論 文 の 内 容 の 要 旨

論文題目　A Study on Thermophilic Bioelectrochemical Systems for CO_2–to–Methane Conversion Technology
（二酸化炭素-メタン変換技術の開発へ向けた好熱性バイオ電気化学的システムの研究）

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Bioelectrochemical systems (BESs) are promising technologies which use living microorganisms as biocatalysts to drive the reactions at the electrodes and have wide applications in wastewater treatment, electricity generation, H_2 production, biosensor and bioelectronics. Recently, it has been reported that CO_2 could be converted to CH_4 in a process called “electromethanogenesis”, in which methanogens attached on the cathode act as biocatalyst. Based on this technology, our lab proposed a promising application for BESs: to build a sustainable carbon cycle system by combining BESs and CO_2 dioxide capture and storage (CCS) technology together, aiming to convert the CO_2 stored in CCS reservoir to CH_4. Up to now, however, BESs still cannot be commercialized due to their low performance, not even the sustainable carbon cycle systems. Operating BESs at elevated temperatures is one measure to improve the performance of BESs, but there are only a few studies investigated thermophilic BESs. Compared with more than 20 species of mesophilic electrochemically-active microorganisms, there are only two species of thermophilic electrochemically-active microorganisms, *Thermincola potens* JR and *T. ferriacetica*, were reported to be capable of transferring electrons to anode. Therefore, expanding our knowledge of thermophilic BESs is desirable to improve the performance of thermophilic BESs. My thesis is mainly focused on the fundamental studies of thermophilic BESs, including the exploration of novel thermophilic electrochemically-active microorganisms and their application on H_2 production and CO_2 conversion to CH_4.

Firstly, to study the mechanism of CO_2 conversion to CH_4, we built a mesophilic single-chamber BES reactor aiming to produce CH_4 by inoculating the effluent of a mesophilic microbial fuel cell and adding 1.0 V voltage into the circuit. After inoculation, the current was generated in the single-chamber BES reactor, while there was no current observed in the abiotic control reactor with 1.0 V voltage. After one batch cycle, only CH_4 was produced in the inoculated BES reactor while there was no CH_4 or H_2 was observed in the abiotic control reactor

with 1.0 V voltage and inoculated control reactor (without applied voltage). The results showed that CH_4 was probably produced due to the electromethanogenesis reaction:

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \qquad \text{(Eq. 1)}$$

We also presented the first comprehensive phylogenetic analysis of both the biocathodic and bioanodic communities by constructing 16S rRNA clone libraries. The results showed that the composition of the cathodic microorganisms was significantly different with that in a previous report: no methanogen of the *Methanobacteriales* class was detected, and instead, a methanogen closely related to *M. bavaricum* of the *Methanomicrobia* class was the dominant methanogen. Moreover, it was suggested that an exoelectrogenic bacteria, *G. sulfurreducens*, was enriched on the biocathode. These observations indicated the possibility that diverse species of methanogens could catalyze electromethanogenesis on the biocathode. It has been shown that *G. sulfurreducens* is also capable of catalyzing hydrogen production using an electrode (cathode) as the electron donor. Thus, we think it is possible that, in the following stage of incubation, *G. sulfurreducens* established a cooperative relationship with the methanogen for the electromethanogenic reaction by first receiving electrons from the cathode for hydrogen formation and then providing the resulting H_2 to the methanogens for hydrogenotrophic methanogenesis. Alternatively, because it has recently been shown that *G. sulfurreducens* and related *Geobacter* species can directly transfer electrons to other microorganisms (including methanogens), it is also possible that *G. sulfurreducens* provided electrons (not molecular H_2) directly to the methanogen, which utilized the electrons in the electromethanogenic reaction (Eq. 1). The detailed mechanism needs to be further investigated by using pure culture as inoculum.

Secondly, to explore and identify novel thermophilic electrochemically-active microorganisms, two-chamber microbial fuel cells were built in this study. Thermophilic microorganisms from various sources, including thermophilic digestive sludge and oilfield formation water under different temperature, were used as the inoculum. These MFCs started up successfully and showed substantial power density generation, suggesting that electrochemically-active microorganisms (exoelectrogens) were enriched in the anode chambers of these microbial fuel cells. The maximum power density was obtained in the thermophilic MFC inoculated with Yabase oilfield formation water (1003 mW m^{-2}), higher than those reported with thermophilic MFCs in several previous studies (generally $\leqq$ 400 mW m^{-2}) and comparable to that of a thermophilic MFC under continuous mode of operation (1030 ± 340 mW m^{-2}). The electron transfer mechanisms between the electrochemically-active microorganisms and anodes were investigated by using medium exchange experiment and electrochemical methods (cyclic voltammetry). The results showed that all the electron transfer mechanisms (except the hyperthermophilic bioanodes) were direct electron transfer. The

microbial analyses of the bioanode in each reactor was analyzed by constructing gene-clone libraries. The results showed that *Firmicutes* and *Deferribacteres* phylum accounted for the majority in the microbial analyses of bioanodes. Based on the microbial analyses, two novel thermophilic exoelectrogens, *Caloramator australicus* strain RC3 and *Calditerrivibrio nitroreducens* Yu37-1, were tested in the experiment and proven to be capable of transferring electrons to anodes. Furthermore, a hyperthermophilic MFC was successfully started up by inoculating the hyperthermophilic microorganisms from the produced water of an oilfield. As the hyperthermophilic MFC could operate at the elevated temperature range between 75°C and 98°C, it has a potential application in industrial processes under extreme conditions. The microbial analysis showed that *Caldanaerobacter subterraneus* (subspecies *subterraneus* and *tengcongensis*, respectively) are the dominating bacteria. These results largely expanded our knowledge of thermophilic electrochemically-active microorganisms.

Thirdly, a thermophilic biocathode capable of H_2 production was for the first time built in this study. A single-chamber microbial electrolysis cell (MEC) reactor was firstly started up by inoculating the effluent of a thermophilic MFC inoculated with the thermophilic digestive sludge. At an applied voltage of 0.8 V, H_2 was produced in the inoculated single-chamber MEC reactor, while there was no H_2 measured in the abiotic control reactor (with 0.8 V) and the inoculated control reactor (without applied voltage), suggesting both the microorganisms and voltage are needed for the H_2 production. The cyclic voltammogram of the biocathode showed that the cathodic current of the cathode was significantly more negative than that of the anode, suggesting that the cathode have a relatively higher catalyzing activity for H_2 production. Thus the cathode in the single-chambered MEC was transferred into a two-chamber MEC reactor and further analyzed by using electrochemical methods. The linear sweep voltammetry (LSV) showed that the biocathode had a significant higher reducing activity than the control electrodes (bioanode or non-inoculated electrode). At the potential of –0.8 V vs. SHE, the thermophilic biocathode produced a current density of 1.28 A m^{-2} and an H_2 production rate of 376.5 mmol day^{-1} m^{-2}, which were around 10 times higher than those of the non-inoculated electrode, with the cathodic H_2 recovery of *ca*. 70 %. The molecular-phylogenetic analysis of the bacteria on the biocathode indicated that the community was comprised of six phyla, in which *Firmicutes* was the most populated phylum (77% of the clones in the 16S rRNA library). It was the first report of thermophilic biocathode capable of producing H_2, largely expanding our knowledge of thermophilic BESs.

Last, a thermophilic biocathode capable of converting CO_2 to CH_4 was for the first time built and its electron transfer mechanisms was investigated in this study. This biocathode was

firstly started up in a single-chamber reactor using the effluent of a thermophilic MFC inoculated with Yabase oilfield formation water as the inoculum. After start-up, the maximum CH_4 production rate of the biocathode was around 1103 mmol day^{-1} m^{-2}, which was much higher than that in previous studies (lower than 656 mmol day^{-1} m^{-2}) and the mesophilic biocathode (450 mmol day^{-1} m^{-2}) reported in this study. In addition, the current to CH_4 conversion efficiency was around 100% in the single-chamber BES reactor, suggesting a directly electron transfer mechanism. Then the biocathode was transferred into a two-chamber reactor for further analysis. At a set potential of –0.7 V vs. SHE, the biocathode was capable of converting CO_2 to CH_4 with an abiotic anode as the courter electrode and CO_2 as sole carbon source. The cyclic voltammogram (CV) of the biocathode showed a catalytic wave with a midpoint potential of –0.34 V vs. SHE in the range of –0.6 V ~ –0.3 V vs. SHE. In contrast, there was no significant peaks observed in the CV of the cell-free spent medium of the biocathode and the abiotic control electrode. In addition, the biocathode can produce CH_4 at a rate of 14 mmol day^{-1} m^{-2} with CO_2 as the sole carbon source at a set potential of –0.4 V vs. SHE. As the theoretical redox potential for H_2 production was –0.456 V at pH 7 at 55°C and no CH_4 or H_2 was detected in the absence of CO_2, it suggested that the H_2 evolution was not necessary for the conversion of CO_2 to CH_4 and the electron transfer was in a direct manner. Correspondingly, the midpoint potential of –0.34 V vs. SHE was responsible for the CO_2 reduction, which was probably due to the redox components (e.g. enzyme) on the surfaces of microorganisms. The morphology of the biocathode was also analyzed by the scanning electron microscopy (SEM), which showed that a thin layer of biofilm with relative homogeneous shape of microbial cells was formed on the biocathode. The microbial analyses showed that *Methanothermobacter thermautotrophicus* and *Thermincola ferriacetica* were the dominant species of archaea and bacteria, respectively. To investigate the functional role of the pure culture methanogen, *M. thermautotrophicus* was inoculated into a two-chamber BES reactor and the result showed that this pure culture was capable of accepting electrons from the cathode for CO_2 reduction by itself. However, the CH_4 production rate was lower than that of the mixed culture, which was probably due to the lack of supporting functions of other microorganisms, such as exoelectrogens.