BIOGAS POTENTIALS FROM MIXED SUBSTRATES: **EFFECT OF PRE-TREATMENT AND CO-DIGESTION**

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Author: Radziah Wahid
Department of Engineering – Biological and Chemical Engineering, Aarhus University

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The aim of this study is to improve biogas potentials through pre-treatment and co-digestion processes. Pre-treatment is important as it can increase the accessibility of microorganisms to cellulose during anaerobic fermentation, especially for highly lignified substrate, and thus increase the biogas potential. Different substrates such as agricultural crops, algae and animal manures are used in this research. Briquetting and extrusion are two main pre-treatment techniques that will be analyzed in depth. Different control parameters are manipulated to find the optimal settings and configuration of the machinery for the highest biogas yield and lowest costs in terms of energy. The influence of co-digestion of plant materials with animal manures is another focus area as it may offer a range of process benefits. Animal manures provide buffering capacity and a wide range of nutrients while plant material with high carbon content balances the carbon to nitrogen (C/N) ratio, thus reducing the risk of ammonia inhibition. Fundamental knowledge about anaerobic digestion of animal manures is investigated first before co-digestion with different substrates is initiated. This is important to fully understand the synergies of anaerobic digestion involved in biogas production from animal manures alone.

Keywords: Biogas, Co-digestion, Lignocellulosic Materials, Pre-treatment

Referee/Supervisor: Henrik Bjarne Møller

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Radziah Wahid
Aarhus University, Department of Engineering

Abstract

The aim of this study is to improve biogas potentials through pre-treatment and co-digestion processes. Pre-treatment is important as it can increase the accessibility of microorganisms to cellulose during anaerobic fermentation, especially for highly lignified substrate, and thus increase the biogas potential. Different substrates such as agricultural crops, algae and animal manures are used in this research. Briquetting and extrusion are two main pre-treatment techniques that will be analyzed in depth. Different control parameters are manipulated to find the optimal settings and configuration of the machinery for the highest biogas yield and lowest costs in terms of energy. The influence of co-digestion of plant materials with animal manures is another focus area as it may offer a range of process benefits. Animal manures provide buffering capacity and a wide range of nutrients while plant material with high carbon content balances the carbon to nitrogen (C/N) ratio, thus reducing the risk of ammonia inhibition. Fundamental knowledge about anaerobic digestion of animal manures is investigated first before co-digestion with different substrates is initiated. This is important to fully understand the synergies of anaerobic digestion involved in biogas production from animal manures alone.

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1. Introduction

1. 1 Background

Production of biogas from manure and crops offer great advantages for energy generation and may serve as a substitute for fossil fuels. Biogas could potentially help reduce global climate change by minimizing waste from animal farms and agricultural crops. In Denmark, animal manure is a large and almost unexploited energy resource. The environmental benefits of using manure in biogas plants is much higher than for any other substrate due to the combined effect of production of methane as a non-fossil fuel, and the corresponding reduction in the emissions of methane to the atmosphere from unwanted anaerobic degradation during storage and application on the fields (Sommer et al., 2001). Co-digestion of plant materials with animal manures may offer interesting results of biogas productions. Animal manures provide buffering capacity and a wide range of nutrients, while the addition of plant material with high carbon content balances the carbon to nitrogen (C/N) ratio of the feedstock, therefore reducing the risk of ammonia inhibition (Lehtomaki et al., 2007). The positive synergy effects often observed in co-digestion, due to the balancing of several parameters in the co-substrate mixture, have offered potential for higher methane yields (Mata-Alvarez et al., 2000). Finding new crops to boost biogas production at biogas plants is vital, as manure alone has a low methane yield (Cavinato et al., 2010). The ultimate goal is to find crops that produce maximum methane yield per hectare with low environmental impact and that are economical for farmers. Maize is a common co-substrates used in agricultural biogas plant operated with fermentation of manure, especially in Germany (Britz & Delzeit, 2013). Low lignin content in maize is the main advantage for efficient biogas conversion, but maize is not favorable for long term use as severe competition between energy and food supplies is created. For this reason, interest in using agricultural waste and high yielding perennial crops that may be produced on environmentally sensitive or marginal land has increased in recent years. Advantages of using perennial grass are less nutrient and pesticides requirement, less energy to plant and cultivate perennial grass than annual crops and higher energy conversion efficiency than annual crops due to a longer growing season (Uellendahl et al., 2008).

Lignocellulosic biomass is a renewable and carbon-neutral resource that can be found abundantly and low in cost however, the characteristics of the materials itself are the major barrier for efficient conversion of cellulose and hemicelluloses into monosaccharide that can be subsequently fermented into biogas. Pretreatment of biomass can be an efficient way to increase the biogas production but it is also associated with cost for energy and maintenance. Hydrolysis is the first steps involved in anaerobic digestion, where hydrolytic bacteria will break down the insoluble compounds such as particulate and colloidal waste into soluble monomers and dimers. Hydrolysis rate depends on parameters such as pH, size of particles, production of enzymes, diffusion and adsorption of enzymes on the particles of wastes subjected to the digestion process. Hydrolysis is a crucial process where, inhibition in this stage will cause insufficient substrates for the methanogens leading to decrease in methane production. Hydrolysis is considered as a rate-limiting step in anaerobic digestion and it can be significantly improved by removal of lignin and hemicelluloses, reduction of cellulose crystallinity and increase of porosity through pretreatment processes (Nizami et al., 2010). Pre-treatment alter the size and structure of lignocellulosic materials, as well as chemical composition to improve hydrolysis of carbohydrate fraction to simple sugars during anaerobic digestion (Kumar et al., 2009).

1. 2 Objectives

This study embarks on the following objectives:

- To understand the basic anaerobic digestion of cattle manure for biogas production
- To evaluate the potentials of different crops (miscanthus, red clover, caraway, ribwort plantain and chicory) in producing biogas through anaerobic digestion and near - infrared (NIR) spectroscopy.
- To investigate the effects of extrusion pre-treatment of different biomasses for biogas potentials
- To examine the effect of co-digestion of briquetted and macerated straw by using cattle manure as a base for biogas production
- To investigate the effects of pre-treatments of cattle manure and its effects toward hydrolysis process during anaerobic digestion.

2. General descriptions of methodology

The first part of PhD study is focusing on the fundamental concepts of biogas production from cattle manure. A full-scale experiment (10 m^3) and a pilot scale experiment (16 L); in which the two temperature ranges (50°C and 35°C) and two hydraulic retention times (HRT) (16 and 20 days) were tested in this research. Digestate physicochemical composition, methane (CH_4) yield and microbial composition were determined from reactors in each experimental period. Ultimate CH_4 yield and residual CH_4 emission were determined in a batch assay. Second part of the project is screening the biogas production from different crops such as miscanthus, caraway, chicory, red clover and ribwort plantain. Effects of different harvesting times, genotypes and plant fraction on biogas production are evaluated in this study. Anaerobic digestion and near-infrared spectroscopy are used to estimate methane yield from the crops. Chemical compositions of the samples are analyzed. The project was collaborated with other PhD students from Department of Agroecology, Aarhus University. Third part of the study evaluated on the pre-treatment of lignocellulosic materials such as straw and artificial deep litter using extrusion and briquetting process. In extrusion pre-treatment, effect of screw configurations and feeding velocity on biogas production and sugar availability are examined. For briquetting pre-treatment, three 16 L pilot reactors namely, reactor 1 (R1), reactor 2 (R2) and reactor 3 (R3) were working during 64 days with 20 days of hydraulic retention time in continuous stirring conditions (100 rpm) at 49±1^{o}C and two reactors (30 m^3) was running at 52 ^{o}C with 20 days of hydraulic retention time. Different substrates were added to each reactor; R1 – cattle manure (CM), CM + macerated wheat straw (MCM) and CM + briquetted wheat straw (BCM). pH, total and volatile solids (VS), total nitrogen, total ammonium and volatile fatty acids and biogas composition were analyzed once per week. The last part of the study is focusing on hydrolysis process during anaerobic digestion. Different pre-treatments of cattle manure and the effects of each pretreatment on hydrolysis process will be evaluated. The experiment will be done at Research & Technology Food & Agriculture Institute (IRTA), Barcelona. During preparation of this midterm report, the following experiments; 1, 2, 3 and 4 were already completed. However, only manuscript for experiment 2 is completed and is included in this report. For experiment 1, 3 and 4, only an abstract of each experiment will be included in the report.

2.1 Experiment 1: Anaerobic digestion of cattle manure in terms of methane productivity and microbial composition: Thermophilic *vs.* mesophilic range

The objective of this work was to determine the optimal temperature range for anaerobic digestion of animal manure founding on productive, microbiological and environmental criteria. For this purpose two experiments were designed: a full-scale experiment (10 m^3) and a pilot scale experiment (16 L); in which the two temperature ranges (50°C and 35°C) and two hydraulic retention times (HRT) (16 and 20 days) were tested. Digestate physicochemical composition, methane (CH_4) yield and microbial composition were determined from reactors in each experimental period. Ultimate CH_4 yield and residual CH_4 emission were determined in a batch assay. Thermophilic anaerobic digestion of cattle manure resulted in higher CH_4 yield and lower residual CH_4 emission during digestate storage. The highest differences between temperatures ranges were obtained in pilot reactors working at 16 days, meaning that HRT can be reduced only under thermophilic conditions. Thermophilic conditions showed a lower microbial diversity. Reads of Euryachaeota increased in reactors when comparing with cattle manure. The major percentage of reads belonged to Bacteroidetes in cattle manure and Firmicutes in mesophilic and thermophilic reactors. Dominant percentage of Euryachaeota reads in cattle manure belonged to Methanovebribacter and Methanocorpusculum genus and Methanosarcina and Methanobacterium genus in both groups of reactors.

2.2 Experiment 2: Methane potentials from *Miscanthus sp.*: Effect of harvesting time, genotypes and plant fractions

Abstract

The perennial C_4 grass miscanthus was evaluated as a potential energy crop for methane production when harvested green in autumn. *Miscanthus x giganteus* (*M. x giganteus*) and *Miscanthus sinensis* (*M. sinensis*) were harvested at five harvesting times, from August to November 2012 and methane yield from stems and leaves were analyzed by a batch assay of 90 days digestion. Estimated dry matter yields were highest at harvest 1st October for *M. x giganteus* and 13th September for *M. sinensis.* Cellulose and lignin contents were higher in *M. x giganteus* than *M. sinensis* and low lignin content in leaves led to rapid degradation during the early fermentation period of the anaerobic batch assay. At 90 days of anaerobic digestion, cumulative specific methane yields of *M. x giganteus* for stem and leaf varies from 285-333 and 286-314 NL (normalized liter) $(kg\ VS)^{-1}$ while 291-312 and 298-320 NL (kg VS)$^{-1}$ for *M. sinensis* stem and leaf respectively. Estimated methane yields per ha were positively correlated with the dry matter yields of miscanthus (r=0.92) and optimal harvesting time was suggested between September - October. The methane yield of *M. x giganteus* at the optimal harvest time was estimated at 3824 Nm^3 ha^{-1} (stem) and 1605 Nm^3 ha^{-1} (leaf) while 3507 Nm^3 ha^{-1} (stem) and 2957 Nm^3 ha^{-1} (leaf) for *M. sinensis*. However, the estimation of miscanthus dry matter yield by sampling of single shoots showed a discrepancy from whole plot harvesting, and needs to be further analyzed and optimized.

Keywords: *Miscanthus; Harvest time; Genotypes; Plant fractions; Methane Potentials*

Introduction:

Finding new crops to boost biogas production at biogas plants is vital, as manure alone has a low methane yield [1]. The ultimate goal is to find crops that produce maximum methane yield per hectare with low environmental impact and that are economical for farmers. Several factors that influence the methane yield are types of crop used, harvest time and chemical composition [2]. Maize is a common co-substrate used in agricultural biogas plants operated with fermentation of manure, especially in Germany [3]. Low lignin content in maize is the main advantage for efficient biogas conversion, but maize is not favorable for long term use as severe competition between energy and food supplies is created. For this reason, interest in using agricultural waste and high yielding perennial crops that may be produced on environmentally sensitive or marginal land has increased in recent years. The main obstacle in using perennial crops are their lignocellulosic properties which lead to lower biogas production but, dynamic growth in pre-treatment technologies research may overcome this and offer a wider range of crops as feedstock in the future [4].

Miscanthus is a perennial grass native to the East Asian region and was brought to Europe in 1935 by Aksel Olsen [5]. It was then cultivated and spread throughout Europe as an ornamental and since the 1980s the potential of miscanthus as a bioenergy crop has been investigated. In Asia, miscanthus is often used as animal feed and for roofing material and has never been considered as an energy crop until the end of 20th century. Miscanthus is highly persistent and the estimated life time of a plantation is 20–25 years. About 25 species of the genus *Miscanthus* were listed by various researchers and three species, namely *M. sinensis*, *M. sacchariflorus* and *M. x giganteus* are mainly used for biomass production [6]. Miscanthus is harvested once a year and shoots start to emerge during spring (April) and accumulate rapidly through summer with the highest yield around

September. The yield then starts to decline around October until February as results of the shedding of dead leaves and translocation of nutrient to the rhizomes [7].

In the agricultural sector, the economic feasibility of energy crops used for biogas production partly depends on the biomass yield per hectare harvested and of the necessary amount of nitrogen to apply. Miscanthus has high biomass yield with low or no nitrogen requirement and high adaptability to different soil and climatic environments [8]. Lewandowski et al., [9] reported that nitrogen fertilization is required when miscanthus were planted on soils with low levels of nitrogen available and nitrogen fertilization can be avoided or limited to 50-70 kg/ha/year if miscanthus is planted at locations with sufficient nitrogen mineralization. This is due to the characteristic of miscanthus, where it will translocate nitrogen and other minerals from aboveground biomass to the rhizome in autumn and winter and reuse the nutrients during shoot growth in spring [10].

Genotypes, soil types, nutrients used, crop age, bioclimatic location, and weather during the growing season were found to be factors that affect the biomass yield of miscanthus [11]. Chemical compositions of the crops varied with its development stages [11]. Jørgensen et al., [12], evaluated development and yield quality of four different groups of miscanthus over three years in Denmark. The crops were established in 1997 and harvested during autumn and spring for three years. The yield was low during the establishment year and started to increase in the two subsequent years. Eleven genotypes of miscanthus gave different biomass yields and a hybrid of *M. sacchariflorus* and *M. sinensis* was found to have highest dry matter yield compared to the others. Clifton-Brown et al., [13] reported that dry matter yields of *M. x giganteus* were influenced by crop age and harvesting time. In this study, development of *M. x giganteus* was monitored over sixteen years at a site in Southern Ireland. Results showed an increase in dry matter yields for five years following establishment and started to decline after ten years of development. Yields varied when *M. x giganteus* was harvested in different seasons (autumn and spring). Average autumn and spring yields over the fifteen harvest years were 13.4±1.1 and 9.0±0.7 t DW/ha respectively.

Most research papers available have focused on the establishment, development and yield quality of miscanthus as an energy crop for combustion [7, 8, 12]. Few studies have emphasized the potential of miscanthus as feedstock for biorefinery purposes. Hayes [14], investigated the effect of different harvesting time on mass and compositional changes in *M. x giganteus* relevant for biorefinery purposes in Ireland. In this study, it was found that early harvest (October to December) produced greater yield per hectare than at late harvest (March and April), when leaves had been lost during winter. In contrast with the combustion process, low moisture content of feedstock is not the main concern in a biorefining process, thus early harvest may be a better option.

The potential of miscanthus as an energy crop for ethanol production was also investigated by Zhuang et al., [15]. A data model assimilation analysis was used to estimate land and water requirement for three crops, namely maize, *Miscanthus* and switchgrass, to achieve the US national biofuel target of 79 billion liters of ethanol. It was assumed that the crops will be planted on the current maize producing areas to produce biomass feedstock. Comparison was made between each crop, and *Miscanthus* resulted in higher efficiency in term of land and water usage, followed by maize and switchgrass. It was estimated that about 26.5 million hectares of land and over 90 km^3 of water are needed if maize is used as feedstock to achieve US national biofuel demand. With an advanced biomass-biofuel conversion technology, only 9 million hectares of land and 45 km^3 water are required to fulfill national target if *Miscanthus* is used.

The need for further investigating the potential of miscanthus as biofuel crop is vital especially with the increased concern for finding effective biomass with high energy yield at low production cost and minimal environmental effects. Thus, the purposes of this work were to evaluate *M.* x *giganteus* and *M. sinensis* with respect to (a) dry matter yield, (b) chemical compositions and (c) methane potential. This was done by considering harvest of biomass between August and November 2012 and analyzing leaves and stems separately.

Materials and Methods:

Field experiment

Two miscanthus genotypes; *M. x giganteus* and *M. sinensis,* were harvested in existing field experiments at Research Center Foulum, Aarhus University, Tjele, Denmark in three replicates. The *M. x giganteus* plots were established in 1993 with plots of 13.2 x 12m and the *M. sinensis* (EMI genotype no. 11) plots were established in 1997, with the size 5x5m. All were fertilized with 75 kg/ha nitrogen annually. Description of the *M. sinensis* genotype and establishment can be found in [12] and details for *M. x giganteus* in [16]. From August 2012 till November 2012 stems, *M. x giganteus* and *M. sinensis* were collected every third week, in total five sampling times namely, 29th August (harvest 1), 13th September (harvest 2), 1st October (harvest 3), 22nd October (harvest 4) and 13th November (harvest 5). From each sampling time, a leaf sample consisting of all leaves, and a stem sample, consisting of the internodes adjacent to the gravity centre of the stems, were collected. The leaves were chopped using a communicator (Laborhäcksler, Baumann Saatzuchtbedarf, Germany). Dry matter content of was measured following harvest by drying three representative sub-samples at 60°C until constant weight was achieved and the remainder of the material was frozen for further analysis.

Yield estimation and correction

Single stem harvest

To estimate the yield, an average estimation of the weight of one stem was calculated at each harvest. This was based on cutting of randomly selected stems, the number of stems were chosen to ensure that at least 400 g of both leaves and stems were collected and minimum of 13 stems. For *M. x giganteus,* the number of stem was from 13 to 18 and for *M. sinensis,* the number varied from 33 to 94. This number was then multiplied with the average of four stem counting within a frame with an area of 0.497 m^2. To validate the single stem harvest, a study was carried out to estimate the correlation between single stem harvest and harvest of a bigger area. This was done once for each of the genotypes.

Area harvest and correction factor

On the same day as a single stem harvest, miscanthus at bigger area was also harvested as described in Larsen et al., 2013. It was assumed that harvest of the bigger area results in the most realistic results, so the two yield estimations were then used to estimate the ratio of single stem: area resulting in a factor used as a correction factor for the single stem harvests.

Analytical Methods

For standardization, the samples of miscanthus were dried at 60°C using oven to constant weight. Samples used for biogas production were then chopped using a heavy-duty cutting mill (Retsch SM 2000) with a sieve of 6mm square holes. Dry matter (DM) content and the volatile solid (VS) were determined from ground samples. For ash determination, the dried samples were burned in the muffle furnace at 550°C. The volatile solids were calculated by subtracting the raw ash content from the total solids.

For fiber analysis, the samples were ground to 0.8 mm particle size using a Foss mill (FOSS Cyclotec™ 1093), and only one replication was analyzed. Cellulose, hemicelluloses and lignin composition of *M. x giganteus* and *M. sinensis* were determined by measuring the value of ADF (Acid Detergent Fiber), NDF (Neutral Detergent Fiber) and ADL (Acid Detergent Lignin) of the plant. Cellulose was calculated as the difference between ADF and ADL, hemicelluloses as the difference between NDF and ADF. The analysis followed the Van Soest method, [17]. Samples used for fiber analysis were from taken the same plot.

Inoculum preparation

Inoculum was collected from a mesophilic post digester at the biogas plant in Research Center Foulum, Aarhus University, Denmark. Inoculum was stored for 3 weeks in an incubator at 35^{o}C to ensure the biogas production from inoculum was minimized during the batch assay. The inoculum was filtered using a manual sieve to remove the larger particles. Biophysical and biochemical analysis of filtered inoculum were performed. The average TS and VS of the inoculum were 3.58% and 2.44% respectively. Average pH of inoculum was 8.01 and Total Ammonium Nitrogen (TAN) in the inoculum was 0.71 g/l.

Batch experiment

The batch test was done as described by Møller, Sommer and Ahring [18]. For batch experiment, all samples of *M. x giganteus* and *M. sinensis* used were taken from the same plot. Biogas potential from stem and leaf fractions were analyzed in this study. Inoculum and miscanthus samples were added to 1L bottles in a ratio of 1:1 with respect of weight of volatile solids and closed with butyl rubber stoppers. The bottles were then flushed with N_2 for 2 minutes and shaken before incubation at 35^{o}C (mesophilic conditions) for 90 days. Each sample and a control containing only inoculum were repeated in triplicate. The biogas volume was measured after the first three days, then twice a week in the beginning, and once a week towards the end of the experiment. The biogas compositions of each sample were analyzed by using gas chromatography (Agilent technologies 7890A). Methane produced from each sample was corrected by subtracting the volume of methane produced from the control, containing inoculum only. Specific methane yields were expressed in NL (kg VS)$^{-1}$ (NL=normalized liter, gas volume corrected to 0^{o}C and 1.013 bar) and area based methane yield = Dry matter yield x VS x 100^{-1} x Specific methane yield.

Statistical analysis

Data collected from the experiment were calculated and further analyzed by using online Pearson correlation and Assistat version 7.7 beta. Description on the Pearson correlation can be found in Wessa [19] and Assistat software in Silva and Azevedo [20].

Results and discussion

Crop dry matter yield

Average dry matter yield of *M. x giganteus* obtained from the five harvesting time were in the range of 22-29 tonnes/ha while it was 14-18 tonnes/ha for *M. sinensis* (Figure1). The dry matter yield for *M. x giganteus* was higher than found in other Danish studies from the same site [12, 16]. Previously, dry matter yield of miscanthus above 30 tonnes/ha were recorded in Southern Portugal with proper irrigation, high annual radiation (6200 MJ/m^2) and high average temperature (15.4^{o}C) [10]. Both annual air temperature and radiation values were lower during the field trial in Foulum, 8^{o}C and 3547MJ/m^2 respectively and thus expected to result in a lower crop yield.

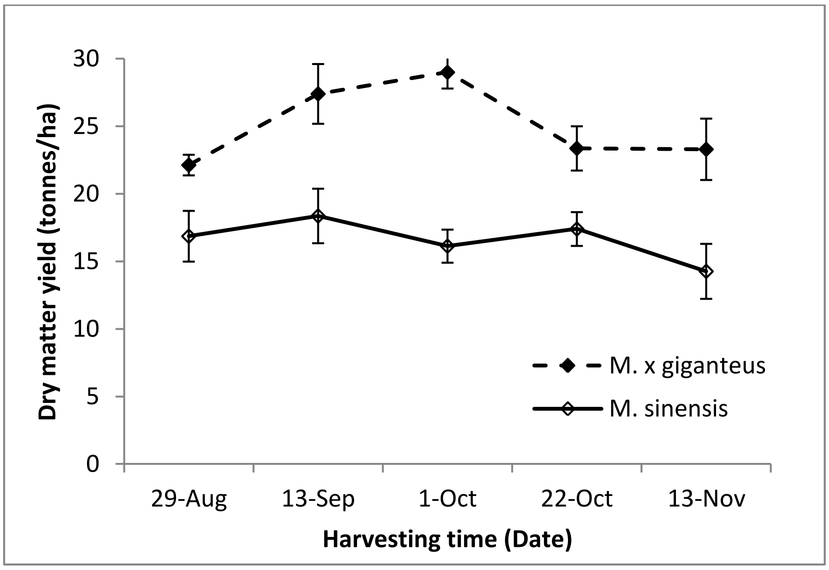


Figure 1: Estimated dry matter yield of *M. x giganteus* and *M. sinensis.* Vertical lines indicate ± standard error of mean.

Comparison was made with data reported by Larsen et al., [16] as *M. x giganteus* used was from the same plots as the *M. x giganteus* used in this study. Biomass yields in both experiments were based on manual harvest of the above-ground part of M. x *Giganteus* with stubble height of 5 to 10 cm. However, Larsen et al., [16] harvested whole plots of 22.1 m^2 (plots with 126 cm row distance) and 36.9 m^2 (plots with 260 cm row distance) for the yield determination. Development of *M. x giganteus* was monitored over 20 years by Larsen et al., and it was observed that dry matter yield throughout these years was always less than 20 tonnes/ha. The difference in yield level estimation is probably due to an overestimation of the number of stems by the counting of small sub-plots, and also that less leaves were lost during harvest in the gentle one-by-one cuttings compared to the harvest in Larsen et al., [16] that was done by a motorized hedge trimmer.

Very high yields of *M. x giganteus* obtained in this study led to further investigation on the effect of different cutting methods (one-by-one cuttings vs. motorized hedge trimmer cuttings done by Larsen et. al) in estimating the biomass production. In this experiment in 2013, one-by-one cutting was done in a similar way to the methods used in 2012, while harvesting with a motorized hedge trimmer was done according to Larsen et al., [16].

Differences in dry matter yields harvested with the two methods were calculated and the correction factors were 0.63±0.07 for *M. x giganteus* and 1.36±0.08 for *M. sinensis*. Dry matter yields obtained in 2012 were recalculated based on the correction factors and are illustrated in Figure 2.

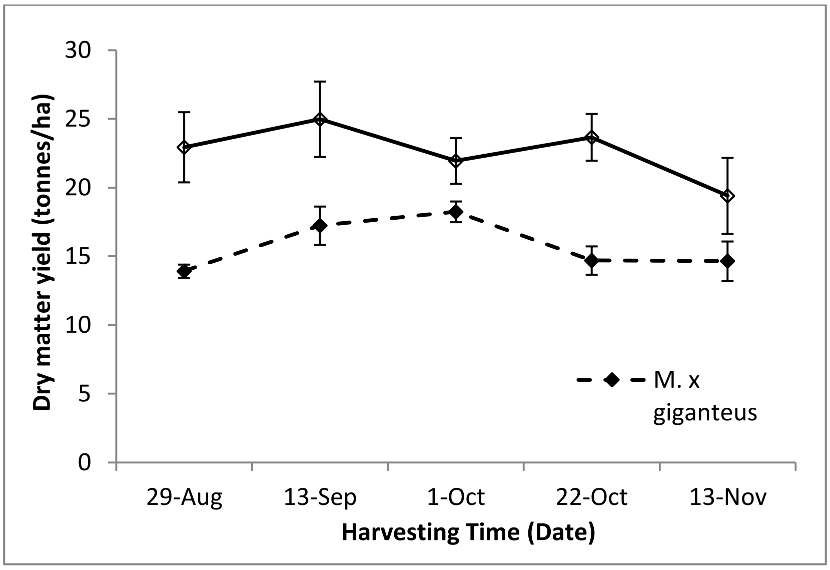


Figure 2: Estimated dry matter yield of *M. x giganteus* and *M. sinensis* (based on correction factor). Vertical lines indicate ± standard error of mean.

Estimated dry matter yields of miscanthus based on the correction factors were in the range of 14.7 to 18.2 and 19.4 to 25.0 tonnes/ha, for *M. x giganteus* and *M. sinensis* respectively. From Figure 2, it was observed that the dry matter yields of *M. x giganteus* increased from the 1st to 3rd harvest and started to decrease in harvest 4 to harvest 5. In contrast, dry matter yield of *M. sinensis* was highest at the second harvesting time (13th September) and no clear trend was observed from harvest 3 to harvest 5. In Jørgensen [21], similar observations were found as dry matter yield of both genotypes were increased from August 1994 and started to decrease after September 1994 for *M. sinensis* and after October 1994 for *M. x giganteus*. The estimated yields of *M. sinensis* from August – November 1994 were in range of 9 to 21 tonnes/ha, while estimated yields of *M. x giganteus* was 11 to 19 tonnes/ha. Highest dry matter yield was observed in September 1994 for *M. sinensis* and October 1994 for *M. x giganteus*.

Chemical Composition of miscanthus

Chemical characteristics of miscanthus at different harvesting times were determined (Table 1). As observed, no significant difference was examined on chemical composition of *M. x giganteus* and *M. sinensis* at different harvesting time. However, differences in cell wall compositions were apparent between genotypes, where, *M. x giganteus* had higher cellulose and lower hemicelluloses concentrations than *M. sinensis*. Also, differences were pronounced at different fraction as cellulose and lignin concentrations were higher in stems than in leaf samples while hemicelluloses content was higher in leaf than stem.

Table 1: Means values of dry matter, ash content, biochemical composition and biomethane potential of two miscanthus genotypes at five harvest time.

Parameters	Factor 1 (Fraction)		Factor 2 (Genotype)		Factor 1 x Factor 2			
	Stem	Leaf	*M. x giganteus*	*M. sinensis*	GS	GL	SS	SL
Cellulose (%DM)	48.58[a]	33.93[b]	43.97[a]	38.54[b]	52.45	35.48	44.71	32.36
Hemicellulose (%DM)	24.10[b]	32.58[a]	25.34[b]	31.35[a]	19.71[bB]	30.97[aB]	28.50[bA]	34.19[aA]
Lignin (%DM)	13.08[a]	9.34[b]	11.82[a]	10.60[a]	14.73[aA]	8.91[bA]	11.43[aB]	9.75[aA]
Ash (%)	2.63[b]	4.98[a]	4.46[a]	3.15[b]	3.16	5.76	2.10	4.20
BMP (NL $(kg\ VS)^{-1}$) at 31 days	223.58[b]	250.57[a]	234.41[a]	239.74[a]	221.40	247.42	225.76	253.72
BMP (NL $(kg\ VS)^{-1}$) at 90 days	302.96[a]	307.48[a]	303.20[a]	307.24[a]	303.17	303.23	302.75	311.73
Dry matter Yield (tonnes/ha)	11.41[a]	7.77[b]	7.88[b]	11.30[a]	10.68[aA]	5.08[bB]	12.14[aA]	10.46[bA]

For Factor 1 & Factor 2:
[a-b] means values bearing different lowercase letter in the same row are significantly different at $P < 0.05$.

For interaction between (Factor 1 x Factor 2):
[a-b] means values bearing different fraction of same genotype (GS & GL; SS & SL) in the same row are significantly different at $P < 0.05$.
[A-B] means values bearing different genotype of same fraction (GS & SS; GL & SL) in the same row are significantly different at $P < 0.05$.

VS = volatile solid; DM = dry matter basis; BMP = biomethane potential
GS – *M. x giganteus* stem; GL – *M. x giganteus* Leaf; SS – *M. sinensis* Stem; SL – *M. sinensis* Leaf

Similar results were obtained by Hodgson et al., [22], where the crops were established in 1997 and harvested at two different periods, November 2005 and February 2006. Five miscanthus species were used in the experiment, namely *M. x giganteus, M. sacchariflorus* and three genotypes from *M. sinensis* species (EMI08, EMI11 AND EMI15). Differences in chemical compositions were pronounced between genotypes where *M. sinensis* had lower content of cellulose and lignin and higher hemicelluloses content than *M. x giganteus and M. sacchariflorus*. Only small variations were observed on chemical compositions of miscanthus at different harvest time in this study.

A pre-study from 2007 investigated the effects of harvesting time on chemical compositions and methane yields of whole crops from *M. x giganteus* at the same site. The crop was harvested three times, 7th September, 9th October and 18th December 2007 and data is presented in Table 2. Each sampling consists of the whole plants (leaf and stem) and the samples were chopped to a size of 20-25 mm using a communicator (Laborhäcksler, Baumann Saatzuchtbedarf, Germany). It was clearly observed that ash content and hemicelluloses concentration decreased with later harvest time while cellulose concentrations increased with late harvest. Positive correlation was also observed in lignin content at different harvest time as r=0.68. In 2007, the effect of harvest time on chemical compositions was significant while, no significant variations obtained in chemical composition of miscanthus at different harvest in 2012 ($P > 0.05$ for cellulose, hemicelluloses and lignin). This may be explained by the fact that in 2007, fresh samples were used while dry samples used in 2012. Also, in the pre-study, harvest time was extend to middle December 2007, similar result may be obtain for this study if harvest time is extend to December 2012.

Specific methane yield

Accumulated specific methane production from *M.* x *giganteus* and *M*. *sinensis* after 90 days incubation at mesophilic conditions was determined (Figure 3). Specific methane yield of *M. x giganteus* stem and leaf varied from 285-333 NL (kg VS)$^{-1}$ (stem) and 286-314 NL (kg VS)$^{-1}$ (leaf), while *M. sinensis* yields were in the range of 291-312 NL (kg VS)$^{-1}$ (stem) and 298-320 NL (kg VS)$^{-1}$ (leaf). Typical cumulative specific methane yield curves were obtained from the batch test [2, 23]. At the beginning of the experiment, production of methane increased rapidly and the production rates became slower and more stable towards the end of incubation period. It was found that major part of methane was gained from both leaf (78-85%) and stem (69-78%) fraction within the first 31 days. The high conversion rate at the initial batch period illustrates that more easily biodegradable biomass produced methane rapidly at the beginning of the assay for both genotypes. As expected, lower lignin content in leaf fractions led to faster degradation during anaerobic digestion. However, continuous methane production was observed from stems towards the end of the fermentation when production from the leaves had reached the maximum suggesting that the maximum biogas potential was similar in the two fractions.

Methane production as a function of harvest time of *M. x giganteus* and *M*. *sinensis* stem and leaf are presented in Figure 4a and 4b. At 31 days, specific methane yield was higher in leaf than in stem fraction while no major difference was observed between fractions at 90 days. As discussed previously, lower lignin content in leaf than in stem fractions led to rapid production of methane during anaerobic digestion in the earlier stage. No significant variation was observed between genotypes, *M. x giganteus* and *M. sinensis* at 31 and 90 days. Effect of harvest time on specific methane yield was pronounced at 31 days but, only small difference was observed.

Results from this study were compared with the data obtained from the experiment done in 2007 (Figure 5). It was found that the cumulative specific methane yields from *M. x giganteus* in 2007 were reduced significantly as harvesting time increased, probably due to higher lignin concentrations at later harvest time. In the recent study, only small variations of methane yield were observed at different harvest time and genotypes. However, in 2007, last harvesting time was in December and it was found that methane yield in harvest 1 and 2 were not much different. This might be related to similar lignin content in the samples at harvest 1 and 2 which led to small differences in methane production.

As observed in Table 1 and 2, methane yield of samples harvested in 2012 were higher than from the preliminary experiment (2007). Potential reason that led to this was difference in the samples used, since fresh samples of whole plant and larger samples size (25 mm) were used in 2007, while dried samples and 6mm grinding size were used in recent study. As reported previously, reduction in samples size and drying process leads to an increase of specific surface area, a reduction in degree of polymerization and cause shearing of materials which increase the total hydrolysis yield of lignocelluloses by 5-25% and reduces digestion time by 23-59% [24, 25]. As hydrolysis is more effective, biogas produced from the digestion process will be higher. Besides, samples in 2007 comprised the whole plant fraction, while stem and leaf fraction were investigated in present study.

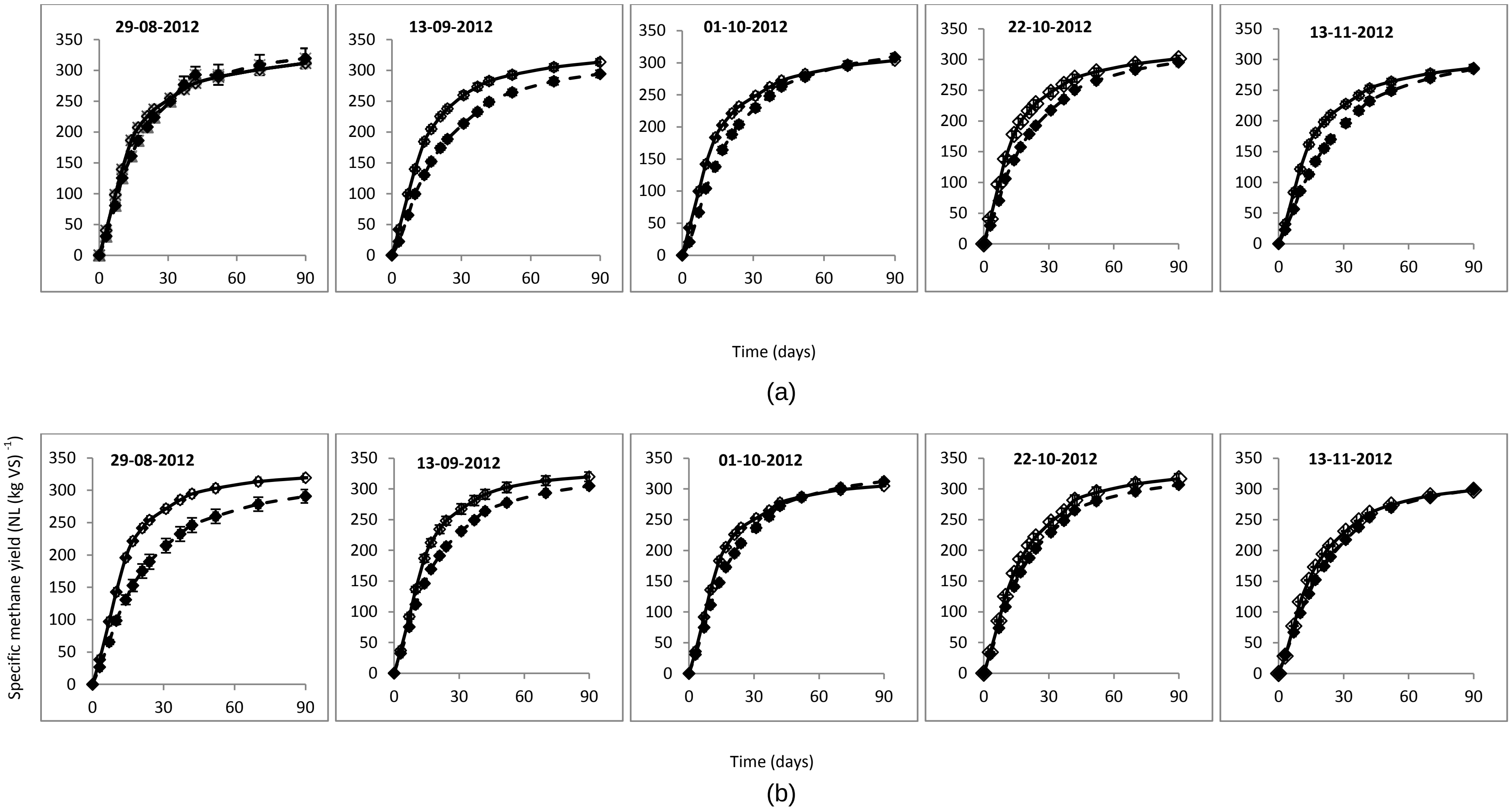
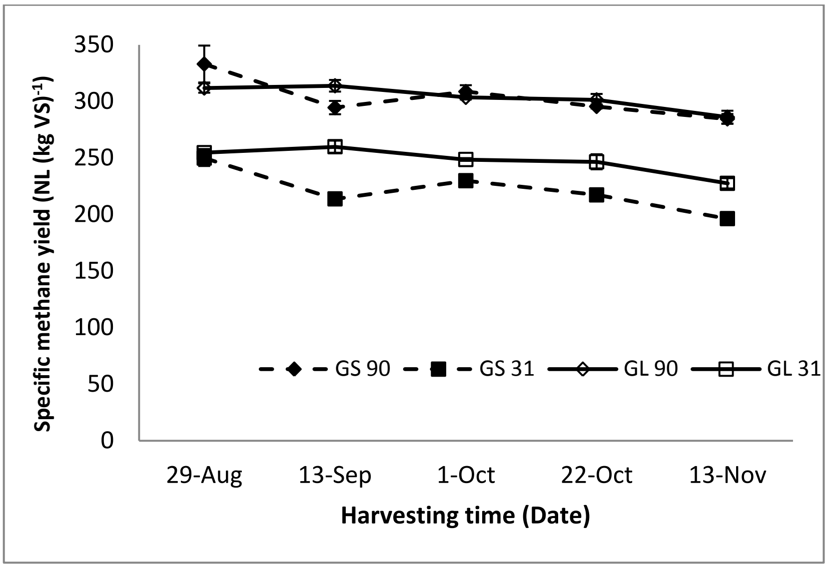
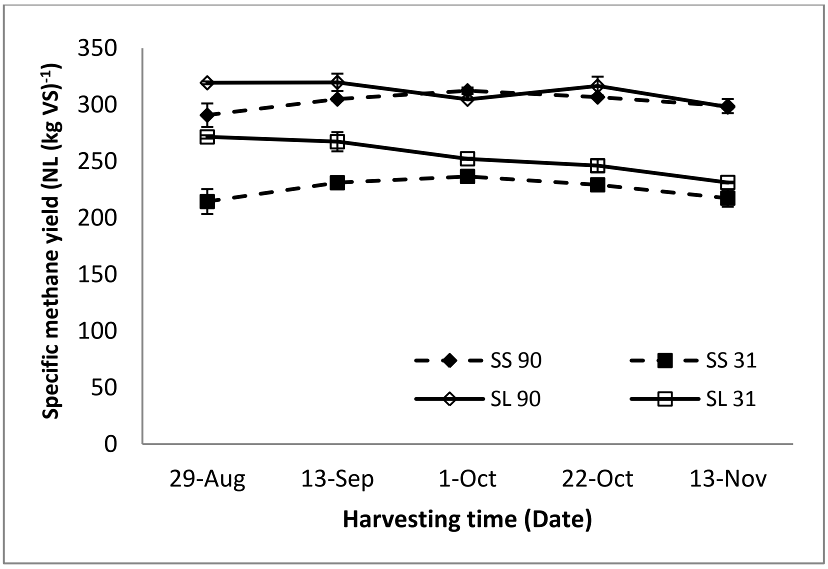


Figure 3: Accumulated specific methane yield after 90 days for all samples at different harvesting times (a) *M. x giganteus;* (b) *M. sinensis.* Vertical lines indicate ± standard error of mean.



(a)



(b)

Figure 4: Methane production from (a) *M. x giganteus* and (b) *M. sinensis* stem and leaf at 31 and 90 days. Vertical lines indicate ± standard error of mean.

GS – *M. x giganteus* stem; GL – *M. x giganteus* Leaf; SS – *M. sinensis* Stem; SL – *M. sinensis* Leaf

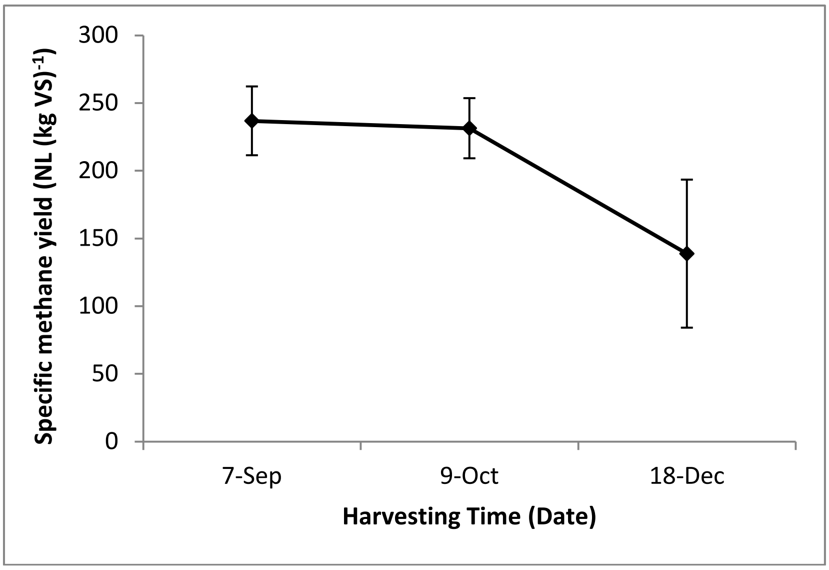


Figure 5: Methane production from *M. x giganteus* at 90 days in 2007 (whole plant). Vertical lines indicate ± standard error of mean.

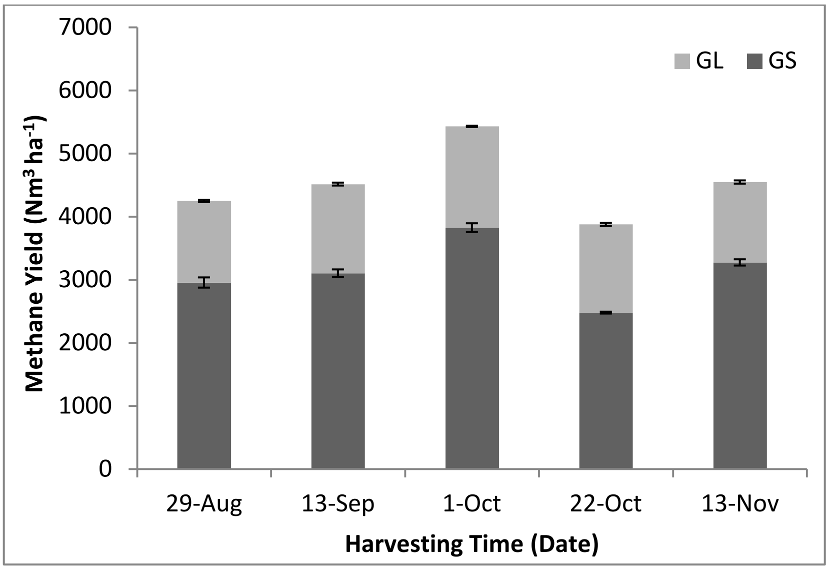
Table 2: Preliminary data of dry matter, ash content, biochemical composition and biomethane potential of *M. x giganteus* in 2007. Data was based on two replications.

Sample ID	% DM				BMP (NL (kg VS)$^{-1}$)
Genotype – harvest date	Ash	Cellulose	Hemicelluloses	Lignin	90 days
M. x giganteus 7-09	4.3	39.7	27.9	7.8	236.7
M. x giganteus 9-10	3.0	43.3	25.1	7.0	231.3
M. x giganteus 18-12	2.5	54.2	20.9	11.6	138.7
Pearson correlation (r):	-0.97	0.94	-0.97	0.68	-0.47

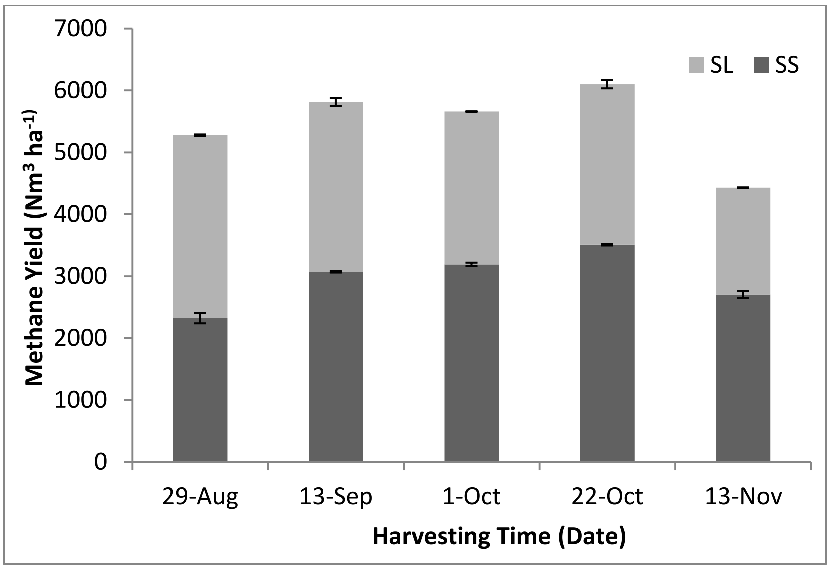
VS = volatile solid; DM = dry matter basis; BMP = biomethane Potential

Methane yield per ha

Estimated methane yield per ha of *M. x giganteus* and *M. sinensis* were calculated and presented in Figure 6a & 6b. The difference in dry matter yields of *M. x giganteus* and *M. sinensis* significantly influenced methane yield per ha since strong positive correlation was determined (r=0.92). Maximum methane yield per ha was obtained at the harvest on 1st October for *M. x giganteus* and on 22nd October for *M. sinensis*. Methane yield per ha estimated for *M. sinensis* were greater to the yield from *M. x giganteus* as a result of higher dry matter yields. Lower leaf fraction contributes to large difference in methane yield per ha of *M. x giganteus* stem and leaf while minor difference were observed for *M. sinensis*.



(a)



(b)

Figure 6: Methane Yield per Ha of (a) *M. x giganteus*; (b) *M. sinensis.* Vertical lines indicate ± standard error of mean.

GS – *M. x giganteus* stem; GL – *M. x giganteus* Leaf; SS – *M. sinensis* Stem; SL – *M. sinensis* Leaf

Calculated methane yield per ha for *M. x giganteus* stems and leaves varied from 2478 to 3824 Nm^3 ha^{-1} and 1274 to 1605 Nm^3 ha^{-1} while 2320 to 3507 Nm^3 ha^{-1} and 1724 to 2957 Nm^3 ha^{-1} from *M. sinensis* stems and leaves, respectively. Kandel et al., [23] found that methane yield per ha of reed canary grass was more strongly influenced by dry matter yield than specific methane yield and the effect of specific methane yield on methane yield per ha was only pronounced at the last four harvesting times as dry matter yields were similar. In Kandel at al., [23], stems fraction was superior in methane yield per ha than leaves as the proportion of stems were higher except in the first two harvesting times. As observed in the results, variations in specific methane yield for both fractions were small and differences in methane yield per ha were mainly due to dry matter yield.

Miscanthus for combustion is usually harvested at plant senescence but harvesting during this time led to low yield, while harvesting too early caused poor quality for combustion [14]. When considering feedstock for biogas production, green and moist miscanthus may be used instead of dry biomass [16]. Thus, instead of waiting for crop senescence time, the time of maximum above-ground biomass (which in Denmark is September-October) may be an option for farmers to harvest miscanthus for biogas production. The variation in the potential methane yield per ha was only limited during September – November which means that high yields can be obtained by direct harvest and delivery to the biogas plant throughout this period and keep storage costs down.

Conclusions:

The overall area specific methane yield per hectare of miscanthus correlated significantly to the dry matter yield (r=0.92). The yield of *M. x giganteus* at the optimal harvest time was estimated at 3824 Nm^3 ha^{-1} (stem) and 1605 Nm^3 ha^{-1} while 3507 Nm^3 ha^{-1} and 2957 Nm^3 ha^{-1} for *M. sinensis* stem and leaf respectively. The dry matter yield estimated from harvest of single stems was probably overestimated, and data were recalculated by considering correction factors (*M. x giganteus*: 0.63±0.07 and *M. sinensis*: 1.36±0.08) obtained by whole plot harvest. This deviation needs to be further analyzed with more proper experimental design. Specific methane yield of *M. x giganteus* varied from 285-333 NL $(kg\ VS)^{-1}$ (stem) and 286-314 NL $(kg\ VS)^{-1}$ (leaf) while M. *sinensis* were in the range of 291-312 NL $(kg\ VS)^{-1}$ (stem) and 298-320 NL $(kg\ VS)^{-1}$ (leaf), respectively. Production of methane was rapid within 31 days and became slower and more stable towards the end of the incubation period.

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2.3 Experiment 3: Extrusion as pretreatment for boosting methane production: Effect of screw configurations and feeding velocity

Interests in converting lignocellulosic materials into biogas have increased as lignocellulosic materials offer an interesting potential as co-substrates with animal manure. Wheat straw is an abundant lignocellulosic containing material that can be used for co-digestion with manure and can lead to positive synergy due to the balancing of several parameters in the co-substrate mixture which lead to higher methane potentials. However, the characteristics of lignocellulosic materials itself are the major barrier for efficient conversion of cellulose and hemicelluloses into monosaccharide that can be subsequently fermented into biogas. Pretreatment of biomass can be an efficient way to increase the biogas production but it is also associated with cost for energy and maintenance. Extrusion is a physical pretreatment where the materials are passing through the extruder barrel, resulting in physical and chemical changes due to heating, mixing and shearing. It is believed that the effects of screw speed and barrel temperature of extruder cause changes in materials structure hence increase the accessibility of cellulose for enzyme action. Increased methane yield with a positive energy budget makes extrusion an interesting technology to further develop. This study investigated the impact of extrusion as pretreatment for increasing sugar availability and methane production by manipulating screw configurations of extruder. Two biomasses namely, straw and water (B1) and artificial deep litter (B2) and five screw configurations namely; mild kneading (A), long kneading (B), reverse elements (C), kneading with reverse elements (D) and kneading with reverse kneading (E) were examined. The feeding velocity of the extruder was also varied during the experiment. During the experiment, raw and extruded biomasses were collected and further analyzed for sugar availability and biogas potential. Sugar availability test was done by using dinitrosalicylic acid (DNS) method and effect of different incubation time (0.5, 25, 46, 70 and 94 hours) on sugar availability was also investigated. Anaerobic batch digestion was performed to examine biogas potential and the experiment was carried out for 90 days under mesophilic conditions (35^{o}C). Results showed increments in sugar availability for all extruded samples compared to untreated material. Increase in incubation time led to increase in sugar availability, however, no differences were observed at 70 and 94 hours incubation. Sugar availability was increased with 8-44% in all biomasses with highest increment (44%) measured from extruded B1 and screw configuration D. Increased sugar availability was observed to accelerate degradation of the biomasses at the early digestion phases resulting in higher yield of methane. About 3-26% increments in methane yield were observed from all samples after 28 days digestion whereas after 90 days the increments in gas yield was lower. The increments in methane yields at 90 days for B1 were 1-15% and 1-5% for B2 except screw configurations D. Increased in feeding velocity had no influence on the ultimate methane yield of the sample. Results from the study indicated that extrusion increased degradation of carbohydrates during anaerobic digestion.

2.4 Experiment 4: Biogas potentials from forbs species (anaerobic digestion and NIR spectroscopy)

In this study, the influence of harvesting frequency on yield (in 2012 and 2013), chemical composition and methane yield of forb species in pure stand and mixture is examined. The main goal of the research is to characterize different forbs species in terms of their suitability as substrate for biogas production in an organic biogas plant. Five samples namely, caraway, chicory, red clover, ribwort plantain and standard mixture are evaluated. Biogas production from each species is tested from batch test, which running for 90 days. Chemical compositions of the samples are analyzed. Beside anaerobic digestion, biogas potentials from the species will be estimated using near-infrared spectroscopy. Figure 1 shows brief methodology for this experiment.

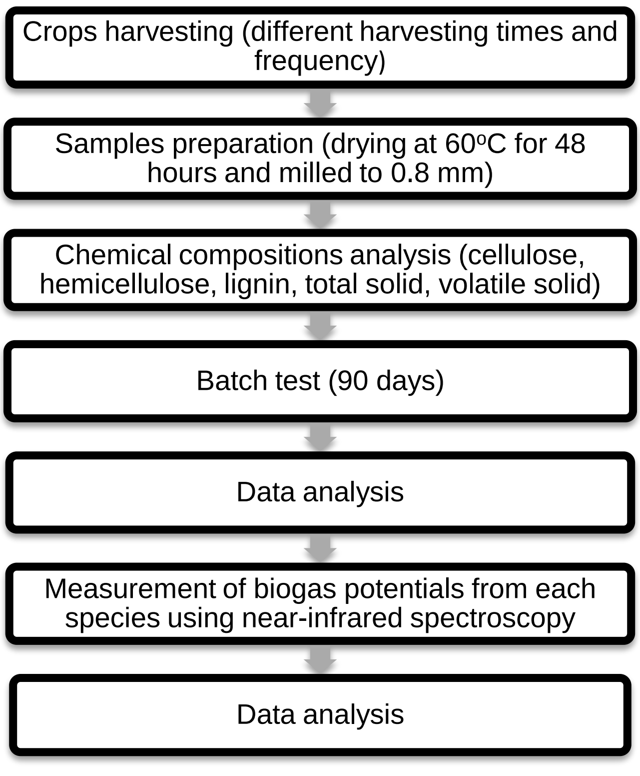


Figure 1: Methodology for Experiment 5

2.5 Experiment 5: Methane production from cattle manure co-digested with briquetted and macerated wheat straw

The aim of this study was to evaluate methane (CH_4) yield from cattle manure (CM) co-digested with briquetted or macerated wheat straw in continuously stirred tank reactors. Three pilot reactors (15 L) were working during 64 days with 20 days of hydraulic retention time in continuous stirring conditions (100 rpm) at 49±1^{o}C and two reactors (30 m^3) was running at 52 ^{o}C with 20 days of hydraulic retention time. Different feeds were added to each reactor: CM (control), CM + macerated wheat straw (MCM) and CM + briquetted wheat straw (BCM). Both straw types were added in a 5% concentration on a fresh matter basis (weight/weight) in the small digesters and 8.2% on a fresh matter basis in the larger digesters. On a weekly basis, pH, total and volatile solids (VS), total nitrogen, total ammonium and volatile fatty acids and biogas composition were analyzed. The measured CH_4 yield was subjected to variance analysis through randomized complete block design at 5% of probability using Dunnett's test for means comparison. The highest CH_4 yield ($P<0.001$) was obtained from BCM (218 L CH_4/kgVS), followed by MCM (211 L CH_4/kgVS). The control reactor showed the lowest ($P<0.001$) CH_4 yield (167 L CH_4/kgVS). Co-digestion of CM with wheat straw resulted in an increase of up to 23% in terms of LCH_4/kg VS and 42% in terms of LCH_4/kg of slurry. In the larger digesters the yield was 277 L CH_4/kgVS from BCM and 205 L CH_4/kgVS from CM. In larger scale the yields is significant higher indicating a scaling effect on the yields. Although briquetting only increased around 3% CH_4 yield compared with macerated straw, briquetting straw is a suitable technology not only to reduce transport and storage costs, but also to improve mixing and handling which can allow increasing the inclusions levels of straw in anaerobic digesters. In order to improve the methane yield from macerated and briquetted straws by further opening up the ligno-cellulosic structure making the fiber more available to the microorganisms, alkaline pretreatment (NaOH and KOH) are carried out in the coming period. These results will be included in the presentation.

2.6 Experiment 6: Pre-treatments of cattle manure and its effects on hydrolysis process during anaerobic digestion

Experiment 6 is planned to be done at IRTA, Barcelona on August – October 2014. A brief description on the experiment is discussed below.

Pretreatment methods include physical, chemical, physicochemical, biological or combination of several methods (Xie et al., 2011). Choosing the suitable pre-treatment method is a challenge as different pre-treatment will result in various substrate characteristics, which will lead to a different effect on hydrolysis process during anaerobic digestion. Besides increasing the biogas production, selection of optimum pre-treatment methods will also based on other factors such as cost, environmental effects and suitability for large scale production. This study will investigate effects of different pre-treatment on cattle manure, focusing on hydrolysis stage during anaerobic digestion. A mathematical model describes the relationship of different parameters that affecting hydrolysis step of anaerobic digestion process will be carry out in this study. Figure 1 shows brief descriptions on experiment 6.

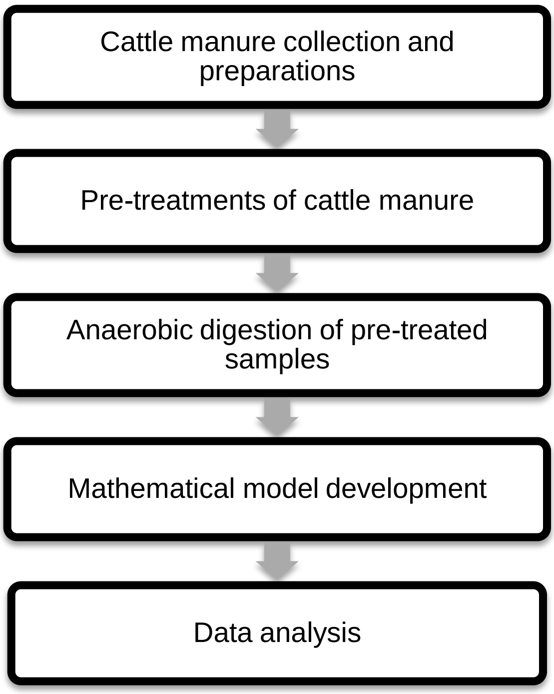


Figure 1: Proposed methodology for Experiment 6

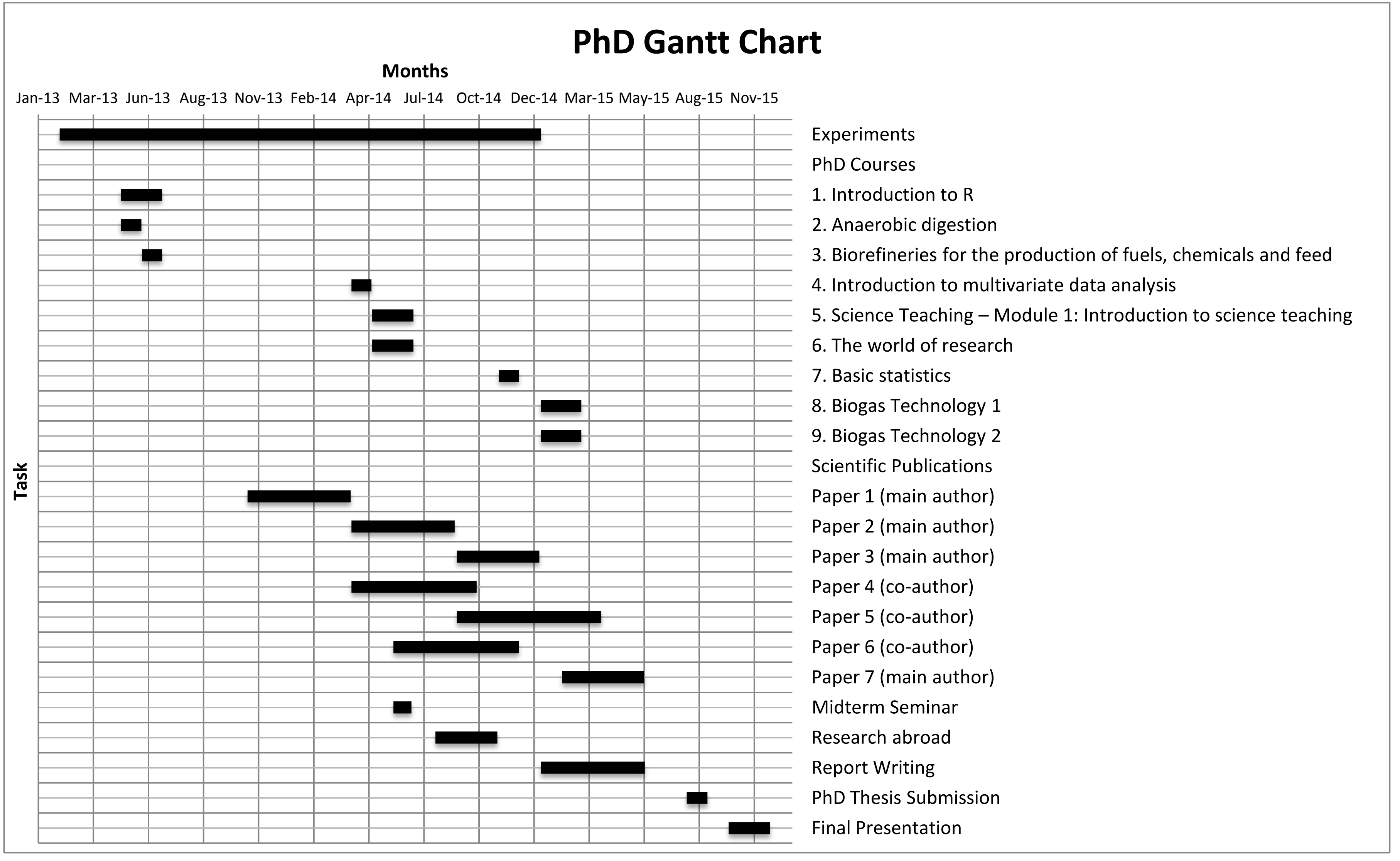
3. PhD courses

Courses name	ECTS	Date	Institution	Status
Introduction to R	1	June 2013	Aarhus University	Completed
Anaerobic digestion for waste treatment and renewable energy production	3	May 2013	Aalborg University	Completed
Biorefineries for the production of fuels, chemicals and feed	4	June 2013	Aalborg University	Completed
Introduction to multivariate data analysis	3	April 2014	Aalborg University	In progress
Science Teaching – Module 1: Introduction to science teaching	3	May 2014	Aarhus University	In progress
Basic statistics	6	Nov 2014	Aarhus University	Planned
Biogas Technology 1	5	2015	Aarhus University	Planned
Biogas Technology 2	5	2015	Aarhus University	Planned
Total	30			

4. Planned publications

Title	Status
Paper 1: Methane potentials from *Miscanthus sp.*: Effect of harvesting time, genotypes and plant fractions (Main author)	Submitted to Biomass & Bioenergy Journal – Under reviewed
Paper 2: Extrusion as pretreatment for boosting methane production: Effect of screw configurations and feeding velocity (Main author)	In progress
Paper 3: Biomethane potentials from forbs species using near-infrared (NIR)spectroscopy (Main author)	Planned
Paper 4: Methane production from cattle manure co-digested with briquetted and macerated wheat straw (Co-author)	In progress
Paper 5: The influence of harvesting frequency on yield (in 2012 and 2013), chemical composition and methane yield of forbs species in pure stand and mixture (Co-author)	Planned
Paper 6: Anaerobic digestion of cattle manure in terms of methane productivity and microbial composition: Thermophilic *vs.* mesophilic range (Co-author)	In progress
Paper 7: Pre-treatments of cattle manure and its effects on hydrolysis process during anaerobic digestion	Planned

5. Time Schedule – Gantt chart



6. Disseminations

No	Activities	Hours	Date
1	Teaching – Biogas Technology	30	2013
2	Teaching – Analytical chemistry Laboratory	80	27/2/2014 – 7/3/2014
3	Research abroad – IRTA, Barcelona	200	1/8/2014 – 31/10/2014
4	Presentation at upcoming conferences	200	2014-2015
5	Supervising internship (2 students)	100	April 2014-July 2014
6	Teaching – Biogas courses	140	2015
7	Instruction/ knowledge sharing with foreign postdoc (helping in explaining on lab equipments and experiments)	150	2013-2014
	Total	900	

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Radziah Wahid, Biogas potentials from mixed substrates: effect of pre-treatment and co-digestion, 2014

Department of Engineering
Aarhus University
Edison, Finlandsgade 22
8200 Aarhus N
Denmark

Tel.: +45 4189 3000