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Evaluation of marine sediments as microbial sources for methane production from brown algae under high salinity

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HIGHLIGHTS

- Marine sediments effectively converted brown algae to methane at seawater salinity.
- All activities needed for methane production were detected under saline conditions.
- The marine sediments possessed higher activity than sources of non-marine origin.
- Marine sediments were microbial sources suitable for the methane production.

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ABSTRACT

Various marine sediments were evaluated as promising microbial sources for methane fermentation of *Saccharina japonica*, a brown alga, at seawater salinity. All marine sediments tested produced mainly acetate among volatile fatty acids. One marine sediment completely converted the produced volatile fatty acids to methane in a short period. Archaeal community analysis revealed that acetoclastic methanogens belonging to the *Methanosarcina* genus dominated after cultivation. Measurement of the specific conversion rate at each step of methane production under saline conditions demonstrated that the marine sediments had higher conversion rates of butyrate and acetate than mesophilic methanogenic granules. These results clearly show that marine sediments can be used as microbial sources for methane production from algae under high-salt conditions without dilution.

1. Introduction

Methane can be produced from biomass by anaerobic microbial conversion. Many organic components included in biomass, e.g., sugar, protein, and lipid, can be utilized for methane production. Among biomass types, marine macroalgae are an attractive feedstock for methane fermentation because they do not compete with agricultural land, and they have a lower lignin content than terrestrial lignocellulose, resulting in facile degradation (Wei et al., 2013). In marine macroalgae, however, the high salt content in the biomass can inhibit microbial methane production (Demirbas, 2010). Wet marine macroalgae contain approximately 3% ash (Roesijadi et al., 2010); thus, their salt concentration can reach 3%, which is similar to the salinity of seawater, during methane production. Dilution of marine macroalgae with water can solve this problem; however, such dilution leads to an increase in the cost of methane production because of the increased use of water (Shi et al., 2014). Thus, it would be ideal to use marine macroalgae as the substrate for methane production without dilution.

To produce methane from marine macroalgae without dilution, salt-tolerant microorganisms are needed. One possible approach is to use marine sediments as the source of the microorganisms. It has been reported that methane was produced from green algae in seawater by using a marine sediment and a sludge of nonmarine origin, and the methane yield obtained by using the marine sediment was higher than that obtained by using an inoculum of nonmarine origin (Schramm and Lehnberg, 1984). Thus, it was considered that marine sediments could be effectively used as microbial sources for methane production from macroalgae at seawater salinity.

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Methane is produced from organic matter in three steps, namely, hydrolysis/acidogenesis, acetogenesis, and methanogenesis. In the first step, organic matter is converted to volatile fatty acids (VFAs) by various bacteria. In the second step, VFAs other than acetate, namely propionate and butyrate, are converted to acetate and hydrogen by propionate- and butyrate-oxidizing bacteria, respectively. In the final step, hydrogen and carbon dioxide are converted to methane by hydrogenotrophic methanogens, and acetate is converted to methane and carbon dioxide by acetoclastic methanogens. Differences in the activity in the three steps affect the production of methane. Thus, it is important to understand the activity involved in methane production. However, for methane production from macroalgae by marine sediments at seawater salinity, there have been no reports on the conversion rate at each step of methane production.

In this study, therefore, various marine sediments were used as microbial sources for methane production from marine macroalgae at seawater salinity to measure the activity in three steps of methane production. *Saccharina japonica*, a brown alga and the most-produced macroalgae in the world (Jung et al., 2013), was used as the substrate for methane production. Methane production was conducted in a medium containing 30 g NaCl/L, which was similar to seawater salinity (Feijoo et al., 1995). Furthermore, the conversion rates of the second and third steps in methane production from the brown alga were measured to identify the rate-limiting step.

2. Methods

2.1. Materials

Dried *S. japonica* was purchased from a commission agent handling seafood. The total solid (TS), volatile solid (VS), and ash content of the brown alga were 94.2, 73.3, and 21.0 wt.%, respectively. The chemical oxygen demand (COD) of the dried seaweed was 1,030 mg/g-TS. Alga milled to a size less than 0.7 mm was used in the experiments. Marine sediments and mesophilic methanogenic granules were used as microbial sources as listed in Table 1.

2.2. Cultivation

Microbial sources were added to a container, and dilution medium in an anaerobic state was added. The anaerobic state was induced by heating in a boiling water bath for 30 min and cooling on ice with continuous bubbling of N_2/CO_2 (80:20) for 30 min. The medium was dispensed to the container containing the sediments with continuous bubbling of CO_2. The container was sealed with butyl rubber and then incubated at 37 °C.

The dilution medium used for the cultivation of microbial sources with the brown alga had the following composition: $(NH_4)_2SO_4$, 5 g/L; $Na_2MoO_4{\cdot}2H_2O$, 0.12 g/L; $Fe(NH_4)_2SO_4{\cdot}6H_2O$, 0.039 g/L; $Co(NO_3)_2{\cdot}6H_2O$, 0.029 g/L; $CaCl_2{\cdot}2H_2O$, 0.021 g/L; $MgSO_4{\cdot}7H_2O$, 0.25 g/L; $NaHCO_3$, 10 g/L; NaCl, 30 g/L; vitamin solution, 10 mL/L; and trace element solution, 10 mL/L. Marine sediments that contained 1 g of TS or mesophilic granules that contained 0.03 g of TS were added to a test tube with 10 mL of the dilution medium that contained the alga at a concentration of 5 g/L. The tube was incubated with shaking. For subculture, 5 mL of the culture was inoculated to 50 mL of medium in a 125-mL vial.

Table 1
Microbial sources used in this study.

Microbial source	Origin	TS[a] content (%)	VS[b] content (%)
Mesophilic granule	UASB[d] reactor	5.5	4.3
MS[c]-1	Ariake sea	34.7	3.6
MS-2	Tokyo Bay site A	79.3	2.0
MS-3	Tokyo Bay site B	69.0	1.4
MS-4	Tokyo Bay site C	67.3	2.9
MS-5	Hiroshima Bay	55.9	5.0

[a] Total solid.
[b] Volatile solid.
[c] Marine sediment.
[d] Upflow anaerobic sludge blanket.

The dilution medium used for cultivation with VFA or hydrogen had the following composition: NH_4Cl, 1 g/L; KH_2PO_4, 0.3 g/L; $CaCl_2{\cdot}2H_2O$, 0.08 g/L; $MgCl_2{\cdot}6H_2O$, 0.1 g/L; $KHCO_3$, 4 g/L; NaCl, 30 g/L; vitamin solution, 10 mL/L; and trace element solution, 10 mL/L.

Acetogenesis was conducted with the dilution medium that contained propionate or butyrate at a concentration of 5 g/L; 10 mL of hydrogen-consuming culture from marine sediments and 5 mL of hydrogen-consuming culture from the mesophilic granules were added to a 125-mL vial with 40 and 20 mL of the medium, respectively. Hydrogen-consuming culture was obtained as described below to prevent inhibition of hydrogen-forming oxidation of VFAs by residual hydrogen partial pressure. The vial was incubated under static conditions.

For hydrogenotrophic methanogenesis, marine sediments that contained 2.5 g of TS and the mesophilic granules that contained 0.63 g of TS were added to a 125-mL vial with 25 and 12.5 mL of the dilution medium, respectively. The headspace of the vial was flushed with N_2/CO_2 (80:20), and then pressurized with H_2/CO_2 (80:20) to 1.5 or 2 atm. The vial was incubated with shaking.

Acetoclastic methanogenesis was conducted with dilution medium that contained acetate at a concentration of 5 g/L. Marine sediments that contained 10 and 60 g of TS were added to a 125-mL vial with 50 mL of the medium and a 750-mL vial with 300 mL of the medium, respectively. Mesophilic granules that contained 0.25 g of TS were added to a 125-mL vial with 25 mL of the medium. The vial was incubated under static conditions.

The specific conversion rate was estimated from the maximum slope calculated using an approximation formula. The formula was obtained by fitting a third-order polynomial trendline to the experimental data points using Microsoft Excel.

The VFAs produced in the subculture were converted to COD, and the amount of VFA–COD per unit amount of VS of the inoculum was plotted against the incubation period to calculate the VFA formation rate. In other conversions, the substrates consumed were converted to substrate-COD per unit amount of VS of inoculum to calculate the substrate consumption rate.

2.3. Analytical methods

TS content was measured by drying wet marine sediment at 105 °C for >4 h. VS content was calculated as the ash-free dry weight. Ash content was measured by heating dried marine sediment at 600 °C for 3 h. COD was analyzed by a commercially available kit according to the manufacturer's protocol (Hach, Loveland, CO, USA).

VFAs and other intermediate organic substances were quantified by high-performance liquid chromatography (LC-2000 Plus HPLC; Jasco, Tokyo, Japan) equipped with a refractive index detector (RI-2031 Plus; Jasco), Shodex RSpak KC-811 column (Showa Denko, Kanagawa, Japan), and a guard column (Shodex RSpak KC-G; Showa Denko) at 60 °C. Ultrapure water containing 0.1% (v/v) phosphoric acid was used as the mobile phase at a flow rate of 0.7 mL/min. Crotonate was used as an internal standard.

The gas composition in the headspace of the container was analyzed by gas chromatography (GC-8A; Shimadzu, Kyoto, Japan) equipped with a thermal conductivity detector and a stainless steel column packed with activated carbon, at 70 °C. Argon was used as