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Hydrolysis and Acidogenesis of Farm Dairy Effluent for Biogas Production at Ambient Temperatures

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

Anaerobic ponds are an established technology for treating farm dairy effluent in New Zealand. These ponds produce a significant amount of methane but because of their large size, they are rarely covered for methane capture. The removal of solids prior to entering the ponds would allow for shorter retention times resulting in smaller ponds that could be covered. However, removal of solids entails loss of organic material and thus methane production. It was proposed that improved hydrolysis of solid content prior to solids separation could increase the organic content of the liquid fraction. No literature was found describing two-stage (acidogenic/hydrolytic and methanogenic) systems which achieve hydrolysis combined with solids separation of manure slurries. Hence, the aim of the present study is to examine the feasibility of such a system.

Five parameters were examined to determine favourable conditions for hydrolysis of solids and acidogenesis in farm dairy effluent. These were: 1) mixing, 2) hydraulic retention time (HRT), 3) liquid to solid ratio (dilution), 4) addition of rumen contents, and 5) reactor configuration.

Continuous mixing of cow manure sludge inhibited net volatile fatty acid (VFA) production, likely due to oxygenation. By comparison, a once-daily brief stirring regime resulted in production of 785 mgVFA/Lsludge compared with 185 mg/L from a continuously stirred reactor. Mixing had little effect on soluble COD yield.

HRTs ranging between 1 and 10 days resulted in greater hydrolysis yields (0.25 to 0.33 gCOD/gVS_{added}) compared with 0.15 gCOD/gVS_{added} for a 15-day HRT. It was hypothesised that the attachment of hydrolytic bacteria to solids prevented washout at shorter HRTs. In contrast, longer HRTs favoured VFA production. This may have been due to the planktonic nature of acidogenic bacteria, making them more vulnerable to washout at shorter HRTs.

The effects of solid:liquid ratio on hydrolysis and acidogenesis were examined with sludge:water ratios ranging from 1:1 to 1:0.25. The addition of larger volumes of

water resulted in improved acidogenesis with the 1:1 sludge:water mixture producing a liquor with 245% more VFA mass (635 mg) than reactors with a 1:0.25 sludge:water mixture (184 mg).

Addition of rumen contents was shown to have little or no effect on either acidogenesis or hydrolysis. This may have been due to a masking effect of an increased organic load through the addition of undigested grass in the rumen.

A mix, settle and decant (MSD) system and an unmixed flow-through leachbed separator system were trialled and compared as hydrolytic/acidogenic reactors. The MSD system produced 0.033gVFA/gTS_{added} and 0.315gCOD/gTS_{added} compared with 0.015gVFA/gTS_{added} and 0.155gCOD/gTS_{added} in the unmixed leachbed separator. It was hypothesised that improved mixing and longer solid-liquid contact times in the MSD system provided greater surface contact and transfer of organics to the liquid phase thereby enhancing hydrolysis.

A two-stage (acidogenic/hydrolytic and methanogenic) system was tested at bench scale. A partially mixed leachbed separator was fed with manure slurry. This retained solids while leaching out a treated feed high in organic content to be fed into a variety of methanogenic systems. The leachbed separator produced a treated feed with a VFA concentration of 562 mg/L, 120% higher than the influent slurry (255 mg/L). Soluble COD increased 60% from 1,085 mg/L in the slurry to 1,740 mg/L in the treated feed.

20-day HRT and 10-day HRT unmixed unheated methanogenic reactors, both fed with treated feed from the leachbed separator, had lower specific methane yields (0.14 and 0.11 LCH_4/gVS respectively) than a 50-day HRT reactor fed with untreated slurry (0.17 LCH_4/gVS). However, both the 20-day HRT reactor and the 10-day reactor had higher volumetric methane yields (0.033 and 0.057 LCH_4/Lreactor/day respectively) than the 50-day HRT reactor fed with slurry (0.024 LCH_4/Lreactor/day). Gas production was shown to rise as the VFA levels in the treated feed rose. Fermentation in the leachbed followed by separation was shown to improve average gas production by up to 57% compared to separation alone.

Field-scale trials of a leachbed separator system followed by a 20-day HRT methanogenic reactor were undertaken. VFA concentrations increased from 100 mg/l in the influent to 1,260 mg/l in the treated feed, while the soluble COD increased from 2,766 mg/L to 5,542 mg/L. The methanogenic reactor produced 0.08 m^3 CH_4/ $m^3_{reactor}$/day, four times higher than that which would be expected from a covered pond of the same size. This was hypothesised to be due to the increased biodigestability of the feed to the tank digester as well the increased organic loading rate.

This study indicates that the use of a leachbed separator would be an effective low-tech strategy for reducing the HRT of farm anaerobic ponds, and reducing the size of covers required for biogas energy recovery.

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1. Introduction

New Zealand's dairy farms produce an estimated 70 million m^3 of effluent annually (Saggar *et al.*, 2004). Most of this comes from manure dropped in the milking shed and on feed pads, as housing herds indoors is not yet common practice in New Zealand. Estimates of the volume of methane, which could be derived from this resource, range from 37 to 120 L/cow/day[1]. This methane would have a total energy (heat and electricity) value of 62 to 200 kWh/cow/year (based on a 270-day season). It has been estimated that the power requirements of an average New Zealand dairy farm equate to 160kWh/cow/year (Wells, 2001). Using this figure, the power output from an on-farm biogas system could cover from 41 to 133% of a farm's power needs.

There has been an 84% increase in the national herd size since 1975 (LIC, 2008). This period has also seen a decrease in the number of herds, which has resulted in an increase in the average herd size to around 350 in 2008. This trend of decreasing herd numbers coupled with increasing herd size is clearly shown in Figure 1-1 below.

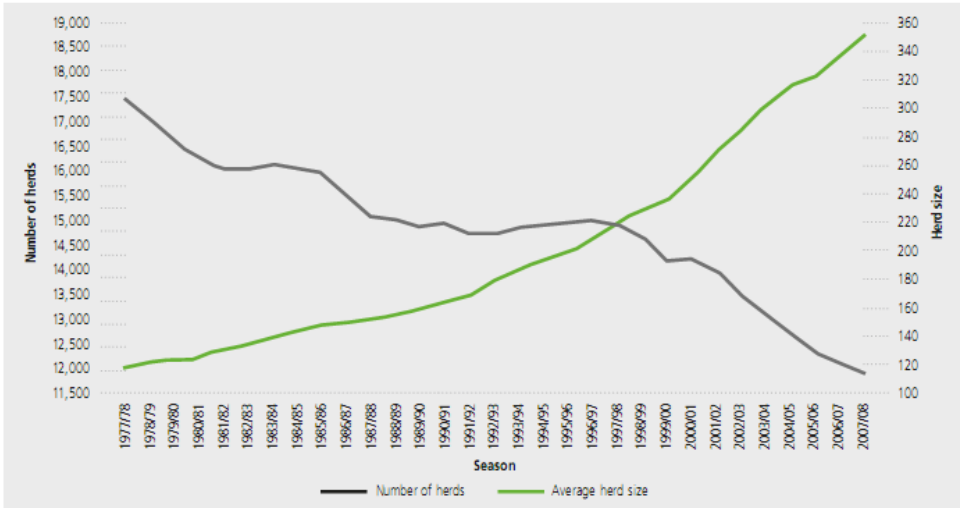

Figure 1-1: Change in number and size of dairy herds 1977-2008. (LIC, 2006)

[1] The variation in the methane yield is due to variations of specific methane yield (L CH_4/kg VS) reported in the literature. These estimates were derived using the highest and lowest specific methane yields presented in section 2.4 below.

A typical 350 head dairy farm in New Zealand produces around 17.5 m^3 of effluent daily, based on typical effluent production of 50 L/cow/day (Dexcel, 2006; Vanderholm, 1984). This effluent has the potential to produce over 40 m^3 of methane per day under ideal conditions. This would be enough to supply the power needs of the modern dairy farm. However the use of methane production from mesophilic digesters in the New Zealand context is thought to only be financially viable for farms that have more than 700 cows and a feed pad (Craggs, 2006).

Many dairy farms in New Zealand use a two-pond system to treat effluent from their milking sheds. Typically, these consist of a 4-metre deep anaerobic pond followed by a shallower aerobic pond. Methane output from these anaerobic ponds has been estimated at 0.02 m^3/m^3 of pond per day (NZ Ministry of Agriculture and Fisheries, 1994). Assuming a typical depth of 4 m for farm anaerobic ponds, this equates to an areal methane production rate of 0.08 m^3/m^2/day. Park and Craggs reported a lower areal methane production of 0.023 m^3/m^2/day from anaerobic ponds on dairy farms (2007). At such low volumetric gas production, it is not economical to cover ponds that typically have an area ranging from 300 m^2 for small herds to over 1000 m^2 for herds of 500 cows. It has been estimated that covering ponds for methane capture is only economically viable for New Zealand dairy farms with herds larger than 1000 cows (Craggs, 2007). Typical anaerobic ponds in New Zealand are designed on an organic loading basis (0.020–0.028 kg BOD_5/m^3/day) which results in long hydraulic retention times of 50 up to 120 days (Dexcel, 2006). The large size of the ponds also allows for the storage of solids for up to five years before desludging. Removal of solids from the effluent ahead of the pond system (a standard practice in the U.S) would result in smaller ponds due to the lower organic load and reduced need for solids storage, but it would also reduce the amount of methane that could be collected.

As can be seen in Figure 1-1 above, the current average sized dairy farm is now of a sufficient size to be energy self-sufficient but not yet of sufficient size to make the capture of this energy economically viable. The main obstacles are the costs of either installing a heated mixed digester or of covering the large surface area of conventionally designed dairy farm anaerobic ponds. A solution to this problem

could be to develop smaller pond reactors that are more efficiently able to convert the organic matter held in farm dairy effluent into biogas. The use of smaller sized ponds results in a reduced hydraulic retention time (HRT). If the HRT is to be reduced, the biomethanation process must be sped up. This requires the optimisation of the rate-limiting step. In psychrophilic (ambient temperature) biomethanation the rate limiting step is hydrolysis (Noike *et al.*, 1985), the transfer of organic matter from the solid to the liquid phase.

The primary aim of this study is to improve hydrolysis in the psychrophilic biomethanation of farm dairy effluent (FDE). The rationale behind this is that improved hydrolysis in the first stage of a two-stage system, which retains solids in the first stage, will result in a liquid feed with a high soluble organic content. This feed can then be fed into the second stage, a methanogenic reactor. By decoupling the hydrolysis process, it is proposed that the HRT of the methanogenic reactor can be substantially shortened. This would have a practical benefit for on-farm pond systems, as shorter HRTs will result in smaller ponds, which can be more economically covered for biogas capture.

The secondary aim of this study is to improve the process of acidogenesis. The enhanced production of VFAs in a two-stage system has a two-fold benefit. The primary benefit is to the performance of the first stage hydrolytic reactor. The build up of VFAs can help to reduce pH thereby providing an environment which is inhibitory to the growth of methanogens. The establishment of methanogenic population would cause the wastage of organic material in the form methane release in an uncovered reactor. An added benefit is that a feed high in VFAs is more readily digestible in a methanogenic reactor and can be more quickly converted to methane provided that the methanogenic reactor is not limited by methanogenic growth. This condition occurs in reactors where biomass is retained such as attached growth systems or UASB reactors.

In order to achieve the above aim, the objectives of this thesis are:

- To investigate the effects the volume of water added to settled solids (dilution) has on hydrolysis and acidogenesis;

- To investigate the effects of mixing on hydrolysis and acidogenesis;
- To investigate the effects of contact time between the water and the settled solids (HRT) on hydrolysis and acidogenesis;
- To investigate the effects of the addition of rumen contents on hydrolysis and acidogenesis;
- To use the findings of the above investigations to design reactor processes that aim to achieve maximum extraction into the liquid phase of soluble organic material,
- To incorporate these reactor processes into a simple low cost farm-scale process to provide a low solid/high soluble organic content feed for ambient temperature anaerobic biogas ponds and digesters.

2. Literature Review

2.1. Introduction

This literature review begins by examining current and past literature relating to the biochemistry and biology of the biomethanation process with a particular emphasis on the hydrolysis stage. The literature on parameters such as residence time, temperature, pH, and inhibitory factors as they relate generally to the biomethanation process will then be reviewed. The physical and chemical characteristics of farm dairy effluent (FDE), particularly in the New Zealand setting will be examined along with the potential of FDE for methane production. Various methods of pre-treatment of FDE, both experimental and mature technologies, will be reviewed. The effect of these pre-treatment methods on hydrolysis and biogas production will be examined. The literature on methanogenic reactors fed with FDE will be reviewed. This literature will provide benchmarks against which the reactor performance of this study can be measured.

2.2. Biochemistry and Microbiology of Anaerobic digestion

2.2.1. Overview

The biochemical conversion of a complex substrate involves the interactions of many different consortia of microorganisms. Tables showing the different species of microorganisms can be found in Appendix 1. The conversion of organic material into methane gas (biomethanation) can be broken down into four major stages; hydrolysis, acidogenesis, acetogenesis and methanogenesis. These stages along with substrates and products are shown schematically in Figure 2-1 below.

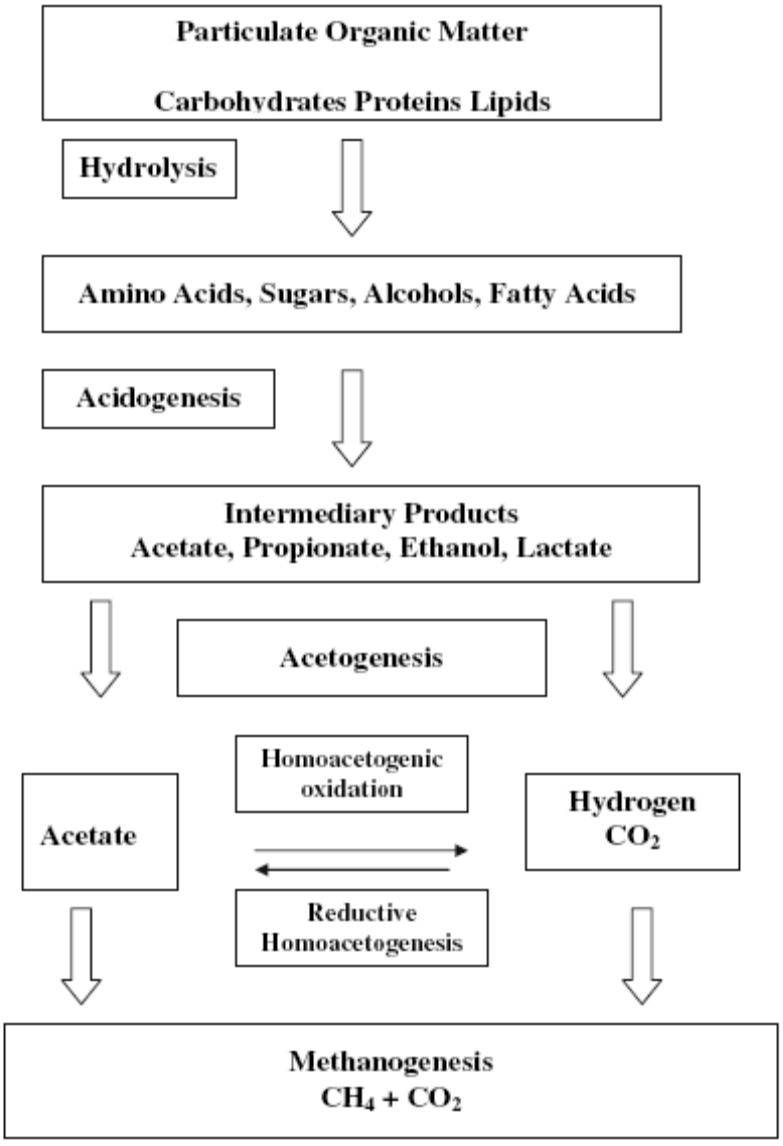

Figure 2-1: Different stages of biomethanation (Demirel & Scherer, 2008).

2.2.2. Hydrolysis

Hydrolysis is first step of anaerobic digestion. It is more accurately called depolymerisation as hydrolysis is just one process of breakdown of macromolecules (Chynoweth & Pullammanappallil, 1996). However most researchers typically refer to hydrolysis so this convention shall be retained. The products of hydrolysis are small soluble molecules which can be assimilated into the microbial cell and metabolized. The hydrolytic bacteria are responsible for the depolymerisation of organic polymers and fermentation to products, including organic acids, alcohols, and the methanogenic substrates. The hydrolytic bacteria are made up of both

facultative and strict anaerobes. For complex substrates with a high solids content, hydrolysis is usually the slowest step and hence the rate-limiting step in the overall anaerobic digestion process (Noike *et al.*, 1985). The depolymerisation process is carried out by extracellular enzymes secreted by microorganisms. The main substrates broken down are cellulose, hemicellulose and lignin (collectively known as lignocellulose), starches, proteins and lipids. The enzymes most employed in hydrolysis are hydrolases and lyases. Hydrolases include lipases, which hydrolyse the ester bonds of lipids to produce fatty acids and glycerol; glycosidases, which hydrolyse the polysaccharide component of plant cell walls; and peptases, which hydrolyse peptide bonds in proteins. Lyases catalyse the non-hydrolytic removal of groups from substrates. Phosphodiesterases hydrolyse the ester bonds of some modified polysaccharides that contain sugars with phosphoryl, acyl or alkyl groups (Chynoweth & Pullammanappallil, 1996).

Overall, the products of the hydrolysis process that can ultimately be converted to methane are carboxylic volatile acids, keto acids, hydroxy acids, ketones, alcohols, simple sugars, amino acids, H_2 and CO_2 (Kashyap *et al.*, 2003).

The hydrolysis step is particularly important in the break down of manure as 40-50 % of the solids in manure consist of biofibres. These biofibres are complex structures, typically comprising of cellulose covered by a layer of hemicellulose tightly associated with lignin. The hemicellulose and cellulose are able to be broken down by extracellular hydrolytic enzymes whereas the lignin is quite resistant to degradation (Angelidaki & Ahring, 2000).

Hydrolysis can be seen as taking placing through two separate methods. Bacteria can release enzymes into the bulk liquid where they are adsorbed onto a particle or react with a soluble substrate (Vavilin *et al.*, 2008). Alternatively (or concurrently) organisms can attach to a particle, produce enzymes in its vicinity and take up the soluble products released by the enzymatic reaction (Vavilin *et al.*, 2008). Studies in the stomachs of cows have shown that the breakdown of fibres is achieved by attached (sessile) bacteria, which attack the exposed ends of the fibres or enter

through damaged surfaces in the lignocellulose covering (Hobson & Wheatly, 1993). In cows, this damage is achieved by initial chewing and cud chewing.

The complete modelling of hydrolysis is coupled to a number of factors; substrate concentration, product concentration, biomass concentration, surface kinetics, temperature and toxicity (Vavilin *et al.*, 2008). The major determining factor is dependent on the type of waste being digested, but for complex substrates, it has been shown that particle size is an important factor. For this reason, a lot of research has involved communition, or particle size reduction, in order to increase available surface area for both enzymatic action and biomass colonisation. Wen *et al*. (2004) showed that decreasing the particle size from 840–590 to 590–350 nm enhanced glucose yield by 29% after 96 h mechanical pre-treatment of animal manure. Various forms of pre-treatment apart from communition, which have been investigated with regard to improving the hydrolysis of lignocellulosic material, have been steam, ammonia or CO_2 explosion, which forces the crystalline structure of the fibres to explode through rapid decompression, pyrolysis, treatment with acids, alkalis, hydrogen peroxide, ozone and acid catalysed organic solvents. All these methods have been shown to have increased soluble products after enzymatic hydrolysis to some extent (Sun & Cheng, 2002). A number of researchers have used biological pre-treatments. The use of brown, white and soft-rot fungi has been shown to enhance the breakdown of cellulose and lignin (Schurz, 1978.). Myint and Nirmalakhandan (2009) reported a 15 % increase in soluble COD production from the use of brown-rot fungi in a leachbed reactor containing fibre from cow manure. The addition of cellulolytic strains of bacteria have been found to improve biogas production from cattle manure by as much as 44% (Yadvika *et al.*, 2004)

2.2.3. Acidogenesis

Acidogenesis is the conversion of soluble organic matter produced by hydrolytic bacteria. The soluble organic matter is converted to simple organic compound such as volatile fatty acids, alcohol, lactic acid and mineral compounds such as carbon hydroxide, hydrogen, ammonia and hydrogen sulphide. In the case of cattle manure the acidogenic biomass grow on the soluble products of hydrolysis consisting of a readily degradable component, hemicellulose; and a slowly degradable component,

cellulose (Myint *et al.*, 2007). Acidification is strongly affected by temperature according with the Arrhenius law, however thermophilic temperatures which result in cell death and higher energy costs may result in sub-optimal temperatures being preferable (Guerrero *et al.*, 1999).

2.2.4. Acetogensis

The organisms that convert intermediates such as propionate, butyrate, lactate and ethanol to acetate are obligate hydrogen producing acetogenic bacteria. These species are syntrophic in that they require the presence of H_2 utilising bacteria to keep hydrogen concentration below a 10^{-3} atm (Wolin 1974 in Mawson 1986). An excess of hydrogen often leads to an increase in longer chain VFAs and lowering of pH and inhibition of acetogenesis. The H_2 utilising bacteria in turn rely on the acetogens for their hydrogen source (Ahring, 2003). There are also organisms present that form acetate and other C-3 or higher volatile acids via back reactions with dihydrogen and carbon dioxide. The following are the principal reactions involved in the conversion of substrates to acetate:

Syntrophic acetogenic reactions:

$Propionate^- + 3H_2O \rightarrow acetate^- + HCO_3^- + H^+ + 3H_2$

$Butyrate^- + 2H_2O \rightarrow 2\ acetate^- + H^+ + 2H_2$

$Propionate^- + 2HCO_3^- \rightarrow acetate^- + 3\ formate^- + H^+$

$Butyrate^- + 2HCO_3^- \rightarrow 2\ acetate^- + 2\ formate^- + H^+$

Homoacetogenic reactions:

$Lactate^- \rightarrow 1\ ½\ acetate^- + ½\ H^+$

$Ethanol + HCO_3^- \rightarrow 1\ ½\ acetate^- + H_2O + ½\ H^+$

$Methanol + ½\ HCO_3^- \rightarrow ¾\ acetate^- + H_2O$

$4H_2 + 2HCO_3^- + H^+ \rightarrow acetate^- + 4H_2O$

2.2.5. Methanogenesis

The methane bacteria are a unique group of organisms belonging to the Archaea kingdom (Woese et al. 1990). Methanogenic substrates include acetate, methanol, dihydrogen/carbon dioxide, formate, methanol, carbon monoxide, methylamines,

methyl mercaptans, and reduced metals. In most non-gastrointestinal ecosystems 70% or more of the methane formed is derived from acetate, depending of the type of starting organic carbon. In contrast, in ruminants where acetic acid, as well as propionic and butyric acids, is removed by absorption through the rumen wall and then metabolized by the host, methane appears to be almost exclusively produced from the reduction of CO_2 by H_2 (Marchaim, 1992).

The following are the principal reactions involved in the conversion of substrates to methane:

$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$

$Acetate^- + H_2O \rightarrow CH_4 + HCO_3^-$

Methanol $\rightarrow$ ¾ CH_4 + + ¼ HCO_3 + ¼ H^+ + ¼ H_2O

2.3. Parameters Affecting Biomethanation

2.3.1. Residence time

As with all biological treatments, the solids retention time (SRT) must not be less than the growth rate of the slowest growing bacteria in a reactor. The growth rate will vary depending on the pH, temperature and available nutrients. The minimum retention time will also vary depending on the nature of the waste. Methanogenesis of a highly soluble waste will generally be limited by the growth rate of acetate degrading methanogens. In this case a maximum specific growth rate (μ_{max}) of 0.4 day^{-1} suggests a minimum SRT of 2.5 days (Mawson, 1986). In a study of the digestion of cattle manure slurry Linke calculated that the critical SRTs for wash-out of methanogenic bacteria at 24 ^oC and 35 ^oC were 7.75 d and 2.76 d respectively (Linke, 1997). Acidogens have much higher growth rates than methanogens, which in turn results in much shorter retention times needed to prevent washout. For simple sugars, a minimum retention time of 2.5 to 3.5 hours is sufficient to prevent washout of acidogenic bacteria (Demirel & Yenign, 2002). The minimum retention time for effective acidogenesis of swine manure has been reported as 0.4 days (Hwang *et al.*, 2001) while stable operation of an acidogenic reactor fed with unscreened dairy manure has been reported with an HRT and SRT of 2 days (Demirer & Chen, 2005). For mixed solid waste, a μ_{max} of 2.0 day^{-1} (minimum SRT of 0.5 days) has been reported (Chynoweth & Pullammanappallil, 1996).

2.3.2. Temperature

The metabolic and growth rates of chemical and biochemical reactions tend to increase with temperature, until the temperature tolerance of the microorganism is met. If the temperature is extreme, denaturation of the cell will occur ending the effective life of the cell. Microorganisms exhibit optimal growth and metabolic rates within a well-defined range of temperatures, which is specific to each species. Psychrophilic organisms thrive in temperatures below 25^{o}, mesophilic between 25 and 40^{o} and thermophilic higher than 45^{o}.

Methanogenic bacteria are more sensitive to changes in temperature than other organisms present in digesters. This is due to the faster growth rate of the other groups, such as the acidogens, which can achieve growth even at low temperatures (Marchaim, 1992) . All bacterial populations in digesters are fairly resistant to short-term temperature upsets, up to about two hours, and return rapidly to normal gas production rates when the temperature is restored. It has been reported that long term adaptation of active psychrophilic microbial communities is required for the efficient digestion of cattle manure at low temperatures (Yadvika *et al.*, 2004).

Cullimore *et al*. in Hill (2001) demonstrated that biogas production from swine wastes was able to occur between 3°C and 9°C and that methane became the primary biogas component at approximately 10°C. Sutter and Wellinger (1985) indicated that gas production increases linearly in the range 10–20°C.

Safley and Westerman (1990) reported acceptable methane yields for loading rates ranging between 0.15 and 0.57 kg VS/m^3/day at 14 and 23^{o}C. They presented the following equation for use in estimating the appropriate loading rate to give a desired methane yield for a specific temperature based on a known loading rate and temperature for a CSTR digester:

$$LR_2 / LR_1 = e^{p(T2-T1)}$$

where: LR_1 and LR_2 are the loading rates (kg VS/m^3/day) at T_1 and T_2 (the temperatures (^{o}C); p = 0.1 is the rate constant ($^{o}C^{-1}$). Based on typical loading rates of

mesophilic digesters operating at 35^{o}C (1–3 kg VS/m^{3}/day) the predicted loading rate for 10^{o}C would be 0.08–0.24 kg VS/m^{3}/day to achieve similar methane yields.

2.3.3. Mixing

Various researchers have found that excessive mixing can have a negative effect on biomethanation. Ong showed that the rate of biomethanation in a continuously stirred digester was inferior to that of a non-stirred one (Ong *et al.*, 2002). They found mixing led to a decrease in production of extracellular polymeric substances (EPS). EPS enables the clumping of consortia of microorganisms into optimal communities for the efficient break down of substrates. This was in agreement with another study which pointed to the need for initiation centres and agglomeration in order to support syntrophic communities (Vavilin & Angelidaki, 2005). It has been proposed that excessive stirring breaks up these initiation centres and inhibits methane production. Stroot *et al* (2001) also showed that high solids reactors which were minimally mixed performed better. They suggested that some degree of agitation of digester contents is likely required to mix the feed and to allow the formation of new spatial associations among different microbial populations and that without mixing, single cells would be surrounded by their own progeny as a result of their growth, resulting in reduced kinetic effectiveness. In terms of acidogenesis a study using primary sludge found a 70% increase in VFA production in an unmixed reactor compared with VFA production in a mixed reactor (Banister & Pretorius, 1998). In contrast to this, others have found that mixing improves methane production from cattle slurries (Kalia & Singh, 2001; Sakar *et al.*, 2009) and that this effect is more pronounced when scale up occurs (Vesvikar & Al-Dahhan, 2006). Their studies found higher levels of VFA in unmixed reactors, indicating incomplete contact between methanogens and VFAs. It may be that in an acidogenic reactor, this build-up of pockets of high concentrations of VFA maybe advantageous in preventing methanogen growth and VFA consumption.

2.3.4. Nutrients

In anaerobic digestion micro-organisms utilize carbon 25–30 times faster than nitrogen. Thus to meet this requirement, microbes need a 20–30:1 ratio of C to N with the largest percentage of the carbon being readily degradable (Hills, 1979) Carbon to phosphorus ratios suggestions range from 75:1 to 113:1 (Speece, 1987) .

2.3.5. Inhibitory factors

Inhibition of the anaerobic processes has often been reported resulting from high concentrations of VFAs, H_2, NH_3 and extremes in pH (Hobson & Wheatly, 1993). Partial pressure of CO_2 can affect conversion of propionate and acetate, with conversion to methane being inhibited at high concentrations. The optimum CO_2 concentration is 20%. The concentration of methane has not been found to affect methanogenesis (Hobson & Wheatly, 1993).

Ammonia can be inhibitory at concentrations higher than 3,000 mg/l. However at higher pH (>7.4) ammonia can be inhibitory at above 1,500 mg/l. This is because free ammonia (NH_3) is more inhibitory than the ammonium ion (NH_4^+). These two forms are in equilibrium and ammonia dominates at higher pH (Hobson & Wheatly, 1993). Ammonia is thought to inhibit the methanogens rather than the fermentative bacteria.

VFA with concentrations over 1,000 mg/l have been reported as having an adverse effect on methanogens (Hobson & Wheatly, 1993). Short chain products appear to inhibit the breakdown of longer chain substrates. For example, high levels of acetate can inhibit propionate and butyrate oxidation. High levels of propionate can also inhibit butyrate oxidation but higher levels of butyrate do not appear to inhibit propionate breakdown (Demirel & Yenign, 2002).

The effect of inhibitory substances on hydrolysis has been less widely studied. There is some evidence that an accumulation of amino acids and sugars can cause inhibition of hydrolysis along with the accumulation of cellubiose, the cellulose break-down intermediate (Duff & Murray, 1996). It was suggested that the selective removal of these products by membrane technology could prevent the inhibitory action. It was shown that cellubiose was inhibitory to enzyme action rather than biological growth. High levels of VFA have been shown to inhibit hydrolysis (Vavilin *et al.*, 2008), though there is some debate as to whether this is in fact due to the lowering of pH that VFAs cause or the actual inhibitory action of the VFAs (Pin-Jing *et al.*, 2006). Veeken et al (2000) concluded that no inhibition by VFA or by non-ionized VFA can be measured at pH values between 5 and 7, and that acidic pH

was the inhibitory factor. They proposed a linear function of pH inhibition in the interval between 5.0 and 7.0.

In studies by Yu and Fang (2001), zinc was found to inhibit acidogenesis at concentrations over 10 mg/L. Copper inhibited acidogenesis at all tested concentrations ranging greater than 5 mg/L. Copper was found to be 1.4-4.3 times more toxic than zinc with regard to production of fatty acids and hydrogen as well as degradation of carbohydrate and protein (Yu & Fang, 2001).

2.3.6. pH

A neutral pH is preferred for combined anaerobic digestion. This is not necessarily the optimum pH for all the micro organisms involved in biomethanation but it suits the widest range (Tchobanoglous *et al.*, 2003). The hydrolysis of readily degradable substrates in landfills was found to be inhibited at pH below 5.6 and the optimum for hydrolysis of polysaccharides is 6.5-7.0 (Vavilin *et al.*, 2008). Fermentation of simple sugars can occur between pH 4.5 and 7.9 with an optimum range between 5.7 and 6.0 (Demirel & Yenign, 2002). Stable acidification of unscreened cattle manure has been reported at pH 6.0 (Demirer & Chen, 2005), while Myint and Nirmalakhandan reported a stable pH of 5.0 for a leachbed reactor containing cattle manure (2009). The optimum pH for the breakdown of VFAs and methanogenesis is 6.5-7.5 (Hobson & Wheatly, 1993). Burke suggests an optimum pH range between 6.8 and 8.5 (Burke, 2001).

2.4. Dairy Shed Effluent Characteristics

The percentage of manure deposited by a dairy cow in the dairy shed has been estimated at between 10 and 20% of its daily manure output (Vanderholm, 1984). The volume of urine and excreta that each cow produces per day is estimated at 54 L. The amount of material that is available for digestion and biogas production is dependent on the amount of time that a cow spends on hardstand areas from which manure can be collected. On farms without feed-pads or stand-off areas this is typically estimated at 2 hours per day (Dexcel, 2006). The amount of manure collected can be estimated as t_s/t_w x V_m, where t_s = time on hardstand, t_w = waking hours (typically 16) and V_m = the volume of manure produced every day (typically

54 L). The estimates of average volumes of effluent per cow per day from wash down dairy sheds vary from 45 L to 80 L per day (Hickey *et al.*, 1989). A large portion of this figure is made up of wash down water. The volume of wash down water is highly variable and dependent on the wash down method; scrape, hose down, flood wash or a combination of the three. A figure for wash down water of 50 L/cow/day (Vanderholm, 1984) is most commonly cited for design purposes. Effluent volumes per cow for larger herds can vary significantly; a herd of 500 cows can have a wash water volume between 30 and 120 L/cow/day.

The average composition of farm dairy effluent (FDE) comprises 10% excreta, 4% teat washings, and 86% wash-water plus other foreign material (Gibson, 1995). Solids content of the effluent can range from .04 to 4.96% with the average content being 0.9% (Longhurst *et al.*, 2000). The higher solids figure may relate to farms with low water usage or feed pad effluent, which is typically higher in solids due to less frequent wash down procedures.

Various mean nitrogen levels in FDE have been reported ranging from 181 mg/l to over 500 mg/L (Longhurst *et al.*, 2000). Nitrogen levels are seasonal and tend to peak in the spring when start of lactation and increased pasture growth coincide. Reported levels of nitrogen have been rising in recent years. This may be due to the increased use of nitrogen fertilisers.

Organic nitrogen is the main N source (80-95%), followed by ammonium (typically 17%) and small amounts of nitrate (<1%) (Longhurst *et al.*, 2000). FDE tends to have high levels of phosphorus (21-82 mg/l) and potassium (164-705 mg/l).

Cow manure has relatively high COD/BOD ratio ranging from about 4:1 to 12:1. It also has a lower fraction of biodegradable volatile solids (VS) compared to other farm manures. This is due to the efficiency of the cow's rumen digestion system and its high fibre diet. It has been estimated that only between 23 to 43 % of VS in cow manure is readily digestible compared to 63% in pig and poultry manure (Wilkie *et al.*, 2004).

The question "how much methane can be derived from a substance?" has many different answers and there are a wide range of different measures and terms used throughout the literature. Methane productivity is usually expressed on a per kg VS added basis, but sometimes it is based on VS removed or destroyed, TS, influent mass, influent volume, COD, BOD or animal unit. The theoretical methane yield (B_u) is a calculation based on conversion of lipids, proteins, carbohydrates, VFAs and lignin to methane using Bushwell's formula (Moller *et al.*, 2004). The ultimate methane yield (B_o) is the methane productivity in terms of VS added (L CH_4 /kg VS_{added}) as residence time approaches infinity. This is typically determined using a specific methane potential test where a substance is digested for up to 90 days under ideal conditions of temperature, inoculum, nutrients and dilution. The specific methane yield is the volume of methane produced per influent VS for experimental set ups, trials and other reactors. The specific methane yield is typically a measure of a reactor's performance whereas the ultimate methane yield is an attempt at characterisation of a starting material. Table 2-1 below shows B_o values for cow manure and other farm wastes by various researchers. Values for pig manure are also shown for comparison purposes. The range presented, from 125 up to 284 LCH_4/kg VS_{added}, is relatively consistent considering the variability that can exist in cow manure due to types of feed, climate, breed and location.

Table 2-1: Literature values for ultimate methane yield of dairy, pig and poultry manures.

Study	Dairy manure LCH_4/kg VS_{added}	Pig manure LCH_4/kg VS_{added}
(Moller *et al.*, 2004)	148 ± 41	356
(Angelidaki & Ellegaard, 2003)	200	300
(Vedrenne *et al.*, 2008)	243 ± 41	297 ± 40
(Amon *et al.*, 2007)	125 - 166	
(Bryant *et al.*, 1976) reported in (Safley & Westerman, 1992a)	170	
(Morris, 1976) reported in (Safley & Westerman, 1992a)	240	

2.5. Pre-treatment of Farm Dairy Effluent

2.5.1. Solids separation

Removing solids reduces the volume needed for storage in ponds and makes the effluent more manageable. It is required for the covered ponds which are typically used in the US (Mattocks *et al.*, 2008). It also removes a proportion of the nitrogen, which is in the solid material, allowing the liquid fraction to be irrigated over a smaller area. Removing solids does have an impact on methane production. Pain *et al* (1984) found that removing 52% of VS from slurry resulted in a 30% reduction in biogas. Hills and Kayhanian found that a 30 minute settling period retained 54% of methane potential in the settled sludge (Wilkie *et al.*, 2004).

2.5.2. Gravity systems

Weeping-wall sludge stores are normally built above ground on a concrete or packed earthen base. Effluent enters in one end of the store and flows out through 50 mm slots between wooden or concrete panelling at the opposite end, while the solids are retained in the sludge store. The excess liquid that drains through the slots is discharged to a pond or applied to land. The weeping-wall store is suitable for wastes containing a lot of fibre such as wastes from feed pads. The walls can be between 1 and 2 metres high. If they are mechanically cleaned once a year, approximately 40 m^3 storage is required per 100 cows per year (Scandrett, 2005).

2.5.3. Mechanical solids separation

These methods can achieve high rates of solids removal from both farm dairy and feed pad effluent. Due to higher capital investment, mechanical solids removal is generally suited to large operations which generate 30-40 m^3 of waste a day. The two types of mechanical solids separators most commonly used in New Zealand for FDE are:

• Screw press separators – the effluent is forced under pressure through one or more layers of fine mesh screens to separate the solids and liquids. Screw press separators are normally built on raised platforms over concrete pads so that solids (15-25% TS) can pile up below for easy removal.

• Belt presses (pressure separators) – these are continuously fed dewatering systems that use chemical conditioning, gravity drainage and mechanically applied pressure to dewater the manure. These belt-pressed solids come out at between 30-50% TS.

Inclined wedge wire screens and rotating screens are other mechanical technologies that can be applied to dairy effluent. While widely used in United States dairy farms, their use is not yet widespread in New Zealand. Table 2-2 below shows the percentage solids capture that can be expected from various technologies.

Table 2-2: Percent capture of total solids for separator technologies

Solid / Liquid Separator Technology	**Total Solids Capture Efficiency**
Static Inclined Screen	10-20%
Inclined Screen with Drag Chain	10-30%
Vibratory Screen	15-30%
Rotating Screen (Drum)	20-40%
Centrifuge	20-45%
Screw Press	30-50%
Settling Basin	40-65%
Weeping Wall	50-85%
Scrape and Dry	50-90%

(Southern-California-Edison, 2005)

2.6. Treatment and Biomethanation of Farm Dairy Effluent

Typical treatment options for dairy farms in New Zealand are direct application to land from a holding tank, application to land from a holding pond, treatment in a two stage pond system followed by discharge to water or land, solids separation before irrigation of liquid effluent, and anaerobic digestion (NZ Ministry of Agriculture and Forestry, 2005). The ultimate fate of effluent (liquids and solids) after treatment tends to be as irrigation to land or incorporation into soil. Some farms still discharge to water but most New Zealand regional councils require discharge to land. In New Zealand, it is still common practice for FDE to be treated using a two-stage pond system. In 1995/96 it was estimated 40% of farmers were using pond systems (Saggar *et al.*, 2004). In the last ten years there has been a move away from treatment and discharge to storage and deferred irrigation (Houlbrooke, 2008). Many regional councils in New Zealand are requiring that farmers have ponds with storage capacity ranging from 4 to 13 weeks depending on soil conditions, rainfall and irrigation methods. Though both deferred irrigation and covered pond biogas systems require

ponds for successful operation, the two systems are not necessarily compatible. To achieve effective biogas production or treatment a pond must have a certain hydraulic residence time; this is relative to the filled volume of the pond. However, effective storage capacity is dependent on available empty space; a full pond has no effective storage capacity. If storage ponds are to be used as anaerobic digestion facilities, this issue must be addressed.

2.6.1. Waste Stabilisation Ponds and Lagoons

The recovery of methane from FDE in lagoons is widely practiced in the USA. EPA guidelines for covered lagoons specify that solids should be separated prior to digestion in a covered lagoon. Energy recovery is only considered viable for unheated lagoons that are in farms south of latitude 40^{o} north. North of this only flaring is employed to remove excess gas, which is typically only produced in low volumes due to cold temperatures. In the USA, the government has supported biogas production from lagoons by offering guidelines for their construction and free downloadable programmes which calculate biogas, solid and liquid effluent production based on geographical location, herd size and type (dairy or piggery), method of farming, and method of effluent management (Mattocks *et al.*, 2008).

In New Zealand, the practice of covering dairy ponds and lagoons for methane recovery has had limited uptake. Since the 1970s many dairy farms in New Zealand have used two-stage waste stabilisation ponds to treat wastewater prior to discharge or application to land. This system typically has an anaerobic pond (4-5 metres deep) followed by a shallower facultative pond (1-1.5 m deep) (Craggs *et al.*, 2004). The anaerobic ponds are usually sized for hydraulic retention times (HRT) of 85-120 days. Methane output from these anaerobic ponds has been estimated at 0.02 m^3/m^3 of pond per day (NZ Ministry of Agriculture and Fisheries, 1994).

Safely and Westerman (1992b) reported satisfactory digester performance for both winter and summer conditions in a low temperature (10.6 -15 ^{o}C) covered lagoon fed with screened FDE with a mean methane yield of 0.322 m^3 CH_4/kg VS_{added}. This compared favourably with other reported values of 0.20 m^3 CH_4/kg VS_{added} (Hills & Kayhanian, 1985) for the liquid fraction of separated FDE (35°C,10-day HRT).

Safely and Westerman attributed the improved performance to the longer HRT used (67 days).

The fermentation pit (Oswald *et al.*, 1994), shown in Figure 2-2 below, is an adaptation of an anaerobic pond. It is essentially a deep pit that has influent fed in from the bottom. Most solids are retained in the pond as a sludge blanket through which all influent must pass. In this sense, it operates as a simplified upflow anaerobic sludge blanket reactor (UASB). The SRT is typically up to 20 years for domestic wastewater (periods between desludging) while the HRT is only 1-3 days. CO_2 and N_2 are effectively removed by passing through the water column so as to produce a gas with a high methane content (90% compared to the typical 60%).

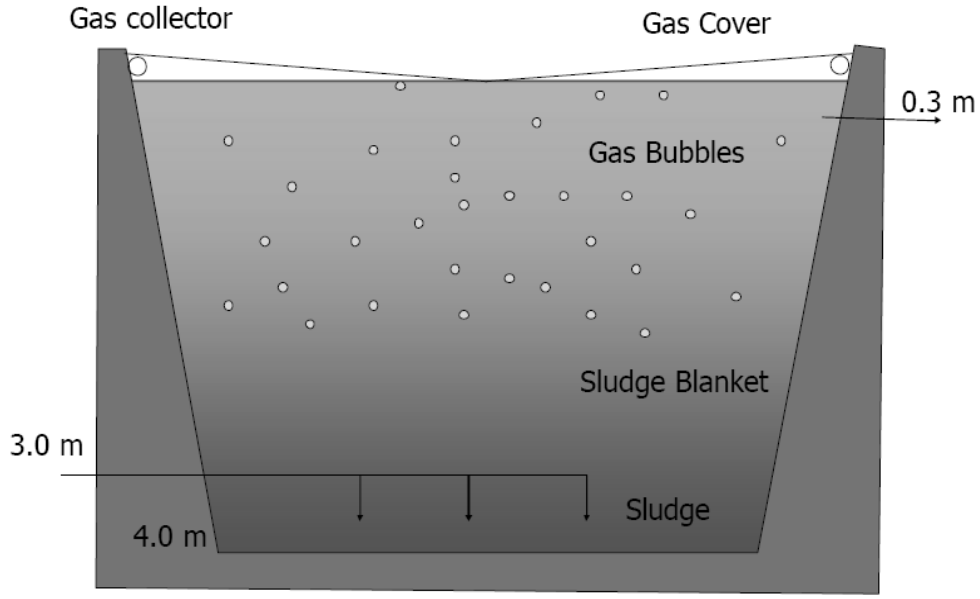

Figure 2-2: Fermentation pit (Craggs, 2006).

2.6.2. Digesters

The continuously stirred tank reactor (CSTR) also known as the complete-mix suspended growth reactor can run in batch mode or continuous mode. A CSTR is suitable for manure that is 3 to 10 % solids such as dairy manure collected by a flush system. Zeeman *et al* (1988) found that start up for psychrophilic CSTRs fed with dairy manure was not achievable below a temperature of 20 ^{o}C without the aid of a large volume of inoculum. With the addition of 50% volume of mesophilic sludge, they were able to establish methanogenic reactors at temperatures as low as 5 ^{o}C. The minimum successful HRT used was 30 days with 50 days being the optimum for

methane production on an influent volume basis. Pain *et al* (1984) compared the operation of two mesophilically operated CSTRs fed with screened and unscreened FDE. They noted a 30% reduction in gross gas production from the screened manure compared to the unscreened manure, meaning that a significant proportion of the methane potential was removed through the action of screening out the solids. However there was a significantly higher specific gas yield for the screened slurry (0.347 m^3/VS_{added}) than for the unscreened slurry (0.255 m^3/VS_{added}). A reduction in HRT from 30 days to 20 days resulted in a 12% lower specific gas yield for screened slurry.

UASBs have been used by a number of researchers for the treatment of FDE (Castrillon *et al.*, 2002; Chen & Shyu, 1996; Luostarinen & Rintala, 2005), but in nearly all of these instances the emphasis has been on COD reduction rather than biogas production. UASBs are particularly efficient at converting soluble waste streams, such as those containing sugars, to methane. Typically, they are run at short retention times (< 3 days), which make them unsuitable for substrates that require significant hydrolysis such as FDE.

The anaerobic plug-flow digester is suited to wastes with a high solids content (TS 11-13%). It is a long trough with roughly 1:5 width to length ratio that is covered by a flexible cover to trap biogas (Lusk, 1998). Waste moves along the trench in plug-flow fashion pushed along by the daily addition of a fresh application of manure and a slight gradient in the trench. These digesters are typically run at mesophilic temperatures and are designed for 20-30 day HRTs. They are not suitable for farms running flush systems as they require a high solids content for stable operation.

The anaerobic filter or attached growth anaerobic reactor or biofilm reactor is a reactor which enables the retention of biomass through addition of growth media to a reactor tank. The growth media is colonized by active biomass and retained in the reactor while the treated liquid phase is allowed out. Early filters in the 1960s employed stones as media. These however had low void volumes and were prone to blockages due to solids and biomass. Other media used have included plastic rings, slag, woodchips, ceramics and various sheeting materials (Tchobanoglous *et al.*, 2003). Psychrophilic (12^o C) anaerobic digestion in a laboratory scale fixed-film

reactor fed with screened dairy manure was successfully attained with a 1 day HRT (Lo & Liao, 1986). That study indicated that a fixed-film reactor could handle a sudden change of temperature without stopping gas production, and also a high loading rate (28.7 g VS/L/d) and a short hydraulic retention time, with no signs of process instability. Hernandez and Rodriguez (1992) treated screened and settled cattle waste in a down-flow anaerobic filter filled with ceramic raschig rings at retention times from 0.5 to 4 days. The methane productivity was exceptionally high at 0.7-2.8 L CH_4/L reactor/day. Vartak *et al* (1997) compared the performance of various psychrophilic packed bed reactors fed with unscreened FDE. They found that methane production was highest with a polyester matting medium compared to a limestone medium or a combination of both. They attributed this to the porosity of the polyester matting.

2.6.3. Leachbeds

Leachbeds are reactors that retain solids while allowing liquid to drain out. Liquid is often applied to the top of leachbeds in order to flow through the amassed solids and remove the products of hydrolysis. Leachbeds can be run as one stage processes, where leachate is recycled through the solids and methanogenesis is allowed to develop in the leachbed, or as a two-stage process where the leachate is fed into a high rate methane reactor such as a UASB. This technology has been trialled for enhancing hydrolysis and acidogenesis of the solid fraction of municipal waste (Chugh *et al.*, 1999; Ghanem *et al.*, 2001; Jiang *et al.*, 2005; Wang & Banks, 2000), grass residues (Lehtomaki *et al.*, 2007; Yu *et al.*, 2002) and a mixture of cotton gin waste and dairy manure (Funk *et al.*, 2005). Lehtomaki et al found that recycling of digestate through a UASB in a two stage process significantly increased methane potential extraction (66%), compared to recycling of the digestate in a one stage process, which extracted only 20 % of the methane potential of the starting material (2007). They attributed this to the removal of soluble products by the UASB, whereas the one-stage process suffered from product inhibition. At the time of the experimental stage of this study, no leachbed trials had been reported using only FDE. Since then Myint and Nirmalakhandan (2009) have reported successful hydrolysis and VFA production using a leachbed packed with pistachios-half-shell as porosity enhancers. The increased porosity of the leachbed was intended to improve contact between liquids and solids in the reactor and enable more efficient removal

of products in the leachate. They reported a 132 % increase VFA yield compared to a control reactor that had no pistachio porosity enhancers.

2.7. System Performance

Table 2-3 below shows the biogas production rates per reactor volume per day of a selection of reactor types fed with dairy farm effluent. Heated lagoons (Pain *et al.*, 1984) and anaerobic filters generally always outperform low temperature reactors and lagoons. The highest gas production is that achieved by an anaerobic filter filled with ceramic rings (Hernandez & Rodriguez, 1992). This reactor was able to achieve biogas production of 4.7 m^3/m^3 per day with HRTs of less than one day. It is not clear if this reactor was heated or not. They achieved methane conversion rates of 0.17 m^3 CH_4/kg VS_{added}.

Table 2-3: Literature values for volumetric gas production rates of various psychrophilic reactor configurations fed with dairy effluent.

Researcher	**System**	**Feed**	**Temperature (^oC)**	**HRT (days)**	**Organic loading (kg VS/m^3/day)**	**CH_4 production (m^3/kgVS_{added}/day)**	**CH_4 production (m^3/m^3 reactor/day)**
Safely and Westerman (1992b)	Lagoon	Screened	10.6 -15	67	0.12	0.39	0.109
(Hernandez & Rodriguez, 1992)	Anaerobic Filter	Screened and settled	Not reported	0.5	16.3	0.17	2.8
(Vartak *et al.*, 1997)	Anaerobic filter (polyester matting)	Unscreened	10	33	0.12	0.08	0.013
(Lo & Liao, 1986)	CSTR	Screened	22	10	2.94	0.06	0.18
(Lo & Liao, 1986)	Fixed film reactor	Screened	12	1	28.7	0.01	0.3
(MAF, 1994)	Typical dairy farm anaerobic pond	Unscreened	Ambient	50-120			0.02

The literature review has revealed that the rate-limiting step of the biomethanation process with regard to substrates with a high fibre content is the hydrolysis step. In order to shorten the overall residence time required for effective biomethanation of farm dairy effluent it is therefore necessary to investigate methods of speeding up and enhancing the hydrolysis step. A number of parameters have been identified in the literature that can have a positive or negative effect on the rate of hydrolysis for lignocellulosic complexes. Temperature, pH, communition, mixing, dilution, SRT, HRT, enzymatic addition, improved liquid-solid contact and various forms of chemical and physical pre-treatments have all been investigated to varying degrees in relation to their effect on hydrolysis. The effect of these parameters on the hydrolysis of farm dairy effluent has been less widely studied, particularly at psychrophilic temperatures. In this study, it was decided to focus the investigation on those parameters which would allow an on-farm biomethanation process to be run as a passive system with a minimum of energy input. Mixing, dilution, HRT, and enzymatic addition were chosen as parameters to be examined in this study due to these parameters being relatively straightforward to manipulate in a farm setting. Leachbed systems have been used successfully by a number of researchers to hydrolyse waste products (Chugh *et al.*, 1999; Ghanem *et al.*, 2001; Lehtomaki *et al.*, 2007). However, there has been little application of this technology to hydrolyse farm dairy effluent solids, hence this study has undertaken to investigate the use of leachbed systems coupled with unheated methanogenic reactors to digest farm dairy effluent for biogas production.

3. Methodology

3.1. Overview

The main purpose of this study was to improve hydrolysis in the psychrophilic biomethanation of farm dairy effluent (FDE). This was chosen because hydrolysis is the rate-limiting step in the biomethanation of fibrous substrates such as FDE. As such, the enhancement of hydrolysis would result in a reduction of the overall retention time required in farm pond digester. In order to quantify hydrolysis it is necessary to first define the starting material, or substrate, and the products of the hydrolysis process. Hydrolysis is the break down of macromolecules and polymers into soluble monomers. As discussed in the literature review above the substrates in FDE are typically lignocellulosic complexes made up of cellulose, hemicellulose and lignin, with some starches and proteins. The soluble products can be carboxylic volatile acids, keto acids, hydroxy acids, ketones, alcohols, simple sugars, amino acids, H_2 and CO_2. The quantification of the all these species of hydrolysis substrates and products was not practical for this type of study so an analytical measure was chosen which encompassed all the starting materials and another was chosen which encompassed all the products but excluded the substrates. The hydrolysis substrates were quantified using the COD of the solid fraction of the material, and volatile solids (VS). Both of these are a measure of the solid organic content of a material. The products of hydrolysis were quantified using a soluble COD test. It is assumed that only hydrolysis brings about an increase in soluble organics and therefore soluble COD. Soluble COD has been used a measure of hydrolytic activity in the determination of hydrolysis constants for cellulose and hemi-cellulose in a study on hydrolysis and acidogenesis of cow manure (Myint & Nirmalakhandan, 2009; Myint *et al.*, 2007). The products of hydrolysis have also been expressed as soluble COD in a study of hydrolysis and acidogenesis of primary sludge (Miron *et al.*, 2000).

In order to have a clear picture of how much organic material was being released into the bulk liquid from the FDE sludge by way of hydrolysis every day it was decided to remove the bulk liquid and replace it with fresh water on a daily basis. Any VFAs and COD detected in the bulk liquid could then be assumed to have come from the solids by way of hydrolysis and acidogenesis in the 24 hours prior to sampling. This

methodology was used for all the batch studies, namely the dilution, mixing and rumen addition experiments. For the HRT experiments, the frequency of the bulk liquid replacement was adjusted in line with the required HRT.

3.2. Terminology

In the discussion section the terms hydraulic residence time (HRT) and solids residence time (SRT) are used. HRT in the context of this study refers to the residence time of the liquid portion of any effluent that is removed from the reactor, while SRT refers to the residence time of the solids that are retained in the reactor. For calculations of SRT and HRT it is assumed that all solids are retained in the reactor when decanting or separation takes place, although it is clear that some solids, and a potentially large microbial population, do move out of reactors with the removal of a liquid portion. For example, if one litre of sludge is added to a vessel and allowed to settle over 24 hours prior to the removal of 900 mL of liquid and then 900 mL of fresh water is added for another 24 hours prior to the removal of 900 mL of liquid and so on for a total of ten days, then the HRT would be reported as 1 day and the SRT as 10 days. For bacteria that are attached to the solids (referred to as sessile bacteria) this SRT would be accurate, however for bacteria existing in the liquid phase (referred to as planktonic bacteria) their SRT might range from 1 to 10 days depending in when they were washed out. For this reason, references to SRT should only be taken to refer to the settleable portion of the sludge rather than any biological population, unless specifically referred to.

In this thesis, a number of terms are used to describe the various portions of farm dairy effluent. The terms are defined below.

Manure refers to a mixture a faeces and urine as excreted from the cow. This was collected by scraping off the surface of the milking shed within an hour of having been dropped by the cows.

Slurry refers to a mixture of water and manure. This was prepared in the lab using 9 parts water to 1 part manure. Slurry has a total solids content ranging from 0.5 to 2%.

Supernatant refers to the liquid portion of the slurry which is removed after settling, either by decanting to reveal the sludge layer underneath or by draining out through a sieve or wire mesh. Supernatant typically has less than 0.5% total solids content.

Sludge refers to the thicker solid portion left remaining after the removal of the supernatant from settled slurry. Sludges have a total solids content greater than 2%.

3.3. Experimental Procedures

3.3.1. Characterisation

Characterisation analysis was carried out to determine the characteristics of FDE. This included analysis of the slurry, and the supernatant and sludge after settling. This was carried out to determine the organic content of the FDE in order to better understand the potential for methane production.

When raw manure is mixed with wash-down water from a milking shed it forms a slurry. This slurry typically comprises 10% excreta, 4% teat washings, and 86% wash-water plus other foreign material (Gibson 1995). For the experiments in this study, slurry was prepared by mixing 1 part of fresh cow manure with 9 parts of tap water. Two methods of separation were employed to derive the portions of sludge and supernatant. Settle and decant (S&D) involves allowing the slurry to settle for at least two hours so that two distinct layers are formed. The supernatant is then poured or pumped off leaving behind the majority of solids in the sludge layer. This method was used to mimic the separation that might occur on farm systems where the liquid portion is removed from a settling basin by overflow or pumping out. Gravity separation involves allowing the slurry to settle and the liquid portion to drain out through the solids, which are held back by a porous wall or a geotextile cloth. This method was used to mimic the separation that might occur on farms with weeping walls or screen separation systems.

The raw manure for all experimental set ups was gathered fresh from the standing area of the Massey University #4 Dairy Unit milking shed. If not used immediately it was stored at 4^{o} C.

Slurry, supernatant and sludge were analysed for analysed for solids content (VS and TS), COD (soluble and total) and VFAs.

3.3.2. Dilution Experiment

These experiments were carried out to determine the effect of dilution (the ratio of sludge to fresh water) on hydrolysis in terms of the transfer of COD from sludge to the liquid phase, and on acidogenesis in terms of VFA production. It was hypothesised that greater levels of dilution could result in greater levels of hydrolysis and acidogenesis due to a decrease in the concentration of hydrolysis and acidogenesis products in the bulk liquid.

100 L of slurry was prepared by mixing 10 L of fresh cow manure with 90 L of fresh tap water. The slurry was well mixed and allowed to settle for two hours. The supernatant was removed by pumping off until the sludge layer (volume 20.5 L) was revealed. 1,000 mL sludge was added to 2,000 mL conical flasks to which were added differing volumes of water (250, 500, 750 and 1,000 mL). On addition, each reactor was briefly stirred and allowed to settle. Reactors had contact times of 24 hours. At the end of contact times, the same respective volumes of supernatant were removed and replaced with the same respective volumes of water. The experiments ran for 15 days. Temperature was controlled at 20 ^{o}C. All dilution conditions were run in duplicate. All reactors were sampled daily and analysed for COD, VFA and pH.

3.3.3. Mixing Experiment

Available literature indicates that some degree of mixing is beneficial to hydrolysis and acidogenesis through the action of improving contact between the bulk liquid and solids. Other literature had indicated that mixing could encourage methanogenesis by reducing the build up of VFAs around hydrolysis initiation centres. Hence the mixing experiments were carried to determine if there is an optimum level of mixing with regard to hydrolysis. The effect of mixing on hydrolysis and acidogenesis was measured in terms of soluble COD and VFA production respectively.

29.5 L of slurry was prepared by mixing 3 L of fresh cow manure with 26.5 L of fresh tap water. The slurry was well mixed and allowed to settle for two hours. The supernatant was removed and the remainder was passed through a 2.5 mm mesh

sieve. This produced 4.8 L of sludge. Samples of slurry, sludge and supernatant were taken and analysed for solids content (VS and TS), COD (soluble and total), VFAs and pH.

400 mL of sludge was placed in a 1,000 mL conical flask with 400 mL fresh reverse osmosis (RO) water. Reactors were closed with rubber bungs. The next day and for each day afterwards for a period of 25 days approximately 400 mL of liquid portion was removed by straining out through a porcelain filter. 400 mL of fresh RO treated water was then added to each reactor. Temperature was controlled at 20^{o}C. All mixing conditions were run in duplicate. The decant was analysed for COD (total and soluble), VFAs and pH.

Three conditions were trialled:
Condition 1: once daily manual stirring by briefly swirling the reactor;
Condition 2: stirring for 15 minutes once every 2 hours; and
Condition 3: continuous stirring.
Stirring was by magnetic stirrer (Heidolph MR3001) set at 200 rpm with a 30 mm magnetic bar.
At the end of the experiment, the remaining sludge was analysed for volume, TS, VS and COD (total and soluble).

3.3.4. Hydraulic Residence Time Experiment

Little literature was found describing the effect of hydraulic residence time on the hydrolysis process. It was hypothesised that adjusting the hydraulic residence time could affect hydrolysis through the removal of both planktonic (water borne) bacteria and hydrolysis products from the hydrolysis reactor. This experiment was carried to determine the effect of the residence time of the liquid phase (HRT) on hydrolysis of the solid phase in terms of soluble COD production, and the effect of HRT on acidogenesis in terms of VFA production.

400 mL of RO water was added to 400 mL of separated sludge in 1 L conical flasks. 400 mL of liquid phase was removed by decanting and replaced with fresh water at varying intervals depending on the assigned HRT. The experiment ran for 30 days and 5 HRTs were trialled; 1, 2, 5, 10 and 15 days. The experiment was carried out in

a temperature-controlled room at 20^{o} C. All HRT conditions were run in duplicate. The decant was analysed for COD (total and soluble), VFAs and pH.

3.3.5. Rumen Dosing Experiment

Two rumen dosing experiments (RD1 and RD2) were carried to see if the addition of rumen contents would have any effect on hydrolysis of cow manure sludge in terms of COD solubilisation and acidogenesis in terms of VFA production. It was hypothesised that the addition of a bacterial population, known to produce hydrolytic enzymes, such as that found in rumen contents could enhance the hydrolysis of fibres found in FDE sludge.

Sheep rumen contents rather than cow rumen contents were used, as no fresh cow rumen was available at the time of experimentation.

RD1: Two batches were run, both in triplicate. Batch RD1b was the control. The only difference from batch RD1a was that the rumen contents were sterilised by autoclaving. Both experiments were run in a constant-temperature room (22^{o} C). 500 mL of sludge, settled from 10% fresh cow manure slurry, was placed in 2 L conical flasks. To this was added 100 mL of rumen contents (autoclaved in the case of the control) which had been freshly sampled from one fistulated sheep. To this was added 600 mL of RO water. The flask was briefly stirred manually to suspend the solid matter. Flasks were then sealed and allowed to stand and settle for 23.5 hours. After this period, the same volume as was added was removed in the form of supernatant. This process was repeated daily for a further 14 days. Temperature was controlled at 20 ^{o}C. Decant was analysed for COD_{total}, TS, VS, pH, and VFAs. Off gas was captured using floating inverted cones and volumes recorded daily.

RD2: This experiment was carried out with the same method as RD1 except that an extra batch was run which had no rumen addition but only 500 mL of sludge. In addition, all reactors were diluted with a buffered artificial saliva solution to replace the RO water which was used in experiment RD1. The saliva solution was used to provide more favourable conditions for the rumen organisms. The saliva solution was sparged with laboratory grade CO_2 prior to use to remove dissolved oxygen. Reactor flasks were flushed with nitrogen gas after replacement of the dilution liquid

and then sealed with rubber bungs. The experiment was run for 15 days. Temperature was controlled at 20 ^{o}C. The decant was analysed for COD, pH and VFAs. In addition, a small sample of reactor mixed liquor was analysed for VS and TS. Batches were run in triplicate.

Artificial Saliva (McDougall, 1948)
1L H_2O, 9.8g $NaHCO_3$, 3.97g Na_2HPO_4 anhydrous, 0.47g NaCl, 0.57g KCl, 0.06g $MgCl_2$, 0.04g $CaCl_2$

All components except $CaCl_2$ were added to the 1L of water and allowed to dissolve overnight with stirring. Next morning the $CaCl_2$ was added and allowed to dissolve (this is required so that the $CaCl_2$ does not form insoluble complexes with the phosphates in the buffer). The solution was sparged with CO_2 for 30 minutes prior to use and pH was measured to check it was around 6.8.

3.3.6. Column Leachbeds

Leachbeds are reactors that retain solids while allowing liquid to drain out. In gravity flow-through leachbeds liquid is applied to the top of leachbeds in order to flow through the amassed solids and remove the products of hydrolysis. Given the success of other researchers in using this type of reactor to hydrolyse waste products (Chugh *et al.*, 1999; Ghanem *et al.*, 2001; Lehtomaki *et al.*, 2007), it was decided to trial leachbeds in the hydrolysis of solids in FDE. A series of leachbed experiments were carried out to determine if this type of reactor would result in improved hydrolysis and acidogenesis as evidenced by an increase in COD and VFA concentration in the liquid portion of FDE. Two trials were conducted; LB1 trialled FDE slurry with further daily applications of slurry, while LB2 trialled an initial volume of sludge with daily applications of fresh water.

Experiment LB1: This was an initial exploratory experiment carried out to determine if cumulative applications of FDE slurry to a leach bed reactor would result in higher COD and VFA in the liquid portion, than could be achieved by simple liquid solid separation. Cumulative application was chosen, rather than running the column as single application of slurry, in order to mimic the daily addition of fresh material that would be expected on a working dairy farm.

3 L of 10% cow manure slurry was poured into a geotextile sock made from weed matting placed in a 15 L Perspex column reactor. The purpose of the geotextile sock was to retain solids while allowing for the liquid portion of the slurry to drain out. The geotextile sock sat on 2 cm of coarse gravel to provide drainage. The liquid portion was allowed to drain out of the bottom of the leachbed to a collection vessel. Every day a further 3 L of slurry was added and the liquid portion drained through the solids of the previous days applications. The sludge in the column was unmixed apart from mixing caused by the once daily pouring on of fresh slurry. This experiment ran for 24 days. The experiment was carried out at ambient temperatures ranging from 5^{o} to 15^{o} C. Daily temperature was not recorded. Volumes of leachate collected were recorded daily. Leachate was analysed for COD_{total}, BOD, VFAs and solids content.

Experiment LB2: This experiment was carried out to examine the extent of hydrolysis and acidogenesis that occurred as a result of the continuous application of fresh water to sludge in a gravity flow-through leachbed reactor. Fresh water rather than FDE supernatant was used as the liquid phase in order to ensure that all organics detected resulted from the resident solid portion rather than the influent liquid. The experiment was also used to compare the effect of leachbed reactor configuration on hydrolysis and acidogenesis in FDE sludge. The two configurations were a gravity flow-through leachbed reactor (LB2) and a settle and decant leachbed reactor (SD1, described in section 3.3.7 below).

12 L of sludge settled from 71 L of 10% slurry was placed in a geotextile sock made from weed matting placed in a 15 L Perspex column reactor. The geotextile sock sat on 2 cm of coarse gravel to provide drainage. The liquid portion was allowed to drain out of the bottom of the leachbed to a collection vessel. Water was applied to the column at a rate of about 2.13 L per day using a timed peristaltic pump and allowed to filter through the solids. The sludge in the column was unmixed. This experiment ran for 28 days. The experiment was carried out at 22^{o} C in a constant temperature room. Volumes of leachate collected were recorded daily. Leachate was analysed for COD_{total}, BOD, VFAs and solids content.

Figure 3-1: Photo of laboratory set-up of experiment LB2.
The reactor can be seen on the left, with the influent water reservoir in the blue bucket.

3.3.7. Settle and Decant Leachbeds

In the settle and decant reactor fresh water is added to the solids, briefly stirred and then allowed to settle before decanting the liquid portion 24 hours later. This configuration was trialled to compare its performance in terms of increase in COD, as a measure of hydrolysis, and VFA, as a measure of acidogenesis, in the liquid phase to that of a gravity flow-through leachbed reactor (LB2).

12L of sludge prepared from 10 % cow manure slurry was prepared and placed in a 15 L Perspex column reactor. It was then briefly stirred (approximately 30 seconds) and left to resettle. This was then allowed to stand overnight before draining and replacing with a fresh volume of water (approximately 2 L per day) Actual volumes added were determined by the output volume of reactor LB2. This experiment ran for 28 days. The experiment was carried out at 22^{o} C in a constant temperature room. Volumes of leachate collected were recorded daily. Leachate was analysed for COD_{total}, BOD, VFAs and solids content.

3.3.8. Bench-scale Leachbed Separator

A bench-scale leachbed separator was operated in order to investigate the degree of hydrolysis (in terms of soluble COD production), acidogenesis (in terms of VFA production) and downstream methane production in methanogenic reactors that could be achieved using manure slurry as an influent. The leachbed separator, which provided the treated feed for methanogenic reactors B and C (see 4.8.2 below), was run as a series of batches. 3.5 L of 9:1 water to manure slurry was mixed daily and added to the leachbed separator. The contents of the leachbed separator were mechanically stirred for 5 minutes after the addition of slurry. Solids were kept in the separator by a wire mesh while the liquid was allowed to drain out. The liquid effluent coming out of the separator is termed the "treated feed". The experiment was carried out in a temperature-controlled room set at 20 ^{o}C. Figure 3-2 below shows the leachbed separator in operation. The HRT of the system was controlled by adjusting the height of the outlet thereby varying the volume in the leachbed separator available for containing the liquid portion. The average SRT of the retained solids was dependent on the length of time for each batch so that SRT = (1 + 2 + 3 …. + n)/n , or more simply SRT = 0.5n + 0.5, where n = the number of days the batch was run. No solids were removed from the leachbed separator until the completion of the batch.

Figure 3-2: Experimental set-up of leachbed separator

Three operating conditions were trialled for the leachbed separator:

Condition 1: less than 1 day HRT and a maximum of 30 days SRT, average SRT of 15.5 days. (batches 1-3).

For the first three batches the solids were allowed to accumulate in the separator for about 30 days before being removed giving the maximum SRT. The treated feed was allowed to drain freely out of the bottom of the separator giving an HRT of less than one day.

Condition 2: 8 day HRT and a maximum of 30 days SRT, average SRT of 15.5 days. (batch 4 and the first 30 days of batch 5).

The outlet of the separator was lifted so that liquid remained within the separator, in contact with the solids, for 8 days. This was done as a result of observations of higher VFA and soluble COD content coming out of the field trial leachbed separator and the findings of the HRT and dilution experiments which indicated that HRTs longer than 2 days and higher liquid to solid ratios favoured hydrolysis .

Condition 3: 8 day HRT and a maximum of 135 days SRT, average SRT of 68 days (batch 5).

For batch 5 the solids were allowed to accumulate in the reactor for 135 days. The change in operation was done to investigate whether the extended SRT for solids in the separator would improve the solubilisation of COD or result in COD consumption due to the establishment of a methanogenic population.

The operating parameters of the leachbed separator are summarised in Table 3-1 below.

Table 3-1: Operating parameters of the leachbed separator.

Parameter	**Value**
Effective reactor volume (L)	22.5
Total reactor volume (L)	30
Feed type	10% cow manure slurry
Volumetric loading (L/L/day)	0.156
HRT (days)	1 and 8
Average organic loading (mgCOD/L/day)	2,110
Average organic loading (mgVS/L/day)	1,180
Temperature (^{o}C)	20

3.3.9. Bench-scale Methanogenic Reactors

The purpose of the bench-scale trials was to determine the effect of the leachbed separator operation on down stream methanogenic reactors in terms of volumetric methane yield (LCH_4/Lreactor/day), methane yield relative to volume of influent slurry (LCH_4/Lslurry), and specific methane yield (LCH_4/gVS_{added}). All methanogenic reactors were run continuously; however, the trial can be described as being divided into 5 phases relating to the 5 batches of the leachbed separator. In the text, these 5 phases are described as "batches" in order to match up with the batches of the leachbed separator. In the bench-scale trials three types of methanogenic reactors were operated. The first type (Reactor A) was designed to operate as a typical anaerobic pond fed with 9:1 water to manure slurry and having a 50-day HRT. The second type (Reactor B) was also designed to operate like a pond but was fed with treated feed from the leachbed. This was initially run at a 20-day HRT and is referred to as B1. However, towards the end of the trial (batch 5) the HRT of this reactor was reduced to 10 days at which point the reactor is referred to as B2. For batch 5, a new reactor was commissioned to run at a 20-day HRT, this reactor took over the function of B1 and is referred to as B1 as well. The third type (Reactor C) was a rock filter designed to act as attached biomass reactor fed with treated feed. River gravel was used as the medium for the biomass attachment. This reactor was initially run at a 10 day HRT and is referred to as C1, the HRT was then reduced to 5 days at which point the reactor is referred to as C2. All reactors were operated unmixed and at 20 ^{o}C in a temperature-controlled room. All reactors were seeded with anaerobic sludge sourced from the anaerobic pond at #4 Dairy Unit of Massey University. Figure 3-3 shows the unstirred methanogenic digesters (reactors A and B) connected up to gas meters.

Figure 3-3: Experimental set-up of bench scale digesters.

A schematic of the combined set-up of slurry inputs, leachbed separator and methanogenic digesters is shown in Figure 3-4 below.

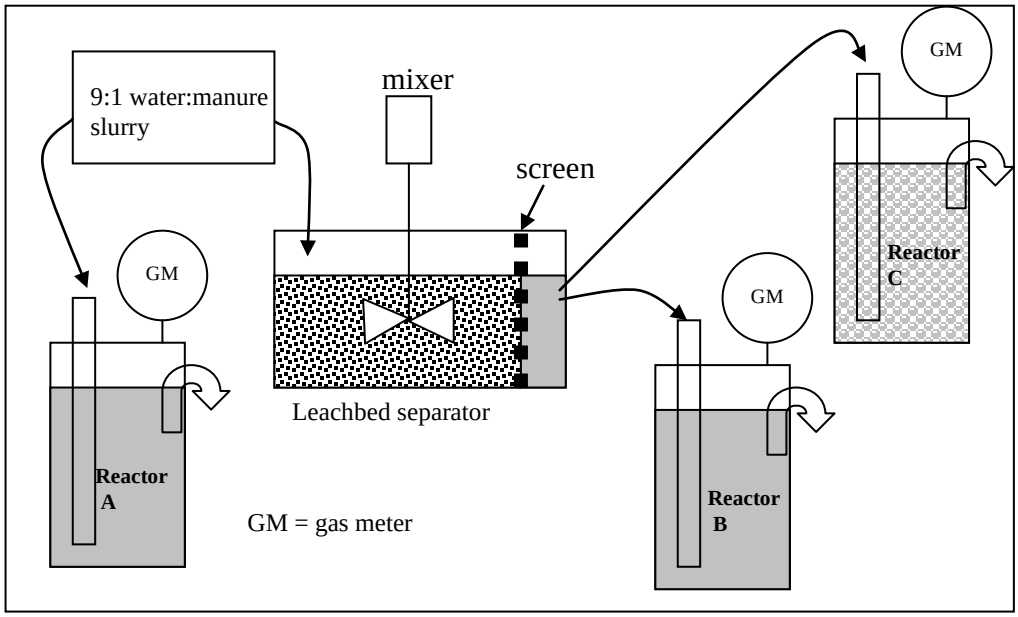

Figure 3-4: Schematic of bench-top experiment.

The operating parameters of the methanogenic reactors are summarised in Table 3-2 below.

Table 3-2: Operating parameters of bench scale methanogenic reactors.

	Reactor				
	A (unstirred reactor)	**B1 (unstirred reactor)**	**B2 (unstirred reactor)**	**C1 (rock filter)**	**C2 (rock filter)**
Available volume (L)	20	20	20	10	10
Feed	400 mL slurry	1,000 mL treated feed	2,000 mL treated feed	1,000 mL treated feed	2,000 mL treated feed
HRT (days)	50	20	10	10	5
Organic loading (mgCOD/L/day)	230	450	900	900	1,800
Organic loading (mgVS/L/day)	150	305	610	610	1,215
Temperature (^{o}C)	20	20	20	20	20

3.3.10. Field Trials

Field trials of a leachbed separator and ambient temperature methanogenic reactor were carried out to investigate the larger scale application of the laboratory findings. Field trials took place at the #4 Dairy Unit of Massey University. This is a dairy farm with a herd of 300 mixed Friesian and Jersey milking cows. The trial started in early October of 2007. The data presented in this study is from late November 2007 to May 2008.

A submersible DW– vox 100 single phase pump transferred dairy shed wash-down effluent from a grit pit across to a leachbed separator which was constructed from a converted 120 m^3 feed pad pit (see Figure 3-5 below). A weeping wall was constructed in the pit to retain the solids within the leachbed separator. The weeping wall was 1.3 m high and 4.8 m wide. It was constructed with 4,800mm X 50mm x

100 mm lengths of tanalised pine stacked with 10 mm gaps between them. The weeping wall was placed 1.5 m from the end concrete wall so that an 8.6 m^3 reservoir was formed to receive effluent from the leachbed separator. Overflow from this reservoir was at a height of 1.2 m. The effective volume of the leachbed separator was approximately 100 m^3. The influent side of the leachbed separator also received a significant volume of waste cattle feed and manure from the feed pad. FDE influent into the leachbed separator was estimated using a volume of 50 L/d/cow (Vanderholm, 1984) resulting in 15 m^3/d. An extra 1.7 m^3 per day of solids from the feed pad was estimated to enter the leachbed separator giving a total influent of 16.7 m^3/d. The estimated organic loading on the leachbed separator was 2,250 gVS/m^3/day.

Figure 3-5: Weeping wall leachbed separator at #4 Dairy Unit.

A submersible 400-watt single-phase vortex pump controlled by programmable timer fed from the weeping wall reservoir to an unstirred 15 m^3 methanogenic reactor. The reactor was fed daily with 0.75 m^3 feed from the leachbed separator. This gave a nominal HRT of 20 days. Temperature was ambient and uncontrolled. The methanogenic reactor was seeded with approximately 5 m^3 of anaerobic sludge derived the farms anaerobic pond. Effluent from the methanogenic reactor was discharged into the existing pond system. Flow from the reactor was measured using a V-notched weir. Gas measurement was made with a model 750 Ampy brand domestic gas meter. A schematic of the leachbed separator and methanogenic reactor system is shown in Figure 3-6 below.

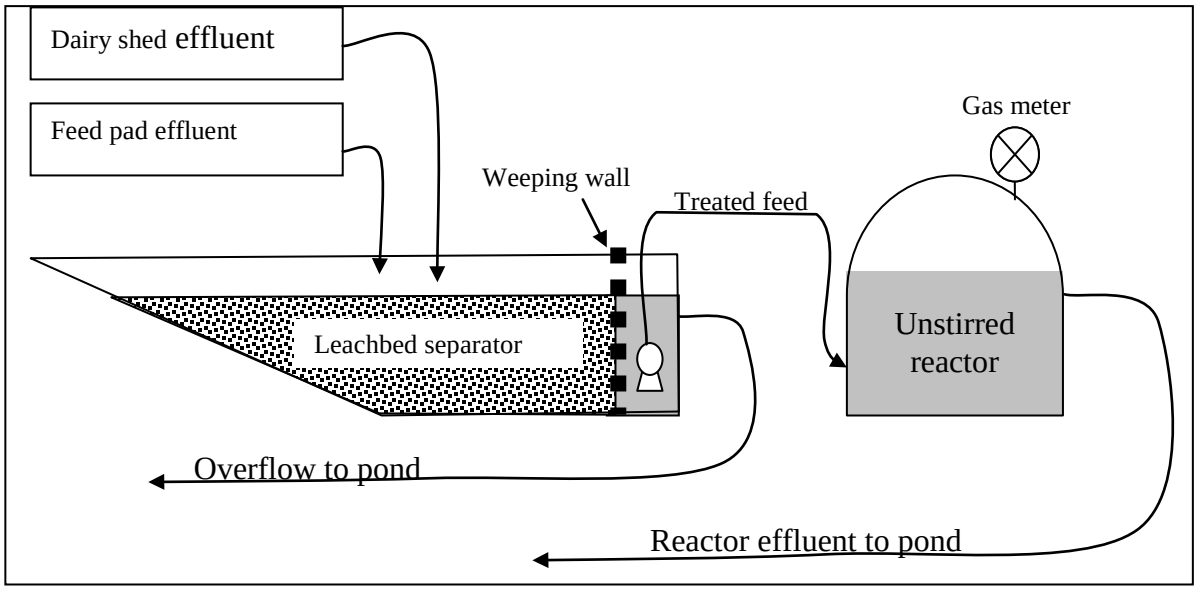

Figure 3-6: Schematic of pilot trial at #4 Dairy Unit.

A summary of the experiments and their objectives is shown in Table 3-3 below along with the chronological order in which they were carried out. Due to time constraints the bench-top trials and field trials were started prior to the completion of some of the batch experiments. Presentation of the results begins with batch experiments, followed by the reactor configuration experiments, then the bench-top experiments and finishing with the field trials.

Table 3-3: Summary of experiments and their objectives.

Chronological order	Experiment	Objectives
1	Characterisation	To determine the organic content of the FDE in order to better understand the potential for methane production.
2	Leachbed LB1	To determine if cumulative applications of FDE slurry to a leach bed reactor would result in higher COD and VFA in the liquid portion, than could be achieved by simple liquid solid separation.
3	Leachbeds LB2 and SD1	To determine the extent of hydrolysis and acidogenesis as a result of the application of water to sludge in leachbed reactors. To compare the effect of leachbed reactor configuration on hydrolysis and acidogenesis in FDE sludge.
4	Dilution	To determine the effect of dilution (the ratio of sludge to fresh water) on hydrolysis and acidogenesis in FDE sludge.
5	Rumen contents addition	To determine the effect of the addition of rumen contents on hydrolysis and acidogenesis in FDE sludge.
6	Bench-top leachbed separator	To investigate the degree of hydrolysis, acidogenesis and downstream methane production in methane reactors that could be achieved using FDE slurry as an influent to a leachbed separator.
7	Bench-top methane reactors	To compare the methane production of two unstirred methane reactors fed with different influents; treated feed and FDE slurry. To compare the methane production of two different types of methane reactors (unstirred and rock filter) fed with treated feed. To compare methane production of methane reactors at varying HRTs.
8	Field trials	To trial a larger scale leachbed separator and ambient temperature methane reactor in an on-farm environment.
9	Mixing	To determine the effect of mixing regimes on hydrolysis and acidogenesis in FDE sludge.
10	HRT	To determine the effect of hydraulic residence time on hydrolysis and acidogenesis in immobilised FDE sludge.

3.4. Analytical Procedures

3.4.1. Total Solids (TS) and Volatile Solids (VS)

TS and VS were determined following the method set out in sections 2540 B and 2540 E of *Standard Methods* (APHA, 1998). All TS and VS analysis was carried out in duplicate.

3.4.2. Volatile Fatty Acids (VFAs)

Volatile fatty acids measured were acetic acid, propionic acid, n-butyric acid, iso-butyric acid, n-valeric acid and iso-valeric acid. Volatile fatty acids were analyzed by ion chromatography using a Dionex, DX-100, ion chromatograph. All samples were filtered through 0.45 μm membrane filters prior to analysis. Single samples were taken for VFA analysis.

3.4.3. pH measurement

The pH of samples was measured using an Orion model 230A pH meter. The pH meter was calibrated prior to use using pH 4 and pH 7 colour key buffer solutions (BDH Laboratory Supplies)

3.4.4. Chemical Oxygen Demand (COD)

The closed reflux colorimetric method as described in section 5220 D of *Standard Methods* (APHA, 1998) was used. Total (COD_{total}) and soluble ($COD_{soluble}$) measurements were undertaken, with soluble COD being defined as the fraction of COD that passed through a 0.45 μm membrane Minisart filter. The COD test was performed as follows:

A 2.5 mL volume of sample was added to Kimax COD tubes, followed by 1.5 mL of digestion solution and 3.5 mL of sulphuric acid reagent. Tubes were closed with caps, briefly shaken and heated at 150^{o} for 120 minutes in a Hach COD digestion block and allowed to cool. All samples and blanks were prepared in triplicate. The absorbance of the digested samples was measured at 600 nm on a Shimadzu UV-1201 spectrophotometer. The spectrophotometer was zeroed using a digested blank prepared using reverse osmosis water in place of a sample.

3.4.5. BOD

BOD was assessed using a HACH BODTrak manometric BOD apparatus. Samples were first diluted down to approximate range and then measured wastewater sample volumes were placed in BOD bottles of the apparatus, and BOD nutrient buffer pillows from HACH were added. Lithium hydroxide sachets (also from HACH) were placed in the bottleneck stoppers to remove any CO_2 given off by the samples. This ensured that pressure measurements accurately reflected oxygen consumption. Sealing caps were then fastened airtight with silicon grease. Samples were stirred by magnetic stirrers to enhance oxygen transfer. During the test, pressure changes due to oxygen uptake by the sample were continuously monitored by manometric sensors. These manometric measurements were then converted to mgBOD/L, which was displayed on the apparatus. Readings were then corrected for dilution factors. Tests were run for 5 days. All samples were prepared in duplicate.

3.4.6. Gas Composition

Gas was extracted from reactors using lock valve syringes and injected into a Shimadzu gas chromatograph.

3.4.7. Data Analysis

Data was analysed using the tools available in Microsoft Excel. Error bars in graphs are variance of results based on maximum and minimum values where samples numbered less than three. Error bars on graphs using samples populations greater than three are 95% confidence limits unless otherwise indicated. Statistical analysis of variance was carried out using one-way and two-way ANOVA with an alpha value of 0.05.

4. Results and Discussion

4.1. Characterisation of Dairy Cow Manure Slurry

Characterisation of dairy cow manure slurry was carried out in order to determine the COD, volatile and total solids and VFA content of the slurry and its liquid and solid fractions. This was done in order to provide a basis for comparison for subsequent experiments on improving hydrolysis and acidogenesis.

4.1.1. Settling Characteristics

The percentage of sludge derived from 1:9 manure to water slurry using the settle and decant technique ranged from 11% to 20.5%. The gravity method through a geotextile cloth produced a sludge, which was 9% of the original volume after draining overnight. Although the gravity method resulted in generally higher volumes of decant and a higher solids content sludge residue than the settle and decant method, the settle and decant method was used in the dilution, HRT, enzyme addition and mixing trials due to the ease of operation (2 hours compared to overnight) and ease of handling for larger volumes of slurry.

4.1.2. Characteristic of the Slurry

A summary of the COD, VFA and solids concentrations found in 1:9 manure to water slurries used in this study can be found in Table 4-1 below. Numbers of samples vary for each parameter as not all parameters were measured for every slurry sample. In the table it can be seen that the COD concentrations of the slurry showed a high degree of variability and were generally higher than those reported in the literature for New Zealand; 6,600 – 11,000 mg/L (Mason & Mulcahy, 2003). BOD measurements of slurry ranged from 1,540 to 3,200 mg/L. These fall within the ranges reported in the literature; 1,500 mg/L (Vanderholm, 1984) and 4,500 mg/L (Saggar *et al.*, 2004). The VFA concentration in the slurry is typically higher than that reported by Mason and Mulcahy (210 mg/L COD_{vfa}).

Table 4-1: Concentrations of total and soluble COD, VFAs, total and volatile solids found in slurries used in this study.

	COD_{tot} (mg/L)	COD_{sol} (mg/L)	COD_{sol}/ COD_{tot} (%)	VFA_{total} (mg/L)	TS (%)	VS (%)	VS/TS (%)
Average	11,354	2,061	19%	308	1.03	0.75	74%
Number of samples	55	44	43	53	30	29	29
Max	22,333	4,952	37%	553	1.48	0.90	81%
Min	3,571	586	6%	53	0.56	0.45	59%
Std Dev	4,332	843	6%	115	0.19	0.12	5%
95% confidence	±1,145	±249	±2%	±31	±0.07	±0.04	±2%

Figure 4-1 below shows the average percentages of 53 samples of 1:9 manure to water slurries used in the bench-top methanogenic reactor experiments. A breakdown of the VFA fractions shows that acetate was the most abundant species, with significant amounts of propionate and butyrate. While the acetate portion is the most readily convertible to methane, it accounts for only 58%. The remaining 42% must undergo acetogenesis before conversion to methane can take place. This means that any biomethanation system must include conditions that are favourable to acetogenesis if full conversion of VFAs to methane is to take place.

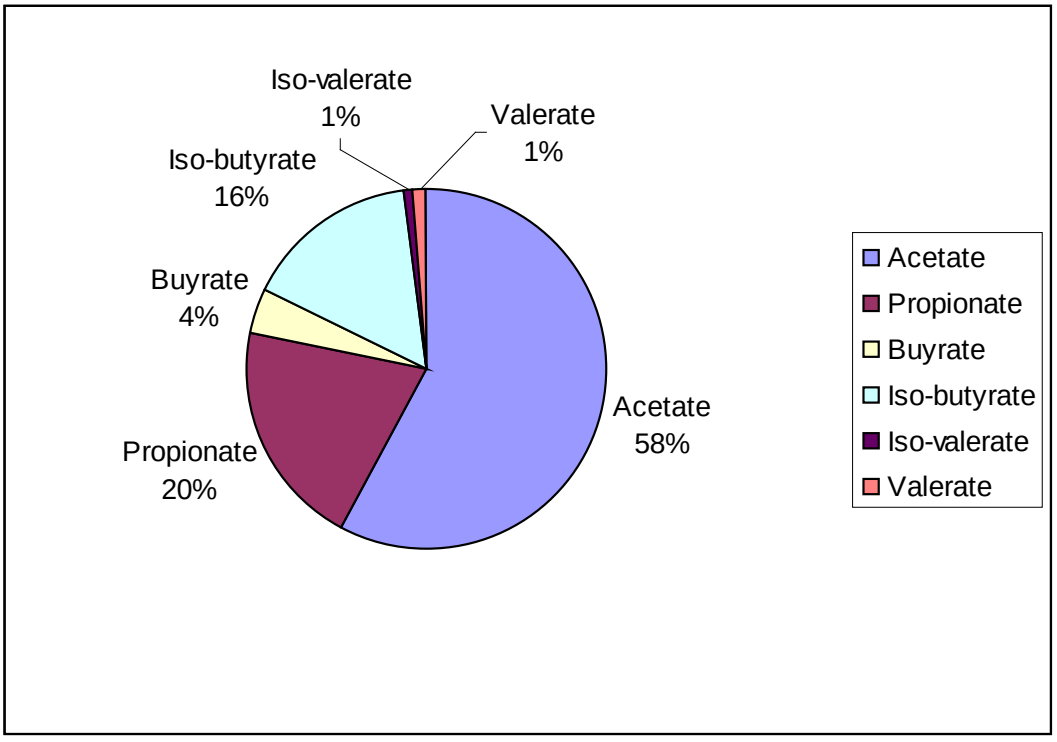

Figure 4-1: VFA percentage distribution in cow manure slurry.

4.1.3. Characteristics of the Sludge and Supernatant

Table 4-2 and Table 4-3 below show the average COD, VFA and solids content of the sludges and supernatants derived from slurry in the dilution, mixing, HRT, rumen addition and leachbed experiments. Numbers of samples vary for each parameter as not all parameters were measured for every sample.

Table 4-2: Concentrations of total and soluble COD, VFAs, total and volatile solids found in sludges used in this study.

	COD_{tot} (mg/L)	COD_{sol} (mg/L)	COD_{sol}/ COD_{tot} (%)	VFA_{total} (mg/L)	TS (%)	VS (%)	VS/TS (%)
Average	46,467	12,149	22%	380	5.57	4.26	72%
Number of samples	6	3	3	3	5	3	3
Max	61,305	20,000	36%	447	7.61	6.43	85%
Min	23,715	5,686	13%	311	4.40	4.06	61%
Std Dev	14,391	7,257	12%	68	1.22	1.27	13%
95% confidence	±11,515	±8,212	±14%	±77	±1.07	±1.44	±15%

Table 4-3: Concentrations of total and soluble COD, VFAs, total and volatile solids found in supernatants used in this study.

	COD_{tot} (mg/L)	COD_{sol} (mg/L)	COD_{sol}/ COD_{tot} (%)	VFA_{total} (mg/L)	TS (%)	VS (%)	VS/TS (%)
Average	6,216	4,503	65%	219	0.43	0.29	66%
No.of samples	8	3	3	6	4	4	4
Max	8,286	6,500	78%	235	0.47	0.35	75%
Min	4,452	3,200	42%	97	0.41	0.26	62%
Std Dev	1,289	1,756	20%	61	0.02	0.04	6%
95% confidence	±893	±1,987	±22%	±49	±0.02	±0.04	6%

Figure 4-2 below shows the relative divisions of supernatant and sludge mass in the original slurry in terms of total COD, VFA and BOD (averaged). The percentage of COD in the supernatant ranged between 36 and 59 % of that found in the slurry. This is a similar range to that reported by Wilkie *et al* (2004) in their review of COD removal efficiencies through screening and settling; they reported that most systems resulted in 40 to 68 % of total COD ending up in the liquid portion. Supernatant BOD accounted for 56 to 64% of the total BOD in the slurry. This is slightly higher than relative portion of COD in the supernatant, possibly indicating a higher biodegradable component in the supernatant.

Figure 4-2: Average COD (total), VFA and BOD contributions (%) of supernatant and sludge fractions in slurries used in this study.

VFA analysis typically showed most of the VFAs to be in the supernatant. This is not surprising as VFAs are only detectible in their soluble form. The implication of this is that much of the BOD in the sludge is in non-VFA form.

Although the characteristics of farm dairy effluent are variable and dependent on various factors such as rations, breed and health of cows, and wash down practices, further research could be carried out in characterisation work. Specifically, there was limited analysis undertaken of the soluble COD, VFA content and solids content of the sludges and supernatant portions of the farm dairy effluent. Characterisation would have benefited from running specific methane potential tests on all portions of the farm dairy effluent. Characterisation would also benefit from standardisation of the separation techniques by, for example, applying gravity separation using screens with a standard mesh size.

4.2. Dilution Experiment

In this experiment, the effect of dilution (the ratio of sludge to fresh water) on the transfer of COD from sludge to the liquid phase, and on VFA production was investigated. Four sludge to fresh water dilution ratios were trialled; 1:0.25, 1:0.5, 1:0.75 and 1:1. Every day respective volumes of liquid were removed and replaced with the same volume of fresh water.

4.2.1. The Effect of Dilution on Hydrolysis

Figure 3-15 shows the change in COD concentration of the effluent liquid over the course of the experiment. The COD concentration was highest for the reactors with lowest dilution and lowest for those with highest dilution. The concentration appeared to level off for all reactors after about day 12. In a perfectly mixed inert system, where there is no hydrolysis or biological activity, constituents will be diluted and washed out so that the concentration of the constituents will approach zero.

The plot of change in concentration over time is called a washout curve and for an inert system will fit the curve: $C_{out}(t)/C_{(0)} = e^{-t/hrt}$.

Washout curves for the highest and lowest dilutions are included in the figure below for comparison. It can be seen that the change in COD concentration for the reactor with the lowest dilution (1:0.25) closely follows the washout curve indicating that there is little release of organic material from the solid phase. In contrast, the COD concentration of the highest dilution reactor (1:1) continues to have organic material in the effluent beyond that predicted by the washout curve. This may indicate that a greater degree of hydrolysis is taking place.

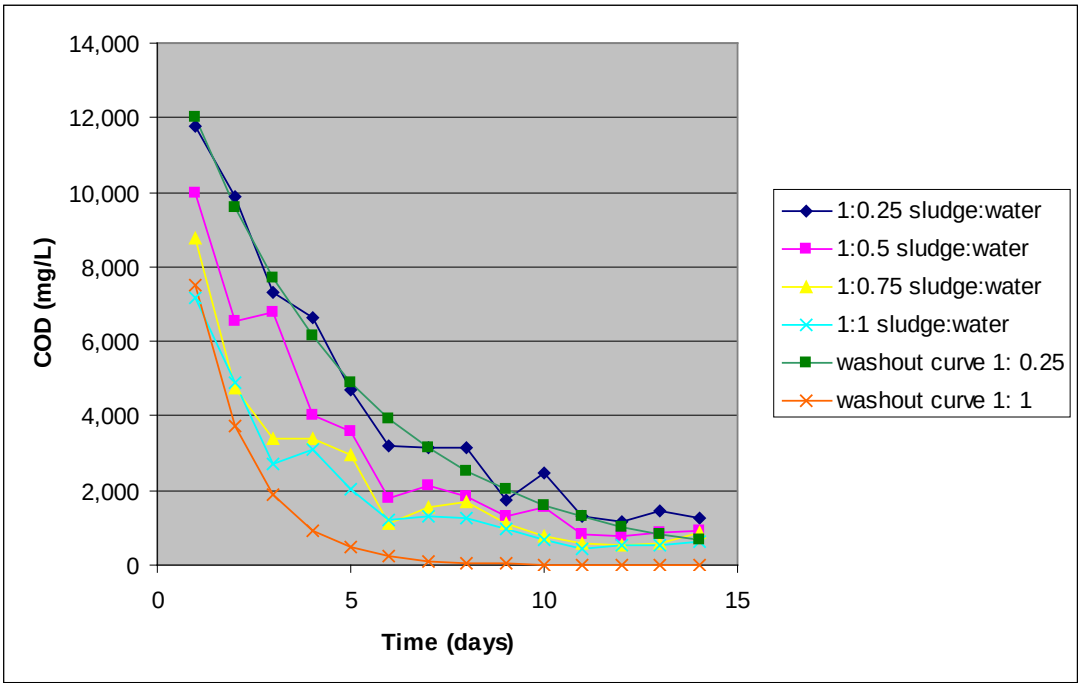

Figure 4-3: COD concentration of effluents from reactors with different sludge:liquid dilutions and theoretical washout curves.

Although COD concentration was highest in those reactors with lower levels of dilution the actual mass of COD extracted was higher in those reactors which had higher dilution ratios (Figure 4-4 below). Those reactors with higher dilution showed increased COD transfer to the liquid phase, beyond what might be expected from simple mass transfer due to dilution. The total mass of COD extracted from the settled solids in the highest dilution in just 15 days represented 44% of the total COD contained in the settled sludge. The 1:1 sludge to water reactor yielded 27,500 mg COD compared to 14,800 mg COD in the 1:0.25 sludge to water reactor, an 86% increase.

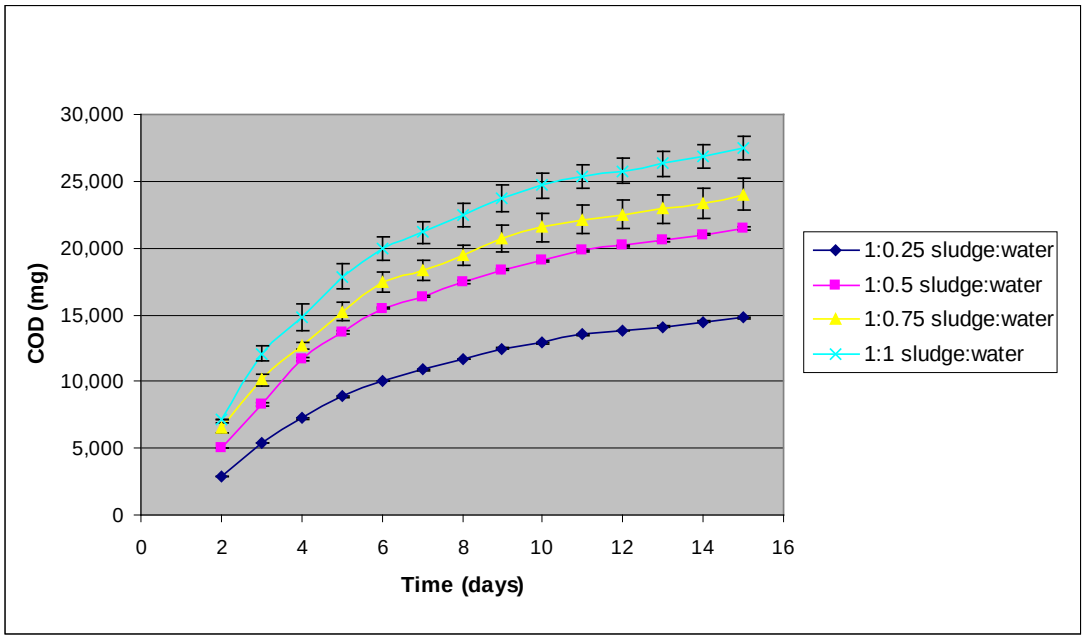

Figure 4-4: Cumulative COD mass (mg) extracted from reactors with different sludge:liquid dilutions and theoretical washout curves.
(Error bars are variance between duplicates)

A COD mass balance is presented in Table 4-4 below. This shows a range of COD losses ranging from 3,022 for the lowest dilution reactor to -4,641 mg COD for the highest dilution reactor. Negative numbers for total losses indicate errors in the determination of the COD mass, as it is not possible to take more COD out of the system than was originally present.

Table 4-4: COD mass balance for the dilution experiment.

	1: 0.25 sludge:water	1: 0.5 sludge:water	1: 0.75 sludge:water	1: 1 sludge:water
Initial COD (mg)	61,048	61,048	61,048	61,048
Total COD extracted (mg)	14,793	21,466	23,995	27,482
Total COD remaining (mg)	43,233	39,511	38,278	38,207
Total COD losses (mg)	3,022	70	-1,225	-4,641

A possible explanation for the increased COD yield for more highly diluted systems is that the mechanisms for COD release from the solid phase, for example hydrolysis, are product inhibited at the lower dilutions. This is unlikely as COD concentrations of the liquid phase were never higher than 12,000 mg/L and other researchers have

achieved sustainable hydrolysis at much higher effluent concentrations up to 100,000 mg/L (Vandeviviere *et al.*, 2002). Alternatively the higher COD yield for those reactors with higher dilution may be attributable to an equilibrium which may exist between substrates and products. In chemistry, equilibriums have an impact on the behaviour of a system when products or substrates are added or removed. Le Chatelier's principle predicts that more end product can be obtained if it is continuously removed from the solution (Atkins, 1998). Although it could be argued that Le Chatelier's principle only applies to closed chemical systems, if the hydrolysis of solids substrates is due largely to enzymatic action then production of soluble COD can be seen as a predominantly chemical rather than biological action. While the production of the enzymes, which bring about hydrolysis, can be seen as a more complex biological reaction, the chemical breakdown of lignins in the bulk solution should vary in theoretically predictable ways depending on changes in pressure, temperature and concentration. The production of the hydrolytic enzymes may itself be governed by changes in product concentration in the bulk liquid. This is a biological form of Le Chatelier's principle known as homeostasis. In this case, enzyme production could be increased due to a positive feedback mechanism caused by low concentrations of a particular product. This can also be seen as principally a mass transfer issue. Mass transfer resistances can occur during transport of enzymes from the microorganism to the bulk fluid, and from there to the substrate surface. Mass transfer resistances can also occur in the transportation of hydrolysis products from the substrate surface to bulk fluid. Jain *et al*. (1992) concluded that mass transfer resistance at the particle scale controlled the rate of hydrolysis. A relationship between the rate of enzyme production and the concentration of the enzyme in the bulk liquid was proposed :

$$\frac{\partial C_b}{\partial t} = K_1 a (C_m - C_b)$$

where K_1a = mass transfer coefficient (hr^{-1})
C_b = concentration of enzyme in the bulk liquid
C_m = concentration of enzyme in a film surrounding the microbe

The same principles may also be responsible for the increased VFA yields observed in the higher dilution reactors (see section 4.2.2 below).

4.2.2. The Effect of Dilution on Acidogenesis

A comparison of total VFA mass extracted is shown in the figure below. The reactors with the highest dilution produced on average 635 mg of VFA compared to only 184 mg for the reactors with the lowest dilution

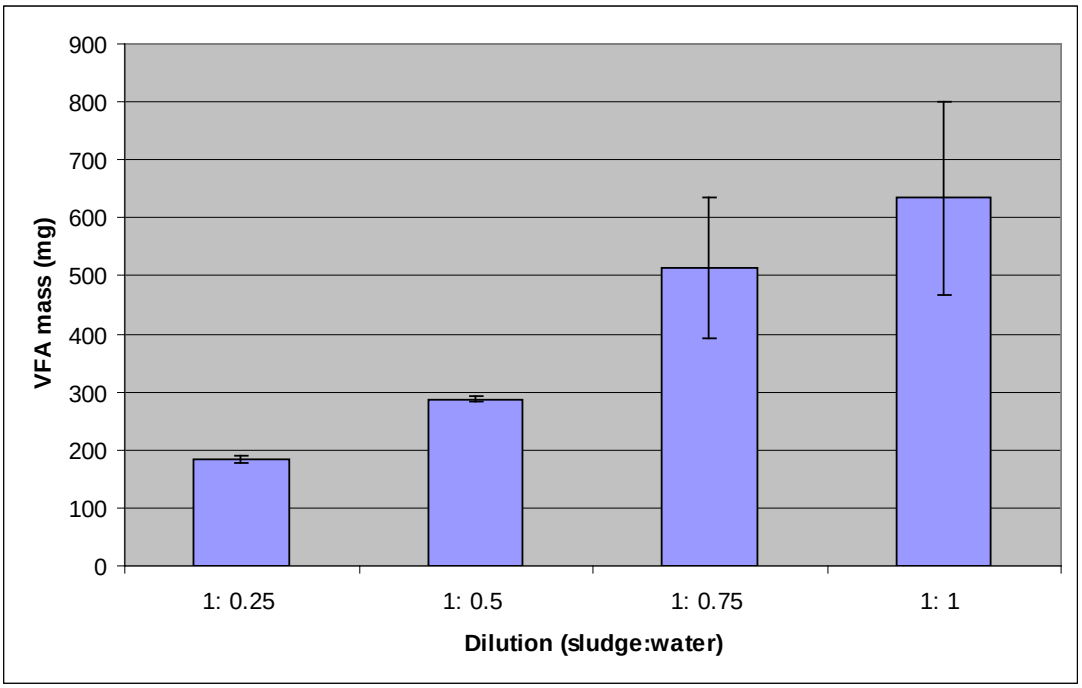

Figure 4-5: Comparison of VFA mass (mg) extracted from reactors with differing sludge:liquid ratios.

The best performing reactor in this experiment (1:1 sludge to water ratio) produced only an extra 250 mgVFA/L of sludge or only an extra 34 mgVFA/L of slurry. This is significantly less than the 1,130 mgVFA/L of sludge produced in settle and decant reactor trials in this study (see section 4.7 below).

Studies in acidogenesis of solid waste (restaurant food waste) showed little difference in VFA production from the same COD influent at different dilutions (Argelier *et al.*, 1998). However, they did find that specific VFA production (VFA/bacteria.d) was over four times higher at lower dilutions (1:5) compared with higher dilutions (1:25). Their dilution range was much higher than in this study but the COD concentration of the influent was comparable, ranging from 17,000 mg/L to 85,000 mg/L. The COD concentration of the reactor contents in this study ranged from 30,000 mg/L to 47,500 mg/L. Barrister and Pretorius (1998) found that a 3-fold

dilution of primary sludge led to a 70% increase in yield of VFAs in an acidogenic digester when compared to digestion without dilution.

Studies involving uninocculated cow manure slurry (Vedrenne *et al.*, 2008) showed a high degree of inhibition of methanogenesis for samples that were not diluted. The causes of inhibition were ascribed to either high VFA (over 5g/L) or free ammonia concentrations over 100 mg/L. In the present study initial VFA concentrations in all reactors were far below the 5g/L threshold suggested for VFA inhibition. Ammonia concentrations were not analysed for this trial but were analysed for other sludges and found to be as high as 550 mg/L NH_3. Depending on the initial concentration, the low dilution reactor may have taken more than six days to achieve non-inhibitory concentrations compared with only two days for the high dilution reactor, based on theoretical washout curves.

4.2.3. pH

The graph below shows the change in pH for each reactor over the course of the experiment. Reactors with 1:0.25 dilution showed little trend of acidification; fluctuating between 6.9 and 6.4. This is not in the range which would inhibit hydrolysis due to denaturing of enzymes, nor is it high enough to favour free ammonia (NH_3) over NH_4^+ (Chen *et al.*, 2008). The other reactors all show a definite downward trend in pH. The extent of acidification seems to correlate with the extent of dilution. The most highly diluted reactors showed the greatest pH drop, reaching about 5.3 on day 14 and 15.

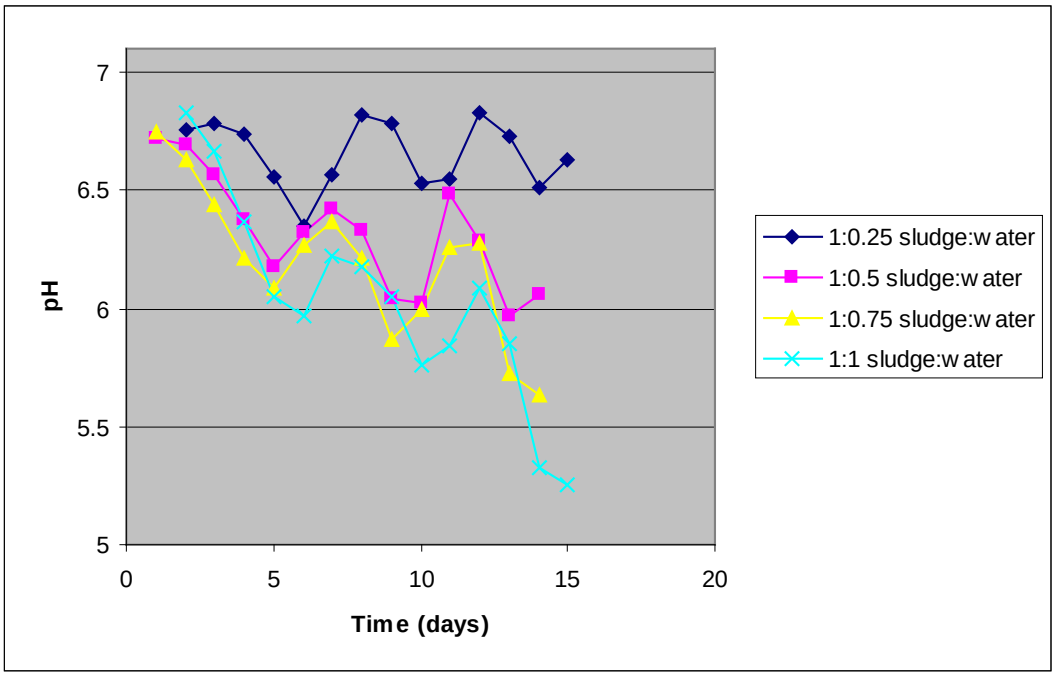

Figure 4-6: Change in pH of reactors with differing sludge to liquid ratios.

There are a number of areas in which the experimental method of this experiment could be improved in future research. While this experiment appears to show that there are higher yields of COD and VFA from those reactors with higher levels of dilution, it is unclear on what the mechanisms are that cause this. Further research is recommended into the nature of the cause of the increased yield, as to whether it is a physical or a biological cause. In addition, increased yield of COD does not necessarily mean increased hydrolysis. The COD may have originated from solids being washed out of the reactors rather than hydrolysis. The method used in this experiment could be improved using additional analysis. The measurement of the solids content in the discharged supernatant would help to determine the contribution of solids to the total COD content. Measurement of soluble COD would provide a clearer indication of hydrolysis than total COD. Daily determination of volatile solids removal in the sludge would also provide some additional evidence for hydrolysis.

The use of daily additions of fresh water and removal of supernatant was used in order to isolate the COD and VFA produced on any one day and prevent build up of these products in the reactor. However, this method may have introduced more uncertainty than it removed. Daily addition and removal of liquid probably resulted

in variations of the volume of resident liquid in the reactors. This error would have had the greatest impact in the determination of total COD and VFA masses removed as the daily mass was calculated based on the daily volume of supernatant removed, and may have been the reason behind the negative values obtained in the COD mass balance. Not removing supernatant daily would also have prevented the washout of suspended solid material from the reactors. The removal of the original supernatant and its daily replacement with water is also a condition which is unlikely to be mimicked in real life applications. A repeat of this experiment could benefit from maintaining the same supernatant throughout the experiment without daily removal. The condition of dilution could be created through variations in the manure to water ratios used in the preparation of the slurry, say 5%, 10%, 25% and 50% manure content slurries. This would have removed the need for daily additions of water and removals of supernatant thereby removing a source of experimental error. The recommendation that future research should use the original supernatant existing in the slurry as the bulk liquid is also applicable to the mixing, rumen dosing and HRT experiments.

4.3. Mixing Experiment

This experiment was carried out to investigate the effect of mixing on hydrolysis and acidogenesis in terms of soluble COD and VFA production respectively. All reactors received a 400 mL volume of sludge on day 1 plus a 400 mL volume of tap water. Each day the liquid portion of each reactor was removed and 400 mL of fresh water was added. The reactors differed only in their mixing conditions;

Condition 1: once daily mixing

Condition 2: once hourly mixing

Condition 3: continuous mixing

4.3.1. The Effect of Mixing on Hydrolysis

Soluble COD was highest for the constantly stirred reactors for the first 3 days but after day 5 the once daily mixed reactor consistently had the highest soluble COD in the decant (Figure 4-7 below).

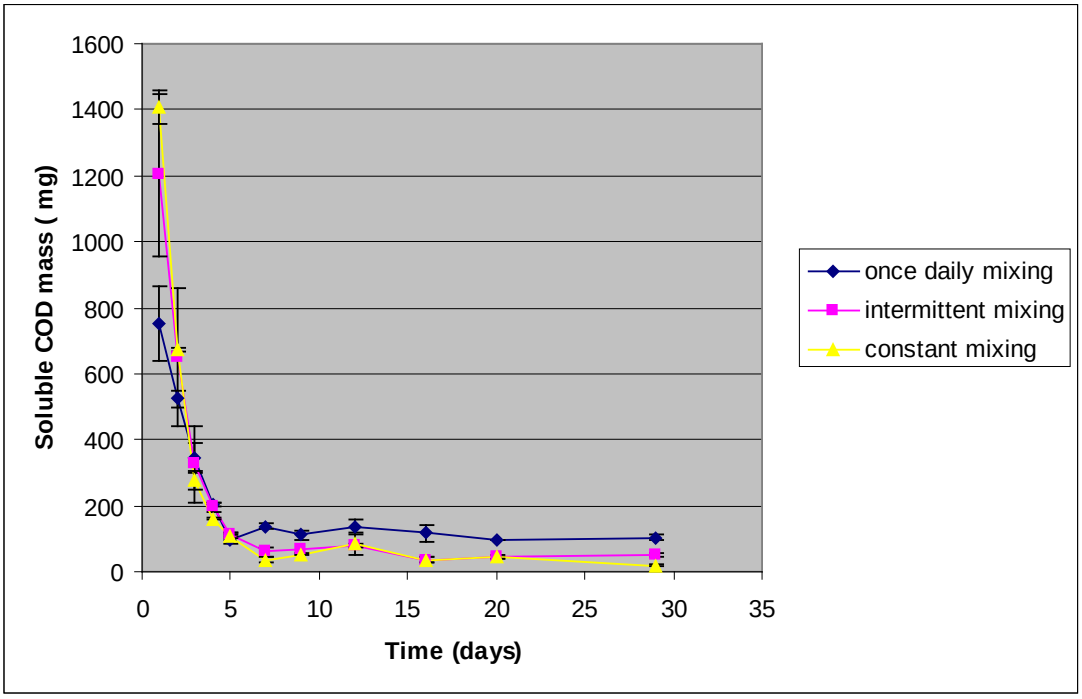

Figure 4-7: Comparison of soluble COD mass removed each day from mixing experiment reactors.

The first 8 days can be considered as washout of already existing soluble COD, while the following 25 days represent a period where solubilisation of COD occurred. At the rate of dilution (1:1, sludge to water) with 50% volumetric replacement with fresh water every day it would be expected that over 99% of any pre-existing soluble COD would be removed within the first 8 days so any significant amounts of soluble COD present after that should be the result of release caused by hydrolysis.

Despite higher soluble COD production for most of the run, the unmixed reactor was not able to make up for the initial lower COD production in the first few days by the mixed reactors as can be seen in the figure below showing cumulative soluble COD removed. Soluble COD production for the constantly mixed and intermittently mixed reactors was negligible for the final ten days of the trial, while the unmixed reactor appeared to be continuing soluble COD production. Extending the trial further may have provided clarification on the ultimate soluble COD yields of the three systems. It is not clear whether the once daily mixed reactor would have surpassed the other reactors in soluble COD production or reached a similar limit.

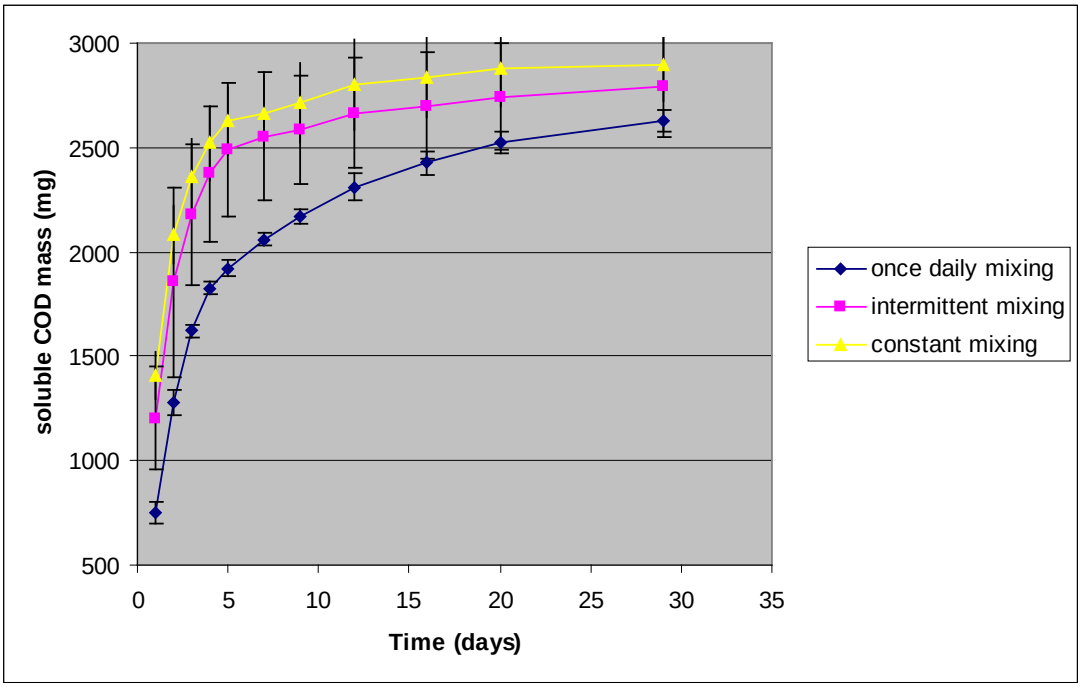

Figure 4-8: Comparison of cumulative soluble COD mass extracted from mixing experiment reactors.

Although a continued increase in soluble COD after day 8 appears to indicate soluble COD production due to hydrolysis, an examination of the mass balance of COD (Table 4-5 below) shows there was no overall increase of soluble COD from that originally in the reactors. The original mass of soluble COD for the 400 mL of sludge was 4,305 mg yet none of the reactors succeeded in removing this amount of soluble COD, with the yield for all reactors being between 2,653 and 2,900 mg. This appears to indicate that COD consumption was taking place. All reactors show some degree of total COD loss in the mass balance, which also may indicate consumption by anaerobic or aerobic microorganisms.

Table 4-5: COD mass balance for the mixing experiment.

	Once daily mixing	Intermittent mixing	Constant mixing
Initial Total COD (mg)	22,590	22,590	22,590
Initial Soluble COD (mg)	4,305	4,305	4,305
Total COD extracted (mg)	7,063	8,637	9,362
Soluble COD extracted (mg)	2653	2795	2900
Total COD remaining (mg)	10,524	9,696	9,927
Total COD losses (mg)	5,003	4,257	3,302

4.3.2. The Effect of Mixing on Acidogenesis

The unmixed reactors were the only reactors which produced any significant amounts of VFA and these levels were not high (<100 mg/l). They produced only 785 mg extra VFA per litre of starting sludge (equivalent to only 62 mg per litre of slurry). This is a modest increase as the slurry already had a VFA concentration of 123 mg/l. The continuously mixed and intermittently mixed reactors showed almost no evidence of VFA production after day 3. The intermittently mixed reactor produced 268 mgVFA/L_{sludge} while the continuously mixed reactor produced 185 mgVFA/L_{sludge}. As most of the VFA was collected at the end of day 1 of the trial it is most likely to be VFA associated with the original sludge rather than the result of production within the reactor.

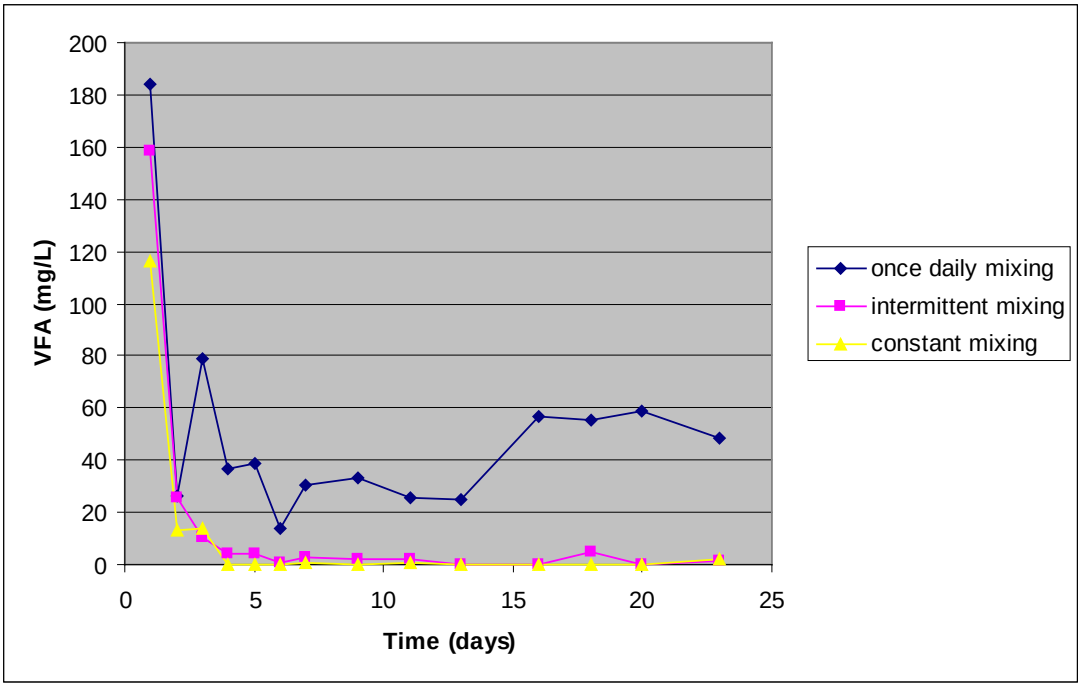

Figure 4-9: VFA concentration (mg/l) in effluents of mixing experiment reactors.

The lack of VFA produced by the stirred reactors was probably due to aeration; dissolved oxygen in the unstirred reactors was less than 0.7ppm, compared to about 2.5 ppm in the intermittently stirred reactors and 4-7 ppm in the constantly stirred reactors. The experiment was not carried out in an oxygen free environment as any mixing on a farm was envisioned to be in an open pit. In a full-scale situation, the level of aeration caused by mixing would be much less, as aeration to a depth of a

few centimetres would not have the same impact as on the laboratory reactors. The addition of dissolved oxygen would have had an inhibitory effect on the acidogens which are typically a mixture of both facultative and strict anaerobes (Chynoweth & Pullammanappallil, 1996), which may have impacted on VFA production. While explanations for the degrees of acidogenesis and hydrolysis exhibited may be hypothesised, the confounding of results due to oxygenation does not allow definite conclusions to be drawn as to effect of mixing. Further research is required in this area. Specifically a number of improvements could be made to the experimental method. It is recommended that future trials be operated as batches over at least 30 days with no replacement of supernatant and under oxygen-free conditions. The suppression of the aerating effect of mixing, through operation in an oxygen-free environment could remove the confounding effect of COD consumption which was most likely brought about through the establishment of aerobic species. It would also remove the inhibitory effect that oxygen can have on acidogenic species. The removal of supernatant and replacement with fresh water in this experiment was carried to extend on the findings of the dilution experiments which indicated that higher COD and VFA yields were possible with greater degrees of dilution. However, in the mixing experiment, the daily replacement of supernatant served little purpose in the determination of the effects of mixing and it may have merely provided another source of experimental error. The removal of supernatant every day and replacement with fresh water may have masked the effects of COD consumption. The use of RO water in this experiment may also have had a negative impact on the bacterial populations. Distilled and deionised water is commonly used in microbiology and medicine to lyse cells. Although concentrations of salts in the FDE sludge would probably have been sufficient to prevent a hypotonic solution in the reactor, these salts may have all been washed out after a few days leaving any attached hydrolytic and acidogenic cells vulnerable to lysis. This could be an explanation for the low degree of COD and VFA production observed.

4.4. Hydraulic Retention Time Experiment

In these experiments, the effect of varying the residence time of the liquid phase on the hydrolysis and acidogenesis of a volume of FDE sludge was studied. 400 mL of water was added to 400 mL of separated sludge. 400 mL of liquid phase was

removed, and replaced with fresh water at varying intervals depending on the assigned HRT. The experiment ran for 30 days and five HRTs were trialled; 1, 2, 5, 10 and 15 days.

4.4.1. The Effect of HRT on Hydrolysis

A comparison of the cumulative soluble COD mass collected, on a COD/VS_{added} basis, from the influent (Figure 4-10 below) shows the 1-day HRT reactors removed the most soluble COD over the first 20 days. After day 25, the 5-day HRT reactors had the highest yield of soluble COD with 0.325 gCOD/gVS_{added}. The 1, 2 and 10-day HRT reactors had similar soluble COD yields while the 15-day HRT reactor produced only 0.147g soluble COD for every g VS_{added}.

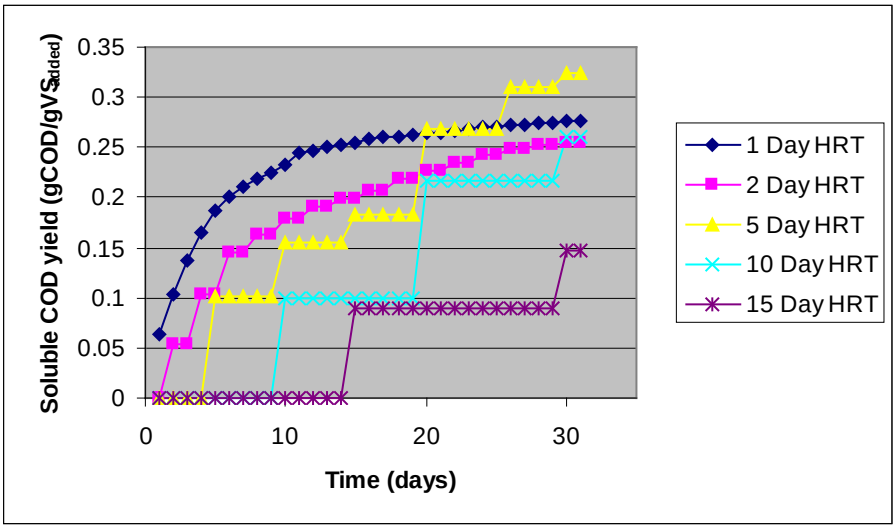

Figure 4-10: Cumulative soluble COD (gCOD/gVS_{added}) extracted from reactors operating at different HRTs.

The 5-day, 10-day and 15-day HRT reactors all reached similar levels of COD mass removal on their first decants (about 1,000 mg) while the 1-day and 2-day HRT reactors took 2 decants to reach this level. The implication of this could be that the longer HRT reactors had reached a limit of soluble COD concentration beyond which hydrolysis could be inhibited. The agent of this inhibition cannot be determined from COD measurements, however its effect can. If an inhibition were occurring this would explain the improved hydrolysis achieved by the 1-day HRT reactor. As products are removed every day, the solution never achieves concentrations that could be inhibitory. An examination of soluble COD concentrations which existed inside the reactors (Figure 4-11 below), shows both the 10-day and 15-day HRT reactors reaching around 3,000 mg/l by day 5 but tended to

remain steady at that level until they were decanted and concentrations dropped due to addition of fresh water. After decanting there were again increases in COD, but a limit appeared to be reached on about day 16 in the cases of the 10 and 15-day HRT reactors. This limit again appears to be around 3,000 mg/l. While it appears that some inhibition of hydrolysis tends to occur above soluble COD concentrations of 3,000 mg/l, this is not in agreement with a hydrolysis study of cattle manure by Myint *et al* (2007). That study found that hydrolysis was only limited when soluble COD concentrations exceeded 6,000 mg/L. However, that study was carried out at 35 ^{o}C, which may be significant. A study in to the hydrolysis of olive pomace (also at 35 ^{o}C) proposed a soluble COD saturation constant of 9,800 mg/L above which hydrolysis began to be limited (Borja *et al.*, 2005).

All the longer HRT reactors (5, 10, 15 day) showed declines in soluble COD concentration after day 18-20 which were not related to decanting. This implies that some consumption of COD was taking place. There may have been effective colonisation of methanogens taking place, which could explain the consumption of COD. Alternatively, the COD consumption could have been due to action of aerobes. Although the reactors were not actively mixed or aerated, there may have been sufficient surface oxygen transport to sustain an aerobic population of bacteria. Surface oxygen transfer effects can be more significant at the laboratory scale where the depth of the reactors is only a few centimetres compared to full-scale reactors where depths are measured in metres. Reactors were not sealed from air and no dissolved oxygen measurements were taken, hence the presence of aerobes cannot be excluded.

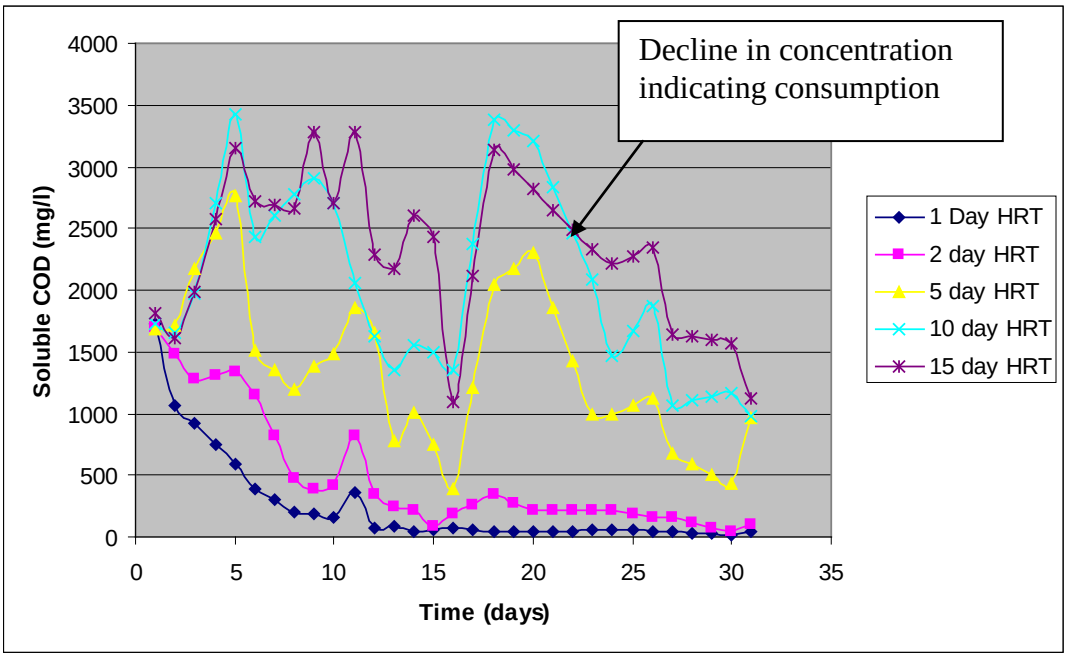

Figure 4-11: Soluble COD concentration in the reactors operating at different HRTs.

4.4.2. The Effect of HRT on Acidogenesis

In terms of total VFA yield, the 15-day HRT reactor had the highest production with 0.033 gVFA/gVS$_{added}$, followed by the 2-day HRT reactor and then the 10 and 1-day reactors following behind that with similar amounts of VFA produced. Figure 4-12 below shows the largest yield coming from the 15-day HRT reactor on day 15.

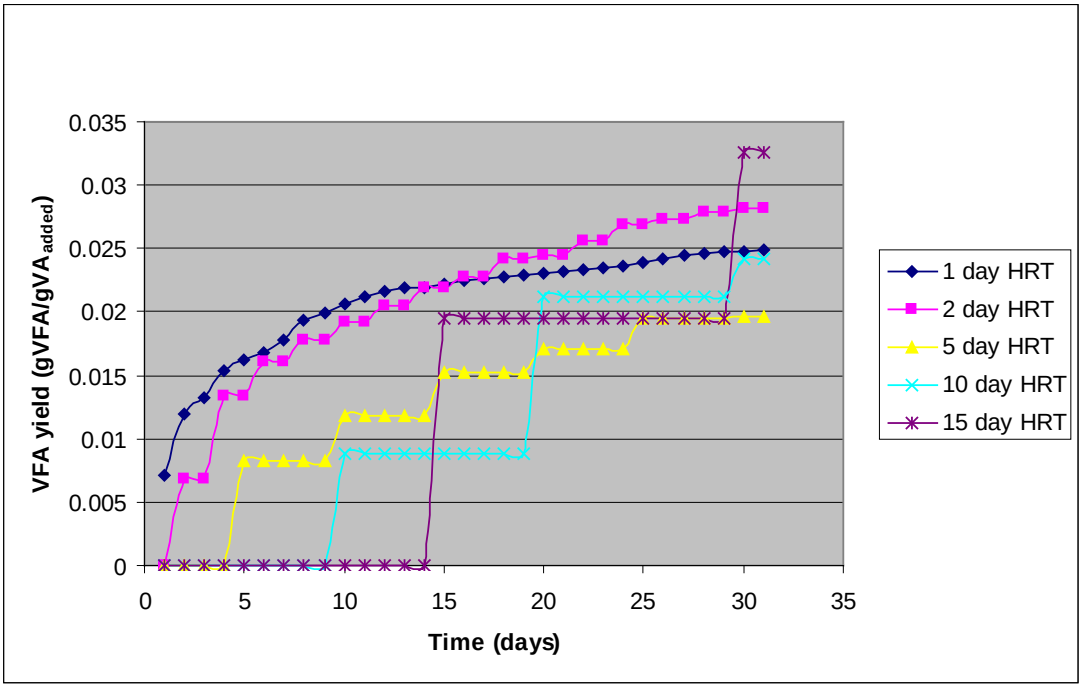

Figure 4-12: Cumulative VFA yield (gVFA/VS$_{added}$) derived from the effluent of reactors operated at different HRTs.

The increase on day 30 for the 10-day HRT reactor is much less than that achieved on day 20. The 5-day HRT reactor shows almost no increase on day 30. The reason for this can be seen more clearly in Figure 4-13 below, which shows the change in VFA concentrations in the reactors. The 10-day HRT reactor shows a general decrease in VFA concentration during its last 10-day cycle. The 5-day HRT reactor and 15-day reactor also show signs of VFA consumption occurring after day 27. The establishment of methanogenic or aerobic populations could explain this as discussed above. However the pH of all the reactors was typically under 6.00 which is at a level which is generally accepted in the literature as being inhibitory for methanogens (Hobson & Wheatly, 1993)

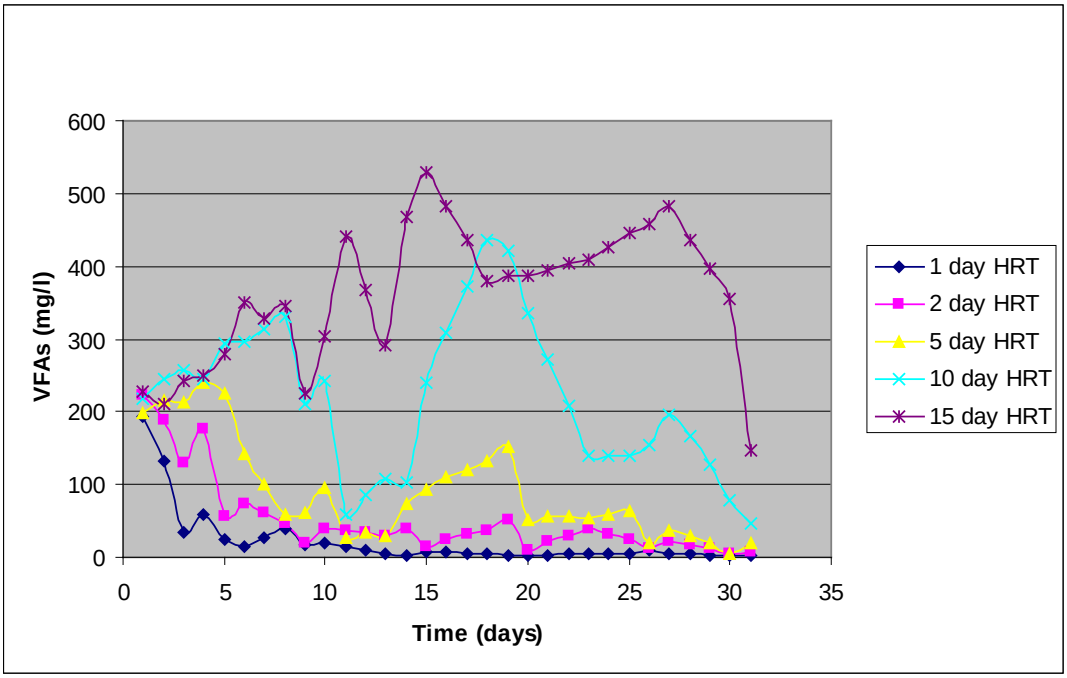

Figure 4-13: VFA concentration in reactors operated at different HRTs.

If the reactors had been decanted on day 27, prior to the consumption of VFAs, the 15 day HRT reactor would have produced over 400 mg VFA and the 15 day HRT reactor would have had a significantly improved yield. Despite the consumption of VFAs, we can see that the longer HRT reactors are producing more VFAs that the 1-day HRT reactor even though the shorter HRT reactors appear to produce more soluble COD. The highest soluble COD yielding reactor (5-day HRT) was the lowest VFA yielding reactor while the lowest soluble COD yield (15-day HRT) also yielded the most VFA. An explanation of this could lie in the morphological phenotypes of

acidogens and hydrolysers. If acidogens are predominantly planktonic then they would be much more susceptible to washout that can occur at low HRTs. Short HRTs would not adversely affect hydrolysers if they are predominantly sessile and attached to the solids which are their food source. We might expect acidogens to be planktonic as their food source is soluble and therefore associated with the bulk liquid and not the solids. In a study of the hydrolysis of crystalline cellulose, Jensen *et al* found that 25% of the bacterial population was attached and that the planktonic fraction dominated (Jensen *et al.,* 2008). They noted that this was in contrast to rumen based studies where particle associated (sessile) bacteria are reported to range from 50% (Merry & McAllan, 1983) to 70% (Cheng *et al.*, 1977). It was suggested that this was possibly due to the functional characteristics of the rumen, where soluble VFAs are removed thereby limiting the substrate available for planktonic organisms (Jensen *et al.*, 2008). A later study by the same authors found that the rate for hydrolysis of cellulose was most strongly correlated with the concentration of sessile biomass rather than with the concentration of total or planktonic biomass (Jensen *et al.,* 2009). This supports the proposition that hydrolysis should be less effected by changes in HRT than acidogenesis.

The implications of shorter HRTs favouring hydrolysis and longer HRTs favouring acidogenesis in leachbed systems are that it must be decided which regime would best favour methane production in the methanogenic digester. As acidogenesis can occur concurrently with methanogenesis in the digester and is not the rate-limiting step, it may be that a higher soluble organic content with reduced VFA content would result in a higher methane yield than a lower soluble organic content with increased VFAs. Opting for shorter HRTs could also help to prevent the establishment of a methanogenic population. With the same amount of influent coming in, a shorter HRT results in a smaller reactor vessel which in turn results in a higher organic loading rate. A higher OLR has been observed to result in a lowering of pH in acidogenic/hydrolytic reactors fed with unscreened dairy manure (Demirer & Chen, 2005). A lowering of pH could help to inhibit methanogens.

Limiting solids retention time to less than 25 days could also help to reduce the consumption of VFAs and soluble COD that occurs in the reactors, by not allowing a methanogenic population to establish. However, limiting the solids retention time

would result in a sacrifice of unhydrolysed solids that could contribute to soluble COD production and greater methane production downstream. Limiting the solids retention would also impact the on-farm operation of a leachbed system as solids removal would be required on a more frequent basis.

4.5. Rumen Dosing Experiment

Two rumen dosing experiments were carried to see if the addition of rumen contents would have any effect on gas production, VFA production, COD solubilisation or hydrolysis of cow manure sludge. The first experiment (RD1) compared cow manure sludge dosed with active rumen contents obtained from fistulated sheep to cow manure sludge dosed with autoclaved rumen contents. There was no discernable difference in VFA or COD extraction between the series. The results of experiment RD1 can be found in Appendix 5. The second experiment (RD2) was adjusted to ensure anaerobic conditions and had an additional control, which was cow sludge with no rumen addition. Experiment RD2 used a buffered solution mimicking cow saliva for dilutions. This solution was used to provide optimal chemical conditions for the rumen organisms. The buffered solution was sparged with CO_2 to ensure anaerobic conditions more favourable to rumen organisms. The headspace of the reactors was also flushed with CO_2 after changing dilution liquid. The experiments used a 1:1 dilution of sludge to buffer solution as this had been shown to be more favourable to both hydrolysis and acidogenesis in the dilution experiments (see section 4.2 above).

4.5.1. The Effect of Rumen Dosing on Hydrolysis

There was little difference in the soluble COD concentration in the effluent from the fresh rumen reactors and autoclaved rumen reactors (Figure 4-14 below). The sludge only control series had slightly lower soluble COD concentrations. All reactors show a slight increase in soluble COD between day 3 and 7.

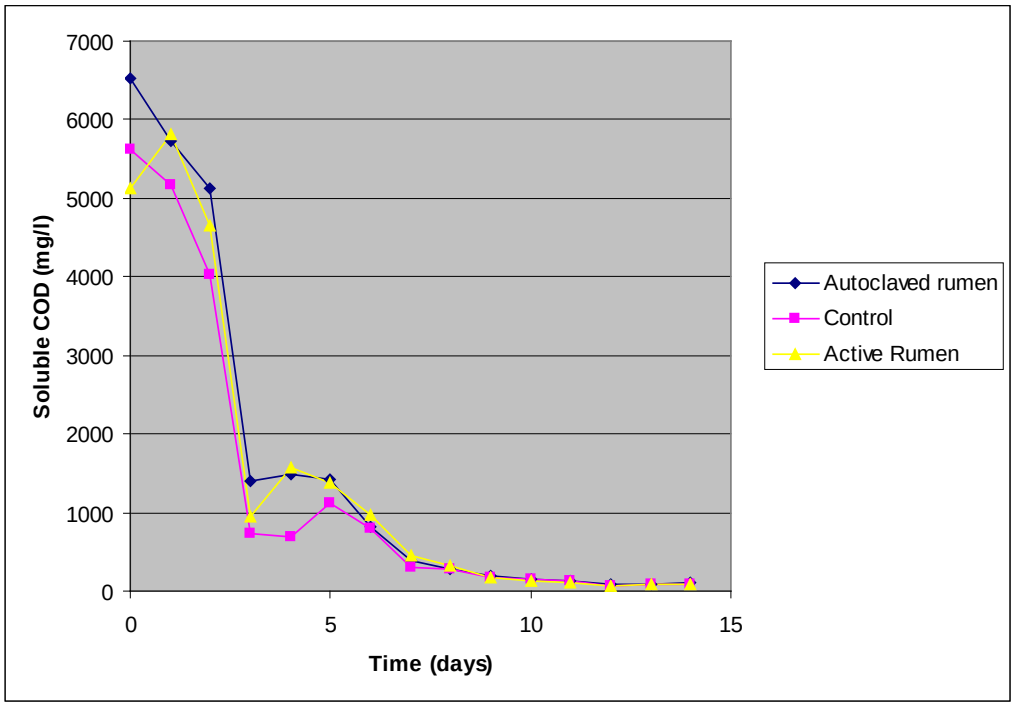

Figure 4-14: Soluble COD concentration of effluents in the rumen dosing experiment.

Table 4-6 below shows the COD mass balance for the mixing experiment. It can be seen that masses of total COD and soluble COD derived from the autoclaved rumen and active rumen are quite similar indicating that there was little if any contribution to hydrolysis from active rumen organisms. The negative numbers for the total COD losses indicate errors in COD measurement, possibly compounded as the Total COD extracted measure is a function of the COD concentration multiplied by the volume of the supernatant removed added together over the 14 days of the experiment.

Table 4-6: COD mass balance for the rumen dosing experiment.

	Autoclaved rumen	Active rumen	Control
Initial COD (mg)	31,601	32,881	28,301
Total COD extracted (mg)	23,457	22,170	18,896
Soluble COD extracted (mg)	8,874	9,194	8,034
Total COD remaining (mg)	14,690	14,079	10,961
Total COD losses (mg)	-6,545	-3,367	-1,555

4.5.2. The Effect of Rumen Dosing on Acidogenesis

The graph (Figure 3-23 below) shows an increase in VFA production in the first 2 days with production steadily falling off after that. The autoclaved rumen shows the highest production followed by fresh rumen and then the control.

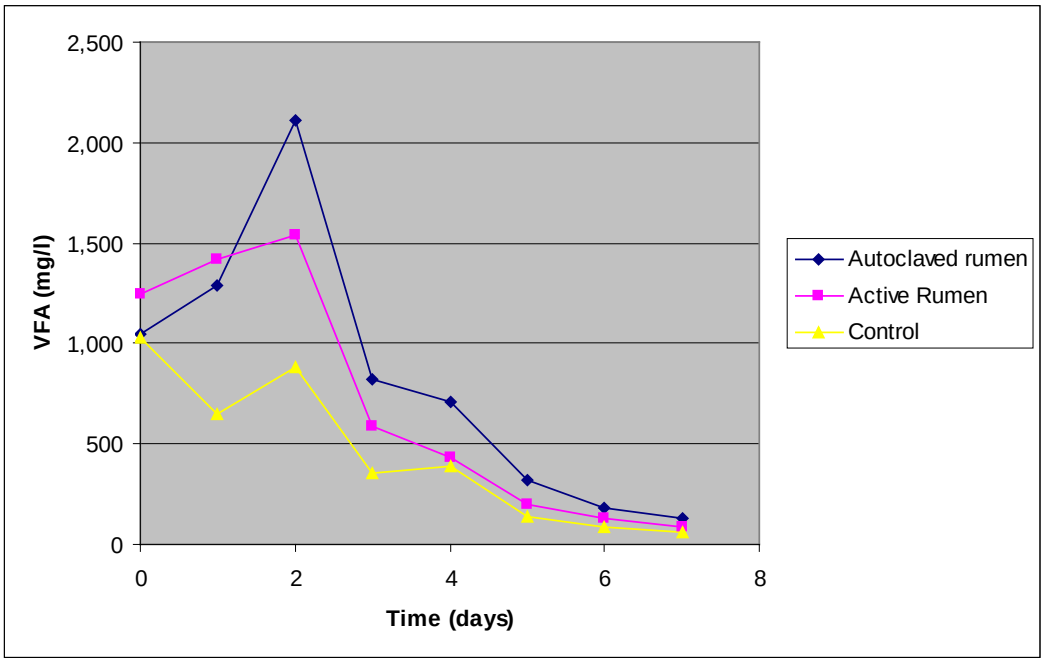

Figure 4-15: VFA concentration in effluent

Total VFA mass production is shown in Table 4-7 below. The autoclaved rumen dosed reactor produced the most VFA, while the sludge only control reactor produced less than half the VFA mass of the reactor dosed with autoclaved rumen contents. The VFA mass extracted was greater for all reactors than the amount present in the starting material indicating that some acidogenesis was occurring.

Table 4-7: Total VFA mass production from rumen dosed and control reactors.

series	Sludge (control)	Sludge + autoclaved rumen	Sludge + fresh rumen
total VFA mass (mg)	2,610	5,740	4,440
total VFA mass – starting VFA mass (mg)	2,165	4,545	3,080

Even though there was a significant difference in VFA concentrations of the rumen and the cow manure starting material (Table 4-8 below) this did not account for the differences in VFA production between the reactors.

Table 4-8: VFA concentration of starting material in rumen addition trials

Starting material	VFA concentration (mg/l)
Rumen contents	9,136
Autoclaved rumen contents	7,552
Start sludge	891

It would not be expected that the autoclaved rumen reactors would have any VFA production due to the action of rumen bacteria or rumen enzymes. All rumen enzymes and bacteria should have been ruptured or denatured by the autoclaving process. The explanation for the higher VFA production in the autoclaved rumen reactor may lie in the addition of partially digested grass, which made up a large proportion of the rumen contents. Grass has a methane potential of 0.4-0.45 m^3/ kg VS (Lehtomaki *et al.*, 2007) which is greater than the methane potential of FDE (125 up to 284 LCH_4/kg VS_{added}). The presence of a greater amount of readily digestible material may explain the higher VFA production in the reactors with rumen contents. In addition, the process of autoclaving the rumen may have resulted in cell breakdown, which would speed up the hydrolysis process. This physical hydrolysis may be masking any biological hydrolysis that occurred in the active rumen reactor. Other researchers have reported effective hydrolysis of grass (Barnes & Keller, 2003) and mixed solid waste (Dencamp *et al.*, 1989) through the addition of rumen contents. Apart from the substrate, both of these studies differed from this one in two aspects; the operation of their reactor was mesophilic and rumen contents were strained to prevent the addition of fibrous material. A study by Angelidaki and Arhing (2000) showed little improvement in methane production through the addition of cellulytic enzymes to cow manure but did record a 30% increase in methane potential with the addition of a culture of hemicellulose-degrading bacterium B4.

The lack of improvement in both hydrolysis and acidogenesis in this study indicate that an on-farm strategy of simply applying fresh rumen contents to digesters may not be an effective strategy to improve methane production. While this study used the whole contents of sheep rumen to farm dairy effluent sludge with little effect, further research should still be carried out to determine if the addition of rumen bacteria would add benefit to the digestion process as some improvements could be

made to the experimental method. Use of cow rumen bacteria rather than sheep rumen bacteria may prove more effective as these bacteria could be more acclimatised to the conditions found in farm dairy effluent. Separation of the rumen bacteria from undigested grass by straining through a sieve would also remove the confounding effect of an additional substrate (the undigested grass).

4.6. Column Leachbeds

Leach bed reactors allow liquid to pass through solids and drain out by way of gravity through a barrier such as a geotextile material or a screen. Solids remain in the reactor while the majority of the liquid passes out to be used in a secondary methanogenic reactor. The passing of liquid through the solids allows solubilised products such as VFAs and soluble sugars to be transferred to the liquid phase and removed from the reactor. In this study, two leachbed batch experiments were carried out.

4.6.1. Experiment LB 1

This experiment was carried out to determine if cumulative applications of cow manure slurry to a leach bed reactor would result in higher COD and VFA yields than could be achieved by simple liquid solid separation. Three litres of slurry (1:9 raw manure: fresh water) was poured into a geotextile sock and the liquid portion was allowed to drain away. Every day a further 3 litres of slurry was added and the liquid portion drained through the solids of the previous days' applications. This experiment ran for 24 days. Unlike the other experiments presented in this trial, this experiment was carried out at ambient temperature in uncontrolled conditions.

BOD and COD of the leachate from the column were measured daily. If hydrolysis was occurring then leachate concentrations of both COD and BOD were expected to increase as organic matter was transferred from the solids to the liquid portion. A decrease in BOD and COD would be an indication of consumption of organic material, either by methanogenic or by fermentative means. Figure 4-16 below appears to show overall consumption of organic material rather than hydrolysis. The COD concentration increased from about 4,000 mg/L to over 7,000 mg/L during the first eight days of the experiment but then it dropped back down to around 4,000

mg/L (apart from a dip down to 2,000 mg/L around day twenty) for the rest of the experiment.

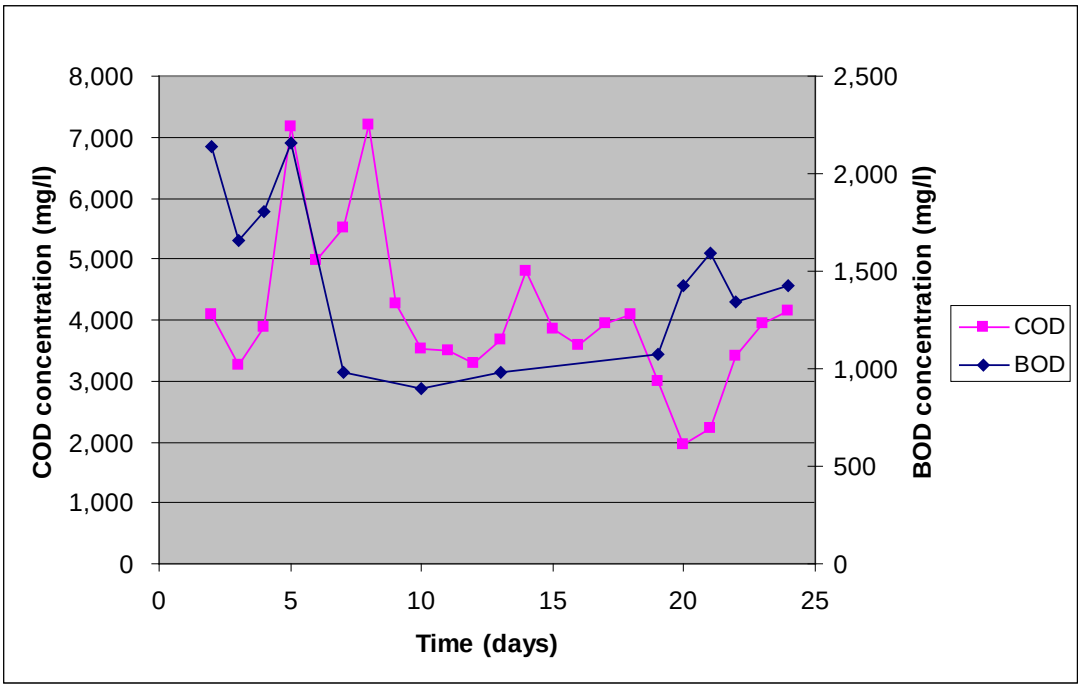

Figure 4-16: COD and BOD concentration (mg/L) of leachate LB1 reactor.

In their leachbed study of hydrolysis from cattle manure solids Myint *et al* noted a steady increase in COD from the leachbed (2009). However, in that study leachate was recirculated so a steady increase would be expected unless COD consumption was taking place.

The BOD concentration appeared to fluctuate between 1,600 and 2,100 mg/L for the first four days before falling to 985 mg/L (refer Figure 4-16 above). No real change was seen until day 20 when it rose to around 1,500 mg/L. As with the COD concentration, BOD production did not increase beyond that initially detected on the first day, indeed for most of the experiment it was substantially less. As fresh material was being added daily, it would be expected that BOD and COD should not decrease unless there was some consumption. The average COD concentration of the leachate was 4,050 mg/L. This was substantially less than was achieved through simple separation techniques. The average COD concentration of the supernatant when using simple separation (with no leaching) was over 6,200 mg/L (see section

4.1.3 above). This points to there being consumption of organic material with limited or no hydrolysis occurring.

In contrast to the COD (Figure 4-16 above) we can see that there was an increase in VFA concentration coming out from the leachbed column over the course of the experiment (Figure 4-17 below). This indicates that VFA production is on-going in the column and that it is not a case of existing material simply being washed out.

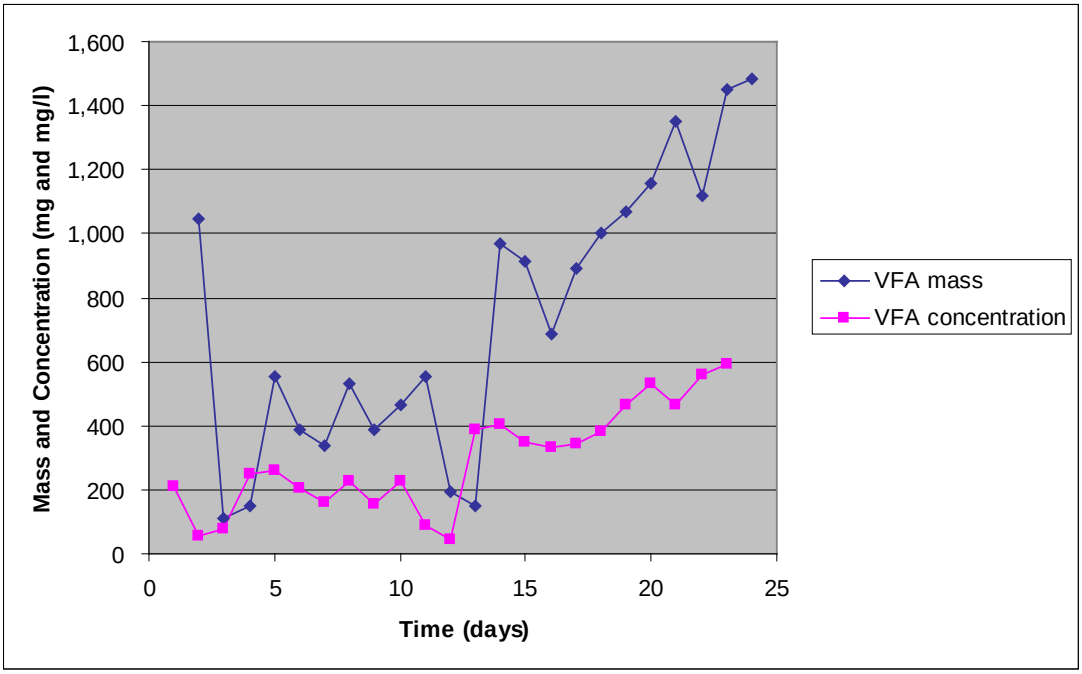

Figure 4-17: VFA mass and concentration in the leachate from LB1.

The starting concentration of VFAs was about 200 mg/l and stayed at that concentration until day 10 when there was a slight drop off before increasing steadily to 600 mg/l on day 23. The implication of this is that a 300 % increase in VFA production can be achieved by allowing sludge to remain in the reactor vessel rather than discarding it as in a typical separation process.

The total mass of VFA collected over the course of the experiment was about 16,950 mg. The TS content of the slurry used in this experiment was measured at 1.35 % indicating that about 778 g of TS was added to the column. This gives a total yield of 0.0218 g VFA/g TS_{added}. Myint *et al* reported that they achieved yield of 0.132 g VFA/g TS_{added}, (2009) which is substantially more than that achieved in this study. Their improved VFA production could be a result of the greater hydolysis, and

subsequent soluble organic content of the leachate in their trials. Mason and Mulcahy reported VFA production from cow manure slurry as a maximum of 0.39 g COD_{vfa}/g BOD_5 in their 20 day batch fermentations of cow manure slurry (Mason & Mulcahy, 2003). The COD_{vfa} extracted in this study was calculated at 0.11 g COD_{vfa}/g BOD_5, significantly less than the Mason and Mulcahy study.

The results of this experiment show that there was little if any hydrolysis achieved over the course of the trial and that there may have been a significant degree of consumption of COD. There was evidence of improved acidogenesis when compared to simple separation but the VFA yields were substantially less than demonstrated in other studies using cow manure as a substrate. Comparison between the three studies (this one, Mason and Mulcahy, Myint *et al*) is not straightforward as there are significant differences in experimental set up. This study used successive additions of substrate, while the other two were batch studies which only had the initial starting material as substrate. Myint *et al* used solids resulting from an initial separation as the starting material, while this study and that by Mason and Mulcahy used a slurry of cow manure. In this study liquid was drained off constantly, while in the Myint study liquid was recycled and contact improved through the use of a pistachio husk medium. In the Mason and Mulcahy study the liquid portion was retained in the vessel over the course of the experiment. It may be that the improved contact in the case of the Myint experiment, and the extended HRT and liquid to solid ratio in the case of the Mason and Mulcahy experiment are the conditions which led to improved hydrolysis and subsequent improved VFA production. These issues are examined in the sections relating to dilution, mixing and HRT.

4.6.2. Experiment LB2

This experiment was carried out to examine the changes in COD concentration and VFA concentration of leachate that occurred as a result of continuous application of fresh water to sludge. The changes in COD concentration were measured to determine hydrolysis while VFA concentration was used to measure acidogenesis. 12 L of sludge settled from 71 L of 1: 9 cow manure to water slurry was placed in a geotextile sock. Fresh water was dripped on the column at a rate of about 2.1 L per day, this being the slowest rate at which the peristaltic pump used was able to pump.

The water was allowed to filter through the solids to an effluent outlet. This experiment ran for 28 days.

Figure 4-18 below shows the change in the concentrations of COD and BOD. There is a sharp decline in both COD and BOD for the first 3-5 days. This is most likely due to wash out of existing suspended organic material and possibly an initial lag phase where hydrolytic and activity was low. The lag period could arise from the time it took to establish a hydrolytic bacterial population. There is then a period of increased concentration which tails off between day 12 and 20. This is then followed by another increase in COD production after day 20.

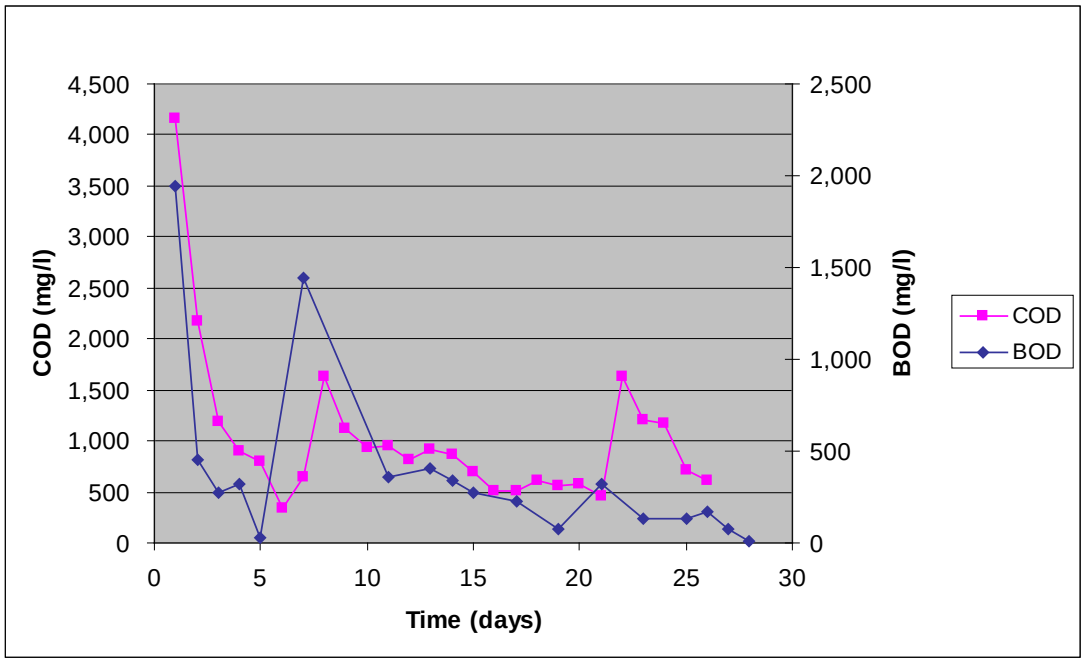

Figure 4-18: COD and BOD concentrations in the leachate from LB2.

The increase in COD and BOD after the initial washout stage can be taken as evidence of hydrolysis. If no hydrolysis were taking place then COD and BOD would be expected to trail off with no increases. Table 4-9 below shows the BOD and COD yields from LB2, including that obtained from the supernatant that was removed prior to the sludge being placed in the column. There were significant increases in the amount of COD and BOD (18.5% and 15.5% respectively) that could be obtained compared to simple separation. The COD derived from the sludge (0.155 g COD/gTS_{sludge}) was comparable to that (0.172 g COD/g TS_{manure}) obtained from the leachbed reactor by Myint and Nirmalakhandan (2009).

Table 4-9: COD and BOD yields from the supernatant and leachate derived from the sludge of experiment LB2.

	Total COD mass (mg)	Total BOD mass (mg)
Original Supernatant	358,642	156,526
Leachate	81,662	28,774
Total extracted	440,304	185,300
% increase due to leaching	18.5 %	15.5%
Production g/gTS$_{added}$	0.155	0.055

VFA concentration of the leachate is shown in Figure 4-19 below. There was a period of declining VFA concentration (days 1 to 7) which may be associated with the wash out of existing VFAs and a period of inactivity. This is followed by an increase in VFA concentration between day 7 and 13 indicating a period of active VFA production. The increase in VFA may be related to the increase on BOD and COD production which occurs around day 7 and 8 (see Figure 4-18 above). BOD measurements include complex carbohydrates that require further breakdown to become VFAs. The production of VFAs would be linked to the production of these soluble organic substrates.

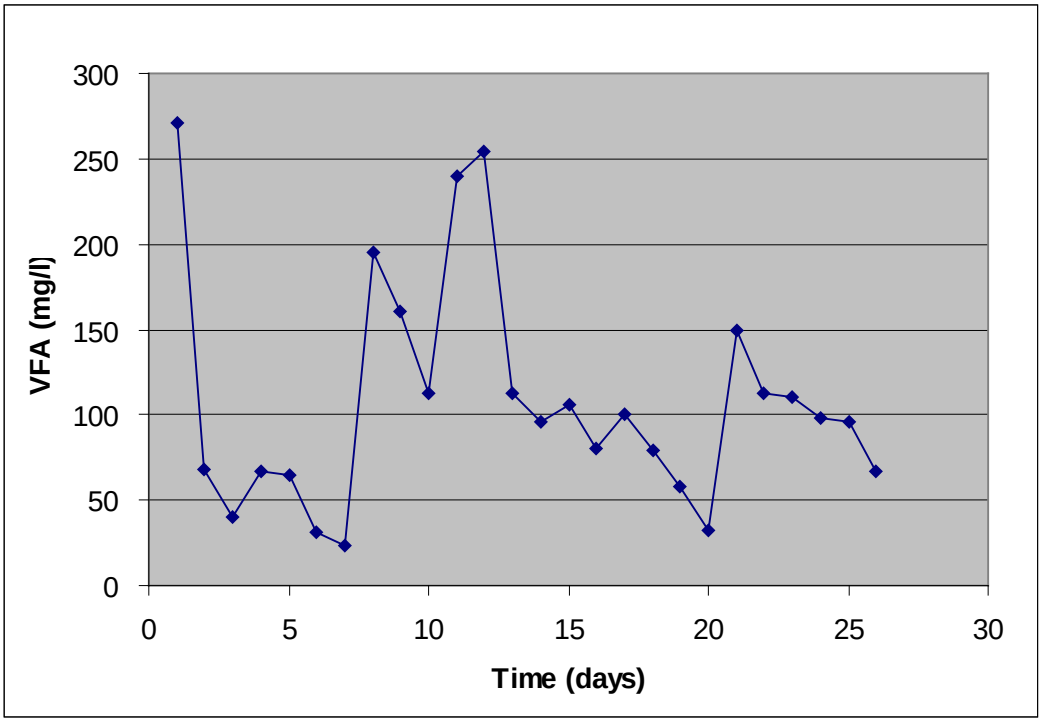

Figure 4-19: VFA concentration from the leachate of LB2.

The additional spike in VFA production at the end of Experiment LB2 may be due to a longer HRT caused by blockage of the column. The blockage caused liquid to remain in the column rather than drain out resulting in a longer HRT over that time period of the experiment. This may indicate that longer residence times result in improved hydrolysis. Alternatively, it may be a second phase of activity. In a study of VFA production from farm dairy effluent, by Mason and Mulcahy, a similar increase was seen in VFA concentration around day 15, though it was not commented upon (Mason & Mulcahy, 2003). A second phase of methane production was noted in a two phase system batch system using unscreened cattle manure (Demirer & Chen, 2008). This was attributed to the digestion of less readily degradable organics after the easily degradable fraction had been digested. The two phase of VFA production may relate to the two main products of hydrolyis from cattle manure, hemicellulose and cellulose (Myint *et al.*, 2007). The first phase of VFA production may arise from the acidogenesis of the readily degradable hemicellulose while the second phase may arise from the more slowly degradable cellulose. The implications of this for on-farm systems is that residence times should be sufficiently long enough to allow both production phases to be completed if full utilisation of the hydrolysis products is to take place.

The total mass of VFA collected over the course of the experiment was about 22,325 mg, with 14,482 mg derived from the original supernatant and 7,843 mg from the leachate. This represents a 35% increase over that which could be achieved by separation alone. The TS content of the sludge used in this experiment was 4.4 % indicating that about 528 g of TS was added to the column. This gives a total of 0.0149 g VFA/g TS_{added} extracted from the column. This does not include the VFAs which were removed by removing the supernantant in the original separation. Myint *et al* reported that they achieved an extraction rate of 0.132 g VFA/g TS_{added}, (2009) which is substantially more than was achieved in this study.

4.7. Settle and Decant Leachbed

For the “settle and decant” reactor in this study, an initial volume of sludge was added to the reactor along with a volume of water. The contents were briefly stirred and then allowed to settle. The resulting supernatant was then removed and a fresh

volume of water was added and stirred into the remaining sludge. This experiment (SD1) was run as a comparison for the leachbed experiment (LB2). The same volume of starting sludge, prepared from the same 1:9 water to manure slurry, was used as in experiment LB2. The volume of water added was the same as that in LB2 and the volume of decant removed was the same as that in LB2.

4.7.1. Hydrolysis in the Settle and Decant Leachbed

SD1 was run for 28 days. Table 4-10 below summarises the key data. This reactor removed 38.2% of the COD from the sludge. The sludge contained about 48% of the original COD of the slurry. The initial settling and decanting of the slurry plus the subsequent treatment in this experiment succeeded in removing 70% of the original COD into the liquid phase. 21% was left in the sludge. The remaining 9% was unaccounted for indicating that some consumption of COD had taken place.

Table 4-10: COD balance for settle and decant leachbed (SD1)

Initial COD mass	358,357 mg
COD mass in leachate	166,230 mg
Theoretical COD remaining	192,127 mg
Measured COD remaining	221,441 mg
% $COD_{measured}$ removal	38.2%

The COD concentration in the decant (Figure 4-20 below) showed a general downward trend as less COD was washed out with subsequent water applications. Again, the spike at the end (COD mass) relates to increased volume of water applied as this was linked to the volumetric output of experiment LB2. The COD concentration appeared to stop its decline around day 17 and remain at around 2,000 mg/L. The BOD concentration shows an almost linear decline throughout the experiment.

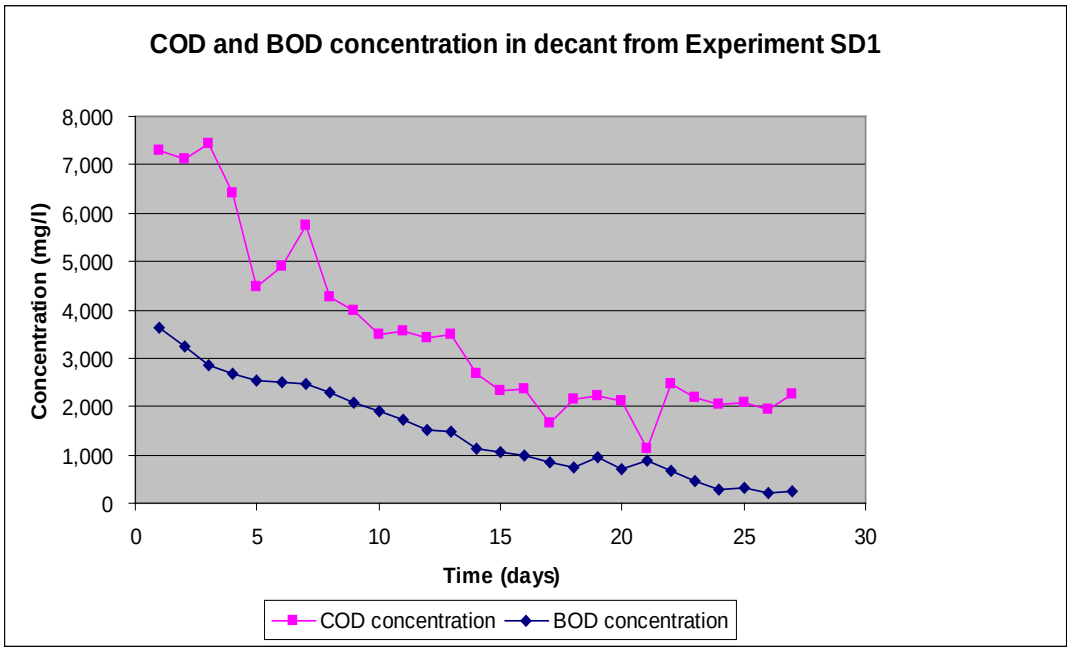

Figure 4-20: COD and BOD concentration in the decant from SD1

There was a measured 11.7% reduction in total solids for Experiment SD1 where reduction = initial mass - (mass in leachate + mass remaining).

Table 4-11: Solids removed from SD1.

Initial TS mass	528 g
TS mass in leachate	116 g
TS mass reduction	62 g
TS mass remaining	350 g
%TS reduction	11.7 %

4.7.2. Acidogenesis in the Settle and Decant Leachbed

A summary of VFA data from experiment SD1 is shown in Table 4-12 below. The leaching process was able effectively double the VFA content derived from the slurry. The concentration of VFAs in the leachate was higher than that in the original supernatant removed from the slurry. This suggests that the leachate would make a suitable feed for a methanogenic reactor. There was 0.033 g VFA/g TS_{added} extracted from the column. This does not include the VFAs that were removed in the supernatant in the original separation. While this was significantly better than that achieved in the leachbed reactor it was still significantly less than that (0.132 g VFA/g TS_{added}) achieved by Myint and Nirmalakhandan (2009).

Table 4-12: VFA data from Experiment SD1.

	Original Supernatant	**Leachate**	**Total**
VFA mass (mg)	16,000	17,400	33,400
VFA extracted per litre slurry (mg/L_{slurry})	225	245	470
Average VFA concentration (mg/L)	271	325	
VFA extracted per g TS (g/gTS_{added})		0.033	

VFA concentration in the leachate (Figure 4-21) increased until about day 8 and then declined. The picture would probably be clearer if a uniform volume of water had been added each day. There appears to be second burst of VFA production between days 18 and 25. It may be that the VFAs in the second peak are derived from substrates, such as cellulose, that take longer to be bio-available or are produced by bacteria that have a slower growth rate than acidogens that use a different substrate, such as hemi-cellulose.

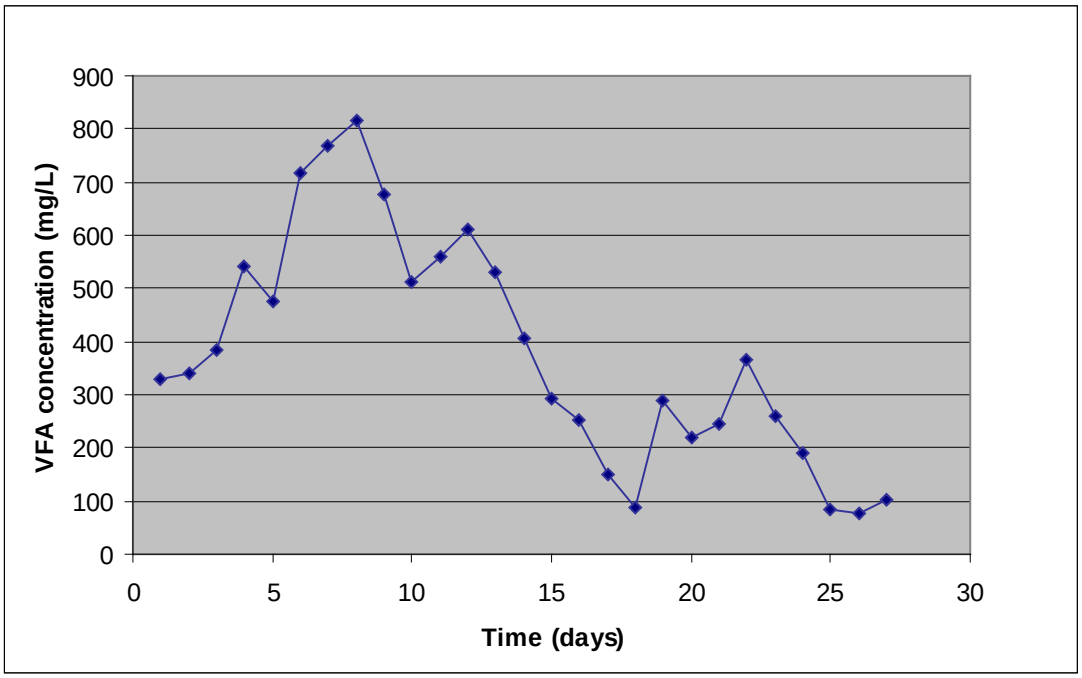

Figure 4-21: VFA concentration in the leachate from experiment SD1.

4.7.3. Comparisons with LB2

As seen in Figure 4-22, for the same amounts of sludge reactor SD1 produced greater amounts of COD, VFA and BOD than reactor LB1. However, reactor SD1 also allowed more TS through to the effluent. This would account for some of the increased COD and BOD mass but not the substantial (over 125%) improvement in COD_{vfa} production. The increase in TS production (52%) is also less than the increase in COD (112%) and BOD (128%), indicating that the increase is due to improved hydrolysis rather than transfer of solids out of the reactor. The improved performance may be due to the mixing; improved contact between solids and liquid compared to possible channelling which occurs in a leachbed reactor. Alternatively, it may be due to increased HRT. Despite the fact that each reactor had the same volume of water pass through it each day the settle and decant system would have had a longer HRT as the whole volume of liquid was allowed to stay in contact with the sludge for the total 24 hours, while the leachbed drained over the 24 hours. This would have resulted in an HRT of 1 day for the settle and decant system but the flow through time for the leachbed system would have been considerably less than this.

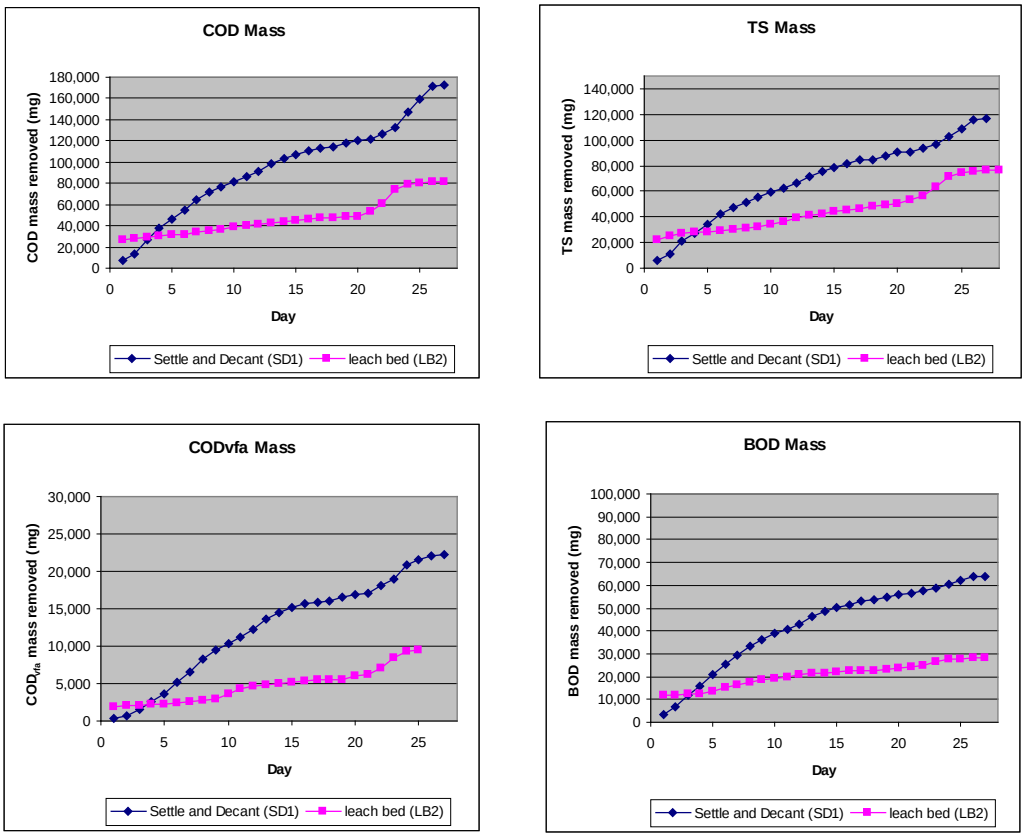

Figure 4-22: Comparisons of COD_{total}, COD_{vfa}, TS and BOD mass removed from the "settle and decant" and leachbed reactors.

4.8. Bench Scale Reactors

4.8.1. Performance of the Leachbed Separator

The leachbed separator was operated to provide a high organic low solid feed for the methanogenic reactors (see section 4.8.2 below). The liquid effluent coming out of the separator is what is referred to as “treated feed”. Three operating conditions were trialled for the leachbed separator to determine which configuration provided the highest degree of hydrolysis in terms of soluble COD production, the highest degree of acidogenesis in terms of VFA production, and ultimately the highest methane production rate in the methanogenic reactors.

Condition 1: less than 1 day HRT and an average SRT of 15.5 days (batches 1-3).

Condition 2: 8 day HRT and an average SRT of 15.5 days (batch 4 and the first 30 days of batch 5).

Condition 3: 8 day HRT and an average SRT of 68 days (batch 5).

Figure 4-23 below shows a comparison of the cumulative production of soluble COD and VFAs from the treated feed and the 9:1 water to manure slurry used. The calculation is based on a production of 1 litre of slurry or treated feed per day in order to enable direct comparison. Soluble COD data was not collected for Batch 1. Typically sampling and analysis were only done every 3-5 days so values for those days between analyses are extrapolated based on a linear variation between the sampling days.

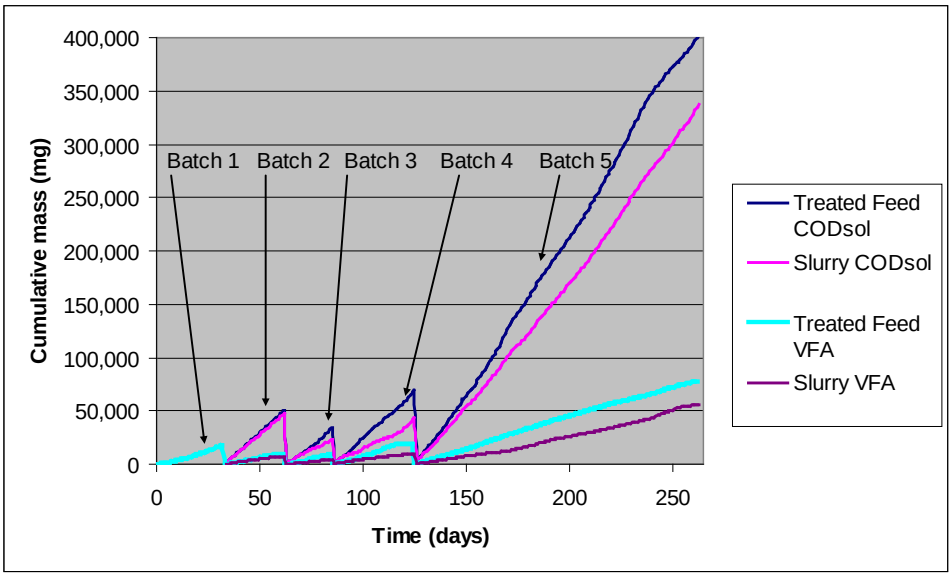

Figure 4-23: Cumulative VFA and soluble COD mass derived from slurry and treated feed (based on 1 litre per day production).

The VFA curve for the treated feed in Batch 1 shown above is based on only two data points (at the beginning and end of the batch) so this should be interpreted with some caution. In Batches 2 and 3, (condition 1 which had an HRT of less than 1 day), the concentrations of both soluble COD and VFA were consistently higher in the treated feed than the slurry. A clear divergence between the soluble COD yield of the slurry and treated feed can be seen in batch 3 in Figure 4-23 above. This divergence can be seen even more clearly for Batches 4 and 5, (conditions 2 and 3 which had 8 day HRTs), with significantly higher VFA and soluble COD yields from the treated feed compared to the slurry. This supports the premise that both extra hydrolysis and acidogenesis are taking place in the leachbed separator. In batch 4 there was a marked improvement in VFA and soluble COD production with a 120% increase in VFA yield and a 60% increase in soluble COD in the treated feed compared to the slurry. However, in batch 5, which can be considered as condition 2 for the first 30 days, the degree of increase does not appear to be significantly higher than the condition 1 batches. Because of this inconsistency, it is not possible credit the improved performance in batch 4 to the increase in HRT from 1 day to 8 days.

The overall yields for VFA and soluble COD for batches 2 to 5 in the slurry and treated feed are shown in Table 4-13 below. A comparison of the percentage increase in VFAs for condition 2 compared to condition 1 shows improved VFA yield. However improved soluble COD yield is only seen in batch 4 and not the condition 2 phase of batch 5 (the first 30 days of the batch)[1]. This may indicate that the longer HRT has a more direct impact on acidogenesis (as evidenced by improved VFA yield for condition 2) than it does on hydrolysis. In batch 5 there was little change in soluble COD yield noted between day 31 (21% increase) and day 135 of the batch (20% increase). This was however a concurrent reduction in the VFA yield, which dropped from 83% to 29% between day 31 and day 85 of the batch. The reduction in VFA yield may be explained by the establishment of a methanogenic population in the separator. If this is so, then the consumption of organic content by the methanogens may be masking an actual increase in soluble COD production. Gas

[1] From this point forward, the numbering of days refers to days in a batch rather than days in the whole trial.

measurements taken from samples of sludge from within the separator during batch 5 showed significant gas production after day 30, averaging 3.9 L per day from day 90 onwards, with at times over 7 litres of gas being released from the leachbed separator per day. This gas was generally of low quality with less than 15% methane content but represented COD losses averaging 1,675 mg per day from the leachbed separator after day 90. This equates to about 500 mg/L readily convertible COD lost from the treated feed effluent. Given that the increase in COD_{sol} in the treated feed compared to the influent slurry for this batch was only 511 mg COD/L, this is a significant energy loss.

Table 4-13: Average VFA and soluble COD concentrations of batches 2-5 from the slurry and treated feed.

Batch	**2**	**3**	**4**	**5 (prior to day 30)**	**5 (after day 30)**
Condition	1	1	2	2	3
Batch length (days)	29	23	40	30	105
Slurry VFA (mg/L)	256	236	255	304	438
Treated feed VFA (mg/L)	353	402	562	557	567
% VFA increase	38%	70%	120%	83%	29%
Slurry COD_{sol} (mg/L)	1,661	1,008	1,085	2,127	2,539
Treated feed COD_{sol} (mg/L)	1,757	1,476	1,740	2,577	3,050
% COD_{sol} increase	6%	46%	60%	21%	20%

Figure 4-24 below shows the changes in VFA concentration occurring in the treated feed coming from the leachbed separator. All batches show an increase in concentration over the first 10 to 15 days indicating an increase in acidogenic activity. Batches 2, 3 and 5 all show an eventual decrease in concentration indicating that VFA consumption is taking place. The start of VFA consumption ranges from day 16 for batch 3 to day 34 for batch 5. VFA consumption was detected in the HRT experiments as well (see section 4.4.2) with consumption starting after day 20 for the 10 day HRT reactor and day 27 for the 15 day HRT reactor.

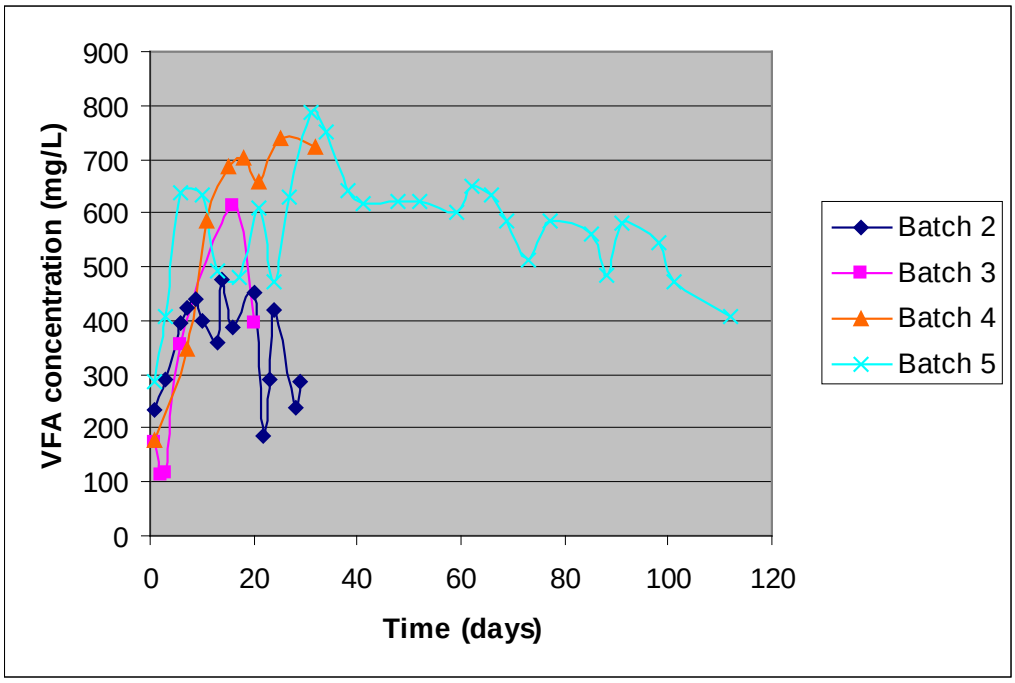

Figure 4-24: Changes in total VFA concentration of treated feed from batches 2 to 5.

As mentioned above the consumption of VFA may be the result of the establishment of a methanogenic population. In most two-stage systems, methanogenesis is controlled by having a low pH and short retention times that are unfavourable for methanogens. In the digestion of solids from farm dairy effluent (FDE) longer SRTs are required in order to achieve sufficient breakdown of the fibres. With a relatively slow release of soluble CODs and the extended retention of the solid fraction of the FDE, this may result in conditions that are favourable for the establishment of methanogenic populations. The pH of the contents of leachbed separator showed a general decrease over the course of each batch from starting points of around 7 to pH ranging from 6 to 6.5 (refer Figures 7-4 to 7-8 in Appendix 1). This pH range is not sufficiently low enough to inhibit the onset of methanogenesis. Lower pH has been achieved in other two-phase studies using cattle manure without the need for pH control. Myint and Nirmalakhandan (2009) maintained a pH below 5. Demirer and Chen (2005) observed that there was a decrease in pH relating to organic loading rate (OLR) in an acidogenic reactor fed with unscreened diary manure. In that study pH dropped from 6.7 to 6 when the OLR was increased from 2.0 to 5.0 gVS/L/day. The OLR used in this study was only 1.1gVS/L/day. Further research is required to investigate whether an increase in OLR would be an effective strategy for the suppression of methanogenesis in the leachbed separator.

The suppression of methanogenesis is of importance to an on-farm system; if it is not possible to prevent the establishment of methanogenesis then batches in the leachbed separator would have to be limited to between 20 and 35 days, depending on when VFA consumption starts. The removal of solids from a biogas system on such a frequent basis could be a large demand on labour resources and would not make such a system attractive to farmers. The frequent removal of solids would also affect the soluble COD and VFA yield. Not only is a possibly significant source of methane potential being removed but also each new batch requires a start-up period lasting from 10 to 15 days where VFA and soluble COD production has not yet reached an optimum steady state.

An examination of the VFA species in the treated feed from batch 5 (Figure 4-25 below) revealed that VFA consumption noted above was primarily of acetate. Acetate concentrations rose to about 450 mg/L on day 35 before steadily decreasing to below 50 mg/L by day 85. This was concurrent with the increased gas production noted above. Propionate production did not appear to be affected and concentrations were observed to increase up to 440 mg/L by day 90. This extra propionate production was also detected in the effluent of reactors B1 and B2 (refer to Figure 7-9 in Appendix 5).

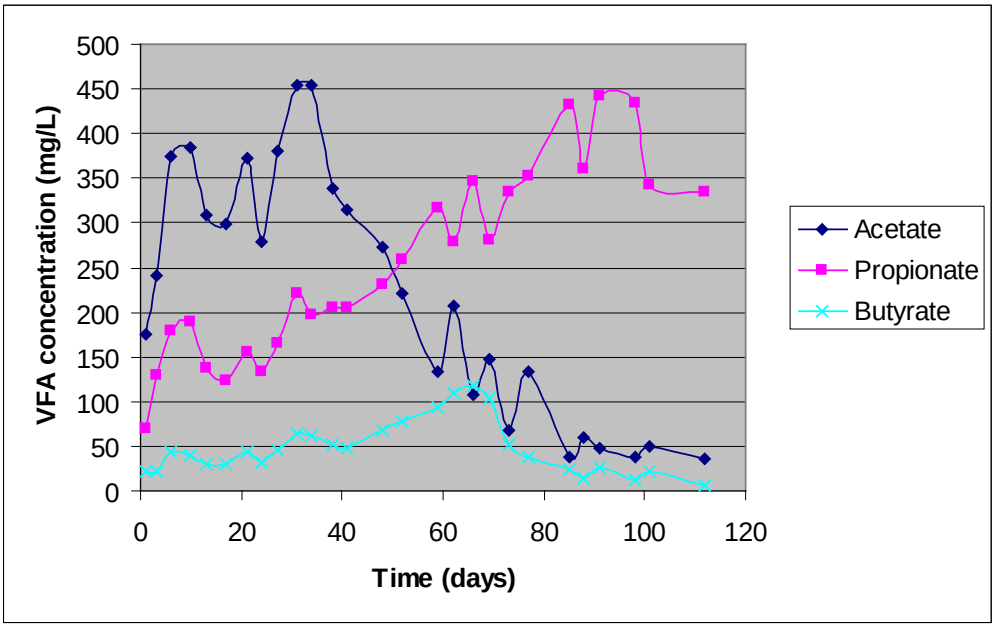

Figure 4-25: Concentration changes of major VFA species during Batch 5.

4.8.2. Performance of the Methanogenic Reactors

The purpose of the bench scale trials was to compare the methane production of three reactor configurations in relation to reactor volume (L CH_4/L reactor/day), influent feed (L CH_4/L slurry), influent VS (L CH_4/gVS_{added}). In the bench scale trials three types of methanogenic reactors were operated. The first type (Reactor A) was designed to operate as a typical anaerobic pond fed with slurry and having a 50-day HRT. Reactor A should be considered as one batch over the whole experiment as organic material in the form of solids builds up in the reactor. Its operation was independent of the batch system even though the cow manure slurry starting material is the same. The second type (Reactor B) was also designed to operate like a pond but was fed with treated feed from the leachbed separator. This was initially run at a 20 day HRT and is referred to as B1. For batch 5 the HRT of this reactor was then reduced to 10 days at which point the reactor is referred to as B2. For batch 5 a new reactor was commissioned to run at a 20 day HRT, this reactor took over the function of B1 and is referred to as B1. The third type (Reactor C) was a rock filter designed to act as attached biomass reactor fed with treated feed. River gravel was used as the medium for the biomass attachment. This reactor was initially run at a 10 day HRT and is referred to as C1, the HRT was then reduced to 5 days at which point the reactor is referred to as C2. A summary of the operating conditions is provided in Table 4-14 for ease of reference.

Table 4-14: Operating parameters of bench scale methanogenic reactors.

	Reactor				
	A (unstirred reactor)	**B1 (unstirred reactor)**	**B2 (unstirred reactor)**	**C1 (rock filter)**	**C2 (rock filter)**
Feed	slurry	treated feed	treated feed	treated feed	treated feed
HRT (days)	50	20	10	10	5

All the reactors produced biogas with methane percentages ranging from 51% up to 73%. Reactor B1 consistently had the highest methane percentage; 60 to 73% compared with 51 to 62% for the pond reactor. The typical methane content of full-

scale digesters is reported as being between 65 and 70% (Tchobanoglous *et al.*, 2003). The average methane content of biogas collected from each trial is shown in Table 4-15 below.

Table 4-15: Average methane content of biogas from the bench-top reactors.

Reactor	A	B1	B2	C1	C2
Batch 1	62 %	73%	-	62 %	-
Batch 2	60 %	66%	-	58%	-
Batch 3	61 %	66 %	-	66%	-
Batch 4	51 %	62%	-	55%	-
Batch 5	56%	60%	65%	-	60%

Figure 4-26 below shows the methane yield for each reactor and for each condition of the leachbed separator, based on the slurry that entered the leachbed reactor. A description of the leachbed separator conditions can be found in section 4.8.1 above. It can be seen that the control reactor (A) has the highest yield throughout the trial with methane production ranging from 0.76 to 1.19 LCH_4/Lslurry compared to 0.53 to 0.74 LCH_4/Lslurry for the 20-day HRT reactor fed with treated feed (B1). This may be a result of a significant portion of the organic content of the slurry being removed in the leachbed, resulting in lower yields for the reactors fed with treated feed. Alternatively, it may be due to the shorter HRTs, under which the reactors fed with treated feed operated. This highlights an area that should be clarified in future research. It is not known whether the variations in gas production can be attributed to organic content removal, improved hydrolysis or reduced HRTs. In terms of testing the effects of hydrolysis in the leachbed separator on methane production, this could be achieved by comparing methane reactors with the same configuration and HRT but fed with different feeds. The feeds being treated feed from the leachbed separator, untreated FDE supernatant and FDE slurry.

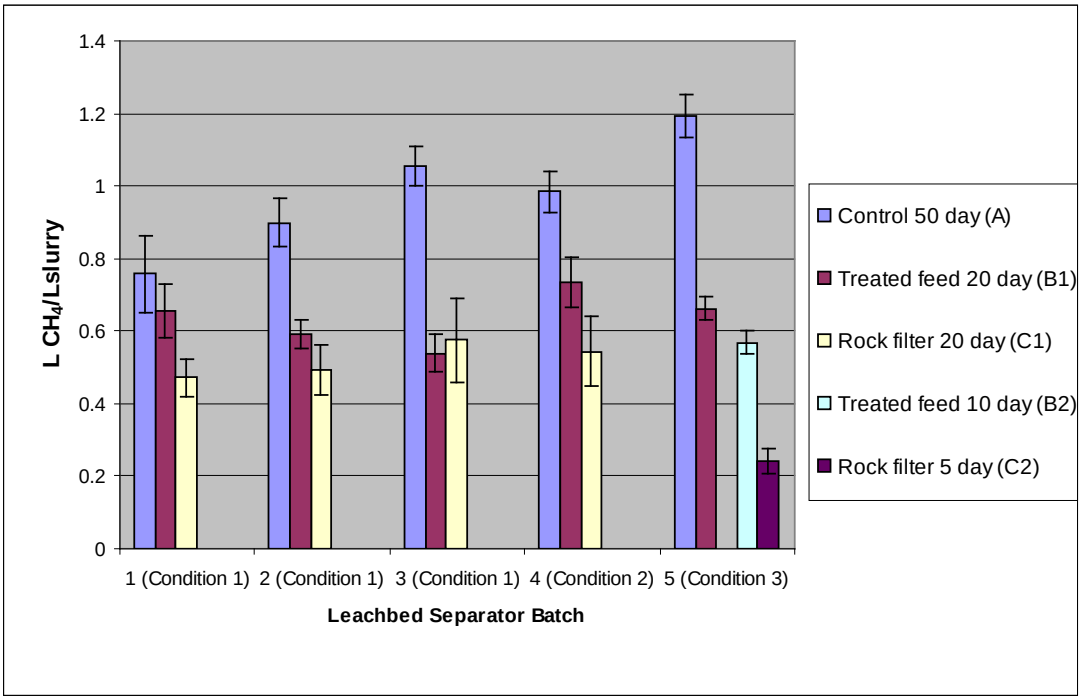

Figure 4-26: Methane yield based on total influent slurry for different reactor configurations and leachbed separator conditions (L CH_4/L slurry).
Error bars are 95% confidence.

There was a steady improvement in the gas production of Reactor A over the course of the experiment. Gas production started at 0.76 L CH_4/Lslurry for batch one and rose to 1.19 L CH_4/L slurry for batch 5. This is probably due to the build up of a large organic sludge layer as discussed above. This sludge layer would have provided an increasing amount of organic substrate resident in the reactor. The organic substrate, in the form of manure fibres and solids would be slowly broken down to release more readily digestible substrates for conversion to methane. Alternatively, the increase may have been the result of microbial acclimation; however, as the seed sludge was from what is presumed to be a well-acclimated dairy farm pond this seems unlikely. It can be seen that volumetric gas production for Reactor B1 is highest for batch 4. The improvement may be related to higher volatile solids (VS) content in the influent slurry and treated feed rather than being due to the change in the leachbed HRT that was implemented for batch 4. The average VS content of the slurry in batch 4 was 7,825 mg/L compared to 6,490 mg/L in batch 3.

Figure 4-27 below shows the specific biogas yield (LCH_4/gVS_{added}) for each reactor based on the type of treated feed used. This relates to VS entering the methanogenic reactors, not VS entering the leachbed separator. There appears to be little significant improvement in the specific yield for reactor B1 in batch 4, based on a change in

conditions in the leachbed reactor, despite the increased biogas yield as illustrated in Figure 4-26 above. Analysis of Variance (one way ANOVA) comparison of the first 20 days of batches 3 and 4 for reactor B1 indicated a 20% increase in average gas production with an F statistic of 9.8. This indicates that the two batches have significantly different means as the F-statistic is greater than the $F_{critical}$ value of 4.1. However, ANOVA comparison between batches 2 and 3 for reactor B1 indicated a 28% increase between batches with an F statistic of 11.7. As batches 2 and 3 were both condition 1 (1 day HRT in the leachbed separator) and showed significant variance, it is not possible to ascribe the increase in specific methane yield in batch 4 to the increase to an 8 day HRT in the leachbed separator. Two-way ANOVA carried out using data from reactor B1 for batches 2, 3, 4 and 5 indicated that an unknown source of variance between successive batches of the same HRT (F-statistic: 15.8) was significant whereas the change in HRT (F-statistic: 1.1, $F_{critical}$: 4.0) was statistically insignificant. The unknown source of variance, i.e the reason for the increases between batches 2 to 5 in reactor B1, could be due to acclimation of biomass, accumulation of organic solids in the reactor or variations in the volatile solids content of the incoming slurry. The later is the most likely as the steady increase exhibited in the LCH_4/gVS_{added} data is not evident in the LCH_4/Lslurry data. Complete ANOVA results are shown in Appendix 6.

When comparing reactor A with reactor B1 it can be seen that they have similar specific methane yields to each other for batches 1 and 4, but for batches 2, 3 and 5 reactor A has higher specific methane yields than reactor B1. This indicates that while the results are variable, the reduction of the HRT from 50 days to 20 days has had a limited impact on the conversion of influent VS to methane.

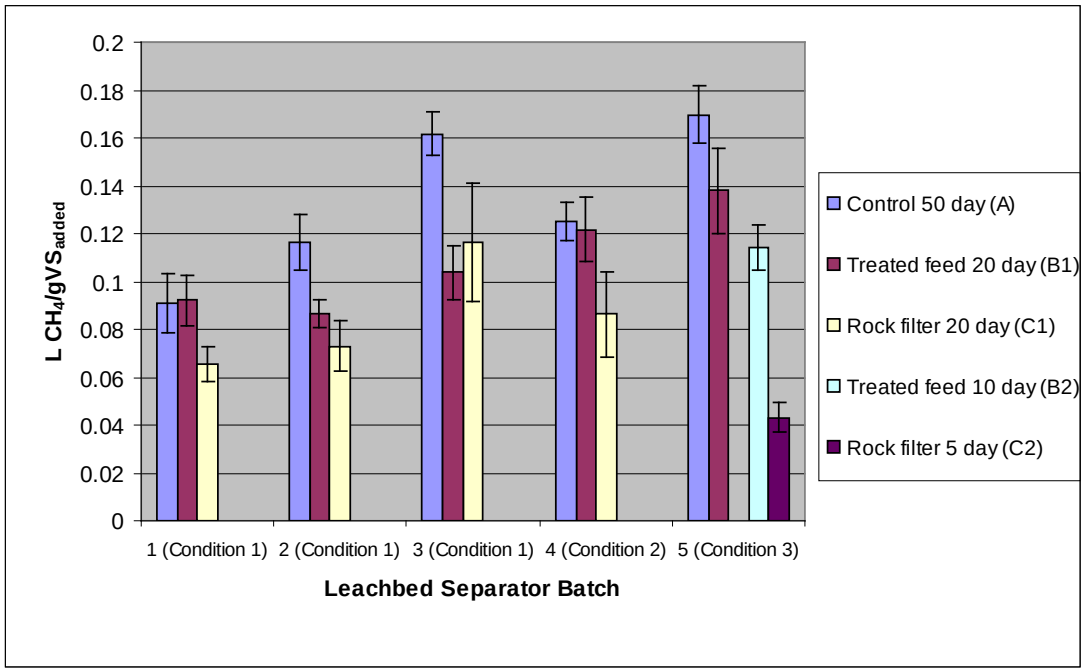

Figure 4-27: : Specific methane yield (LCH_4/gVS_{added}) plotted against condition changes in the leachbed separator.
Error bars are 95% confidence.

Figure 4-28 below show the average methane production for each of the reactors per litre of reactor volume (volumetric gas production). The control reactor (A) generally showed a slight increase for each successive batch. As mentioned earlier, this was most likely due to the accumulation of organic material that occurred in the reactor as the solids settled out. This would have provided an ever-increasing source of substrate for methane production over the period of operation. The disadvantage of this is that the available volume of the reactor is steadily being reduced resulting in a reduced HRT. Eventually it would be expected that the reactor would become full of solids and the majority of methane production would be from the accumulated solids rather than the influent slurry. The 20 day HRT reactor fed with treated feed (B1) showed stable gas production rates when fed with condition 1 treated feed (batches 1-3, 1 day HRT in the leachbed separator) with average methane production for each batch ranging from 0.027 to 0.033 LCH_4/Lreactor/day. The change to an 8 day HRT in the leachbed separator (conditions 2 and 3) may have resulted in improved gas production to over 0.037 LCH_4/Lreactor/day for batch 4. The highest methane production rate was exhibited by the 10 day HRT reactor fed with treated feed (B2) with an average rate of 0.056 LCH_4/Lreactor/day.

The 10-day HRT rock filter had consistently the poorest gas production. The function of the rocks in the filter was to provide a surface for the retention of biomass. It would be expected that the advantage of biomass retention would only be evident when the HRT of the system is less than the minimum SRT of the biomass, about 7.75 days in the case of methanogens at 24^{o} C (Linke, 1997). The poor performance of the rock filter at a 5 day retention, both in terms of LCH_4/gVS_{added} and LCH_4/Lreactor, suggest that there has been little retention of biomass and washout may have been occurring. An examination of the rocks after decommissioning of the reactor showed that the rocks were blackened but that there appeared to be no significant accumulation of biomass on the rock media. Without retention of biomass, the media only wasted available reactor space and provided no advantage with regard to methane production. The poor performance of the rock filter may be attributed to the choice of media as other researchers have shown that attached growth systems can result in significantly improved volumetric methane yields (Hernandez & Rodriguez, 1992; Lo & Liao, 1986). Future research into the effectiveness of using an attached growth system to digest treated feed should investigate the use of alternative media such as raschig rings or porous rocks such as scoria. Hydraulic retention times of less than 5 days should also be trialled in order to determine the effectiveness of the immobilisation of biomass.

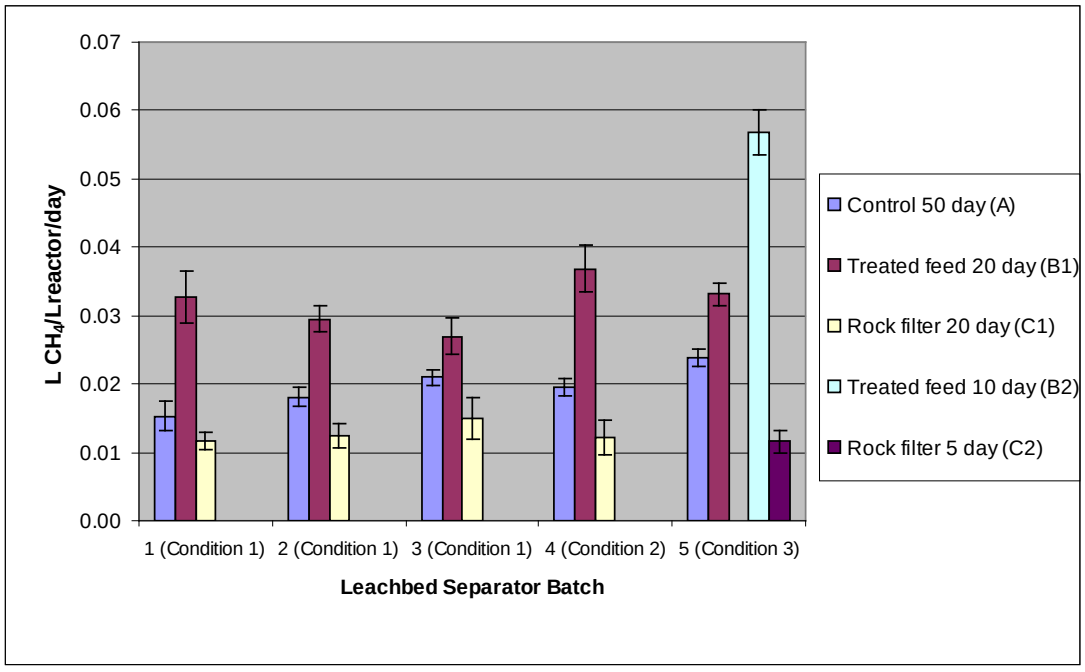

Figure 4-28: Volumetric gas production for different reactor configurations and leachbed separator conditions (L CH_4/Lreactor/day).
Error bars are 95% confidence.

Figure 4-29 below shows total gas production for reactor A and B1 in batch 4. Reactor B1 shows a steady rate of increase with gas production over the first 14 days, rising from 500 mg on day 1 to over 1,200 mg after day 14. This increase appears to mirror the increase in VFAs in the treated feed over the same period. A similar pattern of gas production increasing as VFA increased in the treated feed was observed in the other batches. Plots of those batches can be found in Appendix 5. There is a clear downward trend in pH of the treated feed for the first 15 days, which probably relates to the increase in VFA. The soluble COD of the treated feed shows some increase over the first 15 days but the trend is variable and does not appear to be so closely related to gas production.

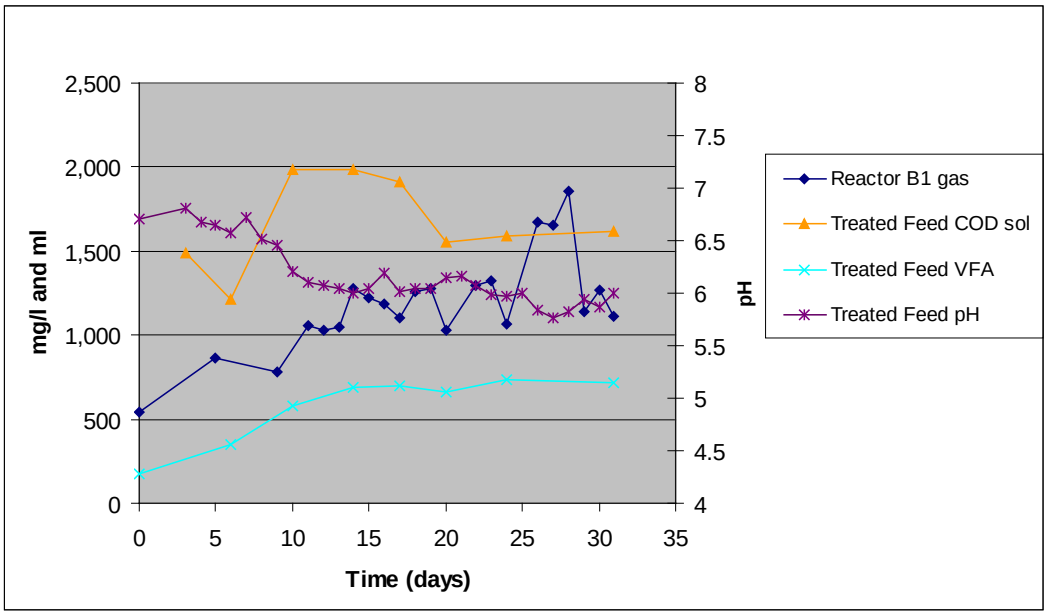

Figure 4-29: Gas production and treated feed characteristics for batch 4.

A similar pattern of increased gas production reflecting increased VFA content can be seen for batch 3 (Figure 4-30 below). In this batch, the increase in the soluble COD of the treated feed is more pronounced and is steady throughout the batch.

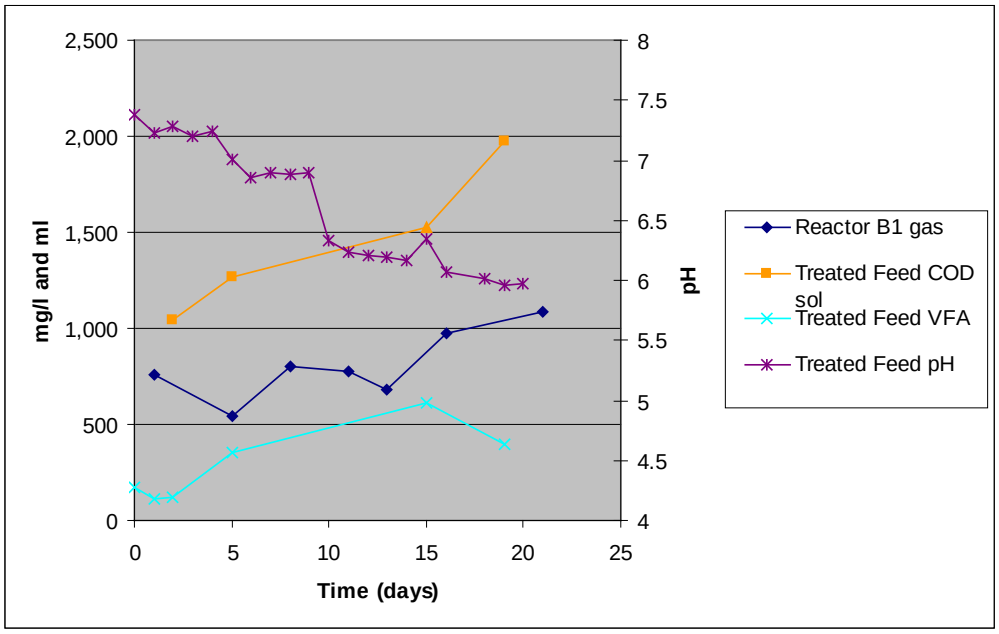

Figure 4-30: Gas production and treated feed characteristics for batch 3.

If it is accepted that increased gas production for reactor B1 (which is shown consistently in all the batches) is due to increased VFA levels and solubilisation that occurs in the leachbed separator we can calculate the improvement that the leachbed separator brings about. The first one or two days in any leachbed separator batch should result in a treated feed which has undergone solids separation but very little of the hydrolysis and fermentation process. Any improvement in gas after day 2 might therefore be attributed to the processes that occur in the leachbed separator. The entry of a readily digestible and convertible substrate in to the methanogenic reactor should result in an increase in gas production independent of the HRT of the system. This is because conversion of the substrate to methane is related to bio-availability of the substrate rather than the length of time the influent is resident in the reactor. Table 4-16 below shows the improvement in gas production for each batch. The results from days 1 and 2 are treated as equivalent to a reactor fed with untreated separated feed. Gas production from days 3-30 is shown as an average and is assumed to have arisen from feed which has undergone treatment. It can be seen that the leachbed separator has brought about an improvement in gas production for all batches, with the greatest improvement being 57% arising from Batch 5. The 8-day HRT in the leachbed separator (batches 4 & 5) does not appear to have increased methane production beyond that which occurred with a 1 day HRT (batches 1-3).

Table 4-16: Improvement in methane production between day 1-2 of a batch (untreated) and days 3-30 of a batch (treated).

Leachbed Separator batch	Leachbed Separator condition	CH_4 production from untreated feed days 1 and 2 (L CH_4/day)	CH_4 production from treated feed days 3 -30 (L CH_4/day)	% increase
Batch 1 (B1)	1 day HRT	0.44	0.68	55%
Batch 2 (B1)	1 day HRT	0.43	0.60	39%
Batch 3 (B1)	1 day HRT	0.40	0.55	38 %
Batch 4 (B1)	8 day HRT	0.54	0.74	36%
Batch 5 (B2)	8 day HRT	0.73	1.15 (days 3 to 135)	57 %

All reactors operated with relatively low pH, typically between 6.3 and 6.5. This is below what is considered optimum for methanogenesis; between 6.5 and 7.5 (Hobson & Wheatly, 1993).

Table 4-17 below shows the operating conditions and methane production achieved in previous studies and the present study. The control reactor (A1) showed good agreement in terms of gas production with a typical anaerobic pond. The reactors fed with treated feed (B1 and B2) had comparable volumetric CH_4 production with the lagoons used by Safely and Westerman (1992b) despite having much shorter HRTs. The highest volumetric CH_4 production of all the studies was 2.8 m^3CH_4/m^3reactor /day (Hernandez & Rodriguez, 1992). However, the temperature was not reported for that study and it may have been in the mesophilic range. The authors attributed some of the performance of the reactor to the high BOD soluble molasses diet of the cows. The CSTR and fixed film reactors of Lo and Liao (1986) also showed high volumetric CH_4 production. This may be attributable to the significantly higher organic loading rates that were used in those studies (2.9 and 28.7 kg VS/m^3/day c.f 0.67 kg VS/m^3/day used in this study). The performance of reactors with high volumetric CH_4 production appears to go with a reduced specific methane yield and reduced HRTs. The fixed film reactors of Lo and Liao had a very short HRT (1 day) and a very low specific methane yield (0.01 $m^3/kgVS_{added}$) compared with that of Safely and Westerman (1992b) who had a 67 day HRT and a high 0.322 $m^3/kgVS_{added}$ specific methane yield. The specific methane yields achieved in reactors B1 and B2

in this study appear be similar to or slightly higher than those reported in the literature with the exception of Safely and Westerman (1992a). The specific methane yield reported in that study is significantly higher than the ultimate methane yields for dairy manure reported in the literature (refer Table 2-1 in the literature review). The authors attribute this to a build-up of organic material which occurred in the lagoon. The rock filter used in this study (reactor C1) had very similar specific methane yields and volumetric methane production rates to those report by Vartak *et al* (1997) despite this study using a significantly shorter HRT (10 days c.f 33 days). However, the temperature used in the Vartak study was also significantly lower (10 ^{o}C c.f 20 ^{o}C).

Table 4-17: Literature values for volumetric gas production rates of various psychrophilic reactor configurations fed with dairy effluent.

Researcher	System	Feed	Temperature (^oC)	HRT (days)	Organic loading (kg VS/m^3/day)	CH_4 production ($m^3/kgVS_{added}$)	CH_4 production ($m^3/m^3_{reactor}$/day)
Safely and Westerman (1992b)	Lagoon	Screened	10.6 -15	67	0.136	0.322	0.044
(Hernandez & Rodriguez, 1992)	Anaerobic Filter	Screened settled	Not reported	0.5	16.3	0.17	2.8
(Vartak *et al.*, 1997)	Anaerobic filter	Unscreened	10	33	0.12	0.08	0.013
(Lo & Liao, 1986)	CSTR	Screened	22	10	2.94	0.06	0.18
(Lo & Liao, 1986)	Fixed film reactor	Screened	12	1	28.7	0.01	0.3
(MAF, 1994)	Typical anaerobic pond	Unscreened	Ambient	50-125	0.15	0.2	0.02
This study	Unstirred tank (A1)	Unscreened	20	50	0.15	0.14	0.021
This study	Unstirred tank (B1)	Treated screened	20	20 (28) *	0.31	0.14	0.037
This study	Unstirred tank (B2)	Treated screened	20	10 (18) *	0.54	0.11	0.056
This study	Rock filter (C1)	Treated screened	20	10 (18) *	0.61	0.12	0.015
This study	Rock filter (C2)	Treated screened	20	5 (13) *	1.11	0.04	0.012

* Numbers in brackets indicate the HRT of the combined system including the leachbed separator and the methanogenic reactor.

Both reactors B1 and B2 showed improved volumetric methane production when compared to the control reactor (A) which is important if the size of ponds is to be reduced to make them more economical to cover. An examination of the gas production rates within batches appears to show that the action of the leachbed separator in improving hydrolysis has contributed to an improved volumetric methane production rate. However, a comparison between reactors B1 and B2 seems to indicate that shorter HRTs in the methanogenic reactors may also result in higher volumetric methane production. What is not clear is the relative contribution of reduced HRT and improved hydrolysis, via the leachbed separator, to the improved volumetric methane production. Future research should separate out these two variables for further examination. This would require a comparison of unscreened, screened and treated FDE digested at various HRTs to determine the effect of HRT on volumetric methane production and specific methane yield. A comparison of treated screened FDE, untreated screened FDE and whole untreated FDE digested at the same HRTs would help to determine the contributions of the leachbed separator treatment and physical separation on the volumetric methane production and specific methane yield.

4.9. Field Trials

An unmixed leachbed separator feeding an unmixed tank digester was trialled at Massey University #4 Dairy Farm. The trial was carried out to test the findings of the laboratory experiments at field scale. The leachbed separator was constructed in the existing feed-pad pit and had a wooden weeping wall built into it to retain solids. The leachbed separator was fed with a combination of feed pad scrapings and FDE form the milking shed. A 15 m^3 black polyethylene tank served as a methanogenic reactor and was fed with about 0.75 m^3 of treated feed from the leachbed separator everyday, giving a nominal HRT of 20 days.

The leachbed separator showed good performance in terms of increased VFA concentration (Figure 4-31) with effluent levels averaging 1,260 mg/L compared to an average influent VFA concentration of 100 mg/L, a 1,160 % increase. This is a significant improvement over the best performing batch (batch 4) in the bench-top

leachbed separator, which had a 120% increase and an average VFA concentration of 562 mg/L. There was an average 100% increase in the soluble COD concentration of the treated feed (5,542 mgCOD_{sol}/L) compared to the influent (2,776 mgCOD_{sol}/L) indicating that a good degree of hydrolysis was occurring. In comparison, maximum COD_{sol} concentration in the bench-top treated feed was 3,050 mg/L. The differences between the soluble organic content of the field treated feed and the bench-top treated feed may well relate to the additional organic load which was received from the feed pad adjacent to the leachbed separator on the farm. Waste feed rations and cow manure were scraped from this feed pad approximately once a week during the period of the trial.

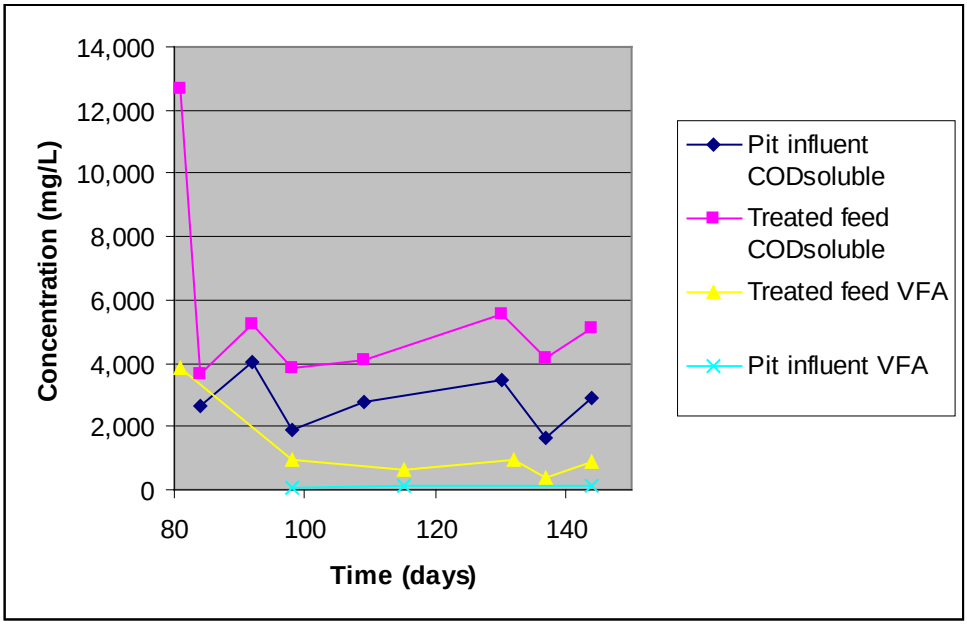

Figure 4-31: VFA and soluble COD concentrations in the influent and effluent from the leachbed separator pit.

Initial gas production was between 1.6 and 4.8 m^3/day for the first 30 days of the trial (Figure 4-32 below). There was, however, a great deal of variability in gas output. Many of the lower readings were due to faults when the line to the reactor became blocked and the reactor was not fed. There appeared to be an increase in gas production for the first 10 to 20 days of a new batch followed by a decline. A chemical cleaning was done of the whole dairy shed between day 70 and 80; this may have affected reactor performance. Beyond these variations there appears to be an overall decline in reactor performance over the whole trial. This did not appear to be as a result of organic overloading as effluent from the reactor typically had VFA

concentrations below 200 mg/L which is, although higher than ideal, within acceptable limits (Hobson & Wheatly, 1993).

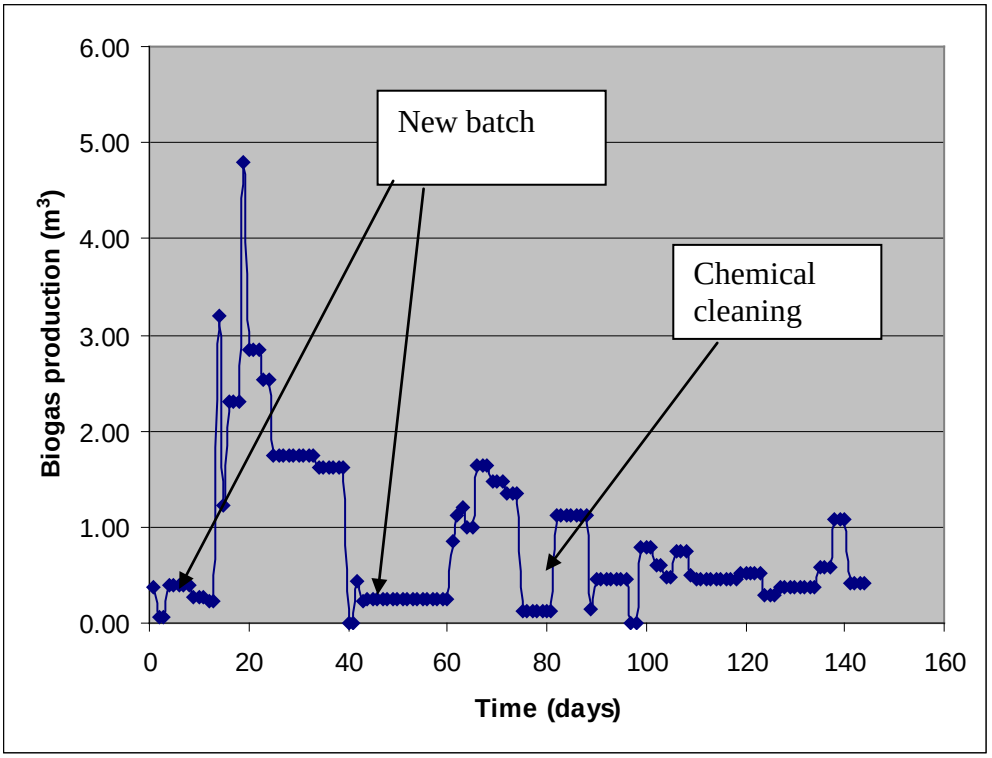

Figure 4-32: #4 Farm daily biogas production.

The higher gas production between day 10 and 20 does not appear to be related to temperature. The average temperature for the first 30 days (December) was 17.9 ^{o} C compared to 19.6 ^{o} C for days 31 to 62 (January). The highest production rate was 0.21 m^3 CH_4/ m^3reactor/day but this was almost twice as high as any other day. During the peak production period (day 15 -40) the reactor was producing an average of 0.08 m^3 CH_4/ $m^3_{reactor}$/day. This is over 4 times that reported for standard anaerobic ponds (MAF, 1994) and over ten times that reported by Park and Craggs (2007) for dairy farm anaerobic ponds, but still under half that achieved by Lo *et al* who achieved 0.18 m^3 CH_4/ $m^3_{reactor}$/day for a 10 day HRT CSTR at 22^{o} C. It was also over twice as high as that achieved in the bench-top experiments (0.037 m^3 CH_4/ $m^3_{reactor}$/day for reactor B1). This was most likely due to the significantly higher $COD_{soluble}$ and VFA contents of the influent to the field reactor.

The short length of the field trial and interruptions to the operation of the reactor caused by technical difficulties and farm practices contributed to a limited and

variable data set. While the results indicate that this technology has the potential to be successfully employed on working dairy farms, further investigation is required to confirm these preliminary findings. Further research would benefit from more extensive characterisation of both influents and effluents. The effects of dilution, HRT and mixing on leachbed operation were not examined during the field trials and these parameters could benefit from additional research. In the farm setting dilution could be controlled by adjusting the flow on the hoses used for wash down and typically attached to a scraper on the backing gate. HRT in the leachbed separator could be controlled through the height of the outlet from the weeping wall reservoir. Mixing can be achieved using effluent mixers mounted on pontoons and controlled through simple timers.

5. Conclusions

A literature review was carried out which found a large body of material on anaerobic digestion and its application to farm dairy effluent. There was, however, very little literature available relating to the hydrolysis and acidogenesis processes that dairy effluent solids have to undergo in order for methanogenesis to occur. Prior to the start of experiments, no study was found in the literature describing a two-stage system incorporating a hydrolytic leachbed with a methanogenic reactor using farm dairy effluent or any other manure products.

A characterisation of farm dairy effluent found between 40 and 50% of the total COD and between 35 and 46% of the total BOD was in the sludge of separated slurry. This indicated that a significant portion of organic matter was available in the solids, which could potentially be converted to methane.

In order to test the effect of dilution, sludge was fermented using different volumes of fresh water. Four sludge to fresh water dilution ratios were trialled: 1:0.25, 1:0.5, 1:0.75 and 1:1. Those reactors with higher volumes of wash water produced greater COD mass in the effluent. The highest COD extraction in this study was achieved with 1:1 sludge to water ratio. This dilution yielded 27,500 mg COD compared to 14,800 mg COD in the 1:0.25 sludge to water reactor, an 86% increase. A similar pattern was exhibited in VFA production with 635 mg VFA produced from the 1:1

sludge to water reactor compared to 184 mg/L produced from the 1:0.25 sludge to water reactor. It was hypothesised that the higher levels of hydrolysis and acidogenesis in the higher dilution reactors was caused by having lower mass transfer resistance between the solid and liquid phase. The lower mass transfer resistance was thought to be brought about by lower concentrations of products in the more highly diluted reactors. The results indicate that by increasing the water added in the leachbed separator a higher degree of VFA and COD extraction can be achieved. While a 1:1 ratio of water to sludge was shown to produce higher levels of COD and VFA than lower dilutions in this study, the addition of extra process water would have implications for the second stage methanogenic digester, requiring a larger digester volume in order to maintain the same HRT. Further research is recommended in this area in order to determine an optimum level of dilution in terms of reactor size and additional methane production.

It was proposed that addition of rumen to cow manure sludge could bring about improved hydrolysis and acidogenesis due to the enzymatic action of the rumen bacteria. Little difference in COD or VFA content of the liquid phase was observed between reactors dosed with active rumen and those dosed with inactive rumen. This indicated that there was no improvement in hydrolysis or acidogenesis due to the addition of active rumen. The autoclaving of the inactive rumen may have had a hydrolysing effect on undigested grass allowing the release of organic material. This may have masked the hydrolysis caused by the active rumen. Although these trials were inconclusive, future research should be carried to investigate whether, under the correct conditions, the addition of rumen can bring about improved hydrolysis. However, the simple addition of rumen contents to hydrolytic dairy manure digesters does not appear to be an effective strategy.

In trials to investigate the effect of mixing on hydrolysis and acidogenesis, a once daily mixing regime was shown to achieve higher levels of VFA production (0.018 $mgVFA/mgVS_{added}$) than intermittent mixing (0.006 $mgVFA/mgVS_{added}$) or constant mixing (0.004 $mgVFA/mgVS_{added}$). In contrast, the constantly mixed reactor, which produced very little VFA, had the highest level of soluble COD production. It was found that constant mixing had an oxygenating effect that may have resulted in inhibition of the anaerobic acidogens. This oxygenation may have had less effect on

hydrolysing species which can include facultative anaerobes. With the confounding effect of oxygenation, it was not possible to draw conclusions as to the effect of mixing on hydrolysis or acidogenesis indicating that further research is required.

In an investigation on the effects of HRT on acidogenesis and hydrolysis, it was found that HRTs shorter than 10 days favoured hydrolysis, as evidenced by increased soluble COD production. A reactor operating with a 5 day HRT yielded 0.325 $gCOD_{soluble}/gVS_{added}$ compared to a 15-day HRT reactor which yielded 0.147 $gCOD/gVS_{added}$. It was hypothesised that this might be due to the sessile nature of hydrolytic bacteria, which causes them to remain attached to the solids fibres and more resistant to washing out at shorter HRTs. Soluble COD concentrations appeared to be limited to 3,000 mg/l, possibly indicating some product inhibition. The more frequent removal of products, which occurred at shorter HRTs, was thought to prevent high concentrations of soluble COD thereby enhancing hydrolysis. In contrast, longer HRTs appeared to favour acidogenesis, with a 15-day HRT reactor producing the greatest mass of VFA (0.033 $gVFA/gVS_{added}$) compared to the other HRTs trialled (ranging between 0.02 and 0.028 $gVFA/gVS_{added}$). It was hypothesised that this could be due to the planktonic nature of acidogenic bacteria, making them more susceptible to wash out at shorter HRTs. By adjusting the HRT to favour hydrolysis rather than acidogenesis, shorter retention times could be employed. This would have a practical implication for on-farm systems as the leachbed separator could be sized for a 5 day HRT, thereby reducing construction costs. Soluble COD consumption was observed in all reactors after 20 days, indicating that methanogenic populations were able to establish. As this was observed in all reactors regardless of HRT, it was hypothesised that the methanogenic populations are associated with the solid phase rather than the liquid phase. If methanogenic populations are becoming established in a leachbed separator within 20 days then this could result in wastage of methane potential. 20-day batches may add a level of operational difficulty, due to the need for frequent solids removal, which could make this technology unattractive to farmers. The suppression of methanogenic activity in the hydrolytic stage is an issue that should be addressed in future research.

A mix, settle and decant reactor was compared with an unstirred gravity drained leachbed reactor in order to determine which system had the highest production in terms of COD and VFAs. The settle and decant reactor exhibited increased hydrolysis compared to gravity drained leachbed reactor, producing 0.315 $mgCOD/VS_{added}$ compared to 0.155 $mgCOD/VS_{added}$ from the leachbed reactor. The settle and decant system also produced more VFA (0.033 $mgVFA/VS_{added}$) than the leachbed reactor (0.015 $mgVFA/VS_{added}$). It was proposed that the higher VFA and COD contents were due to improved mixing and longer HRTs than those in the unstirred gravity drained leachbed reactor. It was hypothesised that improved mixing had the effect of allowing new spatial associations between bacteria and substrate, and of removing inhibitory substances that could accumulate.

In bench-top trials, the use of a partially mixed leachbed separator was shown to increase both the soluble COD and VFA content of the liquid portion of farm dairy effluent. Average VFA levels ranged from 353 to 567 mg/L, which was 38 to 120% increase over that detected in the influent slurry (batch averages ranged from 236 to 438 mg/L). Soluble COD levels were shown to increase by up to 60%, rising from 1,085 mg $COD_{soluble}$ in the influent slurry to 1,740 mg/L in the treated feed. A change in the operating conditions of the leachbed separator, from a 1-day HRT to an 8-day HRT, initially showed improvement in both hydrolysis and acidogenesis. This performance was not sustained in a subsequent batch, which means that it is not possible to ascribe definitively the improved hydrolysis and acidogenesis to the increased HRT. Further research is required to clarify effect of HRT on the system performance.

Both a 20-day HRT unmixed unheated reactor fed with treated feed from a partially mixed leachbed separator and a 50-day HRT unmixed unheated reactor fed with untreated slurry produced similar specific methane yields (0.12 and 0.14 $m^3CH_4/kgVS_{added}$ respectively) indicating that the shortened retention time had only a minor effect on VS conversion to methane. Shortening the retention time further to 10 days resulted in a specific methane yield of 0.11 $m^3CH_4/kgVS_{added}$. In terms of the total slurry entering the two-stage system there was between 14 and 49% less methane produced per litre of slurry from the 20-day HRT reactor fed with treated feed (0.53 to 0.74 $LCH_4/Lslurry$) compared to the 50-day HRT reactor fed with

slurry (0.76 to 1.19 LCH_4/Lslurry). In terms of volumetric yield the 20-day HRT reactor fed with treated feed consistently produced more methane (0.027 to 0.037 LCH_4/Lreactor/day) than the 50-day HRT reactor fed with untreated slurry (0.015 to 0.24 LCH_4/Lreactor/day). This indicates that there is a trade-off between increased volumetric yield and decreased total yield per unit of farm dairy effluent. A 10-day HRT unmixed unheated reactor fed with treated feed was shown to have a 0.057 LCH_4/Lreactor/day volumetric methane production rate, 138% higher than a 50 day HRT reactor fed with slurry (0.024 LCH_4/Lreactor/day). Gas production was shown to rise as the VFA levels in the treated feed rose. As shown in Table 4-16 fermentation in the leachbed followed by separation was shown to improve average gas production in the 20-day HRT unmixed unheated reactor, by up to 55% compared to separation alone.

In field trials on a working dairy farm, an unmixed "weeping wall" leachbed separator produced an effluent with less solids, higher soluble COD and higher VFA content than the influent slurry. The unmixed leach bed separator was shown to increase VFA content of the liquid phase from an average 100 mg/l in the influent to 1,260 mg/l in the treated feed. The soluble COD increased from 2,776 mg/L in the influent slurry to 5,542 mg/L in the treated feed. This was an improvement over the performance of the laboratory-scale leachbed separator and was partly due to the estimated doubling of solid material and organic load resulting from the input of scrapings from an adjacent feeding pad. Treated feed from the leachbed separator was fed into an unstirred unheated 20-day HRT tank digester. During a peak period of 24 days, the average volumetric methane production rate was 0.08 m^3 CH_4/ m^3reactor/day, four times higher than that which would be expected from a covered pond of the same size. However, performance of the reactor was variable and not consistent over the trial. This was attributed to technical and mechanical issues at the farm. Further work is required to improve the day-to-day operation of the on-farm system.

This study has shown that a leachbed system coupled with a pond reactor can result in a reduction of HRT to 18 days from the currently used 50 to 120 days. Of those 18 days, only 10 would be required in a covered system, with the other eight days able

to take place in an uncovered leachbed separator. This would mean a five to 12-fold reduction in the size of the down-stream anaerobic ponds. This would result in lower start-up costs for farm biogas operations. The reduction of HRT and removal of solids is, however, at the cost of total methane production that could be derived from the farm dairy effluent. While there is a trade-off in the total methane production, the reduction in pond size could make the covering of ponds a more economically viable proposition than at present. The study showed that while a leachbed separation system could increase the soluble organic content of the liquid fraction of farm dairy effluent, further optimisation of the process is required to ensure that excessive soluble COD consumption does not occur in the leachbed separator. If the establishment of a methanogenic population in the leachbed separator can be prevented this could result in a higher methane yield from the same amount of farm dairy effluent. It would also have the advantage of reducing the time and labour needed for removal of solids. Further research is needed into the effect of organic loading rates, particularly in respect to the advantageous inhibition of methanogenesis in the leachbed separator. While the results of field trails indicate that this technology has the potential to be successfully employed on working dairy farms, further investigation is required to confirm these preliminary findings and optimise the on-farm plant design.

6. References

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7. Appendices

Appendix 1: Microbiological Species Involved in Biomethanation

Table 7-1: Species of hydrolysers and their respective substrates and products.

Species	**Substrate**	**Product**	**Enzymes**
Clostridium prefringens *C. bifermentans, C.histolyticum, C.sporogenes* (McInerney, 1988)	proteins	Peptides amino acids ammonia carbon dioxide	
Anaerovibrio lipolytica and *Syntrophomonas wolfei* (Cecchi and Mata-Alvarez, 1992).	Simple lipids (fats and oils) Complex lipids (e.g., phospholipids, glycolipids)	saturated and unsaturated long chain fatty acids, glycerol	esterase
Streptococcus bovis, Bacteriodes amylophilus, Selenomonas ruminatium, Succinomonas amylolytica, B.ruminocola and also a number of *Lactobacillus* species (Tsao 1984).	Starch: □ethyla and amylopectin.	Glucose maltose	α-amylase β-amylase Glucoamylase

(Chynoweth & Pullammanappallil, 1996)

Table 7-2 : Species of acetogens and their respective substrates

Species	substrate
Syntrophomonass wolfei, Syntrophobacter wolinii	propionate, butyrate, lactate, and ethanol

Table 7-3: Species of methanogens and their respective substrates.

Species	substrate
Methancoccales	H_2-CO_2 formate
Methanobacteriales	H_2-CO_2 formate
Methanogenium relatives	H_2-CO_2 formate
Methanosarcinaceae	acetate
Methanosarcina plus relatives	Methanol, ethylamines; some use acetate and H_2-CO_2
Methanosarcina	acetate H_2-CO_2, methanol, and methylamines;
Methanosaeta	Use only acetate;

(Raskin *et al.*, 1994)

Appendix 2: Empirical Design Calculations

For an anaerobic pond the volumetric BOD loading (λv, g/m3 d) is given by:

$\lambda v = LiQ/Va$

where *L*i is the BOD5 of the raw wastewater (mg/L = g/m3), *Q* is the wastewater flow (m3/d) and *V*a is the anaerobic pond volume (m3).

The permissible range of λv is 100 g/m3 d at temperatures≤10°C, increasing li nearly to 300 g/m3 d at 20°C, and then more slowly to 350 g/m3 d at 25°C and above. The design temperature is the mean temperature of the coldest month. Once the temperature is known, the value of λv is determined and the value of *V*a calculated. The anaerobic pond area is then determined by dividing *V*a by the pond depth (e.g., 3 m). BOD_5 removal is 40% at temperatures ≤10°C, increasing linearly to 70% at 25°C and above.(Pena Varon & Mara, 2004).

Dexcel guidelines (Dexcel, 2006) suggest sizing ponds based on (BOD_5 loading) + (local rainfall less evaporation data) where BOD_5 loading per cow per day = 0.12 kg/cow/day. The following design specifications are also suggested:

• length to width ratio approximately 2 : 1.

• minimum pond depth is 3 m for ponds serving up to 250 cows. For larger herds, pond depth is 4 m.

• internal batter slope = 2 : 1.

• pond width does not exceed 24 m because of the ‘reach’ limitations of excavator and desludging machinery.

Appendix 3: Process Models

Kinetic models are mathematical expressions that attempt to predict the outputs of anaerobic digestion. This requires the linking together of equations which describe the biochemical process kinetics of anaerobic digestion. A complete model will incorporate the kinetics microbial growth, hydrolysis, acidogenesis, acetogenesis, methanogenesis and various types of inhibition. In microbial growth kinetics key parameters are the maximum specific growth rate (μ_{max}), substrate concentration (S), the saturation constant (K_s) biomass concentration (X), specific decay rate (b) product yield ($Y_{p/s}$) and biomass yield ($Y_{x/s}$) (Gavala *et al.*, 2002). Typically, rates and constants must be derived experimentally and will be different for various substrates, processes and species involved in the processes. In addition expressions must be derived which mimic changes brought about by changes in temperature and sometimes pH.

McCarty derived an expression biomass growth;

$R_x = (\mu_{max}.S/K_s + S).X - b.X$ (McCarty & Mosey, 1991). This incorporated the original Monod expression (Monod, 1949) but also included a decay coefficient. Others who have derived expressions for microbial growth are Moser, Contois, Grau et al, and Chen and Hashimoto (Gavala *et al.*, 2002)

One of the more straightforward models which has found use in the design of on-farm systems was developed by Chen and Hashimoto from the Contois model:

$$Yv = \left(\frac{Bo * VS}{\theta}\right)\left[1 - \left(\frac{K}{\theta * \mu m - 1 + K}\right)\right]$$

where: Yv = volumetric methane production, L CH_4 per influent volume/day

VS = influent total volatile solids concentration, grams per influent volume/day

Bo = ultimate methane yield, L/g VS added as θ approaches infinity

θ = retention time, days

μm = maximum specific microbial growth rate, $days^{-1}$

K = kinetic parameter, dimensionless

The above model is used in the calculation of digesters in the Agstar Farmware package

Chen and Hashimoto defined the relationship between μm and temperature for temperatures between 20 °C and 60 °C based on the analysis of data from several sources as follows: $\mu m = (0.013\ T) - 0.129$

Hashimoto also proposed equations below to describe the relationships between K and VS for dairy (Hashimoto, 1982) and swine manures (Hashimoto, 1984):

$K_{dairy} = 0.8 + 0.016^{(0.06 * VS)}$

$K_{swine} = 0.6 + 0.0206^{(0.051 * VS)}$

where: VS = influent total volatile solids concentration per influent volume, kg/m^3.

Kdairy is less than or equal to 1.64 while Kswine is less than or equal to 1.2.

Table 7-4: B_o values used in Farmware design.

Animal	**B_o**
Grow-Finish	0.48
Farrowing	0.3525
Farrow-Finish	0.3525
Nursery	0.48
Farrowing plus nursery	0.3525
Dairy	0.304

Appendix 4: Rumen Dosing Experiment 1 Results

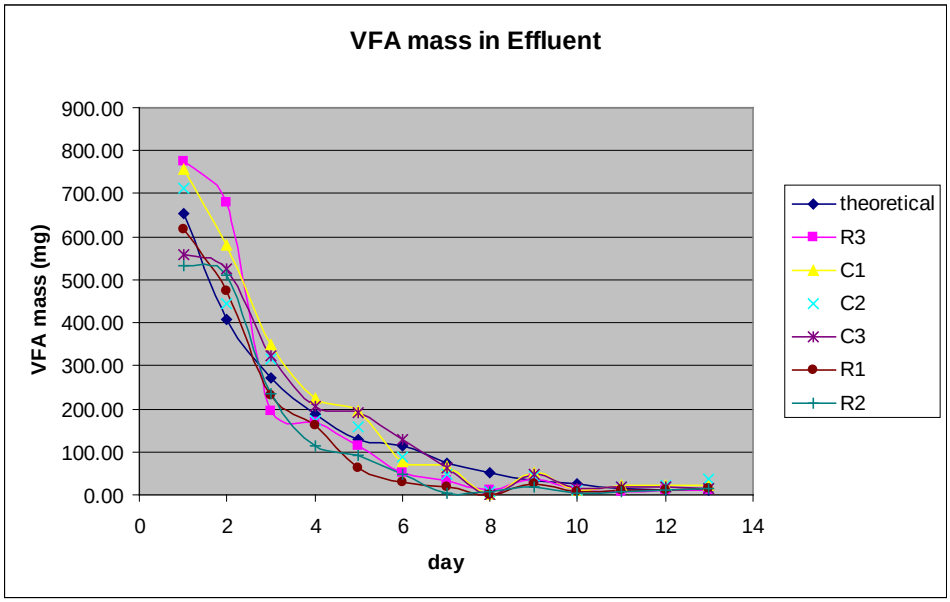

Figure 7-1: VFA mass in effluent

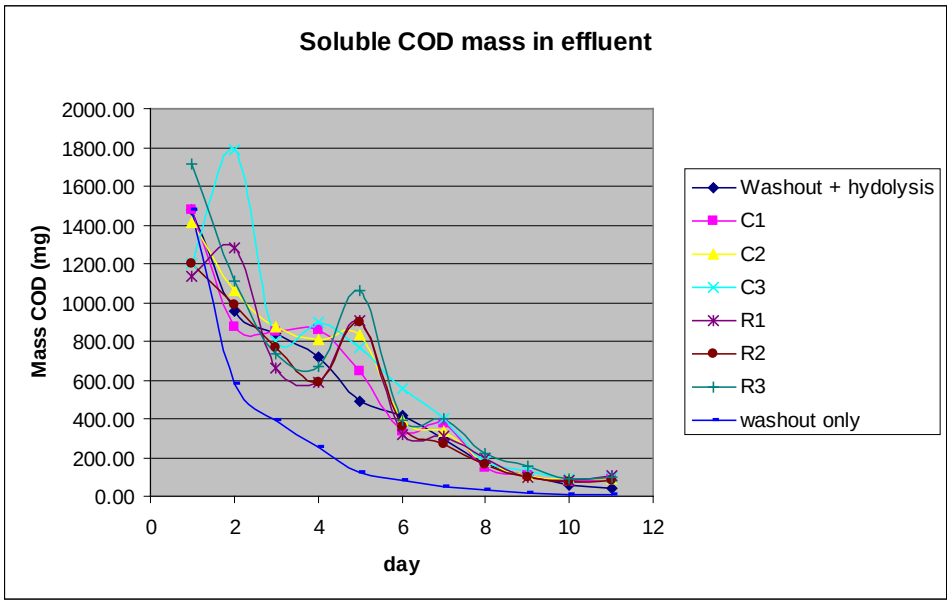

Figure 7-2: Soluble COD mass in effluent

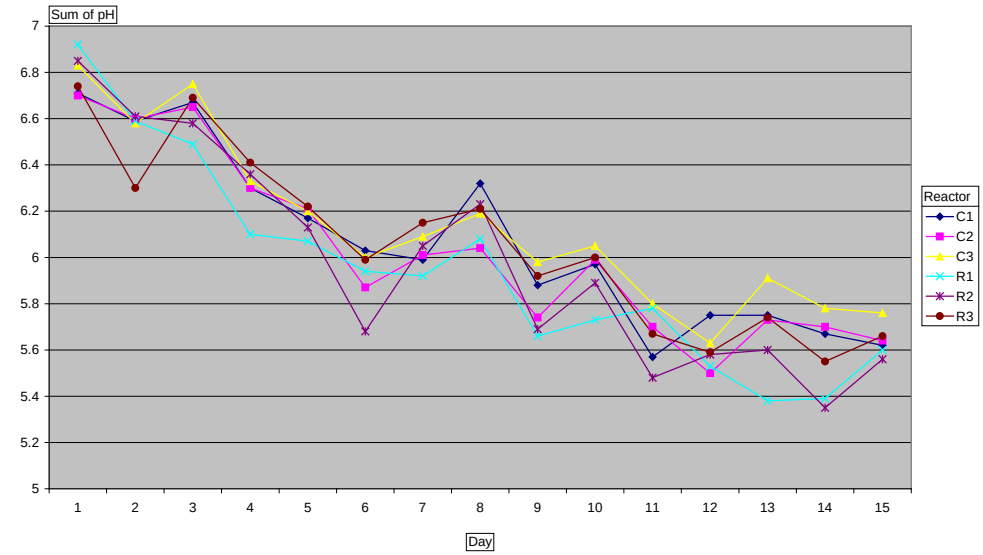

Figure 7-3: pH of reactors

Appendix 5: Bench-top Experiments Supplementary Data

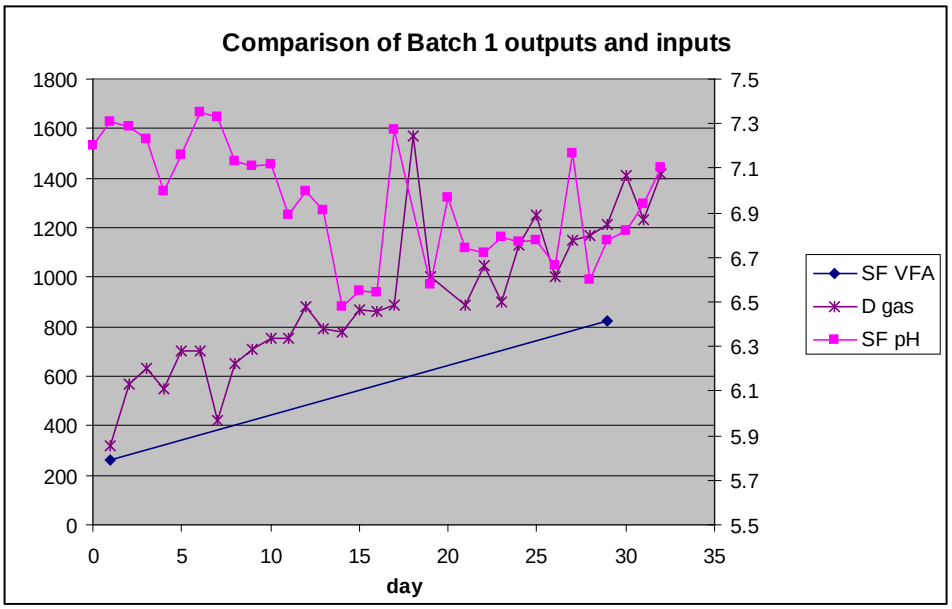

Figure 7-4: Relationship between gas production and treated feed characteristics in Batch 1.

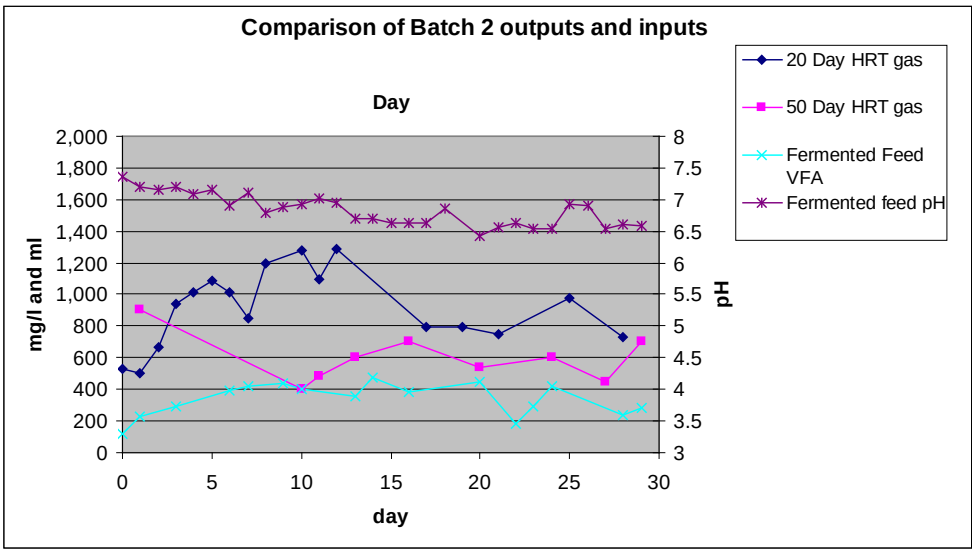

Figure 7-5: Relationship between gas production and treated feed characteristics in Batch 2.

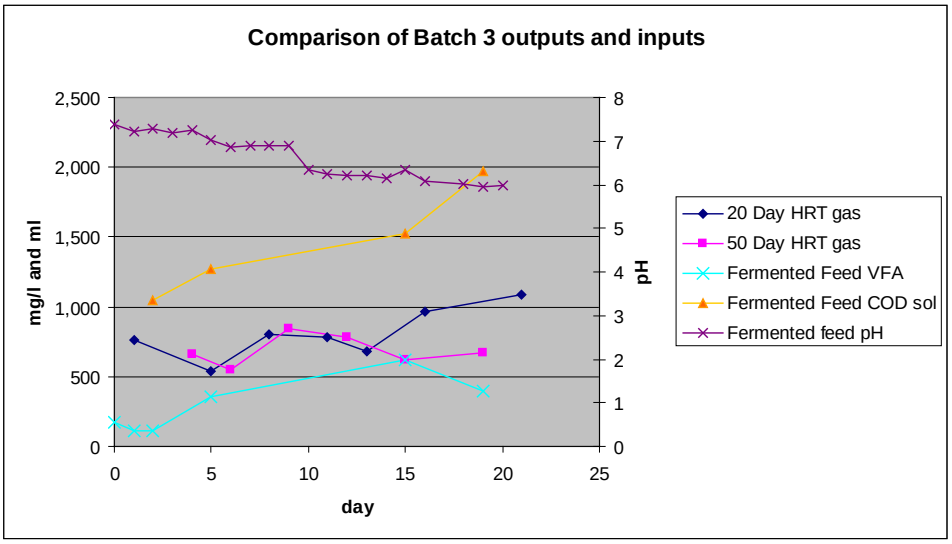

Figure 7-6: Relationship between gas production and treated feed characteristics in Batch 3.

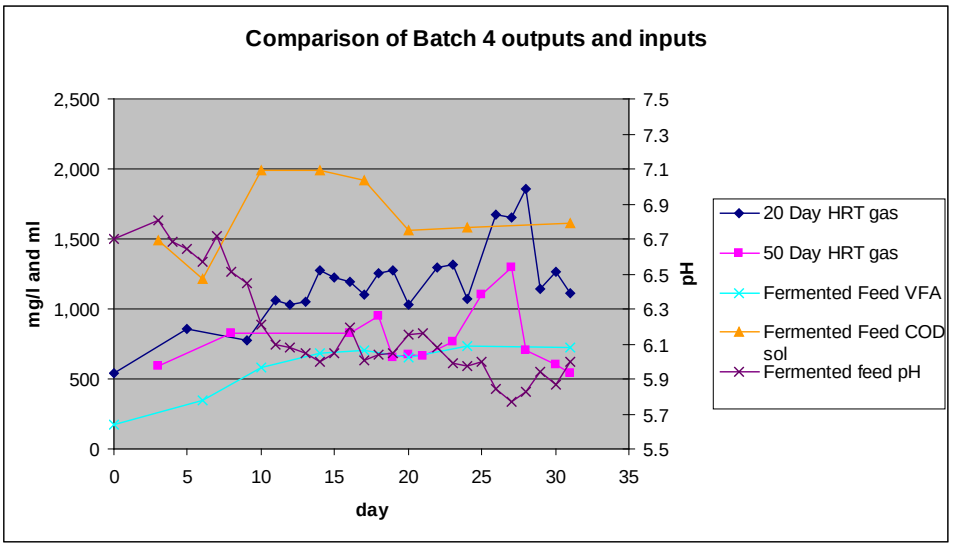

Figure 7-7: Relationship between gas production and treated feed characteristics in Batch 4.

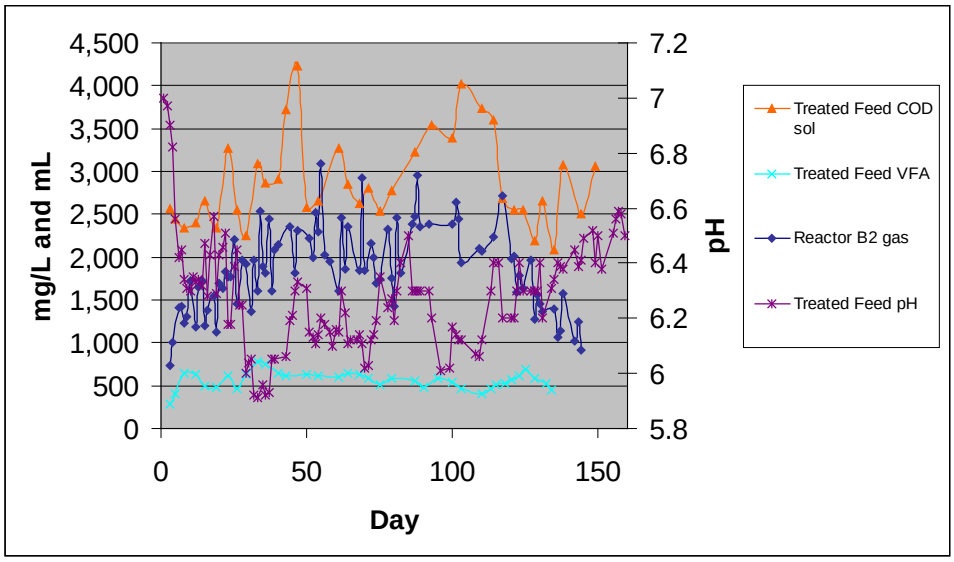

Figure 7-8: Relationship between gas production and treated feed characteristics in Batch 5.

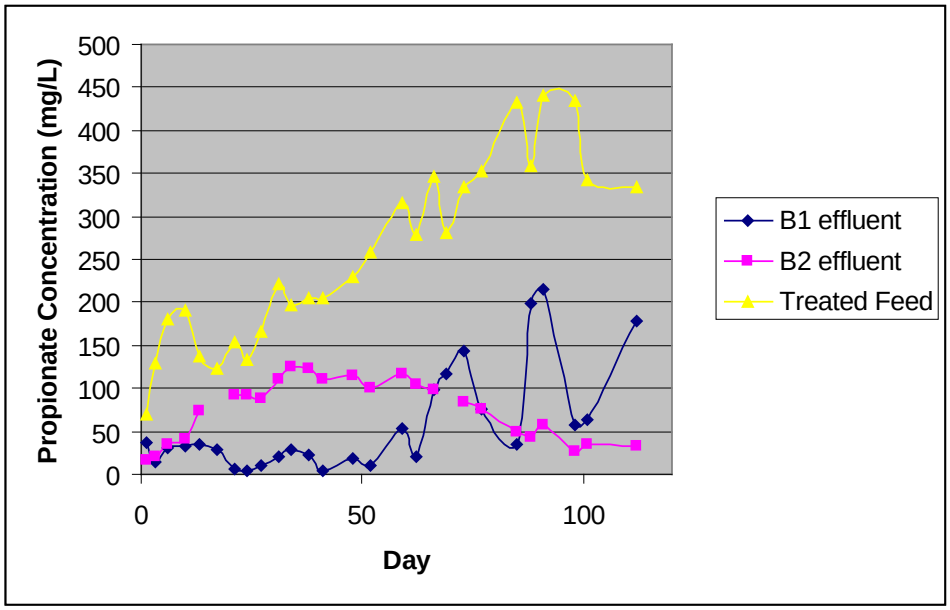

Figure 7-9: Propionate concentrations (mg/L) of methanogenic reactor effluents and treated feed (Batch 5).

Appendix 6: Analysis of Variance (ANOVA) results

Anova: Single Factor Comparison between of LCH_4/g VS between batch 3 and 4

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
1 Day HRT (batch 3)	20	2.077844	0.103892	0.000666
8 Day HRT (batch 4)	20	2.506294	0.125315	0.00027

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.004589	1	0.004589	9.805306	0.003341	4.098169
Within Groups	0.017785	38	0.000468			
Total	0.022375	39				

Anova: Single Factor Comparison between of LCH_4/g VS between batch 2 and 3

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
1 Day HRT (batch 2)	20	1.619047	0.080952	0.000232
1 Day HRT (batch 3)	20	2.077844	0.103892	0.000666

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.005262	1	0.005262	11.71762	0.001497	4.098169
Within Groups	0.017066	38	0.000449			
Total	0.022328	39				

LCH_4/g VS day and 8 day HRT

Anova: Two-Factor With Replication

SUMMARY	1 Day HRT	8 Day HRT	Total
Trial 1	*Batch 2*	*Batch 4*	
Count	16	16	32
Sum	1.295873	1.458855	2.754729
Average	0.080992	0.091178	0.086085
Variance	0.000176	0.000976	0.000585
Trial 2	*Batch 3*	*Batch 5*	
Count	16	16	32
Sum	1.74418	1.791527	3.535707
Average	0.109011	0.11197	0.110491
Variance	0.00068	0.000577	0.00061
Total			
Count	32	32	
Sum	3.040054	3.250382	
Average	0.095002	0.101574	
Variance	0.000617	0.000863	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Batch	0.00953	1	0.00953	15.82002	0.00019	4.001194
HRT	0.000691	1	0.000691	1.147427	0.288378	4.001194
Interaction	0.000209	1	0.000209	0.346828	0.558125	4.001194
Within	0.036144	60	0.000602			
Total	0.046575	63				