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Electrochemical Oxidation of Ammonium ion coupled to Bioelectrochemical Dechlorination of Dichlorobenzoic Acid to Benzoic Acid by Anaerobic Consortium

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We developed a new catalytic electrode capable of oxidizing ammonium, which was used as an anode. Another functional electrode capable of reducing NAD^+ to NADH was used as a cathode, which was modified with neutral red. The modified electrode with neutral red can mediate electron transfer from electrode to bacterial cells. Two functional electrodes were combined as an anode and cathode for electrochemical oxidation of ammonium ion and bioelectrochemical reduction of dichlorobenzoic acid, respectively. Ammonium ion was oxidized to nitrate on anode coupled to reduction of dichlorobenzoic acid to benzoic acid on cathode of two compartments electrochemical reactor.

Key words: Electrochemical oxidation, ammonium ion, bioelectrochemical dechlorination

1. Introduction

Halogenated carbon compounds form an important group of chemicals produced by industrial processes. Due to the halogen substituent, many of these compounds are poorly degraded and hence persist in the environment[1)]. Chlorinated aromatic compounds are wide spread toxic compounds that are included in the U.S. Environmental Protection Agency list of priority pollutants. Mineralization of chloroaromatic compounds in methanogenic environment often starts with reductive dechlorination to phenol and ends with formation of methane and carbon dioxide[2)]. Reductive dehalogenation is the predominant bioconversion responsible for the metabolism of haloorganic compounds under anaerobic conditions and the only know fate process for many highly halogenated pollutants. Research has been restricted largely to enrichments and undefined microbial consortia, and consequently our understanding of the biochemistry and physiology of reductive dehalogenation is constrained[3-7)]. The bacteriological dehalogenation reactions are catalyzed by a membrane-bound reductive dehalogenase[8-9)], which is believed to function as the terminal reductase for anaerobic haloorganic respiration. The biochemical basis of energetic benefit of reductive dehalogenation in other isolates capable of catalyzing these reactions has been investigated to various extents[10)].

Nitrifiers are gram-negative, obligate aerobic chemolitortrophs which oxidize ammonium to nitrite or nitrite to nitrate as their sole energy source and assimilate carbon dioxide via the Calvin Benson Cycle[11)]. Nitrifiers are recognized as important agents in the nitrogen cycles of aquatic and terrestrial ecosystems. Although nitrifiers have been isolated from diverse environments and are generally ubiquitous in soils and freshwater and marine environments[12)], they account for a very small proportion of the total bacterial population in natural environments. This is another reason why the ammonium wasted into aquatic and terrestrial environment needs long time to be completely oxidized to nitrate.

An electrochemical oxidation of ammonium and a

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bioelectrochemical reduction of chlorinated organic carbons may be one of the solutions to solve the problems that are slow oxidation of ammonium and difficulty of reductive dehalogenation by bacteria. We developed a bioreactor with double functions: electrochemical oxidation of ammonium and bioelectrochemical dehalogenation of chlorinated organic carbons. In this research, we tried to perform the coupling reaction between the electrochemical ammonium oxidation and bioelectrochemical chlorinated organic carbon in two compartment bioreactor whose separated by ion-transferring membrane.

2. Materials and Methods

2.1. Chemicals

All chemicals used in this research were purchased from Sigma-Aldrich (USA).

2.2. Organisms

Anaerobic bacterial consortium was obtained from anaerobic digestive reactor located in Jungrang wastewater treatment plant and transported to laboratory by using anaerobic serum bottle. The headspace of anaerobic bottle containing anaerobic bacterial consortium was filled with hydrogen gas for maintaining anaerobic reduction condition until use.

2.3. Preparation of electrode

A graphite-Cd(II) anode was prepared from mixture of 60% (w/w) fine graphite powder (mean particle size is 1-2 mm, Sigma-Aldrich, ST. Louis, Missouri 63178, USA), 37% (w/w) inorganic binder (white clay mainly composed of Kaolin of which mean particle size is 1-2 mm), 3.0% (w/w) copper ion, respectively. Proper amount of distilled water was added to the mixture for making a graphite mixture paste, and the paste was configured to square-shaped plate (20 cm×20 cm×1 cm thickness) by pressing at 44kg/cm^2, drying on air for two weeks at room temperature and solidified by baking at 1200^{o}C for 12 hr under anaerobic condition using an electric Kiln (Red Corona Model 50L, USA). The graphite-Cd(II) electrode functions as ammonium oxidizer. Graphite electrode modified with neutral red was used as cathode which functions as electron mediator from electrode to bacterial cells[13-14].

2.4. Septum between two-compartmented bioreactor

A porcelain membrane was used as a septum between two reactors, which was prepared from mixture of 100% (w/w) white clay powder mainly composed of Kaolin of which mean particle size is 1-2 mm. Proper amount of distilled water was added to the white clay powder for making a clay paste, and the paste was configured to square-shaped plate (20 cm × 20 cm × 5 mm thickness) by pressing at 44kg/cm^2, drying on air for two weeks at room temperature and solidified by baking at 1200^{o}C for 12 hr using an electric Kiln (Red Corona Model 50L, USA). After baking the porcelain membrane was confirmed to absorb water but not to leak water through the micro-pore.

2.5. Cyclic voltammetry

The cyclic voltammograms were obtained using the transformed graphite-Cd(II) into rod type (diameter 5mm, length 4cm) as a working electrode, platinum wire as a counter electrode and Ag/AgCl as a reference electrode in 50 mM Tris-HCl buffer (pH 7.5), respectively. Cyclic voltammetry was performed using a cyclic voltammetric potentiostat (model CV50W, BAS, USA) linked to an IBM personal computer data acquisition system. Prior to use, the electrodes were cleaned using ultrasonic cleaner. The scanning rate was 50mVs^{-1} over the range of +2.0 volt to -2.0 volt. For observation of the reaction between the graphite-Cd(II) electrode and NH_4^+, NO_3^- and NO_2^-, each 1mM of NH_4^+ was added to the reactor after 2 cycles scanning. And then variation of cyclic voltammogram was observed.

2.6. Structure of electrochemical reactor

The electrochemical bioreactor was divided into two compartments by septum as shown in Fig 1. Ammonium can be oxidized to nitrate by catalysis of Cd(II)-anode and reducing power can be transferred from cathode to bacterial cell through neutral red. Oxidation

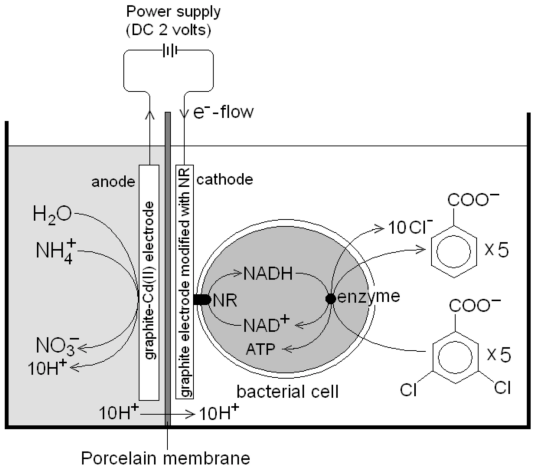

Fig. 1. Schematic structure of electrochemical bioreactor divided into two compartments. Theoretically, oxidation of 1.0 M ammonium is coupled to production of 10 M protons and electrons, which are consumed in coupling with reduction of halogenated hydrocarbon.

reaction in anode compartment and reduction reaction in cathode compartment were coupling each other. Three kinds of bioreactors were prepared according to the anode - cathode combination: 1) graphite-Cd(II) anode - normal graphite cathode, 2) graphite-Cd(II) anode - normal graphite cathode plus soluble neutral red (100 μM), and 3) graphite-Cd(II) anode ñ modified graphite cathode with neutral red. Potential difference between anode and cathode was 2 volt DC. 200 mM phosphate buffer containing 200 mM NaCl and 1000 ppm NH_4^+ was used as anolyte and 50 mM phosphate buffer containing 100 mM dichlorobenzoic acid was used as catholyte. Before inoculation, catholyte was purged with hydrogen gas to remove soluble oxygen for 20 min. No substrates or carbon source was added to the cathode compartment. T

2.7. Analysis

Dichlorobenzoic acid and benzoic acid were analyzed by GC (Varian 3400 star model) equipped with flammable ionized detector and capillary column (DB-1 model, Agilant technology, USA). Inlet split was adjusted to 50 ml/min, and column, injector and detector temperature was adjusted to 100-250°C (rising 10°C/min), 150°C and 250°C, respectively. Hydrogen was used as the carrier gas (20 ml/min). Samples were prepared by butanol extraction of bacterial culture (catholyte).

3. Results and Discussion

3.1. Electrochemical reaction between graphite-Cd(II) and NH_4^+

We tested function of graphite-Cd(II) electrode as NH_4^+-oxidizer by cyclic voltammetry. As shown in Fig 2, the cyclovoltammogram was shifted by addition of ammonium ion and the current variation was observed. The increase of current by addition of ammonium ion indicate that the graphite-Cd(II) electrode can oxidize or reduce ammonium ion under high potential and low potential, respectively. On the basis of this result, we designed the bioreactor with graphite-Cd(II) electrode which was used as anode for electrochemical oxidation of ammonium ion.

3.2. Electrochemical oxidation of ammonium ion to nitrate

The oxidation efficient of ammonium ion was dependent on the cathode reaction as shown in Fig 3. The electrons produced coupled to ammonium oxidation have to be consumed in coupling with the reduction reaction on cathode surface (Fig 1), however the

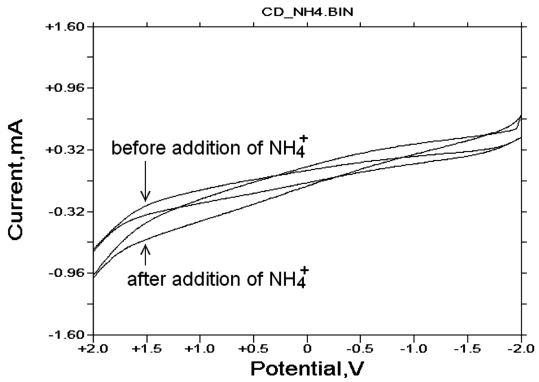

Fig. 2. Cyclic voltammogram of graphite-Cd(II) electrode in a phosphate buffer (50 mM, pH 7.0) without and with ammonium ion. By addition of ammonium ion, the cyclic voltammogram was remarkably shifted. The scan rate was 10mV/s, the working electrode was the modified with cadmium ion, the reference electrode was Ag/AgCl and the counter electrode was platinum wire.

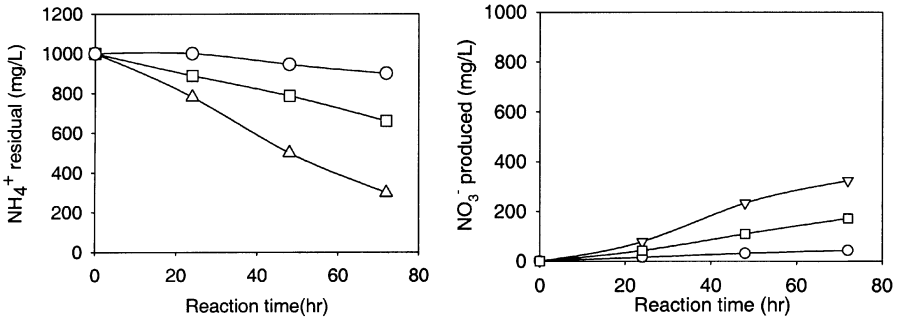

Fig. 3. Electrochemical oxidation of ammonium ion (left side) to nitrate (right side) in anode compartment of bioreactor in coupling with normal graphite cathode (○), normal graphite cathode plus soluble neutral red (□), and modified graphite cathode with neutral red (▽).

electrons cannot be transferred to bacterial cytoplasm without electron mediator, neutral red. For bioelectrochemical reduction of benzothiophene in condition without electron donor except electrons transferred from anode, bacterial cells have to take up the electrons via electron mediator. The circular symbols means that ammonium ion is difficult to oxidize in coupling reaction without electron acceptor, and triangle symbols means that neutral red immobilized to graphite electrode is more effective for electron transfer from electrode to bacterial cells than soluble one. Generally, most of bacterial cells are tendency to absorb to solid materials such as graphite, glass and ceramic. The immobilized neutral red to cathode may be easily reduced and then re-oxidized coupled to reduction reaction of NAD^+ in cytoplasm of bacterial cell absorbed to cathode. However, the soluble neutral red has to contact to cathode for reduction and then contact to bacterial cell for electron transfer. This is reason why the coupling reaction of ammonium oxidation with dichlorobenzoic acid reduction is more activated in the combination of graphite-Cd(II) anode - modified graphite cathode with neutral red. We cannot propose the reason to unbalance between ammonium consumed and nitrate produced, but thought that some ammonium ions may oxidize to dinitrogen (N_2) instead of nitrate.

3.3. Bioelectrochemical reduction of dichlorobenzoic acid

The bioelectrochemical reduction of dichlorobenzoic acid to benzoic acid was most effective in the bioreactor with the modified cathode with neutral red, which is coincident with the results obtained from the coupling reaction for ammonium oxidation. As shown in Fig. 4,

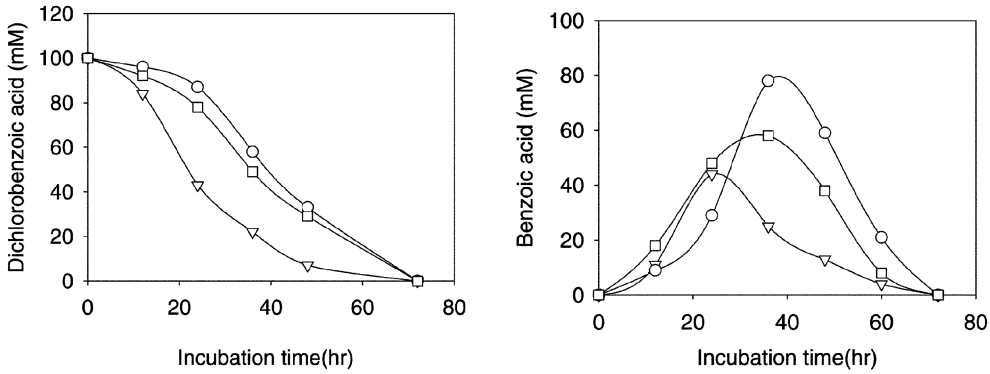

Fig. 4. Bioelectrochemical reduction of dichlorobenzoic acid (left side) to benzoic acid (right side) in cathode compartment of bioreactor with normal graphite cathode (○), normal graphite cathode plus soluble neutral red (□), and modified graphite cathode with neutral red (▽).

the dichlorobenzoic acid was decreased in coupling with increase of benzoic acid. However, the dichlorobenzoic acid consumed was not balanced with benzoic acid produced. This is thought that the benzoic acid may be degraded into other hydrocarbons or spent by bacterial cells as carbon source.

The metabolite produced from reductive dechlorination reaction of dichlorobenzoic acid has to be benzoic acid, which is detected by gas chromatrograpy as shown in Fig. 5. The benzoic acid peak was observed from 20 to 40 hr of incubation time, but the peak was disappeared after 60 hr. This severs a possibility that the benzoic acid may be degraded to other hydrocarbon or consumed by bacterial cell. The most important thing found in this study is that bacterial metabolism can be controlled and the bacteriologically slow-oxidizing materials such as ammonium ion can be rapidly oxidized by electrochemical energy. Ammonium ion is too stable to auto-oxidize to nitrite or nitrate under oxygen environment. General method for oxidation of ammonium ion is dependent on bacterial metabolism, however too slow to apply to large-scale wastewater treatment system. Wastewater containing high concentration of ammonium ion above 100 mgL^{-} has to be diluted for application to bacterial treatment system because the ammonium or nitrite oxidizer cannot grow.

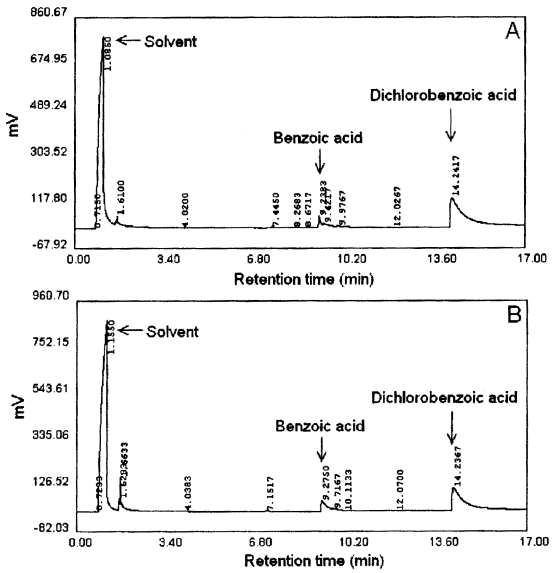

Fig. 5. Chromatograms of dichlorobenzoic acid and benzoic acid. The samples were extracted from bacterial culture with butanol (solvent) before (A) and after reaction (B) for 20 hr.

Since the recognition of biologically catalyzed reductive dehalogenation reaction over a decade ago, the anaerobic metabolism of chlorinated aromatic compounds has been the subject of many investigations. During this period, chloroaromatic compounds, including many chemicals that are known einvironmental contaminants and public health concerns, have been found to be widely susceptible to reductive dehalogenation. We found that the reductive dehalogenation of dichlorobenzoic acid is activated by electrochemical reaction in coupling with ammonium oxidation. The electrochemical method is useful for control of bacterial growth and metabolism, or compulsive oxidation of inorganic compounds such ammonium, nitrite and sulfite. We are developing and testing new electrodes for control various biological or chemical reactors such as dye wastewater treatment system, heavy metal treatment system, excreta treatment reactor and bacterial fermentation.

4. Conclusion

In this research we developed the bioreactor system for electrochemical oxidation of ammonium ion in coupling with reductive dichlorination of dichlorobenzobenzoic acid. The reducing power produced coupled to electrochemical oxidation of ammonium ion can be effectively converted to bacterial reducing power, by which dichlorobenzoic acid was bioelectrochemically reduced to benzoic acid.

Acknowledgments

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