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Development of Electrochemical Sensor for Detection of Ammonium Ion, Nitrite and Nitrate

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1. Introduction

Ammonium ion, nitrite and nitrate were the bacterial metabolites produced in the metabolisms for bacterial oxidation of organic nitrogen compounds or in the anaerobic bacterial respiration with inorganic nitrogen compounds as the electron acceptors. The inorganic nitrogen compounds are absolutely required for growth of the autotrophic microorganism because the nitrogen is the key element for the biosynthesis of microorganism living in natural ecosystem.[1-3] Especially, when the organic carbons are not enough for the heterotrophs and the inorganic nitrogen compounds are flown in the natural water stream without contaminants algae has to be suddenly increased. The outbreak of algae is the most important factor for contamination of natural water such as the rivers, the lakes and the sea. The dead algae containing abundant organic carbon and nitrogen is the first factor for the secondary and tertiary contamination of natural ecosystem.[4] Generally, a big quantity of inorganic nitrogen is difficult to produced from natural ecosystem or flow in the natural ecosystem in the course of ecological cycle of nitrogen. However, the high concentration of inorganic nitrogen compounds over the range needed for ecological recycling have been discharged from wastewater treatment systems or plants. Actually, the results produced from the various researches for reduction of inorganic nitrogen discharged from wastewater treatment system have been progressed and practical, but not completed. For protection against contamination of natural ecosystem by the inorganic nitrogen compounds, which have to be rapidly and precisely detected before the treatment water is discharged from wastewater treatment system to the natural water stream. Once the wastewater containing the inorganic nitrogen compounds was discharged into natural water stream, the inorganic nitrogen compounds cannot be removed from the water stream.[5-8] This is the reason why the measurement time for detection of the inorganic nitrogen compounds has not to be over 10 minutes. If the sample number is 10 the treated water has to be stored in the reservoir for at least 100 minutes during analysis, for which the large scale of reservoir has to be constructed. Another problem can be happened from the concentration of inorganic nitrogen compounds, which can be over the limitation value. In this case, the treated water has to be recycled from the reservoir to treatment system.

The electrochemical sensors for detection of inorganic nitrogen compounds have been developed and have been commonly used in the field and laboratory, for this system, the membrane with selective permeation of inorganic nitrogen compounds was applied to the electrode. This electrode-type sensor is known to be high precise and has high repetition rates in the showing results, however, the function is known to be diminished when used repeatedly. We developed the catalytic electrode for electrochemical oxidation of ammonium ion and nitrite, and reduction of nitrate to compose the simpler electrochemical sensor than the electrode-type sensor. This research was focused on the catalytic electrode with function of oxidation and

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reduction but not precise measurement of inorganic nitrogen compounds. We used the voltammetric method for measurement of oxidation and reduction tendency of inorganic nitrogen compounds. Potential difference between the catalytic electrode and reference electrode (Ag/AgCl) was proportional to the oxidation or reduction tendency of inorganic nitrogen compounds but the inorganic nitrogen compounds are not practically oxidized or reduced on electrode surface. Because the inorganic nitrogen compounds are actually oxidized or reduced in the amperometric system, the current produced coupled to oxidation of the inorganic nitrogen or consumed coupled to reduction of the inorganic nitrogen can be proportional to the inorganic nitrogen compounds only contacting to the electrode surface. This is an advantage of the potentiometric method.

2. Experimental

2.1. Preparation of electrodes

The electrodes modified with metal ions were made from mixture of 60% (w/w) fine graphite power (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% (w/w) metal ions (Cd^{2+}, Cu^{2+} and Mg^{2+}), respectively. Proper amount of distilled water was added to the graphite mixture, and the graphite paste was configured to square-shaped plate (20 cm×20 cm×2 cm) by pressing at 44 kg/cm^2. The square-shaped graphite plate was completely dried on air for 3 weeks and then baked at 1200°C for solidification for 5 hr. The graphite-metallic ion electrode was originally created in our laboratory.

2.2. Construction of electrochemical sensor

The electrochemical sensor was constructed with reference electrode and working electrode as shown in Fig. 1. The graphite-Cd(II), graphite-Cu(II) and graphite-Mg(II) electrode were fixed on the tip of bronze bar with conductive epoxy and coated the outside of graphite electrodes with Teflon. This is the simplest structure of various electrochemical sensors.

2.3. Measurement of inorganic nitrogen compounds

For measurement of the inorganic nitrogen compounds, standard solution containing inorganic nitrogen compounds from 1 to 10 mg/L was used but real wastewater was not applied to the electrochemical sensor. The measurements were 12 times repeated for calculation of standard deviation and equation was obtained from the results.

3. Results and Discussion

Some metal ions being chelated by organic compounds such as proteins (enzymes) have the catalytic function.[9-12)] However, the organic-chelating metal ions can easily lose its function by the destruction of basic structure of organic compounds under even normal environment.[3-14)] This is a negative point for composing sensor using the biological compounds. For solving this problem, we tried to make an inorganic complex composed with metal ions and graphite. The graphite and metal ions are used as an electron conductor and a catalyst of oxidation-reduction, respectively.[15)] As shown

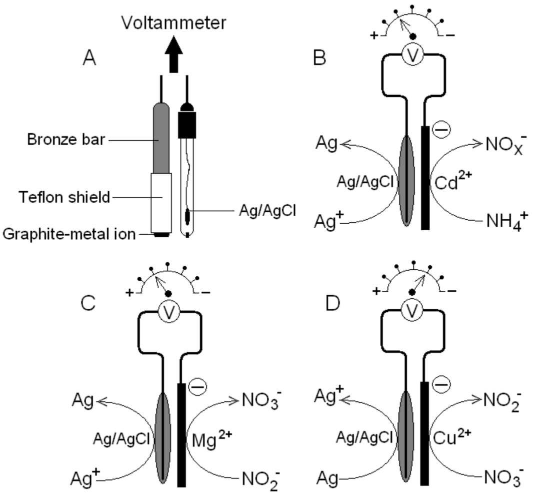

Fig. 1. Schematic structure of sensor structure of which working electrode was composed of graphite and Cd^{2+}, Mg^{2+} and Cu^{2+} ion, respectively. Metal ions are functioning as a catalyst for oxidation of ammonium ion and nitrite, or reduction of nitrate. Graphite is functioning as a matrix for electron transfer. In this system, the ammonium ion, nitrite and nitrate are not actually oxidized or reduced.

in Fig. 1, reference electrode can be easily oxidized or reduced under neutral pH coupled to oxidation of ammonium ion and nitrite, and reduction of nitrate, but Ag/AgCl is not practically oxidized or reduced in the voltammetric circuit.[16] The potential difference between the reference electrode and working electrode (graphite-metallic ion electrode) can be measured with voltammeter, which is not interfered by the oxidation or reduction of samples for analysis.

Before the electrode system was designed, we tested for selection of the catalytic function as shown in Fig. 2, 3 and 4, respectively. The graphite-Cd(II) electrode,

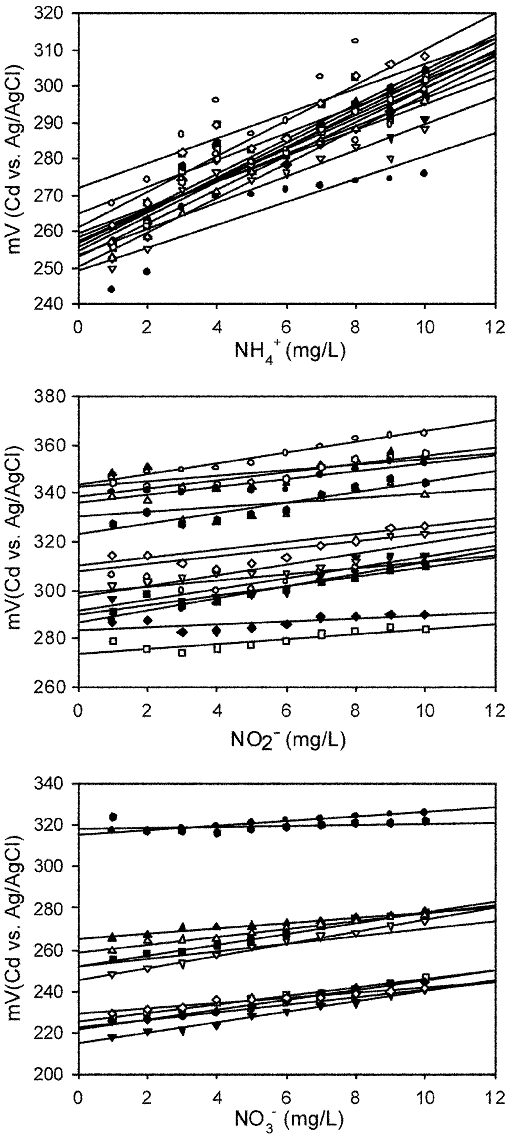

Fig. 2. The potential variation between working electrode (graphite-Cd(II) electrode) and reference electrode (Ag/AgCl) in the ammonium ion, nitrite and nitrate solution, respectively, with different concentration from 0 to 10 mg/L. The potential difference between graphite-Cd(II) electrode and reference electrode was highest in ammonium ion solution.

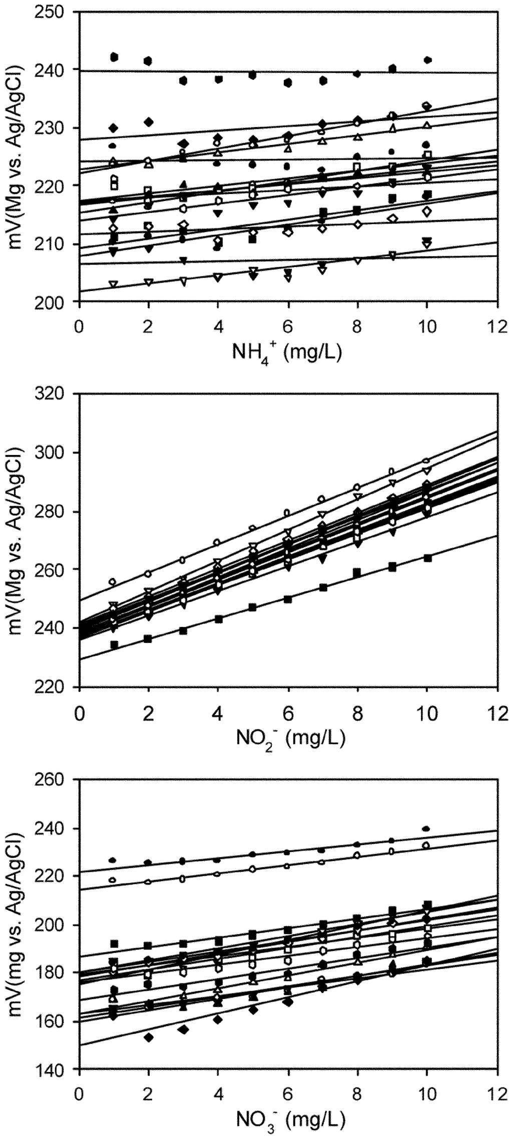

Fig. 3. The potential variation between working electrode (graphite-Mg(II) electrode) and reference electrode (Ag/AgCl) in the ammonium ion, nitrite and nitrate solution, respectively, with different concentration from 0 to 10 mg/L. The potential difference between graphite-Mg(II) electrode and reference electrode was highest in nitrate solution.

graphite-Mg(II) electrode and graphite-Cu(II) electrode were selectively reacted with ammonium ion, nitrite and nitrate, respectively. On the basis of these results, ammonium ion, nitrite and nitrate were measured by using the graphite-metallic ion electrode for the standard solution as shown in Fig. 5, 6 and 7, respectively. In the twelve repeated tests, standard deviation was 11%, 6% and 13% for ammonium ion, nitrite and nitrate, respectively. These results show that the graphite-metallic ion electrodes are not enough to apply to the wastewater treatment system. We are attempting to develop the disposable electrode system to escape from the problem happened by the repeating use of the electrode. The most improved electrochemical sensor was composed by modification of graphite or metal electrodes with enzymes, which have catalytic function

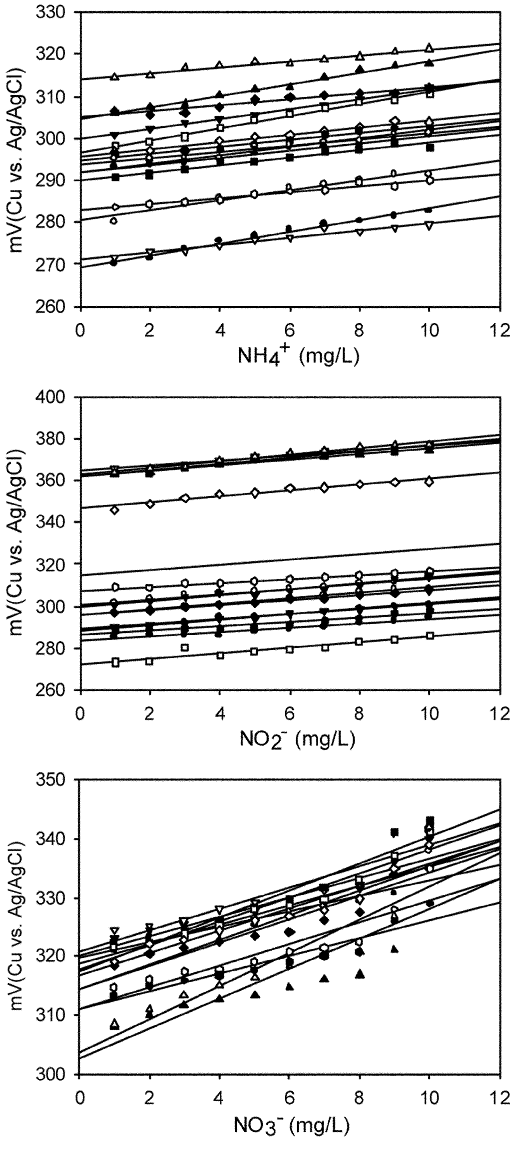

Fig. 4. The potential variation between working electrode (graphite-Cu(II) electrode) and reference electrode (Ag/AgCl) in the ammonium ion, nitrite and nitrate solution, respectively, with different concentration from 0 to 10 mg/L. The potential difference between graphite-Cu(II) electrode and reference electrode was highest in nitrate solution.

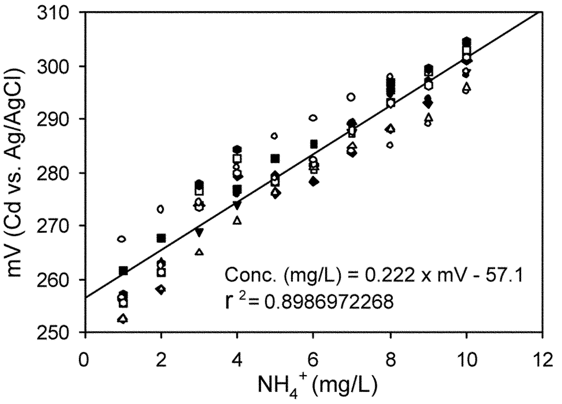

Fig. 5. Statistical analysis of potential difference between graphite-Cd(II) working electrode and reference electrode in ammonium ion solution. Mean deviation among twelve tests under same condition with same electrodes and solution is 11%, which is little bigger than the deviation in manual method.

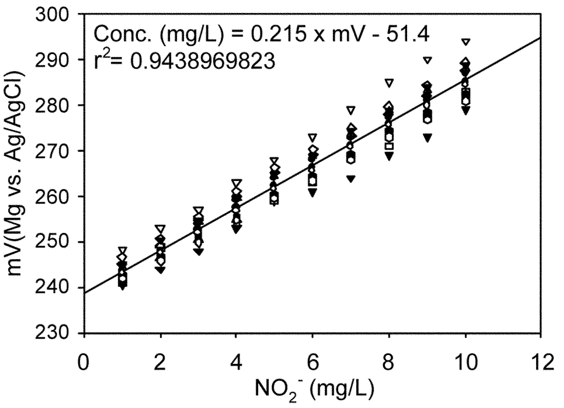

Fig. 6. Statistical analysis of potential difference t graphite-Mg(II) working electrode and reference e in nitrite solution. Mean deviation among twel under same condition with same electrodes and so about 6%, which is similar to the deviation in method.

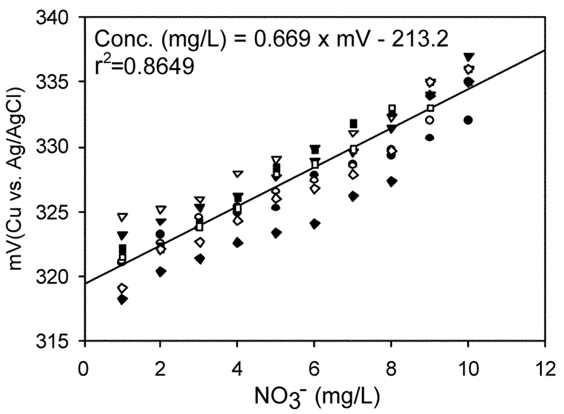

Fig. 7. Statistical analysis of potential difference between graphite-Cu(II) working electrode and reference electrode in nitrate solution. Mean deviation among twelve tests under same condition with same electrodes and solution is about 13%, which is bigger than the deviation in manual method.

for the specific biochemical reaction. However, the function and structure of enzymes tends to be destructed, and its activity was very rapidly decreased in proportional to the storage time under normal condition. Accordingly, the development of stable sensor without the destruction and activity decrease is absolutely required.

Conclusion

The selective electrodes were developed for analysis of ammonium ion, nitrite and nitrate, respectively. Graphite-Cd(II), graphite-Mg(II) and graphite-Cu(II) electrode were confirmed to selectively react with ammonium ion, nitrite and nitrate, respectively. In the results from the tests with the electrode, the standard deviation was shown to be too high to apply to the wastewater analysis. We are attempting development of the functional electrode with higher sensitivity than those developed in this research.

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