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Impact of temperature, microwave radiation and organic loading rate on methanogenic community and biogas production during fermentation of dairy wastewater

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HIGHLIGHTS

- ▶ Dairy sewage treatment in a new reactor joining suspended sludge and trickling bed.
- ▶ Fermentation at 1 or 2 kg COD/(m^3 d) at 35 or 55 °C kept by microwaves or convection.
- ▶ Temperature and microwaves as main factors affecting species succession of *Archaea*.
- ▶ Microwaves promoted methanogens' diversity and supported biogas production.
- ▶ *Methanosarcinaceae* determined *Archaea* diversity and methane-rich biogas production.

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ABSTRACT

This study analyzed dairy wastewater fermentation in convection- and microwave-heated hybrid reactors at loadings of 1 and 2 kg COD/(m^3 d) and temperatures of 35 and 55 °C. The biomass was investigated at a molecular level to determine the links between the operational parameters of anaerobic digestion and methanogenic *Archaea* structure.

The highest production of biogas with methane content of ca. 67% was noted in the mesophilic microwave-heated reactors. The production of methane-rich biogas and the overall diversity of *Archaea* was determined by *Methanosarcinaceae* presence. The temperature and the application of microwaves were the main factors explaining the variations in the methanogen community. At 35 °C, the microwave heating stimulated the growth of highly diverse methanogen assemblages, promoting *Methanosarcina barkeri* presence and excluding *Methanosarcina harudinacea* from the biomass. A temperature increase to 55 °C lowered *Methanosarcinaceae* abundance and induced a replacement of *Methanoculleus palmolei* by *Methanosarcina thermophila*.

1. Introduction

Methane fermentation is the process applied in technologies of industrial wastewater treatment, particularly from the food industry, characterized by high organic concentrations. During anaerobic transformations, a stabilization of organic matter occurs with simultaneous production of methane-containing biogas. Fermentation is a multi-phase process and environmental requirements of microorganisms conducting each phase are different. In practice, the operational conditions should be optimized to prevent the accumulation of acid by-products that are the reasons for methanogenesis failure. To maintain stable methane production, the conditions in the reactor should favor the development of multi-species communities, because the highly diverse assemblages of microorganisms are less vulnerable to fluctuations in the environmental parameters (Saikaly et al., 2005). After an environmental disturbance which results in the elimination of some species, others are able to sustain a particular type of metabolic pathway.

The final phase of the fermentation – methanogenesis – is run by obligatory anaerobes, classified as *Archaea*. Since methanogenic *Archaea* are susceptible to changes in environmental factors (Zinder, 1993), it is necessary to define the operational parameters that promote the optimal conditions for the growth of a numerous and diverse community of these microorganisms in the reactor. Despite the fact that these microorganisms are autotrophic, the growth of most of them is driven by the presence of acetate. The types of

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available organic carbon sources affect the diversity of communities in anaerobic reactors (Ariesyaday et al., 2007). It was observed that methanogenic consortia differed in microbial composition depending on the temperature of the process. Karakashev et al. (2005) investigated 15 full-scale biogas plants operated with either manure or sludge as feedstock and observed that higher methanogenic diversity was noted in the reactors operated under mesophilic conditions compared to those with thermophilic conditions. On the other hand, Goberna et al. (2010) proved that higher temperatures (55 °C vs. 37 °C) during the fermentation of olive mill wastes and cattle excreta fostered methanogenic diversity by promoting the growth of H_2 scavengers.

Literature data indicate that to ensure a high production of biogas with high methane content, the fermentation should be carried out in the heated reactors (meso- or thermophilic process). Reactor heating is usually accomplished by a warming agent whose heat is dispersed by convection. An alternative to this method is the use of microwave radiation, which additionally helps to improve sludge dewatering properties and solubility of the reactants, thereby inducing the rate of fermentation (Ahn et al., 2009; Eskicioglu et al., 2009; Yu et al., 2009).

To date, there have been few reports on the influence of microwaves on the species composition and abundance of methanogenic microorganisms. Apart from heating, the use of microwaves generates so-called 'non-thermal effects', which may affect the structure of the species (Zieliński et al., 2007; Cydzik-Kwiatkowska et al., 2012a) and the functioning of the biofilm, by changing the enzymatic activity, DNA, RNA and protein syntheses in the cells of microorganisms (Pakhomov et al., 1998). These effects depend on the strength, frequency and the length of the exposure (Banik et al., 2003). The application potential of microwave technology in wastewater treatment requires further research on the effects of this heating method on the microbiota of anaerobic digesters.

The hybrid fermenters presented in this study are designed by the authors. They integrated the up- and down-flow reactor with suspended sludge and the trickling filter. The application of the trickling bed, atypical for anaerobic conditions, was connected with the use of microwave heating. Microwave energy was supplied to a part of the reactor with a bed, which warmed the biofilm (and indirectly the whole reactor volume) and facilitated the degassing of wastewater distributed on the surface of the bed.

The study aimed at determining the links between the organic loading rate, fermentation temperature, the type of heating applied (convection vs. microwaves) and the community structure of methanogenic *Archaea* in hybrid reactors treating dairy wastewater. The molecular and technological data were subjected to robust statistical analyses to define the key factors influencing both the total number of species and the relative abundance of microorganisms as components of species diversity. In the research, for the molecular characteristics of microbial consortia, electrophoresis in a denaturant gradient (DGGE) and fluorescence *in situ* hybridization (FISH) were used.

2. Methods

2.1. The experiment organization

The experiment was conducted in hybrid anaerobic digesters operating in a pilot-scale installation. In the upper part of the bioreactors, the biofilm was immobilized on polyethylene particles transparent to microwaves (total bed volume 30 L, surface 0.1256 m^2, specific surface 315 m^2/m^3, diameter of a single particle 13 mm). Suspended biomass was present in the lower part of the bioreactors (with a volume of 70 L). Wastewater up-flowed into the reactors (Fig. 1) and the hydraulic retention time was 1 day.

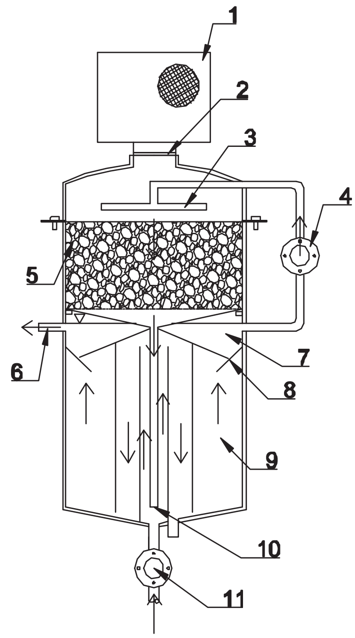

Fig. 1. Scheme of a hybrid reactor heated by microwaves: 1 – microwave generator, 2 – insert for microwave generator sealing, 3 – distribution of wastewater, 4 – upper recirculation pump, 5 – upper section of a bed, 6 – outflow with the water closure, 7 – lower section; zone of sludge clarifying, 8 – sludge trap, 9 – lower section; zone of suspended sludge, 10 – central pipe, 11 – pump for substrate dosing; arrows denote the direction of wastewater flow.

The reactors were inoculated with digested sludge from the municipal wastewater treatment plant in Olsztyn (Poland).

In order to estimate the potential athermal effect of microwaves on the fermentation performance and microbial communities, two reactors were operated in parallel. In the microwave-heated reactor, a magnetron of 1.6 kW was the source of radiation. The microwave frequency was 2.45 GHz and an automatically regulated magnetron emitted the radiation in a continuous manner. The second (control) reactor was heated by convection through an automatically steered water jacket. Both reactors were insulated with a 50-mm thick mineral wool layer to avoid the loss of heat. In each part of the reactors, four temperature sensors (HI 98801, Hanna Instruments, France) were installed enabling constant temperature measurements. The temperature of the influent was maintained at 25 °C ± 1.5 °C by an independent thermostating system.

In the research, synthetic dairy wastewater was prepared based on skimmed powdered milk and tap water. The characteristics of wastewater and operational parameters of six experimental series are given in Table 1. The names of the series were abbreviated according to the following manner: the first two numbers indicate the process temperature in °C, the third number reflects the organic loading rate (OLR) in kg COD/(m^3 d), and the letter corresponds to the heating method: C – convection, M – microwaves, e.g. 35–1-C – reactor exploited at the temperature of 35 °C, at OLR of 1 kg COD/(m^3 d) and heated by convection.

2.2. Analytical methods

After a 30-day period of adaptation, each experimental series was carried out for 30 days. During this time, once per day the influents and effluents were assayed for the concentrations of COD, BOD, total nitrogen and phosphorus, pH according to APHA (1992). The biogas amount was continuously analyzed using the mass flow analyzer (Aalborg, USA) and the biogas composition was determined with a GSF 430 analyzer (Gas Data Ltd, UK). At