OPTIMISATION AND INHIBITION OF ANAEROBIC DIGESTION OF **LIVESTOK MANURE**

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Abstract: The objectives of this study were to:(1) optimise methane production of livestock manure during anaerobic digestion, focusing on the addition of mixed enzymes, thermal pre-treatment and co-digestion, and (2) develop a better understanding of ammonia and sulphide inhibition during digestion of animal manure . Enzyme addition increased methane yield of manure following incubation prior to digestion. Thermal pre-treatment increased methane yield but was only energetically favourable when a surplus of thermal energy was available. Digestion of acidified manure showed sulphide inhibition but no inhibition was measured when processing solid fractions of acidified manure. Co-digestion of non-acidified manure with the solid fractions of acidified manure was found to be a promising method of increasing methane yield in terms of digester volume. Total volatile acids, isobutyric and isovaleric acid and total ammonia nitrogen were found to be useful indicators of ammonia inhibition.

Keywords: Bioenergy, Biomass, Biogas, Environmental engineering, Slurry separation

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OPTIMISATION AND INHIBITION OF ANAEROBIC DIGESTION OF LIVESTOK MANURE

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Abstract

This thesis deals with the optimisation and inhibition of the anaerobic digestion (AD) of animal manure. The optimisation process during this PhD study focused on mixed enzyme (ME) addition, thermal pre-treatment and co-digestion of raw manure with solid fractions of acidified manure, while for inhibition processes, ammonia and sulphide inhibition were studied.

ME addition increased methane yield of both dairy cow manure (DCM) and solid fractions of DCM (by 4.44% and 4.15% respectively, compared to the control) when ME was added to manure and incubated prior to AD. However, no positive effect was found when ME was added to manure and fed immediately to either mesophilic (35°C) or thermophilic (50°C) digesters.

Low-temperature pre-treatment (65°C to 80°C for 20 h) followed by batch assays increased the methane yield of pig manure in the range from 9.5% to 26.4% at 11 d incubation. These treatments also increased the methane yield of solid-fractions pig manure in the range from 6.1% to 25.3% at 11 d of the digestion test. However, at 90 d the increase in methane yield of pig manure was only significant at the 65°C treatment, thus low-temperature thermal pre-treatment increased the rate of gas production, but did not increase the ultimate yield (B_0).

High-temperature pre-treatment (100°C to 225°C for 15 min.) increased the methane yield of DCM by 13% and 21% for treatments at 175°C and 200°C, respectively, at 27 d of batch assays. For pig manure, methane yield was increased by 29% following 200°C treatment and 27 d of a batch digestion test. No positive effect was found of high-temperature pre-treatment on the methane yield of chicken manure. At the end of the experiment (90 d), high-temperature thermal pre-treatment was significantly increasing the B_0 of pig manure and DCM.

Acidification of animal manure using sulphuric acid is a well-known technology to reduce ammonia emission of animal manure. AD of acidified manure showed sulphide inhibition and consequently methane production was 45% lower when compared with the control, but no inhibition was found when treating solid fractions of acidified manure. In addition, it was found that a digester treating non-acidified DCM could operate in a stable state when 30% of the input was substituted with the solid fraction of acidified DCM and that methane production increased by 50% in terms of digester volume. Post-digestion test results showed that methane production of digested slurry increased as the concentration of solid-fraction acidified DCM increased. Therefore in order to gain optimal biogas potential of substrates and reduce the methane emission of digested slurry, post-digestion is needed when digesters process large concentrations of solid fractions of acidified manure.

Of microorganism inhibitors, ammonia is expected to be the most common cause of suboptimal AD process performance when co-digesting animal manure with a proteinaceous substrate. In an experimental digester with a total ammoniacal nitrogen (TAN) value of 2.9 g L^{-1} corresponding to 0.7 g L^{-1} of free ammonia (FA), the methane yield was reduced by 24% compared to a reference digester which had a TAN of 2.2 g L^{-1} (FA 0.48 g L^{-1}). Biogas production, TAN and FA values, total VFA concentration, isovaleric and isobutyric acid concentrations were useful indicators of ammonia inhibition.

Preface

This thesis is presented as partial fulfilment of the requirement for the Ph.D. degree at Department of Engineering, Faculty of Science and Technology, Aarhus University. The research work was conducted from December 2009 to November 2012 under the guidance of my supervisors Henrik Bjarne Møller and Alastair James Ward. The thesis is based on the works that were prepared in the following manuscripts. In the text, they are referred to by their Arabic number.

1. Sutaryo, S., Ward, A.J., Møller, H.B., 2012. The effect of mixed enzyme addition in anaerobic digestion on methane yield of dairy cattle manure. Resubmitted after revision to peer-review journal
2. Sutaryo, S., Ward, A.J., Møller, H.B., 2012. The effect of low temperature thermal pre-treatment on the methane yield of pig manure fractions. Manuscript draft.
3. Raju, C.S., Sutaryo, S., Ward, A.J., Møller, H.B., 2012. Effects of high-temperature isochoric pre-treatment on the methane yields of cattle manure, pig and chicken manure. Environmental Technology, DOI:10.1080/09593330.2012.689482.
4. Sutaryo, S., Ward, A.J., Møller, H.B., 2012. Thermophilic anaerobic co-digestion of separated solids from acidified dairy cow manure. Bioresource Technology, 114: 195–200.
5. Sutaryo, S., Ward, A.J., Møller, H.B., 2012. Anaerobic digestion of acidified slurry fractions derived from different solid – liquid separation methods. Bioresource Technology, 130: 495–501.
6. Sutaryo, S., Ward, A.J., Møller, H.B., 2012. Ammonia inhibition in thermophilic anaerobic digestion of dairy cattle manure. Submitted to peer-review journal.

In addition, the following publications were produced during the PhD study:

1. Moset, V., Cerisuelo., Sutaryo, S., Møller, H.B., 2012. Process performance of anaerobic co-digestion of raw and acidified pig slurry. Water Research. 46: 5019–5027.
2. Raju, C.S., Løkke, M.M., Sutaryo, S., Ward, A.J., Møller, H.B., 2012. NIR monitoring of ammonia in anaerobic digesters using a diffuse reflectance probe. Sensors, 12: 2340–2350.
3. Kandel, T. P., Sutaryo, S., Møller, H.B., Jøgensen, U., Lærke, P, E., 2012. Chemical composition and methane yield of reed canary grass as influenced by harvesting time and harvest frequency. Bioresource Technology. DOI:org/10.1016/j.biortech.2012.11.138, in press.

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The Ph.D. study was supported by Directorate General of Higher Education, Department of National Education, Republic of Indonesia so thanks to everyone who has made this possible.

Finally, I would like thank my wife, children, family, colleagues and friends for their support during the study process.

Foulum, November 2012

Sutaryo

Abstract in Danish

Denne Ph. D. -afhandling omhandler optimering og hæmning af den anaerobe nedbrydning (AD) af husdyrgødning. Optimeringen omhandler enzymtilsætning, forbehandling ved hjælp af varme og samudrådning af gylle med den faste del af forsuret gylle. Hæmningsdelen er fokuseret omkring ammonium- og sulfidhæmning.

Enzymtilsætning øgede metanudbyttet af kvæggylle og den faste del af kvæggylle (FKG) med henholdsvis 4,44% og 4,15% sammenlignet med kontrol når enzymblandingen blev tilsat gyllen og inkuberet før denne blev tilsat til anaerobe reaktorer. Derimod var der ingen effekt af enzymblandingen, når denne blev tilsat gyllen direkte og umiddelbart herefter overført til anaerobe reaktorer, hverken under mesofile (35 °C) eller termofile (50 °C) forhold.

Forbehandling af svinegylle ved opvarmning fra 65 °C til 80 °C i 20 timer efterfulgt af inkubering gav et merudbytte i metanpå mellem 9,5% og 26,4% efter 11 dages inkubation. Denne forbehandling øgede også metanudbyttet af den faste del af svinegyllen fra 6,1% til 25,3% efter 11 dages inkubation. Dog var metanudbyttet efter 90 dage kun signifikant højere ved 65 °C behandlingen. Termisk forbehandling ved disse temperaturer øger altså hastigheden af gasproduktionen, men ikke det totale gasudbytte (B_0).

Forbehandling af svinegylle ved høje temperaturer (100 °C til 225 °C i 15 min) gav et merudbytte i kvæggylle på 13% og 21% ved forbehandling ved henholdsvis 175 °C og 200 °C efter 27 dages inkubering. For svinegylle blev metanudbyttet øget med 29% ved 200°C forbehandlingen efter 27 dages inkubering. For kyllingegødning var der ingen effekt på metanudbyttet ved termisk forbehandling ved høje temperaturer. Efter 90 dage var det total gasudbytte (B_0) signifikant højere for svinegylle og kvæggylle efter termisk forbehandling ved høj temperatur.

Forsuring af gylle ved hjælp af svovlsyre er en velkendt teknologi til at formindske ammoniakudledning. Biogasproduktion af forsuret gylle hæmmes af sulfid, og metan produktionen falder med 45% sammenlignet med kontrollen. Dog ses ingen hæmning af metanproduktionen, når den faste del af forsuret gylle nedbrydes anaerobt. Desuden blev det vist at en reaktor der tilsættes ikke forsuret kvæggylle kunne køre stabilt når 30% af indholdet blev erstattet med forsurede gylle fibre. Med denne blanding blev metanproduktionen øget med 50% set i forhold til reaktorvolumen. Efternedbrydningstest viste at metanproduktionen fra nedbrudt materiale steg med en stigende andel af forsurede gyllefibreFKG. For at opnå optimal biogasproduktion fra substratet og reducere metanudledningen af nedbrudt materiale er efternedbrydning nødvendig i de tilfælde hvor reaktorer behandler en større koncentration af forsuret gødning.

Ammonium er den af de mikrobielt hæmmende stoffer, der anses for at være den mest almindelige grund til hæmning af processen når gylle samudrådnes med proteinrigt materiale. I en reaktor med et total indhold nitrogen på vandlig form (TAN) på 2,9 g L^{-1} svarende til 0,7 g L^{-1} frit ammoniak (FA) blev metan udbyttet reduceret med 24% i forhold til en reference reaktor indeholdende 2,2 g L^{-1} TAN (FA 0,48 g L^{-1}). Biogas produktion, TAN og FA værdier, Total VFA, koncentrationer af syreformen af isovalerat og isobutyrat er alle værdifulde indikatorer for ammoniak hæmning.

Nøgleord: Biogas, forbehandling, samudrådning, fastdel, ammoniak

Table of Content

1. Anaerobic digestion of livestock manure

1.1 Introduction

The capability of anaerobic microorganism consortia to convert low-value organic material into useful renewable energy in the form of biogas has gained attention in the last few decades. The anaerobic digestion (AD) process naturally occurs in anaerobic conditions such as marine and fresh water sediments, sewage sludge, and in the gut of mammals (Angelidaki et al., 2003). Initially AD was implemented to treat sludge from wastewater treatment plants. Lately, this practice was extended to treat animal manure, agricultural and industrial by-products (González-Fernández et al., 2008). Application of this technology offers some advantages such as reducing volatile suspended solids and odours, destroying pathogenic microorganisms, and producing renewable energy. In waste management, AD is more favourable compared to aerobic treatment due to better control of emission and recovery of energy in the form of biogas (Fricke et al., 2005, Cakir and Stenstrom, 2005). Decomposition of organic material through the AD process is ecologically advantageous in two ways: 1) by localising the decomposition process in a closed reactor, where the potential emission of methane, as the main product of biogas, to the atmosphere can be prevented, and the gas can subsequently be used in variety of purposes, for example in combined and heat power production (CHP) or in the transportation sector, 2) energy obtained from this process can displace the utilisation of fossil fuel (Ward, 2008). Moreover, the carbon dioxide released in the AD process is not considered a greenhouse gas emission, since the carbon has recently been used by plants in the photosynthetic process and to be released again to the atmosphere is part of the carbon cycle (Ward, 2008). Taking these advantages into account and the rising price of fossil fuel, particularly since the energy crisis in the 1970s, biogas has become the focus of much attention both in research environments and in the industrial sector (Angelidaki et al., 2003). For instance in 2008, Denmark had 20 full-scale centralised biogas plants of 550-8500 m^3 volume and more than 80 farm-scale biogas plants (Nielsen and Angelidaki, 2008).

Low-value and low-cost substrates that are affordable and can be used sustainably in biogas plant are agriculture by-products such as animal manure, straw and crop residues. Unfortunately, these organic materials are of low biodegradability, therefore AD treatment of these substrates, particularly animal manure which is the most abundant source of organic matter for AD in Europe has been uneconomical (Møller et al., 2007). The common method to solve this problem is pre-treating the recalcitrant substrate prior to AD. Other methods involve co-digestion of manure with other organic materials that have a higher methane potential per volume substrate (Asam et al., 2011) or engineering biogas plants to enable them to cope with such substrates (Bruni, 2010).

Another issue in AD is inhibition during the fermentation process. The common inhibitors in AD are ammonia, sulphide, light metal ions, heavy metal and organic compounds (Chen et al., 2008). Ammonia inhibition can occur when AD-processing proteinaceous substrates (Braun et al., 2003) and solid fractions of pig manure (Møller et al., 2007), whereas sulphide inhibition takes place when AD-treating sulphate-containing wastewater from sources such as the paper and board industry, molasses-based fermentation industries and edible oil refineries (Colleran et al., 1995) and when treating acidified animal manure (Sutaryo et al., 2012). Therefore, the objectives of this PhD study were to:

- evaluate methods to improve methane production from livestock manure focusing on mixed enzyme addition in AD and thermal pre-treatment of manure prior to AD.
- evaluate co-digestion of animal manure with solid-fraction acidified manure from solid-liquid manure separation,
- determine methane production of animal manure fractions derived from different solid-liquid manure separation techniques,
- explore the effect of ammonia inhibition at different levels of inhibition on methane production of dairy cow manure (DCM),
- determine the effect of sulphide inhibition on AD processing acidified livestock manure.

1.2 Livestock manure management through anaerobic digestion

Livestock manure is an abundant biomass substrate for AD (Kaparaju and Rintala, 2008; Nasir et al., 2012). In Denmark, the estimated energy potential of methane from available biomass resources through the AD process is 30 petajoules (PJ) annually and manure contributes 80% to this potential (Angelidaki and Ellegaard, 2003). It is expected that livestock manure production worldwide will continue to increase in the future. This phenomenon is inevitable since increases in welfare and living standards of society are often followed by a dietary shift from carbohydrate sources to protein sources. The consequence of this is a high demand for livestock products, particularly in the developed world. For instance, Denmark with a population size of 5.580.516 in 2012 (Statistics

Denmark, 2012) produced an estimated 25.3 million pigs in 2009 (Annual report of Danish pig production, 2008) and cattle population 1.615 million in 2012 (Statistics Denmark, 2012). The agricultural sector is therefore a significant contributor to the anthropogenic non-carbon dioxide greenhouse gas emissions, particularly methane and nitrous oxide (Monteny et al., 2006) and ammonia and water pollution through leaching mechanisms (Burton and Turner, 2003). Therefore, manure management is urgently needed to reduce these effects. Animal manure management in the AD system has some advantages such as reducing emissions of carbon dioxide by the substitution of fossil fuel with biogas and reducing methane emission from manure in manure storage tanks (Møller et al., 2007), reducing odour emission (Hansen et al., 2006), and improving the fertilizer quality of digested slurry (Angelidaki et al., 2003). Manure management through AD treatment, particularly in Denmark, is also in line with the target of the Danish government for the utilisation by 2020 of 50% of the manure produced in Denmark as a substrate in AD to produce renewable energy in the form of biogas (Aftale om Grøn Vækst, 2009). The AD process is also part of the European Commission's Directive on Renewable Energy that sets a target of 20% of energy production from renewable energy sources by 2020 (European Commission, 2009).

Livestock manure is a substrate well suited for AD because: 1) it has a high water content enabling it to dilute concentrated by-products, thus resolving problems with pumping, 2) the high buffer capacity of manure is very useful to prevent sudden changes in pH value, and 3) it has a wide range of nutrients that are very important for microorganism growth (Angelidaki and Ellegard, 2003). However, livestock manure also has some limitations as a substrate in AD. The high water content of manure, previously characterised as a positive factor , also means it is a very dilute substrate with too little easily degradable carbon to produce much methane per unit volume (Hamelin et al., 2011), and thus more energy is required to maintain the temperature of the biogas reactor and the cost of transporting the manure to a centralised biogas plant is higher (Asam et al., 2011) and animal manure, particularly pig manure, has too low a C/N ratio which can lead to ammonia inhibition during the AD process (Hansen et al., 1998).

1.3 Anaerobic digestion process

Anaerobic digestion is a complex and multistep process, which generally consists of four main phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis involving different microorganism consortia at each step (Fig. 1) (Gujer and Zehnder, 1983).

Furthermore, hydrolysis is an extracellular step, while the rest processes are intracellular (biological process) (Batstone et al., 2002). These steps should be in proper balance to ensure enough products in each step can be used as substrate in the following phase without overproduction (Ward, 2008). For instance, if the rate of hydrolysis phase is higher than the methanogenic rate, this can cause accumulation of volatile fatty acids (VFA). An elevated concentration of these intermediate fermentation products can inhibit the methanogenic microorganisms (Pind et al., 2003) leading to AD process failure.

Hydrolysis in AD is the solubilisation and degradation of biopolymer particulate organic compounds and colloidal wastes into soluble monomeric or oligomeric organic compounds (Gerardi, 2003). This process is catalysed by extracellular enzymes including amylase, cellulase, protease and lipase that are excreted by bacteria (Taherzadeh and Karimi, 2008). Even though a wide range of exocellular enzymes are involved during this process, hydrolysis can be a rate-limiting step, particularly when AD-treating semi-solid waste (Ferrer et al., 2008). Once simple organic compound have been produced during the hydrolysis step, these products can be utilised as a substrate in the next step of AD.

The simple soluble substrate produced in the hydrolysis phase will be absorbed and degraded by different facultative and obligate anaerobic bacteria in the acidogenic step, producing short-chain VFAs, alcohols, hydrogen and carbon dioxide (Chandra et al., 2012). A high concentration of hydrogen produced by acidogenic microorganism during this phase can cause inhibition of the production of acetate by acetogens, as will be discussed latter.

Alcohols, for instance ethanol, and VFAs with more than two carbon atoms are degraded by acetate-forming bacteria with acetate, hydrogen and carbon dioxide as the main products (Parawira, 2012; Gerardi, 2003). Furthermore, hydrogen and carbon dioxide are constantly reduced to acetate by homoacetogenic microorganisms (Chandra et al., 2012). A mutually symbiotic relationship occurs between acetogens and methanogens. Acetogens produce acetate that can be used as substrate by methanogens, yet acetogens also produce hydrogen. Acetogens can survive at very low concentration of hydrogen (Gerardi, 2003). In an environment with high hydrogen partial pressure, acetogens lose their activity to produce acetate. However, methanogens are continuously removing hydrogen during the production of methane, therefore elevated hydrogen partial pressure does not usually occur (Chandra et al., 2012).

Methanogenesis takes places in obligate anaerobic conditions and is considered an exergonic reaction (Chandra et al., 2012). During this phase, carbon dioxide-reducing and hydrogen-oxidizing methanogens convert hydrogen and carbon dioxide producing methane, while acetoclastic methanogens utilize acetate to produce methane (Parawira, 2012). Approximately 70% of methane in AD is derived from this pathway (Parawira, 2012). Methanogenesis is the critical step in AD and methanogenic archaea are the actors here. This phase is critical because methanogens are sensitive to the different environmental conditions, and this phase can therefore have a large impact on AD (De Vrieze et al., 2012).

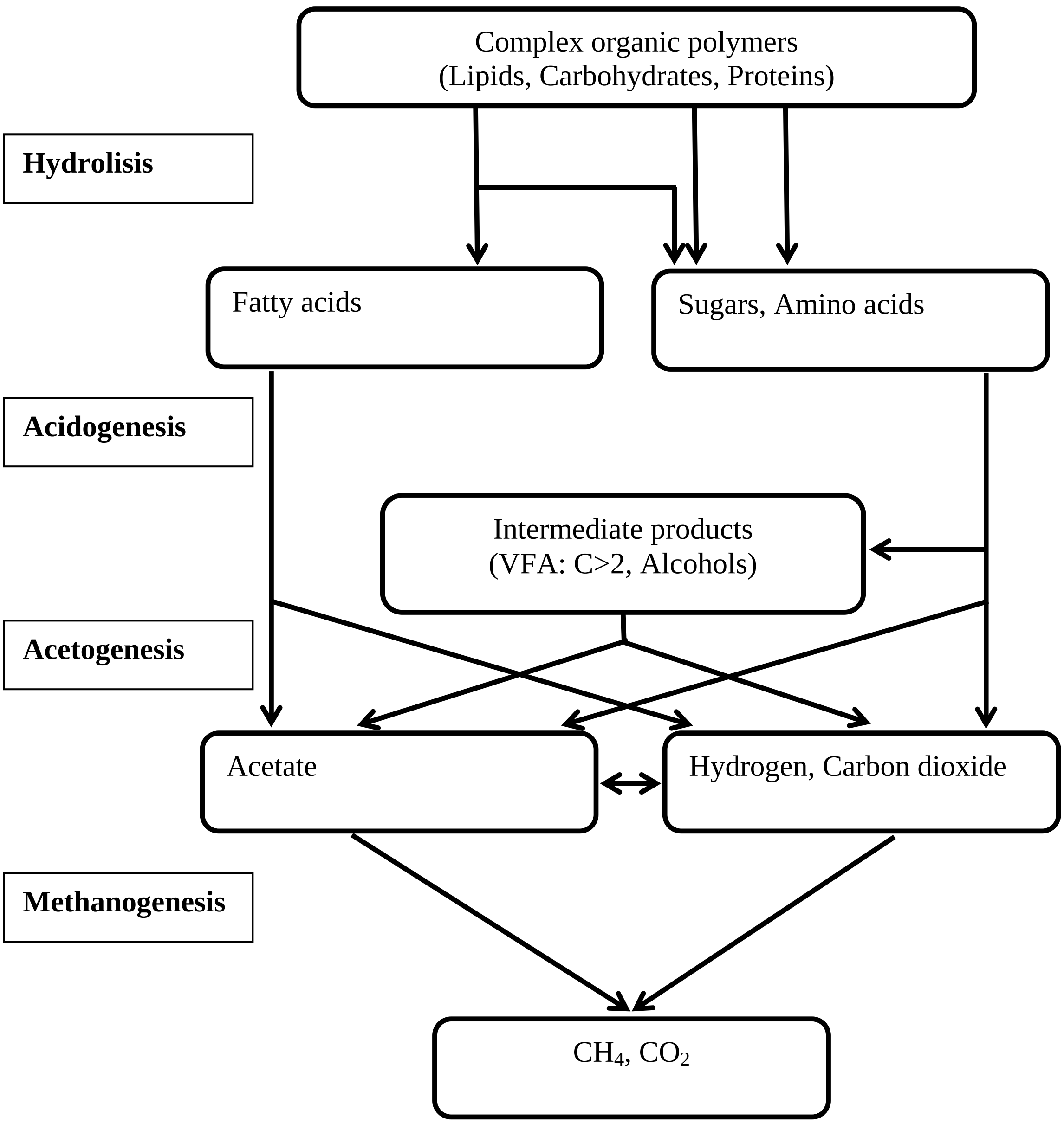


Fig. 1. Simplification of anaerobic digestion process (adapted from Gujer and Zehnder, 1983).

1.4 Lignocellulosic material

Lignocellulose (Fig. 2) is the main organic material in plant cell walls that consists of 30% to 50% cellulose, 15% to 35% hemicellulose and 10% to 30% lignin (Sousa et al., 2009).

Cellulose

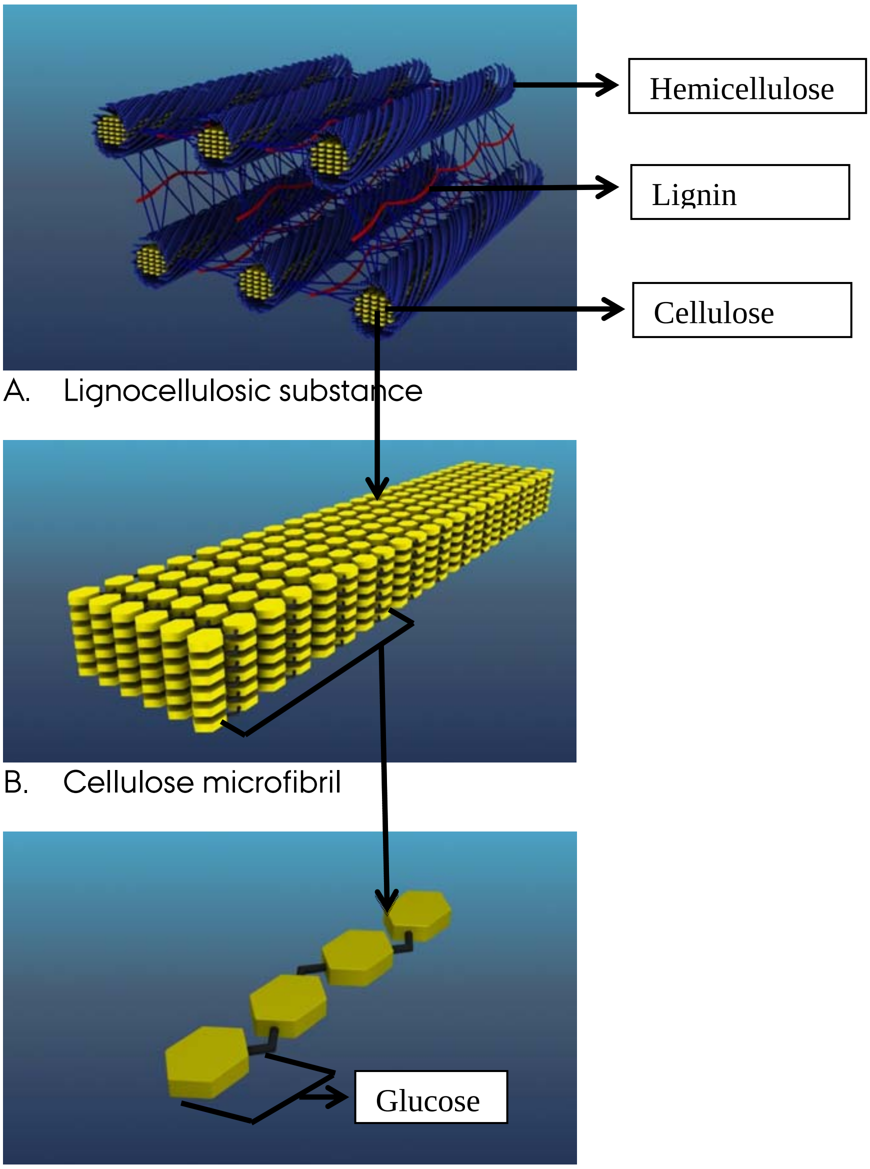
Cellulose is a polymer of glucose molecules that link to form a D-anhydroglucopyranose unit with β-1,4 glycosidic ether bridges, while the repeating unit of cellulose is the disaccharide cellobiose (Bobleter, 1994). The intramolecular hydrogen bonds in cellulose make it more rigid and intermolecular hydrogen bonds with neighbouring cellulose molecules cause it to be water-insoluble and with a stable configuration (Bobleter, 1994). Microfibrils is the group of cellulose chains (20-300) and bunched together to form cellulose fibres (Agbor et al., 2011). The cellulose consists of a crystalline (organized) structure and an amorphous, less well-organized part (Hendriks and Zeeman, 2009). Cellulase favours the amorphous part rather than the crystalline portion for hydrolysis, therefore cellulose with a larger crystalline part will be more resistant to enzymatic attack (Taherzadeh and Karimi, 2008).

Hemicellulose

The monomers making up hemicellulose, which differ from cellulose, are heterogeneous polymers of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose), and sugar acids (Saha, 2003). In agricultural by-products such as straw and grass, hemicellulose mainly consists of xylan, while in softwood it mainly consists of glucomannan (Agbor et al., 2011). Hemicellulose is highly branched and amorphous, therefore hemicellulose is easier to hydrolyse than cellulose (Lee et al., 2007). It is also a physical protector of cellulose, hence removal of hemicellulose by pre-treatment can increase the contact area of cellulose to enzymes and subsequently improve the hydrolysis rate (Taherzadeh and Karimi, 2008).

Lignin

Lignin is an amorphous heteropolymer constructed of three phenyl propane units (p-coumaryl, coniferyl and sinapyl alcohol) interlinked by different types of linkages (Hendriks and Zeeman, 2009). Lignin is known as the 'cement' which binds cellulose and hemicellulose together; thus delignification processes of lignocellulosic organic substances can increase the enzymatic hydrolysis (Taherzadeh and Karimi, 2008)



A. Lignocellulosic substance

B. Cellulose microfibril

C. Sugar molecules

Fig. 2. Representation of a lignocellulosic material (adapted from Ritter, 2008).

2. Optimisation of methane production from livestock manure in the AD process

The methane production in terms of volatile solids (VS) of manure is approximately 290 L kg^{-1} VS for pig manure and 210 L kg^{-1} VS for cattle manure (Burton and Turner, 2003). Since the VS concentration of manure is very low, approximately 5-7% for pig manure and 7-9% for DCM (Angelidaki and Ellegaard, 2003), methane production from these substrates per substrate volume is low. This causes the low economic performance of AD-treatment of animal manure (Møller et al., 2007). The major problems with the utilisation of manure in the AD process are a high water content (Hamelin et al., 2011) and low biodegradability of animal manure due to a high biofibre content that mainly consists of lignocellulosic material (Nielsen et al., 2004). The biodegradability of manure is about 32%, 69% and 52% for DCM, pig manure in the fattening growth stage and sow manure, respectively (Møller et al., 2004). The presence of biofibres, a slowly degradable part of organic substance in livestock manure, impeded the rate of hydrolysis of the AD processing of animal manure (González-Fernández et al., 2008).

There are several factors that contribute to the limitation of enzymatic hydrolysis of the recalcitrant biomass such as crystallinity and degree of polymerisation of cellulose, available surface area/porosity, presence of lignin, protection of cellulose by hemicellulose and fibre strength (Mosier et al., 2005). Therefore, an effective pre-treatment method should increase the surface area, which improves the accessibility of the substrate to enzymes, minimises the loss of substrate and formation of inhibitors and should lower costs (Bruni, 2010). Some pre-treatment methods have been evaluated and developed including physical methods, chemical and physicochemical methods, biological methods and combinations of some pre-treatment method (Taherzadeh and Karimi, 2008; Hendriks and Zeeman, 2009; Agbor et al., 2011). Two pre-treatment methods – biological and thermal pre-treatment of animal manure prior to use as a substrate in AD – were evaluated in this PhD study.

2.1 Biological pre-treatment

Polymeric organic compounds in the organic matter substrate for AD such as proteins, carbohydrates and lipids cannot be taken up by the cells (Mshandete et al., 2005). Therefore, these organic compounds should be broken down to simpler organic structures to facilitate transport through the cell membrane. This process is normally facilitated by enzymes that are excreted by microorganisms in the digester. In the case of lignocellulosic material, the biodegradation process is facilitated by cellulases and hemicellulases (Parawira, 2012). During the PhD study, an enzyme mixture (ME) was added to DCM and used as substrate in AD using continuously fed digesters.

The three ME addition experiments comprised:

1) ME addition to thermophilic digesters (50°C): ME addition to DCM with immediate feeding to the digester and ME addition to DCM in an enzymatic pre-treatment step.
2) ME addition to DCM with immediate feeding to mesophilic (35°C) digesters.
3) ME addition to solid-fraction DCM followed by incubation at 35°C for 20 h prior to mix with liquid-fraction DCM and feeding to thermophilic digesters. Inactivated ME –

by autoclaving it at 121°C for 30 minutes (Yunqin et al., 2010) – was added to control digesters.

Summary of results and discussion

- There was no significant effect on methane production from DCM following ME addition to DCM with immediate feeding either in thermophilic or mesophilic digesters. This was attributed to: microorganisms in the digester degrading the ME since the substrate was fed into the digester immediately after mix with ME (Brule et al., 2007), and extracellular enzymes produced by microorganisms already present in the digester were sufficient to facilitate the hydrolysis of the organic compounds in DCM (Romano et al., 2009). Thus this was not a limiting factor for the hydrolysis rate in AD (**Paper 1**).
- Addition of ME to DCM followed by incubation at 50°C for three days gave a significant (p<0.05) increase in the methane yield (approximately 4.5%) compared with the control digester. This digester operated at the same hydraulic retention time (HRT) as the control digester. Methane production was also detected during the incubation period, and the total sum of methane yield of pre-treatment and digestion was found to be 8.33% higher than in the control. However, since the system had an overall longer HRT than the control, a further experiment to confirm a positive effect of ME addition using the similar process condition is needed (**Paper 1**).
- Addition of ME to solid fractions of DCM followed by incubation at 35°C for 20 h also gave positive effect (p≤0.05) on methane yield of a mixed substrate (30% liquid-fraction DCM and 70% enzyme-treated solid-fraction DCM) compared to the control digester. However, the high cost of enzyme application compared to the extra methane yield of DCM gained due to ME application (approximately 4.2% in this experiment) may still be the limiting factor for enzyme application in full-scale biogas plant, even though some research and genetic engineering to produce low-cost enzymes are addressing this issue (Parawira, 2012).
- **Paper 1** did not evaluate the individual organic matter as a target of ME addition, but the 20% increase in total VFA concentration of enzyme-treated solid-fraction DCM can be an indicator of the role of ME in the hydrolysis process of cellulase activity in the cellulose component of solid-fraction DCM.

- The results of **Paper 1** indicate that in order to increase methane yield of DCM through the AD process, ME should be added in the enzymatic pre-treatment step prior to its use as a substrate in AD.

2.2 Thermal pre-treatment

Of the pre-treatment processes, thermal pre-treatment appears to have a positive effect on the energy balance (Hendriks and Zeeman, 2009). In this pre-treatment, substrate is heated and the composition of the hemicellulose backbone and the branching groups determines the effectiveness of the treatment (Hendriks and Zeeman, 2009). During the PhD study, the thermal pre-treatment was conducted either at low temperature or high temperature. Low-temperature thermal pre-treatment was performed using a water bath in which the sample was placed in a 0.5-L sealed glass bottle, followed by cooling down the sample in a room-temperature water bath (**Paper 2**). High-temperature pre-treatments were conducted in a bench-scale high temperature and pressure reactor (Parr instrument company, USA, model Parr 4524). The main parts of this thermal pre-treatment instrument consist of a 2 L sealed stainless steel reactor, mechanical stirrer and an external electric coil heater. During the thermal pre-treatment process the reactor was completely sealed. After thermal pre-treatment, the reactor was cooled to about 35°C using a water bath (Raju et al., 2012).

Low-temperature pre-treatment

Paper 2 investigated the effect of low-temperature thermal pre-treatment on the methane yield of raw pig manure and solid-fraction pig manure in batch digestion. Application of the low-temperature thermal pre-treatment method in AD is an interesting pre-treatment method since the energy requirement during pre-treatment can be fulfilled by using surplus heat from the CHP plant that is often associated with AD. Therefore this energy source is cheap and an often wasted heat fraction from CHP put to good use (Menardo et al., 2011).

A batch assay experiment to determine the effect of low-temperature thermal pre-treatment on methane yield of pig manure fractions was conducted with the method developed by Møller et al. (2004). Four different thermal treatments (65°C to 80°C with 5°C intervals) were applied for 20 h to both raw and solid-fraction pig manure.

Summary of results and discussion

- Low-temperature thermal pre-treatment gave a slight increase in pH of pre-treated samples compared to untreated samples (**Paper 2**). Total VFA in pre-treated samples also increased significantly compared to control, dominated by acetic acid and butyric acid. Acetic acid and butyric acid in pre-treated pig manure (65°C) increased by 65% and 63%, respectively, compared to the control, while for the solid-fraction pig manure (80°C) the increase was 63% and 126% compared to the control. An increased pH value in the pre-treated sample may be caused by the solubilisation of macromolecules (Carrère et al., 2009) or formation of primary substances such as ammonia nitrogen (Bonmati et al., 2001), while an increase in total VFA in the pre-treated samples may be caused by autohydrolysis or fermentative microorganism activity since low-temperature thermal pre-treatment was conducted for 20 h (**Paper 2**).
- There was a significant improvement in the methane production from pig manure within the range 9.5% to 26.4% at 11 d incubation, but at the end of experiment (90 d) a significant improvement in methane production of pig manure was only seen at the 65°C pre-treatment. This result suggests that low-temperature thermal pre-treatment can increase the reaction rate but has relatively little effect on overall yield at infinite HRT, as represented by B_0 (**Paper 2**).
- A large improvement in methane production in the early stages of the batch digestion test would suggest an increased rate of reaction, which is of interest to a commercial continuous stirred-tank reactor (CSTR) biogas plant (**Paper 2**). Moreover, in Denmark a CSTR processing pig slurry without co-digestion with energy crops typically has an HRT of 12 d (Ward et al., 2010).
- Application of low-temperature thermal pre-treatment gave significant improvement in methane production from solid-fraction pig manure which was linear with increasing pre-treatment temperatures tested in this study.

High-temperature pre-treatment

Paper 3 evaluated the application of high-temperature thermal pre-treatment ranging from 100°C to 225°C at 25°C intervals for 15 min. on biochemical methane potential (BMP) of cattle manure, dewatered pig manure and chicken manure.

Summary of results and discussion

- High-temperature thermal pre-treatment of DCM at 175°C and 200°C for 15 min. gave a significant increase in methane production throughout the 90 d incubation period. At 27 d, the improvements of methane production were 13% and 21% at 175°C and 200°C, respectively.
- For pig manure, the methane production was increased at all temperatures over the 125°C to 200°C range with the largest improvement of 29% at 200°C at 27 d compared to untreated samples.
- The significant methane production of pre-treated samples compared to untreated samples in cattle manure and in dewatered pig manure indicates a change in the structure of the lignocellulosic material in the substrate, giving easier access to microbial enzymes (Bruni et al., 2010).
- There was no positive effect of high-temperature thermal pre-treatment on methane production from chicken manure. Even at 225°C thermal pre-treatment the methane production decreased by 18% compared to the control. This lack of a positive effect may be because of the high biodegradability of chicken manure, since there was no bedding material in the sample, thus providing limited potential for improving the methane production in the pre-treated sample (**Paper 3**).

The result of energy calculation showed that thermal pre-treatment in both low- and high-temperature thermal pre-treatment is a worthwhile method of increasing methane production of livestock manure only when there is thermal energy available that can be utilised in the thermal pre-treatment process (**Paper 2, Paper 3**).

2.3 Co-digestion

Another method to improve methane production of livestock manure on a fresh weight substrate basis is by increasing the VS concentration of manure by substitution some of the manure with other substrates that have a higher VS concentration and methane potential. This strategy is known as co-digestion. The definition of anaerobic co-digestion is treatment that combines different types of waste as substrate in AD with the main aim of improving methane production (Cuetos et al., 2011). This strategy can increase methane production on a fresh substrate weight basis by balancing the nutrient content of the substrate and reducing the negative effects of inhibitor compounds of substrate in the AD process (Cuetos et al., 2011).

The economic balance of AD showed that to be economically effective the substrate in AD should produce a methane yield of more than 20 m^3 CH_4 t^{-1} biomass (Angelidaki and Ellegaard, 2003). For manure the methane yield ranges from 10 to 20 m^3 CH_4 t^{-1}, while from industrial organic by-products it varies from 30 to 500 m^3 CH_4 t^{-1}. Besides increasing methane production of the substrate, the addition of by-products can also stabilise the AD process if added in a controlled manner (Angelidaki and Ellegard, 2003). Therefore, co-digestion of manure and organic industrial by-products seems an attractive method of making biogas plants economically viable. However, in Denmark the availability of high strength organic by-products is limited compared to the amount of manure (Hamelin et al., 2011) and the high prices of this biomass have made it difficult for AD co-digestion of these substrates to be economically attractive (Ward et al., 2010). Therefore, Hamelin et al. (2011) proposed strategies to alleviate this constraint: 1) an external carbon source in the form of energy crops as a co-substrate , 2) to design animal housing systems that separate urine and faeces and produce manure with a higher VS content, 3) to apply solid-liquid separation and use solid manure fractions as co-substrate and 4) to use a bigger digester with longer substrate retention time to compensate for low methane yield of animal manure. In addition, in order to increase volumetric methane yield of manure Møller et al. (2004) suggested the utilisation of straw as bedding material since straw has higher methane yield per unit fresh weight and a higher VS content than manure and solid-fraction animal manure.

Co-digestion experiment during PhD study

Manure separation into solid and liquid fractions was originally developed in order to alleviate the problem specific to livestock production of a surplus of nutrients from manure in relation to crop requirement. The surplus nutrients in animal manure can be transported in the form of a solid nutrient-rich fraction to farms that need to import nutrients (Møller et al., 2000). However, since this organic matter has a high methane potential per unit fresh weight (Hjorth et al., 2010), it can alternatively be used as co-substrate with raw livestock manure in the AD process. Furthermore, using solid-fraction animal manure instead of energy crops for co-digestion in AD can avoid competition for arable land between energy crops and food production (Searchinger et al., 2008).

Paper 4 investigated the co-digestion of raw non-acidified DCM and solid-fraction acidified DCM. Three different levels of substitution – 10%, 20% and 30% (ww^{-1}) – of raw DCM with solid-fraction acidified DCM were tested using four identical intermittent stirred tank reactors. The reference digester treated DCM. The experiment was conducted at a thermophilic (50°C) temperature. Treatment was started after the 21-d start-up period and continued for 56 d, corresponding to four times HRT. This experiment was followed by an evaluation of residual methane potential of the digested material by batch assay. The solid-fraction acidified DCM was obtained from a Danish farm using acidification technology developed by InFarm A/S, Aalborg, Denmark. Solid liquid manure separation was performed using the screw-press solid-liquid separation method with 0.5 mm screen size.

Summary of results and discussion

- Methane production per gram of substrate VS declined significantly as the concentration of solid-fraction acidified DCM rose (Fig. 3A). This phenomenon is expected since solid-fraction acidified DCM is the recalcitrant part of animal manure that has a lower biodegradability (**Paper 4**). This result is in line with the reduction in VS concentration as the concentration of solid-fraction acidified DCM increased.
- However, methane production in terms of digester volume for the substitution of DCM with 30% solid-fraction acidified DCM was about 50% higher than that for the reference digester (Fig. 3B). In addition, the residual methane potential of digested slurry from this digester was almost three times higher than that in digested slurry from the control. Thus, post-digestion of digested slurry with a high concentration of solid-fraction acidified DCM is needed in order to prevent methane emission from digested material and to achieve the full methane potential of the substrate (**Paper 4**).
- Total ammoniacal nitrogen (TAN) and sulphide concentrations were under the inhibition threshold as reported by Hashimoto (1986) and Parkin et al., (1990).
- All digesters ran satisfactorily as indicated by a stable methane production and low VFA concentration after approximately two times the HRT transition period; therefore solid-fraction acidified DCM is suitable as a co-substrate, at least up to 30% substitution (**Paper 4**).
- The mean H_2S content in biogas from the digester processing 30% solid-fraction acidified DCM was almost ten times higher (4100 ppm) than in the control. Therefore in the practical application of co-digestion of this substrate more attention should be paid to the maintenance of scrubber devices for removing H_2S from

biogas (**Paper 4**). As reported by Rasi et al. (2011), the H_2S concentration in the biogas for traditional boilers and internal combustion should low and not exceed 1000 ppm.

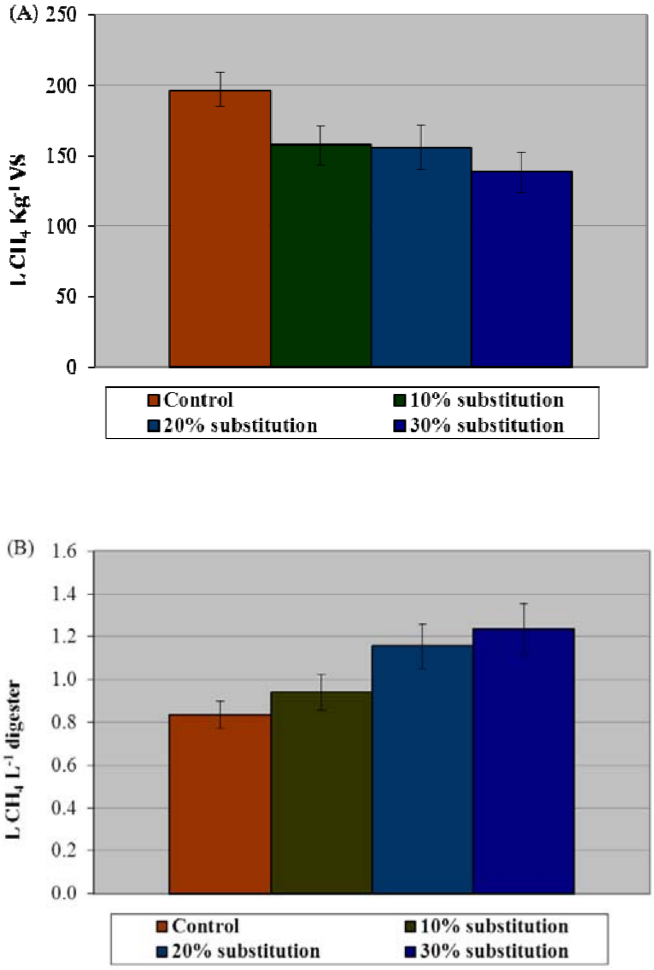


Fig. 3. Mean methane production of digester processing different concentrations of solid-fraction acidified DCM: A. L CH_4 Kg^{-1} VS, B. L CH_4 L^{-1} digester.

2.3.1 Methane production from animal manure fractions derived from acidified manure

In Denmark sulphuric acid is commonly used to acidify animal manure in order to reduce ammonia emissions. The number of farm using this method is expected to increase in the future; therefore information about methane production from acidified manure fractions is needed.

Evaluation of methane yield of manure fractions derived from acidified manure

Paper 5 determined the methane production of acidified livestock manure fractions produced with different solid-liquid manure separation techniques. The screw press (Fig. 4) is the most efficient method for producing solid fractions of manure with a high TS content (Hjorth et al., 2010), also in digested slurry (Menardo et al., 2011). Solid-fraction animal manure with a high TS content not only has a high methane potential per unit fresh weight but can also save on the volume and therefore the transport cost of this substrate. However, the pressure adjustment in terms of screen size and pressure in the press chamber (plate tension) may influence the VS transfer of raw manure to the solid fraction, which can influence the ultimate methane yield (*Bo*) (**Paper 5**). Therefore this paper evaluated the influence of screen size and pressure in the press chamber of screw press manure separation. In addition, this paper also evaluated the methane production of acidified pig manure fractions subjected to drum/rotating screen separation and the methane production of acidified manure fractions and non-acidified DCM fractions subjected to belt press separation, as affected by the acidification process.

The experiment was conducted as a batch digestion experiment with method described by Møller et al. (2004). Batch assay was maintained at mesophilic conditions (35°C) for 90 d. Substrates in this study were: 1) acidified sow manure fractions subjected to screw press separation with four different screen sizes and two different plate tensions, 2) acidified pig manure fractions subjected to drum/rotating screen manure separation and 3) acidified and non-acidified DCM fractions subjected to the belt press slurry separation method.

Fig. 4. Solid-liquid DCM separation process using screw press equipment.

Summary of results and discussion

- The ultimate methane yield of solid-fraction acidified sow manure was significantly (p<0.05) increased with a bigger screen size in screw press manure separation, but plate tensions to the cylinder mesh opening showed an opposite effect. A smaller screen size and a lower plate tension to the cylinder mesh opening may apply more pressure to the raw manure, and therefore smaller and easier degradable material passes into the liquid fraction (**Paper 5**). Moreover, the higher pressure to the raw acidified manure resulted in solid fractions with a higher VS content, but of a seemingly low biodegradability (**Paper 5**) (Table 1).

Table 1. Methane production manure fractions with different manure separation methods.

Manure	Fractions	Treatment	Separation method	Screen size (mm)	Plate tension (mm)	B_0 (L kg VS^{-1})
Sow	Raw	Acidified	-	-	-	177.8 ± 17.7
Sow	Liquid	Acidified	Screw press	0.75	48*	105.6 ± 21.3
Sow	Solid	Acidified	Screw press	0.25	48	265.5 ± 0.9
Sow	Solid	Acidified	Screw press	0.35	48	280.9 ± 4.4
Sow	Solid	Acidified	Screw press	0.50	48	281.3 ± 0.6
Sow	Solid	Acidified	Screw press	0.75	48	288.2 ± 2.7
Sow	Solid	Acidified	Screw press	0.35	25**	269.1 ± 8.6
Sow	Solid	Acidified	Screw press	0.50	25	273.1 ± 3.5
Pig	Raw	Acidified	-	-	-	397.8 ± 10.3
Pig	Liquid	Acidified	Drum screen	1	-	392.2 ± 2.4
Pig	Solid	Acidified	Drum screen	1	-	319.3 ± 12.3
Dairy cow	Raw	Acidified	-	-	-	256.6 ± 19.7
Dairy cow	Liquid	Acidified	Belt press	0.30	-	223.3 ± 15.3
Dairy cow	Solid	Acidified	Belt press	0.30	-	278.4 ± 13.1
Dairy cow	Raw	Non acidified	-	-	-	372.7 ± 15.9
Dairy cow	Liquid	Non acidified	Belt press	0.30	-	384.6 ± 26.7
Dairy cow	Solid	Non acidified	Belt press	0.30	-	289.2 ± 1.2

* : low pressure

** : high pressure

- The ultimate methane yield (*Bo*) from the solid-fraction acidified pig manure from drum screen separation was higher than from solid-fraction acidified sow manure using screw press separation (Table 1). This may be due to the dissimilar compositions of these substrates, plus the solid-fraction acidified pig manure that was drum-screen separated had a higher concentration of smaller and more easily degradable compounds compared to the solid fractions acidified sow manure (**Paper 5**).

- There was no negative effect of the acidification process on the *Bo* of solid-fraction DCM. The *Bo* of solid-fraction acidified DCM using the belt press was 3.3 times higher than that in raw non-acidified DCM in terms of fresh weight substrate; therefore solid-fraction acidified DCM is suitable as a co-substrate to increase methane production in terms of digester volume (**Paper 4**, **Paper 5**).

3. Inhibition of microorganism activity in the AD process

Methane production of livestock manure in terms of fresh weight substrate is low due to the high water content and low biodegradability of manure. Co-digestion of manure with organic matter that has a high methane potential is an alternative way of improving methane yield in the AD of manure. However, this organic material should be added in a controlled manner (Angelidaki and Ellegard, 2003), otherwise methane production in AD will be suboptimal due to inhibition of microorganism activity. Nielsen and Angelidaki (2008) reported that in Danish centralised biogas plants treating animal manure and industrial organic by-products, a high concentration of ammonia and long-chain fatty acids is in most cases expected to cause microbial inhibition. Such inhibition is usually indicated by a decrease in the steady-state rate of methane production and an accumulation of organic acids in the AD process (Kroeker et al., 1979). Some inhibitors and their inhibition thresholds are presented in Table 2.

Table 2. Inhibitors and inhibition thresholds in the AD process.

No.	Inhibitors	Inhibition threshold
1	Ammonia	- TAN : 2.5 g L^{-1} both mesophilic and thermophilic of AD processing cattle manure that not previously acclimated to high ammonia concentration; 4 g L^{-1} to previously acclimated with high ammonia concentration (Hashimoto, 1986). - Increasing FA : 0.55 to 0.65 g L-1 in thermophilic of AD cause decreasing methane yield by 25% of digester processing cattle manure (Angelidaki and Ahring, 1993).
2	LCFAs	Oleic acid and lauric acid, IC_{50} = 4.3 mM (Chen et al., 2008).
3	Sulphide	- 100 – 800 mg L^{-1} as dissolved sulphide or approximately 50 – 430 mg L^{-1} as undissociated H_2S (Parkin et al., 1990). - C/SO_4^{2-} = 1.6 corresponding to 1400 mg SO_4^{2-} L^{-1} (Siles et al., 2010).

Inhibition of microorganism activity in the AD process can be attributed to:

1) inadequate knowledge of the organic substrate composition
2) insufficient knowledge of the substrate degradation characteristics
3) inadequate supervision process, particularly with regard to VFA concentration
4) insufficient substrate storage causing improper mixing and less precision in dosing the different substrates (Nielsen and Angelidaki, 2008).

During the PhD study, the study on the inhibition of the AD process focused on ammonia inhibition and sulphide inhibition to evaluate the AD-processing of acidified manure, a relatively new method to reduce ammonia emission of livestock manure that uses sulphuric acid in the acidification process.

3.1 Ammonia inhibition

Ammonia is a biological degradation product of the nitrogenous content of the organic matter, mostly in the form of proteins and urea (Kayhanian, 1994). Ammonia is essential for microorganism growth, but if present in high concentrations in the substrate, it can cause inhibition in the AD process (Nielsen and Ahring, 2007). The TAN inhibition threshold can be seen in Table 2. The TAN value is a combination of free ammonia nitrogen (NH_3) and ionized ammonium nitrogen (NH_4^+) (Kayhanian, 1994. Free ammonia is known as the active component that causes ammonia inhibition since it is freely membrane-permeable (Siles et al., 2010). Angelidaki and Ahring (1994) found a poor performance of the AD processing of cattle manure under thermophilic conditions when free ammonia exceeded approximately 0.7 g L^{-1}. Whittmann et al. (1995) proposed change in intracellular pH, increase of maintenance energy requirement and inhibition of specific enzyme reaction as mechanisms of ammonia inhibition.

The ammonia inhibition experiment using urea as a source of ammonia was conducted with five different TAN concentrations. The different TAN and free ammonia (FA) concentrations in the digester were obtained by adding urea to the DCM to obtain target level of TAN and FA, and to subsequently maintain this concentration through experiment by daily urea additions. The experiment was performed using five identical continuously fed digesters maintained at 50°C for four times HRT followed by a recovery period in which no urea was added to the DCM for 26 d (**Paper 6**).

Summary of results and discussion

- The result showed a strong negative correlation between methane yield of DCM and TAN and FA concentrations ($Y = -21.798X + 145.06$, $R^2 = 0.98$ and $Y = -46.68X + 117.62$ $R^2 = 0.96$), respectively (**Paper 6**).
- Methane yield during statistical period (the last three weeks experiment or after more than 2.5 digester volume turnover), showed that the methane yield in the digester with TAN 2.93 g L^{-1} corresponding to FA 0.71 g L^{-1} was 23.6% lower than the methane yield in the control digester with a TAN of 2.15 g L^{-1}, corresponding to FA 0.48 g L^{-1} (**Paper 6**).
- Total VFA concentration the day after urea addition was fairly constant in all digesters, but then increased sharply and stabilised at an elevated level (**Paper 6**). Accumulation of acetic acid in the digester processing DCM with urea addition suggests that there was inhibition of methanogen activity while the accumulation of VFA indicated there was product inhibition of acetogenic microorganisms (**Paper 6**). Pind et al. (2003) reported that this phenomenon can occur when acetate is at elevated concentrations, a condition akin to the result of this experiment.
- Isobutyric acid and isovaleric acid accumulated during the experiment. Therefore they are useful indicators in ammonia inhibition. Nakakubo et al. (2008) suggested that isobutyric acid, butyric acid and isovaleric acid could be used as process indicators during ammonia inhibition. After a period of ammonia inhibition, butyric and valeric acid were shown to gradually decrease, suggesting that there was conversion of these organic acids to other acids. The former was converted to isobutyric acid and the latter to propionic acid. A conversion pathway of individual VFAs is presented in Fig. 5 (Tholozan et al., 1988; Wang et al., 1999; Pind et al., 2003 and Nielsen and Ahring, 2007).
- During the recovery period (no urea addition), methane yield in the digester that got the lowest ammonia inhibition was similar to that in the control digester starting from the 23th day after urea cessation (**Paper 6**).

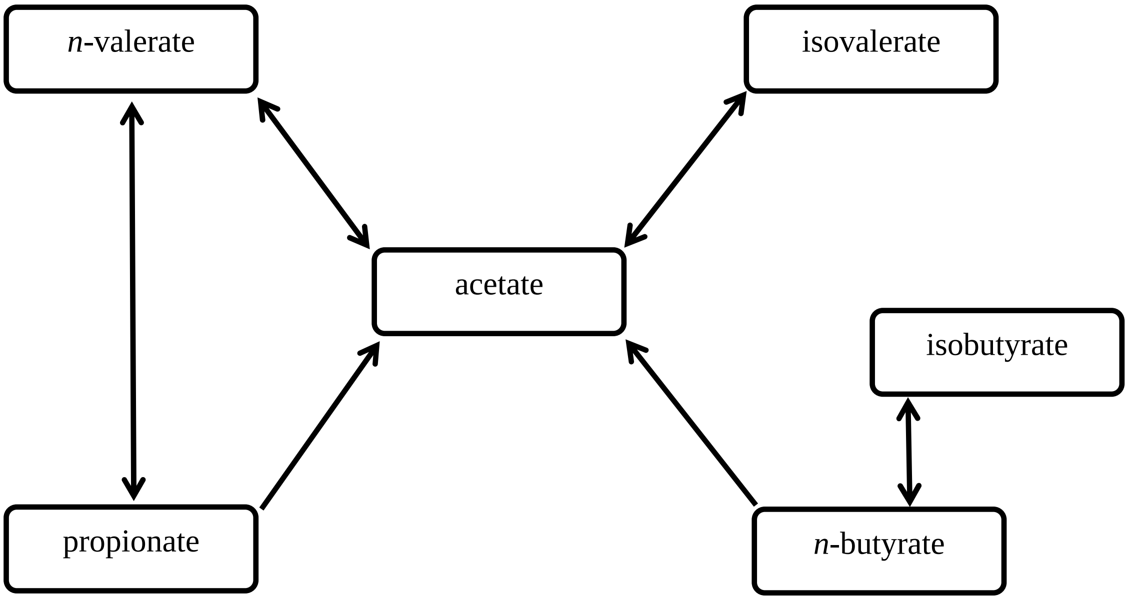


Fig. 5. Conversion and degradation pathway of individual VFAs (Tholozan et al., 1988; Wang et al., 1999; Pind et al., 2003 and Nielsen and Ahring, 2007).

- There are three important parameters that determine FA concentration – these are TAN concentration, pH value and temperature (Hansen et al., 1998). Therefore in the anaerobic digestion of substrates with a high TAN concentration a longer HRT or a lower temperature should be chosen to achieve the optimal methane yield (**Paper 6**). In order to prevent ammonia inhibition, Kayhainan (1994) suggested that the C/N ratio of the substrate should be kept between 22-35 and the pH of the operating digester be controlled. Chen et al. (2008) proposed a method to counteract ammonia inhibition which involved increasing the biomass retention time in the CSTR system by switching off the stirrer half an hour before and after substrate addition, immobilizing microorganisms by inert material (clay, activated carbon, zeolite) (Angelidaki et al., 1990), while Kabdasli et al. (2000) successfully demonstrated the removal of ammonia from the substrate using a chemical precipitation method with magnesium ammonium phosphate and ammonia-stripping by aeration using a diffuser, and volatilisation using stirring.

3.2 Sulphide inhibition

The method currently practised for acidifying animal manure using sulphuric acid to reduce ammonia emission has been developed in Denmark. This method can successfully decrease ammonia emission from pig houses by 70% (Kai et al., 2008). However, a high sulphur concentration in acidified manure may cause inhibition of microorganisms in the AD process. The presence of sulphate in acidified manure can stimulate the growth of sulphate-reducing bacteria (SRB) which leads to competition of SRB with methanogens for substrate (Siles et al., 2010). In the AD process, sulphate is reduced to sulphide by SRB (Gerardi, 2003). H_2S as the main part of dissolved sulphide in the liquid phase can easily penetrate the cell membrane and denature native protein within the cytoplasm, producing sulphide and disulphide cross-links between polypeptide chains (Siles et al., 2010). The sulphide inhibition threshold can be seen in Table 2.

During the PhD study two experiments that had an impact on sulphide inhibition were performed. **Paper 4 and paper 5** evaluated methane production of acidified manure in batch digestion.

Summary of results and discussion

- Batch digestion treating both raw and liquid-fraction acidified manure showed sulphide inhibition, but it seems there was no sulphide inhibition of AD when processing solid-fraction acidified manure. Methane production of solid-fraction acidified manure is much higher than that in raw non-acidified manure, therefore solid-fraction acidified manure is a suitable biomass for co-digestion to increase methane yield in terms of digester volume (**Paper 4 and Paper 5**).
- Sulphur inputs from the substrate to the digester in the raw, liquid and solid-fraction acidified sow manure were 240 mg, 480 mg and 50 mg, respectively (**Paper 5**). Therefore, if the sulphur concentration in the inoculum can be ignored, the sulphur concentrations in the digester treating raw, liquid and solid-fraction acidified sow manure were 1059, 1596, 306 mg L^{-1}, respectively. Siles et al. (2010) evaluated sulphate inhibition using a stirred tank reactor processing a glucose solution supplemented with Na_2SO_4 and found that the ratio C/SO_4^{2-} inhibition threshold was 1.6 corresponding to 1400 mg SO_4^{2-} L^{-1} (Table 2). Moset et al. (2012) found that there was 18% methane reduction in AD-processing of a mixed substrate of 20% acidified pig manure and 80% non-acidified pig manure (ww^{-1}). Moreover, the sulphate concentration in this substrate was 730 mg L^{-1}.
- The ultimate methane yield of acidified DCM was significantly lower (p<0.05) than that from non-acidified DCM. From calculation data from **paper 5** and summarised in Table 1, the ultimate methane yield of acidified DCM was 45% lower than that of

non-acidified DCM. Therefore this result supports the hypothesis that acidified livestock manure can cause sulphide inhibition in the AD process (**Paper 5**).

- Chen et al. (2008) proposed methods to prevent and reduce sulphide inhibition including dilution of substrate and reducing the sulphide concentration in the substrate by sulphide removal (stripping, coagulation, oxidation, precipitation and partial oxidation).

4. Concluding remarks

Livestock manure management through the AD process is a favourable method for recovering energy and preventing methane emission from animal manure in the manure storage tank. Methane production of manure can be improved by pre-treatment prior to AD. However, the pre-treatment method tested during the PhD study was not the ideal method of increasing methane yield of animal manure. For instance, the high cost of mixed enzyme addition is still a limitation factor in full-scale biogas plants and thermal pre-treatment is a worthwhile method of increasing methane yield of manure only when surplus energy for the pre-treatment process is available. Another method to increase methane production of animal manure is by co-digestion of manure with another substrate of a higher methane potential and VS concentration than manure. For example, the co-digestion of DCM with solid-fraction acidified DCM appears a promising method. During the PhD study, it was also demonstrated that there was no negative effect of the acidification process on the methane yield of solid-fraction DCM, but there was sulphide inhibition of the anaerobic digestion of acidified manure. In order to prevent microorganism inhibition during the co-digestion process, substrate with high biogas potential should be carefully added to avoid suboptimal digestion conditions caused by the inhibition of microorganism activity. In the case of ammonia inhibition, total VFA concentration, isobutyric acid, isovaleric acid, TAN value and biogas production can be used as process indicators.

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6. Appendix

1. Sutaryo, S., Ward, A.J., Møller, H.B., 2012. The effect of mixed enzyme addition in anaerobic digestion on methane yield of dairy cattle manure. Resubmitted after revision to peer-review journal
2. Sutaryo, S., Ward, A.J., Møller, H.B., 2012. The effect of low temperature thermal pre-treatment on the methane yield of pig manure fractions. Manuscript draft.
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4. Sutaryo, S., Ward, A.J., Møller, H.B., 2012. Thermophilic anaerobic co-digestion of separated solids from acidified dairy cow manure. Bioresource Technology, 114: 195–200.
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6. Sutaryo, S., Ward, A.J., Møller, H.B., 2012. Ammonia inhibition in thermophilic anaerobic digestion of dairy cattle manure. Submitted to peer-review journal.

The papers are not included in the www-version, but can be obtained from the Library at Department of Engineering, Faculty of Science and Technology
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