Bioresource Technology 245 (2017) 833–840

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Improved methanization and microbial diversity during batch mode cultivation with repetition of substrate addition using defined organic matter and marine sediment inoculum at seawater salinity

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ARTICLE INFO

Keywords:
Defined substrates
Batch mode cultivation with repetition of substrate addition
Methane
Seawater salinity
Microbial community

ABSTRACT

The activation of microbes, which are needed to initiate continuous methane production, can be accomplished by fed-batch methanization. In the present study, marine sediment inoculum was activated by batch mode methanization with repetition of substrate addition using defined organic matter from sugar, protein, or fat at seawater salinity to investigate the potential for application of the activation method to various types of saline waste and microbial community compositions. All substrates had methane potentials close to the theoretical value except for bovine serum albumin (BSA) whose methane potential was lower, but the maximum methane potential reached the value during repeated methanization. Beta diversity analysis revealed that substrate (especially BSA)-fed and non-fed cultures had distinct microbial community compositions. Bacterial members depended on substrate. Thus, marine sediment inocula activated via the methanization method can be used to effectively treat various types of saline waste.

1. Introduction

Treatment of saline wastewater is critical for industries and municipalities. Seafood processing, leather, and petroleum industries discharge saline wastewater (Lefebvre and Moletta, 2006), as do municipalities when seawater is used for toilet flushing in order to conserve freshwater (Vyrides and Stuckey, 2009). Saline wastewater is treated by a biological or physicochemical process; the former is more attractive as it consumes less energy (Lefebvre and Moletta, 2006), although it can be inhibited by salt in the wastewater, especially when the process is anaerobic as opposed to aerobic (Lefebvre and Moletta, 2006).

Saline wastewater, which contains various organic materials, is treated anaerobically to produce biomethane using non-halophilic or halophilic inoculum (Lefebvre et al., 2007). Non-adapted inoculum is inhibited by saline wastewater (Gebauer, 2004) or requires a long acclimation period before it can be used for treatment (Omil et al., 1995). Halophilic inocula have greater potential for wastewater treatment than non-halophilic inocula, which has been demonstrated for fish (Aspé et al., 1997) and pharmaceutical saline (Shi et al., 2015) wastewaters. Moreover, undiluted marine macroalgae, which contain mainly sugars in addition to saline water, were highly methanized semi-continuously by marine as compared to non-halophilic inocula (Miura et al., 2016). However, most of these studies did not analyze microbial community composition; as such, little is known about anaerobic halophilic microbial communities in the treatment of wastewater under saline conditions.

Active microbes are needed to initiate continuous anaerobic treatment; cultures are activated by fed-batch cultivation (Angelidaki et al., 2006), which induces methanization of protein-rich biomass even in the presence of inhibitory substances such as ammonia (Kovács et al., 2013) and marine macroalgae under conditions of high salinity (Miura et al., 2015b). Fed-batch cultivation was recently reported to be more efficient than batch and continuous methods for acclimating microbes to high ammonia levels (Tian et al., 2017). However, the impact of fed-batch methanization of various types of organic matter under saline conditions is unknown.

To address this issue, in this study, we subjected defined components from organic material (sugar, protein, and fat) to batch mode

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http://dx.doi.org/10.1016/j.biortech.2017.09.009
Received 30 June 2017; Received in revised form 1 September 2017; Accepted 2 September 2017
Available online 05 September 2017
0960-8524/

methanization with repetition of substrate addition under saline conditions. The aims were to investigate (1) the potential application of the methanization to various types of saline waste; and (2) microbial community composition in component substrate-fed cultures in order to try to obtain useful information about microorganisms involved in treatment of complex substrates in saline environments. Marine sediments were used as inocula owing to their high capacity for methanization under saline conditions (Aspé et al., 1997; Miura et al., 2016; Shi et al., 2015). Agar and starch were used as marine and food waste-containing sugars, respectively; bovine serum albumin (BSA) was used as a model protein; and glyceryl trioleate was used as a fat, since oleic acid is the major component of lipid fatty acids from food waste (Meng et al., 2017).

2. Materials and methods

2.1. Materials

Agar (Purified powder; Nacalai Tesque, Kyoto, Japan), starch (Soluble; Sigma-Aldrich, St. Louis, MO, USA), BSA (Fatty acid-free; Nacalai Tesque), and glyceryl trioleate (≥97.0%; Sigma-Aldrich) were used as substrates. Sodium bicarbonate (Sigma-Aldrich) was used as the bicarbonate. Marine sediment was collected from Ariake Sea in Japan and stored at 4 °C, and used as a source of microbes. The marine sediment had 33.1 wt% total solid (TS), 3.17 wt% volatile solid (VS), and 51 mS/cm conductivity.

2.2. Chemical analysis

Chemical analyses were carried out as previously reported (Miura et al., 2016). The weight of VS was calculated as ash-free TS weight. Ash weight was measured by treating TS—obtained by drying at 105 °C for over 4 h—at 600 °C for 3 h. Conductivity was measured for the supernatant of the marine sediment obtained by centrifugation at 18,000*g* for 5 min at 4 °C, by using a conductivity meter (LAQUAtwin B-771; Horiba, Kyoto, Japan). Gas composition was analyzed by gas chromatography (GC-8A; Shimadzu, Kyoto, Japan). Gas volume was measured by substitution with NaCl-saturated water. Volatile fatty acids (VFAs) were detected by high-performance liquid chromatography (LC-2000 Plus HPLC; Jasco, Tokyo, Japan). The concentration of total ammonia was measured using a commercial kit (Ammonia; Wako, Osaka, Japan). The pH was measured for the stored supernatant of samples, which were exposed to N_2/CO_2 (80:20) gas bubbles for 1 min to reproduce the pH of the culture prior to the measurement, by using a pH meter (LAQUAtwin B-712; Horiba, Kyoto, Japan).

2.3. Batch mode cultivation with repetition of substrate addition

Marine sediment was transferred from a stock container to a bottle to measure VS content and for inoculation. The bottle was stored at 4 °C after purging with N_2 gas during measurement of VS. Marine sediment (1.8 g VS, 56.8 g in total) was inoculated into a 250-ml vial, which was briefly flushed with N_2 gas and sealed with butyl rubber. The substrate (0.3 g VS) and sodium bicarbonate (0.3 g) were added to the vial and immediately mixed with a N_2 gas-discharging long needle except in the case of oily glyceryl trioleate before sealing the vial with butyl rubber. For glyceryl trioleate, the added sediment was mixed with the N_2-discharging needle immediately after addition of bicarbonate, and the substrate was then added and the vial sealed after brief flushing of the headspace with N_2. The butyl rubber was fastened with an aluminum cap and the vial was purged with N_2/CO_2 (80:20) gas to obtain a completely anaerobic headspace. Cultivation of triplicate samples was carried out at 37 °C with shaking at 200 rpm. When methane production had almost ceased, an aliquot was sampled, substrate without bicarbonate was added to the vial, and cultivation was resumed. Substrate addition was done twice. Samples were stored at −20 °C.

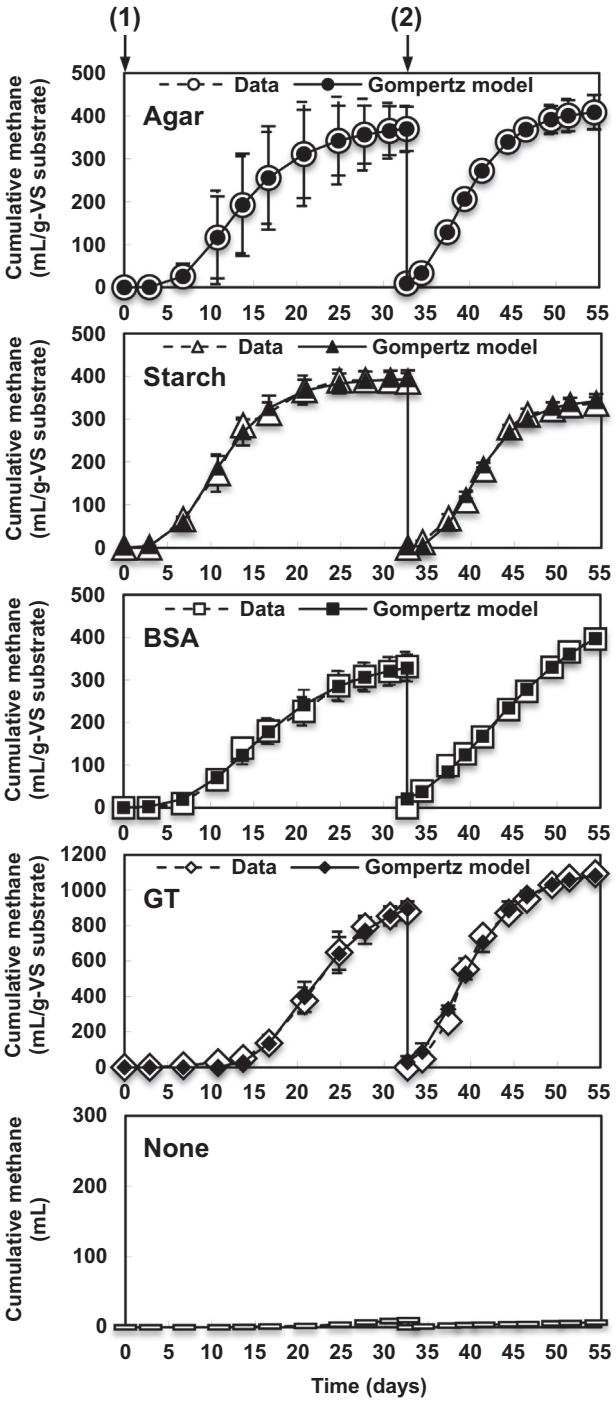

Fig. 1. Batch mode methanization of different substrates with repetition of substrate. BSA, bovine serum albumin; GT, glyceryl trioleate; none, no substrate. Values in parentheses indicate the number of times the substrate was added.

2.4. Genomic DNA extraction and amplification of the 16S rRNA gene

Samples from the second feeding (Section 2.3) were thawed on ice and then subjected to genomic DNA extraction as previously described (Miura et al., 2015b). DNA was extracted from aliquots (about 250 mg) of triplicate cultures using a NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany) and a FastPrep-24 instrument (MP Biomedicals, Solon, OH, USA) for mechanical lysis. The DNA was used as a template for PCR amplification of the 16S rRNA gene as previously reported (Miura et al., 2015b), except that the archaeal 16S rRNA gene-specific primers had the following Illumina (San Diego, CA, USA) overhang