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IMPACT OF MICROWAVE HEATING ON THE EFFICIENCY OF METHANE FERMENTATION OF ALGAE BIOMASS*

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Key words: microalgae, microwave radiation, biomass, methane fermentation, biogas.

Abstract

Providing the optimal temperature is a means of increasing the effectiveness of methane fermentation processes. The use of an electromagnetic microwave field enables energy to be directed to a mixture of anaerobic sludge and processed biomass this reduces energy losses.

The aim of this study was to determine the effect of electromagnetic microwave radiation in stimulating thermal conditions in anaerobic reactors, on the effectiveness of methane fermentation process of microalgae biomass and on the qualitative composition of biogas produced. The quantity of gaseous metabolites of anaerobic bacteria produced in both experimental variants (convective and microwave heating) averaged approximat's 450 cm^3 g^{-1} VS. The electromagnetic microwave radiation proved to have an immediate impact on the improvement in the qualitative composition of biogas produced. The stimulation of thermal conditions using electric heaters resulted in a methane contetnt of 65% in biogas, whereas the use of microwaves assured ca. 69% in sewage gas.

WPŁYW OGRZEWANIA MIKROFALOWEGO NA EFEKTYWNOŚĆ FERMENTACJI METANOWEJ BIOMASY GLONÓW

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Słowa kluczowe: mikroalgi, promieniowanie mikrofalowe, biomasa, fermentacja metanowa, biogaz.

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Abstrakt

Zapewnienie optymalnej temperatury jest sposobem na zwiększenie efektywności procesów fermentacji metanowej. Użycie promieniowania mikrofalowego umożliwia bezpośrednie skierowanie energii do mieszaniny osadu beztlenowego i przetwarzanej biomasy, co znacznie ogranicza straty energii.

Celem badań było określenie wpływu zastosowania elektromagnetycznego promieniowania mikrofalowego jako czynnika stymulującego warunki termiczne w reaktorach beztlenowych na efektywność procesu fermentacji metanowej biomasy mikroglonów i skład jakościowy wytwarzanego biogazu. Ilość produkowanych gazowych produktów metabolizmu bakterii beztlenowych w obu wariantach eksperymentalnych wynosiła średnio (konwekcyjne i mikrofalowe ogrzewanie) 450 cm^3 g s.m.o.$^{-1}$. Udowodniono, że elektromagnetyczne promieniowanie mikrofalowe wpłynęło bezpośrednio na poprawę składu jakościowego uzyskiwanego biogazu. Stymulowanie warunków termicznych za pomocą grzałek elektrycznych pozwoliło na uzyskanie prawie 65% metanu w biogazie, natomiast wykorzystanie mikrofal zapewniło około 69% zawartość tego komponentu gazu fermentacyjnego.

Introduction

The rate of biochemical degradation of organic matter depends on the activity of microorganisms or – to be more specific – on the rate of enzymatic reactions. In turn, the activity of enzymes is determined by environmental factors, including process temperature. For this reason, providing the optimal temperature is a means of increasing the effectiveness of methane fermentation processes (LANGE and AHRING 2001, TAKASHIMA et al. 2006).

The thermal conditions inside anaerobic reactors may be effectively controlled via microwave radiation. The use of an electromagnetic microwave field enables energy to be directed to a mixture of anaerobic sludge and processed biomass (the body absorbing the radiation). This reduces energy losses as a result of absorption by structured elements of the reactor (HA et al. 2011). Our earlier study demonstrated a significant increase in the effectiveness of organic matter degradation and methanogenesis in a microwave-modified system (CYDZIK-KWIATKOWSKA et al. 2012). Genetic studies carried out in systems with conventional heating and with electromagnetic microwave radiation have shown significant changes in the species structure of microorganisms that are affected by the microwave radiation (ZIELIŃSKI and ZIELIŃSKA 2010). Results achieved so far seem to confirm theories of non-thermal effects of microwaves, as changes were observed not only in the activity of particular enzymes but also in entire bacterial populations. These findings are the grounds for investigations aimed at determining the effects of thermal and athermal properties of microwaves on the effectiveness of methane fermentation of typical energetic plants used in agricultural biogasworks (ZIELIŃSKI et al. 2007, ZIELIŃSKI et al. 2009, ZIELINSKI and KRZEMIENIEWSKI 2007).

The aim of this study was to analyze the effect of an electromagnetic microwave radiation, as a factor stimulating thermal conditions in anaerobic reactors, on the effectiveness of methane fermentation process of microalgae biomass and on the qualitative composition of biogas produced.

Experimental procedures

Study design

The study was divided into two experimental stages with different methods of assuring appropriate thermal conditions in the reaction tanks. In stage I, the reactors were placed in a thermostating cabinet in which thermal conditions were provided by electric heaters. In stage II the cabinet was replaced by an electromagnetic microwave field.

Materials

The microalgae that constituted the substrate for the fermentation process originated from the waters of the Vistula Lagoon. The biomass was separated and concentrated with the use of a fractional-technical scale instalation. Before the algae biomass was fed into the anaerobic reactors, the material was concentrated in a laboratory centrifuge. The physicochemical characteristics of the algae biomass used in the study is presented in Table 1.

Table 1

Characteristics and properties of microalgae biomass used in the study

Characteristic	Unit	Mean value	Standard deviation
Dry matter	[%]	10.40	1.49
Organic dry matter	[% TS]	87.69	1.06
Dry mineral matter	[% TS]	12.31	1.06
Total nitrogen	[mg g^{-1} TS]	45.97	3.92
Total phosphorus	[mg g^{-1} TS]	4.36	0.94
TC	[mg g^{-1} TS]	463.82	25.31
TOC	[mg g^{-1} TS]	437.26	19.77
C:N ratio	–	9.51	0.43
Protein	[% TS]	28.73	2.45
Lipids	[% TS]	19.96	1.39
Saccharides	[% TS]	15.84	2.55
pH	8.82–7.03		

The microalgae biomass analyzed in the study was harvested from June till August. The species composition of obtained microalgae was determined with optical microscope equipped with camera MF346 Optech 3MP.

Qualitative analyses of samples of phytoplankton biomass collected in this period demonstrated that the *Cyanoprokaryota* was the predominant taxonomic group (82.6 ± 7.4% of all algae). Taxonomic diversity in the Vistula Lagoon was also affected by algae of the *Chlorophyta* group (11.1 ± 4.7%) and *Bacillariophyceae* (4.7 ± 2.3%). The most abundant species of blue-green algae included *Aphanizomenon flos-aqae* and *Microcystis sp.* Species of other planktonic algae (*Pyrrophyta*, *Euglenophyta*, *Chrysophyta*: *Chrysophyceae*) were in the minority, and their combined contribution did not exceed 1.6 ± 0.4% of all algae.

Anaerobic sludge used as the inoculum of anaerobic reactors in the experiments originated from the closed fermentation tanks of the Municipal Wastewater Treatment Plant "Łyna" in Olsztyn. Its characteristics are presented in Table 2.

Table 2

Characteristics of anaerobic sludge used in the study

Characteristic	Unit	Mean value	Standard deviation
Dry matter	[%]	3.81	0.21
Organic dry matter	[% TS]	68.46	2.53
Dry mineral matter	[% TS]	31.54	2.53
Total nitrogen	[mg g^{-1} TS]	33.08	3.35
Total phosphorus	[mg g^{-1} TS]	1.66	0.23
TC	[mg g^{-1} TS]	309.05	28.37
TOC	[mg g^{-1} TS]	199.42	34.29
C:N ratio	–	9.34	0.08
Protein	[% TS]	20.67	2.77
Lipids	[% TS]	3.12	0.51
Saccharides	[% TS]	1.57	0.36
pH	7.52–6.89		

Experimental equipment

Experiments were conducted using anaerobic reactors with full mixing and an active volume of 4.0 dm^3 (total volume – 5.0 dm^3). The initial concentration of anaerobic sludge in the tanks was kept at ca. 4.0 g TS dm^{-3}. Different heating systems were used depending on the stage of the study, i.e., stage I, electric heaters, and stage II, electromagnetic microwave radiation. A magnetron served as the source of microwave radiation. Radiation was transmitted from the magnetron via a wave-guide to a cabinet with fermentation tanks. The microwave generator (Plazmatronika) applied in the study has a fluent power control that ranges from 0 to 600 W. The frequency of the radiation was 2.45 GHz. The heating systems were controlled via a thermal controller that responded immediately to indications of temperature sensors located inside the reactors. Methane fermentation was conducted at 35°C, with a hysteresis of ± 1°C. The process of anaerobic degradation of organic substrates was run under conditions of mesophilic fermentation, with a load of 2.0 kg VS dm^{-3} d and a hydraulic retention time of 20 days. A scheme of the experimental set-up is presented in Figure 1.

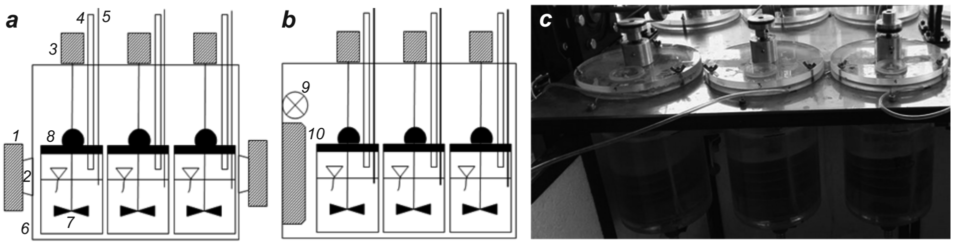

Fig. 1. Design of experimental equipment: *a* – microwave heating; *b* – convective heating (*1* – magnetron, *2* – wave-guide, *3* – stirrer drive, *4* – pressure measurement, biogas collection, *5* – temperature sensor, *6* – steel cabinet, *7* – stirrer, *8* – model fermentation tanks, *9* – ventilator, *10* – heater)

Analytical methods

The substrat composition was determined before and after the anaerobic digestion. The content of dry matter, organic dry matter and mineral dry matter was determined gravimetrically. In samples of biomass dried at 105°C, the content of total carbon (TC) was determined as well as, total organic carbon (TOC) and total nitrogen (N_{tot}). Analyses were carried out with a Flash 2000 elementary particle analyzer (Thermo company). Total phosphorus (P_{tot}) was assayed with the colorimetric method with ammonium(V) metavanadate and ammonium molybdate after sample mineralization in a mixture of sulfuric(VI) acid and chloric(VII) acid at a wavelength of 390 nm using a DR 2800 HACH Lange spectrophotometer. The content of total protein was determined by multiplying the content of N_{tot} by a protein conversion factor (x 6.25). The content of reducing sugars was measured with the colorimetric method with an anthrone reagent at a wavelength of 600 nm using the DR 2800 HACH Lange spectrophotometer. The concentration of lipids was determined with the

Soxhlet method, using Buchi extraction apparatus, whereas the pH of water was measured with a potentiometer.

Temporary and total flow of biogas from the continuous reactors was measured using a flow meter (Allborg SS-Body company). The composition and percentage contents of particular components of biogas were analyzed daily with the use of a GC Agillent 7890 A gas chromatograph.

Statistical analysis

The statistical analysis of experimental results was conducted using a STATISTICA 10.0 PL package. The hypothesis normality of distribution of each analyzed variable was verified using the W Shapiro-Wilk test. One-way analysis of variance (ANOVA) was conducted in order to determine differences between variables. Homogeneity of variance in groups was determined using a Levene test. The Tukey (HSD) test was applied to determine the significance of differences between the analyzed variables. In all tests, results were considered significant at $\alpha = 0.05$.

Results

The algae biomass used in the study was characterized by a organic matter content of 87.69 ± 1.06%, and TC and TOC of 463.82 ± 25.31 mg g^{-1} TS and 437.26 ± 19.77 mg g^{-1} TS, respectively. The mean content of N_{tot} was 45.97 ± 3.92 mg g^{-1} TS, whereas that of P_{tot} was around 4.36 ± 0.94 mg g^{-1} TS. In the analyzed biomass, the C:N ratio – being of great significance to the methane fermentation process – was very low, at 9.51 ± 0.43 on average (Table 1). The content of total protein, lipid substances and carbohydrates in the mixed culture of microalge was 28.73 ± 2.45% TS, 19.96 ± 1.39% TS and 15.84 ± 2.55% TS, respectively.

In stage I, during the period of stable operation, biogas production was 440.41 ± 8.45 cm^3 g^{-1} VS and methane content averaged 65.20 ± 2.04%, (Figure 2, Figure 3). Total production of biogas was 3523.28 ± 67.6 cm^3 d^{-1}, wherein methane was 2297.18 ± 71.87 cm^3 d^{-1}.

In stage II, during the period of stable operation biogas production was 453.11 ± 9.09 cm^3 g^{-1} VS (Figure 2). Total biogas production was higher than in stage I and amounted to 3624.88 ± 72.72 cm^3 d^{-1}. The use of microwave radiation gave a significantly higher concentration of methane, it was on average 68.78 ± 2.03% (Figure 3). Biogas production rate increased from 9.16 cm^3 g^{-1} VS d^{-1} to 21.2 cm^3 g^{-1} VS d^{-1} during the use of electromagnetic microwave radiation in stage II.

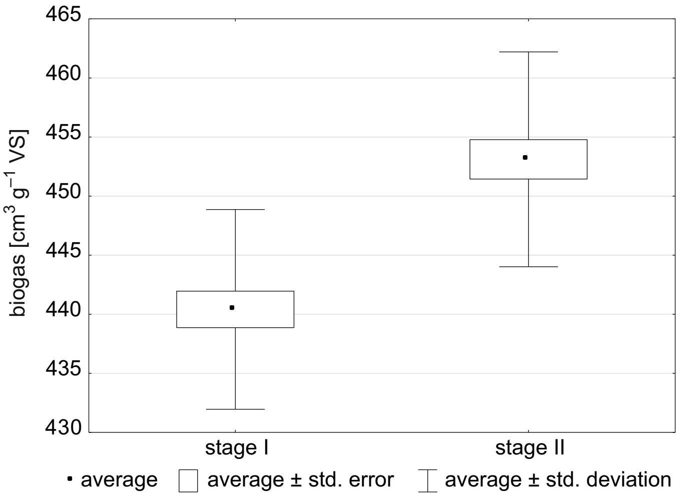

Fig. 2. The percentage content of biogas production after process of anaerobic sludge adaptation

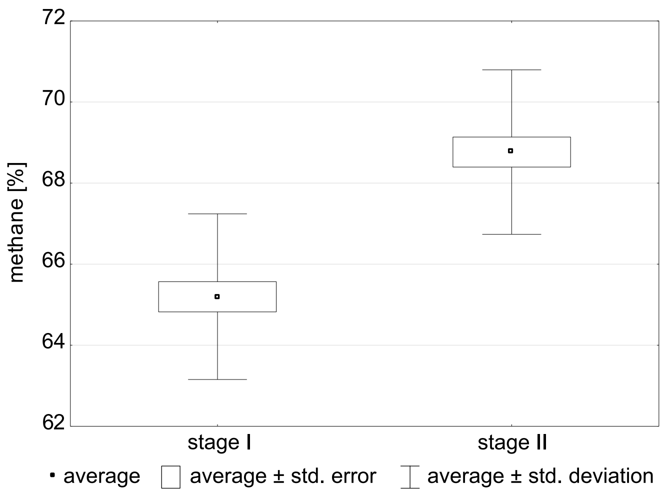

Fig. 3.The percentage content of methane production after process of anaerobic sludge adaptation

The study demonstrated that the use of electromagnetic microwave radiation (stage II of the study) had a direct impact on faster adaptation of the technological system and achieved greater stability of the methane fermentation process. The anaerobic sludge used as the inoculum originated from closed fermentation tanks of a municipal wastewater treatment plant, hence its metabolism was adjusted for sewage sludge degradation. Efficient algae biomass conversion to biogas required a period of sludge adaptation and its metabolism adjustment to a new substrate – in this case: the microalgae biomass. The heating of the technological system with microwave radiation produced stable effects of biogas production as early as 30 days after exploitation (Figure 4). During stage I with convective heating, the process of anaerobic sludge adaptation lasted twice as long and stable effects of biogas production were achieved after nearly 60 days (Figure 4). The study also demonstrated that the impact of the physical factor was reflected more in a equalized concentration of methane in biogas (Figure 5, Figure 3).

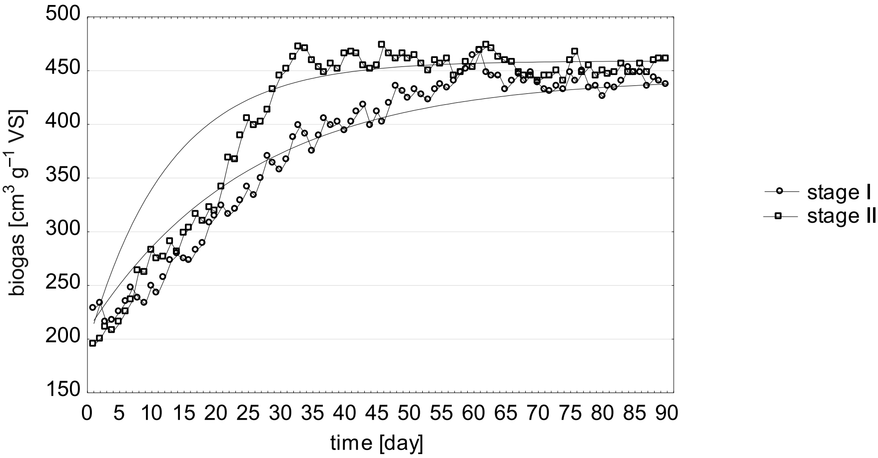

Fig. 4. The biogas production depending on the applied method of heating model fermentation tanks

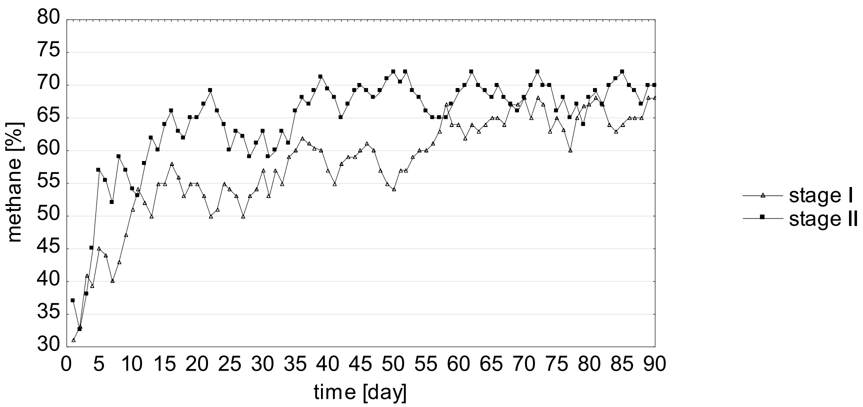

Fig. 5. The percentage content of methane in biogas depending on the applied method of heating model fermentation tanks

Discussion

The literature provides extensive descriptions of the use of microwave radiation in chemical syntheses processes (KAPPE 2002, LIDSTRÖM et al. 2001, OLSSON and JUSLIN 2000, VASS et al. 1999). Microwave heating increased production and quality of biogas from algae biomass. The impact of electromagnetic energy always involves energy transfer and usually leads to an increase of temperature. The term "athermal" refers to effects of microwaves specific to electromagnetic energy and not observed in the case of conventional heating (MUSSGNUG et al. 2010). For instance, PARKER et al. (1996) investigated the effect of microwaves on the activity of the hydrated enzyme – lipase. The rate of the enzymatic reaction heated with microwaves was seen to increase maximally several times more compared to conventional heating.

BANIK et al. (2006) analyzing biological effects of microwave radiation, point to, among other things, a positive impact of the radiation on the process of methane fermentation. The exposure of *Methanosarcina barkei* – DSM 804 bacteria to high frequency radiation between 13.5 GHz to 36.5 GHz produced an increased volume of biogas and increased methane concentration in biogas.

In the presented experiment, a significantly faster adaptation of the technological system was observed, which resulted in stable effects of the fermentation process, and an equalized concentration of methane. It confirms findings of ZIELIŃSKI et al. (2007) regarding the impact of microwave radiation on the activity of a biofilm in reactors with immobilized biomass. In those cases significant enhancement was observed in the activity of microorganisms exposed to microwaving. Significantly increased process efficiency was achieved under identical technological conditions especially in the case of nitrifying activity. At a temperature difference between the control reactor and the irradiated reactor not exceeding 3°C, the yield of the process increased by ca. 30%.

The mean level of biogas production in the present study was at 453,11 cm^3 g^{-1} VS. Comparable values were reported by De Schamphelaire and Verstraete (DE SCHAMPHELAIRE and VERSTRAETE 2009) in their research on the effectiveness of biogas production from mixed cultures of freshwater microalgae. The initial loading of periodical fermentation tanks at 0.6 g VS dm^{-3} d^{-1} enabled methane production of 310 cm^3 CH_4 g^{-1} VS after 45 days of biomass retention in the technological system. Samson and LeDuy (SAMSON and LEDUYT 1986) fermented biomass of blue-green algae (*Spirulina maxima)* in a reactor at a loading of 1.0 g VS dm^{-3} d^{-1} and retention time of 33 days. They noted that methane production in the mesophilic process was 240 cm^3 CH_4 g^{-1} VS on average, and they observed 68.0% – 72.0% effectiveness of organic biomass degradation. RAS et al. (2011) obtained a biogas production yield of 150 cm^3 CH_4 g^{-1} VS and 240 cm^3 CH_4 g^{-1} VS at 16 days and 28 days hydraulic retention time, respectively. This experiment was conducted with biomass of *Chlorella vulgaris*, and fermentation tanks loading at a level of 1.0 g COD dm^{-3} d^{-1}. The effectiveness of biomass conversion to gaseous metabolites of anaerobic bacteria ranged from 29.0% to 49.0%.

In the presented experiment, the COD:N ratio reached 9.51 ± 0.43, however the content of total protein in the case of the mixed culture of microalgae reached ca. 28%. Similar observations were made by ZAMALLOA et al. (2012). In the case of testing biomass of *Spirulina platensis*, the COD:N ratio was higher and reached 10.3 ± 0.6. It was due to a very high contribution of proteins in biomass – reaching up to 60%. For two other analyzed species of algae the ratios of carbonic substances expressed by the COD value to nitrogen were comparable, i.e. 15.6 ± 7.4 for *Scenedesmus obliquus* and 13.4 ± 3.4 for *Phaeodactylum tricornutum*.

The potential of biogas production was proved to be directly determined by species, but no correlation was found between the taxonomic groups and process effectiveness. The fermentation of *Chlamydomonas reinhardtii* algae from the phylum *Chlorophyta* resulted in biogas production at a level of 587 $\pm$ 8.8 cm^3 g^{-1} VS , whereas in the case of *Dunaliella salina* – at a level of 505 $\pm$ 24.8 cm^3 g^{-1} VS. Anaerobic processes applied to blue-green algae *Arthrospira platensis* and *Euglena gracilis* resulted in production effectiveness of gaseous metabolites of fermentation bacteria reaching 481 $\pm$ 13.8 cm^3 g^{-1} VS. and 485 $\pm$ 3.0 cm^3 g^{-1} VS, respectively. Finally, biogas production from *Chlorella kessleri* and *Scenedesmus obliquus* algae was the lowest and accounted for 335 $\pm$ 7.8 cm^3 g^{-1} VS and 287 $\pm$ 10.1 cm^3 g^{-1} VS, respectively (MUSSGNUG et al. 2010).

Conclusions

The conducted experiments demonstrated that the quantities of produced biogas per load of organic substrate fed to the technological systems were similar irrespective of the applied method of heating fermentation tanks. At both experimental stages, the quantity of gaseous metabolites of anaerobic bacteria was at an average level of ca. 450 cm^3 g^{-1} VS. It was proved that the electromagnetic microwave radiation had a direct impact on the improved qualitative composition of biogas produced. The stimulation of thermal conditions using electric heaters produced nearly 65% of methane in biogas, whereas the use of microwaves assured methane content of ca. 69%. In addition, the study demonstrated that the use of electromagnetic microwave radiation had a significant impact on faster adaptation of the technological system and on obtaining stable effects of methane fermentation.

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