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Biogas potential from cow manure – Influence of diet

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Preface

This report is a master thesis work which corresponds to 30 credits at E-level. The project was carried out in partial fulfilment of the requirements for the degree of Master of Science (M.Sc.) for the program Sustainable Technology at the Department of Industrial Ecology, KTH, Stockholm. The work was conducted at the Department of Microbiology, SLU, Swedish University of Agricultural Sciences, Uppsala.

Assoc. Prof. Anna Schnürer and Assoc. Prof. Gunnar Börjesson, Department of Microbiology, SLU, were project supervisors and Prof. Håkan Jönsson, Department of Energy and Technology, SLU was examiner.

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The fear of the Lord is the beginning of knowledge, but fools despise wisdom and instruction. Proverbs 1:7

Abstract

Cow manure is an excellent substrate for biogas production in anaerobic digesters though the gas yield from a single substrate is not high. However, mixing cow manure with other kind of waste materials in co-digestion can optimize the production of biogas. In this thesis work the biogas potential from cow manure as a single substrate was investigated. The questions to be resolved were if 1) the biogas potential was affected by the feeding strategy of the cows and 2) there is correlation between manure methane potential and enteric methane emission from the same feed. Six fistulated Swedish red breed dairy cows were offered three different types of feed mixtures A (high starch and low fibre), B (medium starch and fibre), and C (low starch and high fibre) during three experimental periods (1, 2, and 3). The complete diet was composed of forage and concentrate. The forage was high quality grass silage, and the concentrate consisted of barley, oat, peas, and rapeseed cake. Each cow received only one type of feed mixture during each experiment period. During the last 5 sampling days of each experiment period, the cows manure was collected and frozen at -20 °C. A batch type reactor was then operated at 37 °C to investigate the methane potential of the manures. The result showed that enteric methane emission of the cows was weakly positively correlated with methane potential of their manure (R=0.2). A better fit was found between starch content in the cow diet and methane potential of the manure though it was not significant alone (P=0.19). The result of the present work was against the hypothesis “less enteric methane of the cow will give high gas potential of the manure.”

Keywords: anaerobic digestion, biogas production potential, methane, feed mixture, forage, concentrates, cow manure, enteric methane, batch experiment, multiple regression, correlation and ANOVA.

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1. Introduction

Today, the issue of global warming and climate change are strongly receiving public attention and have become a major environmental concern both at national and international level. The increasing concentration of atmospheric greenhouse gases as a result of culpable human activities represents the major cause for this problem. Human activities, which are responsible for the atmospheric concentration of greenhouse gas include; agricultural expansion (especially livestock husbandry, rice cultivation), industrial activities, fossil-fuel exploitation and use, and waste production and management (landfills and animal wastes) (Lassey, 2008). The most important Greenhouse gases from such activities include carbon dioxide, methane, nitrous oxide, and fluorinated gases such as sulfur hexafluoride and hydrofluorocarbon (US EPA, 2007). The atmospheric concentration of such gases importantly influences the earth's climate and cause global warming.

In the context of global warming, carbon dioxide gas currently gains more public attention than other greenhouse gases. However, it is important to consider also other gases. One of these is methane (CH_4) gas. Methane is produced under anaerobic conditions during degradation of organic materials by certain micro-organisms. The major biological sources of methane include natural wetlands, rice paddies, landfills, ruminants, termites, river beds, and lakes (Immig, 1996). Methane is an important greenhouse gas with the ability of global warming 25 times greater than that of carbon dioxide (IPCC, 2007). It is estimated that more than 60% of the global methane release is connected to human activities. Methane emission accounts for 16% of all global greenhouse gas emissions and the current value of global average atmospheric methane concentration is about 1720 ppbv (parts per billion by volume) which is more than double of the concentration during the pre-industrial period 800 ppbv (Mosier et al., 1998). Methane is therefore important greenhouse gas, which needs a serious consideration for it contributes to global warming.

With regard to source, agriculture appears to be the major contributor of atmospheric methane. Worldwide, 35% of greenhouse gas emission is from agriculture (IPCC, 1996). According to Mosier et al. (1998) as cited by Olesen et al. (2006) agriculture is a responsible sector for 50% of anthropogenic emission of methane. The major sources of agriculture methane are enteric fermentation and rice paddies. Methane from ruminant livestock (enteric fermentation) is the largest biogenic source within agriculture sector (EPA, 2009). Methane is a natural phenomenon in the ruminant rumen and they emit methane as part of their natural digestive processes (EPA, 2009). Rumen is the home of billions of microbes including bacteria, methanogens, protozoa, and fungi. These microbes break down feed to produce volatile fatty acids (VFAs), carbon dioxide, ammonia, and methane. The VFAs are used by the animals as energy source while the produced gases are removed by eructation (EPA, 2009; Iqbal et al., 2008). This way of methane formation by ruminant animals is known as enteric fermentation. Iqbal et al. (2008) and Grainger et al. (2007) reported that about 17-30% of the global methane production is from enteric fermentation. Globally, ruminant livestock produce approximately 80 million tonne of methane annually (EPA, 2009). Cattle can produce 250-500 litre of methane per day and animal (Johnson, 1995a; Johnson, 1995b). Enteric methane emission from dairy cows is twice as large as other cattle such as beef cows, calves, growing steers/heifers, and feedlot cattle (O'Mara, 2004).

Cattle also contribute to the atmospheric methane through their slurry/manure when anaerobically stored. Steed and Hashimoto (1994) as cited by Yamulki (2005) summarized that the total global methane production from manure account to approximately 35.2 million tonne per year, which is around 9% of the total biogenic production. Methane formation from the manure is produced via microbial degradation of soluble lipids, carbohydrates, organic acids, and proteins left in the manure (Yamulki, 2005).

Generally, ruminant livestock has been identified as the largest single source of anthropogenic methane through enteric fermentation and slurry emission (Iqbal et al., 2008). What does this methane release by ruminant animals then represent? Methane emission from ruminants is not only of environmental concern but also means a lot for the animal productivity. A number of previous studies have shown that enteric methane production by ruminant livestock also represents a significant loss of dietary energy and feed inefficiency of the animals. Typically cattle lose 2-15% of their ingested energy as eructated methane (Ginger et al., 2000). Translated to emissions this corresponds to 100 to 300 litre per day. Czerkawski (1986) gave a more tangible term regarding this:

“The energy contained in methane produced by about 12 cows daily would be sufficient to provide an average household with its domestic gas.” Thus, mitigating strategies against ruminant methane emission has three major benefits. Firstly, less livestock methane represents lower atmospheric concentration of greenhouse gas. The second benefit is to increase the income of farmers since less methane means increasing the efficiency of livestock production. A third important benefit comes when livestock manure is converted to biogas.

Many factors are involved in the amount of methane emission by ruminant animal: The main factors include; feed intake level, digestibility of feed, type of carbohydrate in the diet, supplementation of feed with lipid or ionophore, efficiency of feed uptake, type of feed, and animal’s digestive system or alteration of ruminal microflora (Jungbluth et al., 2001; US EPA, 2007; Johnson, 1995a; Johnson, 1995b; IPCC, 2001). Methane emission from cattle can be mitigated through manipulation of these factors.

Current research proposes various mitigation strategies to decrease the amount of methane released from ruminants. The current mitigation strategies can in general be categorized in to two major groups. The first strategy includes improvement of nutrient strategy in order to decrease methane emission and thus increase the animal productivity. The second strategy is to redirect the fermentation of the rumen towards less total methane production (Iqbal et al., 2008). Nutritional strategies include; high proportion of concentrate in the diet (Blaxter and Claperton, 1965; Johnson, 1995a; Johnson, 1995b; Lovett et al., 2003; Beauchemin and McGinn, 2005; Iqbal et al., 2008), forage type and quality (Iqbal et al., 2008) and supplementation of oil and oil seeds to the diet (Jordan et al., 2006). Some of the methods for the latter strategy include; defaunation (removal of protozoa from the rumen) through dietary manipulation, ionophores, synthetic chemicals (copper sulphate, calcium peroxide and detergent etc); and natural compounds like non-protein amino acids and vitamin A etc (Iqbal et al., 2008). However, many of the mitigating strategies are not feasible due to negative effect on livestock health, lack of long term effect, and high cost in spite of their positive effect on suppressing livestock methane emission (Iqbal et al., 2008). For example, high level of concentrate in the feed may result in animal health problem such as acidosis. Concentrate feed also incur high production costs that may limit its use in low cost system. Furthermore, completely defaunated animals may have digestion problems and the methane reduction effect can only be temporary.

It is therefore important to develop sufficient and sustainable mitigation strategies for ruminant methane emission from enteric fermentation as well as manure storage without having negative consequences for the livestock producer (economically), the livestock itself (health), or the environment. According to Iqbal et al. (2008) integrated research that considers animal, plant, microbe, and nutrient level strategies may give a promising long term reduction of livestock methane emission. Genetic selection of animals, vaccination at the microbe level, improvement of plants for livestock feed with further research are some the suggested methane suppressing strategies without negative effect on animal or environment.

Do nutritional strategies against enteric methane affect livestock methane emission from the manure? Strategies to suppress ruminants' enteric methane may be followed by an increase in methane production from their manure. There are few reports regarding this question. Hindrichsen et al. (2005) showed an increase in slurry methanogenesis due to supplementation of concentrate in the cows' diet at the same time as the enteric methane emission was reduced. Külling et al. (2002) also reported a certain tendency for a compensatory increase in methane production from cattle manure in cases of reduced enteric methane release. The increase in methane potential of the slurry is likely caused by the microbial degradation of organic matter left in the manure (Yamulki, 2005). The realistic solution to reduce methane emission from manure storage will be using anaerobic digestion technology in a controlled biogas plant so that methane can be collected as biogas (Külling et al., 2002). In this way atmospheric methane emission from the manure storage could dramatically be decreased. Biogas generated can be utilized for various energy services, such as heat, combined heat, and power. In addition, the biogas can be used as vehicle fuel, after removal of carbon dioxide and hydrogen sulphide in an upgrading system (IAE, 2005). Cows manure for the production of biogas is therefore beneficial both for the environment and the economy; the former when the gas is used as a vehicle fuel replacing petrol/diesel as well as reducing direct emissions from the manure storage (Lantz et al., 2007).

The present work is a continuation of the study investigating the effect of diet composition on dairy cows’ enteric methane emission. The objective of the present work was to evaluate the biogas production potential from the cow manure. The second objective was to correlate the methane production potential of the manure with the feeding mixtures of the cows, milk yield of the cow, and enteric methane emission of the same cows. The hypothesis of the current work was that “low methane emissions of the cow will give high gas potential of the manure.”

2. Background

Biogas is a clean, environmental friendly and renewable form of energy generated when micro-organisms degrade organic materials in an oxygen free environment. The formation of biogas can occur either in natural environment or controlled conditions in constructed biogas plants, so called anaerobic degradation (AD). Swamps, marshes, river beds, rumen of herbivore animal are some of the areas where biogas is formed naturally (Marchaim, 1992). The same microbial activities are achieved in both natural and controlled conditions. The feedstock for biogas production in constructed plants is more or less any organic fractions from household organic waste to dedicated energy crops like maize (Lantz et al., 2007). The potential feedstock for the production of biogas include; municipal solid waste, industrial organic waste, garden waste, agricultural waste (manure and crop residue), energy crops, cellulose rich biomass, algae and seaweed (water based), by-products of ethanol and bio diesel production (Lantz et al., 2007: Demetriades, 2008; Börjesson and Mattiasson, 2007; SGC, 2007).

2.1 Biogas in Sweden

When it comes to renewable energy production and utilization, Sweden has a remarkable position in Europe because of its vast natural resources and water courses. Around 29% of the primary energy supply in the country is of renewable origin (SEA, 2005). Biogas technology is one of the alternative means regarding renewable energy. The biogas industry in Sweden started in 1950-1970's aiming initially at reduction of the volume of sewage sludge (Nordberg, 2006). There are approximately 233 biogas facilities in Sweden with a total biogas production of 1.3 TWh /year (SEA, 2005). Many of these biogas facilities (139) are located at municipal sewage treatment plants (WWTP) (Lantz et al., 2007; SEA, 2005) and there almost 43% of the Swedish biogas production takes place (SGC, 2007). The theoretical potential of biogas production in Sweden is however around 14-17 TWh/ year, which is more than 10 times of the present production (Lantz et al., 2007). Agriculture related biomass represents the largest part of this potential. Presently almost 80% of the potential from the agriculture sector is not used (Lantz et al., 2007). The potential feedstocks from agriculture sector in Sweden include; cultivated crops, crop residue, and animal manure (Lantz et al., 2007). In Sweden, the estimate of animal manure represents a biogas potential of $2.5*10^{-27}$ TWh/year (Lantz et al., 2007). The production of biogas can take place at large scale centralized plants where different feed stocks materials are digested and also at small farm based plants, which mainly digest agricultural feed stocks (Lantz et al., 2007). Denmark was the first country in Europe that tested the idea of centralized anaerobic digestion and 22 co-digesters are presently in function (Angelidaki and Ellegaard, 2003). All digesters use animal manure as the main substrates together with other organic wastes such as source sorted household waste, industrial organic waste, and sewage sludge. Denmark is followed by Sweden, which has around 13 co-digestion plants. In these plants, animal manure is co-digested with other kind of wastes such as industrial organic waste and household waste (SGC, 2007).

Cow manure is an excellent substrate for the production of biogas when co-digested with other kinds of waste materials such as organic industrial waste, household waste and sewage sludge even though its methane yield as a single substrate is low (IEA, 2005) . The reasons for its low methane yield as a single substrate are its high water content and high fraction of fiber (Angelidaki and Ellegaard, 2003). However, cow manure serves as an excellent "carrier" substrate during the mixed digestion of wastes and allows anaerobic digestion of concentrated industrial waste, which would be difficult to treat separately (Angelidaki and Ellegaard, 2003). This suitability of manure to be used as "carrier" substrate is because of; its high water content, which act as solvent for dry waste materials, its high buffering capacity that regulate the optimum pH in the reactor, and the high level of nutrient, a requirement for optimal bacteria growth (Angelidaki and Ellegaard, 2003). Anaerobic digestion of cow manure gives approximately 63% of biogas. The advantages of co-digesting animal manure together with other kinds of waste materials have been reported in different research studies. Angelidaki and Ellegaard (2003) reported the increase in biogas yield due to co-digestion of cattle manure together with waste materials in anaerobic digestion process. Today, co-digestion of different substrate has become a standard technology in most of European countries also in Asia and USA.

Biogas can be utilized in various energy services. For example in Sweden biogas is primarily used for heating purposes followed by use as vehicle fuel and electricity/power generation (Svensson et al., 2008). Biogas has been used as a vehicle fuel in Sweden since the beginning of the 90´s (SGC, 2007). Sweden in general has taken the lead in the production and utilization of biogas as a vehicle fuel. In 2006, almost 24 million normal cubic meters of biogas were used as a vehicle fuel, which is equivalent to 26 million litres of petrol (SGC, 2007) and more biogas was sold as a vehicle fuel than natural gas in the same year for the first time (SGC, 2007). Furthermore, 30 million litre petrol equivalents of biogas were produced in the country in the year 2008 (Avfall Sverige, 2009). In developing countries, however, biogas is mainly used for cooking and lighting purpose. In China for example, approximately 8 million small farm scale biogas digesters are generating gas for cooking and lighting (SGC, 2007). In Ethiopia, there are several farm scale biogas plants where animal manure is used as the main substrate and the gas produced is used for cooking and lighting at the household level.

Anaerobic digestions of organic materials such as cow manure also produce a digestate, which is an excellent agriculture fertilizer. Around 390,000 tons of digestate was produced in the year 2008 in Sweden, of which 96% was used in farming (Avfall Sverige, 2009). The remaining 4% was either dehydrated and/or processed by after-composting.

2.2 Process and mechanism of bio-methanation

In anaerobic digestion, different groups of micro-organisms work in sequence at four different stages. Figure 1 below illustrates the four main stages of anaerobic digestion of organic material. These are hydrolysis, acidogenesis, acetogenesis and methanogenesis (Jarvis, 2004).

2.2.1 Stage 1-Hydrolysis

This step involves the enzyme-mediated alteration of insoluble organic compounds with high molecular mass, i.e. proteins, fats, lipids, and carbohydrate etc, into soluble organic components such as amino acids, fatty acids, monosaccharide, and other simple organic compounds (Yadvika et al., 2004). The insoluble large molecules consist of many small molecules joined together by chemical bonds and thus need to be hydrolyzed before entering the bacterial cell. The hydrolysis step is carried out by several different anaerobic and facultative bacteria (Yadvika et al., 2004).

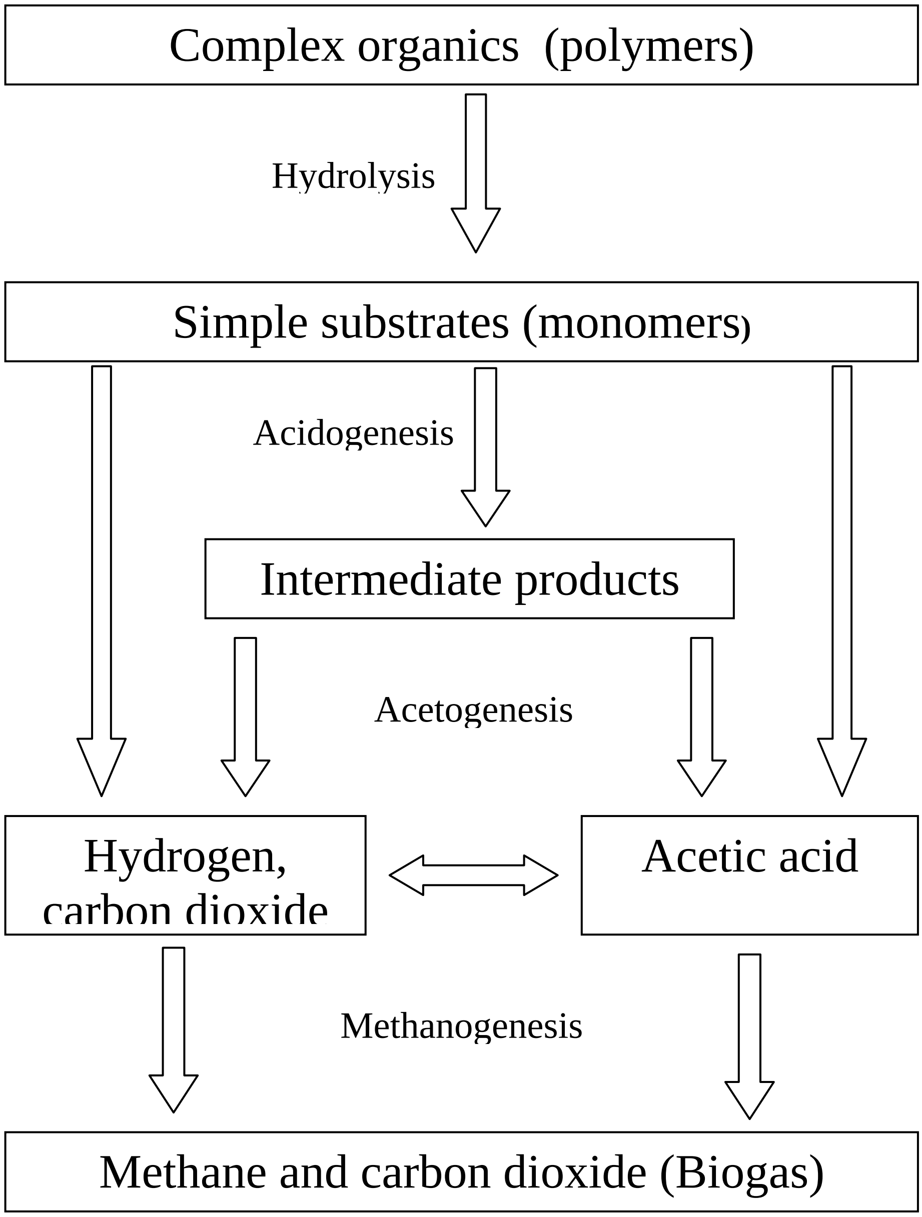

Figure 1. The four main stages in anaerobic fermentation of organic material (Jarvis, 2004)

2.2.2 Stage 2-Acidogenesis

In this stage, soluble compounds produced in the first stage are further degraded by a diversity of different facultative anaerobes through different fermentation processes. The fermentation results in the production of carbon dioxide, hydrogen gas, organic acids, alcohols, some organic-nitrogen compounds and some organic-sulphur compounds etc (Gerardi, 2003). The most important acid here is acetic acid as it is the principal organic acid used as a substrate material for the methane-forming organisms.

2.2.3 Stage 3-Acetogenesis

In this stage, the other intermediate products and acids than acetate that were formed in the fermentation are further converted to acetic acid as well as carbon-dioxide and hydrogen by different anaerobic oxidation reaction involving so called acetogenic bacteria (Jarvis, 2004).

2.2.4 Stage 4-Methanogenesis

During this stage, methanogenic micro-organisms convert acetic acid, hydrogen and carbon dioxide to methane and carbon dioxide i.e. biogas. The remaining compounds like alcohols, organic-nitrogen compounds which methanogens can not degrade will be accumulated in the digestate (Gerardi, 2003).

2.3 Operational parameters for biogas production

The production of biogas is factored by many operational parameters. Some parameters that affect the production of biogas include temperature, pH, pre-treatment, particle size, agitation, rate of organic load, retention time etc. Any rapid change in these parameters can adversely affect the production of biogas (Yadvika et al., 2004).

2.3.1 Temperature

The biogas production process is highly influenced by the temperature inside the digester. In nature the formation of methane occurs at different range of temperatures; psychrophlic (<30 °C), mesophilic (30–40 °C), and thermophilic (50–60 °C) (Yadvika et al., 2004). However, mesophilic and thermophilic temperature ranges are more favourable for anaerobes to be active (Yadvika et al., 2004). In general, high temperature give a higher methane production rate and allows higher loading rates, thus decreasing the reactor volume needed for a specific material. Anaerobic digestion at thermophilic temperature also gives a better sanitation, i.e. killing of pathogens. However, thermophilic processes are more sensitive to high levels of ammonia, released from protein rich materials (Yadvika et al., 2004). Thermophilic processes are also more costly to heat compared to mesophilic processes (Demetriades, 2008). The digestion period in a mesophilic process usually needs comparably longer time, commonly between 20 and 30 days. In thermophilic temperature, however, gas can be produced in much less time comparing to mesophilic temperature. Digesters which process agricultural waste normally operate at mesophilic temperatures. Today, approximately half of the Swedish co-digestion plants run at thermophlic temperature (Avfall Sverige, 2009).

2.3.2 pH

The substrate’s acidity is measured by pH, which is an important parameter affecting the growth of microbes during anaerobic digestion (Yadvika et al., 2004). For optimal performance of the microbes, the pH with in the digester should be kept in the range of 6.8 - 8.0. The pH value below or above this interval may restrain the process in the reactor since micro-organisms and their enzymes are sensitive to pH deviation (Yadvika et al., 2004). There are also situations in anaerobic fermentation which can highly affect the pH in the digester. These include high amounts of volatile fatty acids, acetic acid, and carbon dioxide produced by the microbes and ammonia. These factors can have an impact on the pH in the reactor and might inhibit the activity of the microbes (Nijaguna, 2002; Yadvika et al., 2004).

2.3.3 Carbon: Nitrogen (C/N) ratio

For efficient biogas plant operation, the C/N ratio of the input substrate should be kept within the desired range since the nutrient composition has an impact on the optimal growth and activity of micro organisms (Nijaguna, 2002). Carbon and nitrogen are the main nutrients for anaerobic bacteria. In anaerobic digestion, the carbon utilization of micro-organisms is 25-30 times higher than nitrogen. Thus, for optimum functioning microbes usually need 25-30:1 ratio of C to N with the largest part of the carbon being easily degradable. Any deviation from this ration gives a less efficient process. Co-digestion with different substrate materials can improve the biogas production since a single substrate can be limiting due to its nutrient content. Accordingly, waste materials with low C content can be mixed with other N rich substrates in order to reach the desired C: N ratio (Yadvika et al., 2004) and the optimum mix of feed stocks are necessary to get the optimum C/N ratio (Nijaguna, 2002). For this reason the importance of cow manure as a co-digest substrate with other waste materials such as organic industrial waste and household waste have been suggested by different studies in order to optimize the methane yield (IEA, 2005; Nijaguna, 2002). The C/N ratio of cow dung is around 16 – 25 (Nijaguna, 2002). Manure can also be co digested with different type of plant materials in order to increase the biogas production.

2.3.4 Particle size

The production of biogas is also affected by particle size of the substrate. Too big particle size is problematic for microbes to digest and it can also result in blockage in the digester. Small particle size gives a large surface area for substrate adsorption and thus allows the increased microbial activity followed by increase in the production of gas (Yadvika et al., 2004).

2.3.5 Water content

Water is the vital element for micro-organisms' life and their activity. The movement of bacteria and activity of extra cellular enzyme etc are highly determined by the water content in the digester (Nijaguna, 2002). Optimum moisture content has to be maintained in the digester and the water content should be kept in the range of 60-95 % (Demetriades, 2008). However, the optimum water content is likely to differ with different input materials depending up on the substrates chemical characteristics and bio-degradation rate (Nijaguna, 2002).

2.3.6 Agitation

The close contact between micro-organisms and the substrate material is important for an efficient digestion process. This can be achieved in a number of ways. For example, daily feeding of the substrate instead of long interval provides the desired mixing effect. Installation of certain mixing devices such as propeller, scraper, or piston is also a mechanism for stirring (Yadvika et al., 2004).

2.3.7 Organic loading rate

The rate at which substrate is supplied to the digester is referred to as organic loading rate and is usually expressed in terms of Kg volatile solids per m^3 and day. The gas production rate in the digester is highly dependent on the organic loading rate (Yadvika et al., 2004).

2.3.8 Hydraulic retention time (HRT)

The average time spent by the biomass inside a continuous biogas plant before it comes out from the digester is known as the hydraulic retention time, also abbreviated as HRT. The process of degradation requires at least 10-30 days in mesophilic condition, while in thermophilic environment HRT is usually shorter (Demetrides, 2008).

2.3.9 Seeding

To start up a new anaerobic process, it is critical to use an inoculum of micro organisms to commence the fermentation process. The common seeding materials include digested sludge from a running biogas plant or material from well-rotted manure pit or cow manure slurry (Yadvika et al., 2004).

2.4 Biogas operational techniques

The production of biogas can take place with different operating techniques. Two commonly used methods include batch wise digestion and digestion in a continuous process.

2.4.1 Batch wise process

In the batch type digester, the air tight reactor tank is charged once with substrate, inoculum micro organisms and in some cases a chemical to keep the reactor pH. The reactor is then sealed and fermentation is allowed for some days (Nijaguna, 2002). In this kind of processes, reactors are filled completely and emptied completely after some retention time. Thus, the daily gas production is build up to the maximum level and then decline after some retention days (Nijaguna, 2002). Substrate handling is easy with this method though there is a great variation in the production of biogas both in quality and quantity (Demetriades, 2008). The unsteady gas production in the batch process can be compensated by operating three to four digesters in parallel but filling them at different time. The batch process provides the highest degradation of the substrate material and all degradable materials can be converted to biogas if the retention time is long enough.

2.4.2 Continuous process

In continuous process, the addition and removal of substrate materials may occur between 1-8 times every day (Demetriades, 2008). In this process, the substrate material is pumped regularly into the digester and an equal volume of digested material is displaced and thus the volume in the digester remains constant. Continuous feeding of substrate is possible with this kind of process which at last gives much more even production of gas than the batch process. For smaller digester, the feeding of material is often once or twice a day but the larger digester are operated more continuously with feeding intervals of less than one hour (Demetrides, 2008)

3. Materials and method

3.1 Equipment and supplies

In this study the following equipments and supplies were used

- 1 litre glass bottles with thick rubber septum and aluminium caps as batch reactors
- 37 ° C incubator and shaking table operating at 130 rpm
- Inoculum from Västerås biogas plant
- Cow manure as a substrate for batch experiment
- A 2 ml plastic syringe for gas sampling of a fixed volume from the reactors
- Digital pressure meter for gas pressure measurement in the respective bottles
- Gas chromatograph (GC) to measure methane concentration from the sampled gases
- 20 ml glass vial to collect sampled gas from each test bottles

3.2 Experimental cows and feeding strategy

Six fistulated dairy cows of Swedish red breed in the age of 42±10 [mean ± standard deviation (SD)] months were considered in this study. Fistulated cows are cows with intentional hole in their stomach for scientific research (Figure 2). The cows were stabled at Kungsängen research centre, Uppsala and were part of the project “Sustainable dairy production on high-forage diets.” Three of the cows were in their first (early) lactation, and the rest three cows were in the second (mid) and third (late) lactation periods. The research centre houses 100 dairy cows of the Swedish red breed and 100 heifers. 56 dairy cows are housed in a loose housing system with an automatic milking system (AMS). 46 cows are tied up in individual stalls. Calves and heifers are kept in loose housing systems. The experimental cows in the methane trial were tied up and fed separately with three different feed mixtures during three different periods. The complete diets were composed of forage and concentrate (Table 1). The forage was high quality grass silage containing timothy and meadow fescue. The concentrate consisted of barley, oat, peas, and rapeseed cake. The first mixture, A was composed of equal percentage of grass silage and cereal (50:50). The second treatment, B was a mixture of 70:30 percentage of grass silage to cereal. The third mixture, C composed of a percentage of 90:10 grass silage to cereal. In terms of percentage of starch per Kg dry matter intake of the cow, mixture A contained high starch 17.18±0.6, mixture B medium starch 10.31±0.4, and mixture C contained 3.43±0.1 low starch (mean ± SD). In terms of percentage of neutral detergent fibre (NDF) per Kg of dry matter intake of the cow; mixture A was 29.67±1.5 (low fibre), mixture B was 33.55±2.1 (medium fibre), and mixture C was 37.44±2.7 (high fibre) (mean ± SD) (Patel et al., submitted).

Figure 2. Fistulated experimental dairy cows from Kungsängen dairy farm used in this study. (The photo was taken at the Kungsängen dairy farm, Uppsala, Sweden, March, 2009)

The forages were weighed manually during the sampling days. The cows were fed four times a day at 6 am, 9 am, 12 am, and 5 pm. Each experimental period consisted of 27 days of which 7 days were considered for transition time in order to avoid effects of gut fill from the previous diet. 13-15 days were used for adaptation, and 10 days for measuring/sampling. Each cow was offered one mixture during each period. The dry matter intake (DMI) and the nutrient compositions of the diets in each period and mixture are given in Table 1. DMI is the feed intake of cows in terms of its dry matter after removing the moisture/water content from the feed. The NDF is the neutral detergent fibre and it is a common measurement of fibre (cellulose, hemicelluloses and lignin) content in the animal feed analysis.

Table 1. Diet compositions and DMI of cows

Cows	Experimental Periods	Feed Mixtures	Average feed intake		
			DMI (Kg/day)	NDF (Kg/day)	Starch (Kg/day)
1202	1	B	17.52	5.75	1.85
1222	1	A	12.09	3.53	2.13
1328	1	C	18.38	6.69	0.65
1379	1	B	15.24	5.00	1.61
1381	1	C	16.57	6.04	0.58
1382	1	A	14.89	4.34	2.62
1202	2	A	17.07	4.84	3.00
1222	2	C	12.77	4.47	0.45
1328	2	B	17.89	5.67	1.88
1379	2	C	15.65	5.48	0.55
1381	2	A	15.72	4.46	2.76
1382	2	B	15.28	4.84	1.61
1202	3	C	18.00	7.36	0.59
1222	3	B	12.41	4.49	1.22
1328	3	A	17.43	5.49	2.86
1379	3	A	14.85	4.68	2.44
1381	3	B	16.13	5.84	1.59
1382	3	C	15.70	6.42	0.52

Cow methane emission (enteric methane) had been measured (October 2008 to January 2009) (Patel et al., submitted). Sulfur Hexafluoride (SF_6) tracer technique was employed to collect breath sample. More detail about the measurement technique of enteric emission of cows, which were considered for this study can be referred from the report of Patel et al. (submitted).

3.3 Determination of dry and organic matter

Manure was collected twice a day per cow during the last five sampling days in each period. This means that a total of ten samples were collected per cow and period (2*5*1=10). Sample substrates were placed in plastic bags to avoid movement of materials from the bag and then frozen at -20 °C. At the start of this study the manure samples were thawed and 100 g manure was extracted from each sample and then pooled, giving one sample per cow and period to be analysed in the methane potential batch test.

Prior to analysing the methane potential of the manure, volatile solid (VS) and total solid (TS) of the manures were determined according to standard methods (APHA/AWWA/WEF, 1995). Triplicate samples were analysed for single manure in order to determine VS and TS. Total solid means the weight of sample left after drying the sample at 105 °C for a minimum of twelve hours compared with the total weight of the sample before drying. Volatile solid is the weigh loss of the sample weighted before and after burning the sample at 550 °C for at least 6 hours. The final ash left is equal to the sample mineral content. Total solid and volatile solid measurements of the tested manures are shown in Table 2. Manures from different periods were handled at different occasions.

Table 2. TS and VS values of the manure samples investigated in this study as well as of the inoculums used in the methane potential experiments.

Manures	TS (%)[1]	VS (%)[2]
Period 1		
1202	14.0	11.8
1222	18.8	15.7
1328	16.2	13.9
1379	17.8	15.2
1381	17.3	14.9
1382	18.5	16.2
Inoculum young, 2009- 01- 26	5.5	3.9
Period 2		
1202	14.8	12.4
1222	17.1	14.4
1328	14.2	12.3
1379	14.5	12.5
1381	17.2	14.9
1382	17.1	14.9
Inoculum old, 2009- 01- 26	4.3	2.9
Period 3		
1202	14.2	12.1
1222	17.4	14.5
1328	16.3	14.0
1379	15.3	13.0
1381	15.2	13.1
1382	13.9	12.1
Inoculum young, 2009- 02-17	5.7	4.2

[1] % of total solid or dry matter in the sample substrate

[2] % of volatile solid or organic matter in the sample substrate per total solid

3.4 Inoculum

The inoculum used in the batch tests was collected from Västerås biogas plant at two different occasions. The inoculum was not used directly after collection in the batch experiment but allowed to degas during incubation at 37 °C. The inoculum collected at the first occasion (2009-01-26) was used for the first and second batch experiment after one and two weeks of incubations, respectively. The inoculum collected at second occasion (2009-02-17) was used for the third batch experiment after one week of incubation. The TS and VS measurement of the inoculum, performed at the start of each batch test, are given in Table 2. Inoculum used after one week of incubation is referred to as “inoculum young/new” and inoculum incubated for two weeks before used in a batch test is referred to as “inoculum old.”

3.5 Batch experiment

A batch testing analysis was performed in order to determine the biogas/methane potential of the collected cow manure (Hansen et al., 2004). In total three batch experiments were performed at different occasions. In the tests each manure sample was run in triplicate. Triplicates were also started without any added manure (control bottles) for each batch experiment. Each batch experiment consisted of a total of 6*3 test bottles plus 3 control bottles. The experiment was started by loading the test bottles with 3 g VS from each respective manure as well as 6 g VS from the inoculum. Each control bottles only loaded with 6 g VS of the inoculum. All experiment bottles had a total volume of 1 litre. Nitrogen gas was used to flush the bottles while filled with substrate and inoculum in order to ensure anaerobic conditions in the headspace of test bottle (Hansen et al., 2004). Each bottle was then filled with tap-water up to a total volume of 700 ml, while still N_2 flushing. After closing the bottles with a butyl rubber stopper and an aluminium cap, the bottles were placed on a table shaking at 130 rpm (round per minute) at 37 ^{o}C.

Figure 3. Experiment bottles in the batch wise experiment, incubated at 37 °C and placed on a table shaking at 130 rpm. (The photo was taken at the SLU Laboratory, Department of microbiology, April, 2009)

3.6 Gas measurement

A digital pressure meter (Testo 512, Testo AG, Lenzkirch, Germany) was used for the gas pressure measurement in the bottles. In all batches, the first gas measurement was done one day after the start of the experiment. The following gas pressure measurements were carried out randomly with expectation of gas production over time. After measuring pressure, 2 ml gas sample was extracted with a plastic syringe from each bottle. The sample gas was then injected into an empty glass (contained only air) vial of 23 ml volume. Prior to gas injection in the glass vial, the vials were pre-sealed with a rubber stopper and aluminium cap. After gas sampling, each experimental bottle were depressurized to atmospheric pressure by collecting the gas into a plastic bag. Sampled gas in the glass vial was then analysed with GC for determination of methane concentration in each test bottle. Gas sampling was continued until the gas production from each experiment bottle levelled off/ceased. The gas production from sample manure collected during the first experiment was levelled off after 31 days of incubation. For the second experiment the gas production levelled off after 42 days of incubation and the third experiment after 37 days of incubation.

3.7 Data analysis

3.7.1 Methane analysis

The methane content was analysed using GC (PerkinElmer ARNEL Clarus 500) with helium as carrier gas at a flow rate of 31 ml per minute. The column used was a 7’ HayeSep N 60/80, 1/8’’ SF, and the injection temperature was set to 60 °C using a

Headspace sampler Turbo Matrix 110. Methane was detected using a flame-ionization detector, which operated at a temperature of 250 °C.

Microsoft excel was used to calculate the biogas production per manure and period so that the accumulated mean methane production of the substrates can be read over time as illustrated in Figure 4 below. Methane production potentials between manures were compared and evaluated when the accumulated production had levelled off, i.e. when the methane production ceased.

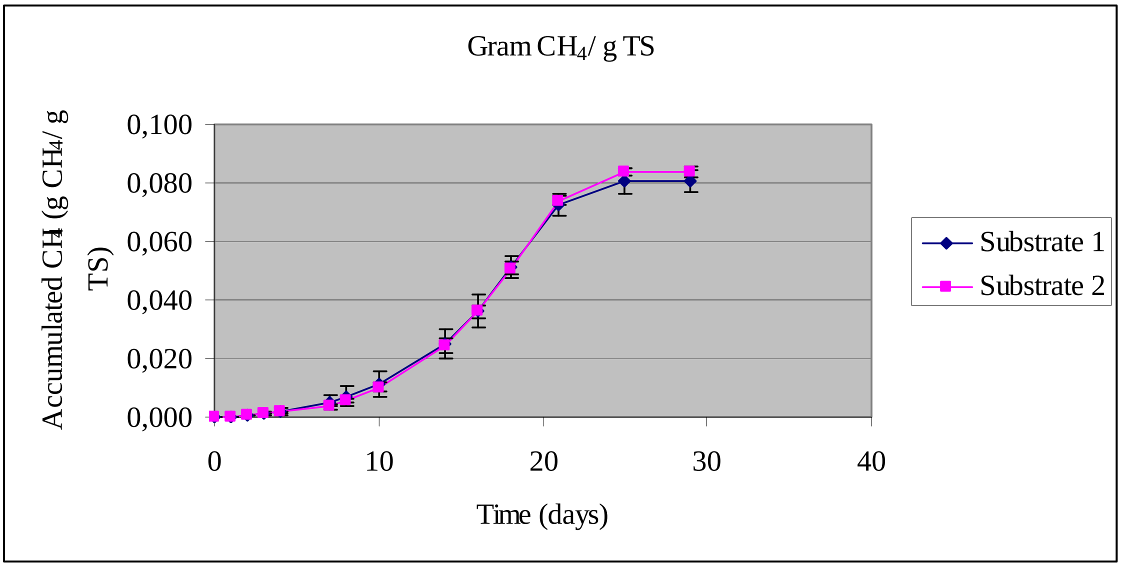

Figure 4. Typical methane production curves of two different substrates over time, in this case cow manure (1 and 2), the bar represent standard deviation of triplicate samples of each substrate. The accumulated gas production levelled off/ceased after 29 days of incubation.

3.7.2 Statistical analysis

A one-factor-ANOVA was run using (Microsoft excel, 2003) to evaluate the effect of different feed mixtures on the cow manure methane potential and enteric methane emission. For this purpose, the accumulated manure methane where the gas production ceased was considered in the analysis as a total methane potential of the manure. Furthermore, a simple correlation (Microsoft excel, 2003) was performed among variables such as starch (% of DM), neutral detergent fibre (% of DM), enteric methane emission, manure methane potential, and milk yield of the cow. Manure methane was calculated in g CH_4/ g TS or dry matter (DM) of the manure in order to make it comparable with the enteric methane emission, which was also calculated in g CH_4/Kg DM. Multiple regression and stepwise analysis was also performed using JMP version (SAS Institute, Cary NC) to formulate the mathematical equation for manure methane production, to see a step variable for manure methane, and carry out multivariate correlation between variables. In the regression model, methane production from the manure was considered as Y (dependent factor).

4. Result and discussion

Figure 5 below shows the comparison summary of methane potential of the cows manure as a result of different feed mixtures A, B, and C between the three experimental periods 1, 2, and 3. Detailed figures of the accumulated mean methane production from the cow manure over time (in days) in the first, second and third experimental periods respectively is illustrated in Figures 6, 7, and 8. The numbers 1202 - 1382 in Figures 5-8 correspond to manure from the cows. The methane potentials of the cow manures, the average daily enteric methane emissions, and average daily milk yield of the cows as a result of different feed mixtures (A, B, C) during three experimental periods (1, 2, 3) and three lactation periods (1,2, and 3) are presented in Table 3.

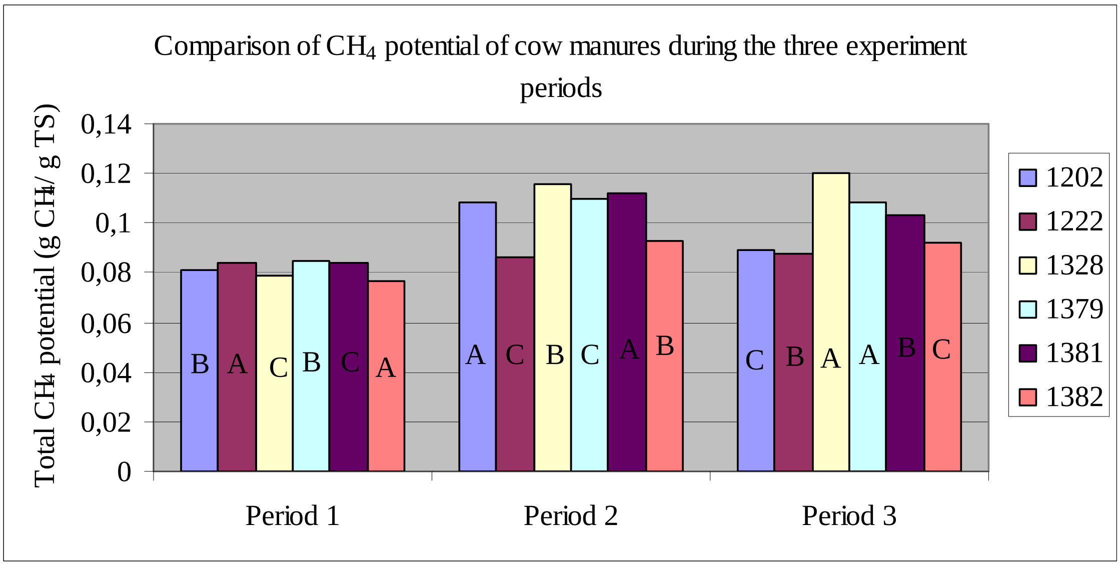

Figure 5. The methane production potential of the cow manure in all experimental periods

During period 1 the methane potential for the different manure samples was quite similar. Possibly, a slightly higher value was obtained for manure from cow 1222 which received feed mixture A. In both period 2 and 3, manure from the cow 1328 produced the highest level of methane with different feed mixtures B and A respectively.

4.1 The effect of feed mixtures on methane production potential of the manure

In all experimental periods, the gas production from the manure increased exponentially with time and ceases after certain days of degradation (Figures 6, 7 and 8). The figures also included the gas production from the control experiment/inoculum. The gas production from the control bottles was subtracted from the methane produced from the bottles with added manure

4.1.1 Period 1 experiment

In this period, the gas production levelled off/ceased after 29 days of incubation. The accumulated mean methane production over time is illustrated in figure 6 below.

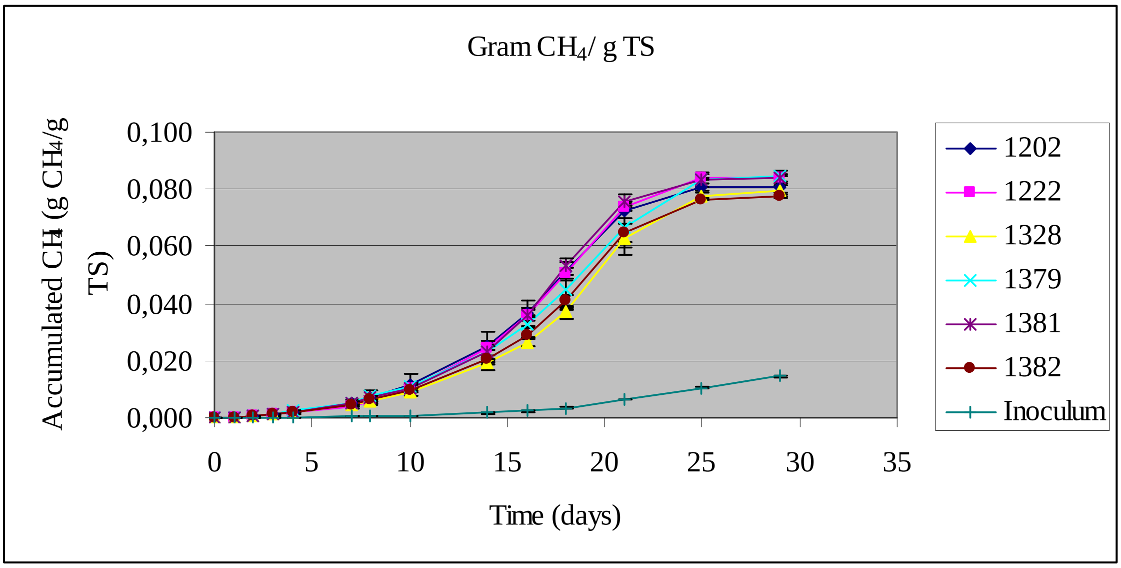

Figure 6. Accumulated mean methane production (with standard error) over time from the first period experiment

Manure from cow 1379 gave the highest methane yield, 0.085 g CH_4/ g TS and this cow had received feed mixture B. The lowest methane production, 0.077 g CH_4/ g TS was from cow manure 1382, received mixture A. Manure from cows 1222 and 1381 resulted in the same value of methane yield, 0.084 g CH_4/ g TS with two different feed mixtures A and C respectively. Manure from cows 1328 and 1202 which offered mixtures C and B, produced methane yield of 0.079 and 0.081 g CH_4/ g TS respectively (see Table 3).

4.1.2 Period 2 experiment

During this period, the gas production ceased after 42 days of incubation. The accumulated mean methane production over different period of time is shown in Figure 7 below.

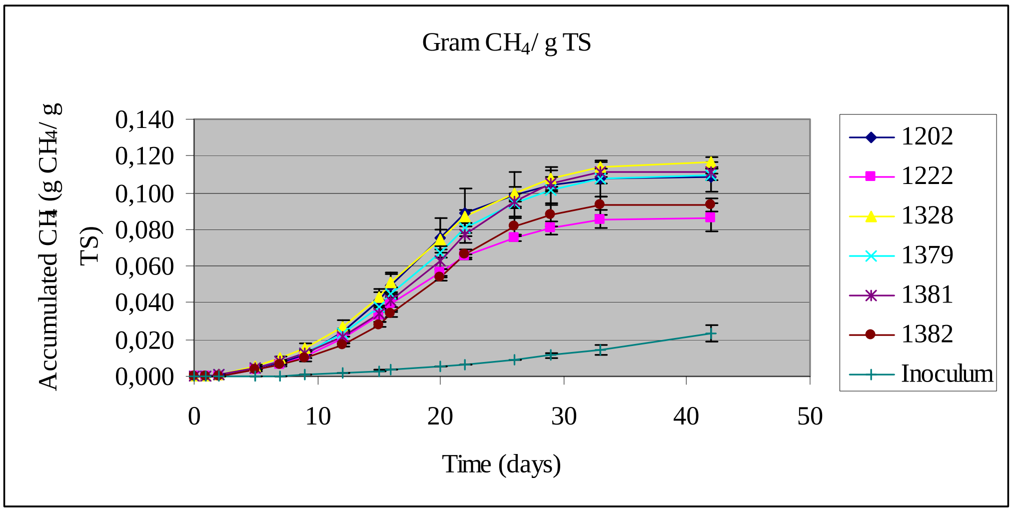

Figure 7. Accumulated mean methane productions (with standard error) over time from the second period experiment

The highest methane yield, 0.116 g CH_4/ g TS was from cow 1328, which received feed mixture B. Manure from cow 1222 produced the lowest methane, 0.086 g CH_4/ g TS and this cow received mixture C. Cow 1381 which received feed mixture A resulted in a methane yield of 0.112 g CH_4/ g TS, which was higher than manures from cows 1379 and 1382 which received feed mixtures C and B respectively (see Table 3).

4.1.3 Period 3 experiment

The methane production levelled off after 34 days of incubation. The accumulated mean methane yield over time in days is illustrated in Figure 8 below.

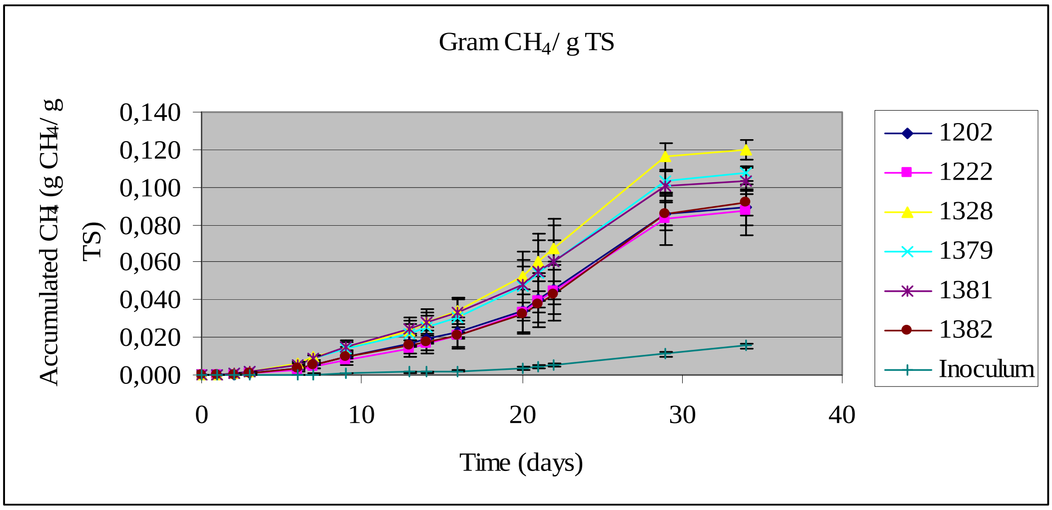

Figure 8. Accumulated methane production (with standard error) over time from the third period experiment

Like the second period experiment, manure from cow 1328 produced the highest accumulated methane production, 0.12 g CH_4/ g TS. However, in this period this cow received mixture A. The lowest production, 0.088 g CH_4/ g TS was from cow manure 1222. This cow was fed with feed mixture B. Manure from the other cows 1379 and 1381 which offered feed mixture A and B had produced a bit more methane yield 0.108 and 0.103 g CH_4/ g TS respectively than those cows 1202 and 1382 which received feed mixture C and the methane yield was 0.089 and 0.092 g CH_4/ g TS respectively (see Table 3).

Cows fed with mixtures A, B, and C resulted in average manure methane potentials (g CH_4/ g TS, mean ± SD) of 0.102 ± 0.017, 0.094 ± 0.013, and 0.090 ± 0.011 respectively. A single factor ANOVA showed that there was no significant differences between feed mixtures A, B, C on the methane production potential of the cow manure ($P>0.05$), even though there was a tendency of larger methane potential for feed mixture A than for B, than for C, i.e. that more feed starch resulted in more manure methane potential.

4.2 The effect of feed mixtures on enteric methane

The average daily enteric methane emissions and milk yield of the cows as a result of different feed mixtures A, B, C, and the percentage of starch and NDF in the dry matter intake of the cows is illustrated in Table 3 below. The manure methane potential was given in three digits in order to see the differences of methane potential between manures because two digits couldn't show the difference in potential between manures.

Table 3. Average enteric methane, milk yield, and manure methane potential as well as average DMI, % of NDF and starch content in the cows feed per its DMI

Cows	Lactation periods	Experimental Periods	Feed mixtures	Averages					Methane production potential of the manure (g CH_4/ g TS)
				DMI (Kg/day)	% of NDF/DMI	% of Starch/DMI	Enteric methane (g CH_4/Kg DM)	Milk yield (Kg milk/Kg DMI)	
									29 days[a]
1202	3	1	B	17.52	32.79	10.56	10.74	1.30	0.081
1222	3	1	A	12.09	29.17	17.60	22.76	0.76	0.084
1328	2	1	C	18.38	36.42	3.52	11.77	1.12	0.079
1379	1	1	B	15.24	32.79	10.56	15.57	1.11	0.085
1381	1	1	C	16.57	36.42	3.52	18.82	1.09	0.084
1382	1	1	A	14.89	29.17	17.60	12.28	1.63	0.077
									42 days[a]
1202	3	2	A	17.07	28.35	17.55	17.16	1.42	0.108
1222	3	2	C	12.77	35.01	3.51	20.23	0.00	0.086
1328	2	2	B	17.89	31.68	10.53	14.67	1.22	0.116
1379	1	2	C	15.65	35.01	3.51	22.99	0.91	0.110
1381	1	2	A	15.72	28.35	17.55	19.12	1.35	0.112
1382	1	2	B	15.28	31.68	10.53	20.03	1.36	0.093
									34 days[a]
1202	3	3	C	18.00	40.89	3.22	25.73	1.14	0.089
1222	3	3	B	12.41	36.20	9.84	29.44	0.00	0.088
1328	2	3	A	17.43	31.50	16.41	17.16	1.30	0.120
1379	1	3	A	14.85	31.50	16.41	21.81	1.05	0.108
1381	1	3	B	16.13	36.20	9.84	27.34	1.09	0.103
1382	1	3	C	15.70	40.89	3.28	18.61	1.37	0.092

[a] Incubation time after which the maximum methane potential of the manure was obtained

4.2.1 Period 1 experiment

In this period, the highest enteric methane emission, 22.76 g CH_4/Kg DM was from cow manure 1222 which offered feed mixture A (high starch and low fiber). The lowest enteric methane emission, 10.74 g CH_4/Kg DM was from manure of 1202 cow which received feed mixture B (medium starch and fibre). Cows 1328 and 1381 which received mixture C (low starch and high fibre) resulted in an enteric methane emission of 11.77 and 18.82 g CH_4/Kg DM respectively. The other cows 1379 and 1382 emitted an enteric methane of 15.57 and 12.28 g CH_4/Kg DM as a result of feed mixtures B and A respectively.

4.2.2 Period 2 experiment

During this period, the highest enteric methane emission, 22.99 g CH_4/Kg DM was from cow 1379, obtaining feed mixture C that contained high fibre and low starch content. The lowest enteric methane emission, 14.67 g CH_4/Kg DM, was from cow 1328, received feed mixture B that contained medium starch and fibre content. Feed mixture A with high starch and low fibre resulted in an enteric methane emission of 17.16 and 19.12 g CH_4/Kg DM from cows 1202 and 1381 respectively. These emissions were less than the emissions from the other cows 1222 and 1382 which was offered feed mixture C and B respectively.

4.2.3 Period 3 experiment

During this period, the highest enteric methane emission, 29.44 g CH_4/Kg DM was from the cow 1222, as a result of feed mixture B. The lowest enteric methane emission, 17.16 g CH_4/Kg DM, was from cow 1328 which received feed mixture A. Cow 1381, offered with mixture B, emitted 27.34 g CH_4/Kg DM. This enteric methane emission was higher compared to levels obtained from cows 1202 and 1382 which received mixture C, as well as from cow 1379 which received feed mixture A.

The average enteric methane emissions of the cows (g CH_4/Kg DM, mean ± SD) were 18.387 ± 3.793, 19.632 ± 7.429, and 19.693 ± 4.739 from feed mixtures A, B, and C respectively. A one-factor ANOVA showed that there was no significant difference between feed mixtures A, B, and C on the enteric methane emission of the cows ($P>0.05$). The tendency of feed mixtures for enteric methane emission was opposite from that for the manure methane potential, but was very slight.

4.3 The effect of feed mixture on milk yield of the cow

4.3.1 Period 1 experiment

The highest milk yield, 1.63 Kg milk/Kg DM of the cow intake, was obtained from cow 1382, as a result of feed mixture A. The lowest average milk yield in this period, 0.76 Kg milk/Kg DM of cow intake, was obtained from cow 1222 which received feed mixture A. Cows 1328 and 1381, which both received feed mixture C produced, a milk yield of 1.12 and 1.09 Kg milk/Kg DM intake of cow, respectively.

4.3.2 Period 2 experiment

The highest average milk yield, 1.42 Kg milk/Kg DM, of this period was from cow 1202 which received feed mixture A. The lowest yield, 0.00 Kg milk/Kg DM, was from cow 1222 with feed mixture C. This cow produced the lowest milk yield also in period 1, but then as a result of feed mixture A. Cows offered with mixture B, 1328 and 1382, produced a milk yield of 1.22 and 1.36 Kg milk/Kg DMI respectively. This yield was comparably higher than the yield from cows which received feed mixture C.

4.3.3 Period 3 experiment

In this period, cow 1382 had the highest milk yield, 1.37 Kg milk/Kg DM, and this cow received feed mixture C. Cow 1222, receiving feed mixture B produced the lowest milk yield, 0.00 Kg milk/Kg DM. Mixture A, with high starch and low fibre content, resulted in 1.3 and 1.05 Kg milk/Kg DM from cows 1328 and 1379, respectively.

The average milk yield of the cow as a result of feed mixtures A, B, and C (Kg milk/Kg DMI, mean ± SD) were 1.251 ± 0.279, 1.013 ± 0.597, and 0.939 ± 0.483, respectively. The difference in milk yield of the cow as a result of different feed mixture was not significant ($P>0.05$). However, there was a rather strong tendency that the higher starch diets produced more milk per Kg DMI, i.e. that mixture A resulted in a higher milk yield compared to feed mixture B, which in turn gave a higher milk yield than feed mixture C.

4.4 The relation among starch, NDF, milk yield, enteric methane, and manure methane

The multivariate correlation among variables such as starch and NDF in the feed, milk yield, enteric methane emission, and manure methane potential can be referred from Table 4 below. Manure methane potential had a positive correlation (R=0.32) with starch whereas the negative correlation (R=0.28) was seen with NDF (% of DM). Enteric methane was negatively correlated with starch (R=0.14), but positively correlated (R=0.34) with NDF (% of DM). The milk yield of the cow was positively associated with starch (R=0.32). However, a negative correlation was obtained (R=0.26) between milk yield and NDF. All of these correlations are in line with the tendencies discussed above. The milk yield of the cow was negatively associated with the enteric emission of the cow (R=0.55), but positively related with the manure methane potential (R=0.19). Finally, enteric methane emission of the cow and methane potential of their manure had a positive correlation (R=0.18).

Table 4. Multivariate correlations between variables

	Milk/DM (Kg/Kg)	Enteric CH_4 (g CH_4/Kg DM)	Starch (% of DM)	NDF (% of DM)	Manure CH_4 (g CH_4/g TS	DMI
Milk/DM (Kg/Kg)	1.0000	-0.5497	0.3168	-0.2625	0.1905	0.6541
Enteric CH_4 (g CH_4/Kg DM)	-0.5497	1.0000	-0.1364	0.3433	0.1819	-0.4667
Starch (% of DM)	0.3168	-0.1364	1.0000	-0.8714	0.3228	-0.1870
NDF (% of DM)	-0.2625	0.3433	-0.8714	1.0000	-0.2823	0.1711
Manure CH_4 (g CH_4/g TS	0.1905	0.1819	0.3228	-0.2823	1.0000	0.2607
DMI	0.6541	-0.4667	-0.1870	0.1711	0.2607	1.0000

Figure 9 below shows the principle component matrix based on correlations. PC1 and PC2 are principle components 1 and 2 respectively. This graph illustrates how much one variable is explained by others or it tells the closeness between variables in such a way that one explains the other better. Starch for example could better explain manure methane potential than the other variables since these variables are found very close to each other. On the other hand, DMI may explain milk yield better than other variables. In contrast to this, NDF and manure methane potential are almost opposite to each other and thus NDF may not better explain manure methane potential and so on.

Moreover, the multiple regression analysis showed that starch (% of DM) could better explain the methane potential of the cow manure than other variables included in the analysis (Table 5).

Table 5. Stepwise fit (Response: Manure (g CH_4/g TS))

Lock	Entered	Parameter	Estimate	nDF	SS	"F Ratio"	"Prob>F"
X	X	Intercept	0.08729484	1	0	0.000	1.0000
		Milk/DMI (Kg/Kg)	0	1	2.839e-5	0.146	0.7073
		Enteric CH_4 (g CH_4/Kg DM)	0	1	0.00017	0.925	0.3515
	X	Starch (% of DM)	0.00077421	1	0.000341	1.861	0.1914
		NDF (% of DM)	0	1	1.457e-8	0.000	0.9932

The regression model predicts the following equation:

Y (manure methane) = starch*0.00077421 + 0.08729484

Where;

0.00077421= constant

0.08729484 = intercept

The step wise analysis of the model chosen starch for its better fit with the manure methane potential than the other variables although it was not significant alone (P= 0.1914).

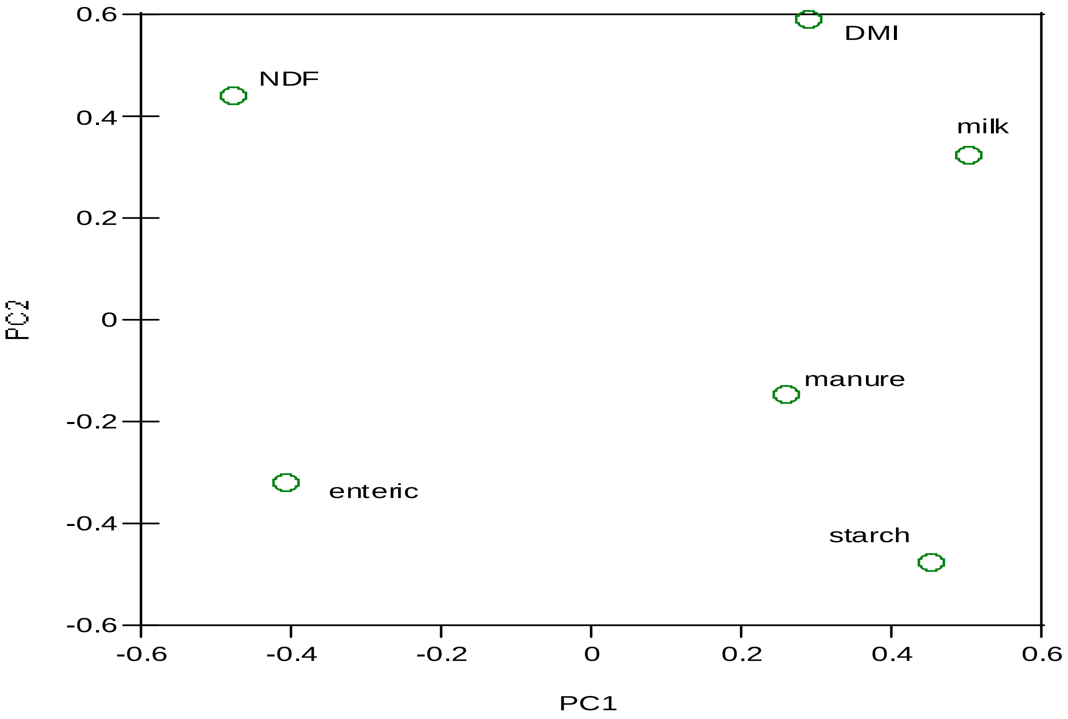

Figure 9. Principle component matrix based on correlations

4.5 Methane production from control experiment

Table 6 gives a summary of methane yield from the control bottles during all the three periods. The gas production in the control bottles originated from the inoculum itself. Methane yields after 29 days of incubation and at the end of the experiment were considered in the analysis because these days were common for all experiment periods.

Table 6. Methane yield (g CH_4/g TS) from the control bottles

Controls	Accumulated mean methane production with standard error	
	Production after 29 days of incubation	Production at the end of the experiment
Inoculum 1	0.015± 0.0003	0.015± 0.0003
Inoculum 2	0.011± 0.00136	0.023±0.00435
Inoculum 3	0.011± 0.00154	0.016 ± 0.0014

A one factor ANOVA showed no significant difference between inoculum ages on the methane production potential of manure substrates ($P>0.05$). The gas production with time from the three control experiments can also be seen in Figures 6, 7, and 8. In the first and third period experiments, when one week inoculum was used, the gas production from the test bottles levelled off after 29 and 34 days respectively. During the second period experiment, the gas production levelled off after 42 days with two weeks old inoculum. In this study, the age of the inoculum was considered as the reason for different levelling off time between periods. This is also supported by Demetriades (2008) who reported about the significant effect of inoculum age on the substrate’s degradation rate in the batch digestion process. However, the methane production potential of substrates remained unaffected with different inoculum age.

5. Concluding discussion

Methane emission from dairy cows through enteric fermentation and manure storage has currently become a serious environment concern. In the present study, focus was given to investigate the relation of these emissions with the diet of the cows as well as with each other.

The result showed that, there is still a tendency that the cow diet may affect both enteric and manure methane though it was not significant and strong in this study. The starch content in the diet (% DM) better explained the methane potential of the manure than the other variables included in this study. In addition to this, manure methane was slightly correlated to the enteric emission of the cows. The correlation was positive between manure and enteric methane though it is very weak. The result therefore goes against the hypothesis “less methane of the cow will give high gas potential of the manure.”

Other results from this study was that both enteric and manure methane might also be influence by the milk yield of the cows, which is affected by DMI. A higher yield of milk was observed from cows with large intake of DM and a low emission of enteric methane. The correlation was strong and negative between milk yield and enteric emission, and strong and positive between DMI and milk yield. This indicates that lactation periods may matter for the emission of methane from the cows (enteric and manure). It is also reported that, lactation stages (early, mid, and late) have effect on cow’s fiber digestibility of their diet (Extension org, 2010), which may also influence the methane potential of the associated manure. If this is true, considering cows with different lactation periods as one experiment group for a study of the effect of different feed mixtures (A, B, and C) on their methane emission (enteric and manure) may lead to unclear result. This may be one of the reasons for the insignificant difference found between feed mixtures and methane (enteric and manure) as well as for the weak correlation between different variables. Another reason might be that the cow forage used in the present study was high quality forage. In most studies, the NDF concentration in the cow diet is negatively associated with DMI, because fiber ferments slowly and stays in the rumen longer than other feed components. However, in the present study the correlation was slightly positive (see Table 4 above). This means that the DMI of cows was higher for feed mixtures, which are high in NDF content. This probably was due to the fact that fiber is more digestible in the rumen of the cow and that this stimulates a higher intake of NDF. If this is the case, no significant difference of methane production (enteric and manure) can be obtained for feed mixtures A, B, and C since the other component of diet was easily digestible concentrates. Previous studies have also showed that methane emission is low for concentrates and high quality forages (Grainger et al., 2008; Holter and Young, 1992; Hindrichsen et al., 2005; Iqbal et al., 2008).

The cow itself might also be of importance for the results, rather than the feed or lactation period. For example, manure from cow 1328 resulted in higher manure methane potential and low enteric methane during two different experiment periods with different feed mixtures A and B.

6. Conclusions

- No significant difference was found between feed mixtures A, B, and C on methane production potential of dairy cow manure per g TS and their enteric methane emission per kg DMI.
- There may be a possible effect of starch in the cow diet on the methane potential per g TS of the associated cow manure although it was, in this study, not significant alone
- Manure methane potential per g TS slightly increases with enteric methane production per kg DMI.
- The milk yield of the cow was negatively correlated with enteric methane production per kg DMI and positively with manure methane potential per g TS.

7. Further studies and possibilities

It would be interesting to carry out more experiments to see the simultaneous effect of different dairy cow feed composition on enteric methane and manure methane potential. The other interesting topic for future investigation would be to look into the cow microbial digestive system and see how it’s population affects the enteric as well as manure methane production. Investigating the effect of organic content in the manure (for example the content of nitrogen, carbon, potassium, or phosphorus, which is needed for optimal growth of micro-organisms) on methane production potential of the manure would also be an interesting topic for future studies. Though, it seems that the inoculum age had no effect on the methane production potential of the cow manure, the effect of different source and type of the inoculum on methane potential need further investigation. The other interesting topic for further study will be to see the effect of cow diet on methane (enteric and manure) production with in a group of cows with the same lactation period. All these recommendations could help to get wider understanding of the subject matter and to add new results to the findings of this research work.

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