Project no. **SES6-CT-2004-502824**

Project acronym: **CROPGEN**

Project title: **Renewable energy from crops and agrowastes**

Instrument: Specific Targeted Research Project

Thematic Priority: SUSTDEV: Sustainable Energy Systems

D11: Optimal pre-treatment methods to increase methane yields under various conditions

Due date of deliverable: Month 25
Actual submission date: Month 25

Start date of project: 01/03/2004 Duration: 39 months

Organisation name of lead contractor for this deliverable

University of Vienna (BOKU-IFA)

Revision [0]

Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)		
Dissemination Level		
PU	Public	PU
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Optimal pre-treatment methods to increase methane yields under various conditions

Introduction

Biogas production energy crops is focused on the production of energy. In this aspect it differs from anaerobic digestion in waste treatment, where the main effort is to produce a stable end product (digestate) and energy is merely a useful by-product. In order to meet the requirements for the efficient production of energy from crops it is necessary to provide a fast and complete conversion of the biomass into biogas. However, biomass is a complex matter consisting of components that vary in their accessibility to anaerobic digestion. Especially the hydrolysis of lignocellulose has been found to be a rate limiting step in the anaerobic digestion of solid biomass (Pavlosthathis and Giraldo-Gomez, 1991; Mata-Alvarez et al., 2000). Lignocellulose is a complex of cellulose, hemicelluloses and lignin. Cellulose and hemicelluloses are polysaccharides and therefore potential substrates for the anaerobic digestion process, while lignin is a complex polymer of phenolic compounds and is recalcitrant to degradation under anaerobic conditions. In order to utilise the high amounts of carbohydrates present in lignocellulosic material, it is necessary to split the polysaccharides cellulose and hemicellulose into monosaccharides that can be further metabolised by the microbial community. However, the polysaccharides are embedded in the lignin matrix and therefore difficult to access for hydrolytic enzymes. Furthermore, cellulose forms crystalline regions that make it resistant to enzymatic attack. Therefore an ideal pre-treatment should increase the surface area by breaking up the lignocellulose complex, decrease the lignin content, and reduce the crystallinity of cellulose (Fan et al., 1981).

Pre-treatment methods can be divided into physical (mechanical and thermal) treatments, chemical treatments, biological treatments, and combinations thereof. Intensive research has been carried out on the pre-treatment of lignocellulosic substrates for improving enzymatic hydrolysis and consequent ethanol production from the released sugars (Fan et al., 1982; Kosaric et al., 1983; Sun and Cheng, 2002). However, requirements for anaerobic digestion may differ in some aspects from those for ethanol production. For example, the utilisation of chemicals like sulfuric acid will cause unwanted effects on gas composition in the anaerobic digestion process.

For the task in the CROPGEN-Project, the objective was to test several pre-treatment methods on their potential to improve methane formation from different energy crops in laboratory scale experiments. The results of these experiments are used to describe optimal pre-treatment conditions. BOKU-IFA-Tulln concentrated on conventional energy crops (maize and maize grains) as they are currently used in Austria, while JyU conducted their experiments with energy crops that can be grown under boreal conditions.

Pre-treatment of maize and maize grains

Materials and Methods

Whole crop maize silage from the harvests of 2004 and 2005 and maize grains from the harvest of 2003 were used as substrates (Table 1).

Table 1: Characteristics of substrates

Substrate	TS [%]	VS [%]	COD [mg O_2/g]
Whole crop maize silage[1]	36.35	35.00	483.93
Whole crop maize silage[2]	37.76	36.44	505.54
Whole crop maize silage[3]	40.09	38.79	--
Whole crop maize silage[4]	32.32	31.13	--
Corn grains	85.26	83.97	1 122.08

[1] Sampled 28 November 2004 (used for grinding)
[2] Sampled 8 March 2005 (used for autoclaving)
[3] Sampled 21 March 2005 (used for alkaline pre-treatment)
[4] Sampled 12 December 2005 (used for steam explosion)

Total solids (TS) were determined by drying the material at 105°C for 24 h. Ash content for the calculation of the volatile solids (VS) was determined by ashing ground dry samples at 550°C over a period of 5 h in a muffle furnace. Chemical Oxygen Demand (COD) was determined according to DIN 38414 H41-1 (Deutsche Einheitsverfahren) guidelines.

Pre-treatment methods

Size reduction. For the grinding of maize grains a farmscale mill (Multicracker Typ HSM 37 E, which is supplied by two motors of 18.5 CV and suitable for the grinding of citrus fruits, tuber, roots, cereals, coffee grains, etc.) was used. Five different particle sizes (1, 3, 5, 7 and 9 mm can be adjusted. The corn grain sample was ground to each of the particle size possibilities of the machine. For control purposes the five ground samples were subjected to a sieve analysis. As shown in Figure 1, the actual particle sizes were generally lower than the values that were adjusted on the machine. There was only a small difference between the theoretically 1 and 3 mm particle size samples (both had nearly 50% of the particles in a range from 1 to 2 mm).
In the case of the whole crop maize silage, a laboratory mill (Retsch ZM 1000) was used achieving two different particle sizes, 4 and 1 mm. Then the potential of the methane production from the 1, 5 and 9 mm particle grain samples and the 1, 4 and 10 mm (initial substrate) particle size whole crop maize samples was evaluated.

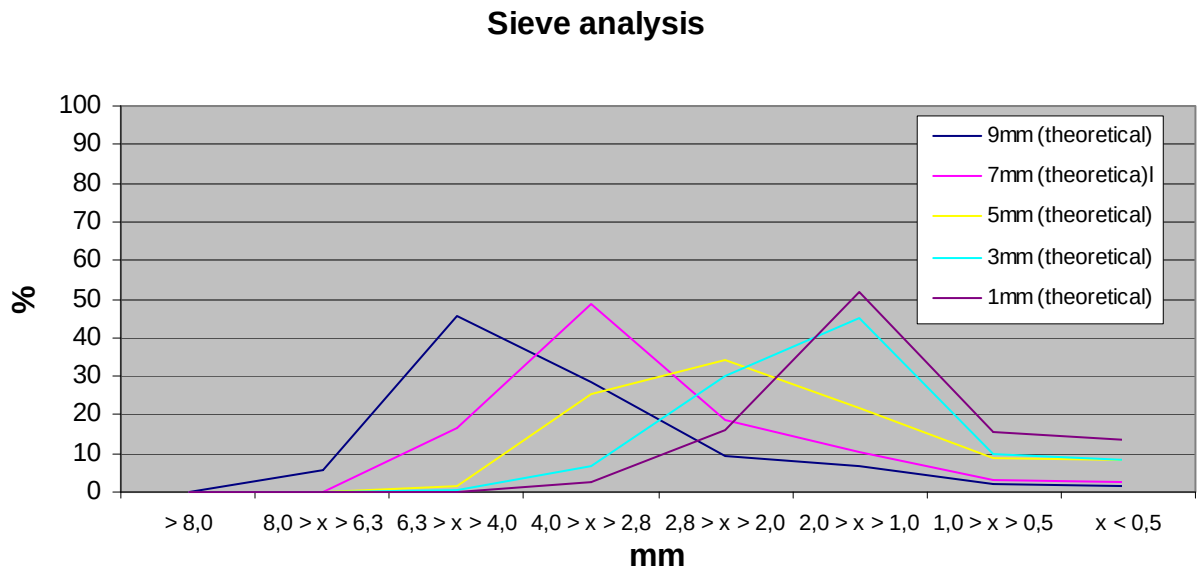

Figure 1: Sieve analysis from the different corn grains samples

Autoclaving. For the autoclaving pre-treatment whole crop maize silage was pressure cooked for 20, 40 and 60 minutes at a temperature of 121°C and 2 bar pressure.

Alkaline incubation. For the alkaline treatment, whole crop maize silage was treated with different concentrations of NaOH and varying incubations times. 500 g of the substrate were incubated with 1 300 ml NaOH (2% (w/w) or 4% (w/w)) for 24 h and 72 h at room temperature in 5 L containers. After the treatment the pH was adjusted with phosphoric acid to a value between 7.7 and 7.9 to perform batch fermentation tests.

Steam explosion. A pressure and heat treatment of the silage with a 20 L hydrolysis reactor was carried out at different temperatures and reaction times.

Table 2: Conditions for steam explosion treatment

Temperature [°C]	Time [min]	TS in reactor [%]
140	5	12.7%
140	20	12.8%
185	5	12.8%
185	20	12.7%
162	12.5	12.8%
162	12.5	15.0%

Characteristics of pre-treated samples

The characteristics of the samples (pre-treated) are shown in Table 3. The ground grains presented the same characteristics as the initial substrate (Table 1).

Table 3: Characteristics of the pre-treated samples

Pre-treatment	**TS [%]**	**VS [%]**	**COD [mg O_2/g]**
Size reduction (whole crop maize silage)			
1 mm	47.99	46.22	638.90
4 mm	38.69	37.27	515.09
10 mm (initial substrate)	36.35	35.00	483.93
Autoclave (121°C) (whole crop maize silage)			
20 min	38.86	37.44	520.27
40 min	39.21	37.80	524.96
60 min	38.73	37.43	518.53
Alkaline treatment (whole crop maize silage)			
2% NaOH, 24 h	11.11	9.58	
2% NaOH, 72 h	11.24	9.74	
4% NaOH, 24 h	11.37	8.83	
4% NaOH, 72 h	9.78	7.71	
Steam explosion (whole crop maize silage)			
140°C, 5 min	13.13	12.65	
140°C, 20 min	14.81	14.25	
185°C, 5 min	14.55	14.03	
185°C, 20 min	13.44	12.91	
165°C, 12.5 min	13.96	13.47	
165°C, 12.5 min	15.79	15.21	

Determination of methane potential

The methane production from all the pre-treated samples was evaluated through anaerobic batch fermentation tests. These tests were carried out under mesophilic conditions according to the modified norm DEV S6, DIN 38 414-S6 (Deutsche Einheitsverfahren guidelines). The test procedure consisted of a 0.5 L glass bottle (reaction bottle) where the sample was incubated at 35 ± 1ºC with 500 ml of inoculum. Every six hours the reaction bottles were stirred by magnetic agitation for 10 minutes. For the determination of the methane outcome, the reaction bottle was connected by a totally gas-impermeable tube to a 1 L bottle filled up with a 4 M KOH solution (for the absorption of CO_2 contained in the biogas). Simultaneously this bottle was connected (gas-impermeable hose) to a 1 L glass bottle filled with a displacement solution. The displaced fluid was measured with a measuring cylinder.

Every sample was carried out in three replicates (batch fermentation tests). In addition two blanks (only inoculum in the reaction bottle) were performed. The period of analysis lasted between 28 and 56 days, with daily measurements of the displaced fluid.

Results

An overview on the methane yields (Nm^3/t VS) from all pre-treated samples is given in Table 4.

Table 4: Overview on methane yields from different pre-treatments

Pre-treatment	**Methane Yield [Nm^3/t VS]**
Size reduction	
Corn grains 1 mm	444.22
Corn grains 5 mm	442.16
Corn grains 9 mm	441.64
Whole crop maize silage 1 mm	350.49
Whole crop maize silage 4 mm	335.44
Whole crop maize silage 10 mm (initial substrate)	319.84
Autoclave (121°C) (whole crop maize silage)	
20 min	431.27
40 min	409.37
60 min	422.56
untreated reference	435.30
Alkaline treatment (whole crop maize silage)	
2% NaOH, 24 h	538.21
2% NaOH, 72 h	584.89
4% NaOH, 24 h	597.84
4% NaOH, 72 h	539.15
untreated reference	391.13
Steam explosion (whole crop maize silage)	
140°C, 5 min	440.53
140°C, 20 min	424.28
185°C, 5 min	446.54
185°C, 20 min	402.24
165°C, 12.5 min	466.33
165°C, 12.5 min	440.48
untreated reference	486.22

Particle size. For technical reason some size reduction of the biomass will be required in any case and is usually state of the art. Preferably a milling or chopping step should be carried out during harvesting or before ensiling of the material. This will improve the storage and reduce problems in feeding the material into the digestor.
Size reduction with the mill had no significant effect on the methane production from maize grains (Fig. 2), it was only noticed that the replicates gave more homogeneous results. Once the grains are opened, the microorganisms and enzymes can accede; therefore neither an influence on CH_4 yields from the 1, 5 and 9 mm samples nor difference in degradation velocity was observed in this experimental setup.

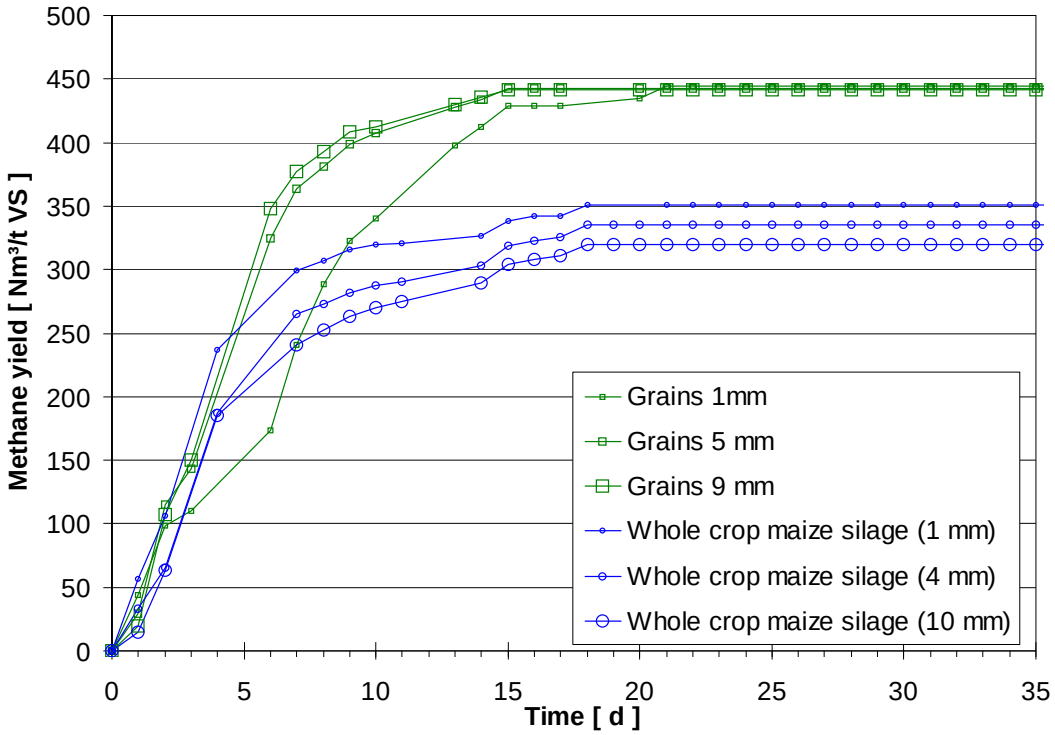

Figure 2: Methane yields from different particle size grains and silage

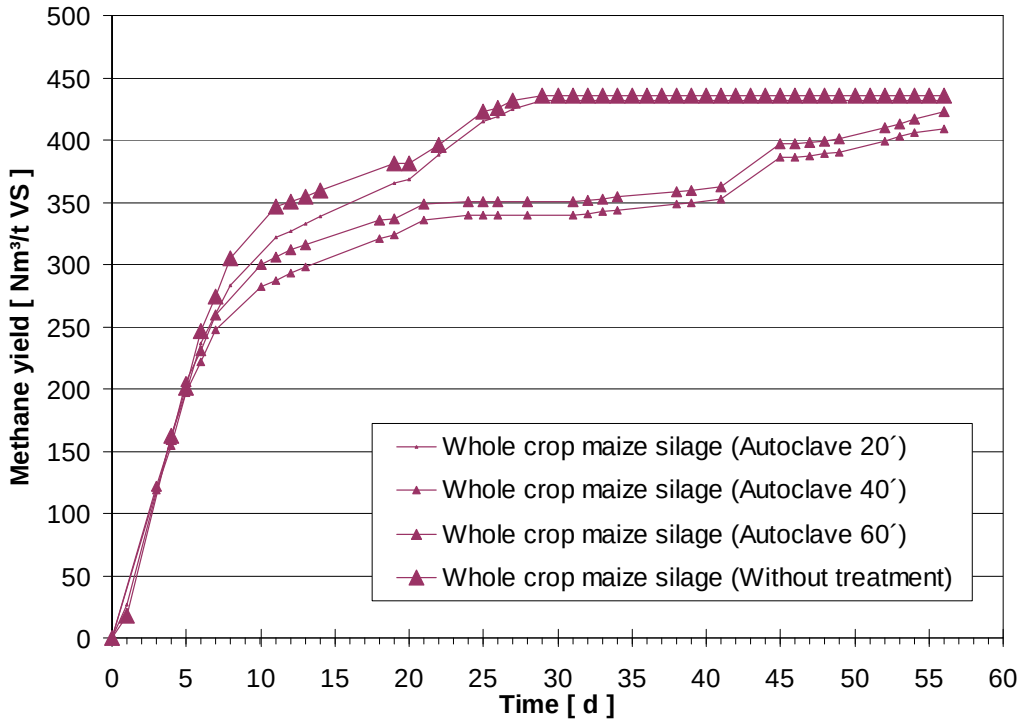

Figure 3: Methane yields from silage samples pre-treated with the autoclave

With whole crop maize a slight effect of the methane yield could be observed (see Fig.2). The outcome from the 1 mm pre-treated sample was 9% higher than that from the untreated one. Methane production and degradation velocity were higher at lower particle sizes.

Autoclave treatment. The whole crop maize silage samples treated with the autoclave did not show any improvement in the methane yield (see Fig. 3). There was no positive influence of pressure and heat. Higher temperature-exposition times did not result in higher methane yield or higher degradation velocity. In fact, both were higher in the untreated samples. The reasons for this phenomenon are probably the same as described in the steam explosion treatment.

Alkaline pre-treatment. Alkaline pre-treatment of the whole crop maize silage samples significantly increased the methane production (Fig. 4): Improved methane yields were observed with concentrated NaOH solutions at short time and low NaOH solutions at longer incubation time. The samples treated with a 4% NaOH solution for 24 h offered an increase in methane production of 34% compared with the untreated sample and the sample treated with a 2% NaOH solution during 72 h showed an increase of 33%. This is in accordance with other studies (Angelidaki and Ahring, 2000) that have found an increase on methane production from lignocellulosic materials after treatment with alkaline agents.

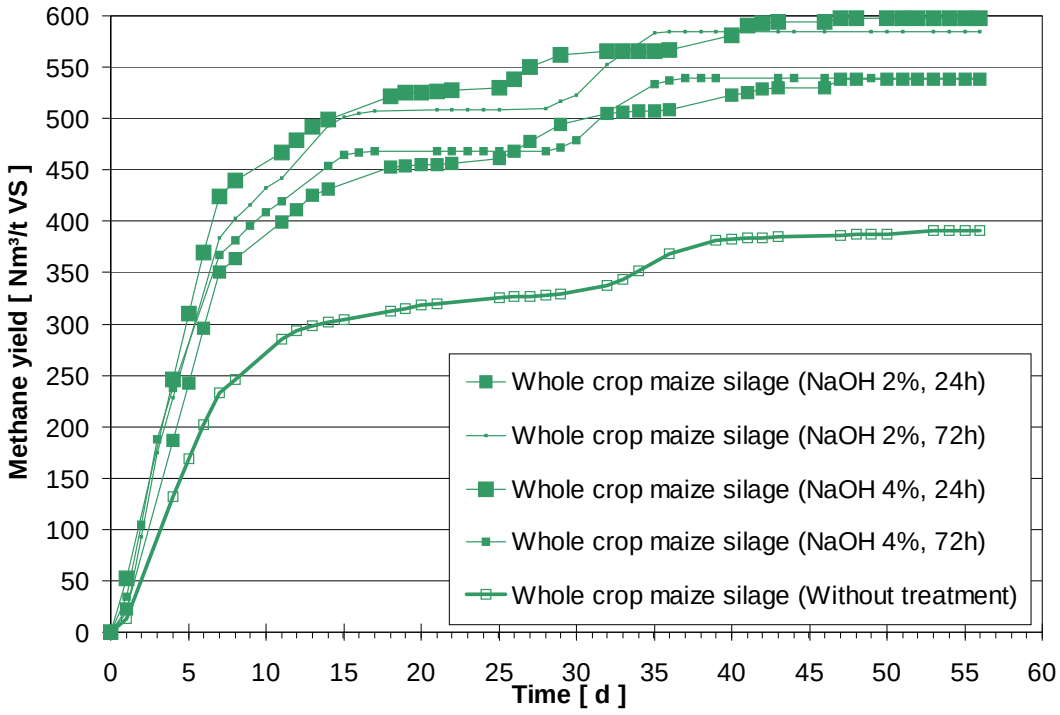

Figure 4: Methane yields from silage samples pre-treated with alkaline solutions

Steam explosion. The whole crop maize silage samples treated in the steam explosion reactor at different reaction times and temperatures did not improve methane yields (Fig. 5). Especially the treatments at sever conditions (high temperatures, long reaction times) produced significantly lower yields that the untreated control. Further chemical analysis of the samples showed, that the starch fraction in the maize silage is almost completely dissolved and partly hydrolysed during the steam pre-treatment. To some extent degradation of the generated monosaccharides takes place. This will result in the formation of unwanted by-products, which leads to losses in fermentable substances. Moreover these by-products, namely Maillard products and sugar degradation products like hydroxymethyl furfural and levulinic acid, are inhibitory to the fermentation process. It could also be shown that disintegration of the lignocellulose can only be expected at severe conditions, high temperatures and long reaction times. Therefore this pre-treatment method cannot be recommended for energy crops containing starch.

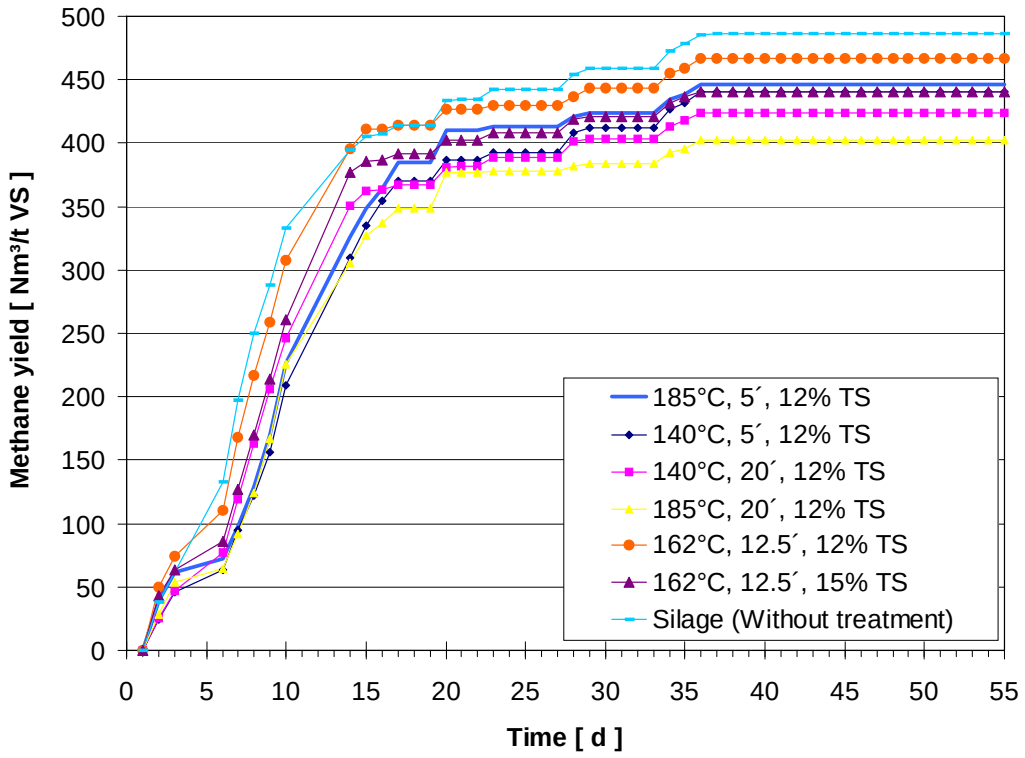

Figure 5: Methane yields from silage samples pre-treated with the hydrolysis reactor

Pre-treatment of energy crops grown under boreal conditions

Materials and Methods

Crops used in these experiments were tops of sugar beet *Beta vulgaris*, grass hay (75% timothy *Phleum pratense*, 25% meadow fescue *Festuca pratensis*) and straw of oats *Avena sativa*. Characteristics of the substrates are given in Table 5.

Table 5: Characteristics of substrates

Substrate	pH	TS [%]	VS [%]	N_{tot} [% TS]	NH_4-N [% N_{tot}]
Sugar beet tops	6.4	11.2	9.2	2.8	0.9
Grass hay	6.4	30.2	27.9	3.9	2.1
Straw	9.5	63.4	59.6	0.8	1.7

Pre-treatment methods

The pre-treatments studied were alkaline treatments (NaOH and mixture of $Ca(OH)_2$ + $NaCO_3$), peracetic acid treatment, autoclaving, water incubation (24 h, at 20ºC), white-rot fungi treatment, enzyme treatment and composting (1 and 2 weeks).

Enzyme treatment. Enzyme treatment was performed in 20 L buckets in 10 % (w/w) total solids (TS) concentration, incubated at 35°C for 24 h with two xylanases (GC 320 and Multifect) and two cellulases (IndiAge MAX L and Primafast 200) (Genencor International Ltd) in enzyme concentration of 0.1 % (w/w).
Before addition of enzymes, the pH of the substrate-water-mixture was adjusted to 5, which, according to the manufacturer, is in the range of optimal activity for these enzymes. The treatments were performed with addition of both active and inactived (autoclaved 20 min at 121oC) enzyme, as well as without enzyme addition. After the treatments, liquid was separated from solids by centrifugation (2800 r/min, 5 min) with a household spin dryer (775 SEC 156 Centrifuge, Thomas) equipped with a nylon-woven fabric bag (pore size 100 µm).

Composting. Composting was performed in 220 L composters (Biolan Oy) for 7 and 14 d. The substrate was inoculated with 500 mL of commercial inoculum (Kompostiheräte, Biolan Oy) per 100 L of substrate in order to secure rapid start-up of composting process. Wood chips in ratio 1:2 (chips:substrate (v/v)) were added to composters as support material. The contents of the composters were manually mixed once a week during incubation

White rot fungi treatment. Sterilised and non-sterilised substrate was inoculated with a commercial mycelial suspension of Pleurotus ostreatus and incubated at 20°C for 21 d in cotton-plugged 2 L bottles.

Chemical pre-treatments. For chemical pre-treatment NaOH (4% and 2% (w/w)) and peracetic acid (20% and 10% (w/w)) were used in 10% TS concentration and $Ca(OH)_2$ + Na_2CO_3 (3% + 4% and 1.5% + 2% (w/w)) was used in 5% TS concentration. The substrates were incubated in 20 L buckets and 20°C for 24 and 72 h.

Methane potential assays

The methane production of treated and untreated plant materials were determined in batch experiments, performed in duplicate 2 L glass bottles (working volume 1.5 L), incubated at 35±1°C for 42 d. 500 mL of inoculum (average values: TS 5.4 %, volatile solids (VS) 4.1 %, total nitrogen (N_{tot}) 3.1 g/L), obtained from a mesophilic farm digester treating cow manure, was added, and $VS_{substrate}/VS_{inoculum}$-ratio of 1 was used in all batch experiments. In treatments where liquid was separated by centrifugation, the substrate added to batch experiments consisted of the separated liquid and solids in the same ratio as in the end of pre-treatment. The only exceptions were peracetic acid treatments, where, due to the possible inhibitory effect of peracetic acid, the separated liquid was not added to batch experiments. Distilled water was added to reach the working volume of 1.5 L, and 3 g/L of $NaHCO_3$ was added as buffer. The bottles were flushed with N_2/CO_2-gas and sealed with rubber stoppers. The gas produced was collected in aluminium gas bags. Bottles were manually mixed before each sampling. Batch experiments with mere inoculum were incubated as blanks.

Results

The respective results are shown in Figures 6 – 7.

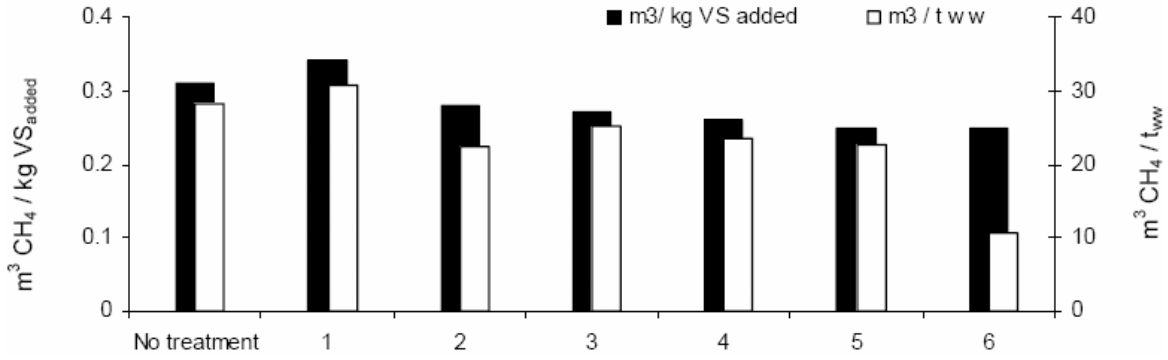

Figure 6: Methane yields of untreated and pre-treated sugar beet tops. 1. 2% NaOH 24 h, 2. Autoclaving, 3. 4% NaOH 24 h, 4. Incubation at 35°C with inactive enzyme, 5. Incubation at 35°C with active enzyme, and 6. 10 % PA 24 h.

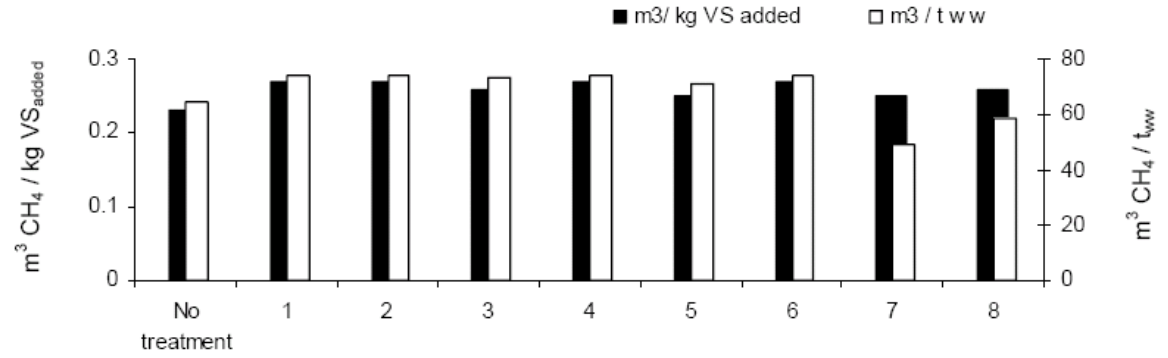

Figure 7: Methane yields of untreated and pre-treated grass hay. 1. Incubation at 35°C with active enzyme, 2. Incubation at 35°C with inactive enzyme, 3. Incubation at 35°C without enzyme, 4. 2 % NaOH 72 h, 5. 2 % NaOH 24 h, 6. 3.0 % Ca(OH)2 + 4.0 % Na_2CO_3 72 h, 7. 20 % PA 24 h, and 8. Autoclaving.

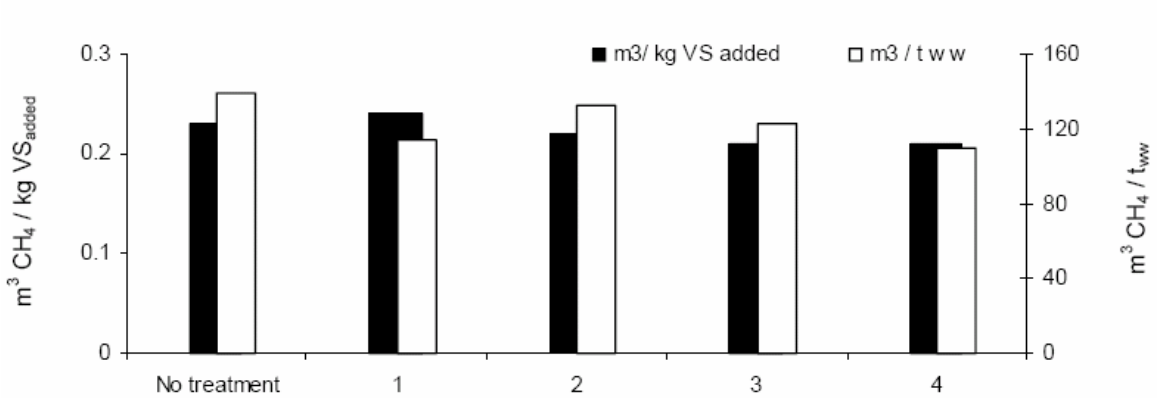

Figure 8: Methane yields of untreated and pre-treated straw. 1. 20 % PA 72 h, 2. 4 % NaOH 24 h, 3. Urea addition, and 4. Incubation in water.

Alkaline treatments and water incubation were the most promising with the investigated crops, increasing the methane yields by 10–20% as compared to untreated crops. However, the effects were highly dependent on the actual conditions, e.g. on the duration of the alkaline treatment and the chemical dosages. Non-optimal conditions and most of the treatments resulted in decreased methane yields under the studied experimental arrangements. Some of the treatments, e.g. composting caused major losses of organic material (up to 60% of volatile solids), resulting in methane yields less than 50% of those of untreated crops.

Conclusions

Considering all tested methods, alkaline pre-treatment gave the most promising results with both starchy energy crops like maize silage and lignocellulosic substrates like grass hay and sugar beet tops. Particularly good results were achieved with whole crop maize silage. In some experiments lower concentration of alkali could be compensated by longer pre-treatment times. An advantage of alkaline treatment is that it is comparably easy to apply. The use of $Ca(OH)_2$ and $NaCO_3$ instead of NaOH might reduce the costs of the treatment.

Although size reduction does not have a large impact on methane production, it is necessary to some extent to ensure the smooth operation of an anaerobic digestion plant. Preferably the size reduction should be combined with the harvesting of the energy crops. Small particle sizes ensure an optimal ensiling process and therefore good storage characteristics. Proper size reduction may also be required by the feeding system of the fermenter. Long fibrous plant material may cause trouble with augers and lead to plugging. Smaller particle sizes also will improve the mixing inside the fermenter. The optimal particle size will be dependent on the feeding system and the digestor design.

Biological pre-treatments like the use of enzymes, composting, inoculation with white rot fungi did not show the desired effects. Especially composting and treatment with white rot fungi can lead to significant losses in VS during pre-treatment.

Besides concerns with respect to the overall energy balance, thermal pre-treatment is not recommended for starch containing materials. It could be shown that the desired effect on lignocellulose can only be achieved at temperatures above 160°C. At the same time less recalcitrant carbohydrates are subject to degradation and can form unwanted by-products. However, such pre-treatments could be effective for crops consisting mainly of lignocellulose. Addition of chemicals like peracetic acid may cause additional problems because of the formation of inhibitive lignin fermentation products and their own toxicity to microorganisms.

Literature

Angelidaki, I. and Ahring, B.K. (2000). Methods for increasing the biogas potential from the recacitrant organic matter contained in manure. *Water Science and Technology* **43**(3), 189–194

Deutsche Einheitsverfahren (2005). DIN 38414 H41-1, Wiley-VCH

Fan, L.T., Gharpury, M.M. and Lee, Y.H. (1981). Evaluation of pre-treatments for enzymatic conversion of agricultural residues. *Biotechnology and Bioengineering Symposium* **11**, 29–45

Fan, L.T., Lee, Y.-H. and Gharpury, M.M. (1982). The nature of lignocellulosics and their pretreatments for enzymatic hydrolysis. *Advances in Biochemical Engineering* **23**,159–187

Kosaric, N., Wieczorek, A., Cosentino, G.P., Magee, R.J. and Prenosil, J.E. (1983), Ethanol fermentation. In: Rehm, H.J., Reed, G. and Dellweg, H. (Eds.): Biotechnology. Vol. 3, Verlag Chemie, Weinheim, pp. 212–239

Mata-Alvarez, J., Macé, S. and Llabres, P. (2000). Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresource Technology* **74**, 3–16

Pavlostathis, S.G. and Giraldo-Gómez, E. (1991). Kinetics of anaerobic treatment: acritical review. *Critical reviews in environmental control* **21**(5,6), 411–490

Sun, Y. and Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production. A review. *Bioresource Technology* **83**,1–11