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# Electromethanogenic $CO_2$ conversion by subsurface-reservoir microorganisms

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**Abstract**

To develop a technological system to add substantial value to CCS operations, we are proposing a system to employ a new bio-electrochemical reaction, called "electromethanogenesis", to convert geologically-stored $CO_2$ into methane, a recycled energy source. In this study, we showed that microorganisms derived from a subsurface reservoir were electromethanogenically active. Moreover, the microbial consortium selectively enriched based on electrochemical activity had the highest electromethanogenic activity reported so far. Thus, our study indicated that, for the electromethanogenic conversion of geologically-stored $CO_2$, recruitment of microorganisms endogenous to the reservoir was an effective strategy.


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Keywords: methane, microbes, electromethanogenesis, CCS, Bioelectrochemical systems

## 1. Introduction

According to the IEA BLUE Map scenario, it is estimated that 100 Carbon dioxide Capture and Storage (CCS) projects need to be deployed by 2020 and over 3000 projects by 2050 to reduce green house gas emissions by 50% by 2050 [1]. However, the deployment of CCS is limited to only eight fully-integrated operations at the present (Sleipner, Snohvit, In Salah, Weyburn, Shute Creek, Val Verde, Enid Fertilizer and Century projects [2]) and largely affected by legal and regulatory aspects, public acceptance and financial issues. To contribute the furtherance of CCS deployment, one long-term goal of our

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laboratory is to develop technological systems to add substantial value to CCS operations. Particularly, we are proposing a system to employ a new bio-electrochemical reaction, called “electromethanogenesis”, to convert geologically-stored $CO_2$ into methane within the reservoir. Resulting methane can be produced from the $CO_2$-storage reservoir and utilized as a fuel gas.

Bio-electrochemical systems (BESs) are emerging biotechnologies applicable to energy generation/conversion, wastewater treatment, biosensor and bioremediation. A common feature of BESs is the utilization of microorganisms to catalyze electrochemical reactions at solid electrodes [3]. “Electromethanogenesis” is a recently-reported process, in which, at the cathode of BESs, methanogenic microorganisms (collectively called methanogens) produce $CH_4$ by reducing $CO_2$ with electrical current as a reducing-power source.

However, molecular mechanism of this reaction is yet to be elucidated. Several studies have supported the hypothesis that methanogenic archaea can directly utilize proton and electron (Eq. 1) [4].

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \quad (1)$$

During the electromethanogenic process, no significant hydrogen evolution was detected. However, there is still a formal possibility that the process involves transient formation of molecular hydrogen (Eq. 2) [5], which is then immediately utilized for $CO_2$ reduction (Eq. 3) by hydrogenotrophic methanogen. The formation of molecular hydrogen (Eq. 2) can occur as an abiogenic electrochemical reaction and/or also be catalyzed microbially.

$$2H^+ + 2e^- \rightarrow H_2 \quad (2)$$

$$CO_2 + 4\mathrm{H}_2 \rightarrow CH_4 + 2H_2\mathrm{O} \quad (3)$$

It has been reported that the conversion efficiency of electrons (current) consumed at the cathode into methane was as high as 96% [6]. Thus, electromethanogenesis is highly-effective energy-conversion reaction, in which electrical energy is used to reduce $CO_2$ and stored in the form of $CH_4$. By applying electromethanogenesis for the conversion of geologically-stored $CO_2$, the $CO_2$-storage reservoir can be used as a storage cite of massive energy. Such technology can be particularly useful for utilization of intermittent electrical energy from renewable power sources (such as solar and wind).

Although the electromethanogenic system can recruit microorganisms either indigenous to the reservoir or exogenously injected, indigenous microorganisms will be practically a better choice to employ, as they are more likely to maintain the activity within the reservoir, their native environment. However, electromethanogenesis has been found only recently [6, 7], so electromethanogenic activity of subsurface microorganisms have never been reported. To examine the technical feasibility of electromethanogenesis for the subsurface application, we examined electromethanogenic activity of microbial consortium derived from a subsurface reservoir. The subsurface microbial consortium, which was selectively enriched based on their electrochemical activity, showed the highest electromethanogenic activity reported so far. Thus, this result suggested that, for the electromethanogenic conversion of geologically-stored $CO_2$, recruitment of microorganisms endogenous to the reservoir was an effective strategy.

## 2. Materials and Methods

### *2.1. Microbial materials*

Formation water was anoxically collected from an almost-depleted petroleum reservoir (located in Akita prefecture, Japan) and used as a microbial source. Subsurface microorganisms in the water sample were first propagated at 55℃ for 2 weeks with pre-sterilized *Methanobacterium* medium [NITE medium #1067; containing 0.136 g $KH_2PO_4$, 0.54 g $NH_4Cl$, 0.2 g $MgCl_2 \cdot 6H_2O$, 0.147 g $CaCl_2 \cdot 2H_2O$, 2.5 g $NaHCO_3$, 0.5 g cysteine · HCl, 0.5 g $Na_2S \cdot 9H_2O$, 0.8 g sodium acetate, 0.2 g yeast extract, 1 mg resazurin, 6.4 mg EDTA, 62 mg $MgSO_4 \cdot 7H_2O$, 5.5 mg $MnSO_4 \cdot H_2O$, 10 mg NaCl, 1 mg $FeSO_4 \cdot 7H_2O$, 1 mg $Co(NO_3)_2 \cdot 6H_2O$, 1 m g $CaCl_2$, 1 mg $ZnSO_4 \cdot 7H_2O$, 0.1 mg $CuSO_4 \cdot 5H_2O$, 0.1 mg $AlK(SO_4)_2$, 0.1 mg $H_3BO_3$, 0.1 mg $Na_2MoO_4 \cdot 2H_2O$, 0.01 mg $Na_2SeO_3$, 0.1 mg $Na_2WO_4 \cdot 2H_2O$, 20 μg biotin, 20 μg folic acid, 100 μg pyridoxine-HCl, 50 μg thiamine-HCl, 50 μg riboflavin, 50 μg nicotinic acid, 50 μg Ca-pantothenate, 10 μg *p*-aminobenzoic acid, 0.1 μg vitamin $B_{12}$ (per liter)] under anoxic condition [$N_2/CO_2$ (80/20)].

### *2.2. Enrichment of electrochemically-active microorganisms by using Microbial Fuel Cell*

Microbial Fuel Cell (MFC) is a type of BESs, in which electrical current is generated from decomposition of organic matter by electrochemically-active microorganisms (Fig. 1) [8]. In this study, MFC was used to enrich electrochemically-active microorganisms in the formation water. Two-chamber MFC reactors were constructed using two 300 ml grass bottles. The anion-exchange membranes (Nafion 117, DuPont Co.) were treated with $H_2O_2$ (10 %), $H_2SO_4$ (5%) and deionized water and inserted between two chambers as a separator. The anode and cathode were both plain carbon felt (40 $cm^2$: Tsukuba Materials Information Laboratory, Tsukuba, Japan), which were connected to the circuit via titanium wires (0.5 mm in diameter: Alfa Aesar, Ward Hill, MA, USA). The resistance between the electrodes and titanium wires were less than 3 Ω.

25 ml of the culture was inoculated into the anode chambers of MFCs, followed by 225 ml fresh anaerobic pre-sterilized *Methanobacterium* medium (from which $Na_2S$, cysteine and resazurin were excluded). 50 mM potassium ferricyanide solution supplemented with 2.5 g $L^{-1}$ $NaHCO_3$ was used as the catholyte. The headspaces of reactors were filled with $N_2/CO_2$ (80/20). An external resistance (100 Ω) was connected between the electrodes. The voltage across the external resistance was monitored automatically by an Agilent 34970A data acquisition unit (Agilent Technologies, Santa Clara, CA, USA) every 5 minutes. Each reactor was operated in a fed-batch culture continuously stirred by a magnetic stir bar and incubated at 55℃. The reactors were then sealed with butyl-rubber stoppers and incubated anaerobically.

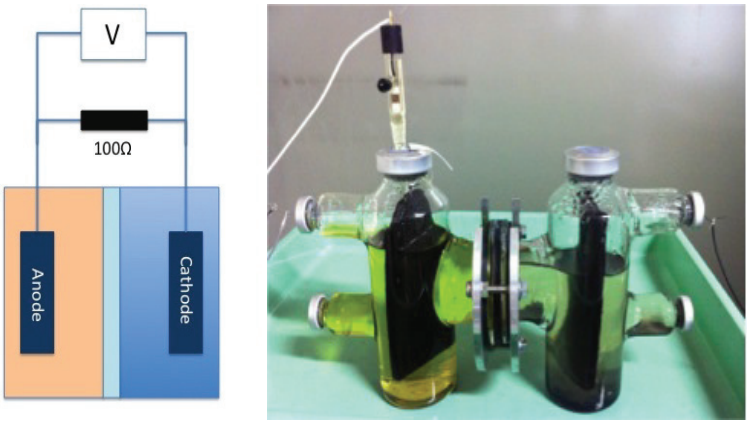


Fig. 1. A schematic drawing (left) and picture (right) of the MFC reactor

### 2.3. Assay of electromethanogenic activity of the subsurface microorganisms

Single-chamber BES reactors were constructed using 250 ml serum bottles (Maruemu, Osaka, Japan). The anode and cathode were both plain carbon felt (40 $cm^2$: Tsukuba Materials Information Laboratory, Ibaraki, Japan), which were connected to the circuit via titanium wires. 100 ml of anoxically-prepared *Methanobacterium* medium ($Na_2S$, cysteine and resazurin were excluded) was aliquoted into the reactors. The medium contained 10 mM acetate as a main organic substrate. 10 ml of the effluent from the MFC anode chamber was inoculated into each reactor. The reactors were then sealed with butyl-rubber stoppers and incubated anaerobically with a gas mixture of 80% $N_2$: 20% $CO_2$ at 55°C without agitation. Constant voltage of 0.75V was applied to the reactor using a digital power supply (Array 3645A: Array Electronics, Nanjing, China) with the positive pole connected to the anode and the negative pole to the cathode of reactor (Fig. 2).

A gas chromatography [GC-2014 with a Shincarbon ST column (6 m × 3 mm ID); Shimadzu, Kyoto, Japan] was used to monitor methane production in the reactors. A fixed external resistance (1.0 Ω) was connected between the anode and the cathode. To measure the current produced in the reactor, the voltage across the fixed external resistance was monitored by using a multimeter (34970A, Agilent Technologies, Santa Clara, CA, USA).

The conversion rate from current to methane was calculated by the following equation [9]. F is Faraday constant (96485 C $mol^{-1}$) and I is the current (A).

$$methane\ recovery\ rate\ (\%) = \frac{the\ amount\ of\ electron\ incorporated\ into\ methane}{the\ amount\ of\ electron\ comsumed\ in\ the\ circuit} \times 100$$

$$= \frac{the\ amount\ of\ methane\ production\ (mol) \times 8}{\int_{t=0}^{t} I / 2F} \times 100 \quad (4)$$

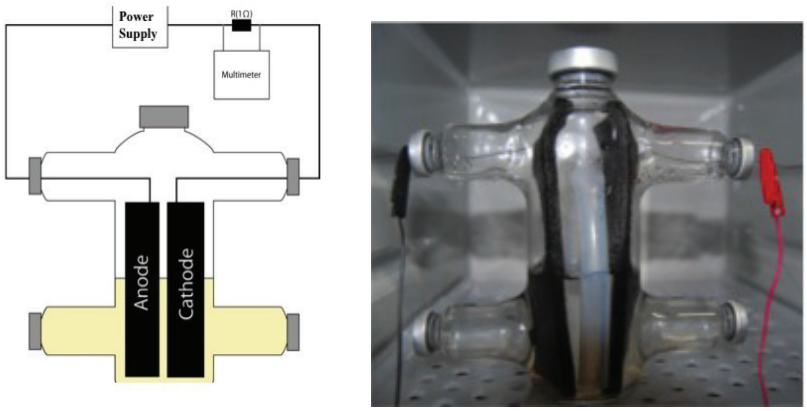


Fig. 2. A schematic drawing (left) and picture (right) of the single-chamber BES reactor

## 3. Results and Discussion

### *3.1. Collection and propagation of the subsurface microorganisms*

To examine electromethanogenic activity of subsurface microorganisms, microbial samples (formation water) were collected from a production well in an oilfield. Currently, the reservoir is almost depleted of crude oil, producing large amounts of water together with crude oil (the ratio of the volume of the produced formation water relative to the total volume of the produced fluid was < 95%), and can be therefore a future reservoir of CCS operation in Japan. The reservoir is a formation of tuffaceous sandstone of Miocene-Pliocene age, located around 1293 to 1436 m under the surface, with *in situ* temperature of 40-82°C and pressure of 5 MPa. The formation waters were directly collected from sampling valves located on the well head into sterile sampling bottles.

To augment the biomass, the formation water was first incubated in the *Methanobacterium* medium. Our previous observation indicated that such incubation step could promote acclimation of subsurface microorganisms to laboratory conditions and significantly shorten the lag phase. Then, the pre-acclimated sample was used as the inoculum.

### *3.2. Enrichment of electrochemically-active microorganisms derived from the reservoir brine*

A two-chamber MFC was used to selectively enrich microorganisms with electrochemical activity. It has been shown that, as the anode electrode was only electron acceptor, the microbes capable of extracellular electron transfer (collectively called “exoelectrogens”) could preferentially propagate in the anode chamber of MFC. Culture derived from the formation water was inoculated into the anode chamber of MFC. The MFC reactor showed active current production from one day post inoculation, indicating propagation of electrochemically-active microorganisms (data not shown). Electrochemically-active microbial consortium was further enriched by repeating fed-batch incubation in MFC and inoculated into the single-chamber BES reactors for electromethanogenesis assay.

### *3.3. Electromethanogenic activity of subsurface microorganisms*

The BES reactors inoculated with the electrochemically-enriched consortium were incubated with an applied voltage of 0.75 V. No molecular hydrogen was exogenously supplied to the reactors. In the first cycle of the fed-batch process (data not shown), methane production was initiated around 26 hours post inoculation. Regardless of the long lag time, the methane production was in an applied-voltage-dependent manner.

At the second cycle of the fed-batch process (Fig. 3a), methane was actively produced directly after the initiation of incubation (i.e. after the medium exchange). The methane production rate was 386 mmol $day^{-1}m^{-2}$ (subjected to cathode surface area), which was the highest electromethanogenic production rate so far documented. No significant amount of methane was produced in control reactors without applied voltage (data not shown). Current generation was nearly proportional to the methane production rate and also depended on the applied voltage (Fig. 3b), suggesting that the current produced in the circuit was consumed for the methane production. The current-to-methane conversion rate was as high as c.a. 98%. No significant production of methane or current was detected in the control reactor without applied voltage (0 V in Fig. 3).

To further examine the effect of applied voltage on the methane production, a range of voltages (from 0.4 to 0.8 V) were applied to the electromethanogenic reactors (data not shown). With an applied voltage of 0.5 V or lower, the methane production rate was indistinguishable from the control with no applied

voltage. With applied voltages of 0.6 to 0.8 V, the methane production rate was significantly higher than that of the no-applied-voltage control and the relationship between the applied voltage level and the methane production rate was linear. The current-to-methane conversion rates were ≧95%, regardless of the applied voltage levels.

Thus, our results indicated that the microbial consortium derived from a subsurface reservoir was highly capable of electromethanogenesis and suggested that microorganisms endogenous to the reservoir could be recruitable for the electromethanogenic conversion of geologically-stored $CO_2$. To establish such subsurface $CO_2$-conversion technology based on electromethanogenesis, we are currently studying the microbial mechanism of the reaction by the reservoir microorganisms. Preliminary analysis of the cathodic microbial population suggested that a hydrogenotrophic methanogen and an exoelectrogenic bacteria were the dominant species (data not shown). This observation suggested an electromethanogenic pathway mediated by a previously unknown cooperative interaction between methanogens and exoelectrogens. BES reactors using the co-cultures of those dominant species will be constructed to examine this hypothesis.

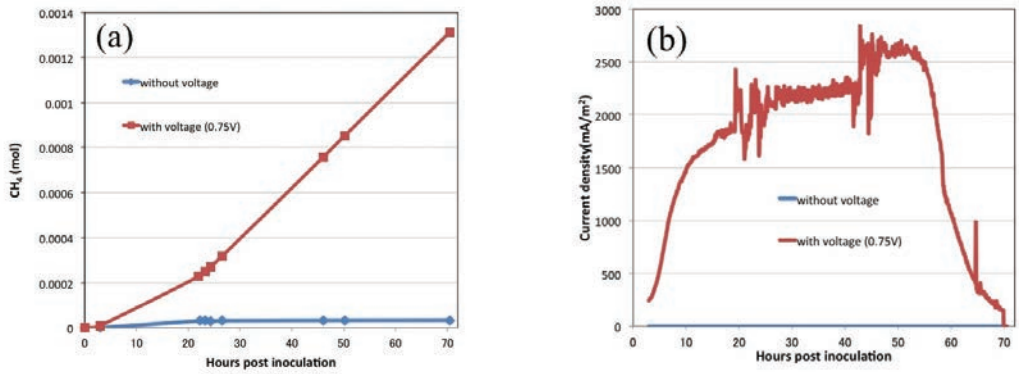


Fig. 3. Methane (a) and current (b) production in the single-chamber BES reactors inoculated with microbial consortium derived from the reservoir brine.

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