主論文の要約

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Thesis title:

Solid-phase Humin as a Versatile Redox Mediator and Bioelectrochemical System Enhancing Pentachlorophenol Degradation (多様な電子伝達能を持つ固体ヒューミンとペンタクロロフェノール分解を促進する生物電気化学システム)

Summary:

Bioelectrochemical systems (BESs), in which the cathodes are employed as direct electron donors for the microbial reduction of oxidized contaminants in subsurface environments, have been attracting attention as a promising technology with environmental benefits. BESs decrease the needs of energy as well as organic matter (as an electron donor) in comparison with conventional biological methods. In BESs, dissolved redox mediators, such as methyl viologen (MV) and anthraquinone-2,6-disulfonate (AQDS) act to facilitate electron transfer between the cathodes and microorganisms, and have been studied as a strategy for fine-tuning environmentally relevant microbial metabolisms such as dechlorination. Dissolved redox mediators, reversibly oxidized and reduced, is very important during electron transfer system of anaerobic microbial respiration. They accelerate reactions by lowering the activation energy, resulting in the enhancement of microbial transformation of pollutants. In naturally occurring humic substances (HSs), dissolved humic and fulvic acids have also been reported as redox-active organic macromolecules with reversible redox sites. However, some dissolved redox mediators (such as MV and AQDS) are toxic to human health and environment, and continuous dosing by dissolved redox mediators would be required in the environmental application of BESs, resulting in a reduced performance. Thus, an insoluble redox mediator that is nature-

originating, ubiquitous and versatile for diversity of oxidized pollutants is needed to explore and utilized in BESs for *in situ* bioremediation.

This thesis aimed to elucidate that solid-phase humin served as a redox mediator of multiple microbial respiration reactions with different terminal electron acceptors, and was immobilized in bioelectrochemical system to enhance the pentachlorophenol (PCP) dechlorination.

In Chapter 2, a solid-phase humin, acting as a redox mediator, was able to enhance multiple reductive biotransformations, including dechlorination of PCP, dissimilatory reduction of amorphous Fe (III) oxide (FeOOH), and reduction of nitrate, in a consortium. In presence of humin, PCP was dechlorinated to monochlorophenol (MCP) or phenol within 10 days, iron reduction was concomitant to 1.47 mM ferrous after 6 days and nitrate (5 mM) was totally reduced to ammonium with 3 days, while no dechlorination activity was observed, only 0.13 mM ferrous was produced after 8 days and large amount of nitrite was accumulated even after 20 days in the absence of humin control. Humin that was chemically reduced by $NaBH_4$ served as an electron donor for these microbial reducing reactions, with electron donating capacities of 0.013 mmol e^-/g for PCP dechlorination, 0.15 mmol e^-/g for iron reduction, and 0.30 mmol e^-/g for nitrate reduction. Two pairs of oxidation and reduction peaks within the humin were detected by cyclic voltammetry analysis. 16S rRNA gene sequencing-based microbial community analysis of the consortium incubated with different terminal electron acceptors, suggested that *Dehalobacter* sp., *Bacteroides* sp., and *Sulfurospirillum* sp. were involved in the PCP dechlorination, dissimilatory iron reduction, and nitrate reduction, respectively. These findings suggested that humin functioned as a versatile redox mediator, donating electrons for multiple respiration reactions with different redox potentials.

In Chapter 3, immobilized humin to the graphite electrode poised at -500mV (vs. SHE) significantly enhanced the microbial reductive dechlorination of PCP as solid electron mediator in bio-electrochemical system. Compared with the suspended system, the immobilized system dechlorinated PCP at a much higher efficiency, achieving 116 μmol Cl^- g $humin^{-1}$ d^{-1} (or 11.6 μmol Cl^- L^{-1} d^{-1}). Fluorescence microscopic observation showed a

conspicuous growth of bacteria adhered on the humin-immobilized electrode which had been negatively poised. Electron balance suggested that the electron required for microbial dechlorination was supplied primarily from the humin-immobilized electrode. Molecular analyses of microbial community structures based on 16S rRNA genes showed that *Dehalobacter* and *Desulfovibrio* grew on the humin-immobilized electrode poised at a negative potential as the potential dechlorinators. The findings that humin, solid-phase redox mediator, was successfully applied to the bio-electrochemistry system extends and improves the potential application of bio-electrochemical method for *in situ* bioremediation, given the wide distribution of humin and its efficiency and stability as a mediator.

After PCP was completely dechlorinated, phenol was still present a significant threat to the environment and human health. In Chapter 4, a microbial fuel cell (MFC), intact graphite electrode was used as both of anode and cathode, was operated for phenol degradation with a soil-free mixed culture inoculum. This MFC showed a comparable current density (120 mA/m^2) and higher coulombic efficiency (22.7%) than previous studies although they use the platinum (Pt) catalyst cathode, sediment or soil as the inoculum and keep air sparging in cathode. In phase II, after renewing the medium containing the plankton bacteria with only anaerobic medium, phenol degradation MFC was successfully maintained just by the anode biofilm and without any decreased performance. Cyclic voltammetry analysis showed distinct oxidation and reduction peaks of anode biofilm. The current generation might be mainly attributed the anode biofilm redox activity. 16S rRNA gene sequencing-based microbial community analysis of anode biofilm and plankton bacteria, suggested that *Geobacter metallireducens* was considered to be the phenol degrader and responsible for the current generation in anode biofilm.

Above all, the results achieved in this study, especially the finding that, humin was successfully immobilized on a graphite electrode as a stable solid-phase redox mediator and significantly enhanced the microbial reductive dechlorination of PCP, have important implications for *in situ* bioremediation.