Applied Energy 202 (2017) 399–407

Contents lists available at ScienceDirect

Applied Energy

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

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

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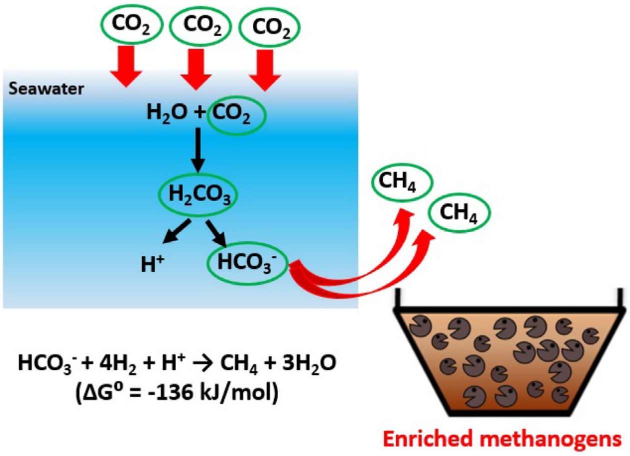
HIGHLIGHTS

- CO_2 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 $^{13}CH_4$ was generated from microbial conversion of $NaH^{13}CO_3$.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 April 2017
Received in revised form 14 May 2017
Accepted 26 May 2017

Keywords:
Carbon dioxide
Ocean acidification
Methanogens

ABSTRACT

The dissolved CO_2 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, Serrati* sp. and methanogens including *Methanobacterium* sp., *Methanosarcina* sp., and *Methanosaeta concillii*. Using a ^{13}C-labeled isotope, we demonstrate that 81% of the methane is derived from microbial conversion of $NaH^{13}CO_2$ in artificial

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http://dx.doi.org/10.1016/j.apenergy.2017.05.171

Waste sewage sludge
Methane

seawater. Therefore, this study shows that oceans, with the largest surface area on Earth, have a potential as a substrate for methane energy production via an acclimated consortium approach.

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 (CO_2) derived from anthropogenic activity which then acts as the main contributor for ocean acidification [2,3]. The amount of dissolved CO_2 has been increasing each year, and it is easier for CO_2 to dissolve in water at lower temperatures [2]. CO_2 dissolution in water produces carbonic acid (H_2CO_3), hydrogen ions (H^+), bicarbonate ions (HCO_3^-), and carbonate ions (CO_3^{2-}) by the following reactions: $CO_2 + H_2O \leftrightharpoons H_2CO_3 \leftrightharpoons H^+ + HCO_3^- \leftrightharpoons 2H^+ + CO_3^{2-}$ which cause excess protons in the form of H^+ which then acidifies the ocean [4]. The increment in CO_2 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 of 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 $CaCO_3 \leftrightarrow CO_3^{2-} + Ca^{2+}$ to form the shells and skeletons of marine organisms [3,6]. In seawater, the ratio of dissolved carbon species is 0.5% $[CO_2]$: 86.5% $[HCO_3^-]$: 13% $[CO_3^-]$ so bicarbonate is the dominant species while dissolved CO_2 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 chemolitotrophic 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 CO_2 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 CO_2 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 ^{13}C labelled isotope of $NaHCO_3$ 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 over 40 days with N_2 every 4 days. Then, the N_2 was replaced by H_2 every 4 days for about 10 days to remove any residual CO_2 in the headspace of the vials. The same procedure was conducted until no methane was detected in the vials. Therefore, the methanogen enrichment procedure takes about 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 $NaHCO_3$

To see the potential of carbonate to produce methane by the enriched methanogens, 4 g/L $NaHCO_3$ 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 $NaHCO_3$ 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 $^{13}C/^{12}C$ ratio in methane from $NaHCO_3$

Two sets of experiments were performed using 0.196 g/L $NaH^{13}CO_3$ in water and 0.196 g/L $NaH^{13}CO_3$ mixed in the artificial seawater according to Dana et al. [21]. Both vials were inoculated