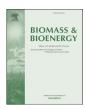
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Research paper

Influence of a light source on microalgae growth and subsequent anaerobic digestion of harvested biomass



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ABSTRACT

The aim of the study was to determine the influence of the light source on the taxonomic structure and chemical composition of the harvested biomass, and on the fermentative biogas/methane production. Cultivation of a mixed microalgae culture was carried out in closed vertical photobioreactors equipped with different light sources. The effectiveness of anaerobic digestion was analysed by respirometric measurements. The harvested microalgae biomass was characterized by various taxonomic structures and varied chemical composition depending on the light source used during cultivation stage. In variants where warm white LED lighting and red light were used, species from the *Cyanoprokaryota* division predominated, characterized by a high concentration of organic compounds and nitrogen in the biomass. TOC values amounted to almost 430 mg/g TS. In the remaining variants, *Chlorophyta* predominated, and TOC values were in the range of 388.0-411.3 mg/g TS. A significantly higher biogas/methane production (p=0.05) was found in variants in which biomass with *Cyanoprokaryota* predominating was tested. The biogas yield was in the range of 383.2 L/kg VS to 400.8 L/kg VS, and the methane content was close to 55%. A lower effectiveness of biogas and methane formation were observed in variants with *Chlorophyta* as a predominating taxonomic group.

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1. Introduction

High photosynthetic effectiveness, rapid growth, resistance to pollution and the ability to adapt to variable environmental conditions allow to state that microalgae are becoming a competition for biomass of typical energetic plants [1-5]. Moreover, the microalgae biomass may be transformed into many types of biofuels: biodiesel obtained from lipids cumulated in cells, hydrogen formed in photobiological transformations, biogas from anaerobic decomposition of organic substances. It may also be intended for a direct combustion [3,6-8].

Abbreviations: LED, light-emitting diodes; VS, volatile solids; TS, total solids; TC, total carbon; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus.

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Literature data indicates that conversion of the algae biomass into biogas is a highly profitable and economical solution, and the amount of the obtained energy is comparable with values obtained during cellular lipid extraction [8–10]. Post-fermentation sludge is an additional product of the process apart from high-energy biogas. The sludge may be used in a direct way as a fertilizer for land plants or, after simple processing, it may be recycled into the algae biomass cultivation system as a component of the nutrient medium [11].

The yield of anaerobic digestion depends on the constant provision of significant amounts of an organic substrate with a homogenous and stable composition. Selection of the algae cultivation technology is a very important element, in many cases decisive for the possibility of utilization of the algae biomass for biogas production [12–14]. Algae cultivation may be carried out using various methods, from advanced technological solutions, in which the process is monitored and controlled in detail, to less

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foreseeable techniques based on the use of open tanks [15]. Closed systems are photobioreactors with various structures, which - as distinct from open systems - provide a possibility to control the temperature of the cultivation medium constantly, limit the access of predators, parasites and competitive algae species, as well as allow for controlling the manner, intensity and time of exposure [14,16].

The way of providing light is one of the most important elements affecting the effectiveness of production of algae biomass directly. In many studies, it was proven that the type of the light source, wavelength and exposure method affect not only the yield of microalgae production, but also the formation of the taxonomic structure and the chemical composition of the obtained biomass [17]. Characteristics of the microalgae biomass, most of all cell wall structure, contents of carbon and nitrogen compounds, and the C/N ratio directly affect the biogas/methane yield during anaerobic digestion [8]. It is assumed that light-emitting diodes (LED), considering their price, operating costs, time of effective operation and reliability, will be one of the most important light sources globally, which may be used in closed systems of microalgae production [17]. It has been proven that the type of the implemented LEDs used in cultivation directly affects the physiology and biochemical composition of the microalgae, allows for regulating the production of biomass belonging to various taxonomic groups and enables accumulation of specific chemical compounds in the cells of microalgae [18-21].

The aim of the study was to determine the influence of the light source on the taxonomic structure and chemical characteristics of harvested biomass and on the fermentative biogas/methane yield.

2. Material and methods

2.1. Microalgae inoculum

The inoculum was a mixed culture of the microalgae originating from own cultivation, carried out in an 300 L open reactor of a race ways type, operated on the pilot plant scale. Cultures were illuminated continuously (500 lux, cool-white fluorescent tube). This process resulted in algae inoculum which was introduced into the eight vertical closed photobioreactors to obtain an initial biomass concentration at a level of 50 mg TS/L. The taxonomic structure of inoculum was as follow: *Chlorophyta* 84.2%, *Cyanoprokaryota* 12.1%, *Bacillariophyceae* 2.4%, other 1.3%.

2.2. Microalgae cultivation in photobioreactors

The microalgae cultivation was carried out using eight vertical closed pipe photobioreactors with an operative capacity of 68 L. They were made of borosilicate glass and had an internal diameter of 180 mm and operative height of 3000 mm. The compressed air in the amount of $400-1200~\text{dm}^3/\text{h}$ was fed continuously to the reactors at their bottom. This operation ensured proper stirring of the cultivation medium and homogeneity of conditions in the whole reactor volume and enabled introduction of CO_2 to the culture.

The experiments were divided into eight variants. The criterion used was the applied light source. The photobioreactors were placed in a chamber covered with aluminium foil and were illuminated continuously. Organization of the experimental variants is shown in Table 1.

At the beginning of the experiment, an inorganic nutrient medium with the chemical characteristics shown in Table 2 was introduced into the photobioreactors. During the experiment, concentrations of nutrient substances were monitored (DR 5000 Hach-Lange, Germany) and their deficiencies were supplemented consecutively.

The cultivation of the microalgae was carried out for 30 days until a concentration of the biomass in the photobioreactors at a level of *ca.* 1000 mg TS/L was obtained. After the cultivation process was ended, the obtained algae biomass was concentrated, separated and dehydrated by initial sedimentation and then by centrifugation for 5 min at 8000 rpm (Rotina 380, Hettich GmbH & Co.KG, Germany). Dehydrated biomass was later subjected to chemical and taxonomic analyses, and then used as the fermentation substrate.

2.3. Anaerobic digestion of microalgae biomass

The obtained microalgae biomass was used as a substrate during anaerobic digestion. The anaerobic batch experiments were done in respirometric Oxi-Top reactors (WTW GmbH, Germany). The measurement equipment consisted of a reaction tank with a working volume of 0.5 L and a measure recorder (Fig. 1). The reactors recorded changes of the partial pressure in the chamber, caused by biogas production. The ideal gas equation of state was the basis for calculations of the biogas amount produced in the respirometric studies. Based on changes in the pressure inside the measurement chamber, volumes of the biogas forming were calculated, converted to normal conditions. Also, the rate of biogas production depending on the experimental variant used, was determined in the respirometric studies. The biogas production rate constants were determined based on the obtained experimental data, by nonlinear regression using Statistica 10.0 PL software.

In every variant of the experiment, 300 cm 3 of the anaerobic sludge inoculum were introduced into the reaction chambers, and then the microalgae biomass was dosed. The anaerobic inoculum originated from closed fermentation chambers of a municipal treatment plant. The concentration of volatile solids (VS) seeded into the reactor was $69.2 \pm 2.8\%$ TS.

In all variants, an initial organic loading rate was on the level of 5.0 g VS/L. In order to ensure anaerobic conditions inside the fermentation chambers, the bottles were flushed with oxygen free nitrogen gas in order to remove atmospheric air and then capped tightly with rubber. The reactors were placed in a thermostatic room and then incubated continuously in 38 °C \pm 0.5 °C for 20 days. Values of the pressure in the reaction chamber were recorded every 24 h. The experiments were carried out in triplicate.

2.4. Analytical methods

The taxonomic identification of microalgae inoculum and microalgae biomass were carried out based on non-solid or semi-solid preparations. The qualitative and quantitative analyses of phytoplankton were conducted at microscope magnifications of: $1.25\times10\times40$ or $1.25\times10\times100$. Once collected and fixed with a 4.0% formalin solution with the addition of ethyl alcohol the samples were concentrated via sedimentation to the volume that enabled determining at least 10 and not more than 25-30 specimens of phytoplanktonic algae in one visual field of the microscope at the magnification of $500\times$.

Determinations of total solids (TS) and volatile solids (VS) were carried out by gravimetric analysis. In the biomass samples dried at $105\,^{\circ}$ C, total carbon (TC), total organic carbon (TOC) and total nitrogen (TN.) were determined by elementary particle analyser Flash 2000 (Thermo Scientific, USA). Determination of total phosphorus (TP) was carried out by colorimetric analysis using ammonium metavanadate (V) and ammonium molybdate (VI) after the sample mineralization in a mixture of sulphuric (VI) and chloric (VII) acids, at a wavelength of 390 nm, using a DR 2800 spectrophotometer (HACH Lange, Germany). The pH value in H_2O was determined by

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