Oceans as bioenergy pools for methane production using activated methanogens in waste sewage sludge

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Highlights

- CO₂ dissolved in seawater can be a carbon source for methane production.
- Methane energy was generated from seawater (carbonate ion) by enriched methanogens.
- Microbial communities adapted to seawater salinity improved methane production.
- 81% of 13CH₄ was generated from microbial conversion of NaH13CO3.

Graphical Abstract

The dissolved CO₂ that causes ocean acidification has great potential for bioenergy production. In this study, we demonstrate that activated methanogens in waste sewage sludge (WSS) are useful for converting bicarbonate in seawater into methane. These activated methanogens were adapted in different seawater sources for methane production through repeated batch experiments that resulted in an increase of 300–400 fold in the methane yield. During these repeated batch experiments, the microbial communities in WSS adapted to the high salinity of seawater to generate more methane. Microbial community analysis showed the dominance of Achromobacter xylosoxidans, Serratia sp. and methanogens including Methanobacterium sp., Methanosarcina sp., and Methanosaeta concilii. Using a 13C-labeled isotope, we demonstrate that 81% of the methane is derived from microbial conversion of NaH13CO3 in artificial seawater.

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1. Introduction

Oceans cover 71% of the Earth’s surface and hold 97% of the terrestrial water [1]. Oceans contain dissolved materials and ions, microorganisms, and dissolved gases from the atmosphere. The oceans absorb one third of the atmospheric carbon dioxide (CO2) derived from anthropogenic activity which then acts as the main contributor for ocean acidification [2,3]. The amount of dissolved CO2 has been increasing each year, and it is easier for CO2 to dissolve in water at lower temperatures [2]. CO2 dissolution in water produces carbonic acid (H2CO3), hydrogen ions (H+), bicarbonate ions (HCO3−), and carbonate ions (CO32−) by the following reactions: CO2 + H2O → H2CO3 → H+ + HCO3− which cause excess protons in the form of H+ which then acidifies the ocean [4]. The increment in CO2 dissolution in seawater is indicated by the reduction in marine pH by 0.3–0.4 pH units since ocean pH is predicted to be reduced from pH 8.1 in 2000 to pH 7.7 in 2100 with the corresponding increase in dissolved organic carbon (11–20%) and bicarbonate (17–20%) [5]. Of course, ocean acidification affects many marine ecosystems [5].

In general, the ocean is the best carbon sink since the dissolved carbon is used to make coral reefs in marine sediments. Calcium carbonate also precipitates biologically by the reaction of CaCO3 − → CO2 + Ca2+ to form the shells and skeletons of marine organisms [3,6]. In seawater, the ratio of dissolved carbon species is 0.5% [CO2]: 86.5% [HCO3−]: 13% [CO32−] so bicarbonate is the dominant species while dissolved CO2 is present in small concentrations [7].

World energy demands require renewable energy sources to replace fossil fuel to facilitate sustainable development [8]. Methane gas is colorless, odorless, safe, and has proven to be a good energy source for electricity and power generation [9,10]. Moreover, methane gas can be used as a substrate for other value-added products such as methanol and other hydrocarbons [11,12]. During anaerobic degradation of high molecular weight organics, methane evolution occurs in four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [13]. Different microbial communities including Bacteria and Archaea are involved by chemolithotrophic activity in order to produce methane [14]. Biological methane production is cost effective by using waste sewage sludge (WSS) that has been enriched with different kinds of microorganisms [15]. In marine environments, many attempts have been made to produce methane using microalgae for oil production [16] as well as in deep ocean basins by taking advantage of the available carbon in marine sediments and their Archaea [17]. Much research has been conducted utilizing organic carbon available in WSS as a source of carbon for methane [18,19]. However, to the best of our knowledge, no studies have been conducted for methane production from seawater by taking advantage of CO2 dissolution and carbonic species accumulation. The usual limitation is the salinity constraints that affect methanogens in seawater [20]. We have developed methods for producing enriched methanogens that capture CO2 gas and convert it into methane [15]. Thus, in this study, we explored the possibility of methane production from bicarbonate in seawater by using our enriched terrestrial methanogens from WSS.

This paper demonstrates that enriched methanogens that were grown under a limited carbon condition (grown for 50 days until all organic carbons were depleted) are capable of capturing carbon from seawater. A 13C labelled isotope of NaHCO3 was used in artificial seawater to show the potential of our enriched consortia in assimilating carbonate species from seawater. Therefore, this work demonstrates that methane production from seawater by enriched methanogens may provide renewable energy as well as provide the benefit of reducing ocean acidification.

2. Materials and method

2.1. Sludge source and preparation of the enriched methanogen inoculum

Waste sewage sludge was obtained from the Hiagari wastewater treatment plant in Kitakyushu, Japan. The sludge was washed three times using distilled water and the supernatant was discarded after centrifugation at 8000g for 10 min. The total solids content in the washed sludge was adjusted to 5% (wet sludge pellet, w/v) with distilled water prior to the preparation of the inoculum (enriched methanogens). The total volume of 30 mL 5% (w/v) waste sewage sludge was added to tightly crimped 66 mL serum vials to provide anaerobic conditions. To enrich the bacterial culture for methanogens in the samples, the inoculum was sparged with hydrogen gas for two minutes. The vials were incubated at 37°C at 120 rpm, and the methane concentration in the headspace of the vials was measured by gas chromatography for 50 days. The details of the procedure and characteristics of raw sludge as well as the enriched methanogens were presented in our previous study [15].

2.2. Methane production at different pH in NaHCO3

To see the potential of carbonate to produce methane by the enriched methanogens, 4 g/L NaHCO3 was adjusted to different pH (6, 7, 8 and 9). Five ml of the enriched methanogens were added to 25 ml of different initial pH of 4 g/L NaHCO3 in independent, 66 ml, tightly-crimped serum vials. The vials were purged with nitrogen gas for two minutes to remove dissolved oxygen followed by hydrogen sparging for another two minutes. The vials were incubated at 37°C at 120 rpm, and the methane concentration in the headspace of vials was measured by gas chromatography for 15 days. The same initial pH conditions were utilized with the same concentration of NaCl as control experiments.

2.3. Seawater sampling and artificial seawater preparation

Seawater samples were taken from four different locations: (i) Port Dickson, Negeri Sembilan, Malaysia (May 1st, 2014), (ii) Ashiya, Kitakyushu, Japan (April 21st, 2014) (iii) Hibikinada, Kitakyushu, Japan (April 21st, 2014) and (iv) Tsunoshima, Yamaguchi, Japan (August 13th, 2014). The pH and metal content of the different seawater samples are shown in Table 1. Artificial seawater was prepared according to Dana et al. with 35 ‰ salinity (35 g/L) [21].

2.4. Detection of 13C/12C ratio in methane from NaHCO3

Two sets of experiments were performed using 0.196 g/L NaH13CO3 in water and 0.196 g/L NaH13CO3 mixed in the artificial seawater according to Dana et al. [21]. Both vials were inoculated...
with 15 mL of active enriched methanogens from WSS. The control was prepared by using autoclaved enriched methanogens. The vials were sparged with nitrogen for two minutes and hydrogen for another two minutes. The vials then were incubated at 37 °C with shaking at 120 rpm. 13CH4 production was determined as the 13C/12C ratio in the headspace of the vials by a stable isotope ratio mass spectrometer (SIRMS) as described by Wang et al. [22].

2.5. Repeated batch fermentations for methane production from seawater

Methane production was conducted in three repeated batch fermentations. Fifteen mL of enriched methanogens were centrifuged at 18,000 g for 10 min at room temperature (25 °C). The supernatant was discarded, and the pellet was mixed with 5 mL of distilled water prior to inoculation into 25 mL of seawater in a tightly crimped serum vial. The vials were purged with N2 gas for 2 min to provide anaerobic conditions followed by H2 for 2 min to provide electrons for methane production. The vials were incubated at 37 °C at 120 rpm for 20 days prior to the methane production assay. For the second batch fermentation, the contents of the vials were centrifuged again at 18,000 g for 10 min at 25 °C. The pellet was mixed with 5 mL of distilled water prior to inoculation into 25 mL of the same seawater sources used in the first batch fermentation in a tightly crimped serum vial. The vials were purged and incubated under the same conditions as the first batch fermentation for methane. This cycle was repeated again for the third batch fermentation.

2.6. Analytical methods

Methane, hydrogen, and carbon dioxide gas were analyzed using a GC-3200 gas chromatograph (GL Sciences, Japan) equipped with a thermal conductivity detector. Helium was used as a carrier gas at 100 kPa while current was set at 100 mA. Methane and hydrogen gas were analyzed by using a Molecular Sieve 13X 60/80 mesh column, SUS 2 m × 3 mm ID (GL Science, Japan). The oven temperature was set at 40 °C, while the injector and detector temperatures were set at 50 °C and 65 °C, respectively. Carbon dioxide was analyzed by a WG-100SUS 1.8 m × ø 0.37 OD column (GL Science, Japan). The oven, injector, and detector temperatures were set at 50 °C, while the injector and detector temperatures were set at 50 °C and 65 °C, respectively. Carbon dioxide was analyzed by using a HG-10SUS 1.8 m × ø 0.37 OD column (GL Science, Japan). The oven, detector, and detector temperatures were set at 50 °C and 65 °C, respectively. Carbon dioxide was analyzed by using a HG-10SUS 1.8 m × ø 0.37 OD column (GL Science, Japan). The oven, detector, and detector temperatures were set at 50 °C and 65 °C, respectively. Carbon dioxide was analyzed by using a HG-10SUS 1.8 m × ø 0.37 OD column (GL Science, Japan). The oven, detector, and detector temperatures were set at 50 °C and 65 °C, respectively. Carbon dioxide was analyzed by using a HG-10SUS 1.8 m × ø 0.37 OD column (GL Science, Japan). The oven, detector, and detector temperatures were set at 50 °C and 65 °C, respectively. Carbon dioxide was analyzed by using a HG-10SUS 1.8 m × ø 0.37 OD column (GL Science, Japan). The oven, detector, and detector temperatures were set at 50 °C and 65 °C, respectively. Carbon dioxide was analyzed by using a HG-10SUS 1.8 m × ø 0.37 OD column (GL Science, Japan). The oven, detector, and detector temperatures were set at 50 °C and 65 °C, respectively. Carbon dioxide was analyzed by using a HG-10SUS 1.8 m × ø 0.37 OD column (GL Science, Japan). The oven, detector, and detector temperatures were set at 50 °C and 65 °C, respectively. Carbon dioxide was analyzed by using a HG-10SUS 1.8 m × ø 0.37 OD column (GL Science, Japan). The oven, detector, and detector temperatures were set at 50 °C and 65 °C, respectively. Carbon dioxide was analyzed by using a HG-10SUS 1.8 m × ø 0.37 OD column (GL Science, Japan). The oven, detector, and detector temperatures were set at 50 °C and 65 °C, respectively.
mer extension was performed at 72 °C for 1 min. The amplification was then subjected to another 18 cycles of denaturation at 94 °C for 1 min, annealing at 57 °C for 1 min, and extension at 72 °C for 1 min. The final extension was then performed at 72 °C for 25 min. The amplification products were analyzed via a 2% agarose gel with ethidium bromide staining prior to their use in DGGE.

DGGE was performed using a NB-1480A instrument (EIDO, Japan) equipped with a water thermostatted temperature control system (Eyela NCB-1200). PCR DGGE samples were loaded on 8% polyacrylamide gels with a denaturant concentration of 30% to 57% gradient in 1× TBE buffer. DGGE was performed at 60 °C for 7 h at 50 V using a V-C Stabilizer (Mitsumi Scientific Industry Co., Ltd). The gel was stained with ethidium bromide for 45 min, viewed under UV light, and photographed using the GelScene Tablet Imaging System (ASTEC). DGGE bands were excised from the gel and stored in 10 µL sterile Milli-Q water overnight at 4 °C. The excised bands then were centrifuged at 13,000 rpm for 30 s. The supernatant was used as a template using primer set 357f without a GC clamp and 518r. The PCR product was purified using the QIAGEN Gel Extraction Kit (cat# 28704, USA). The purified PCR products were sent for sequencing at FASMAC, Japan. The DNA sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) of the GenBank database through the website http://blast.ncbi.nlm.nih.gov/Blast.cgi. The sequences were aligned with ClustalW through the website http://www.genome.jp/tools/clustalw/ to identify the nearest relative.

2.8. Microbial community analysis by Illumina MiSeq

DNA extraction was performed using the PowerSoil DNA Isolation kit (MO BIO Laboratory Inc, Cat#12800-50). PCR amplification used primers 27F and 1492R with the same ingredients and PCR reaction as mentioned above. The V2 region of the samples were amplified using Nextera XT DNA Library Preparation Kit according to the manufacturer’s protocol. The data generated from Illumina MiSeq were deposited into NCBI short read archive database under accession number SRP105375.

3. Results and discussion

3.1. Methane production from bicarbonate

A preliminary study was conducted using 4 g/L NaHCO₃ in water (0.196 g/L NaHCO₃ is available in artificial seawater) [21] at different pH (6.0, 7.0, 8.0, and 9.0) using enriched methanogens from WSS to demonstrate that methane can be produced from carbonate sources at oceanic pH (around pH 7–8) [5]. In a previous study, we showed that enriched methanogens are able to sequester CO₂ gas by converting it to methane [15]. Thus, in this study, we wished to show that our enriched methanogens are able to utilize the NaHCO₃ in seawater as a carbon source for methane production. The concentration of bicarbonate (HCO₃⁻) at different pH were measured before and after the fermentation to ensure that methane was produced from the reduction of bicarbonate. Fig. 1a

Fig. 1. Methane production from bicarbonate species. (a) Methane (CH₄) yields (mol/mol) from bicarbonate (HCO₃⁻) reduction by enriched methanogens after 15 days of fermentation in 4 g/L NaHCO₃ in water at different initial pH, (b) Hydrogen reduction percentage during methane production at different initial pH, and (c) the percentage of 13C/12C [atom] of methane in the headspace of 0.196 g/L NaH13CO3 in water and 0.196 g/L NaH13CO3 in artificial seawater. No methane was produced in the control vials.
shows the conversion rate of methane (mol) from bicarbonate (HCO$_3^{-}$/CO$_3^{2-}$) (mol) reduction after 15 days of fermentation with NaHCO$_3$ in water. This is derived from the conversion of one mol of bicarbonate to one mol of methane [24]. The results show that methane was produced in all fermentation vials with 4 g/L NaHCO$_3$ in water at different pH values. However, no trace methane was found in the control vials when NaCl was used instead of NaHCO$_3$ indicating that no methane can be produced without bicarbonate. The enriched methanogens acted as a catalyst in the reaction to produce methane by the exergonic reaction of HCO$_3^{-}$ + 4H$_2$ + H$^+$ → CH$_4$ + 3H$_2$O ($\Delta G^\circ = -136$ kJ/mol) [24]. Since methanogens are active in a narrow pH range (pH 6.5–8.3) [25], as expected, the highest methane production from bicarbonate occurred at an initial pH of 7 (0.93 ± 0.06 mol CH$_4$/mol HCO$_3^{-}$) compared to an initial pH of 8 (0.72 ± 0.02 mol CH$_4$/mol HCO$_3^{-}$) and an initial of pH 6 (0.70 ± 0.01 mol CH$_4$/mol HCO$_3^{-}$) (Fig. 1a). Hence, these results demonstrate the potential of using seawater as for methane production since oceanic pH is around pH 7–8 [5].

Fig 1(b) shows the extent of hydrogen reduction throughout the fermentation. The active methanogens utilized hydrogen faster at pH 7 which corroborates the higher conversion rate of bicarbonate ion to methane at this pH. As expected, the slowest hydrogen reduction was found in fermentations with an initial pH of 6. For the overall experiment, hydrogen was completely utilized starting from 9 days of fermentation. Throughout this experiment, low amounts of CO$_2$ were produced (in the range of 17–55 mol/L) during days 1–3 of the fermentation for all of the experiments, then CO$_2$ was not present in the headspace after 3 days of fermentation. This corroborates our previous studies that showed the enriched methanogens assimilate CO$_2$ for methane production during fermentation [15]. Due to very low amount of CO$_2$ being produced, the methane yield was calculated based on bicarbonate ion reduction during this experiment.

In the preliminary study, it was found that relatively high concentrations of bicarbonate in water can be converted to methane (Fig. 1a). Next, we investigated whether the enriched methanogens can produce methane from more realistic concentrations of NaHCO$_3$ in seawater. To corroborate that methane comes from carbonate in seawater, two sets of experiments were conducted using a carbon isotope ($^{13}$C) with two different media, both containing 0.196 g/L NaH$^{13}$CO$_3$: the first experiment was conducted using water, while the second experiment was conducted using artificial seawater. Both experiments were inoculated with active enriched methanogens from WSS. The dead cell negative control was prepared using autoclaved enriched methanogens. Fig. 1c shows the percentage of $^{13}$C/$^{12}$C (isotopic fractionation of stable isotope carbon-13 ($^{13}$C) and carbon-12 ($^{12}$C)) in the headspace of methane in water and artificial seawater. The results show that the percentage of $^{13}$C/$^{12}$C in the headspace from the enriched methanogens was 100% and 81% atom $^{13}$C/$^{12}$C methane from water and artificial seawater, respectively. The lower methane conversion in artificial seawater shows that the salt content in artificial seawater inhibited the methanogenesis process [20,26]. Therefore, our study shows that active methanogens in WSS are able to utilize
carbonate species in NaHCO₃ for the production of methane. No trace of ¹³CH₄ was found in the control vials indicating that no bicarbonate was converted to methane without active methanogens.

3.2. Repeated batch fermentations for methane production

High salt content in seawater inhibits methane production by methanogens since Zhang et al. [26] reported that methanogenesis is inhibited when the salt content exceeds 55 g/L. Therefore, seawater with 35‰ (35 g/L) [21] salt content was considered feasible as a feedstock for methane production. To favor the production of methane from carbon dissolved in seawater, the microorganisms in the enriched WSS were acclimatized to the higher salinity by using repeated batch reactor fermentations and different sources of seawater. The tightly crimped vials were anaerobically incubated for 20 days for methane production for each batch fermentation cycle. Then, the enriched methanogens adapted to the high salinity of seawater were collected and subjected to a new cycle containing fresh seawater. Distilled water was used instead of seawater as a negative control. In the repeated batch fermentation experiments (three cycles), very low methane was detected in the control vial. In contrast, methane production improved from batch to batch for all the seawater sources (Fig. 2a).

Fig. 2b shows the changes of hydrogen and methane during the repeated batch fermentations with artificial seawater. The methane production corresponded with the amount of hydrogen reduction for each repeated batch fermentation. In addition, not all of the supplied hydrogen was consumed during the repeated batch fermentations. During the first batch fermentation, 156 ± 3 μmol of hydrogen was consumed and 24 ± 8 μmol of methane was produced indicating that 6 mol of hydrogen was utilized for production of 1 mol of methane. However, the pattern changed during the second and third batch fermentations when 500 ± 91 and 553 ± 27 μmol of hydrogen were consumed, evolving 197 ± 17 and 230 ± 18 μmol of methane, respectively. These results indicate that only 2.5 and 2.4 mol of hydrogen were required for 1 mol of methane during the second and third batch fermentations, respectively. Therefore, the expected theoretical yield 1 mol of methane produced from 4 mol of hydrogen through the reaction of HCO₃⁻ + 4H⁺ + H⁺ → CH₄ + 3H₂O did not support the experimental yield directly due to several biological factors. First, the diverse anaerobic microbes might consume hydrogen at the first stage and able to convert it to methane during the second and third stage of methane production. In addition, carbonic species in artificial seawater might produce hydrogen ions by the reaction of H₂CO₃ (aq) → H⁺ + HCO₃⁻ (aq) → 2H⁺ + CO₂⁻ (aq) which then can contribute to methane evolution during the second and third batch fermentations [4,7].

Table 2 summarizes the overall process (inputs and outputs) for methane production (per liter of artificial seawater) at the different stages of the repeated batch fermentations. The results indicate the first stage of methane production involves the adaptation of the enriched methanogens to the saline conditions of seawater, thus consuming low hydrogen (140 mL H₂/L seawater) and producing low methane (20 mL CH₄/L seawater). However, the system was stable during the second and third stage of the repeated batch fermentations which require 450–470 mL of H₂ to evolve 190–210 mL of CH₄ from 1 L of seawater. The calculated inputs and outputs show that this system can be profitable when the cost of hydrogen energy is $3/L and generates methane with a maximum net profit of $4.10 (when the methane price is $17.13/L) from 1 L of seawater. Even though the cost of hydrogen might be an issue for methane production in this system, hydrogen can be produced more economically by using wind turbines, solar panels, water electrolysis, or photovoltaic cells [15]. In addition, in the case when hydrogen storage and hydrogen purification systems are not available, hydrogen can be made directly from seawater. To make this system more profitable, H₂ can also be produced through ionized hydrogen ions using seawater as a feedstock [4]. Another advantage of our system is that the enriched methanogens can sequester CO₂ [15]. Therefore, the mixture of hydrogen and CO₂ necessary for this process can be directly injected into the system without the requirement of a hydrogen purification system. In the proposed process, the best HRT (hydraulic retention time) for methane production from seawater is 15–20 days for stable operation. Unlike other methods for methane production such as gasification [27], hydrothermal pretreatment of biomass [28] and pressurized biofilm anaerobic reactors [29] which require high temperatures and pressures, the cost of methane production from seawater using enriched methanogens can be considered as inexpensive. Thus, this system can be considered in the future for the solution of ocean acidification impact and energy production.

3.3. Microbial community analysis

In our previous study in which we produced methane from CO₂ captured by active methanogens in WSS instead of seawater, we showed that enriched methanogens are able to sequester CO₂ to methane [15]. We found through RNA-based quantitative real time PCR that the archaeal community in the enriched methanogens are still active even with the limited amount of carbon. Here, 16S rRNA microbial community analysis of the enriched methanogens was investigated by Illumina MiSeq to understand more details regarding the bacterial community during methane production from seawater.

The presence of large amounts of salt is generally known to inhibit the growth of non-marine microorganisms [26]. However, after repeated batch fermentations (Fig. 2a), the non-marine microorganisms of the WSS adapted to the high salinity of the seawater. We examined the microbial community in (i) the control, (ii) 0.196 g/L NaHCO₃ in water, (iii) 0.196 g/L NaHCO₃ in artificial seawater, and other seawater sources taken from (iv) Port Dickson, Malaysia, (v) Hibikinada, Japan, and (vi) Ashiya, Japan during first.
cycle in the batch reactors by denaturing gradient gel electrophoresis (DGGE) (Fig. 3a). The DGGE results show that different band patterns and intensities were observed. Detailed analyses of each band shows that the bacterial communities can be grouped into four phyla that include Proteobacteria, Firmicutes, Bacteria, and Bacteroidetes (Fig. 3b). Each phylum has been seen previously in other environmental samples, particularly in activated sludge systems [30]. Furthermore, the phylum Firmicutes which is dominated with Tissierella sp. and Clostridium sp. in the fermentation culture, are well-known hydrogen producing bacteria in the anaerobic reaction mixture [31,32].

The selected samples were then subjected to Illumina MiSeq analysis for further detailed analysis. Overall, 365,000 high quality reads were obtained with an average of 73,000 ± 26,000 reads per sample. Fig. 4 shows the relative abundance of each microbial taxa from the MiSeq analysis from the five different samples after the first batch cycle in (i) the control, (ii) artificial seawater, and seawater from (iii) Ashiya, Japan, (iv) Tsunoshima, Japan, and (v) Port Dickson, Malaysia. The results demonstrate that all samples were comprised of different types of microbial taxa. Control samples (without the addition of any seawater) show the diverse types of microbes in the community. Meanwhile, the microbial community in seawater after the first batch fermentation contains the dominant species of Achromobacter xylosoxidans and Serratia sp. Different kinds of methanogens were also represented such as Methanobacterium sp., Methanosarcina sp., and Methanosaeta concilii. A. xylosoxidans which were dominant in artificial seawater (38%), seawater from Ashiya, Japan (37.5%), and seawater from Port Dickson, Malaysia (37.7%). A. xylosoxidans is a marine halotolerant bacterium which was used to degrade polycyclic aromatic hydrocarbons (PAHs) [33]. The increasing amount of Serratia sp. also can be seen from its increase from 9% in the control to 21%, 29%, and 12% in artificial seawater, seawater from Ashiya, Japan, and seawater from Port Dickson, Malaysia, respectively. Gupta and Thakur [34] reported that Serratia sp. is one of the indigenous microorganisms in sewage sludge that degrades wastewater contaminants. An increment in methanogens was also observed in the enriched methanogens in seawater. No dominant methanogens were detected in the control sample. However, the number of methanogens increased to 36% in seawater from Tsunoshima, Japan which comprised of 18% of Methanobacterium sp., 15% of Methanosarcina sp., and 3% of Methanosaeta concilii. The growth and performance of the methanogenic bacteria is crucial in influencing the anaerobic digestion process for methane production [35].

3.4. Economic and environmental benefits

The ocean is the biggest carbon sink in the world [36]. The increased amount of atmospheric CO2 dissolved in the ocean has increased the amount of carbon available in the ocean basin for methane production. The total volume of carbon in the form of dissolved organic matter in the oceans is about 700 teragrams which is 200 times more than the total carbon in marine organisms [37]. The amount of total dissolved carbon species then is about 4 teragrams of CO2, 606 teragrams of HCO3−/CO2, and 90 teragrams of CO3−/CO2 in the oceans [7,37]. Therefore, the total amount of carbon in the ocean is 8 times higher than that in fossil fuels including coal, oil, and gas [38]. Hence, the use of carbonate in the ocean for...
methane production is an exciting approach since it will be a renewable resource; i.e., methane from ocean bicarbonate will be combusted to CO₂, which will then be re-dissolved into the ocean. The current technologies, such as pyrolysis, gasification, and liquefaction, that use non-renewable resources, create resource scarcity and increase the impact of global warming. In addition, the thermal and energy input for these processes is another disadvantage [39]. In contrast, our process does not require high energy input, high temperature, or electricity. Also, the current trend in industry is methane production from renewable resources such as from industrial biomass to create a ‘zero-discharge’ strategy through fermentation [40]. Methane production from carbon species available in seawater is a natural renewable resource, and our approach is also useful for mitigating ocean acidification. Hence, our proposed technology has the benefits of economic sustainability via a long term energy source and reduced global warming. This study also shows the diverse and unique features of using a microbial community in WSS for carbonate ion assimilation in seawater to produce methane.

4. Conclusions

The study successfully demonstrates that methane may be produced from seawater as a carbon source. Methane production from carbonate reduction was proven with 100% and 81% [¹³C/¹²C] atom basis] methane from NaH¹³CO₃ in water and artificial seawater, respectively. Furthermore, the methane was produced at a realistic marine pH. We also show that around 300–400 fold higher methane yield is achieved from seawater sources compared to the control experiments indicating that the methane produced stems with available carbonate in seawater. Also, the salinity constraints of the methanogens was overcome by repeated batch cycles of methane production in the high salinity of seawater. Thus, methane from seawater may make a significant contribution in regard to economic and environmental benefits for a low carbon footprint society.

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