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Technical Feasibility of Anaerobic Co-digestion of Dairy Manure with Chicken Litter and Other Wastes

A Thesis
Presented for the
Master of Science Degree
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Esteban Manuel Zamudio Cañas
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ABSTRACT

The largest waste stream from agricultural livestock activity is manure. Efforts herein focus on the improvement of anaerobic digestion of animal wastes which creates a stable solid residue and recovers energy in the form of methane. Co-digestion of chicken litter (CL) and dairy manure (DM) was studied using stirred reactors at mesophilic temperature (35 °C) to evaluate the feasibility of co-digesting these two substrates by varying the organic loading rate (OLR) using increasing amounts of chicken litter. The results indicate that chicken litter and dairy manure can be successfully co-digested with chicken litter present at up to 33% of Volatile Solids (VS) in the feedstock (OLR $1.5(\pm 0.03) \text{ gVS L}_{\text{reactor}}^{-1} \text{ day}^{-1}$). Synergistic and/or antagonistic effects were not observed in terms of methane production. It was also found that reactors reach a dynamic stability 7 days after increasing the organic loading rate. While both total and free ammonia tolerance of the bioreactors solids improved by combining these two substrates, true adaptation was only observed for free ammonia which increased as the proportion of CL was increased. No improvement in pathogen indicator removal was detected.

Other co-digestion experiments were performed in batch reactors using filtered dairy manure solids (FDMS), grease trap waste (GTW), and sawdust (S). Manure solids (0.417 and 0.842 mm) was present at up to 70% as VS in feedstock and increased total methane production by $114 \pm 2 \%$, but decreased efficiency (methane yield) by $59 \pm 14 \%$. Grease trap waste alone was difficult to degrade, but co-digestion improved efficiency and VS removal of dairy manure alone by $111 \pm 9 \%$ and $76 \pm 4 \%$, respectively, for all additions tested. In contrast, sawdust could not be degraded reducing efficiency in all additions tested.

Finally, adaptation to different temperatures was evaluated in batch reactors. Microbial population could adapt to lower temperatures down to 19 °C with an acceptable decrease in methane production, but longer retention times were needed. At a 20 days retention time, methane production decreased by only 10% when the temperature decreased from 35 to 25 °C.

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CHAPTER 1 Introduction and General Information

1.1 Introduction

“The building of a sustainable society will require reduction of dependency on fossil fuels and lowering of the amount of pollution that is generated. Waste treatment is an area in which these two goals can be addressed simultaneously. As a result, there has been a paradigm shift recently, from disposing of waste to using it” (Angenent et al. 2004).

An increase in large scale livestock farms in both developed and developing countries presents a major worldwide environmental problem (Güngör-Demirci et al., 2004). The largest waste stream coming from agricultural livestock activity is manure (Lehtomäki et al., 2007). The huge amount of animal manure that is being produced in small areas requires carefully designed and improved disposal and/or treatment solutions to reduce harmful impacts on environment.

Several treatment and disposal alternatives have been studied, including: land application (Araji et al., 2001; Sommer et al., 2001), pond systems (Wang et al., 1996), composting (Guerra-Rodriguez et al., 2001; Imbeah, 1998; Tiquia et al., 2002), constructed wetlands (Clarke et al., 2002; Knight et al., 2000), ground injection (Morken et al., 1998), reverse osmosis (Thörneby et al., 1999) and anaerobic digestion (Alvarez et al., 2006; Bujoczek et al., 2000; Callaghan et al., 2002; Krylova et al., 1997; Misi et al., 2001a). Anaerobic digestion has been widely studied because it can produce renewable energy, reduce organic and pathogen content, and create a stable residual waste that can be used as soil fertilizer (El-Mashad et al., 2004; Keshtkar et al., 2001; Uludag-Demirer et al., 2008).

Anaerobic digestion coming from livestock activity (cattle, pigs and poultry), mostly involves the utilization of liquid wastes such as swine and cattle manure. More commonly, those wastes are stored in lagoons (or ponds) or in stockpiles which are emptied twice or three times at year. Solid manure can create several problems such as odor, runoff, and potential pathogen contamination; liquid manure in ponds releases considerable amounts of uncontrolled methane gas into the atmosphere. Methane gas is 20

times more active as a greenhouse gas than carbon dioxide (USDA, 2008; Hasslberger, 2005), and contributes to global warming. In the United States, the third greatest source of methane emission is agriculture, emitting approximately one-quarter of total national CH₄ emission (Chianese et al., 2008). The Environmental Protection Agency (EPA) has focused its efforts on creating regulations for those emissions, but there is a lack of environmental friendly disposal options for the large amounts of manure produced.

Anaerobic digestion of swine or poultry manure has previously shown a deficient performance due to inhibitory ammonia levels (Gelegenis et al., 2007a; Hansen et al., 1998; Kelleher et al., 2002; Krylova et al., 1997). Further, poultry manure is a solid waste which is hard to handle for treatment. Research has demonstrated that a possible solution to both problems is to dilute poultry wastes with water to approximately 3% total solids (Angenent et al., 2002; Kelleher et al., 2002). In contrast, anaerobic digestion of liquid dairy wastes (Total Solids < 10%) has been successfully performed worldwide (Wase et al., 1992; Ahring et al., 1992; Speece, 1996; Munch et al., 1999).

Mixing different agrowastes (Callaghan et al., 1997; Callaghan et al., 2002; Gelegenis et al., 2007a; Misi et al., 2001a; Rushbrook, 1990; Tafdrup, 1994) to improve physical-chemical properties prior to anaerobic digesting is not a new idea (Dagnall, 1995; Forster et al., 1976). Co-digesting can reduce problematic characteristics such as high solid content and high ammonia concentrations.

The goal of this study was to find the maximum methane production rate by adding broiler (meat chicken) litter in a semi-continuous reactor digesting liquid dairy wastes. Synergistic or antagonistic effects, ammonia adaptation and/or inhibition threshold and pathogen indicator removal were evaluated at different organic load rates. In addition, substrates other than chicken litter were investigated for co-digesting with cow manure.

1.2 Hypothesis and Objectives

The primary hypothesis of this research was dry chicken litter can be mixed with liquid dairy manure in order to increase methane gas production in anaerobic digestion. A

second hypothesis was that the digester microbes would adapt to an increasing organic loading rate up to a threshold.

The objectives of this research were to:

1. Find the maximum methane production rate by increasing the organic loading rate with chicken litter in semi-continuous reactors digesting dairy manure.
2. Analyze the presence of either synergistic, antagonistic or no side effects when co-digesting chicken litter and dairy manure.
3. Evaluate co-digestion of dairy manure with other wastes such as filtered dairy manure solids, grease trap wastes, and saw dust.
4. Quantify temperature adaptation in anaerobic digestion of dairy manure.

CHAPTER 2 Literature Review

2.1 Anaerobic Digestion Overview

Anaerobic Digestion is a natural biological process by which organic matter is reduced to methane under oxygen free environments roughly according to equation 1 (Salminen et al., 2002; Kelleher et al., 2002; Chen et al., 2008). The degradation pathways to produce methane can be summarized with a 4-stage model (Figure 2.1).



Each stage in Figure 2.1 is performed by a different group of microbes that use as a starting point the final product of the previous stage. The stability of the process is determined by the coordination of all groups of microbes metabolizing their own substrates under preferred reactors conditions (Poliafico, 2007).

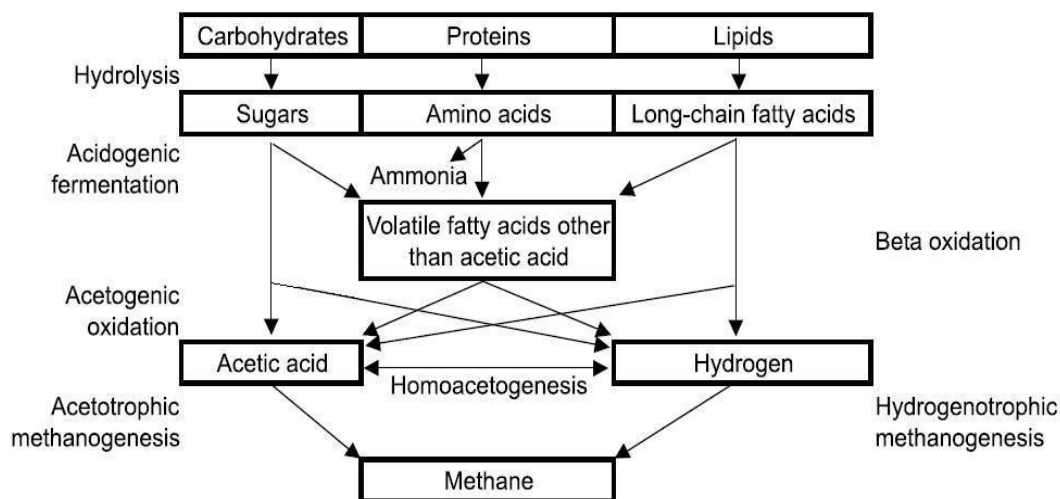


Figure 2.1: Anaerobic pathways in anaerobic degradation (Salminen et al., 2002).

Thus, digester conditions must maximize methane production without negatively affecting any group. Usually the weakest group of microbes is the methanogenic consortium (Gerardi, 2003). However, the rate-controlling stage depends on operational conditions such as wastewater characteristics, hydraulic retention time, organic loading, and temperature, and it is not always the same (Alvarez, 2006; Garcia-Ochoa et al., 1999; Gerardi, 2003; Lyberatos et al., 1999; Hashimoto, 1982).

2.1.1 Stages

The four different stages in anaerobic digestion are: hydrolysis, in which insoluble complex molecules are broken down by enzymes to obtain soluble simple compounds; acidogenesis (or fermentation), in which the soluble compounds are in turn converted into volatile acids, alcohols, hydrogen and carbon dioxide; acetogenesis, in which acetate is created from volatile fatty acids other than acetate, alcohols and hydrogen; and finally methanogenesis, in which methane is produced from either acetate or hydrogen (Angelidaki et al., 2007; Gerardi, 2003; Karia et al., 2006).

2.1.1.1 Hydrolysis

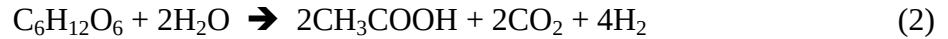
In this step insoluble complex organic matter is broken down into their backbone constituents in order to allow their transport through microbial cell membrane (Madigan et al., 2008; Gerardi, 2003). The process is catalyzed by novel enzymes excreted mostly by facultative fermentative microbes. Proteases, secreted by proteolytic microbes, convert proteins into amino acids; cellulases and/or xylanases, produced by cellulytic and xylanolytic microbes, hydrolyze cellulose and xylose (both complex carbohydrates) into glucose and xylan (both sugars), respectively; finally lipases, created by lipolytic microbes, convert lipids (fats and oils) into long-chain fatty acids and glycerol (Angelidaki et al., 2007; Gerardi, 2003; Salminen et al., 2002; Sterling et al., 2001). Table 2.1 summarizes the hydrolysis step and gives specific examples of the bacterial genera involved.

Table 2.1: Enzymes utilized during the hydrolysis step of anaerobic digestion (Gerardi, 2003).

Substrate to be degraded	Exoenzyme Needed	Example	Microbe	Product
Polysaccharides	Saccharolytic	Cellulase	Cellulomonas	Simple Sugar
Proteins	Proteolytic	Protease	Bacillus	Amino acids
Lipids	Lipolytic	Lipase	Mycobacterium	Fatty acids

2.1.1.2 Acidogenesis (fermentation)

In this stage, the products of the hydrolysis (e.g. glucose) are converted into acetate, other volatile fatty acids (VFAs), alcohols, hydrogen and carbon dioxide by facultative and anaerobic bacteria. Even though a simple substrate such as glucose can be fermented, different products are produced by the diverse bacterial community. Equations 2, 3 and 4 show the conversion of glucose to acetate, ethanol and propionate, respectively.

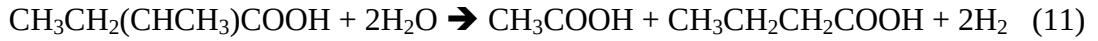
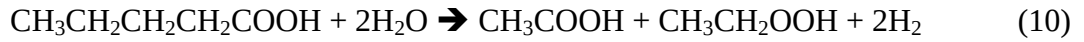
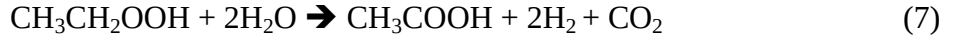
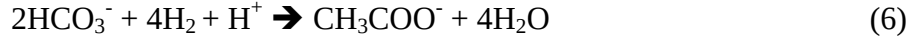
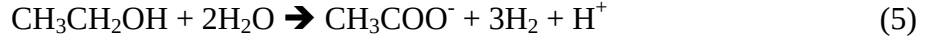


In an equilibrated system, most of the organic matter is converted into readily available substrates for methanogenic microbes (acetate, hydrogen and carbon dioxide), but a significant part (approximately 30%) is transformed to short chain fatty acids or alcohols (Angelidaki et al., 2007; Gerardi, 2003). Degradable organic matter is removed in this stage (Angelidaki et al., 2007). By-product of amino acids fermentation, ammonia and hydrogen sulphide are released (Salminen et al., 2002) that can be inhibitory for anaerobic digestion.

2.1.1.3 Acetogenesis

In this stage, which is sometimes considered to be a part of acidogenesis, acetate-forming microbes convert alcohols, VFAs other than acetate, CO_2 , and a part of hydrogen

to acetate. Typical reactions of this step are expressed in equations 5 to 12, wherein acetate is created from ethanol, bicarbonate, propionate, n-butyrate, iso-butyrate, n-valerate, 2-methylbutyrate and iso-valerate, respectively (Angelidaki et al., 2007; Pind et al., 2003).

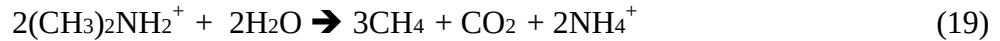
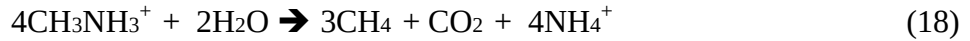
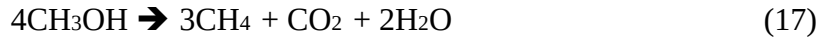
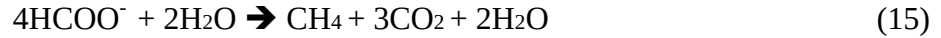


While hydrogen-producing acetogenic bacteria produce acetate, H_2 and CO_2 from volatile fatty acids and alcohol, homoacetogenic bacteria create acetate from CO_2 and H_2 (Sterling et al., 2001; Lübken et al., 2007). However, most of the acetate is created by hydrogen-producing acetogenic bacteria (Angelidaki et al., 2007). Thermodynamically, most of these reactions have low or even positive $\Delta G_o'$ (at standard conditions), so hydrogen has to be continuously depleted to enable these reactions to occur (Madigan et al., 2008). In contrast with fermentative microbes, obligatory hydrogen-producing acetogenic bacteria have no alternative metabolic pathways and cannot survive at high hydrogen partial pressures (Angelidaki et al., 2007).

2.1.1.4 *Methanogenesis*

Methanogenesis is conducted exclusively by obligate anaerobic microbes. Approximately 70% of the methane is produced from acetate (Kugelman et al., 1965; Jeris et al., 1965; Smith et al., 1966), while the remaining 30% is produced from the reduction of carbon dioxide by hydrogen and other electron donors (Hashimoto et al., 1981). Other substrates that can be converted into methane are methanol, methylamines, methylsulfides

and some alcohols, but these biological reactions are insignificant (Angelidaki et al., 2007; Gerardi, 2003). Equations 13 to 22 below illustrate reactions by which methane is produced. While equation 13 presents methane production from hydrogen and carbon dioxide by hydrogen-utilizing methanogenic microbes, equations 14 to 16 show methane production from acetate (and acetogenic compounds) and carbon monoxide by acetoclastic methanogens (Angelidaki et al., 2007; Poliafico, 2007). Equations 17 to 22 present methanogenesis from methanol (and methylamines) by methylotrophic methanogens (Gerardi, 2003). Note that as a consequence of the consumption of VFA and the production of ammonia (NH₃), the pH becomes slightly alkaline, which is optimal for methanogenic microbes (Angelidaki et al., 2007)



2.1.2 *Parameters of Anaerobic Digestion*

Several different groups of microbes are involved in anaerobic digestion, so it is crucial to maintain digester stability. An understanding of the parameters that can adversely affect the process is critical, because even small changes in conditions can disturb or permanently damage microbial interactions and performance.

2.1.2.1 *Important nutrients and the effect of feedstock composition*

Carbon, oxygen, nitrogen, hydrogen and phosphorus are the main components in organic wastes, and microbial cell material is approximately 50, 20, 12, 8 and 2 percent of those elements, respectively (Gerardi, 2003). In addition, sulphur is required to synthesize vital proteins in metabolic and anabolic pathways (Madigan et al., 2008). These are generally considered macro nutrients and must be present in digester feedstock at 10^{-4} M. A feedstock C/N ratio of 25:1 produces optimal gas production (Gerardi, 2003). If the C/N ratio is low too much nitrogen is present leading to ammonia (NH_3) accumulation that causes either high pH values or methanogenic inhibition (Salminen et al., 2002). If the C/N ratio is high nitrogen is rapidly depleted and results lower gas production (Poliafico, 2007).

Elements in trace concentration such as potassium, calcium, sodium, magnesium and iron are required as co-factors and micronutrients for enzymatic activity and as components in metal complexes. In addition, nickel and cobalt are vital for growth of methanogenic organisms. For example, F_{430} is an enzyme involved in acetoclastic methanogenesis which is activated by nickel (Gerardi, 2003; Poliafico, 2007). Most micronutrients and co-factors are inhibitory at high concentrations to fermentative and methanogenic microbes (Poliafico, 2007; Chen et al., 2008). However, in low concentrations they can stimulate methane production (Kungelman et al., 1971; Krylova et al., 1997).

A common problem with feedstock used for anaerobic digestion is the presence of recalcitrant compounds, or compounds that present physiochemical challenges. For example, lipids tend to form floating scum and long-chain fatty acids (LCFA), while lignin can be considered non-degradable compound under anaerobic digestion (Lehtomäki et al., 2007; Poliafico, 2007). Furthermore, some feedstock components contain toxic compounds (Chen et al., 2008, Poliafico, 2007). For instance, the degradation of proteins releases ammonia which can be inhibitory for both fermentative and methanogenic microbes (Chen et al., 2008; Krylova et al., 1997).

2.1.2.2 *pH and Alkalinity*

After gas production, pH is the best indicator of future digester instability (Poliafico, 2007). Initially, pH will decrease as organic matter undergoes acetogenesis, but methanogens rapidly consume those acids increasing pH and stabilizing digester performance. Enzymatic activity of fermentative bacteria requires a pH higher than 5.0. Methanogenic activity occurs at pH between 6.2 and 8 with an optimum range between 7.0 and 7.2 (Gerardi, 2003; Poliafico, 2007). In addition, other phenomenons such as the dissociation of important compounds (ammonia, sulphide and organic acids, among others) are directly affected by pH (Angelidaki et al., 2007). Alkalinity is crucial in pH control and enhances digester stability. Alkalinity is mainly present in the form of bicarbonates in equilibrium with carbon dioxide gas at a given pH (Gerardi, 2003). Therefore, pH depends on the partial pressure of CO₂ and balance between acid and alkaline components in the liquid phase and can be used as indicator of methanogenic consortium performance (Chen et al., 2008; Gerardi, 2003; Poliafico, 2007).

2.1.2.3 *Temperature*

The gas production rate during anaerobic digestion is affected by temperature. There are two temperature ranges at which most methanogenic microbes are active: mesophilic, between 20 and 37°C, and thermophilic, between 50 and 60°C. At temperatures between 40 and 50°C methanogens are inhibited. (Gerardi, 2003). Optimum biogas production occurs at 35 and 55°C for mesophilic and thermophilic organisms, respectively (Angelidaki et al., 2007).

Mesophilic and thermophilic conditions present different reactor design and operational advantages and drawbacks. During thermophilic digestion both greater destruction of pathogens and higher substrate degradation (and biogas production) can be achieved, but the microbe population is more sensitive to environmental conditions such as concentration of inhibitory compounds, temperature fluctuations, changes in organic loading and pH (Angelidaki et al., 2007; Chen et al., 2008; Gerardi, 2003; Poliafico, 2007; Van Lier, 1995). In addition, thermophilic conditions require a large amount of heat energy that reduces the net energy production (El-Mashad et al., 2004).

2.1.2.4 *Retention Time*

The hydraulic retention time (HRT) is the theoretical time that the influent liquid phase stays in the digester, while the solids retention time (SRT) is generally the ratio between solids maintained in the digester and solids wasted in the effluent. In conventional single stage completely mixed or plug flow digesters, the HRT matches the SRT, but in other systems the SRT can be greater than the HRT. The conversion of organic matter to gas is more closely related to SRT rather than HRT (Poliafico, 2007). SRT has a critical value at which wash out occurs (Poliafico, 2007; Madigan et al., 2008). Research has focused on increasing the SRT while decreasing the HRT, so better biogas yield can be achieved using smaller digester (Collins et al. 2006; Sakar et al., 2009; Yetilmezsoy et al., 2008).

Anaerobic digestion retention times range from 14 and 30 days (Borja et al., 2002; Callahan et al., 2002; Hashimoto, 1982; Poliafico, 2007). A short retention time will produce higher biogas per volume, but less organic matter will be degraded. Although a short retention time is desired for reducing the digester volume, a balance must be made to achieve the desired operational conditions, for example maximizing either methane production or organic matter removal.

2.1.2.5 *Organic Loading Rate*

The organic loading rate (OLR) is the organic matter flowing into the digester per time, expressed as mass of organic matter over digester volume over time. Typical values of OLR ranges between 0.5 and 3 kg VS*m⁻³*d⁻¹ (Poliafico, 2007; Gerardi, 2003). Higher organic loading rates trend to increase organic acids production and decrease reactor design volumes, but overloading conditions can permanently damage non-adapted methanogenic consortia and reduce organic matter removal rates.

2.1.2.6 *Mixing*

Mixing the digester contents will enhance the anaerobic digestion process by distributing substrate, nutrients, and bacteria and equalizing temperature (Gerardi, 2003). The benefits of mixing include:

- Eliminates or reduces scum buildup.
- Eliminates thermal stratification or localized pockets of depressed temperature.
- Maintains digester sludge chemical and physical uniformity throughout the tank.
- Stimulates the rapid dispersion of metabolic wastes produced during substrate digestion, that could otherwise inhibit methane production.
- Stimulates the rapid dispersion of any toxic material entering the tank (minimizing toxicity).
- Prevents deposition of grit.

Methanogenic populations, especially the hydrogenotropic consortium, grow in a syntrophic relationship with acetate and hydrogen-forming microbes (Madigan et al., 2008) that require close contact between both species which mixing ensures. Hydrolytic and acid-forming bacteria perform more efficiently in suspended mixed organic matter, because a larger surface area to volume promotes hydrolases and acid-forming microbial activity (Gerardi, 2003).

2.1.3 *Advantages and Drawbacks of Anaerobic Digestion*

The main technical advantages provided by the use of anaerobic digestion as a wastewater treatment technology are summarized in Table 2.2. Most important for animal wastes, anaerobic digestion recovers plant nutrients such as nitrogen and phosphorus. Nitrogen and phosphorus are recovered as ammonia and orthophosphate, respectively (Poliafico, 2007). As a consequence, the digestate has significant value as a slow fertilizer release (Alvarez, 2006; El-Mashad et al., 2004; Keshtkar et al., 2001; Uludag-Demirer et al., 2008).

Also in regard to animal wastes, it is important to note that pathogens destruction during anaerobic digestion is extensive (Albhin et al., 2007; Nicholson et al., 2002). *Salmonella* and *E.Coli* (belonging to bacteria group) are used as pathogen indicator organisms (Albhin et al., 2007; Horan et al., 2004; Smith et al., 2005). Previous research have shown that thermophilic conditions are more effectively destroy pathogen indicators

Table 2.2: Advantages and drawbacks of aerobic and anaerobic digesters (Gerardi, 2003).

Feature	Digester	
	Aerobic	Anaerobic
Alkalinity additional	If nitrifying	Yes
Degradation rate of organics	Rapid	Slow
Degradation of xenobiotics	No	Yes
Design and constructino costs	Higher	Lower
Heating requirements	No	Yes
Malodor production	Yes	No
Methane production	No	Yes
Nutrient requirements	Higher	Lower
Operating costs	Higher	Lower
Oxygen requirement	Yes	No
Pathogen destruction	Less	More
Sensitivity to changes	Less	More
Sludge disposal costs	Higher	Lower
Sludge production	Higher	Lower
Solids retention time	Lower	Higher
Start-up time	Lower	Higher

and presumably pathogens also (Albhim et al., 2007; Salminen et al., 2002a; Shih et al., 1987; Sahlström et al., 2004). A reduction in total coliforms (which includes *E.Coli*) of between 98.5 and 100 % occurs during thermophilic digestion (Salminen et al., 2002a; Song et al., 2004), while specific pathogen reduction (including *Listeria monocytogenes*, *Salmonella senftenberg* and *Escherichia coli*) is around a 99 % in mesophilic conditions (Horan et al., 2004).

2.2 Anaerobic Co-digestion

Co-digestion is the simultaneous digestion of two or more organic waste feedstock. The use of this technology has advantages and limitations which are discussed below. Specific examples of co-digestion studies with animal wastes are also presented.

2.2.1 *Co-digestion advantages and limitations*

Although most of bio-wastes can be successfully digested (Braun et al., 2004), co-digesting can improve nutrient balance, enhance pH buffer capacity, and optimize rheological qualities (Lehtomäki et al., 2007; Li et al., 2009) that improve overall gas production in a synergistic fashion (Alvarez et al., 2008a; Gelegenis et al., 2007b; Kaparaju et al., 2005; Kugelman et al., 1971; Macias-Corral et. al., 2008; Magbauna Jr. et al., 2001; Misi et al., 2001a; Mshandete et al., 2004). It may be that the synergistic effect results from complementary microbial consortia coming from different wastes (Macias-Corral et. al., 2008). Furthermore, mixing different wastes can dilute toxic compounds that may inhibit digestion, such as ammonia, sulfides, light metal ions, heavy metals and some organic compounds (benzenes, phenols, alcohols, ethers, oils and fats, lignin and lignin-related compounds, among others) (Chen et al., 2008; Kelleher et al., 2002; Mata-Alvarez et al., 2000; Salminen et al., 2002a). Finally, economic advantages can result from shared equipment, easier handling of feedstock, and a more stable process in general (Hatmann et al., 2005; Li et al., 2009; Misi et al., 2001a; Mshandete et. al., 2004).

The main disadvantage of co-digestion is that it still remains largely unstudied (Callaghan et al., 1997; Misi et al., 2001a; Mshandete et. al., 2004). Also, practical limitations include transport cost and difficulties complying with regulations and policies for different types of waste streams (Mata-Alvarez et al., 2000).

2.2.2 *Co-digestion of Chicken Litter with Dairy Manure*

In the United Kingdom, poultry litter has been anaerobically digested in full-scale plants, but methane production is lower than predicted. Poultry litter has a high fraction of biodegradable organic matter including carbohydrates, proteins, oils and fats (Kelleher et al., 2002). However, high ammonia concentrations occur as a result of degradation of organic nitrogen present in proteins and urea and could explain the low biogas production and stability problems. Although methanogenic activity can be stimulated when the ammonium ions concentration (NH_4^+) ranges from 40 to 660 mg N-NH₃/L (Kayhanian, 1994), higher ammonia concentrations can inhibit the overall anaerobic digestion process

with the methanogenic consortium being the most sensitive group (Chen et al., 2008; Kayhanian, 1994). There are three possible mechanisms for ammonia inhibition (Callahan et al., 2002; Chen et al., 2008; Krylova et al., 1997; Madigan et al., 2008; Sterling Jr. et al., 2001; Whittmann et al., 1995):

- The hydrophobic and highly membrane permeable unionized ammonia molecule (NH_3) diffuses into cell membranes creating proton imbalance and/or potassium deficiency. As a consequence, energy production is not feasible.
- The amination of α -ketoglutaric acid from the Citric Acid Cycle (microbial respiration) can impair oxidation of organic compounds leading to an accumulation of fermentation products (mostly volatile fatty acids and alcohols) that lower pH and reduces gas production.
- Unionized (free) ammonia can directly inhibit the enzymatic reaction which synthesizes methane.

Conflicting literature exists concerning the inhibitory concentration of ammonia and the sensitivity of different digester microbes. Reduction in methane production due to ammonia inhibition has been reported when total ammonia ($\text{NH}_3 + \text{NH}_4^+$) ranges from 1.2 to 15 g N- NH_3 /L (Angelidaki et al., 1993a,b; Angelidaki et al., 1994; Boardman et al., 1997; Borja et al., 1994; Bujoczek et al., 2000; Chamy et al., 1998; Hendriksen et al., 1991; Gallert et al., 1997; Gallert et al., 1998; Guerrero et al., 1997; Hansen et al., 1998; Kayhanian, 1994; Krylova et al., 1997; Poggi-Varaldo et al., 1997; Soubes et al., 1994; Sung et al., 2003). In addition, it has been suggested that free or uncharged ammonia (NH_3) is the most toxic form of ammonia, especially for methanogens (Angelidaki et al., 1993a,b; Mata-Alvarez et al., 2000). Concentrations as low as 66 mg N- NH_3 /L have been proposed as the starting inhibitory level (Burton et al., 2003; Koster et al., 1984; De Baere et al., 1984) and a range between 99 and 150 mg N- NH_3 /L is significantly inhibitory

(Angelidaki et al., 1993b; Webb et al., 1985a,b; McCarty et al., 1961; Braun et al., 1981). The significant differences in inhibitory concentration for both total ammonia and free ammonia can be explained by differences in substrates, inocula, operational conditions (temperature, pH, retention time, among others) and acclimation periods (Callaghan et al., 1999; Mata-Alvarez et al., 2000). However, after a certain period of time, methanogens may adapt to several times to initial threshold (Salminen et al., 2002a). It was suggested that this acclimation is more likely due to the growth of new methanogens rather than metabolic changes in actual methanogens (Angelidaki et al., 1993a; Koster, 1986). It remains unclear whether acetoclastic or hydrogenotropic methanogens are the most sensitive consortium. While some researchers have reported acetoclastic methanogens are more severely affected by ammonia (Angelidaki et al., 1993a; Calli et al., 2005; Bhattacharya et al., 1989; El-Mashad et al., 2004; Robbins et al., 1989; Sprott et al., 1986), other investigations have concluded that hydrogenotropic methanogens are more sensitive (Koster et al., 1988; Wiegant et al., 1986; Zeeman et al., 1985).

When digesting chicken litter, co-digestion has been suggested as a means to prevent ammonia inhibition (Callahan et al., 1999; Chen et al., 2008; Gelegenis et al., 2007a; Kelleher et al., 2002; Krylova et al., 1997). Poultry manure has been co-digested with hog waste (Magbauna Jr. et al., 2001), fruit and vegetable waste (Callaghan et al., 2002), molasses (Misi et al., 2001a), sheep and goat manure (Misi et al., 2001a), organic fraction of municipal solid waste (Hartmann et al., 2005), cheese whey (Gelegenis et al., 2007b), olive-mill wastewater (Gelegenis et al., 2007a) and dairy manure (Callahan et al., 1999; Callahan et al., 2002; Mata-Alvarez et al., 2000; Misi et al., 2001a).

Co-digestion of dairy and poultry wastes can be an attractive solution for waste treatment (Güngör-Dermici et al., 2004). Less transportation expenses are required for solid chicken litter (Mata-Alvarez et al., 2000). When these wastes are mixed, chicken litter is dissolved by water present in dairy manure reducing total solids contents (with an optimal between 0.5 and 3% for chicken litter) and improving its rheological properties (Angenent et al., 2002; Kelleher et al., 2002; Mata-Alvarez et al., 2000; Salminen et al., 2002a). In fact, it has been reported that chicken litter with dairy manure could be one of

the most promising alternatives for co-digestion (Bousfield et al., 1979; Callaghan et al., 1999; Callaghan et al., 2002).

Güngör-Dermici et al. (2004) studied in batch reactors the effect of several parameters such as initial COD, nutrient addition, temperature and microbial acclimation when co-digesting broiler and dairy manure. They concluded that nutrients in manures are sufficient for digestion if the solids content is appropriately adjusted. However, they observed that performance of digestion decreases as the fraction of broiler manure increases, and speculated that the decline in performance was due to ammonia inhibition. Other researchers (Callaghan et al., 1999; Misi et al. 2001a,b) have performed batch experiments co-digesting dairy manure with chicken litter reporting that a decrease in methane production seems to be linked to ammonia inhibition. A similar study by Callaghan et al. (2002) in continuous reactors at 35 °C and 21 HRT revealed that as chicken litter was gradually increased higher organic matter removal was obtained, with the maximum removal obtained when chicken litter was about a 30% as wet volume of the feed ($OLR\ 4.44 \pm 0.21 gVS\ L_{reactor}^{-1}\ day^{-1}$). However, those researchers concluded that chicken litter was not a good co-substrate because it produced high free ammonia concentrations. Further research is necessary to determine the quantity and the maximum organic loading rate that chicken litter could be applied to a working digester treating dairy manure without adversely affecting its performance.

2.3 Summary

Anaerobic digestion is a well establish technology for manure wastewater treatment and it can produce renewable energy, reduce organic and pathogen content, and create a stable residual waste that can be used as soil fertilizer. However, some manures present problems such as a high solids content or inhibition by toxic compounds released in the process. Co-digestion can not only reduce these problems, but also could improve the overall performance.

Co-digestion of chicken litter with dairy manure may improve methane production and solve problems such as the high total solids and ammonia inhibition problems associated with chicken litter. A failure of anaerobic co-digestion seems to be linked to ammonia inhibition, but further research is required to obtain a better insight when co-digestion of these wastes is successfully achieved.

CHAPTER 3 Materials and Methods

3.1 Substrates

3.1.1 *Co-digestion of Dairy manure with chicken litter*

Dairy manure (DM) was collected from a 800 head farm located in Loudon County, Tennessee, USA and stored at 4 °C in a closed container. This dairy fed a complete ration containing a mixture of corn silage, soy bean hulls, and straw. Chicken litter (CL) was collected from a broiler (meat chicken) farm in Greene County, Tennessee, USA and stored at 4 °C in a closed container. The litter was a mixture of kiln dried wood shavings, bird feces, spilled feed and feathers.

Periodically (twice a month approximately), DM was filtered through a 0.417 mm screen to remove straw and solids that otherwise clogged reactor influent lines. DM was then diluted to 20 g/L of Volatile Solids (VS), capped and stored at 4 °C as it was continuously fed into a control reactor or mixed with CL. Similarly, chicken litter was macerated using a domestic blender and sieved through a 2 mm mesh screen to remove feathers, and degraded wood shavings and stored at 4 °C. The composition of both ready-to-use substrates (dairy manure and chicken litter) is presented in Table 3.1. Parameters illustrated in characterization (Table 3.1) are in the expected range according with literature for both dairy manure (Alvarez et al., 2006; Ahring et al., 2001; Callahan et al., 2002; Güngör-Demirci et al. (2004); Kaparaju et al., 2008; Keshtkar et al., 2003; Møller, et al., 2004) and chicken litter (Abouelenien et al., 2009; Callahan et al., 2002; Güngör-Demirci et al., 2004; Kelleher et al., 2002; Salminen et al., 2002a). Deionized water (conductivity of 5.5×10^{-6} S/m) was used for dilution when needed.

Table 3.1: Composition of dairy manure and chicken litter as used.

Parameter ^a	unit	Dairy Manure (DM)		Chicken Litter (CL)	
		mean	sd	mean	sd
pH		7.3	0.1	-	-
Total Solids (TS)	% wet	2.3	0.0	55.75	0.15
Total Suspended Solids (TSS)	% TS	35.6	4.1	-	-
Volatile Solids (VS)	% TS	57.6	0.6	60.6	1.2
Suspended Volatile Solids (VSS)	% TS	46.9	1.8	-	-
Total COD	mg/L O ₂ /g VS	1460	234	389 ^b	33
Soluble COD	mg/L O ₂ /g VS	587	18	-	-
Total VFAs (as acetic acid)	mg/g VS	68.4	2.1	UDL ^b	UDL
Free Ammonia	mg [N-NH ₃]/g VS	0.4	0.0	-	-
Total Ammonia	mg [N-NH ₃]/g VS	18.4	0.8	14.1 ^b	1.0
Total Kjeldahl Nitrogen	mg N/g VS	56.1	1.9	70.7 ^b	4.2
Total Nitrogen	mg N/g VS	74.4	3.7	81.3 ^b	5.8
Total Phosphorus	mg P/g VS	22	1	279 ^b	7
Total Alkalinity	mg as CaCO ₃ /g VS	270	4	83 ^b	39

^a Each parameter was analyzed in triplicate.

^b Analyzed for three different samples created by adding 0.5, 1 and 1.5 g of CL to a total volume of 1 liter using deionized water. UDL = under detection limit.

3.1.2 Co-digestion of dairy manure with other substrates

Saw dust (SD), grease trap waste (GTW) and filtered dairy manure solids (FDMS) were co-digested with dairy manure. Screened (0.417 mm mesh) effluent was used as co-digestion inocula and was collected from continuous lab scale reactors at: 35(±2) °C, fed with DM at an organic loading rate of 1.01(±0.04) gVS L_{reactor}⁻¹ day⁻¹, presenting stable (±5%) methane yield (216±12 ml CH₄ /g VS added), methane percentage (70.6±2%), VS removal (31.4±1.8%), pH (7.56±0.04), and low VFAs (< 30 mg/L as acetic acid) (Kaparaju et al., 2009; El-Mashad et al., 2004). Waste and inocula characterization are presented in Table 3.2. Filtered dairy manure solids was obtained by collecting the material retained between sieves 0.417 and 0.842 mm mesh size. Sawdust was collected from particles passing through a 0.417 mm sieve mesh size. After sieving, solids wastes were rinsed and immersed for 12 hours with DI water. Then, they were rinsed again and dried in an oven at 105±2 °C until constant mass and stored in desiccators at 20 °C.

Table 3.2: Inocula and characterization of other substrates.

Parameter ^a	unit	Inocula		Sawdust ^b		Filtered dairy manure solids ^b		Grease trap waste	
		mean	sd	mean	sd	mean	sd	mean	sd
pH		7.56	0.04	-	-	-	-	4.51	0.05
Total Solids (TS)	% wet	2.3	0.0	100	0	98.2	0.9	13.8	0.4
Volatile Solids (VS)	% TS	62.3	0.2	99.6	0.1	96.4	0.2	98.8	0.1
Total Kjeldahl Nitrogen	mg N/g VS	83.7	8.5	2.8	0.3	14.9	0.3	1.2	0.2
Total Phosphorus	mg P/g VS	30.0	3.0	UDL		10.0	1.0	1.0	0.0
Total Alkalinity	mg as CaCO ₃ /g VS	650	19	-	-	-	-	51	1

^a Each parameter was analyzed in triplicate.

^b This solids substrates were immediately dried and stored in desiccators. UDL = under detection limit.

3.2 Continuous reactor experiment

Two sets of three semi-continuously stirred tank reactors (CSTR) were configured to evaluate co-digestion feasibility (Appendix A, Figures A.1, A.2). Control (digestion of dairy manure alone) and test (co-digestion) experiments were carried out in triplicate at 35(±2) °C using one-gallon plastic bottles (Nalgene LDPE) with a working volume of 3.6 L. These test reactors were inoculated with screened (0.417 mm mesh) effluent from continuous lab-scale reactors at 35(±2) °C fed with dairy manure at an organic loading rate of 1.01(±0.04) gVS L_{reactor}⁻¹ day⁻¹ and presenting a stable (±5%) methane yield (225±12 ml CH₄/gVS added), methane percentage (70.5±1.3%), VS removal (30.5±1.4%), pH (7.52±0.03), and low VFAs (< 30 mg/L as acetic acid). All of the reactors were mixed via a magnetic stirrer. Each reactor had three ports: two upper ports, one for feeding and the other for gas collection, and a lower effluent port. A set volume of digested manure was withdrawn from each reactor daily and replaced (over 40 minutes) with the same volume of fresh feedstock, setting the hydraulic retention time at 20 days (Bousfiled et al., 1979; El-Mashad et al., 2004; Pain et al., 1988; Sakar et al., 2009). Biogas was collected in hermetically sealed plastic bags. In addition, a water seal device was placed in the tubing system carrying biogas from the reactors to a collector which maintained a constant atmospheric pressure inside the reactors and prevented oxygen leaking into reactors while

the daily gas production was measured. A gas sampling port was placed in this tubing system. Gas volume measurement was made by the water displacement method.

All reactors were fed at an organic loading rate (OLR) of $1.01(\pm 0.04) \text{ gVS L}_{\text{reactor}}^{-1} \text{ day}^{-1}$ until they presented stable ($\pm 5\%$) biogas and methane yields, methane percentage, VS removal, pH and low VFAs ($< 30 \text{ mg/L}$ as acetic acid). The experiment involved increasing the OLR in test digesters to $1.28(\pm 0.03)$, $1.5(\pm 0.03)$ and $1.77(\pm 0.05) \text{ gVS L}_{\text{reactor}}^{-1} \text{ day}^{-1}$ by adding increasing amounts of chicken litter (section 3.1.1). Before the organic loading increased, digesters were run either two retention times or until stable ($\pm 5\%$) methane yield, methane percentage, VS removal, pH and low VFAs were obtained (Kaparaju et al., 2009; El-Mashad et al., 2004). Organic matter coming from dairy manure ($1.01(\pm 0.04) \text{ gVS L}_{\text{reactor}}^{-1} \text{ day}^{-1}$) was kept constant along all stages in control reactors and as the background organic loading in co-digesting reactors. The feedstock used in co-digestion was prepared as follows. The ready-to-use CL (section 3.1.1) was mixed with filtered and diluted CM to increase the VS concentration, and filtered again through a 0.417 mm mesh screen to ensure the same maximum particle size of feedstock in both control and co-digesting reactors. Then, it was stored at 4°C as it was continuously fed into co-digesting reactors.

3.3 Batch reactor experiments

Experiments were conducted in 160 ml serum bottles (100 ml effective volume) capped with butyl rubber stoppers and secured with tear-off aluminum seals (Appendix A, Figure A.3). Control reactors containing only inocula were also run in all experiments to determine the background gas production. Digested manure of different organic loading rate stages from continuous reactors was used as inocula depending on the experiment. After seeding with inocula and the desired substrate, the reactors were flushed 5 minutes with a 99.9% of purity N_2 passed through copper filings at 300°C to remove all traces of O_2 (Bryant 1972; Shelton et al., 1984). Bottles were maintained at $35(\pm 2)^{\circ}\text{C}$ in a dark shaking-plate incubator unless other conditions are specified. Gas volume was measured by water displacement method. All experiments were performed in triplicate. A schematic

chart with the proposed experimental procedure and sampling points is illustrated in Figure 3.1.

3.3.1 *Methane potential and substrate degradability experiments*

Four replicate bottles were seeded with 80 ml of digested dairy manure (OLR 1.01 gVS L_{reactor}⁻¹ day⁻¹) and either 20 ml of different liquid dairy manure concentrations or various known mass of solid chicken litter. Different concentration of organic matter measured as Volatile Solids (VS) were used in order to ensure a methane potential value in a non-overloaded, and consequently non-inhibited, process. Methane potentials were measured at days 20 (four replicates) and 72 (two replicates) when methane production was less than 10%. Substrate degradability (two replicates) was measured at day 20.

3.3.2 *Methane production capacity and ammonia inhibition concentration experiments*

Biomass from the different organic loading stages during continuous digestion and co-digestion were collected and used as inocula for testing ammonia adaptation and methane production capacity. Bottles were seeded with 57 ml of the biomass and 13 ml of dairy manure. Substrate/seed ratios were based on VS and kept below 0.5 to avoid organic overloading (Lehtomäki et al., 2007). Three replicates were used in all tests. To ensure anaerobic conditions after N₂ flushing a Na₂S*9H₂O solution was added as a reducing agent at a final concentration of 0.1 mM (Christiansen et al., 1996; Bryant 1972; Kettunen et al., 1997; Khan et al., 1978; Shelton et al., 1984).

Biomass methane production capacity (ml CH₄ gVS added⁻¹ gVS inoc⁻¹) was measured in one set of experiments using biomass from the different organic loading stages of the continuous reactors (Figure 3.1). In another set of experiments, ammonia (NH₄Cl in deionized water) was added to determine the IC₅₀ value, defined as the concentration resulting in 50% inhibition of the control vial (no addition of ammonia). Gas production IC₅₀ was calculated using a linear regression of specific methane activity (SMA) (ml CH₄ gVS inoc⁻¹ d⁻¹) over time (Cavaleiro et al., 2008; Collins et al., 2006; Kettunen et al., 1997).

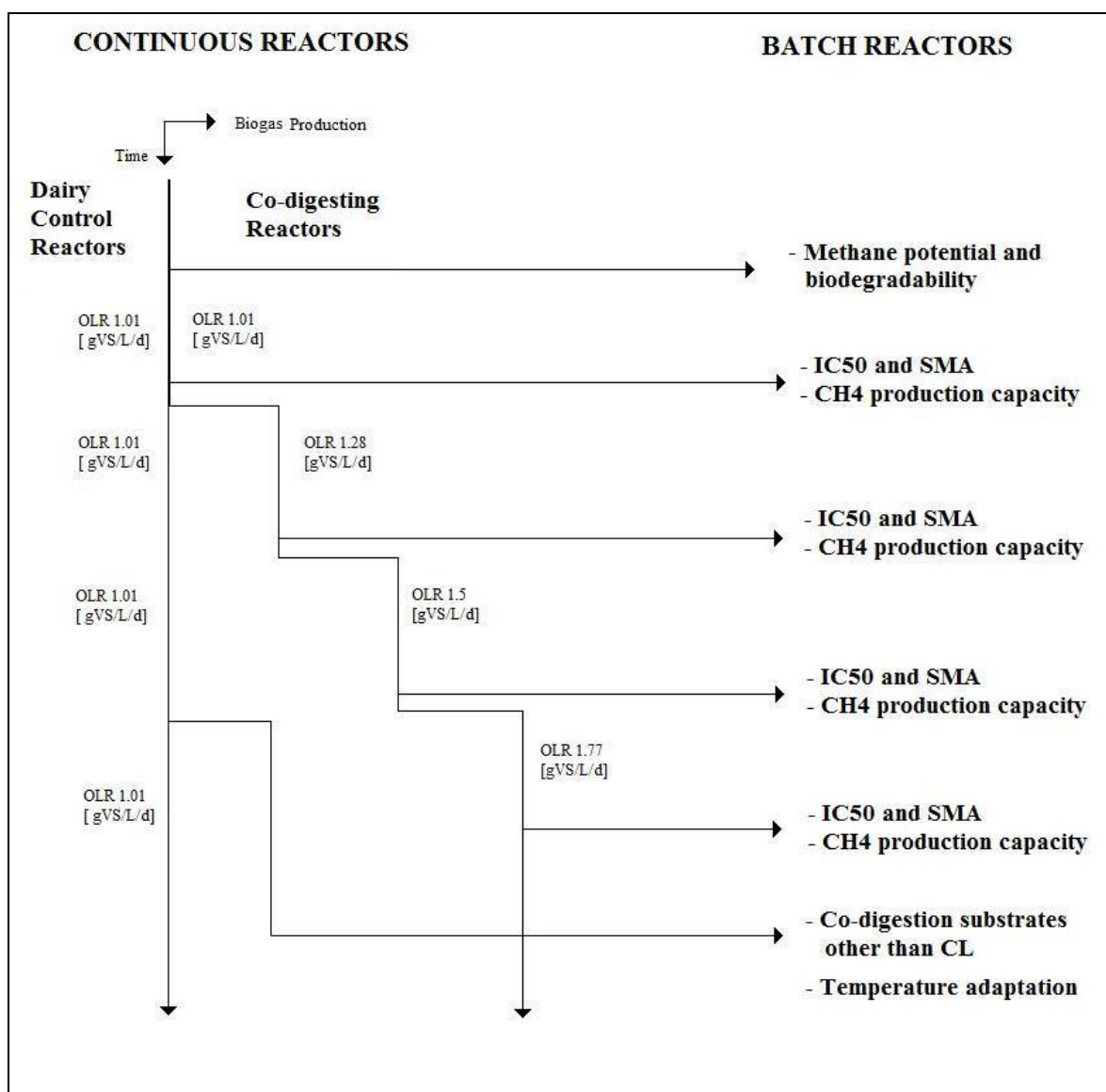


Figure 3.1: Experimental procedure and sampling point diagram.

3.3.3 Methane potential from other substrates and co-digestion

Eighty milliliters (80 ml) of digested dairy manure ($\text{OLR } 1.01 \text{ gVS } L_{\text{reactor}}^{-1} \text{ day}^{-1}$) was used as seed to determine methane potential of different wastes. While for methane potential single substrates were added with inocula, in co-digestion experiments co-substrates and a fixed volume of dairy manure were added with inocula. Different organic loadings were obtained by increasing only the co-substrate amounts for the co-digesting

experiments. Three replicates of each concentration were tested until the increase in methane production was less than 10%. Measurements of VS were performed at the beginning and at the end of the experiment.

3.3.4 *Temperature adaptation of anaerobic digestion*

Digested dairy manure (80 ml) and either raw dairy manure or deionized water (20 ml) were evaluated for methane production at four temperatures: 35, 25, 19, and 9°C.

3.4 Analytical methods

3.4.1 *Measured data*

The pH measurements were taken with a pH meter (Thermo Scientific ORION model 720-A) and probe (Thermo Scientific ORION 9156DJWP). Total Solids (TS) in liquid and solid samples was determined by using Standard Method 2540 B and G, respectively; Volatile Solids (VS) was measured using Standard Method 2540 E (APHA; 2005). Gas composition was analyzed with a Hewlett Packard 5890 Series II GC provided with TCD and a Supelco packing column (60/80 carbonxen-1000, 4.57 m, 2.1 mm inner diameter) using high purified Argon gas as carrier gas at 5 ml/min. Operational conditions were: oven 125 °C, injection port 150 °C, detector 170 °C. A pressure lock syringe was used in all gas sampling. VFAs were also measured using a Hewlett Packard 5890 provided with flame ionization detector (FID) and the Restek Stabilwax®-DA column (15m, 0.53mmID, 0.5um) using Nitrogen as a carrier gas at 2.4 ml/min with a split ratio of 1:11. Operational conditions were: oven 110 °C, injection port 250 °C, and detector 250 °C. Prior to injection, samples for VFAs analysis were centrifuged 10 min at 13,300 rpm, the supernatant was acidified with 3M HCl, and centrifuged 10 min at 13,300 rpm. Chemical Oxygen Demand (COD) and Total Alkalinity were measured according to Standard Methods 5220 C and 2320-B, respectively (APHA; 2005). Total Kjeldahl Nitrogen and Total Ammonia ($\text{NH}_3 + \text{NH}_4^+$) were measured using Standard Methods 4500-

N_{org} C, D and 4500-NH₃ D, respectively (APHA; 2005). An ion selective probe was used to measure Total Ammonia (ORION 9512HPBNWP) and an ORION model 250 meter. Total Nitrogen was analyzed by a calibrated total nitrogen measurement unit (TNM-1, Shimadzu) displaying a coefficient of variance less than 2.5%. For Total Phosphorus measurements, samples were digested according to QuickChem Method 13-115-01-1-B (Zellweger Analytics, INC.; 1998) to convert all phosphorus to orthophosphate, and then an inductive coupled plasma atomic emission spectroscopy (ICP-AES) was used to determine the final concentration. The ICP instrument was a Spectroflame-P (Spectro Analytical Instruments) equipped with an Alitea-pump (0-50 rpm) and adjusted to a wavelength of 178.2 nm. The samples for Total Kjeldahl Nitrogen, Total Nitrogen, Total Phosphorus and soluble COD (SCOD) determination were filtered with 1.6µm glass fibre filter papers. *Escherichia coli* was determined using Method 1604 (EPA; 2002).

3.4.2 Calculated data

Free ammonia, which is the most toxic form of ammonia, depends on total ammonia concentration, temperature and pH. A simple formula was used to compute this parameter (Angelidaki et al., 1997).

$$\frac{[free\ NH_3]}{[NH_3 + NH_4^+]} = \left(1 + \frac{10^{-pH}}{10^{\left(0.09018 + \frac{2729.92}{T(K)}\right)}} \right) \quad (23)$$

The % of Volatile Solids reduction was computed according to the following formula:

$$\%VS_{reduction} = \frac{VS_{influent} - VS_{effluent}}{VS_{influent}} \cdot 100 \quad (24)$$

3.4.3 *Statistical methods*

Mean and standard deviation of the data were determined with the Microsoft Excel version 2003. Other statistical analyze was performed with the JMP software version 7.0.1. ANOVA was used to determine significant differences in the means at a level of confidence of 5%. Tukey-Kramer Honestly Significant Difference (HSD) was used to define groups of statistically different values (Sall et al., 2005). Normal distribution of data was checked by Shapiro-Wilk test. Homogeneity of variance was analyzed by Levene test statistic. For unequal variances, sample sizes between groups no different than 50% were used because F-test yields similar results when compared with equal variances (Ketchum et al., 2009).

CHAPTER 4 Dairy Manure and Chicken Litter Co-digestion

4.1 Temperature adaptation of dairy manure anaerobic digestion

It is well known that temperature directly affects the kinetics of enzymatic reactions (Snoeyink et al., 1980) and that the limiting step for anaerobic digestion is enzymatic hydrolysis (Angelidaki et al., 1993b, Garcia-Ochoa et al., 1999). In a hydrolytic rate-limited process, the specific methane activity (the rate at which methane is produced) measures the rate of hydrolysis. When the enzyme concentration is not rate-limited such as the case of dairy manure, the temperature dependence of hydrolysis can be described by the Arrhenius equation (Kettunen, et al., 1997; Madigan et al, 2008, Veeken et al., 1999). However, microbial populations can adapt to lower temperature (Kettunen, et al., 1997). Thus an experiment was performed to test the microbial adaptation to low temperatures and quantify the influence in methane production (Table 4.1).

Figure 4.1 shows specific methane production over time at different temperatures. As expected, at 35 °C specific methane activity was higher than at lower temperatures, though the specific methane production at 25 and 19°C approached the production at 35°C (54.7 ± 0.7 and 47.7 ± 0.5 ml gVS inoc⁻¹ versus 56.6 ± 0.7 at day 28). In fact, at 37 days, methane production at 25 °C was similar to that at 35 °C. Previous research (Rebac et al., 1996; Yan et al., 1996) has reported that biomass adaptation to temperature can significantly enhance methanogenic activity. Adaptation to a temperature of 19 °C occurred, but specific methane production (47.7 ± 0.5 ml gVS inoc⁻¹ at day 28) was always lower than at temperatures 25 and 35°C. In contrast, at a temperature of 9 °C microbial population was considerably inhibited (13.5 ± 0.7 ml gVS inoc⁻¹ at day 28). However, at 9 °C methanogenic consortium was active. This finding is in agreement with previous research (Huser et al., 1982; Kettunen et al., 1997; Westermann et al., 1987; Kotsyurbenko et al. 1993) in which methane production was observed at temperatures from 3 to 6 °C.

Table 4.1: Experimental design investigating temperature adaptation of dairy manure anaerobic digestion.

Inoculum Addition (ml)	DI Water (ml)	Cow manure (ml)	Total g VS/L	Temperature
80	20		10.59	Inoc 35 °C
80		20	14.66	CM 35 °C
80	20		10.59	Inoc 25 °C
80		20	14.66	CM 25 °C
80	20		10.59	Inoc 19 °C
80		20	14.66	CM 19 °C
80	20		10.59	Inoc 9 °C
80		20	14.66	CM 9 °C

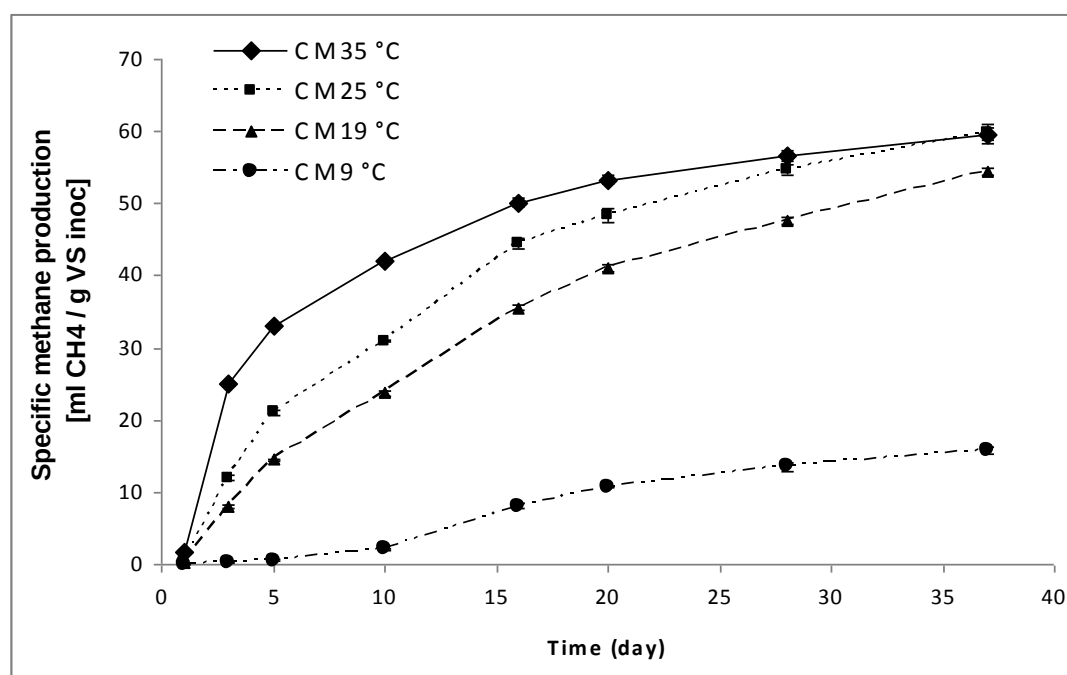


Figure 4.1: Specific methane production at different temperatures.

An Arrhenius model was applied to the data (Figure 4.2). The activation energy (E_a) for this study (67.39 kJ/mol) was comparable with the average activation energy (64 ± 14 kJ/mol) that Veeken et al. (1999) measured for different biowastes such as wholewheat bread, leaves, bark, straw, orange peelings, grass and filter paper. These activation energy values are typical for enzymatic kinetic reactions (Roels, 1983), perhaps confirming that hydrolysis during digestion mimics the kinetics process when an enzyme concentration exceeds the concentration of degradable substrate surface sites (Veeken et al., 1999).

4.2 Methane potential from substrates

The first step before co-digestion is to determine methane potential from singles substrates. Figures 4.3 and 4.4 present the results for DM and CL. Table 4.2 presents the biodegradability and methane recovery at 20 days. Volatile Solids (VS) was chosen as the parameter to measure the organic strength of substrates, because Chemical Oxygen Demand (COD) is subjected large errors with heterogeneous substrates such as DM and CL, and because large dilution factors (100) would have been required to conduct the COD test (Angelidaki et al., 2007).

Because digested dairy manure was used as inocula, methane production from dairy manure presented just one day of lag phase (Figure 4.3) indicating a fast adaptation of inocula to new conditions. In contrast, an adaptation period of 3 days was observed when digesting chicken litter (Figure 4.4). Constant methane production rate was achieved between days 1 and 4 for dairy manure and between days 3 and 5 for chicken litter. Thereafter, methane production decreased until reaching a maximum value at day 72.

The biodegradability of chicken litter was considerably higher than biodegradability of dairy manure. While dairy manure volatile solids were approximately 30% biodegradable, chicken litter was almost twice that value at 58%. Kelleher et al. (2002) indicate that chicken litter has a higher fraction of biodegradable organic matter than other livestock wastes, including high levels of organic nitrogen due to the high

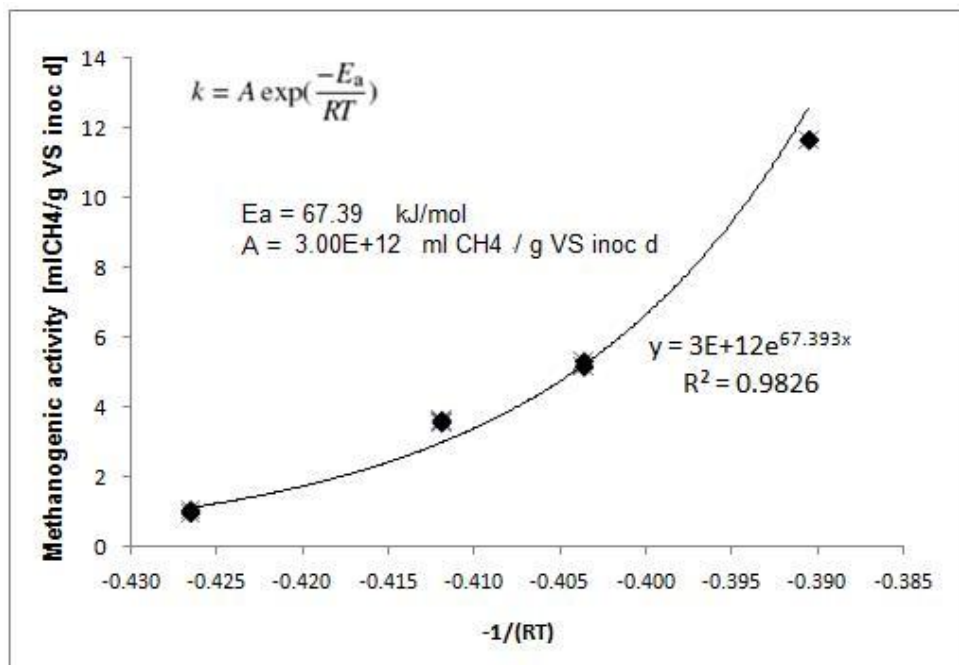


Figure 4.2: Arrhenius model for gas production at different temperatures.

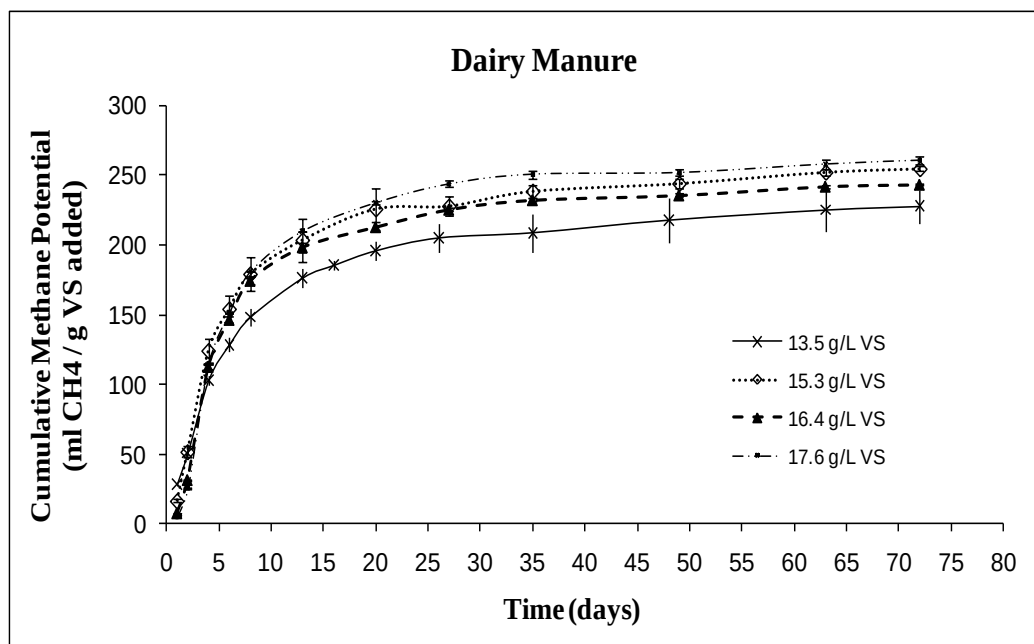


Figure 4.3: Dairy manure methane potential test.

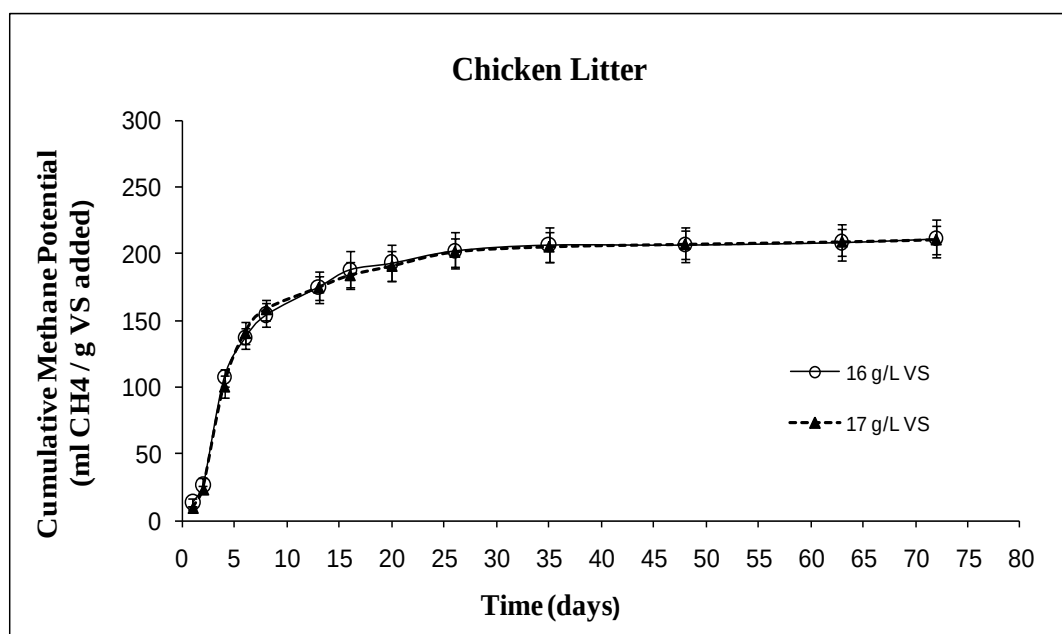


Figure 4.4: Chicken litter methane potential test.

Table 4.2: Methane potential and biodegradability for dairy manure and chicken litter.

Feedstock	20 days Potential						Ultimate Potential			
	ml CH ₄ / g VS added		ml CH ₄ / g VS removed		VS Removal (%)		ml CH ₄ / g VS added		(%) CH ₄ recovered in 20 days	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Dairy Manure	223.2	21.7	747.8	26.0	29.8	2.6	246.7	8.9	90.3	7.9
Chicken Litter	192.0	14.5	330.1	29.3	58.2	1.1	211.0	14.3	90.9	1.0

content of protein and urea. This is in agreement with these results.

Dairy manure and chicken litter differ in their chemical composition (Table 3.1) and biodegradability, so different methane productions per unit of VS removed were expected. In 20 days, dairy manure produced more than twice methane gas than chicken litter (approx. 748 versus 330 ml CH₄ / gVS removed). Nonetheless, the methane production per unit of VS added (or loaded) at 20 days for chicken litter and cow manure were not significantly different (ANOVA, $p > 0.05$). A possible explanation is that chicken litter was degraded faster than dairy manure (Callaghan et al., 1999), so in 20 days more VS were converted to methane generating a similar volume of methane than dairy manure. If neither synergistic nor antagonistic effects occur during co-digesting, methane production per unit of VS added would be same for all organic loading rates.

On the other hand, when methane production over time was less than 5% (day 72), it is assumed that most of the biodegradable fraction was already converted to methane. Ultimate methane production is independent of degradation speed and directly related to manure composition and biodegradable fraction. Thus, different ultimate methane production from chicken litter and dairy manure were expected. Ultimate methane potentials of dairy manure and chicken litter were 246 and 211ml CH₄ / gVS added, respectively.

Several authors have reported methane potential for cow manure and chicken litter which are presented in Table 4.3. Difference in properties, and consequently in methane potentials for the same waste, depends on factors such as animal species, diet, digestibility, protein and fibre content; other factors include animal age, housing, environment, stage of production among others (Alvarez et al., 2006; Lorimor et al., 2000; Sakar et al., 2009). Values of methane potentials for both wastes found in this study are in agreement with values previously reported by literature.

Table 4.3: Methane potentials from literature.

Feedstock	Methane potential (ml CH ₄ / g VS added)	Temperature (°C)	Retention time (days)	Reference
Dairy Manure	201 - 245	35	20	This study (2009)
	188 - 220	35	20	Lehtomäki et al. (2007)
	191 - 257	37	15	Mladenovska et al. (2003)
	191	36	15	Demirer et al., (2005)
	50 - 131	35	20 - 50	Alvarez et al. (2006)
	178	25	15	Pain et al. (1988)
	180	35	22	Kaparaju et al. (2002)
	140 - 266	25 - 40	15 - 30	Braun et al. (1982)
	177 - 206	35	20	This study (2009)
Chicken Litter	220	35	30	Fantozzi et al. (2009)
	140 - 220	35	12 -29	Webb et al. (1985a)
	240 - 260	35	14 -29	Webb et al. (1985b)
	220	35	22 - 24	Safley et al. (1987)
	200	34	40	Pechan et al. (1987)
	225	35	21	Callaghan et al. (2002)

4.3 Performance in continuous co-digestion

4.3.1 *Biogas and Methane production*

The highest performance in anaerobic digestion equates the maximum methane gas production. Methane production can be increased by increasing the organic loading rate. Methane production from anaerobic digestion of dairy manure alone yields low net energy that may not be economically attractive at current oil prices (Braun et al., 2004). Dry chicken litter (56% TS) provides extra organic matter when mixed with liquid dairy manure (2% TS) creating a suitable feedstock. However, there is a limit for the increase in organic loading rate. A collapse of reactors or a low efficiency characterized by low methane yields reveals this limit; it could be caused by OLR overloading or release of toxic compounds such as ammonia. The objective of this section was to find the maximum

methane production rate by increasing the organic loading rate using chicken litter in semi-continuous reactors digesting dairy manure.

The first organic loading rate was $1.01(\pm 0.04)$ gVS L_{reactor}^{-1} day⁻¹ digesting dairy manure alone in all reactors. At stable conditions (Figure 4.5) control reactors produced 1062 ± 12 and 828 ± 26 ml day⁻¹ for biogas and methane production rates, respectively, while test reactors produced 1183 ± 25 and 836 ± 34 ml day⁻¹ for biogas and methane production rates, respectively. Volatile fatty acids (VFAs) always remained below 30 mg/L as acetic acid at this organic loading rate. Note that only acetic acid was detected. Normalized methane production between control and test reactors was not significantly different (ANOVA, $p > 0.05$) at this initial organic loading rate.

Co-digestion experiment started by increasing OLR from $1.01(\pm 0.04)$ to $1.28(\pm 0.03)$ gVS L_{reactor}^{-1} day⁻¹. The change in biogas and methane production rate were the best indicators of the instability created by the OLR increase (Figure 4.5A,B). At this organic loading rate stable (variation less than 5%) biogas and methane production rate were achieved from day 52 at 1543 ± 29 and 1079 ± 32 ml day⁻¹, respectively. VFA concentration was low (< 30 mg/L as acetate) and only acetate was detected. The small increase observed in both biogas and methane production rate at the end of this stage from days 72 to 77 (Figure 4.53A, B) can be explained by small variability of organic strength (VS) in the base feedstock. However, this variability was small enough to be considered unimportant.

The next organic loading rate fixed at $1.5(\pm 0.03)$ gVS L_{reactor}^{-1} day⁻¹ started at day 77 and finished at day 98. Stable gas production was achieved from day 87 yielding biogas and methane production rates of 1859 ± 25 and 1236 ± 34 ml day⁻¹, respectively. VFA concentration was low (< 30 mg/L as acetate) and only acetate was detected.

However, when OLR was increased to $1.77(\pm 0.05)$ gVS L_{reactor}^{-1} day⁻¹ the system collapsed after approximately 18 days producing a maximum VFAs concentration of 2360 ± 223 mg/L as acetate (Figure 4.5C) at day 140. In addition, propionate was detected at a maximum concentration of 188 ± 29 mg/L as acetate at day 140. Propionate is used as an imbalance indicator because its oxidation ceases earlier than other VFAs when digestion instability is created (Angelidaki et al., 2007). It is important to mention that in all stages

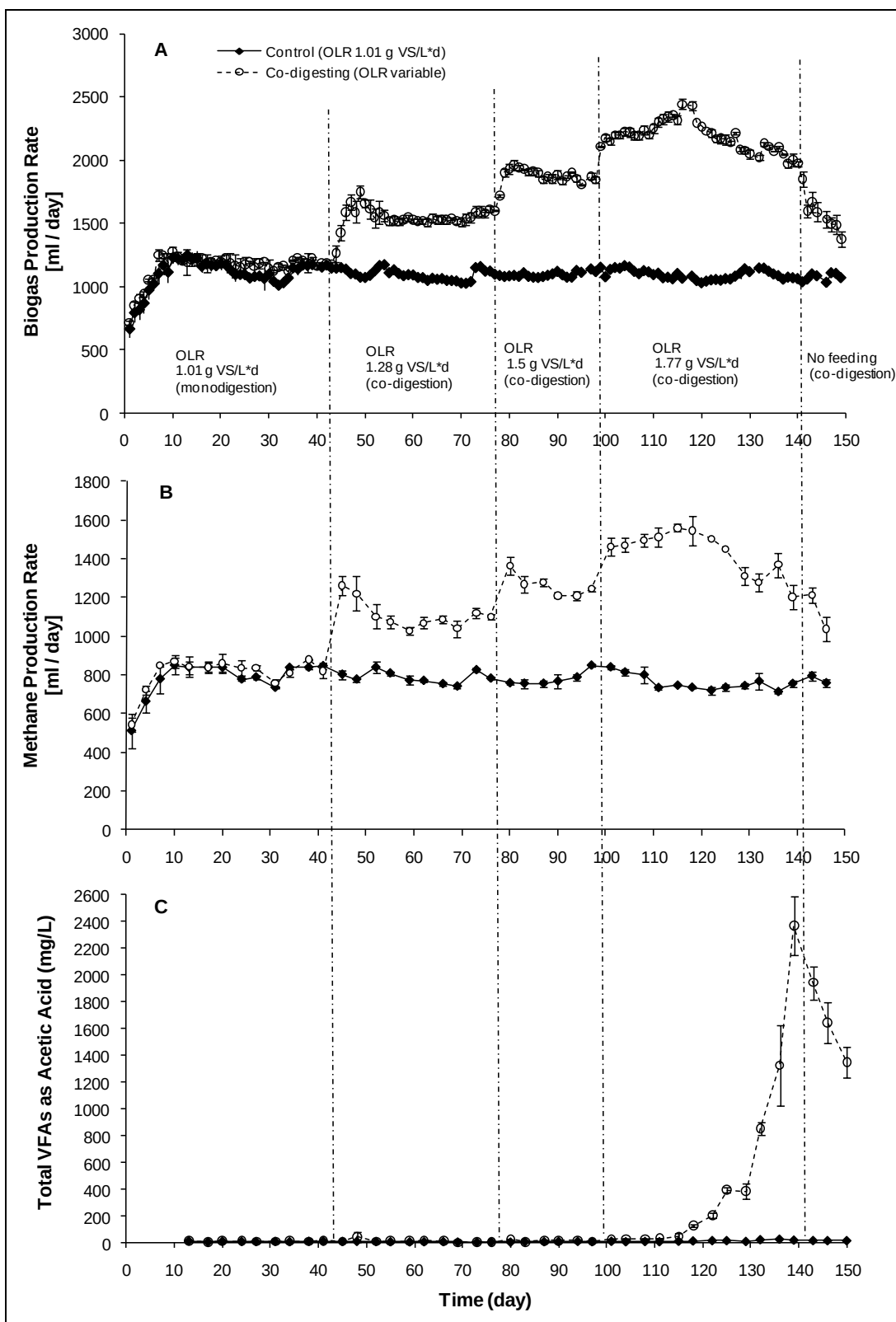


Figure 4.5: Performance in co-digestion.

when co-digestion was successfully achieved VFAs concentrations were always low (< 30 mg/L as acetic acid), indicating a good and stable performance. However, VFAs showed a change only when the imbalance was irreversible after 18 days from the increase in loading rate up to $1.77(\pm 0.05)$ gVS L_{reactor}^{-1} day $^{-1}$. This makes VFAs a non-reliable stability indicator when co-digesting animal waste because of the buffer capacity provided by a high alkalinity (Angelidaki et al., 1993b, 2007). At day 140, a collapse of the system was assumed because VFAs concentration was about 100 times than at the lower organic loading rates (Ahring et al., 1995; Gerardi 2003; Mshandete et al., 2004), so the influent was shutoff leading to a decrease in VFAs.

Surprisingly, 13 days after the organic loading rate was increased to $1.77(\pm 0.05)$ gVS L_{reactor}^{-1} day $^{-1}$ (day 111), the biogas production rate increased until reaching a peak at day 116 (2442 ± 40 versus 2216 ± 22 ml day $^{-1}$ from previous days). Perhaps one retention time at an OLR of $1.5(\pm 0.03)$ gVS L_{reactor}^{-1} day $^{-1}$ was insufficient to reach real stability, although stable biogas and methane production rate and low VFAs were achieved. Although the biogas production rate was increasing from day 111, methane production rate was similar between days 111 and 116 (1537 ± 33 ml day $^{-1}$), indicating that methanogenic microbes were the first group affected by the imbalance leading to VFA accumulation (Angelidaki et al., 2007). From day 117, the biogas production rate decreased reflecting a total collapse of the system until VFAs reached their maximum value. Thus, gas and methane production rate appear to be the most responsive and reliable instability indicators. Dataset and additional test considered from this last organic loading stage only includes samples taken between days 107 to 110 (9 and 12 days after increasing OLR for this last stage) when reactors seemed to perform similar and stable. In summary, biogas and methane production rates at an OLR $1.77(\pm 0.05)$ gVS L_{reactor}^{-1} day $^{-1}$ were assumed to be 2222 ± 28 and 1484 ± 12 ml day $^{-1}$, respectively.

Chicken litter can be safely added up to a 33% as Volatile Solids (VS) in the feedstock (OLR $1.5(\pm 0.03)$ gVS L_{reactor}^{-1} day $^{-1}$) increasing methane production by 49.3%. Callaghan et al. (2002) found a similar maximum chicken manure percentage in feedstock, reporting a maximum of 33% of chicken manure based on VS added for non-acclimated reactors at 21 days retention time when increasing the OLR from 3.19 ± 0.14 to

4.44±0.21gVS L_{reactor}⁻¹ day⁻¹. These authors started dairy manure digestion (control) with inocula acclimated to higher OLR (3.19±0.14 21gVS L_{reactor}⁻¹ day⁻¹) than this study, so a higher OLR as the optimum co-digestion would be expected. In addition, while they achieved the best performance after increased 1.4 times the baseline OLR by adding chicken litter, in this study it was reached after increased 1.49 times the baseline OLR. On the other hand, Magbauna et al., (2001) found a similar percentage (between 30 and 35% as VS added) for poultry when added with hog manure in batch reactors using as parameters methane yield based on VS destroyed and VS removal. Misi et al., (2001b) concluded that poultry manure should be kept between 10 to 40 % as VS added for any co-digestion mixtures to ensure the best methane yield in batch reactors.

A considerable number of previous research have reported a successful anaerobic digestion of dairy manure at higher organic loading rates than in this study (Ahring et al., 2001; Callaghan et al., 2002; Demirer et al., 2005; Mladenovska et al., 2003; Nielsen et al., 2004; Sung et al., 2001). Moreover, a range of optimal organic loading rates between 2.5 - 3.5 gVS L⁻¹ day⁻¹ for dairy manure and between 5 - 7 gVS L⁻¹ day⁻¹ for dairy manure with co-substrates have been proposed (Burton et al., 2003; Sakar et al., 2009). It can be observed that proposed ranges are greater than the organic loading rate at which reactors in this study collapsed (1.77(±0.05) gVS L_{reactor}⁻¹ day⁻¹). However, the selection of the initial OLR is related to inocula acclimatation and waste composition depending of a wide number of factors (Lorimor et al., 2000; Sakar et al., 2009).

On the other hand, the time needed for reactors to become stable after increase the OLR was 7 days (Figure 4.6) after when there was no significant difference in the methane production rate (ANOVA, p > 0.05). Percentage of the average methane production rates after 7 days were 100±36, 100±39, 100±41, and 100±25 % for an OLR of 1.01(±0.04), 1.28(±0.03), 1.5(±0.03), and 1.77(±0.05) gVS L_{reactor}⁻¹ day⁻¹, respectively. A possible explanation could be that microbial population grew fast enough to handle this increment in organic matter within 7 days.

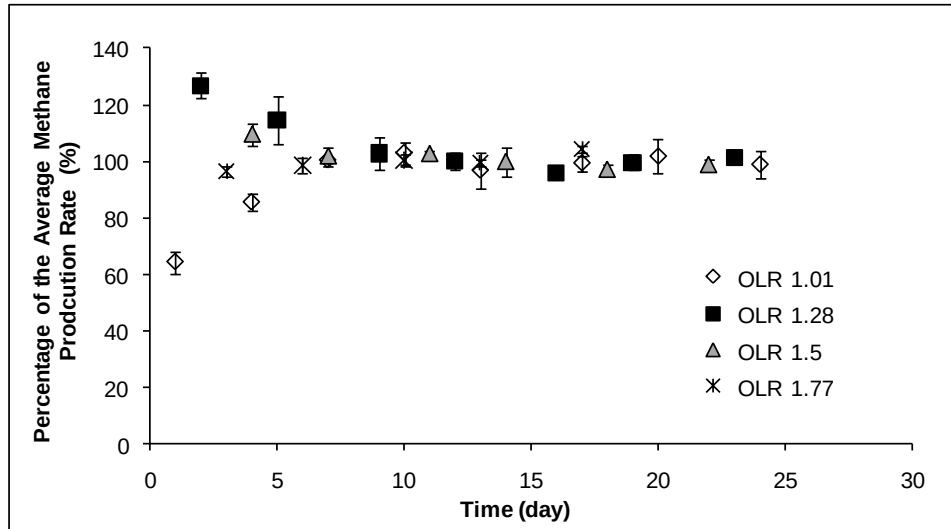


Figure 4.6: Methane production rate after increasing OLR. Percentage of the average methane production rate (%) was computed as the ratio between daily methane production and the average production at stable conditions for each OLR. A value of 100% illustrates that methane production at a specific day reached the average production at stable conditions for that OLR.

4.3.2 Synergistic or Antagonistic effects

It is impossible to determine the amount of organic matter (measured as VS) that is degraded from each substrate during co-digestion. For example, one substrate could eventually reduce the biodegradability of another one. As a consequence, it is not possible to estimate a methane production from methane potentials based on VS removed. In contrast, known amounts of different wastes are combined, so an expected methane production from potentials of single substrates based on VS added can be estimated. In this way, normalized methane production rate per unit of VS added allows the efficiency and the presence of synergistic or antagonistic effects when co-digesting to be evaluated (Alvarez et al., 2008a; Amon et al., 2007; Callaghan et al., 2002; Kaparaju et al., 2005; Li et al., 2009; Macias-Corral et al., 2008; Mshandete et al., 2004). Normalized methane production rate per unit of VS added or methane yield based on VS added was used to analyze efficiency of co-digestion at different organic loading rates (Figure 4.7). Methane yields were 229 ± 8 , 234 ± 5 , 232 ± 3 , and 229 ± 1 ml gVS added⁻¹ day⁻¹ at OLR of 1.01(± 0.04),

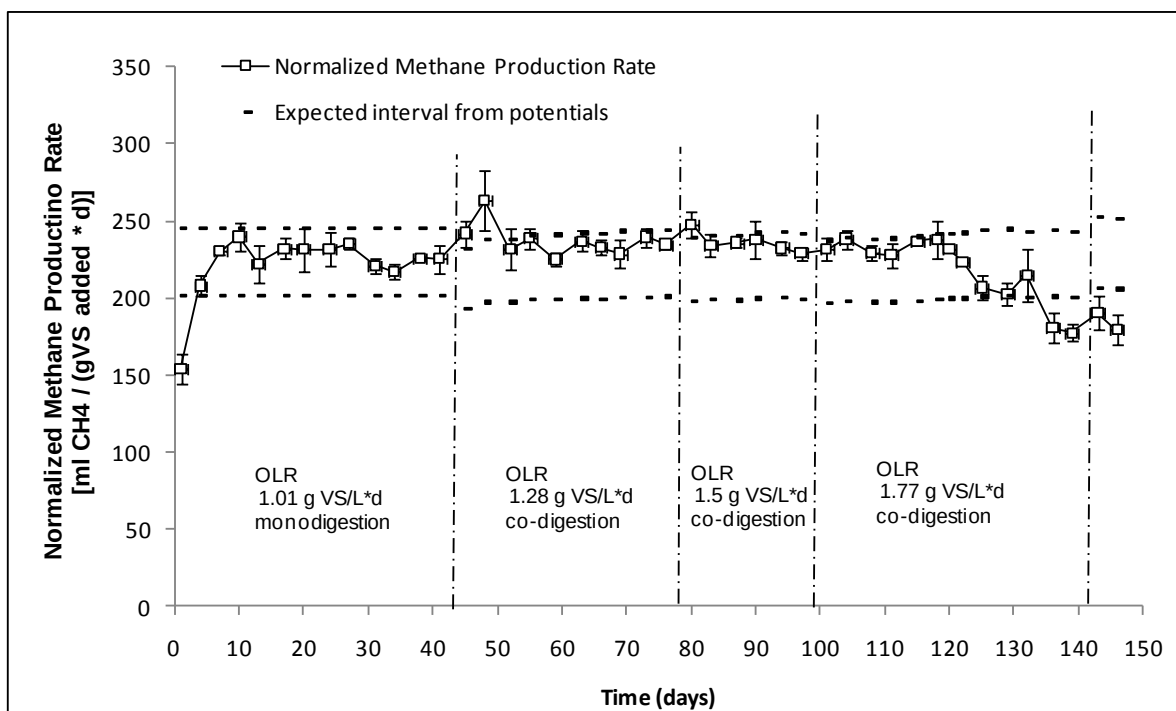


Figure 4.7: Methane yield at different organic loading rates during co-digestion. Expected interval at each OLR was computed using the 95% confidence limits from methane potentials based on VS added at 20 days.

1.28(± 0.03), 1.5(± 0.03), and 1.77(± 0.05) gVS $L_{\text{reactor}}^{-1} \text{ day}^{-1}$, respectively. No significant differences (ANOVA, $p > 0.05$) were found in methane yields between all organic loading stages including the OLR of 1.77(± 0.05) gVS $L_{\text{reactor}}^{-1} \text{ day}^{-1}$ when samples were taken the first 12 days. This constant methane yield indicates neither synergistic nor antagonistic effects occurred during co-digestion. Instead, just additive effects were produced. Even so, a batch experiment was developed by which microbial methane production capacity was tested for the different organic loading stages. It was hypothesized that if just additive effects were present, the same amount of biomass taken from each stage would generate the same methane yield when the same amount of a single substrate (dairy manure) was added. Figure 4.8 presents the results of this experiment. For days 2 to 5, methane productions per unit of biomass at different organic loading rates are summarized in Table 4.4. No significant difference (ANOVA, $p > 0.05$) among different OLR for days 2 and 4 was found indicating that the biomass presents no improvement in its capacity for degra-

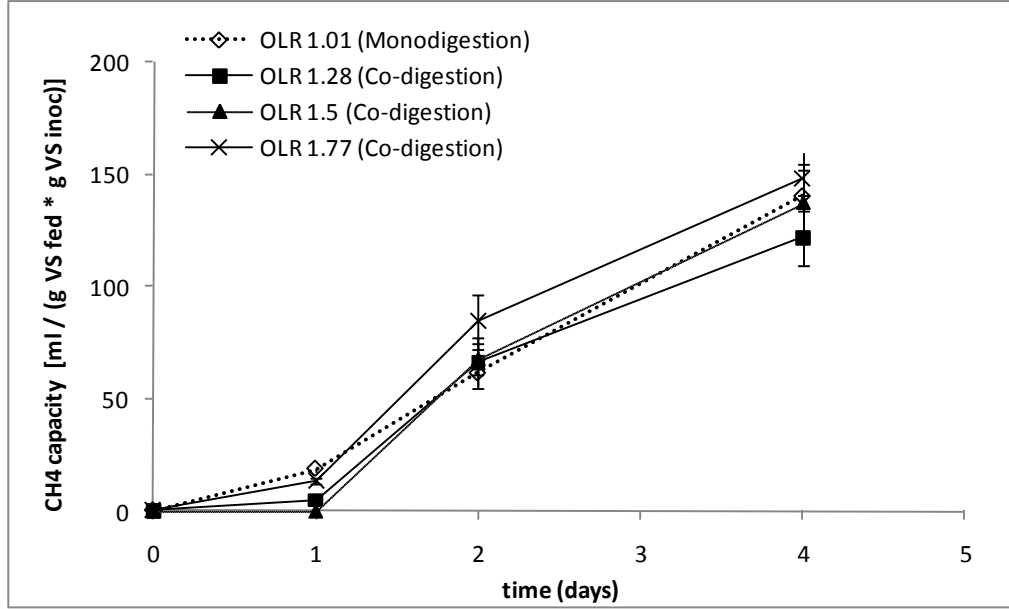


Figure 4.8: Specific methane production capacity batch experiment.

Table 4.4: Specific methane production capacity batch experiment.

Organic Loading Rate Stage [gVS/(L*d)]	CH4 production capacity [ml/(gVSinoc * gVSfed)]			
	Day 2		Day 4	
	mean	sd	mean	sd
1.01 (± 0.04)	61	6	141	19
1.28 (± 0.03)	66	11	129	10
1.5 (± 0.03)	68	7	138	15
1.77 (± 0.05)	75	6	146	6

ding dairy manure. These results also indicate that neither synergistic nor antagonistic effects occurred when co-digesting DM with CL.

There is discord in the poor reviewed literature about whether co-digestion of chicken litter with dairy manure produces either synergistic, antagonistic or additive effects. While some research has concluded synergistic effects exists (Misi et al., 2001a,b),

other reports have demonstrated antagonistic effects possibly due to ammonia inhibition (Callaghan et al., 1999; Callaghan et al., 2002; Güngör-Demirci et al., 2004). These results can be explained by differences in both waste compositions (Lorimor et al., 2000; Sakar et al., 2009). However, chicken litter requires water addition to be digested, so positives effects were achieved co-digesting these wastes in the sense that no water addition was needed. The substrates by themselves appear to contain all nutritional requirements for anaerobic digestion. Güngör-Demirci et al. (2004) tested the impact of adding a basal medium containing all necessary micro and macro nutrients for an optimal microbial growth in a batch experiment co-digesting cow manure and chicken litter mixed at different ratios. They concluded that enough water is present to dissolve all needed nutrients.

A linear relationship between biogas and methane production rates ($\text{mL L}_{\text{reactor}}^{-1} \text{day}^{-1}$) and substrate organic loading rate has been reported at mesophilic temperatures for some animal wastes (Alvarez et al., 2006; Gelegenis et al., 2007a; Hill, 1990; Webb et al., 1985a). This linear relationship exists up to a maximum organic loading rate that varies with the type of waste, and above which inhibition occurs resulting in a VFAs buildup (Alvarez et al., 2006; Husain, 1998; National Academy of Sciences, 2001). Such a linear relationship is clearly observed for the co-digestion experiments herein (Figures 4.9 and 4.10). Researchers have reported values for the slope for dairy manure ($76 \pm 25 \text{ mL CH}_4 \text{ gVS}^{-1}$) and for chicken manure ($251 \pm 83 \text{ mL CH}_4 \text{ gVS}^{-1}$) (Table 4.5). The slope for co-digestion of chicken litter with dairy manure in this study was between the reported slopes for digestion of single substrates. Slopes referred to g VS removed were 159 ± 10 and $97 \pm 8 \text{ mL CH}_4 \text{ L}^{-1} \text{ gVSremoved}^{-1} \text{ day}^{-1}$ for biogas and methane, respectively. Organic matter removal is discussed in section 4.3.3. On the other hand, the difference in slopes for biogas and methane (Figures 4.9 and 4.10) is explained by the slight but constant decrease in biogas methane concentration as the loading rate was increased (Figure 4.11). Methane percentage in biogas at stable conditions were decreasing 70.9 ± 2.2 , 69.6 ± 1.2 , 67.7 ± 1.7 and 66.8 ± 1.5 at OLR of $1.01(\pm 0.04)$, $1.28(\pm 0.03)$, $1.5(\pm 0.03)$, and $1.77(\pm 0.05) \text{ gVS L}_{\text{reactor}}^{-1} \text{ day}^{-1}$, respectively. Gas composition revealed that carbon dioxide (CO_2) was the main gas displacing methane as the loading rate increased, indicating a decrease in performance

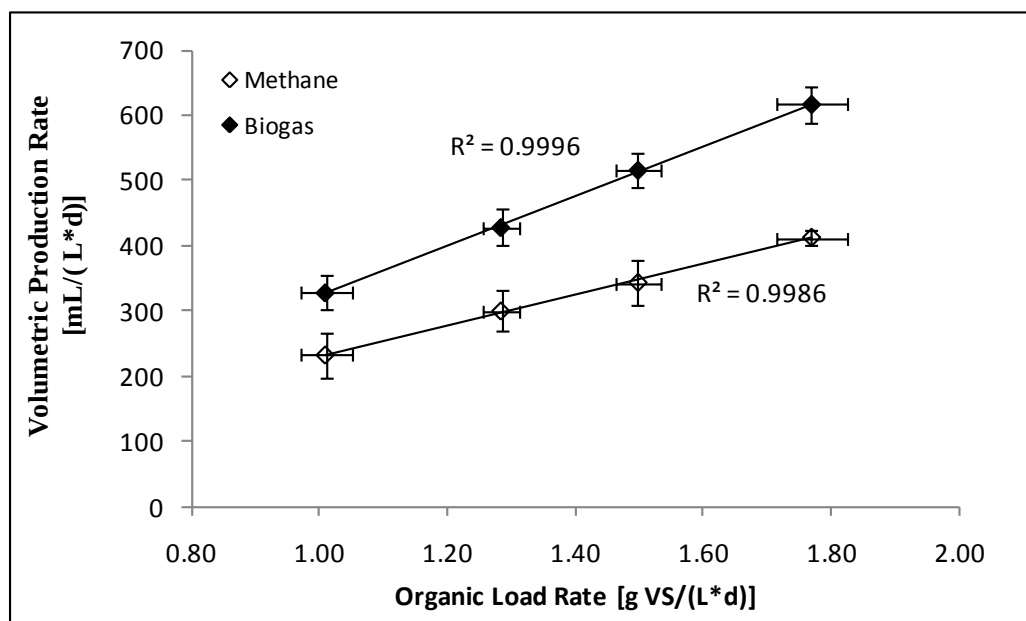


Figure 4.9: Production rate v/s organic loading rate in co-digestion. Note that data for OLR 1.77 corresponds to samples taken before the failure.

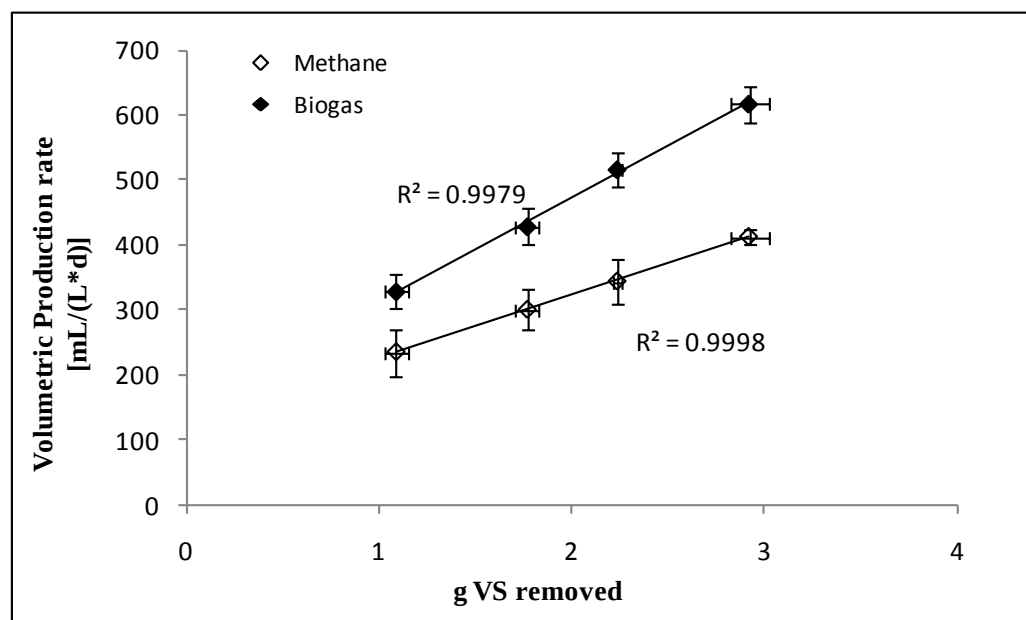


Figure 4.10: Production rates versus VS removed during co-digestion. Note that data for OLR 1.77 corresponds to samples taken before the failure.

Table 4.5: Slopes for dairy manure and chicken litter. Slopes are obtained from relationship between Volumetric Methane Production Rate (mL/L/d) and Organic Loading Rate (gVS/L/d).

Feedstock	HRT (days)	Temperature (°C)	Slope [ml CH ₄ / g VS]		Reference
			mean ^a	sd ^a	
Dairy Manure	50	35	50	8	Alvarez et al., 2006
	10.2 -15	35	79	37	Converse et al.1977
	6.2	60	100	12	Hill, 1990
Chicken manure	18	35	227	147	Hill, 1983
	52.5	35	343	4	Hill, 1990
	12 - 29	35	182	7	Webb et al., 1985
Co-digestion of chicken litter with dairy manure	20	35	233	8	This study, 2009

^a Mean and Standard deviation from linear regressions were estimated using the statistical software JMP version 7.0.1.

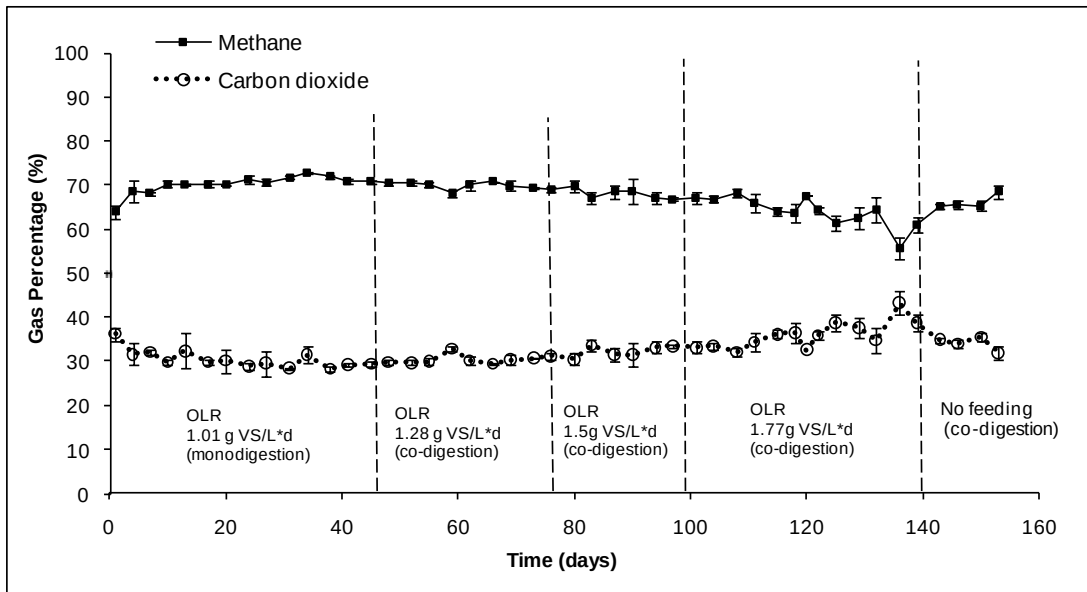


Figure 4.11: Gas composition over time in co-digestion.

(Figure 4.11). At stable conditions carbon dioxide percentages were 29.7 ± 2.2 , 30.2 ± 1.2 , 31.4 ± 1.8 and 32.6 ± 1.7 % at OLR of $1.01(\pm 0.04)$, $1.28(\pm 0.03)$, $1.5(\pm 0.03)$, and $1.77(\pm 0.05)$ gVS $L_{\text{reactor}}^{-1} \text{ day}^{-1}$, respectively. The decreasing methane fraction could have been presage the reactor failure.

4.3.3 *Organic matter removal*

As chicken litter is a more biodegradable substrate than dairy manure, an increase in overall organic matter removal was expected as the OLR was increased. Figure 4.12 shows that the % VS removal increased as organic loading was increased. At stable conditions, volatile solids removals were 30 ± 1.2 , 38.3 ± 0.7 , 41.6 ± 0.3 , and 46.1 ± 0.6 at OLR of $1.01(\pm 0.04)$, $1.28(\pm 0.03)$, $1.5(\pm 0.03)$, and $1.77(\pm 0.05)$ gVS $L_{\text{reactor}}^{-1} \text{ day}^{-1}$, respectively. A change in VS removal showed the imbalance caused by an increase in organic loading rate. Although at the collapse (day 118) a decrease in VS removal occurred, reaching a value of 40.3 ± 0.3 %, in next 6 days the removal increased. This can be explained by a change in base feedstock (dairy manure) which is reflected in control reactors.

4.3.4 *Alkalinity, pH and Ammonia*

Total alkalinity, pH and ammonia are interrelated. Depending on pH, carbon dioxide reaches equilibrium with carbonic acid, bicarbonates and carbonates, and ammonia can be found as free ammonia (NH_3) or ammonium ion (NH_4^+). Both carbon dioxide and ammonia are released during anaerobic digestion, but only ammonia directly affects total alkalinity. Figures 4.13A and B, respectively, illustrate increasing ammonia and total alkalinity as the organic loading rate was increased, while the pH was increased only from 7.5 to 7.8. At stable conditions total ammonia concentrations were 580 ± 30 , 1180 ± 24 , 1313 ± 45 , and 1361 ± 81 mg N- NH_3/L ; free ammonia concentrations reached values of 32 ± 2 , 53 ± 2 , 65 ± 2 , and 81 ± 6 mg N- NH_3/L (Figure 4.13A); and total alkalinity were 9344 ± 168 , 10832 ± 124 , 12742 ± 325 , and 13330 ± 948 mg/L as CaCO_3 (Figure 4.13B) at

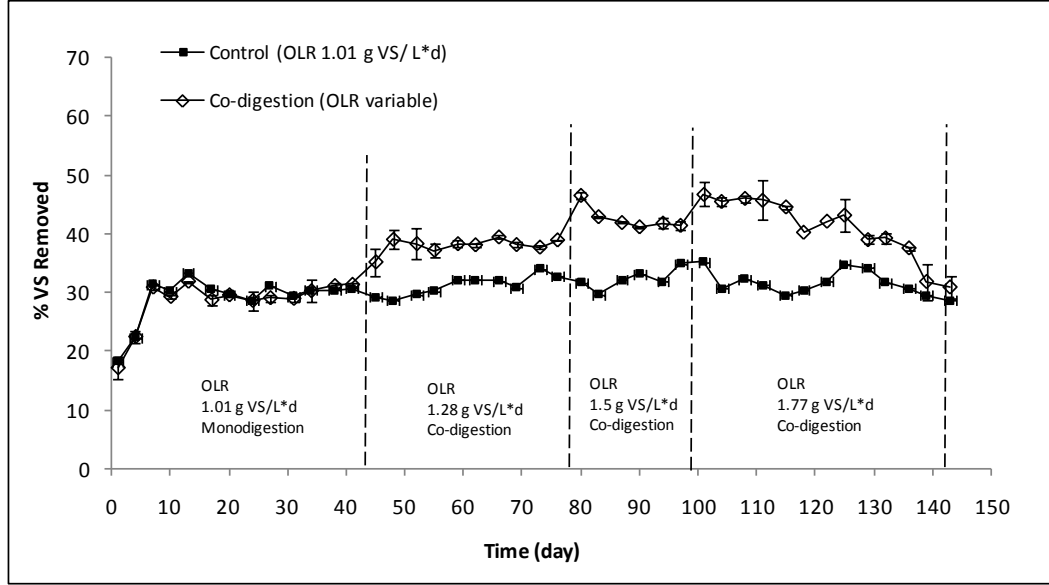


Figure 4.12: Percentage of removal at different organic loading rates in co-digestion.

OLR of $1.01(\pm 0.04)$, $1.28(\pm 0.03)$, $1.5(\pm 0.03)$, and $1.77(\pm 0.05)$ $\text{gVS L}_{\text{reactor}}^{-1} \text{ day}^{-1}$, respectively. However, it can be seen that an OLR $1.5(\pm 0.03)$ no real stability was reached by these parameters suggesting that one retention time may not be enough to achieve stable conditions.

On the other hand, due to the large alkalinity, pH and VFA were steady and low, respectively. Therefore, pH and VFAs were not good indicators of reactor instability at high total alkalinity (Angelidaki et al., 2007).

Research indicates that for unadapted sludge working under mesophilic conditions, a total ammonia concentration of $1.4 \text{ g N-NH}_3/\text{L}$ is the inhibitory threshold concentration for methanogenic group (Gonzalez-Fernandez et al., 2009). From Figure 4.13A it can be seen that total ammonia concentration in test reactors is extremely close to that threshold at the last stage when organic loading rate was $1.77(\pm 0.05) \text{ gVS L}_{\text{reactor}}^{-1} \text{ day}^{-1}$.

In addition, as a consequence of the increasing total ammonia concentration and pH, free ammonia concentration (NH_3) in the last stage increased considerably when comparing with previous stages (Figure 4.13A). Free ammonia is considered as the most toxic form of ammonia for methanogenic consortium (Angelidaki et al., 1993a,b; Mata-

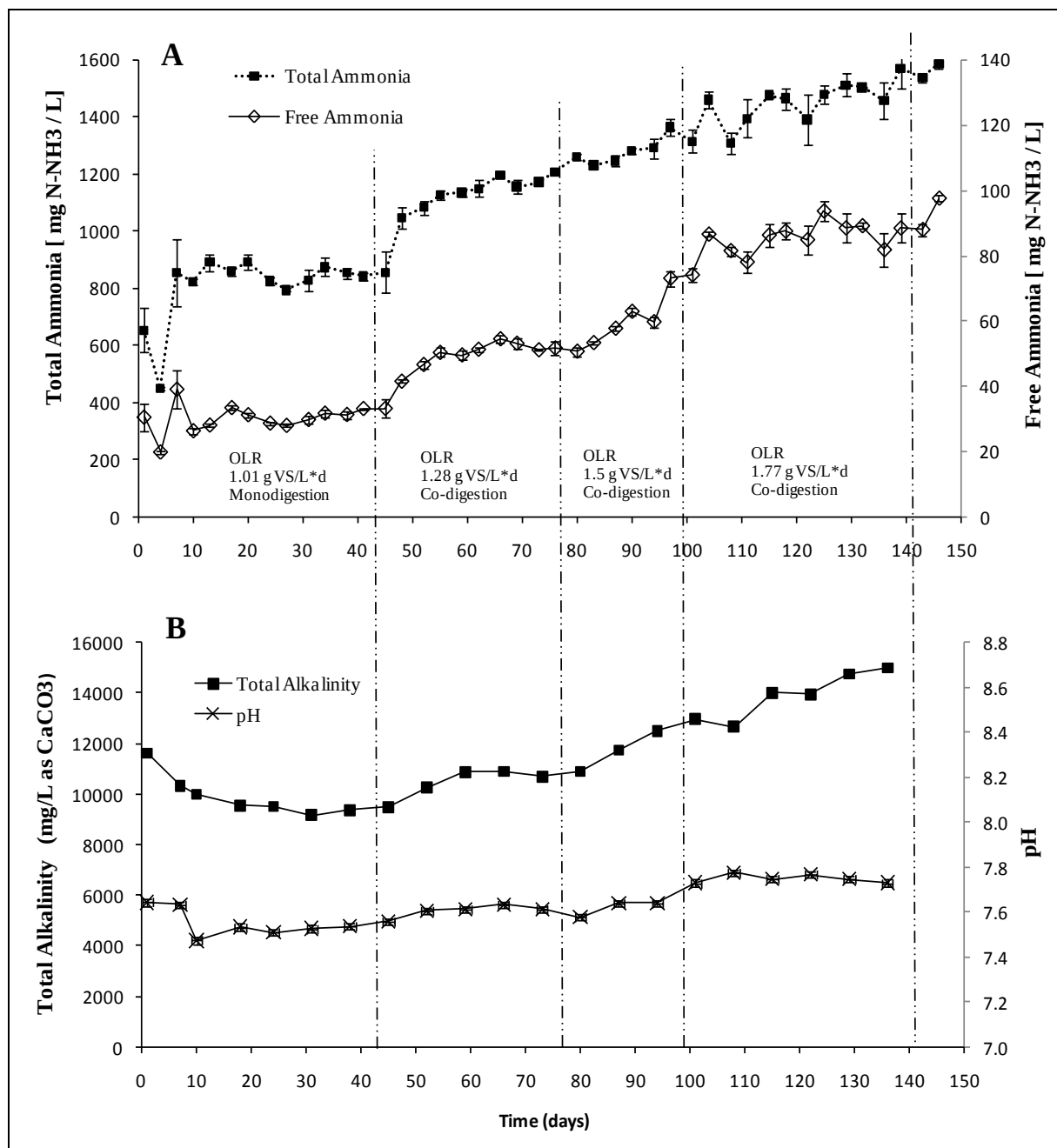


Figure 4.13: Total alkalinity, pH and ammonia concentration at different organic loading rates during co-digestion.

Alvarez et al., 2000) with an initial threshold for inhibition about 66 mg N-NH₃/L (Burton et al., 2003; Koster et al., 1984; De Baere et al., 1984). Other studies have reported that a range between 99 and 150 mg N-NH₃/L is inhibitory for methanogens (Angelidaki et al., 1993; Webb et al., 1985a,b; McCarty et al., 1961; Braun et al., 1981). The difference in inhibitory concentration is due to differences in substrates, inocula, operational conditions, and acclimation periods (Callaghan et al., 1999; Mata-Alvarez et al., 2000). Thus, both total and free ammonia reached inhibitory levels at the last stage according to the literature.

An experiment was conducted to evaluate whether or not adaptation to higher ammonia levels occurred as the OLR was increased. More specifically, the ammonia concentration that produced a reduction of 50% in methanogenic activity with dairy manure (IC₅₀) was measured. If IC₅₀ increased with the OLR, then adaptation occurred. Previous research has concluded that, depending on acclimation time, methanogens may adapt to several times the initial inhibitory threshold (Salminen et al., 2002a). Figures 4.14 and 4.15 present the results of this experiment with respect to total and free ammonia, respectively. It can be seen that for total ammonia (Figure 4.14), adaptation occurred only when co-digestion started, after which point the microbial population presented a relatively constant ammonia tolerance (2.76±0.04 g N-NH₃/L). Increase ammonia tolerance may be more likely due to the growth of a new population rather than the adaptation of an existing population (Angelidaki et al., 1993a; Koster, 1986). Figure 4.14 suggests that new microbial population added as a result of co-digestion, may have improved the resistance capacity to total ammonia. However, Figure 4.15 shows increasing adaptation for free ammonia, with the largest increase observed when co-digestion started. This trend in adaptation correlates (Pearson correlation coefficient = 0.99956) with the increasing concentration at each organic loading stage. From Figures 4.14 and 4.15 it can be observed that specific methane activity with dairy manure (ml CH₄ g VS inoc⁻¹ day⁻¹) kept practically constant, even though resistance to ammonia increased.

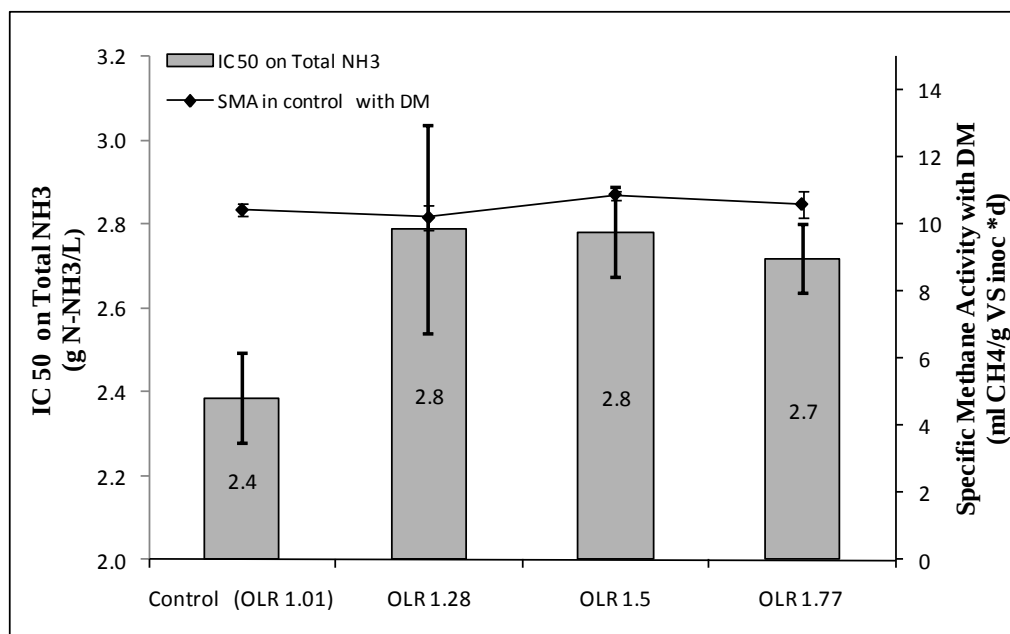


Figure 4.14: IC₅₀ for total ammonia at different organic loading stages in co-digestion. Error bars estimated as 95% confidence interval from linear regression of SMAs versus NH₃ concentrations.

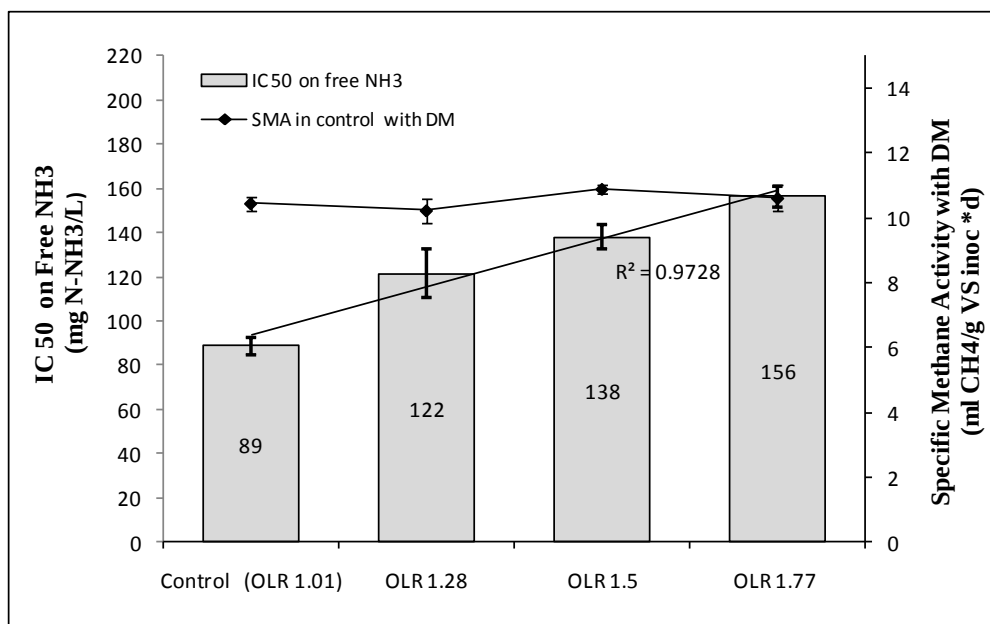


Figure 4.15: IC₅₀ for free ammonia at different organic loading stages in co-digestion. Error bars estimated as 95% confidence interval from linear regression of SMAs versus NH₃ concentrations.

4.3.5 Pathogens Removal

Temperature is an important parameter affecting pathogen removal, but there are other factors involved such as retention and/or treatment time, pH, VFAs, batch or continuous digestion, bacterial species, availability of nutrients and initial concentration of pathogens in biowaste (Sahlström, 2003). Although pathogen removal is well known to be achieved under thermophilic anaerobic digestion (Albhim et al., 2007; Salminen et al., 2002a; Shih et al., 1987; Sahlström et al., 2004), little research has documented pathogens reduction when co-digesting under mesophilic conditions. Figure 4.16 shows pathogen indicator (*Escherichia coli*) concentration over time at different organic loading rates. Removal values were between 68 ± 4 and 97 ± 2 %, which are in the range of values reported by Horan et al. (2004) for total coliform removal under mesophilic anaerobic digestion.

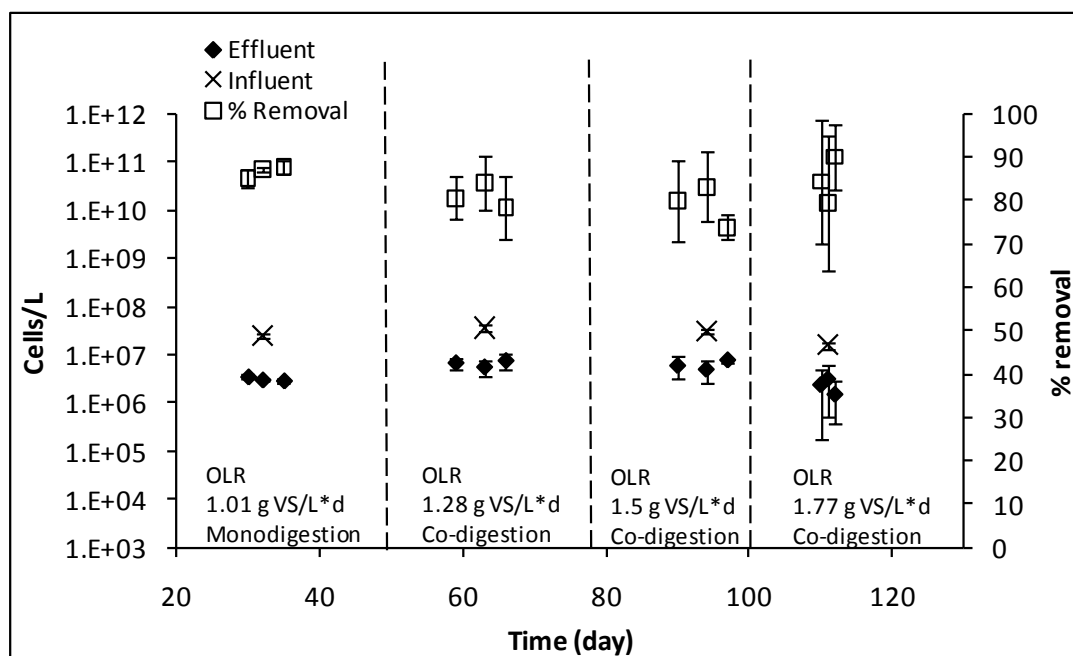


Figure 4.16: *E.coli* at different organic loading stages in co-digestion.

4.4 Conclusions

Methane production from anaerobic digestion of dairy manure alone yields low net energy that may not be economically attractive at current oil price (Braun et al., 2004). The results of the present study indicate that chicken litter can be added into a digester treating dairy manure to increase the organic loading rate leading in a higher methane production rate. Chicken litter can be safely added up to a 33% as Volatile Solids (VS) in the feedstock (OLR $1.5(\pm 0.03) \text{ gVS L}_{\text{reactor}}^{-1} \text{ day}^{-1}$) increasing methane production by 49.3%. Previous research (Callaghan et al. 2002; Magbauna et al., 2001; Misi et al., 2001b) have reported a similar maximum addition percentage of chicken manure feedstock for continuous and batch reactors.

Similar normalized methane yields were achieved for both co-digestion and dairy manure alone, indicating a lack of synergistic effects. However, chicken litter requires water addition to be digested, so positives effects were achieved co-digesting these wastes in the sense that no water addition was needed.

Although the reactors reached stable (variation less than 5%) biogas and methane production, VS removal, pH and low VFAs ($< 30 \text{ mg/L}$ as acetic acid) within 7 days following an increase in organic loading rate, ammonia and total alkalinity at OLR $1.5(\pm 0.03) \text{ gVS L}_{\text{reactor}}^{-1} \text{ day}^{-1}$ needed a longer time to reach stability. From OLRs at 1.01 and $1.28 \text{ gVS L}_{\text{reactor}}^{-1} \text{ day}^{-1}$, all parameters seemed to become stable in 2 instead 3 or 4 retention times previously suggested to achieve steady states conditions (Callaghan et al., 2002).

Perhaps, a combined effect of high ammonia concentration and an overloading resulting from the late response to an increase in ORL from previous stage may have been the reason for digester failure at an OLR of $1.77(\pm 0.05) \text{ gVS L}_{\text{reactor}}^{-1} \text{ day}^{-1}$. More research needs to be done evaluating the influence of high ammonia concentration under different organic loading rates. Thus, a longer operating baseline is recommended when co-digesting chicken litter with dairy manure to achieve a better microbial adaptation to both higher substrate and ammonia concentrations.

Because of the large total alkalinity in the system, pH and VFAs were not good indicators of instability. Instead, gas production should be followed closely in order to detect any symptom of imbalance.

Total and free ammonia tolerance could be improved just by simply combining dairy manure with chicken litter. New microbial population added as a result of co-digestion may have improved the resistance capacity to total ammonia, but a proportionally increasing adaptation for free ammonia was detected when increasing free ammonia concentrations in reactors.

By establish the retention time at 20 days it was possible to recover up to approximately 90% of methane from substrates. In addition, this large retention time allowed the microbial population to better develop free ammonia adaptation.

No trend in pathogen indicator (*E.coli*) removal was observed in co-digestion. Removal values reached typical values ranging from 68 ± 4 and 97 ± 2 %.

The microbial population in this study could adapt to lower temperatures down to 19 °C with an acceptable decrease in methane production. However, a 37 day retention time would be required to obtain a similar methane production at 25 °C to that at 35 °C, making this economically unattractive for continuous reactors. At 20 days of retention time, methane production decreased by 10% when temperature decreased from 35 to 25 °C.

CHAPTER 5 Dairy Manure Co-digestion with Other Substrates

5.1 Dairy Manure and its Filtered Solids (FDMS)

Separating solids from manure before digestion has significant impact on methane production. It has been found that screened manure leads a higher methane production (Liao et al., 1984), but usually liquid-solid pretreatments are not an economically attractive option. Commonly, unfiltered dairy manure is used in farm-scale anaerobic reactors. In this study, batch reactor experiments were carried out to evaluate the maximum solids content without adversely affecting methane production of dairy manure. Filtered dairy manure solids (FDMS) characteristics are given in Table 3.2. Between 30-50% of solids in manure are fiber (Angelidaki et al., 2000) creating a heterogeneous material (Figure 5.1). In this study, different amounts of manure solids were added (as VS by mass) to a fixed volume of liquid dairy manure (Table 5.1). Note that total solids should be less than 10% for a successful anaerobic digestion (Bujoczek et al., 2000; Kelleher et al., 2002; Sakar et al., 2009) and, in this study, total solids were always less than 4.4%.

Table 5.1: Experiment design in co-digestion of dairy manure (DM) with filtered dairy manure solids (FDMS).

A										
Inoculum Addition (ml)	DI Water (ml)	Dairy manure (ml)	g VS DM	FDMS (g)	g VS FDMS	volume added (ml)	Na ₂ S solution (ml)	Total volume (ml)	Total g VS/L	Observation
80	20				0.00		1.70	101.7	10.94	Inocula Control
80		20	0.402		0.00		1.70	101.7	14.90	Cattle Manure
80				0.346	0.33	1.29	1.30	82.6	17.48	FDMS
80		20	0.402	0.346	0.33	1.29	1.70	103.0	17.92	45% FDMS + 55% DM
80		20	0.402	1.037	0.99	3.87	1.70	105.6	23.75	71% FDMS + 29% DM
80		20	0.402	2.073	1.98	7.73	1.70	109.4	31.98	83% FDMS + 17% DM

B										
Inoculum Addition (ml)	DI Water (ml)	Dairy manure (ml)	g VS DM	FDMS (g)	g VS	volume added (ml)	Na ₂ S solution (ml)	Total volume (ml)	Total g VS/L	Observation
80	20				0.00		1.70	101.7	10.94	Inocula Control
80		20	0.402		0.00		1.70	101.7	14.90	Dairy Manure
80				0.346	0.33	1.23	1.30	82.5	17.49	FDMS
80		20	0.402	0.346	0.33	1.23	1.70	102.9	17.93	43%FDMS + 57%DM
80		20	0.402	1.037	0.99	3.70	1.70	105.4	23.79	70%FDMS + 30%DM
80		20	0.402	2.073	1.98	7.40	1.70	109.1	32.07	82%FDMS + 18%DM



Figure 5.1: Filtered dairy manure solids (FDMS) used as co-substrate.

Although the experiment was performed in triplicate, a second experiment was made to corroborate the results. The manure solids were anaerobically digested, but, as expected, they produced a much smaller normalized methane yield than liquid dairy manure (Figure 5.2). While no lag phase for dairy manure is observed for both experiments, a lag phase of 4 days was detected for FDMS. During the lag phase, the microbial population in inocula acclimatizes to a new substrate, so it makes sense that no lag phase was observed for dairy manure. After the lag phase, methane is generated at a constant rate. As a consequence of the heterogeneity of FDMS, normalized methane yield was different (122 ± 2 versus 214 ± 4 ml gVS added⁻¹ at last day). Normalized methane yield decreased with addition of FDMS when compared with dairy manure alone. For example, 71% as VS of FDMS in feedstock provided a methane yield of 149 ± 33 versus 254 ± 16 ml gVS added⁻¹ compared with dairy manure. It can be noted that normalized methane yield (or methane potential) for liquid dairy manure in 20 days (229 ± 1 and 242 ± 5 ml CH₄ gVS added⁻¹) is in agreement with methane potential obtained for continuous mono-digestion of dairy manure reported in chapter 4.2 (223 ± 22 ml CH₄ gVS added⁻¹). This experiment was carried out in the last third of continuous reactors experiment, so it can be concluded that the storage at a temperature of 4 °C is an adequate procedure to preserve both samples and feedstock. The ultimate methane yield of FDMS were 50 ± 1 and 80 ± 3 % of the ultimate methane yield for dairy manure for the two experiments (Figure 5.2).

To estimate the presence of synergism or antagonism during co-digestion, actual methane production and normalized methane yield (based on VS added) of single substrates were compared. This comparison indicates lower methane production than expected with individual substrates (Figure 5.3). The antagonistic effect observed in both experiments increased with increasing manure solids. In addition, the process was inhibited when FDMS were 83% as VS of feedstock (Figure 5.3A), with a lower % VS removal. No considerable improvement in methane production was obtained when the manure solids percentage was increased from 71 to 83% in feedstock. Note that this maximum addition produced an increase in the average methane production (ml) by 114 ± 2 % over DM alone.

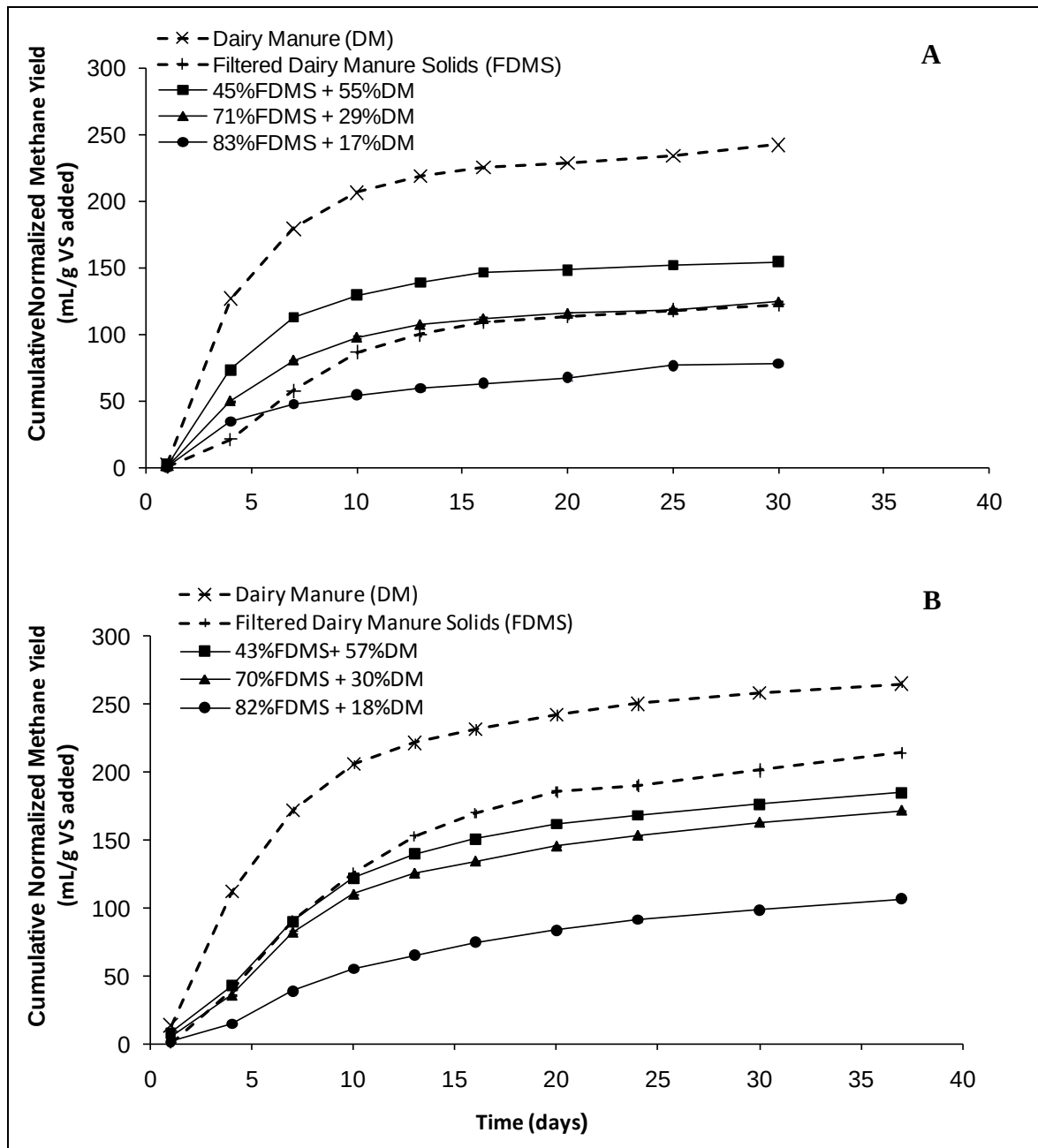


Figure 5.2: Cumulative Normalized methane yield in two co-digestion experiments (A and B) of dairy manure (DM) with filtered dairy manure solids (FDMS). Note that background methane production from inocula was subtracted.

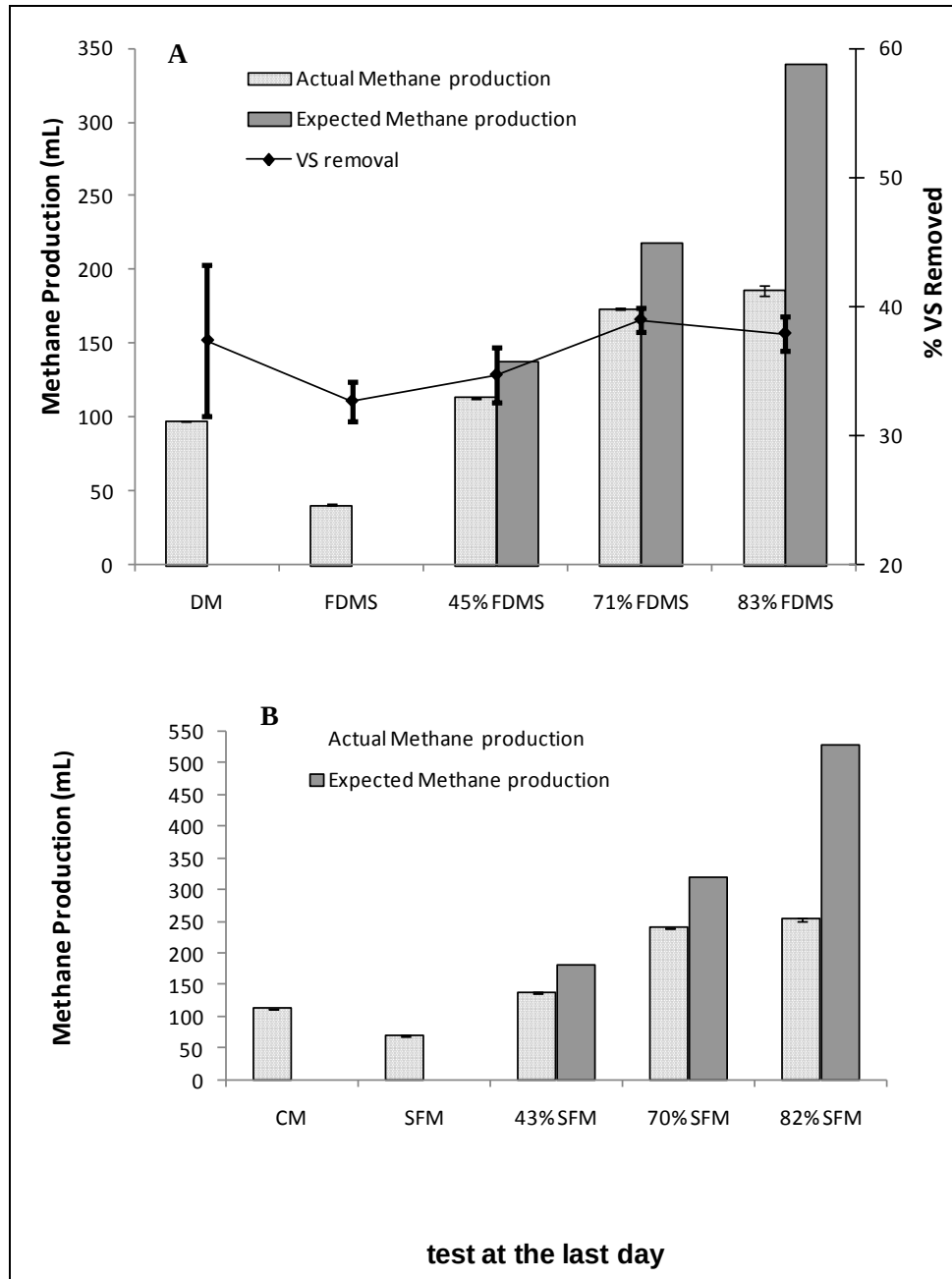


Figure 5.3: Antagonistic effects and VS removal in two co-digestion experiments (A and B) of dairy manure (DM) with filtered dairy manure solids (FDMS). Note that VS removed from inocula were subtracted.

Similarly, Kaparaju et al. (2008) studied the post-methanation feasibility of solids and liquid fractions from effluents of farm-scale anaerobic digesters treating dairy manure. They concluded that solid fraction (particle size $>0.25\text{mm}$) was degraded having a methane potential of approximately 41% of the liquid fraction between 30 and 50 days. Moreover, they reported that the ultimate methane yield of the solid fraction (incubation between 250 and 340 days) was similar to methane yield for raw dairy manure reported for farm-scale digesters. In the present study methane yield of solids at days 30 and 37 increased 50 ± 1 and $80\pm 3\%$, respectively, of the normalized methane yield over liquid dairy manure alone. Note that Kaparaju et al. (2008) used larger particle size and already digested wastes, so a smaller methane potential could be expected when comparing their results with results from the present study.

It can be concluded that the highest percentage of manure solids in the feedstock was about 70% as VS. Total methane production increased by $114\pm 2\%$, but the process efficiency (methane yield) decreased by $59\pm 14\%$. The microbial population adapted to different concentrations of VS from manure solids, but longer retention time would be needed to digest separated solids. Moreover, antagonistic effects are present. More research needs to be done to determine a maximum particle size of filtered dairy manure solids that can be anaerobically digested. Finally, storing at a temperature of $4\text{ }^{\circ}\text{C}$ is an adequate procedure to preserve both samples and feedstock.

5.2 Dairy Manure and Grease Trap Waste (GTW)

Lipids (oils, fats and grease) are the main components of grease trap waste (Camarota et al., 2006; Davidsson et al., 2008; Luostarinen et al., 2009) and yield after a slow degradation glycerol (Demirel et al., 2005) and long chain fatty acids (LCFAs) that can inhibit the methanogenic process (Davidsson et al., 2008; Demirel et al., 2005; Luostarinen et al., 2009). However, by co-digesting dairy manure and grease trap waste (GTW), both grease hydrolysis and LCFAs consumption may be improved.

Different amounts of grease trap waste were anaerobically digested to measure the methane production potential. Subsequently, co-digestion of dairy manure and grease trap waste was tested (Table 5.2).

The lag phase at an addition of 1.24 gVS of GTW alone was longer than in smaller additions (14 versus 7 and 5 days), but co-digesting with dairy manure diminished this lag phase (9 days) despite higher VS were added (Figure 5.4). In the first 7 days, methane yield for both mono (275 ± 3 and 131 ± 19 ml gVS added⁻¹ for 0.41 and 0.83 GTW added as g VS, respectively) and co-digesting GTW (247 ± 5 and 158 ± 4 ml gVS added⁻¹ for 0.41 and 0.83 GTW added as g VS, respectively) was similar for the smaller additions. These last two facts are in fully agreement with results found by Davidsson et al. (2008) and Luostarinen et al., (2009) and also support the conclusions reported by pervious research (Beccari et al., 1996; Kuang, 2002) which states that toxicity of LFCAs can be reduced in presence of carbohydrates and proteins.

The addition of 0.83 and 1.24 gVS of GTW as single substrate caused inhibition of methane yield (680 ± 22 and 650 ± 9 ml CH₄ gVS added⁻¹ at last day), compare with just 3% GTW (801 ± 24 ml CH₄ gVS added⁻¹ at last day). Researchers (Davidsson et al., 2008; Luostarinen et al., 2009; Salminen et al., 2002b) have reported ultimate methane potential for grease trap waste between 844 and 928 ml gVS added⁻¹. They concluded that those potentials are relatively close to the theoretical methane potential of fats or lipids (C₅₇H₁₀₄O₆) computed by the Buswell formula (approx. 1014 ml CH₄ gVS⁻¹). Normalized methane yield reported in the literature is higher than the yields obtained in this study

Table 5.2: Experiment design in co-digestion of dairy manure with grease trap waste.

Inoculum Addition (ml)	DI water addition (ml)	Dairy manure (ml)	g VS from DM	Grease trap waste (ml)	g VS from GTW	Total Volatile Solids (g/L)	Observation (as g VS added)
80	20	0	0	0	0	12.04	Inocula control (no addition)
80	10	10	0.21	0	0	14.17	0.21 Dairy manure
80	17	0	0	3	0.41	16.19	0.41 GTW
80	14	0	0	6	0.83	20.34	0.83 GTW
80	11	0	0	9	1.24	24.48	1.24 GTW
80	7	10	0.21	3	0.41	18.32	0.41 GTW + 0.21 CM
80	4	10	0.21	6	0.83	22.46	0.82 GTW + 0.21 CM
80	1	10	0.21	9	1.24	26.61	1.24 GTW + 0.21 CM

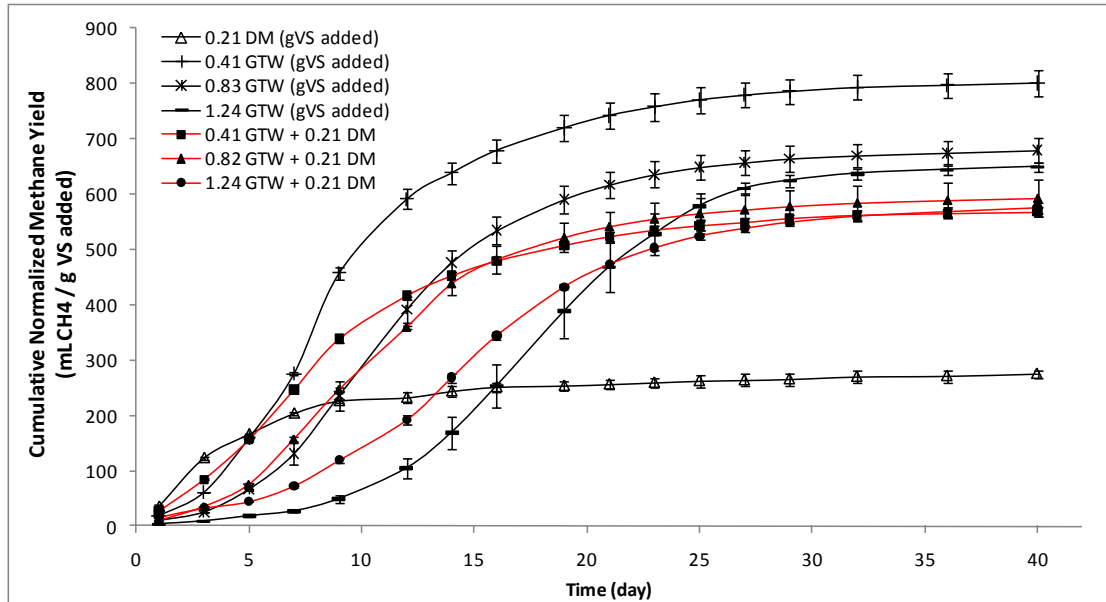


Figure 5.4: Cumulative Normalized methane yield in co-digestion of dairy manure with Grease Trap Waste. Note that background methane production from inocula was subtracted.

suggesting that LCFA could have inhibited the process at the lower GTW additions. Smaller additions on GTW should have been included in this study.

For single substrate, on day 40, organic matter removal decreased as the percentage of GTW increased (Figure 5.5) reaching 86.1 ± 2.4 , 81.8 ± 0.4 , and 80.9 ± 1.3 % for additions of 0.41, 0.83, and 1.24 gVS added, respectively. Inhibition could be explained by problems related with an excess of oil and grease such as a reduction in substrate transfer rates into microbial cells, the development of filamentous microorganisms that created floating scum, and a deficient vertical mixing causing loss of biomass concentrated at the reactor bottom (Cammarota et al., 2006). Organic matter removal (% VS removal) and methane yield were similar in all co-digesting combinations. While VS removal was 72 ± 4 %, methane yield reached $579 \pm 21 \text{ mL CH}_4 \text{ gVS added}^{-1}$. Co-digestion increased VS removal and methane yield (efficiency) of dairy manure alone by 111 ± 9 and 76 ± 4 %, respectively. Research needs to be done to find the optimal GTW addition at which the highest methane production and efficiency (methane yield) are achieved, but using smaller additions.

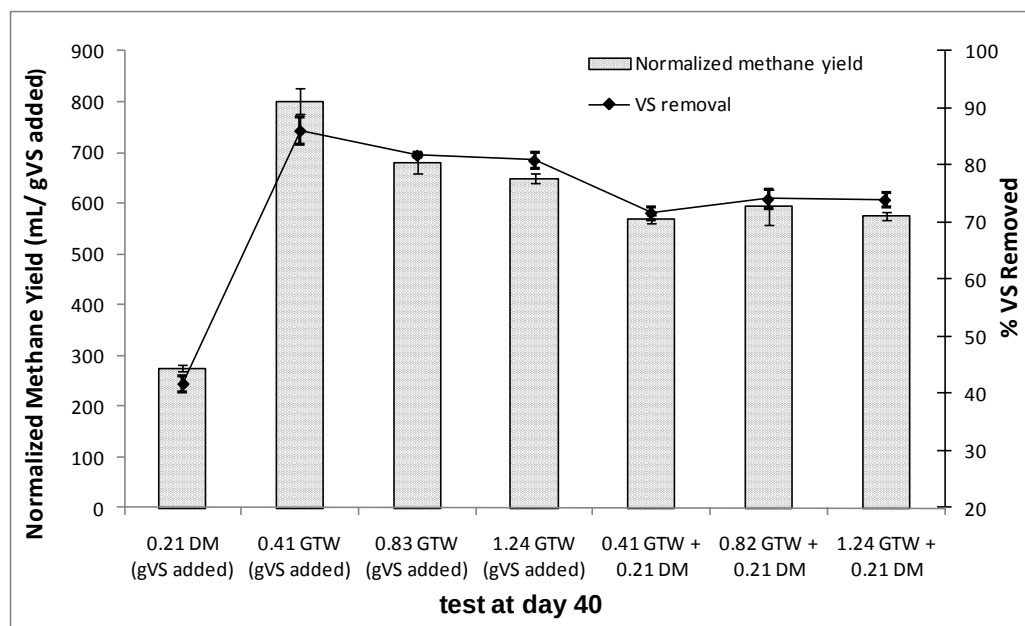


Figure 5.5: Ultimate normalized methane yield and VS removal in co-digestion of dairy manure with Grease Trap Waste. Note that background methane production from inocula was subtracted.

Although GTW was difficult to digest as single substrate, it could be digested when mixed with dairy manure improving methane yield and VS removal of dairy manure alone. Research needs to be done to find the optimal GTW addition at which the highest methane yield is achieved.

5.3 Dairy Manure and Sawdust

Sawdust is a complex lignocellulosic material which hydrolysis slowly thereby is limiting anaerobic digestion (Adney et al., 1991; Kaparaju et al., 2002; Op den Camp et al., 1988; Vinzant et al., 1990). In fact, lignin is considered as anaerobically undegradable (Chynoweth et al., 1987; Op den Camp et al., 1988) and is present at 25% and 30% by dry weight in woody materials (Banks, et al., 1998; Op den Camp et al., 1988; Chynoweth et

al., 1987). In fact, it has been also proven that an addition of isolated lignin presents no degradability by rumen microorganisms (Akin 1982). Research has used fluids from the rumen of cattle and sheep as inocula to provide microorganisms able to hydrolyze lignocellulosic materials (Adney et al., 1991; Op den Camp et al., (1988). Op den Camp et al. (1988) concluded that high lignin or lignin monomers concentrations reduce anaerobic biodegradability of lignocellulosic materials due to inhibition of cellulases enzymes. In addition, cellulosic and hemicellulosic materials lowers methane yields (Adney et al., 1991; Banks, et al., 1998; Chen et al., 1987). Other factors also affect woody materials biodegradation such as low moisture content; lignin, cellulose and hemicellulose fraction and their degree of association; proportion of structural and non-structural carbohydrates; cellulose crystallinity (degree of structural order of their backbones); particle size; and wood-to-bark ratio (Gunaseelan, 1997). In the present experiment, screened sawdust was co-digested with dairy manure under anaerobic, mesophilic conditions. It was hypothesized the dairy manure could provide buffer capacity needed for sawdust digestion, as well as supply the needed moisture for anaerobic digestion. In Table 5.3 is summarized the experiment design.

Although no lag phase was noted for dairy manure, a lag phase of maximum 1 day was detected for co-digesting assays (Figure 5.6). Microbial population never acclimated to pure sawdust. Methane yield at 20 days for dairy manure and sawdust reached 88 ± 9 and 49 ± 6 % of yield at last day, respectively. At 20 days co-digesting test achieved 88 ± 9 , 88 ± 9 , and 85 ± 9 % of yield at last day for sawdust addition of 52, 76 and 87 % as VS, respectively. Normalized methane yield from sawdust (6 ± 1 ml gVS added⁻¹) was $2 \pm 2\%$ of dairy manure methane yield (277 ± 4 ml gVS added⁻¹) at the last day. Adding sawdust to dairy manure only decreased methane yield. Maximum methane yields (day 20) for co-digestion were 150 ± 2 , 94 ± 1 , and 56 ± 1 ml gVS added⁻¹ for sawdust additions of 52, 76 and 87 % as VS, respectively. Very little sawdust was degraded (2 ± 1 % VS removal), so organic matter removal considerably decreased when sawdust was added (22 ± 3 , 9 ± 3 , and 7 ± 1 compared with $37 \pm 1\%$ VS removed) (Figure 5.7).

Table 5.3: Experiment design in co-digestion of dairy manure with sawdust.

Inoculum Addition (ml)	DI Water (ml)	Cow manure (ml)	g VS CM	Straw (g)	g VS	volume added (ml)	Na2S solution (ml)	Total volume (ml)	Total g VS/L	Observation
80	20						1.70	101.7	10.71	Inocula Control
80		20	0.401				1.70	101.7	14.65	Cow Manure Control
80				0.435	0.43	1.08	1.30	82.4	18.47	Saw Dust Control
80		20	0.401	0.435	0.43	1.08	1.70	102.8	18.71	52%Sawdust + 48%CM
80		20	0.401	1.305	1.30	3.25	1.70	104.9	26.57	76%Sawdust + 24%CM
80		20	0.401	2.610	2.60	6.49	1.70	108.2	37.77	87%Sawdust + 13%CM

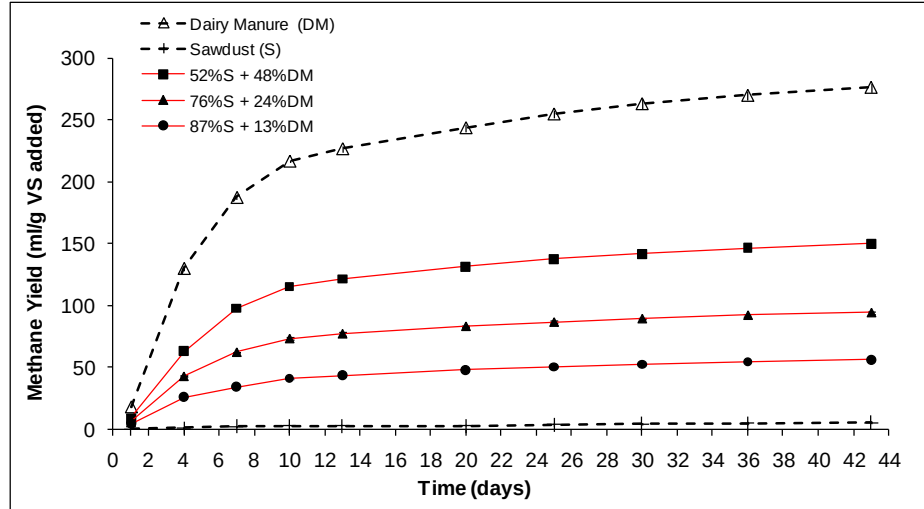


Figure 5.6: Cumulative methane yield of co-digestion of dairy manure with sawdust. Note that background methane production from inocula was subtracted.

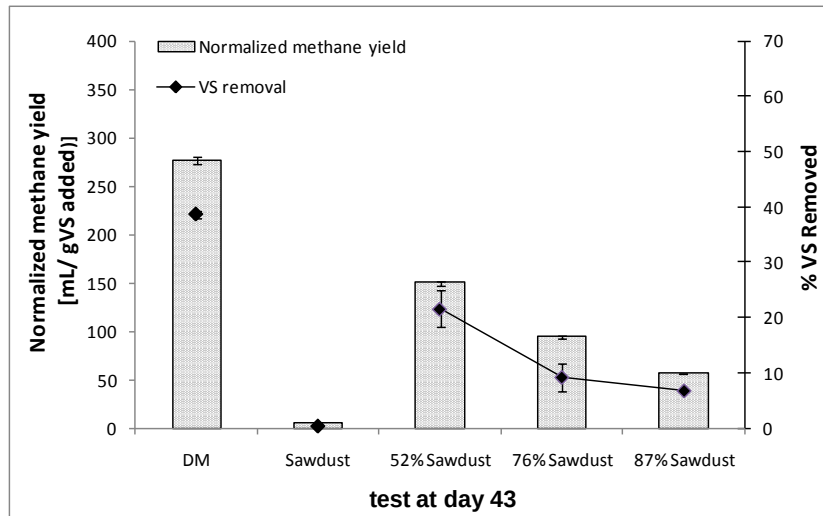


Figure 5.7: Methane production of co-digestion of dairy manure with Sawdust. Note that background methane production from inocula was subtracted.

Finally, it can be said that sawdust couldn't be degraded by co-digesting with dairy manure. Moreover, the addition of large amounts of sawdust can create high concentrations of lignin and lignin monomers that could inhibit hydrolytic enzymes (Op den Camp et al., 1988).

5.4 Conclusions

Filtered solids from dairy manure (0.417 and 0.842 mm) can be co-digested up to a maximum percentage in the feedstock of 70% volatile solids. Total methane production increase by 114 ± 2 % as a consequence of an increase in organic loading, but the process efficiency (methane yield) decreased by 59 ± 14 %. Antagonistic effects were found. More research needs to be done to determine a maximum particle size that can be digested.

GTW is hard to digest as single substrate, but it can be co-digested improving methane yield (efficiency) and VS removal of dairy manure alone by 111 ± 5 and 76 ± 4 %, respectively, for all combination tested. Research needs to be done to find the optimal GTW addition at which the highest methane yield is achieved.

Sawdust alone is considered anaerobically undegradable and its co-digestion with dairy manure was unsuccessful. Moreover, the addition of large amounts of sawdust can create high concentrations of lignin and lignin monomers that could inhibit hydrolytic enzymes (Op den Camp et al., 1988).

In addition, from all co-digestion experiments the methane potential for dairy manure in 20 days was similar to methane potential obtained for mono digestion studied in chapter 4.2, indicating both an optimal performance and a closed mass balance on carbon of those continuous reactors. By storing the substrates at a 4 °C both samples and feedstock will preserve properly.

CHAPTER 6 Conclusions and Recommendations

6.1 Conclusions

The following conclusions were drawn from this study:

1. Chicken litter can be added into a digester treating dairy manure to increase the organic loading rate leading in a higher methane production rate. Chicken litter can be safely added up to a 33% as Volatile Solids (VS) in the feedstock increasing methane production by 49.3%. Other researches (Callaghan et al., 2002; Magbauna et al., 2001; Misi et al., 2001b) found a similar maximum chicken manure percentage in feedstock for continuous and batch reactors.
2. No synergistic effects were detected when co-digesting chicken litter with dairy manure. However, chicken litter requires water addition to be digested, so positives effects are achieved co-digesting these wastes in the sense that no water addition is needed.
3. The selection of the initial OLR is related to inocula acclimatation and waste composition and depends on a wide number of factors.
4. For dairy manure, 2 retention times seems to be enough to reach stable conditions. However, it has previously been suggested 3 or 4 retention times are required to reach steady state conditions.
5. Perhaps, a combined effect of high ammonia concentration and overloading resulted in reactors collapse. More research needs to be done evaluating the influence of high ammonia concentration under different organic loading rates.

6. Because of the large total alkalinity in the system, pH and VFAs are not good indicators of instability. Instead, gas production should be followed closely in order to detect any symptom of imbalance.
7. Total and free ammonia tolerance could be improved just by simply combining dairy manure with chicken litter. However, microbial adaptation for free ammonia occurs when increasing free ammonia concentrations in reactors.
8. By establish the retention time at 20 days it is possible to recover up to approximately 90% of methane from substrates. In addition, this large retention time allows the microbial population to better develop free ammonia adaptation.
9. Co-digestion seems have no influence in pathogen indicator (*E.coli*) removal. Removal values reached typical values ranging from 68 ± 4 and 97 ± 2 %.
10. The microbial population can adapt to lower temperatures down to 19 °C, but at longer retention times making this economically unattractive for continuous reactors. At 20 days of retention time, methane production decreases by 10% when temperature decreases from 35 to 25 °C.
11. Filtered solids from dairy manure (0.417 and 0.842 mm) can be co-digested up to a maximum percentage in the feedstock of 70% volatile solids to increase methane production by $114\pm2\%$ as a consequence of an increase in organic loading, but the efficiency (methane yield) decreases by 59 ± 14 %. Antagonistic effects are found.
12. GTW can be co-digested improving methane yield (efficiency) and VS removal of dairy manure alone by 111 ± 5 and 76 ± 4 %, respectively in all addition tested.
13. Co-digestion of sawdust with dairy manure is unsuccessful.

14. By storing the substrates at a 4 °C both samples and feedstock will preserve properly.

6.2 Implications

1. Uncontrolled methane emissions from dairy and poultry feeding operations can be reduced by anaerobic co-digestion.
2. Revenue for farmers can be obtained by selling biogas and reducing fees related to wastewater disposal.
3. A reduction of pathogens can be achieved by anaerobic digestion diminishing surface and groundwater contamination.
4. Sludge from anaerobic digestion can be used as a more stable fertilizer.

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APPENDIX

Appendix A



Figure A.1: 3.6L semi-continuous reactor treating dairy manure alone.



Figure A.2: 3.6L semi-continuous reactor co-digesting chicken litter with dairy manure.



Figure A.3: 160-mL serum bottle used for different batch experiment after experiments were finish.

VITA

Esteban Manuel Zamudio Cañas was born on September 2nd, 1982 in the city of Santiago, Chile. He graduated from Colegio De La Salle high school in December of 2000. Then he was accepted and enrolled by the Department of Civil Engineering in Public Works at the University of Santiago of Chile (USACH). He obtained his bachelor degree in Civil Engineering by September of 2007. By August of 2008 he was accepted by the Department of Civil and Environmental Engineering as a Master of Science student at The University of Tennessee, Knoxville. Through one year and a half of research and study, he completed the requirements for his Master of Science degree in Civil Engineering (environmental concentration) in December of 2009.