Biogas Production from Energy Crops and Agriculture Residues

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Abstract (max. 2000 char.):

In this thesis, the feasibility of utilizing energy crops (willow and miscanthus) and agriculture residues (wheat straw and corn stalker) in an anaerobic digestion process for biogas production was evaluated. Potential energy crops and agriculture residues were screened according to their suitability for biogas production. Moreover, pretreatment of these biomasses by using wet explosion method was studied and the effect of the wet explosion process was evaluated based on the increase of (a) sugar release and (b) methane potential when comparing the pretreated biomass and raw biomass. Ensiling of perennial crops was tested as a storage method and pretreatment method for enhancement of the biodegradability of the crops. The efficiency of the silage process was evaluated based on (a) the amount of biomass loss during storage and (b) the effect of the silage on methane potential. Co-digestion of raw and wet explosion pretreated energy crops and agriculture residues with swine manure at various volatile solids (VS) ratio between crop and manure was carried out by batch tests and continuous experiments. The efficiency of the co-digestion experiment was evaluated based on (a) the methane potential in term of ml CH_4 produced per g of VS-added and (b) the amount of methane produced per m^3 of reactor volume.

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PAPERS

SUMMARY (English)

In this thesis, the feasibility of utilizing energy crops (willow and miscanthus) and agriculture residues (wheat straw and corn stalker) in an anaerobic digestion process for biogas production was evaluated. Potential energy crops and agriculture residues were screened according to their suitability for biogas production. Moreover, pretreatment of these biomasses by using wet explosion method was studied and the effect of the wet explosion process was evaluated based on the increase of (a) sugar release and (b) methane potential when comparing the pretreatd biomass and raw biomass. Ensiling of perennial crops was tested as a storage method and pretreatment method for enhancement of the biodegradability of the crops. The efficiency of the silage process was evaluated based on (a) the amount of biomass loss during storage and (b) the effect of the silage on methane potential. Co-digestion of raw and wet explosion pretreated energy crops and agriculture residues with swine manure at various volatile solids (VS) ratio between crop and manure was carried out by batch tests and continuous experiments. The efficiency of the co-digestion experiment was evaluated based on (a) the methane potential in term of ml CH_4 produced per g of VS-added and (b) the amount of methane produced per m^3 of reactor volume.

Many crops can be used as substrates for biogas production. Within this project, the agriculture i.e. residues wheat straw and corn stalker and energy crops i.e. willow and miscanthus were primarily selected due to their biomass production rate (i.e. t/ha) and most importantly these crops are easy to cultivate in the northern part of Europe. The methane potential of these crops, as determined in laboratory methane potential test, indicated that corn stalker gave the highest methane potential, which was 399 ml per g VS-added. For wheat straw and miscanthus was 260 and 268 ml per g VS-added, respectively. Crop willow had the lowest methane potential among these crops, which was only 150 ml per g VS-added.

The optimal wet explosion pretreatment condition for each crop was carried out by adjusting the parameters of the wet explosion process i.e. temperature, pressure, retention time, amount of oxidation agent (H_2O_2) and the total solids (TS) concentration of the biomass. The results showed that after wet explosion pretreatment 46, 27, 19 and 12% of the contained sugars became soluble for corn stalkers, miscanthus, wheat straw and willow, respectively. Subsequently, the methane potential of these pretreated crops was tested. It was found that, although a high release of soluble sugars was observed after wet explosion, the methane yield (CH4 per g VS-added) obtained from the wet-exploded crops was slightly lower compared to the raw biomass. Only willow showed an increase of 80% in methane potential. Taking the energy consumption during the wet explosion process into account, this pretreatment method is still energetically profitable for biogas production from willow.

Ensiling of willow and miscanthus with and without biological additives (lactic acid bacteria LAB) was tested in silage periods between 1 to 5 months. Within 5 months, willow and miscanthus lost 2 and 3% of its weight, respectively. The methane potential test on silage willow indicated that the biodegradability of willow was enhanced during the ensiling process, and the methane potential of silage willow (3 months) was 20% higher than that of raw willow. No significant change of the methane potential was observed when ensiling miscanthus. Moreover, ensiling of willow and miscanthus with additives showed no advantages in either biomass losses or methane potential compared to the ensiling process without additives.

Batch experiments on co-digestion of swine manure with various biomasses at different VS ratio indicated that the balance of carbon: nitrogen (C: N ratio) played the most important role in the co-digestion system. When the VS concentration of manure was fixed, the lower crop input, the higher biogas yield in term of ml CH_4 per g VS-$_{added}$, and higher crop input, the higher volumetric reactor productivity in term of m^3 CH_4 produced per m^3 of reactor working volume. However, continuous reactor experiments using manure richer in nitrogen than the manure used in the batch tests showed an increase in both methane yield and volumetric reactor productivity. Co-digestion with a TS ratio of 1:1.5 (manure: wheat straw) resulted in a 23% higher methane yield and 111% higher productivity, when compared to those of a TS ratio of 1:1.

RESUME (Dansk)

Brug af energiafgrøder (pil og elefantgras) og landbrugsafgrøder (hvedehalm og majsstængler) i samudrådning med gylle til biogasproduktion er evalueret i denne afhandling. Mulige energiafgrøder og afgrødeaffald blev screenet for deres anvendelighed i biogasproduktion. Forbehandling af afgrøder med våd explosion (WE) blev studeret og effektiviteten evalueret baseret på (a) sukkerfrigørelse og (b) metanpotentialet. Ensilering af flereårige afgrøder blev testet som lagrings- og forbehandlings –metode for at øge bionedbrydeligheden og effektiviteten af ensileringsprocessen. Effektiviteten blev evalueret baseret på (a) biomassetabet og (b) metanpotentialet af den behandlede biomasse sammenlignet med den rå biomasse. Samudrådning af rå eller WE-energiafgrøder med svinegylle med varierende VS-forhold (volatile solids) fra afgrøde og gylle blev udført med batch forsøg og kontinuerlig eksperimenter. Effekten af samudrådning blev evalueret baseret på (a) metanpotentialet i form af ml $CH4$ / VS_{tilsat} og (b) den totale metanproduktion per m^3 reaktorvolumen.

Mange afgrøder kan bruges som substrat til biogasproduktion. I dette projekt blev affald som hvedehalm og majsstængler samt energiafgrøder som pil og elefantgras primært valgt pga. deres biomasseproduktionrate og da de nemt kan kultiveres i Nordeuropa. Metanpotentialet af disse afgrøder, bestemt ved laboratorietest, indikerede at majsstængler gav 399 ml per g VS. For hvedehalm og elefantgræs var resultatet henholdsvis 260 ml og 268 ml. Pil havde det laveste potential af 150 ml.

Den optimale WE betingelse for afgrøderne blev fundet ved justering af parametre som temperatur, tryk, opholdstid, mængde af $H2O2$ (oxidant) og biomassetørstof (TS). Resultaterne viser at for majsstængler, elefantgræs, hvedehalm og pil, blev frigjørt henholdsvis 46%, 27%, 19% og 12% sukkerinholdet. Efterfølgende blev metanpotentialet af de WE behandlede biomasser bestemt. Det blev fundet at, selvom høj frigørelse af opløselig sukker blev opnået efter WE, var metanpotentialet lavere i forhold til den rå biomasse med undtagelse af pil, for hvilket metanpotentialet blev øget med 80%, som gør det enregimæssigt rentabelt at bruge WE for pil.

Ensilering af pil og elefantgras med eller uden biologiske additiver (mælkesyrebakterier LAB) blev testet i ensileringsperioder mellem 1 og 5 måneder. Både pil og elefantgræs tabte 2 og 3% af vægten inden for 5 måneder. Målingen af metanpotentialet på ensileret pil viste, at bionedbrydeligheden af pil blev forbedret under ensilerings-processen. Metanpotentialet af ensileret pil (3 måneder) var 20% højere end af rå pil. Ingen væsentlige ændringer af metanpotentialet af ensileret elefantgræs blev observeret i forhold til rå elefantgræs. Desuden viste ensilering af pil og elefantgræs med additiver ingen fordele i både biomassetab og metanpotentiale i sammenligning med ensileringsprocessen uden tilsætningsstoffer.

Batch eksperimenter med samudrådning af svinegylle med forskellige afgrøder i forskellige VS forholdet mellem svinegylle og afgrøder viste, at balancen mellem kulstof og kvælstof (C: N-forhold) har spillet den vigtigste rolle: Ved en fastsat mængde af tilsat gylle, steg metanudbytten (i form af ml CH4 / g VS) i takt med lavere tilføjelse af afgrøder. Derimod gave en højere tilføjelse af afgrøde en højere volumetriske reactor produktivitet i form af m3 CH4 produceret per m3 reaktorvolumen. Dog kan en højere input af afgrøder også forhøje både metanudbyttet og den volumetriske reaktorproduktivitet, hvis kvælstof koncentrationen af gyllen er høj: En kontinuerlig reaktorforsøg med samudrådning som brugte gylle med et højere kvælstofindhold end gyllen brugt i batch forsøgene, viste at et TS forhold af 1:1.5 (gylle: hvede halm) gav en 23% højere methanudbytte og en 111% højere volumetrisk reaktor produktivitet sammenlignet med et TS forhold af 1:1.

INTRODUCTION TO THE THESIS

Methane production through anaerobic digestion of organic materials is a robust technology for renewable energy Methane can be used for the replacement of for fossil fuels in both heat and power generation and as well as a vehicle fuels, thus contributing to the greenhouse gas emission and slowing down the climate change. Currently, 22 large-scale biogas plants are under operation in Denmark and the construction of new large-scale facilities is on the way. These plants have been playing and will still play the major role in cleaning up the environment. All these plants are based on manure as main substrate, but their economical profitable operation relies on the addition of other biomass products with a high biogas yield. The biogas yield from raw manure alone is only 20–30 m^3/t while the operation is only profitable when biogas yields higher than 30 m^3/t can be achieved. This is currently done by addition of industrial organic waste materials such as fat sludge or fish oil. However, the volume of these waste streams is limited and almost all of these wastes available in Denmark have been currently treated in the existing biogas plants and some of the biogas plants have started to import high potential organic wastes (Danish Energy Agency, 1995).

Especially with the installation of further biogas plants, the positive economy will rely on the addition of high potential waste types. The addition of energy crops or crop residues with a high biogas potential is, therefore, very attractive in order to improve the economy of the plants. Furthermore, using the existing biogas plants for the production of bio-fuel from energy crops or crop residue is a low-cost way of renewable energy production. Introducing energy crops or crop residues into the biogas production in combination with manure does, furthermore, ensure the valorization of the process effluent as fertilizer product. However, to implement this idea for industrial scale production, there is a need for detailed investigation on the effect of the addition of the crop biomasses and the optimization of the anaerobic digestion process.

The main objectives of this study are:

o Reviewing the different control parameters of anaerobic digestion process and different pre-treatment methods of energy crops in the last decades
o Investigating the energy balance and cost-benefit analysis of biogas production from perennial energy crops pretreated by wet oxidation
o Characterizing and identifying the most promising agriculture residues and energy crops suitable for farm- and centralized-scale biogas production
o Evalating the effect of wet explosion as a pretreatment method on biogas production from different crops

- Investigating the biogas yield of co-digestion of swine manure with agriculture residue wheat straw at different ratio
- Investigating the effects of ensilage process as a pretreatment and storage method on methane production from perennial crops.

According to the objectives, the results of this study were included in the following papers:

Paper 1 is an introduction to the anaerobic digestion process and different pretreatment methods of crops by reviewing previous studies within this research filed. (The results included in the paper were carried out by both literature review and laboratory experiments by the student).

Paper 2 presents the experimental results of wet-explosion pretreatment of wheat straw under various conditions, and the results of co-digestion of wheat straw together with swine manure under thermophilic conditions (55 °C).

Paper 3 displays the experimental results for co-digestion of swine manure with different crops (with and without wet explosion pretreatment) at various TS ratio between manure and crops. The results were obtained in both batch and continuous experiment.

Paper 4 presents the results for strategies, energy balance and cost-benefit analysis of biogas production from perennial energy crops pretreated by wet oxidation.

Paper 5 presents the experimental results for ensilage of perennial crops willow and miscanthus for 1, 3 and 5 months with and without biological additives.

Paper 1

Biogas production from energy crops and agriculture residues: a review

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Biogas production from energy crops and agriculture residues: a review

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1. Introduction

Due to that the amount of fossil fuels is limit and and the relative price are increasing, alternative renewable resources such as bioenergy, wind or solar power have been developed or promoted from last decades. For wind or solar power, they are often limited by climate or location and seasons. Therefore, bio-energy will be one of the most significant renewable energy sources for next few decades[4]. Bioenergy can for example be obtained by anaerobic digestion of organic compounds to methane rich biogas. Biogas can be used in replacement for fossil fuels in both heat and power generation and as a vehicle fuel. In comparison with the other renewable energy sources, the advantages of the technology are: 1) environmental friendly due to the reduction of the CH_4 and CO_2 emission; 2) suitable for most locations and climates around the world; 3) mature technology and easy to operate the process. In 2007, was there approximate 4,242 farm-scale and more than 26 centralized biogas plants in EU treating agricultural residuals alone or codigested together with different industrial organic waste for the purposes of waste treatment and biogas production. The total energy production from these biogas plants in EU in 2007 was estimated to approx 50 PJ[71]. In Europe, several type of anaerobic reactor are designed and applied for treatment of different organic waste and wastewater. For instance, conventional continuously stirred tank reactors (CSTR) are broadly extensively used for treatment of liquid livestock waste[72], sewage sludge and the organic fraction of household solid waste[73]. Conventional reactors with recycling like the contact process is typically used for treatment of sewage and other wastewaters [74]. Upflow anaerobic sludge blanket (UASB) reactors are typically applied for treatment of waste water from food and paper industries [75, 76], while anaerobic filters with fixed or suspended beds are used for industrial waste water treatment or sanitation of landfill leachates [77, 78].

At present, most of the biogas plants are run either at mesophilic temperature (35-37°C) or thermophilic temperature (52-55°C). Initially, thermophilic process was tend to be more sensitive to different process parameters like high ammonia content in the digester or organic loading rate compared to mesophilic process[71]. However, increasing knowledge about the microbiology of the thermophilic anaerobic microorganisms improves the possibility of controlling the operation at thermophile process. Previous studies have confirmed that the thermophilic process is as efficient and stable as the mesophilic process if maintained correctly [72, 80, 81]. The advantage of thermophilic process is that degradation of the substrate is substantially faster under thermophilic conditions than mesophilic. This means that the sludge retention time can be shorter in a thermophilic plant than in a mesophilic plant[71]. Moreover, themophilic digestion has been demonstrated as an efficient mean to destroy pathogens [64, 68, 69] and in coherence with the new and more strict legislation in some countries

concerning the level of pathogenic microorganisms in the digested effluent when applied as fertilizer, the thermophilic anaerobic digestion will probably be more promoted in the future[82].

In Denmark, there are about 22 centralized and 56 farm scale biogas plants currently under operation. Most of these plants use animal manure (mainly swine and cattle) as the primary feedstock, and more than 2.1 million tons of animal manure is treated annually [71].Due to the low organic matter content (around 5% for swine manure and 8% for cattle manure) of the animal manure[79], the economically profitable operation of these biogas plants relies on the addition of other biomass products with high biogas yields. The biogas yield from manure typically ranging from 10 to 20 m^3/t while the operation is only profitable when biogas yields higher than 30 m^3/t of treated material can be achieved under Danish conditions[83]. This is currently done by addition of industrial organic waste such as fat sludge or fish oil. Therefore, the investigation of other biomasses to be codigested with manure is of great interest. In addition, many wastewater plants in Denmark use anaerobic digestion to stabilize the sewage sludge.

Recently, codigestion of animal waste with energy crops or crops residues is gaining attention in many parts of the world. Crop residues such as corn stalker and straws are produced in large quantities in both European countries and rest of the world every year, it has been estimated that within the agricultural sector in EU, 1,500 million tons of crops could be anaerobically digested each year, and half of this potential accounted for energy crops [84]. Crops like corn stalker or straw, due to their organic nature, can be valuable alternative feedstock for biogas production [85]. The concept of codigestion animal manure with different crops is not new. In early 1980s Hills, Roboert and Hashimoto reported that in codigestion process, manure could provide buffering capacity and wide range of nutrients, while the added plant material with high carbon content could improve the carbon/nitrogen ratio, and hereby decreasing the risk of ammonia inhibition to the digestion process [86, 87, 88]. These positive synergistic effects were considered providing potential for higher methane yields[89]. Because of its large unexploited potential for biogas production via anaerobic digestion, crop residues certainly deserve more research attention for being used as a feedstock for co-digestion with animal manure[90]. On the other hand, cultivation of perennial energy crops such as switchgrass, willow and miscanthus has been recognized require much less of energy to plant, nutrient and pesticide supply compared to annual crop like corn. Initially, energy crop willow was burnt for energy production and miscanthus for bioethanol production [91, 92]. But using willow and miscanthus for biogas production has rarely been investigated. However, one of the major problems encountered while digesting these crops is the low digestibility due to their lignocellulosic composition. Therefore, to improve methane production from these crops, a suitable pretreatment method is needed to break the lignocellulosic structure and make the embedded sugar polymer bio-available [12, 13, 16, 19].

Preset study covers some of the recent research in biogas production from different energy crops and agriculture residues with main focuses on: 1) the important parameters of choosing crops for biogas production; 2) the different pr-treatment methods for crops intended for biogas production i.e., pretreatment of the crops physically, chemically, biologically or as combination of theses; 3) strategies for codigestion of swine manure with energy crops and other agriculture residues.

2. Anaerobic digestion (AD) of biomass

2. 1. Anaerobic digestion

Anaerobic digestion includes a series of biological processes in which microorganisms breakdown biodegradable material in the absence of oxygen. It has been used for industrial or domestic purposes to treat waste and to produce energy. The process produces a methane and carbon dioxide rich biogas suitable for energy production. After anaerobic digestion, the nutrient-rich digestate can be used as fertilizer. There are a number of microorganisms that are involved in the anaerobic digestion system. During anaerobic digestion, the biomass undergoes a numbers of different processes and is converted to intermediate molecules such as sugars, hydrogen, and acetic acid before converted to biogas. Fig. 1 shows the different stages of anaerobic digestion occurring in anaerobic biogas reactors [36, 37, 38, 39]. In Stage I, hydrolytic and fermenting bacteria break down large organic polymers into their smaller constituent parts. These constituent parts or monomers such as sugar are readily available by other bacteria. The process of breaking these chains and dissolving the smaller molecules into solution is called hydrolysis. Acetate and hydrogen produced in Stage I can be used directly by methanogens. Other molecules such as volatile fatty acids (VFAs) with a chain length that is longer than acetate –e.g. propionate and butyrate must first be catabolised into compounds that can be directly utilised by methanogens. This takes place in Stage II, which is called acidogenesis, where there is further breakdown of the remaining components by acidogenic (fermentative) bacteria. Here VFAs are created along with ammonia, carbon dioxide and hydrogen sulfide as well as other by-products. The third stage of anaerobic digestion is called acetogensis. In this stage, simple molecules is created and further digested by acetogens to produce largely acetic acid as well as carbon dioxide and hydrogen. Stage VI is the last stage which is called methanogenesis. Here methanogens utilise the intermediate products of the preceding stages and convert them into methane, carbon dioxide and water. It is these components that make up the majority of the biogas emitted from the system.

In reactors with well balanced microbial communities, anaerobic degradation of complex organic matter occurs without accumulation of reduced intermediates. It has been shown that a sudden increase

of hydrogen concentration in the reactor can cause buildup of volatile fatty acids creating instabilities and lead to digester failure [43].
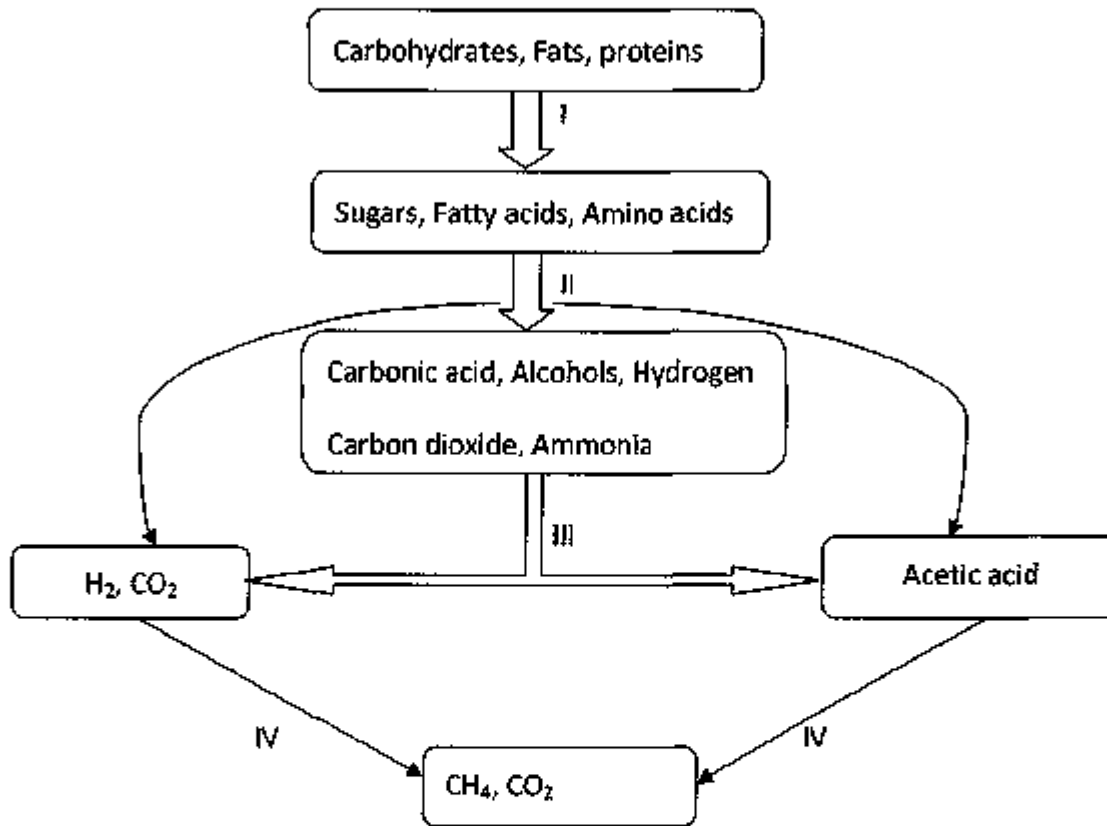
Fig.1. Biological and chemical stages of anaerobic digestion occurring in anaerobic biogas reactors

I. Bacterial hydrolysis of the input materials in order to break down insoluble organic polymers such as carbohydrates and make them available for other bacteria. II. Acidogenic bacteria then convert the sugars and amino acids into carbon dioxide, hydrogen, ammonia, and organic acids. III. Acetogenic bacteria then convert these resulting organic acids into acetic acid, along with additional ammonia, hydrogen, and carbon dioxide. IV. methanogens convert hydrogen and acetic acid to methane and carbon dioxide[36].

2.2 Anaerobic digestion reactors

2.2.1. Continuous stirred tank reactor (CSTR)

Anaerobic CSTR reactor (**Fig. 2 a**) is a continuous reactor, which is used most frequently with liquid substrate in the range of 2-8% of total solids (TS) concentration, but it can handle more solid reactions as well in the range of 16-22% of TS concentration[4]. However, all the anaerobic CSTR reactors have the same components, an inlet part that brings all the substrate into the digester at a regulated rate, a digester with a stirrer inside that rotates around to mix the substrates, and finally an outlet part, through which the digested substrate can be exited from the reactor. The rates of the inlet and outlet must be the same to keep the working volume of the digester steady. To run an anaerobic CSTR reactor for biogas production, there are several indicators such as biogas production, pH and concentration of VFA that can be used to exam the initial failure of the reactor. The rationale for the use of theses parameters is that the imbalance due to adverse operation normally gives accumulation of intermediary products, especially VFA and decreased gas production [48, 49, 50]. A lowering of the pH can be caused by increased concentration of organic acids in the absence of sufficient buffering capacity. These indicators thus provide information on an ongoing reactor failure, which may have progressed beyond the point which would allow for control measures. The indicators may be adequate for detection of gradual changes leading to slowly progressing reactor stress, but may not be suited in cases of more harmful condition requiring a prompts corrective response[51].

2.2.2 Upflow anaerobic granular sludge (UASB) reactor

UASB reactor (**Fig. 2 b**) is one of the most popular high-rate reactors for anaerobic biological treatment of wastewater. UASB reactors represented 41% of all full scale industrial anaerobic wastewater treatment plants in 1983. Similar to CSTR reactor, UASB reactor includes an inlet and an outlet, but the digester is divided into four compartments: 1) the granular sludge bed, 2) the fluidized zone, 3) the gas-solids separator, and 4) the settling compartment[52]. Granular sludge can be naturally formatted during treatment of soluble substrate in reactors operating in up-flow manner[53]. Organisms of both genus *methanosarcina* and *methanosaeta* have been found to play an important role in formation and maintainance of methanogenic granules in UASB reactor[52].

UASB reactors are most frequently applied for industrial wastewater[93], and typically suited to dilute waste water streams or liquid manure (3% TS with particle size < 0.75mm) [94]. Therefore, in Denmark most of the organic wastes with high TS concentration (manure slurry or primary sludge) are treated by CSTR reactors.
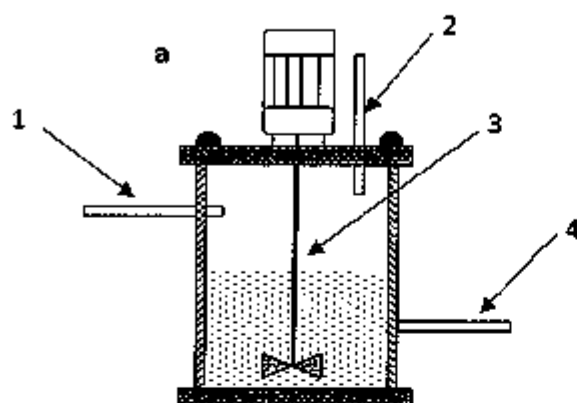
1. Inlet, 2. Biogas release port
3. Stirrer, 4. Outlet
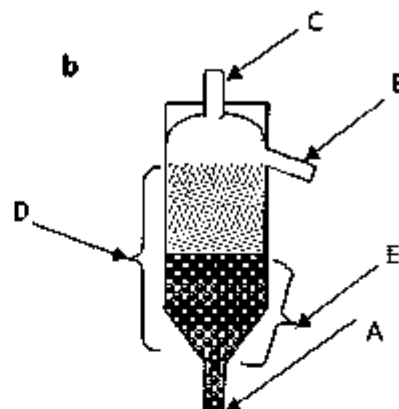
A. Inlet,   B. outlet,   C. Gas outlets
D. liquid phase,   E. Granular sludge bed

Fig. 2. CSTR and UASB reactor illustrate figure

2.2.3 Configuration of anaerobic digester

- **Feeding methods:** Biomass substrates with TS of 12% or less normally are handled and fed into the digesters by pumps; these feeding pumps can be used for external stirring digesters. Biomass substrates with TS higher than 12% are typically fed into digesters by screws.

- **Pasteurization:** Besides the effect of the temperature in the digester on pathogens and weed seeds, additional pasteurization maybe needed - especially for centralized biogas plants, where typically the digestate is distributed to farms. For some animal by-products pressure sterilization is needed in order to use these as substrate in biogas plants[95].

- **Stirring systems:** For many years the biomass inside the digester has been one of the critical points for optimizing the operation of biogas units. The many different substrate used do indeed have different characteristics, potentially resulting in the formation of floating layer or sedimentation. So the stirring system must be designed in accordance with the planned use of biomass substrates.

- **Handling of digestate**: The traditional way of utilizing digestate as a bio-fertilizer is well-documented, the breakdown of the organic substance in the substrates typically resulting in the mineralization of the nutrients, thus making these more easily available to the plants. Hansen et al.[96] reported that in the biogas reactor biomass is broken down and convert into biogas. During that process organic nitrogen is converted into plant available ammonium, which means that content of plant available ammonium in digested manure is higher than in untreated manure. Moreover, when organic matter is broken down in the biogas reactor, the viscosity of the manure decreases, and manure becomes a very fluid, that infiltrates the soil relatively faster application. In the soil the ammonium is protected from ammonia volatilization, and the fertilizer value in the manure is maintained.

The information above is mostly concluded by Danish agriculture advisory service 2007.[71]

2.3 Single-phase and two–phase treatment

In a two-phase digester, the undigested substrates from the first phase can be digested in the second-phase digester, carrying out the same reactions as the first phase but running at a different retention time or temperature. For easily digesting biomass, a two-phase reactor can have a lower overall retention time than a single-phase. The second phase could be a stirred tank or a plug-flow digester or an anaerobic filter[4]. A two-phase digester is a mechanically similar system of two stirred-tank digesters. In this process, fermentation and methanogenesis are separated by using different retention times. Liquefaction and acidification of the substrate are accomplished in the first reactor, while only methanogenesis takes place in the second reactor. It was first promoted by Ghosh et al.[65] in 1975 for the combined digestion of sewage sludge and municipal solid waste. The total digestion time was considerably lower than the conventional single-phase digestion. Some kinetic considerations argue in favor of the two-phase approach when optimal growth conditions for hydrolytic and methanogenic bacteria are considered[66]. Two-phase AD of the organic fraction of MSW was studied by Hofenk et al.[67], who concluded that there was no difference in the biogas yields between single-stage and two-phase systems. Unless the hydrogen produced in the fermentative phase can be collected and transferred to the methanogenic phase, a loss of potential CH_4 occurs. This two-phase system is technologically feasible, but an assessment of the economic feasibility is more complex and has to be reviewed for any given situation.

Recently, Winterberg, Seela and Wilke 2006[97] defined three main concepts for digester/ reactor configuration: *1) One stage methanogenic system*. This system can be used for both plants and animal manure. The biomass is pumped directly from a storage tank (in the case of animal manure) to the

digester, whereas the more solid energy crops are typically fed directly into the digester (s). And the bio-fertilizer is transferred/ pumped directly from the digester (s) to the post storage tanks; *2) Two-stage methanogenic system.* In this system, the biogas production takes place in two digesters running serially (main and post digester). The main digester has a high organic loading rate and short retention time. The main function of the main digester is equalization, homogenization and solids degradation, while the post digester is typically run with longer retention time, thereby obtaining the remaining part of biogas potential. *3) Two stages configuration with separated acidification phase.* This system can be applied on anaerobic treatment of waste water or in the field of municipal waste digestion, but has hardly ever been applied in the agriculture sector.

2.4 Temperature

There are two conventional operational temperature levels for anaerobic digesters, which are determined by the species of methanogens in the digesters[61]. Mesophilic digestion takes place optimally around 37-41°C or at ambient temperatures where mesophiles are the primary microorganism present. Thermophilic digestion takes place optimally around 50-55°C and even at elevated temperatures up to 70°C where thermophiles are the primary microorganisms present.

Traditionally, AD was mostly applied in the mesophilic temperature range of ambient temperature and up to 37°C. Thermophilic process was once believed to be less stable and more rapidly to process failure. Throughout the recent 20 years, however, more and more thermophilic biogas plants have been established and nowadays most of the centralized biogas plants in Denmark are operated under thermophilic conditions, proving that stable thermophilic digestion is no longer a problem[62]. Thermophilic operation offers the advantage of a higher reaction rate, causing a more profitable process with a lower retention time.

Operation at higher temperatures facilitates greater sterilization of the end digestate. In countries where legislation, such as the Animal By-Products Regulations in the European Union, requires end products to meet certain levels of reduction in the amount of bacteria in the output material, this may be a benefit[63]. Thermophilic operation leads to a better hygenisation of the waste material than mesophilic treatment. Typical pathogens found in manure are eliminated within some hours of thermophilic treatment at the biogas plant, while they may survive for longer periods in digester tanks which operate at mesophilic temperatures. The 90% decimation time for a number of pathogenic bacteria was less than 1.2 hours at 53°C, while it was between 0.9 and 7.1 day at 35°C[64]. Fecal coliforms could not be detected in the effluent of the thermophilic process whereas the original waste contained 3×10^3 CFU/g TS (CFU: colony forming units) and conventional aerobic compost produced in windrows from the

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same original material still counted 2×10^2 CFU/g TS^{68} . Kubler[69] has reported that after addition of pathogen seeds of *Salmonella typhimurium*, *Escherichia coli* and *Candida albicans* none of these indication microorganisms were detected after 11 h of thermophilic treatment at 55°C while up to 10^7-10^9 CFU/ ml were detected in a reference batch of the pathogen-infected waste after storage at room temperature. The thermophilic process was also shown to be useful for weed seed elimination[69]. Additional treatment at higher temperatures can be beneficial for further sanitation. In Denmark, a treatment of household waste at temperatures more than 70°C for 1 hour is required if the waste is used as fertilizer for consumable crops[64].

3. Selections of biomass for methane production

In general all kind of organic substances can be used for biogas production. Different input materials, however, strongly differ in energy content and digestion suitability, both of which affect biogas yield. **Fig. 3** shows the typically chain for producing methane through anaerobic digestion from energy crops and agriculture residues: from the production, animal feed, manure collection and harvested of crop biomass, to storage and pretreatment of the biomass, production and utilization of biomass, storage, post-treatment of the digestate, and finally returning the digestate back to the crop production area as fertilizer and soil-improvement medium.
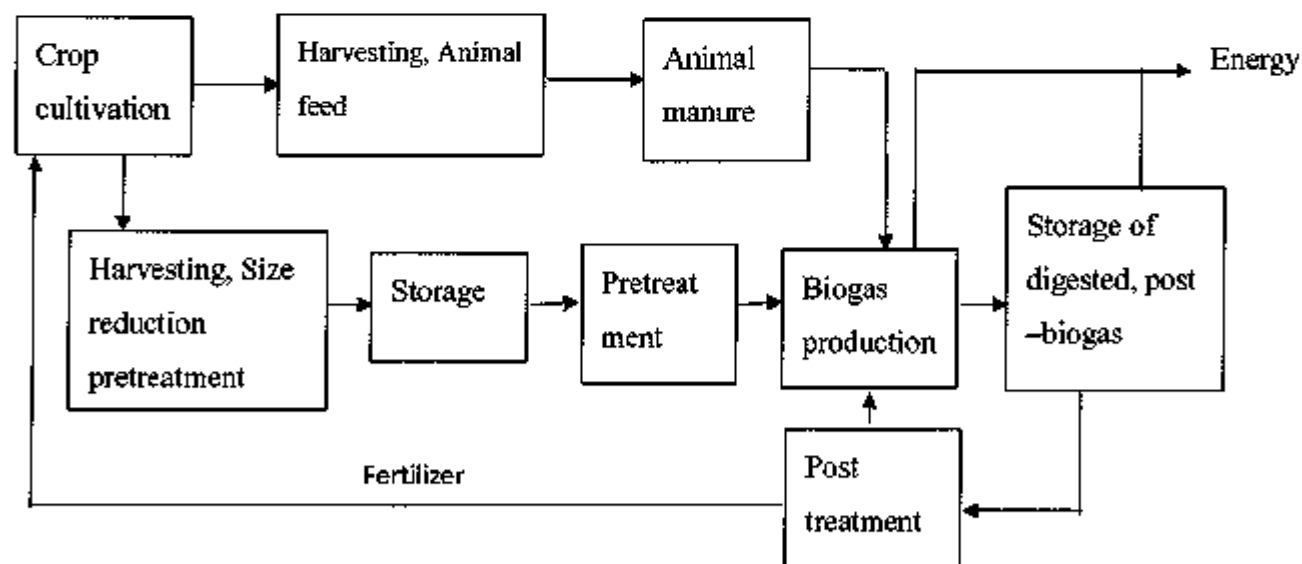
Fig. 3. The chain for producing methane through anaerobic digestion from energy crops and agriculture residues.

For choosing energy crops for methane production, the most important parameter is the net energy yield per hectare, which is defined mainly by biomass yield and convertibility of the biomass to methane, but the energy cost of cultivation and pretreatment has to be take into account. The methane yield on VS basis is also an important parameter to identify the promising crops (**Table 1**), but solid content and crop yield on the field are what will matter at the end when choosing a crops for biogas gas production. For instance, there are some crops have high methane yield on a VS-added basis, such as rhubarb at 0.49 m^3 CH_4/kg VS added[16], but it has low solid content, that results in a lower methane yield per hectare in the range of 800-1,700 m^3 CH_4/ha. Compare the methane yield on VS basis, corn stalker gives much lower methane yield than rhubarb in the range of 0.270-0.298, but 5,300-12,390 m^3 methane can be obtained per hectare. Crops can be obtained in two ways[1]. The first one is from agriculture residues such as wheat straw, corn stalker or garden waste, these biomass residues and waste are the by-products from agriculture, forestry, forest, or agriculture industries and households. Crops like cornstalk and wheat straw are easy to cultivate and produce in large amounts. Moreover, they have the advantage of being familiar to farmers and suitable for harvesting and storing with the existing methods and machinery. The second way is from dedicated energy crops. These crops are grown first and foremost for energy. The ideal energy crop has efficient solar energy conversion resulting in high yield, needs low agrochemical inputs, has low water requirement, and has low moisture level at harvest. Plants with perennial growth habits such as miscantus and switchgrass are particularly promising, and have the advantages of low establishment cost (when average across the rotation) and greater resilience in drought. Perennial crops like willow and miscanthus have been in focus in Denmark. They have a high yield potential, low need of fertilizer and in an established crop there are none or very little needs of pesticides. Both crops are able to reduce the leaching of nitrate and thereby protect the ground water. So far willow is the only crop grown at a commercial scale in Denmark[3].

In the southern part of Demark, crop residues (corn stalker and wheat straw) and energy crops (willow and miscanthus) were collected in the middle of august. The methane potential of each crop was carried out under the condition of 0.5 cm (particle size), 55°C, 6% (TS concentration), 60 days (retention time) and 117 ml (volume of the batch reactor). As shown in **Table** 1, all these crops show high methane potential except willow, which gives only 0.12 m^3 CH_4/kg VS added. For crop corn stalker, wheat straw and miscanthus, methane potential is 0.40, 0.26 and 0.27 m^3 CH_4/kg VS added, respectively. Therefore, crops like willow needs to be more pretreated in order to have higher methane potential. (See chapter 4)

Table 1. Methane potential from different crops and crop residues

Biomass	Reactor type [1,2]	$m^3 CH_4 kg-1 VS-added$	Country	References
Alfalfa	BA	0.24	DE	41
Clover	BA	0.29–0.39	AT	42
Oats, straw	BA	0.32±0.02	FI	43
Sugarbeet leaves	CSTR	0.17–0.22	DE	41
Corn stalker	BA	0.27–0.29	DE	41
Ryegrass and clover	CSTR	0.49±0.05	NZ	44
Lupine	BA	0.36±0.04	FI	43
Marrow kale	BA	0.31±0.02	FI	43
Tall fescue	BA	0.33–0.34	AU	45
Vetch	BA	0.32	DE	41
Giant knotweed	BA	0.17	FI	46
Festlolium	BA	0.32–0.35	AU	45
Rapeseed	BA	0.24±0.02	FI	43
Grass	BA	0.29–0.31	DE	41
Cocksfoot	BA	0.33–0.34	AU	45
Vetch - oat	BA	0.41±0.02	FI	47
Grass, lawn	BA	0.30±0.04	FI	47
Grass, fresh	BA	0.23±0.03	FI	47
Sugarbeet leaves	BA	0.29	DE	41
Corn stalker	BA	0.40±0.05	DK	*
Wheat straw	BA	0.26±0.02	DK	*
Miscanthus	BA	0.27±0.03	DK	*
Willow	BA	0.12±0.01	DK	*

*[1] Batch assays [2] Continuously stirred tank reactors. * Results carried out in Risø-DTU*

4. Pretreatment of crops intended for methane production

The structure of energy crops or agriculture residues mainly consists of cellulose, hemicellulose and lignin (**Fig. 4**), i.e. lignocellulose. In addition to these compounds, crop biomass can contain e.g. non-structural carbohydrates such as glucose, fructose, sucrose and fructans, proteins, lipids, extractives and pectin[12]. Lignin is not degraded in anaerobic conditions, and the rate and extent of lignocellulose utilization is severely limited due to the intense cross-linking of cellulose with hemicellulose and lignin. Moreover, the crystalline structure of cellulose prevents penetration by micro-organisms or extracellular enzymes[13]. As the rate-limiting step in anaerobic digestion of solid materials such as energy crops and crop residues is hydrolysis of complex polymeric substances[14,15], one way of

improving the methane production from anaerobic digestion of lignocellulosics is to pre-treat the substrate in order to break the polymer chains to more easily accessible soluble compounds.
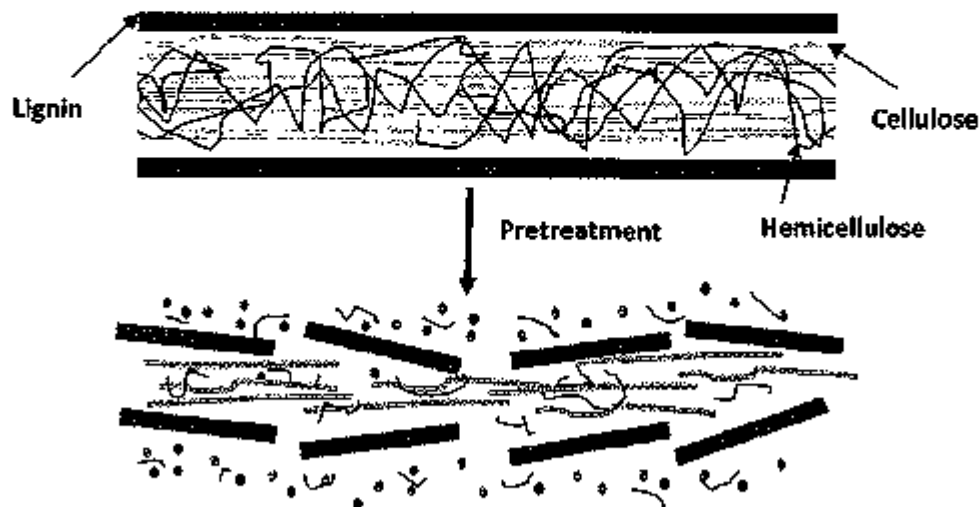
Figure 4: Lignocellulose Crops structure and pretreatment

An ideal pre-treatment would increase surface area and reduce lignin content and crystallinity of cellulose[16]. Pre-treatments can be carried out either physically, chemically or biologically, or as combinations of these [19]. Pre-treatments have been quite intensively studied for facilitating the enzymatic hydrolysis and consequent ethanol production from lignocellulosic substrates[29], but there is less information available on the effects of pretreatment crops biomass for biogas production.

4.1. Review on different pretreatment methods

- *Size reduction*: Biogas yield can be improved by reducing the particle size of the crop biomass because size reduction can increase the available surface area and release the intracellular components[24]. The studies on the effect of particle size reduction on biogas potential conducted by Sharma et al.[25] and Kaparaju et al.[26] (**Table 2**) indicated that, in batch experiment with wheat straw, rice straw, mirabilis leaves and dump grass, biogas yields increased with decrease in particle size, but the difference between the smallest particle sizes tested (0.088-0.40 mm) was

small. Biogas potential test on oats, grass hay and clover indicated that there was no difference of biogas production observed between particle size of 5, 10 and 20 mm on oat, whereas the 10

Table 2 Biomass size reduction pretreatments associated with improvement in methane yield for ligmocellulosic biomass.

Pretreatment methods	Crops	Particle side (mm)	CH4 potential (m3 CH4/kg VS added)	Reference
	Wheat straw	150	0.13	25
	"	6	0.20	"
	"	1	0.24	"
	"	0.4	0.23	"
	"	0.088	0.23	"
	"	20	0.26	"
	"	0.5	0.33	"
	Rice straw	150	0.24	"
	"	6	0.35	"
	"	1	0.36	"
	"	0.4	0.37	"
	"	0.088	0.37	"
	Mirabilis leaves	80*50	0.29	"
	"	6	0.33	"
Mechanical	"	1	0.33	"
size reduction	"	0.4	0.34	"
(mm)	"	0.088	0.34	"
	Dhup grass	30	0.14	"
	"	6	0.21	"
	"	1	0.21	"
	"	0.4	0.23	"
	"	0.088	0.23	"
	Oats	20	0.25	26
	"	10	0.25	"
	"	5	0.26	"
	Grass hay	20	0.27	"
	"	10	0.35	"
	"	5	0.32	"
	Clover	20	0.21	"
	"	10	0.14	"
	"	5	0.2	"

mm particle size was found optimal for grass hay and least optimal for clover. However, all these tests were performed in laboratory biogas potential tests, on the basis of which it is difficult to determine the importance of particle size for full scale operation.

- *Steam pretreatment/steam explosion:* It is one of the pretreatment in thermal pretreatment category. During steam pretreatment the biomass is put in a large vessel and steam with high temperature (up to 240°C) and corresponding pressure, is applied for few minutes. After a set time, the steam and pressure is released and biomass is quickly cooled down. The objective of the steam pretreatment/steam explosion is to solublize the hemicellulose to make the cellulose better accessible for enzymatic hydrolysis and to avoid the formation of inhibitors [98]. A recent study from Alexander Bauer et al.[99] reported that steam pretreatment of wheat straw under the condition of 170°C and10 minutes can increased the biogas yield by 31% compared to untreated straw[99]. Another study form Liu et al.[100], steam explosion pretreatment of municipal solid waste under the condition of 240°C and 5 minutes biogas yield increased 40%. However, steam pretreatment includes a risk on production of compounds like furfural, HMF, and soluble phenolic compounds. These compounds are inhibitory to the methane production. The biogas producing bacteria are however capable of adapting, at least to a certain concentration, to such compounds[101, 102, 103]. Moreover, steam pretreatment includes a risk on condensation and precipitation of soluble lignin components, making the biomass less digestible, and therefore reducing the biogas production.

- *Acid pretreatment:* The pretreatment can be done with dilute or strong acids. The main reaction that occurs during acid pretreatment is the hydrolysis of hemicellulose, especially xylan as glucomannan is relatively acid stable. Solublized hemicelluloses (oligomers) can be subjected to hydrolytic reactions producing monomers, furfural, HMF and other (volatile) products in acidic environments[104,105]. During acid pretreatment solublized lignin will quickly condensate and precipitate in the acidic environments[106,107]. The solublization of hemicellulose and precipitation of solubilized lignin are more pronounced during strong acid pretreatment compared to dilute acid pretreatment. For methane production, acid pretreatment of lignocelluloses material is one of the attractive methods because methanogens can handle compounds like furfural and HMF to a certain concentration. Study from Weiping Xiao and WilliamW. Clarkson[108] reported that acid pretreatment of newsprint under the condition of acetic acid concentration of 35%, 2% nitric acid and boiling for 30 min. The biogas yield from treated newsprint bioconversion increased nearly three times over that of untreated newsprint in a 60-day test. However, the major drawback of this pretreatment method, particularly at low

pH, is the formation of different types of inhibitors such as carboxylic acids, furans and phenolic compounds[109, 110]. If the concentration of these compounds is high they usually inhibit the microbial growth and fermentation, and this will result in lower productivity of biogas. Therefore, the pretreatments at low pH should be selected properly in order to avoid or at least reduce the formation of these inhibitors [111].

- *Alkaline pretreatment*: During alkaline pretreatment the first reactions taking place are solvation and saphonication. This causes a swollen state of the biomass and makes it more accessible for bacteria[102]. Application of alkaline solutions such as NaOH, $Ca(OH)_2$ (lime) or ammonia to remove lignin and a part of the hemicellulose, and efficiently increase the accessibility of enzyme to the cellulose. The alkaline pretreatment can result in a sharp increase in saccharification with manifold yields[112]. Using alkaline pretreatment of biomass for biogas production was studied by several authors, e.g. a treatment of waste-activated sludge with 0.3 g NaOH/g volatile solids (VS) at 130°C for 5 min resulted in 40-50% solubilization of VS and more than 200% improvement in methane production compared to the control experiment [113, 114]. Experiment from Lehtomäki et al. [19] reported that pretreatment mixture of sugar beet tops grass hay straw at condition: 2% NaOH 24 hours and 72 hours, the biogas production was increased 9 and 17% respectively, condition at 3% $Ca(OH)_2$+4% Na_2CO_3 72h, the biogas production was also increased 17% compared to untreated biomass (**Table 3**). Study from Frigon J-C et al.[60] reported that alkaline pretreatment of summer and winter switchgrass under the condition of 7 g/L NaOH for 3 hours can increase the biogas production maximum 32% (**Table 3**).

Table 3 Chemical pretreatments associated with improvement in methane yield for ligmocellulosic biomass.

Pretreatment methods	Crops	Pretreatment condition	CH4 potential (m3 CH4/kg VS added)		% CH4 increase	Reference
			Before pretreatment	After pretreatmen		
	Sugarbeet tops					
	Grass hay					
	Straw	Alkalis	0.23	0.25	9	19
	"	2% NaOH 24h	0.23	0.25	9	"
	"	2% NaOH 72h	0.23	0.27	17	"
Chemical pretreatment	"	3% Ca(OH) 2+4% Na2 CO3 72h	0.23	0.27	17	"
	"	xylanases, cellulases	0.23	0.27	17	"
	Summer switchgrass	7 g/L NaOH 3h	n.r.	0.38	0-+32	60
	Winter switchgrass	7 g/L NaOH 3h	n.r.	0.13		"

- *Wet-oxidation pretreatment*: Wet oxidation has been applied as pretreatment for both ethanol and biogas production. In this process, the biomasses are treated with water and oxygen or oxidation agent such as hydrogen peroxide (H_2O_2) at high temperature above $120^\circ C$ and for a period of e.g. 5 minutes [115, 116, 117]. The temperature followed by reaction time, oxygen/the mount of oxidation agent and pressure are the most important parameters in wet oxidation [21]. The objective of the pretreatment is to breakdown the hemicellululose and lignin structures to increase the accessibility of the cellulose. During the wet oxidation pretreatment several reactions can take place such as electrophilic substitution, displacement of side chains, cleavage of alkyl aryl ether linkage or the oxidative cleavage of aromatic nuclei[118]. Previously, wet-oxidation pretreatment of several type of bio-wastes was studied by Lissens et al.[119]. They used wet oxidation to improve anaerobic biodegradability and biogas yields of food waste, yard waste and digested bio-wastes treated in a full-scale biogas plant with pretreatment condition: $185-220^\circ C$, oxygen pressure 0-12 bars and 15 minutes. They reported that the wet oxidation process increased biogas yields by approximately 35-70% from raw and digested lignocelluosic bio-wastes.

- *Wet-explosion pretreatment:* Most recently, a new wet explosion pretreatment equipment has been developed by Ahring and Munck (2006)[21]. The principle of the treatment is to heat the biomass with water on a high temperature of at least 170°C, and provide an oxidation reaction under high pressure by addition of an oxidizing agent (H_2O_2). In subsequent step, the material undergoes a sudden pressure release (steam explosion). The method is a combination of steam explosion, and wet oxidation and it enables operating with high biomass concentrations and handling of big particle sizes, thereby, avoiding initial energy intensive mechanical milling. Moreover it is an easy controllable process with low total energy consumption[21]. The wet explosion method has so far been successfully employed for the pretreatment of wheat straw for ethanol production[22, 23]. Therefore, it was interesting to investigate whether the wet explosion pretreatment also has a beneficial effect on the biogas productivity when wheat straw and others crops is used as a feedstock. The most important parameter of evaluation the wet explosion process is to exam the amount of soluble sugar released from lignocellulosic biomass. The results of pretreatment different biomass can be very due to the structure and lignin content. Previously we have optimized the pretreatment condition for agriculture residue corn stalker, wheat straw and energy crop willow and miscanthus by manipulating the parameters of the wet explosion process. The optimal process conditions was found by changing retention time from 2 to 8 minutes, amount of oxidizing agent added 3 to 8% of TS concentration, temperature from 150 to 195 °C and pressure from 5 to 14 bars. The above changes was made based on the best process conditions for pretreatment of wheat straw, which was 5 minutes retention time, 12 bar, 185°C, and the H_2O_2 concentration was 6% of TS concentration[27]. **Table 4** shows the soluble sugar released amount of corresponding pretreatment conditions. The characterization results of soluble sugar concentration were significantly high after wet explosion pretreatment compared to raw biomass. The maximum soluble sugar released amount of wheat straw, corn stalker, willow and miscanthus were 19, 46, 12 and 25% higher than the raw crops (**Table 4**).

- **Table 4** Parameters for adjusting optimal pretreatment conditions of crops and soluble sugar released amount.

	g of soluble sugar released/100g (TS)				
Energy crops and Crop residues	Retention time (min) 2/5/8	TS concentration (%) 10/15/20	H_2O_2 concentration (% of TS) 3/6/8	Temperature (°C)150/180/195	Pressure (bar) 5/10/14
	12.07±0.2	19.53±0.20	13.13±0.20	13.13±0.30	13.13±0.30
	15.83±0.53	15.83±0.53	15.83±0.53	15.83±0.53	15.83±0.53
Wheat straw	13.86±0.45	15.40±0.22	15.22±0.60	0.92±0.60	0.92±0.60
	29.84±1.34	28.54±1.55	33.66±1.15	30.29±1.83	30.29±1.83
	29.89±1.45	29.89±1.38	29.89±1.23	29.89±1.10	29.89±1.10
Corn stalker	31.92±1.35	46.62±1.33	18.28±1.27	11.71±1.37	11.71±1.37
	6.51±0.54	9.08±0.33	7.01±0.33	1.35±0.25	1.35±0.25
	12.23±1.02	12.23±1.02	12.23±1.02	12.23±1.02	12.23±1.02
Willow	9.57±0.45	9.21±0.55	9.06±0.55	4.87±0.38	4.87±0.38
	7.56±0.52	25.09±1.15	9.00±0.66	5.15±1.23	5.15±1.23
	13.51±.98	13.51±0.98	13.51±0.98	13.51±0.98	13.51±0.98
Miscanthus	18.26±1.35	14.56±0.85	16.20±0.35	15.13±0.93	15.13±0.93

However, even the wet explosion pretreatment released significant amount of soluble sugar, the biogas yields of pretreated wheat straw, corn stalker and miscanthus still far too low compared to these fresh crops (the results was conducted by 60 days of batch experiment). The only positive effect was occurred from willow, biogas precaution from wet explosion pretreated willow was 80% higher than fresh willow (**Table 5**). The reason of biogas decreased in wheat straw, corn stalker and miscanthus was probably due to their structure are softer and easier to digest compared to woody crop willow. Therefore, when pretreatment these types of crops at high temperature and acidic condition, more soluble sugar are released meanwhile also more inhibitors are formed during the pretreatment, such as furfural, 5-hydroxymethylfurfural and phenolic compounds, this is a commonly known fact [120].

Table 5 Wet oxidation pretreatment associated with improvement in methane yield for ligmocellulosic biomass.

Pretreatment method	Crops	Pretreatment condition	CH_4 potential (m^3 CH_4/kg VS added)		% CH4 increase	Reference
			Before pretreatment	After pretreatment		
	Corn stalker	15%TS/6% H2O2/180oC/5 mins	0.56	0.36	-36	
	"	15%TS/3% H2O2/180oC/5 mins	0.56	0.46	-18	
	"	20%TS/3% H2O2/180oC/5 mins	0.56	0.39	-31	
	Wheat straw	15%TS/6% H2O2/180oC/5 mins	0.26	0.24	-6	
Wet oxidation	"	10%TS/6% H2O2/180oC/5 mins	0.26	0.23	-11	
	Willow	15%TS/6% H2O2/180oC/5 mins	0.15	0.27	80	
	Miscanthus	15%TS/6% H2O2/180oC/5 mins	0.27	0.21	-20,	
	"	15%TS/6% H2O2/180oC/8 mins	0.27	0.16	-39	Results from our lab.
	"	20%TS/6% H2O2/180oC/5 mins	0.27	0.19	-29	

- *Silage pretreatment*: Ensiling is a biological process that has been used to preserve forages for animal feed for centuries. Ensilage is also a convenient process for protecting the moisture of crops for biogas production because dying is not a favorable method when crops are used for biogas production. Instead, methods based on ensiling are often preferred[121]. For example, previously studies on willow **(paper 2 and 5)** have showed that biogas yield of willow harvested in winter time is 25% lower than the willow harvested in the summer. In the ensiling process, the soluble carbohydrates contained in biomass undergo lactic acid fermentation. This reaction will lead to a drop in pH and to inhibition of the growing detrimental microorganisms. At the same time, the acidification produces intermediates for methanogenic fermentation. In this way the ensiling process can be considered as a pretreatment which simultaneously has potential to promote methane production from plant matter [122]. Study from H. Vervaeren et al.[57] reported that ensiling of maize for 7 weeks influenced the methane production per VS in subsequent anaerobic digestion by up to 22.5%. The methane potential of silage rye (3 months), barley, milky (3 months) and maize (4 months), increase more than 20% compared to that fresh crops [58, 123]. However, many authors have reported various annual crops stored as silage have equal or higher methane potential to those of fresh crops **(Table 6)**, but studies on ensilage of perennial crops such as willow or miscanthus for methane production are very limited. However, ensilage of perennial crop willow and miscanthus was tested in our study **(Paper 5)**. The study shows that ensilage of willow and miscanthus with biological additives has no significant influence on the biomass losses compared to the non additives. The biomass losses amount of willow and miscanthus can be controlled under 2 and 3% with ensiling process, respectively. Ensilage of willow as pretreatment method for methane production can increase the methane potential. Taking the biomass losses into account, ensilage of willow for 1, 3 and 5 months can increase the total methane production by 12, 22 and 22% compared to fresh willow, respectively. The methane potential of all silage miscanthus was ranged within 181-189 ml CH_4 per g-VS original, giving no significant increase of methane production compared with fresh miscanthus. Moreover, due to the higher biomass losses, the total methane production from silage minscanthus after 5 months was 3% lower than the fresh miscanthus.

Table 6 Ensilage pretreatments associated with improvement in methane yield for ligmocellulosic biomass.

Pretreatment methods	Crops	Duration of ensilage (months)	CH₄ potential (m³ CH₄/kg VS added)		% CH₄ increase (Compared to original VS)	Reference
			Fresh crop	silage crop		
	Mixture of timothy, red clover meadow desue grass	2	0.36	0.42	17	56
	"	6	0.36	0.43	19	"
	Rey grass	2	0.41	0.47	15	"
	"	6	0.41	0.45	10	"
	Maize	1,6	0.52	0.58	12	57
	"	4	0.38	0.48	25	123
Ensilage (months)	Barley, flowering	3	0.43	0.46	5	58
	Barley, milky	3	0.50	0.65	31	"
	Ray, flowering	3	0.37	0.47	29	"
	Ray, milky	3	0.41	0.49	20	"
	Willow	1	0.14	0.16	15	59
	"	3	"	0.22	54	"
	"	5	"	0.22	56	"
	Miscanthus	1	0.19	0.19	-1	"
	"	3	0.19	0.19	-2	"
	"	5	0.19	0.19	-1	"

4.2. Summary of pre-treatment methods

Various pretreatment methods have been presented or developed for lignocelluloses and waste material in order to improve biogas production. All the pretreatmetn method in some of the point are approved that they can enhanced the biodegradedability of lignocelluloses material such as crops. But due to a lack of large scale experience and economical studies, the feasibility and applicability of most of methods cannot be reliably evaluated. Nevertheless, previously study (**Paper 4**) on energy balance and cost-benefit analysis of biogas production from perennial energy crops (willow) pretreated by wet oxidation/explosion was shown that the energy consumption of the pretreatment can be covered by the extra biogas it prouduced[124]. Amount all these pretreatment methods, silage pretreatment has the advantages of low energy and low or non chemical requirement, low or non inhibitors produced during the pretreatment process, easy can cheap to apply on both farm and centralized scale biogas plants, and mild environmental.

5 Co-digestion of swine manure with crops residues or energy crops

The concept of anaerobic co-digestion is a cost effective waste treatment method, in which two different types of organic wastes are mixed and treated tighter in single facility[34]. By doing so, one takes advantage of the abundance of special compound in one waste type to compensate for its shortage in other waste type, and consequently increase biodegradability and biogas production [34]. Using such approach, satisfactory results were obtained with several combinations of agriculture waste, e.g. swine and poultry waste or energy crops and cow wastes [35, 36]. In both cases, it was shown that co-digestion not only facilitated biodegradation of the organic compounds but also enhanced biogas production. In Denmark most of agriculture biogas plants use manure as primary feed stock, but it has been recognized that using animal manure alone may not represent the most efficient way to produce biogas due to its inherent deficiency of carbon source[90]. To increase the biogas yield per ton of animal manure, one of the options was to adding carbon rich crops [86, 87, 88]. In the past, co-digestion of animal manure with various crops has been intensively studied, i.e. co-digestion swine or cow manure with corn stalker or wheat straw at various VS ratio (manure: crop) (**Table 7**). But most of these studies were only focused on the mesophilic temperature in the range 37-39°C and annual crops such as corn stalker or wheat straw. Therefore, investigation of the effect of co-digestion swine manure with annual and perennial crops on thermophilic temperature could be interest.

Table 7. Co-digestion of swine and cow manure with plants biomass in CSTR reactor, results reported from literature.

Feedstock	Temperatur e (oC)	Manure: crop (VS ratio)	Feed TS (%)	HRT. Hydrolic retention time (d)	ml CH_4 per g-VS added	Ref .
Swine manure, corn stalker	39	75:25	8	16	210	125
Swine manure	35		n.r.*	39	140	126
Swine manure, potato waste	35	85:15	n.r.	26	220	126
''	35	80:20	n.r.	39	315	126
''	35	80:20	n.r.	25	290	126
Swine manure	35		n.r.	15	320	128
Swine manure, wheat straw	35	75:25	n.r.	15	240	128
''	35	50:50	n.r.	15	220	128
Cow manure	35		7.3	15	350	127
Cow manure, wheat straw	35	50:50	7.8	15	100	127
''	35	25:75	7.6	15	70	127
''	35	10:90	7.9	15	30	127
Cow manure	n.r.		10	40	107	129
Cow manure, wheat straw	n.r.	80:20	10	40	109	129
''	n.r.	60:40	10	40	113	129
''	n.r.	40:60	10	40	103	129
''	n.r.	20:80	10	40	97	129

*n.r. not reported

From previously study, the efficiency of co-digestion swine manure with both fresh and wet-explosion pretreated corn stalker, wheat straw, miscanthus and willow with different VS ratio (manure: crop)were tested (paper 3). The efficiency of the experiment was evaluated by two parameters: 1) The methane potential of the mixture biomass. 2) The biogas productivity per reactor volume. For wet-oxidized crops, there was a significant of decrease methane potential observed from wet oxidized corn compared with raw corn, and the cause of this is probably due to the inhibitory compounds releasement during the wet oxidation pretreatment[130], the similar results was also observed from wet-oxidized wheat straw and miscanthus (**Table 8**).

Table 8:

Results of methane potential test on different crops at different co-digestion ratio with swine manure.

Substract	Crop: manure (TS ratio)	Duration of the batch assay	Total CH$_4$ production	ml CH$_4$ per g-VS added[c]	ml CH$_4$ per g-TS added	% of total CH$_4$ increased than swine manure alone	% of total day 20	M^3 CH$_4$ produced/m^3 working volume/day (HRT 20 days)
Control	I[a]	75	21±0.5	0	0	0	0	0
	II[b]		140±3	362±4	238±3	0	87	0.28
Raw willow	17/83		187±3	282±3	277±3	34	93	0.37
	29/71		198±2	258±3	253±3	41	97	0.40
	38/62		218±2	251±2	246±2	56	90	0.44
	50/50	45	237±5	220±2	216±2	69	90	0.47
Wet oxidized willow	17/83		233±4	335±3	321±3	66	97	0.47
	29/71		264±3	328±4	315±4	88	99	0.53
	38/62		302±2	333±3	320±3	116	90	0.60
	50/50		313±5	277±2	266±2	124	92	0.63
Raw miscanthus	17/83		210±3	324±3	315±3	50	85	0.42
	29/71		248±1	334±5	324±5	77	76	0.50
	38/62		290±3	346±1	336±1	107	67	0.58
	50/50	55	357±2	346±2	336±2	155	62	0.71
Wet-oxidized miscanthus	17/83		195±3	238±6	228±6	39	71	0.39
	29/71		222±3	235±4	226±4	59	69	0.44
	38/62		263±2	248±5	239±5	88	83	0.53
	50/50		337±5	259±4	249±4	141	80	0.67
Raw corn stalker	17/83		257±3	410±3	394±3	84	84	0.51
	29/71		275±3	378±5	363±5	96	83	0.55
	38/62		336±5	410±2	394±2	140	70	0.67
	50/50	60	391±3	385±3	370±3	179	59	0.78
Wet-oxidized corn stalker	17/83		211±2	260±8	251±8	51	89	0.42
	29/71		201±2	211±4	205±4	44	88	0.40
	38/62		63±3	43±3	42±3	-55	85	0.13
	50/50		41±1	17±1	16±1	-71	95	0.08
Raw straw	17/83		200±2	320±3	298±3	43	82	0.40
	29/71		224±3	311±4	290±4	60	74	0.45
	38/62		266±5	329±5	306±5	90	78	0.53
	50/50	85	317±3	318±2	296±2	126	82	0.63
Wet oxidized straw	17/83		184±1	261±3	251±3	31	81	0.37
	29/71		206±1	254±4	244±4	47	75	0.41
	38/62		250±2	275±3	264±3	79	80	0.50
	50/50		297±2	266±2	255±2	112	83	0.59

[a] Inocula. [b] Inocula+pigmanure. [c] calculated basis on the original TS and VS before pretreatment.

No significant evidence of inhibition was observed when the TS ratio of wet-oxidized or raw crops and swine manure at 50:50 or below 50:50[120] except wet-oxidized corn stalker, when the TS concentration of wet-oxidized corn stalker increased to 0.75 and 1 to 1 of swine manure the methanogenesis was stopped. The reason of this is the wet-oxidized corn stalker contains higher soluble sugar among the four wet-oxidized crops. Therefore, when co-digestion this biomass with swine manure the soluble sugar are quickly convert to volatile fatty acids (VFA) by acidogenesis bacteria, the sudden VFA concentration increasing leading the pH dropped to below to 4.5, the methanogenesis reaction was inhibited by the lower pH [48, 49, 50]. This will not appeared in a CSTR reactor due to the large buffering capacity [131]. However, there was a large amount of CH_4 increasing observed from wet-oxidized willows, more than 90% methane was obtained from wet-oxidised at TS ratio (willow 38:62 manure) than from raw willow, this is probably because the structure of crop willow are more woody compared with wheat straw or corn stalker. There are two major effects on willow after wet-oxidation pre-treatment, one is the releasement of sugar and other easily biodegradable compounds from lignocellulose, and another is that the particle size of willow was reduced to under 0.5 cm. Particle size reduction of the biomass by chipping, milling and grinding can increase the available surface area and intracellular component of the biomass and therefore increase the biodegradability of the biomass[6]. However, co-digestion of wet-oxidation pretreated miscanthus, corn stalker and wheat straw with swine manure, the methane potential was much slightly lower in comparison with that raw crop, which was in the range 290-316, 211-360 and 254-275 ml CH_4 g^{-1} VS_{added} in the all different TS ratio, respectively. However in some of the case wet-oxidized of these three crops shows the fast methane conversion rates, but it was not so encouragement for further investigation due to the energy consuming of pretreatment. On the other hand, pretreatment of willow with wet-explosion method increased more than 90% of methane potential, according to the previously study on energy balance and cost-benefit analysis of biogas production from perennial energy crops pretreated by wet oxidation[124] approved that wet-oxidation of willow for biogas production is economically profitable. Although, the biogas potential in term of ml CH_4 produced per g-VS was not increased, but in term of CH_4 produced per m^3 of reactor volume, supply willow to swine manure (2% of VS) basis reactor at VS ratio 1:1(crop: manure) can increase 66 % of total biogas production than treatment of swine manure alone, for miscanthus, corn stalker, and wheat straw are 155, 179 and 126% respectively (Table 8), these results

indicated that by increase certain amount of organic solids concentration in manure basis reactor can increases the bioreactor productivity, which means more biogas production per ton of treated material.

6. Conclusions

Laboratories throughout the world are continuing research on AD to evaluate different types of waste streams and biomass feedstocks as substrate for various reactor configurations and to develop with improved reaction kinetics and biogas yields. Based on this literature study and performed experiments in our laboratorium, the following conclusions can be draw: 1) Crop residues or energy crops can be a significant main or co-digestion substrates for biogas production due the large quantity supplement and high biogas production yields, perennial energy crops are rather recommended from economic point of view (low energy and chemical inputs compared to annual crops), but the biomass production rate per ha and suitable harvesting season needs to be further optimized. 2) The biodegradability of crops can be enhanced by different pre-treatment, but most of these present pre-treatment methods require certain amount of energy and chemicals inputs, also they have the potential risk of formation inhibitory compounds to anaerobic bacteria. Therefore these factor should be considered when apply the suitable pre-treatment methods for treating crops. 3) Ensilage is one of the most promising methods for storage of crops for biogas production, the water content and the weight of the biomass can be well protected during the process. Moreover, ensilage can be also used as a pre-treatment method for enhancement some of the crops biodegradability. Therefore, ensilage crops as storage and pre-treatment methods are highly recommended. 4) Co-digestion of swine manure with miscanthus, corn stalker and wheat straw can be one of the promising methods to boost the biogas yield in swine manure based anaerobic reactor. The amount of crop input can affect the biogas production in two aspects, the lower crop input the higher biogas yield in term of ml CH_4 per g VS_{added}, and higher crop input, the higher volumetric reactor productivity in term of m^3 CH_4 produced per m^3 of reactor working volume. However, continuous reactor experiments using manure richer in nitrogen than the manure used in the batch tests showed an increase in both methane yield and volumetric reactor productivity. Co-digestion with a TS ratio of 1:1.5 (manure: wheat straw) resulted in a 23% higher methane yield and 111% higher productivity, when compared to a TS ratio of 1:1. **(Paper 3)** [132]. Moreover, the biodegradability of these tested crops, except willow, was significant, no further pretreatment is needed beside size reduction.

REFERENCES

1. Francesco C., Neil D. B., Annette C. 2009 Energy-and greenhouse gas-based LCA of bioenergy system. Resources, conservation and recycling 53(2009) 434-447.

2. Börjesson, P. & Berglund, M. 2003. Environmental analysis of biogas systems. 80 p., Reporet No. 45 Environmental and Energy Systems Studies, Lund University, Lund.

3. Kristensen, EF & Sambra, A 2009, 'All year supply of willow biomass to power plant', I Energy conversion from biomass production: EU -AgroBiogas, NJF s. 48-49.

4. Gunaseelan, V. N. 1997. Anaerobic digestion of biomass for methane production. A review. Biomass Bioenergy 13 83-114

5. Zauner, E. & Kuntzel, U. 1986. Methane production from ensiled plant material. Biomass 10: 207–223.

6. Zubr, J. 1986. Methanogenic fermentation of fresh and ensiled plant materials. Biomass 11: 156–171.

7. Weiland, P. 2003. Production and energetic use of biogas from energy crops and wastes in Germany. Appl. Biochem. Biotechnol. 109: 263–274

8. Shiralipour, A. & Smith, P. H. 1984. Conversion of biomass into methane gas.Biomass 6: 85–92.

9. Chynoweth, D. P., Turick, C. E., Owens, J. M., Jerger, D. E. & Peck, M. W. 1993. Biochemical

10. Methane potential of biomass and waste feedstocks. Biomass Bioenergy 5: 95–111. Badger, D. M., Bogue, M. J. & Stewart, D. J. 1979. Biogas production from crops and organic wastes. 1. Results of batch digestions. New Zealand J. Sci. 22:11–20.

11. Stewart, D. J., Bogue, M.J. & Badger, D. M. 1984. Biogas production from crops and organic wastes. 2. Results of continuous digestion tests. New Zealand J. Sci. 27: 285–294.

12. Mcdonald, P., Henderson, N. & Heron, S. 1991. The Biochemistry of Silage. 2ⁿᵈ ed. Chalcombe Publications, Marlow UK.

13. Fan, L. T., Gharpuray, M. M. & Lee, Y.-H. 1981. Evaluation of pretreatments for enzymatic conversion of agricultural residues. Biotechnol. Bioeng. Symp. 11: 29–45.

14. Noike, T., Endo, G., Chang, J. E., Yaguchi, J. I. & Matsumoto, J. I. 1985. Characteristics of carbohydrate degradation and the rate-limiting step in anaerobic-digestion. Biotechnol. Bioeng. 27: 1482–1489.

15. Mata-Alvarez, J., Martinez-Viturtia, A., Llabrés-Luengo, P. & Cecchi, F. 1993. Kinetic and performance of a batch two-phase anaerobic digestion of fruit and vegetable wastes. Biomass Bioenerg. 5: 481–488.

16. Fan, L. T., Gharpuray, M. M. & Lee, Y.-H. 1981. Evaluation of pretreatments for enzymatic conversion of agricultural residues. Biotechnol. Bioeng. Symp. 11: 29–45.

17. Zubr, J. 1986. Methanogenic fermentation of fresh and ensiled plant materials. Biomass 11: 156–171.

18. Madhukara, K., Nand, K., Raju, N. R. & Srilahta, H. R. 1993. Ensilage of mango peel for methane generation. Process Biochem. 28: 119–123.

19. Lehtomäki, A., Viinikainen, T. A., Ronkainen, O. M., Alen, R. & Rintala, J. A. 2004. Effect of pre-treatments on methane production potential of energy crops and crop residues. In: Guiot, S. G., Pavlostathis, S. G. & Lier, J. B. van (eds), Proc. the 10th World IWA Congress on Anaerobic Digestion: 1016–1021. IWA Publishing, London.

20. M.Galbe. G. Zacchi 2007. Pretreatment of lignocellulosic materials for efficient bioethanol production. Adv Biochem Engin 108: 41-65.

21. Ahring, B.K., Munck, J., 2006. Method for Treating Biomass and Organic Waste with the Purpose of Generating Desired Biologically Based Products. Patent No. WO 2006-032282 A1.

22. Georgieva, T.I., Hou, X.R., Hilstrom, T., Ahring, B.K., 2008a. Enzymatic hydrolysis andethanol fermentation of high dry matter wet-exploded wheat straw at low enzyme loading. Applied Biochemistry and Biotechnology 148 (1–3), 35–44.

23. Georgieva, T.I., Mikkelsen, M.J., Ahring, B.K., 2008b. Ethanol production from wet exploded wheat straw hydrolysate by thermophilic anaerobic bacterium Thermoanaerobacter BG1L1 in a continuous immobilized reactor. Applied Biochemistry and Biotechnology 145 (1–3), 99–110.

24. M.Galbe. G. Zacchi 2007. Pretreatment of lignocellulosic materials for efficient bioethanol production. Adv Biochem Engin/ bioechnol (2007) 108: 41-65

25. Sharma, S. K., Mishra, I. M., Sharma, M. P. & Saini, J.S. 1988. Effect of particle size on biogas generation from bimass residues. Biomass 17: 251–263.

26. Kaparaju, P., Luostarinen, S., Kalmari, E., Kalmari, J. & Rintala, J. 2002. Codigestion of energy crops and industrial confectionery by-products with cow manure: Batch scale and farm-scale evaluation. Wat. Sci. Technol. 45:275–280.

27. Lissens, G., Klinke, H., Verstraete, W., Ahring, B.K., Thomsen, A.B. (2004,2) Wet oxidation treatment of organic household waste enriched with wheat straw for simultaneous saccharification and fermentation into ethanol. *Environmental Technology - London*, 25(6), 647-656

28. Danmarks Energistyrelsens Energiforskningsprogram (DEE): Udredning vedrørende forøgelse af husdyrgødnings biogaspotentiale. Institut for Bioteknologi, Danmarks Tekniske Universitet (1993)

29. Sun, Y. & Cheng, J. 2002. Hydrolysis of lignocellulosic materials for ethanol production. A review. Biores. Technol. 83: 1–11.

30. Sehoon K, Holtzapple MT 2005. Bioresour Technol 96:1994

31. Chang VS, Kaar WE, Burr B, Holtzapple MT 2001. Biotechnol Lett 23:1337

32. Palmqvist E, Hahn Hågerdal B 2000 Bioresour Technol 74:17

33. Larsson S, Palmqvist E, Hahn Hågerdal B 1999. Enzyme Microb Twchnol 24:151

34. Hartmann. H, Angelidaki. I and Ahring. B. K. 2002. Co-digestion of organic fraction of municipal waste with other types. J. Mata-Alvarez (ed). IWA Publishing ISBN 1 900222 14 0.

35. Hsu TA 1996 in: Wyman CE Handbook on bioethanol production and utilization. Taylor & Francis, Washington, P 179

36. G Evans 2001. Biowaste and Biological waste treatment P 89-114

37. Ahring, B. K. 1991 Methanogenesis during thermophilic anaerobic digestion with focus on acetate, P275-283

38. Sleat, R. & Mah, R. (2006) Hydrolytic Bacteria in Anaerobic digestion of biomass, p15

39. Boone, D. & Mah, R. (2006) Transitional bacteria in anaerobic digestion of biomass, p35

40. boone, D. R. 1982. Terminal reactions in the anaerobic digestion of animal waste. Appl. Environ. Microbial. 43:57-64

41. Zauner E and Kuntzel U, Methane production from ensiled plant material. Biomass Bioenerg 10:207–223 (1986).

42. Amon T, Amon B, Kryvoruchko V, Machmüller A, Hopfner-Sixt K, Bodiroza V, et al., Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. Biores Techn 98:3204–3212 (2007)

43. Lehtomäki A, Viinikainen TA and Rintala JA,2008. Screening boreal energy crops and crop residues for methane biofuel production. Biomass Bioenergy 32:541-550

44. Stewart DJ, Bogue MJ and Badger DM, Biogas production from crops and organic wastes 2. Results of continuous digestion tests. New Zealand Journal Science 27:285–294 (1984).

45. Seppälä M, Paavola T and Rintala J, Methane yields of different grass species on the second and third harvest in boreal conditions. 11th I WA World Congress on Anaerobic Digestion; 23–27 September 2007 Brisbane, Australia.

46. Lehtomäki A, Viinikainen TA and Rintala JA, Screening boreal energy crops and crop residues for methane biofuel production. Biomass Bioenerg 32:541–550 (2008).

47. Lehtomäki A, Biogas production from energy crops and crop residues, P hD Thesis.Univeristy of Jyväskylä, Jyväskylä, Finland (2006).

48. Ahring, B.K., M. Sandberg., I. Agelidaki. 1995b Volatile acids as indicators of process in anaerobi8c digestors. Appl. Microbio. Biotechnol. 43: 559-565.

49. Mathrani, I. M., K. Johansen., Ahring, B.K. 1994. Experience with thermophilic anaerobic digestion of manure, organic industrial and househoud waste at the large scale plant in Vegger, Denmark, p, 365-374.

50. Zinder, S. H. T. Anguish,. S. Cardwell. 1984b. Methangenesis in a thermophilic anaerobic digestor: Methanotrix sp. as an important aceticlastic methanogen. Appl. Environ. Microbio. 47:796-807.

51. Switzenbaum, M. S., E. Giraldo, R. F. Hickey. 1990. Monitoring of the anaerobic methane fermentation process. Enzyme Microb. Technol. 12:722-730

52. Schmidt JE, Ahring BK. 1996. Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors. Biotechnol Bioeng. 1996 Feb 5;49 (3):229-46.

53. Lettinga, G. 1995. anaerobic digestion and wastewater treatment systems. Antonie ban Leeuwenhoek 67:3-28

54. Delgenés et al., 2002 J.P. Delgenés, V. Penaud and R. Moletta, Pretreatments for the enhancement of anaerobic digestion of solid wastes Chapter 8, Biomethanization of the Organic Fraction of Municipal Solid Wastes, IWA Publishing (2002), pp. 201–228.

55. Fan et al., 1987 L.T. Fan, M.M. Gharpuray and Y.H. Lee, Cellulose hydrolysis, Springer-Verlag, Berlin, Germany

56. Outi Pakarinen, Lehtomäki A, Sanna Rissanen, Jukka Rintala.2008 Storing energy crops for methane production: Effects of solids content and biological additive Bioresource Technology 99:15 pp.7074-7082

57. Vervaeren. H, Hostyn. K, Ghekiere. G, Willems. B 2009. Biological ensilage additives as pretreatment for maize to increase the biogas production.

58. eiermann, M., Plöchl, M., Linke, B. & Scheile, H. 2002. Preliminary evaluation of some cereals as energy crops for biogas production. In: Sayigh, A. A. M. (ed.), Proc. World Renewable Energy Congress VII, CD-version. Pergamon, Cologne.

59. Ensilage of perennial crop willow and miscanthus for biogas production paper 5

60. Frigon J-C, Mehta P and Guiot SR, The bioenergy potential from the anaerobic digestion of switch grass and other energy crops. Growing the margins: Energy, Bioproducts and Byproducts from farm and food sectors;April 2–5 2008; London, Canada.

61. Song, Y.C., Kwon, S.J., Woo, J.H. (2004) Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic- and thermophilic digestion of sewage sludge, Water Res. 2004 Apr;38(7):1653–6

62. Ahring, B.K. (1994). Status on science and application of thermophilic anaerobic digestion. Water Science and Technology, 30(12), 241–249.

63. Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption

64. Bendixen, H.J. (1994). Safeguards against pathogens in Danish biogas plants. Water Science and Technology, 30(12), 171–180.

65. Ghosh, S., Conrad, J R . and Klass. D. L,. anaerobic acidogenesis of waste water sludge. Jour. Water Pull. Contr. Fed ., 1975, 47, 30-45

66. Ghosh, S., Ombregt, J.P,. and Pipyn. P. Methane production from industrial waste by two phase digestion. Wat. Res., 1985, 19, 1083-1088

67. G. Hofenk, S lips. B.A. rijkens and J.W Voetberg, Two-phase of anaerobic digestion of solid organic wastes yields biogas and compost. EC contract final report 57(1984).

68. Six, W. and De Baere, L. (1992). Dry anaerobic conversion of municipal solid waste by means of the Dranco process. Water Science and Technology, 25(7), 295–300.

69. Ku¨bler, H. (1994). Versuch zur thermophilen Faulung von kommunalem Bioabfall. Mu¨ ll und Abfall, 26(8), 478–488.

70. Kayhanian, M. (1999). Ammonia inhibition in high-solids biogasification: an overview and practical solutions. Environmental Technology, 20(4), 355–366.

71. Birkmose. T, Henning. L.F, Jøgen. H, 2007. State of biogas plants in European agriculture (Danish agriculture advisory service)

72. Ahring, B. K. 1994. Status on science and application of thermophilic anaerobic digestion. Wat. Sci. Tech. 30: 241-249.

73. Gellens, V., J. Boelens, and W. Verstraete. 1995. Source separation, selective collection and in reactor digestion of biowaste. Antonie van Leeuwenhoek 67:79-89

74. Grady, C. P. L. J., and H. C Lim. 1980. anaerobic digestion and anaerobic contact, p. 833-886. In biological wastewater treatment. Marcel Dekker, Inc. New York.

75. Lettinga, G. 1995. Anaerobic digestion and wastewater treatment systems. Antonie van Leeuwenhoek 67: 3-28.

76. Rintala, J. A., and J.A puhakka 1994. Anaerobic treatment in pulp- and paper-mill waste management: a review. Biores. Techbol. 47: 1-18.

77. Kobayashi, H. A., E. Conway de Macario, R. S. Williams, and A. J. L. Macario. 1998. Direct characterization of methanogens in two high-rate anaerobic reactor. Appl. Environ. Microbio. 54 693-698.

78. Daniels, L. 1992. Biotechnological potential of methanogens, P181-193. In M. J. Danson, D. W. Hough and G. G. Lunt (ed.), Biochem. Soc. Sym. 58.

79. Hartmann. H and Ahring B. K., The Future of Biogas Production. http://vbn.aau.dk/files/14922223/Hartmann_future_biogas.pdf

80. Dinsdale, R. M., F. R. Hawkes , and D. L. Hawkes. 1997. Comparison of mesophilic and thermophilic upflow anaerobic sludge blanket reactors treating instant coffee production wastewater. Wat. Res. 31: 163-169

81. Mackie, R. I., and M. P. Bryant. 1995. anaerobic digestion of cattle waste at mesophilic and thermophilic temperatures. Appl. Microbiol. Biotechnol. 43: 346-350

82. Aitken, M. D., and R. W. Mullennix. 1992. Another look at the thermophilic anaerobic digestion of wastewater sludge. Wat. Environ. Res. 64: 915-919.

83. Angelidaki, I., Ellegaard, L., 2003. Codigestion of manure and organic wastes incentralized biogas plants. Applied Biochemistry and Biotechnology 109, 95–105.

84. Amon T., Hackl E., Jeremic D., Amon B. & Boxberger J. 2001. Biogas production from animal wastes, energy plants and organic wastes. In: van Velsen, A. F. M. & Verstraete, W. H. (eds), Proc. 9th World Congress on Anaerobic Digestion: 381–386. Technologisch Instituut zw, Antwerp.

85. Li, X., Li. Q., Zheng., M., Fu, G.Z., Lar, J.S., 2009. Anaerobic codigestion of cattle manure with corn stover pretreated by sodium hydroxide for efficient biogas production. Energy Fuels 23, 4535-4639.

86. Hashimoto, 1993 A.G. Hashimoto, Conversion of straw–manure mixtures to methane at mesophilic and thermophilic temperatures, Biotechnol. Bioeng. 25 (1983), pp. 185–200.

87. Hills, 1979 D.J. Hills, Effects of carbon–nitrogen ratio on anaerobic-digestion of dairy manure, Agric. Wastes 1 (1979), pp. 267–278.

88. Hills and Roberts, 1981D.J. Hills and D.W. Roberts, Anaerobic-digestion of dairy manure and field crop residues, Agric. Wastes 3 (1981), pp. 179–189.

89. Mata-Alvarez. J, Mace. S and Llabres. P, Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives, Bioresour. Technol. 74 (2000), pp. 3–16.

90. Wang. H, Lehtomaki. A, Tolvanen. K, Puhakka. J and Rintala. J, Impact of crop species on bacterial community structure during anaerobic co-digestion of crops and cow manure, Bioresour. Technol. 100 (2009), pp. 2311–2315.

91. Mola-Yudego, B; Aronsson, P. (2008). "Yield models for commercial willow biomass plantations in Sweden" (PDF). Biomass and Bioenergy 32 (9): 829–837.

92. Hodsman, L., Smallwood, M., Williams, D. "The promotion of non-food crops", National Non-Food Crops Centre, 2005-11-30. Retrieved on 2009-05-11.

93. Reith. J. H, R. Wijffels. H and Barten. H., 2003. Status and perspectives of biological methane and hydrogen production. Dutch Biological Hydrogen Foundation.

94. Bal AS, Dhagat NN. Upflow anaerobic sludge blanket reactor--a review. Indian J Environ Health. 2001 Apr;43(2):1-82.

95. Commission regulation (EC) No 1334/2003. amending the condition for authorization of a number of additives in feeding stuffs belonging to the group of trace elements.

96. Hansen, W. N., Birkmose, T. S., Mortensen, B and Skaaningm, K. 2004. Miljøeffekten af bioforgasning og separering af gylle. Grøn Viden markbrug nr. 296. Danmarks Jordbrugs Forskning. (Environmental effects of digestion and separation of slurry. Green Knowledge fieldwork No. 296th Danish Institute of Agricultural Research.)

97. Winterberg, R. Seela, V and Wilke, A 2006. Evaluation of current and upcoming technological system for AD. Internal working document inn agrobiogas project.

98. Hendriks. A.T.W.M., and Zeeman. G 2008. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource Technology 100: 10-18.

99. Alexander Bauer Æ Christian Leonhartsberger Æ., Peter Bo"sch Æ Barbara Amon Æ Anton Friedl Æ., Thomas Amon., 2009. Analysis of methane yields from energy crops and agricultural by-products and estimation of energy potential from sustainable crop rotation systems in EU-27. Clean Techn Environ Policy (2010) 12:153–161

100. Liu, H.W., Walter, H.K., Vogt, G.M., Vogt, H.S., Holbein, B.E., 2001. Steam pressure disruption of municipal solid waste enhances anaerobic digestion kinetics and biogas yield. Biotechnology and Bioengineering 77, 121–30.

101. Benjamin, M.M., Woods, S.L., Ferguson, J.F., 1984. Anaerobic toxicity and biodegradability of pulp mill waste constituents. Water Res. 18 (5), 601–607.

102. Fox, M.H., Noike, T., Ohki, T., 2003. Alkaline subcritical-water treatment and alkaline heat treatment for the increase in biodegradability of newsprint waste. Water Sci. Technol. 48 (4), 77–84.

103. Noike, T., Niigata Engineering, 2001. Micromolecularization of undegradable organic substances in methane fermentation. In: Development of the Waste Treatment System for Recycling Society. Waste Research Foundation, Tokyo.

104. Fengel, D., Wegener, G., 1984. Wood: Chemistry, Ultrastructure, Reactions. De Gruyter, Berlin.

105. Ramos, L.P., 2003. The chemistry involved in the steam treatment of lignocellulosic materials. Quim. Nova. 26 (6), 863–871.

106. Liu, C., Wyman, C.E., 2003. The effect of flow rate of compressed hot water on xylan, lignin and total mass removal from corn stover. Ind. Eng. Chem. Res. 42, 5409–5416.

107. Shevchenko, S.M., Beatson, R.P., Saddler, J.N., 1999. The nature of lignin from steam explosion/enzymatic hydrolysis of softwood. Appl. Biochem. Biotechnol., 867–876.

108. Xiao, W., Clarkson, W.W., 1997. Acid solubilization of lignin and bioconversion of treated newsprint to methane. Biodegradation 8, 61–66.

109. Taherzadeh, M.J.; Karimi, K. Enzymatic-based hydrolysis processes for ethanol from lignocellulosic materials: A review. BioResources 2007, 2, 707-738.

110. Delmer, D.P.; Amor, Y. Cellulose biosynthesis. Plant Cell 1995, 7, 987-1000.

111. Mohammad J. Taherzadeh , and Keikhosro Karimi 2008. Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. Mol. Sci. 2008, 9, 1621-1651

112. Kassim, E.A.; El-Shahed, A.S. Enzymatic and chemical hydrolysis of certain cellulosic materials. Agr. Wastes 1986, 17, 229-233.

113. Tanaka, S.; Kobayashi, T.; Kamiyama, K.; Bildan, M. Effects of thermochemical pretreatment on the anaerobic digestion of waste activated sludge. Water Sci. Technol. 1997, 35, 209-215.

114. Tanaka, S.; Kamiyama, K. Thermochemical pretreatment in the anaerobic digestion of waste activated sludge. Water Sci. Technol. 2002, 46, 173-179.

115. Palonen, H.; Thomsen, A.B.; Tenkanen, M.; Schmidt, A.S.; Viikari, L. Evaluation of wet oxidation pretreatment for enzymatic hydrolysis of softwood. Appl. Biochem. Biotechnol. 2004, 117, 1-17.

116. Garrote, G.; Dominguez, H.; Parajo, J.C. Hydrothermal processing of lignocellulosic materials. Holz Als Roh-und Werkst. 1999, 57, 191-202.

117. Azzam, A.M. Pretreatment of cane bagasse with alkaline hydrogen peroxide for enzymatic hydrolysis of cellulose and ethanol fermentation. J. Environ. Sci. Heal. 1989, 24, 421-433.

118. Hon, D.N.S., Shiraishi, N., 2001. Wood and Cellulosic Chemistry, second ed. Dekker, New York.

119. Lissens, G.; Thomsen, A.B.; De Baere, L.; Verstraete, W.; Ahring, B.K. Thermal wet oxidationimproves anaerobic biodegradability of raw and digested biowaste. Environ. Sci. Technol. 2004, 38, 3418-3424.

120. Palmqvist, E., Hahn-Hägerdal, B., 2000. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioresource Technology 74, 25–33.

121. Egg, R., Coble, C., Engler, C and Lewis, D. 1993. Feedstock storage, handling and processing. Biomass Bioenergy 5: 71-94.

122. Zubra. J. 1986. Methanogenic fermentation of fresh and ensiled plant materials. Biomass 11: 156-171.

123. Gunnarson, S., Malmberg, A., Mathisen, B., Theander, O., Thyselius, L. & Wunsche, U. 1985. Jerusalem atrichoke (Helianthus tuberosus L.) for biogas production. Biomass 7: 85–97.

124. Uellendahl. H, Wang. G, Møller. H. B., Jørgensen. U, Skiadas. I. V, Gavala. H. N and Ahring. B. K 2008. Energy balance and cost-benefit analysis of biogas production from perennial energy crops pretreated by wet oxidation. Water Science & Technology—WST. 58.9

125.   Fujita, M., Scharer, J. M. & Moo-Young, M. 1980. Effect of corn stover addition on the anaerobic digestion of swine manure. Agric. Wastes 2: 177–184.

126.   Kaparaju, P. & Rintala, J. 2005. Anaerobic co-digestion of potato tuber and its industrial by-products with pig manure. Res. Conserv. Recy. 43: 175–188.

127.   Hashimoto, A. G. 1983. Conversion of straw-manure mixtures to methane at mesophilic and thermophilic temperatures. Biotechnol. Bioeng. 25: 185–200.

128.   Fischer, J. R., Iannotti, E. L. & Fulhage, C. D. 1983. Production of methane gas from combinations of wheat straw and swine manure. Transactions ASAE 26: 546–548.

129.   Somayaji, D. & Khanna, S. 1994. Biomethanation of rice and wheat straw. W. J. Microbiol. Biotechnol. 10: 521–523.

130.   Galbe. M, Zacchi. G, 2007. Pretreatment of lignocellulosic materials for efficient bioethanol production. Adv Biochem Engin/ bioechnol (2007) 108: 41-65

131.   Hartmann. H, Angelidaki. I, and Ahring. B. K. 2002. Co-digestion of organic fraction of municipal waste with other waste type. J. Mata- Alvarez. (ed) IWA Publishing ISBN 1 900222 14 0

132.   Guangtao Wang, Jens Ejbye Schmidt. Co-digestion swine manure with raw and pretreated crops at different total solids ratio (manure: crops) paper 3 in this thesis.

Paper 2

Wet explosion of wheat straw and co-digestion with swine manure: Effect on the methane productivity

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Wet explosion of wheat straw and codigestion with swine manure: Effect on the methane productivity

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ABSTRACT

The continuously increasing demand for renewable energy sources renders anaerobic digestion to one of the most promising technologies for renewable energy production. Twenty-two (22) large-scale biogas plants are currently under operation in Denmark. Most of these plants use manure as the primary feed-stock but their economical profitable operation relies on the addition of other biomass products with a high biogas yield. Wheat straw is the major crop residue in Europe and the second largest agricultural residue in the world. So far it has been used in several applications, i.e. pulp and paper making, production of regenerated cellulose fibers as an alternative to wood for cellulose-based materials and ethanol production. The advantage of exploiting wheat straw for various applications is that it is available in considerable quantity and at low-cost. In the present study, the codigestion of swine manure with wheat straw in a continuous operated system was investigated, as a method to increase the efficiency of biogas plants that are based on anaerobic digestion of swine manure. Also, the pretreatment of wheat straw with the wet explosion method was studied and the efficiency of the wet explosion process was evaluated based on (a) the sugars release and (b) the methane potential of the pretreated wheat straw compared to that of the raw biomass. It was found that, although a high release of soluble sugars was observed after wet explosion, the methane obtained from the wet-exploded wheat straw was slightly lower compared to that from the raw biomass. On the other hand, the results from the codigestion of raw (non-pretreated) wheat straw with swine manure were very promising, suggesting that 4.6 kg of straw added to 1 t of manure increase the methane production by 10%. Thus, wheat straw can be considered as a promising, low-cost biomass for increasing the methane productivity of biogas plants that are based mainly on swine manure.

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1. Introduction

A big number of large-scale biogas plants are currently under operation around the world. Most of these plants are based on wastes (solid or liquid) like manure, sewage sludge or municipal waste as main feedstock. The continuously increasing demand for renewable energy sources such as methane renders anaerobic digestion to one of the most promising technologies for renewable energy production. Twenty-two (22) large-scale biogas plants are currently under operation in Denmark. Most of these plants use manure as the primary feedstock but their economical profitable operation relies on the addition of other biomass products with a high biogas yield. The biogas yield from manure is typically rang-ing from 10 to 20 m^3/t while the operation is only profitable when biogas yields higher than 30 m^3/t of treated material can be achieved (Angelidaki and Ellegaard, 2003). This is currently done by the addition of industrial organic waste materials like, for example, fat sludge or fish oil. However, the volume of these waste streams is limited and some biogas plants have started to import organic waste of high methane potential. Therefore, the investigation of other, abundant biomasses to be codigested with manure is of great interest.

Wheat straw is the major crop residue in Europe and the second largest agricultural residue in the world (Kim and Dale, 2004). Wheat straw, which consists mainly of cellulose (33–38%), hemicelluloses (26–32%) and lignin (17–19%), has traditionally been used for pulp and paper making as well as to produce regenerated cellulose fibers as an alternative to wood for cellulose-based materials (Reddy and Yang, 2005). Also, wheat straw is one of the most important global feedstocks for ethanol production (Kim and Dale, 2004). The advantage of exploiting wheat straw for various applications is that it is available in considerable quantity and at

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low-cost. Thus, wheat straw can also be considered as one of the best options for increasing the methane production through biomass digestion. However, the efficiency of methane production from lignocellulosic residues can be limited by the low biodegradability of the lignocellulose. The destruction of the lignocellulosic structure will release the sugars contained in the biomass and therefore can possibly increase the production of methane.

Several pretreatment technologies such as wet oxidation (Schmidt and Thomsen, 1998), steam explosion (Ballesteros et al., 2006), dilute acid (Saha et al., 2005) and hydrothermal (Thomsen et al., 2006) have been used for the pretreatment of wheat straw mainly for ethanol production. Recently, a new pretreatment technology, wet explosion, has been developed by Ahring and Munck (2006). The method is a combination of thermal hydrolysis, wet explosion, and wet oxidation and it enables operating with high biomass concentrations and handling of big particle sizes, thereby, avoiding initial energy intensive mechanical milling. Moreover it is an easy controllable process with low total energy consumption (Ahring and Munck, 2006). The wet explosion method has so far been successfully employed for the pretreatment of wheat straw for ethanol production (Georgieva et al., 2008a,b). Therefore, it was interesting to investigate whether the wet explosion pretreatment also has a beneficial effect on the biogas productivity when wheat straw is used as a feedstock.

The present study investigates the codigestion of swine manure with untreated wheat straw in a continuous operated system, as a method to increase the efficiency of biogas plants that are based on anaerobic digestion of swine manure. Also, the pretreatment of wheat straw was investigated with the wet explosion method, that is, increase of temperature and pressure, addition of hydrogen peroxide as oxidizing agent and finally instant pressure release by flashing the biomass to atmospheric pressure. The efficiency of the wet explosion process was evaluated based on (a) the sugars release and (b) the methane potential of the pretreated wheat straw compared to that of the raw biomass.

It was found that, although a high release of soluble sugars was observed after wet explosion, the methane obtained from the wet-exploded wheat straw was slightly lower compared to that from the raw biomass. On the other hand, the results from the codigestion of raw (non-pretreated) wheat straw with swine manure were very promising, suggesting that 4.6 kg of straw added to 1 t of manure increase the methane production by 10%. Thus, wheat straw can be considered as a promising, low-cost biomass for increasing the methane productivity of biogas plants that are based mainly on swine manure.

2. Materials and methods

2.1. Raw biomasses

Wheat straw was chopped with a hammer mill to a particle size of approximately 3–5 cm and stored at −20 °C. Swine manure that was obtained from a biogas plant in Denmark, was homogenized and kept at −20 °C until it was used.

2.2. Analytical methods

Determinations of the total (TS) and volatile solids (VS), total suspended (TSS) and volatile suspended solids (VSS), chemical oxygen demand (COD), Kjeldahl nitrogen and NH_4^+–N were carried out according to Standard Methods (APHA, 1989). For the quantification of VFA, acidified samples with 17% H_3PO_4 were analysed on a gas chromatograph (Hewlett Packard 5890 series II) with a flame ionisation detector and a capillary column (Hewlett Packard FFAP 30 m, inner diameter 0.53 mm, film 1 mm). Biogas composition

in methane was quantified with a gas chromatograph (Shimadzu GC-8A) equipped with a flame ionisation detector and a packed column (Porapak Q, 80/100-mesh).

Three groups of carbohydrates were analyzed in wheat straw biomass: the first group was the total carbohydrates, including those bound in the lignocellulosic biomass, the second group was the soluble carbohydrates and the last one was the sugar monomers. For total carbohydrates determination, a representative sample of the material was first solubilised in strong acid at 30 °C for 60 min and then hydrolysed in dilute acid at 121 °C for 60 min. The detection and quantification of the released sugar monomers, which were mainly glucose, xylose and arabinose, was made by HPLC. A sample free of solids was either hydrolyzed in dilute acid and then passed through the HPLC in order to determine the soluble carbohydrate content or directly passed through the HPLC for sugar monomers determination. Detection and quantification of the sugar monomers, glucose, xylose and arabinose was made by HPLC isothermally at 60 °C with a BioRad Aminex HPX-87H column using 4 mM H_2SO_4 as eluent at a flow of 0.6 ml/min. The content in lignin was determined as the ash-free residue after two steps hydrolysis with strong acid (72% w/w H_2SO_4, 12 M) at 30 °C and dilute acid (2.5% w/w H_2SO_4, 0.42 M) at 121 °C, respectively.

2.3. Wet explosion of wheat straw

Wet explosion took place in a 2.8 L reactor. Standard wet explosion conditions were 180 °C and 10 bar pressure with H_2O_2 as oxidizing agent as a dose of 6 g per 100 g TS straw. Different TS content, H_2O_2 concentration and temperatures were also tested as shown in Table 1. The efficiency of the process was evaluated based on (a) the sugars release and (b) the methane potential of the pretreated material compared with that of the raw biomass.

2.4. Methane potential of raw and wet-exploded wheat straw

Methane potential tests of the raw and pretreated wheat straw were carried out in triplicates in 117 ml serum vials sealed with butyl rubber stoppers and aluminum crimps. An amount equivalent to 0.5 g-TS of wheat straw was added as substrate together with 20 ml inoculum from a Continuous Stirred Tank Reactor (CSTR) type anaerobic digester fed with mixture of swine manure and wheat straw. Triplicate vials with no substrate added served as controls. The vials were incubated at 55 °C and the methane production was followed throughout the experiment. Methane potential was calculated as the volume of methane produced per g of TS of biomass added as substrate. Thus, the suitability of the wet explosion as a pretreatment method for enhanced methane production was assessed.

2.5. Continuous codigestion experiments

Continuous thermophilic (55 °C) codigestion experiment of a mixture of swine manure and wheat straw at a 1:1 TS-based ratio was carried out in a lab-scale unit. The unit consisted of one 7-L active volume CSTR and two influent compartments interconnected with computer-controlled valves (for the unit set-up see Fig. 1). The one influent compartment was filled up with swine manure obtained after filtration (20 g/L final TS concentration) and the other one was filled up with wheat straw. Swine manure and wheat straw were added in the digester four times a day at regular time intervals at a Hydraulic Retention Time (HRT) of 15 days. The reactor was started-up with anaerobic mixed culture from a thermophilic lab-scale digester fed with 40% swine manure, 40% cow manure and 20% manure fibres. The reactor performance was monitored daily through pH, biogas and methane production measurements, while complete characterization took place once a steady

Table 1
Wet explosion conditions tested.

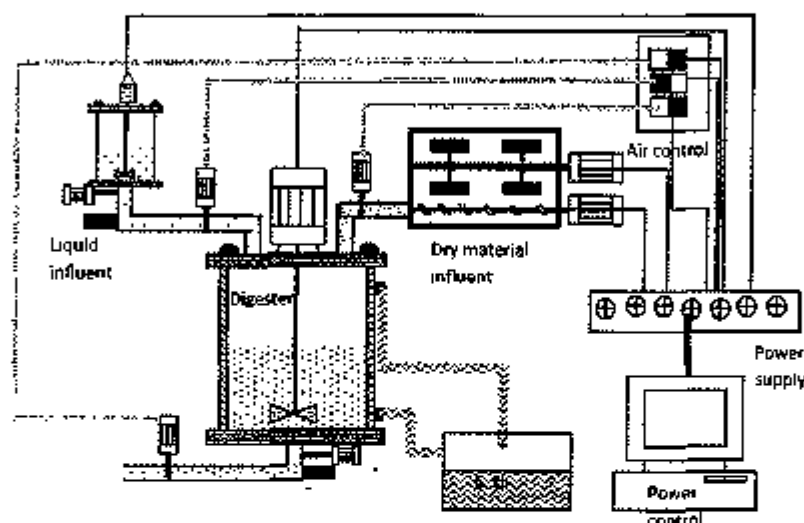| Conditions description		H_2O_2 (initial C) (g/100 g TS)	T (°C/P, bar)	HRT (min)	TS (%)
Standard		6	180/10	5	15
1	Lower H_2O_2 concentration	3	180/10	5	15
2	Higher H_2O_2 concentration	9	180/10	5	15
3	Lower T	6	150/5	5	15
4	Higher T	6	195/14	5	15
5	Shorter HRT	6	180/10	2	15
6	Longer HRT	6	180/10	8	15
7	Lower TS concentration	6	180/10	5	10
8	Higher TS concentration	6	180/10	5	20

Fig. 1. Set-up of the codigestion unit.

state was reached. All liquid samples were taken directly from the interior of the reactor. TSS and VSS were measured in both reactor and effluent in order to make sure that no accumulation of solids occurred in the reactor.

3. Results and discussion

3.1. Characterization of the biomasses

Wheat straw and swine manure were characterized regarding their total and volatile solids concentration (TS and VS, respectively), chemical oxygen demand content (COD) and total and ammonium nitrogen (total-N and NH_4^-–N) concentration. Additionally, carbohydrate and lignin content was determined in wheat straw biomass. The characterization results are shown in Table 2.

Table 2
Characteristics of wheat straw and swine manure.

	Wheat straw	Manure
TS (%)	93 ± 0.03	2.1 ± 0
VS (%)	86 ± 1.67	1.4 ± 0
Total COD (g/g-TS)	0.96 ± 0.01	0.92 ± 0.01
Total-N (mg/g-TS)	6.79 ± 0.10	105.55 ± 0.90
NH_4^-–N (mg/g-TS)	0.82 ± 0.02	87.09 ± 2.80
Total carbohydrates (% of TS)	48 ± 0.15	ND[a]
Lignin (% of TS)	20 ± 0.40	ND[a]

[a] Not determined.

As anticipated, wheat straw was rich in organics (VS) and carbohydrates content while swine manure was characterized by high nitrogen content. Moreover the wheat straw size reduction described in Section 2.1 is not expected to have a significant effect on the bioavailability of the substrate and consequently on the methane potential tests (see Section 3.3) since the original straw size was a few cm longer. Furthermore, the effect of size reduction could only be significant if it was down to a few mm (Ahring and Angelidaki, 1998; Hartmann et al., 2000).

3.2. Wet explosion of wheat straw – release of sugars

Wet explosion of wheat straw was investigated as a pretreatment method in order to enhance the organic matter biodegradability and consequently the methane production through the release of sugars and other easily biodegradable components. Sugar monomers and soluble sugars content in pretreated wheat straw under different wet explosion conditions is shown in Fig. 2. Characterization of the wet-exploded wheat straw regarding to carbohydrates availability showed a high release of soluble sugars with sugars monomers being a minor part of them. The higher release of soluble sugars was observed in straw treated under standard condition and condition 7 (lower TS). Furthermore, condition 7 was the only one that resulted in higher (24%) sugar release in comparison to standard conditions (see Fig. 3). Therefore the methane potential of pretreated straw under conditions 7 and standard was assessed and compared to that of raw straw.
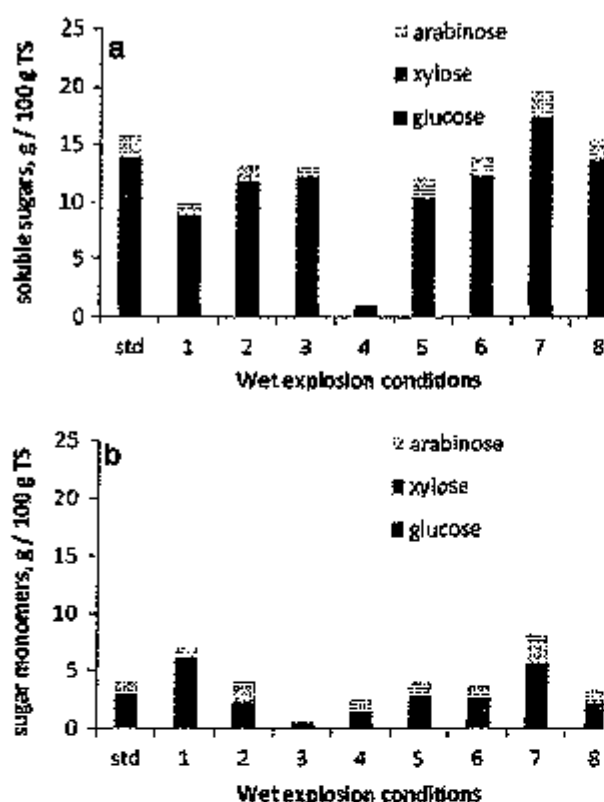
Fig. 2. Wet-exploded wheat straw content in soluble (a) and free (b) sugars.
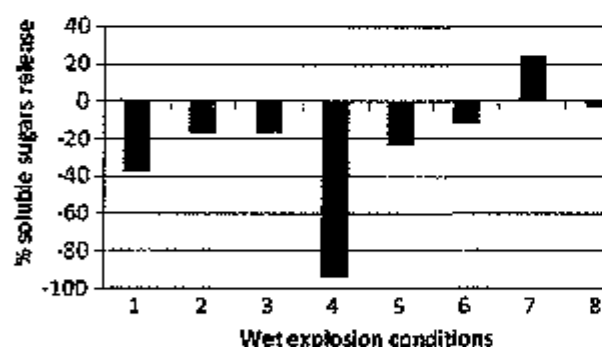

Fig. 3. Relative release of soluble sugars at different wet explosion conditions compared to the standard conditions.

In general, wet oxidation fractionates lignocellulose into a solid cellulose-rich fraction and a liquid hemicelluloses-rich fraction (Bjerre et al., 1996; McGinnis et al., 1983). This was also the case with the wet explosion method applied in this study since the soluble sugars fraction measured after the pretreatment was rich in xylose, which is the main component of hemicellulose. Significant factors that affect the pretreatment efficiency are the oxidant concentration, the temperature and duration of the reaction as well as the total solids concentration. The oxidant (the H_2O_2 in this case) participates in the degradation reactions; excess use of oxidant may cause complete oxidation of the solubilisation products while limited use may result in incomplete degradation and solubilisation. This was most probably the reason of the lower soluble sugars concentration after the pretreatment under conditions 1 and 2 compared to the standard conditions (Fig. 3). The same more or

less role has the temperature and the reaction time combined with the use of an oxidant; lower temperatures and lower reaction times may result in incomplete degradation while higher temperatures and longer reaction times may result in complete oxidation of the solubilisation products (pretreatment conditions 3–6 in Fig. 3). The results of the present study showed that only a reduction of the initial total solids concentration enhanced the solubilisation of wheat straw. This can be possibly attributed to the higher ratio of oxidant to biomass solids compared to that under standard conditions.

3.3. Methane potential of raw and wet-exploded wheat straw

Following the results obtained from the analysis of soluble and free sugars after pretreating wheat straw with wet explosion, the methane potential of pretreated straw under conditions 7 and standard was assessed and compared to that of raw straw. The methane potential of raw and wet-exploded wheat straw under conditions 7 and standard is shown in Fig. 4. Methane productivities were calculated as the mean value of three consecutive measurements and as soon as the methane production in the vials ceased, which happened after about 18 days of incubation. Despite the high release of soluble sugars (Fig. 2a) the methane obtained from the wet-exploded wheat straw was slightly lower compared to the raw biomass (232 ± 13 and 244 ± 11 compared to 261 ± 1 ml CH_4 per g TS, respectively). This, most probably, was due to the formation of inhibitory compounds, which is a very common problem when pretreatment at elevated temperatures is employed prior to fermentations (Klinke et al., 2004). The same phenomenon was reported by Penaud et al. (2000) where the methane production declined after thermochemical pretreatment although the biomass was solubilised at a high degree. In the study of Eskicioglou et al. (2007) inhibition of methane production was also observed after microwave pretreatment of waste activated sludge but subsequent acclimation of anaerobic biomass was possible and finally solubilisation of the substrate led to increased methane production as well. Inhibitors formed during pretreatment of lignocellulosic biomass at high temperatures and acidic conditions, i.e. furfural, 5-hydroxymethylfurfural and phenolic compounds, is also a commonly known fact (Palmqvist and Hahn-Hägerdal, 2000). Taking into consideration the low differences between methane potential values and the standard deviation we can safely conclude that the wet explosion pretreatment did not have any significant effect on methane production. In fact, wet explosion resulted in a slight reduction of methane production. In any case, wet explosion (which is an energy consuming process) is not suitable as pretreatment of straw for codigestion
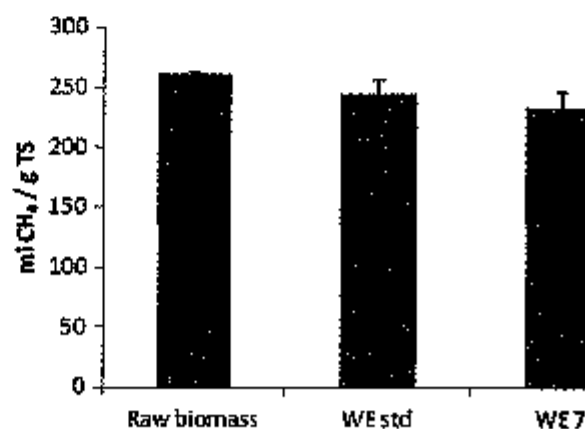

Fig. 4. Methane potential of raw and wet-exploded wheat straw.

with manure at the conditions tested because the additional required energy consumption is not counterbalanced by any increase in methane production.

3.4. Continuous codigestion experiments

Raw straw and manure were codigested in a continuous reactor since wet explosion of wheat straw did not give higher methane yields. The reactor performance in terms of biogas and methane production, volatile fatty acids and total and volatile suspended solids concentration is shown in Figs. 5–7, respectively (samples for VFA and solids determination were collected only after day 75, when biogas production was established in the system). Steady state, as shown in Figs. 5–7, was reached after approximately 15 retention times, which was mainly attributed to machinery failures (i.e. the digester cooled down at the day 95 because of a broken water bath, etc.). TSS and VSS concentration in the interior and effluent of the reactor were the same throughout the duration of
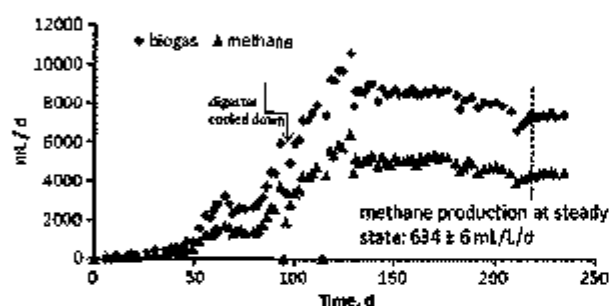
Fig. 7. Total suspended and volatile suspended solids concentration profile throughout the duration of the continuous experiment (measurements were performed on sampling inside the reactor).

the experiment and thus no accumulation of solids occurred. pH value was also measured and it was 7.4 at steady state. Methane production at steady state equaled 222 l per kg TS fed. Assuming that raw wheat straw gave 261 l CH_4 per kg TS (Section 3.3) and given that manure to straw TS ratio was 1:1, manure contributed with 183 l CH_4 per kg TS, given the 10 g TS per day loading rate in both straw and manure. The methane production from the continuous reactor, where swine manure and straw were codigested in, was somewhat lower than the values obtained in the study of Llabrés-Luengo and Mata-Alvarez (1987). In the latter, 319–366 l CH_4 per kg VS were produced after 60 days of digestion in batches, while the corresponding figure obtained in the present study was 271 l CH_4 per kg VS (the TS and VS content of straw and manure presented in Table 2 was used for the calculation). The slightly increased methane productivities in the study of Llabrés-Luengo and Mata-Alvarez (1987) can obviously be attributed to the fact that they were obtained after 60 days of batch digestion (long incubation time) while in the present study a hydraulic retention time

Fig. 5. Biogas and methane production throughout the duration of the continuous experiment
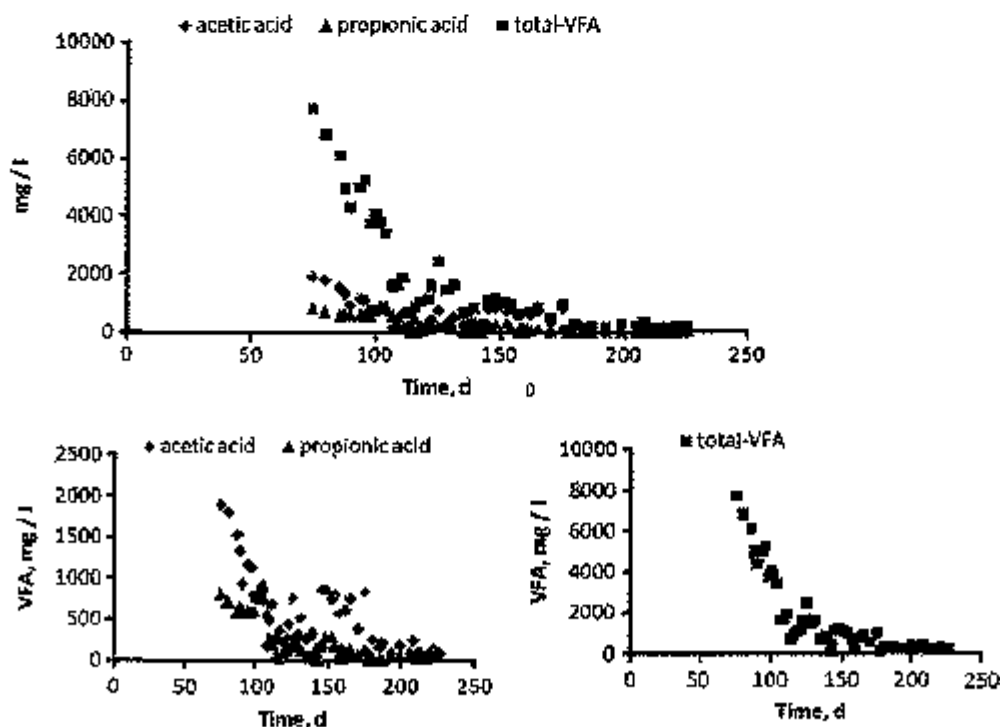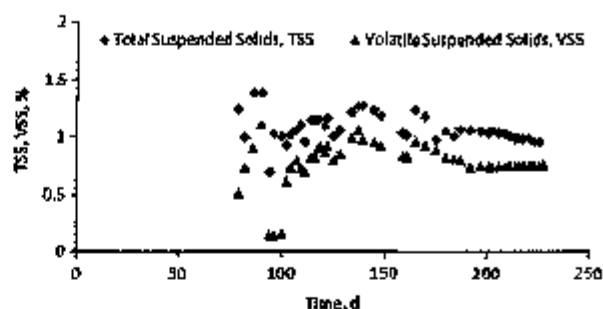
Fig. 6. Acetic and propionic acid and total-VFA (including acetic, propionic, isobutyric, butyric, isovaleric and valeric acids) concentration profile throughout the duration of the continuous experiment

of only 15 days was applied to a continuous system. Based on the results obtained, a swine manure with 6% TS (as an average representative value) and a methane potential of 183 l CH₄ per kg TS will give us approximately 11 m³ CH₄ per t manure. This is also consistent with the expected 10–20 m³ of CH₄ per t of manure treated according to Angelidaki and Ellegaard (2003).

In the study of Møller et al. (2004) it was theoretically calculated that 10 kg of straw added to 1 t of manure will increase the methane production by approximately 10%. From the present experimental study it can be concluded that 1 t of manure supplemented with 4.6 kg of straw (240 m³ CH₄ per t straw) will result in a 10% increase of methane produced compared to that expected from manure alone. This value is considered close to the value obtained in the study of Møller et al. (2004) taking into account the differences in composition that usually occur in such biomasses. It is worth mentioned that in the study of Møller et al. (2004) the calculation of the theoretical methane yield was based on the composition of manure and straw in carbohydrates, lipids and proteins.

4. Conclusions

In the present study, the codigestion of swine manure with wheat straw in a continuous operated system was investigated, as a method to increase the efficiency of biogas plants that are based on anaerobic digestion of swine manure. Also, the pretreatment of wheat straw with a wet explosion method was studied and the efficiency of the wet explosion process was evaluated based on (a) the sugars release and (b) the methane potential of the pretreated wheat straw compared to that of the raw biomass. It was found that, although a high release of soluble sugars was observed after wet explosion, the methane obtained from the wet-exploded wheat straw was slightly lower compared to that from the raw biomass. Therefore, wet explosion (which is an energy consuming process) is not suitable as pretreatment of straw for codigestion with manure at the conditions tested because the additional required energy consumption is not counterbalanced by any increase in methane production. On the other hand, the results from the codigestion of raw (non-pretreated) wheat straw with swine manure were very promising, suggesting that to get a 10% increase in methane production one would need to add only 4.6 kg of straw to 1 t of manure. Thus, wheat straw can be considered as a promising, low-cost biomass for increasing the methane productivity of biogas plants that are based mainly on swine manure.

Acknowledgments

References

Ahring, B.K., Angelidaki, I., 1998. Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. Biomass for Energy and Industry, 145–148.

Ahring, B.K., Munck, J., 2006. Method for Treating Biomass and Organic Waste with the Purpose of Generating Desired Biologically Based Products. Patent No. WO 2006-032282 A1.

Angelidaki, I., Ellegaard, L., 2003. Codigestion of manure and organic wastes in centralized biogas plants. Applied Biochemistry and Biotechnology 109, 95–105.

APHA (American Public Health Association, American Water Works Association, Water Pollution Control Federation), 1989. Standard Methods for the Examination of Water and Wastewater. 17th ed. APHA, Washington, DC.

Ballesteros, I., Negro, M.J., Oliva, J.M., Cabanas, A., Manzanares, P., Ballesteros, M., 2006. Ethanol production from steam-explosion pretreated wheat straw. Applied Biochemistry and Biotechnology – Part A – Enzyme Engineering and Biotechnology 129–132, 496–508.

Bjerre, A.B., Olesen, A.B., Fernqvist, T., Ploeger, A., Schmidt, A.S., 1996. Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose. Biotechnology and Bioengineering 49, 568–577.

Eskicioglou, C., Terzian, N., Kennedy, K.J., Droste, R.L., Hamoda, M., 2007. Athermal microwave effects for enhancing digestibility of waste activated sludge. Water Research 41 (11), 2457–2466.

Georgieva, T.I., Hou, X.R., Hilstrom, T., Ahring, B.K., 2008a. Enzymatic hydrolysis and ethanol fermentation of high dry matter wet-exploded wheat straw at low enzyme loading. Applied Biochemistry and Biotechnology 148 (1–3), 35–44.

Georgieva, T.I., Mikkelsen, M.J., Ahring, B.K., 2008b. Ethanol production from wet-exploded wheat straw hydrolysate by thermophilic anaerobic bacterium Thermoanaerobacter BG1L1 in a continuous immobilized reactor. Applied Biochemistry and Biotechnology 145 (1–3), 99–110.

Hartmann, H., Angelidaki, I., Ahring, B.K., 2000. Increase of anaerobic degradation of particulate organic matter in full-scale biogas plants by mechanical maceration. Water Science and Technology 41 (3), 145–153.

Kim, S., Dale, B.E., 2004. Global potential bioethanol production from wasted crops and crop residues. Biomass and Bioenergy 26 (4), 361–375.

Klinke, H.B., Thomsen, A.B., Ahring, B.K., 2004. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. Applied Microbiology and Biotechnology 66, 10–26.

Llabrés-Luengo, P.L., Mata-Alvarez, J., 1987. Kinetic study of the anaerobic digestion of straw-pig manure mixtures. Biomass 14, 129–142.

McGinnis, G.D., Wilson, W.W., Mullen, C.E., 1983. Biomass pretreatment with water and high pressure oxygen. The wet oxidation process. Industrial and Engineering Chemistry Product Research and Development 22, 352–357.

Møller, H.B., Sommer, S.G., Ahring, B.K., 2004. Methane productivity of manure, straw and solid fractions of manure. Biomass and Bioenergy 26, 485–495.

Palmqvist, E., Hahn-Hägerdal, B., 2000. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioresource Technology 74, 25–33.

Penaud, V., Delgenes, J.P., Moletta, R., 2000. Influence of thermochemical pretreatment conditions on solubilisation and anaerobic biodegradability of a microbial biomass. Environmental Technology 21 (1), 87–96.

Reddy, N., Yang, Y., 2005. Biofibers from agricultural byproducts for industrial applications. Trends in Biotechnology 23 (1), 22–27.

Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V., 2005. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. Process Biochemistry 40 (12), 3693–3700.

Schmidt, A.S., Thomsen, A.B., 1998. Optimisation of wet oxidation pretreatment of wheat straw. Bioresource Technology 64 (2), 139–151.

Thomsen, M.H., Thygesen, A., Jørgensen, H., Larsen, J., Christensen, B.H., Thomsen, A.B., 2006. Preliminary results on optimization of pilot scale pretreatment of wheat straw used in coproduction of ethanol and electricity. Applied Biochemistry and Biotechnology 130 (1–3), 448–460.

Paper 3

Co-digestion swine manure with raw and pretreated crops at different total solids ratio (manure: crops)

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Abstract

To enhance biogas productivity, co-digestion of swine manure with raw and wet oxidation pretreated agriculture residue or energy crops, i.e., wheat straw, corn stalker, willow and miscanthus was investigated in this study. Methane potential of co-digestion of swine manure with each biomass at TS ratio (swine manure: raw or wet-oxidized crop) 1:0.2, 1:0.4, 1:0.6 and 1:1, respectively, was examined in batch experiment. Co-digestion swine manure with wheat straw which is the largest agriculture residue in Europe at TS ratio (swine manure: wheat straw) 1:1 and 1:1.5 was also tested in a continuous operated system. The methane potential and productivity was calculated in terms of ml CH_4 per g-TS of biomass added and ml CH_4 per L of reactor working volume per day, respectively. All the experiments were done under thermophilic anaerobic conditions. The results from batch experiment showed that addition of crops significantly increased methane production and the methane potential was not influenced by increase the amount of crops in the range (swine manure: wheat straw) 1:0.2 to 1:1. Wet-oxidization pre-treatment of crops released significant amount of soluble sugar, but higher biogas yield was only obtained with willow, which was more than 90% higher than raw willow. The continuous co-digestion experiment indicated that adding 60 to 90 kg-TS of wheat straw to a swine manure based reactor (55°C, 6%-TS of swine manure) could increases the total methane productivity of the reactor, in term of m^3 CH_4 produced/m^3 reactor working volume/day, 92 and 192% respectively.

Keywords: co-digestion, agriculture residue, energy crop, wet-oxidation, biogas.

1. Introduction

Presently, most of the large-scale biogas plants in Europe are operated based on waste (solid or liquid) like manure, sewage sludge or municipal waste as substrate and their economical profitable relies on the addition of other biomass products with a higher biogas yield such as industrial organic waste materials. Typically, for example, in Denmark there are twenty-two biogas plants which use manure as the primary feedstock for biogas production, and the biogas yield is in the range of 10-20m^3/t of treated manure, while if take the reactor operation cost into account, it is only profitable when biogas yields higher than 30 m^3/t of treated material can be achieved[9]. This is currently done by the addition of industrial organic waste such as fat-sludge or fish oil[9]. However the volume of these waste streams is limited and some biogas plants have starts to import organic waste with high methane

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potential. Therefore, the investigation of other alternative biomasses to be co-digestion with manure is of great interest.

Recently, co-digestion of animal manure with carbon rich crops is getting more attention in many parts of the world. Agriculture residues, such as wheat straw or corn stalker can be considered as one of the best options for increasing the methane production since theses two crops are produced in large quantities in EU annually. A report, published by the Danish company, Novozymes, estimates that the 27 EU member states will have somewhere between 250 and 300 million tonnes of agriculture residues available annually by 2020 to convert into bio-products, and wheat straw will contribute 50% of total biomass from agriculture sector[8]. Beside of agriculture residues, some of the perennial energy crops such as miscanthus, switchgrass and willow has been recognized since it takes far less energy and nutrient to plant and cultivate (seen over the whole crop life-time)[10], and their solar energy conversion efficiency is often higher than annual plants due to a longer growing season. Furthermore, perennial crops provide a better environment for more diverse wildlife habitation[11]. Similar comments were reported by Schmidt et. al, that perennial crops such as miscanthus and switchgrass needs low agrochemical inputs, has low water requirement and low moisture levels at harvest and has greater resilience in drought[12]. All these factors increase the sustainability of cultivation of perennial energy crops and make perennial crops favourable candidates for energy production from biomass in the long run.

However, the efficiency of methane production from lignocellulosic crops can be limited by low biodegradability of lignocellulose. Therefore breaking the lignocellulosic structure will release the sugars contained in the biomass and this can possibly increase the production of methane.Several pretreatment technologies such as wet oxidation, steam explosion, dilute acid and hydrothermal treatment have been used for the pretreatment of wheat straw mainly for ethanol production[12,13,14,15]. In 2006, a new pretreatment wet-oxidation equipment has been developed by Ahring and Munck[16]. The method is a combination of thermal hydrolysis, wet explosion, and wet oxidation and it enables operating with high biomass concentrations and handling of big particle sizes, thereby, avoiding initial energy intensive mechanical milling. Moreover it is an easily controllable process with low total energy consumption[16]. The wet explosion method has so far been successfully employed for the pretreatment of wheat straw for ethanol production[2]. Therefore, it is interesting to investigate whether the wet explosion pretreatment also has a beneficial effect on the biogas production when wheat straw is used as a feedstock.

The objective of this study was to test the co-digestion of swine manure with different crops for the enhancement of biogas production and the biogas productivity of the anaerobic reactor. Therefore, assess the viability and feasibility of co-digestion willow, miscanthus (dedicated crops for energy production), corn stalker and wheat straw (agriculture residues) with swine manure in term of methane production. Different amount of raw and pretreated crops added to swine manure was tested in batch experiment, respectively. The applicability of co-digestion wheat straw with swine manure at two TS ratio was evaluated in a continuous experiment through 14 months period.

2. Material and methods

2.1 Raw biomasses and inoculm

Crops wheat straw, corn stover (agriculture residues), miscanthus and willow (energy crops) were collected in the middle of August and chopped with a hammer mill to a particle size of approximately 3-5 cm and stored at -20°C. Two types of swine manure were obtained from a pig farmer plant and a biogas plant in Denmark was homogenized and stored at -20°C until it was used. The inoculum applied in the experiment was obtained from the effluent of a steady-state lab-scale thermophilic anaerobic CSTR reactor which was for co-digestion of swine manure and wheat straw at TS ratio 1:1.

2.2 Analytical methods

Total solids (TS) and volatile solids (VS), chemical oxygen demand (COD), kjeldahl nitrogen and NH_4^+-N were analysed according to the standard methods (APHA, 1989). Lignin content was carried out by strong acid hydrolysis method[17]. For the quantification of VFA, acidified samples with 17% H_3PO_4 were analysed by a gas chromatogragh (Hewlett Packard series II) with a flame ionisation detector and a capillary column (Hewlett Parkard FFAP 30 m, inner diameter 0.53 mm, film).Methane content in biogas was quantified by a gas chromatograph (Shimadzu GC-8A) equipped with a flame ionisation detector and packed column (Porapak Q 80/100mesh).

Three groups of carbohydrates were analyzed in all crop biomasses: group one was the total carbohydrates, including those bound in the lignocellulosic biomass, the second and last groups was the soluble carbohydrates and sugar monomers. For total carbohydrates determination, a representative sample of the material was first solubilised in the strong acid at 30°C for 60 min. The released glucose, xylose and arabinose were measured by HPLC. A sample free of solids was either hydrolyzed in dilute acid and then passed through the HPLC in order to determine the soluble carbohydrate content or directly passed through the HPLC for sugar monomers determination. Detection and quantification of the sugar monomers, glucose, xylose and arabinose was made by HPLC isothermally at 60 °C with BioRad Aninex HPX-87H column using 4mM H_2SO_4 as eluent at a flow of 0.6 ml/min. The content in was determined as the ash-free residue after two steps hydrolysis with strong acid (72% w/w H_2SO_4, 12M) at 30°C and diluted acid (2.5% w/w H_2SO_4, 0.42M) at 121 °C, respectively.

2.3 Wet oxidation of crops

Wet oxidation of crops was performed in a 2.8 L reactor. The reactor was mainly constructed by six parts: (1).An oil heating pump can heat the digester up to 250°C. (2). A 2.8 L digester with a pressure safety device on the top of the reactor, the digester can hold maximum pressure up to 20 bars. (3) A 1200 rpm stirring stirrer connected to the digester, to mixing the biomass during the operation. (4) A 50 L flash tank connected to the bottom of the digester, to receive the treated biomass. (5) A gas bottle with maximum 100 bars of pressure supplies pressure to inject the liquid chemical into the digester. (6)

A computer control panel to control and monitoring of the stirring speed, temperature and pressure of the reactor (for the wet oxidation unit Fig.1). Wet oxidation condition was 180°C and corresponding 10 bars of pressure with H_2O_2 as oxidizing agent as dose of 6% of total solid concentration of crops. The efficiency of the process was evaluated based on the sugar release amount and the methane potential of the pretreated material compared with that biomass. i.e., content of soluble sugar, TS, VS and lignin in both raw and pretreated crops were analysed, respectively. And the methane potential of each raw and pretreated crops codigestion with manure were tested by batch experiment at different TS ratio (crops: manure) respectively.
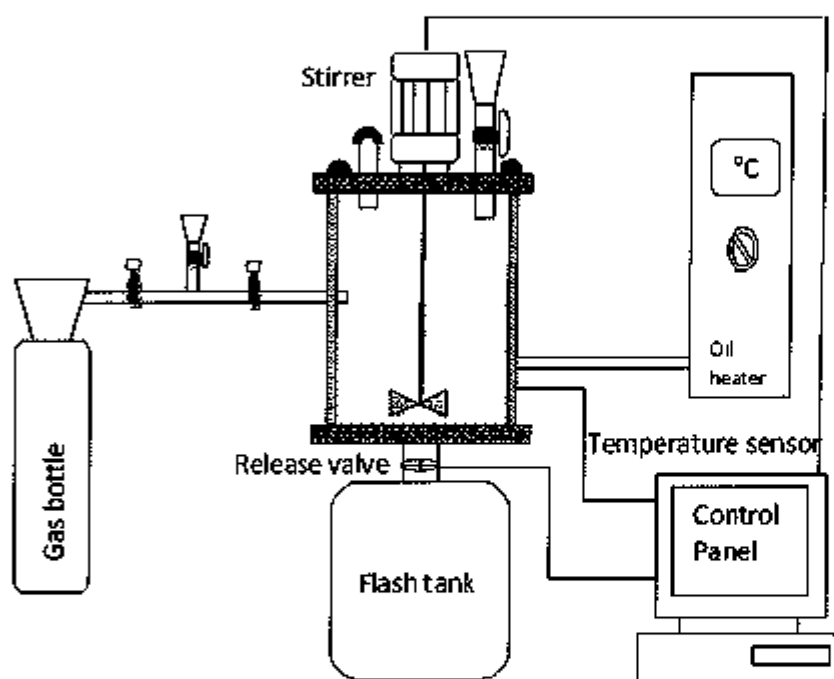
Fig.1 Wet oxidation unit

2.4 Co-digestion of raw and wet-oxidized crops with swine manure at various mixing ratio

The optimal co-digestion feed ratio between crops (i.e, wheat straw, corn stalker, willow and miscanthus) with swine manure at different TS ratio was carried out in triplicates in 117 ml serum vials sealed with butyl rubber stoppers and aluminium crimps under anaerobic condition. The vials contained 5 ml of inoculum obtained from a continuous stirred tank reactor (CSTR) type anaerobic digester fed with swine manure and wheat straw, 25 ml of swine manure proximally equivalent to 0.5 g-TS. 0.1, 0.2, 0.3 and 0.5 g-TS of raw and wet-oxidized crops were added separately, i.e., the ratio between swine manure and crop was, 1:0.2, 1:0.4, 1:0.6 and 1:1. There are triplicate vials with only inoculums served as control I, triplicate vials with 5 ml inoculums and 25 ml of pig manure as control II (see more

detail) Table 1. The vials were incubated at 55°C and the methane production was followed throughout the experiment. Methane production yield was calculated as the volume of methane produced per g of TS biomass added as substrate.

Table 1

Co-digestion test with various mixing ratio of crops and swine manure.

Set-up	Pig manure (g·TS)	Crop* (g-TS)	Inoculum (ml)	Total TS added g	Crop and swine manure TS ratio %	Working Volume (ml)
Control I	0	0	5	0	0	5
Control II	0.5	0	5	0.5	0/100	30
A	0.5	0.1	5	0.6	17/83	30
B	0.5	0.2	5	0.7	29/71	30
C	0.5	0.3	5	0.8	38/62	30
D	0.5	0.5	5	1	50/50	30

Four crops,i.e., raw and wet-oxidized crops wheat straw, corn stalker, miscanthus and willow were tested.

2.5 Continuous co-digestion of swine manure with wheat straw

Continuous co-digestion of a mixture of swine manure and wheat straw at 1:1 and 1:1.5 TS-based ratio was carried out in a lab-scale unit. The unit was operated at thermophilic temperature (55°C), consisted of one 7-L active volume CSTR and two influent compartments. One influent compartment was filled up with swine manure (20g/L TS concentration) and the other one filled up with wheat straw (TS content 93%), Swine manure and wheat straw were added in the digestion four times a day at regular time intervals at hydraulic retention time (HRT) of 15days. All the automatic stirrers, influent and effluent valves was controlled by a computer system. The reactor was started-up with anaerobic mixed 40% cattle manure, and 40% of swine manure, and 20% manure fibres. The pH, total biogas production and CH_4 content was monitored daily, while complete characterization took place once the steady state reached. TS and VS were measured both in the reactor and in the effluent in order to make sure that no accumulation of solids occurred in the reactor.

3. Results and discussion

3.1 Characterization of the raw and wet-oxidized biomasses

Raw and wet-oxidized crops and swine manure were characterized regarding their total and volatile solids (TS and VS) concentration respectively, chemical oxygen demand content (COD) and total and ammonium nitrogen (total-N and NH_4^+-N) concentration. The total and soluble carbohydrate and lignin content were also determined in all the biomasses, although the soluble sugar was very low, which was

lower than 1% of TS concentration. The characterization results of raw biomasses are shown in Table 2, and the results for wet-oxidized biomasses are shown in Table 3. The characterization result shown that all the raw and wet-oxidized crops were rich in organics (VS) and carbohydrates content but poor in nitrogen. Present results (Table 2) were shown for swine manure, which was rich in nitrogen but poor in VS, therefore relatively lower carbon carbohydrates content.

Table 2. Characterization results of raw crops and swine manure

	Wheat straw	Corn stalker	Miscanthus	Willow	Swine[1] manure	Swine[2] manure
TS (%)	92.1±0.1	28.3±0.4	36.3±0.2	53.2±1.9	2.3	2.1
VS (%)	86.3±1.7	27.4±0.4	35.2±0.2	52.1±1.8	1.5	1.4
Total-COD (g/g-TS)	1.0±0.1	1.3	1.2±0.1	1.3±0.1	1.1	1
Total-N (mg/g-TS)	6.8±0.4	15±1	7.4±0.5	4.3±0.5	98±1	107±3
NH4-N (mg/g-TS)	0.8±0.1	2.2	1.1±0.1	0.7±0.1	39±0.1	87±1
Total Carbohydrate(% of TS)	48±2	63±3	62±3	45±2	12.2±0.1	14
Soluble sugar (% of TS)	<1	<1	<1	<1	<1	<1
Lignin (% of TS)	20±1	14±1	23.2±0.3	23.2±0.3	11.7±0.1	10.1
Residues (% of TS)	18.5±2	23±2	15±1	30±2	75±3	75

[1] swine manure used for batch test. [2] swine manure used for continuous test.

The methodology of wet oxidation pre-treatment was to enhance the biodegradability of crops by breaking the polymer chain to easily accessible soluble compounds, therefore, increasing the methane production yield(1). Comparison of the characterization results Table 2 and Table 3 shows that, after wet-oxidation pre-treatment, the soluble sugar content was significantly increased in all the crops. Especially for corn stalker which was increased nearly 30%, this is because the corn stalker was harvested when it was fresh and therefore, there was high water and lower lignin content in it. The soluble sugar content was also in wheat straw, miscanthus and willow, which was 15.8, 13.5 and 12.2%, respectively.

Table 3. Characterization results of wet-oxidized crops

	Wheat straw	Corn stalker	Miscanthus	Willow
TS (%)	13.8±0.3	11.6±0.6	11.8±0.2	13.6±0.2
VS (%)	13.2±0.4	11.2±0.6	11.3±0.2	13.1±1.1
Total-COD (g/g-TS)	1.1±0.1	1.3±0.1	1.3±0.1	1.3
Total-N (mg/g-TS)	3.5±0.1	13.3±0.6	3.8±0.2	3.8±0.2
NH4-N (mg/g-TS)	6.0±3.2	1.1±0.1	0.7±0.1	0.7±0.1
Total Carbohydrate	46.4±0.9	64.7±4.1	55.3±5.2	58.4±3.3
Soluble sugar (% of TS)	15.8	29.9	13.5	12.2
Lignin (% of TS)	34.8±1.4	24.3±0.8	31.7±0.8	33.9±1.9
Residues (% of TS)	20±1	12±1	16±2	9±1

There were three major sugar monomers content in wet-oxidized crops, which were arabinose, xylose and glucose. The sugar monomers composition for each crop was different. For corn stalker consists of proximally 60% of glucose, 30% xylose and 10% of arabinose. The rest crops wheat straw, willow and miscanthus consists proximally 70% of xylose and 20% glucose and 10% arabinose (See Fig 2, the soluble sugar composition of wet-oxidized crops). The wet-oxidation condition applied in this study was the standard condition for pre-treatment of wheat straw applied on bio-ethanol production study[2]. In the previously study[18], different concentration of oxidation agent, TS, temperature and pre-treatment retention time was tested on each crop based on the standard wet oxidation condition, but the other pre-treatment conditions, either cost too much energy or the soluble sugar releasement amount too low.
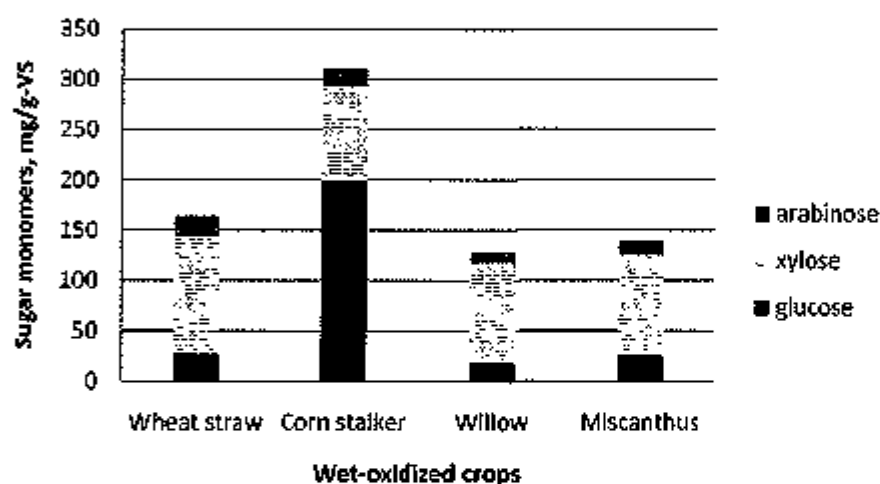

Fig.1. Soluble sugar composition of wet-oxidized crops.

3.2 Selection of raw and wet-oxidized crops co-digestion with swine manure at various mixing ratio

After proximally three month's co-digestion of swine manure and crops in a 55°C incubator, the methane potential of biomasses varied from 220 to 410 ml CH_4 g^{-1} VS_{added} (Table 4), the highest methane potential was determined from Raw corn stalker-A and C (410 ml CH_4 g^{-1} VS_{added}), the total methane production was 84 and 140% higher than digest swine manure alone. Methane potential of willow, miscanthus and wheat straw was in the range 220-282, 324-346 and 311-329 ml CH_4 g^{-1} VS_{added} in the all different TS ratio, respectively.

For wet-oxidized crops, there was a significant of decrease methane potential observed from wet oxidized corn compared with raw corn, and the cause of this is probably due to the inhibitory compounds releasement during the wet oxidation pretreatment[3], the similar results was also observed from wet-oxidized wheat straw and miscanthus (Table 4). No significant evidence of inhibition was observed when the TS ratio of wet-oxidized or raw crops and swine manure at 50:50 or below 50:50[4] except wet-oxidized corn stalker, when the TS concentration of wet-oxidized corn stalker increased to

0.75 and 1 to 1 of swine manure the methanogenesis was stopped. The reason of this is the wet-oxidized corn stalker contains higher soluble sugar among the four wet-oxidized crops. Therefore, when co-digestion this biomass with swine manure the soluble sugar are quickly convert to volatile fatty acids (VFA) by acidogenesis bacteria, the sudden VFA concentration increasing leading the pH dropped to below to 4.5, the methanogenesis reaction was inhibited by the lower pH [19]. This will not appeared in a CSTR reactor due to the large buffering capacity [5,6]. However, there was a large amount of CH_4 increasing observed from wet-oxidized willows, more than 90% methane was obtained from wet-oxidised-C than from raw willow-C, this is probably because the structure of crop willow are more woody compared with wheat straw or corn stalker. There are two major effects on willow after wet-oxidation pre-treatment, one is the releasement of sugar and other easily biodegradable compounds from lignocellulose, and another is that the particle size of willow was reduced to under 0.5 cm. Particle size reduction of the biomass by chipping, milling and grinding can increase the available surface area and intracellular component of the biomass and therefore increase the biodegradability of the biomass[6]. However, co-digestion of wet-oxidation pretreated miscanthus, corn stalker and wheat straw with swine manure, the methane potential was much slightly lower in comparison with that raw crop, which was in the range 290-316, 211-360 and 254-275 ml CH_4 g^{-1} VS_{added} in the all different TS ratio, respectively. Although in some of the case wet-oxidized of these three crops shows the fast methane conversion rates, but it was not so encouragement for further investigation due to the energy consuming of pretreatment. On the other hand, pretreatment of willow with wet-explosion method increased more than 90% of methane potential, according to the previously study on energy balance and cost-benefit analysis of biogas production from perennial energy crops pretreated by wet oxidation[7] approved that wet-oxidation of willow for biogas production is economically profitable.

In Denmark, the hydraulic retention times (HRT) of most thermophilic anaerobic digester are operated within 20day[4]. Therefore, the quality and quantity of the biogas, i.e., the increases of maximum CH_4 per g-biomass added and increases of percentage CH_4 than swine manure, the biomass conversion rate to biogas and methane production efficiency m^3 produced per m^3 reactor working volume per day must take into account when choosing crops for co-digestion with swine manure. In all the raw and wet-oxidized crops expect wet-oxidized corn stalker, at Biomass-A gives the highest methane production yield in the term of CH_4 per g-TS added, i.e., best biogas yields and less crop demand. Biomass-D shows the highest methane production rate in the term of m^3 CH_4 per reactor working volume per day, i.e., best volumetric reactor productivity and large crop demand also bigger amount of fertilizer. Basis on the currently results also it shows that co-digestion raw or pretreated willow with swine manure at day 20, the methane potential reached more than 90% of total biogas production, i.e. highest biomass conversion rate and less operation cost. For the rest of raw or pretreated crops, it was around 80% but high methane production per reactor volume per if the HRT is 20 days.

3.3 Continuous co-digestion experiment

Co-digestion raw wheat straw and swine manure in a CSTR reactor was divided into two phases, phase one, co-digestion raw wheat straw and swine manure was at TS based ratio 1:1, and phase two at 1.5:1. The whole experiment was completed in 415days, which was approximately 27 retention times, of which 17 retention times was used for phase one and 10 retention times for phase two. The reactor performance in terms biogas and methane production, volatile fatty acids and total and volatile solids concentration is shown in Figs 2-5, respectively (samples for VFA and solids determination was collected only after day 75, when biogas production was established in the system). The steady state of phase one was reached after 15 retention times, which was mainly attributed to machinery failures. The TS and VS concentration in the interior and effluent of the reactor were the same throughout the duration of the experiment. This indicated that there is no accumulation of solids occurred inside the reactor.
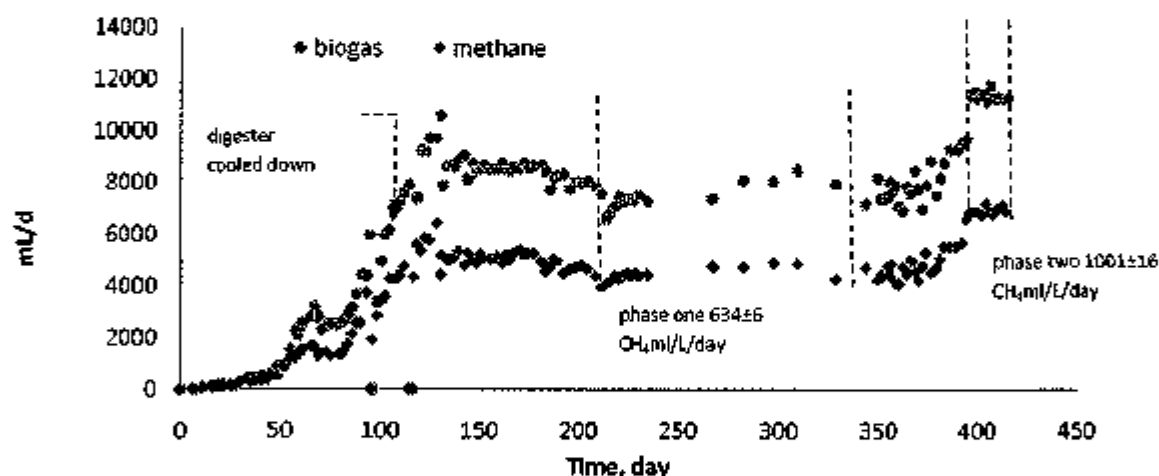

Fig. 2. Biogas and methane production of the CSTR reactor.
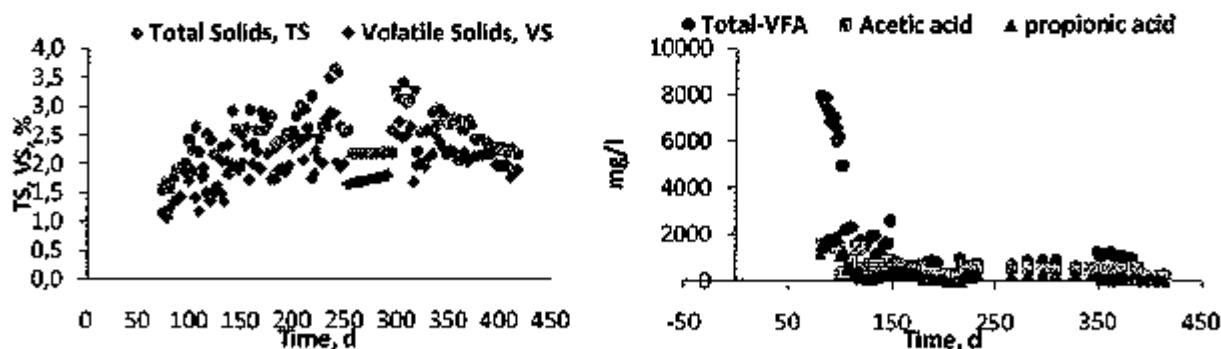

Fig.3. Total and volatile solids concentration (measurements were performed on sampling inside the reactor)

Fig.3. Acetic, propionic acid and total −VFA (including acetic, propionic, isobutyric, butyric, isovaleric and valeric acids)

Table 4:

Results of methane potential test on different crops at different co-digestion ratio with swine manure.

Substract	[a]	Duration of the batch assay	Total methane potential			% of total CH$_4$ increased than swine manure alone	Short-term methane potential	
			Total CH$_4$ production	ml CH$_4$ per g-VS added[b]	ml CH$_4$ per g-TS added		% of total day 20	M^3 CH$_4$ produced/m^3 working volume/day (HRT 20 days)
Control	I	75	21±0.5	0	0	0	0	0
	II		140±3	362±4	238±3	0	87	0.28
Raw willow	A		187±3	282±3	277±3	34	93	0.37
	B		198±2	258±3	253±3	41	97	0.40
	C		218±2	251±2	246±2	56	90	0.44
	D	45	237±5	220±2	216±2	69	90	0.47
Wet oxidized willow	A		233±4	335±3	321±3	66	97	0.47
	B		264±3	328±4	315±4	88	99	0.53
	C		302±2	333±3	320±3	116	90	0.60
	D		313±5	277±2	266±2	124	92	0.63
Raw miscanthus	A		210±3	324±3	315±3	50	85	0.42
	B		248±1	334±5	324±5	77	76	0.50
	C		290±3	346±1	336±1	107	67	0.58
	D	55	357±2	346±2	336±2	155	62	0.71
Wet-oxidized miscanthus	A		195±3	238±6	228±6	39	71	0.39
	B		222±3	235±4	226±4	59	69	0.44
	C		263±2	248±5	239±5	88	83	0.53
	D		337±5	259±4	249±4	141	80	0.67
Raw corn stalker	A		257±3	410±3	394±3	84	84	0.51
	B		275±3	378±5	363±5	96	83	0.55
	C		336±5	410±2	394±2	140	70	0.67
	D	60	391±3	385±3	370±3	179	59	0.78
Wet-oxidized corn stalker	A		211±2	260±8	251±8	51	89	0.42
	B		201±2	211±4	205±4	44	88	0.40
	C		63±3	43±3	42±3	-55	85	0.13
	D		41±1	17±1	16±1	-71	95	0.08
Raw straw	A		200±2	320±3	298±3	43	82	0.40
	B		224±3	311±4	290±4	60	74	0.45
	C		266±5	329±5	306±5	90	78	0.53
	D	85	317±3	318±2	296±2	126	82	0.63
Wet oxidized straw	A		184±1	261±3	251±3	31	81	0.37
	B		206±1	254±4	244±4	47	75	0.41
	C		250±2	275±3	264±3	79	80	0.50
	D		297±2	266±2	255±2	112	83	0.59

[a] A, B, C and D indicated different TS ratio composition (see table 1). [b] calculated basis on the original TS and VS before pretreatment.

pH valve was also measured and it was 7.4 at steady state. The methane production at steady state of phase one and phase two was stabilized at 634 and 1001±16 CH_4 /L active volume/day, which is equals to 222 and 271L CH_4 per kg TS fed. The methane production yield increase of phase two than that of phase one was probably because the carbon source and nitrogen concentration (C/N) were more balanced than a phase one. A similar study form Li et al. (2009)[6] reported that co-digestion corn stalker with cattle manure at four mixing ratio (manure/corn stalks: 1:1, 1:2, 1:3 and 1:4) for biogas production. The highest methane production yield was obtained at 1:3 from their study. Another study from Wu et al. (2010)[7] reported that co-digestion swine manure with wheat straw C/N ratio (manure/wheat straw: 16:1, 20: 1 and 25:1), the C:N ratio of 20:1 was found gives the highest methane production yield. Based on our batch methane potential experiment, digestion swine manure alone with 6% TS (as average reprehensive value) for 15days give approximately 232ml CH_4 per g-TS added which equals about 14 m^3 per t of swine manure (section 3.2). This is also consistent with the expected 10-20 m^3 of CH_4 per t of swine manure treated according to Angelidaki and Ellehaard (2003). However, from the present experiment study it can be concluded that add 60 and 90kg-TS of straw to a swine manure CSTR reactor which is operated at 15days retention time will results in 92 and 192% increase of methane produced compared to that expected manure alone, respectively.

3. Conclusions

The results of the present study has ensured that adding carbon rich crops to the co-digestion process with swine manure can increases the methane production significantly. Wet-oxidation pre-treatment of crops indicates that the soluble sugar content of each tested crops can be increased after pre-treatment, but intent for higher methane production was only occurred in willow. Among the four tested crops, willow has the fastest conversion rate to biogas, more than 90% of total methane production at day 20. Even though raw miscanthus, corn stalker and wheat straw has lower biomass conversion rate, which was varied from 60 to 89% methane produced of total methane production at day 20, but since these crops has much higher methane potential when they co-digestion with swine manure, the total methane production per m^3 working volume per day was still higher than raw willow. The methane potential in the term of ml CH_4 per g-VS added was not significantly influenced by changing the amount of crops in the range of 0.2 to 1 g-VS of crop per 1 g-VS of swine manure. Co-digestion swine manure with wheat straw indicate that supply 60 to 90kg-TS of straw to a thermophilic swine manure reactor which is operated at 15days retention time and 6% TS concentration of manure will increases the total methane production 92 and192%, respectively.

Acknowledgements

Reference

1. Fan, L. T., Gharpuray, M. M. & Lee, Y.-H. 1981. Evaluation of pretreatments for enzymatic conversion of agricultural residues. Biotechnol. Bioeng. Symp. 11: 29–45.
2. Georgieva TI., Hou XR ., Hilstrom T ., Ahring BK. 2008 Enzymatic hydrolysis and ethanol fermentation of high dry matter wet-exploded wheat straw at low enzyme loading. APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY 148: 35-44
3. M.Galbe. G. Zacchi 2007. Pretreatment of lignocellulosic materials for efficient bioethanol production. Adv Biochem Engin/ bioechnol (2007) 108: 41-65
4. Biogas Plant for the Treatment of Organic Waste and Cattle Slurry in Aalborg Municipality http://ec.europa.eu/energy/renewables/bioenergy/doc/anaerobic/015bm_352_1992.pdf
5. Palmqvist, E., Hahn-Hägerdal, B., 2000. Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioresource Technology 74, 25–33.
6. Li, X.,Li,Q., Zhang, M.X., Fu,. G.Z., Lar,. J.S, 2009 Anaerobic co-digestion of cattle manure with corn stover pretreated by sodium hydroxide fro efficient biogas production. Energy Fuels 23, 4635-4639.
7. Xiao, U., Wangying, Y, Jun, Zhu,. Curtis, M. (2010) Biogas and CH_4 productivity by co-digestion with tree crop residues as anexternal carbon source. Bioresource Technology 101: 4042-4047
8. Farm waste: Worth its weight in gold? http://www.euractiv.com/en/cap/farm-waste-worth-its-weight-gold-news-497922
9. Angelidaki, I., Ellegard, L., 2003. Codigestion of manure and organic waste in centralized biogas plant. Applied Biochemisty and biotechnology 109, 95:105
10. U.S. DOE 2006 breaking the biological barriers to cellulosic ethanol: A joint research agenda. DOE/SC-0095, US. department of energy office of science and office of energy efficiency ad renewable energy (www.doegenomestolife.org/biofuels/).
11. Semere, T. & Slater, F. M. 2007. Ground flora, small mammal and bird species diversity in miscanthus and reed canary-grass fields. Biomass Bioenergy 31: 20-29.
12. Schmidt, A.S., Thomsen, A.B., 1998. Optimisation of wet oxidation pretreatment of wheat straw. Bioresource Technology 64 (2), 139–151.
13. Ballesteros, I., Negro, M.J., Oliva, J.M., Cabanas, A., Manzanares, P., Ballesteros, M., 2006. Ethanol production from steam-explosion pretreated wheat straw. Applied Biochemistry and Biotechnology – Part A – Enzyme Engineering and Biotechnology 129–132, 496–508.
14. Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V., 2005. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. Process Biochemistry 40 (12), 3693–3700.
15. Thomsen, M.H., Thygesen, A., Jørgensen, H., Larsen, J., Christensen, B.H., Thomsen, A.B., 2006. Preliminary results on optimization of pilot scale pretreatment of wheat straw used in coproduction of ethanol and electricity. Applied Biochemistry and Biotechnology 130 (1–3), 448–460.
16. Ahring, B.K., Munck, J., 2006. Method for Treating Biomass and Organic Waste withthe Purpose of Generating Desired Biologically Based Products. Patent No. WO 2006-032282 A1.
17. Kaar, W.E., Cool, L.G., Merriman, M.M., Brink, D.L., 1991. The complete analysis of wood polysaccharides using HPLC. J. Wood Chem. Technol. 11, 447-463
18. Wang G, Gavala HN, Skiadas IV, Ahring BK., 2008.Wet explosion of wheat straw and codigestion with swine manure: effect on the methane productivity. Waste management. 11, 2830-2835

19. Uellendahl. H, Wang. G, Møller. H. B., Jørgensen. U, Skiadas. I. V, Gavala. H. N and Ahring. B. K 2008. Energy balance and cost-benefit analysis of biogas production from perennial energy crops pretreated by wet oxidation. Water Science & Technology—WST. 58.9

Paper 4

Energy balance and cost-benefit analysis of biogas production from perennial energy crops pretreated by wet oxidation

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Energy balance and cost-benefit analysis of biogas production from perennial energy crops pretreated by wet oxidation

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ABSTRACT

Perennial crops need far less energy to plant, require less fertilizer and pesticides, and show a lower negative environmental impact compared with annual crops like for example corn. This makes the cultivation of perennial crops as energy crops more sustainable than the use of annual crops. The conversion into biogas in anaerobic digestion plants shows however much lower specific methane yields for the raw perennial crops like miscanthus and willow due to their lignocellulosic structure. Without pretreatment the net energy gain is therefore lower for the perennials than for corn. When applying wet oxidation to the perennial crops, however, the specific methane yield increases significantly and the ratio of energy output to input and of costs to benefit for the whole chain of biomass supply and conversion into biogas becomes higher than for corn. This will make the use of perennial crops as energy crops competitive to the use of corn and this combination will make the production of biogas from energy crops more sustainable.

Key words | anaerobic digestion, energy crops, lignocellulose, miscanthus, perennial crops, pretreatment, wet oxidation

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INTRODUCTION

Anaerobic digestion of energy crops has in recent years expanded extensively throughout Europe. Especially in Germany where a minimum price is guaranteed for electricity generated from renewable energy resources, large areas of agricultural land are cultivated predominantly with corn for energy production in biogas plants. Annual crops like corn are, however, cultures that need significant energy and fertilizer input for their growth. It has been recognized that perennial crops like miscanthus, switchgrass, and willow take far less energy to plant (seen over the whole crop lifetime) and to cultivate and require less nutrient and pesticide supply (U.S. DOE 2006; European Environment Agency 2007). At the same time, their annual solar energy conversion efficiency is often higher than that of annual plants due to a longer growing season. Furthermore, perennial crops

provide a better environment for more diverse wildlife habitation (U.S. DOE 2006; Semere & Slater 2007), and reduce nutrient losses (Aronsson & Bergstrom 2001; Jørgensen 2005). These factors increase the sustainability of cultivation of perennial crops and make perennial crops favorable candidates for energy production from biomass in the long run. The microbial degradation of the raw perennial crop biomass and its microbial conversion into for example biogas is, however, limited since these crops consist of lignocellulose. Therefore, a suitable pretreatment is needed to break the lignocellulosic structure and make the embedded sugar polymers bioavailable. The wet oxidation pretreatment is a thermal pretreatment method under high pressure with addition of oxygen. Wet oxidation has been successfully applied for the pretreatment of

lignocellulosic biomass for subsequent bioethanol fermentation (Lissens *et al.* 2004*a*), and has been tested for the pretreatment of different organic waste fractions for subsequent anaerobic digestion (Lissens *et al.* 2004*b*). This pretreatment method has been further developed at BioCentrum-DTU for treating biomass at high dry matter concentration and with a subsequent pressure release (flash); therefore this pretreatment method is also denoted wet explosion. This pretreatment method has previously been applied for increasing the biogas yield of manure fibers showing that the process has its highest potential for treating concentrated lignocellulosic biomass (Uellendahl *et al.* 2007). The combination of wet oxidation together with acid presoaking and enzymatic hydrolysis has shown that 64% of glucose and 95% of xylose can be released from miscanthus for the subsequent conversion into bioethanol (Sørensen *et al.* 2008). Most recently the wet oxidation pretreatment has been applied for enhancing the degradability of different perennial crops in order to increase their biogas yield. For energy crops like miscanthus the pretreatment efficiency is related to the degree of lignification of the plant, which is highly dependent on the harvest time. This paper compares the energy balance and cost-benefit analysis of the perennial crops with the energy balance and cost-benefit analysis of corn as a typical annual energy crop.

MATERIAL AND METHODS

The energy balance and cost-benefit analysis for perennial energy crops performed in this study implies the whole chain of plant cultivation (field preparation, planting, fertilizer and pesticide application), harvesting and conversion of the plant material at a centralized biogas plant (Figure 1). This enables the comparison of the cost-benefit of perennials to the annual crop corn, the effect of low and high yielding perennial crops and to evaluate the effect of the additional wet oxidation pretreatment. In order to compare the different scenarios independent of the market prices an energy balance has been developed in the first place, based on the energy input of each cultivation and process step and the final output as biogas, respectively. The cost-benefit analysis is performed based on the prices for seeds, fertilizer, pesticides, soil application and transportation and for electricity sales prices from biogas in Denmark. These costs and sales prices are also applied for those scenarios with higher biomass yields as achieved for example in Southern Germany. The energy inputs and costs for the cultivation are directly given as kWh/ha and €/ha, respectively. Plant propagation and transportation is only taken into account as cost factor, not for the energy balance. The energy input and costs for the biogas process and the pretreatment are calculated as kWh/ha and €/ha by combining the process input/costs in kWh/t-TS, and €/t-TS with the respective yields of energy crops (t-TS/ha).

Energy input and costs for plant cultivation

The energy input for the fertilizer used for cultivation of the different crops is based on the different fertilizer needs of each crop and the specific energy needed to produce 1 kg of the specific fertilizer (Table 1). The energy input for the
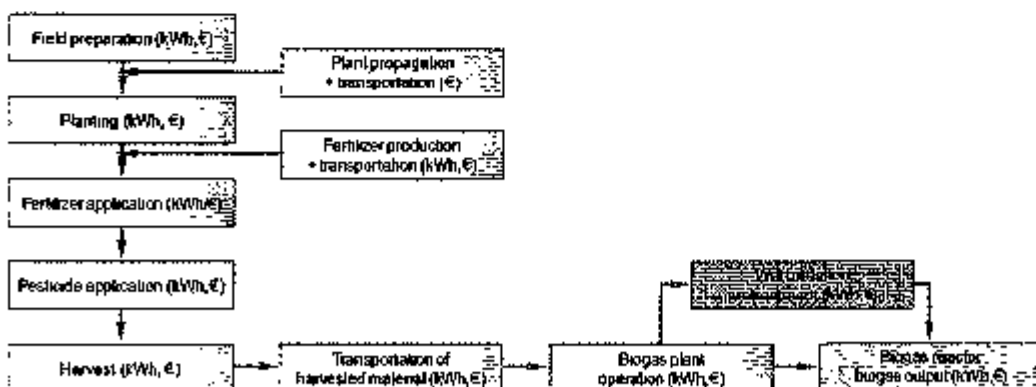

Figure 1 | The different steps in the cultivation and biogas conversion of energy crops taken into account for the energy balance and cost benefit analysis.

			Corn	Miscanthus	Willow
Fertilizer input[*]	N	kg/ha/year	146	90	100
	P	kg/ha/year	42	6	6
	K	kg/ha/year	111	45	45

[*]Energy consumption for production of N 50 MJ/kg, P 12 MJ/kg and K 7 MJ/kg (Dalgaard et al. 2001)

different steps in plant cultivation and harvest is displayed in Table 2. For corn the numbers are based on calculations by Møller et al. (2008). For the cultivation of willow the total energy input for the cultivation over the whole cultivation period given by Heller et al. (2003) is divided by the total cultivation period. The input for miscanthus is estimated from the data on corn and willow. Cost calculations are based on current prices in Denmark for seed, fertilizer, pesticides and fuel for machinery used for field preparation. The data are valid at crop yields of 10–15 ton dry matter per ha. At higher yields both energy use and costs for harvest and transport will increase.

Energy input and costs for biogas process and pretreatment

The energy used for the operation of the biogas plant and for the wet oxidation pre-treatment is displayed in Table 3. The calculations for the pre-treatment are based on the treatment of 20,000 ton solid biomass per year. The energy consumption per ton of solid biomass will be lower for pre-treatment installations with a higher capacity. Investment costs are not regarded for the biogas plant which is assumed

		Corn[*]	Miscanthus[†]	Willow[‡]
Field preparation	MJ/ha/year	953	100	300
Planting	MJ/ha/year	108	100	100
Fertilizer application	MJ/ha/year	72	72	50
Pesticide application	MJ/ha/year	108	25	25
Harvest + transport[§]	MJ/ha/year	1,795	2,190	1,150
Total	MJ/ha/year	3,016	2,487	1,625

[*]Møller et al. (2008)
[†]20 years cultivation
[‡]Heller et al. (2003). 23 years cultivation
[§]For biomass yields achieved in Denmark

		Biogas plant operation[*]	Wet oxidation pretreatment[†]
Heat	MJ/t	96	4.9
Electricity	MJ/t	96	0.1
Total	MJ/t	193	5.0

[*]Møller et al. (2008).
[†]Christensen et al. (2007)

as pre-existing. Investment costs for the wet oxidation pretreatment have been estimated to 725,000 € (Christensen et al. 2007) for equipment with a capacity of 20,000 ton solid biomass per year. The payback time is set to 10 years.

Biomass yield and energy output from biogas production

The energy output per ha from the conversion of the crop into biogas depends on both the growth yield of each crop on the field (Table 4) and the biogas yield achieved in the biogas process (Table 5).

The anticipated crop yields are those achieved or expected under practical commercial conditions and not yields from controlled experiments, which are often 10–30% above yields in practice (Venendaal et al. 1997). Miscanthus for biogas conversion is expected harvested in autumn with a high water content and app. 35% higher dry matter yield than at spring harvest (Jørgensen et al. 2003; Lewandowski & Heinz 2003). Finally, the degree of lignification is lower for earlier harvest times thereby enhancing the microbial degradability under anaerobic conditions. For comparison of the effect of higher crop yields the biomass yields achieved in climate with a higher average temperature than in Denmark were taken. Average yields of 25.5 t-DM/ha were achieved for Miscanthus x giganteus genotypes in field trials harvested in autumn in Southern Germany following the third growing season (Clifton-Brown & Lewandowski 2002). These yields were achieved without irrigation and with application of the same amount of fertilizer as used in Denmark (Table 1). For the present cost-benefit analysis a 30% lower value was anticipated under commercial conditions. This value was also anticipated for corn for regions with higher average temperature.

The biogas yield per ton of organic matter (volatile solids, VS) is influenced by the pretreatment. The different

Table 4 | Biomass yields of perennial and annual crops

		Corn DK	Miscanthus DK	Willow DK	Corn DK	Miscanthus DK
Biomass yield	kg-TS/ha	10,120[*]	12,700[+]	11,180[+]	17,850[$]	17,850[$]
	TS	31%	42%	53%	–	–
	VS/TS	96%	97%	98%	–	–

[*]Landscener (2006)
[+]Graversen & Gylling (2002) and Jørgensen et al. (2003)
[*]2008 data from Danish Agricultural Advisory Service
[$]Practical yield calculated based on Clifton-Brown and Lewandowski (2002)

Table 5 | Methane yields achieved in batch experiments for the different crops with and without pretreatment

			Corn	Miscanthus	Willow
Methane yield	Raw material	L-CH$_4$/kg-VS	360	200	200
	After wet oxidation	L-CH$_4$/kg-VS	360	360	360

methane yields per ton of organic matter with and without pretreatment are currently investigated. The preliminary results are given in Table 5. For these experiments *Miscanthus x giganteus* was harvested in autumn. The wet oxidation process was optimized for achieving the highest increase in biogas yield at low process operation costs. For the biogas yield achieved per ha of cultivated land it is taken into account that part of the organic matter is oxidized during the wet oxidation process, reducing the VS content by 5%. The benefit from the biogas production is calculated as net electricity production with 40% efficiency of electricity production in a combined heat and power plant. The sales price for electricity produced from biogas is fixed at 0.10 €/kWh in Denmark from 2008. For calculation of the net energy production the energy consumption for operation of the biogas plant and pretreatment is subtracted from the total energy production.

RESULTS AND DISCUSSION

The results are distinguished between energy in- and output and cost-benefit for the biomass supply and the biogas production for the different energy crops. For the energy balance the energy needed for cultivation, harvest and transport is taken as input and the total energy output in the form of methane in the biogas plant is taken into account. For the cost-benefit analysis calculations are based on the

benefit from electricity produced from the biogas and the costs for cultivation and harvest by the farmer and the costs for the transport of the harvested biomass by either of these two partners. Any kind of further profit is not included in these calculations. Therefore, this model can only directly be applied for scenarios where the biogas plant together with the CHP unit is owned by the farmers.

Energy balance

The energy in- and output and net energy gain for cultivation and biogas production from the different energy crops with and without pretreatment is displayed in Table 6. The energy input for cultivation and harvest is 82% for miscanthus and 54% for willow of the energy needed for the growth of corn in Denmark. The energy input needed for transportation of the harvested biomass to the biogas plant and for processing at the biogas plant is lower for biomass with a higher dry matter concentration. These values are therefore lowest for willow. The energy input for the biomass supply increases with higher biomass yields due to higher costs per ha for transportation of the harvested biomass and treating it at the biogas plant. The energy input for miscanthus compared to corn with the same higher biomass yield is slightly lower due to a slightly lower energy input for harvesting 1 ton of miscanthus. Due to the significantly lower use of fertilizer for the two perennial crops the energy input for the fertilizer is for miscanthus

Table 6 | Energy and output for biomass cultivation and biogas production for miscanthus, willow and corn without and with pretreatment

Crop		Corn DK yield	Miscanthus DK yield	Willow DK yield	Corn S EU yield	Miscanthus S EU yield
Energy input						
Cultivation + harvest[*]	MWh/ha	0.84	0.69	0.45	1.22	0.94
Fertilizer — production + transport	MWh/ha	2.43	1.36	1.50	2.43	1.36
Biogas plant	MWh/ha	1.75	1.62	1.13	3.09	2.28
Pretreatment (operation)	MWh/ha	0.05	0.06	0.05	0.08	0.08
Total input + pretreatment	MWh/ha	5.06	3.73	3.13	6.82	4.66
Energy output						
Total	MWh/ha	36.04	25.36	22.55	63.69	35.67
Net energy gain	MWh/ha	31.02	21.69	19.47	56.95	31.10
Output/input	GJ/GJ	7.2	6.9	7.3	9.5	7.8
With pretreatment						
Total	MWh/ha	34.23	43.37	38.56	60.50	61.00
Net energy gain	MWh/ha	29.17	39.64	35.43	53.68	56.34
Output/input	GJ/GJ	6.8	11.6	12.3	8.9	13.1

[*]Transportation costs are assumed to be proportionally higher with biomass yield

and willow only 56% and 62%, respectively, of the energy needed for the fertilizer used for corn cultivation. The energy needed for the wet oxidation pretreatment is about 1–2% of the energy needed for the biomass supply and the operation of the biogas plant.

Without pretreatment the net energy gain is 43% and 85% higher for corn than for miscanthus under Danish standard yields and high yielding conditions, respectively, due to the lower methane yields of the raw miscanthus. The net energy gain for willow is lower than for corn due to its lower biomass yield. Supplying the biogas plant with raw material without pretreatment the energy output/input ratio is accordingly higher for corn than for miscanthus and willow.

Applying the wet oxidation pretreatment the methane yield of the perennial crops is significantly higher and the net energy gain and energy output/input ratio becomes significantly higher for the perennial crops compared to the untreated corn for biomass yields achieved in Denmark. This shows that the positive effect of increasing the biogas yield for miscanthus and willow through the wet oxidation pretreatment is much higher than the additional energy input needed for the pretreatment. It can be calculated that an increase of the methane potential from 200 L-CH_4/kg-VS to 211 L-CH_4/kg-VS would be sufficient to cover energy

input and loss of volatile solids during the pretreatment. According to these calculations corn should not be pretreated by wet oxidation since its specific methane yield per kg-VS is not increased but the pretreatment results in a loss of organic matter and thereby a loss of biogas yield. With the same higher biomass yields for miscanthus and corn in Southern Europe the net energy gain for pretreated miscanthus is almost as high as for untreated corn and the energy output/input ratio is remarkably higher for pretreated miscanthus. If miscanthus is not pretreated the net energy gain and the energy output/input ratio is lower for miscanthus than for corn. For the non-treated willow, the energy output/input ratio is, however, as high as for corn, which is mainly because of to the lower transportation and processing costs of willow due to its higher dry matter concentration.

Cost-benefit analysis

The costs for the biomass supply to the biogas plant and the benefit from electricity production at the biogas plant combined with a combined heat and power (CHP) plant is displayed in Table 7. The costs for field application of the different energy crops are about the same. The material

Table 7 | Cost-benefit for miscanthus, willow and corn without and with pretreatment

Crop		Corn DK yield	Miscanthus DK yield	Willow DK yield	Corn DK yield	Miscanthus DK yield
Costs						
Material (Seeds, fertilizer, ensilage plastics, pesticides)	€/ha	€ 483	€ 148	€ 148	€ 483	€ 148
Application (machinery + fuel)	€/ha	€ 436	€ 430	€ 430	€ 436	€ 430
Transport*	€/ha	€ 492	€ 456	€ 319	€ 870	€ 642
Total	€/ha	€ 1,411	€ 1,033	€ 896	€ 1,789	€ 1,219
Pretreatment – investment	€/ha	€ 122	€ 122	€ 122	€ 122	€ 122
Benefit for biogas plant						
Net el. Production (40% eff.)	MWh/ha	14.06	9.82	8.79	24.86	13.81
Net benefit	€/ha	−€ 5	−€ 51	−€ 16	€ 697	€ 162
Output/input	€/€	1.00	0.95	0.98	1.39	1.13
With pretreatment						
Net el. Production (40% eff.)	MWh/ha	13.34	17.02	15.20	23.58	23.94
Net benefit	€/ha	−€ 199	€ 547	€ 502	€ 447	€ 1,053
Output/input	€/€	0.87	1.47	1.49	1.23	1.79

*For 15 km average distance to biogas plant, price for transportation: 1.00 €/t km

costs for the cultivation are however only about one third for the perennial crops mainly due to a lower need for fertilizer and pesticides. While the costs for field application and materials are assumed independent of the biomass yields, transportation costs of the harvested material will be larger with higher biomass yields, but lower per ton of dry matter for biomass harvested with a higher TS content.

For moderate biomass yields and an average distance of 15 km to the biogas plant the transportation costs will be about as much as the costs for field application, but they become the largest cost factor for longer distances and higher biomass yields. The transportation costs for willow are lowest because of its high dry matter concentration. The investment costs for the pretreatment are relatively high and are between 7% and 14% of the biomass supply costs. It is assumed that the investment costs for the pretreatment (in €/ha) are independent of the biomass yield per hectare since the investment costs per ton treated material will be lower for higher treatment capacities.

Taking only the benefit from electricity sales into account the calculations show that for relatively low biomass yields as achieved in Denmark there is no net benefit neither for corn nor for untreated miscanthus and willow. Without treatment the net benefit becomes only positive for higher biomass yields, and is much lower for untreated miscanthus than for untreated corn.

For pretreated miscanthus and willow, however, the net benefit from electricity production via biogas from the perennial energy crops becomes positive even for the biomass yields achieved in Denmark. Also for higher biomass yields as in Southern Germany the net benefit is higher for the perennial crops than for corn since the costs for cultivation are much lower. Both the net benefit and the benefit/cost ratio are highest for the pretreated perennial crops at high biomass yields. The benefit/cost ratios are, however, much lower than the energy output/input ratios for the current material and energy sales prices.

CONCLUSIONS

The perennial crops miscanthus and willow have a much lower specific methane yield than corn when treated under anaerobic conditions without pretreatment. The net energy gain is therefore lower for the perennials than for corn used as energy crops in a biogas plant without applying any pretreatment. Increasing the specific methane yield of lignocellulosic biomass like miscanthus and willow by the

wet oxidation pretreatment does, however, increase the methane yield significantly and the ratio of energy output to input and of benefit to costs of the whole chain of biomass supply and conversion into biogas is higher than for corn. Indeed, for biomass yields achieved in Denmark, only the conversion of perennial crops via wet oxidation and biogas achieve a positive net benefit from electricity sales. This shows that pretreatment of miscanthus and willow is essential for making their use as energy crops for biogas production competitive to the use of corn. The pretreatment will enable the economically competitive use of perennial crops which have a lower environmental impact during cultivation and are thereby more sustainable.

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REFERENCES

Aronsson, P. G. & Bergstrom, L. F. 2001 Nitrate leaching from lysimeter-grown short-rotation willow coppice in relation to N-application, irrigation and soil type. *Biomass Bioenergy* 21, 155–164.

Christensen, J., Hjort-Gregersen, K., Uellendahl, H., Ahring, B. K., Lau Baggesen, D., Stockmarr, A., Møller, H. B. & Birkmose, T 2007 Fremtidens biogasfællesanlæg – nye anlægskoncepter og økonomisk potentiale. Fødevareøkonomisk Institut, Rapport nr. 188 (in Danish with English summary).

Clifton-Brown, J. C. & Lewandowski, I 2002 Screening *Miscanthus* genotypes in field trials to optimize biomass yields and quality in Southern Germany. *Eur. J. Agronomy* 16, 97–110

Dalgaard, T., Halberg, N. & Porter, J. R. 2001 A model for fossil energy use in Danish agriculture used to compare organic and conventional farming. *Agric. Ecosyst. Environ.* 87(1), 51–65.

European Environment Agency 2007 Estimating the environmentally compatible bioenergy potential from agriculture. EEA Technical report 12/2007, p 134.

Graversen, J. & Gylling, M 2002 Energiafgrøder til fastbrændselsformål–produktionsøkonomi,

håndteringsomkostninger og leveringsplaner. FØI Working Paper no. 07/2002 (in Danish).

Heller, M. C., Keoleian, G. A. & Volk, T. A. 2003 Life cycle assessment of a willow bioenergy cropping system. *Biomass Bioenergy* 25, 147–165.

Jørgensen, U., Mortensen, J. V., Kjeldsen, J. B. & Schwarz, K. 2003 Establishment, development and yield quality of fifteen Miscanthus genotypes over three years in Denmark. *Acta Agric. Scandinavica, Section B–Plant Soil Sci.* 53, 190–199.

Jørgensen, U. 2005 How to reduce nitrate leaching by production of perennial energy crops? In: Zhu, Z., Minami, K. & Xing, G. (eds) *3rd Nitrogen Conference*, Nanjing, China, pp. 513–518.

Landscenter 2006 Budgetkalkuler for de enkelte produktionsgrene. Dansk Landbrugsrådgivning, Landscentret (in Danish).

Lewandowski, I. & Heinz, A. 2003 Delayed harvest of Miscanthus–influences on biomass quantity and quality and environmental impacts of energy production. *Eur. J. Agronomy* 19, 45–63.

Lissens, G., Thomsen, A B., de Baere, L., Verstraete, W. & Ahring, B. K. 2004a Thermal wet oxidation improves anaerobic biodegradability of raw and digested biowaste. *Environ. Sci. Technol.* 38, 3418–3424.

Lissens, G., Klinke, H., Verstraete, W., Ahring, B. K. & Thomsen, A B. 2004b Wet oxidation pre-treatment of woody yard waste: parameter optimization and enzymatic digestibility for ethanol production. *J. Chem. Technol. Biotechnol.* 79(8), 889–895.

Møller, H. B., Nielsen, A. M., Murto, M., Christensson, K., Rintala, J., Svensson, M., Seppälä, M., Paavola, T., Angelidaki, I. & Kaparaju, P. L. 2008 Manure and energy crops for biogas production. Status and barriers. Accepted for publication by Nordic council of Ministers, MJS–Miljöstrategier for jord- och skogsbruk (www.norden.org/mjs).

Semere, T. & Slater, F. M 2007 Ground flora, small mammal and bird species diversity in miscanthus (*Miscanthus x giganteus*) and reed canary-grass (Phalaris arundinacea) fields. *Biomass Bioenergy* 31, 20–29

Sørensen, A., Teller, P. J., Hilstrøm, T & Ahring, B. K. 2008 Hydrolysis of Miscanthus for bioethanol production using dilute acid presoaking combined with wet explosion pre-treatment and enzymatic hydrolysis. *Bioresour. Technol.* 99(14), 6602–6607.

Uellendahl, H., Mladenovska, Z. & Ahring, B. K. 2007 Wet oxidation of crude manure and manure fibers. Substrate characteristics influencing the pretreatment efficiency for increasing the biogas yield of manure. *Proceedings 11th World Congress on Anaerobic Digestion*, 23–27 September, 2007, Brisbane, Australia

U S DOE 2006 Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda. DOE/SC-0095, U.S. Department of Energy Office of Science and Office of Energy Efficiency ad Renewable Energy (www.doegenomestolife.org/biofuels/).

Venendaal, R., Jørgensen, U. & Foster, C. A. 1997 European energy crops: a synthesis. *Biomass Bioenergy* 13(3), 147–185.

Paper 5

Biological ensilage of perennial crops willow and miscantus as pretreatment to increase the biogas production

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Abstract

Biological ensilage with and without additives i.e., lactic acid bacteria were tested on willow and miscanthus substrate for their effect on biogas production and preservation of volatile solids content. In general, ensilage of willow and miscanthus with addition of biological additives had no significant influence on the biomass losses compared to that without addition of the additives. During ensiling process, the amount of biomass losse could be controlled under 2% and 3% for willow and miscanthus, respectively. Ensilage of willow as pretreatment method could increase methane potential. Taking the biomass losses into account, methane production from willow was increased by 12%, 22% and 22% after ensilage for 1, 3 and 5 months, respectively, compared to fresh willow. Ensilage of miscanthus gave no significant increase in methane production compared with fresh miscanthus. Moreover, due to biomass losses, the total methane production from silage minscanthus after 5 months was 3% lower than that from fresh miscanthus.

1. Introduction

As a technology for renewable energy, anaerobic digestion of different organic materials for methane production has in recent years expanded extensively throughout Europe, as methane can be used in replacement for fossil fuels in both heat and power generation as well as a vehicle fuel. In Germany, the fastest growing sector of bio-farming has been in the area of renewable energy crop on nearly 500,000 ha land (2006)[6]. This rapid growth has occurred only with substantial government support, as in the Germany bonus system for renewable energy. However, large areas of agriculture land are cultivated predominantly with corn for energy production in biogas plants[7]. Annual crops like corn needs significant energy and fertilizer input for their growth. On the other hand, perennial crops such as willow, switch grass and miscanthus have been recognized to require much less energy to plant, cultivate and nutrient. At same time, their annual solar energy conversion efficiency is often higher than that of annual plants due to the longer growing season[8]. Furthermore, cultivate perennial crops can provide a better environment for more diverse wildlife habitation and reduce nutrient losses [9, 10]. These factors increase the sustainability of cultivation of perennial crops and make perennial crops favourable candidates for energy production from biomass in the long run. However, the efficiency of methane production from lignocellulose crops can be limited due to low biodegradability of the lignocellulose. The destruction of the lignocelluosic structure will release the sugars contained in biomass and therefore increase the amount of organic matter converted to methane.

In recent years, several pretreatment technologies such as steam explosion[11], wet oxidation[12, 13] and hydrothermal treatment[14] have been used for the pretreatment of lignocellulose biomass mainly for ethanol production. All those pretreatment method require large amount of electricity or chemicals. Therefore, investigation on low energy or less chemical requirement pretreatment methods is necessary.

Ensiling is a biological process that has been used to preserve forages for animal feed for centuries. In the ensiling process, the soluble carbohydrates contained in biomass undergo lactic acid fermentation, this reaction will leading to a drop in pH and to inhibition of the growing detrimental microorganisms[1], at the same time the acidication produces intermediates for methanogenic fermentation. In this way the ensiling process can be considered as a pretreatment which simultaneously has potential to promote methane production from plant matter[15]. Previously, study from H. Vervaeren et al.,[2] reported that ensiling of maize for 7 weeks influence the methane production per VS in subsequent anaerobic digestion by up to 22.5%. The methane potential of silage rye (3 months), sugar beet tops (3 months) and maize (4 months), increase more than 20% compared to that fresh crops [16, 17, 18], but studies on ensilage of perennial crops such as willow or miscanthus for methane production are very limited.

In the present study, biological ensilage (with and without additives) of perennial crops willow and miscanthus as pretreatment to increase the biogas production was investigated. The efficiency of the ensilage process was evaluated based on (a) the biomass lost amount during the ensilage and (b) the methane potential of the silage crops compared to that fresh crop.

2. Material and methods

2.1. Substrate and inoculua

Perennial crops willow and miscanthus was harvested in the middle of December from southern part of Denmark. To minimize any handling losses, the crops was freshly chopped with a hammer mill to particle size of approximately 3-5 cm and immediately processed for ensilage. The inoculua used in this experiment was obtained from the effluent of a 7-l lab-scale thermophilic anaerobic reactor which had been in steady stage and used for treating swine manure and straw.

2.2. Analytical methods

Total solids (TS) and volatile solids (VS), kjeldahl nitrogen and NH_4-N were measured according to the standard methods (APHA, 1989). Methane was quantified with a gas chromatograph (Shimadzu GC-8A) equipped with flame ionisation detector and packed column (Porapak Q 80/100-mesh). pH was measured by diluted biomass with willipore water at concentration 1:1 (w/w).

The composition of the biomass was measured by strong acid hydrolysis of the carbohydrates. Dried and milled samples (160mg) were treated with 72% (w/w) H_2SO_4 (1.5 ml) at 30°C for 60

minutes. The solutions were diluted with 42 ml of water and autoclaved at 121 °C for 60 minutes. The hydrolysates were filtered, and the Klason lignin content was determined as the weight of the filter cake subtracted the ash content. The filtered (5ml) were mixed with 0.50 g $Ba(OH)_2$ $8H_2O$ and after 5 minutes, the samples were centrifuged with approx. 3000g for 5 minutes. The recovery samples of glucose, xylose and arabinose were determined by standard addition of sugars to samples before autoclavation. The sugars were determined after separation on a HPLC-system (Shimadzu) with a Rezex ROA column (Phenomenex) at 63°C using 4 mM H_2SO_4 as eluent and a flow rate of 0.6 ml/min. Detection was done by a refractive index detector (Shimadzu Corp., Kyoto, Japan). Conversion factors for dehydration on polymerization was 162/185 for glucose and was 132/150 for xylose and arabinose (Kaaret al., 1991; Thygesen et al., 2005) [19, 20].

2.3 Ensiling process

Willow and miscanthus was chopped and mixed with and without Biomax Si (Chr. Hansen A/S, Denmark) forage additive containing lactic acid bacteria (stain of *Lactobacillus Plantarum*, to confirm stable silage production). 1 g of powder of forage additive was dissolved in 10 liters of water and sprayed over raw biomass samples in the amount equal 40 ml per 1 kg of biomass. Subsequently, the biomass was homogenously distributed into several 2L plastic bags and 100% vacuumed by a vacuum-packing machine (Model MVS35, Minipark Torre, UK), and 0.1L of CO_2 was pumped into the bags to ensure the bacteria was not stressed. 1 kg of chopped willow and miscanthus without additive and were prepared as fresh biomass control, respectively. All the packed biomasses and controls were kept at room temperature for 150 days. Sample for characterization and batch test was taken at day 0, 30, 90, and 150 respectively. The biomasses were weighed before and after silage to determine the changes in biomass during the storage.

2.4. Methane potential of raw and silage treated willow and miscanthus

The sample of willow and miscanthus for methane potential tests were taken from day 0, and after silage day 30, 90 and 150 respectively. The tests were carried out in triplicates in 117 ml serum vials. An amount equivalent to 0.5g-TS of biomass was added as substrates together with 20 ml inoculum. Triplicate vials with no substrate added served as control. The headspace of the vials was flushed with mixture gas of 80% N_2 and 20% CO_2 before sealed with butyl rubber stoppers. The vials were incubated at 55°C and the methane potential was calculated as the volume of methane produced per g of added volatile biomass after being normalized to the amount before silage. Thus the suitability of the silage as a pretreatment method for the enhancement of methane production was assessed.

3. Results and discussion

3.1 Characterization

Willow and miscanthus were stored as silage with additives containing lactic acid bacteria (stain of *Lactobacillus Plantarum*, to confirm stable silage production) for 1, 3 and 5 months at room temperature (approx. 20°C). The raw and silage biomasses were analyzed for several parameters to characterize the biomass (Table 1).

As can be seen, the pH of the fresh willow and miscanthus were similar which was 6.5 and 6.7 respectively. Whereas after ensilage, the pH ranged from 4.2 to 4.7 with willow and from 4.0 to 4.4 with miscanthus, no significant changes of pH were found between 1, 3 and 5 months silage in both biomasses. The TS and VS concentration of the silage crops were in general the same with fresh crops in both crops (Table 1). The total biomass loss was calculated by subtracts the weight of biomass before and after silage. In general the biomass losses for willow and miscanthus were kept below 2 and 3% respectively, the highest biomass losses was occurring from miscanthus after 5 months silage which was 2.5% in both with and without additives bags (Table 2). Concentration of total-N in fresh crops was 4.7 mg/g-TS in willow and 7.5 mg/g-TS in miscanthus, and they changed little during the silage (4.38-4.87 mg/g-TS in silage willow and 7.21-7.77 mg/g-TS in silage miscanthus). The concentration of NH_4-N shown in (Table 1), indicated that after silage the concentration of NH_4-N was slightly higher than fresh corps in both willow and miscanthus(0.66-0.78 mg/g-TS in silage willow and 0.90-1.02 mg/g-TS in silage miscanthus) (0.58 mg/g-TS in fresh willow and 0.89 mg/g-TS in fresh miscanthus), the highest increase was found in silage willow without additives after 5 months. Concentration of lignin in fresh willow and miscanthous was 29.5 and 26.4 g/100g-TS respectively, it was little lower in silage biomass (27.5-28.34 g/100g-TS in silage willow and 25.7-26.14 g/100g-TS in silage miscanthus). The concentration of total carbohydrates in both crops was significantly high, 54.4 and 58.5 g/100g-TS of total carbohydrates was measured in fresh willow and miscanthus, for silage willow and miscanthus was in the ranged 52.9-54.3 and 56.3-59.1 g/100g-TS respectively. Moreover, soluble sugar such as fructose, glucose were found to be low or below detection. The concentration of inorganic compounds such as ash and minerals was under 20% in both crops. In general, characterization results of silage sample with additives were similar with that sample without additives, no significant advantages were observed in both crops (Table 1).

3.2 Methane potential test

The methane potential of fresh and silage substrates were determined in 50 days batch assays at 55°C (Fig.1). The methane potential of fresh willow and miscanthus was 141 and 187 ml CH_4 per g-VS, respectively. The methane potential of all silage willow was significantly increased compared with fresh willow, the highest methane potential was found in willow silage with additives after 5 months, which was 177 ml CH_4 per g-VS added, take 1.7% of biomass lost into account equals to 175 ml CH_4 per g-VS $_{original}$, and the total methane production was about 23% higher than the fresh willow. Ensilage

willow with and without additives had slightly different effects on biomass losses and methane production, but in general was the same, biomass lost more with ensilage period increases, 1.6-1.9% of biomass was lost during 1 to 5 months ensilage period in willow without additives and for willow with additives was 1.3-1.7%. Taking the biomass losses into account, the methane potential of silage willow without additives was in the range 161-176 ml CH_4 per g-VS $_{original}$ and the total methane production was about 12-22% higher than the fresh willow. The total methane production of silage willow with additives was about 18-23% higher than the fresh willow (Table 3).

On the other hand, the methane production results of silage miscanthus shown there was no significant methane increases was determined in both silage miscanthus with and without additives. And the biomass losses were in generally higher than willow. Especially after 5 months ensilage, 2.5% of biomass was lost in both with and without additives conditions. The methane potential of all silage miscanthus was ranged 186-194 ml CH_4 per g-VS added. But take the biomass losses into account, methane production from ensilaged miscanthus with additives can be 3% lower than fresh miscanthus.

According to our results, willow and miscanthus can be stored as silage at room temperature for several months without significant losses in biomass. The total biomass can be controlled lewer than 3% for both crop, this value was much lower than annual crops such as sugar beet tops or grass, Outi Pakarinen et al. reported ensilage grass and sugar beet tops for 3 months, the total biomass can be lost

Table 3. Methane potentials of fresh and silage willow and miscanthus.

| Crop	Duration (months)	Loss of VS (%)	ml CH_4/g-VS added	ml CH_4/g-VS original	CH_4 increased (%)
Raw willow fresh	0	ND	141	141	0.00
Willow silage (without addtive)	1	1.6	161	158	12.06
	3	1.7	177	173	22.70
	5	1.9	176	172	21.99
Willow silage (with additive)	1	1.3	168	166	17.63
	3	1.5	175	172	22.25
	5	1.7	177	175	23.40
Raw miscanthus fresh	0	ND	187	187	0.00
Miscanthus silage (without addtive)	1	1.8	189	186	-0.72
	3	1.8	193	189	1.32
	5	2.5	194	189	1.13
Miscanthus silage (with additive)	1	1.8	187	184	-1.80
	3	2.2	188	184	-1.70
	5	2.5	186	181	-3.07
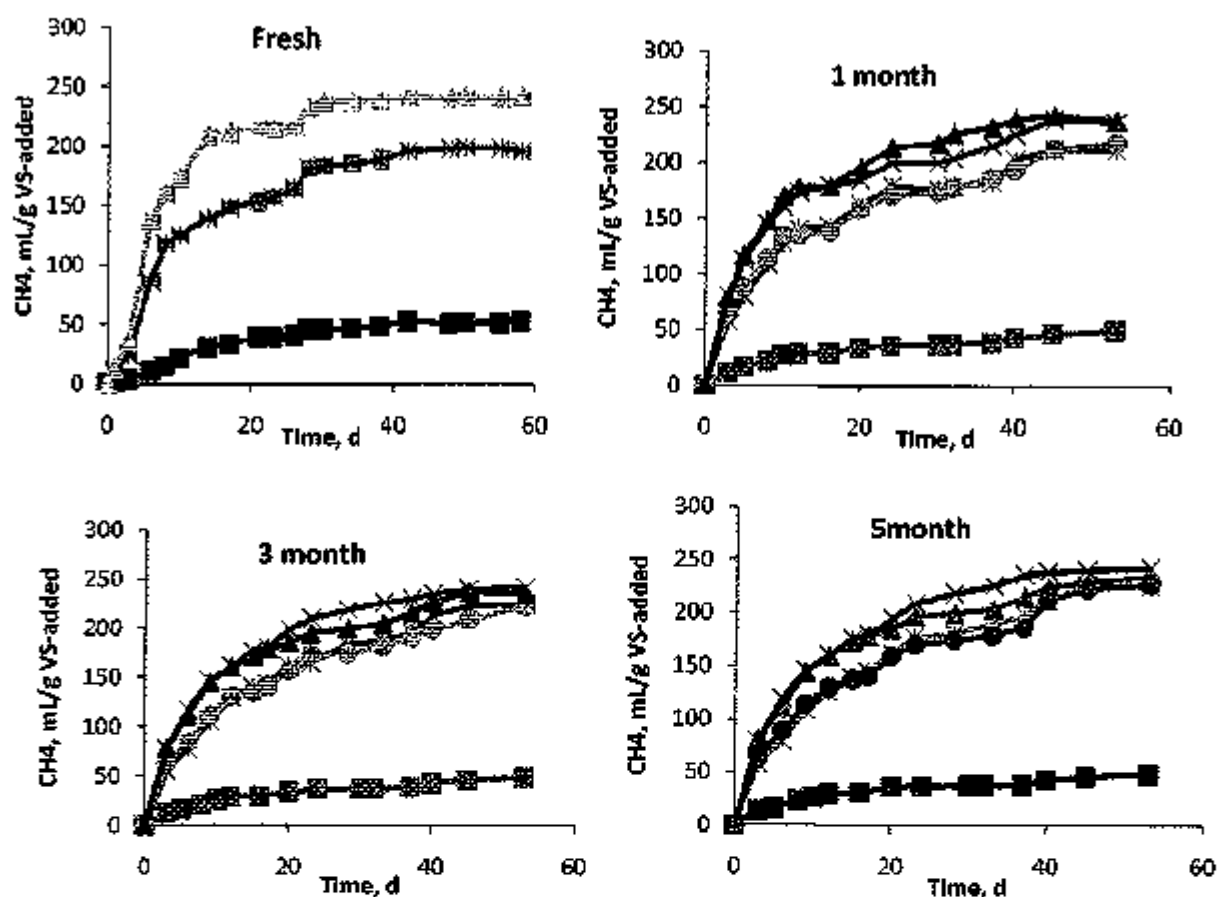
Fig.1. Methane potential test.

■ = inoculums, ж = willow, ● = willow with additives
▲= miscanthus, x = miscanthus with additives.

at minimum 19%[1]. The higher losses of biomasses during ensilage of miscanthus than willow was apparently partly due to the fact that the miscanthus contained more easily degradable compounds compared with willow, as shown by the higher methane potential of fresh miscanthus. The similar results were reported by Lehtomäki et al[1]. when compared sugar beet tops with grass. However, ensilation is a complex process, during which several factors are critical like the absence of oxygen, the availability of readily biodegradable carbohydrates, the absence of inhibitors and ambient temperature conditions[2]. Many studies have previously reported various crops stored as silage with or without additives to have equal or higher methane potentials than fresh crops; for instance, with rye and maize, increases 20 and 25% respectively [3,4], but if taking into account the losses of VS the true methane potential was not significantly high or even lower than the fresh crop.

There were no significant different observed between ensilage with or without additives in biomass losses and methane potential. An increase in methane potential is linked to the degradation of complex sugar structures (polysaccharides) to more readily biodegradable intermediates[5]. However, the methane

potential increases of willow were occurred after 1 month ensilage, but the higher increases were occurred after 3 months ensilage.

4. Conclusions

The present study shows that ensilage of willow and miscanthus with addition of biological additives has no significant influence on the biomass losses compared to the non additives. The biomass losses amount of willow and miscanthus can be controlled under 2 and 3% with ensiling process, respectively. Ensilage of willow as pretreatment method for methane production can increase the methane potential. Taking the biomass losses into account, ensilage of willow for 1, 3 and 5 months can increase the total methane production 12, 22 and 22% compared to fresh willow respectively. The methane potential of all silage miscanthus was range within 181-189 ml CH_4 per g-VS $_{original}$, silage miscanthus gives no significant increase of methane production compared with fresh miscanthus. Moreover, due to the higher biomass losses, the total methane production from silage minscanthus after 5 months was 3% lower than the fresh miscanthus.

Table 1. Chemical characterization of willow and miscanthus before and after ensilage.

Crop	Duration (months)	pH	TS (%)	VS (%)	Total-N (mg/g-TS)	NH_x-N (mg/g-TS)	Lignin (g/100gTS)	Total carbohydrate (g/100gTS)	Soluble sugar	Extractives/ residues
Fresh willow	0	6.5	48.4±0.2	47.6 ±0.2	4.7±0.4	0.58±0.03	29.5±0.4	54.4±1.2	<1	15
Willow silage (without addtive)	1	4.7	49.1±0.4	48.2±0.3	4.9±0.3	0.73±0.03	27.8±0.2	53.9±0.8	<1	18
	3	4.3	48.2±0.1	47.2±0.1	4.6±0.2	0.74±0.05	27.6±0.3	52.7±0.7	<1	19
	5	4.2	48.3±0.2	47.3±0.3	4.4±0.2	0.78±0.06	28.0±0.3	54.3±0.5	<1	18
Willow silage (with additive)	1	4.3	48.4±0.3	47.4±0.2	4.7±0.3	0.69±0.02	28.3±0.4	52.9±0.3	<1	19
	3	4.3	48.2±0.2	47.2±0.2	4.4±0.2	0.68±0.3	27.9±0.3	53.2±1.1	<1	19
	5	4.4	48.1±0.1	47.1±0.1	4.6±0.3	0.66±0.4	27.5±0.2	53.8±0.6	<1	18
Fresh miscanthus	0	6.7	58.9±0.3	57.5±0.3	7.5±0.2	0.89±0.01	26.4±0.3	58.5±1.3	<1	16
Miscanthus silage (without addtive)	1	4.4	59.5±0.4	58.1±0.2	7.2±0.2	0.97±0.06	25.9±0.5	57.8±0.8	<1	16
	3	4.1	58.8±0.1	57.0±0.4	7.2±0.2	0.99±0.05	25.7±0.4	58.3±0.1	<1	16
	5	4.3	58.7±0.2	56.9±0.2	7.8±0.2	0.90±0.04	25.3±0.8	58.5±0.3	<1	16
Miscanthus silage (with additive)	1	4.0	58.8±0.3	56.9±0.2	7.6±0.2	0.90±0.03	26.1±0.7	56.3±0.8	<1	17
	3	4.2	58.6±0.2	56.8±0.3	7.6±0.2	0.96±0.04	25.7±0.1	57.3±1.2	<1	16
	5	4.2	58.3±0.2	56.6±0.3	7.5±0.2	1.02±0.02	25.7±0.6	59.1±1.5	<1	16

Table 2. Characterization of biomass losses.

Crop	Duration (month)	Biomass *			
		Before (g)	After(g)	loss(g)	loss (%)
Willow silage (without addtive)	1	400.8	394.5	6.3	1.6
	3	400.2	393.8	6.7	1.7
	5	401.3	393.5	7.8	1.9
Willow silage (with additive)	1	400.3	395.2	5.1	1.3
	3	400.5	394.5	6.0	1.5
	5	400.0	393.4	6.6	1.7
Miscanthus silage (without addtive)	1	400.5	393.4	7.1	1.8
	3	398.5	391.2	7.3	1.8
	5	400.7	390.6	10.1	2.5
Miscanthus silage (with additive)	1	400.0	392.8	7.2	1.8
	3	400.2	391.3	8.9	2.2
	5	400.6	390.4	10.2	2.5

*Data was the average of duplicate sample

References

1. Outi Pakarinen, Annimari Lehtomäki, Sanna Rissanen and Jukka Rintala. Storing energy crops for methane production: Effects of solids content and biological additive. Bioresource Technology 2008. 99, 7074-7082.

2. Vervaeren. H, Hostyn. K, Ghekiere. G, and Willems. B. Biological ensilage additives as pretreatment for maize to increase the biogas production 2009. Renewable Energy 35: 2089-2093

3. Heiermann, M., Plöchl, M., Linke, B. & Schelle, H. 2002. Preliminary evaluation of some cereals as energy crops for biogas production. In: Sayigh, A. A. M. (ed.), Proc. World Renewable Energy Congress VII, CD-version. Pergamon, Cologne.

4. Neureiter, M., dos Santos, J. T. P., Lopez, C. P., Pichler, H., Kirchmayr, R. & Braun, R. 2005. Effect of silage preparation on methane yields from whole crop maize silages. In: Ahring, B. K. & Hartmann, H. (eds), Proc. 4th Int.

5. Egg RP, Coble CG, Engler, Lewis DH. Feed storage, handling and processing. Biomass Bioenergy 1993:71-94

6. Michael Köttner, C. 2009. Energy Crops – Creating Markets for Heat and Electricity: Biogas from Energy Crops in Germany.

7. Uellendahl. H, Wang. G, Møller. H .B, Jørgensen. U, Skiadas I. V, Gavala. H. N and Ahring B.K, Energy balance and cost–benefit analysis of biogas production from perennial energy crops pretreated by wet oxidation, Water Sci. Technol. 58.9 (2008), pp. 1841–1847.

8. Finch J W, Karp A, McCabe D P M, Nixon S, Riche A B and Whitmore A P. 2009. Miscanthus, short-rotation coppice and the historic environment. Centre for Ecology & Hydrology (http://nora.nerc.ac.uk/7566/1/EngHerit_Report_final.pdf)

9. Semere, T. & Slater, F. M. 2007 Ground flora, small mammal and bird species diversity in miscanthus (Miscanthus x giganteus) and reed canary-grass (Phalaris arundinacea) fields. Biomass Bioenergy.31, 20–29.

10. Aronsson, P. G. & Bergstrom, L. F. 2001 Nitrate leaching from lysimeter-grown short-rotation willow coppice in relation to N-application, irrigation and soil type. Biomass Bioenergy 21, 155–164.

11. Ballesteros, I., Negro, M.J., Oliva, J.M., Cabanas, A., Manzanares, P., Ballesteros, M., 2006. Ethanol production from steam-explosion pretreated wheat straw.Applied Biochemistry and Biotechnology – Part A – Enzyme Engineering and Biotechnology 129–132, 496–508.

12. Palonen H, Thomsen AB, Tenkanen M, Schmidt AS, Viikari L. Evaluation of wet oxidation pretreatment for enzymatic hydrolysis of softwood. Appl. Biochem. Biotechnol. 2004;117:1–17

13. Schmidt A, Thomsen A. Optimization of wet oxidation pretreatment of wheat straw. Bioresource Technol. 1998;64:139–151.

14. Thomsen, M.H., Thygesen, A., Jørgensen, H., Larsen, J., Christensen, B.H., Thomsen, A.B., 2006. Preliminary results on optimization of pilot scale pretreatment of wheat straw used in coproduction of ethanol and electricity. Applied Biochemistry and Biotechnology 130 (1–3), 448–460.

15. Zubra. J. 1986. Methanogenic fermentation of fresh and ensiled plant materials. Biomass 11: 156-171

16. Heiermann, M., Plöchl, M., Linke, B. & Schelle, H. 2002. Preliminary evaluation of some cereals as energy crops for biogas production. In: Sayigh, A. A. M. (ed.), Proc. World Renewable Energy Congress VII, CD-version. Pergamum, Cologne.

17. Chynoweth, D. P., Turick, C. E., Owens, J. M., Jerger, D. E. & Peck, M. W. 1993. Biochemical methane potential of biomass and waste feedstocks. Biomass Bioenergy 5: 95–111.

18. Neureiter, M., dos Santos, J. T. P., Lopez, C. P., Pichler, H., Kirchmayr, R. & Braun, R. 2005. Effect of silage preparation on methane yields from whole crop maize silages. In: Ahring, B. K. & Hartmann, H. (eds), Proc. 4th Int. Symp. On Anaerobic Digestion of Solid Waste, Vol. 1: 109–115.BioCentrum-DTU, Copenhagen.

19. Kaar, W.E., Cool, L.G., Merriman, M.M., Brink, D.L., 1991. The complete analysis of wood polysaccharides using HPLC. J. Wood Chem. Technol. 11, 447-463

20. Thygesen, A., Oddershede, J., Lilholt, H., Thomsen, A.B., Ståhl. K., 2005. On the determination of crystallinity and celluluse content in plant fibres. Cellulose 12, 563-576.

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