Agronomy Research **15**(5), 1956–1970, 2017
https://doi.org/10.15159/AR.17.063

Influence of chemical composition on the biochemical methane potential of agro-industrial substrates from Estonia

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Abstract. Batch trials were carried out to evaluate the Biochemical Methane Potential (BMP) of 61 different substrates collected from agricultural farms and industrial sites in Estonia. Tests were performed in 500 mL plasma bottles at 36°C. The highest methane yield from all tested substrates was obtained from unconsumed dairy products (557 ± 101 L kg^{-1} VS) while the lowest was obtained from animal slurries (238 L kg^{-1} VS ± 42). From tested energy crops, foxtail millet achieved the highest methane yield (320 L kg^{-1} VS). Silages from different crops presented methane yields from 296 ± 31 L CH_4 kg^{-1} VS to 319 ± 19 L CH_4 kg^{-1} VS. The influence of chemical composition and kinetic rate constants (k) on methane potential was analyzed. Anaerobic digestibility of selected agro-industrial substrates was markedly influenced by their organic content, i.e. total proteins and lignin concentrations. Rate constants were found to correlate negatively with hemicellulose, cellulose and lignin ($p < 0.05$). Results from this study suggest that an appropriate characterization of the chemical composition of the substrates is important not only for predicting BMP and the kinetics rates, but also for identifying possible inhibitors during the anaerobic digestion process. Results on the BMP and national availability of studied substrates indicate that herbal biomass and agro-industrial residues are promising substrates for biogas production in agricultural biogas facilities in Estonia.

Key words: Biogas, Biomass, Biochemical Methane Potential, Kinetic rate, Agro-industrial, Wastes.

INTRODUCTION

Climate change mitigation is a matter of great interest worldwide, in which renewable energy systems have gained high interest due to their low carbon footprint and high environmental sustainability. Energy from biomass stands at the fourth largest energy source in the world, due to resource availability in rural and urban areas and pollution reduction in the case of municipal solid waste (Frank et al., 2016). Data on the energy potential of different organic substrates have been widely studied, resulting in more efficient reactors with process and inhibition control (Munoz et al., 2015).

However, bioreactors with incorporated instrumentation, control and automation are very rare in developing countries, due to economic and training skills limitations.

Research projects in northern European countries have extensively study biomass availability, chemical characteristics and its influence on the energy potential to identify substrates with high potential for the energy industry. Among the most suitable substrates are forestry and agricultural residues, sewage and industrial organic wastewater, municipal solid wastes and livestock and poultry slurries (Gissen et al., 2014).

Several studies have been conducted on the assessment and comparison of biogas and methane potential of different substrates at different experimental scales (lab, bench, pilot and industrial scale). However, few studies have been conducted on the comparison of different biomass residues and its relation between the chemical composition and the bioenergy potential. Those studies usually report data on no more than 10 different substrates. Correlation of methane potential with different parameters of biomass composition have widely been studied. Results obtained have consolidated predictive models of specific methane yields from different sources of biomass. The most studied correlations between methane yield and chemical composition of biomass are cellulose, hemicellulose, crude fat, acid detergent lignin (ADL), acid detergent fiber (ADF) (Herrmann et al., 2016). However, results from those studies are limited to: 1) few datasets mainly from residual biomass and 2) correlations between methane yield of different substrates ratios or samples of the same crop species.

Studies from different protein-rich substrates have shown to have great potential for methane production. Unfortunately, high concentration of such materials may cause anaerobic digestion instability due to the release of ammonia nitrogen (NH_3-N). Ammonia, which is released from the degradation of amino acids during acidogenesis, has been identified as a common inhibitor for different microorganisms involved during the anaerobic digestion process, in which the most sensible are the methanogens (Yuan & Zhu, 2016). Low methane yields have been attributed to insufficient ammonia nitrogen (500 mg L^{-1}) due to low microbial activity and buffering capacity, while excessive concentration (> 5,000 mg L^{-1}) may result in biogas inhibition (Yenigün & Demirel, 2013).

Anaerobic degradation of biomass is reduced by enfolded cellulose and hemicellulose in lignin. This is explained by limited accessibility of particulate substrate by microorganisms during the hydrolytic phase (Herrmann et al., 2016). The synthesis of complex polymers is fundamental for enzyme penetration and efficient biodegradation during all steps of anaerobic digestion: hydrolysis, anaerobic fermentation and methanogenesis.

Nutrients are categorized as micronutrients (also known as trace elements) and macro-nutrients. It is very important to check nutrient concentration at first to avoid poor performance of anaerobic digestion or biogas inhibition. Trace elements are one of the significant factors for micro-organisms growth and activity as they play an essential role for many physiological and biochemical processes. Lack of understanding of metabolic behavior of trace element requirements of methanogens could result in low methane yields, acidification and process instability during anaerobic digestion of energy crops. Therefore, proper nutrient concentrations in the digester is important for enhancement of methane and biogas production and process stability. Poor management of process control could derivate in high concentration of trace elements in the digestate triggering possible toxicity risks during its disposal and/or utilization as biofertilizer.

Degradation rate of compounds can be described by a means of a differential kinetic equation. Methane prediction of a specific substrate can be achieved by knowing the biodegradation kinetics. The first order kinetic constant (k) represents a measure of biodegradability rate. The higher the k value, the higher the biodegradability of the substrate in the digestate.

In Estonia, there is estimated an area of around 286 thousand hectares of abandoned agricultural land that can be considered for cultivation of energy crops and around 128 thousand hectares of semi-natural grasslands (Astover et al., 2008). The calculated theoretical herbal biomass production is up to 2 billion tons per year (Roostalu & Melts, 2008). In Estonia, there are other agro-industrial sources of biomass that can also be considered for the production of biogas, such as fermentation slops from brewery industry, unconsumed milk products, grain mill residues, etc. Nowadays, Estonia has 18 biogas plants, in which 5 are based on agro residues.

The novelty of the present research study stands on the correlation analysis of dataset obtained from the chemical composition of 61 different agro-industrial residues and its relation with the methanogenic potential and the kinetic rate constants. The aim of this study is to evaluate methane yield of the main agro-industrial substrates of Estonia and to identify main chemical parameters that affect methane yield.

MATERIALS AND METHODS

Inoculum

The inoculum was collected from an anaerobic reactor from a facility located at Tallinn, Estonia. The reactor works as part of a wastewater treatment plant (Estonia). Sludge samples collected were gently stirred and filtered with a 2 mm mesh to remove large particles. For the trials, the sludge was previously incubated for one week at mesophilic temperature (36 °C) under a headspace of N_2/CO_2 (80:20) for degasification (consumption of residual organic matter). The main characteristics of the inoculum were as follows: total solids (TS) 22.1 g L^{-1}, suspended solids (SS) 15.7 g L^{-1}, volatile suspended solids (VSS) 598 g kg^{-1} SS.

Feedstock

Based on biomass availability, 61 substrates were collected from different locations (agricultural farms and industrial sites) in Estonia. The substrates selected were: energy crops (jerusalem artichoke with and without flowers, sunflower collected at 2 different periods, hemp collected at 2 different periods, Amur silvergrass, energygrass and millet), silages (grass, maize, alfalfa, timothy grass and red clover), hay, animal slurries (cattle and pig) and agro-industrial residues such as brewery residues (distillery slops) and grain mill residues (aspiration dust, bran and flour) and unconsumed milk products. For the case of energy crops, i.e. silage and hay samples, they were conditioned by milling to achieve particles size of 1 mm. All samples were stored in plastic boxes in a fridge at 4 °C before use.

Experimental procedure

The Biochemical Methane Potential (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 using 575 mL plasma bottles containing 150 mL of inoculum (in-reactor biomass concentration 7.26 g VSS L^{-1}) and 0.3 g TS of each substrate. Distilled water was added to reach an effective volume of 200 mL. A set of 3 bottles without substrate were prepared for each batch to study the methane production of inoculum (blank test). Previous work (Luna del-Risco et al., 2011) has indicated that inoculum collected from Tallinn wastewater treatment plant is sufficient in providing the nutrients necessary for operating a successful BMP test and thus no additional nutrient medium was added. The bottles were closed and the headspace was flushed with N_2/CO_2 (80:20). Test bottles were incubated at 36 °C in a set of Mermet isothermal chambers. Samples were incubated for up to 78 days, and stirred manually twice a day. Biogas production and gas composition were determined periodically. Cumulative methane yield was calculated as the sum of methane produced over the incubation period minus the methane yield in blank test. Biogas production was expressed at standard conditions (0 °C, 1 atm.) per kilogram of TS or VS of substrate added to the test.

The rate of degradation of substrates was assumed to follow the first–order kinetics as done by Gunaseelan (2009). Methane production was modeled by fitting the experimental data with the first-order decay rate model (Eq. 1) in GraphPad 5.0.

$$B = B_{max} \cdot [1 - \exp(-k \cdot t)], \quad (1)$$

where B is the cumulative methane yield (L kg^{-1} TS or L kg^{-1} VS) at time t (days); B_{max} is the maximum methane yield (L kg^{-1} TS or L kg^{-1} VS) and k is the first-order decay rate constant (1 d^{-1}).

Analytical methods

Substrates were analyzed for pH, total solids (TS), volatile solids (VS), total organic carbon (TOC), total nitrogen (TN), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (ADL), calcium (Ca), phosphorus (P), magnesium (Mg) and potassium (K). pH was measured by a Sentron 1001pH to check samples. TS was measured by drying substrates for 24 hours at 105 °C and VS by incineration at 550 °C for 2 hours. TOC was determined by catalytically-aided platinum 680 °C combustion technique (Shimadzu TOC-V), TN was determined by copper catalyst Kjeldhal method using a Kjekltec Auto 1030 and total proteins (TP) were calculated by multiplying total nitrogen values by a factor of 6.25 (TP = TN*6.25) in the case of plant biomass and by a factor of 6.38 for milk proteins (Merrill & Watt, 1955, Merrill & Watt, 1973). NDF and ADF were determined using a Foss Tecator Fibertec 1020. Lignin was determined as described by AOAC 973.18 method. On the basis of NDF, ADF and ADL analysis, hemicellulose (NDF-ADF) and cellulose (ADF-ADL) concentrations were calculated as proposed by Van Soest et al. (1991). Ca, P and Mg were determined using a Fiastar 5000 following the o-Cresolphthalein complexone method (Connerty & Briggs, 1966), the stannous chloride method (ISO/DIS 15681-1, 2001) and the titan yellow method (Heaton, 1960), respectively. Total fat and proteins concentrations of unconsumed milk products were taken from the manufacturer.

Biogas production was measured by pressure increase in test bottles using a calibrated pressure transducer (0–4 bar, Endress & Hauser), equipped with needle and a valve to avoid gas leakages during measurements.

Methane content was analyzed chromatographically using a gas chromatograph (CP-4900 MICRO-GC, Varian Inc., Palo Alto, CA 94304) that was equipped with an ultra-low volume thermal conductivity detector (TCD) and two columns (molecular sieve 5A and Porapak Q), with the former for analyzing gaseous hydrogen (H_2), oxygen (O_2), methane (CH_4), carbon monoxide (CO), and nitrogen (N_2), and the latter for carbon dioxide (CO_2). The operating conditions for the micro-GC were 10 s for stabilization, 100 ms for sample injection, 30 s for sampling, 120 s for running, and 8 s for backflushing. The temperatures for the sampling line, columns, and the injector were set at 50, 80, and 110 °C, respectively. Argon at a pressure of 4.2 kg cm^{-2} was used as the carrier gas and its flow rate was automatically controlled by the micro-GC. Methane yield was expressed as normal L (273 K and 1,013 mbar) per kg of VS (kg^{-1} VS).

Statistical analysis

The dependence of methane potential, (i.e. highest cumulative methane yield achieved in the BMP test, and rate constant values, k) on the chemical composition of substrates was studied by correlation analysis. Statistical analyses were performed with STATISTICA version 8.0.360.0 (Statsoft, Inc.) using the Shapiro–Wilk's test for normality. Correlation analysis was done by calculating Pearson's correlation coefficients (r) and their significance levels *p*. *p*-values below 0.05 were regarded as significant.

RESULTS AND DISCUSSION

Chemical composition of substrates

Results on the chemical composition of substrates analyzed are presented in Tables 1, 2 and 3. Due to a wide variety of substrates from different sources, a specific set of analyses were considered for each group independently. Lack of information for some parameters was due to errors in the chemical characterization.

Overall, results obtained in this study are consistent with the findings of other authors. Chemical composition of silages and hay (Tables 1 and 2) is very similar to that reported by Amon et al. (2007) and Dinuccio et al. (2010). Concentrations of macro and micro-nutrients found in this study (Table 1) are similar to the findings of Baležentienė & Mikulionienė (2006) for timothy silages (P: 2.8 g kg^{-1} TS; Ca: 2.1 g kg^{-1} TS; Mg: 0.4 g kg^{-1} TS; K: 27.1 g kg^{-1} TS). Organic content and fiber concentrations found in animal slurries (Table 2) appear to be consistent with the findings of Thygesen et al. (2014). Chemical composition of energy crops (Table 2) is within the same range of that found by other authors (Mursec et al., 2009; Klimiuk et al., 2010; Pakarinen et al., 2011; Uusitalo et al., 2014). Chemical composition of unconsumed dairy products and selected agro-industrial residues (Table 3) was similar to the results from Dubrovskis et al. (2009) and Dinuccio et al. (2010).

Table 1. Chemical composition of different silages from Estonia

	n	TS (%)	SD	VS (%)	SD	Total Proteins ($g\ kg^{-1}$ TS)	SD	Hemicellulose ($g\ kg^{-1}$ TS)	SD	Cellulose ($g\ kg^{-1}$ TS)	SD	Lignin ($g\ kg^{-1}$ TS)	SD	P ($g\ kg^{-1}$ TS)	SD	Ca ($g\ kg^{-1}$ TS)	SD	Mg ($g\ kg^{-1}$ TS)	SD	K ($g\ kg^{-1}$ TS)	SD
Grass silage	4	31.4	6.7	92.7	0.5	114	9.2	219	15.9	ND	-	ND	-	2.55	0.3	6.11	0.8	1.44	0.15	24.5	1.8
Maiz silage	3	17.4	0.6	95.2	0.5	98.5	8.5	266	37.7	ND	-	ND	-	1.97	0.4	4.82	0.3	1.59	0.19	14.8	0.9
Silage mix*	19	29.4	8.6	92.0	1.8	147	25	178	42	ND	-	ND	-	2.81	0.5	9.22	2.3	1.95	0.3	23.5	4.9
Hay	4	91.3	0.4	93.7	1.5	99.2	16	272	53.5	354.6	39	58	21	ND	-	ND	-	ND	-	ND	-

n: number of samples tested for same substrate (each sample was analyzed in triplicate); * Mixture of different ratios of grasses and legumes silages; Mix rate not specified; ND: Not Determined.

Table 2. Chemical composition of animal slurries, some energy crops and hay from Estonia

Substrate	n	TS (%)	SD	VS (%)	SD	Hemicellulose ($g\ kg^{-1}$ TS)	SD	Cellulose ($g\ kg^{-1}$ TS)	SD	Lignin ($g\ kg^{-1}$ TS)	SD
Animal slurries											
Pig slurry	1	7.0	2.7	79.4	2.8	145	18	104	12.5	72	5.8
Cattle slurry*	9	7.8	2.8	78.2	3	107	13	167	7	112	10
Energy crops											
Jerusalem Artichock	2	21.4	1.3	95	4	49.8	7	234.6	36	53.8	5
Sunflower	2	25.8	1.5	89	2.4	62.4	15	307	47.1	80	3.9
Energy grass	1	27.2	1.6	93	3.1	273.3	20	378.5	32.1	96.5	4.9
Hemp	2	30.2	0.9	94	6	107	1.6	544	8	79.5	11.4
Amur Silvergrass	1	36.4	2.1	95	4.8	301	15	420	21.6	70	12.8
Foxtail millet	1	22	1.3	92	6.2	316	32	330	15.4	53.4	8.7

n: number of samples tested for same substrate (each sample was analyzed in triplicate); * TN = 4.32 (0.34) $g\ kg^{-1}$ TS.

Biochemical Methane Potential (BMP) and kinetic rate constants

BMP and kinetics rates constants obtained in this study were compared with similar studies conducted in countries such as Finland, Denmark, Sweden, Hungary, Austria, among others.

BMP results are grouped according to their origin and presented in Table 4. Cumulative methane yields for grass silage, maize silage and mix silage were 319 L CH_4 kg^{-1} VS, 307 L CH_4 kg^{-1} VS and 296 L CH_4 kg^{-1} VS, respectively. In a study conducted by Lehtomäki et al. (2008), they obtained a methane yield of grass silage of 300-372 L CH_4 kg^{-1} VS. Those results are consistent with our findings.

For maize silage, Pobeheim et al. (2010) found methane potentials ranging from 295 to 370 L CH_4 kg^{-1} VS. Our results from methane yield of hay (286 L CH_4 kg^{-1} VS, Table 4) are similar to the result from Kaparaju et al. (2002) who found a value of 270 L CH_4 kg^{-1} VS. Cattle and pig slurry presented a methane potential of 238 ± 42 L CH_4 kg^{-1} VS and 317 L CH_4 kg^{-1} VS, respectively. Vedrenne et al. (2008) found methane potential for pig slurry of 175–350 L CH_4 kg^{-1} VS. For cattle slurry, a methane potential of 243 L CH_4 kg^{-1} VS was found in the study conducted by Steffen et al. (1998). Results on the methane potential of studied energy crops are presented in Table 4. Heiermann et al. (2009) found an average methane potential of 280 ± 30 L CH_4 kg^{-1} VS and 297 ± 108 L CH_4 kg^{-1} VS for hemp and Jerusalem artichoke, which are in agreement with the results of this study (289 L CH_4 kg^{-1} VS and 310 L CH_4 kg^{-1} VS, respectively). For sunflower, Antonopoulou et al. (2010) found a methane potential of 260 L kg^{-1} VS, slightly lower than the value measured in this study (296 L CH_4 kg^{-1} VS).

Pokój et al. (2010) studied amur silver grass and obtained a methane potential of 210 L kg^{-1} VS which is much lower than the result from this study (317 L CH_4 kg^{-1} VS). Similarly, the methane yield of millet (323 L CH_4 kg^{-1} VS) was lower than those observed by Mahamat et al. (1989) (257 L CH_4 kg^{-1} VS). This variation on the methane potential of sunflower, amur silver grass and millet could be explained by differences in harvesting time or chemical composition. For energy grass (Szarvasi-1), Janowszky & Janowszky (2002) have reported methane potential of 300–350 L CH_4 kg^{-1} VS, slightly higher than the value of this study (290 L CH_4 kg^{-1} VS).

To our knowledge, only few studies on dairy derived products have been conducted on the methane potential of unconsumed milk products (whey, expired milk, poor quality industrial leftovers). The authors have found a study conducted by Alkanok et al. (2013), in which results from dairy product wastes obtained was 350 L CH_4 kg^{-1} VS. Dinuccio et al. (2010) found a methanogenic potential of 501 L CH_4 kg^{-1} VS for whey. This result appears to be within the same range of our findings (480–660 L CH_4 kg^{-1} VS).

For grain mill residues, the methane yield observed in this study (328 L CH_4 kg^{-1} VS) was much higher than the results reported by Dubrovskis et al. (2009) who obtained a methane yield of 130 L CH_4 kg^{-1} VS from grain mill wastes. This variation can be explained by the difference in the chemical composition of the substrate. Methane potential of distillery slops (358 L CH_4 kg^{-1} VS, Table 4) was in the same range as the results obtained by Steffen et al. (1998) for fermentation slops (338 L CH_4 kg^{-1} VS).

To characterize the conversion rate of selected substrates during anaerobic digestion, kinetic rate constants *k* were calculated and the values obtained are shown in Table 4. Kinetic rate constants are key elements to quantify the speed of substrate biodegradation.

Table 3. Chemical composition of unconsumed milk products and selected agro-industrial residues

Substrate	n	TS (%)	SD	VS (%)	SD	TOC (g kg^{-1} TS)	SD	Hemicellulose (g kg^{-1} TS)	SD	Cellulose (g kg^{-1} TS)	SD	Lignin (g kg^{-1} TS)	SD	Total Proteins (g kg^{-1} TS)	SD	Fats (g kg^{-1} TS)	SD
Unconsumed Cheese*	3	36.4	17.1	98	1.6	ND	-	ND	-	ND	-	ND	-	334	200	495	234
Unconsumed Milk	4	11.7	9	99	0.2	ND	-	ND	-	ND	-	ND	-	295	53	277	63
Grain mill residues	3	86	6	92	2.2	415	41	313.1	96	140	64	50.7	10	ND	-	ND	-
Distillery slops	2	75	2.8	92	1.4	455	50	ND	-	ND	-	ND	-	ND	-	ND	-

n: number of samples tested for same substrate (each sample was analyzed in triplicate); ND: Not Determined; *: includes sour cream.

Table 4. Kinetic rate constants are key elements to quantify the speed of substrate biodegradation

Substrate	n	CH_4 kg^{-1} TS	SD	CH_4 L kg^{-1} VS	SD	k d^{-1}	SD
Grass silage	4	296	19	319	19	0.172	0.02
Maiz silage	3	292	21	307	21	0.150	0.02
Silage mix*	19	272	31	296	31	0.230	0.05
Hay	4	268	33	286	33	0.086	0.01
Pig slurry	1	252	21	317	21	0.139	0.13
Cattle slurry	9	186	42	238	41	0.092	0.04
Jerusalem Artichocke	2	294	4	310	7	0.179	0.02
Sunflower	2	262	8	296	15	0.154	0.04
Energy grass	1	270	17	290	19	0.061	0.06
Hemp	2	272	9	289	11	0.095	0.01
Amur Silvergrass	1	300	32	317	38	0.064	0.06
Foxtail millet	1	296	27	323	29	0.101	0.10
Unconsumed Cheese**	3	644	60	658	56	0.260	0.07
Unconsumed Milk	4	478	24	481	24	0.344	0.03
Grain mill residues	3	300	38	328	49	0.160	0.03
Distillery slops	2	331	35	358	33	0.131	0.03

n: number of samples tested for same substrate (each sample was analyzed in triplicate); * Mixture of different ratios of grasses and legumes silages. Mix rate not specified; ** Includes sour cream.

The fastest kinetic rate constant was found for unconsumed milk products (0.344 ± 0.03 d^{-1}) while the slowest was found for energy grass (0.061 d^{-1}). As for agricultural biomass, k for grass silage, maize silage, silage mix and hay varied between 0.086 and 0.230 d^{-1}. Chynoweth et al. (1993) found conversion rate constants for different ensiled substrates (millet, energycane, napiergrass) ranging from 0.072 to 0.106 d^{-1}. In the case of animal slurries, k values for pig slurry were higher than for cattle slurry. Conversion rate constant for cattle manure (0.092 d^{-1}, Table 4) is similar to the result from Sánchez et al. (2000) who found a value of 0.086 ± 0.004 d^{-1}.

As for energy crops, the highest k value was found for Jerusalem artichoke (0.179 ± 0.02 d^{-1}). This variation between the kinetic rates obtained could be explained by the concentration of the lignocellulosic fraction of the substrates. For agro-industrial substrates, the lowest rate was found for distillery slops (0.131 ± 0.03 d^{-1}). In a study conducted by Jiménez et al. (2004) on the anaerobic digestion of untreated molasses, a conversion rate constant of 0.14 d^{-1} (9g COD added) was found. Conversion rates of unconsumed dairy products (0.260–0.344 d^{-1}, Table 4) were slightly lower than results obtained by Najafpour et al. (2009) for cheese whey (0.358 d^{-1}). Different chemical composition of the substrates could explain the difference in the rates. All results are explained by the influence of biomass composition with the biodegradation rate. Substrates with high concentrations of compounds such as lignin will result in process slow-down. In our results, chemical composition variance between cattle and pig slurry can explain the difference on the kinetic rates.

Correlations between chemical composition and BMP

Correlations between the cumulative methane production (in L CH_4 kg^{-1} TS) and the methane production rate constant with the chemical characteristics of substrates are presented in Table 5 and Figs 1 to 4.

Table 5. Pearson's correlation of cumulative methane yields and kinetic rate constants with the chemical composition of agro-industrial substrates

Variable	n	Cumulative methane yield		Kinetic rate constant	
		r	*p*	r	*p*
TS	60	-0.168	0.221	-0.109	0.413
VS	60	0.785	< 0.001*	0.033	0.8
TOC	7	0.36	0.427	0.425	0.401
Total Proteins	37	0.767	< 0.001*	0.249	0.136
Fats	7	0.365	0.421	-0.139	0.765
Hemicellulose	45	0.343	0.029*	-0.514	< 0.001*
Cellulose	20	-0.1	0.722	-0.505	0.023*
Lignin	18	-0.917	< 0.001*	-0.789	0.008*
P	26	-0.473	0.016*	0.741	< 0.001*
Ca	26	-0.563	0.002*	0.702	< 0.001*
Mg	26	0.059	0.771	0.513	0.007*
K	26	-0.613	<0.001*	0.764	<0.001*

* Statistically significant correlations ($p < 0.05$).

Among the different chemical parameters, only VS, total proteins, hemicellulose, lignin, P, Ca and K showed significant influence on the methane yield as single independent variables (Table 5). As expected, one of the main parameters influencing methane yield was organic matter, i.e. VS content, whose correlation with methane production was significantly positive. Proteins, at optimal concentrations, are also known to stimulate methane formation positively and therefore high methane yield can be attained from substrates rich in proteins (Nielfa et al., 2015).

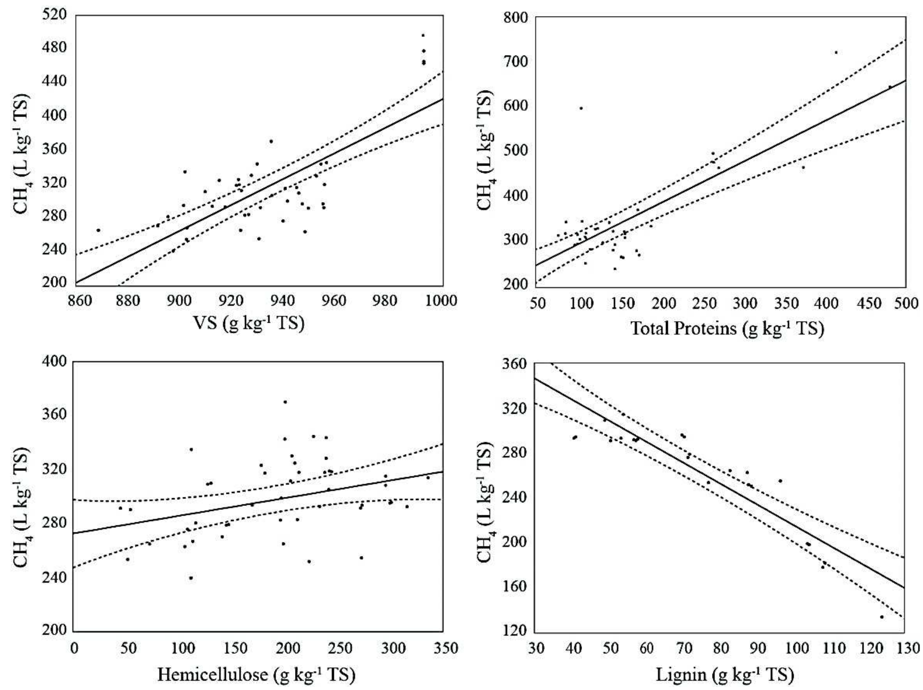

Figure 1. Pearson's correlation between methane yield and chemical parameters ($p < 0.05$). 95% confidence intervals are presented in dash lines. Y-axis values may vary compared with others.

In the case of biomass fiber composition, hemicellulose correlated positively with methane production ($p < 0.05$), although the correlation was poor. For cellulose, no significant correlation was found. Previous studies confirm that cellulose and hemicellulose can be bioconverted into methane and carbon dioxide during anaerobic digestion. However, degradation rate of cellulose depends mainly on whether it is lignin-incrusted or in a crystalline form (Klimiuk et al., 2010). Lignin content presented a strong negative correlation with methane production. Our results appear to be consistent with the findings of other authors (Klimiuk et al., 2010; Triolo et al., 2011; Pecorini et al., 2016).

Macronutrients (P, Ca, and K) were only measured for silages and their Pearson's correlations with methane yield were found negative and statistically significant. P and Ca are known for being essential for metabolic reactions and growth of anaerobic bacteria, but they can become toxic when present in high concentrations (Van Langerak et al., 1998; Chen et al., 2008). In our study, concentrations of these elements in the biomass were not excessively high to provoke a negative effect on methane production. So, it can be assumed that variations on the chemical composition of crops samples such as grasses, silages and hay and its different ratios affected the methane yield and therefore attributed for the negative correlation obtained.

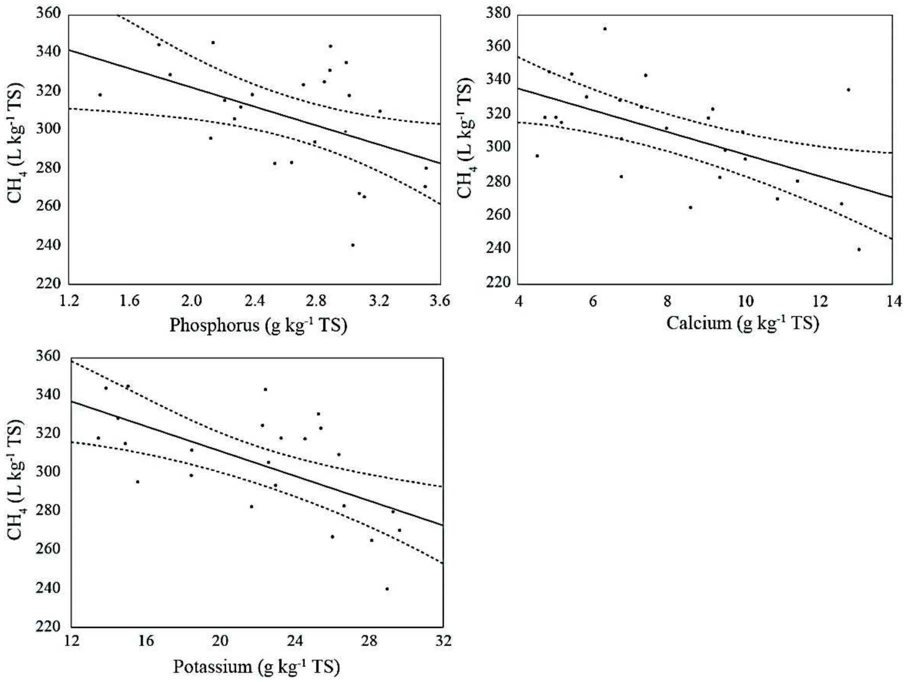

Figure 2. Pearson's correlation between methane yield and chemical parameters ($p < 0.05$). 95% confidence intervals are presented in dash lines. Y-axis values may vary compared with others.

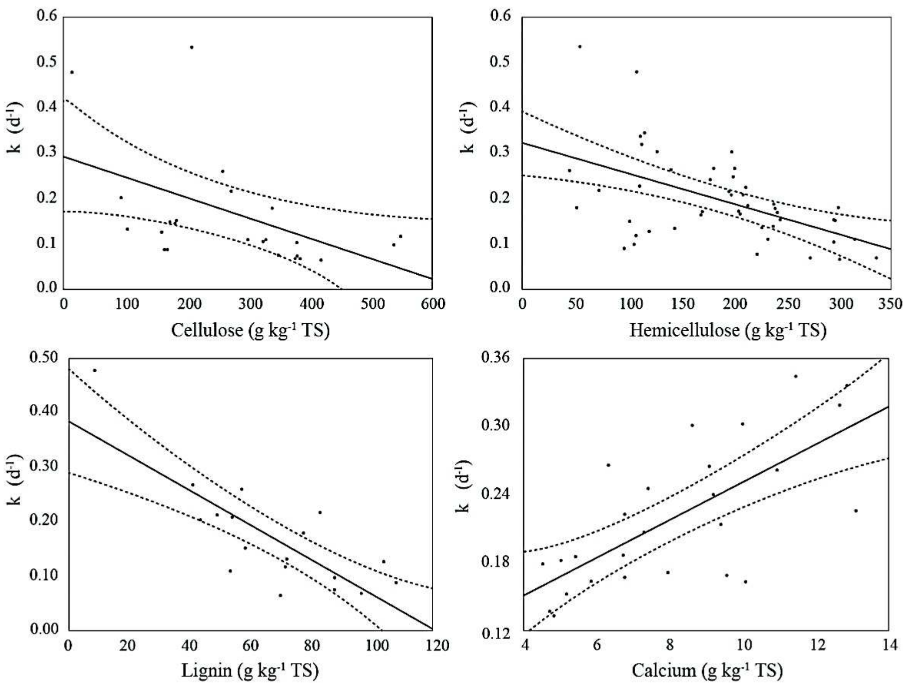

Figure 3. Pearson's correlation between methane production rate constant and chemical parameters ($p < 0.05$). 95% confidence intervals are presented in dash lines. Y-axis values may vary compared with others.

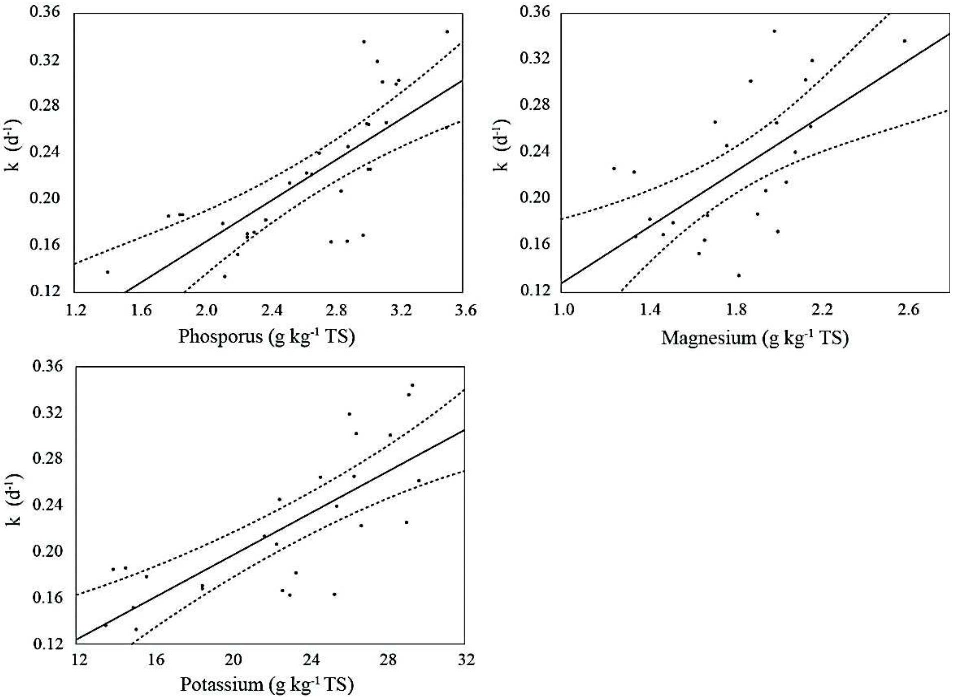

Figure 4. Pearson's correlation between methane production rate constant and chemical parameters ($p < 0.05$). 95% confidence intervals are presented in dash lines. Y-axis values may vary compared with others.

Accumulation of mineral elements in plants depends on soil properties, cultivation and fertilization, climate, harvesting time as well as plant properties (Juknevičius & Sabienė, 2007). Various plant species have a different ability to accumulate mineral elements, therefore content of Ca, P and K can differ significantly in different crops, especially between legume and grass species (Baležentienė & Mikulionienė, 2006). Concerning the methane production rate constant (k), positive correlations ($p < 0.05$) were only found for P, Ca, Mg and K (Table 5, Figs 3 and 4). These results suggest that P and light metal ions enhance the speed of the anaerobic biodegradation process. The most rapid bioconversion of studied substrates occurred in the tests with unconsumed milk products which contained high amount of proteins. In contrast, Figs 3 and 4 showed that high concentration of lignocellulosic material (hemicellulose, cellulose and lignin) in the substrate, resulted in low rate of methane production.

CONCLUSIONS

This study confirmed that studied Estonian substrates are suitable for bioenergy production by means of anaerobic digestion. Methane potential from unconsumed milk products should be considered for its integration into the Estonian energy market for bioenergy production. However, special attention on inhibitors control shall be considered from this kind of biomass to avoid process failure. Herbal biomass such as energy crops, silages, and hay presented also relatively high biochemical methane potential. Due to their high availability in Estonia, these substrates could be considered as potential source for biogas production in rural areas, and be considered as suitable co-substrates to animal slurries to increase biogas yield. As biogas is produced mainly from landfills and sewage sludge, methanisation of agro-industrial wastes could represent a potential effort to reach the established goal to cover 3% of transport energy use by 2020.

Correlation of chemical composition parameters identified lignin with the highest influence on specific methane yields. Methane yield decreases when lignin content and fibre fractions increases. Although, anaerobic digestion of agro-industrial wastes is extensively used in countries such Denmark, Germany, Austria, Sweden, in Estonia the utilization of such substrates in anaerobic digestion plants have not been widely applied. The results of this study positively highlight the bioenergy potential of studied substrates for the Estonian renewable energy mix.

ACKNOWLEDGEMENTS. We would like to thank the Archimedes Foundation for the PhD grant and Eesti Energia for co-funding the research of bioconversion of Estonian wastes and crops. This research was co-funded by the Estonian Targeted Funding project SF0690063s08. We greatly thank our laboratory specialist Liina Nurk for helping us with the technical work. We would also like to thank Prof. Henri-Charles Dubourguier (RIP) for his expertise and support during the development of this research. We would also like to thank the Research Center of Universidad de Medellin for providing sources for completing this work.

REFERENCES

Alkanok, G., Demirel, B. & Onay, T.T. 2013. Determination of biogas generation potential as a renewable energy source from supermarket wastes. *Waste Management* **34**, 134–140.

Amon, T., Amon, B., Kryvoruchko, V., Werner Zollitsch, W., Mayer, K. & Gruber, L. 2007. Biogas production from maize and dairy cattle manure-Influence of biomass composition on the methane yield. Agriculture. *Ecosystems and Environment* **118**, 173–182.

Antonopoulou, G., Stamatelatou, K. & Lyberatos, G. 2010. Exploitation of rapeseed and sunflower residues for methane generation through anaerobic digestion: the effect of pretreatment. *Chemical Engineering Transactions* **20**, 253–258.

Astover, A., Roostalu, H., Kukk, L., Muiste, P., Padari, A., Suuster, E. & Ostroukhova, A. 2008. Potentsiaalne maaressurss bioenergia tootmiseks (Potential land resource for bioenergy production). *Eesti Põllumees* **38**, 17.

Baležentienė, L. & Mikulionienė, S. 2006. Chemical composition of Galega mixtures silages. *Agronomy Research* **4**, 483–492.

Chen, Y., Cheng, J.J. & Creamer, K.S. 2008. Inhibition of anaerobic digestion process: A review. *Bioresource Technology* **99**, 4044–4064.

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.

Connerty, H.V. & Briggs, A.R. 1966. Determination of serum calcium by means of ortho-cresolphthalein complexone. *American Journal of Clinical Pathology* **45**, 290–296.

Dinuccio, E., Balsari, P., Gioelli, F. & Menardo, S. 2010. Evaluation of the biogas productivity potential of some Italian agro-industrial biomasses. *Bioresource Technology* **101**, 3780–3783.

Dubrovskis, V., Plume, I., Kotelenecs, V. & Straume, I. 2009. Investigation of biogas production from relatively dry biomass. *In: Proceedings 8th International Scientific Conference on Engineering for Rural Development*, Jelgava, LV, pp. 239–242.

Frank, S., Bottcher, H., Gusti, M., Havlik, P., Klaassen, G., Kindermann, G. & Obersteiner, M. 2016. Dynamics of the land use, land use change, and forestry sink in the European Union: the impacts of energy and climate targets for 2030. *Climate change* **138**, 253–266.

Gissen, C., Prade, T., Kreuger, E., Nges, I.A., Rosenqvist, H., Svensson, S.E., Lantz, M., Mattsson, J.E., Borjesson, P. & Bjornsson, L. 2014. Comparing energy crops for biogas production Yields, energy input and costs in cultivation using digestate and mineral fertilization. *Biomass & Bioenergy* **64**, 199–210.

Gunaseelan, V.N. 2009. Biomass estimates, characteristics, biochemical methane potential, kinetics and energy flow from Jatropha curcas on dry lands. *Biomass & Bioenergy* **33**, 589–596.

Heaton, F.W. 1960. Determination of magnesium by the titan yellow and ammonium phosphate methods. *Journal of Clinical Pathology* **13**, 358–360.

Heiermann, M., Plöchl, M., Linke, B., Schelle, H. & Herrmann, C. 2009. Biogas crops – Part I: Specifications and suitability of field crops for anaerobic digestion. *Agricultural Engineering International: the CIGR Ejournal*. Manuscript **187** (XI).

Herrmann, C., Idler, C. & Heiermann, M. 2016. Biogas crops grown in energy crop rotations: Linking chemical composition and methane production characteristics. *Bioresource Technology* **206**, 23–35.

ISO/DIS 15681-1. 2001. Determination of orthophosphate and total phosphorus contents by flow analysis (FIA and CFA) – Part 1: Method by Flow Injection Analysis (FIA).

Janowszky, J. & Janowszky, Z. 2002. 'Szarvasi-1' energygrass. *Agricultural Research Development Institute P.U.C.*, *http://www.energiafu.hu/nemesit_en.html, 2017*.

Jiménez, A.M., Borja, R. & Martin, A. 2004. A comparative kinetic evaluation of the anaerobic digestion of untreated molasses and molasses previously fermented with Penicillium decumbens in batch reactors. *Biochemical Engineering Journal* **18**, 121–132.

Juknevičius, S. & Sabienė, N. 2007. The content of mineral elements in some grasses and legumes. *Ecologija* **52**, 44–52.

Kaparaju, P., Luostarinen, S., Kalmari, E., Kalmari, J. & Rintala, J. 2002. Co-digestion of energy crops and industrial confectionery wastes with cow manure: Batch-scale and farm-scale evaluation. *Water Science & Technology* **45**, 275–280.

Klimiuk, E., Pojój, T., Budzński, W. & Dubis, B. 2010. Theoritical and observed biogas production from plant biomass of different fibre contents. *Bioresource Technology* **101**, 9527–9535.

Lehtomäki, A., Viinikainen, T.A. & Rintala, J.A. 2008. Screening boreal energy crops and crop residues for methane biofuel production. *Biomass & Bioenergy* **32**, 541–550.

Luna-delRisco, M., Normak, A. & Orupõld, K. 2011. Biochemical methane potential of different organic wastes and energy crops from Estonia. *Agronomy Research* **9**, 331–342.

Mahamat, A.Y., Gourdon, R., Leger, P. & Vermande, P. 1989. Methane recovery by anaerobic digestion of cellulosic materials available in Sahel. *Biological Wastes* **30**, 181–197.

Merrill, A.L. & Watt, B.K. 1955. Energy value of foods, basis and derivation. Agriculture Handbook No. 74. *Unites States Department of Agriculture*, Washington D.C, 105 pp

Merrill, A.L. & Watt, B.K. 1973. Energy value of foods: basis and derivation. Agriculture Handbook No. 74. *United States Department of Agriculture*, Washington D.C, 105 pp

Munoz, R., Meier, L., Diaz, I. & Jeison, D. 2015. A review on the state-of-the-art of physical/chemical and biological technologies for biogas upgrading. *Reviews in environmental science and bio-technology* **14**, 727–759.

Mursec, B., Vindis, P., Janzekovic, M., Brus, M. & Cus, F. 2009. Analysis of different substrates for processing into biogas. *Journal of Achievement in Materials and Manufacturing Engineering* **37**, 652–659.

Najafpour, G.D., Tajallipour, M., Komeili, M. & Mohammadi, M. 2009. Kinetic model for an up-flow anaerobic packed bed bioreactor: Dairy wastewater treatment. *African Journal of Biotechnology* **8**, 3590–3596.

Nielfa, A., Cano, R., Perez, A. & Fdez-Polanco, M. 2015. Co-digestion of municipal sewage sludge and solid waste: Modelling of carbohydrate, lipid and protein content influence. *Waste Management and Research* **33**, 241–249.

Owen, W.F., Stuckey, D.C., Healy, J.B., Young, L.Y. & McCarty, P.L. 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Research* **13**, 485–492.

Pakarinen, A., Maijala, P., Stoddard, F.L., Santanen, A., Tuomainen, P., Kymäläinen, M. & Viikari, L. 2011. Evaluation of annual bioenergy crops in the boreal zone for biogas and ethanol production. *Biomass & Bioenergy* **35**, 3071–3078.

Pecorini, I., Baldi, F., Carnevale, E.A. & Corti, A. 2016. Biochemical methane potential tests of different autoclaved and microwaved lignocellulosic organic fractions of municipal solid waste. *Waste Management* **56**, 143–150.

Pobeheim, H., Munk, B., Johansson, J. & Guebitz, G.M. 2010. Influence of trace elements on methane formation from a synthetic model substrate for maize silage. Bioresource Technology **101**, 836–839.

Pokój, T., Klimiuk, E., Gusiatin, Z.M. & Bulkowska, K. 2010. Methane yield of biomass plants estimated on their chemical composition and continuous digestion studies. Proceedings Venice 2010. In: *Third International Symposium on Energy from Biomass and Waste*, Venice, IT, A15 pp.

Roostalu, H. & Melts, I., 2008. Põllumajanduses tekkiva biomassi ressursi hindamine (Assessment of the resource of agricultural biomass). *Eesti Põllumees* **38**, 18.

Sanchez, E., Borja, R., Weiland, P., Travieso, L. & Martin, A. 2000. Effect of temperature and pH on the kinetics of methane production, organic nitrogen and phosphorus removal in the batch anaerobic digestion process of cattle manure. *Bioprocess Engineering* **22**, 247–252.

Steffen, R., Szolar, O. & Braun, R. 1998. Feedstocks for Anaerobic Digestion. Institute of Agrobiotechnology Tulin. University of Agricultural Sciences, Vienna, AT, 29 pp.

Thygesen, O., Sommer, S.G., Shin, S.G. & Triolo, J.M. 2014. Residual biochemical methane potential (BMP) of concentrated digestate from full-scale biogas plants. *Fuel* **132**, 44–46.

Triolo, J.M., Sommer, S.G., Moller, H.B., Weisbjerg, M.R. & Jiang, X.Y. 2011. Influence of lignin on biochemical methane potential of biomass for biogas production. *Current Opinion in Biotechnology* **22**, S146–S146.

Uusitalo, V., Havukainen, J., Manninen, K., Hohn, J., Lehtonen, E., Rasi, S., Soukka, R. & Horttanainen, M. 2014. Carbon footprint of selected biomass to biogas production chains and GHG reduction potential in transportation use. *Renewable Energy* **66**, 90–98.

Van Langerak, E.P.A., Gonzales-Gil, G., Van Aelst, A., van Lier, J.B., Hamelers, H.V.M. & Lettinga, G. 1998. Effects of high calcium concentrations on the development of methanogenic sludge in upflow anaerobic sludge bed. *Water Research* **32**, 1255–1263.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597.

Vedrenne, F., Béline, F., Dabert, P. & Bernet, N. 2008. The effect of incubation conditions on the laboratory measurement of the methane producing capacity of livestock wastes. *Bioresource Technology* **99**, 146–155.

Yenigün, O. & Demirel, B. 2013. Ammonia inhibition in anaerobic digestion: A review. *Process Biochemistry* **48**, 901–911.

Yuan, HP. & Zhu, NW. 2016. Progress in inhibition mechanisms and process control of intermediates and by-products in sewage sludge anaerobic digestion. *Renewable & Sustainable Energy Reviews* **58**, 429–438.