Casein whey as booster for anaerobic co-digestion of primary sludge

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We need to leave oil before oil leaves us.

Fatih Birol, Chief Economist

International Energy Agency

Abstract

Spare capacity found in many municipal primary sludge digesters could be used to improve the biogas production through the addition of other organic waste. This work investigates the potential of casein whey as an additional substrate. The amount of whey required for maximum biogas production and stable reactor performance was tested, along with the use of cow manure as an additional substrate to enhance reactor stability.

Bench-scale continuously stirred tank reactors were operated at 38 °C with an initial hydraulic retention time of 20 days. Biogas production was recorded daily and compared to a control reactor. To assess reactor stability, pH, alkalinity, chemical oxygen demand (COD) and volatile fatty acid concentration were measured.

To manage seasonal production, whey (W) was stored at ambient temperature prior to utilisation. This caused 74 % of the lactose to ferment to mainly L-lactate, accompanied by a pH drop from initially 4.5 to 3.6 and decreased COD. While fresh whey co-digested with primary sludge (PS) did not improve the biogas production, stored whey utilised at the ratio 10:3 (PS:W) improved the biogas production to 150 % of the control.

Cow manure (CM) co-digested with primary sludge and fresh whey at the ratio 10:7:1 (PS:W:CM) improved the biogas production by up to 200 % after slow acclimatisation to the whey. The addition of cow manure to primary sludge and stored whey did not improve the biogas production beyond the 150 % achieved without cow manure.

Investigation into why cow manure improved biogas production in primary sludge and whey co-digestion established that fungi found in cow manure could play an important role in the hydrolysis of complex material and therefore the biogas production.

Improved biogas production from fresh whey was only achieved when cow manure was provided. It appeared that additional lactic acid bacteria supplied by cow manure was required to ferment the high lactose concentration in fresh whey.

This work has shown how the seasonal availability of whey can be effectively used to improve the biogas production from municipal sludge digestion. During peak milk production fresh whey could be co-digested with primary sludge and cow manure at the ratio 10:5:1 (PS:W:CM) achieving 178 % biogas production. If cow manure is difficult to obtain, the ratio 10:3:0.1 is recommended, achieving 138 % biogas production. When the availability of fresh whey decreases, stored whey at the ratio 10:3 (PS:W) is recommended without cow manure, producing 150 % biogas compared to primary sludge alone.

Utilising whey as a viable substrate would improve productivity of municipal sludge digesters as well as alleviating environmental issues associated with whey disposal.

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Abbreviations

%	-	Percent
°C	-	degree Celsius
atm	-	Standard atmosphere
ATP	-	Adenosine triphosphate
BOD_5	-	Biochemical oxygen demand
$C_{12}H_{22}O_{11}$	-	Lactose
$C_2H_3O_2^-$	-	Acetate
C_2H_6O	-	Ethanol
$C_3H_3O_3^-$	-	Pyruvate
$C_3H_5O_3$	-	Lactic acid
$C_3H_5O_3^-$	-	Lactate
$C_5H_7O_2N$	-	Bacterial tissue
$C_6H_{12}O_6$	-	Galactose
$C_6H_{12}O_6$	-	Glucose
$CaCO_3$	-	Calcium carbonate
CH_3COOH	-	Acetic acid
CH_4	-	Methane gas
CHO_2^-	-	Formate
CL	-	Compost leachate
CM	-	Cow manure
CO_2	-	Carbon dioxide
$CoCl_2$	-	Cobalt(II) chloride
COD	-	Chemical oxygen demand
COD:N:P	-	Chemical oxygen demand to nitrogen to phosphorus ratio

CSTR	-	Continuously stirred tank reactor
d	-	Days
DNA	-	Deoxyribonucleic acid
EMP pathway	-	Embden-Meyerhof-Parnas pathway
FW	-	Fresh whey
$FeCl_2$	-	Iron(II) chloride
g	-	Gramm
GC	-	Gas chromatograph
H^+	-	Hydron
H_2	-	Hydrogen gas
H_2O	-	Water
H_2S	-	Hydrogen sulphide
H_2SO_4	-	Sulphuric acid
HRT	-	Hydraulic retention time
HS^-	-	Hydrosulphide ion
IC	-	Ion chromatograph
K	-	Potassium
$K_2Cr_2O_7$	-	Potassium dichromate
K_2O	-	Potassium oxide
kg	-	Kilogram
kWh	-	Kilowatt hour
l	-	Litre
LAB	-	Lactic acid bacteria
LDH	-	Lactate dehydrogenase
L_N	-	Norm litre
m	-	Metre

m^3	-	Cubic metre
mg	-	Milligram
MJ	-	Megajoule
ml	-	Millilitre
mm	-	Millimetre
mmol	-	Millimole
mol	-	Mole
N	-	Normality of a solution
N	-	Nitrogen
NAD^+	-	Nicotinamide adenine dinucleotide
NADH	-	Reduced form of nicotinamide adenine dinucleotide
NH_3	-	Ammonia
NH_4^+	-	Ammonium
NH_4^+-N	-	Dissolved ammonium as nitrogen
$NiCl_2$	-	Nickel(II) chloride
NIWA	-	National Institute of Water and Atmospheric Research
nm	-	Nanometre
ODM	-	Organic dry matter
OLR	-	Organic loading rate
P	-	Phosphorus
pH	-	Minus the decimal logarithm of the hydrogen ion activity in a solution
PS	-	Primary sludge
Q	-	Volumetric flow rate (m^3/s)
RNA	-	Ribonucleic acid
RO	-	Reverse osmosis
rpm	-	Revolutions per minute

sCOD	-	Soluble chemical oxygen demand
SiO_2	-	Silicon dioxide
SRT	-	Solid retention time
StW	-	Stored whey
t	-	Tonne
tCOD	-	Total chemical oxygen demand
TKN	-	Total Kjeldahl nitrogen
TS	-	Total solids
UASB	-	Upflow anaerobic sludge blanket
V	-	Volume
VFA	-	Volatile fatty acids
vol	-	Volume
VS	-	Volatile solids
W	-	Whey
ww	-	Wet weight
WWTP	-	Wastewater treatment plant
$ZnCl_2$	-	Zinc chloride

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Chapter 1 Introduction

Background

As the demand for energy keeps increasing and energy resources become scarcer, the use of renewable energy resources is of increasing interest. One of the renewable energies to consider is biogas generated from organic matter, including purpose grown energy crops as well as organic waste and by-products.

Biogas production from energy crops is not necessarily sustainable, profitable or good for the environment (Shilton & Guieysse, 2010). Depending on the crop itself, the location and the agricultural production method, it can be energy- and cost-intensive and is often only profitable when subsidised (Prochnow et al., 2009). Further, it is an ethical dilemma whether to grow crops for energy production or to feed people (Walker, 2009).

On the other hand, large amounts of organic waste, in particular livestock waste, often pose a potential threat to the environment due to pollution of adjacent land and water bodies as well as air pollution through odour and gas emissions (Ahring, 2003). The use of waste and by-products as a fuel source for biogas production may play a particularly important role in the energy generation from non-fossil fuel resources. A potential organic waste source is whey, a by-product of cheese and casein manufacturing. Worldwide about 177 x 10^9 litres of whey are produced each year (3A Business Consulting, 2007). In the season 1996/1997 the whey production in New Zealand was 5 x 10^9 litres per year (Kellam, n.d.). On-site utilisation or the transport of whey to a whey processing facility is often not feasible, in particular for smaller production sites. As a result a significant proportion of it is disposed of without treatment (Gelegenis,

Georgakakis, Angelidaki & Mavris, 2007; Ghaly & Singh, 1985; González Siso, 1996; Leite, Guimarães, de Araújo & Silva, 2000; Zhou & Kosaric, 1995). The risk posed by whey in the environment is huge due to its low acidity and very high organic pollution load (very high BOD_5 and COD) (Ghaly, Mahmoud, Rushton & Arab, 2007), which exceeds the strength of domestic wastewater by about 100 times (Mawson, 2003).

If viable in anaerobic digestion, whey could be turned into a valuable substrate, providing energy and alleviating environmental pollution problems. Though the organic load in whey is easily degradable, anaerobic digestion of whey is difficult due to its extremely low pH and alkalinity (Ghaly & Ramkumar, 1999; Gelegenis et al., 2007; Jihen, Hassib, Mohtar & Said, 2010; Malaspina, Cellamare, Stante & Tilche, 1996; Lo & Liao, 1986; Venetsaneas, Antonopoulou, Stamatelatou, Kornaros & Lyberatos, 2009). To overcome these issues and improve stability, the digestion of cheese whey in CSTR and other reactor types with pH control through chemical dosing of alkalinity (Ghaly, 1996; Ghaly & Ramkumar, 1999; Malaspina et al., 1996; Venetsaneas et al., 2009) and with co-substrates such as dairy (Lo, Liao & Chiu, 1988; Lo & Liao, 1989) or poultry (Gelegenis et al., 2007) manure has been investigated.

This research focuses on the potential biogas production increase from the anaerobic co-digestion of primary sludge with casein whey in a CSTR. Many existing municipal digesters have spare capacity, with the possibility of co-digesting more organic material (Alatriste-Modragón, Samar, Cox, Ahring & Iranpour, 2006). Utilising the acidic casein whey as a co-substrate instead of cheese whey has not been reported on. As whey production is often seasonal, the storage of whey in order to provide a constant supply may be important. The effects of storing whey and utilising it in anaerobic digestion have not been reported on in the literature. Chemical dosing is not applied in this research, as this can be very cost-intensive. Instead, additional co-substrates that are available are trialled to improve process stability and their influence on co-digestion of primary sludge with casein whey is examined. Cow manure is used, as the literature suggests it improves digester stability and provides alkalinity. Compost leachate is tested as an alternative to cow manure, as it has a similar alkalinity.

The following benefits could result if the co-digestion of casein whey in municipal digesters improves the biogas production from primary sludge:

- lower treatment costs for whey,
- reduction of environmental pollution,
- whey could be turned from a by-product into a valuable resource,
- efficiency of municipal digesters could be improved through increased biogas production,
- increased energy generation would result in more profitable primary sludge treatment.

Objectives

The objective of this research is to improve the biogas production from primary sludge, through anaerobic co-digestion with casein whey while operating at a stable pH.

Specific objectives of this research are to investigate:

- Biogas production from co-digestion of primary sludge with casein whey in a CSTR
- The need for acclimatisation of anaerobic bacteria in digesters to casein whey
- The influence of fresh and stored casein whey on the anaerobic co-digestion process
- The effect of the co-substrate cow manure on the co-digestion of primary sludge with casein whey
- The minimum manure requirement to achieve a stable reactor pH when co-digesting fresh and stored casein whey
- The possibility of co-digesting compost leachate as an alternative to cow manure in anaerobic co-digestion of primary sludge with casein whey
- The effects resulting from the combination of casein whey with primary sludge and cow manure or compost leachate

Chapter 2 Literature review

2.1 What is anaerobic digestion?

Anaerobic digestion is the breakdown of biodegradable material, mostly complex organic material, into methane, carbon dioxide and water by microorganisms in the absence of oxygen (Ahring, 2003; Ghaly, 1996; Hansen & Cheong, 2007). The process requires limited input of energy and nutrients (Mawson, 2003).

The anaerobic digestion process is carried out by a consortium of interdependent and symbiotic microorganisms. A wide spectrum of substrates are consumed by the heterotrophic microorganisms and synthesised to new cellular material while various end products are produced (Ghaly, 1996).

The digestion process is divided into four stages. The different groups of microorganisms responsible for the different process steps are named in Figure 2.1.

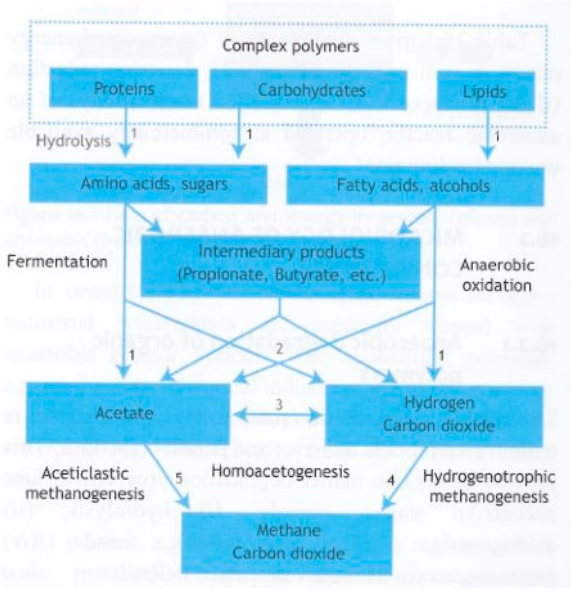

Figure 2.1: Anaerobic digestion of complex polymers. Numbers indicate the groups of microorganisms involved: 1. Hydrolytic and fermentative bacteria, 2. Acetogenic bacteria, 3. Homo-acetogenic bacteria, 4. Hydrogenotrophic methanogens, 5. Acetocalstic methanogens. (van Lier, Mahmoud & Zeeman, 2008)

1. Hydrolysis/Liquefaction

Insoluble complex materials and large organic polymer chains are broken down into soluble materials and monomers (Ghaly, 1996). These are transportable into microbial cells and can be metabolised (Hansen & Cheong, 2007). This process step is catalysed by extracellular enzymes such as cellulase, protease and lipase (Hansen & Cheong, 2007).

2. Acidogenesis

Monomers from the hydrolysis are fermented into a variety of end products including volatile fatty acids (VFA), carbon dioxide (CO_2) and hydrogen (H_2) (Ghaly, 1996). The end products of this step depend on the type of bacteria predominant in the reactor. Examples are:

- Bacteroides
- Bifidobacterium
- *Clostridium* (Hansen & Cheong, 2007).
- Lactobacillus
- Streptococcus

3. Acetogenesis

Fatty acids and alcohol are digested into hydrogen, acetic acid and carbon dioxide by acetogenic bacteria such as *Syntrobacter wolini* and *Syntrophomonas wolfei* (Hansen & Cheong, 2007).

4. Methanogenesis

Methanogenic microorganisms, classified as archae, produce methane gas from acetate, carbon dioxide and hydrogen. Around 70 % or more of the methane produced in this step is directly derived from the conversion of acetate to methane by acetoclastic methanogens.

$$CH_3COOH \rightarrow CH_4 + CO_2$$

The secondary route is the reduction of carbon dioxide to methane gas by hydrogenotrophic methanogens that utilise hydrogen as an electron donor. These methanogens can also use formate as an electron donor to produce methane.

$$4\ H_2 + CO_2 \rightarrow CH_4 + H_2O$$

$$4\ HCO_2H \rightarrow 3\ CO_2 + CH_4 + 2\ H_2O$$

(Ferry, 1997; Hansen & Cheong, 2007; van Lier et al., 2008; Yang, Tang & Okos, 1987)

Low growth rate, high susceptibility to external conditions and limited substrate utilization range are unique physiological characteristics of methanogens (Hori, Haruta, Ueno, Ishii & Igarashi, 2006).

A balance between the fast-growing acidogens and the slow-growing methanogens is necessary to achieve successful anaerobic digestion (Hansen & Cheong, 2007). Due to different growth speeds of microorganisms it is suggested that the methanogenesis is the rate-limiting step in the overall process (Göblös, Portöto, Bordás, Kálmán & Kiss, 2008; Hansen & Cheong, 2007; Lee, Kim, Hwang, O'Flaherty & Hwang, 2009). However, when complex/semi-solid substrates are digested, hydrolysis is considered to be the rate-limiting step. Acidogenesis is generally seen as the most rapid step in the conversion of biomass to biogas (van Lier et al., 2008).

Microbial growth of anaerobic microorganisms and the resulting biogas production occur naturally wherever high concentrations of wet organic matter accumulate without the presence of dissolved oxygen (Hashimoto, Chen & Prior, 1979; Lusk, 1998). It is also employed in man-made digesters to treat organic matter of various origins. "Anaerobic digestion is considered as the most efficient of biological treatment." (Speece, 1996) and is believed to be the most common method for sludge stabilisation

(Baertsch et al., 2007, as cited in Lee et al., 2009). Operational economy and generation of usable gas as well as the reduction of pollutants in the wastewater make anaerobic digestion a particularly interesting treatment solution for high strength wastewater (Dareioti, Dokianakis, Stamatelatou, Zafiri & Kornaros, 2009). A large number of different anaerobic digestion technologies and plants are found throughout the world. The largest number stabilises primary and secondary sludge from wastewater treatment, often with the main focus on the treatment of sludge rather than biogas production. A widely employed application is the treatment of heavily polluted wastewater from the food processing industry (Ahring, 2003). Another approach is to apply anaerobic digestion as a first biological treatment to stabilise agricultural and food processing wastes (manure, whey, etc.) prior to disposal with the benefit of producing energy in form of biogas (Ghaly, 1996).

2.1.1 Biogas

In general, biogas contains between 50 and 75 % methane and 25 to 45 % carbon dioxide, 2 to 7 % water vapour, under 2 % oxygen and nitrogen, under 1 % hydrogen sulphide (Fachagentur Nachwachsender Rohstoffe e.V., 2009). Traces of mercaptans and amines are found also in biogas (Ghaly, 1996). Raw biogas is humid and the moisture content of the biogas increases exponentially with the temperature (Burke, 2001).

The quantity of produced biogas is expressed as conversion/destruction of volatile solids to gas, as COD converted/removed to gas or as converted organic dry matter. The amount of methane gas produced depends on the temperature and pressure. Under standard conditions (0 °C and 1 atm) 0.35 litres methane gas are produced per unit COD converted under anaerobic conditions. At other than standard conditions, the amount of methane produced can be determined using the universal gas law to calculate the volume of gas occupied by one mole of methane gas at the given temperature. For a temperature of 35 °C the amount of methane produced is 0.40 litres per unit COD converted (Tchobanoglous, Burton & Stensel, 2003).

For the biogas/methane yield from crops and manure, the substrate composition, in particular the protein, fat, fibre, cellulose, hemicellulose, starch and sugar content, is

important (Comino, Rosso & Riggio, 2009). The amount of biogas produced is also dependent on the organic compounds present in the feedstock as shown in Table 2.1.

Table 2.1: Specific biogas yield of main compounds

Gas yields			
	l biogas /g $VS_{destroyed}$	l biogas /kg ODM	l methane /g $COD_{removed}$
Carbohydrates	0.83	790	0.35
Lipids	1.45	1250	-
Proteins	0.99	700	-
Reported by	Gelegenis et al. (2007)	Pesta (2007)	Hansen and Cheong (2007)

The typical energy value of biogas is reported to be 21 MJ/m^3. Methane gas has a calorific value of 55 MJ/kg (Hansen & Cheong, 2007; Lusk, 1998).

2.1.2 Important parameters/favourable conditions

As indicated, temperature plays an important role for anaerobic digestion. There are also other parameters and conditions that affect the anaerobic digestion process and are used to describe and evaluate the process and its stability.

2.1.2.1 pH

The pH is a measure for the activity of hydrogen ions in solution defined as -log [H^+]. It indicates at a given temperature the intensity of the acidic or basic character of a solution (Clesceri, Greenberg & Eaton, 1998).

The pH is seen as one of the most important control parameters of anaerobic digestion, as the process is limited to a relatively narrow pH interval. The favourable pH ranges between six and eight with an optimum near neutral pH seven. Several studies report a good digester performance and stability at pH between 6.5 and 7.5 (Hansen & Cheong, 2007).

In general the different steps of the anaerobic digestion process (see Figure 2.1) and their rate depend on pH and temperature as well as composition and concentration of intermediate compounds. However, each process step has its own favourable

conditions and optima. The pH optimum for hydrolysis and acidogenesis lays just below 6.5 while acetogenesis and methanogenesis operate at on optimum pH above 6.5 (Janke, 2002). Acidogenesis will even occur at low pH, which means even when methanogens are not active any more, acidification will continue (van Lier et al., 2008).

2.1.2.2 Temperature

Temperature plays an important role as it influences the rate of bacterial action and the quantity and moisture content of the biogas produced (Burke, 2001).

Anaerobic digestion is divided into psychrophilic, mesophilic and thermophilic temperature ranges. Hansen and Cheong (2007) define the psychrophilic temperature range for temperatures from 15 to 20 °C, the mesophilic range from 30 to 40 °C with an optimum approximately at 35 °C and the thermophilic range from 50 to 65 °C.

To achieve a certain temperature regime, heating of the digester is usually required. To ensure good process operation and to prevent instability, the reactor needs to be continuously held at a certain temperature (Hansen & Cheong, 2007).

2.1.2.3 Alkalinity

The total alkalinity is the sum of all titratable bases measuring the ability of the reactor to neutralise excess organic acids and maintain a constant pH. Measured through titration it varies significantly with the pH endpoint used (Ghaly & Ramkumar, 1999). Alkalinity is comprised of different species of salts of weak acids, therefore it is conventionally expressed in $CaCO_3$ equivalent (Speece, 1996).

Alkalinity can be generated from the metabolism of organic compounds when a cation is released. It is measured as the concentration of organic compounds that release a cation (such as proteins) and the salts of organic acids. The reduction of sulphate/sulphite can also form alkalinity. In an anaerobic digester, the efficiency of sulphate reduction can be between 50 and 98 %, therefore the impact on the alkalinity in the digester can be profound. Further alkalinity can be formed during the degradation of nitrogenous organic compounds, proportional to the quantity of ammonium released. The metabolism of carbohydrates, sugars, organic acids, aldehydes, ketones

and esters does not result in the creation of alkalinity as no cation is released during biodegradation (Speece, 1996).

The main consumer of alkalinity in an anaerobic digester is carbon dioxide produced during the fermentation and methanogenesis (Speece, 1996). Carbonic acid is formed in the digester due to carbon dioxide solubilising, which is caused by partial pressure of the gas in a digester. The carbon dioxide concentration in the biogas produced is therefore a reflection of the alkalinity requirements of the digester (Tchobanoglous et al., 2003).

According to Tchobanoglous et al. (2003), typical alkalinity concentrations from 2,000 to 3,000 mg/l as $CaCO_3$ are required to maintain the pH in the digester at or near neutral. For an operational digester Ghaly and Ramkumar (1999) and Ghaly, Ramkumar, Sadaka and Rochon (2000) recommend a total alkalinity of 2,500 to 5,000 mg/l.

In most cases the alkalinity measured is the total alkalinity, which does not always represent the available buffering capacity of an anaerobic system. The total alkalinity measures all alkalinity regardless of its origin including the bicarbonate alkalinity and approximately 80 % of the VFA (Anderson & Yang, 1992; Speece, 1996). Free VFA require buffering while VFA salts at neutral pH contribute to the total alkalinity (Speece, 1996). However, this VFA alkalinity is not available for neutralisation of additional free volatile acids. The bicarbonate alkalinity represents the total alkalinity minus the alkalinity contributed by VFA. At neutral pH the bicarbonate alkalinity can be seen as the true buffering capacity of an anaerobic system (Anderson & Yang, 1992; Speece, 1996).

2.1.2.4 Nutrient requirements

The influent material for a functional anaerobic digestion process needs to contain carbon, nitrogen, phosphorus, hydrogen, oxygen, water and various other elements, as those are essential for the bacteria.

After carbon, the nutrients demanded in the highest concentration are nitrogen and phosphorus (Hansen & Cheong, 2007; Pesta, 2007). Carbon is used to form cellular

material as well as being converted into biogas. For synthesis of protein, enzymes, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), nitrogen is required. Phosphorus is used to produce energy-storing compounds as well as RNA and DNA. The common empirical formula of the bacterial composition of the feed is $C_5H_7O_2N$ (Parkin & Owen, 1986).

Sulphur precursors in the fed substrate may be required as well (Rajeshwari, Balakrishnan, Kansal, Kusum Lata & Kishore, 2000; Zehnder et al., 1980, as cited in Hansen & Cheong, 2007). The empirical formula of the bacterial composition is considered to be $C_5H_7O_2NP_{0.06}S_{0.1}$ (Speece, 1996). Reported sulphur content for optimal growth and methane production is of approximately 0.001 to 1.0 mg/l (Zehnder et al., 1980, as cited in Hansen & Cheong, 2007). According to Speece (1996), nitrogen, phosphorus and sulphur in concentrations of 50, 10 and 5 mg/l, respectively are desirable to maintain a maximum methanogenic activity.

In addition, some trace elements stimulate the activity and growth of microorganisms and promote the methane production (Hansen & Cheong, 2007; Rajeshwari et al., 2000; Speece, 1996). Trace elements/micronutrients are essential for all microorganisms. The different species of microorganisms involved have a variety of unique environmental requirements and need different trace elements (Wood & Tchobanoglous, 1975). The three most important trace elements/trace metals for methanogens are iron, nickel and cobalt (Rajeshwari et al., 2000; Speece, 1996). Nickel is required for acetogens as well as for methanogens. The latter incorporates it into the factor F_{430}, which contains substantial amounts of nickel. It is unique to methanogens and probably involved in the methane formation process (Diekert, Konheiser, Piechulla, & Thauer, 1981).

Stimulating effects are also reported for

- Zinc,
- Manganese,
- Molybdenum,
- Tungsten.
- Copper,
- Boron,
- Selenium,

Iron, cobalt, nickel and zinc are recommended in the order of 0.02, 0.004, 0.003 and 0.02 mg/g acetate produced, respectively. This results in a recommended amount per litre of reactor volume of 1.0 mg $FeCl_2$, 0.1 mg $CoCl_2$, 0.1 mg $NiCl_2$, and 0.1 mg $ZnCl_2$ (Speece, 1996).

The presence of such nutrients, in particular trace elements, in an anaerobic digester does not assure their bioavailability to the bacteria. Trace elements can occur in complex forms inhibiting the uptake by bacteria (Fytili & Zabaniotou, 2008; Speece, 1996).

A nutrient deficiency can be indicated by a high VFA concentration in the reactor. However, increased VFA concentrations can also indicate a nutrient or other toxicity (Speece, 1996).

2.1.2.5 Chemical oxygen demand

The chemical oxygen demand (COD) is a measure of organic compounds contained in a solution. It measures the amount of oxygen equivalent of organic material that can be oxidised chemically using dichromate in an acid solution (Clesceri et al., 1998).

The COD can be divided into total and soluble COD with the first being higher than the second. The organic matter that is contributing to the total COD is first converted into volatile acids that are the main contributor to the soluble COD (Ghaly & Ramkumar, 1999). These are converted into biogas in the following steps. The reduction in soluble COD is higher than in total COD due to the conversion of soluble material into non-soluble microbial cells (Ghaly, 1996). The more organic material removed, the higher the expected methane/biogas production. Per unit of COD removed, 0.35 litres of methane is produced anaerobically under standard conditions (Tchobanoglous et al., 2003) (see page 8).

The COD can be used to measure the strength of wastewater and to quantify the success of the anaerobic digestion process (Ghaly & Ramkumar, 1999). A low COD in the process effluent indicates degradation of the influents organic matter and therefore good process performance (Göblös et al., 2008).

For anaerobic digestion, the substrate needs influent COD concentrations greater than 1,500 to 2,000 mg/l, otherwise aerobic treatment might be more preferable (Tchobanoglous et al., 2003). For a highly loaded anaerobic process a theoretical minimum COD:N:P ratio of 350:7:1 and for a lightly loaded system a ratio of 1,000:7:1 is required (Hansen & Cheong, 2007).

2.1.2.6 Retention time

Hydraulic retention time

The hydraulic retention time (HRT) is representing the rate of the liquid flow into the reactor and out of the reactor. It is calculated by the total volume of the digester divided by the daily flow and usually expressed in days (see Equation 2.1) (Hansen & Cheong, 2007).

Equation 2.1: Hydraulic retention time (Burke, 2001; Hansen & Cheong, 2007)

$$HRT = \frac{V}{Q}$$

Where $V = Volume\ in\ l$

$Q = Daily\ flow\ in\ l/d$

The HRT required for an operational anaerobic digestion process is depending on the type of reactor employed as well as on the type of substrate digested.

Solid retention time

The solid retention time (SRT) is important in maintaining digester stability. It is representing the average time that a solid particle remains in the reactor and therefore the time available for the bacterial growth in the reactor. The SRT equals the mass of total biomass in the reactor divided by the biomass wasted from the reactor every day (see Equation 2.2) (Hansen & Cheong, 2007).

Equation 2.2: Solid retention time (Burke, 2001)

$$SRT = \frac{V \times C_d}{Q_w \times C_w}$$

Where $V = Volume\ in\ l$

$Q_w = Volume\ wasted\ each\ day\ in\ l$

$C_d = Solid\ concentration\ in\ digester$

$C_w = Solid\ concentration\ in\ effluent$

In a successfully operated digester, the minimum SRT must allow the working microorganisms to regenerate themselves, which is mostly influenced by the

temperature (Hansen & Cheong, 2007). Hansen and Cheong (2007) suggest a minimum SRT for methane forming microorganisms of three to five days at 35 °C.

2.1.2.7 Loading rate

The amount of organic matter that must be handled by the system is characterised by the loading rate. It represents the rate at which substrate is added to the digester and measured in organic influent (g or kg) to the system per unit volume (l or m^3) per time (usually days) (see Equation 2.3). It is also an index of stress imposed on the microbial population in the reactor (Hansen & Cheong, 2007).

Equation 2.3: Loading rate (Burke, 2001)

$$L = \left(\frac{1}{HRT}\right) \times C_i = \left(\frac{Q}{V}\right) \times C_i$$

Where HRT = *Hydraulic rentention time in d*

C_i = *Initial feedstock concentration* (*COD*)

Q = *Daily flow in l / d*

V = *Volume in l*

2.1.2.8 Mixing

Mixing distributes organisms and contents uniformly throughout the digester and transfers heat (Cantrell, Ducey, Ro & Hunt, 2008) allowing a homogenous mixture of bacteria and feedstock (Karim, Hoffmann, Klasson & Al-Dahhan, 2005).

Mixing can be necessary to avoid dead zones, thermal stratification and grit, scum and solid accumulation. When feeding thick influent material such as manure, it is especially important. Consequently, the intensity as well as the duration of mixing are important (Karim et al., 2005). A lack of sufficient mixing can result in a floating layer of solids accumulating at the top even in low solids reactors (Karim et al., 2005). Poor mixing and accumulation of material anywhere in the reactor can reduce the reactor volume and subsequently may also lead to a decrease in HRT.

2.1.3 Inhibition

Inhibition of the anaerobic digestion process or of one or more of its stages can occur due to components and toxic substances contained in the feed substrate as well as process intermediates and end products.

Inhibition is usually indicated by decreased pH and lower biogas production as well as reduced methane content in the gas and low COD removal (Ghaly & Ramkumar, 1999; Hansen & Cheong, 2007). Inhibition of the methanogenesis in particular is usually indicated by reduced biogas production and high concentration of VFA (Hansen & Cheong, 2007).

A low pH inhibits the activity of the process and especially of the methanogens (Ghaly & Ramkumar, 1999). If the pH drops to six or lower and remains there, the digester may fail (Hansen & Cheong, 2007).

2.1.3.1 Nitrogen and sulphur compounds

Feed material with high concentration of nitrogenous and sulphuric compounds can result in the production of end products such as ammonia (NH_3), ammonium (NH_4), ammonium-nitrogen (NH_4^+-N), and hydrogen sulphide (H_2S) as well as sulphuric acid (H_2SO_4). High concentrations can be toxic and can inhibit the anaerobic digestion process (Burke, 2001; Hansen & Cheong, 2007).

Ammonia is formed by the mineralisation of organic nitrogen in substrates rich in protein or urea and free ammonia is more toxic than ammonium. Ammonia in the digester has not only a potential inhibitory effect but may lead to a build-up of ammonium-nitrogen, which is dissolved ammonium measured as nitrogen (Hansen & Cheong, 2007). The generated ammonium bicarbonate will keep the pH at neutral or above seven (Speece, 1996; Hansen & Cheong, 2007), while an undetected accumulation of VFA may occur causing increasing reactor instability. Ammonium-nitrogen is inhibitory at levels of 1,500 to 3,000 mg/l (Hansen & Cheong, 2007).

Sulphide toxicity can occur when the influent material contains high concentrations of sulphate. The presence of sulphate and subsequent formation of sulphide can lead to

precipitation of some essential trace elements. This reduces their availability for microorganisms and will affect their growth (Isa, Grusenmeyer & Verstraete, 1986; Zehnder, Ingvorsen & Marti, 1982). Concentrations of soluble sulphide between 50 and 100 mg/l may be tolerated by the system with little or no acclimation. Concentrations above 200 mg/l are toxic (McCarty, 1964, as cited in Hansen & Cheong, 2007). At a pH near neutral during anaerobic treatment, most soluble sulphide exists in form of hydrogen sulphide or HS^- (Rittmann & McCarty, 2001, as cited in Hansen & Cheong, 2007). Acetogenic bacteria are less sensitive to hydrogen sulphide than methanogenic bacteria (Hansen & Cheong, 2007).

2.1.3.2 Volatile fatty acids

Generally the concentration of volatile fatty acids (VFA) in an anaerobic digester can be used as an indication for digester stability but these process intermediates can also be responsible for inhibition or even digester failure (Hansen & Cheong, 2007). A low total concentration of VFA indicates a stable digester operation (Ghaly & Ramkumar, 1999). An increased level of VFA is generally an indicator for an imbalance in the process and may even indicate impending digester failure (Burke, 2001; Ghaly & Ramkumar, 1999; Göblös et al., 2008; Hansen & Cheong, 2007). Ghaly and Ramkumar (1999) suggest a healthy and well-balanced digester has a total VFA concentration under 1,000 mg/l. The concentration of VFA in the reactor is influenced by the temperature, the type of material digested and the organic loading rate. The higher the temperature and/or the longer the retention time, the lower the resulting concentration of VFA (Ghaly, 1996).

Under stable and well-balanced conditions the acidification of the digester, which is caused by the organic acid production, is buffered by the bicarbonate production of the methanogens. Disadvantageous circumstances for methanogenic bacteria can be caused by high organic loading rates. Particularly when easily degradable substrates such as sugars and simple organic monomers are digested, the rapid conversion into VFA can cause an imbalance between acidogens and methanogens. This may result in the accumulation of organic acids requiring reserve alkalinity to buffer increased acidity (Speece, 1996). The level of VFA in the reactor can have a major influence on the pH and an accumulation of VFA is generally accompanied by a rapid decrease in pH as illustrated in Figure 2.2. This can result in an inhibition of the biogas production and

may cause cessation of methane production and digester failure (Burke, 2001; Ghaly & Ramkumar, 1999; Göblös et al., 2008; Hansen & Cheong, 2007).

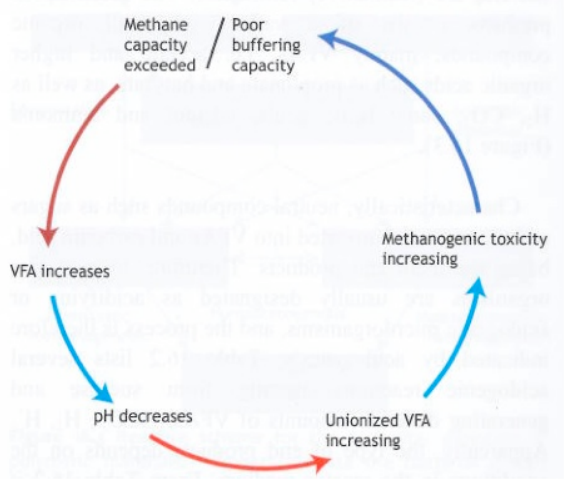

Figure 2.2: Reactor pH drop as a result of accumulating VFA (van Lier et al., 2008)

2.1.3.3 Other toxicity

Toxic materials such as fungicides and antibacterial agents (Burke, 2001) as well as heavy metals and salts can have an adverse effect on the process (Hashimoto et al., 1979). Small amounts of toxic materials can be handled by the system and may even stimulate the process, but excess amounts can have adverse effects on anaerobic digestion (Burke, 2001; Hansen & Cheong, 2007). It has also been reported that a cation toxicity can occur (Hansen & Cheong, 2007).

2.1.4 Reactor design

2.1.4.1 CSTR

There are different types of reactors used for anaerobic digestion. One of the most common configurations is the completely stirred tank reactor (CSTR).

Liquid or slurry is continuously introduced to the reactor and liquid content is continuously removed. All solids are in suspension and exit with the effluent in a well-mixed reactor. The biomass growing in the reactor continuously replaces the biomass removed with the effluent. The substrate and biomass concentration leaving the reactor is the same as in the reactor. In a completely mixed system like a CSTR all content of

the system has the same retention time and the solids' retention time equals the hydraulic retention time (Hansen & Cheong, 2007).

The typical loading rate of a CSTR is between 0.5 and 2.5 kg COD/m^3/day according to Hansen and Cheong (2007) and between 1 and 5 kg COD/m^3/day according to Tchobanoglous et al. (2003). A hydraulic retention time of minimum ten days must be provided depending on the digested waste while 10 to 30 days is more reasonable (Hansen & Cheong, 2007). To provide sufficient process stability and operation safety, Parkin and Owen (1986) advise a hydraulic retention time of 15 to 30 days.

CSTR are commonly used for high concentration organic mixtures, wastewater, biological sludge and high strength industrial waste (Hansen & Cheong, 2007). The mesophilic temperature regime is more reliable in a CSTR than the thermophilic process. As all materials in a completely mixed reactor are kept in suspension, successful operation with a minor concentration of foreign material is possible (Burke, 2001).

The complete suspension of substrate and biomass in the reactors can cause a loss of microbial cells with the effluent when the HRT is too low (Ghaly, 1996). Loss of degradable material in a CSTR can occur, with the effluent leading to a low methane recovery (Kaparaju, Ellegaard & Angelidaki, 2009).

2.2 Substrates

Almost all kinds of organic material can be converted into biogas (Holm-Nielsen, Al Seadi & Oleskowicz-Popiel, 2009). Organic waste contains many different populations of anaerobic or facultatively anaerobic microorganisms (Ahring, 2003). Even if only a single substrate is provided, a variety of microorganisms coexist in the anaerobic digester (Lee et al., 2009).

Various studies show that the sensitivity and performance of anaerobic digestion may be improved by combining several waste streams. The co-digestion of different organic by- or waste products may overcome problems like nutrient imbalance, rapid acidification, the presence of inhibitory compounds and other factors. Also, as well as

the process profitability, the biogas/methane yield may improve with co-digestion (Alvarez & Liden, 2009; Dareioti et al., 2009).

In any system, the composition and origin of substrates and co-substrates must be known in order to evaluate the suitability for anaerobic digestion. The acidity (pH and alkalinity), the COD and the BOD_5 is also important. The biodegradability of the input materials affects the biogas/methane yield (Hashimoto et al., 1979). Lignin is considered a key influencing factor on the digestibility of organic substrates (Amon, Hackl, Jeremic & Amon, 2002; Scherer, 2002; Wellinger, Edelmann, Favre, Seiler & Woschitz, 1984) as it is poorly degraded in anaerobic digestion. For an effective digestion, the presence of cellulase, as a catalyst for the hydrolysis, is required (Lehtomäki, Huttunen & Rintala, 2007; Wen, Liao & Chen, 2005a).

The following sections describe four possible feed materials for anaerobic digestion:

- Primary sludge,
- Whey,
- Cow manure and
- Compost leachate.

As no other substrates were used, the above are the only ones described and characterised.

2.2.1 Primary sludge

The wastewater treatment process and the production of primary, secondary and tertiary sludge is illustrated in Figure 2.3.

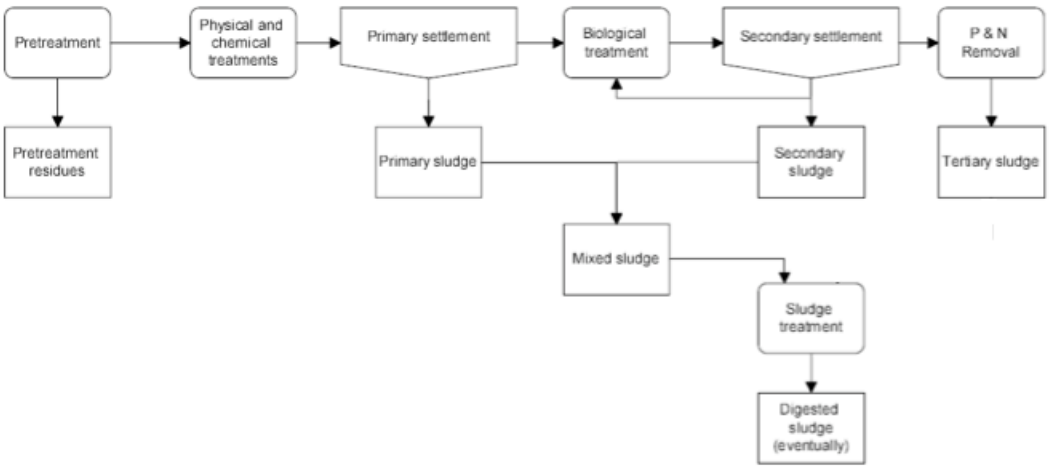

Figure 2.3: Wastewater treatment and sludge treatment (Arthur Andersen LLP & Sede, 2001)

Primary sludge is generated during mechanical/physical wastewater treatment when organic solids are removed from the wastewater through sedimentation and floatation. Suspended organic solids settle due to gravity while grease, fat and oil float due to their density and weight. It contains mainly water, organic matter, un-degradable particles and living organisms (Bouskova, Dohanyos, Schmidt & Angelidaki, 2005) as well as complex and diverse carbon sources (Lee et al., 2009). According to Tchobanoglous et al. (2003) primary sludge contains on average six percent solids by weight, which can vary due to different treatment methods. The sources of solids/particles removed differ according to the type of treatment plant and the operational method (Fytili & Zabaniotou, 2008). Primary sludge is considered high in organic matter, 70 % of primary the solid content are organic while 30 % are inorganic (Mudrack & Kunst, 1988, as cited in Werther & Ogada, 1999). The characteristics of sludge also vary in different countries and areas due to water consumption and local industry (Luostarinen, Luste & Sillanpää, 2009).

Primary sludge is reported to provide a number of trace elements in varying concentrations (Eriksson, 2001). The complete report on 61 trace elements in sewage sludge by Eriksson (2001) is provided in Appendix A. Some of them are listed with other characteristics of sewage sludge and primary sludge reported by different authors in Table 2.2.

Table 2.2: Characteristics of sewage/primary sludge

		Primary sludge		Sewage sludge	Primary sludge
TS	% w/w	6	-	-	-
	g/l	-	-	-	12
VS	% TS	65	-	-	65
	g/l	-	-	-	7.8
Protein	% TS	25	-	-	24
	g/kg	15	-	-	-
	g/l	-	-	-	2.88
Carbon	% VS	-	-	-	51.5
	g/l	-	-	-	4.017
Hydrogen	% VS	-	-	-	7
	g/l	-	-	-	0.546
Oxygen	% VS	-	-	-	35.5
	g/l	-	-	-	2.769
Nitrogen	% TS	2.5	-	-	-
	% VS	-	-	-	4.5
	g/l	-	-	-	0.351
	g/kg	1.5	-	-	-
Phosphorus	% TS	1.6	P_2O_5	-	2
	g/l	-	-	-	0.24
	g/kg	0.96	-	27	-
Sulphur	% VS	-	-	-	1.5
	g/l	-	-	-	0.117
	g/kg	-	-	9	-
Potassium	% TS	0.4	K_2O		0.3
	g/l	-	-	-	0.036
	g/kg	0.24	-	4.4	-
Magnesium	% TS	-	-	-	0.6
	g/l	-	-	-	0.072
	g/kg	-		3.4	-
Sodium	g/kg	-		3.5	-
Calcium	% TS	-	-	-	10
	g/l	-	-	-	1.2
Iron	% TS	-	-	-	2
	g/l	-	-	-	0.24
	g/kg	-	-	49	-
Aluminium	% TS	-	-	-	0.2
	g/l	-	-	-	0.024
	g/kg	-	-	40	-
Cadmium	g/kg	-	-	0.0014	-
Silicon	% TS	15-20	SiO_2	-	-
	g/kg	10.2	-	-	-

Chloride	% TS	-	-	-	0.8
	g/l	-	-	-	0.096
Reported by		Tchobanoglous et al. (2003)		Eriksson (2001)	Arthur Andersen LLP and Sede (2001)

2.2.1.1 Anaerobic digestion of primary sludge

In order to reduce the biological activity of primary sludge before disposal, it has to be treated and anaerobic digestion is the favoured method. It offers a valuable option to stabilise the sludge, reduce its volume, decrease organic content, lower the number of harmful microorganisms and to reduce air pollution through odour (van Lier et al., 2008).

About half of the organic material in primary sludge can be converted into biogas (Ahring, 2003) with a biogas/methane yield as reported in Table 2.3.

Table 2.3: Biogas/methane yield from primary sludge

Biogas yield	Type of digester	Reported by
0.75-1.12 m^3/kg $VS_{destroyed}$	Not stated	Tchobanoglous et al. (2003)
1-2 m^3/ m^3 reactor vol/day	Not stated	Ahring (2003)

The production of about 13.5 MJ of energy per kg COD removed is possible, yielding 1.5 kWh electricity (assuming 40 % electric conversion efficiency) (van Lier et al., 2008).

2.2.2 Whey

Whey is the by-product and most important waste stream of cheese or casein manufacturing. It is the liquid residue obtained from whole, cream or skim milk after separation of the coagulum. There are basically two types of whey, cheese and casein whey. Cheese whey can be separated into two types depending on the manufacturing process, sweet whey from coagulation with rennet and sour (acidic) whey from coagulation with acid (Bylund, 1995; Frigon, Breton, Bruneau, Moletta & Guiot, 2009).

Commercial casein whey originates from the separation of curds and liquid from pasteurised skim milk after coagulation by rennet (rennet casein whey) or precipitation

by acid (acid casein whey). It is drained in a decanter from the raw material milk after a series of heating and cooling processes (Bylund, 1995; Southward, n.d.). There are basically three types of acid casein named after the acid used for manufacturing, therefore there are also three types of acidic casein whey. New Zealand primarily produces lactic acid casein whey, although production of sulphuric acid casein whey has increased in recent years. Hydrochloric acid casein whey is uncommon in New Zealand as the small chemical industry makes it cost intensive It is most commonly produced in Australia and Europe. All rennet casein whey is produced through coagulation using rennet of different origins (Southward, n.d.).

Whey represents about 85 to 95 % of the milk volume and also retains about 55 % of the milk nutrients (Göblös et al., 2008; González Siso, 1996). While during cheese production six to nine litres of cheese whey per kg cheese are obtained (Ghaly et al., 2000; Mawson, 2003; Rodgers et al., 2004), the manufacturing of a kg of casein results in the production of about 25 to 30 kg of casein whey (Mawson, 2003). However, the amount of both can vary at individual manufacturing sites and in different countries. In Australia and New Zealand, about 7.6 kg of whey are produced per kg cheese and about 25 kg whey per kg casein (Sienkiewicz & Riedel, 1990). In countries with a pasture based dairy industry, the production of whey is seasonal due to the seasonal production of milk (Mawson, 2003).

The current world's whey (cheese and casein) production accounts for 177 million tons per year (3A Business Consulting, 2007) (see Figure 2.4) .

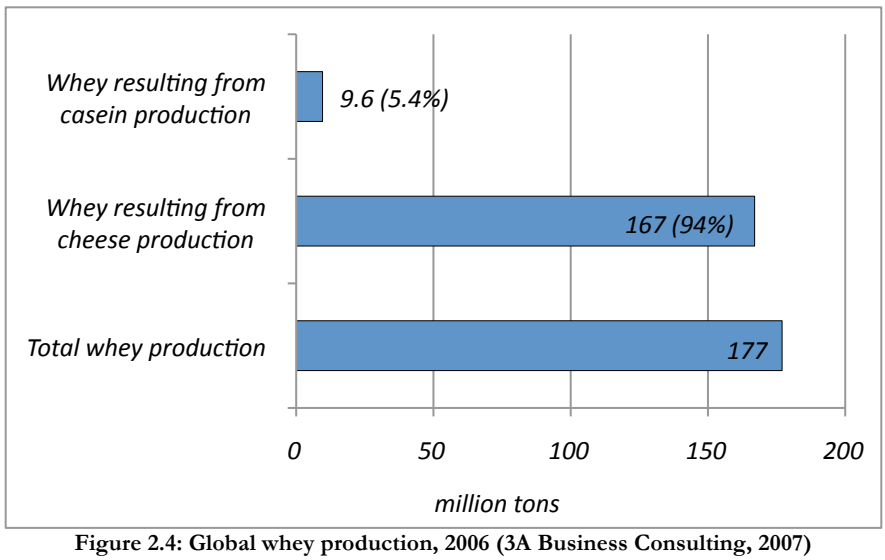

Figure 2.4: Global whey production, 2006 (3A Business Consulting, 2007)

In New Zealand, the annual production of whey amounts to about four billion litres. In the 1996/1997 season estimated around five billion litres of whey were produced (Kellam, n.d.).

Due to increasing production and consumption, especially of cheese, over the years, the amount of whey assembled has risen (Ghaly & Ramkumar, 1999). The growth of globally produced whey is expected to be two percent per year (3A Business Consulting, 2007). Yet only half of the world's produced whey is utilised or processed; the remaining half is not treated but discarded (Gelegenis et al., 2007; Ghaly & Singh, 1985; González Siso, 1996; Leite et al., 2000; Zhou & Kosaric, 1995). Ghaly et al. (2007) found that the production of whey increased between 1979 and 2007 by 165 % while the utilisation and disposal rate essentially remained the same. Due to high organic load and the high pollution potential of whey and the acidity, it poses a serious threat to the receiving environment (Ghaly et al., 2007).

2.2.2.1 Characteristics of whey

The main difference between the different types of whey is the varying pH, shown in Table 2.4.

Table 2.4: pH of different types of whey

Cheese whey		Casein whey		Reported by
Sweet	**Acid**	**Rennet**	**Acid**	
5.9-6.6			4.3-4.6	Bylund (1995)
5.6	5.1 or below			Zall (1992)
	Under 5.1			Mawson (1994)
6.1	4.6		4.4	Pesta, Meyer-Pittroff and Russ (2007)
6.1	4.7		4.5-4.7	Sienkiewicz and Riedel (1990)
		6.5-6.7	4.3-4.6	Southward (n.d.)
		6.5	4.5	Jelen (2002)

After water (93 to 94 % of whey), the main component of whey is lactose, accounting for about 70 to 72 % of whey solids (Ghaly & Ramkumar, 1999; Ghaly et al., 2000; Jelen, 2002). The precise composition depends on the method of manufacturing of the cheese and casein products as well as on the location (Mawson, 1994). It also depends on the milk used due to varying characteristics of mammalian milk as well as seasonal

differences in the composition (Zall, 1992). The typical composition of different whey produced in New Zealand is listed in Table 2.5 below.

Table 2.5: Compostition of New Zealand whey (Matthews, 1978 and Clark, 1988 as cited in Mawson, 1994)

		Ceddar cheese whey	Casein whey		
			Lactic acid	Sulphuric acid	Rennet
Total solids	g/kg	56	56-64	63	58-65
True protein	g/kg	5.5	5.6	5.6	6.2-7.3
Lactose	g/kg	40	38-44	47	45-52
Ash	g/kg	5.0	6.6-7.6	8.0	4.2-4.9
Lactate	g/kg	0.8	6.3-7.3	n.a.	0.2

Whey in general is relatively rich in calcium, phosphorus, sodium, potassium and chlorine. The concentration of the last three elements is constant in whey irrespectively of its origin. The concentration of calcium and phosphorus is greater in acidic than in sweet whey. Acidic whey is also richer in lactic acid than sweet whey. Acidic whey has a higher ash content but lower protein content than sweet whey (Bylund, 1995; González Siso, 1996; Mawson, 1994; Thivend, 1978; Zall, 1992). As a result of using sulphuric acid as a coagulant, the sulphate levels in sulphuric acid casein whey can be more than twice as high as in milk.

Acetate is the predominant volatile fatty acid in whey (Ghaly & Ramkumar, 1999). The suspended solids of whey mainly contain organic compounds, mostly nitrogenous compounds (Ghaly & Ramkumar, 1999) and account for 1.2 g/l (Rodgers et al., 2004). Nitrogen in whey is of soluble nature, subject to biological transformation and accounts for 1.5 % (Ghaly et al., 2007). Thivend (1978) reports 0.5-0.8 % of dry matter to be non-protein nitrogen and 12-13 % of dry matter to be protein, which consists of mainly nitrogen.

The characteristics of acid and rennet casein whey differ noticeably. After pH the main difference between rennet and acid casein whey is the mineral content and the composition of the whey proteins (Jelen, 2002). Table 2.6 lists some differences between rennet and acid casein whey.

Table 2.6: Characteristics of rennet and acid casein whey (Jelen, 2002)

		Casein whey	
		Rennet	**Acid**
TS	g/l	63-70	63-70
Lactose	g/l	46-52	44-46
Protein	g/l	6-10	6-8
Calcium	g/l	0.4-0.6	1.2-1.6
Phosphate	g/l	1-3	2-4.5
Lactate	g/l	2	6.4
Chloride	g/l	1.1	1.1

The different acid casein whey compared are very similar (see Table 2.5) and for most applications interchangeable, as are the rennet casein whey (Southward, n.d.).

All whey has a very high BOD_5 and COD in common with roughly 100 times the strength of domestic wastewater (Mawson, 2003). Consequently, the wastewater from cheese and casein manufacturing has a high pollutant potential with high loading from mainly carbohydrates (Venetsaneas et al., 2009). Table 2.7 shows BOD_5 and COD reported in the literature.

Table 2.7: BOD_5 and COD of whey as reported

BOD_5	**COD**	**Reported by**
30,000-50,000 mg/l	60,000-100,000 mg/l	Gelegenis et al. (2007)
50,000-70,000 mg/l	60,000-80,000 mg/l	Göblös et al. (2008)
30,000-50,000 mg/l	60,000-80,000 mg/l	González Siso (1996)
30,000-40,000 mg/l	-	Thivend (1978)

High biodegradability of up to 99 % and high organic load are key characteristics of whey as well as the extremely low alkalinity of roughly exceeding 50 meq/l (Gelegenis et al., 2007; Malaspina et al., 1996; Rodgers et al., 2004). As a result, whey has little or no buffering capacity. These characteristics make whey difficult to treat. In the wastewater treatment process, the high organic load of whey can disrupt the biological process. Consequently, application onto land is often used for final disposal (Ghaly et al., 2007).

2.2.2.2 Lactose: The main component in whey

Lactose is a disaccharide with the formula $C_{12}H_{22}O_{11}$. Large amounts of lactose are only found in milk. Depending on the type of mammal, lactose comprises between 2 and 7.3

% (by weight). It represents the major carbohydrate in milk, synthesised in the mammary glands from the monosaccharides galactose and glucose. Small amounts of lactose are found in the blood and urine of mammals as well as in complex forms found in some plants (Harper, 1992).

Lactose fermentation

The lactose fermentation process consists of three basic steps plus the conversion of the fermentation products to methane. The entire fermentation process of lactose into methane is described in Figure 2.5.

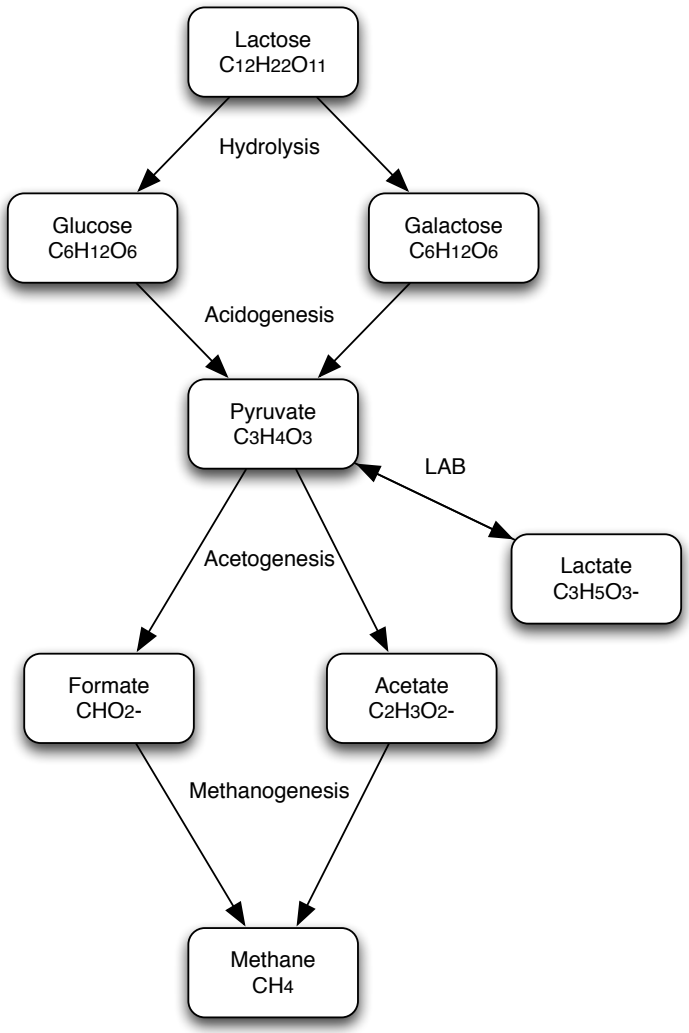

Figure 2.5: Lactose fermentation to methane in presence of lactic acid bacteria

Once lactose is transported into the cells by the enzyme lactose permease, the sugar is hydrolysed into its two sub-units, glucose and galactose (Madigan & Martinko, 2006; Walstra, Wouters & Geurts, 2006).

$$\text{Lactose } C_{12}H_{22}O_{11} \rightarrow \text{Glucose } C_6H_{12}O_6 + \text{Galactose } C_6H_{12}O_6$$

The hydrolysis is performed by the enzyme lactase (β-galactosidase), which breaks down the chemical bond between galactose and glucose freeing both for adsorption (Miller, Jarvis & McBean, 2007). Lactase is contained in lactic acid bacteria (LAB) (Bylund, 1995) as well as found in animals, plants, bacteria, fungi and yeast (González Siso, 1996; Seyis & Aksoz, 2004). As the optimum pH of micro fungal lactase is acidic, its utilisation is restricted to acidic solutions such as whey. The enzyme can also be inhibited by lactose (González Siso, 1996).

Glucose (and galactose) is then fermented into pyruvate gaining two ATP in the glycolysis (EMP pathway) (Madigan & Martinko, 2006; Walstra et al., 2006).

Glucose $C_6H_{12}O_6$ → 2 Pyruvate $C_3H_3O_3^-$

Pyruvate is further digested into different products depending on the environmental conditions and the enzymes catalysing the fermentation (Madigan & Martinko, 2006).

2 Pyruvate $C_3H_3O_3^-$ → 2 Ethanol C_2H_6O + 2 CO_2 (in presence of yeast)

2 Pyruvate $C_3H_3O_3^-$ ↔ 2 Lactate $C_3H_5O_3^-$ + 2 H^+ (in presence of LAB)

2 Pyruvate $C_3H_3O_3^-$ → 2 Acetate $C_2H_3O_2^-$ + 2 Formate CHO_2^-

(Madigan & Martinko, 2006)

In a further reaction some formate breaks down into CO_2 and hydrogen (Madigan & Martinko, 2006).The fermentation products acetate, formate, CO_2 and hydrogen can be digested into methane. Two thirds of methane are produced from the methyl-group of acetate and about one third from the reduction of CO_2 with electrons derived from hydrogen or formate (Ferry, 1997).

Acetate $C_2H_3O_2^-$ + H^+ → Methane CH_4 + CO_2

CO_2 + 4 H_2 → Methane CH_4 + 2 H_2O

4 Formate CHO_2^- → 3 CO_2 + CH_4 + 2 H_2O

(Ferry, 1997)

Lactate can be converted into acetate and then into methane after being converted back to pyruvate. The enzyme lactate dehydrogenase (LDH) catalyses the conversion from pyruvate to lactate as well as lactate to pyruvate as shown in Figure 2.6. NAD^+, a

coenzyme essential for the transport of electrons and hydrogen, is regenerated from its reduced form NADH when pyruvate is reduced to lactate (Walstra et al., 2006).

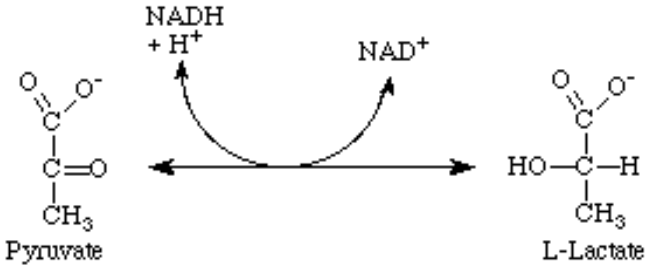

Figure 2.6: Pyruvate to lactate conversion (Paselk, 2008)

Yang and Guo (1990) found that one gram of lactose results in the production of about 300 ml of methane gas. According to Ghaly and Ramkumar (1999) the consumption of one gram lactose results in the production of 0.849 litres of gas. Ghaly et al. (2000) calculated based on Buswell's formula that one gram lactose produces 0.786 litres of gas from:

Equation 2.4: Buswell's formula

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2$$

Lactose $C_{12}H_{22}O_{11} + H_2O \rightarrow 6\ CH_4 + 6\ CO_2$

1 mole = 22.2 litres at 25 °C mol weight of lactose = 342 g

1 mole of lactose → 268.8 l of gaseous products (CH_4+CO_2)

→ 0.786 l of gas from 1 g lactose (Ghaly et al. 2000)

Lactic acid bacteria

Lactic acid bacteria (LAB) are widely distributed in natural environments and exist in whey of all types as well as in

- Plant surfaces,
- Decaying material from plants,
- Food products such as milk, meat, fish, soy sauce,
- Gastrointestinal tracts of humans and animals
- Manure and sewage.

The variety of habitats LAB are isolated from suggests that they are highly competitive bacteria (Vandevoorde, Vande Woestyne, Bruyneel, Christiaens & Verstraete, 1992). This diverse group consists of twelve genera of bacteria; the most important in fermentation processes are *lactococcus*, *leuconostoc*, *streptococcus* and *lactobacilli*. All LAB are:

- Rods or cocci,
- Nonmotile,
- Anaerobic but aerotolerant and
- Gram-positive,
- Non-sporeforming,
- Catalase negative bacteria.

(Walstra et al., 2006; Wood, 1992).

All groups of LAB require organic carbon as source for growth and energy. They obligatorily ferment sugars to acids, especially lactic acid, as end products (Walstra et al., 2006). Thus they are restricted to habitats containing sugar (Madigan & Martinko, 2006).

For their growth LAB demand a high nutritional composition of their growth media. The presence of a number of

- Amino acids,
- Vitamins,
- Minerals,
- Peptides,
- Nucleic acids and derivates

as well as a proteolytic enzyme system and sufficient amounts of immediately available nitrogenous compounds are required (Vandevoorde et al., 1992; Walstra et al., 2006). LAB can adapt to the protein casein as source of nitrogen, which degrades into peptides fulfilling their amino acid requirement (Walstra et al., 2006).

Lactic acid/Lactate

Lactic acid ($C_3H_6O_3$) is a hydroxy carboxylic acid with the systematic name 2-hydroxypropanoic acid. Lactate ($C_3H_5O_3^-$) is the salt or ester of lactic acid (Madigan & Martinko, 2006). Lactic acid/lactate is the major or sole product of sugars fermented by LAB (Earnshaw, 1992; Walstra et al., 2006). It is chiral with two optical isomers; L-(+)-lactic acid and D-(-)-lactic acid as well as a racemic mixture DL-lactic acid. L-lactic acid is produced in mammals and their metabolic system while both isomers are found in

bacterial systems (Södergård & Stolt, 2002). Most LAB produce only one of the isomers of lactic acid depending on the isomeric form of the enzyme lactate dehydrogenase (LDH) present that catalyses pyruvate reduction (Hofvendahl & Hahn-Hägerdal, 2000; Södergård & Stolt, 2002). In acid whey the relative proportion of D-lactic acid of the total lactic acid concentration accounts for about 15 % (Wielonek, 1988 as cited in Renner, 1992).

The rate of lactic acid production varies greatly with growth conditions for the LAB, such as composition and pre-treatment of the solution, temperature and oxygen pressure. The lower the pH, the higher the concentration of undissociated lactic acid present at a given total concentration (Walstra et al., 2006).

The methane yield for lactate is 1.45 mole of methane gas per mole of lactate converted (360 ml CH_4/g lactic acid) (Yang & Tang, 1991). According to Yang and Guo (1990) the methane yield is around 300 ml of methane per g of lactic acid, which is similar to that from lactose (see page 30).

When pyruvate is reduced to lactate, the pH in the medium drops (Bylund, 1995; Davies, Grego & Kenworthy, 1974; Earnshaw, 1992; Fernandes et al., 1992; Madigan & Martinko, 2006; Martin, 1996). Continued production and accumulation of lactate occurs when the pH is below 6 (Yang et al., 1987) and leads to a pH reduction to as low as pH 4.0 to 4.5. This activity is used by the food industry to preserve milk and other dairy products by inhibiting the growth of spoilage microorganisms (Bylund, 1995; Fernandes et al., 1992; Madigan & Martinko, 2006; Martin, 1996).

2.2.2.3 Anaerobic digestion of whey

Even though whey's main component lactose is easily degradable, treatment of whey including using anaerobic digestion is difficult due to the high BOD_5 and COD, the tendency to acidify very quickly and the low alkalinity (González Siso, 1996; Kisaalita, Lo & Pinder, 1990). Chartrain and Zeikus (1986) have demonstrated that during hydrolysis of whey about 82 % of lactose is transformed into lactate. This results in a rapid formation of VFA and acidification characterised through a drop in pH and reduction of alkalinity. This has been reported to lead to lower COD removal, less

biogas produced and lower methane yield found in different reactor configurations (Frigon et al., 2009; Ghaly, 1996; Göblös et al., 2008; Rodgers et al., 2004).

During stable anaerobic digestion lactate never accumulates (Cohen, Zoetemeyer, van Deursen & Van Andel, 1979; Yang et al., 1987). The lactate production rate does not exceed the lactate removal rate. However, if a high concentration of an end product occurs, there can be a feedback inhibition on the enzyme catalysing the reaction, slowing down the end product production rate (Dyson, 1974).

Another inhibition in biogas production may be caused by the relatively high protein levels of acid whey which might lead to ammonia production which in turn inhibits the biogas production (Göblös et al., 2008) (see page 16).

The digestion of in particular sulphuric acid casein whey can lead to high levels of sulphide in a reactor. The resulting sulphate formation can precipitate trace elements, which might lead to a deficiency in the reactor and can affect the growth of microorganisms (Isa et al., 1986; Zehnder et al., 1982),

Also, the high levels of carbohydrates in whey (about four to five percent) promote the growth of acid forming bacteria, while having a negative effect on methane forming bacteria which can lead to an imbalance and then to acidification. (Gelegenis et al., 2007).

Despite its relatively unsuitable characteristics, anaerobic digestion of whey is an excellent treatment approach (Ghaly & Ramkumar, 1999; Jihen et al., 2010; Lo & Liao, 1986; Venetsaneas et al., 2009). Loading rates up to 30 kg COD/m^3/d have successfully been treated in whey digesting reactors with COD removal efficiencies greater than 95 % (Mawson, 1994). In 2003 the same author reports of about 90 % COD removal for loading rates of 20 kg COD/m^3/d to be possible while on large scale the typical load is 1.5 to 10 kg COD/m^3/d. Mawson (2003) concludes that high strength effluent such as whey can be treated with significantly shorter hydraulic retention times than biological solids residence time in UASB reactors. He also reports that digester operation under mesophilic conditions from 30 to 37 °C is favoured.

Research carried out by Lo and Liao (1986) with cheese whey in an anaerobic rotating biological contact reactor showed that retention times as short as five days can be achieved. At retention times below five days, VFA accumulate and lead to a pH drop to below six. Longer retention times can minimise the acidification effect of cheese whey (Lo & Liao, 1986).

Most published research papers have studied the influence of cheese whey on the anaerobic digestion process. Most also regard supplementing of alkalinity or co-digestion with other substrates as necessary to achieve stable digestion of cheese whey (Gelegenis et al., 2007; Ghaly, 1996; Malaspina et al., 1996; Lo et al., 1988; Lo & Liao, 1989; Wildenauer & Winter, 1985; Venetsaneas et al., 2009; Yan, Lo & Pinder, 1993).

Without pH control, Ghaly (1996) found that two-stage, unmixed anaerobic digestion of acid cheese whey is not feasible. In a pH controlled reactor, Ghaly (1996) found that acid cheese whey can increase the biogas production rate, the methane yield and the pollution potential reduction significantly. Further he established that an increase in temperature and/or decrease of the hydraulic retention time increases the biogas production rate for reactors digesting acid cheese whey (Ghaly, 1996). Ghaly and Ramkumar (1999), Malaspina et al. (1996) and Venetsaneas et al. (2009) also advise pH control through supplementing alkalinity for the stable anaerobic digestion of whey.

Stable co-digestion of whey with chicken manure has been reported by Gelegenis et al. (2007), and with cow manure by Lo et al. (1988) and Lo and Liao (1989). Lo et al. (1988) found that anaerobic digestion of cheese whey and cow manure eliminates the need for pH control as cow manure acts as a buffer for the whey. As the manure also provides nutrients, no further addition of these is required. The resulting improvement in stability is the only advantage Lo et al. (1988) reported for the digestion of cheese whey and cow manure over the digestion of cheese whey alone. No advantages were found in terms of methane production. Lo and Liao (1986) found that cheese whey digestion could not be operated below a HRT of five days, while Lo et al. (1988) found that cheese whey and cow manure could be digested at a HRT as low as two days. However, the methane production of the mixture was lower than that of cheese whey alone as the biodegradable fraction was lower due to the addition of cow manure (Lo et al., 1988).

The biogas and methane yield as well as the typical methane content reported from anaerobic digestion of whey are listed in Table 2.8.

Table 2.8: Biogas/methane yield and methane content from anaerobic digestion of whey

Biogas yield	Methane content	Methane yield	Digested material	Type of digester	Reported by
m^3 gas /m^3 whey		m^3 CH_4 /kg ODM			
35-38	60-62 %		Sweet and acid whey		Mawson (1994)
37	50-60 %				Zall (1992)
39			Not stated	Not stated	Organic Resource Management Inc. (2010)
		0.5-0.6	Not stated	Not stated	Pesta et al. (2007)

2.2.3 Cow manure

Typically 54 kg of raw manure is produced per cow per day. When cows are housed, the manure will contain a certain amount of foreign material, such as dirt and bedding material. If cows are in paddocks, as commonly occurs in New Zealand, all collectable manure originates from the milking shed and yard. Most of the faeces and urine is deposited in patches in the field and usually not collected (Vanderholm, 1984). Dairy cows come into the milking shed usually twice a day for milking. During the time in the shed and on the yard, approximately ten percent of the manure is deposited (Stewart, 2008).

The majority of dairy farmers in New Zealand hose down the shed and yard with water after milking, producing a dilute manure slurry. The liquid content depends on the amount of fresh water used for the dairy operations (Dareioti et al., 2009). An amount of 50 litres of yard washwater per animal and day is reported in the Agricultural Waste Manual (Vanderholm, 1984). It is usually collected in effluent pond systems and the supernatant is irrigated onto land (Stewart, 2008).

As the main feed source of dairy cows is grass, which is primarily composed of fibre, cow manure is mostly made up of fibrous materials (cellulose, hemicellulose and lignin).

Anaerobic bacteria and fungi in the ruminant's digestive tract produce the hydrolytic enzymes cellulase and hemicellulase, which degrade some of the cellulose and hemicellulose to glucose (Bauchop & Mountfort, 1981; Davies, Theodorou, Lawrence & Trinci, 1993; Theodorou, Mennim, Davies, Zhu, Trinci & Brookman, 1996; Trinci et al., 1994). The amount of fibrous material in the manure still accounts for about 50 % or more of the dry matter (Chen et al., 2005; Wen, Liao & Chen, 2005b).

Most of the energy rich substances (i.e. carbohydrates and proteins) contained in feed are removed in the ruminant's digestive tract (Lehtomäki et al., 2007). Carbon is the most abundant element in cow manure, followed by nitrogen, potassium and calcium (Chen et al., 2005; Wen et al., 2005b). Table 2.9 shows the composition of cow manure and the some of the elements it contains, including heavy metals and trace elements, as reported by several papers.

Table 2.9: Characteristics of cow manure

Cow manure										
						Manure	Freshly voided, cow on pasture			
TS	% w/w	6.5	-	-	14.6	-	-	-	12	-
	g/l	-	-	-	-	-	-	-	120	-
	g/kg	-	-	-	-	55.45	-	81.5	-	-
VS	% w/w	5.3	-	-	-	-	-	-	-	-
	% TS	-	-	-	-	-	-	73	-	-
	g/kg	-	-	-	-	-	-	59.495	-	-
	g/l	-	-	-	-	-	-	-	102	-
Protein	% TS	-	-	-	-	-	-	-	12.5	-
	g/kg	-	-	-	-	-	-	-	15	-
Carbon	% TS	-	-	-	50.51	45.37	-	-	-	-
	g/kg	-	-	-	73.745	25.158	158	-	-	-
Nitrogen	% TS	-	-	-	3.03	3.03	-	-	-	-
	g/l	-	-	-	-	-	-	-	5.294	TKN
	g/kg	-	-	-	4.424	1.680	9	4.44	-	-
	mg/g TS	41.5	15.8	NH_4-N	-	-	-	-	-	-
Phosphorus	% TS	-	-	-	0.81	0.48	-	-	-	-
	g/l	-	-	-	-	-	-	-	0.824	-
	g/kg	-	-	-	1.183	0.266	1.6	0.463	-	-

Sulphur	% TS	-	-	-	0.496	0.31	-	-	-	-
	g/kg	-	-	-	0.724	0.172	-	-	-	-
Potassium	% TS	-	-	-	1.24	2.86	-	-	-	-
	g/kg	-	-	-	1.810	1.586	15	5.74	-	-
Magnesium	% TS	-	-	-	0.966	0.55	-	-	-	-
	g/kg	-	-	-	1.410	0.305	3.7	-	-	-
Sodium	% TS	-	-	-	0.243	0.47	-	-	-	-
	g/kg	-	-	-	0.355	0.261	1.4	-	-	-
Calcium	% TS	-	-	-	2.41	1.2	-	-	-	-
	g/kg	-	-	-	3.519	0.665	9.8	-	-	-
Iron	% TS	-	-	-	0.134	0.03	-	-	-	-
	g/kg	-	-	-	0.196	0.017	4.288	-	-	-
Aluminium	% TS	-	-	-	-	0.014	-	-	-	-
	g/kg	-	-	-	-	0.008	-	-	-	-
Cadmium	g/kg	-	-	-	-	-	<0.0002	-	-	-
Reported by		Lehtomäki et al. (2007)			Wen et al. (2005a)	Chen et al. (2005)	Garcia-Gil, Plaza, Soler-Rovira and Polo (2000)	Vanderholm (1984)	Burke (2001)	

The following macronutrients and heavy metals can also be found in cow manure:

- Manganese
- Zinc
- Copper
- Chromium
- Nickel
- Lead

(Chen et al., 2005; Wen et al., 2005a)

A detailed report on 61 trace elements by Eriksson (2001) can be found in Appendix A.

About 65 to 80 % of the nitrogen and phosphorus content of the animal's feed is excreted in the faeces and urine of a dairy cow (Ryser Walther & Flisch, 2001). More than 50 % of phosphorus in cow manure is present in its inorganic form, the rest in organic form (Barnett, 1994; McAuliffe & Peech, 1949). Only 25 % of the nitrogen consumed is retained in the body, the rest is excreted, with an estimated 52 % in the urine and 48 % in the faeces (Sanchez, Borja, Weiland, Travieso & Martin, 2000). The carbon to nitrogen ratio is reported to be 11 to 14 (Hashimoto, 1983; Hills & Roberts, 1981).

Cow manure contains a number of anaerobic bacteria and fungi (McGranaghan, Davies, Griffith, Davies & Theodorou, 1999; Milne, Theodorou, Jordan, King-Spooner & Trinci, 1989; Trinci et al., 1994), which are involved in the hydrolysis of plant material in the rumen (Bauchop & Mountfort, 1981; Davies et al., 1993; Theodorou et al. 1996; Trinci et al., 1994). Anaerobic bacteria found in cow manure are reported to be *Bacillus*, *Enterococcus* ssp., *Clostridium*, faecal coliforms, faecal *streptococci*, *E. coli* and lactic acid bacteria (Östling & Lindgren, 1991; Unc & Goss, 2004; Wang, Mankin & Marchin, 2004).

Anaerobic fungi are of the family *Neocallimastigaceae* (Bauchop, 1989) and suggested to be ubiquitous among mammalian herbivores (Bauchop, 1989; Theodorou et al. 1996). They obtain energy through fermentation of carbohydrates, like lactose and glucose, into formate, lactate, acetate, succinate, ethanol, CO_2 and H_2 (Bauchop & Mountfort, 1981; Trinci et al., 1994). Theodorou et al. (1996) and Trinchi et al. (1994) reported that the production of lignin degrading enzymes by anaerobic fungi is unlikely. Anaerobic fungi have been isolated from fresh faeces (Lowe, Theodorou & Trinci, 1987), desiccated faeces stored up to 128 days (Milne et al., 1989), faeces dried for 210 days

(Theodorou et al., 1990) and in manure stored at temperatures from -20 to 39 °C including freezing and thawing (McGranaghan et al., 1999).

2.2.3.1 Anaerobic digestion of cow manure

There is a notable difference between the manure of different animals and the resulting biogas production. Most of the biodegradable carbon of a cow's diet is already digested in the rumen and the intestines, therefore the biogas production potential and the methane concentration in biogas from ruminants is lower than from other animal manure (Comino et al., 2009).

The animal's diet is one of the influencing factors for the composition of the cow manure as well as for the biogas production (Amon, Amon, Kryvoruchko, Zollitsch, Mayer & Gruber, 2007). It is also important to monitor whether the cows are treated with antibiotics. Some substances can influence the microorganisms responsible for the biogas production (Masse, Lu, Masse & Droste, 2002).

Dairy waste degrades slower than swine or poultry manure due to higher lignin content. Therefore, the lignin content in cow manure is considered the key-influencing factor for the digestibility of manure (Amon et al., 2002; Scherer, 2002; Wellinger et al., 1984). Lignin, which is made up of long chains of monosaccharides, is difficult for anaerobic bacteria to digest due to its complex structure. A shielding effect of lignin occurs due to its intense cross-links with cellulose and hemicellulose (Fan et al., 1981, as cited in Lehtomäki et al., 2007). The anaerobic digestion of cow manure is therefore limited to the ability of microorganisms to utilise fibre (Wen et al., 2005a; Wen et al., 2005b). The higher the amount of fibre in the manure, the lower the resulting biogas potential (Lehtomäki et al., 2007).

Acids such as sulphuric acid can be employed to obtain mono-sugars from fibre, this process is known as acid hydrolysis (Chen et al., 2005). Weak acids tend to remove lignin, however, this results in poor hydrolysis of cellulose. Strong acids, due to corrosive properties, require expensive equipment (Howard, Abotsi, Jansen van Rensburg & Howard, 2003).

Some fungi and bacteria can break down cellulose and other fibres by producing the enzymes cellulase and hemicellulase (Chen et al., 2005; Howard et al., 2003; Trinci et al., 1994). Anaerobic fungi are reported to produce hydrolytic enzymes, including cellulase, hemicellulase, protease, amylase and others (Trinci et al., 1994).

The performance of a digester fed with cow manure is primarily affected by the amount of biodegradable matter in the manure, the feeding rate and the hydraulic retention time (Karim, Klasson, Drescher, Ridenour, Borole & Al-Dahhan, 2007).

Mixing also becomes of greater importance when reactors are fed with thick manure (Karim et al., 2005). Foreign material in the cow manure can cause stratification in the reactor. Sand accumulates at the bottom, while undigested straw accumulates as a thick mat on the top of the reactor. To a certain degree the risk of stratification can be reduced with intense mixing (Burke, 2001).

A feedstock with high solids concentration such as cow manure can also lead to a high concentration of end products, which can inhibit the anaerobic digestion. Dilution of the feedstock can reduce these risks and reduce the concentration of nitrogen and sulphur, resulting in a positive effect on the process (Burke, 2001).

Experiments have resulted in different methane potentials and biogas production rates from the digestion of cow manure as the only substrate as shown in Table 2.10.

Table 2.10: Biogas/methane yield from cow manure

Biogas yield	Methane content	Methane yield	Type of digester	Digested material	Reported by
25 m^3/t	60 %	-	Not stated	Liquid cow manure	Fachagentur Nachwachsender Rohstoffe e.V., (2009)
45 m^3/t	60 %	-	Not stated	Cow manure	
25 m^3/t	-	-	Not stated	Liquid cow manure	Organic Resource Management Inc. (2010), Weiland (2000)
-	-	233 ± 20 l/kg VS_{added}	calculated methane potential	-	Lehtomäki et al. (2007)
-	-	190 ± 16 l/kg TS_{added}		-	
-	-	12 ± 1 l/t ww		-	
208.2-267.7 L_N/kg VS	-	125.5-166.3 L_N/kg VS	batch digester	-	Amon et al. (2007)
1-2 m^3/m^3 reactor/d	-	-	Not stated	-	Ahring (2003)

Amon et al. (2007) achieved in their experiments with dairy cow manure a gross energy yield of 14.6 to 19.3 MJ/kg VS. Burke (2001) states that biogas derived from cow manure typically contains 55 to 65 % methane, 35 to 45 % carbon dioxide and traces of hydrogen sulphide and nitrogen. Further he found that 65 to 67 % of the COD of dairy manure can be converted into gas with long retention times in order to allow cellulose to be converted. A conversion rate of 50 % of the volatile solids to gas can be achieved with an organic loading rate of 5.6 to 6.4 $kg/m^3/d$. Mesophilic digesters appear to achieve better conversion rates at lower loading rates, while thermophilic digesters achieve better conversion of volatile solids to gas at high loading rates (Burke, 2001).

The co-digestion of cow manure has been reported to be beneficial. Cow manure is considered to provide buffering capacity and add nutrients when co-digested with plant material (Lehtomäki et al., 2007) and whey (Lo et al., 1988; Lo & Liao, 1989).

2.2.4 Compost leachate

Leachate originates from precipitation that percolates through compost as well as percolation of groundwater through waste that dissolves soluble substances (Savage & Tyrrel, 2005). It can also be generated from high moisture content waste such as fruit and vegetables as they decompose (Environment Agency, 2001). The run-off from hard surfaces and machinery in composting facilities may be collected with the leachate (Savage & Tyrrel, 2005). "Leachate is known as wastewater whose treatment is very complicated, expensive and involving various processes." (Dichtl, Kayser & Steensen, 1997) In the UK, the production of compost leachate is considered a potential environmental hazard associated with composting of organic waste (Tyrrel, Seymour & Harris, 2008).

Various factors influence the composition of compost leachate, such as the type of composted material, the maturity of the compost it is derived from, the composting technology employed, the degree of cover and the weather. Therefore, the characteristics and the chemistry of leachate are variable (Tyrrel et al., 2008). Fischer (1996) suggests compost leachate to be highly variable in composition and very strong with a BOD_5 of 10,000 to 50,000 mg/l and NH_3/NH_4^+ of 300 to 1,200 mg/l. Compost leachate derived from green waste composting facilities was found to have similar concentrations of readily biodegradable organic compounds and nutrients as untreated urban wastewater (Tyrrel et al., 2005, as cited in Tyrrel et al., 2008).

Chapter 3 Materials & Methods

3.1 Research purpose

The aim of this research was to mimic existing municipal anaerobic digesters at the Palmerston North wastewater treatment plant to optimise the biogas production through addition of casein whey to the main feedstock primary sludge.

3.2 Reactor set-up

Erlenmeyer glass vessels with a volume of 2,000 and 500 ml were operated as batch-fed CSTR. They were placed on stirring plates and equipped with magnetic rods to ensure constant mixing. The speed of stirring depended on the stirring plates used and varied between roughly 150 and 180 rpm.

To create an oxygen free environment, the reactors were closed off with a rubber bung as seen in Figure 3.1. Sealed into the lid were Y-shaped plastic tubes with one side fitted with tubing connecting to a gas meter and the other side prepared with a rubber septum for gas sampling. In order to provide a mesophilic temperature regime in the reactors, the room was constantly heated to 36 to 38 °C.

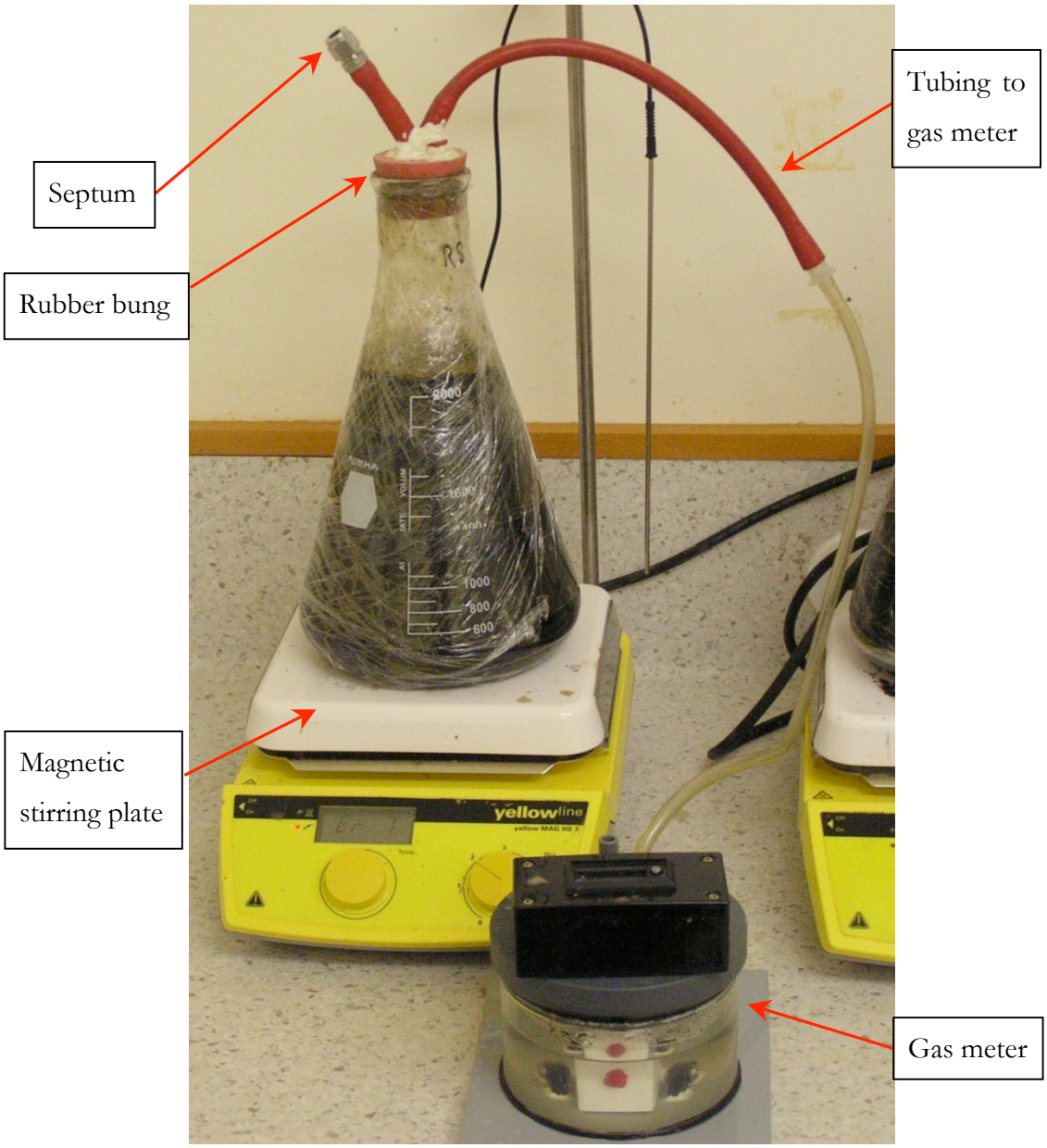

Figure 3.1: Anaerobic bench-scale biogas reactor on stirring plate connected to gas meter

3.3 Daily monitoring

The pH and the gas production of the biogas reactors were monitored daily and recorded in spreadsheets. The pH was measured with a pH meter, type TPS WP-91 Dissolved Oxygen-pH S 7432. The gas produced was monitored daily with gas meters, built and supplied by NIWA. The following picture (Figure 3.2) shows a gas meter used in the experiment.

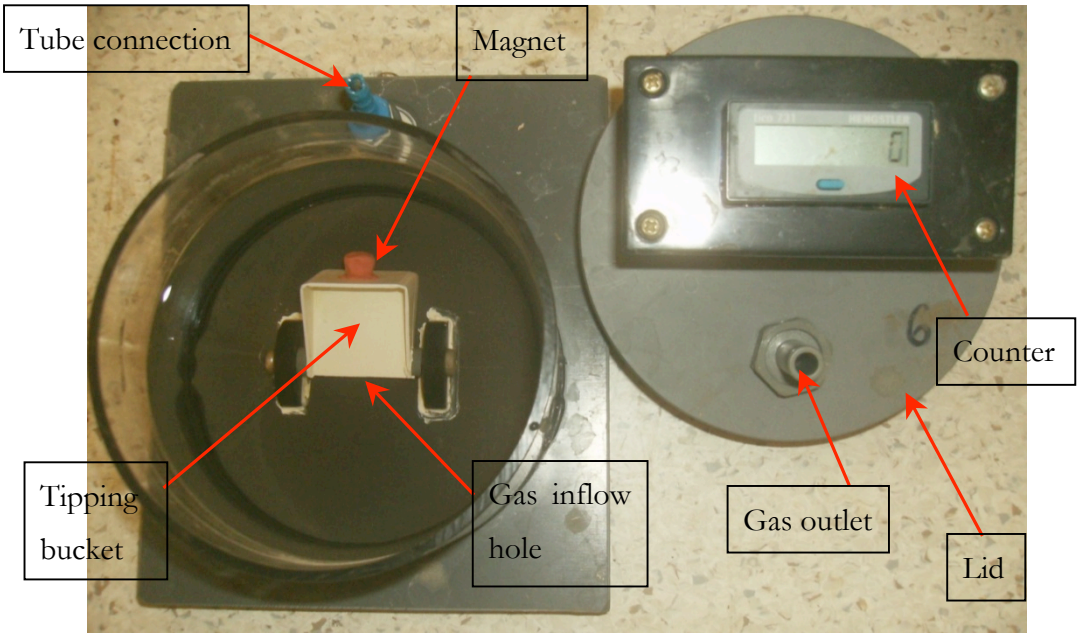

Figure 3.2: Gas meter (open) and gas meter lid aside

Gas produced in the digesters entered the water-filled gas meter through a hole in the bottom plate. A tipping bucket installed above the hole and tipped over when enough gas had entered. On the top of the tipping device a magnet was placed that passed a second magnet, installed in the bottom middle of the lid. A counter mounted on the top of the lid counted each time the magnets passed each other in the meter. Each tip accounted for two ml of gas, thus the reading of the meter has to be doubled.

3.4 Start-up

All reactors were started from digester effluent from the Palmerston North wastewater treatment plant. Two litres of liquid from the digesters were introduced into the flasks and 50 ml of primary sludge were immediately fed into the reactors. For the following two days no feed was introduced into the reactors. For the next three days the reactors received 50 ml of primary sludge, then 75 ml for another three days and eventually 100 ml of primary sludge after discarding the same amount as fed. After the change of the environment from the digesters at the wastewater treatment plant to much smaller glass vessels, the anaerobic bacteria had to be slowly acclimatised to the different feeding regime. While at the wastewater treatment plant the primary sludge is introduced to the digesters for 15 minutes every hour, the substrate was only introduced once a day in the experiment carried out.

3.5 Feeding

Feed was introduced to the reactors on a daily basis. The feed substrates stored in the fridge were warmed up in hot water prior to feeding to avoid cold shock for the anaerobic bacteria. Frozen feed was thawed 24 to 48 hours before usage in the fridge.

The same amount that was fed into each reactor was discarded daily by pouring it out in order to keep a constant volume. Primary sludge was the main feedstock, all reactors received 100 ml (or 25 ml daily for smaller reactors with a volume of 500 ml). The resulting HRT of 20 days was the same as in the digesters at the Palmerston North wastewater treatment plant. When co-substrates were fed, these were introduced with the primary sludge. The HRT changed accordingly. Feeding of cow manure in amounts lower than ten ml was achieved by adding ten ml of an appropriate dilution.

3.6 Organic substrates used

3.6.1 Primary sludge

Primary sludge for the experiment was obtained in batches from the Palmerston North wastewater treatment plant. Acquired in plastic vessels the sludge was poured into 500 ml plastic bottles with a lid for storage purposes. The primary sludge was analysed before utilisation.

During the experiments with acclimatisation (described under 3.8.2.1, 3.8.3.1 Co-digestion with acclimatisation and 3.8.3.3) the primary sludge was stored frozen. It was thawed roughly 24 to 48 hours before feeding and each batch used for about ten days. Research carried out by Montusiewicz, Lebiocka, Rozej, Zacharska and Pawlowski (2010) showed that freezing changed the characteristics of primary sludge. It was found that COD, TS and VS decreased while VFA and sCOD increased. However, the biogas production from frozen and thawed sludge (31 l biogas/day) was found to be only eleven percent higher than from fresh sludge (28 l/d) (Montusiewicz et al., 2010).

The average from weekly analysis and analysis of each new batch of the primary sludge used for the experiment is displayed in Table 3.1.

Table 3.1: Characteristics of primary sludge used for experiments with acclimatisation

		Average	Min	Max
Alkalinity	mg/l	542	192	802
tCOD	mg/l	27,861	10,850	67,121
sCOD	mg/l	7,869	380	19,319
	% of tCOD	28	3	29
VFA	mg/l	653	0	1,975
Acetate	mg/l	266	0	769
	% of total	41	-	39

For all other experiments, the primary sludge was obtained twice a week and stored in the fridge. As the biogas production depends on the COD removed from the influent material (Tchobanoglous et al., 2003), the primary sludge was analysed prior to utilisation and only used when the total COD was between 45,000 and 70,000 mg/l to avoid high variations in the resulting biogas production. The characteristics are displayed in Table 3.2.

Table 3.2: Characteristics of primary sludge used for experiments without acclimatisation

		Average	Min	Max
Alkalinity	mg/l	604	351	1,073
tCOD	mg/l	57,186	39,664	76,726
sCOD	mg/l	1,882	848	2,966
	% of tCOD	3	2	4
Lactose	mmol/l	0.42	0.00	2.49
D-glucose	mmol/l	1.51	0.03	15.63
L-lactate	mmol/l	1.07	0.01	15.44
Acetate	mmol/l	6.42	1.66	14.35
VFA	mg/l	845	185	1,705
Acetate	% of total	45	-	51

3.6.2 Casein whey

The casein whey used in all experiments with acclimatisation (see 3.8.2.1, 3.8.3.1 Co-digestion with acclimatisation and 3.8.3.3) was produced by the local Fonterra Casein plant in Longburn and supplied in ten litre buckets. The liquid was poured into 250 and

500 ml plastic bottles with lids and stored in the freezer as Ghaly (1996) states that freezing of cheese whey did not change its composition. Analysis was carried out once a week and the results are displayed in Table 3.3.

Table 3.3: Characteristics of fresh whey used for experiments with acclimatisation

		Average	Min	Max
pH		4.52	-	-
Alkalinity	mg/l	431	116	695
tCOD	mg/l	54,538	28,186	86,972
sCOD	mg/l	53,238	21,822	83,638
	% of tCOD	98	77	96
VFA	mg/l	626	0	2,195
Acetate	mg/l	277	0	806
	% of total	44	-	37

For all other experiments and the whey storage experiment, the casein whey was obtained at the Fonterra Casein plant in Reporoa on the 17th of March 2010. The whey was bottled, labelled and stored in the freezer as fresh whey. For the whey storage experiment, some of it was stored in the incubator (as described in Whey storage experiment on page 58). Both types of whey were analysed weekly. The characteristics of fresh whey are displayed in Table 3.4.

Table 3.4: Characteristics of fresh whey used for experiments without acclimatisation

		Average	Min	Max
pH		4.54	4.37	4.82
Alkalinity	mg/l	458	347	575
tCOD	mg/l	72,907	54,579	137,971
sCOD	mg/l	72,874	52,209	133,903
	% of total	100	96	97
Lactose	mmol/l	98.40	57.26	162.14
D-glucose	mmol/l	0.05	0.00	0.13
L-lactate	mmol/l	1.03	0.03	9.69
Acetate	mmol/l	7.30	0.03	58.23
VFA	mg/l	553	0	3,759
Acetate	% of total	79	-	93

Bacteria found in casein whey

In order to assess the bacteria present in the casein whey used, it was cultivated anaerobically on plate count agar at 30 °C, and gram staining of the cultured microorganisms was applied according to Collins and Lyne (1984). The pictures below (see Figure 3.3) show the gram stain of stored casein whey under the microscope.

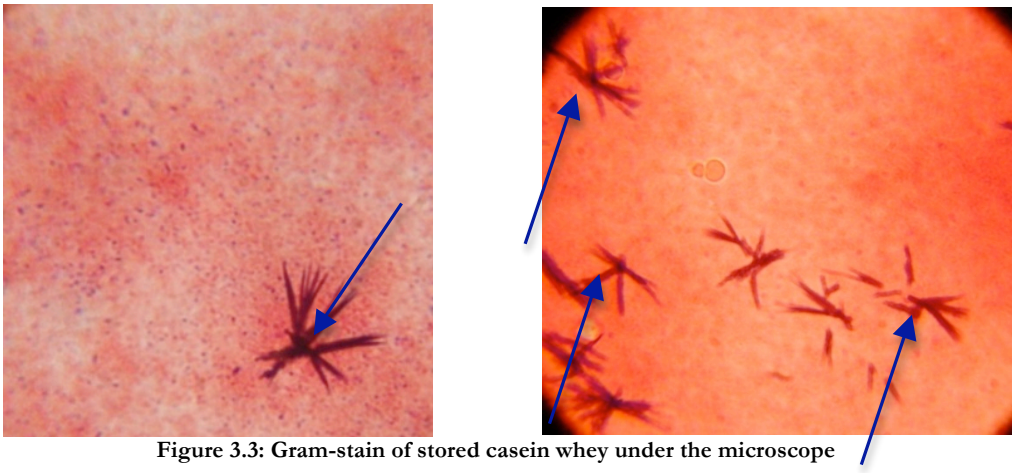

Figure 3.3: Gram-stain of stored casein whey under the microscope

In casein whey a mixed bacteria population of gram-positive (darker spots) and gram-negative (red spots) bacteria was found as well as yeast (blue arrows) as seen in Figure 3.3.

3.6.3 Cow manure

The cow manure used was scraped of the yard of the milking shed at Massey University Dairy Farm No.1. For the experiments with acclimatisation (described under 3.8.2.1, 3.8.3.1 Co-digestion with acclimatisation and 3.8.3.3), the manure was stored in the fridge at around four °C in a sealed container. The average characteristics from weekly analysing of the manure are displayed in Table 3.5.

Table 3.5: Characteristics of cow manure used for experiments with acclimatisation

		Average	Min	Max
pH		6.84	-	-
Alkalinity	mg/l	4,517	1,264	24,038
tCOD	mg/l	118,879	36,214	288,824
sCOD	mg/l	29,029	9,925	53,640
	% of tCOD	24	27	19
VFA	mg/l	449	0	2,922
Acetate	mg/l	91	0	691
	% of total	20	-	24

For all further experiments, manure was obtained and analysed weekly. It was stored in a plastic container in the incubator at 15 °C to achieve conditions as close as possible to the average temperature of Palmerston North. The characteristics of fresh cow manure are displayed in Table 3.6.

Table 3.6: Characteristics of cow manure used for experiments without acclimatisation

		Average	Min	Max
pH		6.91	6.60	7.19
Alkalinity	mg/l	5,910	3,257	9,921
tCOD	mg/l	142,978	96,733	229,841
sCOD	mg/l	27,739	14,920	54,242
	% of tCOD	19	15	24
Lactose	mmol/l	1.17	0.00	2.94
D-glucose	mmol/l	0.46	0.00	0.92
L-lactate	mmol/l	0.49	0.00	1.91
Acetate	mmol/l	0.93	0.07	1.75
VFA	mg/l	97	0	206
Acetate	% of total	53	-	-

Microorganisms found in cow manure

To assess the presence of microorganisms, cow manure was spread on plate count agar and cultivated anaerobically at 30 °C according to Collins and Lyne (1984). Figure 3.4 shows the growth found on the agar plates with cow manure.

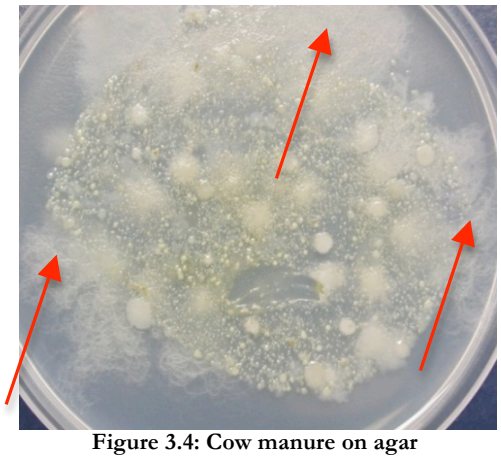

Figure 3.4: Cow manure on agar

It was found that mould is present in cow manure as seen in Figure 3.4 (red arrows).

Storing cow manure at an ambient temperature of 15 °C for thirteen weeks also showed the presence of fungi. This was revealed through growth of fruiting body of fungi (red arrow) and mould (blue arrow) as shown in Figure 3.5. The manure shown in the picture was not used for any experiments. Only freshly obtained manure was used as described above.

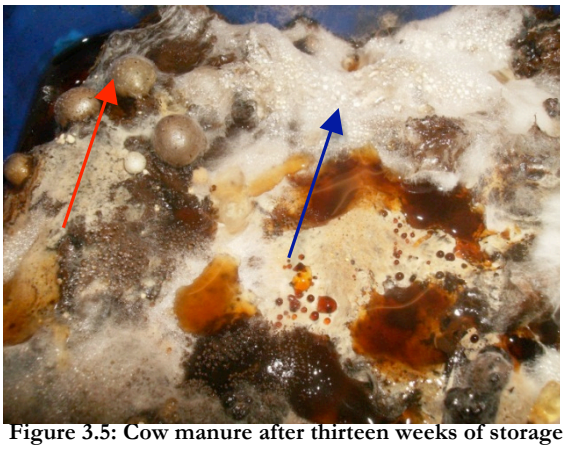

Figure 3.5: Cow manure after thirteen weeks of storage

As the cow manure was not stored under sterile conditions, the fungi in the manure could have been the result of contamination through contact with the atmosphere, the milking shed and yard, etc. However, the presence of fungi was clearly shown through long-term storage as seen in Figure 3.5 as well as reported in the literature. The presence of anaerobic fungi and bacteria in manure, even after drying and storage under different conditions including freezing, has been reported by Davies et al. (1993), Lowe et al. (1987), McGranaghan et al. (1999), Milne et al. (1989), Theodorou et al. (1990) and Trinci et al. (1994).

To assess the type of bacteria present in cow manure, gram-staining of the growth found on the agar plates was applied according to Collins and Lyne (1984). The result of gram-staining samples is shown in Figure 3.6.

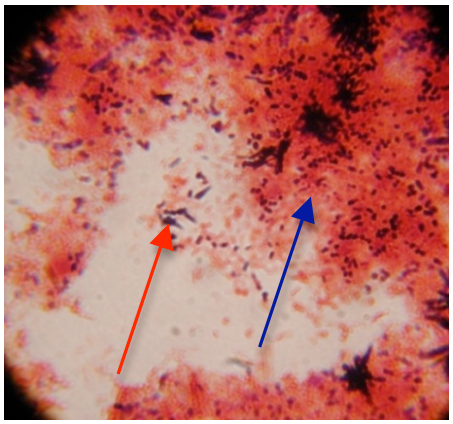

Figure 3.6: Cow manure under the microscope

Cow manure was found to have a mixed population of gram-positive (red arrow in Figure 3.6) and gram-negative (blue arrow in Figure 3.6) rods of various sizes.

3.6.4 Compost leachate

Compost leachate is generated at the Green Waste site at the Awapuni Recycling Centre in Palmerston North. Rainwater percolating through green waste compost piles at the site runs off into a drain. The liquid was scooped from the drain into 250 ml plastic bottles with lid. The leachate was stored in the freezer and thawed prior to usage. The leachate was analysed weekly and the results are shown in Table 3.7.

Table 3.7: Characteristics of compost leachate used

		Average	Min	Max
pH		3.59	-	-
Alkalinity	mg/l	4,295	3,387	5,477
tCOD	mg/l	13,637	5,304	24,622
sCOD	mg/l	12,893	3,637	22,728
	% of tCOD	95	69	92
VFA	mg/l	876	0	2,767
Acetate	mg/l	285	0	1,111
	% of total	33	-	40

3.7 Analysis

3.7.1 Reactors

As well as daily monitoring of pH and gas production, the effluent from the reactors was analysed weekly for the following parameters:

- Alkalinity
- Total and soluble chemical oxygen demand (tCOD and sCOD)
- Volatile Fatty Acids (VFA)
- L-lactate and D-glucose
- Lactose

The methane content in the produced biogas was analysed by taking a sample through the septum in the reactor lid. Monitoring enabled the health, stability and productivity of the reactors to be assessed.

3.7.2 Substrates

The different substrates were sampled and analysed weekly for the following parameters:

- Alkalinity
- Total and soluble chemical oxygen demand (tCOD and sCOD)
- Volatile Fatty Acids (VFA)
- L-lactate and D-glucose
- Lactose

As primary sludge and cow manure were too thick to sample, 50 ml of primary sludge and 20 ml of cow manure were each ten times diluted. With these ten times diluted samples, analysis was carried out. Different dilutions of the samples were used for the different analytical parameters. All dilutions for analysing purposes were achieved by using water filtered through a reverse osmosis filter (RO water).

3.8 Experiments

Purpose

The purpose of the experiments was to determine whether the anaerobic co-digestion of primary sludge with additional casein whey produced more biogas than primary sludge alone and under which circumstances an improved biogas production was achievable.

Set-up

All reactors in this experiment were inoculated and started up as described in 3.4 Start-up. Initially a daily amount of 100 ml of primary sludge corresponding to an HRT of 20 days was fed to all reactors. Experiments were started once the reactors reached steady state on a feed of 100 ml of primary sludge for three weeks constantly. The process is classified as steady state when a constant pH as well as consistent values of biogas produced and COD are found (Ghaly, 1996). Before new feed materials were introduced, the pH had to be above 6.75, the biogas production stable around one litre per litre reactor volume daily, the methane content and COD removal around 60 % and the concentration of VFA very low, indicating that the reactors were healthy.

3.8.1 Control reactors

In order to compare the performance, in particular the biogas production of the experimental reactors to the performance of an anaerobic primary sludge digester, a control reactor fed with primary sludge only was run and analysed. At least one control reactor was running during each of the experiments. The control reactors are listed in Table 3.8.

Table 3.8: Control reactors

Reactor		Volume	Amount of feedstock added daily
		ml	Primary sludge
C1	Control I	2,000	100 ml
C2	Control II	2,000	100 ml
C3	Control III	500	25 ml

3.8.2 Can casein whey improve the biogas production from primary sludge?

3.8.2.1 Primary sludge co-digested with fresh whey

Purpose

This experiment was designed to examine whether the co-digestion of primary sludge with fresh casein whey yielded more biogas than the digestion of primary sludge alone. It was also investigated whether acclimatisation by slowly increasing the amount of whey affected the biogas production and reactor stability.

Co-digestion with fresh whey

The experiment consisted of the control reactor and a reactor fed with primary sludge and fresh casein whey. The whey intake was slowly increased to avoid pH decrease in response to the high acidity and low alkalinity of whey (Gelegenis et al., 2007; Malaspina et al., 1996; Rodgers et al., 2004) and the resulting negative influence on the anaerobic bacteria. Over the 25 weeks of the experiment the amount of whey fed into the reactors was increased by ten ml every three to four weeks from an initial 20 ml. The maximum whey intake was 70 ml, as more whey would result in a HRT below the recommended minimum HRT of ten days (Hansen & Cheong, 2007). Table 3.9 shows the composition of the daily feed into the reactors and the ratio of primary sludge to whey.

Table 3.9: Daily feedstock composition in reactor FW1 and the control reactor C1

Reactor	Volume	Amount of feedstock added daily		Ratio
	ml	Primary sludge	Fresh whey	PS:W
C1	2,000	100 ml	-	1:0
FW1	2,000	100 ml	20-70 ml	10:2-10:7

3.8.2.2 Will storing whey change the outcome when it is co-digested with primary sludge?

Purpose

Whey may not be available all year around, as it is often only produced during peak milk season. In order to achieve a constant supply for anaerobic digestion, storage for up to

ten months could be required. This experiment was designed to examine changes occurring during the storage of whey and the consequences this could have for the anaerobic digestion process.

Whey storage experiment

The whey storage experiment was started when the whey was obtained from the Fonterra Casein plant in Reporoa on the 17th of March 2010. At full-scale, whey would most likely be stored in ponds, open to air and influenced by the weather. To mimic full-scale storage, 60 litres of the whey were stored in three buckets in the incubator at ambient temperature of 15 °C, the average temperature of Palmerston North. A lid was loosely placed over each bucket, and the whey was left to undergo a natural aging process. The stored whey was sampled and analysed weekly and the results were compared to the fresh whey, which was stored frozen.

The fresh and stored whey was analysed weekly for the following parameters:

- pH
- Alkalinity
- Total and soluble chemical oxygen demand (tCOD and sCOD)
- Volatile Fatty Acids (VFA)
- L-lactate and D-glucose
- Lactose

Primary sludge co-digested with <u>stored</u> whey

In this experiment a reactor was run co-digesting primary sludge with stored whey. The performance of this reactor was compared to the control reactor C3. The whey had been aging for 19 weeks when the experiment was started. The amount of whey added was not increased slowly to find out whether acclimatisation was required. The total volume of the reactors used was 500 ml, thus the amount of feedstock added had to be adjusted to achieve an HRT of 20 days in the control reactor. The substrate feed ratio was 10:3 as this could be compared to the reactor with primary sludge and fresh whey (page 57). The comparison was expected to show whether stored whey produced more or less biogas than fresh whey in primary sludge co-digestion. Table 3.10 shows the amount of the feed material entering the reactors daily.

Table 3.10: Daily feedstock composition in reactor StW1 and the control reactor C3

Reactor	Volume	Amount of feedstock added daily		Ratio
	ml	Primary sludge	Stored whey	PS:W
C3	500	25 ml	-	1:0
StW1	500	25 ml	7 ml	10:3

3.8.3 Can cow manure help to improve biogas production from primary sludge and casein whey?

Purpose

The reported literature suggests that the addition of cow manure can improve the stability of anaerobic whey digestion (Lo et al., 1988; Lo & Liao, 1989). In this experiment the influence of cow manure on the co-digestion of primary sludge with both fresh and stored whey was examined to determine if cow manure improved reactor stability and biogas production. The amount of cow manure was also minimised to achieve maximum biogas production with the least transport and storage of the manure possible.

3.8.3.1 What ratio of manure to fresh whey is required?

Purpose

In this experiment, the co-digestion of different amounts of fresh whey with 10 and 20 ml of cow manure was examined. It was tested whether a slow increase of the amount of fresh whey fed (acclimatisation) allowed a different maximum intake than without.

Co-digestion with acclimatisation

This experiment consisted of three reactors. One reactor remained on primary sludge as only feedstock for the time of the experiment and was used as the control reference for changes. The other reactors received 20 ml of whey at the start of the experiment. The amount of whey fed into the digesters was increased every three to four weeks by ten ml to allow acclimatisation of the bacteria to whey. The maximum whey intake was 70 ml, as more whey would result in a HRT below the recommended minimum HRT of ten days (Hansen & Cheong, 2007). In addition the reactors received 10 and 20 ml of

cow manure respectively and the amount remained the same over the time of the experiment. As 20 ml of cow manure had been successfully used in experiments previously carried out (Shilton, Powell, Broughton, Pratt, Pratt & Pepper, n.d. unpublished) it was regarded as a reliable starting quantity and was repeated in this experiment. A lesser amount of ten ml was chosen as well to study the influence of less cow manure on the co-digestion. Table 3.11 shows the composition of the daily feed into the reactors and the resulting ratios of primary sludge to whey (increasing) to cow manure in the feedstock.

Table 3.11: Daily feedstock composition in reactors FW+CM1, FW+CM2 and the control reactor C1

Reactor	Volume	Amount of feedstock added daily			Ratio
	ml	Primary sludge	Fresh whey	Cow manure	PS:W:CM
C1	2,000	100 ml	-	-	1:0:0
FW+CM1	2,000	100 ml	20-70 ml	10 ml	10:2:1-10:7:1
FW+CM2	2,000	100 ml	20-70 ml	20 ml	10:2:2-10:7:2

Co-digestion <u>without</u> acclimatisation

As acclimatisation is time consuming, this experiment was designed to study the response of the anaerobic bacteria to the addition of fresh casein whey at different amounts without prior acclimatisation. A reactor with primary sludge was operated as the control reactor (C2). As 20 ml of fresh whey started without any problems in the previously described experiment, a higher amount of 30 ml was chosen to start with in two reactors. As the minimum HRT of ten days (Hansen & Cheong, 2007) does not allow a higher whey intake than 70 ml, this amount was tested as maximum amount. To examine a medium amount, 50 ml was tried as well. Initially a daily amount of ten ml of cow manure was added to all reactors. A higher amount of 20 ml had previously been tested and was known to improve reactor stability (Shilton et al., n.d. unpublished). After nine weeks of stable performance, the amount of cow manure in the duplicate reactor of 30 ml of whey (reactor FW+CM4) was reduced from ten to one ml of cow manure to study the influence of minimising cow manure. The same was applied after 14 weeks to reactor FW+CM6. The exact feedstock composition is shown in Table 3.12.

Table 3.12: Daily feedstock composition in reactors FW+CM3, FW+CM4, FW+CM5, FW+CM6 and the control reactor C2

Reactor		Volume	Amount of feedstock added daily			Ratio
		ml	Primary sludge	Fresh whey	Cow manure	PS:W:CM
C2		2,000	100 ml	-	-	1:0:0
FW+CM3		2,000	100 ml	30 ml	10 ml	10:3:1
FW+CM4		2,000	100 ml	30 ml	10 ml	10:3:1
	CM dropped	2,000	100 ml	30 ml	1 ml	10:3:0.1
FW+CM5		2,000	100 ml	70 ml	10 ml	10:7:1
FW+CM6		2,000	100 ml	50 ml	10 ml	10:5:1
	CM dropped	2,000	100 ml	50 ml	1 ml	10:5:0.1

3.8.3.2 Can cow manure further improve co-digestion of primary sludge with stored whey?

Purpose

As cow manure is thought to improve reactor stability, it was tested in the co-digestion of primary sludge and stored whey. This experiment aimed to investigate whether cow manure could improve the biogas production from stored whey and primary sludge.

Co-digestion with stored whey and cow manure

In this experiment the reactors were fed with primary sludge, varying amounts of stored whey and, initially, ten ml of cow manure. The whey from the whey storage experiment, described on page 58, was used. The whey had been aging for 19 weeks when the experiment was started. The feed substrate ratios were similar to those applied in the experiment with fresh whey and cow manure (3.8.3.1 Co-digestion without acclimatisation). There were two reactors receiving 30 ml (reactor StW+CM1 and StW+CM2) and one receiving 70 ml of stored whey (reactor StW+CM3). In the reactors StW+CM2 and StW+CM3, the amount of cow manure was reduced from ten ml to one ml daily after nine and ten weeks of stable performance. The feedstock composition is shown in Table 3.13.

Table 3.13: Daily feedstock composition in reactors StW+CM1, StW+CM2, StW+CM3 and the control reactor C2

Reactor		Volume	Amount of feedstock added daily			Ratio
		ml	Primary sludge	Stored whey	Cow manure	PS:W:CM
C2		2,000	100 ml	-	-	1:0:0
StW+CM1		2,000	100 ml	30 ml	10 ml	10:3:1
StW+CM2		2,000	100 ml	30 ml	10 ml	10:3:1
	CM dropped	2,000	100 ml	30 ml	1 ml	10:3:0.1
StW+CM3		2,000	100 ml	70 ml	10 ml	10:7:1
	CM dropped	2,000	100 ml	70 ml	1 ml	10:7:0.1

3.8.3.3 Can compost leachate work as an alternative to cow manure?

Purpose

Due to its local availability and similar alkalinity to cow manure, compost leachate was tested as an alternative to cow manure in the co-digestion process aiming to improve the biogas production from primary sludge and fresh whey. Cow manure was found to have an alkalinity of 4,517 mg/l (see Table 3.5 page 52) while compost leachate averaged at 4,295 mg/l (see Table 3.7 page 54).

Co-digestion with whey and compost leachate

In this experiment a reactor was run co-digesting primary sludge with casein whey and compost leachate. The amount of whey fed into the digester, 20 ml initially, was increased every three to four weeks by ten ml to acclimatise the bacteria to a higher whey intake. Additionally 20 ml of compost leachate was fed, the amount remained stable. The performance of the reactor was compared to the control reactor C1. Table 3.14 shows the composition of the daily feed.

Table 3.14: Daily feedstock composition in reactor FW+CL1 and the control reactor C1

Reactor	Volume	Amount of feedstock added daily			Ratio
	ml	Primary sludge	Fresh whey	Compost leachate	PS:W:CL
C1	2,000	100 ml	-	-	1:0:0
FW+CL1	2,000	100 ml	20-60 ml	20 ml	10:2:2-10:6:2

3.8.4 What happens when casein whey and cow manure are combined?

The combination of cow manure and casein whey was further investigated to discover what influences the biogas production from primary sludge and this co-substrate combination. Acid hydrolysis of cow manure with casein whey was tested. The influence of cow manure by itself on the biogas production was examined. The addition of alkalinity, trace elements and microorganisms with cow manure as digestion process influencing factors were also considered and studied.

3.8.4.1 Is it possible that whey hydrolyses manure?

Purpose

Cow manure contains a high amount of fibrous material and acid has the ability to hydrolyse such materials (Chen et al., 2005; Howard et al., 2003). As whey is acidic, a test was run to determine if whey could hydrolyse cow manure.

Acid hydrolysis experiment

To establish if acid hydrolysis of cow manure with whey occurred when both were added into a measuring tube immediately before being fed into the reactors, the total and soluble COD of the substrates themselves and the mixture was measured. An increase in measured soluble COD in comparison to the estimated soluble COD of the mixture would indicate that complex material from the cow manure is hydrolysed.

Thirty ml of whey and ten ml of cow manure were mixed in a beaker and a sample was taken immediately. This sample was analysed for total and soluble COD and compared to the calculated total and soluble COD that the combination was expected to have based on the individual components COD before mixing them.

3.8.4.2 Is it possible that cow manure alone improves biogas production from primary sludge?

Purpose

This experiment was designed to determine whether cow manure co-digested with primary sludge yielded more biogas than primary sludge alone. It was carried out to

exclude the possibility that manure alone could be responsible for changes in the reactors, which were fed with manure and whey in other experiments.

Co-digestion with cow manure

The cow manure reactor was fed with 100 ml primary sludge and 10 ml cow manure daily; no whey was added to this reactor. The feedstock composition is shown in Table 3.15.

Table 3.15: Daily feedstock composition in reactor CM1 and the control reactor C2

Reactor	Volume	Amount of feedstock added daily		Ratio
	ml	Primary sludge	Cow manure	PS:CM
C2	2,000	100 ml	-	1:0
CM1	2,000	100 ml	10 ml	10:1

3.8.4.3 Does cow manure provide alkalinity that whey is lacking?

Purpose

According to the literature, whey lacks buffering capacity as it has a very low alkalinity, a little above 50 meq/l (Gelegenis et al., 2007; Malaspina et al., 1996; Rodgers et al., 2004). As the literature suggests that cow manure can provide stability to the anaerobic digestion process through adding alkalinity (Lo et al., 1988; Lo & Liao, 1989), the possibility that cow manure supplies alkalinity was examined.

Alkalinity comparison

The alkalinity added with the different feedstock was compared in relation to the amounts fed. Results from testing the total alkalinity in the different reactors fed with primary sludge, casein whey (fresh and stored) and ten ml of cow manure were compared to the alkalinity found in the reactor fed with primary sludge and ten ml cow manure only.

3.8.4.4 Does cow manure provide phosphorus, nitrogen and trace elements?

Purpose

According to Speece (1996), nitrogen, phosphorus and sulphur in concentrations of 50, 10 and 5 mg/l, respectively are desirable to maintain a maximum methanogenic activity. The key trace elements/metals for anaerobic digestion are reported to be iron, nickel, cobalt and zinc, recommended in the order of 0.02, 0.004, 0.003 and 0.02 mg/g acetate produced respectively (Speece, 1996). This results in a recommended amount per litre of reactor volume of 1.0 mg $FeCl_2$, 0.1 mg $CoCl_2$, 0.1 mg $NiCl_2$, and 0.1 mg $ZnCl_2$ (Speece, 1996).

Whey is found to have a deficiency of both key nutrients phosphorus and nitrogen, as well as the trace elements iron, nickel and cobalt (Speece, 1996). In contrast, cow manure is considered carbon-rich and the nutrients contained in the highest abundance after carbon are phosphorus and nitrogen (Chen et al., 2005; Lehtomäki et al., 2007; Ryser et al., 2001; Wen et al., 2005a). Lo et al. (1988) and Lo and Liao (1989) suggest that the addition of cow manure to the co-digestion of whey overcomes the need for additional nutrients.

To determine whether the co-digestion of primary sludge with whey was lacking phosphorus, nitrogen, other macronutrients and/or trace elements, the availability of nutrients in the reactors was investigated.

Nutrient comparison

The theoretical amount of phosphorus and nitrogen available in the reactors was calculated. The amount of phosphorus and nitrogen contained in cow manure reported in the literature was compared to those values reported for primary sludge to investigate the sources for available nutrients.

The concentration of other macronutrients and trace elements contained in primary sludge and cow manure were compared as well to investigate the availability of those in the reactors.

3.8.4.5 Do microorganisms from cow manure influence co-digestion of whey?

Purpose

Cow manure was found and reported to inhabit anaerobic bacteria and fungi (see Microorganisms found in cow manure on page 53). These may have an influence on the anaerobic digestion process. In order to establish whether, and possibly which microorganisms from cow manure influenced the biogas production from primary sludge, whey and cow manure, the manure was sterilised through autoclaving. To investigate the effect on the resulting biogas production, sterilised cow manure was co-digested with primary sludge and stored whey.

Autoclaving cow manure

In order to inactivate bacteria and fungi in cow manure, it was autoclaved at 121 °C for 15 minutes under high pressure of 15 psi. The autoclave used was not equipped with a temperature probe, therefore it was not guaranteed that the sample reached the required temperature of 121 °C for at least 15 minutes.

Co-digestion with whey and autoclaved cow manure

In this experiment the performance of a reactor fed with primary sludge, stored casein whey and autoclaved cow manure (StW+autoclCM) was examined. A reactor co-digesting stored whey and non-autoclaved cow manure (StW+CM4) with the same HRT was also operated as well as a control reactor (C3). The composition of the feed material added to each reactor and the resulting feedstock ratio is shown in Table 3.16.

Table 3.16: Daily feedstock composition in reactors StW+CM4, StW+autoclCM and the control reactor C3

Reactor	Volume	Amount of feedstock added daily				Ratio
	ml	Primary sludge	Stored whey	Cow manure	Autoclaved cow manure	PS:W:CM
C3	500	25 ml	-	-	-	1:0:0
StW+CM4	500	25 ml	7 ml	2.5 ml	-	10:3:1
StW+autoclCM	500	25 ml	7 ml	-	2.5 ml	10:3:1

Microorganism removal from cow manure

To ensure that all microorganisms were removed from the cow manure through the autoclaving process applied, autoclaved cow manure samples were cultured

anaerobically on plate count agar at 30 °C and gram-stained for identification according to Collins and Lyne (1984).

3.8.5 Why do fresh and stored casein whey reactors not result in the same performance?

Purpose

The aim of this comparison was to find out why the co-digestion of primary sludge with fresh casein whey did not result in the same pH and biogas production as the reactors co-digesting primary sludge with stored casein whey.

3.8.5.1 Do lactic acid bacteria from whey influence the co-digestion?

Purpose

As whey contains lactic acid bacteria, their addition to the digesters could have an influence on the biogas production. This was to be examined in the experiment.

Sterilising whey

To remove lactic acid bacteria from stored whey, the substrate was centrifuged at 10,000 rpm for 25 minutes to remove any solids. It was then filtered with a sterilised vacuum-filter apparatus through sterile filters of pore size 0.45 μm to remove microorganisms.

Microorganism removal from whey

To ensure that all microorganisms were removed from the stored whey through the sterilising method used, a sample was cultured anaerobically on plate count agar at 30 °C, according to Collins and Lyne (1984).

Co-digestion with sterile whey and cow manure

For the sterilised whey experiment, the reactor StW+CM1 from a previous experiment was used and the stored whey in the feed material was replaced by sterilised stored whey. For comparison, a control reactor (C3) was run as well as a reactor fed with the same substrate ratio but non-sterile whey (StW+CM4). The composition of the feed material added to each reactor is shown in Table 3.17.

Table 3.17: Daily feedstock composition in reactors StW+CM4, sterileStW+CM and the control reactor C3

Reactor	Volume	Amount of feedstock added daily				Ratio
	ml	Primary sludge	Stored whey	Sterile stored whey	Cow manure	PS:W:CM
C3	500	25 ml	-	-	-	1:0:0
StW+CM4	500	25 ml	7 ml	-	2.5 ml	10:3:1
sterileStW+CM	2,000	100 ml	-	30 ml	10 ml	10:3:1

3.8.5.2 Why does stored whey co-digested result in a higher reactor pH than fresh whey co-digested?

pH comparison

The processes occurring during the storage of casein whey and the resulting changes were observed. Their influence on the pH in the reactors was examined through comparison of the reactors fed with fresh whey to those fed with stored whey.

3.8.5.3 Why does fresh whey co-digested result in a higher biogas production than stored whey co-digested?

The biogas production from the fresh whey and stored whey reactors fed at the same ratio was compared. As the biogas production is the result of the COD removed (Tchobanoglous et al., 2003), the incoming COD as well as the COD removal in the reactors was compared with the biogas production.

3.8.5.4 Why does the co-digestion of <u>fresh</u> whey require cow manure for improved biogas production?

This subject was addressed using the results of the previous experiments and the results of the whey storage experiment. The incoming concentration of lactose, D-glucose, L-lactate and acetate from the substrates fresh and stored whey were compared, and the concentrations found in the effluent of the fresh and stored whey reactors were reviewed. The reported literature discussing anaerobic bacteria, lactic acid bacteria and their sources was assessed.

3.9 Methods & Equipment

All results from weekly and fortnightly testing as well as the daily readings of pH and biogas production were entered into spreadsheets. For comparison reasons, a weekly average of pH and gas production was calculated. The data was also used to make various calculations and to evaluate health and stability as well as productivity and efficiency of the reactors and the process.

pH

The pH was measured with the aforementioned pH meter, type TPS WP-91 Dissolved Oxygen-pH S 7432 (see 3.3 Daily monitoring on page 46).

Total alkalinity

The total alkalinity was measured with a titrator, type Mettler DL25 Titrator with the titration start point of pH seven and end point of pH four. The reagent used was hydrochloric acid solution (N=0.1). For the analyses, the effluent of all reactors and feedstock was ten times diluted with RO water. For whey no dilution was used.

The readings of the titrator in mmol/l were multiplied by the dilution factor and the molar mass of calcium carbonate (100.0869 g/mol) resulting in the total alkalinity in mg/l as $CaCO_3$.

Bicarbonate alkalinity

Bicarbonate alkalinity was measured by titration to a pH end point 5.8, at which about 80 % of the bicarbonate alkalinity is used but only a small part of the VFA alkalinity is titrated (Speece, 1996). Titration was carried out manually by dropping hydrochloric acid (N=0.1) into 50 ml samples of the reactor effluent (1:10 dilution) and into 50 ml of cow manure (1:10 dilution). The bicarbonate alkalinity of each sample was calculated according to the APHA Standard Methods, Method 2320 B (Clesceri et al., 1998).

Equation 3.1: Alkalinity

$$Alkalinity\ (mg\ CaCO_3 / l) = \frac{A \times N \times 50000}{ml\ sample}$$

Where $A = ml\ standard\ acid\ used$

$N = Normality\ of\ standard\ acid$

The results accounted for 80 % of the bicarbonate alkalinity, from which 100 % of the bicarbonate alkalinity was calculated.

Total and soluble COD

For all reactors as well as for all feed substrates the total and soluble COD was determined weekly or in the case of primary sludge and cow manure, upon collection as well. The reactor and whey samples were analysed in duplicate, the primary sludge and cow manure samples in triplicate. The closed reflux, colorimetric method (Method 5220 D) as proposed in the APHA Standard Methods (Clesceri et al., 1998) was used to determine COD. The recorded results are the average measured absorption expressed as COD in mg/l.

Table 3.18 shows the dilutions applied for the total and soluble COD analysis of the reactors and the feed materials.

Table 3.18: Dilutions applied for COD measurements

	Dilution tCOD	Dilution sCOD
Reactors	50-100 x	10 x
Primary sludge	100 x	10 x
Whey	100 x	100 x
Cow manure	400 x	200 x
Compost leachate	50 x	50 x

To determine the total COD the sample was prepared to the appropriate dilution. For measuring the soluble COD, the diluted sample was pressed through a filter, pore size 0.45 μm, with a syringe to remove insoluble matter. The volume of prepared sample used for the analyses was 2.5 ml. To each of the samples 3.5 ml of sulphuric acid (H_2SO_4) and 5.5 ml of potassium dichromate ($K_2Cr_2O_7$) as oxidant was added to oxidise the organic compounds in the sample chemically. After mixing on a Vortex, the samples were left to digest for 120 min at 150 °C in a HACH COD reactor, all according to the Standard Methods. Two blanks with 2.5 ml RO water and two standards with 1:2 dilute COD standard solution were prepared as well. The COD standard solution (1,000 mg/l) was prepared according to the Method 5220 Chemical Oxygen Demand in the APHA Standard Methods (Clesceri et al., 1998)

To avoid precipitation interfering with measuring the absorption, the samples were mixed again after digestion and left for at least 30 min in order to precipitate out any solids. The absorption of the samples was measured in a spectrophotometer, type Helios Y v.7.03 Serial no. 114409, at a wavelength of 600 nm. The absorption was

converted into a COD result in mg/l with the standard curve for a COD standard solution as displayed in Figure 3.7.

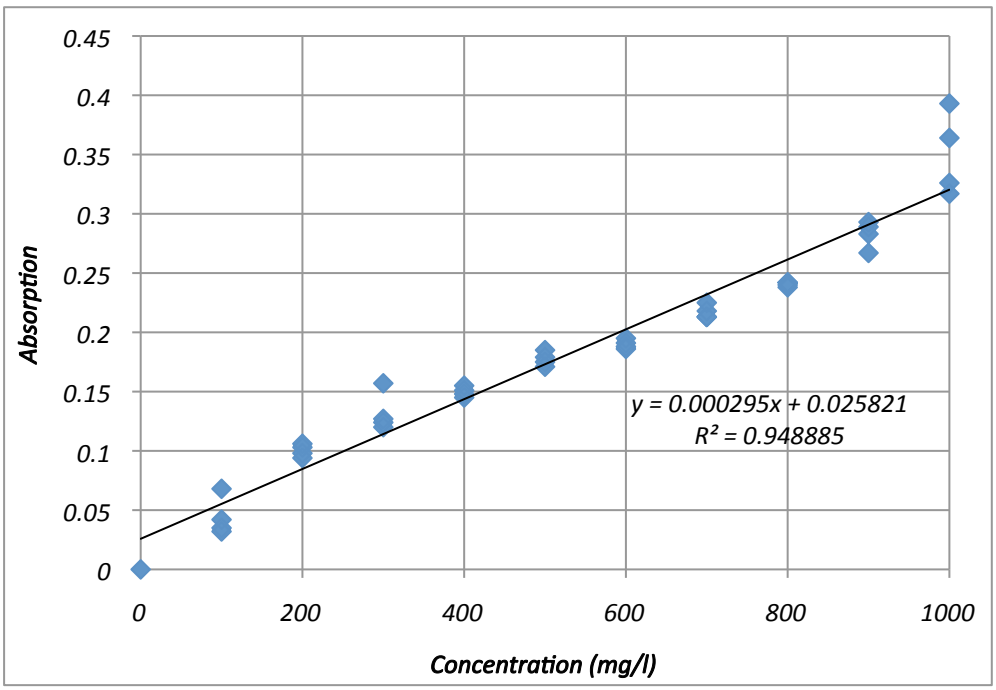

Figure 3.7: COD standard curve prepared

The standard curve determines the factors used to calculate the COD from the absorbance read on the spectrophotometer. (see Equation 3.2)

Equation 3.2: COD

$$COD = \left(\frac{Absorbance}{0.000295} + 0.025821 \right) \times Dilution\ factor$$

The resulting COD values were used to calculate the COD removal (%) in each of the reactors as a result of incoming and outcoming COD (see Equation 3.3).

Equation 3.3: COD removal

$$COD\ removal\ (\%) = \frac{COD_{in} - COD_{out}}{COD_{in}}$$

Volatile fatty acids

The volatile fatty acids in the effluent and the feedstock substrates were measured in an Ion Chromatograph (IC), type Dionex ICS-2000 Ion Chromatograph System serial no. 02110022 with an Dionex AS50 Autosampler serial no. 04030204 with a IonPac® AS11-HC analytical column, a IonPac® AG11-HC guard, a ASRS 300 4 mm self-regenerating suppressor, potassium hydroxide as eluent and deionised water as the mobile phase. The computer converting the results from the IC used the program Chromeleon IT 017534 Version 6.50 SP2 Build 968 serial no. 45810.

The IC is calibrated at 2.5, 5.0, 10.0, 15.0, 20,0, 25.0, 30.0, 35.0, 40.0, 45.0 and 50.0 mg/l to detect following VFA:

- Acetate,
- Propionate,
- I-Butyrate,
- Butyrate,
- 2-methyl-Butyrate,
- I-Valerate and
- Valerate.

In preparation, the samples were centrifuged for ten min at 13x1,000 rpm and the supernatant was filtered to ensure that no solids remained. Two drops of concentrated H_2SO_4 were added to each sample to preserve the rapidly decreasing VFA concentration in the sample. This preservation method was applied after tests were carried out as described in Appendix B. The concentration of each abovementioned VFA measured by the IC and the total concentration of VFA was expressed in mg/l. The concentration of acetate was also converted into mmol/l.

For the reactor's effluent the dilution was based on the results of the previous week. When the reactors were healthy no VFA were found in undiluted samples and analysis was carried out every third week only. When the pH of the reactor dropped below 6.8 for more than one day, VFA analysis was applied weekly and dilution was adjusted appropriately.

For the feedstock the dilution had to be adjusted over the time of the experiment in order to achieve satisfying results. The primary sludge was analysed with 1:2, 1:10 and 1:20 dilutions, cow manure with a 1:20 dilution, the fresh whey with no dilution and 1:10 dilution and the stored whey 1:10 and 1:100.

When results showed a very high acetate concentration and low concentration of the other VFA, more than one dilution of a sample had to be prepared in order to detect all VFA more accurately. The higher dilution was used to detect the acetate peak, which would be too high for the more concentrated or undiluted sample.

L-lactate, D-glucose and lactose

The concentration of L-lactate, D-glucose and lactose in the feed substrates and the reactors was measured weekly. The analysis was carried out with a biochemistry analyser YSI Model 2700 SELECT with a YSI 2710 Turntable. For the analysis of L-lactate and D-glucose, the system buffer YSI 2357, the calibrator standard YSI 2776 and the linearity standard YSI 1531 and YSI 1530 was used. The white probe was equipped with a YSI 2365 dextrose membrane and the black probe with a YSI 2329 L-lactate membrane. For lactose analysis, a YSI 2705 system buffer, a YSI 2783 calibrator standard and a YSI 2784 linearity standard was utilised while a YSI 2702 Galactose Oxidase Membrane was used.

In an anaerobic digester, considerable amounts of D-lactate and DL-lactate can occur but L-lactate will predominate. Only the concentration of L-lactate was measured. Thus the total concentration of lactate is assumed to be higher than the measured L-lactate concentration. According to Wielonek (1988 as cited in Renner, 1992), the concentration of D-lactate in acid whey can account for about 15 % of the total lactate concentration.

Of the two isomers of glucose, only D-glucose occurs naturally (Vaclavik & Christian, 2007), thus L-glucose was not measured.

Gas content

A gas sample (20 ml) was taken with a syringe from the reactors fortnightly or weekly. To detect the percentage of methane gas in the biogas produced, the biogas was analysed in a gas chromatograph (GC), type Shimadzu Gas Chromatograph GC-2014 serial no. C11484439013 CS with nitrogen gas as the carrier gas with a TDC-L thermal conductivity detector operated at 45 °C and an Alltech® CTR I packed column 1.8 m long and an inner diameter of 6 mm. The program Shimadzu GCSolution Analysis Version 2.30.00 SU6 was run on the computer. The methane concentration was given in % methane.

3.9.1 Calculations

To judge the significance of important parameters, statistical t-test for two samples assuming unequal variances with a hypothesised mean difference of zero and an alpha level of 0.05 were performed. The one-tailed p-value was used to state the significance of data sets compared. Results are reported as significant at 95 % confidence (p-value equal to or less than 0.05). The number of independent samples used to calculate the p-value differed depending on the experiment carried out. The lowest number of independent samples used to calculate a p-value was 26 observations.

Chapter 4 Results & Discussion

In this chapter the results from the different experiments are presented and discussed. The control reactors will be described, followed by the reactors co-digesting primary sludge and casein whey. In the next section, the influence of the additional co-substrates cow manure and compost leachate on the co-digestion of primary sludge and casein whey is portrayed. The combination of casein whey and cow manure is then examined further and finally the differences found between the co-digestion with fresh and stored casein whey are presented and discussed.

4.1 Control reactors

During all experiments a control reactor was run with primary sludge as the only feedstock to mimic the local digesters at the wastewater treatment plant. This ensured that changes in the conditions of the primary sludge would be detected and enabled a comparison to be made between the digestion of just primary sludge and primary sludge with co-substrates. There was at least one control reactor run throughout every experiment. As the experiments were carried out at different times, there were three different control reactors.

The HRT in the control reactors was 20 days to mimic the full-scale digesters. The organic loading rate was dependent on the total COD of the primary sludge and averaged around 1.5 kg/m^3/day in the reactor C1 and around 2.9 kg/m^3/day in the reactors C2 and C3 as the primary sludge had a higher COD during that later period.

The average biogas production, pH and alkalinity, as well as VFA concentration of the control reactors are presented in Table 4.1.

Table 4.1: Average characteristics found in the control reactors

	pH	Alkalinity	VFA	Biogas production	Methane content	Methane production
		mg/l	mg/l	ml/l/day	%	ml/l/day
C1	6.81	3,091	4.2	1,279	57.84	739
C2	6.92	3,165	0	1,046	62.07	649
C3	6.95	3,611	0	998	65.37	652

The pH and alkalinity in the control reactors was found to be in the advised range of pH between 6.5 and 7.5 (Hansen & Cheong, 2007) and the alkalinity between 2,500 and 5,000 mg/l (Ghaly & Ramkumar, 1999; Ghaly et al., 2000) of an operational digester. The methane content was found to be, as expected, between 58 and 65 % (see Table 4.1).

On average there were 4.2 mg of VFA per litre found in the control reactor C1, while the control reactors C2 and C3 did not contain any VFA as seen in Table 4.1.

The biogas production depends on the COD converted (Tchobanoglous et al., 2003). Table 4.2 shows the total and soluble COD removed in the control reactor C2 in mg/l and day HRT as well as a fraction of the incoming COD.

Table 4.2: Total and soluble COD removed in the control reactor C2

	Total COD		Soluble COD	
	Removed	Removal	Removed	Removal
	mg/l/d HRT	%	mg/l/d HRT	%
C2	1,524	52.6	41	42.7

As only three percent of the primary sludge was found soluble (see Table 3.2 page 49), the total COD removal was found higher than the soluble COD removal as seen in Table 4.2.

All three control reactors showed a similar performance. They were found to be healthy and very stable and gave consistent results in biogas production, methane content, alkalinity and VFA concentration. The biogas production was comparable to reported one to two m^3 of biogas per day and m^3 of reactor volume for the digestion of primary

sludge (Ahring 2003). The mimicked digesters at the Palmerston North wastewater treatment plant produced about 660 ml/l/d. The results from the bench-scale reactors were expected to be higher as mixing was better.

4.2 Can casein whey improve the biogas production from primary sludge?

In this section the experiments carried out to study the influence of casein whey on the digestion of primary sludge are described and discussed. As the production of whey can be seasonal due to pasture based milk production systems, storing whey could allow a constant supply for anaerobic digestion all year around. Therefore, changes occurring during storage and the effect of those on the anaerobic co-digestion process of primary sludge were investigated and compared to the co-digestion with fresh whey.

4.2.1 Primary sludge co-digested with fresh whey

In this experiment a reactor with a volume of 2,000 ml was fed with primary sludge and fresh casein whey (FW1). The amount of whey the reactor received was slowly increased from 20 ml to a maximum whey intake of 70 ml. The performance of the reactor was compared to the control reactor C1.

Table 4.3 displays the feed ratio, the HRT, OLR as well as the performance in terms of biogas production and pH of the reactor FW1 compared to the control reactor over the increases of fresh casein whey in the feedstock.

Table 4.3: Characteristics of reactor FW1 compared to the control reactor C1

		Ini-tial	Week 5-8	Week 9-12	Week 13-15	Week 16-18	Week 19-21	Week 22-24	Ave-rage
Amount of feedstock added (ml)	**PS**	100	100	100	100	100	100	100	-
	W	0	20	30	40	50	60	70	-
Ratio (PS:W)	**C1**	1:0	1:0	1:0	1:0	1:0	1:0	1:0	-
	FW1	1:0	10:2	10:3	10:4	10:5	10:6	10:7	-
HRT (days)	**C1**	20	20	20	20	20	20	20	-
	FW1	20	16.7	15.4	14.3	13.3	12.5	11.8	-
OLR ($kg/m^3/day$)	**C1**	1.42	1.09	1.45	1.33	1.38	1.83	1.03	1.36
	FW1	1.42	1.48	2.54	2.12	2.42	3.62	3.63	2.64
pH	**C1**	6.79	6.80	6.84	6.80	6.76	6.79	6.86	6.81
	FW1	6.80	6.79	6.75	6.72	6.67	6.65	6.63	6.70
Biogas production (ml/l/day)	**C1**	1,076	1,052	1,290	1,219	1,071	1,395	1,854	1,313
	FW1	1,170	1,195	1,194	1,227	1,317	1,406	1,916	1,346
Biogas prod. (% of control)	**C1**	100	100	100	100	100	100	100	100
	FW1	109	114	93	101	123	101	103	106

Variations in the organic loading rate of the control reactor (as seen in Table 4.3) were due to changes in the COD of primary sludge. As the loading rate of the reactor FW1 increased with increasing amounts of whey, the HRT consequently decreased from 20 days initially to 11.8 days (see Table 4.3). The pH in the reactor FW1 was found to decrease with each increase of whey, however it did not reach levels of concern for the stability of the process.

Comparing the biogas production of the control reactor C1 and the whey reactor FW1 over each increase of whey (see Table 4.3) showed that the biogas productivity of the anaerobic bacteria did not increase significantly (p-value=0.32) with the addition of increasing amounts of whey. On average the reactor with fresh casein whey as a single co-substrate at ratios 10:2-10:7 (PS:W) produced 106 % of the biogas generated in the control reactor (see Table 4.3).

4.2.2 Will storing whey change the outcome when it is co-digested with primary sludge?

Whey storage experiment

The characteristics of whey stored at ambient temperature in comparison to those of fresh whey are presented in Table 4.4. Initial refers to the measurements taken upon obtaining the whey before it was frozen (fresh whey) or stored (stored whey).

Table 4.4: Average characteristics of fresh and stored casein whey

		Initial	Fresh Whey	Stored Whey
pH		4.6	4.54	3.59
Alkalinity	mg/l	503	458	Not measureable
tCOD	mg/l	104,073	72,907	52,347
sCOD	mg/l	92,886	72,874	50,991
	% of total	89	99.9	97
Lactose	mmol/l	Not measured	98.40	13.70
D-glucose	mmol/l		0.05	0.15
L-lactate	mmol/l		1.03	71.38
Acetate	mmol/l	32.03	7.30	47.10
VFA	mg/l	2,028	553	3,317
Acetate	% of total	95	79	85

The storage of casein whey at 15 °C in non-sealed containers was found to result in changes of all observed parameters. As a result of storage the pH of the whey dropped from an initial level of 4.5 to an even more acidic pH of 3.6 (see Table 4.4). With the dropping pH, the stored whey's alkalinity dropped beyond the measurable level as the pH was below the titration end point of 4.2. In fresh whey the pH remained fairly constant around 4.54, while the alkalinity averaged around 458 mg/l (see Table 4.4).

The initial VFA concentration in the whey upon obtaining was 2,028 mg/l as Table 4.4 shows. Changes were found to occur under different storage conditions. In fresh whey it was found that the initial VFA concentration decreased and reached an average of 553 mg/l. Acetate accounted for the majority of VFA, on average 79 % in fresh whey (see Table 4.4). In stored whey the opposite was observed. It was found that the VFA concentration increased to concentrations between 1,711 and 9,998 mg/l, while

averaging at 3,317 mg/l (see Table 4.4). Of the VFA found in stored whey, 85 % was acetate (see Table 4.4).

The lactose concentration in fresh whey was on average 98.4 mmol/l and almost no D-glucose and L-lactate and little acetate was found (see Table 4.4). The lactose to L-lactate ratio in fresh whey was 96:1. Stored whey contained less lactose (average 13.7 mmol/l) but more L-lactate and acetate (see Table 4.4). Only 14 % of the lactose contained in fresh whey remained in the stored product. The ratio of lactose to L-lactate in stored whey was 1:5. This indicates that lactose in stored whey was fermented into L-lactate. The lactose fermentation process is described in Figure 2.5 on page 28. The increase in VFA as described above was another indication of the lactose fermentation process, as VFA, in particular acetate, are products of the lactose fermentation (see Lactose fermentation on page 28). As the pH of fresh as well as stored whey was acidic, the VFA occurred in their acid form. Therefore with the increase of L-lactate and VFA in stored whey, the pH dropped as described above.

As the amount of L-lactate and acetate did not increase and accounted for less than ten mmol/l (see Table 4.4), it is assumed that the lactose fermentation process did not occur in fresh whey. As a result the pH in fresh whey remained close to the initial pH of the whey upon obtaining and before freezing (see Table 4.4).

The COD content of casein whey under different storage conditions was found to have changed as well. Most organic matter responsible for the COD in whey was soluble, thus the total and soluble COD were very similar for fresh and stored casein whey. Both fresh and stored whey had an initial COD of 104,073 mg/l (see Table 4.4) and it was found to decrease over the time of the experiment. On average the total COD of fresh whey was 72,900 mg/l with a soluble portion of 99.9 % as seen in Table 4.4. Even though the stored whey was of the same origin as the fresh whey, its COD dropped to a lower level than the COD in fresh whey. The average COD of the stored whey over the time of the experiment was 52,350 mg/l with an average of 97 % soluble COD (see Table 4.4). It is assumed that this drop was a result of the partial fermentation of lactose to intermediates and end-products such as L-lactate, acetate, formate, CO_2 and hydrogen during storage. The production of hydrogen was assumed to lead to a loss in COD of stored whey as any produced gas would be lost. Assuming that fermentation of

lactose in fresh whey did not occur, no hydrogen would be produced or lost and therefore the COD of fresh whey was higher than of stored whey.

Primary sludge co-digested with stored whey

In this experiment a reactor (StW1) was fed with primary sludge and stored casein whey instead of fresh whey. The amount of whey was not slowly increased. The performance was compared to the control reactor C3. The feed substrate ratio and the resulting HRT and organic loading rate are described in Table 4.5

Table 4.5: Combination of used feed substrates and resulting HRT and OLR in reactor StW1 and the control reactor C3

Reactor	Volume	Amount of feedstock added daily		Ratio	HRT	OLR
	ml	Primary sludge	Stored whey	PS:W	days	kg/m^3 /day
C3	500	25 ml	-	1:0	20.0	3.18
StW1	500	25 ml	7 ml	10:3	15.6	3.59

The average pH and biogas production from the daily recording over the time of the experiment are displayed in Table 4.6 as well as the average alkalinity measured weekly.

Table 4.6: Average characteristics found in reactor StW1 compared to the control reactor C3

Reactor	Ratio	pH	Alkalinity	Biogas production	
	PS:W		mg/l	ml/l/day	% of control
C3	1:0	6.94	3,611	998	100
StW1	10:3	6.99	3,625	1,494	150

It was observed that the addition of stored whey to the reactor previously fed with primary sludge resulted in a higher pH than in the control reactor (see Table 4.6). The average alkalinity in the control reactor's effluent was found to be 3,611 mg/l, while the reactor receiving primary sludge and whey (StW1) had an almost unchanged average alkalinity of 3,625 mg/l (see Table 4.6). This shows that the process was stable despite the addition of acidic whey.

The experiment showed that the anaerobic bacteria in the digesters did not require acclimatisation to cope with the addition of stored whey at the ratio of 10:3 (PS:W). The co-digestion process with stored casein whey (StW1) resulted in a significantly (p-value=0.00) increased biogas production (150 %) in comparison to the control reactor

(see Table 4.6). The fresh whey reactor FW1 at the same ratio produced 93 % of the control's biogas production (see Table 4.3). The stored whey reactor StW1 produced significantly (p-value=0.00) more biogas than the fresh whey reactor FW1 at the same ratio (10:3). This suggests that the lactose fermentation during storage of stored whey into intermediates like L-lactate, acetate and formate (see page 80) may have improved the biogas production. These intermediates could have served as a more direct substrate for the production of methane, making hydrolyses of lactose unnecessary.

4.3 Can cow manure help to improve biogas production from primary sludge and casein whey?

As the literature suggests that cow manure can add stability through adding alkalinity (controlling pH) and nutrients (Lo et al., 1988; Lo & Liao, 1989) to the anaerobic digestion of whey, experiments were carried out to study the influence on the co-digestion of primary sludge with fresh as well as stored casein whey and cow manure.

4.3.1 What ratio of manure to fresh whey is required?

Co-digestion with acclimatisation

In this experiment reactors were fed with primary sludge, fresh casein whey and two different amounts of cow manure, 10 and 20 ml. The amount of whey fed into the reactors was increased from 20 to 70 ml to allow acclimatisation of the anaerobic bacteria to acidic whey.

The performance of the reactors was compared to the control reactor C1. The exact feedstock combination is described in Table 4.7.

Table 4.7: Characteristics of reactors FW+CM1 and FW+CM2 compared to the control reactor C1

		Ini-tial	Week 5-8	Week 9-12	Week 13-15	Week 16-18	Week 19-21	Week 22-24	Ave-rage
Amount of feedstock added (ml)	**PS**	100	100	100	100	100	100	100	-
	W	0	20	30	40	50	60	70	-
	CM in FW+CM1	0	10	10	10	10	10	10	-
	CM in FW+CM2	0	20	20	20	20	20	20	-
Ratio (PS:W:CM)	**C1**	1:0:0	1:0:0	1:0:0	1:0:0	1:0:0	1:0:0	1:0:0	-
	FW+CM1	1:0:0	10:2:1	10:3:1	10:4:1	10:5:1	10:6:1	10:7:1	-
	FW+CM2	1:0:0	10:2:2	10:3:2	10:4:2	10:5:2	10:6:2	10:7:2	-
HRT (days)	**C1**	20	20	20	20	20	20	20	-
	FW+CM1	20	15.4	14.3	13.3	12.5	11.8	11.1	-
	FW+CM2	20	14.3	13.3	12.5	11.8	11.1	10.5	-
OLR (kg/m^3 /day)	**C1**	1.42	1.09	1.45	1.33	1.38	1.83	1.03	1.36
	FW+CM1	1.42	1.78	2.78	2.60	3.03	4.49	4.68	3.23
	FW+CM2	1.42	2.08	3.01	3.09	3.65	5.35	6.44	3.43
pH	**C1**	6.79	6.80	6.84	6.80	6.76	6.79	6.86	6.81
	FW+CM1	6.75	6.78	6.81	6.78	6.68	6.70	6.70	6.74
	FW+CM2	6.77	6.80	6.86	6.80	6.75	6.70	4.98	6.78
Biogas production (ml/l/day)	**C1**	1,076	1,052	1,290	1,219	1,071	1,395	1,854	1,313
	FW+CM1	1,107	1,662	1,877	1,860	1,925	2,356	3,705	2,071
	FW+CM2	1,007	1,594	2,223	2,057	2,305	2,718	489	1,984
Biogas prod. (% of control)	**C1**	100	100	100	100	100	100	100	100
	FW+CM1	103	158	145	153	180	169	200	158
	FW+CM2	94	152	172	169	215	195	26	166

The fluctuations in the loading rate of the control reactor were a result of fluctuating primary sludge COD (see Table 4.7). The whey reactors FW+CM1 and FW+CM2 had a higher loading rate than the control reactor and with increasing amounts of whey, the loading rate increased further as seen in Table 4.7. As FW+CM2 received more whey and therefore more feed substrate, it had the highest loading rate (see Table 4.7). The HRT decreased from the initial 20 days to 11.1 days in FW+CM1 and to 10.5 days in FW+CM2 (see Table 4.7).

Figure 4.1 displays the biogas production achieved in the reactors compared to the control reactor's biogas production for each increase in the amount of whey fed.

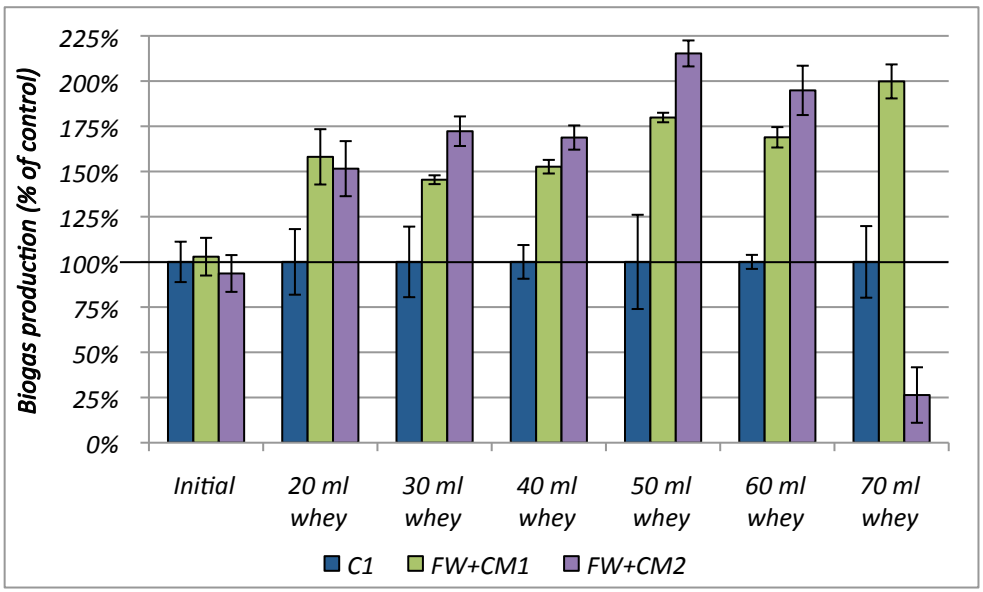

Figure 4.1: Average biogas production of FW+CM1 and FW+CM2 over increasing amounts of whey in % of biogas production achieved in the control reactor C1 with indication of standard deviation.

Where: C1 1:0:0
FW+CM1 10:2:1 – 10:7:1
FW+CM2 10:2:2 – 10:7:2

It was found that the addition of increasing amounts of fresh casein whey and cow manure improved the biogas production from primary sludge as seen in Figure 4.1. The reactor FW+CM1 (10:2:1-10:7:1) was found to have a significantly improved average biogas production (p-value=0.00) in comparison to the average of the control reactor. The biogas production reached a maximum of 200 % of the control when 70 ml of whey was added to the reactor (see Table 4.7). A higher biogas production was found in the reactor FW+CM2 (10:2:2-10:7:2) with the higher cow manure intake (Figure 4.1), however the reactor became instable. After 20 weeks of the experiment it failed, as described later. In reactor FW+CM2, the highest whey loading achieved during stable operation was 50 ml and the resulting biogas production reached 215 % of the control (see Table 4.7). It was found that the highest whey intake under stable operation resulted in the highest biogas production (see Figure 4.1).

The quality of the biogas produced showed that the addition of the co-substrates fresh whey and cow manure to primary sludge resulted in a significantly (p-value=0.01 and 0.00) higher concentration of methane in the biogas produced from FW+CM1 and FW+CM2 than in the control reactor. The highest average methane concentration was found in the reactor fed with whey and 20 ml of cow manure (FW+CM2) with 62.8 %

methane. The concentration in the whey with ten ml cow manure reactor (FW+CM1) was 60.9 %. The control reactor produced biogas with an average of 57.8 % methane. The increase in whey over the time of the experiment did not have an influence on the methane content.

The pH in the reactors receiving primary sludge, increasing amounts of fresh whey and cow manure (FW+CM1 and FW+CM2) is displayed in Figure 4.2. For comparison purposes the pH in the control reactor C1 is shown as well.

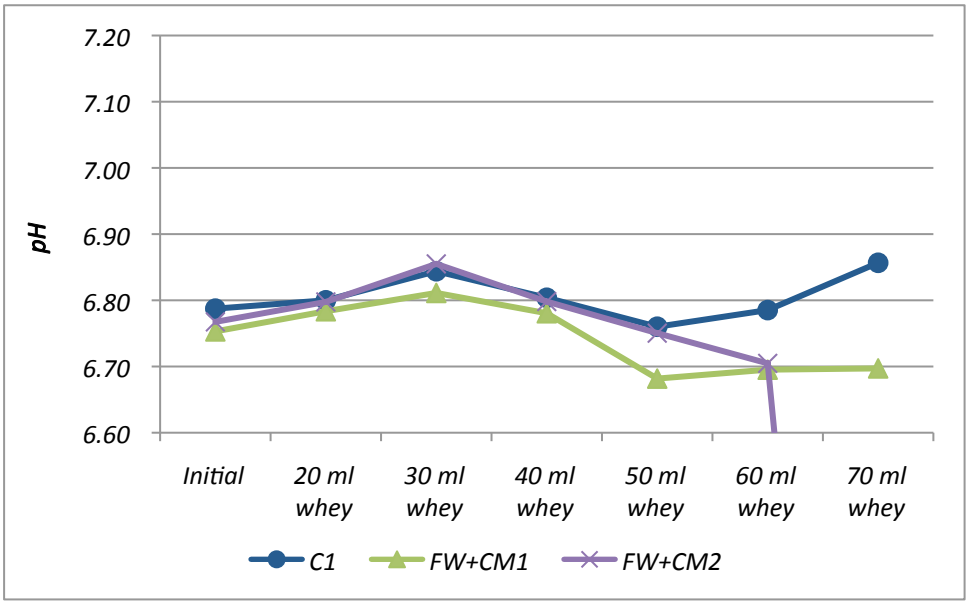

Figure 4.2: Average pH of the reactors FW+CM1, FW+CM2 with different amounts of fresh whey added and the control reactor C1

Where: **C1** **1:0:0**
FW+CM1 **10:2:1 – 10:7:1**
FW+CM2 **10:2:2 – 10:7:2**

The pH in both reactors, FW+CM1 and FW+CM2, followed the fluctuations of the control reactor (C1) for most of the time and remained fairly stable with increasing amounts of whey added as seen in Figure 4.2. As primary sludge was the only substrate added to all reactors, it appeared to be mainly responsible for the pH. The reactor FW+CM2 followed this trend until 60 ml of whey was added. At this point the pH in the reactor dropped suddenly and did not recover (see purple line in Figure 4.2).

The reactor FW+CM2 fed with 100 ml primary sludge, increasing amounts of casein whey and 20 ml cow manure failed when 60 ml of whey was added. The strongest indication for failure was the pH, which eventually dropped to 4.88. The pH as well as the biogas production and the concentration of VFA are displayed in Figure 4.3 and Table 4.8.

Table 4.8: Decrease of biogas production, methane content and COD removal in response to the drop of pH in reactor FW+CM2

	Amount of whey added	pH	VFA	Biogas production	Methane content
	ml		mg/l	ml/l/day	%
Week 17	50	6.73	0	2,496	64.4
Week 18	50	6.77	45	2,198	-
Week 19	60	6.81	not measured	2,928	62.3
Week 20	60	6.84	196	2,937	-
Week 21	60	6.47	4,228	2,290	55.9
Week 22	70	5.08	7,602	542	15.3
Week 23	70	4.89	11,533	436	-

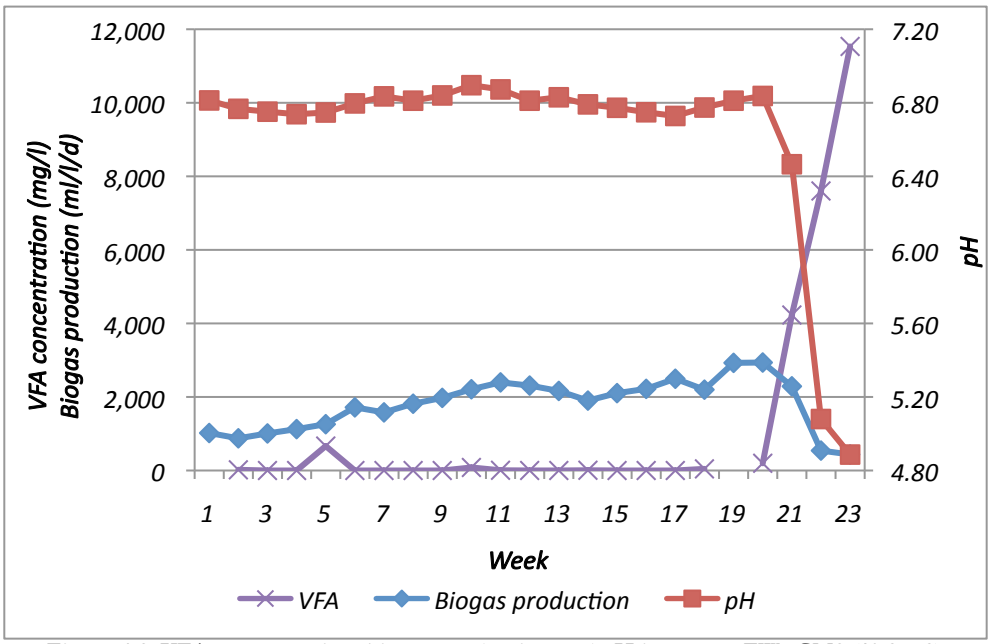

Figure 4.3: VFA concentration, biogas production and pH in reactor FW+CM2 (10:2-7:2)

Concentrations of total VFA below 1,000 mg/l are suggested to be healthy, and higher concentrations can be seen as an indication for an imbalance occurring in the process (Ghaly & Ramkumar, 1999). A significant increase in VFA from 45 mg/l (week 18) to well above 7,500 mg/l (week 22 onwards) was observed in the reactor FW+CM2 (see Table 4.8 and Figure 4.3), indicating imbalance. The continued addition of whey, which is easily degraded to acetate and other VFA may have contributed to the steep increase in unfermented VFA in the reactor. High levels of VFA in the reactor can inhibit the anaerobic digestion process as seen in Figure 2.2 (page 18). The pH was observed to start dropping shortly after the VFA increase from 6.84 (week 20) to 5.08 (week 22) (also seen in Table 4.8 and Figure 4.3). This was seen as a result of excess VFA in the

reactor, which could not be buffered any more, and the reactor failed. Reactor failure after an initial indication of increased VFA concentration to well above the recommended level has also been described by Burke (2001), Ghaly and Ramkumar (1999), Göblös et al. (2008) and Hansen and Cheong (2007).

As a result of the increasingly unstable reactor performance, a decrease in biogas production (see Figure 4.3) and the methane content in the gas was observed (see Table 4.8). The biogas production started to drop at the same time as the pH did as illustrated in Figure 4.3 and Table 4.8. Within two weeks of the first indication of failure, the daily biogas production had dropped to 500 ml/l/day. The decreased methane content may have contributed to the steep pH drop in the reactor as well. It is assumed that with decreasing methane content more CO_2 was produced. Increasing amounts of CO_2 in the reactor lead to further decrease in pH as CO_2 in the reactor consumes alkalinity. The reduction in pH would shift the bicarbonate equilibrium to CO_2, which would make even more CO_2 available. Increasing amounts of CO_2 would also lead to an increase in partial pressure in the reactor, which could have triggered the drop in pH (Speece, 1996).

The failure of the reactor FW+CM2 was not solely attributed to the increasing amounts of whey. The cause for the break down was also associated with the amount of cow manure added. It was observed that there was an increasingly thick layer of solid, fibrous material floating on the surface of the reactor, which could be controlled only by more rapid mixing. Blocking of the mixing device occurred occasionally resulting in stratification in the reactor and as a result the reactor performance suffered. Eventually the accumulation of floating material in the reactor blocked the outlet. This caused a build-up of gas underneath resulting in such high gas pressure that the lid blew off and the reactor content was dispersed.

In comparison, ten ml of cow manure as co-substrate with primary sludge and increasing amounts of fresh casein whey (FW+CM1) showed improved biogas production as well as good reactor health. Even though the overall gas production was lower than when 20 ml of manure was added, the reactor acclimatised to increasing amounts of whey and was found to have a stable pH level (Figure 4.2) even when the amount of whey reached 70 ml.

Co-digestion <u>without</u> acclimatisation

In this experiment, reactors were fed with 100 ml primary sludge and 10 ml of cow manure as well as 30, 50 or 70 ml of fresh casein whey respectively. Acclimatisation to whey was not applied. In two reactors the amount of cow manure added was dropped from ten to one ml after nine weeks of stable operation.

The performance of the reactors was compared to the control reactor C2 fed with primary sludge only. The feeding scheme of the reactors and the resulting HRT and organic loading rate are described below in Table 4.9.

Table 4.9: Combination of used feed substrates and resulting HRT and OLR in reactors FW+CM3, FW+CM4, FW+CM5, FW+CM6 and the control reactor C2

Reactor		Volume	Amount of feedstock added daily			Ratio	HRT	OLR
		ml	Primary sludge	Fresh whey	Cow manure	PS:W:CM	days	kg/m^3 /day
C2		2,000	100 ml	-	-	1:0:0	20.0	2.92
FW+CM3		2,000	100 ml	30 ml	10 ml	10:3:1	14.3	4.53
FW+CM4		2,000	100 ml	30 ml	10 ml	10:3:1	14.3	4.44
	CM dropped	2,000	100 ml	30 ml	1 ml	10:3:0.1	14.3	4.23
FW+CM5		2,000	100 ml	70 ml	10 ml	10:7:1	11.1	5.53
FW+CM6		2,000	100 ml	50 ml	10 ml	10:5:1	12.5	5.08
	CM dropped	2,000	100 ml	50 ml	1 ml	10:5:0.1	12.5	4.69

The more whey and cow manure fed into the reactors, the lower the HRT and the higher the OLR. Thus the highest loading of 5.3 kg/m^3/day and the lowest HRT of 11.1 days was applied to reactor FW+CM5.

Table 4.10 shows the average pH, biogas production, methane content in the biogas produced and the alkalinity of the fresh whey reactors FW+CM3, FW+CM4, FW+CM5, FW+CM6 and the control reactor C2.

Table 4.10: Average characteristics found in the reactors FW+CM3, FW+CM4, FW+CM5, FW+CM6 compared to the control reactor C2

Reactor	Ratio	pH	Alkalinity	Biogas production		Methane content	Methane production
	PS:W:CM		mg/l	ml/l /day	% of control	%	ml/l/day
C2	1:0:0	6.92	3,165	1,046		62.07	649
FW+CM3	10:3:1	7.00	4,168	1,590	152	62.83	999
FW+CM4	10:3:1	Not measured		1,698	161	Not measured	
	10:3:0.1	6.94	3,452	1,439	138	63.49	914
FW+CM5	10:7:1	Failed					
FW+CM6	10:5:1	6.98	4,078	1,860	178	64.58	1,201
	10:5:0.1	Failed					

It was found that the applied ratios of 10:3:1, 10:3:0.1 and 10:5:1 (PS:W:CM) operated at stable pH and led to improved biogas production (see Table 4.10). It was observed that the addition of fresh whey and cow manure to primary sludge (FW+CM3 and FW+CM4) at the ratio 10:3:1 resulted in a significantly higher (p-value=0.00 and 0.00) biogas production compared to the control reactor. 152 to 161 % of the biogas production achieved in the control reactor was found in FW+CM3 and FW+CM4 (see Table 4.10).

The highest biogas production rate of 178 % of the biogas production of the control reactor was achieved in the reactor FW+CM6 when fed with 100 ml primary sludge, 50 ml of whey and 10 ml cow manure (see Table 4.10). More whey, such as in reactor FW+CM5 with 70 ml of fresh whey (10:7:1) failed within a short time. Thus it appeared that at this ratio (10:7:1) without acclimatisation, the whey intake was too high while 10:5:1 was stable and produced more biogas than the control reactor and the reactor with less whey. In the reactor FW+CM1, the ratio of 10:7:1 was achieved successfully after slow increase of the whey intake and produced 200 % of the biogas produced in the control reactor as seen in Table 4.7.

Ten times less cow manure in the feed resulted in 23 % less biogas produced. In the reactor FW+CM4 it was found that the biogas production changed significantly (p-value=0.00) when the amount of cow manure was reduced from ten to one ml. It dropped from 161 % before to 138 % after the reduction (see Table 4.10). At the ratio

10:5:1 it was found that lowering the amount of cow manure to the ratio 10:5:0.1 resulted in reactor failure.

No correlation between the different feedstock ratios was found for the methane content in the biogas produced by the reactors as seen in Table 4.10.

As the biogas production depends on the COD removed (Tchobanoglous et al., 2003), the average total and soluble COD removal in the fresh whey reactors in comparison to the control reactor is displayed in Table 4.11.

Table 4.11: Average COD removed in the reactors FW+CM3, FW+CM4, FW+CM5, FW+CM6 compared to the control reactor C2

Reactor	Ratio	Total COD		Soluble COD	
		Removed	Removal	Removed	Removal
	PS:W:CM	mg/l/d HRT	%	mg/l/d HRT	%
C2	1:0:0	1,524	52.56	41	42.70
FW+CM3	10:3:1	2,005	44.60	1,053	89.16
FW+CM4	10:3:1	Not measured			
	10:3:0.1	2,081	49.28	1,058	92.54
FW+CM5	10:7:1	Failed			
FW+CM6	10:5:1	2,259	45.94	1,282	90.93
	10:5:0.1	Failed			

The addition of casein whey and cow manure to primary sludge increased the amount of total and soluble COD entering the whey reactors. However, it was found that the amount of total and soluble COD removed (expressed in mg of COD removed per litre reactor volume per day HRT) was higher in all fresh casein whey reactors than in the control reactor (see Table 4.11). This was to be expected after it was found that the biogas production from the whey reactors was higher than the control (see Table 4.10). The highest portion of COD removed (mg/l/d HRT) was found in the reactor FW+CM6, which also produced the most biogas (see Table 4.10).

The total COD removal efficiency was found to be lower in the fresh whey reactors than in the control while the soluble COD removal efficiency more than doubled (see Table 4.11). As fresh whey was found almost 100 % soluble (see Table 4.4) an improved soluble COD removal was to be expected, however, a lower total COD removal could not have been due to the addition of fresh whey to the reactors. It was noticed that the

reactor FW+CM4 (10:3:0.1), which received only ten percent of the cow manure the other fresh whey reactors received, had the highest total and soluble COD removal efficiency (see Table 4.11). Therefore, the addition of cow manure with only 19 % of its high total COD of 142,978 mg/l (see Table 3.6 page 9) must be held responsible for lower total COD removal efficiencies in the fresh whey reactors compared to the control.

Figure 4.4 shows the daily measured pH on a weekly average in the fresh whey reactors FW+CM3, FW+CM4, FW+CM5, and FW+CM6 compared to the control reactor C1.

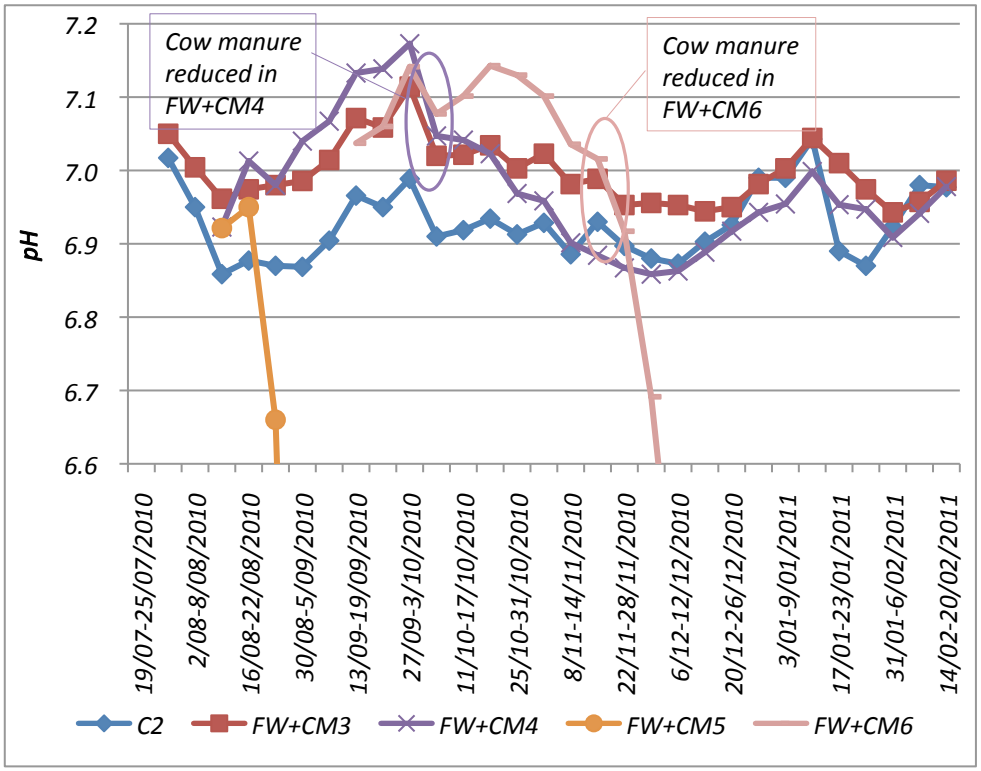

Figure 4.4: Daily measured pH on weekly average in the fresh whey reactors FW+CM3, FW+CM4, FW+CM5 and FW+CM6 in comparison to the control reactor C2.

Where:	**C2**	**1:0:0**
	FW+CM3	**10:3:1**
	FW+CM4	**10:3:1 → 10:3:0.1**
	FW+CM5	**10:7:1**
	FW+CM6	**10:5:1 → 10:5:0.1**

As seen in Figure 4.4, the addition of fresh whey and cow manure to primary sludge resulted in a stable pH of on average seven or above in the reactors FW+CM3, FW+CM4 and FW+CM6 before the cow manure reduction. This was higher than the control reactor's average pH of 6.92 (see Table 4.10). No acclimatisation was found necessary for the amount of fresh whey added to the reactors at the ratios 10:3:1 and 10:5:1.

The reactor FW+CM5 receiving 70 ml of fresh casein whey in a substrate ratio of 10:7:1 failed within three weeks of start-up. A significant drop in pH within a week of introduction of co-substrates was observed as displayed in Figure 4.4 and the pH continued to drop to 4.88 and the reactor was declared to have failed. This suggests that 70 ml of fresh whey cannot be digested without prior acclimatisation at the ratio tested.

When the amount of cow manure in reactor FW+CM4 was reduced to one ml (indicated by the purple circle in Figure 4.4) after nine weeks of successful operation, the pH in the reactor dropped in response to an average pH of 6.94 (see Table 4.10), which was not an alarming level. In reactor FW+CM6, the cow manure was reduced after ten weeks of stable operation (pink circle in Figure 4.4) and again the pH dropped in response. In this case the reactor pH kept dropping to a pH below six and reactor failure was established within 26 days from reducing cow manure. Due to failure of FW+CM6 once cow manure was reduced from ten to one ml, it was observed that the correct whey to cow manure ratio is important.

To achieve improved biogas production from primary sludge, fresh casein whey and cow manure without acclimatisation, it was found that a ratio of 10:5:1 was the upper limit of the ratios tested. More whey (10:7:1) could only be achieved with acclimatisation otherwise led to failure. Less cow manure could only be applied when less whey (10:3:1) was utilised.

4.3.2 Can cow manure further improve co-digestion of primary sludge with stored whey?

Reactors were fed with different ratios of primary sludge to stored whey to cow manure. Stored whey was added to the reactors without prior acclimatisation. The performance of the reactors was compared to the control reactor C2. Table 4.12 shows the HRT and organic loading rate resulting from the different feed ratios.

Table 4.12: Combination of used feed substrates and resulting HRT and OLR in the reactors St+CM1, St+CM2, St+CM3 and the control reactor C2

Reactor		Volume	Amount of feedstock added daily			Ratio	HRT	OLR
		ml	Primary sludge	Stored whey	Cow manure	PS:W:CM	days	kg/m^3 /day
C2		2,000	100 ml	-	-	1:0:0	20.0	2.92
StW+CM1		2,000	100 ml	30 ml	10 ml	10:3:1	14.3	4.18
StW+CM2		2,000	100 ml	30 ml	10 ml	10:3:1	14.3	4.13
	CM dropped	2,000	100 ml	30 ml	1 ml	10:3:0.1	14.3	3.84
StW+CM3		2,000	100 ml	70 ml	10 ml	10:7:1	11.1	4.90
	CM dropped	2,000	100 ml	70 ml	1 ml	10:7:0.1	11.1	4.39

With greater amounts of stored casein whey, the HRT decreased while the loading rate increased as seen in Table 4.12. Dropping the amount of cow manure in StW+CM2 and StW+CM3 from ten to one ml consequently meant a lower loading rate. The highest achieved loading was 4.9 kg/m^3/day and an HRT of 11.1 days in the reactor StW+CM3 (10:7:1) (see Table 4.12).

The average pH and biogas production from the daily recording over the time of the experiment is displayed in Table 4.13 as well as the average alkalinity measured weekly.

Table 4.13: Average characteristics found in the reactors St+CM1, St+CM2, St+CM3 compared to the control reactor C2

Reactor	Ratio	pH	Alkalinity	Biogas production		Methane content	Methane production
	PS:W:CM		mg/l	ml/l vol/day	% of control	%	ml/l vol/day
C2	1:0:0	6.92	3,165	1,046	100	62.07	649
StW+CM1	10:3:1	7.08	4,713	1,377	132	62.81	865
StW+CM2	10:3:1	Not measured		1,448	137	Not measured	
	10:3:0.1	7.02	4,241	1,200	115	62.70	752
StW+CM3	10:7:1	7.00	4,172	1,490	142	63.30	943
	10:7:0.1	Failed					

It can be seen in Table 4.13 that with the addition of stored whey and cow manure at a ratio of 10:3:1 the biogas production increased to 132 to 137 % of the control's biogas production. Significantly (p-value=0.02) more biogas (142 %) was produced in the reactor receiving more whey and the same amount of cow manure StW+CM3 (10:7:1) when compared to StW+CM1 (10:3:1) (see Table 4.13).

Lowering cow manure from ten to one ml in the reactors StW+CM2 and StW+CM3 resulted in both reactors in a significant reduction in biogas production (StW+CM2 p-value=0.01 and StW+CM3 p-value=0.00). In reactor StW+CM2 with ten times less cow manure (ratio 10:3:0.1) 115 % of the biogas produced in the control reactor was achieved (see Table 4.13). In reactor StW+CM3 (10:7:1) the biogas production dropped and the reactor failed when cow manure was reduced (see Table 4.13).

As found for the reactors fed with fresh whey, no correlation between the ratios of primary sludge to whey to cow manure and the resulting methane content in the biogas produced was observed. There was also no increase found in the methane content when comparing the whey reactors to the control reactor as seen in Table 4.13.

The removal of total and soluble COD achieved in the stored casein whey reactors is displayed in Table 4.14.

Table 4.14: Average COD removed in the reactors St+CM1, St+CM2, St+CM3 compared to the control reactor C2

Reactor	Ratio	Total COD		Soluble COD	
		Removed	Removal	Removed	Removal
	PS:W:CM	mg/l/d HRT	%	mg/l/d HRT	%
C2	1:0:0	1,524	52.56	41	42.70
StW+CM1	10:3:1	1,759	42.17	663	82.93
StW+CM2	10:3:1	Not measured			
	10:3:0.1	1,951	50.24	617	87.65
StW+CM3	10:7:1	1,291	26.42	1,325	79.08
	10:7:0.1	Failed			

Despite the increased incoming COD into the stored whey reactors due to the addition of stored casein whey and cow manure, the COD removed, both total and soluble, was higher in the whey reactors than in the control (see Table 4.14). As a consequence of more COD removed in the whey reactors the biogas production of those was higher (see Table 4.13). Only in the reactor StW+CM3 (10:7:1), the total COD removed was lower than in the control reactor (see Table 4.14). The removal efficiency was only about half of the control (see Table 4.14). The lower COD removal did not match the relatively high biogas production from this reactor (see Table 4.13).

As the influent COD into the whey reactors was higher but the amount removed only accounted for 230 and 430 mg/l/d (StW+CM1 and StW+CM2), the COD removed efficiency was lower than the removal rate in the control reactor (see Table 4.14). However, as stored whey was found to be 97 % soluble (see Table 4.4), the amount of soluble material removed from the reactors increased by more than 550 mg/l/d in StW+CM1 and StW+CM2 and by 1,280 mg/l/d in StW+CM3, which resulted in an removal efficiency almost double compared to the control reactor (see Table 4.14).

Figure 4.5 displays the daily measured pH on weekly average in the stored whey reactors StW+CM1, StW+CM2 and StW+CM3 in comparison to the control reactor C2. The day on which the amount of cow manure was reduced is marked with ovals.

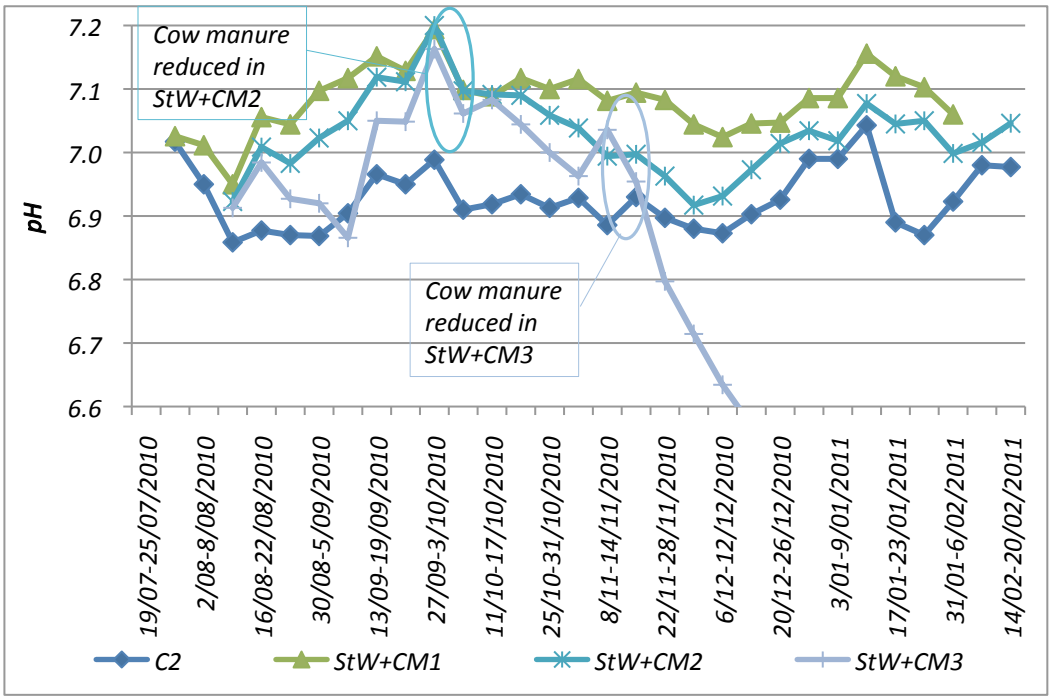

Figure 4.5: Daily measured pH on weekly average in the stored whey reactors StW+CM1, StW+CM2 and StW+CM3 in comparison to the control reactor C2.

Where:	**C2**	**1:0:0**
	StW+CM1	**10:3:1**
	StW+CM2	**10:3:1 → 10:3:0.1**
	StW+CM3	**10:7:1 → 10:7:0.1**

The average pH over the time of the experiment in the control reactor was 6.92, while all stored whey and cow manure reactors displayed a pH of at least 7 and higher at stable operation (see Table 4.13). It was observed that the reactor StW+CM3 (10:7:1) was stable until the amount of cow manure was reduced, which eventually resulted in failure. When the amount of cow manure was dropped from ten to one ml (indicated by the light blue circle in Figure 4.5), the pH started decreasing within a week and after four weeks the pH had dropped below 6.6 (see Figure 4.5). Eventually reaching pH 5.4,

the reactor was declared failed. This was attributed to the reduction of cow manure. The co-digestion of stored casein whey at a ratio of 10:7:1 was stable but could not be operated at ten times less cow manure. Thus as already found for the fresh whey reactors (compare to page 88), a certain amount of stored whey also requires a certain amount of cow manure for stable operation.

This experiment showed that the anaerobic bacteria in the digesters did not require acclimatisation to stored casein whey at the ratios applied. In contrast to the fresh casein whey reactors it was found possible to digest up to 70 ml of stored whey in a ratio of 10:7:1 without acclimatisation, while the reactor with fresh whey failed at this ratio (see Table 4.10).

In comparison, the reactor StW1 with primary sludge, stored casein whey and no manure at a ratio of 10:3 produced 150 % of the control's biogas production (see Table 4.6) while the highest biogas production from primary sludge, stored casein whey with additional cow manure StW+CM3 accounted for 142 % at the ratio 10:7:1 (see Table 4.13). Even though this difference was not significant (p-value=0.44), it showed that for an improved biogas production from stable anaerobic co-digestion of primary sludge with stored casein whey, cow manure was not essential. Lo et al. (1988) and Lo and Liao (1989) assumed cow manure would provide stability to the co-digestion of whey. However, this was found not important when co-digesting primary sludge with stored casein whey at the ratio tested. The addition of cow manure to stored whey and primary sludge was actually found to result in a lower biogas production than without.

4.3.3 Can compost leachate work as an alternative to cow manure?

In this experiment a reactor was fed with primary sludge, increasing amounts of fresh casein whey and compost leachate instead of cow manure. Table 4.15 displays the feedstock ratio and the resulting HRT and OLR of the reactor FW+CL1 in comparison to the control C1 as well as the performance of both.

Table 4.15: Characteristics of the reactor FW+CL1 compared to the control reactor C1

		Ini-tial	Week 9-12	Week 13-15	Week 16-18	Week 19-21	Week 22-24	Ave-rage
Amount of feedstock added (ml)	**PS**	100	100	100	100	100	100	-
	W	0	20	30	40	50	60	-
	CL	0	20	20	20	20	20	-
Ratio (PS:W:CL)	**C1**	1:0:0	1:0:0	1:0:0	1:0:0	1:0:0	1:0:0	-
	FW+CL1	1:0:0	10:2:2	10:3:2	10:4:2	10:5:2	10:6:2	-
HRT (days)	**C1**	20	20	20	20	20	20	-
	FW+CL1	20	14.3	13.3	12.5	11.8	11.1	-
OLR ($kg/m^3/day$)	**C1**	1.09	1.45	1.33	1.38	1.83	1.03	1.41
	FW+CL1	1.09	2.30	2.04	2.39	3.46	3.39	2.72
Biogas production (ml/l/day)	**C1**	1,052	1,290	1,219	1,071	1,395	1,854	1,313
	FW+CL1	1,171	1,132	1,271	1,229	1,281	1,658	1,314
Biogas prod. (%)	**C1**	100	100	100	100	100	100	100
	FW+CL1	111	88	104	115	92	89	100

With the increasing organic loading rate, the HRT in the reactor FW+CL1 decreased from 20 days initially to 11.8 days (see Table 4.15).

The biogas production achieved in the reactor FW+CL1 co-digesting whey and compost leachate is displayed in Figure 4.6 and compared to the control reactor C1 as well as the reactors FW+CM1 and FW+CM2.

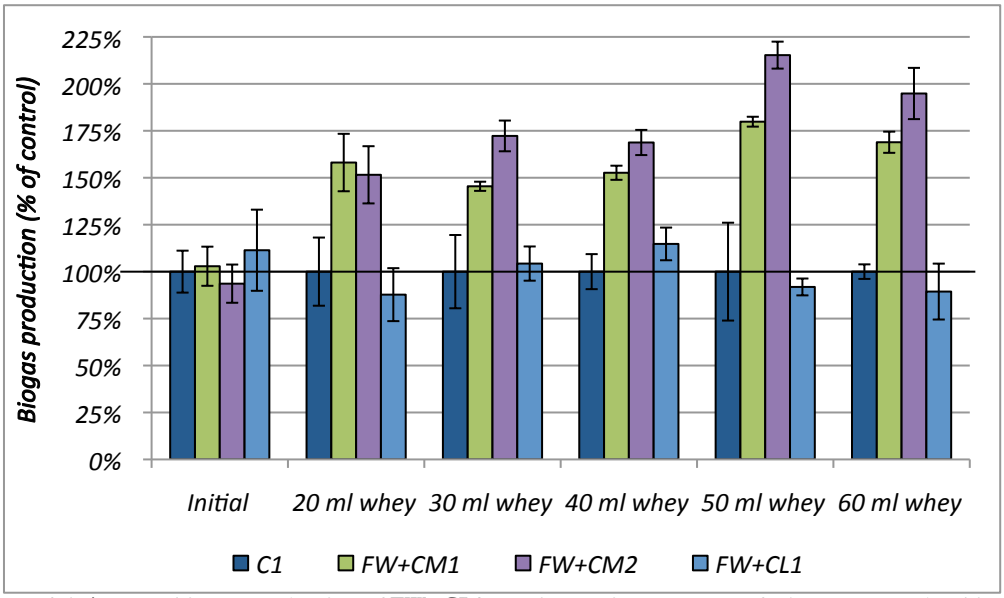

Figure 4.6: Average biogas production of FW+CL1 over increasing amounts of whey compared to biogas production in FW+CM1 and FW+CM2 in % of biogas production achieved in the control reactor C1 with indication of standard deviation.

Where:	**C1**	**1:0:0**
	FW+CM1	**10:2:1 – 10:6:1**
	FW+CM2	**10:2:2 – 10:6:2**
	FW+CL1	**10:2:2 – 10:6:2**

It was found that the biogas production in the reactor did not improve when casein whey with compost leachate (FW+CL1) was added to the main feed substrate primary sludge in comparison to the digestion of primary sludge alone as seen in Table 4.15 and Figure 4.6. Comparing the reactor with compost leachate FW+CL1 to those with cow manure FW+CM1 and FW+CM2 showed that significantly (p-value=0.00 and 0.00) more biogas was produced in the reactors receiving cow manure than in the reactor receiving compost leachate (see Figure 4.6).

The pH in the compost leachate reactor was stable over the time of the experiment and did not change notably with the addition of whey and compost leachate to the main feedstock primary sludge. The average pH and alkalinity of the reactor receiving compost leachate is displayed in Table 4.16 in comparison to the control reactor and the reactors FW+CM1 and FW+CM2.

Table 4.16: Average alkalinity in the reactors FW+CM1, FW+CM2, FW+CL1 and the control reactor C1

Reactor	Ratio	pH	Alkalinity
	PS:W:CM/CL		mg/l
C1	1:0:0	6.81	2,951
FW+CM1	10:2:1 – 10:7:1	6.74	2,928
FW+CM2	10:2:2 – 10:7:2	6.78	3,195
FW+CL1	10:2:2 – 10:6:2	6.79	2,957

When compost leachate was added to the feedstock, the pH as well as the alkalinity remained at similar levels to the pH and alkalinity in the control reactor. As seen in Table 4.16, the whey with ten ml manure reactor (FW+CM1) also had a similar average alkalinity to the control reactor. Although the buffering capacity provided by both additional co-substrates cow manure (see Table 3.5 page 52) and compost leachate (see Table 3.7 page 54) as well as the alkalinity found in the associated reactors was found to be similar, the compost leachate reactor never performed as successfully in terms of biogas production as the cow manure reactors. Comparing compost leachate with cow manure showed that the COD of the leachate (13,637 mg tCOD/l and 12,893 mg sCOD/l as seen in Table 3.7 on page 54) was much lower than of the manure (118,879 mg tCOD/l and 29,029 mg sCOD/l as seen in Table 3.5 on page 52). This could be a reason for the difference in biogas production.

Compost leachate in co-digestion of primary sludge with casein whey did not improve the biogas production and therefore is not a good alternative to cow manure.

4.4 What happens when casein whey and cow manure are combined?

As it was found that casein whey and cow manure co-digested in primary sludge digesters improved the biogas production, the following section describes the results from the research undertaken to scrutinise the reasons for such an improvement.

4.4.1 Is it possible that whey hydrolyses manure?

This experiment was designed to establish whether casein whey can act as acid solvent, hydrolysing cow manure when mixed together before fed into the reactors. The total and soluble COD of cow manure and stored whey were measured separately before they were mixed as well as the COD of the combination of the two substrates once they were mixed. The results of the COD measurements are displayed in Table 4.17

Table 4.17: Results of the acid hydrolysis experiment

	Ratio		**tCOD**	**sCOD**
	CM:W		mg/l	mg/l
Cow manure		measured	142,383	28,028
Stored whey		measured	31,698	32,884
Cow manure + stored whey	1:3	measured	66,107	33,904
		calculated	59,369	31,670

The comparison of the soluble COD measured and calculated showed that 6.6 % more soluble COD was available in the combination of whey and cow manure than expected. This would suggest that some of the total COD was hydrolysed into soluble COD. However, it was found that the measured total COD of the combination of stored casein whey and cow manure was 10.2 % higher than the expected theoretical total COD calculated. An increase of total COD was not expected and was unexplained, however, this indicates that no material from manure was hydrolysed through the addition of acidic whey.

As the difference in measured and expected soluble COD accounted only for 6.6 %, acid hydrolysis of cow manure through casein whey was assumed not to be significant.

4.4.2 Is it possible that cow manure alone improves biogas production from primary sludge?

In order to investigate the possibility that the improved biogas production found in any of the casein whey and cow manure reactors was a result of adding cow manure, a reactor was fed with primary sludge and manure only (CM1). The performance of this reactor was compared to the control reactor C2 fed with primary sludge only. The HRT

and organic loading rate in the reactor CM1 as well as the average pH, biogas production, methane concentration and alkalinity are displayed in Table 4.18.

Table 4.18: Average characteristics of the reactor CM1 compared to the control reactor C2

Reactor	Volume	HRT	OLR	pH	Biogas production	
	ml	days	kg/m^3/day		ml/l/day	%
C2	2,000	20.0	2.92	6.89	1,046	100
CM1	2,000	18.2	3.38	6.95	1,117	107

As more feedstock and COD was added to the cow manure reactor than to the control reactor, the HRT was shorter and the loading rate higher as seen in Table 4.18. The pH was with an average of 6.95 higher than in the control reactor (6.89) (see Table 4.18).

No significant increase (p-value=0.25) in biogas production resulted from the addition of cow manure to primary sludge. The cow manure reactor (CM1) produced on average 107 % of the biogas produced in the control reactor (see Table 4.18). Cow manure as a single co-substrate for anaerobic co-digestion of primary sludge was not found to result in an improved biogas production.

4.4.3 Does cow manure provide alkalinity that whey is lacking?

The literature suggests that cow manure can add alkalinity to the co-digestion of whey (Lo et al., 1988; Lo & Liao, 1989). In order to investigate this, the alkalinity of the feedstock was compared as well as the alkalinity found in the reactors. Table 4.19 shows the average alkalinity of the different feedstock and the amount fed into the reactors.

Table 4.19: Average total alkalinity of the feedstock used in the experiment

Feedstock	Total alkalinity	Amount fed
	mg/l	ml
Primary sludge	604	100
Fresh casein whey	458	30
Stored casein whey	not measureable	30
Cow manure	5,910	10/1

The amount of cow manure fed daily into the casein whey reactors accounted for ten and one percent of the amount of primary sludge added. The average alkalinity of the substrates showed that 10 ml of cow manure provided almost the same alkalinity as 100

ml primary sludge. The reactor CM1, receiving primary sludge and cow manure, was compared to selected fresh and stored whey reactors with either ten or one ml of cow manure or no cow manure to detect differences in the alkalinity. The reactors and the substrate ratios as well as the average pH, total alkalinity and bicarbonate alkalinity found in the reactors are listed in Table 4.20.

Table 4.20: Average pH, total alkalinity and bicarbonate alkalinity in the reactors CM1, FW+CM3, FW+CM4, StW+CM1, StW+CM2, StW1 and the control reactor C2

Reactor	Ratio	pH	Total alkalinity	Bicarbonate alkalinity
	PS:W:CM		mg/l	% of total
C2	1:0:0	6.92	3,165	75
CM1	10:0:1	6.95	3,469	74
FW+CM3	10:3:1	7.00	4,168	69
FW+CM4	10:3:0.1	6.94	3,452	77
StW+CM1	10:3:1	7.08	4,713	74
StW+CM2	10:3:0.1	7.02	4,241	79
StW1	10:3:0	6.99	3,625	74

When both primary sludge and cow manure were fed into reactor CM1 the alkalinity was increased compared to the control reactor C2 as seen in Table 4.20. The comparison between two reactors receiving the same amounts of primary sludge and stored whey but ten and one ml of cow manure (StW+CM1 and StW+CM2) showed that there was a significant (p-value=0.02) difference in alkalinity as a result of less manure (see Table 4.20). As the degradation of nitrogenous compounds can contribute to alkalinity (Speece, 1996), it is suggested that cow manure had an influence on the alkalinity found in the reactors.

The suggestion by the reported literature that cow manure could add alkalinity and therefore stability to reactors co-digesting whey (Lo et al., 1988; Lo & Liao, 1989) was found to be partially true. As described above, cow manure was contributing to alkalinity. Yet as the reactors co-digesting whey only or a combination of whey and cow manure were found to have a higher alkalinity than the reactor co-digesting manure only (as seen in Table 4.20), the increased alkalinity cannot solely originate from cow manure. Comparing the reactor fed with primary sludge and stored whey (StW1) without any manure with the cow manure reactor CM1 and the control reactor C2 shows that the reactor without manure had a significantly (p-value=0.35 and 0.14)

higher alkalinity than CM1 and C2 (see Table 4.20). This indicates that casein whey contributed to alkalinity.

Casein whey contains high concentration of VFA and the salts of VFA can act as alkalinity (Speece, 1996). However, VFA alkalinity does not buffer excess acids, as it does not contribute to the bicarbonate alkalinity (Speece, 1996). Casein whey also contains protein, a nitrogenous compound, nitrogen and sulphur (see 2.2.2.1 Characteristics of whey page 25ff), which degraded can add buffering capacity to the system (Speece, 1996).

However, Table 4.20 shows that, regardless of the co-substrates digested with primary sludge, the bicarbonate alkalinity, which is regarded the true alkalinity by Anderson and Yang (1992) and Speece (1996), averaged around 76 %. In the control reactor without co-substrates, the bicarbonate alkalinity accounted for 75 % of the total alkalinity. Therefore, it was assumed that the true alkalinity was coming from primary sludge. Again the presence of protein and nitrogen as well as sulphuric compounds in primary sludge (see Table 2.2 on page 22) could have provided alkalinity as nitrogen degradation and reduction of sulphate and sulphide produces alkalinity (Anderson & Yang, 1992; Speece, 1996).

4.4.4 Does cow manure provide phosphorus, nitrogen and trace elements?

Availability of phosphorus and nitrogen

Table 4.21 shows the amount of the two essential nutrients, phosphorus and nitrogen, and the total solids for cow manure and primary sludge as reported in the literature (see Table 2.9 on page 37 for cow manure and Table 2.2 on page 22 for primary sludge).

Table 4.21: TS, phosphorus and nitrogen of primary sludge reported in the literature

	Cow manure				Primary sludge		
	Freshly collected		Freshly voided	Slurry	Sewage sludge	Dry solids untreated	
	g/l	% of TS	g/kg	g/l	g/l	g/l	% of TS
TS	146	-	-	-	-	60	-
Phosphorus	-	-	-	24	27	-	-
Nitrogen	4.42	3.03	4.44	-	-	1.5	2.5
Reported by	Wen et al. (2005b)		Vanderholm (1984)	Eriksson (2001)		Tchobanoglous et al. (2003)	

The amount of phosphorus in cow manure and in primary sludge are reported to be very similar (as seen in Table 4.21). The nitrogen content of cow manure is reported to exceed the amount in primary sludge by about three times (see Table 4.24).

The theoretical calculated amounts of phosphorus and nitrogen available in the reactors FW+CM3, FW+CM4 and StW+CM1, StW+CM2 provided by primary sludge and cow manure are shown in Table 4.22. It is assumed that whey was not contributing to the amount of phosphorus and nitrogen in the reactors.

Table 4.22: Amount of phosphorus and nitrogen in the amounts of primary sludge and cow manure fed

		Reactor	
		10:3:1	**10:3:0.1**
Phosphorus	g/140 ml feedstock	2.94	2.724
	g/l reactor volume	20.99	20.79
Nitrogen	g/140 ml feedstock	0.194	0.1544
	g/l reactor volume	1.39	1.18

The amount of phosphorus and nitrogen theoretically available in the reactors exceeded the recommended levels by Speece (1996) of 10 mg of phosphorus and 50 mg of nitrogen per litre. Taking into account the amount of cow manure and primary sludge fed into the reactors on a daily basis, it was found that 10 ml of cow manure theoretically accounted for 8 % of the phosphorus and 29 % of the nitrogen fed with 100 ml primary sludge. Even without the addition of cow manure, the phosphorus and nitrogen levels in the reactors would exceed the level recommended.

Availability of trace elements and macronutrients

Table 4.23 displays some of the macronutrients and trace elements contained in cow manure and primary sludge as reported in the literature (also see Table 2.9 on page 37 for cow manure and Table 2.2 on page 22 for primary sludge).

Table 4.23: Macronutrients and trace elements reported to be found in cow manure and primary sludge

	Cow manure		**Primary sludge**	
		Slurry	Sewage sludge	Dry solids untreated
	mg/kg	mg/kg	mg/kg	mg/kg
Iron	4,288	-	49,000	17,000
Nickel	3	4.6	20	80
Cobalt	-	0.85	6.2	30
Zinc	28	154	550	1,700
Sulphur	-	4,900	9,000	-
Boron	-	52	61	-
Chromium	8	2.3	33	500
Copper	<3	24	390	800
Manganese	86	234	280	260
Molybdenum	-	4.5	6.7	4
Reported by	Garcia-Gil et al. (2000)	Eriksson (2001)		Tchobanoglous et al. (2003)

None of the trace elements or macronutrients available in cow manure exceeded the concentration found in primary sludge (see Table 4.23). This has also been reported by Eriksson (2001) and Speece (1996). The presence of trace elements, which can occur in complex forms, does not guarantee their bioavailability to microorganisms (Speece, 1996).

As the concentration of phosphorus and nitrogen as well as trace elements, in particular iron, nickel, cobalt and zinc in primary sludge is reported to be higher than in cow manure, it is suggested that the biogas production from the co-digestion of primary sludge with casein whey and cow manure was not improved by the addition of nutrients and/or trace elements from cow manure.

4.4.5 Do microorganisms from cow manure influence co-digestion of whey?

Co-digestion with whey and autoclaved cow manure

Cow manure was autoclaved without a temperature probe as described under Autoclaving cow manure on page 66 to remove inhabiting anaerobic bacteria and fungi. The autoclaved manure (without a temperature probe) was used in a reactor co-digesting primary sludge with stored casein whey (StW+autoclCM) at the ratio 10:3:1. The performance of the reactor was compared to the control reactor C3 and to the reactor StW+CM4 fed with primary sludge, stored whey and cow manure (non-autoclaved) at the ratio 10:3:1. The feedstock combination is described in Table 4.24.

Table 4.24: Combination of used feed substrates in reactors StW+CM4, StW+autoclCM and the control reactor C3

Reactor	Volume	Amount of feedstock added daily				Ratio
	ml	Primary sludge	Stored whey	Cow manure	Autoclaved cow manure	PS:W:CM
C3	500	25 ml	-	-	-	1:0:0
StW+CM4	500	25 ml	7 ml	2.5 ml	-	10:3:1
StW+autoclCM	500	25 ml	7 ml	-	2.5 ml	10:3:1

To guarantee that the conditions in the reactors StW+CM4 and StW+autoclCM remained as similar as possible, the COD, the VFA concentration and the alkalinity of the manure were observed before and after autoclaving of the manure. The difference was found to be insignificant for COD (p-value=0.29), VFA concentration (p-value=0.33) and alkalinity (p-value=0.19).

The HRT and organic loading rate as well as the pH and biogas production found in the reactors StW+CM4, StW+autoclCM and the control C3 are displayed in Table 4.25.

Table 4.25: OLR, HRT, pH and biogas production of the reactors StW+CM4 and StW+autoclCM compared to the control reactor C3

Reactor	HRT	OLR	pH	Biogas production	
	days	kg/m^3/day		ml/l/day	%
C3	20.0	3.18	6.94	998	100
StW+CM4	14.5	4.15	6.96	1,468	147
StW+autoclCM	14.5	4.18	6.97	1,159	116

The pH in all reactors was found to be stable and the introduction of autoclaved cow manure to the reactor did not cause a response in pH.

The reactor StW+CM4 produced 147 % of the biogas that the control reactor produced (see Table 4.25). The reactor StW+CM4 had the same HRT as the reactor StW+CM1 at a total volume of 500 ml. Designed to show that the experiment was repeatable; the reactor StW+CM4 produced more biogas and had a lower pH than StW+CM1 at the same ratio of 10:3:1. StW+CM1 produced 132 % (see Table 4.13). As the reactor StW+CM4 was run at a different time small changes in COD and pH of the primary sludge were held responsible for the differences found. The organic loading rate applied to the reactor StW+CM4 was found to be higher than the one applied to StW+CM1. This appears to be the reason for the increased biogas production.

The biogas production in the reactor co-digesting autoclaved cow manure (StW+autoclCM) was significantly (p-value=0.00) higher than in the control reactor C3 (see Table 4.25). Compared to the reactor with non-autoclaved cow manure (StW+CM4) it had a significantly (p-value=0.00) lower biogas production (see Table 4.25), even though the amount of COD available to the anaerobic bacteria in both was the same. When compared to reactor StW1, co-digesting stored whey only at the ratio 10:3, which produced 150 % of the control's biogas (see Table 4.6), it was found that no cow manure in the feed combination (StW1) resulted in a better biogas production than autoclaved cow manure (StW+autoclCM). The anaerobic bacteria in both the autoclaved cow manure reactor (StW+autoclCM) and the stored whey reactor (StW1) had the same amount of COD available from primary sludge and easily degradable whey, while the first had additional COD available from cow manure. Despite this the autoclaved cow manure reactor (StW+autoclCM) performed worse in terms of biogas production than the stored whey reactor without any cow manure (StW1). Therefore, the autoclaving process and thus the removed microorganisms appeared to have had an influence on the biogas production and in particular on the digestion of the cow manure itself.

Microorganism removal from cow manure

In order to assess whether all or which microorganisms were removed from the cow manure through the autoclaving process applied, further tests were carried out. Non-

autoclaved cow manure and manure autoclaved without a temperature probe was spread on plate count agar and cultivated anaerobically at 30 °C according to Collins and Lyne (1984). Figure 4.7 and Figure 4.8 show the growth found on the agar plates with cow manure and autoclaved cow manure.

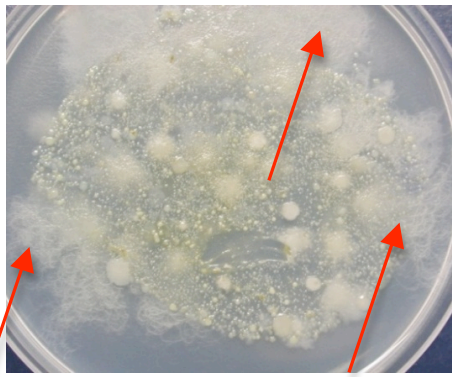

Figure 4.7: Non-autoclaved cow manure on agar

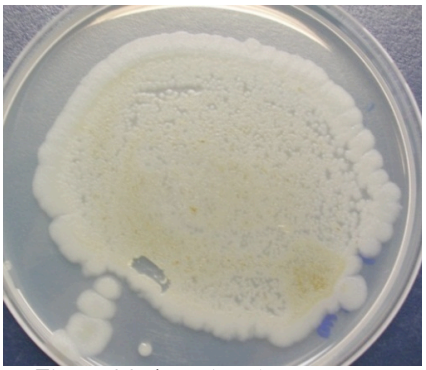

Figure 4.8: Autoclaved cow manure (without a temperature probe) on agar

Mould was found on the agar with non-autoclaved cow manure (see arrows in Figure 4.7) but not on the agar plate inoculated with cow manure autoclaved without a temperature probe (Figure 4.8). This indicated that the autoclaving process applied eliminated the fungi from cow manure.

To assess the type of bacteria present in the manure autoclaved without a temperature probe, gram-staining of the growth found on the agar plates was applied according to Collins and Lyne (1984). The results of gram-staining samples of both cow manure and autoclaved cow manure for comparison are shown in Figure 4.9 and Figure 4.10.

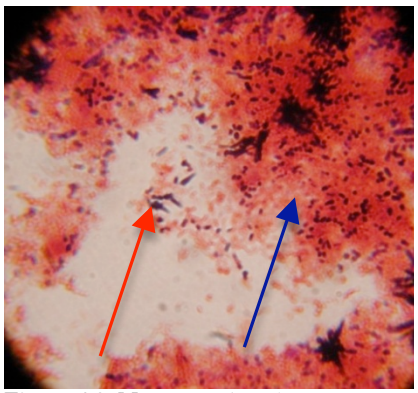

Figure 4.9: Non-autoclaved cow manure under the microscope

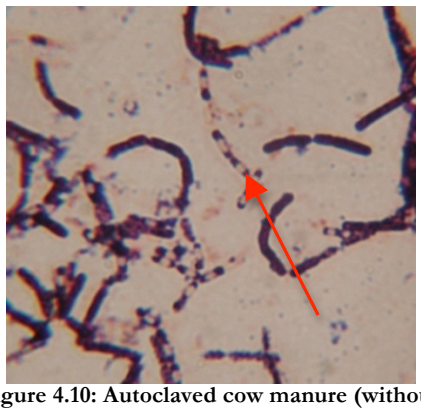

Figure 4.10: Autoclaved cow manure (without a temperature probe) under the microscope

Non-autoclaved cow manure was found to have a mixed population of gram-positive (red arrow in Figure 4.9) and gram-negative (blue arrow in Figure 4.9) rods of various sizes (as described under Microorganisms found in cow manure on page 53). In the cow manure autoclaved without a temperature probe only gram-positive rods with spores

were found to be present (see arrow in Figure 4.10). This suggests that, next to fungi, gram-negative and non-spore forming gram-positive bacteria were removed from the manure by autoclaving without a temperature probe. The vegetative cells of the spore-forming gram-positive bacteria were most likely destroyed as well, but the spores survived and germinated because of the heat treatment, thus gram-positive bacteria were present.

It is assumed that the reason for the partial and not total removal of microorganisms from the cow manure through the autoclaving process applied was due to the thickness of the manure and the lack of a temperature probe in the autoclave used. It is most likely that when autoclaving thick manure in the volumes of 200 or 300 ml, the centre of the sample did not maintain the required temperature of 121 °C for 15 minutes. Therefore some microorganisms, namely spores of gram-positive bacteria, were able to survive the autoclaving process.

The results from the co-digestion with autoclaved cow manure (StW+autoclCM) showed that the biogas production was lower than with non-autoclaved cow manure (StW+CM4) (see Table 4.25). Thus the fungi and/or the gram-negative bacteria removed through the applied autoclaving process may have been important for improving the biogas production from co-digesting primary sludge with stored whey and cow manure.

Fungi from cow manure belong to the family of anaerobic *Neocallimastigaceae* (Bauchop, 1989). They are reported to produce the enzymes cellulase and hemicellulase, which are required for effective digestion of fibre as they catalyse the hydrolysis (Hansen & Cheong, 2007; Lehtomäki et al., 2007; Wen et al., 2005a). In particular, they are responsible for the break down of cellulose and hemicellulose (Bauchop & Mountfort, 1981; Davies et al., 1993; Theodorou et al. 1996; Trinci et al., 1994). These, next to lignin, are the main components in cow manure (Chen et al., 2005; Wen et al., 2005b).

Cow manure co-digested with primary sludge without whey (CM1) did not result in a significant (p-value=0.25) increase in biogas production (see 100 and Table 4.18), but co-digestion of primary sludge with whey did (see Table 4.10 on page 89 and Table 4.13 on page 93). This suggests that whey added key elements necessary for the fungi.

Whey contains large amounts of easily degradable sugars, namely lactose and fermentation intermediates (see Table 4.4). Sugars serve as a good growth environment for fungi (Chen et al., 2005; McGranaghan et al., 1999; Wen et al., 2005a). Therefore, the growth conditions for fungi inhabiting cow manure may have improved when combined with whey due to sugar availability. Subsequently the production of enzymes from anaerobic fungi may have increased, resulting in improved cellulose and hemicellulose degradation into smaller monomers and therefore better cow manure utilisation and improved biogas production.

4.5 Why do fresh and stored casein whey reactors not result in the same performance?

This section will expand on the result that the reactors co-digesting fresh casein whey performed differently in terms of biogas production and pH from reactors co-digesting stored casein whey. Initially, the differences noted are highlighted, and then proposed reasons for the differences are presented.

Difference between fresh and stored whey reactors

A comparison of the fresh casein whey reactors (FW+CM3 and FW+CM4) with those at the same ratio but fed with stored casein whey (StW+CM1 and StW+CM2) is displayed in Table 4.26. It shows the average pH and alkalinity found in the reactors' effluent, as well as the average amount of biogas produced and the methane content of the biogas produced in the reactors receiving fresh and stored whey.

Table 4.26: Average characteristics in fresh whey reactors FW+CM3 and FW+CM4 and stored whey reactors StW+CM1 and StW+CM2 compared

			10:3:1	**10:3:0.1**
				Less manure
pH		**Fresh whey**	7.00	6.94
		Stored whey	7.08	7.02
Alkalinity	mg/l	**Fresh whey**	4,168	4,169
		Stored whey	4,713	4,643
Biogas	ml/l/day	**Fresh whey**	1,590	1,439
		Stored whey	1,377	1,200
Biogas	% of control	**Fresh whey**	152	138
		Stored whey	132	115
Methane content	%	**Fresh whey**	62.83	63.49
		Stored whey	62.81	62.70

The results show that the fresh whey reactors produced more biogas than the stored whey reactors, which had a higher pH and alkalinity (see Table 4.26). As the only difference between the fresh and stored whey reactors at the same ratio was the type of whey used, the changes resulting from the storage of whey must be held responsible for the variations between the fresh and stored whey reactors.

From the methane content in the biogas produced, no correlation between the different type and amounts of whey used was found (see Table 4.26). None of the reactors continuously produced more methane gas than another.

In comparing the two different types of whey, it was found that fresh whey contained significantly (p-value=0.00) more lactose and significantly (p-value=0.00) less L-lactate than stored whey. The lactose to L-lactate ratio in fresh casein whey was 96:1, while stored casein whey contained five times more L-lactate than lactose (see page 79). The concentration of lactose, D-glucose, L-lactate and acetate in the fresh whey reactors (FW+CM3 and FW+CM4), the stored whey reactors (StW+CM1 and StW+CM2) and the control reactor C2 is displayed in Table 4.27. It also displays the total concentration of lactose and intermediates in the substrates fresh and stored whey.

Table 4.27: Concentration of lactose, D-glucose, L-lactate and acetate in primary sludge, fresh and stored casein whey as well as the fresh whey reactors FW+CM3 and FW+CM4, stored whey reactors StW+CM1 and StW+CM2 and control reactor C2 compared

			Substrate	**1:0:0**	**10:3:1**	**10:3:0.1**
				Control reactor		Less manure
Lactose	mmol/l	**Control/PS**	0.42	0.67	-	-
		Fresh whey	98.40	-	0.21	0.34
		Stored whey	13.70	-	0.41	0.52
D-glucose	mmol/l	**Control/PS**	1.51	0.58	-	-
		Fresh whey	0.05	-	5.78	6.51
		Stored whey	0.15	-	7.53	9.26
L-lactate	mmol/l	**Control/PS**	1.07	0.39	-	-
		Fresh whey	1.03	-	3.27	2.96
		Stored whey	71.38	-	4.54	4.69
Acetate	mmol/l	**Control/PS**	6.42	0.00	-	-
		Fresh whey	7.30	-	0.00	0.00
		Stored whey	47.10	-	0.00	0.00
Total	**mmol/l**	**Control/PS**	9.42	1.65	-	-
		Fresh whey	106.77	-	9.26	9.81
		Stored whey	132.33	-	12.47	14.47

The results from measuring the lactose and D-glucose concentration in the fresh and stored whey reactors compared to the amounts added initially with the substrates fresh and stored whey showed that the concentration of lactose decreased in the reactors, while D-glucose increased (see Table 4.27). This indicates that lactose was fermented.

In the fresh whey reactors as a result of the lactose fermentation, an increase in the amount of L-lactate was found (see Table 4.27). The fermentation of one mole of lactose results in four moles of lactic acid (Fernandes et al., 1992; Walstra et al., 2006). In the fresh whey reactors 98.19 mmol of lactose was fermented but only 2.24 mmol of L-lactate produced (see Table 4.27). This indicates that lactose was not only fermented into L-lactate but also into acetate. However, as no acetate was found in the fresh whey reactors (see Table 4.27), all acetate added with the substrates and produced during the lactose fermentation was converted into methane.

In the stored whey reactors, there was a notably higher amount of L-lactate added with the substrate than in the fresh whey reactors (see Table 4.27). In contrast to the fresh whey reactors, the concentration of L-lactate in the stored whey reactors decreased (see Table 4.27) indicating a conversion back into pyruvate and then into acetate. None of the acetate, either added with the substrate or produced during the lactose fermentation process, remained in the stored whey reactors (see Table 4.27) but was converted into methane.

4.5.1 Do lactic acid bacteria from whey influence the co-digestion?

The fresh whey reactors FW+CM3 and FW+CM4 received 11.3 times more lactose, D-glucose, L-lactate and acetate collectively than the control reactor C2 (see Table 4.27). The stored whey reactors StW+CM1 and StW+CM2 received 14 times more lactose, D-glucose, L-lactate and acetate collectively than the control reactor C2 (see Table 4.27). As there was only a small amount of lactose and intermediates available in the control reactor C2 and much more in the reactors receiving fresh and stored whey (see Table 4.27), it was to be expected that more lactose and fermentation intermediates were removed in the fresh and the stored whey reactors than in the control reactor. In the control 7.77 mmol of lactose and intermediates were removed, while 97 mmol in the fresh whey reactors and 118 mmol in the stored whey reactors were removed (see Table 4.27). This leads to the assumption that the anaerobic bacteria present in the whey reactors utilised lactose and intermediates at a very effective rate, possibly more effective than the anaerobic bacteria present in the control reactor. This could be a result of more lactic acid bacteria present in the whey reactors due to whey addition, producing more lactase to hydrolyse lactose. In order to examine the influence of lactic acid bacteria from whey on the digestion process, stored whey was sterilised and then added to a reactor in replacement of non-sterile stored whey.

Microorganism removal from whey

Stored casein whey was sterilised through filtering as described under Sterilising whey on page 67 and cultured anaerobically on standard plate count agar (see Microorganism removal from whey page 67) to ensure microorganism removal. The pictures below

show the agar plate with stored whey (see Figure 4.11) and the plate with sterilised whey (see Figure 4.12) in comparison.

Figure 4.11: Stored whey on agar

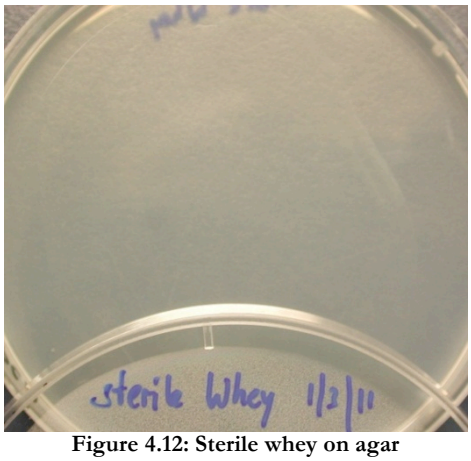

Figure 4.12: Sterile whey on agar

The results of culturing microorganisms anaerobically on agar plates showed that sterilising whey through filtering removed all microorganisms including lactic acid bacteria from the whey (see Figure 4.11 and Figure 4.12). It was found that the sterilising process did not result in a significant difference between the soluble COD (p-value=0.39) of stored whey and the sterilised whey. Comparing the VFA concentration showed that there was no significant (p-value=0.14) difference between stored whey and sterilised stored whey.

Co-digestion with sterile whey and cow manure

A reactor was fed with primary sludge, sterilised stored casein whey and cow manure (sterileStW+CM) at the ratio 10:3:1. The performance of the reactor was compared to the reactor StW+CM4 with non-sterile stored whey and the control reactor C3. The feedstock combination added to the different reactors is described in Table 4.28.

Table 4.28: Combination of used feed substrates in the reactors sterileStW+CM, StW+CM4 and the control reactor C3

Reactor	Volume	Amount of feedstock added daily				Ratio
	ml	Primary sludge	Stored whey	Sterile stored whey	Cow manure	PS:W:CM
C3	500	25 ml	-	-	-	1:0:0
StW+CM4	500	25 ml	7 ml	-	2.5 ml	10:3:1
sterileStW+CM	2,000	100 ml	-	30 ml	10 ml	10:3:1

The resulting HRT and organic loading rate as well as the pH and biogas production found in the reactors are described in Table 4.29.

Table 4.29: OLR, HRT, pH and biogas production of the reactors StW+CM4 and sterileStW+CM compared to the control reactor C3

Reactor	HRT	OLR	pH	Biogas production	
	days	kg/m^3/day		ml/l/day	%
C3	20.0	3.18	6.94	998	100
StW+CM4	14.5	4.15	6.96	1,468	147
sterileStW+CM	14.3	4.18	7.06	1,020	102

The addition of sterilised whey to the reactor sterileStW+CM did not affect the daily measured pH, which was stable throughout the time of the experiment (see Table 4.29).

With an average biogas production of 102 %, the reactor sterileStW+CM did not produce significantly (p-value=0.34) more biogas than the control reactor C3 but significantly less (p-value=0.00) biogas than the stored whey reactor StW+CM4 fed at the same ratio (10:3:1) as seen in Table 4.29.

Lactic acid bacteria, such as *lactobacillus* and *streptococcus*, are the same bacteria as the anaerobic species already present in an anaerobic digester (compare Hansen & Cheong, 2007 on page 5 and Walstra et al., 2006; Wood, 1992 on page 30). As lactose is easily degradable, the anaerobic bacteria are assumed to ferment this substrate first over more complex substrates. With the addition of fresh and stored casein whey, not only more lactose and L-lactate was added to the reactors but also additional LAB. It is suggested that the additional LAB helped to ferment the lactose and L-lactate added with whey. Therefore less biogas was produced in the reactor sterileStW+CM as a result of removing the LAB from the casein whey fed. It appeared that the population of bacteria available in the reactor sterileStW+CM did not match the amount of incoming lactose from whey, and therefore less biogas was produced. Thus it is proposed that lactic acid bacteria from whey play an important role in the co-digestion of primary sludge with casein whey.

4.5.2 Why does stored whey co-digested result in a higher reactor pH than fresh whey co-digested?

In comparing the pH in the reactors receiving 30 ml of casein whey and 10 or 1 ml of cow manure, it was discovered that the reactors fed with fresh casein whey FW+CM3 (10:3:1) and FW+CM4 (10:3:0.1) had a consistently lower pH than those fed with

stored casein whey StW+CM1 (10:3:1) and StW+CM2 (10:3:0.1) (see Table 4.26). This was unexpected given that the stored whey had an average pH of 3.59 and the fresh whey an average pH of 4.54 (see Table 4.4). As the partial fermentation of lactose to L-lactate in stored whey was found to be responsible for the pH drop in the substrate (see page 79), the lactose and L-lactate concentrations in the reactors were investigated.

The concentration of lactose fed into the fresh whey reactors decreased to less than 0.4 % of the incoming concentration remaining. It was fermented into intermediates, as described on page 112 (also see Table 4.27). One of the intermediates is pyruvate, which is converted into acetate, formate and lactate, as seen in Figure 4.13. The pyruvate to lactate conversion is accompanied by a drop in pH (indicated by red circle in Figure 4.13) as described by Bylund (1995), Earnshaw (1992), Madigan and Martinko (2006) and others (see Lactic acid/Lactate on page 32). It is suggested that the lower pH observed in the fresh whey reactors in comparison to the stored whey reactors (see Table 4.26) was a result of the lactose fermentation process occurring in the reactor, in particular of the pyruvate to lactate conversion.

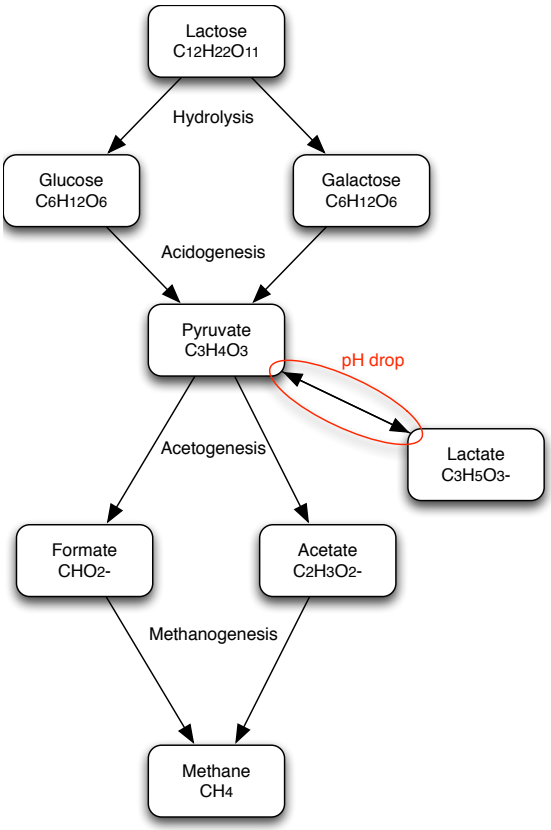

Figure 4.13: pH drop in lactose fermentation process

For stable anaerobic digesters, it is reported that lactate does not accumulate and the production rate does not exceed the removal rate (Cohen et al., 1979; Yang et al., 1987). In stored whey, most of the lactose was fermented to L-lactate during storage, which caused the pH in the substrate to drop (see page 79 and Table 4.4). When added to the stored whey reactors, it is suggested that an imbalance could have occurred due to the high L-lactate concentration. The L-lactate concentration in the stored whey reactors was found to have decreased in comparison to the incoming concentration (see Table

4.27) indicating the removal of L-lactate. Thus it is assumed that the enzyme lactate dehydrogenase primarily catalysed the removal of lactate instead of the production of lactate from pyruvate. This requires NAD^+ to be reduced to NADH (Walstra et al., 2006). It is suggested that all existing or produced pyruvate was converted into acetate and formate and then into methane without more L-lactate being produced (see Figure 4.13). Hence L-lactate production and its associated pH drop in the stored whey reactors would be avoided (as indicated by the red circle in Figure 4.13). This could explain the higher pH observed in the stored casein whey reactors, in comparison to the fresh casein whey reactors at the same ratio (see Table 4.26).

The acidification avoided in the stored whey reactors as described above may have made it possible to digest more stored whey without acclimatisation in a reactor than fresh whey. The fresh whey reactor with the ratio 10:7:1 without acclimatisation (FW+CM5) failed within a very short time (see Table 4.10 page 89), while the stored whey reactor (StW+CM3) at the same ratio achieved an improved biogas production rate of 142 % and had a stable pH (see Table 4.13 page 93). After reducing the amount of cow manure, the latter failed as well.

4.5.3 Why does fresh whey co-digested result in a higher biogas production than stored whey co-digested?

It was found that the fresh whey reactors FW+CM3 and FW+CM4 produced more biogas than the stored whey reactors StW+CM1 and StW+CM2 at the same ratio (10:3:1 and 10:3:0.1) (see Table 4.26). As the only difference between the fresh and stored whey reactors at the same ratio was the type of whey used, the processes occurring during storage must be responsible for the different biogas production. As seen in Table 4.4 on page 79, the fresh whey had a higher average total and soluble COD than the stored whey. Therefore, the COD going into the reactors as well as the COD removed from the reactors was investigated. Table 4.30 displays the average of the incoming total and soluble COD into the reactors FW+CM3, FW+CM4, StW+CM1 and StW+CM2 as well as the total and soluble COD that had been removed in the reactors.

Table 4.30: Average total and soluble COD fed into as well as average total and soluble COD removed in the fresh whey reactors FW+CM3 and FW+CM4 and stored whey reactors StW+CM1 and StW+CM2

			10:3:1	**10:3:0.1**
				Less manure
Total COD in	mg/l	**Fresh whey**	64,232	60,377
		Stored whey	59,648	55,526
Soluble COD in	mg/l	**Fresh whey**	16,868	16,342
		Stored whey	11,442	10,060
Total COD removed	mg/l/d HRT	**Fresh whey**	2,005	2,081
		Stored whey	1,759	1,951
Soluble COD removed	mg/l/d HRT	**Fresh whey**	1,053	1,058
		Stored whey	663	617

Comparing the incoming COD into the reactors shows that more COD was available in the fresh whey reactors than in the stored whey reactors (see Table 4.30) due to higher COD of fresh whey than stored whey (see Table 4.4 on page 79). Also, more COD was removed in the fresh whey reactors than in the stored whey reactors at the same ratio (see Table 4.30). Consequently, more biogas was produced in the fresh whey reactors as the biogas production depends on the COD removed (Tchobanoglous et al., 2003).

As the lactose fermentation product H_2 in stored whey was lost and not available as substrate for methane production, the incoming COD into the stored whey reactors was lower and consequently the biogas production was lower than in the fresh whey reactors. Therefore the lactose fermentation and subsequently the storage conditions of stored whey were considered the reason for a lower biogas production in the stored whey reactors in comparison to the fresh whey reactors.

In both the fresh and stored whey reactors, the most efficient COD removal (total and soluble) was found in the reactors receiving the lowest amount of cow manure at the ratio 10:3:0.1 without taking the control reactor into account (see Table 4.30, Table 4.11 on page 90 for the fresh whey reactors and Table 4.14 on page 94 for the stored whey reactors). Thus the amount of cow manure fed into the reactors had an important effect on the removal of total and soluble COD.

4.5.4 Why does the co-digestion of fresh whey require cow manure for improved biogas production?

Primary sludge co-digested with fresh whey resulted in an improved biogas production only when combined with cow manure. The fresh whey reactor with cow manure at the ratio 10:3:1 (FW+CM3) produced 152 % and at the ratio 10:3:0.1 (FW+CM4) produced 138 % of the biogas production from primary sludge only (see Table 4.26). Interestingly, the reactor co-digesting primary sludge with stored casein whey and no cow manure at the ratio 10:3 (StW1) produced 150 % of the biogas production from primary sludge only (see Table 4.6 on page 81). It is proposed that the reactors fed with fresh whey need elements from the cow manure, which the stored whey reactors do not require.

Cow manure is reported to contain anaerobic rumen bacteria in addition to anaerobic fungi (McGranaghan et al., 1999; Milne et al., 1989; Trinci et al., 1994). Adding anaerobic bacteria with the cow manure could have led to an increase in the bacteria present in the reactors. This could have enhanced the anaerobic digestion process, in particular the acidogenesis carried out by bacteria such as *Lactobacillus*, *Streptococcus* and *Clostridium* (Hansen & Cheong, 2007). These as well as lactic acid bacteria are reported to be contained in cow manure (Östling & Lindgren, 1991; Unc & Goss, 2004; Wang et al., 2004). The addition of LAB could have resulted in an increase in lactase production.

Fresh whey contained a high concentration of lactose (see Table 4.4 on page 79), which required hydrolysing in the digester. From the results of the co-digestion experiment with sterile whey (LAB removed), it is proposed that the additional lactose added with fresh whey required more lactase than the LAB present in the digester (sterile StW+CM) could provide (described on page 114f).

Therefore, the combination of LAB from fresh whey and from cow manure may have provided an adequate amount of lactase to hydrolyse the high lactose concentration present in the fresh whey reactors.

In contrast, no improvement in biogas production was seen when cow manure was added to the stored whey reactors. Stored whey contained more L-lactate and less lactose than fresh whey (see Table 4.4 on page 79), therefore less lactase was required

for lactose hydrolysis. It would appear that the LAB present in stored whey were able to provide sufficient quantities of lactase to hydrolyse the available lactose. Therefore it is assumed that cow manure was not required to improve biogas production from stored whey co-digested with primary sludge.

An unexpected result was the reduction in biogas production when cow manure was added to the stored whey reactors. The stored whey reactor without cow manure at the ratio 10:3 (St1) produced 150 % (see Table 4.6 on page 81) while the stored whey reactor with cow manure at the ratio 10:3:1 (StW+CM1) produced 132 % and at 10:3:0.1 (StW+CM2) produced 115 % of the control reactor's biogas production (see Table 4.13). It appears that the addition of cow manure reduced the biogas production from primary sludge and stored whey.

In the stored whey reactors with ten ml of cow manure (StW+CM1), the total incoming COD was higher than the reactor with one ml of cow manure (StW+CM2) (see Table 4.30). However, the total COD removed was higher in the reactor with less manure (StW+CM2) (see Table 4.30). This indicates that a lower cow manure intake was better for the removal of COD as less non-digestible material was present.

As discussed on page 109, fungi in cow manure find good growth conditions in sugar containing environments. However, stored whey contained little lactose and glucose (see Table 4.4) that could serve as a growth environment for fungi. Thus the amount of sugar available to the anaerobic fungi from cow manure may have been inadequate. In consequence, little or no cellulase and hemicellulase may have been produced and therefore fibrous material from cow manure could not be degraded to more accessible materials. As a result, non-digestible material from cow manure accumulated in the reactors, resulting in lower COD removal (see Table 4.30) and less biogas produced (see Table 4.26) in the stored whey reactors with cow manure.

Chapter 5 Conclusions

5.1 Can casein whey improve the biogas production from primary sludge?

5.1.1 Primary sludge co-digested with fresh whey

It was found that primary sludge co-digested with fresh casein whey of up to a ratio of 10:7 (PS:W) after slow acclimatisation to whey, produced only 106 % of the biogas produced by primary sludge without whey. The reactor did not fail, however, the pH decreased as the amount of whey added increased, and the concentration of VFA remained low. Thus, co-digesting primary sludge with casein whey even after acclimatisation to whey did not improve the biogas production.

5.1.2 Will storing whey change the outcome when it is co-digested with primary sludge?

As casein whey may not be available all year around, the possibility of digesting whey stored at ambient temperature was examined. While the lactose to L-lactate ratio in fresh casein whey was 96:1, it was found that after 37 weeks of storage, an average of 14 % of lactose remained in stored whey. The rest was fermented into lactic acid (lactose to L-lactate ratio 1:5), resulting in a pH decrease in the substrate from 4.5 to 3.6, as well as acetate, formate, CO_2 and hydrogen. The COD of stored whey decreased as the gaseous fermentation product hydrogen was lost.

Co-digesting primary sludge with stored casein whey at a ratio of 10:3 (PS:W) produced 150 % of the control reactor's biogas production. The pH in the reactor was found to be higher in comparison to the co-digestion with fresh casein whey, possibly caused by the fermentation of lactose to intermediates in stored whey. Thus, storing casein whey appears to improve the biogas production from casein whey as a single co-substrate with primary sludge.

5.2 Can cow manure help to improve biogas production from primary sludge and casein whey?

5.2.1 What ratio of manure to fresh whey is required?

As the co-digestion of primary sludge with fresh casein whey was not found to improve the biogas production, cow manure was added to improve reactor stability as proposed in the literature.

At a ratio of 10:3:1, the pH remained stable and a biogas production of 152 to 161 % of the control reactor was achieved. As the transport of cow manure could be cost intensive or it might be difficult to obtain, the amount of cow manure was reduced by ten times, resulting in 138 % biogas production. The stability of the reactor was not negatively affected by the reduction of cow manure.

Adding more casein whey initially was only found to work at a primary sludge to cow manure ratio of 10:1. Without prior acclimatisation, the highest biogas production achieved was 178 % of the control's biogas production at a ratio of 10:5:1 (PS:W:CM). Addition of more casein whey was found to result in decreasing pH and eventually led to reactor failure. However, after slowly increasing the amount of fresh casein whey to a ratio of 10:7:1 (PS:W:CM) a biogas production of 200 % of the control reactor's gas production was achieved at a stable pH.

If the amount of cow manure is limited, the recommended feed ratio is 10:3:0.1 (PS:W:CM). At this ratio, the pH was stable and the biogas production increased to 138 % of the biogas production from primary sludge alone. If the supply of cow manure is

not constrained, a ratio of 10:5:1 is suggested. At this ratio the biogas produced was 178 % of the primary sludge alone.

5.2.2 Can cow manure further improve co-digestion of primary sludge with stored whey?

The addition of cow manure was investigated to determine if it would increase the biogas production beyond the 150 % found in the co-digestion of primary sludge and stored whey without manure. The highest biogas production from stored whey and cow manure co-digested was achieved at the ratio 10:7:1, producing 142 % of the biogas from primary sludge alone. Thus the co-digestion of primary sludge and stored casein whey without cow manure is more effective for biogas production.

5.2.3 Can compost leachate work as an alternative to cow manure?

Compost leachate was tested as an alternative to cow manure in the co-digestion of primary sludge with casein whey. It was found that the addition of compost leachate at all ratios tested resulted in a biogas production of 99.9 % of the control. As no improvement was found, compost leachate does not appear to be a worthwhile alternative co-substrate for co-digestion of primary sludge and casein whey.

5.3 What happens when casein whey and cow manure are combined?

Despite the addition of high VFA and lactose concentrations to the reactors, neither acetate nor L-lactate accumulated in stable reactors. This suggests that the methanogenesis worked at a highly effective rate and is not the rate-limiting step in anaerobic digestion of primary sludge, casein whey and cow manure at the levels tested.

5.3.1 Is it possible that whey hydrolyses manure?

As fibre can be hydrolysed by acids, it was investigated whether acidic casein whey could hydrolyse cow manure. As the soluble COD of cow manure and casein whey

mixed together was only 6.6 % higher than expected, acid hydrolysis of cow manure through acidic casein whey is proposed to be insignificant.

5.3.2 Is it possible that cow manure alone improves biogas production from primary sludge?

The digestion of primary sludge with cow manure at a ratio of 10:1 without casein whey did not notably improve biogas production (107 %). This suggests that the improved biogas production is primarily due the combination of casein whey and cow manure rather than cow manure alone.

5.3.3 Does cow manure provide alkalinity that whey is lacking?

It was found that cow manure contributes to reactor alkalinity. However, whey, in combination with cow manure and by itself, was also found to contribute to alkalinity. Therefore, cow manure is not required to buffer the low alkalinity of whey as the literature suggests.

5.3.4 Does cow manure provide phosphorus, nitrogen and trace elements?

No indication of phosphorus or nitrogen deficiency through high VFA concentration (Speece, 1996) was found in any of the stable reactors. The theoretically available amount of phosphorus and nitrogen was calculated to exceed the levels recommended by Speece (1996) with or without the addition of cow manure.

Primary sludge has been reported to provide more trace elements than cow manure (Eriksson, 2001; Speece, 1996). Therefore, co-digestion of primary sludge with casein whey did not require nutrients and/or trace elements found in cow manure.

5.3.5 Do microorganisms from cow manure influence co-digestion of whey?

As microorganisms in cow manure may play an important role in biogas production, the substrate was autoclaved to remove microorganisms. Co-digestion with autoclaved cow

manure at the ratio 10:3:1 produced 116 % biogas compared to the control. With non-autoclaved cow manure at the same ratio (10:3:1), a biogas production of 132 to 137 % was achieved. Stored whey without cow manure at the ratio 10:3 produced 150 %. This suggests that microorganisms in cow manure are important for the biogas production.

Cultivating autoclaved cow manure on agar showed that anaerobic fungi were removed through the autoclaving process used. Therefore, fungi in cow manure may play a specific role in the biogas production, possibly through their ability to produce the hydrolytic enzymes cellulase and hemicellulase.

The addition of cow manure to primary sludge did not improve the biogas production, as adequate amounts of sugar for fungal growth were not available. When whey was added the necessary sugars were provided, resulting in enhanced production of the enzymes cellulase and hemicellulase essential for the breakdown of fibrous components in the cow manure. As a result of better cow manure hydrolysis, the biogas production was improved.

5.4 Why do fresh and stored casein whey reactors not result in the same performance?

5.4.1 Do lactic acid bacteria from whey influence the co-digestion?

Casein whey was sterilised to investigate the influence of lactic acid bacteria on the co-digestion of primary sludge with whey and cow manure. It was found that the biogas production was lower (102 %) than with non-sterile whey (132 to 137 %) at the same ratio (10:3:1). Thus the lactic acid bacteria affect the performance of the digesters. With the addition of lactic acid bacteria to the reactors, more lactase to hydrolyse lactose was produced and as a result the lactose utilisation and biogas production improved.

5.4.2 Why does stored whey co-digested result in a higher reactor pH than fresh whey co-digested?

The pyruvate to lactate conversion step of the lactose fermentation causes a drop in pH and therefore acidification of stored casein whey occurred. Pyruvate to lactate conversion occurred to a lesser extent or did not occur in the stored whey reactors due to a high pre-existing L-lactate concentration. Therefore, the pH remained higher in the stored whey reactors than in the fresh whey reactors. The high lactose concentration from fresh casein whey was partially fermented into lactate in the reactors, resulting in a pH drop, and subsequently lower pH than in the stored whey reactors.

5.4.3 Why does fresh whey co-digested result in a higher biogas production than stored whey co-digested?

Fresh whey supplied more COD, in particular soluble COD, than stored whey, and COD removal in the fresh whey reactors was higher than in the stored whey reactors. As the COD removal is directly related to the biogas production, the fresh whey reactors achieved a higher biogas production than the stored whey reactors.

5.4.4 Why does the co-digestion of <u>fresh</u> whey require cow manure for improved biogas production?

The availability of lactase is crucial for the utilisation of lactose. Lactase can be provided by lactic acid bacteria and other types of bacteria. The LAB in fresh casein whey may not have been able to provide sufficient lactase to hydrolyse the high concentration of lactose. Therefore, the fresh whey reactors required the addition of LAB from cow manure to provide adequate amounts of lactase and to produce more biogas than from primary sludge alone.

In contrast, stored casein whey co-digested without cow manure produced more biogas than stored whey and cow manure combined in co-digestion of primary sludge. This suggests that enough lactase was available or provided by LAB to hydrolyse the lactose remaining after fermentation during storage of whey.

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Appendix A

The report by Eriksson (2001) on 61 trace elements in sewage sludge and cow manure shows that both provide a variety of trace elements in different amounts. The key trace elements for anaerobic digestion – iron, nickel and cobalt – are found in primary sludge in concentrations exceeding those in cow manure (Eriksson, 2001). (see Table Appendix A.5.1)

Table Appendix A.5.1: Macro- and micronutrients in cow manure and sewage sludge (Eriksson, 2001)

		CM slurry	Stable manure	Sewage sludge	
		mg/kg	mg/kg P	mg/kg P	mg/kg
B	**Boron**	52	3,000	2,700	61
Ba	**Barium**	44	3,300	11,900	310
Cd	**Cadmium**	0.12	12	44	1.4
Ce	**Cerium**	2.3	180	1,000	24
Co	**Cobalt**	0.85	51	310	6.2
Cr	**Chromium**	2.3	350	1,300	33
Cu	**Copper**	24	4,800	14,000	390
La	**Lanthanum**	1.4	110	650	16
Mn	**Manganese**	234	17,000	9,300	280
Mo	**Molybdenum**	4.5	307	260	6.7
Ni	**Nickel**	4.6	294	720	20
Pb	**Lead**	0.98	81	1,500	33
Rb	**Rubidium**	29	1,600	550	15
Sn	**Tin**	0.98	75	870	22
Sr	**Strontium**	51	3,200	6,900	170
Ti	**Titanium**	38	2,400	63,000	1,800
V	**Vanadium**	2.5	270	650	18
Zn	**Zinc**	154	24,000	25,000	550
Fe	**Iron**	-		-	49,000
P	**Phosphorus**	24,000	-	-	27,000
S	**Sulphur**	4,900	-	-	9,000
Si	**Silicon (SiO_2)**	-	-	-	45,000
K	**Potassium (K_2O)**	-	-	-	4,400
Mg	**Magnesium**	-	-	-	3,400
Na	**Sodium**	-	-	-	3,500
Ca	**Calcium**	-	-	-	28,000
Al	**Aluminium**	-	-	-	40,000

Appendix B

It was found that the concentration of volatile fatty acids (VFA) in unpreserved, filtered samples decreased at a rapid rate as seen in Figure Appendix B.5.1.

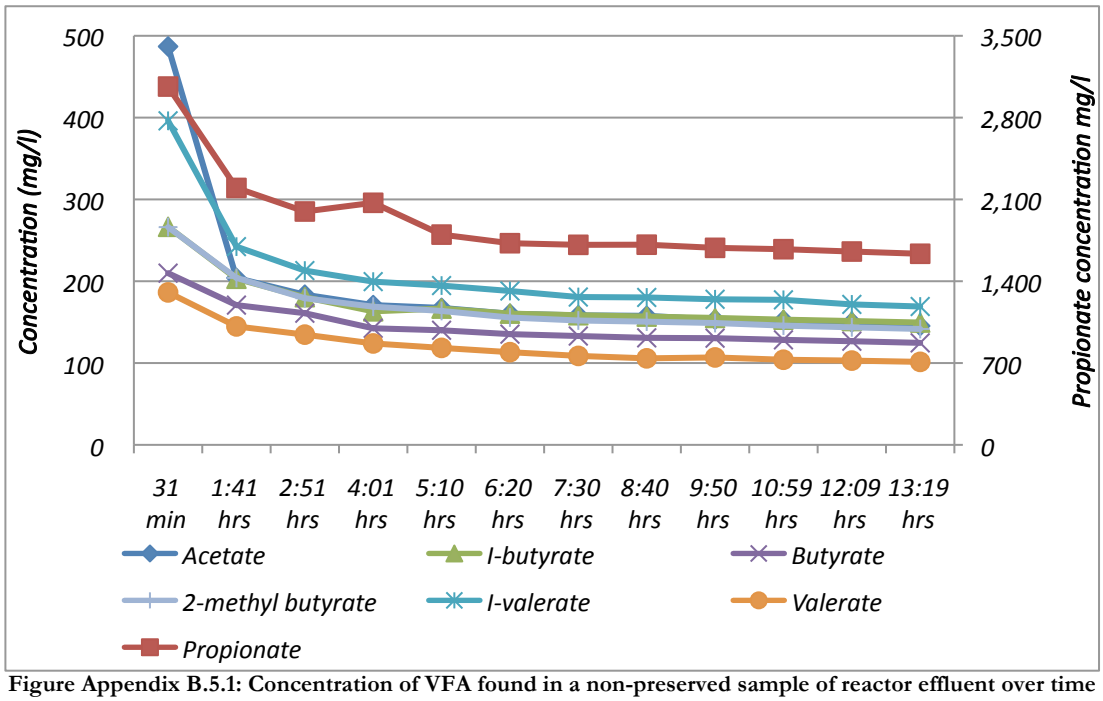

Figure Appendix B.5.1: Concentration of VFA found in a non-preserved sample of reactor effluent over time

Within only two hours of storage in closed vials, the concentration of VFA had already decreased, in some cases by 50 %, as seen in Figure Appendix B.5.1. As the ion chromatograph (IC) used needs more than three hours to run conditioning samples, any concentrations measured were not reflecting the actual concentration of VFA present. Therefore preservation of samples was investigated.

Test carried out have shown that the addition of two drops of H_2SO_4 to the samples resulted in the pH dropping to below two, and as a result the concentration of VFA decreased less rapidly as seen in Figure Appendix B.5.2.

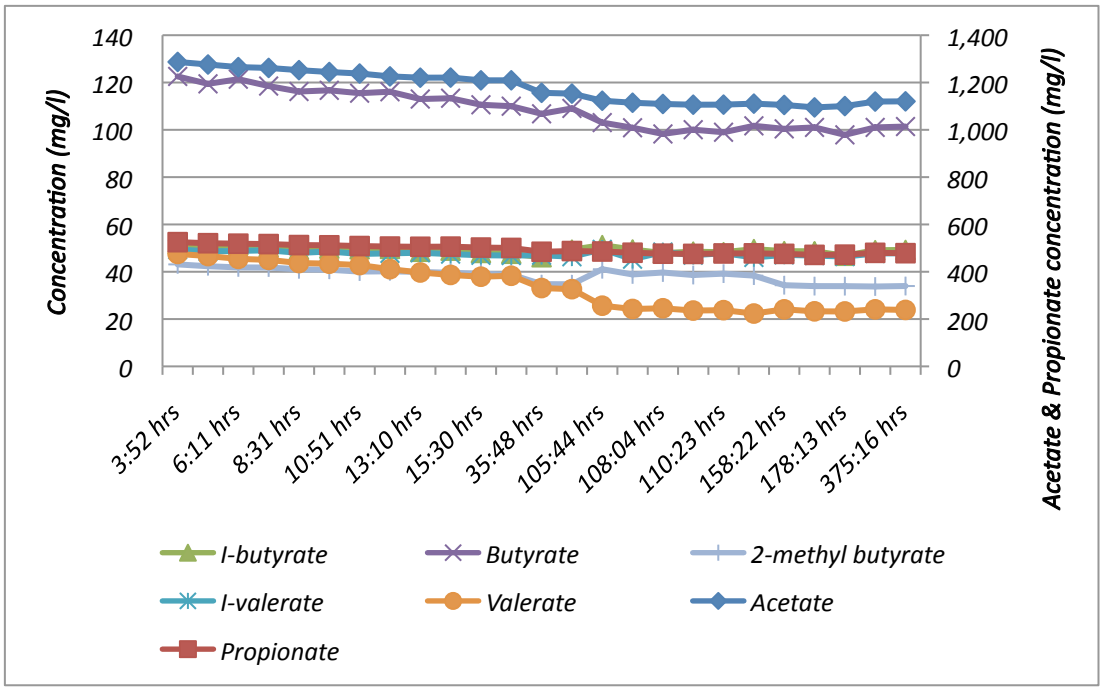

Figure Appendix B.5.2: Concentration of VFA found in a with H_2SO_4 preserved sample of reactor effluent over time

Figure Appendix B.5.2 shows that the concentration of VFA found in samples preserved with two drops of H_2SO_4 did not decrease as notably as in the samples without preservation. After 375 hours, the concentration was still above 50 %. Therefore the addition of H_2SO_4 was applied to preserve VFA samples in this research.

Appendix C

Reactor	Volume	Amount of feedstock added daily				Ratio
		Primary sludge	Fresh whey	Stored whey	Cow manure	PS:W:CM
C1	2,000 ml	100 ml	-	-	-	1:0:0
C2	2,000 ml	100 ml	-	-	-	1:0:0
C3	500 ml	25 ml	-	-	-	1:0:0
CM1	2,000 ml	100 ml	-	-	10 ml	10:0:1
FW1	2,000 ml	100 ml	20-70 ml	-	-	10:2-10:7
FW+CM1	2,000 ml	100 ml	20-70 ml	-	10 ml	10:2:1-10:7:1
FW+CM2	2,000 ml	100 ml	20-70 ml	-	20 ml	10:2:2-10:7:2
FW+CM3	2,000 ml	100 ml	30 ml	-	10 ml	10:3:1
FW+CM4	2,000 ml	100 ml	30 ml	-	10 ml	10:3:1
		100 ml	30 ml	-	1 ml	10:3:0.1
FW+CM5	2,000 ml	100 ml	70 ml	-	10 ml	10:7:1
FW+CM6	2,000 ml	100 ml	50 ml	-	10 ml	10:5:1
		100 ml	50 ml	-	1 ml	10:5:0.1
StW1	500 ml	25 ml	-	7 ml	-	10:3
StW+CM1	2,000 ml	100 ml	-	30 ml	10 ml	10:3:1
StW+CM2	2,000 ml	100 ml	-	30 ml	10 ml	10:3:1
		100 ml	-	30 ml	1 ml	10:3:0.1
StW+CM3	2,000 ml	100 ml	-	70 ml	10 ml	10:7:1
		100 ml	-	70 ml	1 ml	10:7:0.1
StW+CM4	500 ml	25 ml	-	7 ml	2.5 ml	10:3:1

Reactor	Volume	Amount of feedstock added daily				Ratio
		Primary sludge	Fresh whey	Stored whey	Compost leachate	PS:W:CL
FW+CL1	2,000 ml	100 ml	20-60 ml	-	20 ml	10:2:2-10:6:2

Reactor	Volume	Amount of feedstock added daily					Ratio
		Primary sludge	Stored whey	Sterile stored whey	Cow manure	Autoclaved cow manure	PS:W:CM
StW+ autoclCM	500 ml	25 ml	7 ml	-	-	2.5 ml	10:3:1
sterileStW+ CM	2,000 ml	100 ml	-	30 ml	10 ml	-	10:3:1