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EFFECT OF HYDROTHERMAL DEPOLYMERIZATION AND ENZYMATIC HYDROLYSIS OF *MISCANTHUS GIGANTEUS* BIOMASS ON THE YIELD OF METHANE FERMENTATION

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Abstract

The present study determined the effect of preliminary hydrothermal depolymerization and enzymatic hydrolysis of *Miscanthus giganteus* biomass on the yield of methane fermentation in terms of the quantity and composition of biogas produced. Enzymatic hydrolysis of the substrate led to an increase in the volume of biogas produced from 0.12 dm^3/g substrate in the samples without enzymes to 0.17 dm^3/g substrate in variant I, as well as a significant increase in methane. In addition, there were noticeable decreases in dry matter content in all variants to which the enzymatic multicomplex had been added.

Introduction

Increasing the technological effectiveness of methane fermentation of organic substrates with various characteristics is one of the key tasks faced by scientists, technologists, operators and designers of biogas system. Currently, this process is proving very difficult. Thus, alternative technological solutions are still being searched for that would exert a direct impact on the end-point results, namely on the volume and qualitative composition of biogas produced as well as on the characteristics of fermented feedstock (CARRÉRE et al. 2010). By shortening the fermentation process, equipment and invest-

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ment costs can be reduced. To improve methane fermentation, work is ongoing on constructing new reactors, modifying the technological conditions of the process, and implementing new techniques for preliminary preparation, preconditioning and pre-treatment of substrates (SHEHU et al. 2012, YONGZHI et al. 2011, SHIRSATH et al. 2012). For example, enzymatic pre-treatment improve the anaerobic decomposition of biomass from energy crops. In suitable climate or weather conditions, hydrolytic bacteria decompose complex organic compounds to simpler compounds, such as amino acids, fatty acids, glycerine and sugar. This first phase of methane production affects the efficiency of the process. To improve this stage, the use of enzymes that hydrolyze cellulose, hemicellulases and cellobiase has been studied (NEVES et al. 2006, EDER, GUNTHERT 2002, KIM et al. 2003, DHAR et al. 2012). These hydrolytic enzymes are produced by a number of fungi and bacteria, which can be used for cost effective production of cellulose biofluels. Because cellulases, hemicellases and cellobiases break down lignin-cellulose biomass, they are widely used to produce biofuels, food products, chemicals and many other products (SIMONES et al. 2007). The aim of the present study was to determine the effect of preliminary hydrothermal depolymerization and enzymatic hydrolysis of *Miscanthus giganteus* biomass on the quantity and composition of biogas produced by methane fermentation.

Materials and Methods

The experiment was conducted with biomass of *Miscanthus giganteus* used as fermentation substrate. Irrespective of the stage of experiment, the substrate was disintegrated mechanically with a Robot Coupe Blixer 3, and subjected to preliminary hydrothermal depolymerization. Particle size after fragmentation, was between 3–5 mm. Fragmentation was carried out in a pressure reaction with a active volume of 2.3 dm^3. The closed, steel pressure vessel consisted of three elements: the combustion chamber, a steel cover and 4 bolts, which allowed precise joining of the components and tightening of the equipment. In brief, 300 g of *Miscanthus giganteus* biomass with hydration of 55% and an organic matter content of 33.8% of fresh weight were put in the reactor. Next, the reactor was incubated at a temperature of 200°C, at a 1,7 MPa pressure, for 120 minutes in a muffle furnace.

In the subsequent stage of the experiment, the processed biomass of *Miscanthus giganteus* was put into open reactors with an active volume of 0.5 dm^3 and equipped with a mixing system, after which an enzymatic multicomplex (Celluclast 1.5 L, Novozym 188 and Hemicellulase) was added. For maximum enzyme activity the hydrothermally-processed of *Miscanthus* *giganteus* biomass was hydrated to 98.0% and the pH was reduced to 5.23 before the enzymes were added.

The reactors for enzymatic hydrolysis were then incubated at 37°C for 24 h. The experiment was divided into three variants depending on the doses of the enzymes used (Tab. 1).

Table 1

Doses of enzymes in experimental variants

Enzyme name	Declared activity [U/g]	Declared activity [U/g d.m.]	Enzyme dose [g/g d.m]			
			variant 0	variant I	variant II	variant III
Celluclast 1.5 L	700	30	–	$9.61 \cdot 10^{-3}$	$19.23 \cdot 10^{-3}$	$38.46 \cdot 10^{-3}$
Novozym 188	250	25	–	$13.45 \cdot 10^{-3}$	$26.9 \cdot 10^{-3}$	$53.8 \cdot 10^{-3}$
Hemicellulase	1500	30	–	$4.425 \cdot 10^{-3}$	$8.85 \cdot 10^{-3}$	$17.7 \cdot 10^{-3}$
Total dose of enzymes			–	$27.5 \cdot 10^{-3}$	$55.0 \cdot 10^{-3}$	$109.9 \cdot 10^{-3}$

Incubation of a mixture of plant substrate and a specified dose of enzymes was followed by methane fermentation. To this end, the substrate and anaerobic sludge were added to reaction tanks with an active volume of 0.5 dm^3. The characteristics of anaerobic the sludge used in the experiment are presented in Table 2.

Table 2

Characteristics of the anaerobic sludge used in the experiment

Parameter	Unit	Min. value	Max. value	Mean value	Standard deviation
pH	–	7.16	7.43	7.3	0.14
Hydration	[%]	98.4	98.7	98.6	0.15
Dry matter	[mg d.m/g f.m]	130	160	150	15.28
Organic matter	[mg o.d. m./g d.m]	63.87	83.14	75.75	9.72
Mineral substances	[mg m.d.m./g d.m]	63.45	81.39	74.25	9.56
CST (capillary suction time)	[s]	579	611	595	16.0

The process of methane fermentation was conducted at a loading of 1.0 g o.d.m./$dm^3 \cdot$ d and a temperature of 35°C. At the beginning of the experimental cycle, 25% of the total feedstock of the tested biomass of *Miscanthus giganteus* was added to fermentation tanks for sludge adaptation. The other part of the substrate was added on the fifth day of incubation. The kit consisted of a reaction chamber and bags for biogas, connected with each other in a sealed system. The analysis of biogas was carried out after incubation. In order to provide anaerobic conditions, the reactor was deoxygenated by blowing through with nitrogen before starting fermentation. The reaction tanks were equipped with a system for biogas discharge and accumulation and a system for substrate addition. Complete mixing was assured by use of a laboratory shaker operating at 100 rpm. Thermal stability at 35°C was achieved by fixing the system of reactors in a thermostatic cabinet.

The time of substrate retention in the reactors was 20 days. Samples were collected every five days. Analyses were conducted to determine the quantity and composition of biogas produced (Gas Data xi – a portable analyser designed for the analysis at the main ingredients of biogas, the measurement accuracy of CH_4, CO_2: 3%, others 5%) and the extent of organic substances removed determined by measuring COD in the dissolved phase (Hach Lange GMBH LCK 514). Additionally, changes in carbohydrate content were determine with anthrone reagent, as were changes in dry residues (WES 523 gravimetric method).

Results

When the enzymatic mixtures were used, removal of organic compounds from the plant substrate (expressed as COD) was significantly more effective. After five days of incubation, the highest effectiveness 62.5%, was observed in variant III. In the first variant, removal was 58.0%, and a similar value was obtained in variant II.

During methane fermentation, the utilization of dissolve organic carbon depended on the enzyme mixture that was used. After 20 days of plant substrate retention in model fermentation tanks, ranged from 2541 mg O_2/dm^3 in variant III to 3592 mg O_2/dm^3 in the enzyme-free variant. With a greater dose of enzymes, organic compound removal (expressed as COD) was significantly larger (Fig. 1).

During 24-hour incubation of hydrated biomass with the enzymes, glucose concentration in the dissolved phase increased: 59.3% in variant I, 69.9% in variant II, and 76.9% in variant III. The initial concentration of glucose in the technological system fed with *Miscanthus* biomass after thermal depolymerization was 3.87 mg/dm^3. In variant I, the mean glucose concentration was 5.06 mg/dm^3, whereas in variants II and III, it was 5.74 mg/dm^3 and 5.50 mg/dm^3, respectively (Fig. 2). Measurements of glucose concentration confirmed that pre-treatment by enzymatic hydrolysis significantly increased hydrocarbon utilization during fermentation. In variants II and III no glucose was detected in the dissolved phase after 20 days of substrate retention. In contrast with substrate subjected only to preliminary thermal depolymeriz-

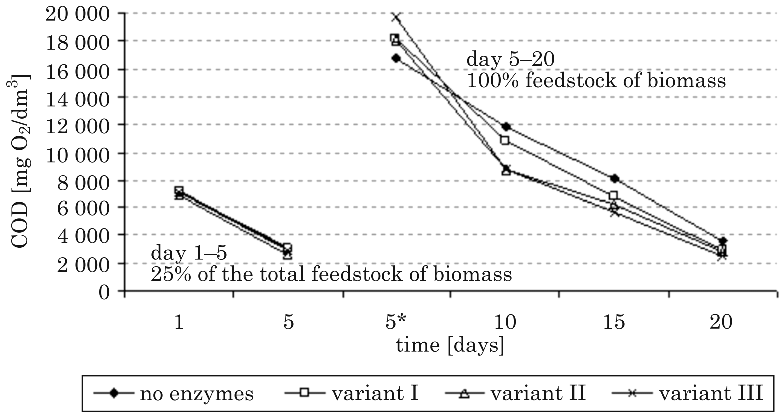

Fig. 1. Changes in COD value in the dissolved phase over the experimental period with different technological variants (5* – start of methane fermentation)

ation glucose, content after 20 days of retention was 0.17 mg glucose/dm^3. In variant I, with the lowest dose of the enzymatic multicomplex, the concentrations was 0.04 mg glucose/dm^3 (Fig. 2).

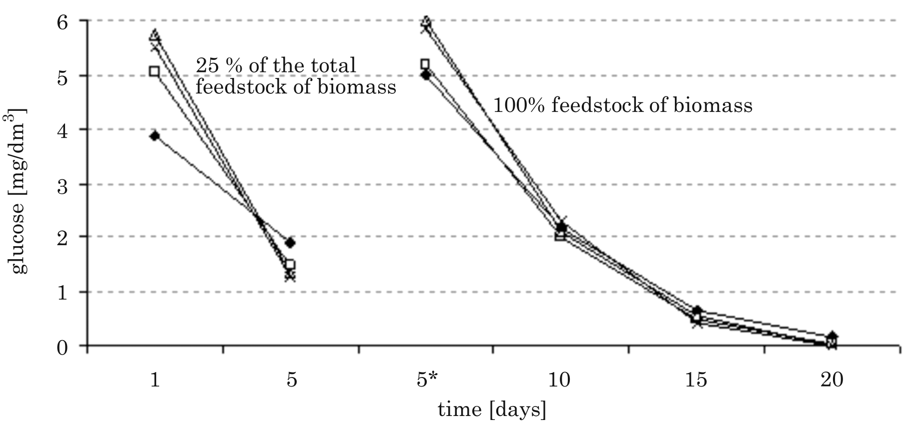

Fig. 2. Changes in the concentration of glucose in the dissolved phase over the experimental period with different technological variants applied (5* – start of methane fermentation)

The greatest decrease in the concentration of dry matter of the plant substrate during the fermentation process was observed in variant III. It was 50.3% on average and was greater by 5.0% than when only thermal depolymerization was used as pre-treatment. In variants I and II, statistically significant changes were also observed in the concentration of dry matter when compared to the samples without enzymatic pretreatment. Content of dry matter at the beginning and at the end of the experimental cycle are presented in Table 3.

Table 3

Changes in the concentration of dry matter over the experimental period

Variant	Total dry matter [mg/dm^3]		Decrease in total dry matter content [%]
	day 1	day 20	
no enzymes	18,310	10,062	45.0
I		9,141	50.1
II		9,316	49.1
III		9,104	50.3

Enzymatic pretreatment significantly increased total biogas production by about 30%; no significant differences were observed with different doses of enzymes. Table 4 presents the characteristics of the biogas produced.

Table 4

Characteristics of the quantity and composition of biogas as affected by the experimental variant

Characteristic	Variant			
	no enzymes	I	II	III
C content in gaseous phase [mol]	0.00534	0.00619	0.00613	0.00648
CO_2 content in gaseous phase [mol]	0.002075	0.00198	0.00203	0.00179
CH_4 content in gaseous phase [mol]	0.003267	0.00421	0.00410	0.00469
CO_2 content [%]	38.8	32.1	33,1	27.6
CH_4 content [%]	61.2	67.9	66,9	72.4
Gas production under process conditions [dm^3]	0.12	0.17	0.16	0.16

Discussion

Results achieved in this study confirmed the necessity of applying the pretreatment.

In the reported experiment, changes in COD concentration in the dissolved phase were monitored during fermentation of *Miscanthus giganteus* biomass. In all experimental variants COD was reduced over 85%. As shown in research described by WEILAND (2003), mesophilic fermentation of mangold roots enabled 90.0% removal of organic contaminants expressed as COD. DINUCCIO et al. (2010) investigated the yield and methane content of biogas production with various substrates including maize, grapes, straw, rice or tomato peels. In all cases, the content of methane in biogas was around 50% to 60%, which was significantly less than with Virginia fan petals.

An enzymatically-enhanced fermentation process has been described in a study by EDER and GUNTHERT (2002), who investigated extraction of the intracellular substance from microorganisms of excess sludge under technical conditions in wastewater treatment plants in Augsburg and Holzkirchen. They achieved a 25% increase in biogas production and also increased loss of organic matter in the digested sludge. A preparation called Encosol-FT (produced from cellulase) has been analyzed at laboratory and technical scale in a wastewater treatment plant in Aachen-Soers. The dry matter content of the sludge decreased by 9.0%, whilst biogas production increased by 23%. In additions KIM et al. (2003), have studied the effects of processing organic wastes originating from restaurants with various methods, namely enzymatic (for 24 hours with doses of enzymes ranging from 0.05 to 0.5%), thermal (30–120 minutes) and a combined thermal-enzymatic method. The aim was to enhance the acidogenic phase of fermentation to obtain volatile fatty acids (VFAs) that would next be used in a wastewater treatment plant as a source of organic carbon for bio-denitrification. In the case of the dissolved substances, the greatest recovery of VFA from COD (VFA/COD = 0.55) was observed on the third day of fermentation of food wastes, whose lysis was enhanced thermally (temp. 121°C, 60 min) and enzymatically (0.1% dose of a multicomplex of enzymes).

In the present study, changes in the concentration of glucose confirmed the significant effect of preliminary enzymatic hydrolysis on hydrocarbons utilization. These results resemble those of MICHALSKA at al. (2012). In their study plant material oxidized under optimal conditions was subjected to enzymatic hydrolysis using cellulase and cellobiase to determine the influence of this pretreatment step. They reported that without chemical pretreatment, no monosaccharides were present in the hydrolysates. This indicates that when cellulose is not hydrolysed by enzymes, biogas production is impossible. As reported by Neves and co-authors (NEVES 2006), such great differences between the values determined for the non-hydrolyzed samples and those subjected to pre-treatment are due to the fact that pre-treatment of lignincellulose materials leads to disruption of the cross-linking between esters of uronic acids and xylane chains. This a considerably increases process yield by facilitating interactions between the enzyme and the substrate. The results indicate that the application of enzymatic hydrolysis may substantially improve the extent of organic compounds biodegradation during anaerobic fermentation and contribute to considerably enhanced production of methane.

YANGA et al. (2009) focused on the use of a rush plant *Spartina alterniflora*, as a substrate for methane fermentation. Their study found that the methane content of biogas increased from 53% after 3 days to ca. 62% after 13 days of fermentation. The rate of the processes was impaired by hydrolysis of lignincellulose substances. The efficiency of organic compounds biodegradation obtained by these authors reached 45%, which was considerably less than that obtained with *Miscanthus giganteus*. The content of methane in biogas reported by these authors, i.e. 358 m^3/t o.d.m., was also remarkably lower than in the experimental variants in our study.

In the presents study, the content of methane increased from 61.2% in the variant without enzymatic hydrolysis to 72.4% in variant III with hydrolysis. The results are in accordance with those reported by MICHALSKA at al. (2012), who treated biomass from *Miscanthus giganteus*, *Sida hermaphrodita* and *Sorghum Moensch* with Fenton;s reagent. The highest biogas production with 75% methane content was obtained with *Sorghum Moensch*. The results of this three-step process of biomass degradation show the necessity of chemical pretreatment, such as oxidation with Fenton's reagent.

NEVES et al. (2006) examined enhanced production of methane from barley wastes from a coffee production process. They compared two methods of biogas production. The first consisted in subjecting the wastes to alkaline hydrolysis before mixing them with sludge from a wastewater treatment plant. This increased gas volume from 25 m^3 CH_4/t o.d.m. obtained after fermentation of barley wastes without pre-treatment to 225 m^3 CH_4/t o.d.m., and in a dry matter content decrease from the initial value of 31% to 67% after the above-described fermentation process. The second method involved mixing barley wastes with organic (household) wastes, which increased methane volume to 363 m^3 CH_4/t o.d.m. and a decreased dry matter content to 61%. In both cases the content of methane in biogas reached ca. 70% and the experiment lasted for 180 days. In our study, pre-treatment of the substrate resulted in a similar increase in biogas production and decrease in dry matter content.

Thermal hydrolysis substantially improves performance, with a substantial consumption of thermal energy. It is likely that low impact pretreatment methods such as mechanical and thermal phased improve the speed of degradation, while high impact methods such as thermal hydrolysis or oxidation improve both the speed and extent of degradation (CARRERA 2010). Differences in results obtained with thermal decomposition are probably due to differences in alterations of the structure of the biomass samples. The temperature and pressure of the steam explosion can influence the effectiveness of the pretreatment process (SEBESTYÉN 2013). REQUE at al. (2012) our results showed a maximum solubilisation and delignification of 53% and 86% respectively at 200°C and a biomass/solvent ratio of 1:100, i.e., 2.5 g in 250 ml of water:ethanol mixture (50:50).

The attention of supporters of methane fermentation is not only focused on the acquisition of cost-effective substrates, but also on the search for methods of intensifying the technological process (RAS 2011). Improvement of the effectiveness of biochemical degradation of organic matter affords the possibility of shortening the fermentation process, and thus of reducing equipment and investment costs (SIALVE 2009). Increasing the production of biogas and the degree of mineralization of the substrate poses a contemporary challenge to scientists and technologists. In particular, the anaerobic decomposition of substrates is limited by the rate and effectiveness of hydrolysis, the first phase of fermentation.

The tests were performed on a laboratory scale. The results do not allow to assess the energy efficiency of the presented pretreatment method. It has been shown, the use of pretreatment allow to increase energy value obtained from 1 g of the substrate from 0.67 W/g to 1.12 W/g. On a laboratory scale even such a significant increase in energy yield is not balanced inputs. Provides a basis for exploration of solutions for use in technical scale, where you can reduce energy expenditure through the use of, for example, heat exchangers to preheat the substrate.

Conclusion

Enzymatic hydrolysis of the substrate increased the volume of biogas produced from 0.12 dm^3/g substrate in the samples without enzymes to 0.17 dm^3/g substrate in variant I, as well as significantly improving its methane content (61,2% – without enzymes; 67,9% – variant I; 72,4% – variant III).

The use of the enzymatic complex resulted in significantly more effective removal of organic compounds, as expressed by COD glucose concentration in the dissolved phase of the plant substrate. There was also a noticeable decrease in the dry matter content of the fermented feedstock in all variants in which the enzymatic multicomplex was administered.

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