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Methanogenic archaeon as biogas producer in psychrophilic conditions

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The aim of the study was to determine the effectiveness of biogas production as a result of degradation of various organic compounds by psychrophilic methanogenic archaeon *Methanococcoides burtonii*. Depending on experimental variant, fermented glucose, whey, bovine liquid manure and sewage sludge were used as substrates. The effectiveness of methane fermentation was measured using 500 mL respirometers incubated at a temperature of 20 °C for 50 days. The highest yield of biogas production was noted in the variant with sewage sludge provided in the quantity that ensured the initial tanks loading at 0.2 g COD/L (133 mL CH_4/g COD_{fed}). In this variant, analyses showed also one of the highest concentrations of CH_4 in biogas produced (53.4%). The high content of methane and high production yield of this biogas component were also reported for the variants with sewage sludge at the loading of 2.0 g COD/L, with bovine liquid manure at the loading of 5.0 g COD/L and with glucose at the loading of 1.0 g COD/L. In these variants, the content of CH_4 in biogas produced reached from 37.6 to 57.1%. Taking into account the quantity of produced biogas and CH_4 concentration from various organic substrates during fermentation process in psychrophilic conditions run by *M. burtonii* strain, the results of this study may be considered promising for the perspective application.

1. Introduction

Today energy systems based mainly on fossil fuels cannot be regarded as sustainable. In view of the expected increase of global energy demand, there is a growing concern about energy supply security, increasing prices of energy carriers, local air pollution and global climate change (Urbaniec et al., 2010). The production of renewable energy from organic waste streams is one of the most important aspects in the concept of sustainable development. It has been projected by the European Union that by the year 2020 approximately 20% of the European energy demands should originate from renewable energy sources. The production of biogas from organic materials should contribute for about 25% towards the total budget of renewable energy sources (Holm-Nielsen et al., 2009). Anaerobic digestion can be considered as one of the most important techniques to convert organic waste streams into renewable energy in the form of methane (Banks et al., 2010; Kastner et al., 2012; Lew et al., 2011). Because of the many advantages of anaerobic digestion over conventional aerobic biological processes such as biogas production, lower sludge production as well as the fact that no energy for aeration is required, anaerobic digestion can be regarded as one of the most promising elements of wastewater treatment systems to meet the desired criteria for the future technology in environmentally-sustainable development (McCarty, 2001). Typically, anaerobic reactors operate under the mesophilic (25–37 °C) or thermophilic (45–60 °C) temperature ranges to ensure optimal microbial activity (McKeown et al., 2012; Ueno et al., 2007; Zhang et al., 2012a), and most rarely at psychrophilic temperature ranges (Dębowski et al., 2014; McHugh et al., 2006; Zhang et al., 2012b). Nevertheless, variations in operating temperature in waste treatment plants are common due to climatic conditions of the geographic area where the plant is located. In temperate regions, the ambient temperature of municipal wastewaters is considerably lower than the optimum value for anaerobic treatment processes (Connaughton et al., 2006a; Dhaked

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et al., 2010). Therefore, with the exception of tropical countries, a significant amount of energy is required to heat wastewater streams up to the optimal temperature range (Lettinga et al., 2001). Heating of the wastewater for mesophilic or thermophilic anaerobic digestion increases energy consumption. Furthermore an elevation in temperature could be especially detrimental because it can potentially kill a population of microorganisms which play a vital role in the digester (Leitão et al., 2006). Although most fermentative bacteria are generally considered to be inactive under psychrotolerant conditions (Scherer and Neuhaus, 2006), the anaerobic treatment of wastewaters at ambient or low temperatures has recently been successfully demonstrated (Alvarez et al., 2008; Kayranli and Ugurlu, 2011). However, further studies are required regarding anaerobically-treated municipal wastewaters and food wastes at low temperatures. Increasingly higher biogas production is reported for the commonly applied and exploited installations based on mesophilic fermentation compared to the process based on psychrophilic fermentation. This is corroborated by research works carried out so far (Dhaked et al., 2010; Kashyap et al., 2003). However, Connaughton et al. (2006b), who were comparing psychrophilic and mesophilic fermentation of brewery effluent, demonstrated a similar efficiency of its treatment and a similar methane content in produced biogas, but also twofold lower effectiveness of biogas production under psychrophilic conditions. Alike effects were demonstrated during fermentation of bovine manure (Zhua and Jha, 2013). Achieving similar values of biogas production during fermentation run at low temperatures is linked with, among other things, the necessity of reducing substrate load or elongating hydraulic retention time, which in many cases is economically-unjustifiable and eliminates the possibility of applying this solution in the technical scale (Dhaked et al., 2010; Kashyap et al., 2003). In addition, psychrophiles are very susceptible to even small changes of process conditions, which may have a negative effect upon process stability. It is, therefore, advisable to search for such strains of psychrophilic microorganisms whose methanogenic activity would allow achieving comparable effects concerning the quantity and quality of produced biogas. According to authors, it is feasible through the use of microorganisms with a high methanogenic activity and isolated from the natural environment. Such a possibility has also been demonstrated in previous literature works (Dębowski et al., 2014; Dhaked et al., 2010; Kashyap et al., 2003). The economic feasibility of the long-term low-temperature anaerobic treatment relies on sufficient microbial activity to ensure reliable wastewater treatment. In contrast to investigations presented in this manuscript, the majority of literature data refer to experiments performed at some systems of mesophilic methanogenic communities that adapt to low temperatures, thus contributing to methanogenesis. Unfortunately, methane (CH_4) production rates of these microbial consortia are low owing to a lower microbial activity under psychrophilic (<20 °C) conditions (Bandara et al., 2012; Kundu et al., 2012). Microorganisms have developed sophisticated adaptation strategies, which enable them to function under suboptimal conditions. Cultivating microbial biomass under sub-optimal conditions may result in a lag phase of a reduced metabolic activity during acclimatization. However, cold-tolerant microorganisms often produce enzymes having the optimum activity at moderate to low temperatures, which might be used in application at low temperature (Feller and Gerday, 2003).

Therefore in the present study, the feasibility of improved low-temperature anaerobic digestion was studied using psychroactive *Methanococcoides burtonii* in laboratory bioreactor systems for anaerobic treatment of glucose, sludge, liquid manure and some food waste (cheese whey powder) at low temperatures. Use of this methanogenic archaeon, which is characterized by a wide range of growth temperatures, in biogas production at low temperatures could potentially reduce the costs of producing energy.

2. Methods

2.1. Respirometric measurements

Experiments were carried out in a laboratory scale using respirometric kits by WTW company, with a total volume of 500 mL (Dębowski et al., 2014). The fermentation process was run at a temperature of 20 °C and retention time of 50 days. The applied anaerobic respirometers recorded an increase of the partial pressure induced by biogas production. The biomass of the test bacteria and organic substrate was used as the inoculum (100 mL). To provide anaerobic conditions, all activities involving substrate feeding to respirometric tanks and inoculation with the analyzed microorganisms were performed in a BACTRON chamber, in the atmosphere of: 5% − CO_2, 5% − H_2, and 90% − N_2.

Once the incubation process had been completed, the composition of biogas produced was determined using a GC Agillent 7890A gas chromatograph that enabled monitoring concentrations of the following components: CH_4, CO_2, O_2, H_2, H_2S and NH_3. Chromatograph was equipped with two Agilent packed columns. The simultaneous analysis of all investigated components of biogas was feasible owing to a valve system that enabled independent injection of the sample onto two columns. Chromatograph was also equipped with TCD detector (thermal conductivity detector). Detector temperature was 190 °C, columns temperature was 45 °C, and analysis duration was 9 min. The value of pressure measured inside the anaerobic respirometers was used to calculate the volume of produced biogas in respect of normal conditions (0 °C; 1013 hPa). Calculations performed in respirometric analyses (the content of biogas and methane) are based on the equations according to Dębowski et al. (2013).

2.2. Organic substrate

Experiments regarding biogas production by *M. burtonii* were carried out with various organic substrates and at varying values of model fermentation tanks loading with a feedstock of organic compounds. Design of the experiment is presented in Table 1.

Solutions of glucose and whey were prepared from powdered substrates and tap water. Sewage sludge used in the experiments originated from a secondary settling tank of a municipal wastewater treatment plant "Łyna" in Olsztyn, where the treatment technology is based on an integrated system of activated sludge that enables the removal of carbon, nitrogen and phosphorus compounds. Before being fed to respirometric tanks, the sludge was diluted with tap water. Bovine liquid manure originated from a farm of the Research Station of the University of Warmia and Mazury in Olsztyn. Before being used in the experiments, the manure was also diluted with tap water.

Table 1
Organization of the study.

Substrate	Variant	Load	Symbol
Glucose (GL)	I	1.0 g COD/L	GL1
	II	10.0 g COD/L	GL10
Cheese whey (WH)	III	1.0 g COD/L	WH1
	IV	10.0 g COD/L	WH10
Liquid manure (LM)	V	5.0 g COD/L	LM5
Sewage sludge (SS)	VI	0.2 g COD/L	SS0.2
	VII	2.0 g COD/L	SS2

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