

Effects of Hydrothermal Depolymerization and Enzymatic Hydrolysis of Algae Biomass on Yield of Methane Fermentation Process

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Abstract

Our study was undertaken in order to determine the effects of preliminary hydrothermal depolymerization and enzymatic hydrolysis of macroalgae biomass originating from the Vistula Lagoon on yield of the methane fermentation process in terms of quantity and quality of produced biogas. The process of enzymatic hydrolysis was conducted with a mixture of enzymes: Cellulast 1.5 L, Novozym 188, and Hemicellulase. In turn, the process of hydrothermal depolymerization was run for 120 minutes at 200°C under a pressure of 1.7 MPa. The processed plant substrate was next subjected to mesophilic fermentation. The application of enzymatic hydrolysis contributed to an increased quantity of and improved qualitative composition of biogas produced.

Keywords: algae biomass, methane fermentation, enzymatic hydrolysis, hydrothermal depolymerization, biogas

Introduction

Biological conversion of biomass to biogas in recent years has become a popular research topic. Researchers have studied the production of methane in almost 100 types of fruits and vegetables, solid waste, leaves, trees, weeds, and marine and freshwater biomass [1]. Unfortunately, nowadays the use of typical, terrestrial energy crops originating from oriented cultivation poses increasing difficulties. In this aspect, an interesting alternative is the possibility of applying aquatic plants, including algae, as biomass in the biogasing process. The use of this type of substrate determines new directions of renewable energy development. Literature data confirm the feasibility of energetic utilization of algae biomass originating from both closed systems as well as natural aquifers [2]. Acquisition of

aquatic plants biomass from open water bodies enables us to accomplishing two objectives: the first linked directly with the acquisition of energetic raw material, and the second with minimization of the eutrophication process.

Algae is a large and diverse group of simple autotrophic organisms, from unicellular to multicellular forms [3]. Microalgae are the raw materials used on a large scale in the food and cosmetic industries. They also are a promising source of renewable energy [4] recently tested as a source of biogas and biooil [3]. The process of methane fermentation of microalgae has a research subject since the 1950s. The first study was published in 1957 [5]. Algae are a relatively good substrate for energy due to their high levels of lipids, starch, and protein and the absence of lignin [6]. The key to proper diagnosis of the biogas potential of algae is to know the mineral composition of algae. In addition to major components such as carbon, nitrogen, and phosphorus in the composition of algae, we encountered iron,

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Table 1. Doses of enzymes in particular experimental variants.

Enzyme	Declared activity [U/g]	Declared activity [U/g d.m.]	Enzyme dose [g/g d.m.]		
			Variant I	Variant II	variant III
Cellulast 1.5 L	700	30	6.54×10^{-3}	$13,09 \times 10^{-3}$	$26,18 \times 10^{-3}$
Novozym 188	250	25	9.52×10^{-3}	$19,05 \times 10^{-3}$	$38,1 \times 10^{-3}$
Hemicellulase	1500	30	3.27×10^{-3}	$6,55 \times 10^{-3}$	$13,1 \times 10^{-3}$
Total dose of enzymes			$19,35 \times 10^{-3}$	$38,7 \times 10^{-3}$	77.4×10^{-3}

cobalt, and zinc, which stimulate the process of fermentation. Protein depends on the type of algae from 6 to 52%, fat from 7 to 23%, and carbohydrates from 5 to 23%. The high share of the protein influences the low C/N ratio compared with terrestrial plants. This ratio for freshwater algae is 10.2 and 36.0 for terrestrial plants. The composition of algal cells is closely associated with the environment in which they occur. Qualitative changes in the composition can affect the efficiency of anaerobic digestion [7].

Anaerobic digestion of microalgae is a promising process, but there are factors that inhibit performance of the process. In the case of certain species of algae, compounds found in the cell wall are not very susceptible to the biodegradation process. Furthermore, high concentrations of ammonia formed during the decomposition of high nitrogen content of microalgae inhibit the growth of methane bacteria [4]. But algae have many advantages compared to energy crops. They are able to double its mass within 24 h and do not constitute competition with food crops, taking into account the needs of a large area of land management. Microalgae require no fresh water, can be grown in salt water and even sewage, and do not require plant protection products [6].

The attention of supporters of methane fermentation is, however, focused not only on the acquisition of substrate, the use of which will be cost-effective and thus substantiated, but also on the search for methods of intensifying the technological process [8]. Improvement of the effectiveness of biochemical degradation of organic matter affords the possibility of shortening the fermentation process, and thus of reducing the cubature of equipment and investment costs [7]. Increasing the production of biogas and degree of mineralization of the substrate poses a contemporary challenge to scientists and technologists. Anaerobic decomposition of substrates is limited, especially by the rate and effectiveness of the first, hydrolytic, phase of fermentation.

Effective conversion of plant substrate into biogas poses a challenge due to the complex structure of the cell wall of plants. Fast and effective hydrolysis of carbohydrates may be facilitated by the preliminary treatment of biomass [9]. The pre-treatment of lignocellulose raw materials may be conducted with physical methods (mechanical disintegration, pyrolysis), chemical methods (diluted acid, alkaline treatment), with physicochemical methods (vapour explosion), and finally with biological methods (with the use of fungi-producing hydrolytic enzymes) [10].

Intensification of this phase may be achieved as a result of substrate pre-treatment, also with the use of commercial enzymatic multicomplexes [11].

This study was undertaken to determine the effect of preliminary hydrothermal depolymerization and enzymatic hydrolysis of macroalgae biomass obtained in Vistula Lagoon on the yield of the methane fermentation process in terms of the quantity and quality of biogas produced.

Experimental Procedures

The materials used in the experiment were algae from the class of brown and green algae collected in the summer period from waters of the Vistula Lagoon. Out of these, the most numerous group was constituted by filiform brown algae of the genus *Pilayella* (90% of all algae) and *Ectocarpus* (8%), followed by a less numerous group of green algae from the genus *Enteromorpha*.

Irrespective of the stage of the experiment, plant substrate disintegrated mechanically with a Robot Coupe Blixer 3 disintegrating device, was subjected to preliminary hydrothermal depolymerization. It was carried out in a pressure reactor with active volume of 2.3 dm³. In brief, 300 g of algae biomass with dry matter content 33% and organic matter content of 24% of fresh weight were administered to the reactor. Next, the reactor with the plant substrate was incubated at 200°C under a pressure of 1.7 MPa for 120 minutes in a muffle furnace.

In the subsequent stage of the experiment, the processed algae biomass was applied into open reactors with active volume of 0.5 dm³ and equipped with a mixing system, and afterwards an enzymatic multicomplex of Cellulast 1.5 L, Novozym 188, and Hemicellulase was dosed in. In order to achieve the maximum activity of the enzymes applied, before being added to the hydrothermally-processed biomass of algae they were hydrated to 98.0% and the pH value was reduced to 5.23. Reactors used for enzymatic hydrolysis were then incubated at 20°C for 24 h. The experiment was divided into three variants depending on doses of the enzymes applied into the technological system (Table 1).

Incubation of a mixture of plant substrate and a specified dose of enzymes was followed by the process of methane fermentation. To this end, the substrate was applied to reaction tanks with anaerobic sludge with vol-

Table 2. Characteristics of 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
Dry matter	[%]	1.3	1.6	1.5	0.15
Organic matter	[% d. m.]	49.13	51.96	50.5	1.42
Mineral substances	[% d. m.]	48.04	50.87	49.5	1.41

ume of 0.05 dm³. The characteristics of the anaerobic sludge used in the experiment are presented in Table 2.

The process of methane fermentation was conducted by applying the following technological parameters: loading of 1.0 g o.d.m./dm³·d and temperature of 35°C. At the beginning of the experimental cycle, 25% of the total feedstock of the tested algae biomass were applied into model fermentation tanks for sludge adaptation. The other part of the substrate was applied on the fifth day of incubation.

In order to provide anaerobic conditions, the whole reactor's volume was deoxidized by blowing through with nitrogen before launching fermentation. The reaction tanks were equipped in a system for biogas discharge and accumulation and a system of substrate insertion. Complete mixing was assured by the use of a laboratory shaker operating with an intensity of 100 rpm. Thermal stability at 35°C was achieved owing to fixing the system of reactors in a thermostatic cabinet.

The time of substrate retention in the reactors reached 20 days. Samples were collected every five days. Analyses were conducted to determine the quantity and composition of biogas produced (Gas Data LMS xi) and the extent of removing organic substances described with the Chemical Oxygen Demand (COD) from the filtered sample (with the Hach Lange GMBH LCK 514 method). Assays were carried out additionally to determine changes in contents of carbohydrates (using the method with anthrone reagent) and dry residue (with the gravimetric method).

Results

The initial value of COD in the filtered sample of the applied substrate ranged from 4,420.0 mg O₂/dm³ in variant III to 4,940.0 mg O₂/dm³ in variant II. A significantly lower mean value of this index, i.e. 3,560.0 mg O₂/dm³, was determined in the filtered sample of algae biomass subjected to preliminary enzymatic digestion. In the first five days of the experiment, the effectiveness of utilization of organic compounds expressed by the COD from the filtered sample in the fermentation process oscillated around 70.0-77.5%, irrespective of the experimental variant. The lowest mean value of this parameter, 1,080.0 mg O₂/dm³, was recorded in variant II. The COD value determined in the filtered sample immediately after the total load of plant substrate (resulting from the technological design of the experiment) was introduced to the technological system ranging from

15,360.0 mg O₂/dm³ in variant I to 15,860.0 mg O₂/dm³ in variants II and III. In the variant without preliminary enzymatic hydrolysis, the value of this index accounted for 8,960.0 mg O₂/dm³ on average, which points to the immediate effect of the applied method of substrate conditioning on the content of organic compounds in the filtered sample.

The recorded values of COD in the samples collected in the subsequent days of the experiment enable us to conclude that the process of utilization of the dissolved fraction of organic carbon proceeded similarly, irrespective of the dose of enzymes multicomplex used in biomass pre-treatment. In variant I, organic substances characterized with COD were removed in 91.0%, whereas in variants II and III the effectiveness of their removal reached 92.5%. In the sample in which the algae biomass was not subjected to preliminary biochemical treatment, the recorded efficiency of organic compound utilization from the filtered sample accounted for 85.0%. Changes in COD values were presented in Fig. 1.

The 24-h incubation of the investigated biomass of algae and enzyme multicomplex resulted in a nearly twofold increase in the concentration of carbohydrates in the dissolved filtered sample, as compared to the sample subjected only to the process of thermal depolymerization. The initial concentration of glucose in the technological system without the multicomplex of enzymes reached 5.4 g/dm³. In variant I, the mean concentration of glucose accounted for 11.9 g/dm³, whereas in variants II and III they were 11.0 g/dm³ and 11.9 g/dm³, respectively. After five days of fermentation run in the model reactors, the highest effectiveness of its utilization was observed in variant II. In that case, the final concentration of glucose reached 1.0 g/dm³.

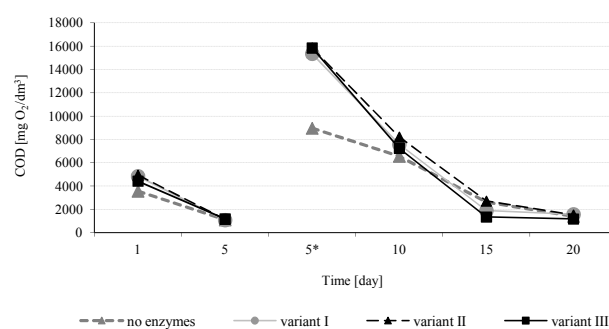


Fig. 1. Changes in COD value in the filtered sample over the experimental period as affected by the technological variant applied (5* – moment of substrate administration).

Table 3. Changes in concentrations 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	14,410	12,270	14.85
I	14,410	8,830	38.7
II	14,410	9,890	31.4
III	14,410	9,720	32.54

In the sample without preliminary enzymatic hydrolysis, the content of saccharides was at a level of 3.7 g/dm³. A significant effect of applying the preliminary enzymatic hydrolysis was confirmed by glucose concentrations assayed in the filtered sample after the introduction of another dose of the substrate to the technological system. In the variant with only thermal depolymerization used as pre-treatment, the content of glucose reached 4.4 g/dm³ on average. In the other experimental variants, its concentration in the filtered sample of the analyzed substrate was observed to increase from 9.8 g/dm³ to 10.0 g/dm³, proportionally to the applied dose of the enzyme multicomplex. The enzymatic conditioning was found to significantly enhance the process of carbohydrate utilization by microorganisms running the fermentation process, which resulted in complete depletion of these compounds after 20 days of the experiment. In the variant in which algae biomass was fermented without enzymatic pre-treatment, the concentration of carbohydrates on termination of the experiment accounted for 3.0 g/dm³. Changes in glucose concentrations are presented in Fig. 2.

The greatest decrease in dry matter content of the analyzed substrate during the fermentation process was observed in variant I. It accounted for 38.7%, on average, and was greater by 14.0% than in the variant with only hydrothermal depolymerization used as the pre-treatment process. In variants II and III, dry matter content of the biomass was observed to decrease by 32.0% on average. Contents of dry matter at the beginning and at the end of the experimental cycle are presented in Table 3.

The highest intensity of biogas production was determined for variants II and III. The volume of biogas produced in these variants reached 0.040 dm³/g of substrate and 0.054 dm³/g of substrate, respectively. The sample with the lowest dose of the enzymes enabled producing 0.022 dm³ of biogas/g substrate. The content of methane determined in all experimental variants was diversified and ranged from 63.0% in variant I to 73.2% in variants II and III. Apart from the composition and quantity of biogas produced, analyses were also conducted for the quantity of carbon produced in the gaseous phase of the process, being an indicator of digestive respiration. Exogenous respiration proceeds as a result of the addition of plant substrate, which is a source of activated sludge to microorganisms. It determines the removal of organic contaminants and nitrogen compounds. The best effects were achieved in the case of variants with the two highest doses of enzymes applied. The quantity of carbon in these variants was twofold higher compared to the variant with the lowest dose of the enzymes and that without added enzymes. Results obtained in this respect are presented in Table 4.

Discussion

Reference data indicate that considerable quantities of biogas may be produced from biomass of root crops, cereals, and fodder crops. Biogas production achieved under

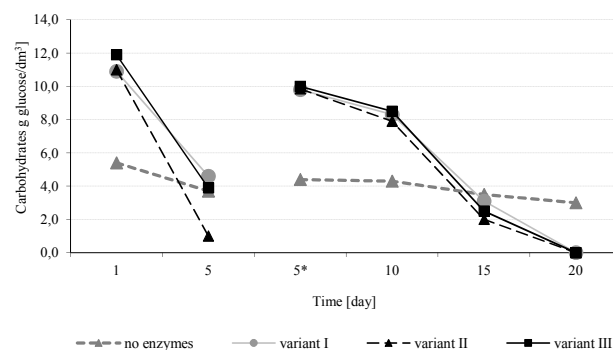


Fig. 2. Changes in concentrations of glucose in the filtered sample over the experimental period as affected by the technological variant applied (5* – moment of substrate administration).

Table 4. Characteristics of the quantity and composition of biogas as affected by the experimental variant.

Parameter	Variant			
	No enzymes	I	II	III
C content in gaseous phase [mol]	0.004829	0.003743	0.006497	0.007023
CO ₂ content in gaseous phase [mol]	0.00136	0.001378	0.001605	0.00188
CH ₄ content in gaseous phase [mol]	0.00347	0.002634	0.004805	0.005122
CO ₂ content [%]	28.2	36.8	25.0	26.8
CH ₄ content [%]	71.8	63.2	73.2	73.2
Volume of gas produced under normal conditions [dm ³ /g]	0.033	0.021	0.045	0.054

laboratory conditions and in installations operating on a technical scale have been reported to range from 200.0 m³ CH₄/t o.d.m. to 400.0 m³ CH₄/t o.d.m. [12], which is more than in the reported experiment. Thermal depolymerization of algae biomass enabled the production of ca. 100.0 m³ CH₄/t o.d.m. after 20 days of biomass retention in the technological system. Interesting results were achieved by Dinuccio et al. in the process of biogas production from maize, dried tomato peels, barley straw, and rice. The production of methane from rice accounted for 416.0 m³/t o.d.m., whereas that from straw – for 360.0 m³/t o.d.m. owing to the lower content of cellulose and hemicelluloses [13].

In studies focused on anaerobic fermentation of man-golds, mesophilic fermentation assured the removal of 90.0% of organic compounds expressed as COD [12]. In the fermentation process of algae biomass, the utilization of organic compounds from the filtered sample accounted for 85.0% in the case of substrates after thermal depolymerization and for 92.0% in the case of substrates after additional enzymatic hydrolysis.

Bauer et al. investigated the yield of methane production from various energy crops used as substrates, including: silages of maize, sunflower, alfalfa, sorghum, barley, and wheat straw. The application of thermal depolymerization of straw at a temperature of 170°C for 10 min, under a pressure of 2.0 MPa, enabled producing 361.0 m³ CH₄/t o.d.m. In an analogous process of fermentation in which the substrate was not subjected to this pre-treatment process, biogas production reached 276.0 m³ CH₄/t o.d.m. [14]. The quantity of methane produced from the fermentation of algae after thermal pre-treatment and without the addition of enzymes accounted for 100.0 m³ CH₄/t o.d.m.

According to Krzemieniowski et al., in the process of methane fermentation of algae it is feasible to produce 280.0 dm³ biogas/kg COD, and the content of methane may reach even 83.0%. In the reported experiment, methane content of the biogas produced reached 72.0% [15]. In turn, Hong-Wei Yen et al. attempted to produce biogas from algae with the addition of waste paper. They demonstrated that waste paper addition to the algae sludge affected, to a great extent, the quantity of biogas produced. This was due to the increased C/N ratio. This experiment was conducted in 5 variants, including: fermentation of algae, fermentation of waste paper, and fermentation of algae with waste paper addition of 25.0%, 50.0%, and 75.0%. The best effects were achieved in the variant of algae fermentation with 50.0% waste paper addition. It enabled producing ca. 1100.0 ml CH₄/dm³·d, which was twofold less than in the case of algae and waste paper fermented alone. According to these authors, the likely reason of these results was enhanced activity of cellulases caused by the increased C/N ratio [16]. The same phenomenon occurred in the case of enzymatic hydrolysis of algae, when the addition of enzymes degrading cellulose and hemicelluloses had an immediate effect on methane production in the fermentation processes of the applied substrates.

Cited articles deal mainly with anaerobic digestion of energy crops that are characterized by a higher content of cellulases and hemicellulases. These plants have a much higher ratio of C/N than algae. These two properties show that biogas plant lignocellulosics contribute to the formation of larger quantities of biogas.

Conclusions

The applied pre-treatment methods turned out to be highly effective, for the enzymatic hydrolysis of algae biomass triggered the release of a considerable quantity of carbohydrates to the filtered sample, which became more available and more rapidly consumed during the process of methane fermentation. Increasing the dose of enzymes had no direct effect on the removal of organic compounds. The enhanced activity of the enzymes contributed to the increased production and improved qualitative composition of biogas. The content of methane in the biogas ranged from 63.0% in variant I to 73.0% in II and III. Variants of experience in employing enzymatic hydrolysis yielded better results in comparison with variants in which the hydrolysis was not applied. Given the cost of enzymes, the best dose turned out to be a dose of 1.0, containing 1.5 Cellulast, Novozym 188, and Hemicellulase: 13.09, 19.05, and 6.55 mg/g·dm.

Analyses conducted in the experiment also showed a decrease in dry matter content of the fermented feedstock. It was especially tangible in the variants in which enzymatic multicomplex was administered to the algae biomass.

In the face of mounting problems related to energy crop plantations, large capital investment costs, and the need for a large area of land management, note the use of this type of raw material for anaerobic digestion. Especially since the results obtained are satisfactory. As shown in the discussion, greater amounts of biogas can be obtained from energy crops than from algae, but the many advantages of algae as a feedstock for anaerobic digestion should lead this process using microalgae.

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