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Semi-continuous methane production from undiluted brown algae using a halophilic marine microbial community

Toyokazu Miura [a,c], Akihisa Kita [a,c], Yoshiko Okamura [a,c], Tsunehiro Aki [a,c], Yukihiko Matsumura [b,c], Takahisa Tajima [a,c], Junichi Kato [a], Yutaka Nakashimada [a,c,*]

[a] Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8530, Japan
[b] Division of Energy and Environmental Engineering, Institute of Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashi-Hiroshima 739-8527, Japan
[c] CREST, JST, Japan

HIGHLIGHTS

- Acclimated marine sediment-derived culture was used for methanization of algae.
- Methane was produced continuously from equivalent of undiluted raw brown algae.
- Methane yield was above 300 mL/g VS at all organic loading rates (OLRs) tested.
- Hydrogenotrophic methanogens predominated among archaea during the highest OLR.

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ABSTRACT

Acclimated marine sediment-derived culture was used for semi-continuous methane production from materials equivalent to raw brown algae, without dilution of salinity and without nutrient supply, under 3 consecutive conditions of varying organic loading rates (OLRs) and hydraulic retention time (HRT). Methane production was stable at 2.0 g VS/kg/day (39-day HRT); however, it became unstable at 2.9 g VS/kg/day (28-day HRT) due to acetate and propionate accumulation. OLR subsequently decreased to 1.7 g VS/kg/day (46-day HRT), stabilizing methane production beyond steady state. Methane yield was above 300 mL/g VS at all OLRs. These results indicated that the acclimated marine sediment culture was able to produce methane semi-continuously from raw brown algae without dilution and nutrient supply under steady state. Microbial community analysis suggested that hydrogenotrophic methanogens predominated among archaea during unstable methane production, implying a partial shift of the methanogenic pathway from acetoclastic methanogenesis to acetate oxidation.

1. Introduction

Marine macroalgae are receiving increasing attention as a feedstock for biomethane production because they do not compete with food for farm land, have a higher growth rate than terrestrial energy crops, and are less resistant to degradation than lignocellulosic feedstocks (Montingelli et al., 2015). Methane can be produced by either thermal or biological gasification of biomass (Chynoweth et al., 2001). Marine macroalgae are suitable for biological gasification by anaerobic microbes because marine macroalgae have a high water content of around 90% (Roesijadi et al., 2010), which is not suitable for the thermal process requiring feedstocks with a low water content of less than 50% to achieve the high temperature needed for the process (Chynoweth et al., 2001). However, marine macroalgae contain high levels of salts (Roesijadi et al., 2010), which inhibit methane production (Feijoo et al., 1995; Liu and Boone, 1991; Miura et al., 2014; Mottet et al., 2014; Rinzema et al., 1988). Therefore, the use of diluted feedstocks or halophilic microbes is needed to allow methane production from these feedstocks.

Methane has been produced from marine macroalgae under semi-continuous conditions using diluted feedstocks (Briand and Morand, 1997; Hanssen et al., 1987; Hinks et al., 2013). In these conditions, the salinity of the feedstock decreases; however, the organic matter in the feedstocks concomitantly decreases because of increased water content, resulting in decreased organic loading rate (OLR) at the same hydraulic retention time (HRT). To obtain

* Corresponding author at: Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8530, Japan. Tel./fax: +81 82 424 4443.
E-mail address: nyutaka@hiroshima-u.ac.jp (Y. Nakashimada).

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the same OLR, the HRT must be kept shorter under diluted than under undiluted conditions, which can result in excessively short HRT causing washout of microbes in traditional single-stage methane production. Therefore, methane should be produced under undiluted conditions to obtain a high OLR without excessively shortening the HRT.

Miura et al. (2015) reported the acclimation of a microbial community from marine sediments by fed-batch cultivation for high-rate, continuous methane production from undiluted raw brown algae. The acclimation of the sediment by fed-batch cultivation enabled obtaining an increased methane production rate compared to the initial marine sediment slurry despite the presence of excess salts in the raw brown algae in addition to the 3% NaCl present in the initial slurry. However, it remains to be evaluated whether the acclimated microbial community is actually applicable to continuous methane production from raw brown algae.

The aim of this study was to evaluate the ability of the acclimated culture to produce methane continuously from brown algae under undiluted conditions. For this purpose, anaerobic digestion was conducted in a semi-continuous regime, using materials equivalent to undiluted raw brown algae, without nutrient supply. The OLR was varied during the production, which was continued beyond steady state.

2. Methods

2.1. Experimental design

Semi-continuous cultivation was carried out using acclimated marine sediment-derived culture as the starter culture. The starter culture was initiated in triplicate in 700-mL vials with intermittent replacement with fresh materials (dried brown algae and anaerobic water) with a total solid (TS) content of 10 wt%, which is equivalent to that of raw brown algae, for semi-continuous methane production. The cultures removed from the triplicate cultures at replacement were combined to measure the volatile solid (VS) content. An aliquot was taken at the beginning of cultivation with fresh materials to measure the pH and salinity. Sampling was also conducted at the end of the cultivation to measure the volatile fatty acids (VFAs) and to analyze microbial community.

2.2. Materials

Dried and milled *Saccharina japonica* was used as substrate (Miura et al., 2015). The TS, VS, and chemical oxygen demand (COD) of the substrate were 94.2 wt%, 73.3 wt%, and 1030 mg/g TS, respectively.

2.3. Semi-continuous methane production using a fed-batch acclimated culture

The starter culture for semi-continuous methane production was prepared in accordance with a previously described protocol for fed-batch acclimation (Miura et al., 2015). In brief, marine sediment and dried *S. japonica* were used as the microbial source and substrate, respectively. Microbes in the marine sediment were acclimated by fed-batch cultivation with repeated addition of dried brown algae to the sediments at 1 wt% of water amount. Thirteen cycles of cultivation were used; anaerobic water was added at the beginning of the 11th round to decrease the salinity and to examine the effect of salinity on the rate of methane production. The cultivation was conducted in triplicate. The salinity at the beginning of 13th round culture was equivalent to approximately 4% NaCl.

Batch cultivation was conducted before starting semi-continuous cultivation to examine the methane productivity. The substrate was added at 1.5 g TS to the starter culture (approximately 200 g) in 700-mL vials. Anaerobic water prepared under N_2/CO_2 (80:20) was added so that the materials added had a TS content of 10 wt%, equivalent to the TS content of raw brown algae. Cultures were incubated statically at 37 °C in triplicate.

Semi-continuous cultivation was conducted under 3 consecutive conditions of varying OLR and HRT as follows: condition 1, OLR of 2.0 ± 0.1 g VS substrate/kg culture/day and HRT of 39.2 ± 1.9 days; condition 2, OLR of 2.9 ± 0.2 g VS substrate/kg culture/day and HRT of 27.8 ± 1.6 days; and condition 3, OLR of 1.7 ± 0.02 g VS substrate/kg culture/day and HRT of 45.8 ± 0.8 days (Fig. 1a). Culture was removed semi-continuously using a tube (3-mm internal diameter) connected with a 20-mL syringe, and replaced with fresh materials (substrate and anaerobic water) with a substrate-TS content of 10 wt%. The cultures were incubated statically at 37 °C and conducted in triplicate.

The OLR was calculated from the VS of substrate added, the weight of the culture measured at the start of cultivation with fresh materials, and the period of cultivation. The OLR at each cultivation with fresh materials was the average of triplicate cultures. The total OLR during cultivation in each of the cultivation conditions was expressed as the average ± standard deviation (SD). The HRT was calculated from the ratio of the weight of fresh materials to the weight of culture measured at the start of cultivation and the period of cultivation. The HRT at each cultivation with fresh materials was the average of triplicate cultures. The total HRT during cultivation in each of the cultivation conditions was expressed as the average ± SD.

The methane yield in the semi-continuous process was calculated as the slope of the plot of the cumulative amount of methane produced against the cumulative amount of substrate added.

For measurement of acetoclastic methanogenic activity, 9.4 mL of medium containing sodium acetate used as substrate (Miura et al., 2014) and 0.6 mL of culture from the first round of semi-continuous cultivation under condition 3 were mixed to make up 10 mL of culture containing 5 g/L of sodium acetate. Triplicate cultures were incubated statically in a test tube at 37 °C. The activity was measured under different salinity levels of 0%, 1%, 2%, 3%, and 4% NaCl. The ratio of substrate to inoculum was 5 to 1 on the basis of the VS. The acetoclastic methanogenic activity was calculated from the plot of acetate concentration against cultivation day by fitting a third-polynormal trendline. The maximum slope of the trendline was calculated as the activity.

2.4. Chemical analyses

The VS, COD, VFAs, methane, the rate of methane production, and salinity were analyzed as reported previously (Miura et al., 2015). Briefly, the VS content of the substrate and culture was measured after incineration at 600 °C of the TS, which was dried at 105 °C. The VFAs were analyzed by high-performance liquid chromatography using crotonate as the internal standard. The methane content was measured using a gas chromatograph and the total gas volume was determined by substituting the gas with water in a cylinder. The rate of methane production was calculated using the modified Gompertz equation from plots of the methane yield. The salinity was calculated from the standard curve of conductivity and the concentration of NaCl. The pH of the culture supernatant was measured by using a pH meter (LAQUAtwin B-712; Horiba, Kyoto, Japan).

2.5. Microbial community analysis

Triplicate culture samples were taken from the starter culture and at the end of culture under conditions 1, 2, and 3 using an 18-gauge needle, and they were stored at −20 °C. These samples