Abstract of Dissertation

Title: Biological methane production from electricity without organic substrates
(無機環境下での電気エネルギーからの生物学的メタン生成)

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The physical phenomena of generating electricity from natural minerals and the catalytic ability of microbes to bio-electrochemically produce methane through CO_2 reduction, may establish a new hypothesis about the possibility of biological methane production from the thermorelectricity in a natural environment. To evaluate this hypothesis, a two-chamber microbial electrosynthesis system (MES), in which no organic substrate was supplied, was applied at very low voltages. Initially, two electrodes of an MES were connected to a DC power supply. After that, the electric power will be developed due to the Seeback effect, which converts temperature gradients to electricity. In MES, the voltage applied to produce methane depends not only on the biocathode potential but also on the anode potential. When coupled with the oxidation of inorganic compounds such as NH_4^+ and HS^- with low potential at the anode, MES for methane production at very low applied voltage can be theoretically established without organic substrates. However, many MES studies have not provided sufficient information about the oxidation reaction on the anode, with experiments conducted at higher applied voltages (> 1.0V). To the best of our knowledge, this study is also the first to report the coupling of NH_4^+ and/or HS^- oxidation on the abiotic and/or biotic anode in a methane-producing MES. In addition, microbial community analysis of the biomass collected from bio-electrodes on the last day of MES operation, revealed the interactions between microbes and bio-electrodes as well as interspecies in bioelectrical methane production.

The more specific objectives corresponding to different experimental conditions of this study were:

a. To investigate the possibility of bioelectrical methane production coupled with abiotic NH_4^+ oxidation in an applied voltage range of 0.05–3.0 V

b. To investigate the possibility of bioelectrical methane production coupled with abiotic HS^- oxidation (MES; abiotic anode) at a low applied voltage.

c. To investigate the possibility of bioelectrical methane production coupled with biotic HS^- oxidation (MES; biotic anode) at a low applied voltage

The three MESs used for the three above objectives (corresponding with RUN 1, RUN 2, and RUN 3) consisted of two glass chambers, namely cathode and anode chamber. Two chambers were connected through a salt bridge (2% agar and 20% KCl) in RUN 1, and by the cation exchange membrane (Nafion EC-NM-211) in RUN 2 and RUN 3. A carbon cloth was

installed in both chambers as electrodes. The electrodes were connected to a DC power supply, and the generated current in the circuit was measure through a 100 Ω resistor. In all three MESs, a small amount of anaerobic sludge collected from a laboratory-scale UASB reactor was inoculated on the surface of the electrode, which worked as a biocathode. For the anode, the platinum powder was coated on the surface of the electrode as a catalyst in RUN 1 and RUN 2. However, in RUN 3, a small amount of the mix of anaerobic and aerobic sludge was inoculated on the surface of the bio-anode. The three MESs were filled with the inorganic substrates and operated in batch mode at 30°C. After each run, the volume of produce gas and their compositions were measured by a gas chromatograph (GC). Besides the recorded current value during a batch, analysis of the liquid samples at the start and end of each batch was performed. Particularly, microbial community analyses were performed for the biomasses taken from the bio-electrodes on the last day of the three MES operations.

The detailed results of RUN 1, RUN 2, and RUN 3 are described as follows:

Bioelectrical methane production coupled with an abiotic ammonia oxidation

Bioelectrical methane production was possible even at a very low voltage of 0.05 V in the MES without organic substrates, when coupling of NH_4^+ oxidation at the anode coated with platinum (Pt) powder. Combined with the useful information from other studies, the anodic product of electrolyzing ammonia on a Pt-anode was mainly NO_3^-. The salt bridge allowed the produced NO_3^- move to the cathode chamber for N_2 production. Additionally, microbial community in the biocathode analysis revealed that methane production occurred simultaneously with biological denitrification, through the three keys players: that are hydrogenotrophic methanogens of *Methanobacterium* and *Methanobrevibacter, denitrifiers of Azonexus*, and hydrogen-producing bacteria *Petrimonas*. This microbial community suggests that methane is indirectly produced without the use of electrons by methanogens.

Bioelectrical methane production coupled with an abiotic sulfur oxidation

Bio-electrical methane production occurred in the biocathode even in a very low applied of 0.1–0.8 V, where abiotic HS^- oxidation to SO_4^{2-} was achieved at the Pt anode. By using a cation exchange membrane placed between the biocathode and anode chamber, differing from the results of RUN 1, only methane gas was detected on the biocathode chamber. Based on the microbial community analysis of biomass enriched on the biocathode, the scheme of electron flow in methane production was proposed. First, electrotrophic H_2 was produced by syntrophic bacteria, such as *Syntrophorhabdus*, *Syntrophobacter*, *Syntrophus*, *Leptolinea*, and *Aminicenantales*, with direct acceptance of electrons at the biocathode. Subsequently, most of the produced H_2 was converted to acetate by homoacetogens, such as *Clotridium* and *Spirochaeta 2*. The detected hydrogenotrophic methanogens, including *Methanobacterium* and *Methanolinea* also used H_2 in methane production. However, the most domain microbes were

Methanosaeta, an acetoclastic methanogens and the main pathway to produce methane was acetate.

Bioelectrical methane production coupled with a biotic sulfur oxidation

The microbial anode was used as an alternative anode catalyst for sulfur oxidation. As expected, methane was also detected on the biocathode chamber in a low applied voltage range of 0.2–0.8 V. The microbial community of biomass collected from biocathode is the same as in RUN 2. This means that not only the most probable pathways for methane production can be explained like in RUN 2, but also the correctness of the results under the same experimental conditions was verified. For the bioanode, a microbial community was also analyzed to identify the process of bio-electrical sulfide oxidation. There are three processes of HS^- oxidation with the assistance of microbial catalyst: (i) HS^- was oxidized to sulfur cycle intermediate (SCI) by sulfur-oxidation microorganisms (SOM) such *as SB-5*, *Anaerolineaceae,* and *Spirochaetes;* (ii) *SCI* was oxidized to SO_4^{2-} by also sulfur-disproportionation microorganisms (SDM); and (iii) HS^- was oxidized to SO_4^{2-} by SOM such as *Rhodocyclaceae*, *Thiobacillacaeae*. Based on the high detection of the related microbes, the (i) and (ii) processes were predominant. In summary, the microbial communities on the biocathode and bioanode were detailed analyzed to propose the processes of methane production and sulfur oxidation in MES.

This study demonstrated that bioelectrical methane production at the biocathode was achieved even at low voltage (0.05, 0.1 V) when coupled with the oxidation of inorganic compounds such as NH_4^+ and HS^- with lower anodic potential than H_2O oxidation. The results of microbial community analysis with useful information from many previous studies indicated the roles of the microorganisms, which are abundant on the electrodes, in methane production. Combined with the feasibility of small thermoelectricity generation through the natural minerals that can function as thermoelectric materials, the findings of this study suggest the possible pathway of bio-electrical methane production in natural environments.