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Biogas production from different size fractions separated from solid waste and the accompanying changes in the community structure of methanogenic *Archaea*

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ABSTRACT

There is a need to study the biogas production of waste substrates using routine tests because the characteristics of these substrates influence the kinetics of methane fermentation. In this study, biogas production from different size fractions of solid waste (0–20 mm, 20–40 mm, 20–80 mm, and 40–100 mm) was measured using a 90 days gas production test in mesophilic conditions. How the methanogenic community structure during fermentation corresponds to the chemical composition of the size fractions was determined.

Biogas production strongly negatively correlated with the biogas production rate constants (k_{biogas}) due to differences in the availability of organic substances. Microorganisms in the 20–80 mm size fraction produced the most biogas (252 ± 11 L/kg TS, $k_{biogas} = 0.16 \pm 0.04$ day^{-1}), which had the highest methane content (ca. 50%), probably because this size fraction had the highest organics content and the most diverse microbial community. In this size fraction, Methanosarcinaceae (acetoclastic microorganisms) and Methanobacteriaceae (hydrogenotrophic microorganisms) were more abundant than in other fractions. The 0–20 mm size fraction produced the least amount of biogas (65 ± 8 L/kg TS); however, its k_{biogas} was the highest (0.32 ± 0.05 day^{-1}), suggesting that organic matter was easily accessible to the microorganisms. Although the 0–20 mm size fraction is considered to be a mineral fraction that can be used for recultivation, the results of this study suggest that this fraction should be processed first to avoid environmental contamination.

1. Introduction

In order to comply with the European Landfill Directive, the mass of the biodegradable fraction of municipal solid waste (MSW) that was landfilled had to be reduced. This has been accomplished by using different technologies and methods such as mechanical–biological pre-treatment (MBP), for example. As part of this process, the waste is sieved to produce different size fractions, some of which can be anaerobically pretreated. During methane fermentation, stabilization of organic matter from MSW occurs simultaneously with production of a large amount of methane-rich biogas per kilogram of volatile fatty acids, and a high percentage of the waste is biodegraded.

The effectiveness of biogas production and organic matter biodegradation is highly dependent on the type of substrate. The morphological and physicochemical characteristics of size fractions may differ according to size. Therefore, it is useful to define and characterize more precisely the size fraction that contains the most organic material that is easily biodegraded and converted to biogas to optimize the recovery of biogas during anaerobic biological treatment. To characterize hard-to-define substrates, such as solid waste, aerobic respiration tests (respiration activity (AT4), dynamic respiration (DR4) or oxygen uptake rate (OUR)) and anaerobic respiration tests (biological methane potential (BM 100), gas generation sum (GS21), gas evolution (GB21)) are used [1]. The choice of test determines the length of the procedure. For example, when comparing the DR4 and BM 100 tests, apart from the DR4 being aerobic and the BM 100 anaerobic, it is important to note that the DR4 test indicates only the amount of readily biodegradable organic matter present in waste, whereas the BM 100 test also measures the amount of more resistant materials [2]. One of the

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most commonly used method is the biogas production test carried out for 21 days (GP21), however de Araújo Morais et al. [3] have found that in fact solid waste biodegradation may last much longer. According to the authors, an incubation period of at least 90 days ensures a very high level of anaerobic degradation (more than 90% of the potential total amount of biogas is produced in this period). In addition, Sánchez [1] has stated that biogas produced at 21 days corresponds to only 73% of the total potential biogas production. Therefore, the time of measurement in the present study was extended to 90 days in order to better determine the total biogas production during anaerobic stabilization of size fractions that could contain organic matter that is difficult to degrade.

The transformation of organic compounds to methane and carbon dioxide is affected by the presence of various microbial groups. Metabolic pathways in which microorganisms are involved may be divided into four basic successive phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The methanogenic microorganisms are sensitive to changes in operating conditions. For this reason, to maintain stable methane production, the conditions in this phase should favor the development of multispecies communities, which are less vulnerable to fluctuations in environmental conditions than communities with low diversity [4–6]. Methanogenic microorganisms, classified as *Archaea*, are obligatory anaerobes, and can produce methane via either the autotrophic or the heterotrophic metabolic pathways. In the former, hydrogenotrophic methane microorganisms (HMB) transform hydrogen and carbon dioxide into methane. In the latter, acetoclastic methane microorganisms (AMB), e.g., *Methanosarcina* spp. and *Methanosaeta* spp. use acetic acid as a substrate. HMBs can double their number in 4–6 h; AMB multiply more slowly (their number doubles after 24 h), and they are intolerant of hydrogen and sulphate [7]. Despite the fact that there are only a few species of AMB, they produce the majority of methane in fermentation reactors [8]. Only 30% of methane is produced from carbon dioxide reduction carried out by autotrophic methane microorganisms. During CO_2 reduction, hydrogen is used, which creates good conditions for the development of acid bacteria. This gives rise to short-chain organic acids in the acidification phase, and consequently, insufficient production of hydrogen in the acetogenic phase. As a result, the biogas may be rich in carbon dioxide, because only an insignificant amount will be converted into methane [9,10]. Although the efficiency of methane fermentation derives from the biochemical activity of these microorganisms, there have been only a few studies on the dynamics of the methanogenic community in anaerobic stabilization of municipal solid waste [11,12].

The aim of this study was to characterize the size fractions of mechanically pretreated solid waste in terms of their composition and biogas production during 90 days of measurement. In addition, the community structure of methanogenic *Archaea* during methane fermentation of the size fractions was analyzed. To characterize the microbial consortia, electrophoresis in a denaturant gradient (DGGE) and fluorescence in situ hybridization (FISH) were used. Finally, the correspondence between the methanogenic community structure during fermentation and the chemical composition of the separated size fractions of solid waste was determined.

2. Materials and methods

2.1. Substrate

In the present study, four size fractions of municipal solid waste were used: 0–20 mm, 20–40 mm, 20–80 mm and 40–100 mm. The size fractions were collected after the mechanical sorting stage at two Waste Treatment Plants in the Warmia and Mazury region of Poland. During this sorting, large stones or particles of metal are removed. Mixed waste is collected at these plants. At each plant, two sieves are used to separate the waste into size fractions. At one plant, the openings in the sieves have a diameter of 40 mm and 100 mm to create fractions of 0–40 mm, 40–100 m and >100 mm. At the second, the sieves are 20 mm and 80 mm, to give fractions of 0–20 mm, 20–80 mm and >80 mm. The 0–40 mm fraction was manually sorted to create a 20–40 mm fraction. Thus, in the present study, these fractions were used: 0–20 mm, 20–40 mm, 20–80 mm and 40–100 mm.

2.2. Inoculum

The inoculum was fermented sludge with characteristics: pH 7.4, total solids (TS) 1.7%, volatile solids (VS) 67.1% of TS, total nitrogen 33.1 mg/g TS, total phosphorus 1.7 mg/g TS, total carbon 309.1 mg/g TS, total organic carbon 199.4 mg/g TS. Inoculum was obtained from a closed mesophilic digester chamber used for sludge digestion at the Municipal Wastewater Treatment Plant (Poland).

2.3. Biogas production measurement

Samples were prepared and the biogas production of each size fraction was determined with batch assays in glass bottles (OxiTop system) in triplicate, according to Heerenklage and Stegmann [13]. Each bottle possessed its own head that recorded and measured the changes in the pressure for 90 days that were caused by formation of biogas during anaerobic fermentation [3]. By using the ideal gas law, the pressure was used to calculate the volume of biogas produced.

100 mL of the inoculum was added to each bottle along with about 3 g TS of the size fraction. In order to determine the biogas production potential of the inoculum, bottles with only inoculum were incubated under the same conditions. The biogas production potential of the fraction itself was determined by taking the difference between the biogas production of the inoculum combined with a sample of the size fraction, and of the biogas production of the inoculum only. The contents of each bottle were flushed with N_2-gas and the lateral connections of the bottles were sealed with rubber stoppers. For the purpose of taking samples for physicochemical analysis, glass bottles with the same volume but without a recording-measuring head were prepared at the same time and in the same way as the bottles with the recording–measuring heads (OxiTop system). All bottles were kept in mesophilic conditions at $36 \pm 1\,^{\circ}C$ in a thermostatic incubator. The contents of bottles were mixed once a day.

2.4. Kinetic evaluations

The anaerobic biogas production may be assumed to follow pseudo first-order kinetics. Biogas production would follow:

$$B_{t;\mathrm{biogas}} = B_{0;\mathrm{biogas}} \times (1 - e^{k_{\mathrm{biogas}} \times t})$$

where $B_{t;\mathrm{biogas}}$ (L/kg TS; L/kg VS) was the cumulative biogas yield at digestion time t (days); $B_{0;\mathrm{biogas}}$ (L/kg TS; L/kg VS) was the maximal biogas yield; k_{biogas} (days^{-1}) was the biogas production rate constant. The $B_{0;\mathrm{biogas}}$ and k_{biogas} values were obtained by nonlinear regression analysis with Statistica software, version 10.0 (StatSoft). Presented values of $B_{0;\mathrm{biogas}}$ and k_{biogas} are the averages with standard deviations.

2.5. Analytical procedures

To characterize the size fractions obtained after mechanical separation, moisture content, total organic carbon compounds (TOC), inorganic carbon (IC), total solids (TS), loss after ignition (volatile