of compacted biomass was transferred from a CSTR into the AF. This inoculum was recirculated from the outlet back into the inlet by gravity several times to allow the unattached biomass to become lodged into the packing material. A peristaltic pump was set up on a timer to manage the hydraulic flow for the reactor.

3.2 Substrates preparation

Three types of substrate were used in this experiment: first, a readily biodegradable wastewater; second, a solid-free leachate of organic fraction of municipal solid waste (OFMSW); and third, the actual leachate from OFMSW. Kinetic studies of leachate treatment by anaerobic digestion process are a relatively a new area. Using a readily biodegradable wastewater would assist the development of kinetic modelling for the leachate treatment since both substrates are similar in providing a readily biodegradable substrate.

3.2.1 Readily biodegradable wastewater

A readily biodegradable wastewater was prepared by dilution of a concentrated mixed fruit cordial to simulate wastewater typical of a soft drinks manufacturing operation (Borja and Banks, 1994a). It contains most of the required nutrients such as vitamin and trace elements, as well as a carbon source. The substrate was diluted with distilled water to give a series of COD strength of 4000 - 8000 mg.l⁻¹. This was to simulate a range of medium strength wastewater. Later the substrate was supplemented with additional buffer and ammonium salts to make it suitable for an anaerobic microbial population.

3.2.2 Leachate of OFMSW

Leachate from OFMSW was supplied by a rapid anaerobic hydrolyser for OFMSW. The hydrolyser is also known as the first stage of two-phase anaerobic digestion. This experiment focused on treatment of leachate as the second stage for phase-separation process. OFMSW was hand sorted to pick out the non-organic material, wood and cardboard from a stock of municipal waste collected from Hampshire Waste Transfer Point, Southampton, UK. The sorted OFMSW was then shredded and turned into a slurry containing 10% total solids. The 10 l anaerobic hydrolyser was operating at 1d solid retention time (SRT) and 3 d hydraulic retention time (HRT). To achieve these operating parameters, 1 1 of the reactor content was wasted and replaced with same volume of OFMSW slurry stock, followed by removal of another 3.33 l of filtered digestate. Filtration was through a mesh with a size of 60 µm. The extracted leachate was then replaced with same volume of distilled water. The separated solids from the mesh were recycled back to the hydrolyser. The stock of filtered leachate was then kept in the freezer at -5° C as the stock for second stage of phase-separation anaerobic digestion. To obtain the solid-free leachate, the stock of filtered leachate was defrosted and filtered again with Whatman-GF/C filter paper.

3.3 Start-up operation

The start-up operation was the acclimatisation of seed-sludge to the specific substrate in the CSTR. Once the seed-sludge was confirmed as a viable inoculum, a small volume of the specific substrate was introduced into each reactor. The seed-sludge had first to be stabilised, however, because it was taken from a fresh digested sludge. The stabilisation could be monitored by the declining production of CH_4 without any addition of substrate. The acclimatisation towards substrates was monitored by production of CH_4 and total COD mass reduction in the reactor.

Acclimatisation to the readily biodegradable wastewater was carried out with caution because its biocompatibility was not confirmed, although it has most of the

required nutrients. 100 ml of substrate with a strength of 5000 - 20 000 mg.l⁻¹ was added when the seed-sludge had been stabilised (as shown by a consistently low CH₄ production). On the following days, the substrate strength was increased by approximately 10% to induce a rapid acclimatisation of the seed-sludge. Effluent strength, pH and CH₄ production were monitored closely. During this acclimatisation phase, which lasted 15 days a final organic loading rate of 570 mg.l-1.d-1 COD, was reached in a reactor with a total working volume of 4 l. At the end of the acclimatisation phase, the contents of the three reactors were mixed together and redistributed equally back to ensure uniformity at the start of the experimental run for the following batch operation.

Acclimatisation of the next inoculum to the OFMSW leachate was much simpler because it could be assumed biocompatible since it also originated from the anaerobic bioreactor. The acclimatisation procedure was carried out by daily addition of 250 ml substrate into 2.5 l of stabilised seeded-sludge. The amount of substrate added was maintained until it reached the required working volume. When the effluent strength and the amount of CH₄ production were consistent, the seed sludge was considered ready for the next operation.

3.4 Batch operation

Batch operation is a basic mode of operation in the anaerobic digestion process for the treatment of any organic substances. In this study, it was only carried out for the readily biodegradable wastewater, in order to obtain information on how the anaerobic digestion process would response to the substrate. Such information would be useful before proceeding to steady-state operation.

Operation of the CSTR system required the removal of a specific volume of spent substrate and replacement with fresh substrate. To achieve this, the mixing was stopped for biomass sedimentation to take place and the biomass was separated from the effluent before its removal. The settling period was fixed at two hours. The volume of effluent withdrawal in each batch cycle was equal to the volume of feed to be introduced. Initially, the feed volume was 0.5 l, followed by 0.8 l, 1.0 l and 1.33 l. Each batch operation was based on a 72-hour cycle. Measurements of cumulative CH_4 production, COD concentration, biomass, pH and alkalinity were taken during each cycle.

3.5 Fed-batch operation

Fed-batch operation is also known as pseudo steady-state operation, and occurred when substrate was added on a 24-hour cycle. The daily volumetric loading and removal were considered as the flow rate (Q l.d⁻¹), and the working volume (V l) was used to calculate the hydraulic retention time (HRT = V/Q). The method of influent loading and effluent withdrawal were same as for batch operation, where the mixing was stopped for two hours for biomass sedimentation to take place. This mode of operation was used for a series of different strengths of the readily biodegradable wastewater and also on both types of OFMSW leachate in the CSTR system.

An initial HRT of 20 d was introduced after the acclimatisation of each substrate. Stabilisation of reactors was indicated by consistent CH_4 production and S_e strength. When stabilised, data for biogas production, S_o and S_e strength, biomass, pH and alkalinity were taken on a daily basis for 3 consecutive days. The pH was closely monitored and sodium bicarbonate was added to raise the pH if it dropped below 6.5. Except where stated in the results section the experimental runs used values of HRT of 20, 18, 16, 14, 12, 10, 8, 6 and 4 days with a stabilisation period in between step change.

3.6 Continuous operation

Continuous operation involves continuous feeding of substrate into the system. Simultaneously the same amount of spent substrate was replaced to accommodate the requirement for steady-state operation. In this experiment, the reactor design was changed from CSTR to AF in order to sustain the biomass population at a higher volumetric loading. The experimentation only involved the treatment of leachate.

It was not possible to provide continuous feeding, however, because the experimental set up could not provide a suitable volumetric flowrate (Q) according to reactor size, ie. Q should be 42 ml.hour⁻¹ in order for a reactor with a 3 l working volume to achieve an HRT of 3 d. An ordinary peristaltic pump could not provide such a flowrate. Thus, the system was operating similarly to a plug-flow system. A series of batch feeds were given to the reactor during each day, ie. to achieve an HRT of 3 d, 4 batches of substrate with a feed volume of 250 ml were pumped into the reactor every 6 hours to make up Q equal to $11.d^{-1}$. Biogas production and composition was recorded and analysed for each batch. The numbers of batches and volume of each one was adjusted according to required Q at each of the HRT's. The effluent in each case was collected after the hydraulic flush and after the alkalinity analysis, the samples were kept in the fridge at 4 °C for further chemical analysis.

3.7 Chemical analysis

Chemical analysis was carried out in accordance with standard methods provided by the American Public Health Association (1991). Measurement of chemical oxygen demand (COD) and volatile solids were undertaken for both influent and effluent. The effluent was analysed for pH, alkalinity, and suspended solids. The same analyses were also conducted on the reactor content during mixing, except for suspended solids. Analysis of volatile solids was carried out for biomass measurement. Besides these standard analyses, another series of analyses for the concentration and profile of volatile fatty acids (VFAs) and biogas was also conducted.

3.7.1 COD analysis

COD analysis is used as a measure of the oxygen demand equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical

oxidant. Closed reflux with titrimetric method was used for the analysis. Samples of influent and effluent were taken under the same conditions, ie. if the influent was filtered, then the effluent would also be filtered for a solid-free leachate. For readily biodegradable wastewater and actual leachate, the influent was analysed in its original form, while the analysed effluent was first separated from the biomass by sedimentation process.

Apparatus

- a. Borosilicate culture tubes, 16 mm x 150 mm with TFE-lined screw cap
- b. Heating block, cast aluminium, 45 mm deep with holes sized for close fit of culture tubes
- c. Block heater to operate at 150 ± 2 °C.

Reagent

- a. Readily available digestion solution (Ficodox, BDH Ltd.)
- b. Ferroin indicator solution: 1.485 g of 1,10-phenanthroline monohydrate and 675 mg FeSO₄.7H₂O were dissolved in 100 ml of H₂O.
- c. Standard ferrous ammonium sulphate (FAS) titrant, 0.01 M.
 3.92 g FAS was dissolved in H₂O with 20 ml of concentrated H₂SO₄ and made up to 1000 ml

Procedure

- a. 2 ml of diluted sample (4 times dilution) was placed in culture tube, followed by addition of 4 ml Ficodox and closed with the screw cap
- b. $2 \text{ ml of } H_2O$ was also prepared according to the previous procedure as the blank
- c. The tubes were placed into the reactor block, refluxed for two hours and cooled to room temperature
- d. 1 to 2 drops of Ferroin indicator was added to the digested solution and titrated with FAS. The end point is a sharp colour change from blue-green to reddish brown
- e. Calculation of COD as mg $O_2 L^{-1} = (A B) \cdot M \cdot 4000$ A = ml FAS used for blank, B = ml FAS used for sample, and M = molarity of FAS

3.7.2 Alkalinity analysis

Alkalinity is the acid-neutralising capacity of a sample of water. It is often expressed as the concentration of calcium carbonate determined from a titration with acid to a pH of 4.5. Fresh sample was used for the analysis.

<u>Apparatus</u>

- a. pH meter
- b. Magnetic stirrer

Reagent

 H_2SO_4 (0.1 N)

Procedure

- a. 20 ml of sample was well mixed and the pH measured
- b. The sample was titrated with H₂SO₄ till pH 4.5
- c. Calculation: mgL^{-1} CaCO₃ = <u>A . N . 50 000</u>

ml sample

 $A = ml \text{ of } H_2SO_4 \text{ used}$ N = normality of $H_2SO_4 \text{ used}$

3.7.3 Volatile solid analysis

Volatile solid (VS) in the mixed reactor content was assumed to be the biomass content, while VS in influent and effluent was assumed to be the degradable organic substances. A well-mixed sample was evaporated in a weighed dish, and then ignited at high temperature for measurement of the volatile solids content. A sample volume that will yield a residue between 10 and 200 mg is sufficient for the analysis.

Apparatus

- a. Crucible dishes
- b. Drying oven, for operation at 103 to 105 °C
- c. Desiccator, provided with a desiccant containing a colour indicator of moisture concentration

- d. Muffle furnace, for operation at 500 ± 50 °C
- e. Analytical balance

Procedure

- a. 10 ml of sample was taken from each reactor, dispensed to the crucible dish, evaporated in the drying oven to a constant weight and kept in the desiccator.
- b. Dry weight for the dried residue and dish (A) was taken before igniting in the muffle furnace for two hours and cooling down in the desiccator
- c. Weight for the ignited residue and dish was measured (B) to a constant reading
- d. Calculation: mg volatile solid $L^{-1} = (A B) \cdot 1000$ sample volume (L)

3.7.4 Suspended solid analysis

Suspended solid analysis of the effluent was carried out to determine the amount of biomass being wasted during the process. The effluent was filtered through a standard glass-fibre filter.

Apparatus

- a. Standard glass-fibre filter (GF/C Whatman filter paper)
- b. Crucible dishes
- c. Drying oven, for operation at 103 to 105 °C
- d. Desiccator, provided with a desiccant containing a colour indicator of moisture concentration.
- e. Muffle furnace, for operation at 500 ± 50 °C
- f. Analytical balance

Procedure

- a. 50 ml of sample was taken from the effluent, dispensed through a glass-fibre filter connected to a vacuum pump to speed up the process. The filter paper was placed in the crucible dishes, evaporated in the drying oven to a constant weight, and kept in the desiccator
- b. Dry weight for the dried filter paper and dish (A) was taken before igniting in the muffle furnace for two hours and cooling down in the desiccator

- c. Weight for the ignited filter paper and dish was measured (B) to a constant reading
- d. Calculation: mg suspended volatile solid. $L^{-1} = (A B) \cdot 1000$ sample volume (L)

3.7.5 Biogas analysis

Beside using the infrared spectrum gas analayser (GA94A, Geotechnical Instruments UK Ltd), the composition of biogas was also analysed by a gas chromatograph (Cathometer head space analyser, Fisons Scientific, UK) with a dual column, one for O_2 and N_2 and another one for CH_4 and CO_2 . The column was injected with samples of collected biogas. The composition was calculated according to the standard peak provided by a standard gas with a 65:35 ratio of CO_2 :CH₄.

3.7.6 Volatile fatty acid analysis

Volatile fatty acids (VFA) were determined using a gas chromatograph (GC) with a 15 m free fatty acid phase (FFAP) column packed with Chrom G (80-100 mesh), together with a flame ionisation detector (FID). Oven temperature was programmed from 145 °C to 220 °C at a rate of 8 °C min⁻¹. Nitrogen was used as the carrier gas at a flow rate of 30 cm³.min⁻¹. The samples were initially centrifuged at a speed of 20 000 rpm, acidified with 10% (v/v) formic acid, and injected into the instrument. Composition of the VFA profiles was calculated according to the standard peaks provided by a known sample containing eight profiles of VFA from C2-C8.

CHAPTER IV RESULTS

4.0 General introduction

This chapter includes two major sections of experimental results for the anaerobic digestion of a readily degradable wastewater and leachate. All of the experimental work was conducted in triplicate in CSTR reactors, except for the final work involving treatment of leachate by a single anaerobic filter (AF). As well as using reactors in triplicate, samples were also taken in triplicates to reduce the source of error. In general, all of the data obtained showed a high reliability with most showing less than 10% probability of error by statistical calculation of standard deviation. The few data sets outside this limit showed a probability of error between 10 - 20%. This data was not omitted as it was strongly related to a further data set used to verify data replication.

The design of experiments had to be on the basis of trial and error as the experimental programme unravelled, this was because it was impossible to predict with any certainty how conditions within the reactor would respond to experimental variables. Some of the experiments did not work because of lack of control and these failures are not all reported, but they led to adopting precautions that led to repeatable and more reliable experiments in the future. For example, maintaining pH at an optimum level was a major problem at the beginning of the work, but by trial and error, a solution to this was found. A step by step approach was also taken so as to develop experimental techniques and plant operating conditions, first using a simple substrate before moving on to the more complex leachate. This was done in order to become familiar with the anaerobic digestion processes and some of the pitfalls in its operation.

4.1 Readily degradable wastewater

A readily degradable wastewater was prepared by diluting a concentrated fruit cordial (VIMTO). In its undiluted state, this had a COD of approximately 750,000 mg.l⁻¹. This substrate was chosen as it had previously been shown to be readily digestable without any further nutrient additions, it was also useful as the substrate strength could be manipulated to a predefined strength. Analysis of the substrate using gas chromatography

showed no detectable VFA in diluted samples and it was free of suspended solids. The only drawback of this substrate was due to the natural acidity of the fruit concentrate, when diluted it had a pH less than pH 4.0 with no measurable alkalinity.

4.2 Batch operation for treatment of the simulated wastewater

Before starting the batch operation, a rapid acclimation stage was introduced for the seed-sludge in the reactor. This was achieved by incrementing the substrate strength from 5000 to 20,000 mg.1⁻¹ of COD, whilst maintaining the volumetric loading rate at $0.1 \ 1.d^{-1}$ until a working volume of 4l was reached from a start volume of 2.5 l. During acclimatisation, the anaerobic reactor responded well, the COD of the wastewater was converted to CH₄ with a conversion factor of between 0.30 to 0.38 10^3 m³CH₄. kg⁻¹ COD removed. The COD concentration in the reactor following each loading reached an almost consistent value in the range 550 - 750 mg.1⁻¹. All measurements indicated that a rapid acclimatisation could be achieved.

After acclimatisation, the substrate strength was adjusted to a COD of 4700 mg. I^{-1} which is typical of a low strength wastewater. Initial batch operation was started using a feed volume (FV) of 0.5 1 which gave a COD mass loading of 2350 mg COD for each feed addition. This was roughly equal to the final substrate mass loading used during the acclimatisation stage. Incremental feed volumes of 0.8 l, 1.0 l and 1.33 l were introduced as the experiment progressed, but these were all at the same substrate strength and the feed cycle time was maintained at 72 hours.

Reactor performance was measured by COD mass removal and CH₄ production. Tables 4.1 - 4.4 show the COD concentration and COD mass removal at time intervals of 1, 12, 24, 48, 72 hours for each series of feed volumes (FV's). No measurement was taken at 0 hours except the initial COD content. COD mass at time-*i* (COD*t_i*) in the reactor was calculated by multiplying the COD concentration (*St_i*) by the working volume (*Vt_i*). Total COD removal was calculated using Equation 4.1. Initial COD load (COD*t₀*) was derived using equation 4.2 rather than by direct COD analysis, while COD*t_i* was calculated using Equation 4.3. Data was derived by analysis of COD on both feed substrate and reactor contents before the batch loading took place.

COD mass removal at time $i = [CODt_0] - [CODt_i]$ (Equation 4.1) $CODt_0 = S_{FV}.FV_i + S_{t0}.V_{t0}$ (Equation 4.2)

 FV_i = feed volume (1)

 S_{FV} = feed concentration (mg.l⁻¹)

 $S_{t\theta}$ = COD content in the reactor before batch loading (mg.l⁻¹)

 V_{t0} = reactor working volume before batch loading (1)

 $CODt_i$

(Equation 4.3)

Where:

 $St_i = \text{COD content at time-}i \text{ (mg.l}^{-1}\text{)}$

 V_{t_i} = reactor working volume at time-*i* (l)

 $= St_i Vt_i$

The data in Tables 4.1 - 4.4 are plotted in Figs. 4.1 - 4.8 using the average value for each parameter measured against the corresponding time. Figure 4.1 shows that the COD content in the reactor decayed exponentially after each feed volume addition. However, at the highest feed volume (FV 4 = 1.33 l), the COD content (Figure 4.1) remained high at the end of the batch time cycle. Figure 4.2 shows the total COD mass removed during each 72 hour of batch cycle for all four feed volumes. Total COD mass removal increased with the increment of feed volume with the exception of FV4.

 Table 4.1 Results from the batch operation of anaerobic CSTR at a feed volume of 0.5 l (FV 1) using a low medium-strength readily degradable wastewater.

Time (hour)	COD content (mg.l ⁻¹)	COD removed (mg)	Cumulative CH ₄ (ml)	CH₄ yield	pН	Alkalinity (mg.l ⁻¹ CaCO ₃)	Biomass (mg.l ⁻¹)
0	820 <u>+</u> 80	0	0	0			
1	770 <u>+</u> 80	230 <u>+</u> 20	110 <u>+</u> 5	0.46 <u>+</u> .06	7.4 <u>+</u> .1	2310 <u>+</u> 20	13510 <u>+</u> 370
12	460 <u>+</u> 60	1450 ± 110	590 <u>+</u> 60	0.41 <u>+</u> .04	7.3	2030 <u>+</u> 30	13120 <u>+</u> 530
24	370 <u>+</u> 40	1810 <u>+</u> 240	730 <u>+</u> 40	0.41 <u>+</u> .04	7.5 <u>+</u> .1	2340 <u>+</u> 90	13580 <u>+</u> 700
48	330 <u>+</u> 20	2000 <u>+</u> 280	880 <u>+</u> 110	0.44 <u>+</u> .04	7.5 <u>+</u> .1	2310 <u>+</u> 40	13720 <u>+</u> 190
72	220 <u>+</u> 60	2440 <u>+</u> 220	940 <u>+</u> 120	0.39 <u>+</u> .05	7.5 <u>+</u> .1	2470 <u>+</u> 200	13580 <u>+</u> 120

Time (hour)	COD content (mg.l ⁻¹)	COD removed (mg)	Cumulative CH ₄ (ml)	CH₄ yield	pH	Alkalinity (mg.l ⁻¹ CaCO ₃)	Biomass (mg.l ⁻¹)
0	1330 <u>+</u> 40	0	0	0			
1	1320 <u>+</u> 40	50	20	0.34 <u>+</u> .03	7.4	2160 <u>+</u> 120	13800 <u>+</u> 390
12	690 <u>+</u> 100	2560 <u>+</u> 270	320 <u>+</u> 30	0.13 <u>+</u> .02	7.2 <u>+</u> .1	1970 <u>+</u> 100	13130 <u>+</u> 60
24	610 <u>+</u> 120	2900 <u>+</u> 350	700 <u>+</u> 50	0.25 <u>+</u> .05	7.1 <u>+</u> .1	2050 <u>+</u> 190	12700 <u>+</u> 720
48	420 <u>+</u> 50	3670 <u>+</u> 40	1110 <u>+</u> 60	0.30 <u>+</u> .02	7.2 <u>+</u> .1	2260 <u>+</u> 100	12850 <u>+</u> 250
72	320 <u>+</u> 30	4060 ± 250	1240 ±70	0.30 <u>+</u> .01	7.2	2410 <u>+</u> 260	13120 <u>+</u> 430

Table 4.2 Results from the batch operation of anaerobic CSTR at a feed volume of 0.751 (FV 2) using a low medium-strength readily degradable wastewater.

 Table 4.3 Results from the batch operation of anaerobic CSTR at a feed volume of 1.01 (FV 3) using a low medium-strength readily degradable wastewater.

Time (hour)	COD content (mg.l ⁻¹)	COD removed (mg)	Cumulative CH ₄ (ml)	CH₄ yield	pН	Alkalinity (mg.l ⁻¹ CaCO ₃)	Biomass (mg.l ⁻¹)
0	1400 <u>+</u> 20	0	0	0			
1	1370 <u>+</u> 30	130 <u>+</u> 70	20 <u>+</u> 5	0.26 <u>+</u> .04	7.4	1880 <u>+</u> 30	13160 <u>+</u> 440
12	1000 <u>+</u> 30	1630 <u>+</u> 460	450 <u>+</u> 60	0.28 <u>+</u> .06	7.4 <u>+</u> .2	1690 <u>+</u> 150	12040 <u>+</u> 630
24	850 <u>+</u> 40	2240 <u>+</u> 200	850 <u>+</u> 120	0.38 <u>+</u> .06	7.4 <u>+</u> .1	1670 <u>+</u> 140	12060 <u>+</u> 920
48	370 <u>+</u> 60	4160 <u>+</u> 260	1330 <u>+</u> 100	0.32 <u>+</u> .04	7.2 <u>+</u> .1	1730 <u>+</u> 80	12200 <u>+</u> 550
72	290 <u>+</u> 90	4480 <u>+</u> 400	1560 <u>+</u> 100	0.35 <u>+</u> .05	7.1 <u>+</u> .1	1840 <u>+</u> 30	12220 <u>+</u> 60

 Table 4.4 Results from the batch operation of anaerobic CSTR at a feed volume of 1.33 l (FV 4) using a low medium-strength readily degradable wastewater.

Time (hour)	COD content (mg.l ⁻¹)	COD removed (mg)	Cumulative CH ₄ (ml)	CH₄ yield	pH	Alkalinity (mg.l ⁻¹ CaCO ₃)	Biomass (mg.l ⁻¹)
0	1690 ± 10	0	0	0			
1	1670 <u>+</u> 10	100	20 <u>+</u> 5	0.21 <u>+</u> .02	6.8 <u>+</u> .2	1270 <u>+</u> 60	9220 <u>+</u> 290
12	1150 <u>+</u> 30	2170 ± 150	660 <u>+</u> 100	0.30 <u>+</u> .06	5.8 <u>+</u> .1	950	8960 <u>+</u> 530
24	1110 <u>+</u> 50	$\begin{array}{r} 2370 \\ 200 \end{array} \pm$	710 <u>+</u> 110	0.25 <u>+</u> .04	6.3 <u>+</u> .2	1100	8620 <u>+</u> 950
48	950 <u>+</u> 110	$\begin{array}{r} 3000 \pm \\ 380 \end{array}$	730 <u>+</u> 110	0.25 <u>+</u> .03	6.1 <u>+</u> .2	1030 <u>+</u> 130	6340 <u>+</u> 650
72	890 <u>+</u> 70	$\begin{array}{r} 3260 \\ 240 \end{array} \pm$	770 <u>+</u> 140	0.24 <u>+</u> .03	6.0 <u>+</u> .1	840 <u>+</u> 230	7330 <u>+</u> 660



Figure 4.1 COD content during the 72 hours of batch operation for each feed volume



Figure 4.2 COD mass removal during the 72 hours of batch operation for each feed volume



Figure 4.3 Cumulative CH₄ production during the 72 hours of batch operation for each feed volume

Figure 4.3 shows the cumulative CH_4 production curve which mirrors that of COD mass removal in Figure 4.2. Production of CH_4 increased with each increment of feed volume, except at FV4. In this case, CH_4 production ceased after 12 hours of batch operation. When compared to COD mass removal during the same period, the result indicates that methanogenesis was probably impeded, this would explain the low CH_4

yield for FV4 in Figure 4.4. Overall CH₄ yield was not consistent during the batch operation, which was probably due to incomplete absorption of the CO₂ in the biogas by the alkaline solution. This inconsistency led to the use of a gas analyser to determine the ratio of CO₂:CH₄ in biogas in subsequent experiments. However, the final CH₄ yield in the experiment was in the range 0.30 to 0.39 10^3 m³CH₄. kg⁻¹ COD removed, which is within the acceptable range considering a theoretical value of 0.35 10^3 m³CH₄. kg⁻¹ COD removed.



Figure 4.4 CH₄ yield during the 72 hours of batch operation for each feed volume



Figure 4.5 pH value during the 72 hours of batch operation for each feed volume

Low pH was the major factor which contributed to the failure of anaerobic operation at the highest COD mass load (FV4). The recommended pH range for an anaerobic methanogenic reactor is between pH 6.5 - 7.5 for optimum performance; at FV4 the pH dropped below this optimum as shown in Figure 4.5. Analysis showed that the pH dropped because of a low alkalinity in the reactor. Buffering capacity was reduced following each feed volume (FV) addition as shown in Figure 4.6 indicating that each increment of FV had reduced the alkalinity level. Maintaining alkalinity at a higher



Figure 4.6 Alkanity during the 72 hours of batch operation for each feed volume



Figure 4.7 Biomass content during the 72 hours of batch operation for each feed volume

level would have helped to reduce the acidifying effect of VFA during the early stages of acidification. This can be observed during the first 12 hours of batch operation where there is a noticeable drop in pH and alkalinity during this time. The reactors recovered from this in those receiving a lower mass load (FV 1-3) where a rise in pH was observed. However, at FV4 all the reactors turned "sour" and ceased producing CH₄ which indicates that the methanogenic activity was retarded by either low pH or VFA toxicity. The susceptibility of the reactors to a fall in pH suggests that the use of supplemental pH control, or the use of chemicals to increase the buffering capacity would be an advantage for the anaerobic treatment of this substrate. Figure 4.7 indicates that in a soured reactor the microbial population in the reactor could not be maintained, as the biomass content decreased rapidly.

4.2.1 Modelling the kinetics of batch operation

Figures 4.2 and 4.3 both clearly show a non-linear relationship between COD removal and CH₄ production as a function of time. Additional data points relating to methane gas production at each of the feed volumes are shown in Table 4.5. These were used to derive the kinetic equations, as the gas production curve is more accurately represented by the increased number of data points. A plot of the data is shown in Figure 4.8. To determine the relationship that exists, a linearised form of Equation 4.4 is representative of a general equation for that function. The variable Y represents the dependent variable, which, in this case, is the CH4 production value, while variable T represents the independent variable, which is the time value. A transformation of the function, which exists for the data set of cumulative CH4 from the experiment, is required.

 Table 4.5 Experimental results for CH4 production during 72 hours of batch operation in an anaerobic CSTR using a low strength readily degradable wastewater.

Time (hour)	FV 1 (0.5L)	FV 2 (0.8 L)	FV 3 (1.0 L)	FV 4 (1.33 L)
1	110 <u>+</u> 5	20 <u>+</u> 5	20 <u>+</u> 10	20 <u>+</u> 5
2	160 ± 5	30 <u>+</u> 10	60 <u>+</u> 10	30 <u>+</u> 10
3	200 <u>+</u> 5	50 <u>+</u> 20	80 <u>+</u> 5	50 <u>+</u> 5
5	290	70 <u>+</u> 20	100 <u>+</u> 10	160 <u>+</u> 20
6	350 <u>+</u> 5	80 <u>+</u> 25	140 <u>+</u> 20	350 <u>+</u> 30
7	420	110 ± 20	180 <u>+</u> 20	370 <u>+</u> 50
11	570 <u>+</u> 50	250 <u>+</u> 20	360 <u>+</u> 50	640 <u>+</u> 100
12	590 <u>+</u> 60	320 <u>+</u> 30	450 <u>+</u> 60	660 <u>+</u> 100
23	740 <u>+</u> 70	680 <u>+</u> 50	820 <u>+</u> 120	700 <u>+</u> 100
24	750 <u>+</u> 60	700 <u>+</u> 50	850 <u>+</u> 110	710 <u>+</u> 110
28	820 <u>+</u> 100	770 <u>+</u> 50	960 <u>+</u> 80	720 <u>+</u> 110
48	880 <u>+</u> 110	1110 <u>+</u> 60	1330 <u>+</u> 100	730 <u>+</u> 110
72	940 <u>+</u> 120	1240 ± 70	1560 <u>+</u> 100	770 <u>+</u> 140

$$Y = b_0 + b_1 T_1$$

(Equation 4.4)

Y = dependent variable

 b_0 = general coefficient for cut-off value

 b_1 = general coefficient for slope value

T = independent variable

A graphic representation as used by Daniel and Wood (1979) was used to identify the type of function that existed in a graph plot of dependent variable against independent variable. The results suggest that the plot in Figure 4.8 is a specific function as shown in Equation 4.5, rather than a power or exponential function. So as to maintain the same nomenclature the symbols representing variables y and α , as described by Daniel and Wood (1979), were changed to G and G_m , respectively. These changes are shown in Equation 4.6, and in the linear form, Equation 4.4 is transcribed as Equation 4.7. The coefficient k is the slope of the line, while the coefficient G_m is the maximum G value taken from the experimental data. To determine these coefficients, $\ln (G/G_m)$ was plotted against l/t as shown in Figure 4.9; the transformation values for this plot are listed in Table 4.6.

$$y = \alpha^{k/t}$$

y = dependent variable $\alpha =$ maximum y value (Equation 4.5)

1800 1600 ⁴ production (ml) 000 0001 (ml) 000 0001 (ml) D FV1 X & FV2 CH4 ∆ FV3 400 α x FV4 12 24 36 48 60 72 Time (hour)





Figure 4.9 Linear estimation of the specific function ($G = G_m^{k/t}$) by straight line fitting for the plot of $\ln (G/G_m)$ against 1/time

Transformation of data in Table 4.5 to obtain a linear function $G = G_m^{k/t}$ for hourly Table 4.6 CH_4 production. The data is subsequently used to calculate coefficient k

1/time	$\frac{\ln (G/G_m)}{FV 1}$	$\frac{\ln (G/G_m)}{FV 2}$	$ \frac{\ln (G/G_m)}{FV 3} $	$\frac{\ln (G/G_m)}{FV 4}$
1	- 2.145	- 4.127	- 4.357	- 3.651
0.500	- 1.771	- 3.722	- 3.258	- 3.245
0.333	- 1.548	- 3.211	- 2.970	- 2.734
0.200	- 1.176	- 2.874	- 2.747	- 1.571
0.167	- 0.988	- 2.741	- 2.411	- 0.788
0.143	- 0.806	- 2.422	- 2.160	- 0.732
0.091	- 0.500	- 1.601	- 1.466	- 0.185
0.083	- 0.466	- 1.355	- 1.243	- 0.154
0.043	- 0.239	- 0.601	- 0.643	- 0.095
0.042	- 0.226	- 0.572	- 0.607	- 0.081
0.036	- 0.137	- 0.476	- 0.486	- 0.067
0.021	- 0.066	- 0.111	- 0.160	- 0.053
0.014	0	0	0	0

 $G = G_m^{k/t}$

(Equation 4.6)

 $ln G = ln G_m + k/t$

(Equation 4.7)

- G = cumulative CH₄ production G_m = maximum CH₄ k = slope coefficient in linear equation
- t = time

FV	Linear equation	Inverse transformation
0.5 L	$\ln G = \ln 940 - 2.8/t$	$G = 940^{-2.8/t}$
0.8 L	$\ln G = \ln 1240 - 6.0/t$	$G = 1240^{-6/t}$
1.0 L	$\ln G = \ln 1560-5.8/t$	$G = 1560^{-5.8/t}$
1.33 L	$\ln G = \ln 770 - 4.6/t$	$G = 770^{-4.6/t}$

Table 4.7 Linear equations representing the specific function $G = G_m^{k/t}$ using fixed values of G_m for each feed volume (FV)

The linear regression lines as shown in Figure 4.9 do not pass through all the data points, but it was still possible to make an estimate of the coefficient k using the spreadsheet function. The linear equation for each of the experimental feed volumes (FV's) is listed in Table 4.7. The specific function, which is the inverse of the linearised equation, gives the actual specific function, which is also listed in Table 4.7. Production of CH₄ (*G*) can be estimated by incorporating any *t*-value into the equation; these have been calculated and the values shown in Table 4.8 and plotted in Figure 4.10. The shape of the derived graph in Fig 4.10 is similar to that in Figure 4.8, which shows experimentally derived values, indicating the kinetics of CH₄ production from a batch feed can be represented by the specific function in Equation 4.6.

Time (hour)	FV 1 (ml)	FV 2 (ml)	FV 3 (ml)	FV 4 (ml)
1	57	3	5	8
2	232	62	86	77
3	370	168	226	166
5	537	373	489	307
6	589	456	593	358
7	630	526	681	399
11	729	719	921	507
12	744	752	962	525
23	832	955	1212	630
24	836	966	1225	636
28	851	1001	1268	653
48	887	1094	1382	700
72	904	1141	1439	722

Table 4.8 Estimation of hourly CH₄ production with the specific function $G = G_m^{k/t}$ with experimentally derived G_m values



Figure 4.10 Estimation of CH_4 production by the specific function using experimental G_m values

The G_m value is not always represented by the experimental maximum G value at the end of a batch feed cycle and reference to Figure 4.8 would indicate that actual G_m values tend to be higher. Ideally, the value taken for an independent variable, which represents a time dependent maximum, should be determined at time value of infinity. For practical reasons this is not possible, but by using a plot of Equation 4.7 the G_m value can be obtained from the intercept of the line in a plot of ln G against 1/time. It is therefore possible to refine the estimation of the coefficient k in Equation 4.6 by deriving the value of G_m as described above. Table 4.9 shows the transformed values of ln Gagainst 1/time, which are then plotted in Figure 4.11.

1/time	ln (G) FV 1	ln (<i>G</i>) FV 2	ln (<i>G</i>) FV 3	ln (G) FV 4
1	4.700	2.996	2.996	2.996
0.500	5.075	3.401	4.094	3.401
0.333	5.298	3.912	4.382	3.912
0.200	4.248	4.248	4.605	5.075
0.167	4.382	4.382	4.942	5.858
0.143	4.700	4.700	5.193	5.914
0.091	5.521	5.521	5.886	6.461
0.083	5.768	5.768	6.109	6.492
0.043	6.522	6.522	6.709	6.551
0.042	6.551	6.551	6.745	6.565
0.036	6.646	6.646	6.867	6.579
0.021	7.012	7.012	7.193	6.593
0.014	7.123	7.123	7.352	6.646

Table 4.9 Transformation of data to plot the specific function $G = G_m^{k/t}$ from hourly CH₄ production data so as to calculate G_m and k coefficients


Figure 4.11 Linear estimation of the specific function $(G = G_m^{kt})$ using a straight line fit for the data plotted as *ln G* against 1/time

There is an omission of some data points in Figure 4.11 so as to give the best straight line fit of the graph to the majority of the points. The intercept and slope were derived from the graph and used as the coefficient G_m and k, respectively. The complete set of linear equations and inverse transformations of the equation are given in Table 4.10. From these equations, a revised set of hourly CH₄ production can be estimated, these are given in Table 4.11 and shown in Figure 4.12. The graph shows that the derived curves for CH₄ production are similar to the experimental data, which is shown in Figure 4.8. However, using both experimental and derived values of G_m for the specific function gave lower values of CH₄ production at the beginning of the batch operation.

FV	Linear equation	Inverse transformation
1: 0.5 1	$\ln G = \ln 1000 - 6.21/t$	$G = 1000^{-6.2/t}$
2: 0.8 1	$\ln G = \ln 1380 - 16.21/t$	$G = 1380^{-16.2/t}$
3: 1.0 1	$\ln G = \ln 1640 - 14.81/t$	$G = 1640^{-14.8/t}$
4: 1.33 1	$\ln G = \ln 980 - 7.23/t$	$G = 980^{-7.2/t}$

Table 4.10 Linear equation and inverse transformation for the specific function $G = G_m^{k/t}$ with calculated G_m values for each FV

Time (hour)	FV 1 (ml)	FV 2 (ml)	FV 3 (ml)	FV 4 (ml)
1	2	0.0001	0.0006	1
2	45	0.36	1	26
3	126	6	12	88
5	289	51	85	231
6	355	88	139	294
7	412	130	198	349
11	569	308	427	508
12	596	349	477	536
23	763	673	861	716
24	772	694	885	725
28	801	765	966	757
48	879	978	1205	843
72	917	1097	1335	886

Table 4.11 Estimation of hourly CH₄ production using the specific function $G = G_m^{k/t}$ with calculated G_m values



Figure 4.12 Estimation of CH₄ production by the specific function with calculated Gm values

4.2.2 Study of residuals

A statistical method was needed in the study that would allow graphical data generated by an equation to be compared with that produced experimentally. In reality this means finding a method of quantifying how similar the shapes of curves are. The method chosen is a simple technique that in fact compares the position of each point on the data curve by taking the square of the difference between that point (y) and the same temporal point on the curve calculated from the model (Y). The value obtained is called the residual and is representative of the deviation between the two points, the higher the

residual the less similar are the two points in terms of the determinant - in our case methane. The sum of the residuals gives a single value, but more importantly a plot of the residuals will show at which points the curve deviate from each other most significantly. The technique was used to compare the graphs of gas production from a batch culture over time with the calculated gas yield determine using the specific function. The data sets, with their residuals, were then compared to evaluate which equation was most accurate in estimating CH₄ production during batch operation. The method adopted is also used by Daniel and Wood (1979) in their work. This method is sometimes also known as 'remainders, discrepancies, and deviations'. Figure 4.13 and Figure 4.14 show the scattered data plots for the residuals estimated for CH₄ production by the specific function using experimental G_m values and calculated G_m values, respectively. Comparing both figures, it is clear that using calculated G_m for the specific function is more accurate as the residuals were less obvious. Experimental G_m values gave a higher residuals value at all feed volumes (FV's) except for an FV of 1.33 l. While residuals which used the calculated values of G_m generally gave a value of less than 150 units, except at FV3 (1.0 l), where it had a discrepancy up to 250 units at the end of the cycle.



Figure 4.13 Distribution of residuals against observed data for the specific function $G = G_m^{k/t}$ using experimental G_m values



Figure 4.14 Distribution of residuals against observed data for the specific function $G = G_m^{k/t}$ using calculated G_m values

As a further check on the accuracy of using the specific function with calculated values of G_m to estimate cumulative CH₄ production, a multiple correlation coefficient squared (R^2) was calculated; this is sometimes known as the coefficient of correlation (Daniel and Wood, 1979). The calculation of R^2 is derived from the total of sum of squares and the total reduction in total sum of squares due to the calculated equation which in listed in Equation 4.8.

$$R^2 = 1 - [rr] / [yy]$$

(Equation 4.8)

$$R^2$$
 = coefficient of determination

$$[rr] = \Sigma (y_i - Y_i)^2$$

= residual sum of squares a measure of the squared scatter of the observed data and the estimated data

 $[yy] = \Sigma (y_i - mean \underline{y})^2$ = total sum of squares

Table 4.12 gives the results from the statistical analysis in which CH₄ production was estimated by the specific function with calculated G_m values. The values included in this table, for each of the feed volumes (FV's), are considered to give an accurate representation of gas production since more than 90% of the estimated data (Y) are acceptable. It might be concluded that an accurate determination of G_m in the specific function of $G = G_m^{k/t}$ is crucial if accurate modelling of gas production in a batch reactor is to be undertaken..

Variable	FV 1	FV 2	FV 3	FV 4
[<i>rr</i>]	32413	46069	83549	71309
[<i>yy</i>]	996325	2239032	3250572	1085525
R^2	0.97	0.98	0.97	0.93

Table 4.12 Statistical properties of residuals for estimating $G = G_m^{k/t}$ with calculated G_m values

4.3 Fed-batch operation for the treatment of a simulated wastewater

Fed-batch operation is also known as "pseudo" steady-state operation. The substrate is given daily and as such a hydraulic retention time (HRT) can be calculated using Equation 4.9. This can also be regulated by changing the daily hydraulic flow rate (Q) of the influent and effluent.

HRT		V/Q	(Equation 4.9)
HRT	=	hydraulic retention time (d)	
V		working volume (l)	
Q	=	hydraulic flow rate (l.d ⁻¹)	

The following experiment was undertaken after a new seed sludge had been introduced into reactors. The method of acclimatisation followed the same procedure as had previously been used (section 4.2) until the working volume (V) of 4 l was reached. At this point 0.25 l of a low strength substrate was added daily until a volume of 5 l was reached. Reduction in the head space volume of the reactors was introduced during this experiment to minimise the volume that would be replaced with atmospheric air during the daily feeding process. By doing this, it was thought that the biogas composition would be more consistent and rapidly displaced by fresh biogas production, thus influencing the reactor contents less by minimising exposure to atmospheric gases.

4.3.1 pH and alkalinity control

As a preliminary experiment, a test for ammonia toxicity to the seed sludge was undertaken. This utilized a portion of seed sludge withdrawn immediately after acclimatisation, and before starting on the daily feeding regime. The test was undertaken as a control to assess the effect of ammonium bicarbonate (NH_4HCO_3) additions which it

was planned to use should be use to overcome the problem of pH fluctuations which had led to biomass loss in the previous batch experiment (section 4.2). The HCO₃⁻ would increase alkalinity whilst the NH₄⁺ would supplement nitrogen within the growth medium as it was thought this was possibly limiting to growth in the previous experiments. However, because of the potential for ammonia to be toxic to anaerobic systems the critical concentration to the acclimated biomass had to be assessed using a toxicity test. The results of this test are given in Table 4.13 and biogas production is plotted as a function of time in Figure 4.15. The test showed no significant impact at NH₄HCO₃ concentrations in the range 10 - 40 mg.l⁻¹ NH₄HCO₃, although maximum biogas production was observed at the concentration of 10 mg.l⁻¹. In all subsequent batch feed experiments 50 mg of NH₄HCO₃ was added daily, giving a reactor concentration of 10mg.l⁻¹ in the 51 working volume. Sodium bicarbonate (NaHCO₃) was also used to increase pH during operation but always using the minimal amount necessary to maintain the operating pH at 6.5. No more than 2000 mg.d⁻¹ NaHCO₃, giving a reactor concentration not more than 400 mg.l⁻¹ of NaHCO₃.

 Table 4.13
 Production of biogas (ml) at different time intervals during the toxicity testing using ammonium bicarbonate at various concentration

Time		Ammonium concentration						
(min)	0 mg/l	10 mg/l	20 mg/l	30 mg/l	40 mg/l			
0	0	0	0	0	0			
15	1.6	1.7	1.7	1.4	1.4			
30	2	2.2	2.3	1.8	1.8			
45	2.3	2.6	2.6	2.1	2.2			
60	2.6	3	2.9	2.5	2.6			
75	3	3.5	3.3	2.8	2.9			
90	3.5	3.8	3.7	3.1	3.2			
105	3.9	4.1	4	3.4	3.6			
120	4.2	4.5	4.2	3.9	4			
135	4.5	4.7	4.5	4.1	4.2			
150	4.8	5	4.8	4.4	4.5			
165	5	5.2	5	4.6	4.7			
180	5.3	5.4	5.2	4.8	4.8			



Figure 4.15 Ammonium bicarbonate toxicity test at various concentration based on biogas against time

4.3.2 Operation performance

The low-strength substrate used had a COD in the range 4000 - 5000 mg.l⁻¹. The feeding regime started at a HRT of 20d, once steady state had been achieved at this retention time then the feed volume was sequentially increased to reduce HRT by 2days at each step. Sufficient time was maintained at each feed rate and HRT to reach a 'psuedo' steady state. The experiment was designed so that the feed addition (Q) would increase exponentially to allow the reduction of HRT. With V equal to 51, the values calculated for Q are listed in Table 4.14 and the exponential relationship is shown in Figure 4.16.

Table 4.14Relationship between flow rate (Q) and hydraulic retention time (HRT) for a fed-batch
operation with a working volume of 51

Q (l.d ⁻¹)	HRT (d)		
0.25	20		
0.28	18		
0.31	16		
0.36	14		
0.42	12		
0.5	10		
0.62	8		
0.83	6		
1.25	4		



Figure 4.16 Exponential relationship between the feed rate (Q) and HRT for the fed-batch operation using a working volume of 5l

Table 4.15 lists the averaged results for the fed-batch operation. Maintaining reactor pH at 6.5 by bicarbonate buffer addition led to successful operation. Effluent pH was consistently in the range of 6.5-6.8 at all HRTs , and effluent alkalinity remained in the range of 500-600 mgl⁻¹ CaCO₃ except at a HRT of 4d. A slight increment of alkalinity level was observed at this point which was attributed to the addition of 2000 mg.d⁻¹ NaHCO₃ so as to maintain an optimum pH level. An addition of 1000 mg.d⁻¹, equivalent to 200mg.l⁻¹.d⁻¹ for *V* equal to 5l, was normally sufficient to maintain the pH at 6.5. Alkalinity results from in the previous batch experiment (section 4.2) appear much higher than those reported here, this is due to previous tests having been carried out on the reactor mixed liquor whereas in this case they pertain to the settled effluent from the fed-batch operation. Alkalinity remained within the acceptable range (IWPC, 1979) for conventional digestion.

HRT (d)	S _o (mg.l ⁻¹)	$\begin{array}{c} S_e \\ (\text{mg.l}^{-1}) \end{array}$	pH	Alkalinity (mg.l ⁻¹)	CH ₄ : CO ₂	CH ₄ (ml.d ⁻¹)	Biomass (mg.l ⁻¹)	Wastage (mg.l ⁻¹)
20	4620 <u>+</u> 200	250 <u>+</u> 30	6.8 <u>+</u> 0.1	620 <u>+</u> 50	80:20	390 <u>+</u> 20	8290 <u>+</u> 430	650 <u>+</u> 40
18	4650 <u>+</u> 250	220 <u>+</u> 50	6.8 <u>+</u> 0.1	600 <u>+</u> 40	80:20	430 <u>+</u> 40	8520 <u>+</u> 400	610 <u>+</u> 40
16	4530 <u>+</u> 440	280 <u>+</u> 50	6.6 <u>+</u> 0.1	510 <u>+</u> 50	80:20	430 <u>+</u> 20	8520 <u>+</u> 380	360 <u>+</u> 50
14	4600 <u>+</u> 360	290 <u>+</u> 50	6.7 <u>+</u> 0.1	510 <u>+</u> 30	80:20	520 <u>+</u> 30	8320 <u>+</u> 340	310 <u>+</u> 40
12	4620 <u>+</u> 380	280 <u>+</u> 30	6.6 <u>+</u> 0.1	570 <u>+</u> 30	80:20	570 <u>+</u> 20	8290 <u>+</u> 230	320 <u>+</u> 70
10	4530 <u>+</u> 260	270 <u>+</u> 60	6.6 <u>+</u> 0.1	520 <u>+</u> 30	80:20	690 <u>+</u> 50	7790 <u>+</u> 290	270 <u>+</u> 40
8	4660 <u>+</u> 490	350 <u>+</u> 90	6.6 <u>+</u> 0.1	520 <u>+</u> 50	60:40	800 <u>+</u> 30	7390 <u>+</u> 510	200 <u>+</u> 30
6	4350 <u>+</u> 420	400 <u>+</u> 80	6.5 <u>+</u> 0.1	590 <u>+</u> 20	60:40	1000 <u>+</u> 50	7370 <u>+</u> 310	240 <u>+</u> 130
4	4280 <u>+</u> 310	950 <u>+</u> 200	6.6 <u>+</u> 0.1	820 <u>+</u> 50	60:40	1270 <u>+</u> 120	7110 <u>+</u> 510	230 <u>+</u> 50

 Table 4.15
 Averaged results from the fed-batch operation with increasing feed rate (Q) leading to progressively shorter hydraulic retention times (HRT's)

Figures 4.17 to 4.19 show the trend of measured parameters against HRT. At a consistent influent strength in the range 4300-4700 mg.l⁻¹ COD (Figure 4.17) the effluent strength also remained consistently in the range of 220-300 mg.l⁻¹ until the HRT reached 8 days when it increased to 350 mgl⁻¹. Reducing the HRT to 6 days showed a further increase in effluent strength to 400 mg.l⁻¹ followed by a sharp increment to 950 mg.l⁻¹ when a HRT of 4d was utilised.



Figure 4.17 Influent and effluent strength at each pseudo steady state value of HRT.

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Figure 4.18 CH₄ production at each HRT.

Figure 4.18 shows that CH₄ production increased exponentially with a reduction in HRT. There appears to be a strong correlation to feed rate (Q), which also exponentially increased as shown in Figure 4.16. The data on CH₄ production, as listed in Table 4.15, was determined from volumetric measurement of gas coupled with gas analysis as described in section 3.7.5. The data also shows that the fraction ratio, CH₄ and CO₂ changed at HRT's of 8days and below, with the percentage CH₄ dropping from 80% to 60%.

Figure 4.19 shows the average biomass concentration in the reactor and the settled effluent, which was wasted at each HRT. The feed substrate was free of solid, thus total volatile solids analysis of these two components gave a measure of biomass yield within the reactor. There was an increment in biomass concentration from 7110 mg.l⁻¹ to 8550 mg.l⁻¹ with decreasing values of HRT, it then decreased to 8290 mg.l⁻¹ at a HRT of 20d, indicating a potential kinetic relationship between these two parameters as discussed later (section 4.4.1). The volatile suspended solids in the effluent was observed to increase with HRT, and there was some indication that granulation of the seed-sludge improved with higher feed rates (Q).



Figure 4.19 Biomass concentration in the reactors and the daily effluent biomass concentration

4.4 Modelling the kinetics of fed-batch operation

The experimental results, which are given in Table 4.15, were used to later validate kinetic equations and the parameters derived below are deduced from the primary data:

Removal efficiency = $S_o - S_e$	(Equation 4.10)
$S_o = \text{influent strength (mg.l-1 COD)}$ $S_e = \text{effluent strength}$	
Total COD mass loading = $S_o.Q$ Q = hydraulic flow rate (1.d ⁻¹)	(Equation 4.11)
OLR = Total COD mass loading V	(Equation 4.12)
OLR = organic loading rate V = working volume	
Total mass COD out = $S_{e.}Q$	(Equation 4.13)
Total COD mass removal = total COD mass loading	
- total mass COD out	(Equation 4.14)

CH_4 yield = CH₄ total COD mass removal

(Equation 4.15)

The parameter $S_o - S_e$ (Table 4.16) is not of any significance in itself, it is included in the table of results as it is later used for the determination of substrate utilisation rate (r_s) . COD removal efficiency remained constant at 94% until an operating HRT of 8d was reached after which point it began to drop. As both parameters share similar variables, it is not surprising that similar curves were revealed when they were plotted against HRT in Figure 4.20. Figure 4.21 shows the daily COD load entering and leaving the reactor as based on Equation 4.11 and 4.13. These both show exponential increments with a reduction in HRT, which indicates they are strongly related to the feed rate (Q). It appears that calculation of daily COD removal, on a daily mass basis, would give a similar curve as it involves the same variables as shown in Equation 4.14. Calculation of CH₄ yield, as listed in Equation 4.15, gives almost consistent values, between 0.30 to 0.36 10³m³CH₄.kg⁻¹COD removed at all HRTs. To determine the overall CH₄ yield the slope of the line in a plot of CH₄ production against COD removed at each HRT (Figure 4.22) was determined which gave a value of 0.31 10³m³CH₄.kg ⁻¹COD removed.



Figure 4.20 COD removal as a function of HRT

HRT (d)	$S_o - S_e$ (mg. Γ^1)	Removal efficiency (%)	Total COD loading (mg.d ⁻¹)	OLR (g.m ⁻³ .d ⁻¹)	Total COD out (mg.d ⁻¹)	Total COD removal (mg.d ⁻¹)	CH4 yield
20	4380 +210	94	1160 <u>+</u> 50	230 <u>+</u> 10	60 <u>+</u> 10	1090 <u>+</u> 50	0.36 <u>+</u> .01
18	4430 <u>+</u> 270	95 <u>+</u> 1	1300 <u>+</u> 70	260 <u>+</u> 10	60 <u>+</u> 10	1240 <u>+</u> 80	0.35 <u>+</u> .02
16	4260 <u>+</u> 450	94 <u>+</u> 1	1410 <u>+</u> 140	280 <u>+</u> 30	90 ± 20	1320 <u>+</u> 50	0.33 <u>+</u> .03
14	4310 <u>+</u> 380	94 <u>+</u> 1	1550 <u>+</u> 140	330 <u>+</u> 30	110 <u>+</u> 20	1550 <u>+</u> 140	0.34 <u>+</u> .03
12	4340 <u>+</u> 390	94 <u>+</u> 1	1940 <u>+</u> 160	390 <u>+</u> 30	120 <u>+</u> 20	1820 <u>+</u> 160	0.31 <u>+</u> .03
10	4260 <u>+</u> 270	94 <u>+</u> 1	2270 <u>+</u> 130	450 <u>+</u> 30	140 <u>+</u> 20	2130 <u>+</u> 140	0.33 <u>+</u> .02
8	4310 <u>+</u> 500	92 <u>+</u> 2	2890 <u>+</u> 300	580 <u>+</u> 60	220 <u>+</u> 60	2670 <u>+</u> 310	0.30 <u>+</u> .03
6	3950 <u>+</u> 460	91 <u>+</u> 3	3610 <u>+</u> 350	720 <u>+</u> 70	330 <u>+</u> 70	3280 <u>+</u> 390	0.31 <u>+</u> .03
4	3330 <u>+</u> 360	80 <u>+</u> 3	5350 <u>+</u> 390	1070 ± 80	1190 <u>+</u> 220	4160 <u>+</u> 450	0.31 <u>+</u> .03

Table 4.16 Calculated parameters at pseudo steady state



Figure 4.21 COD load entering and leaving the reactors at each of the pseudo steady state hydraulic retention times



Figure 4.22 Overall CH₄ yield determined from the methane production and COD removal for each of the HRT investigated

4.4.1 Michaelis-Menten relationship

The kinetics of a fed-batch operation can be described by a Michaelis-Menten relationship. To perform this the substrate utilisation rate (r_s) for each HRT has first to be determined using the First Order Model for r_s as listed in Equation 2.19

Mass-balances model:

 $\partial S/\partial t.V = Q.(S_o - S_e) - r_S.V$

where $\partial S / \partial t = 0$ at a steady-state condition

thus $r_S = (S_o - S_e)/HRT$.

The derivation of the simplified Michaelis-Menten kinetic relationship was shown in section 2.3.2.

Simplified Michaelis-Menten relationship:

 $r_S = \frac{r_m \cdot S_e}{K_m + S_e}$: Not a first order or zero order kinetic reaction at intermediate concentration of S_e

 r_m = maximum substrate utilisation rate

 K_m = Michaelis constant

 $r_S = k.S_e$: First order kinetic reaction $k = r_m / K_m$; at low concentration of $S_e << K_m$ $r_S = r_m$: Zero order kinetic reaction

at high concentration of $S_e >> K_m$

Mtz-Viturtia and Mata-Alvarez (1996) suggested a fast kinetic model could be achieved by plotting ($S_o - S_e$) against HRT to determine the r_s . However this kind of approach is not applicable as Figure 4.20 shows that this plot does not take a linear form. Table 4.17 shows the calculated r_s values, which increased with HRT. To determine the Michaelis-Menten relationship, each r_s value is plotted against S_e as shown in Figure 4.23 and it is apparent that neither a first order nor zero order kinetic relationship is shown. To further elucidate the nature of the relationship a Lineweaver-Burk plot was constructed.



Figure 4.23 Michaelis-Menten relationship for the fed-batch operation of a low medium -strength wastewater

The Lineweaver-Burk plot is based on the arrangement of the Michaelis-Menten equation into a linear form (y = mx + c). Inversion of the equation gives Equation 4.16. By cross multiplying the equation and separating the terms give Equation 4.17. The plot $1/r_s$ versus $1/S_e$ would give the slope as Km / r_m and the intercept on the y-axis as $1/r_m$. Inversed values for the transformation are listed in Table 4.17 and plotted in Figure 4.24 and give a linear equation of y = 0.9814x - 0.0004. The information derived from this plot could not determine the kinetic relationship, as the cut off value for r_m was less than zero, where the opposite should be true. Hypothetically, it should have shown a first order kinetic relationship.

$r_m = \frac{r_m}{r_s}$	$= K_m + S_e$ $= S_e$		(Equation 4.16)
$1 = r_s$	$\frac{K_m}{r_m} \cdot \frac{1}{S_e}$	$+\frac{1}{r_m}$	(Equation 4.17)

$$r_{S-} = r_m \cdot S_e$$

$$\underbrace{r_{M-} \cdot S_e}_{X \cdot (K_m + S_e)}$$
(Equation 4.18)

X = biomass concentration

Table 4.17 List of r_s , S_e and the inverted values of both parameters at each HRT

HRT (d)	r_{S} (d ⁻¹)	S_e (mg.l ⁻¹)	1/S _e	1/ <i>rs</i>
20	832	250	0.00105	0.0012
18	658	220	0.00251	0.00152
16	539	280	0.00285	0.00185
14	426	290	0.00365	0.00235
12	361	280	0.00358	0.00277
10	308	270	0.00342	0.00325
8	266	350	0.00362	0.00376
6	246	400	0.00452	0.00406
4	219	950	0.00402	0.00457



Figure 4.24 Lineweaver-Burk plot for the fed-batch operation of a low medium-strength wastewater

Ramalho (1977) incorporated biomass concentration as an additional parameter into Michaelis-Menten relationship when using this to describe the anaerobic treatment of digested sludge; this modification is shown in Equation 4.18. Table 4.18 shows values used in the plot of r_S . X against S_e and Figure 4.25 shows the same kinetic data plot as in Figure 4.23 except for values of the y-axis. By using this manipulation, which takes account of the changes in the biomass content during the experiment, no significant difference in the kinetic coefficients for the operation was found. A Lineweaver-Burk plot using the additional X parameter is shown in Figure 4.26, but again it shows the cutoff value for $1/r_m$ at less than zero; this is similar to Figure 4.24 and again indicates that the inclusion of the X parameter as suggested by Ramalho (1977) has little influence.

HRT	$r_s.X$	Se	$1/r_{S.}X$	$1/S_e$
(d)	$(mg.l^{-1}.d^{-1})$	$(mg.l^{-1})$		
20	5918848	250	5.5E-07	0.00402
18	4848232	220	4.8E-07	0.00452
16	3982516	280	4.4E-07	0.00362
14	3317906	290	3.9E-07	0.00342
12	2997590	280	3.3E-07	0.00358
10	2559917	270	3E-07	0.00365
8	2267119	350	2.5E-07	0.00285
6	2097567	400	2.1E-07	0.00251
4	1813852	950	1.7E-07	0.00105

Table 4.18List of r_s , X, S_e and the inverted values of both parameters at each HRT for Michaelis-
Menten relationship according to Ramalho (1977)

It appears that the concentration of substrate used in the experiment (COD approximately 4500mgl⁻¹) is too low to yield values suitable for a reciprocal plot to determine r_m or K_m values. At very low concentrations, the kinetic reaction is essentially first order for a Michaelis-Menten relationship; in which case the a straight line relationship would have been apparent in Figures 24 and 25 rather than slight curvature of the line as shown by the experimental data.



Figure 4.25 Michaelis-Menten relationship with modification according to Ramalho (1977)



Figure 4.26 Lineweaver-Burk plot with modification according to Ramalho (1977)

Higher concentrations of feed substrate (medium-strength and high strength) were later used in a subsequent experiment described. In addition an HRT of 10d was used at both substrate strengths in order to obtain a higher organic loading rate (OLR). It was considered unnecessary to use shorter HRT's since the removal efficiency, as shown in Table 4.16, decreased at an OLR of 0.58 kg.m⁻³.d⁻¹. In a reactor volume (V) of 51 operating with a HRT of 10d, these conditions were met when the substrate concentration was 5500 mg.l⁻¹, as was the case with the medium strength wastewater.

It is necessary to choose a substrate concentration with a value in the "neighbourhood" of the half saturation constant K_m in experiments to determine the kinetic relationships associated with the substrate (Segel, 1976). However, where these "neighbourhood" values are not known, as was the case with this study, then they have to

be determined by trial and error. Tables 4.19 to 4.22 show the average and calculated parameters for a medium-strength substrate (COD 5000 - 6500 mg.l⁻¹), and a high strength substrate (COD 7500 - 8500 mg.l⁻¹). The same parameters were taken except for biomass content and wastage content. As shown in Figure 4.26, there is no significant difference on the kinetic performance when the biomass content was in the range of 7800 - 8500 mg.l⁻¹.

The same method of controlling pH and alkalinity was used as for the lower strength wastewater (section 4.3.1). Constant pH and alkalinity levels were found in the effluent at each HRT as shown in Tables 4.19 and 4.21. During the experiment the HRTs was reduced incrementally by 1 day steps rather than the 2 day steps used in the previous experiment (section 4.3.2). This was a precaution to ensure proper acclimating of the bioreactor by reducing any shock induced by rapid changes to the OLR. Figure 4.27 shows the plot of S_e against OLR for all three substrate strengths. In general, S_e strength decreased with higher strength of substrate although higher OLR were given to the bioreactor. A general pattern exhibited at all three substrate strengths was the sharp increment in S_e with an OLR higher than 1kg.m⁻³.d⁻¹.

HRT (d)	S_o (mg.l ⁻¹)	S_e (mg.l ⁻¹)	pН	Alkalinity (mg.l ⁻¹)	CH ₄ : CO ₂	CH ₄ (ml)
10	5260 <u>+</u> 530	340 ± 170	6.8 <u>+</u> .1	750 ± 10	80 :20	880 <u>+</u> 130
9	5580 ± 290	370 ± 130	6.8 <u>+</u> .1	740 <u>+</u> 20	80 :20	950 <u>+</u> 90
8	5390 ± 160	320 <u>+</u> 80	6.8 <u>+</u> .1	750 <u>+</u> 10	60 :40	1190 ± 270
7	5240 <u>+</u> 560	420 <u>+</u> 110	6.8 <u>+</u> .1	720 <u>+</u> 10	60 :40	1230 <u>+</u> 280
6	6510 <u>+</u> 890	600 <u>+</u> 90	6.7 <u>+</u> .1	720 <u>+</u> 10	60 :40	1520 ± 100
5	6270 ± 820	920 <u>+</u> 150	6.8 <u>+</u> .1	720 ± 20	60 :40	1780 <u>+</u> 180

Table 4.19Experimental results from a fed-batch operation using a medium-strength readily
degradable wastewater at different hydraulic retention times (HRT's)

HRT (d)	$S_o - S_e $ (mg.l ⁻¹)	Removal efficiency (%)	Total COD in (mg)	OLR (g.m ⁻³ .d ⁻¹)	Total COD out (mg)	Total COD removal (mg)	CH₄ yield
10	4920 <u>+</u> 580	94 <u>+</u> 3	2630 <u>+</u> 260	530 ± 50	170 <u>+</u> 90	2460 <u>+</u> 270	0.36 <u>+</u> .02
9	5210 <u>+</u> 330	93 <u>+</u> 2	3070 <u>+</u> 160	610 <u>+</u> 30	200 <u>+</u> 70	2870 <u>+</u> 180	0.33 <u>+</u> .02
8	5070 <u>+</u> 210	94 <u>+</u> 2	3370 <u>+</u> 100	670 <u>+</u> 20	200 <u>+</u> 50	3170 <u>+</u> 130	0.38 <u>+</u> .07
7	4830 <u>+</u> 590	92 ± 2	3750 <u>+</u> 400	750 <u>+</u> 80	300 <u>+</u> 80	3450 <u>+</u> 420	0.36 <u>+</u> .09
6	5910 <u>+</u> 890	91 <u>+</u> 2	5400 <u>+</u> 740	1080 <u>+</u> 150	500 <u>+</u> 70	4900 <u>+</u> 740	0.31 <u>+</u> .04
5	5350 <u>+</u> 590	85 <u>+</u> 1	6270 <u>+</u> 820	1260 <u>+</u> 160	960 <u>+</u> 50	5200 <u>+</u> 800	0.34 <u>+</u> .02

Table 4.20Loading and yield related parameters for fed-batch operation using a medium-strength
wastewater at different hydraulic retention times (HRT's)

 Table 4.21 Experimental results for fed-batch operation of a high strength wastewater at different hydraulic retention times (HRT's)

HRT (d)	S _o (mg.l ⁻¹)	$\begin{array}{c} S_e \\ (\text{mg.l}^{-1}) \end{array}$	pH	Alkalinity (mg.l ⁻¹)	CH ₄ : CO ₂	CH4 (ml)
10	8000 <u>+</u> 270	250 ± 30	6.8 <u>+</u> .1	810 <u>+</u> 20	60:40	1180 ± 100
9	7760 <u>+</u> 310	260 ± 40	6.8 <u>+</u> .1	820 <u>+</u> 10	60:40	1350 <u>+</u> 100
8	8060 <u>+</u> 380	450 <u>+</u> 80	6.8 <u>+</u> .1	810 <u>+</u> 20	60:40	1650 <u>+</u> 60
7	7860 <u>+</u> 440	460 <u>+</u> 60	6.8 <u>+</u> .1	810 ± 10	60:40	1870 <u>+</u> 90
6	8210 <u>+</u> 330	720 <u>+</u> 120	6.7 <u>+</u> .1	810 <u>+</u> 10	60:40	2190 <u>+</u> 70
5	8540 <u>+</u> 360	1300 <u>+</u> 200	6.8 <u>+</u> .1	810 <u>+</u> 10	60:40	2270 <u>+</u> 100



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Figure 4.27 Effluent strength against OLR for the fed-batch operation using 3 strengths of wastewater (low, medium and high).

HRT (d)	$S_o - S_e$ (mg.l ⁻¹)	Removal efficiency (%)	Total COD in (mg)	OLR (g.m ⁻³ .d ⁻¹)	Total COD out (mg)	Total COD removed (mg)	CH4 yield
10	7750 <u>+</u> 290	97	4000 <u>+</u> 140	800 <u>+</u> 30	130 <u>+</u> 10	3880 <u>+</u> 150	0.31 <u>+</u> .03
9	7500 <u>+</u> 320	97 <u>+</u> 1	4270 <u>+</u> 170	850 <u>+</u> 30	140 <u>+</u> 20	4120 <u>+</u> 170	0.33 <u>+</u> .04
8	7620 <u>+</u> 520	94 <u>+</u> 3	5040 <u>+</u> 240	1010 <u>+</u> 50	280 <u>+</u> 90	4760 <u>+</u> 330	0.35 <u>+</u> .03
7	7400 <u>+</u> 390	94 <u>+</u> 1	5620 <u>+</u> 310	1120 <u>+</u> 60	330 <u>+</u> 50	5290 <u>+</u> 280	0.35 <u>+</u> .03
6	7490 <u>+</u> 500	91 <u>+</u> 4	6820 <u>+</u> 280	1360 <u>+</u> 60	600 <u>+</u> 130	6220 <u>+</u> 420	0.35 <u>+</u> .03
5	7250 <u>+</u> 650	85 <u>+</u> 5	8540 <u>+</u> 360	1710 <u>+</u> 70	1300 <u>+</u> 200	7250 <u>+</u> 650	0.31 <u>+</u> .02

 Table 4.22
 Loading and yield related parameters for fed-batch operation using a high strength wastewater at different hydraulic retention times (HRT's)

Tables 4.23 and 4.24 show values of S_e and calculated values of r_S for both, medium and high strength substrate. Figure 4.28 shows the Michaelis-Menten relationship derived using this data. The line of best fit through the points representing the high strength waste shows a curvature, flattening off with increasing effluent substrate concentration. This would indicate a change from first order to zero order of reaction. The line of best fit through the points representing the medium strength wastewater was almost linear and as such indicates that the reaction is still first order. Figure 4.29 shows the combination of r_s for low and medium-strength wastewaters against S_e , which is indicative of no change of order in the kinetic reaction. While Lineweaver-Burk plot for all substrates strengths was undertaken using the data in Table 4.18, 4.23 and 4.24 and the resulting plot is shown in Figure 4.30. The value of r_m based on the inverted cut-off point for a high strength wastewater was the most acceptable solution, which yielded a value 1670 mg.l⁻¹.d⁻¹. The slope representing the coefficient [K_m $/r_m$] gave a value of K_m equal to 310 mg.1⁻¹. Using the high strength wastewater the HRT could not be reduced to less than 5 days to determine r_m at higher OLR due to the potential for biomass loss during effluent withdrawal. In the previous experiment, using a low strength wastewater using an HRT of 4d necessitated partial centrifugation of the effluent in order to maintain biomass; it was considered not worth the additional work to pursue this option for the medium and high strength wastewaters.

HRT (d)	r_{S} (d ⁻¹)	S_e (mg.l ⁻¹)	1/ <i>r</i> _s	1/S _e
10	492	340	0.0020	0.00294
9	579	370	0.0017	0.0027
8	634	320	0.0016	0.00313
7	690	420	0.0014	0.00238
6	985	600	0.0010	0.00167
5	1070	920	0.0009	0.00109

Table 4.23Values of r_S , S_e and their inverted values corresponding to different HRT's in fed-
batch operation of a medium-strength wastewater

Table 4.24

Values of r_s , S_e and their inverted values corresponding to different HRT's in fedbatch operation of a high strength wastewater

HRT (d)	r_{S} (d ⁻¹)	S_e (mg.l ⁻¹)	1/ <i>r</i> _s	1/S _e
10	775	250	0.0013	0.004
9	833	260	0.0012	0.00385
8	953	450	0.0010	0.00222
7	1057	460	0.0009	0.00217
6	1248	720	0.0008	0.00139
5	1450	1300	0.0007	0.00077



Figure 4.28 Michaelis-Menten relationship for the fed-batch operation of both the medium and high strength wastewater



Figure 4.29 Michaelis-Menten relationship for the fed-batch operation for the three strengths of wastewater used.



Figure 4.30 Lineweaver-Burk plot for the fed-batch operation for the three strengths of wastewater used.

4.5 Leachate from organic municipal solid waste (OFMSW)

Treatment of the organic fraction of municipal solid waste (OFMSW) using a two-phase anaerobic process has been considered by Wang and Banks (1998). This process incorporates the principle of a hydraulic flush reactor (HFR) in which the solids and liquids retention time in the hydrolysis acidification phase are uncoupled. The flush water, which is rich in soluble precursors for methane production, then passes to a secondary methanogenic reactor. The following section of the work considers this second phase treatment.

The HFR experiment was repeated with similar operating parameters. A loading rate of 10 kg.m⁻³.d⁻¹ total solid (TS) of OFMSW was fed to a semi-continuous stirred tank

reactor (CSTR) at 35 °C, with working volume of 10 l. Three grades of leachate strength were produced. Low-strength leachate contained about 4000 mg.l⁻¹ COD, medium-strength leachate was in the range 4000 - 6000 mg.l⁻¹ COD, and high-strength leachate contained more than 7000 mg.l⁻¹ COD.

The different strengths of leachate were due to the amount of food waste in the OFMSW that was used as the substrate for HFR. In approximate terms, OFMSW with 5% (w/w) of food waste when fed to the HFR produced a low-strength leachate, while from 5% to 10% (w/w) of the food waste fraction led to production of medium-strength and high-strength leachate. Thus during the preparation of substrates for HFR, only OFMSW with a high content of food waste was selected.

Table 4.25 lists the chemical composition of each grade of leachate. All compositions were different except for the solids content. TS and volatile solid (VS) content were in the range 6500 - 9500 and 3000 - 5000 mg.l⁻¹, respectively. It seems that hydrolysis of OFMSW into smaller particles was not affected by the percentage of food waste.

The analysis of the solid-free leachate would explain the extended hydrolysis of the particulate material into soluble substances and the acidogenesis of solutes into VFA. Soluble COD and VFA content increased with the leachate strength. pH and alkalinity level were related to the presence of VFA. Typically, higher VFA content reduced both parameters. VFA analysis showed that acetate concentration was the highest in comparison with other fractions. The higher acetate concentration shows that the methanogenic phase was impeded, while the presence of other VFAs, ie. propionate to caproate, at lower concentrations indicates that the acidogenesis and acetogenesis phases were taking place.

Chemical composition	Low-strength	Medium-strength	High-strength
pH	6.0 - 7.0	5.0 - 6.0	4.0 - 5.0
Alkalinity (mg.l ⁻¹ CaCO ₃)	1000 - 1500	500 - 1000	0 - 500
$COD (mg.l^{-1})$	4000	4000 - 6000	> 6000
Total solid (mg.l ⁻¹)	6500 - 9500	6500 - 9500	6500 - 9500
Volatile solid (mg.l ⁻¹)	3000 - 5000	3000 - 5000	3000 - 5000
Solid-free leachate			
COD (mg.l ⁻¹)	500 - 1000	1000 - 2500	2500 - 5000
VFA (mg.l ⁻¹)	10 - 250	500 - 800	1500 - 2000
Acetate (mg.l ⁻¹)	10 - 100	100 - 300	500 - 1000
Propionate (mg.l ⁻¹)	0 - 30	100 - 300	100 - 200
Butyrate (mg.l ⁻¹)	0 - 30	10 - 110	110 - 250
- iso butyrate	0	0 - 10	10 - 50
- n butyrate	0 - 30	10 - 100	100 - 200
Valerate (mg.l ⁻¹)	0 - 30	50 - 100	110-250
- iso valerate	0	0 - 10	10 - 50
- n valerate	0 - 30	10 - 100	100 - 200
Caproate (mg.l ⁻¹)	0	10 - 50	50-150

Table 4.25 Chemical composition of leachate according to COD strength grading

4.6 Fed-batch operation for the treatment of a solid-free OFMSW leachate

An interesting observation was that the solid content of the leachate completely separated by gravitational settlement after defrosting. Based on this observation, it was decided that the following anaerobic process for leachate treatment should be started with solid-free leachate. As little was known regarding the effect of leachate on the anaerobic system this was also a safer way in which to proceed as the chances of toxicity would be reduced. Solid-free leachate was assumed to contain only soluble organic substances that would presumably not affect the bioreactor. In addition, the seed-sludge from previous experimental work could be reused since it was also used to treat a readily biodegradable wastewater, which was also a solid-free substrate.

The seed-sludge from the previous experiment was maintained and fed with the same substrate. New seed-sludge was also added to supplement the existing sludge and increase the biomass content up to 10 000 mg.l⁻¹. At the same time, acclimatisation with solid-free leachate did not show any complications. The solid-free high-strength leachate

was selected as it had the highest content of soluble organic substances. An operational HRT of 20 d during acclimatisation until production of CH_4 was used consistently before collecting the experimental data. The same data measurements were made as for the previous fed-batch operation.

4.6.1 Operation performances

Table 4.26 shows the average of 9 replicates for each daily fed-batch operation from an HRT of 20 d to 2 d. The pH and alkalinity of the effluent were consistent at 7.3-7.4 and 1300 - 2000 mg.l⁻¹ respectively, at all HRTs and without any additional pH controlling substances. The ratio of $CH_4:CO_2$ in the biogas fraction was also consistent at 80:20. This was in spite of the fact that the influent strength fluctuated with high standard deviation values, due to inconsistent food content in OFMSW.

Meanwhile, the effluent strength was consistent at each HRT, and was in the range 240-340 mg.l⁻¹. This indicates that almost all of the biodegradable substances were fully digested at all HRTs, and only the residual of recalcitrant materials was left. Production of CH₄ as shown in Figure 4.31 occurred at an exponential rate. Reduction of biomass content was observed at the beginning of the operation. However these losses seemed to be recovered at HRT of 8 d and less. It was also noted that the biomass wastage began to decrease, from 250 mg.l⁻¹ to 70 mg.l⁻¹ at an HRT of 8 d and below. This means that optimisation of the granulation process was beginning to occur at this loading. As calculated in Table 4.27, the OLR for a HRT of 8 d was 0.44 kg.m⁻³.d⁻¹, suggesting that this is the minimum OLR that should be used for the treatment of solid free leachate in a fed batch anaerobic reactor. This was the necessary loading in order to achieve the optimum biomass flocculation and to regain the biomass growth. There was no biomass wastage when the operational HRT was less than 4 d and at this point the biomass had to be recovered by centrifugation during effluent withdrawal.

HRT (d)	$\begin{array}{c c} S_o \\ (\text{mg.l}^{-1}) \end{array}$	$\begin{array}{c c} S_e \\ (\text{mg.I}^{-1}) \end{array}$	pH	Alkalinity (mg.l ⁻¹)	CH ₄ : CO ₂	CH ₄ (ml.d ⁻¹)	Biomass (mg.l ⁻¹)	Wastage (mg.l ⁻¹)
20	2680 <u>+</u> 270	310 <u>+</u> 20	7.4	1880	80:20	240 <u>+</u> 30	10210 <u>+</u> 860	220 <u>+</u> 60
18	3400 <u>+</u> 520	340 <u>+</u> 20	7.5	1870	80:20	220 <u>+</u> 20	9990 <u>+</u> 740	220 <u>+</u> 60
16	2710 <u>+</u> 350	340 ± 30	7.4	1880	80:20	200 ± 30	8280 <u>+</u> 790	290 <u>+</u> 60
14	3290 <u>+</u> 230	310 ± 30	7.4	1600	80:20	300 <u>+</u> 20	8110 <u>+</u> 390	190 <u>+</u> 60
12	2670 <u>+</u> 240	320 <u>+</u> 40	7.3	1780	80:20	330 <u>+</u> 30	8090 <u>+</u> 270	210 ± 10
10	2470 <u>+</u> 210	340 <u>+</u> 30	7.4	1880	80:20	380 <u>+</u> 50	7500 <u>+</u> 460	250 <u>+</u> 30
8	3520 <u>+</u> 640	290 <u>+</u> 20	7.4	2050	80:20	730 <u>+</u> 150	7900 <u>+</u> 540	70 <u>+</u> 10
7	3400 <u>+</u> 580	280 <u>+</u> 20	7.4	1990	80:20	780 <u>+</u> 150	9270 <u>+</u> 670	80 <u>+</u> 10
6	4060 <u>+</u> 560	310 <u>+</u> 20	7.3	1990	80:20	1110 <u>+</u> 130	8430 <u>+</u> 790	70 <u>+</u> 10
5	3440 <u>+</u> 730	270 <u>+</u> 10	7.4	1970	80:20	1080 <u>+</u> 230	8840 <u>+</u> 670	60 <u>+</u> 10
4	3920 <u>+</u> 440	240 <u>+</u> 10	7.3 <u>+</u> 0.1	1680 <u>+</u> 100	80:20	$\begin{array}{r}1530 \pm \\120\end{array}$	8220 <u>+</u> 560	70 <u>+</u> 10
3	4050 <u>+</u> 600	260 <u>+</u> 10	7.3 <u>+</u> 0.1	1880 <u>+</u> 60	80:20	2280 <u>+</u> 350	8370 <u>+</u> 510	-
2	3350 <u>+</u> 480	250 <u>+</u> 10	7.3 <u>+</u> 0.1	1340 <u>+</u> 70	80:20	2680 <u>+</u> 460	8950 <u>+</u> 520	-

Table 4.26 Average parameters for the treatment of a solid-free leachate by anaerobic CSTR associated with increasing hydraulic flow rate (Q)



Figure 4.31 Production of CH4 at each HRT for the fed-batch operation of a solid-free leachate

The CH₄ yield as shown in Table 4.27 was slightly reduced immediately after start up when the HRT was 20 d. The yield was about 0.40 $10^3 \text{m}^3 \text{CH}_4.\text{kg}^{-1}$ COD removed at a HRT of 20 d, a significantly high value, then reduced to 0.26-0.27 $10^3 \text{m}^3 \text{CH}_4.\text{kg}^{-1}$ COD removed to an HRT of 12 d, and increased again to the normal range up to the end of the experiment. A possible explanation is that higher production of CH₄ at the beginning was due to residual undigested organic content in the supplementary seedsludge that was added during acclimatisation to increase the biomass population. It also could be assumed that an OLR of less than 0.25 kg.m⁻³.d⁻¹for the fed-batch operation with solid-free leachate might lead the reactor to starvation as indicated by loss of biomass and lower yield of CH₄ during the operating HRTs from 20 d to 10 d. CH₄ production during the same operational HRT period follows curve line, as shown in Figure 4.31. This might also support the assumption of starvation. No further investigation was undertaken on this matter. Meanwhile, Figure 4.32 shows the overall CH₄ yield for this operation at a value of 0.35 $10^3 \text{m}^3 \text{CH}_4.\text{kg}^{-1}\text{COD}$ removed according to the slope of CH₄ production against COD mass removal.



Figure 4.32 Overall CH₄ yield for the fed-batch operation of a solid-free leachate

HRT (d)	$S_o - S_e$ (mg.l ⁻¹)	COD Removal efficiency (%)	Total COD loading (mg.d ⁻¹)	OLR (g.m ⁻³ .d ⁻¹)	Total COD out (mg.d ⁻¹)	Total COD removal (mg.d ⁻¹)	CH₄ yield
20	2370 <u>+</u> 270	88 <u>+</u> 1	670 <u>+</u> 70	130 <u>+</u> 10	80 <u>+</u> 5	590 <u>+</u> 70	0.40 ±.04
18	3060 <u>+</u> 520	89 <u>+</u> 2	950 <u>+</u> 150	190 <u>+</u> 30	100 <u>+</u> 10	860 <u>+</u> 150	0.26 <u>+</u> .04
16	2370 <u>+</u> 340	87 <u>+</u> 2	840 <u>+</u> 110	170 <u>+</u> 20	110 <u>+</u> 10	740 <u>+</u> 100	0.27 <u>+</u> .05
14	2980 <u>+</u> 230	90 <u>+</u> 1	1180 <u>+</u> 80	240 <u>+</u> 20	110 <u>+</u> 10	1070 <u>+</u> 80	0.28 <u>+</u> .02
12	2350 <u>+</u> 230	88 <u>+</u> 2	1120 <u>+</u> 100	220 <u>+</u> 20	130 <u>+</u> 20	990 <u>+</u> 100	0.34 <u>+</u> .04
10	2130 <u>+</u> 220	86 <u>+</u> 2	1230 <u>+</u> 110	250 <u>+</u> 20	170 <u>+</u> 20	1060 <u>+</u> 110	0.36 <u>+</u> .03
8	3230 <u>+</u> 640	92 <u>+</u> 2	2190 <u>+</u> 390	440 <u>+</u> 80	180 <u>+</u> 20	2000 <u>+</u> 390	0.37 <u>+</u> .05
7	3120 <u>+</u> 590	93 <u>+</u> 1	2420 <u>+</u> 410	480 <u>+</u> 80	200 <u>+</u> 10	2220 <u>+</u> 420	0.36 <u>+</u> .07
6	3750 <u>+</u> 580	93 <u>+</u> 1	3370 <u>+</u> 470	670 <u>+</u> 90	260 <u>+</u> 20	3110 <u>+</u> 480	0.36 <u>+</u> .03
5	3170 <u>+</u> 730	91 <u>+</u> 3	3440 <u>+</u> 730	680 <u>+</u> 150	270 <u>+</u> 10	3170 <u>+</u> 730	0.34 <u>+</u> .05
4	3680 <u>+</u> 440	94 <u>+</u> 1	4900 <u>+</u> 550	980 <u>+</u> 110	300 <u>+</u> 10	4590 <u>+</u> 550	0.34 <u>+</u> .03
3	3790 <u>+</u> 600	94 <u>+</u> 1	6760 <u>+</u> 1000	1350 <u>+</u> 200	440 <u>+</u> 20	6320 <u>+</u> 1000	0.36 <u>+</u> .02
2	3110 <u>+</u> 470	90	8390 <u>+</u> 1200	1680 <u>+</u> 240	620 <u>+</u> 30	7770 <u>+</u> 1190	0.34 <u>+</u> .01

 Table 4.27 Calculation of average performance for fed-batch operation of a solid-free leachate by fed-batch anaerobic CSTR

4.6.2 Modelling the kinetics for treatment of a solid-free leachate

The same approach of using the Michaelis-Menten relationship was taken to determine the kinetics of solid-free leachate treatment. However, it was unable to determine any of the three types of kinetic relationship, as shown in Figure 4.33 where the plot of r_S against S_e shows that an almost vertical line could be drawn through the data plots. This could be interpreted as showing that the S_e was very much lower than the K_m value. All operational HRTs produced a minimum strength of effluent. It can be

concluded that the fed-batch operation is able to treat the solid-free leachate up to an OLR of 1.7 kg.m⁻³.d⁻¹with maximum efficiency. It is therefore likely that even higher OLR could be used with a solid-free leachate, however, this could only be achieved by using higher substrate strength or by changing the system design, i.e. continuous feeding.



Figure 4.33 Michaelis-Menten relationship for the fed-batch operation of a solid-free leachate

4.7 Fed-batch operation for the treatment of an OFMSW leachate

Before proceeding with the anaerobic treatment of the OFMSW leachate, a new seed-sludge was introduced because the previous seed-sludge failed to digest the unfiltered leachate. The same acclimatisation procedure was carried out as for the low-strength leachate. A high-strength leachate was subsequently used for the following experiment after preliminary tests had been run on all three types of leachate at an HRT of 20 d. Table 4.28 compares the results for the preliminary tests. It shows that high strength leachate can be treated and if possible this should be used within a treatment system as it gave a greater COD removal efficiency and CH₄ yield. Furthermore, there was no significant difference in the VS removal efficiency. Thus, the following experiment concentrated on the treatment of high-strength leachate. Furthermore based on the results of the previous operation, higher substrate strength was required to give higher OLRs, in order to determine the Michaelis-Menten kinetic relationship for such readily degradable substrates.

Parameter	Low strength	Medium strength	High strength
	leachate	leachate	leachate
S_e (pH)	7.2	7.2	7.2
S_e (alkalinity)	2100	2100	2100
$S_o \text{COD} (\text{mg.l}^{-1})$	4050 <u>+</u> 610	7280 ± 750	9680 <u>+</u> 670
Se COD (mg.l ⁻¹)	430 <u>+</u> 20	420 ± 10	460 <u>+</u> 20
COD removal efficiency	89 <u>+</u> 1 %	94 <u>+</u> 1 %	95 %
$CH_4:CO_2$	80:20	80:20	80:20
CH ₄	150 <u>+</u> 10	330 <u>+</u> 20	500 <u>+</u> 70
Total COD loading (mg.d ⁻¹)	1010 ± 150	1820 ± 200	2420 <u>+</u> 170
Total COD out (mg.d ⁻¹)	110 ± 10	110 <u>+</u> 10	120 ± 10
Total COD removal (mg.d ⁻¹)	900 <u>+</u> 150	1720 <u>+</u> 190	2310 <u>+</u> 160
CH ₄ yield	0.17 <u>+</u> .03	0.19 <u>+</u> .02	0.22 <u>+</u> .07
S_o VS (mg.1 ⁻¹)	3290 <u>+</u> 200	4880 <u>+</u> 180	4820 <u>+</u> 120
S_e VS(mg.l ⁻¹)	400 <u>+</u> 20	450 <u>+</u> 60	470 <u>+</u> 80
VS removal efficiency	88 <u>+</u> 1 %	91 <u>+</u> 1 %	90 <u>+</u> 2 %

 Table 4.28
 Comparison of operation performances for three types of leachate by fed-batch operation at HRT of 20d

4.7.1 Operation performances

Table 4.29 shows the results for the fed-batch operation of a high-strength leachate treatment. Operational HRT's varied from 20 d to 4 d. The HRT was reduced by 0.5 d after the experiment reached a HRT of 6.5, in order to allow a greater degree of acclimatization to the increasing OLR and thus reduce the risk of damaging the reactor through a shock load induced by too great an increment in loading rate. As usual, the operating HRT ended at 4d when the biomass could no longer be collected by gravitational settlement. A slightly longer settlement period of three hours rather than two hours was required to separate the biomass at the HRT of 4 d.

The effluent at all HRTs showed consistent pH and alkalinity levels of 7.2 and 2100 mg.l⁻¹ CaCO₃ respectively, without any addition of pH controlling substances. Although the influent strength to the reactor was inconsistent, the effluent strengths obtained at all HRTs were more consistent, in the range of 420-590 mg.l⁻¹. This indicates that the process was operating at optimum efficiency. It also means that the CH₄ production should be at an exponential rate as was shown for such optimum substrate removal in the previous experiment. It is also possible to assume that the substrate contains the nitrogen for biomass growth. It has been well established that OFMSW leachate contains a high concentration of ammonia nitrogen and organic materials as well as other inorganic compounds (Bae *et al.*, 1997), which probably explains this.

HRT (d)	$\begin{vmatrix} S_o \\ (mg.l^{-1}) \end{vmatrix}$	$\begin{cases} S_e \\ (\text{mg.I}^{-1}) \end{cases}$	CH ₄ : CO ₂	CH ₄ (ml.d ⁻¹)	$\begin{array}{c c} VS in \\ (mg.\Gamma^{1}) \end{array}$	VS out (mg.l ⁻¹)	Biomass (mg.l ⁻¹)
20	7700 <u>+</u> 570	420 <u>+</u> 60	80:20	480 <u>+</u> 20	4600 <u>+</u> 410	510 <u>+</u> 40	12810 <u>+</u> 470
18	7920 <u>+</u> 300	440 <u>+</u> 50	80:20	530 <u>+</u> 20	4500 <u>+</u> 190	510 <u>+</u> 20	13870 <u>+</u> 1600
16	7370 <u>+</u> 340	460 <u>+</u> 50	80:20	720 <u>+</u> 40	4600 <u>+</u> 410	510 <u>+</u> 40	12810 <u>+</u> 470
14	7490 <u>+</u> 330	450 <u>+</u> 10	80:20	840 <u>+</u> 40	4740 <u>+</u> 460	510 <u>+</u> 20	13870 <u>+</u> 1600
12	9630 <u>+</u> 640	510 <u>+</u> 20	60:40	1170 <u>+</u> 100	5040 <u>+</u> 550	500 <u>+</u> 70	12670 <u>+</u> 900
10	9840 <u>+</u> 630	510 <u>+</u> 10	60:40	1650 <u>+</u> 230	5600 <u>+</u> 610	490 <u>+</u> 90	13160 <u>+</u> 830
8	9610 <u>+</u> 590	510 <u>+</u> 10	60:40	2050 <u>+</u> 70	4730 <u>+</u> 640	450 <u>+</u> 30	12970 <u>+</u> 450
6.5	8740 <u>+</u> 320	500 <u>+</u> 50	60:40	2230 <u>+</u> 200	4720 <u>+</u> 520	500 <u>+</u> 90	13410 <u>+</u> 1350
6	8690 <u>+</u> 600	560 <u>+</u> 80	60:40	2450 <u>+</u> 260	4680 <u>+</u> 230	410 <u>+</u> 100	13450 <u>+</u> 1270
5.5	8290 <u>+</u> 1060	500 <u>+</u> 50	60:40	2430 <u>+</u> 270	4290 <u>+</u> 620	560 <u>+</u> 170	13920 <u>+</u> 520
5	8130 <u>+</u> 520	550 <u>+</u> 70	60:40	2520 <u>+</u> 200	4490 <u>+</u> 770	570 <u>+</u> 140	14380 <u>+</u> 390
4.5	7420 <u>+</u> 270	570 <u>+</u> 90	60:40	2350 <u>+</u> 140	3010 <u>+</u> 290	480 <u>+</u> 30	14720 <u>+</u> 800
4	7810 <u>+</u> 160	590 <u>+</u> 70	60:40	2860 <u>+</u> 100	3510 <u>+</u> 410	470 <u>+</u> 30	14040 <u>+</u> 460

Table 4.29 Average parameters for the treatment of a high-strength leachate by anaerobic CSTR associated with increasing hydraulic flow rate (Q)

No biomass wastage analysis is listed in the table. In fact, the effluent VS concentration is another form of biomass wastage measurement. Consistently low VS in the effluent indicates that good granulation of biomass population can be achieved with this leachate as the substrate. The low concentration of VS in the effluent also indicates that a high degree of VS removal had been achieved. Another indication of high VS removal was the biomass content which was also measured as volatile solids (VS). As there was no significant increment of biomass, as shown in Table 4.29, then the indication is that solids were not being accumulated throughout the operation

Table 4.30 shows the operational performance. Substrate removal was above 90% although the OLR reached about 2.0 kg.m⁻³.d⁻¹. This suggests a higher OLR could be applied for the treatment of such a substrate. The CH₄ yield was below 0.30 10^3 m³CH₄. kg⁻¹ COD removed at the beginning of the experiment. The overall CH₄ yield for this operation was 0.33 10^3 m³CH₄. kg⁻¹ COD removed, however, as shown in Figure 4.34 by the slope of CH₄ production against COD mass removal.



Figure 4.34 Overall CH4 yield for the fed-batch operation of a high strength leachate

4.7.2 Modelling the kinetics for treatment of an OFMS leachate

Determination of the Michaelis-Menten relationship for the treatment of OFMSW leachate is shown in Figure 4.35. The effluent strength appears to be too low to reach the substrate saturation point. However a first order kinetic relationship was confirmed by the Lineweaver-Burk plot in Figure 4.36. The cut-off value for the maximum substrate

utilisation constant (r_m) gave a value of less than zero, as was shown in the previous experiment with readily biodegradable wastewater for first order kinetics. Higher OLRs should be used to determine the kinetic constant for the treatment of leachate.

HRT (d)	$\begin{array}{c} S_o - S_e \\ (\text{mg.l}^{-1}) \end{array}$	COD Removal efficiency (%)	Total COD loading (mg.d ⁻¹)	OLR (g.m ⁻³ .d ⁻¹)	Total COD out (mg d ⁻¹)	Total COD removal $(mg d^{-1})$	CH4 yield
20	7280 <u>+</u> 580	95 <u>+</u> 1	1920 <u>+</u> 140	390 <u>+</u> 30	110 <u>+</u> 20	$1820 \\ \pm 140$	0.27 <u>+</u> .03
18	7480 <u>+</u> 350	94 <u>+</u> 1	2220 <u>+</u> 80	440 <u>+</u> 20	120 <u>+</u> 20	2100 <u>+</u> 390	0.25 <u>+</u> .01
16	6910 <u>+</u> 350	94 <u>+</u> 1	2280 <u>+</u> 100	460 <u>+</u> 20	140 <u>+</u> 20	2140 <u>+</u> 110	0.34 ±.02
14	7040 <u>+</u> 340	94	2700 <u>+</u> 120	540 <u>+</u> 20	160 <u>+</u> 5	2530 <u>+</u> 120	0.33 <u>+</u> .02
12	9120 <u>+</u> 650	95 <u>+</u> 1	4040 <u>+</u> 270	810 <u>+</u> 50	210 <u>+</u> 10	3830 <u>+</u> 270	0.31 <u>+</u> .04
10	9330 <u>+</u> 620	95	4920 <u>+</u> 310	980 <u>+</u> 60	260 <u>+</u> 10	4660 <u>+</u> 310	0.35 <u>+</u> .04
8	9100 <u>+</u> 580	95	5960 <u>+</u> 360	1190 <u>+</u> 70	320 <u>+</u> 10	5640 <u>+</u> 360	0.36 <u>+</u> .02
6.5	8240 <u>+</u> 310	94	6730 <u>+</u> 250	1350 <u>+</u> 50	380 <u>+</u> 40	6340 <u>+</u> 240	0.35 <u>+</u> .03
6	8130 <u>+</u> 600	94 <u>+</u> 1	7210 <u>+</u> 490	1440 <u>+</u> 100	460 <u>+</u> 60	6750 <u>+</u> 500	0.36 <u>+</u> .02
5.5	7790 <u>+</u> 1050	94 <u>+</u> 1	7550 <u>+</u> 960	1510 <u>+</u> 190	460 <u>+</u> 50	7090 <u>+</u> 960	0.34 <u>+</u> .02
5	7580 <u>+</u> 530	93 <u>+</u> 1	8120 <u>+</u> 520	1620 <u>+</u> 110	550 <u>+</u> 70	7580 <u>+</u> 530	0.33 <u>+</u> .04
4.5	6850 <u>+</u> 260	92 <u>+</u> 1	8230 <u>+</u> 300	1650 <u>+</u> 60	630 <u>+</u> 100	7600 <u>+</u> 280	0.31 <u>+</u> .03
4	7220 <u>+</u> 180	92 <u>+</u> 1	9760 <u>+</u> 200	1950 <u>+</u> 40	730 <u>+</u> 80	9030 <u>+</u> 230	0.32 <u>+</u> .02

 Table 4.30 Calculation of average performances for fed-batch operation of an OFMSW leachate by fed-batch anaerobic CSTR



Figure 4.35 Michaelis-Menten relationship for the fed-batch operation of a high strength leachate



Figure 4.36 Lineweaver-Burk plot for the fed-batch operation of a high strength leachate

4.8 Continuous-feeding operation for the treatment of an OFMSW leachate

The reactor design was changed from a CSTR to an anaerobic filter (AF) to provide a higher OLR in order to pursue the kinetic investigation. The AF with a 3 1 working volume was set up and fed continuously. The hydraulic flow rate (Q) was measured by the volume of substrate fed into the reactor in 24 hours. Adjustment of Qdetermined the operational HRT. Since only a single AF unit was available, more replicate samples were taken and the 'continuous feed' was given as a series of prequantified volumes within 24 hours to make up the volume required for a constant Q. Mass-balances for each batch of influent and effluent were carried out, and the CH₄ production volume was examined. The average of data summation for all batches in 24 hours was recorded as the operational data for one day and this was repeated for four days for each operational HRT after acclimatisation.

4.8.1 Operation performances

Table 4.31 shows the operational performances for the treatment of leachate by AF. By chance, a slightly lower strength leachate was being produced by the HFR, and this strength as calculated in Table 4.32 would give an OLR of 1.8 kg.m⁻³.d⁻¹ for starting up the AF at an HRT of 3 d. This OLR was similar to the last OLR during CSTR operation, which was 1.9 kg.m⁻³.d⁻¹. Such a precaution was taken to avoid shock loading the reactor because the biomass used in the AF was originally from the CSTR. 2.5 l of concentrated biomass from the CSTR were collected and transferred to the AF. Concentrated biomass was obtained by removing the supernatant in the CSTR after 24 hours of sedimentation. pH and alkalinity measurements of the effluent dropped due to changes in operating design. The most probable reason was lacked of dilution for the AF content. Most of the liquor in the reactor was 'crammed' with biomass and substrate. After being digested, it was immediately replaced with new substrate since the reactor was fed almost continuously.

HRT (d)	Q (L.d ⁻¹)	$\begin{array}{c}S_{o}\\(\mathrm{mg.I}^{-1})\end{array}$	$\frac{S_e}{(\text{mg.I}^{-1})}$	pH	alkali -nity	CH ₄ : CO ₂	CH ₄ (ml.d ⁻¹)	VS in (mg.l ⁻¹)	VS out (mg.l ⁻¹)
3	1.0	5470 <u>+</u> 170	550 <u>+</u> 20	6.8	850	60:40	470 <u>+</u> 40	$\begin{array}{r} 2780 \pm \\ 380 \end{array}$	270 <u>+</u> 10
2.5	1.22	5600 <u>+</u> 110	$\begin{array}{c} 600 \pm \\ 70 \end{array}$	6.8	850	60:40	590 <u>+</u> 70	3450 <u>+</u> 660	440 <u>+</u> 80
2	1.5	6860 <u>+</u> 970	$\begin{array}{r}1400 \pm \\ 380\end{array}$	6.7	800	60:40	820 <u>+</u> 90	3900 <u>+</u> 670	660 <u>+</u> 180
1.8	1.71	5860 <u>+</u> 60	$\begin{array}{r} 2540 \\ 600 \end{array} \pm$	6.3	800	60:40	570 <u>+</u> 60	3960 <u>+</u> 730	690 <u>+</u> 170
1.5	2.0	6240 <u>+</u> 80	6100 <u>+</u> 100	5.5	450	-	-	4240 <u>+</u> 450	4020 <u>+</u> 500

Table 4.31 Average parameters for the treatment of an OFMSW leachate by anaerobic filter with 3.0L working volume (V) associated with increasing hydraulic flow rate (Q)
HRT (d)	$S_o - S_e$ (mg. Γ^1)	Removal efficiency (%)	Total COD loading (mg.d ⁻¹)	OLR (g.m ⁻³ .d ⁻¹)	Total COD out (mg.d ⁻¹)	Total COD removal (mg.d ⁻¹)	CH4 yield
3	4920 ± 190	90 <u>+</u> 1	5470 <u>+</u> 410	1820 <u>+</u> 160	$\begin{array}{c} 1820 \\ 40 \end{array} \pm$	1630 <u>+</u> 120	0.31
2.5	4990 <u>+</u> 100	90 <u>+</u> 1	6830 <u>+</u> 280	2280 <u>+</u> 110	2260 <u>+</u> 100	2030 <u>+</u> 70	0.30 <u>+</u> .1
2	5460 <u>+</u> 1150	79 <u>+</u> 7	10280 ± 1550	3430 <u>+</u> 520	$\begin{array}{r} 3320 \pm \\ 600 \end{array}$	2680 <u>+</u> 550	0.32 <u>+</u> .1
1.8	3320 <u>+</u> 540	57 <u>+</u> 10	$\begin{array}{r}10030 \pm \\170\end{array}$	3340 <u>+</u> 60	5580 <u>+</u> 1650	1890 <u>+</u> 290	0.31

Table 4.32 Calculation of average performance for fed-batch operation of an OFMSW leachate by anaerobic filter

The effluent strength increased with the reduction of COD removal efficiency when Q increased. The calculation as presented in Table 4.32 shows that the anaerobic process for the treatment of OFMSW leachate was able to operate with 90% removal efficiency with an OLR of up to 2.3 kg.m⁻³.d⁻¹, then the efficiency dropped to 60% when the OLR reached 3.4 kg.m⁻³.d⁻¹. The results are therefore in agreement with previous work in which an anaerobic filter (AF) had been reported to achieve 90% of removal efficiency at an OLR of 2.0 kg.m⁻³.d⁻¹ for a medium strength carbohydrate based substrate with low content of suspended solids (Mosey, 1977, see Barnes and Fitzgerald, 1987). Nevertheless, the CH₄ yield remained consistent at 0.31 10³m³CH₄.kg⁻¹COD removed in spite of the drop in efficiency. At an HRT of 1.5, the reactor had completely failed and the effluent pH, alkalinity, and strength were as the same as the influent. At this point, no CH₄ was produced, and the COD and VS removal were zero. The reactor had turned 'soured'. Figure 4.37 shows the VS removal efficiency of the whole anaerobic operation for leachate treatment was consistently between 80-90 % until the operation failed



Figure 4.37 VS removal efficiency for anaerobic treatment of OFMSW leachate

4.9 Modelling the kinetics for combined anaerobic treatment of an OFMSW leachate

Data from the AF experiment were not sufficient to allow a kinetic analysis to be carried out. Thus, data from the CSTR operation was combined with AF data. Referring to Figure 4.35 for the Michaelis-Menten relationship of CSTR operation, the first cluster of data points indicates a zero order kinetic relationship, while the second cluster indicates a similar case to the treatment of solid-free leachate. In both cases, the substrate concentration was considered to be very much lower in order to reach the substrate saturation concentration. The last cluster of data points indicates a first order kinetic relationship, however. Meanwhile the effluent strength in the AF operation indicates that it was reaching the substrate saturation point. Figure 4.38 shows the combination of both data sets for determination of the Michaelis-Menten kinetic relationship. The plot does not show a clear-cut manifestation of the kinetic relationship. More data points are required to represent the Michaelis-Menten relationship. However, it was difficult to tune the flow rate (Q) for a 3 l working volume reactor as the HRT decreases exponentially with Q. A Lineweaver-Burk plot was drawn to determine the kinetic constants values for the Michaelis-Menten relationship as listed in Equation 4.17. The plot is shown in Figure 4.39, without the last data point from AF operation because, as was shown in Figure 4.38, the last data point did not represent the change from first order to zero order kinetic relationship when the r_s value went below the maximum substrate removal rate (r_m) . Table 4.33 shows the transformed data for plotting. The reciprocal plot for this equation in Figure 4.39 determines the constant values of 5000 mg.l⁻¹.d⁻¹ for r_m , and 1330 mg.l⁻¹ for the Michaelis-Menten constant K_m , or as it is also known the half substrate saturation point.





Table 4.33 List of r_s , S_e and the inverted values of both parameter at each HRT in combined operation of an OFMSW leachate

HRT (d)	r_{S} (d ⁻¹)	S_e (mg.l ⁻¹)	1/ <i>r</i> s	$1/S_e$
6	1360	560	0.00074	0.00179
5.5	1420	500	0.00071	0.002
5	1520	550	0.00066	0.00182
4.5	1520	570	0.00066	0.00175
4	1810	590	0.00055	0.00169
3	1640	550	0.00061	0.00182
2.5	2000	600	0.0005	0.00167
2	2730	1400	0.00037	0.00071
1.8	1840	2540	0.00054	0.00039



Figure 4.39 Lineweaver-Burk plot for combined operation of anaerobic CSTR and AF for OFMSW leachate treatment.

4.10 VFA analysis

No VFA was present in the effluent during the fed-batch operation of readily biodegradable wastewater, OFMSW solid-free leachate, and OFMSW leachate. Table 4.34 shows the profiles and concentration of VFA in the effluent for each operational HRT associated with the anaerobic filter. During the continuous-feeding operation the VFA concentration started to increase in the effluent with the reduction of HRT. There was no change in the VFA concentration profiles in the effluent at HRTs from 1.8 d to 1.5 d. These VFA profiles and concentrations were similar to the influent values, meaning VFA removal efficiency had reduced almost to 0%. The reactor was still producing CH₄ at the HRT of 1.8 d, however, which indicates that methanogenesis was still occurring. A possible explanation is, these VFA's were being produced from the continuation of simultaneous hydrolysis, acidogenesis and acetogenesis of VS content as the VS removal efficiency was not affected. This might explain the lower r_s value at this HRT which is shown in Figure 4.38, whereas the rate of methanogenesis was possibly being restricted by the high VFA load and low pH and alkalinity environment, as shown in Table 4.31. At an HRT of 1.5 d the reactor failed, and the VFA and VS concentration in the effluent were the same as in the influent.

Table 4.34

VFA analysis in the effluent of each HRT for AF operation

HRT	VFA analysis
3.0	Total VFA 0
2.5	Total VFA 240 mg.l ⁻¹ Acetate 200 mg.l ⁻¹ Propionate 20 mg.l ⁻¹ Butyrate 20 mg.l ⁻¹
2.0	Total VFA 300 mg.l ⁻¹ Acetate 200 mg.l ⁻¹ Propionate 40 mg.l ⁻¹ n-butyrate 40 mg.l ⁻¹ n-valerate 10 mg.l ⁻¹
1.8	Total VFA 1250 mg.l ⁻¹ Acetate 500 mg.l ⁻¹ Propionate 200 mg.l ⁻¹ n-butyrate 200 mg.l ⁻¹ iso-valerate 50 mg.l ⁻¹ n-valerate 200 mg.l ⁻¹ caproate 100 mg.l ⁻¹
1.5	Total VFA 1250 mg.l ⁻¹ Acetate 500 mg.l ⁻¹ Propionate 200 mg.l ⁻¹ n-butyrate 200 mg.l ⁻¹ iso-valerate 50 mg.l ⁻¹ n-valerate 200 mg.l ⁻¹ caproate 100 mg.l ⁻¹

CHAPTER V DISCUSSION

5.0 General introduction

This chapter considers data extraction from experimental work for the derivation of kinetic expressions. It compares the work carried out here with other similar studies on the modelling of anaerobic treatment processes. As well as using the data to derive the kinetic constants, it is also used to predict the operation of the anaerobic treatment plant. How the combination of CH₄ production and substrates removal provides the input for a kinetic model, which is then written as, a mathematical equation is also explained in this chapter. The discussion also includes supplementary experimental data, which was not included in the previous results chapter, and this was used to verify the application of the kinetic models. A general assumption for the kinetic modelling is that the mixed population of biomass is regarded as one unit of population, and distributed continuously through the reactor; this makes the mathematical approach simpler (Horan, 1990). It is however appreciated that within an anaerobic digester there is a balanced population structure comprising hydrolytic bacteria, acidogens, acetogens and methanogens whose activities impact upon each other creating a complex microbial ecosystem of a more diverse nature than envisaged in most kinetic models that attempt to describe the process. By simplification, some information will thus be lost in the modelling process, but one of the aims of the work was to establish those models, which are most useful in describing the system accurately with the minimum of input data.

5.1 Application of the specific function of CH₄ production in batch operation

A similar study on the anaerobic digestion of readily degradable wastewater in batch-operation to that undertaken here was conducted by Borja and Banks (1994a); this made use of Equation 5.1 as the basic kinetic expression concerning the cumulative production of biogas. This equation was reported as a First-Order model commonly used to describe the anaerobic digestion of organic substrates. As shown in Figure 4.8, CH₄ production from a batch fed anaerobic reactor shows a good agreement, with zero gas produced at t = 0 (high production rate), and maximum amount of gas when t is equal to infinity (zero production rate). The graph of gas production from batch fed reactor thus

exhibits a line with a decreasing slope with increasing time. To check this equation with experimental data, Equation 5.1 was transformed into a linear form as shown in Equation

5.2.
$$G = G_m \cdot [1 - \exp^{-K_0 \cdot t}]$$
 (Equation 5.1)
 $G = CH_4$ cumulative production
 $G_m =$ maximum CH₄ cumulative production
 $t =$ digestion time
 $K_o =$ specific rate constant
 $\ln [G_m / (G_m - G)] = K_o \cdot t$ (Equation 5.2)

Table 5.1 shows the linearised data for each feed volume (FV), these have then been plotted in Figure 5.1 to allow determination of K_o . The previously determined G_m values by the specific function ($G = G_m^{k/t}$) were refitted into Eq 5.2. K_o values, which were given by linear estimation in Figure 5.1, are also listed in the same Table 5.1.

Table 5.1 Ln $(G_m / (G_m - G))$ at time - t_i and K_o constant for each FV of readily degradable wastewater batch operation by anaerobic CSTR

Time (hour)	FV1 (0.5L)	FV2 (0.8L)	FV3 (1.0L)	FV4 (1.33L)
0	0.00	0.00	0.00	0.00
1	0.12	0.01	0.01	0.02
2	0.17	0.02	0.04	0.03
3	0.22	0.04	0.05	0.05
5	0.34	0.05	0.06	0.18
6	0.43	0.06	0.09	0.44
7	0.54	0.08	0.12	0.47
11	0.84	0.20	0.25	1.06
12	0.89	0.26	0.32	1.12
23	1.35	0.68	0.69	1.25
24	1.39	0.71	0.73	1.29
28	1.71	0.82	0.88	1.33
48	2.12	1.63	1.67	1.37
72	2.81	2.29	3.02	1.54
K _o	0.05	0.03	0.04	0.03

The K_o values were virtually constant within the range 0.03 - 0.05. Borja and Banks (1994a) reported that variation of K_o with different initial COD load indicates that the kinetic constant was not significantly influenced by the concentration of substrates. However, slightly higher K_o values were obtained by Borja and Banks (1994a), which were in the range of 0.04-0.05, the difference might be due to the G_m value, which was used during the linear estimation of Equation 5.2. Borja and Banks (1994a) used a value for G_m which was estimated using a non-linear regression program with 95% confidence limit whereas K_o values were estimated in the current study by making use of the specific function of $G = G_m^{-k/t}$.



Figure 5.1 Determination of coefficient K_o for First Order Model according to Borja and Banks (1994a)

The K_o values between both studies are comparable and application of the specific function for the estimation is simpler as it only involves straight line-fitting on the transformed data as shown in the previous chapter. It also appears that, the non-linear program method estimated slightly higher G_m values based on the calculated K_o values. There are other mathematical approaches to estimate the G_m value. Eckenfelder (1970 see Ramalho, 1977) used a method of Log-Difference, while Metcalf and Eddy (1991) used the method of Fujimoto and the method of Least-Squares. These different methods are compared below.

5.1.1 Method of Log-Difference

This method was formulated to estimate the ultimate O_2 consumption during BOD analysis. Modification of this approach could be used to estimate the maximum G_m , utilizing the expression given in Equation 5.3.

$$G_m = \frac{A}{2.303 |B|}$$
 (Equation 5.3)

A = intercept value for the plot of $\partial G/\partial t$ against time

$$B = \frac{\left[\log\left(y'_{1}/y'_{2}\right)\right]}{t_{1} - t_{2}}$$
$$y' = \frac{\partial G}{\partial t}$$
$$y'_{1} = \frac{\partial G}{\partial t} \text{ at } t_{1}$$
$$y'_{2} = \frac{\partial G}{\partial t} \text{ at } t_{2}$$
$$t = \text{time}$$

To determine the coefficients for estimation of G_m , a smooth best-fit curve of G values was plotted through the time data points. The tangent $(\partial G/\partial t)$ for the curve at *t*-time reveals the rate of CH₄ production, which, in this case, was gradually decreasing with each FV. Values of $\partial G/\partial t$ (also known as y') were estimated at $\partial t = 4$, and are shown in Figures 5.2 - 5.4 with the calculated data listed in Table 5.2. However, estimation of y' for FV4 proved to be problematic as the curve was not sufficiently smooth as shown in Figure 5.5. Straight line-fitting of y' against time as shown in Figure 5.6-5.8 was undertaken to determine the coefficients in Equation 5.3. y' which was less than 10 in Figure 5.6 was disregarded which allowed a more smooth line fitting to be achieved. The derived coefficients and values of G_m using Equation 5.3 are listed in Table 5.3.



Figure 5.2 Smooth curve-fitting for CH₄ production of FV1

Table 5.2Estimation of tangent of CH_4 production rate ($\partial G/\partial$) according to smooth curve fitting
for each FV

Time	<i>G</i> - FV1	<i>Æ[.a.</i> - FV1	G-FV2	<i>&GA</i> -FV2	G-FV3	<i>∂G/∂</i> -FV3
0	0		0		0	
4	263	66	125	31	150	38
8	450	47	250	31	275	31
12	575	31	350	25	425	38
16	663	22	450	25	550	31
20	725	16	550	25	675	31
24	763	10	650	25	800	31
28	800	9	725	19	925	31
32	825	6	825	25	1025	25
36	850	6	875	13	1125	25
40	863	3	950	19	1225	25
44	875	3	1025	19	1325	25
48	885	3	1075	13	1400	19
52	895	3	1125	13	1450	13
56	910	4	1175	13	1500	13
60	920	3	1200	6	1525	6
64	930	3	1225	6	1550	6
68	940	3	1250	6	1575	6
72	950	3	1275	6	1600	6



Figure 5.3 Smooth curve-fitting for CH_4 production of FV2



Figure 5.4 Smooth curve-fitting for CH₄ production of FV3



Figure 5.5 Smooth curve-fitting for $\rm CH_4$ production of FV4

Coefficient	FV 1 (0.5L)	FV2 (0.8L)	FV3 (1.0L)
A	70.2	32.4	41.3
ו'ע	70.2	32.4	41.3
y'2	16	6	13
	0	0	0
<i>t</i> ₂	20	68	56
В	0.032	0.011	0.009
G_m	950	1310	1990

Table 5.3Coefficients of Method Log-Difference and estimation of G_m according to the plot of y'
against time



Figure 5.6 Determination of coefficients for Method of Log-Difference: FV1



Figure 5.7 Determination of coefficients for Method of Log-Difference: FV2



Figure 5.8 Determination of coefficients for Method of Log-Difference: FV3

5.1.2 Method of Fujimoto

Metcalf and Eddy (1991) also used the Method of Fujimoto to estimate ultimate O_2 consumption in the BOD test. This method involves an arithmetic plot of gas G_{t+1} against G_t , whilst, on the same graph, a standard line with a slope of 1 is drawn. The value of intersection of the two lines corresponds to the maximum gas production. Figures 5.9 to 5.12 show the plot of G_{t+1} against *G* for each feed volume (FV). Some data has been omitted from the graphs to make the intersection possible. Again, different values of G_m were obtained using this method as follows: 1080ml for FV1, 1450 ml for FV2, 3500 ml for FV3, and 830 ml for FV4.

5.1.3 Method of Least-Square

The Method of Least-Square as used by Metcalf and Eddy (1991) is again a method originally applied to the BOD test. It involves fitting a curve through a set of data points in such way that the sum of the squares residuals (the difference between observed data and value of fitted curve) must be a minimum. It is similar to the study of residuals, however the method involves the estimation of G_m with the time series of CH₄ production. The following equations represent the mathematical procedure of estimation.

 $\partial G/\partial t_i = m (G_m - G_i)$ m = constant (Equation 5.4)

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In this equation, both m and G_m are unknown. It is assumed that $\partial G/\partial$ represents the value of the slope of the curve to be fitted through all the data points for a given m and G_m value. Because of the experimental error, the two sides of Equation 5.4 are equal but differ by an amount equal to R, hence Equation 5.4 can be rewritten as:

$$R = m (G_m - G_i) - \partial G / \partial t_i \qquad (Equation 5.5)$$

The equation is simplified, y is noted for G and y' as $\partial G/\partial$, this gives:

 $R = m (G_m - y) - y'$

(Equation 5.6)



Figure 5.9 Estimation of G_m by Method of Fujimoto for FV1



Figure 5.10 Estimation of G_m by Method of Fujimoto for FV2



Figure 5.11 Estimation of G_m by Method of Fujimoto for FV3



Figure 5.12 Estimation of G_m by Method of Fujimoto for FV4

Substituting α for $m.G_m$ and - β for m

$$R = \alpha + \beta y - y'$$
 (Equation 5.7)

If the sum of the squares of the residuals R is to be minimum, the following equations must hold:

$$\partial \partial \alpha \Sigma R^2 = \Sigma 2r \partial R / \partial \alpha = 0 \qquad (Equation 5.8)$$

$$\partial \partial \beta \Sigma R^2 = \Sigma 2R \partial R / \partial \beta = 0 \qquad (Equation 5.9)$$

If the indicated calculation in Equations 5.8 and 5.9 are carried out using the value of the residual R as defined by Equation 5.7, then the following set of equations result:

$$n\alpha + \beta \Sigma y - \Sigma y' = 0$$
 (Equation 5.10)

$$\alpha \Sigma y + \beta \Sigma y^2 - \Sigma y' y = 0$$

where $n = number of data points$

(Equation 5.11)

where n = number of data points

For determination of y and y', values from the fitted curve of CH₄ production as shown in Figure 5.2-5.4 and listed in Table 5.2 were reused. No determination at FV4 was carried out since the fitted-curve was not smooth. Table 5.4 shows the coefficients used in Equations 5.10 and 5.11 to derive G_m for each FV and the final estimated values of G_m .

Coefficient	FV 1 (0.5L) (n = 19)	FV2 (0.8L) (n = 19)	FV3 (1.0L) (n = 19)
Σy	14080	15100	19100
Σy^2	11612930	14983750	24186250
Σy'	240	320	400
Σу'.у	129880	217030	342030
β	- 0.04	- 0.01	- 0.01
α	40	25	33
G _m	1000	2500	3300

 Table 5.4
 Acquired coefficients and estimated G_ms by Method of Least-Square

5.1.4 Comparison of estimated G_m

Table 5.5 shows the comparison of estimated G_m values obtained by each of the methods described above for each data set corresponding to a specific FV. These estimated values of G_m were further fitted into Equation 5.2 and plotted in Figure 5.13 to 5.15. Acquired values of K_o taken from these figures are also listed Table 5.5 and enable a comparison of G_m values being fitted into the First Order Model by Borja and Banks (1994a) to be made. It shows that for FV1, the estimations made by all the methods are similar and give a value of G_m similar to that obtained using the specific function. Likewise, the values obtained for K_o were also similar except where calculated using the method of Fujimoto. However at higher loadings, corresponding to FV2, 3, and 4, only

the estimation of G_m by the Method of Log-Difference was similar to the estimation obtained using the specific function. The other methods all resulted in a higher estimation of G_m . In addition to a higher estimation of G_m , lower values of K_o were also obtained when data was fitted to the First Order Model.

Estimation by specific function could be used as the standard as it has shown a high correlation accuracy (R^2) for predicting of cumulative CH₄ production during batch operation. The specific function also offers a straight-forward method compared to those that have been previously suggested and does not require computer software capable of non-linear curve fitting.

Table 5.5 Comparison of estimated G_m by each method and K_o for each FV of batch operation

FV	FV 1		F	V2	FV3		FV4	
	G_m	Ko	Gm	Ko	Gm	K _o	Gm	Ko
Log -Difference	950	0.06	1310	0.04	2000	0.02	n.a.	n.a.
Fujimoto	1080	0.03	1450	0.04	3500	0.01	830	0.05
Least-Squares	1000	0.05	2500	0.01	3250	0.01	n.a.	n.a.
Specific function	1000	0.05	1380	0.03	1640	0.04	980	0.03



Figure 5.13 Determination of the coefficient K_o for the First Order Model by Borja and Banks (1994a) with G_m acquired from Method of Log-Difference



Figure 5.14 Determination of the coefficient K_o for the First Order Model by Borja and Banks (1994a) with G_m acquired from Method of Fujimoto



Figure 5.15 Determination of the coefficient K_o for the First Order Model by Borja and Banks (1994a) with G_m acquired from Method of Least-Square

Also non-linear curve fitting has been described by other researchers as not entirely satisfactory because it is sensitive to the initial guesses for the numerical values of coefficients to be calculated (Dinopoulou *et al.*, 1988). However the coefficient k in the specific function $(G = G_m^{k/t})$ has little physical meaning.

5.2 Application of Michaelis-Menten kinetic model

Most of the kinetic models utilized so far to describe anaerobic operation are based on the Michaelis-Menten relationship. As previously noted (section 2.3.2) this kinetic relationship is as in Equation2.13

$$r_S = \frac{r_m \cdot S_e}{K_m + S_e}$$

 r_S = substrate utilisation rate (mg.l⁻¹.d⁻¹) r_m = maximum substrate utilisation rate (mg.l⁻¹.d⁻¹) S_e = effluent strength (mg.l⁻¹.) K_m = Michaelis-Menten constant (mg.l⁻¹)

and expression of the coefficient r_s depends on a mass-balance of the operation. It has been expressed in Equation 2.1 as:

$$\partial S/\partial t . V = Q.(S_o - S_e) - r_S . V$$

 $\partial S/\partial t$ = net substrate concentration (mg.l⁻¹.d⁻¹)

V =working volume (l)

 S_o = influent strength (mg.l⁻¹)

If it is a batch operation, the coefficient Q is equal to zero. Thus,

 $\partial S/\partial t = r_S$

If it is a fed-batch operation, in which pseudo steady-state operation is achieved, then $\partial S/\partial A$ is equal to zero and gives the Equation 2.19:

$$r_s = (S_o - S_e) / HRT$$

HRT = hydraulic retention time

5.2.1 Application of batch operation kinetic model for a readily biodegradable wastewater

The CH_4 production from a batch operation using the readily degradable wastewater behaved in accordance with a first order kinetic relationship and substrate removal can be represented by Equation 2.17:

$$r_S = \partial S / \partial t = k \cdot S$$

S = substrate concentration

k = constant

Integrating this equation gives:



$$\ln\left(S_e / S_o\right) = k \cdot t$$

From which a prediction of S_e could be made by rearrangement of the integrated equation 5.13

 $S_e = S_o^{kt}$ (Equation 5.13)

The coefficient k for this equation can be determined by plotting ln (S_e / S_o) against time as shown in Figure 5.16. The transformed data are listed in Table 5.6. S_o was taken at time zero, while S_e was measured at each of the sampling times for each feed volume (FV). The values of the constant k derived (for each value of FV) from the plot are also included in Table 5.6. These are all around 0.02 h⁻¹ except in the case of FV4. where substrate removal failed due to the reactor turning "sour".

Table 5.6 Prediction of the constant k using transformed data from the equation $S_e = S_o^{kt}$ for each feed volume.

Time (hour)	$ \operatorname{Ln}\left(S_e/S_o\right) \\ : \mathrm{FV1} $	Ln (<i>S_e/S_o</i>) : FV2	Ln (S _e /S _o) : FV3	$ \begin{array}{c} \operatorname{Ln}(S_e/S_o) \\ : \mathrm{FV4} \end{array} $
0	0	0	0	0
1	- 0.06	- 0.01	- 0.02	- 0.01
12	- 0.58	- 0.66	- 0.34	- 0.38
24	- 0.80	- 0.78	- 0.50	- 0.42
48	- 0.91	- 1.15	- 1.33	- 0.58
72	- 1.32	- 1.42	- 1.57	- 0.64
k	- 0.02	- 0.022	- 0.024	- 0.011



Figure 5.16 Determination of the constant k for batch operation based on a first order model.

Table 5.7 compares the observed data and estimated data for S_e for FV1, FV2, and FV3 with the constant k set to 0.02 h⁻¹ for the batch operation. A study of residuals on the overall data shows the estimation of S_e is disputable. Calculation of the coefficient of determination value (R^2) shows the overall data was 71% accurate (if $R^2 = 1$ it is considered as 100% accurate). Considering each individual FV data set then the estimation for FV1 was satisfactory as it gave R^2 t be 0.75 indicating the data to be 75% accurate. While for FV2 and FV3, the accuracy was only at 67% and 69% respectively. As such the result shows that Equation 5.13 does not provide a good estimate of S_e strength at infinite time.

5.2.2 Application of the fed-batch operation kinetic model to a readily biodegradable wastewater

The kinetic of a CSTR fed-batch anaerobic bioreactor treating a readily degradable wastewater was confirmed to behave according to a first order kinetic relationship. Thus, the mathematical equation can be written using a simplified Michaelis-Menten relationship such as:

$$r_S = k \cdot S_e$$

Assuming the fed-batch operation is at a steady-state condition, then this equation can be expanded to:

$$(S_o - S_e) = k \cdot S_e$$
HRT

and if the constant k is known, the equation could be used to predict the operation of the treatment processes. With modification of the First Order Model then estimation of S_e can be achieved when other parameters i.e. influent strength (S_o) and HRT are known using Equation 5.14,

$$S_e = S_o / (1 + k . HRT)$$
 (Equation 5.14)

Time (hour)	Observed S_e (mg.l ⁻¹)	Estimated S_e (mg.l ⁻¹) with Eq. 5.13; $k = 0.02 \text{ h}^{-1}$	Residuals		
$FV1: S_o = 820 \text{ mg.}\Gamma^1$					
1	770	760	10		
12	460	360	100		
24	370	230	140		
48	330	130	200		
72	220	50	170		
	Σ[<i>yy</i>]= 388200	$R^2 = 0.75$	$\Sigma [rr] = 98600$		
	FV	$V2: S_o = 1330$	······································		
1	1320	1290	30		
12	690	540	150		
24	610	380	230		
48	420	160	260		
72	320	80	240		
	Σ[<i>yy</i>]= 611500	$R^2 = 0.67$	$\Sigma [rr] = 201500$		
	FV	$3: S_o = 1400$			
1	1370	1340	30		
12	1000	790	210		
24	850	530	320		
48	370	140	230		
72	290	70	220		
	Σ[<i>yy</i>]= 810500	$R^2 = 0.69$	$\Sigma [rr] = 248700$		
Overall	Σ[<i>yy</i>]= 1910600	$R^2 = 0.71$	$\Sigma [rr] = 548800$		

Table 5.7Comparison of observed data and estimated data for S_e using the First Order Model
applied to batch operation

The constant k can be obtained from a straight line-fitting of the data plot of r_s against S_e as shown in Figure 5.17, this gives a value of 1.25 d⁻¹. The high strength substrate was excluded from the determination as it had previously been shown not conform to the First Order Model. Comparisons between estimated and observed data are listed in Table 5.8 and are used to verify Equation 5.14. The study of residuals gave a satisfactory result with coefficient R^2 equal to 0.77.



Figure 5.17 Determination of the constant k in a First Order Model of Michaelis Menten relationship over a range of strengths of readily degradable wastewater

Table 5.8 Comparison of observed data and estimated data of S_e by First Order Model of Michaelis-
Menten relationship with an overall constant k

HRT (d)	$\left \begin{array}{c} S_o \\ (\text{mg.l}^{-1}) \end{array} \right $	Observed S_e (mg. Γ^1)	Estimated S_e (mg.l ⁻¹) with Eq. 5.14; k = 1.25 d ⁻¹	Residuals
		Low strength	n influent	1,
20	4620	250	180	70
18	4650	220	200	20
16	4530	280	220	60
14	4600	290	250	30
12	4620	280	290	10
10	4530	270	340	70
8	4660	350	420	70
6	4350	400	480	80
4	4280	950	710	240
		Σ[<i>yy</i>]= 407200	$R^2 = 0.78$	$\Sigma [rr] = 90100$
		Medium-streng	th influent	
10	5260	340	390	50
9	5580	370	460	90
8	5390	320	490	170
7	5240	420	540	120
6	6510	600	770	170
5	6270	920	870	50
		Σ[<i>yy</i>]= 267700	$R^2 = 0.68$	$\Sigma [rr] = 85300$
	Overall	Σ[<i>yy</i>]= 735000	$R^2 = 0.76$	$\Sigma [rr] = 175400$



Figure 5.18 Determination of the constant k in a First Order Model using the Michaelis-Menten relationship for each specific strength of readily degradable wastewater

To check the use of an overall constant k, another approach was taken into consideration. This obtained the individual constant k according to substrate strength the results of which are shown in Figure 5.18. It was found that k was equal to 1.07 d⁻¹ and 1.41 d⁻¹ for low and medium substrate strengths respectively. The values of k were incorporated into Equation 5.14 for verification. The study of residuals was carried out to compare the estimates with the observed data as listed in Table 5.9. This showed that it was more satisfactory to use values for the individual constant k as this gave an overall R^2 equal to 0.8.

Estimation of an individual value for k for the medium-strength substrate gave an R^2 of 0.8 compared to an R^2 0.68 using a general value for k. In the case of the low strength substrate the R^2 equal to 0.78 irrespective of whether an individual value for k was used or not. Although the application of individual calculated values of k is more accurate for prediction, an overall constant k was more useful as it could be used for any substrate strength, except with high strength substrate. There was not a great difference in accuracy by application of an overall constant k compared to the individual constant.

HRT (d)	$\begin{array}{c} S_o \\ (\text{mg.I}^{-1}) \end{array}$	Observed S_e (mg. Γ^1)	Estimated S_e (mg. Γ^1) with Eq. 5.14	Residuals				
	Low strength influent; $k = 1.07$							
20	4620	250	210	40				
18	4650	220	230	10				
16	4530	280	250	30				
14	4600	290	290	0				
12	4620	280	330	50				
10	4530	270	390	120				
8	4660	350	490	140				
6	4350	400	590	190				
4	4280	950	810	140				
		Σ[<i>yy</i>]= 407200	$R^2 = 0.77$	$\Sigma [rr] = 94800$				
		Medium-strength in	1fluent; <i>k</i> = 1.41					
10	5260	340	370	30				
9	5580	370	390	20				
8	5390	320	430	110				
7	5240	420	600	180				
6	6510	600	660	60				
5	6270	920	990	70				
		Σ[<i>yy</i>]= 267700	$R^2 = 0.80$	$\Sigma [rr] = 54300$				
	Overall	Σ[<i>yy</i>]= 735000	$R^2 = 0.80$	$\Sigma [rr] = 149100$				

Table 5.9 Comparison of observed data and estimated data of S_e by First Order Model of Michaelis-Menten relationship with a specific constant k assigned to each group of S_e

For the high strength substrate or when using a readily degradable wastewater it appears that the kinetic is between first and zero order . It yields values for the coefficient $r_{S max}$ and K_m of 1670 mg.l.d⁻¹ and 310 mg.l⁻¹, respectively. These values are similar to those derived in the study by Borja and Banks (1994b) on the same substrate with a different reactor design; here the values for r_m and K_m were 900 mg.l.d⁻¹ and 250 mg.l⁻¹, respectively. To verify these values, they were fitted back into Michaelis-Menten equation:

$$r_S = \frac{r_m \cdot S_e}{K_m + S_e}$$

Table 5.10 gives a comparison between the observed r_s and the estimated r_s , verifies the values for the coefficients $r_{S max}$ and K_m , when coefficient R^2 in the studies of residual was equal to 0.97.

Table 5.10Comparison between the observed and estimated r_s according to a Michaelis-Menten
kinetic relationship for the fed-batch operation of readily degradable wastewater at high
strength

HRT (d)	S_e (mg.l ⁻¹)	Observed r _s (mg.l ⁻¹ .d ⁻¹)	Estimated r_s with Eq. 2.13; K_m = 310; r_m = 1670	Residuals
10	390	770	750	20
9	430	820	760	60
8	430	950	990	40
7	520	1060	1000	60
6	570	1200	1170	30
5	690	1580	1350	230
		$\Sigma[yy] = 1904000$	$R^2 = 0.97$	$\Sigma [rr] = 63000$

5.2.3 Application of the fed-batch operation kinetic model to a solid-free OFMSW leachate

Kinetic studies on a solid-free OFMSW leachate showed that it could not be represented by a Michaelis-Menten kinetic relationship as shown in Figure 4.33 (section 4.6.1). Mtz-Virtutia and Mata-Alvarez (1996) suggested a simple method of fitting a first order kinetic model from a mass-balances equation:

$$\partial S/\partial t = Q(S_o - S_e) - r_S \cdot V$$

and from this they determined the coefficient r_s as a constant. For a steady-state condition, the equation can be rearranged as Equation 5.15. However, Figure 5.19 shows the constant r_s for this equation could not be determined by the plot of $S_o - S_e$ against HRT using the data from Table 4.27.

$$S_o - S_e = r_s$$
. HRT (Equation 5.15)

Although the kinetic for a solid-free leachate could not be determined by both kinetic approaches, there appeared to be another way of estimating this by acquiring the specific substrate removal efficiency. As shown in Table 4.27, the reactors gave an almost consistent COD removal efficiency. Rearrangement of the mathematical equation for the specific COD removal efficiency (Equation 4.10):

$$E_r = 100 (S_o - S_e) / S_o$$

gives Equation 5.16

$$S_e = 100 S_o - [E_r \cdot S_o]$$
 (Equation 5.16)
100

 E_r = specific COD removal efficiency (%)

Table 5.11 shows a comparison of observed data and estimated data with the application of constant E_r equal to 90%, which was the average of COD removal efficiency during the operation. Studies of residuals could not determine the accuracy of the estimation as R^2 gave a negative value, though the residuals among both data sets remained relatively low. A most probable reason for this was the low variation in observed S_e data which were represented by [yy]/n. To proceed with statistical comparison, the *t*-*test* was used to compare two groups of data. There was no significant difference between the means of both the observed S_e and estimated S_e according to the *t*-test at level $\alpha = 5\%$. Thus, anaerobic CSTR operation for the treatment of a solid-free OFMSW leachate can be predicted with a specific constant E_r up to an organic loading rate (OLR) of 1.7 kg.m⁻³.d⁻¹.

HRT	$\begin{array}{c}S_{o}\\(\mathrm{mg.I}^{-1})\end{array}$	Observed S_e (mg.l ⁻¹)	Estimated S_e (mg.l ⁻¹) with Eq. 5.16; $E_r = 90\%$	Residuals
20	2680	310	270	40
18	3400	340	340	0
16	2710	340	270	70
14	3290	310	330	20
12	2670	320	270	50
10	2470	340	250	90
8	3520	290	350	60
7	3400	280	340	60
6	4060	310	410	100
5	3440	270	340	70
4	3920	240	390	150
3	4050	260	410	150
2	3350	250	340	90
	Average	297	332	
	[<i>rr</i>] =	92700		
	[<i>yy</i>]=	14600		
	$R^2 =$	-5.4		
	t-test (2 tails)	0.14		
	t -inverse $\alpha = 0.05$	2.06		

Table 5.11 Comparison of observed data and estimated data for S_e by specific removal efficiency from a solid-free OFMSW leachate

4000 - 3500 - (55 2500 - 8 200 - 9 200 - 9 200 - 9 200 - 9 1500 - 9 100 - 50 -				0		
0	4	8	12	16	20	24
			HRT (d)			

Figure 5.19 Determination of a fast first-order kinetic constant as suggested by Mtz-Viturtia and Mata-Alvarez (1996)

5.2.4 Application of fed-batch operation kinetic model to OFMSW mixed leachate

Fed-batch operation of an OFMSW mixed leachate at OLR up to 1.95 kg.m⁻³.d⁻¹ was shown using a Michaelis-Menten kinetic analysis (section 4.7.2) to be of a first order. Equation 5.14:

$$S_e = S_o / (1 + k. \text{ HRT})$$

and modification of the First Order Model was applicable. The plot shown in Figure 5.20 was used to determine the constant k for the equation. Previous discussion on determination of k over a wide-range of S_o strengths showed an overall constant k could represent the kinetic of the model for a specific S_o . However, the straight-line fitting for a plot of r_s against S_e in Figure 5.20 was not satisfactory, as it did not represent the data.

Table 5.12 shows a comparison of observed S_e and estimated S_e using the derived constant k from the straight line-fitting which yielded a value of 2.11d⁻¹. The coefficient R^2 could not determine the accuracy of the estimation as it gave a negative value. However, as there was no significance difference shown by the *t*-test at $\alpha = 5\%$ this supports the use of the First Order Model to accurately represent a fed-batch operation of OFMSW leachate. However, the COD removal efficiency as listed in Table 4.30 showed that it remained above 90% until the end of the operation. The average removal efficiency was 94% and this value was used as the specific COD removal in Equation 5.16 for the estimation of S_e .



Figure 5.20 Determination of constant k in First Order Model of Michaelis-Menten relationship for fed-batch operation of OFMSW leachate

HRT (d)	S _o (mg.l ⁻¹)	Observed S _e (mg.l ⁻¹)	Estimated S_e (mg.l ⁻¹) with Eq. 5.14; k = 2.11 d ⁻¹	Residuals
		OFMSW	leachate	
20	20 7700 420		180	240
18	7920	440	200	240
16	7370	460	210	250
14	7490	450	250	200
12	9630	510	370	140
10	9840	510	450	60
8	9610	9610 510 540		30
6.5	8740	500	590	90
6	8690	560	640	80
5.5	8290	500	660	160
5	8130	550	700	150
4.5	7420	570	710	140
4	7810	590	930	340
	Average			
	Σ[<i>yy</i>]=	33400		
	$\Sigma [rr] =$	439600		
	R^2	-12.16		
	<i>t</i> -test (2 tails)	0.84		
	t -inverse $\alpha = 0.05$	2.06		

Table 5.12Comparison of observed data and estimated data for S_e by a First Order Model of the
Michaelis-Menten relationship for treatment of an OFMSW leachate

Table 5.13 gives a comparison of observed and estimated values of S_e determined using Equation 5.16. A study of residuals could not determine the difference between the observed and estimated data as the coefficient R^2 produced a negative value. However, with a *t*-test at $\alpha = 5\%$ no significant difference between the mean of observed data and the estimated data was detected. The observed data remained consistently lower than the estimated data at all HRT's. Estimation of the batch fed operation can be done with the specific constant E_r reliably up to an OLR of 1.95 kg.m⁻³.d⁻¹.

HRT	So (mg.l ⁻¹)	Observed S_e (mg.l ⁻¹)	Estimated S_e (mg. Γ^1) with Eq. 5.16; $E_r = 94\%$	Residuals
20	7700	420	540	120
18	7920	440	550	110
16	7370	460	520	60
14	7490	450	520	70
12	9630	510	670	160
10	9840	510	690	180
8	9610	510	670	160
6.5	8740	500	610	110
6	8690	560	910	350
5.5	8290	500	580	80
5	8130	550	570	20
4.5	7420	570	520	50
4	7810	590	550	40
	Average	510	810	
	[<i>rr</i>] =	264100		
	[<i>yy</i>] =	33400		
	$R^2 =$	- 6.9		
	<i>t</i> -test	0.001		
	t-inverse α = 0.95	2.06		

Table 5.13Comparison of observed data and estimated data for S_e using the specific removal
efficiency for an OFMSW leachate

5.2.5 Development of a kinetic model for an OFMSW leachate treatment using a combination of fed-batch and continuous operation data.

Kinetic studies on the anaerobic digestion of OFMSW leachate were carried out at higher OLR using a combination of fed-batch operation and continuous-fed operation with an anaerobic filter (AF) reactor. The same concept of using a Michaelis-Menten kinetic relationship was applied to the combined operation using Equation 2.13 as follows:

$$r_S = \frac{r_m \cdot S_e}{K_m + S_e}$$

 r_m = maximum substrate utilisation rate K_m = Michaelis-Menten constant

This equation represents the kinetic relationship between first order and zero order. Borja *et al.* (1995) states that such an equation contains no term to relate the input S_o with the output S_e , implying this kinetic model is unable to estimate the S_e . However to validate the kinetic model, a comparison between observed and estimated values of the coefficient r_s was undertaken. The kinetic analysis derived from Figure 4.39 showed that the constant r_m and K_m were equal to 5000 mg.l⁻¹.d⁻¹ and 1300 mg.l⁻¹, respectively. The observed coefficient r_s was calculated using Equation 2.19:

$$r_S = (S_o - S_e) / HRT$$

Table 5.14 shows the comparison of observed and estimated values of the coefficient r_s for the combined anaerobic operation. An analysis of residuals showed the coefficient R^2 was equal to 0.78 indicating an excellent agreement between the two data sets. The data generated from the combination anaerobic operation could be used to verify the application of the model to the treatment of OFMSW leachate. It also confirmed that the kinetic relationship changes from first order to zero order at an OLR between 2.3 and 3.4 kg.m⁻³.d⁻¹as shown in Figure 4.39.

5.3 Application of the Monod Model

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The Monod Model was introduced to study microbial growth rate in relation to limitation of substrate concentration. The effect of limiting substrate upon the specific growth rate (μ) equation is shown in Equation 2.20:

$$\mu = \frac{\mu_m \cdot S_e}{K_s + S_e}$$

$$\mu = \text{specific growth rate}$$

$$\mu_m = \text{maximum specific growth rate}$$

$$S_e = \text{concentration of the growth-limiting substrate}$$

$$K_s = \text{substrate concentration at half-velocity of the growth rate}$$

HRT (d)	$\begin{array}{c} S_o \\ (\text{mg.I}^{-1}) \end{array}$	$\begin{array}{c} S_e \\ (\text{mg.I}^{-1}) \end{array}$	Observed r_s (mg.l ⁻¹ .d ⁻¹)	Estimated r_S with Eq. 2.13; K_m = 1300; r_m = 5000	Residuals
6	8690	560	1360	1510	150
5.5	8290	500	1420	1390	30
5	8130	550	1520	1490	30
4.5	7420	570	1520	1520	0
4	7810	590	1810	1560	250
3	5470	550	1640	1490	150
2.5	5600	600	2000	1580	420
2	6860	1400	2730	2590	140
			$\frac{\Sigma[\ yy\]}{1405400}$	$R^2 = 0.78$	$\sum [rr] = 305300$

Table 5.14Comparison of coefficients for observed and estimated r_s values using the Michaelis-
Menten kinetic relationship on a OFMSW leachate treatment system.

Lawrence and McCarthy (1969) modified the equation to take into account the biomass yield (Y), this is shown in Equation 2.23:

$$r_S = \frac{\mu_m \cdot S_e \cdot X}{Y \cdot [K_S + S_e]}$$

and where $[\mu_m / Y = k]$, the coefficient k being known as the maximum substrate utilisation rate per unit of biomass, as listed in Equation 2.24:

$$r_S = \frac{k \cdot S_e \cdot X}{[K_S + S_e]}$$

Meanwhile, Equation 5.17 is the equation used by Lineweaver-Burk plot to obtain a straight-line fit for the Monod Model,

$$\frac{X}{r_s} = \frac{K_s}{k} \cdot \frac{1}{S_e} + \frac{1}{k}$$
 (Equation 5.17)

The Monod Model is similar to the Michaelis-Menten kinetic relationship except it incorporates biomass growth factors, i.e. the coefficients of Y and μ_m , whereas the coefficient K_S is the substrate concentration at half of μ_m . In the Michaelis-Menten relationship the same concept is used for deriving K_m , this being the substrate concentration at half of r_m . Most of the experimental results behaved according to first order Michaelis-Menten kinetic relationship except for high strength readily biodegradable wastewater and the continuous-feeding operation of OFMSW leachate.

5.3.1 Relationship between the Monod Model with biomass retention

Uniform changes in biomass concentration were observed for each fed-batch operation, this can be seen in Figure 4.19 and indicates an interdependence exists between the coefficients, r_s and μ . To investigate this relationship a different equation for calculating the mean cell retention time (MCRT) was applied; this is shown in Equation 5.18

$$MCRT = \frac{X \cdot V}{Q_e \cdot X_e + Q_w \cdot X_w - Q_o \cdot X_o}$$
(Equation 5.18)

MCRT = solid retention time

X =biomass concentration

V = working volume

 Q_e = effluent hydraulic flow rate

 X_e = biomass concentration in effluent

 Q_w = wastage hydraulic flow rate

 X_w = biomass concentration in wastage

 Q_o = influent hydraulic flow rate

 X_o = biomass concentration in influent

The MCRT is defined as the average retention time of the biomass in the waste treatment system and is analogous to the sludge age concept of activated sludge (Metcalf and Eddy, 1991). In the experimental work undertaken the influent feed was considered free from biomass ($X_o = 0$) and no biomass was wasted ($X_w = 0$). Thus, Equation 5.18 can thus be modified to Equation 5.19.

$$MCRT = \frac{X \cdot V}{Q_e \cdot X_e}$$
 (Equation 5.19)

A mass-balance for biomass can be written as in Equation 5.20 and rearrangement of the equation with the assumption that $\partial X/\partial = 0$ for fresh influent and there is a negligible endogenous decay rate, this gives Equation 5.21. Most models incorporate the decay rate to take into account biomass loss. However, the decay rate was omitted in this study because the loss was not significant when the biomass were recycled at its maximum rate by using sedimentation within the process. It was also considered better to keep the mathematical model simple.

$$\partial X/\partial A \cdot V = Q_o \cdot X_o - Q_e \cdot X_e + r_G \cdot V$$
 (Equation 5.20)

$$r_G = \left[Q_e \cdot X_e \right] / V$$

(Equation 5.21)

 $\partial X/\partial =$ net biomass concentration in the reactor at time - t

V = working volume

 Q_o = influent hydraulic flow-rate

 X_o = biomass concentration in influent

 Q_e = effluent hydraulic flow-rate

 X_e = biomass concentration in effluent

 r_G = biomass growth rate

If we use Equation 2.21 ($r_G = Y.r_S$) instead of Equation 5.21 and divide by the biomass concentration this gives Equation 5.22. Therefore the right-hand side of Equation 5.22 is equal to the inverted equation used for calculating MCRT (Equation 5.19). Combination of both equations gives Equation 5.23.
$[r_{S} \cdot Y]/X = [Q_{e} \cdot X_{e}]/[V \cdot X]$ [r_{S} \cdot Y]/X = 1/[MCRT] r_{S} = substrate removal rate Y = biomass yield (Equation 5.22) (Equation 5.23)

Table 5.15 gives values of MCRT for the fed-batch operation of a low strength readily degradable wastewater, together with its biomass concentration (X) and calculated values of r_s . The slope of the line in a plot of [1/MCRT] against [r_s /X], from Equation 5.23, is shown in Figure 5.21 and yields the value of the coefficient Y in Equation 5.23, which is equal to 0.067. This approach is thus able to relate the biomass growth in a fedbatch operation with the Monod Model, although the coefficient r_s is represented by the First Order Model. Tables 5.16 and 5.17, along with Figure 5.22 and Figure 5.24 list the prerequisite parameters necessary to determine the coefficient Y which was determined to equal 0.026 and 0.063 for solid-free leachate and high strength leachate, respectively. These values fall within the acceptable range according to Metcalf and Eddy (1991), who list typical Y values for the anaerobic treatment process; these are 0.04-0.07 for fatty acid substrate, 0.025-0.035 for carbohydrate and 0.04-0.1 for domestic sludge.

HRT (d)	MCRT (d)	1/MCRT	r_S / X
20	255	0.0264	0.0039
18	251	0.0289	0.0040
16	379	0.0312	0.0026
14	376	0.0370	0.0027
12	311	0.0436	0.0032
10	289	0.0547	0.0035
8	296	0.0729	0.0034
6	184	0.0893	0.0054
4	124	0.1171	0.0081

 Table 5.15
 Parameters used to determine the coefficient Y for a fed-batch operation to treat a low strength wastewater

HRT (d)	MCRT (d)	1/MCRT	r_S/X
20	928	0.0011	0.0011
18	817	0.0012	0.0012
16	457	0.0022	0.0022
14	598	0.0017	0.0017
12	462	0.0022	0.0022
10	300	0.0033	0.0033
8	903	0.0011	0.0011
7	811	0.0012	0.0012
6	723	0.0014	0.0014
5	737	0.0014	0.0014
4	470	0.0021	0.0021

 Table 5.16
 Parameters used to determine the coefficient Y for a fed-batch operation to treat a solid-free OFMSW leachate

 Table 5.17 Parameters used to determine the coefficient Y for the fed-batch operation treating a high-strength OFMSW leachate

HRT (d)	MCRT (d)	1/MCRT	r_S/X
20	502	0.0020	0.0284
18	490	0.0020	0.0300
16	400	0.0025	0.0339
14	452	0.0022	0.0306
12	304	0.0033	0.0600
10	269	0.0037	0.0709
8	231	0.0043	0.0877
6.5	174	0.0057	0.0945
6	196	0.0051	0.1010
5.5	137	0.0073	0.1018
5	126	0.0079	0.1054
4.5	138	0.0072	0.1034
4	119	0.0084	0.1286



Figure 5.21 Determination of the biomass yield coefficient for a fed-batch operation using a low strength readily degradable wastewater



Figure 5.22 Determination of the biomass yield coefficient for a fed-batch operation using a solidfree leachate

Rearrangement of Equation 5.23 as Equation 5.24 can be used to determine the biomass concentration when the rest of the required parameters are known. When this equation is incorporated into the Monod equation, it gives Equation 5.25, which is useful for the prediction of effluent substrate concentration according to the Monod Model.

$X = r_s \cdot Y \cdot MCRT$	(Equation 5.24)
$S_e = K_s / [\mu_m . MCRT - 1]$	(Equation 5.25)

The Michaelis-Menten kinetic equation only describes the coefficient r_S and it cannot be used for prediction of effluent quality. But, by incorporating the equation for MCRT into the Monod Model then this can to be used to estimate the effluent quality when the kinetic relationship lies between first and zero order. However, as shown in Equation 5.19 the coefficient MCRT is dependent on the parameters X, V, Q_e and X_e . Also, for a reactor design such as the anaerobic filter (AF), a value for X is technically difficult to achieve because of the attached nature of the biomass within the reactor. Mtz.-Viturtia *et al.* (1995) applied Equation 5.26 to calculate the active biomass at time-*t* in a reactor of this nature.

$$X_t = X_o + r_G$$
 (Equation 5.26)
$$X_t = \text{biomass at time-}t$$

 X_o = initial biomass

 r_G = biomass growth rate

Substituting from Equation 2.21 [$r_G = Y.r_S$] then Equation . 5.26 gives:

$$X_t = X_o + Y.r_S$$
 (Equation 5.27)
 r_S = substrate removal rate
 Y = biomass yield

Table 5.18 gives the values of those parameters required in Equation 5.25. Values of X for HRT's of 3 days and below were estimated using Equation 5.27. The initial value taken for X_o was at 14 000 mg.l⁻¹ which was equal to the biomass concentration when it was transferred from the CSTR to the AF. The coefficient μ_m , was determined using the relationship $\mu_m = Y$. k which is derived from the Monod Model in Eq 2.23. The Lineweaver- Burk plot for the Monod Model using data from the combined OFMSW leachate treatment operation is shown in Figure 5.24. The cut-off value determines the value of coefficient k in Equation 5.17 and this was found to have a numerical value equal to 0.42. The slope of the graph determines the coefficient K_s and was found to be equal to 1580 mg.l⁻¹. Using a value of 0.067 (Figure 5.22), for the coefficient γ then the coefficient μ_m is equal to 0.027.

The results of the estimation of S_e using Equation 5.25 are shown in Table 5.18 and reveal that the effluent quality predicted by the Monod Model is dependent on the parameter MCRT, and not on the parameters S_o or HRT. However, both HRT and S_o are required to determine the prerequisite coefficients Y, K_s , k and μ_m . Andrews and Graef (1971) noted that the independence of parameter S_e from S_o in this model denotes that influent strength should be as high as possible for the most efficient utilisation of reactor volume.

The accuracy of estimation could not be evaluated using a study of residuals as a negative value was obtained. However, using the *t*-test at $\alpha = 5\%$, the difference between observed and estimated values of S_e were found to be insignificant. At a higher loading, i.e. at a HRT of 2.5 and 2.0 days, higher residual values were obtained and this might be due to the method of measurement of the biomass concentration in the effluent and subsequently used in the calculation of MCRT using Equation 5.19. The problem lies in

that it is impossible to differentiate between the active biomass and undigested VS content of the reactor, as VS analysis was initially considered to give a good approximation of biomass content. Even with this potential for error the estimated results imply that in order to maintain maximum treatment efficiency of the OFMSW leachate the MCRT should be at least 140days. There was a relatively low value of S_e residuals associated with a HRT of 3d, this indicates that the estimation of active biomass content (X_t) using Equation 5.27 is applicable when the measured VS content in the effluent consists mainly of biomass rather than undigested organic solids.

HRT	rs	X	Xe	MCRT	Observed S_e	Estimated S _e	Residuals
(d)	$(mg.l.d^{-1})$	$(mg.l^{-1})$	$(mg.l^{-1})$	(d)	$(mg.l^{-1})$	$(mg.l^{-1})$	
6	1360	13450	410	197	560	370	190
5.5	1420	13920	560	137	500	590	90
5	1520	14380	570	126	550	660	110
4.5	1520	14720	480	138	570	580	10
4	1810	14040	470	120	590	710	120
3	1640	14100	270	157	550	490	60
2.5	2000	14130	440	80	600	1360	760
2	2730	14170	660	43	1400	9810	8410

Table 5.18 Parameters used to estimate S_e for the combined operation to treat OFMSW leachate

ر]Σ	y]	71380100
Σ[1	r]	10041600
R	2	- 6.1
t-te	st	
(2 ta	ils)	0.30
t-inve	erse	
$(\alpha = 0)$).05)	2.13



Figure 5.23 Determination of the biomass yield coefficient for a fed-batch operation using an OFMSW leachate

$$S_o - \frac{CH_4 \text{ production}}{V \cdot CH_4 \text{ yield}} = S_e \qquad (Equation 5.29)$$

Table 5.19 shows a comparison of the observed and estimated (Equation 5.29) S_e values. The study of residuals showed that the coefficient R^2 varied according to the feed volume used, but in general showed this to be a satisfactory means of estimating effluent strength. Prediction of S_e for FV3 was almost perfect with the coefficient R^2 indicating a 98% accuracy; the accuracy for other FV's was less than 80%. The equation, in terms of its application, shows good potential as a calculation tool because the highest residuals were calculated to be at 400 mgl⁻¹ COD, whilst the rest of residuals were less than 250 mg.l⁻¹.

5.4.2 Estimation for a fed-batch operation

Calculation of CH₄ yield for a fed-batch operation was carried out in the same manner. Figure 5.25 shows the overall CH₄ yield for all strengths of the readily degradable wastewater and Figure 5.26 shows the data for OFMSW leachate. All strengths of the readily degradable wastewater gave a consistent value of 0.33 10^3 m³CH₄.kg⁻¹COD removed and the CH₄ yield for solid-free leachate (Figure 4.32) gave a value of 0.35 10^3 m³CH₄.kg⁻¹COD removed.

Equation 5.30 represents the total COD mass removal during a steady-state operation. Substitution of the equation for CH₄ yield into Equation 5.30 yields Equation 5.31. The left-hand side of Equation 5.31 is representative of substrate removal rate $[r_S]$. Thus if the kinetic of the substrate removal is a first order reaction, then Equation 5.32 represents the first order model based on CH₄. By rearranging the equation as Equation 5.33, then an estimation of effluent substrate concentration (*S_e*) can be made.

Table 5.19Comparison between observed and estimated values of S_e calculated from the CH4
yield and CH4 production data of a batch operation for the treatment of a readily
degradable wastewater

Time (hour)	CH ₄ (ml)	Observed S_e (mg.l ⁻¹)	Estimated S_e (mg.l ⁻¹)	Residuals
0	FV1	$S_o = 820$	-	-
1	110	770	740	30
12	590	460	370	90
24	730	370	270	100
48	880	330	150	180
72	940	220	110	110
		[<i>yy</i>] = 74200	[<i>rr</i>] = 63500	$R^2 = 0.64$
0	FV2	$S_o = 1330$	-	-
1	20	1320	1320	0
12	320	690	1090	400
24	700	610	800	190
48	1110	420	490	70
72	1240	320	390	70
		[<i>yy</i>] = 611500	[<i>rr</i>] = 205900	$R^2 = 0.66$
0	FV3	$S_o = 1400$	-	-
1	20	1370	1390	20
12	450	1000	1060	60
24	850	850	760	90
48	1330	370	390	20
72	1560	290	220	70
		[<i>yy</i>] = 809600	[<i>rr</i>] = 17400	$R^2 = 0.98$
0	FV4	$S_o = 1690$	-	-
1	20	1670	1680	10
12	660	1150	1190	40
24	710	1110	1150	40
48	730	950	1140	190
72	770	890	1110	220
		[<i>yy</i>] = 379600	[<i>rr</i>] = 87800	$R^2 = 0.77$



Figure 5.25 Overall CH₄ yield for a readily degradable wastewater at all strengths experimentally used



Figure 5.26 Overall CH₄ yield from OFMSW leachate treatment

 $[S_o - S_e] \cdot V/[HRT] = \text{Total COD mass removal} \quad (Equation 5.30)$ $\frac{S_o - S_e}{HRT} = \frac{CH_4 \text{ production}}{CH_4 \text{ yield } \cdot V} \quad (Equation 5.31)$ $[CH_4 \text{ production}] / [CH_4 \text{ yield } \cdot V] = k \cdot S_e \quad (Equation 5.32)$ $S_e = [CH_4 \text{ production}] / [CH_4 \text{ yield } \cdot V \cdot k] \quad (Equation 5.33)$ $S_e = effluent$ V = working volume HRT = hydraulic retention time

k =first order constant

Table 5.20 shows a comparison between the observed values for S_e and those estimated according to Equation 5.33. The overall constant k was derived from the previous First Order Model based on substrate removal rate (r_s) and was taken to equal $1.25d^{-1}$. The accuracy of the calculated values was shown to have a coefficient R^2 only equal to 0.48 on the medium strength wastewater.

Table 5.21 shows a comparison between estimated and observed S_e values for the treatment of OFMSW leachate. The study of residuals failed to determine the accuracy of the estimation since the coefficient R^2 was negative. A 2-tails *t*-test at $\alpha = 5\%$ showed that there was insignificant difference between the observed and estimated S_e values indicating the applicability of using the First Order Model based on CH₄.

Table 5.20	Comparison of observed and estimated values of S_e calculated using CH ₄ yield and
	daily CH ₄ production data from a fed-batch operation treating a readily degradable
	wastewater at different strengths

HRT (day)	CH ₄ (ml .d ⁻¹)	Observed S _e (mg.l ⁻¹)	Estimated S_e with Eq. 5.33; $k = 1.25 \text{ d}^{-1}$	Residuals
$S_o = low$				
20	300	250	100	60
18	430	230	210	10
16	430	220	210	70
10	520	200	250	10
12	570	290	250	40
12	600	200	230	70
0	800	270	340	10
6	1000	400	190	40
0	1000	400 -	400	220
	1270	[<i>yy</i>] = 407200	$P^2 = 0.68$	[rr] =
$S_o = \text{medium}$ -strength			<u> </u>	152000
10	880	340	430	90
9	950	370	460	90
8	1190	320	580	460
7	1230	420	600	180
6	1520	600	740	140
5	1780	920	860	60
		[<i>yy</i>] = 267700	$R^2 = 0.48$	[<i>rr</i>] = 139400
	Overall	[<u>yy</u>] = 762200	$R^2 = 0.64$	[<i>rr</i>]= 271400

HRT	CH ₄ (mg.l	Observed S _e	Estimated S _e	Residuals
	'.d'')	(mg.l ⁻¹)	with Eq. 5.33; $k = 2.11 \text{ d}^{-1}$	
20	480	420	140	280
18	530	440	240	200
16	720	460	320	140
14	840	450	370	80
12	1170	510	520	10
10	1650	510	740	230
8	2050	510	910	400
6.5	2230	500	990	490
6	2450	560	1090	530
5.5	2430	500	1080	580
5	2520	550	1120	570
4.5	2350	570	1050	480
4	2860	590	1280	690
	Average			

Table 5.21Comparison of observed and estimated S_e values calculated using CH₄ yield and daily
CH₄ production data from a fed-batch operation treating an OFMSW leachate

Average	
[<i>rr</i>] =	2246200
[yy] =	33400
$R^2 =$	- 66.3
<i>t</i> -test (2 tails)	0.021
t -inverse $\alpha = 0.05$	2.06

CHAPTER VI CONCLUSIONS

6.0 Conclusions

The phase-separation of the anaerobic digestion process for the treatment of the organic fraction of municipal solid waste (OFMSW), was shown to be viable. The leachate produced in the first phase proved to be readily degradable, and was more digestible than the readily degradable soft drink wastewater simulant that was used to derive test data for kinetic model evaluation. This was born out by the value of the substrate removal constant (k) being higher than that obtained for the soft drink wastewater. However, a complete phase-separation within the anaerobic digestion process is not possible due to the diphasic nature of the process, i.e. there will always be some conversion of the leachate from the hydrolysis-acidogenesis phase before transferring the flush liquors to the second stage digestion where the acetogenesis-methanogenesis phase predominates.

Rapid acclimatization to the readily degradable wastewater was successful using an initial inoculum taken from a reactor treating municipal wastewater sludge. The acclimatization required a period of about 10d after seeding before for stable conditions were reached. This acclimatized sludge was subsequently used as a seed for the OFMSW leachate in both the continuous stirred tank reactor (CSTR) and anaerobic filter (AF). In all cases acclimatization was rapid, irrespective of the deisgn or operational mode of the reactor, which supports the claim that the leachate was easily degradable. Although the range of reactor designs used to treat the leachate in the present study was limited, the fact that acclimatization in all the types tried was rapid supports the idea that other reactor designs, such as those detailed in section 2.1, could be adopted for leachate treatment.

The importance of operationally induced environmental factors in the stability of the process was apparent by the failure in digestion during the batch operation of the readily degradable wastewater (based on a soft drinks wastewater). The problem was identified to be an increase in acidity within the reactor which necessitated additional pH buffering substances to be added. The acidity itself was partially as a result of the natural acidity of the substrate, which comprised mainly of grape juice, fermentation products and a lack of natural buffering agents. As well as lacking buffering capacity, the wastewater also showed a deficiency in nitrogen which is necessary for biomass growth. A successful control regime was introduced to solve these two problems simultaneously by the having a daily addition of ammonium bicarbonate and sodium bicarbonate.

The experimental results from the study using a readily biodegradable wastewater (soft drinks wastewater) confirmed that this type of wastewater could be treated by fedbatch operation or in an anaerobic CSTR. The wastewater from other beverage manufacturing industries may differ considerably from that used in this study, and care should be taken in applying the results of this study to 'similarily named' wastewaters without first undertaking a characterization study. For example, wastewater from soft drink manufacturer may also have a substantial load of volatile solids if the process includes fruit pulping and a subsequent separation process. The results from this study aid in the selection of the design load, and even help in predicting the gas yield which can be useful in making preliminary economic assessments as to the viability of the process. But, the running of a pilot scale trial with the actual wastewater is usually the only definitive way to confirm the feasibility of an anaerobic treatment process.

The pH of the reactor proved not to be a problem when using leachate from a first stage hydrolysis/acidification reactor as the digestion substrate, it was also not necessary to add additional nitrogen during the CSTR fed-batch operation. The laboratory scale experiments showed that digestion of this substrate was relatively easy, especially if it was solid-free. Even using a mixed leachate (which contained solids), 90% of volatile solids (VS) content was removed yielding a consistent concentration of VS in the effluent (*S_e*), within the range of 450-550 mg.l⁻¹ was achieved. Again, it must be emphasized, that although laboratory based studies are indicative of likely performance they are no substitute for pilot scale treatability studies to verify final design.

Preliminary design figures that have been derived from the study show a CSTR system could treat both the readily degradable (simulated soft drink wastewater) and

solid-free leachate up to organic loading rate (OLR) of 1.7 kg.m⁻³.d⁻¹. Using mixed leachate a loading of up to 2.0 kg.m⁻³.d⁻¹ could be achieved without affecting the COD removal efficiency. For both types of wastewater the level of COD removal was above 85%. In the continuous flow feed experiment using a single AF reactor for the treatment of leachate it was possible to increase the loading up to an OLR of 2.3 kg.m⁻³.d⁻¹ without compromising the COD removal efficiency which remained at about 90%. At higher loadings efficiency was reduced to 80%, 57% and 0% in the OLR range between 3.4 - 4.2 kg.m⁻³.d⁻¹. It is likely that other modern anaerobic treatment plant utilizing the principal of biomass retention could also be used at high volumetric loadings, unfortunately, time did not permit an evaluation of these.

Kinetic studies on the batch operation of soft drink wastewater lead to the use of a specific mathematical equation for predicting the CH₄ production. The specific function, as listed in Equation 4.6, could be used to estimate the maximum production of CH₄; this overcomes the problem of having to run experiments for unduly long periods in an attempt to approach a theoretical infinite time. The method involves determining the cut-off value for the plot of *ln* gas production against unit of time⁻¹ and is simpler to achieve experimentally than other methods reported in the literature. The results of the estimation of *G_m* using this method was also found to be statistically better than other reported method such as Method of Log-Difference, Method of Fujimoto and Method of Least-Square. These alternative methods were originally derived for estimating the ultimate oxygen consumption in Biochemical Oxygen Demand (BOD) test, and this is where their weakness may lie.

Kinetic studies based round the CSTR and AF reactor systems were developed by application of Michaelis-Menten kinetic relationship and Monod Model. All the substrates used with the exception of the solid-free leachate showed a First Order kinetic relationship (Equation 2.16) up to critical loading. Analysis of the kinetic data in this way showed that the OFMSW leachate was more rapidly degradable than the original readily degradable (soft drink wastewater) used. The speed of biodegradation is expressed by means of the substrate removal constant (k), the value of which equaled to $2.11d^{-1}$ for the OFMSW leachate compared to $1.25d^{-1}$ for the soft drink wastewater.

Table 6.1 summarizes the key constants and equations identified as part of the kinetic studies which can be used for the estimation of effluent strength (S_e). These equations are applicable to all type of operation used in the study. R^2 values showed the accuracy of the estimation, and the *t*-*test* was used when R^2 analysis was not applicable for data interpretation, due to low variation of data values.

Type of operation	Equation	Accuracy
Batch operation:	$S_e = S_o^{kt}$ (Eq. 5.13)	
Soft drink wastewater	$k = 0.02 \text{ h}^{-1}$	$R^2 = 71\%$
Fed-batch operation:	$S_e = S_o / [1 + k.HRT]$	
Low OLR of soft drink	(Eq. 5.14)	$R^2 = 76 \%$
wastewater	$k = 1.25 \mathrm{d}^{-1}$	
Low OLR of leachate	$k = 2.11 \text{ d}^{-1}$	Statistically acceptable at $\alpha = 0.05$
Fed-batch operation: Low OLR of solid-free	$S_e = [100.S_o - E_r S_o] / [100]$ (Eq. 5.16) $E_r = 0.09/$	Statistically
Low OLR of leachate	$E_r = 94\%$	at $\alpha = 0.05$
<u>Continuous-feeding</u> <u>operation</u> : High OLR of leachate	$S_e = [K_s] / [\mu_m \cdot MCRT - 1]$ (Eq. 5.25) $K_s = 1580 \text{ mg.l}^{-1}$ $\mu_m = 0.027 \text{ d}^{-1}$	Statistically acceptable at $\alpha = 0.05$

Table 6.1 Application of the developed kinetic models to estimate the effluent strength

For batch operation, the kinetic follows a first order reaction as described by the Michaelis-Menten relationship, *Se* can be most successfully derived using Equation 5.13.

For fed-batch operation at a low organic loading rate (OLR), the kinetics were also first order. In this case, Equation 5.14 could be used to estimate S_e for both the soft drink wastewater and the leachate. As the equation suggests, the effluent strength is strongly influenced by the hydraulic retention time (HRT) and this must therefore be considered as a primary design parameter. A marginally better estimation of Se can be obtained for a fed batch reactor at a low OLR by making use of the E_r (substrate removal efficiency, which is a function of the influent wastewater strength). Equation 5.16 can thus be used for making predictions of *Se* in the low OLR fed batch reactor. The estimation of S_e for both substrates using both equations 5.14 and 5.16 were statistically acceptable and the latter was shown to be applicable up to an OLR of 1.9 kg.m⁻³.d⁻¹ and 2kg.m⁻³.d⁻¹ for solid-free leachate and mixed leachate, respectively.

In order to achieve higher OLR's with the strength of wastewaters used continuous flow studies were necessary. In this case the kinetic of the reaction changed from First Order to Zero order, and the estimation of S_e can better be derived from the Monod Model as described by Equation 5.25. However, the method requires values for the coefficients μ_m , Y and k to be determined from multi-step calculation. Other models to calculate S_e are rare and it appears much more complicated to experimentally derive values for the coefficients needed for their use, for example the Inhibition Model and the Haldane Model.

Table 6.2 shows the equations that can be usefully used for the estimation of S_e using gas production data.

Type of operation	Equation	Accuracy
Batch operation: Lower OLR of soft drink wastewater	$S_e = S_o - [CH_4 \text{ production } / CH_4 \text{ yield } . V]$ (Eq. 5.29) CH ₄ yield = 0.33	77%
<u>Fed-batch operation</u> : Low OLR of soft drink wastewater	$S_e = CH_4 / [CH_4 \text{ yield. } V. k]$ (Eq. 5.33) $CH_4 \text{ yield} = 0.31$ $k = 1.25 \text{ d}^{-1}$	64 %
Fed-batch operation: Low OLR of leachate	$S_e = CH_4 / [CH_4 \text{ yield. } V. k]$ (Eq. 5.33) $CH_4 \text{ yield} = 0.33$ $k = 2.11 \text{ d}^{-1}$	Statistically acceptable at $\alpha = 0.05$

Table 6.2 Application of developed kinetic models to estimate the effluent strength based on the CH₄ production

In an anaerobic system, it is possible to consider the volume of methane produced to be a linear function of the amount of substrate used. Therefore, for a batch operation, by replacing COD removal with CH_4 yield an estimate of S_e could be made using Equation 5.29. The results of the work presented support the view held by Marsili and Nardini (1985) that the relationship between CH_4 production and soluble organic influent is in many cases almost linear. This great advantage of this approach is that it yields a much simpler solution as it does not require the calculation of constants other than that for the CH_4 yield.

For a fed batch reactor the results presented show that the approach can be used if the kinetics of the substrate removal follow the first order reaction as described by the Michaelis-Menten relationship. CH_4 production and its yield are incorporated into a First Order Model for estimating the value of S_e making use of the substrate removal constant (k), as listed in Equation 5.33. In this case the advantage is more limited as it is again necessary to calculate a kinetic constant which gives a less accurate estimation of *Se* than the models shown in Table 6.1 for a similar reactor application.

The results presented support the potential use of a two-phase digestion system for the efficient treatment of the OFMSW yielding a good gas yield and low strength final effluent. Operational conditions within each phase can be controlled and adjusted to optimise its performance. The feasibility of operating a first stage 'flushing bioreactor' is supported in that the leachate from the hydrolysis-acidogenesic phase was shown to be readily biodegradable, even when compared to a soft drink wastewater. In the treatment of this leachate no additional nutrients were required to maintain the optimum reactor conditions and an efficiency of 92%, even when running up to an HRT of 4 days. The use of attached growth systems may give further advantages and a preliminary study using AF showed the system could treat an OLR up to 2.3 kg.m⁻³.d⁻¹ before the removal efficiency was reduced. In reference to substantiate these treatability results, further work is needed at a pilot scale as well as the testing of other commercial designs of high rate reactor systems to the treatment of these leachates.

Rather than working the kinetic modelling based on trials and errors, this study found that the application of the specific function $(G = G_m^{k/t})$ should be considered in a fed-batch operation. Considering the interval between feeding as a batch operation, a maximum potential gas production can be estimated during feeding. Application of $S_e =$ $S_o - [CH_4 \text{ production / CH}_4 \text{ yield } . V]$ is also worth considering in the same case of fedbatch operation. Estimation of the S_e can be preceded between feedings once the CH₄ production and its yield have been established. Application of batch operation kinetic model in fed-batch operation in future kinetic modeling for any substrate should be taken as the preliminary test. Estimation of the performance for each feeding will be an aid to monitor the stability of the operation before increasing OLR. That is, if the estimated S_e is much lower than the observed Se during feeding, the OLR should be retained to check whether the system is reaching the substrate limitation value, or perhaps still acclimatizing to the new loading. Attaining the range of substrate limitation values will further assist the development of mathematical models for those kinetic reactions in between first order and zero order. Verification of the substrate limitation should be done using a continuous-feeding design.

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