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Development of Neo-electrode with Catalytic Activity for Oxidation-Reduction of Nitrite and Nitrate Coupled to Electricity Production and Consumption

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Graphite electrode containing Cu^{2+} was made from clay, graphite powder and Cu^{2+} by baking at 1200°C. The electrode was named Cu(II)-graphite electrode. The Cu(II)-graphite electrode functioned as a catalyst for oxidation of nitrite without any external oxidant. The nitrite was spontaneously oxidized on Cu(II)-graphite electrode coupled to electricity production. The Fe(III)-graphite electrode was used as a counter electrode. In the meanwhile, the nitrate was electrochemically reduced coupled to electricity consumption. The electricity production and consumption on Cu(II)-graphite electrode was proportional to the nitrite and nitrate concentration. This is a useful discovery for development of nitrite or nitrate measurement system and the catalyst electrochemically controlled.

Key words: Catalytic Cu(Ii)-graphite Electrode, Electrochemical Nitrate Reduction, Electrochemical Nitrite Oxidation

1. Introduction

Nitrate and nitrite were produced as a final metabolite or metabolic intermediate from ammonium ion by oxidative metabolism of the nitrifying bacterial community in natural environment.[1-3] Ammonium ion was biologically produced from organic nitrogen compounds such as amino acid and nucleic acid by deamination metabolism of anaerobic bacteria and animals.[4] Accordingly, the nitrate and nitrite were a kind of natural compounds ecologically circulated in all biosphere such as water stream, lake, sea or soil. However, the high concentration of nitrite and nitrate accumulated in the natural environment may malign both animals and plants and especially, nitrite may function for animal as a kind of toxin. This is a reason why the nitrate and nitrite were controlled for rapid-detection within several minute before the wastewater contaminated with nitrate or nitrite was discharged into natural water stream such as river and sea from wastewater treatment system.[5] We tried to develop the biosensor for detection of nitrate and nitrite by modifying electrode with nitrite oxidase and nitrate reductase but the enzymes were too unstable to apply to biosensor and the activity was very fastly diminished by immobilization or drying on the electrode.[6] Up to the present, the biosensor commercially produced and merchandised is only glucose sensor for the patients with diabetes using glucose oxidase which is very stable under even extreme circumstance.

For removal of the problem of nitrate and nitrite sensor using the enzymes nitrite oxidase and nitrate reductase we tried to make a catalytic electrode with function of nitrite oxidation and nitrate reduction. Ten transitional metal ions (Ti^{3+}, Mg^{2+}, Mn^{2+}, Ca^{2+}, W^{2+}, Mo^{2+}, Al^{3+}, Co^{2+}, Sn^{2+}, Sb^{2+}) were cyclovoltammetrically tested for selection of proper metal ion with catalytic activity and the Cu^{2+} was confirmed to be a proper metallic ion for composing catalytic electrode. Nitrite was auto-oxidized on Cu(II) graphite electrode coupled to electricity production and nitrate was compulsively reduced on Cu(II) graphite electrode coupled to electricity consumption. $Cu^{2+}$was reported to act as catalyst for selective reduction of NO_2 to NO[7] and Torres *et al.*[8-9] reported that the copper centering on laccase could be reduced by nitric oxide.

Copper-ammonia, Copper-ethylenediamine or Cu-methylamine complex were reported to induce oxidative degradation of pyrocatechol violet by hydrogen peroxide[10)] and Cu(II) were reported to catalyze amination of aryl iodides.[11)] Nicholas et al.[12)] developed copper-dependent catalytic antibodies. As shown in various papers reported before, the copper ion is confirmed to be a function as a catalyst. In this research we tested some graphite electrode modified with metallic ions for selection of a proper metallic ion for composing chemical sensor capable of detecting nitrate and nitrite. Nitrate and nitrite is easily reduced and oxidized under mild condition such room temperature and neutral pH by some reductant and oxidant, respectively. We used electrochemical energy as the oxidant and reductant and transient metal ion as the catalyst.

2. Experimental

2.1. Chemicals

All chemicals used in experiment were Sigma grade or reagent grade.

2.2. Preparation of electrodes

A Cu(II)-graphite electrode was made from mixture of 60% (w/w) fine graphite powder (mean particle size was below 600 mesh), 37% (w/w) inorganic binder (white clay mainly composed of Kaolin for porcelain of which mean particle size was below 400 mesh), 3.0% (w/w) cupric ion, respectively. The graphite-Fe(III) electrode was made from mixture of 60% (w/w) fine graphite powder, 37% (w/w) inorganic binder, 3.0% (w/w) ferric ion, respectively. An unmodified electrode was made from mixture of 60% (w/w) fine graphite powder and 40% (w/w) inorganic binder (Kaolin, White clay), respectively. Proper amount of distilled water was added to the mixture for making a graphite paste, and the paste was configured to square-shaped plate (20 cm × 20 cm ×1 cm thickness) by pressing at 1.0 kg/cm^2, drying on air for one to two weeks at room temperature and solidified by baking at 1200°C for 12 hr under anaerobic condition using an electric Kiln (Red Corona Model 50 L, USA). In all experiments the graphite-Cu(II) electrode was used as a catalyst and graphite-Fe(III) electrode was used as a counter electrode.

2.3. Composition of bioreactor

The bioreactor was composed of catalytic and counter electrodes which are modified graphite with Cu(II) and Fe(III), respectively. Graphite-Cu(II) was used as a catalyst and graphite-Fe(III) was used as a counter electrode. The catalytic electrode was equipped at one compartment separated from counter electrode compartment by porcelain membrane (3 mm thickness) The total volume of bioreactor was 20 ml and electrode area was 20 cm^2.

2.4. Measurement of electricity production and consumption

The electricity produced coupled to oxidation of nitrite to nitrate in the bioreactor (Fig. 2) equipped with catalytic Cu(II)-electrode and counter electrode was measured with automatic data acquisition system. The external variable resistance was not connected between catalytic electrode and cathode for control of electron flow rates because the electricity production is too low to flow through resistant. Normal carbon electrodes were equipped in bioreactor used for control test. Amperemeter was directly connected between catalytic electrode and counter electrode. The electricity consumed coupled to reduction of nitrate to nitrite in the bioreactor with Cu(II) and counter electrode. Direct current of 2.0 volt electricity was supplied between the catalytic electrode and counter electrode. Cathode of power supply was connected to the catalytic electrode and anode was connected to negative junction of amperemeter and positive junction of amperemeter was connected to counter electrode as shown in Fig. 2, by which the current was automatically measured coupled to reduction of nitrate to nitrite.

2.5. Cyclic voltammetry

The cyclic voltammogram was obtained using graphite working electrode modified with Mn^{4+} and Fe^{3+} which was transformed into rod type (diameter 5 mm, length 4 cm), platinum wire counter electrode and an Ag/AgCl reference electrode in 50 mM phosphate buffer (pH 7.0). 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 used was 25 mVs^{-1} over the range of +2.0 volt to 2.0 volt.

2.6. Construction of biosensor

The biosensor was constructed with catalytic electrode and counter electrode as shown in Fig. 1. For nitrate measurement, catalytic electrode was used as cathode and Fe(II)-graphite was used as anode. The electricity was reducing power and cathode functions as catalyst. For nitrite measurement, catalytic electrode was used as anode and Fe(III)-graphite was used as cathode. The electricity was automatically produced coupled to auto-oxidation of nitrite to nitrate. The electrode area was adjusted to 9 mm^2.

2.7. Analysis of nitrate and nitrite

Nitrate and nitrite were analysed by standard methode using Sulfanilamide and Naphthylethyldiamine.[13] Nitrate was reduced to nitrite by passing through cadmium column. The red color was produced by reaction of Naphthylethyldiamine with azo dye which was produced by reaction of nitrite with Sulfanilamide.

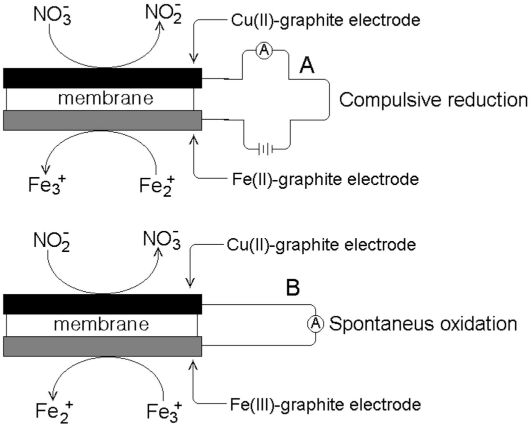

Fig. 1. Schematic structure of chemical sensor made from metallic graphite electrodes. Cu(II)-graphite electrode functions as both electrode and catalyst for oxidoreduction of nitritte and nitrate and Fe(II)-graphite and Fe(III)-graphite electrode functions as counter electrode, respectively.

3. Results and Discussion

The catalytic function of copper ion used for composing electrode was tested by cyclic voltammetry for measurement of the activity reacted with nitrate or nitrite. As shown in Fig. 3, in the phosphate buffer the Cu(II)-graphite electrode was not react and was not shown current variation during scanning from -2.0 volt to +2.0 volt or at reciprocal direction. However, by addition of nitrate the current was positively increased during scanning from -2.0 volt to +2.0 volt but by addition of nitrite at the plus (+) voltage range the current was negatively increased and at the minus (-) voltage range the current was positively increased.

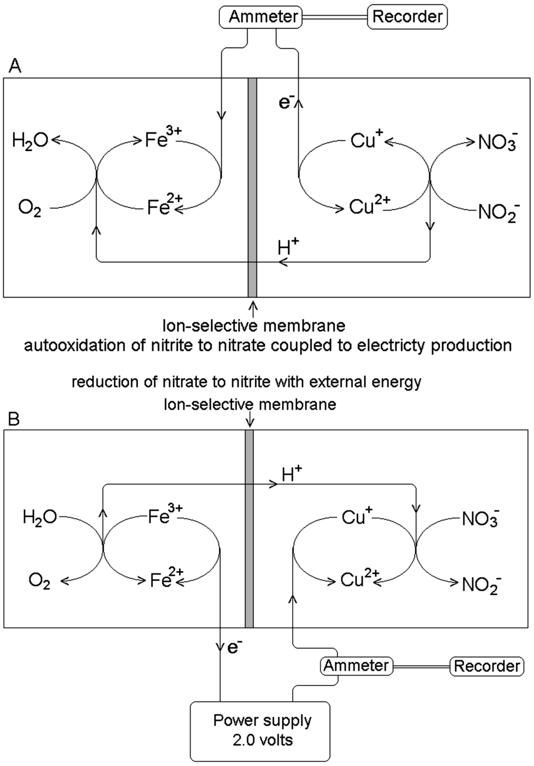

Fig. 2. Schematic structure for electrochemical reaction system for nitrite oxidation (A) coupled to electricity production and nitrate reduction (B) coupled to electricity consumption.

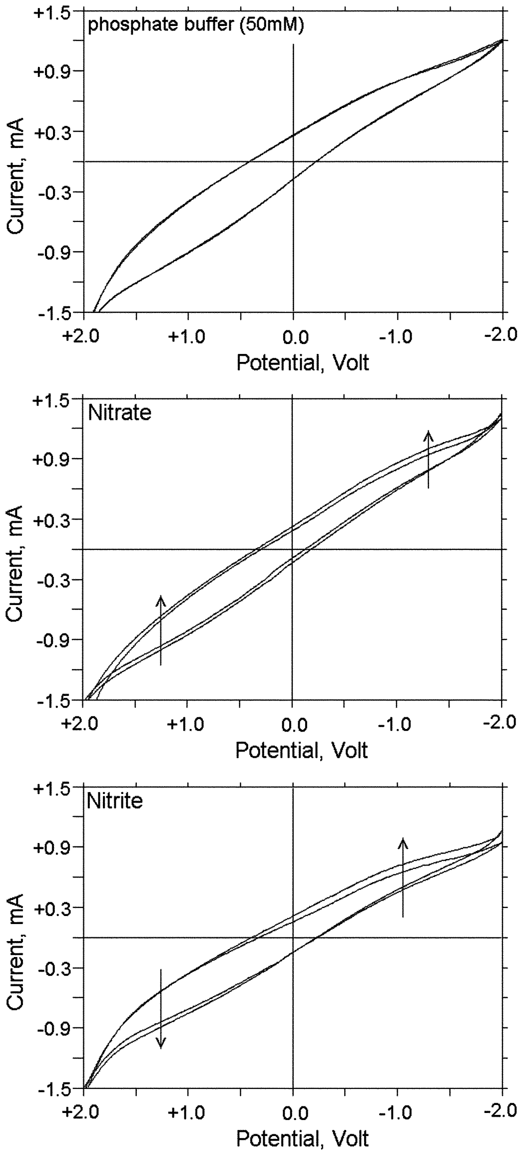

Fig. 3. Cyclovoltammogram of Cu(II)-graphite electrode on phosphate buffer with and without nitrate or nitrite. After addition of nitrate and nitrite the currents of cyclovoltammetry were altered as arrow markes are indicating. By addition of nitrate the current at both positive (+) and negative (-) potential was increased (reduced) and by addition of nitrite the current at positive (+) potential was decreased (oxidized) and at negative (-) potential was increased (reduced).

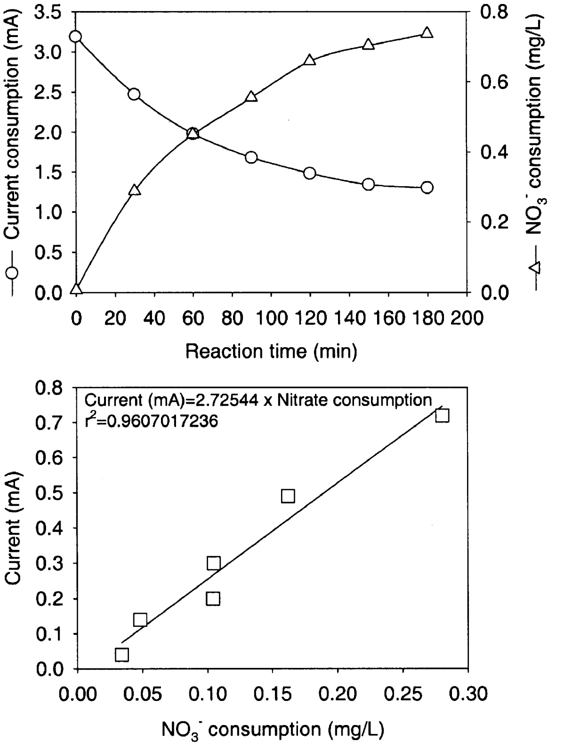

Fig. 4. Electrochemical reduction of nitrate to nitrite coupled to electricity consumption on Cu(II)-graphite cathode and Fe(III)-graphite anode. The current consumption was proportional to NO_3^- consumption but the accuracy estimated by regression was about 0.96.

These shows that the nitrate was uni-directionally reduced to nitrite but the nitrite might be oxidized at plus (+) voltage range but re-reduced at minus (-) voltage range. On the basis of this results we designed the reactor as shown in Fig. 2 and tested nitrite oxidation and nitrate reduction, respectively. As shown in Fig. 4 (upper), nitrate reduced to nitrite on Cu(II)-graphite electrode coupled to electricity consumption, and the nitrate consumption was proportional to the electricity consumption. The correlation between nitrate consumption and electricity consumption was about 98% of coincidence. By which it is possible that nitrate concentration may be quantitatively analyzed. Fig. 5 shows that the nitrite can be auto-oxidized coupled to electricity production. As shown in Fig. 5 (upper) the electricity production was very lower than electricity consumption coupled to nitrate reduction. This is thought that 2 volts of electricity was supplied to Cu(II)-graphite electrode as an external reducing power for compulsive reduction of nitrate to nitrite and part of electric energy may be converted to entropy. Accordingly, the oxidation-reduction balance between supplied

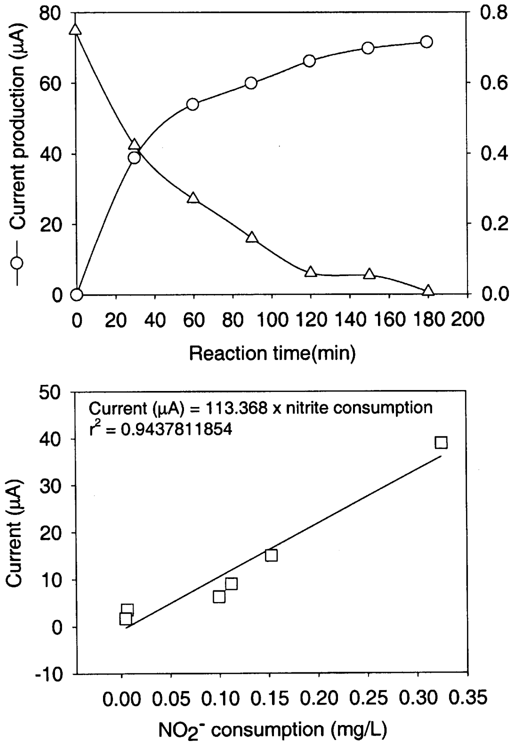

Fig. 5. Electrochemical oxidation of nitrite to nitrate coupled to electricity production on Cu(II)-graphite cathode and Fe(III)-graphite anode. The current production was proportional to NO_2^- consumption but the accuracy estimated by regression was about 0.94.

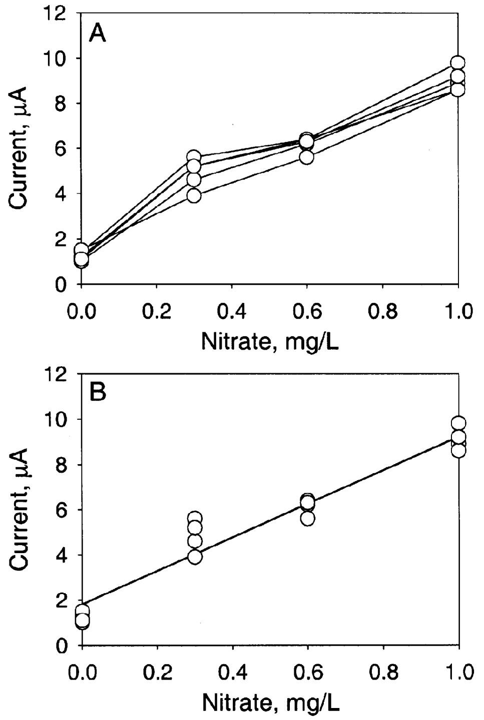

Fig. 6. Amperometric consumption coupled to nitrate reduction to nitrite on Cu(II)-graphite cathode at pH 7.0. Fe(II)-graphite electrode was used as a anode (counter electrode). This test was done by using the system shown Fig. 5A. The current was measured 4 times as shown in figure A and the results were statistically regressed as shown in figure B.

current and nitrate reduction may be not coincident. As shown if Fig. 5 (lower) the correlation between electricity production and nitrite consumption was about 97% of coincidence but the plots at the cross points between current and nitrite concentration was too irregular and unsystematic to be used for quantitative analysis of nitrite. The reason why the correlation between the electricity production and nitrite consumption and the electricity consumption and nitrate reduction are unsystematic is thought that the electrode area is too big to react with low concentration of nitrate or nitrite. For making more precise measurement system we made the sensor type reactor as shown in Fig. 1. As shown Fig. 6, the correlation between nitrate reduction and current consumption was relatively more precise than those obtained from bioreactor (Fig. 2) but the correlation between nitrite oxidation and electricity production was relatively less precise than the correlation between nitrate reduction and electricity consumption. All electrodes and systems used in this research were made by hand in this laboratory for development of new electrode with catalytic function by which the systems and electrodes may have different quality at different processing. We have tried to make functional electrode under quality control system and many apparatus are equipped in this laboratory. The functional or catalytic electrode is very useful tool for bioreactor and chemical reactor for development of environmental treatment system, control of oxidation-reduction reaction of biological compounds and efficient transformation of useful chemicals such as drugs, foods and plastics. For induction of a specific chemical or biochemical reaction *in vitro*, various enzymes have been used for a long

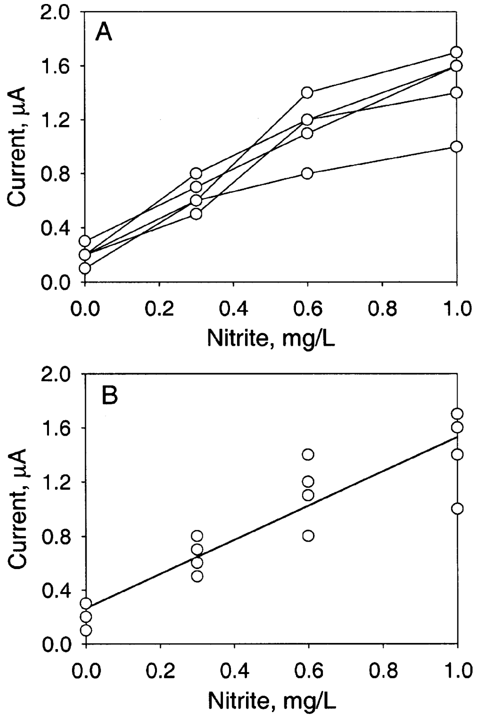

Fig. 7. Amperometric production coupled to nitrate oxidation to nitrate on Cu(II)-graphite anode at pH 7.0. Fe(III)-graphite electrode was used as a cathode (counter electrode). This test was done by using the system shown Fig. 5B. The current was measured 4 times as shown in figure A and the results were statistically regressed as shown in figure B.

time but the enzyme activity is a major limited factor for development of bioreactor or biochemical reaction. The catalytic electrode has two functions which are catalysis of biochemical reaction such oxidation, lyation, phosphorylation, degradation and kination and electron transfer from electrode to chemicals or from chemicals to electrode.

Conclusion

The catalytic Cu(II)-graphite electrode was developed for application to environmental system, biochemical reaction and electrochemical oxidation-reduction of inorganic compounds such nitrate, nitrite and ammonium ion. In test using the catalytic electrode we obtained some useful and reasonable results for quantitative analysis of nitrate and nitrite by electrochemical methode without enzyme. The results in this paper serve the possibility that the more active and functional catalytic electrode may be developed by using various materials such graphite, clay, transient metal ions, biological compounds except enzymes in future.

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