主　論　文　の　要　約

論文題目　Humin-assisted CO_2 Reduction to Acetate by Anaerobic Microorganisms
(腐植ヒューミンで支援された嫌気性微生物による CO_2 の酢酸への還元)

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論　文　内　容　の　要　約

Gradual decrease in fossil fuel as the most currently used energy resource and the uncontrollable and negative impact of CO_2 emission on world climate change is urging researchers to find and develop alternative and sustainable technologies for mitigating anthropogenic CO_2 emissions and not excessively rely on fossil fuels. One of which, carbon capture and utilization technology that captures and converts CO_2 into value-added products such as acetate used as biofuel through catalytic or biological CO_2 conversion is an attractive and emerging technological strategy. Acetogens, strictly anaerobic microbes, reduce CO_2 to acetate using hydrogen as electron donor via Wood-Ljungdahl pathway. The Wood-Ljungdahl pathway does not consume energy, which is increasingly attracting many researchers. Microbial electrochemical synthesis (MES), a promising approach, has been typically designed for conversion CO_2 into acetate by employing electric energy from solar, wind, hydro, etc. as reducing equivalents and

electroactive acetogens as biocatalyst. In the MES, acetogens obtain electrons directly or indirectly from cathode to synthesize acetate from CO_2. The direct electron transfer based on MES has low performance of acetate production and less stability due to the limitations of the surface area and biocompatibility of the cathode for the development of biofilms. The indirect electron transfer based on MES using extracellular electron mediators including hydrogen or artificial soluble mediator for CO_2 reduction may avoid these issues. However, using artificial soluble mediators (2-hydroxy-1,4-naphthoquinone and neutral red), toxic chemicals, for improving efficiency of MES could affect negatively the health of operator, and have difficulties in collecting target products. In previous studies, humin, an innocuous and insoluble material, serve as extracellular electron donor for CO_2-fixing acetogenesis via the Wood-Ljungdahl pathway. Humin as electron mediator was employed in the enhancement of the microbial dechlorination of pentachlorophenol and denitrification in bioelectrochemical systems. Therefore, the study aimed to evaluate and elucidate CO_2-fixing acetogenesis using humin as extracellular electron donor in acetogens and then develop a humin-assisted MES system.

In chapter 2, effects of various chemical factors including iron sulfide (FeS), magnesium sulfate ($MgSO_4$), and sodium chloride (NaCl) on CO_2-fixation in the presence and absence of humin using two different consortia with acetogenic and methanogenic activities was evaluated. Only one consortium is capable of co-utilizing humin and hydrogen as electron donors to reduce CO_2 to acetate but preferring using electrons from humin than from hydrogen. Another consortium utilized hydrogen, not humin as electron donor, suggesting that humin was not a universal electron donor for CO_2-fixing acetogenesis. FeS accelerated the CO_2-fixing acetogenesis at 0.2 g/L as the optimal

concentration, but $MgSO_4$ inhibited at a concentration higher than 0.02 g/L. NaCl appeared to be inhibitory at concentrations higher than 6 g/L. Comparative amplicon sequence analysis suggested minor groups of *Clostridia* were the potential CO_2-fixing acetogenic bacteria using extracellular electrons from humin. Furthermore, the study found CO_2-fixing methanogenesis with *Methanobacterium* utilizing electron from humin only when hydrogen is present. The methanogenesis was accelerated by FeS at 0.2 g/L or higher concentrations, especially without humin, and with NaCl at 2 g/L or higher concentrations regardless of the presence of humin, while no significant effect was observed with $MgSO_4$.

In chapter 3, various acetogens including *Moorella thermoacetica* JCM 9320, *Clostridium aceticum* JCM 15732, *Acetobacterium woodii* DSM 1030, *Sporomusa ovata* DSM 2662, and *Sporomusa sphaeroides* DSM 2875 were selected to examine and evaluate CO_2-fixing acetogenesis using humin as extracellular electron donor. Humin could not donate electrons to acetogens in the absence of either yeast extract or hydrogen. Promotion of CO_2-fixing acetogenesis utilizing humin as electron donor was observed in all examined strains in presence of yeast extract. While *M. thermoacetica* JCM 9320 was the highest acetate producer from CO_2, *A. woodii* DSM 1030 produced the least acetate when yeast extract is present. Only two strains including *A. woodii DSM* 1030 and *S. sphaeroides* DSM 2875 could simultaneously utilize electron supply from humin and hydrogen.

In chapter 4, promoting effect of humin on CO_2-fixing acetogenesis by *M. thermoacetica* JCM 9320 in suspended humin-assisted MES and immobilized humin-assisted MES was investigated. Compared with humin-free MESs, humin enhanced the

CO_2 reduction to acetate in both humin-assisted MESs in the presence of yeast extract. At cathode potential of -510 mV (vs. Ag/AgCl), humin-assisted MESs produced acetate 10-fold higher than humin-free MESs, with more than 90% of the coulombic efficiency. Although the amount of humin used in immobilized humin-assisted MESs (3 g/L) was much lower than in suspended humin-assisted MESs (13 g/L), the acetate production rate of two MESs was almost the same, approximately 20 mg-acetate/L/day. As the cathodic potential became more negative (-810 mV vs. Ag/AgCl), the acetate production rate of MES with addition of humin (39.3 mg-acetate/L/day) was only 1.7 times higher that of non-addition of humin (23.1 mg-acetate/L/day).

Overall, in this study, promoting effect of humin on CO_2-fixing acetogenesis was found in minor group of *Clostridia* in mixed culture and representative acetogenic strains (*M. thermoacetica* JCM 9320, *C. aceticum* JCM 15732, *A. woodii* DSM 1030, *S. ovata* DSM 2662, and *S. sphaeroides* DSM 2875). MES system utilizing humin as extracellular electron donor for converting CO_2 into acetate was developed successfully at higher potential (-510 mV vs. Ag/AgCl) (without water electrolysis) in lab-scale experiment. Based on these findings, the humin-assisted CO_2 reduction system by anaerobic microorganism could be further developed and become a promising energy-efficient and sustainable CO_2 conversion technology toward net-zero CO_2 emission.