ESTIMATION OF BIOCHEMICAL METHANE POTENTIAL OF HIGH PROTEIN *SPIRULINA* BIOMASS

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ABSTRACT

Biomass from microalgae is a promising feedstock for energy production through anaerobic digestion. The aim of this study was to determine the biochemical methane potential of Spirulina biomass. The investigated biomass is characterized by a high protein content and a low carbon/nitrogen ratio. In order to improve the biodegradability of the biomass, the fresh biomass was pre-treated by thermolysis. Although a yield of 0.295 L $CH_4 g^{-1}$ VSS was obtained in the initial experiments, subsequent batch processes showed a drop in yield, slowing until the complete cessation of the methanation process. The main reason for the problems is the inhibition from high concentration of ammonium ions. After regeneration of the methanogenic consortium with vinasse substrate, an increase in yield to 0.445 L $CH_4 g^{-1}$ VSS was observed. The use of pretreated biomass resulted in an unexpected yield reduction effect. Application of vinasse as a co-substrate for methanation showed an increase in yield to 0.529 L $CH_4 g^{-1}$ VSS and stabilization of the process.

Keywords: BMP, microalgae, Spirulina, pre-treatment, bioenergy, biogas.

INTRODUCTION

Global energy consumption has been increasing for more than two centuries since the beginning of the industrial revolution. Still the main part of the energy used is obtained from fossil fuels. This leads to a decrease in easily accessible fossil fuel reserves and an increase in energy prices. In addition, the burning of oil, gas and coal leads to increased emissions of carbon dioxide and environmental problems such as air pollution and the greenhouse effect. The only solution to these problems is the use of renewable energy sources. It is currently considered that the use of biomass to produce renewable energy will solve environmental problems and will allow the global energy goals to be met [1].

Various fuels such as bioethanol, biodiesel and biogas can be produced from biomass. First and second-generation biofuels use plant biomass as the feedstock. Despite the large availability of waste plant biomass (such as agricultural waste) as feedstock for second-generation biofuels, this technology is still under development. Algal biomass is considered to have a number of advantages compared to higher plants because of faster growth rates and the possibility of cultivation on non-arable areas, in lakes and seas [2]. The anaerobic digestion of algae to methane is an affordable way to obtain energy [3]. Although the production of biogas from algal biomass has been studied for a long time, there are still only a few studies on the anaerobic digestion process of microalgae [4, 5]. A probable reason for this is the difficulty in anaerobic digestion of microalgae due to their different composition, the need for pre-treatment and the different composition and amount of biogas produced [3, 6]. In the methanation

of *Spirulina* biomass, a number of authors report low biogas yields, due to the low carbon/nitrogen ratio and the accumulation of ammonium ions in the medium, as well as a negative influence of biomass pretreatment on methane yield [6, 7]. The aim of this investigation was to determine the biochemical methane potential of *Spirulina* biomass at optimal conditions.

EXPERIMENTAL

Substrates

The *Spirulina* biomass was kindly provided to us by colleagues from the University of Mining and Geology "Saint Ivan Rilski" - Sofia, Bulgaria. The biomass was suspended in distilled water and centrifuged at 3000 rpm for 20 min to separate the remaining components of the culture medium. High in COD (270 $gO_2 L^{-1}$) and relatively acidic (pH 3.17) vinasse obtained from wine distillation was used as co-substrate in the methanation of biomass. The main characteristics of vinasse are described by Velichkova et al. [8].

Preparation of substrates

For anaerobic digestion, 10 grams of fresh biomass was suspended in 20 mL of distilled water. In order to obtain a pre-threated substrate, the resulting suspension was subjected to thermolysis by autoclaving for 20 minutes at 121°C and then used in biomethanation.

Methanogenic consortia

Methanogens were obtained as activated sludge from a factory producing bioethanol "Almagest", Verinsko village, Bulgaria.

Analytical methods

The total suspended solids (TSS) of feedstocks were analyzed by drying the samples at 105°C to constant weight. Volatile suspended solids (VSS) contents were determined by burning the dried samples two hours at 550°C. In order to determine the TSS in the spirulina hydrolysate, it was centrifuged at 3000 rpm for 20 minutes, suspended in distilled water and centrifuged again. The contents of elemental carbon (C), nitrogen (N) and hydrogen (H) were measured using an automatic elemental analyzer EuroEA 3000. The content of total protein was calculated by multiplying the value of total nitrogen by 6.25. The dissolved protein content was determined according to the method of Lowry et al. [9]. An ammonia medium range photometer Milwaukee was used to estimate the concentration of ammonium ions in digesters after every batch process.

Total sugar amounts were determined by Anthrone method. Briefly, 4 mL of anthrone reagent (0.2 g of anthrone per 100 mL of concentrated sulfuric acid) was added to 2 mL of a diluted microalgal suspension, and the solutions were heated at 92°C for 8 min. After cooling, the absorbance at 585 nm of the resulting solution was measured spectrophotometrically. The absorbance was compared against a calibration curve prepared with glucose standards that ranged from 10 μg to 100 μg of glucose per 1 mL of solution. Reducing sugars were determined as glucose by using dinitrosalicylic acid (DNS) reagent by the method described by Miller [10]. Gas production rate measurements were performed using a manual constant pressure liquid displacement system. The methane content in biogas composition was determined by Optima 7 gas analyzer. Biochemical methane potential was determined by batch process described by Velichkova et al. [8].

Biomethanation and estimation of biochemical methane potential (BMP)

In this investigation the BMP-test was performed in batch mode at 35°C. Test bottles (500 mL) were filled with 300 mL activated sludge mixed methanogenic consortium and 30 mL microalgae suspension, flushed out with nitrogen for 15 minutes and then placed in a thermostat at 35°C. The amount of biogas generated was determined by water displacement in calibrated glass cylinders. Scheme of laboratory biomethanation system used in BMP test experiments is shown in Fig. 1. The modified Gompertz mathematical model was used to describe the biogas accumulation curves at different experimental conditions [11]. A nonlinear least square regression analysis was used to fit the nonlinear equation to cumulative methane production data, obtained from BMP assays. The Maple software version 15 (Waterloo Maple inc. 2014) was used to determine the values for maximal biogas yield and maximal rate of biogas production in the model. The obtained maximal biogas yield was used for BMP determination according to the following Eq. (1):

$$BMP = (V_{max\,biogas}.C_{methane})/(V_{sub}.VSS_{sub}.100) \quad (1)$$

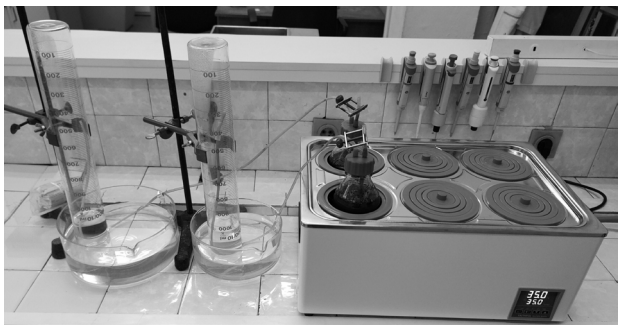

Fig. 1. Laboratory system for BMP determination.

Table 1. Elemental composition of dry spirulina biomass.

Element	% of TSS
Nitrogen	11.23
Carbon	46.71
Hydrogen	7.88

Table 2. Main characteristics of feedstocks used in the BMP tests.

Characteristic	Fresh Spirulina suspension	Termolyzed Spirulina
TSS (%)	6.72	2.68
VSS (% TSS)	95.70	96.10
Total protein (% TSS)	70.18	68.15
Total carbohydrates (% TSS)	11.00	10.50
pH	7.8	7.9
C/N	4.16	4.16
Free protein (% TSS)	0.38	49.30
Free carbohydrates (% TSS)	0.55	5.10
Free reducing sugars (% TSS)	0.04	0.55

where: BMP - biochemical methane potential, L CH_4 g^{-1}VSS; $V_{max\ biogas}$ - maximal biogas yield, L; $C_{methane}$ - methane concentration, %; V_{sub} - volume of the substrate used in the BMP-test, L; VSS_{sub} - volatile suspended solids of substrate, g L^{-1}.

RESULTS AND DISCUSSION

Characteristics of *Spirulina* biomass

In order to characterize *Spirulina* biomass as substrate for biomethanation the most important characteristics were examined. The results from element analysis are summarized in Table 1. The results obtained for the content of the element carbon and nitrogen are approximately equal to those of other studies of spirulina biomass, such as in the study of Herrman et al. [12] the carbon content was 48.3 % TSS and nitrogen 11,2 % TSS.

The characteristic of feedstocks (fresh microalgae biomass suspension and hydrolysate) used in this study is presented in Table 2. The values for the content of protein, carbohydrates and reducing sugars in the thermolyzed biomass were calculated relative to the initial amount of TSS in the microalgae suspension. The data in Table 2 show that the biomass used is characterized by high protein content, over 70 % TSS, and low carbohydrate content, compared to other microalgae species [3]. The main problem for the anaerobic digestion of biomass is the low C/N ratio. The high values of the content of free protein and carbohydrates in the hydrolyzed biomass show that in the process of thermolysis the biomass has undergone significant changes and the cell wall has been destroyed.

The results for biogas accumulation in the first series of batch experiments of methanation of fresh *Spirulina* biomass are shown in Fig. 2. In the first three batch experiments, approximately the same yields and rate of biogas accumulation were observed. However, the yield of biogas decreases from 0.660 L to 0.617 L. In the fourth and fifth (not shown in the figure) periodic processes, the yield drops significantly (to 0.461 L) and in the fifth process the yield is negligible. Although a fresh methanogenic consortium was used when the experiments were repeated, a retardation and stopping

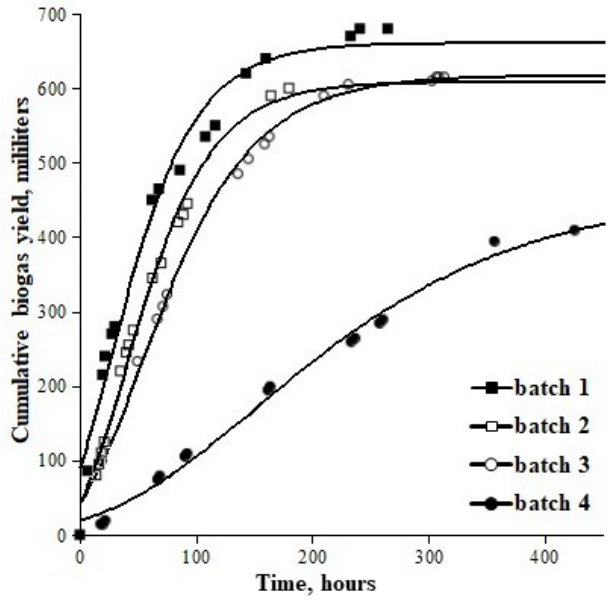

Fig. 2. Biogas accumulation in BMP test of fresh *Spirulina* biomass. Symbols - experimental results; solid line - modified Gompertz model.

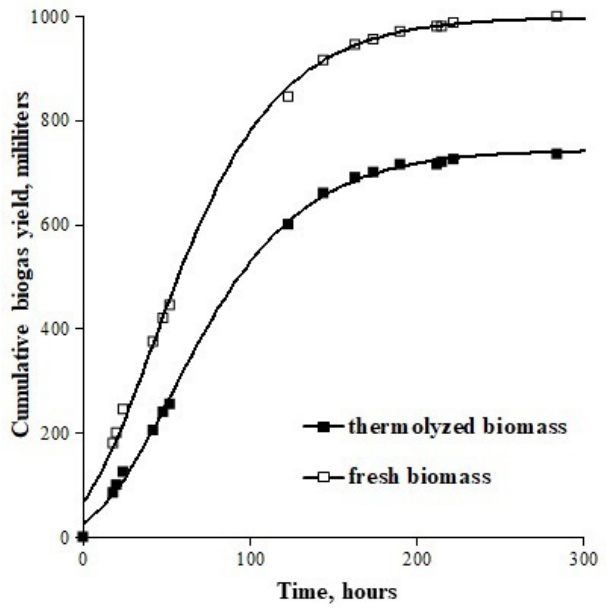

Fig. 3. Biogas accumulation in BMP test of fresh and thermolyzed *Spirulina* biomass. Symbols - experimental results; solid line - modified Gompertz model.

effect of the process was again observed. The main reason for the stoppage of the biomethanation process is most probably the accumulation of ammonium ions in the medium. During the first periodic processes, a

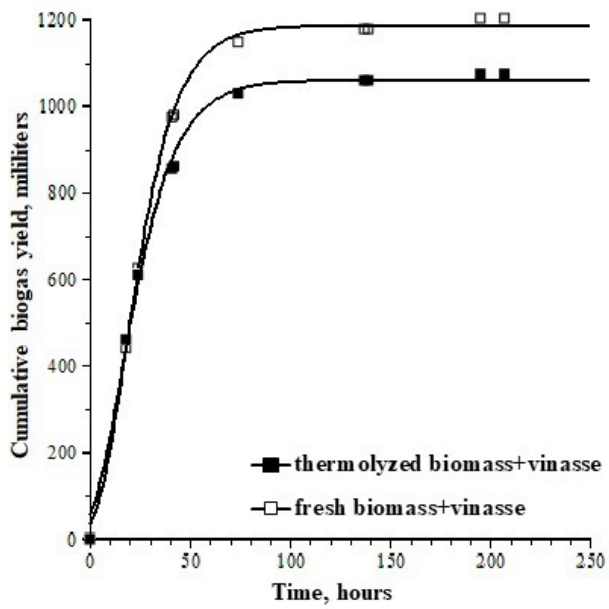

Fig. 4. Biogas accumulation in batch co-digestion of *Spirulina* and vinasse. Symbols - experimental results; solid line - modified Gompertz model.

slight increase in their concentration was found, but after the fourth process, a value of 6.5 g L^{-1} was measured. In order to restore the activity of the methanogenic consortium, regeneration was carried out several times by adding vinasse and as seen in Fig. 3 this leads to an increase in biogas yield and process speed. It was found that regeneration with vinasse after each batch process ensured the stability of the process and the achievement of the same biogas yields. The results shown in Fig. 3 for biogas accumulation with fresh and thermolyzed biomass substrate clearly demonstrate that the pretreatment used leads to a decrease in the yield and the rate of the process. The increase of the methanogenic activity, by regenerating the methanogenic consortium with vinasse, showed that it is appropriate to use co-methanization with the aim of process stability and higher yields.

Fig. 4 clearly demonstrates the effect of co-digestion with vinasse on biogas yield and process rate. In this process, the maximum rates of methanation of the fresh and hydrolyzed biomass are significantly higher than those reached without vinasse as a co-substrate. In less than one hundred hours the maximum yield is reached and the process stops, while in the previous experiments the maximum yield is reached after more than two hundred hours. Although only 15 mL of microalgae

Table 3. Calculated from modified Gompertz model maximum biogas yield, maximal rate of biogas accumulation and BMP.

Substrate	Maximal Yield, mL	Maximal rate, mL h^{-1}	BMP, L CH_4 g^{-1} VSS
Fresh Spirulina Batch1	660.4	6.0	0.295
Fresh Spirulina Batch2	608.0	5.5	0.271
Fresh Spirulina Batch3	616.9	4.2	0.275
Fresh Spirulina Batch4	461.0	1.3	0.205
Fresh Spirulina	996.7	8.7	0.445
Thermolyzed Spirulina	742.0	6.2	0.331
Fresh Spirulina + vinasse	1186.2	31.3	0.529
Thermolyzed Spirulina + vinasse	1049.1	24.8	0.461

suspension was used as substrate, the biogas yields achieved were significantly higher than those without using vinasse as co-substrate. No accumulation of ammonium ions was observed in this experiment, which is probably the main reason for the higher yields. In order to determine the biogas yield from the microalgae alone, experiments were conducted with 15 mL of vinasse, and then the yield was recalculated based on the amount of VSS introduced with the microalgae biomass. The methane content in the biogas obtained in all experiments was 86 %. The values calculated by the modified Gompertz model for the maximum rate of biogas accumulation and the maximum yield, as well as the determined by equation (1) and recalculated per gram VSS results for BMP are summarized in Table 3.

The results in Table 3 show a significant reduction in BMP in batch experiments without regeneration of the methanogenic consortium. Nevertheless, the results obtained in the first three experiments are comparable to a number of other studies on the methanation of microalgae biomass [2, 3]. It is noteworthy that after the regeneration of the methanogenic consortium, the yield of biogas during methanation of fresh biomass reaches 996.7 mL, which is 50 % more than obtained in the first experiment. In both experiments with thermolyzed spirulina as monosubstrate and vinasse co-substrate, a decrease in BMP was observed. The yield reduction in biomass pretreatment found in our study was 15 % to 25 %, but other studies have found yield reductions of more than 50 % [6]. The BMP values in Table 3 are calculated relative to the VSS in the microalgae suspension, and in the experiments with the co-substrate vinasse, they must be recalculated taking into account the amount of biogas that is obtained from the vinasse. After the recalculation, the obtained BMP values for fresh biomass are 0.476 L CH_4 g^{-1} VSS and for thermolyzed 0.415 L CH_4 g^{-1} VSS. Based on the recalculated values, it can be concluded that vinasse does not significantly increase the BMP of the microalgal biomass, but increases the speed and stability of the process.

CONCLUSIONS

The study showed that *Spirulina* biomass can be used as substrate for anaerobic digestion and methane production, but when the batch processes are repeated, a stoppage of the process is observed due to inhibition by the high concentration of ammonium ions. Regeneration of the methanogenic consortium with vinasse increases biogas yields and process stability. Pretreatment of biomass by thermolysis leads to a decrease in biogas yield. The application of vinasse as a co-substrate in anaerobic processing increases the speed and stability of the process, and the increase in BMP is insignificant. The obtained results show that the selected biomass can be successfully used to produce methane, but in order to guarantee the stability of the process and increase the yield, the use of a carbon-rich co-substrate is necessary.

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