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Simultaneous methanogenesis and acetogenesis from the greenhouse carbon dioxide by an enrichment culture supplemented with zero-valent iron

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ABSTRACT

The microbial reduction of CO_2 into value-added products is gaining considerable attention and can play a significant role in the field of environment and energy research. A novel strategy for biotransformation of CO_2 was tested with zero valent iron (ZVI) and enrichment cultures for methane and acetate production under anaerobic conditions at room temperature. The favorable performance of CO_2 conversion (81.67% of conversion rate) was achieved in ZVI-amended treatments by enhanced methanogenesis and acetogenesis simultaneously. The enrichment consortium of microorganisms containing *Methanosarcina* spp. and *Clostridiaceae* was responsible for methane and acetate production, and accounted for 25.89% and ~4.83% of CO_2 conversion, respectively. Scanning electron microscopy (SEM) observation and mass balance analysis of hydrogen detected in the headspace indicated that direct electron transfer and utilization possibly occurred with these microbes, especially methanogens. Interestingly, X-ray Photoelectron Spectroscopy (XPS) confirmed carbonation mineral ($FeCO_3$) as the major strategy of CO_2 consumption under the experimental conditions. These observations collectively revealed that supplementation of ZVI can be a favorable electron donor to stimulate and accelerate the biotransformation of CO_2 into methane and acetate by the enrichment culture of microorganisms, and the information presents available alternative biochemical pathways for energy recovery from greenhouse gas under anaerobic conditions.

1. Introduction

Carbon dioxide (CO_2), as a main greenhouse gas with a continuous increase of its concentrations in the atmosphere, contributes to global climate change, and requires major measures to reduce CO_2 emissions [1–3]. CO_2 can be converted into chemical compounds for use in further synthesis or value-added products for reuse and long-term storage; these strategies play an extremely important role in addressing this emerging green carbon science issues [4] and achieving the sustainable development goal [5–8]. Among different strategies available currently, biological transformation of CO_2 into the clean energy methane attracts considerable attention in recent years and also plays a significant role in the knowledge of geomicrobiology and biogeochemistry [1,9], in which economical and environmentally friendly means of CO_2 reduction can be realized for energy recovery from greenhouse emission gas [10]. From this point of view, CO_2 is also a readily available carbon resource on the Earth [11], and each 1 kt of CH_4 consumed would be translated into the conversion product of 2.8 kt of CO_2 [1].

Methanogenesis is the terminal process of anaerobic biomass degradation with the ubiquitous occurrence in a wide range of niches and conditions [12,13]. Methanogens are ubiquitous microorganisms that are found in a wide range of anaerobic environments on the Earth [14] including Antarctica [15], hot spring [16], oil reservoir [12,17] and wastewater [18]. H_2 and CO_2 are the most common and important substrates for methanogenesis and they

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are converted into methane by hydrogenotrophic methanogens ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$, $\Delta G^{\circ\prime} = -131$ kJ/mol) as the most favorable reaction in comparison with acetoclastic and methylotrophic methanogenesis [13,19]. Interestingly, both H_2 and CO_2 can also be the precursors for acetate synthesis as an alternative and competing pathway for H_2 sink and energy conservation [20,21], which is mediated by homoacetogens ($4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$, $\Delta G^{\circ\prime} = -95$ kJ/mol). It is generally known that these two pathways occur simultaneously for carbon reducing and energy conservation [13,21]. A microbial consortium capable of methanogenesis and homoacetogenesis containing diverse methanogens *Methanosarcina* spp. and *Clostridiaceae* for acetate production from CO_2 was enriched from Xinjiang oil reservoir (21 °C) and was tested for transformation of CO_2 to variety of value-added products. Using the mixed consortia as an inoculum for CO_2 bioreduction has the major advantage in economic cost and efficiency for simultaneous product yields (CH_4 and CH_3COOH) compared with pure cultures [11].

Available electron donors are obligatory and crucial for the two biochemical CO_2 conversion pathways [1,14,21]. Zero valent metals, specifically zero valent iron (ZVI) as reductive materials, can serve as alternative electron donors for methanogenesis to improve performance of methane production by variety of methanogens [22–24]. For the past few years, ZVI has been widely used as the promoter in both anaerobic digestion and environmental contaminant remediation, and was believed to enhance methanogenesis [25–32]. Moreover, ZVI can lower the oxidation-reduction potential (ORP), buffer organic acids and thus provide a suitable environmental condition for the growth of anaerobic microorganisms [25,33–35]. Fe^{2+} generated from the reaction between Fe^0 and H_2O under anaerobic conditions is a positive participant in carbonate mineral formation as one of the major processes in the long-term management of global carbon cycle [36]. Therefore, it is reasonably assumed that ZVI is capable of facilitating simultaneous methanogenesis and acetogenesis from CO_2 by the enrichment culture, and possibly allows CO_2 utilization through multiple biochemical pathways under anaerobic conditions. However, the available knowledge about ZVI-driven CO_2 transformation under anaerobic conditions is very limited.

The objectives of the present research were to gain a better understanding on the performance of ZVI for CO_2 reductive transformation under anaerobic conditions at ambient conditions and potential biological energy recovery from greenhouse gas CO_2 by microbial enrichment consortium from oilfield. To accomplish these objectives, several microcosms were established with the enrichment culture as inoculum not previously acclimated to the ZVI-amended system. The activities of methanogenesis and acetogenesis were periodically assessed, and morphology and transformation products of ZVI in the enrichment cultures were monitored at different stages of the entire incubation period.

2. Materials and methods

2.1. Samples and enrichment

The inoculum for anaerobic incubation experiments was enriched from production water of Kexia No. 6 block in Xinjiang oilfield of China under strictly anaerobic conditions. Geochemical characteristics of this water from oilfield production are summarized in Table S1. The oil reservoir is located 480 m below the sea level and has a pressure of 7.58 MPa. The *in situ* temperature is about 21 °C and the mineralization of the sample is 4212 mg/l. An archaea affiliated to *Methanosarcina* and two kinds of bacteria belonging to *Clostridiaceae* and *Porphyromonadaceae* were identified in the enrichment consortium. The archaeal sequence was compared to the NCBI database (https://www.ncbi.nlm.nih.gov/) by using Basic Local Alignment Search Tool (BLAST) and showed high identity (100%) to members of the species *Methanosarcina mazei* affiliated to the order *Methanosarcinales*. Within the bacterial identified, sequences exhibited the highest similarity with 16S rRNA gene sequence from *Clostridiaceae* (100%) [37] from oil sands tailings (*Youngiibacter multivorans* at 97%) and *Proteiniphilum* sp. S2 (99%) in the phylum of *Firmicutes* and *Bacteroidetes*. The obtained 16S rRNA gene sequences of the microbial consortium were submitted to GenBank database under accession numbers MF623316-MF623318.

2.2. Enrichment culture

The above enriched microbial consortium was cultured as inoculum for the further research use. Before the experimental transfer, a basal medium containing (g/l): NaCl, 2.0; $MgCl \cdot 6H_2O$, 0.4; NH_4Cl, 0.25 and KCl, 0.5 was prepared and autoclaved. Then, it was placed on magnetic stirring hot plate to cool under continuous flushing of pure N_2 gas stream, followed by addition of 0.0001 g/l of resazurin. When cooled to the room temperature, the remaining ingredients (g/l): $CaCl_2 \cdot 2H_2O$, 0.1; KH_2PO_4, 0.75; $K_2HPO_4 \cdot 3H_2O$, 1.375; $Na_2S \cdot 9H_2O$, 0.5; vitamin solution (1 ml/l) and trace elements (10 ml/l) after filtration through the 0.22-μm-pore-size membrane filter were added into the above medium and the final pH was adjusted to 7.0 with dilute HCl or NaOH. The trace elements stock solution contained (g/l): $CoCl_2 \cdot 6H_2O$, 0.50; $CuSO_4 \cdot 5H_2O$, 0.01; $FeCl_2 \cdot 4H_2O$, 1.50; H_3BO_3, 0.02; $MnCl_2 \cdot 4H_2O$, 0.50; $Na_2MoO_4 \cdot 2H_2O$, 0.01; $NiCl_2 \cdot 6H_2O$, 0.20; $CaCl_2 \cdot 2H_2O$, 0.10 g; $AlCl_3$, 0.01; $ZnCl_2$, 0.50 and HCl (25%), 4 ml. The vitamin stock solution contained (mg/l): vitamin B_{12}, 100.0; D(+)-biotin, 20.0; folic acid, 20.0; nicotinic acid, 50.0; *p*-aminobenzoic acid, 100.0; Ca-D(+)-pantothenate, 50.0; pyridoxin·2HCl, 500.0; riboflavin, 50.0; thiamin·2HCl, 100.0 and thioctic acid, 50.0. Thirty ml of the basal medium were transferred into an autoclaved serum bottle (internal volume 120 ml) after flushing with pure N_2 gas and then sealed with a butyl rubber stopper (Bellco Glass, Inc., Vineland, NJ, USA) and aluminum crimp seal according to the Hungate technique [38]. About 2 ml of the inoculum were taken from a stock and injected into the above serum bottles by sterile syringes.

In this study, a total of six treatments were prepared: 1) $Fe^0 + CO_2$ (G1): 0.165 g of ZVI powder were added into the medium as an alternative electron donor with a mixture gas (CO_2:N_2 = 20%:80%) filling the headspace of each serum bottle (five replicates); 2) CO_2 (G2): the headspace was filled with the same mixture gas (CO_2:N_2 = 20%:80%) without addition of ZVI powder (five replicates); 3) Fe^0 (G3): the headspace was filled with pure N_2 amended with 0.165 g of ZVI powder (two replicates); 4) Blank control (G4): without addition of ZVI powder and flushed with pure N_2 in the headspace (two replicates); 5) $Fe^0 + CO_2$ as abiotic control (G5): 32 ml of the basal medium were transferred into a serum bottle with subsequent addition of 0.165 g of ZVI powder flushed with the mixture gas (CO_2:N_2 = 20%:80%) without injection of inoculum (two replicates); 6) CO_2 − abiotic control (G6): 32 ml of basal medium were transferred into a serum bottle flushing with the mixture gas (CO_2:N_2 = 20%:80%) in the headspace without addition of ZVI and injection of inoculum (two replicates). The specific surface area of ZVI powder (Sigma-Aldrich Co., Milwaukee, WI) in this study was 0.0741 m^2/g by using Brunauer-Emmett-Teller (BET) with an average particle diameter of 10–50 μm. All of the cultures were incubated at room temperature in the dark under strictly anaerobic conditions.

2.3. Quantitative PCR

The total genome DNA extracted from 10 ml of G1 at different