Fermentation of plant residues to produce biogas

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Abstract. In this paper, we consider the process of producing biogas with a high methane content when used as a co-substrate for fermentation of plant residues of microalgae. Microalgae Chlorella sorokiniana are a valuable source for obtaining valuable components such as lipids, pigments, proteins, chlorophyll and others. After the extraction of valuable components, residual biomass is formed, which requires further disposal. In this experiment, the digestion process is carried out using an inoculant — lyophilically dried activated sludge from sewage treatment plants in Hamburg in the amount of 450 ml and residual biomass of the microalga Chlorella sorokiniana in the amount of 2.1 g. The studies were carried out in the Anaerobes Test system AMPT-II system. Fermentation produces 205 ml of methane gas.

1 Introduction

Microalgae of the genus Chlorella have long been studied by Russian and foreign scientists and are widely used in various industries: pharmaceuticals, food processing, cosmetology and energy.

As a result of the fermentation of microalgae biomass in special bioreactors under the influence of the inoculant, biogas is formed, which consists of methane, carbon dioxide and a small amount of associated gases [1-3].

The process of anaerobic digestion consists of four main successive stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The first stage proceeds with the help of hydrolytic bacteria, which turn complex compounds (polysaccharides, proteins and lipids) into simpler substances. After this, these products are fermented to propionate, acetate, butyrate, lactate, ethanol, formate and hydrogen at the acidogenic stage.

Hydrogen producing bacteria convert these compounds to acetate, hydrogen, and carbon dioxide (acetogenesis). Methane can be produced in two different ways during methanogenesis: from acetate, as a result of the metabolism of acetophilic bacteria, or from H2 and CO2, due to hydrogenophilic methanogens.

The biogas potential is highly dependent on the microalgae strains and the pretreatment to which they are subjected.

Other factors, such as biomass composition, biomass quality, and digestion process parameters, may affect AD performance. Temperature, pH, time, solid nutrient availability (SRT), redox potential, the presence of toxic compounds, substrate particle size and availability are the most important parameters that need to be controlled during the process.

Methanogenic microorganisms are sensitive, and a small change in this parameter can stop their activity [4-5] . Regarding pH, each stage of the AD process works in different ranges: the optimum hydrolysis and acidogenesis is between 4.5 and 7, and the acetogenesis and methanogenesis are between 6.8 and 8.2 [6].

During bioprocessing, anaerobic digestion is preferable to the production of biodiesel and bioethanol, due to the fact that you can use directly wet biomass, avoiding the drying phase [7].

However, some difficulties may arise with microalgae if cell wall resistance is particularly high, which limits the availability of substrates or if methanogenic bacteria are inhibited by ammonia production due to the low C: N ratio of microalgae

The remainder from biogas production is called “digestat” and can be used as a fertilizer in agriculture for a rich content of minerals and nutrients (such as potassium and phosphates), or it can also be used as a nutrient source for algae culture [8-10].

2 Materials and methods

The residual biomass of the microalgae Chlorella sorokiniana (RBCh) after lipid extraction was used as an object of study. The lipid recovery process was carried out by the Soxhlet method using a hexane: ethanol solvent system in a ratio of 10:90, the product yield was 20% [11]. Before using the residual biomass as an additive for fermentation, the residual biomass was thermally treated in an oven at a temperature of 300^0C for 30 minutes to remove residual solvent.

Microalgae have a very dense cell wall and to increase the output of lipids it is necessary to disintegrate it. The most effective disintegration technologies were selected using a microwave mineralizer at a power of

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120 W, processing time of 5-15 minutes, at a pressure of 1 atm, processing temperature was 52-58^{0}C [12].

To calculate the amount of the required additive, the values of the content of organic (oTS) and inorganic (TS) carbon were determined by calcination, and the following values were obtained - TS=3.01, oTS= 96.46.

As an inoculant, lyophilized activated sludge from a treatment plant in Hamburg was used, which was previously dissolved in a nutrient medium developed by the TUHH staff (table 1).

Table 1. The composition of the environment for the cultivation of activated sludge.

№	Substance	Amount
1	NH_4Cl	2.0 g
2	NaCl	0.2 g
3	$MgCl_2$ x $6H_2O$	0.2 g
4	$CaCl_2$ x $2H_2O$	0.1 g
5	K_2HPO_4 x 3 H_2O	0.8 g
6	Rezazurin	1.0 mg
7	Microelements	2 ml
8	Vitamins	2 ml

Experimental studies were performed using Anaerobes Testsystem AMPT-II. Bioreactors composition were loaded, which are shown in table 2.

Table 2. Bioreactors composition.

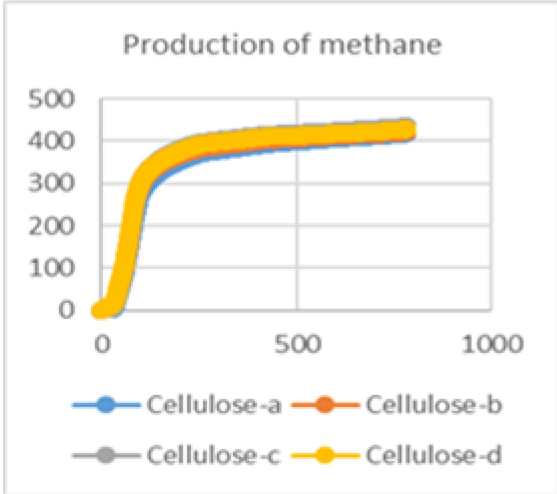
Bioreactors	Amount of Additive RBCh, g	Inoculant amount, ml	The amount of microcellulose, g
MC (a)	-	450	1.6
Chlorella (b)	2.1	450	-
Blank (c)	-	450	-

To control the correct operation of the system, 1.6 g of microcellulose (MC) was loaded into the bioreactor. Data on the volume of methane formed in each bioreactor were continuously recorded in the form of a graphical dependence of the CH_4 volume on the fermentation time for 25 days. The temperature of methanogenesis is 37 ° C.

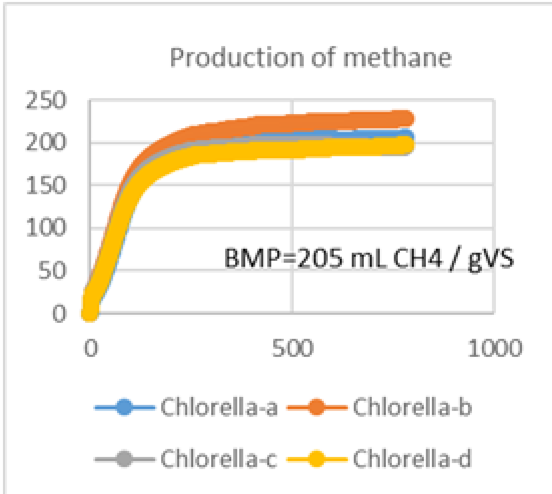
The experiment was carried out in triplicate.

3 Results and Discussion

As a result of the studies, methane emission schedules were obtained using OBX additives (Fig. 1) and without additives. For samples without additives, a methane yield of about 45 ml was obtained, which is significantly lower than for samples with additives. The introduction of RBCh additives significantly increases the amount of methane produced within 25 days. Figure 1 shows the effect of supplements on biogas emissions. A significant increase in methane emissions is also due to the fact that the microalgae biomass was disintegrated to increase lipid yield. A preliminary procedure for the destruction of the cell wall promotes the release of nutrients that contribute to an increase in the formation of methane.



(a)



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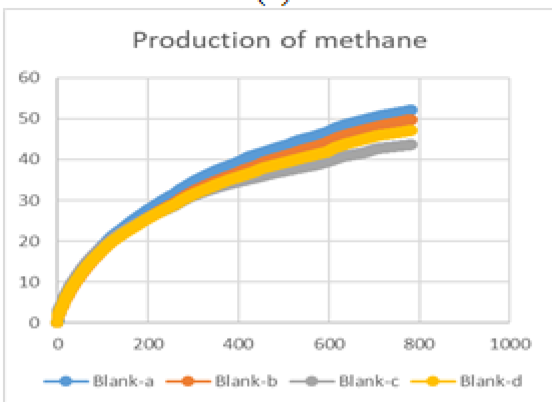

(c)

Fig. 1. The effect of the additive on biogas emission.

For samples with the addition of RBCh and MC, the process of methanogenesis proceeds actively for 180 hours.

The increase in the amount of biogas produced is due to the fact that the microalga Chlorella sorokiniana contains cellulose, lignin, hemicellulose. The cell membrane contains polysaccharides, a secondary polymerized carotenoid, sporopollenin, and cellulose. But the most important thing is that chlorella contains protein, hydrocarbons and a residual lipid content. As a result of the breakdown of fats (lipids), carbohydrates and proteins, biogas is formed and the amount of biogas depends on the concentration of components. The nutrient content in the microalgae biomass depends on the growing method.

It is known that in the dry biomass of chlorella contains 40-55% protein, 35% carbohydrates and up to 10% minerals, lipids 5-10%. Directed cultivation allows one to obtain biomass with a lipid content of more than 20% [13-14]. The residual biomass contains about 5-7% lipids. As a result of the breakdown of fats (lipids), carbohydrates and proteins, biogas is formed and the amount of biogas depends on the concentration of components. The specific biogas yield during the fermentation of fats is 1.5 times higher than carbohydrates and proteins [15].

A significant increase in methane emissions is also due to the fact that the microalgae biomass was disintegrated to increase lipid yield. A preliminary cell wall destruction procedure allows nutrients to be made available to the digestion process.

It is advisable to obtain a larger amount of biogas to use the residual biomass of microalgae after the treatment of pigments, proteins, flavonoids, while maintaining a high lipid content.

4 Conclusions

A method for the disposal of residual biomass of the microalgae Chlorella sorokiniana as an additive in the processes of fermentation of organic waste is proposed.

The addition of RBCh in the amount of 2.1 g made it possible to obtain biogas emission with a methane yield of 205 ml; in the sample without the addition of RBCh, 45 ml of methane is formed.

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