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Biochemical methane potential of different organic wastes and energy crops from Estonia

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Abstract. The biochemical methane potential (BMP) of different Estonian substrates as alternative sources for biogas production was studied. For this purpose, the BMP test was carried out in batch mode at mesophilic temperature (36°C). Substrates were divided into 2 groups: agricultural substrates (silage, hay, cattle and pig slurry) and food industry residues (milk, brewery and cereal industry residues). Methane yields obtained were between 286–319 L $kgVS^{-1}$ for silage and hay, 238–317 L $kgVS^{-1}$ for animal slurry and 272–714 L $kgVS^{-1}$ for agro-industrial wastes. The highest methane yield was obtained from sour cream (714 L $kgVS^{-1}$), the lowest (238 L $kgVS^{-1}$) from cattle slurry. In overall, our results suggest that all tested substrates can be treated anaerobically and are potential sources for the production of methane.

Keywords: methane potential, ultimate methane yield, silage, hay, slurry, residues

INTRODUCTION

Due to the rising cost of fuels and increased pollution, the implementation of renewable energy systems have become an attractive alternative for fossil fuels in many countries worldwide.

In the past few years, Estonia has considered the implementation of different renewable energies as a strategy to reduce its dependence to fossil fuels. In 2010, the Renewable Energy Action Plan (REAP) was published according to Renewable Energy Directive 2009/28 (RED). According to the targets set by RED, by 2020 the renewable energy usage should account for 25% of the total energy consumption in Estonia. To reach this goal, share of renewable energy in the sectors of heat/cooling, electricity, and transport should achieve 17.6, 4.8 and 2.7% of the total energy consumption in these sectors respectively. The development of the biogas sector in Estonia is considered among the REAP actions. Target for annual biogas production was set to 0.5 PJ in 2020. Estonia has great potential for production of biogas using manures, herbal biomass and organic residues. There are about 286 thousand hectares of abandoned agricultural land in Estonia that is suitable for cultivation of energy crops, and 128 thousand hectares of semi natural grasslands (Astover et al., 2008).

Theoretical herbal biomass resources for biogas production are 2 billion tons per year (Roostalu & Melts, 2008). Renewable electricity potential in the agricultural biogas sector is estimated to produce 190 GWh and 690 GWh for manures and herbal biomass respectively (Kask, 2008). Nowadays, there is only one agricultural biogas plant that generates an annual electricity production of 2 GWh/y. Most of biogas renewable energy potential is not used in Estonia.

During the last decades, applications of anaerobic digestion has become very popular for production of renewable energy because of its known energy potential, low maintenance costs and, primarily, to its environmental benefits such as the bioconversion of organic waste into organic fertilizers and biogas (Tafdrup, 1995; Ward et al., 2008; Ahring et al., 1992). Anaerobic digestion is a process that consists of a set of microbial interactions in an oxygen-free environment, in which biogas is produced by means of degradation of organic matter (Schink, 1997; Pain & Hepherd, 1985). Some of the advantages of anaerobic digestion are: wastes with less than 40% of total solids are easily treatable, minimization of sludge, odors and pathogens reduction during the process, compliance with waste management legislation (Mata-Alvarez, 2002; Sahlstrom, 2003; Smet et al., 1999).

Biochemical methane potential (BMP) assays have been widely used to determine the methane yield of organic substrates in specific conditions (Owen et al., 1979; Nallathambi Gunaseelan, 1997; 2004).

In this study the methane potential of 51 substrates from Estonia was determined using BMP assay. The substrates were chosen according to the national availability. In Estonia, the most potential substrates for the production of biogas are silages (grass, maize, and alfalfa), hay and animal manures (cattle and pig). Some other substrates like milk products, brewery residues and grain mill were selected to assess their biogas potential due to their potential to be used as co-substrates in farm-scale anaerobic digesters. Based on the observed methane yields and substrate characteristics the substrates potential for biogas production was estimated.

MATERIALS AND METHODS

Inoculum

The inoculum was collected from the anaerobic reactor of a wastewater treatment plant in Tallinn, Estonia. The inoculum was stored at room temperature in 35 liter tanks, sieved through a 2 mm mesh and pre-incubated at mesophilic range (36°C) 5 days before use to ensure activation and degasification of the sludge. Total solids (TS) and volatile solids (VS) of the inoculum were measured each time before test set-up. TS was adjusted to 20 g per kg of the inoculum by adding distilled water.

Feedstock

Samples were collected in Estonia from 2008 to 2010. 4 samples of grass silage, 4 of maize silage, 18 of different mix silages (grasses and legumes, mix rate is not specified) and 4 of hay were collected from different grasslands, 6 samples of cow slurry and 1 sample of pig slurry were collected from a local farm, 1 sample of fermentation slops and 3 different samples of grain mill residues, i.e. aspiration dust, bran and flour were collected from local industries.

For homogenization, silage and hay samples were conditioned by drying at 65°C and milled to achieve particles size of less than 1mm. Then, samples were packed into plastic boxes and stored in a freezer at 4°C before use. All other samples were used without any treatment.

Experimental procedure

The BMP test performed in this study was based on a modified version of the guidelines described by Owen et al., 1979. The experiment was carried out in triplicate with each sample using 575 ml plasma bottles filled with 150 ml of inoculum and 0.3 g TS of substrate. 50 ml of distilled water was added to reach an effective volume of 200 ml. No additional nutrients were added to the test. It was assumed that nutrients required for anaerobic microorganisms were provided by the inoculum as previous trials with addition of nutrients have not shown any significant difference. Before starting the experiment, the test bottles were flushed for 10 minutes with N_2/CO_2 (80/20). Test bottles were incubated at 36°C in a set of Mermet isothermal thermo chambers during 42–78 days. Initial basal pressure in the test bottles was measured after acclimation at incubation temperature. For each substrate the duration of the BMP test was specifically determined. The methane production from inoculum was determined in blank tests where no substrate was added. Biogas production and gas composition were determined periodically. Mixing was done by shaking the bottles manually regularly once a day.

Analytical methods

Total solids (TS) and volatile solids (VS) were analyzed according to method 1684 (U.S. Environmental Protection Agency – EPA). TS were determined after drying the sample at 105°C overnight. VS in organic wastes were measured as total solids minus the ash content after ignition at 550°C. pH was measured by a Sentron pH-meter 1001pH. Gas samples were taken by connecting the test bottles to the gas chromatograph through a plastic tube attached to a needle. Gas production was analyzed by measuring the increase in pressure in the gas phase of test bottles using an absolute pressure transducer (0–4 bar, Endress & Hauser). Gas composition of biogas samples were analyzed chromatographically using a gas chromatograph (Varian Inc., Model CP-4900) equipped with 2 columns: a Molsieve 5A Backflush heated column (20 m x 0.53 mm), and a PoraPLOT U heated column (10 m x 0.53 mm). Argon and Helium were used as carrier gases in columns 1 and 2, respectively. Injection temperature, column temperature and column pressure were set to 110°C, 120°C and 50 Psi respectively for column 1, and 110°C, 150°C and 22 Psi for column 2, respectively.

Calculation

Methane produced was calculated by subtracting the methane produced by the inoculum from the methane produced in the test with substrate and inoculum. Cumulative methane yield was calculated as the sum of methane produced over the incubation period and expressed as liters per kilogram of TS or VS of substrate added to the test. The volume of methane was calculated to standard temperature and pressure conditions (0°C and 1 atm). The methane production was modeled by fitting the experimental data with two non-linear regression models in GraphPad 5.0. The models tested were one-phase exponential association (Model 1):

$$B=B_{max}\,(1-e^{-k\cdot t}) \tag{1}$$

where B is the cumulative methane yield at time (t), B_{max} is the maximum methane yield, k is the rate constant, expressed in reciprocal of the X-axis time units (d^{-1}), and the two-phase exponential association model (Model 2):

$$B=B_1\,(1-e^{-k1\cdot t}) + B_2\,(1-e^{-k2\cdot t}) \tag{2}$$

where B represents the methane production as a function of time (t), B_1 is the methane yield associated to the bioconversion of readily degradable organics, B_2 is the methane yield associated to the bioconversion of less readily degradable material, k_1 and k_2 are the respective rate constants.

Ultimate methane yields were calculated using above-described models as cumulative methane yield for time t = 100 days. Incubation time required to achieve 60, 70 and 80% of methane yield were calculated from the ultimate methane yield.

One-way analysis of variance (ANOVA) was used to determine statistical significance *($P<0.05$)* of differences between substrate groups.

RESULTS AND DISCUSSION

Agricultural Substrates

Silages and hay. Ultimate methane yields were calculated by fitting measured data with two different models. For methane production modelling the first order degradation model (Model 1, equation 1) has been widely used in different studies (Hashimoto, 1986; Nallathambi Gunaseelan, 2004; 2009,). However, analyzing our data with this model indicated poor fitting results for biomass substrates (Fig. 1). Since Rao et al., 2000 and Rincon et al., 2010 found that biogas production from solid organic substrates were best fitted by the pseudo-parallel first order model, similar two-phase exponential model (Model 2, equation 2) was also tested in this study. It is considered that the methane production curves correspond to the rapid bioconversion of readily degradable components followed by a slower bioconversion of fibrous portion of the substrates. Correlation coefficients obtained after data fitting were in the range of 0.987–0.999, 0.985–0.996, 0.957–0.999 and 0.994–0.998 for grass silage, maize silage, mixed silage and hay respectively.

The chemical characteristics and methane yields for the determination of the methane potential of hay and different silages are presented in Table 1. Cumulative methane yields were calculated to be 319 ± 19, 307 ± 21, 296 ± 31 and 286 ± 33 L $kgVS^{-1}$ for grass silage, maize silage, silage mixture and hay, respectively. These results appear to be consistent with the findings of other authors (Table 1) even though silage samples used in this study have been previously pre-treated. The methane production from all samples started actively after incubation. Time to reach 80% of ultimate methane yield was 15 days for grass silage, 14 days for maize silage, 13 days for mix silage and 19 days for hay (Table 3).

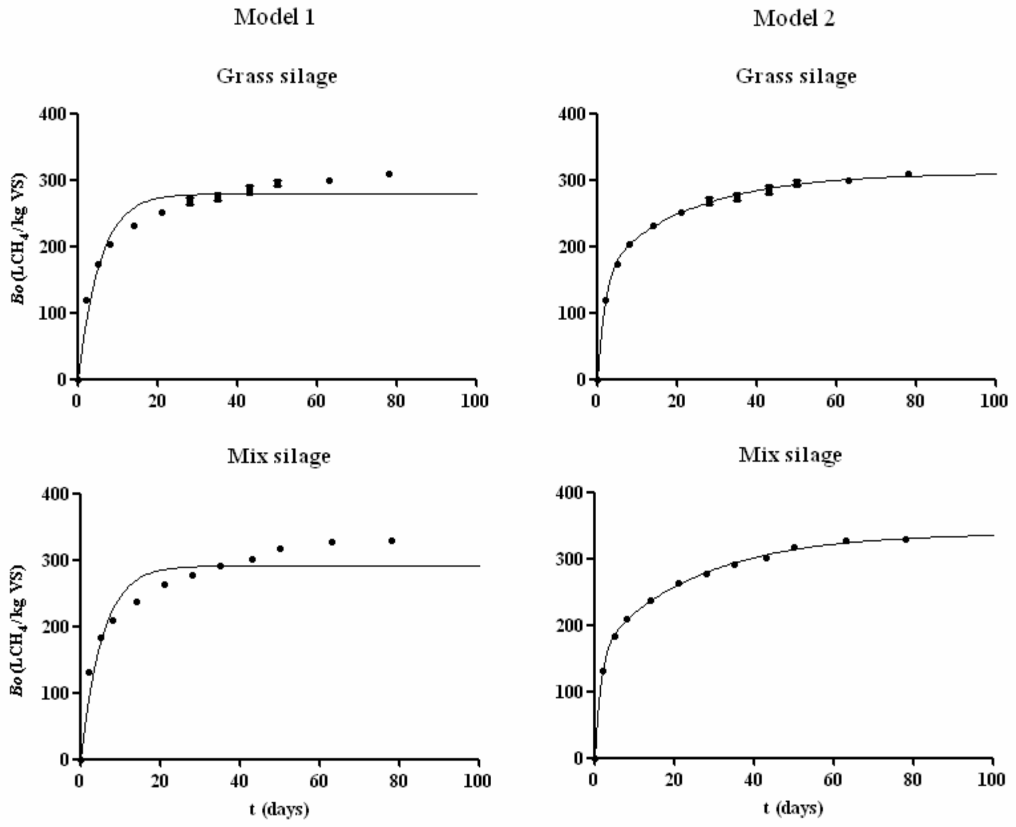

Figure 1. Fitting comparison between two different models tested for silages.

Animal slurries. Results from BMP assay with cattle and pig slurry as substrates are presented in Table 1. As for silages and hay, methane production started actively in all test bottles. Ultimate methane yields for animal slurries were calculated by fitting our data to the one-phase exponential association model (Fig. 2). Correlation coefficient for pig slurry data was 0.946 and for cattle slurry varied from 0.906 to 0.995.

Our results present that during the first 23 days of incubation 80% of the ultimative methane yield has occurred when cattle slurry was used as substrate. In case of pig slurry 80% of the ultimate methane yield occurred within the first 12 days of incubation (Table 3). Tests with cattle slurry presented a methane yield of 238 ± 42 L kgVS^{-1}. Our results appear to be within the same range as results from other studies conducted by different authors (Table 1). BMP results showed that pig slurry produced 30% more methane than cattle slurry. Results obtained for pig slurry appear to be consistent with the findings of Steffen et al., 1998 and Vedrenne et al., 2008.

Ultimate methane yields from agricultural substrates analyzed in numerous trials are presented in Fig. 2. We found no significant statistical difference between the ultimate methane produced from biomass samples, even though their TS content varied significantly *($P<0.05$*; Table 1).

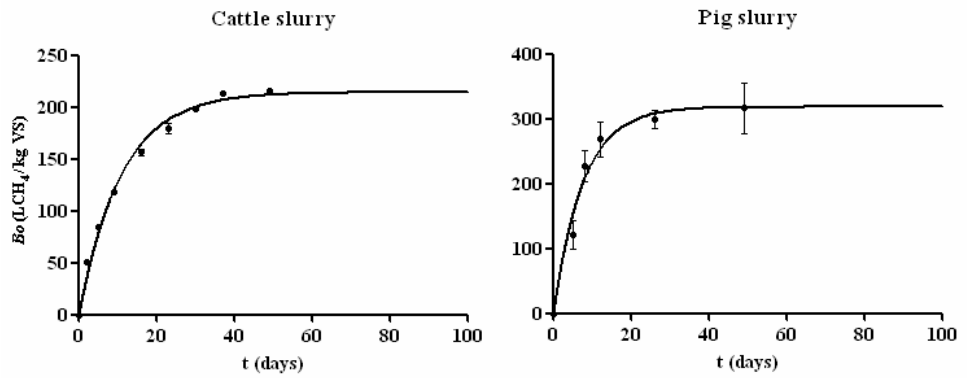

Figure 2. One-phase exponential association fitting curves for cattle and pig slurry.

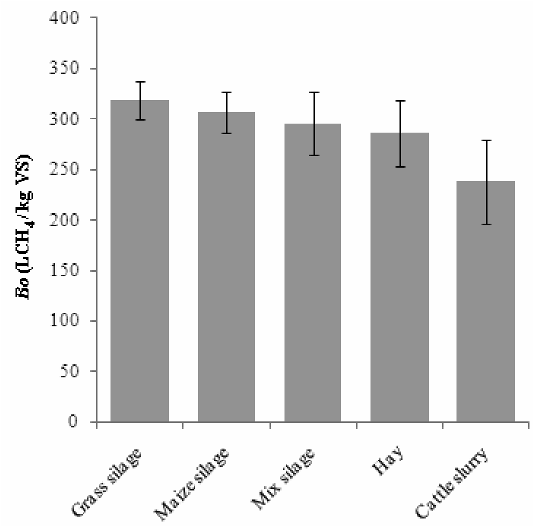

Figure 3. Ultimate methane yield of selected agricultural substrates.

Food industry residues

Milk products. Methane potential of different unconsumed milk products was analyzed during the study, since milk products represent a potential source of biogas in the milk industries as considerable amounts are frequently discharged from factories worldwide. Chemical characteristics and cumulative methane yields of the selected products are presented in Table 2. All milk products presented significantly high methane yields, effect that can be explained by their high content of proteins of dry matter (Frigon et al., 2009).

Methane yields obtained during this experiment were between 458 and 714 L kgVS^{-1}. The methane yield obtained from sour cream presented the highest potential of all tested products, while milk containing 2.5% fat presented the lowest methane yield. Bioconversion of milk wastes occurred very rapidly. 80% of ultimate methane yield was reached after only 3–8 days of incubation (Table 3). However, although milk products could represent high methane potential, special care needs to be taken to avoid inhibition by ammonia in anaerobic digesters (Callaghan et al., 1997).

Table 1. Chemical composition and cumulative and ultimate methane yields of tested substrates (standard deviation of substrates tested more than once are presented between brackets).

Substrate	n	pH	TS	VS	Cumulative methane yield		Ultimate methane Yield	Source
			g kg^{-1}	g $kgTS^{-1}$	L $kgTS^{-1}$	L $kgVS^{-1}$	L $kgVS^{-1}$	
Grass silage	4	4.5	314	928	296 (19)	319 (19)	320 (22)	Our study
		-	318	877	-	372	-	Lehtomäki & Björnsson, 2006
		-	310	871	-	270	-	Cirne et al., 2007
		4.1	259	926	-	300	-	Lehtomäki et al., 2008
Maize silage	3	4.2	174	952	292 (21)	307 (21)	339 (26)	Our study
		3.76	349.2	961	-	338	-	Neureiter et al., 2005
		-	73	836	-	295	-	Dubrovskis et al., 2009a
		-	-	-	-	335–370	-	Pobeheim et al., 2010
Mix silage	18	4.4	294	920	272 (31)	296 (31)	307 (28)	Our study
Hay	4	-	913	937	268 (33)	286 (33)	292 (30)	Our study
		7.9	-	911	-	270	-	Kaparaju et al., 2002
Pig slurry	1	7.0	69.9	794	252	317	321	Our study
		-	69.3	704	-	244–343	-	Vedrenne et al., 2008
		-	30–80	700–800	-	175–350	-	Steffen et al., 1998
Cattle slurry	9	7.7	78	782	186 (42)	238 (42)	247 (58)	Our study
		-	120	850	-	243	-	Steffen et al., 1998

-: not determined

n: number of samples tested for same substrate (one sample represents the average of three replicas)

Table 2. Chemical composition and cumulative and ultimate methane yields of food industry wastes.

Substrate	n	pH	TS g kg^{-1}	VS g kgTS^{-1}	Cumulative methane yield L kgTS^{-1}	 L kgVS^{-1}	Ultimate methane yield L kgVS^{-1}	Source
Cheese	1	-	562	960	633	659	659	
Sour cream	1	-	265	992	708	714	717	
Cottage cheese	1	-	265	980	590	602	602	
Buttermilk	1	-	109	992	485	489	489	
Milk 2,5 % Fat	1	-	110	993	455	458	458	
Milk 3,5 % Fat	1	-	120	993	463	466	468	
Raw milk	1	-	128	993	508	512	517	Our study
Whey		5.2	68.6	911	-	501	-	Dinuccio et al., 2010
Distillery slop	1	3.3^{a}	95.5^{a}	931^{a}	355^{a}	381^{a}	400^{a}	
	1	3.2^{b}	55.4^{b}	912^{b}	306^{b}	335^{b}	385^{b}	Our study
Fermentation slops		-	50	950	-	338	-	Steffen et al., 1998
Grain mill - Aspiration dust	1	4.1	874	940	256	272	274	
Grain mill – Bran	1	4.5	794	914	300	328	330	
Grain mill – Flour	1	-	912	896	344	384	386	Our study
Grain mill waste		-	564	917	-	130	-	Dubrovskis et al., 2009

[a] Before centrifugation (11,000 rpm)

[b] After centrifugation (11,000 rpm)

-: not determined

n: number of samples tested for same substrate (one sample represents the average of three replicas)

Table 3. Time to reach corresponding percentages of ultimate methane yield.

Substrate	n	60% *Bo*		70% *Bo*		80% *Bo*	
		L kgVS^{-1}	Days	L kgVS^{-1}	Days	L kgVS^{-1}	Days
Grass silage	4	196	6	222	9	256	15
Maize silage	3	209	7	239	10	272	14
Mix silage	18	193	5	215	7	247	13
Hay	4	179	11	206	15	233	19
Pig slurry	1	194	7	225	9	260	12
Cattle slurry	9	150	12	173	16	198	23
Cheese	1	396	5	463	6	530	8
Sour cream	1	434	2	502	3	570	4
Cottage cheese	1	361	4	423	5	481	6
Buttermilk	1	296	3	343	3	391	5
Milk 2,5% Fat	1	277	3	321	3	367	5
Milk 3,5% Fat	1	284	2	325	2	372	3
Raw milk	1	308	2	360	3	409	3
Distillery slop (a)	1	249	7	289	9	324	12
Distillery slop (b)	1	232	9	275	13	310	17
Grain mill - Aspiration dust	1	171	6	205	9	235	13
Grain mill - Bran	1	208	5	253	7	281	11
Grain mill - Flour	1	234	4	279	6	315	10

n: number of samples tested for same substrate (one sample represents the average of three replica)

Brewery wastes. Residues from brewery were analysed without pretreatment and after centrifugation at 11,000 rpm for 20 minutes. Cumulative and ultimate methane yields are presented in Table 2. Our results show a reduction of the concentration of VS in test samples that were pre-treated with centrifugation leaded to decreased production of methane. Methane production started actively after incubation. 80% of the ultimate methane yield was already reached on the 12th and 17th day of incubation for samples without and with pretreatment respectively (Table 3). Methane yield in our experiment resulted in similar values compared with Steffen et al. (1998).

Cereal industry residues

Production of methane from three different grain mill residues was studied. Samples consisted of residues of aspiration dust, bran and flour from a grain mill industry. Results of the chemical composition analyses and the methane potentials are shown in Table 2. Cumulative methane yields were 272, 328 and 384 L kgVS^{-1}L for aspiration dust, bran and flour, respectively. Test bottles with flour produced 38% and 10% more methane than test bottles with aspiration dust and bran, respectively. 80% of the ultimate methane yield was reached after 11, 10 and 13 days for bran and flour, and aspiration dust, respectively (Table 3). Dubrovskis et al., 2009b who also tested the methane yield of grain mill wastes found a methane yield of 130 L kgVS^{-1}, which is much lower than our results. This variation can be explained by the difference in the composition of the substrate, as TS concentration reported in their study was much lower than in the substrates analyzed in this study.

CONCLUSIONS

Cattle slurry is planned to be used as the main substrate in many biogas plants in Estonia. However, it was found that cattle slurry is not the most attractive substrate for the production of biogas and therefore co-digestion with other substrates should be considered. Pig slurry presented higher methane potential than cattle slurry, but its low solid content demands additional input of organic dry matter to increase capacity of digesters.

Due to high availability and their methane potential, silages and hay could be considered as possible substrates in rural areas.

Milk wastes presented the highest cumulative methane yield from all tested substrates with a range of 458–714 liters per kilo of VS added.

Fermentation slops are also of great interest for the production of biogas, as high methane yields were obtained. However, centrifugation as a pre-treatment of the samples is not recommended as a decrease in the methane yield was found.

Residues from the cereal industry such as aspiration dust, bran and flour were found suitable for the production of biogas. We suggest it would be valuable to analyze their methane potential in co-digestion with other substrates like animal slurry or with fermentation slopes due to their high dry matter content.

The most rapid bioconversion of substrate to methane occurred in the BMP tests with milk wastes. 80% of the ultimate methane yield occurred barely after only 3–8 days of incubation. The longest period to achieve 80% of the ultimate methane potential was found for cattle slurry with a retention time of 23 days. These results suggest that anaerobic digesters such as the continuous stirred tank reactors (CSTR) can be considered as an option for the production of methane since they can be operated with hydraulic retention times of more than 25 days.

The results of this experiment suggest that herbal biomass and agro-industrial residues are promising substrates for the production of renewable energy. We believe the results presented in this article will contribute to the selection of the most suitable substrates in different projects related to anaerobic digestion in Estonia.

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