Short communication

Polymer-insertion effect on urea conversion kinetics via enzymes immobilized on magnetic microparticles

ABSTRACT

Urease was covalently immobilized on the magnetic particles without and with polymer between the urease and the particle. The polymer insertion enhanced the conversion from urea to bicarbonate. The results of this study seem to be useful in designing a reactor for recycling wastewater generated in the semiconductor process.

Keywords: Urea; Enzyme immobilization; Magnetic microparticles; Polymer-insertion; Conversion kinetics

1. INTRODUCTION

Ultrapure water is critical in high-tech industries such as microstructure manufacturing, fine chemicals, and pharmaceuticals, and is produced through various processes such as adsorption, ion exchange, degassing, membrane filtration, and ultraviolet oxidation [1]. In the process of producing ultrapure water, it is recognized that non-volatile, non-oxidative, ozone-resistant, non-ionic, highly water-soluble, and low-molecular-weight reagent such as urea cannot be easily removed by conventional physicochemical methods [2]. Furthermore, urea is often used as an excellent deicing-compound due to the above-mentioned physical properties, and there is always a possibility that water resources are contaminated with urea because fertilizer essential for agriculture is a potential source of urea [3,4].

Currently, the ideal way to remove urea from water is to break it down into compounds that can be easily removed by conventional water purification processes. Currently, UV oxidation and advanced oxidation process (AOP) are mainly used to remove urea. So far, it has been found that the combination of UV and persulfate is effective, and Evoqua's Vanox AOP technology has been reported to be able to stably maintain the TOC of treated water below 0.5 μg/L [2,5]. And, it has been also reported that urea can be oxidized in hypochlorous acid-based oxidation processes that are 100 times more reactive than ozone [6].

Since the current approaches have required power consumption and compound addition, a more economical process has been pursued. Urease, which acts in the pH range of 5 to 10, promotes the conversion of urea into bicarbonate anion and ammonium cation [7]. Previously, the kinetics of the urea conversion via the immobilized urea immobilized was investigated [8]. Generally, faster kinetics results in shorter residence time in the reactor. In this research, the effect of the polymer insertion between the urease and the magnetic microparticle on the kinetics was investigated.

2. MATERIAL AND METHODS

The ureases were immobilized on the magnetic microparticles through covalent bonds. 150 μL of stock solution of 3 μm diameter particles was washed three times with 20 mM MES rubbing solution at pH 6.0. These particles were reacted with 2.5% (v/v) ethyl(dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide for 45 minutes and then immersed in 50 mL of 20 mM MES containing 50 U urease for 3 hours. For the purpose of the polymer insertion, the particles was treated with 50 mL MES of 50 μM NH2-Polyethyleneglycol(PEG)-COOH(Mn 20K) for 2 hours prior to the urease immersion. To confirm the immobilization, X-ray photoelectron spectroscopy was used. To confirm enzyme immobilization, X-ray photoelectron spectroscopy was used. Concentrations of injected and unbound enzymes were identified using Bradford reagent. The amount of the immobilized enzyme was estimated to be 1.0 μM and 8.0 ng-protein/mg-particle [9].

Cyclic voltammetry (CV) experiments were performed with a Zive electrochemical workstation. After reacting by adding an aqueous solution of urea to the particles on which urease was immobilized, the aqueous solution was extracted over time and transferred to a Pyrex glass cell. An Ag/AgCl reference electrode, a Pt wire counter electrode, and a glassy carbon working electrode were placed in a buffer solution, and the current was measured. The potential was cycled at a scan rate of 0.05 mV/s in the range of 800 to -200 mV relative to the reference electrode.

3. RESULTS AND DISCUSSION

The current from the urea conversion via the immobilized enzyme is shown in Fig. 1. A 200 ppm aqueous solution of urea was measured before and after reacting with the enzyme, respectively. It is observed that the width of the current change is expanded by the reaction. This is analyzed as a change due to an increase in bicarbonate anions converted from the urea.

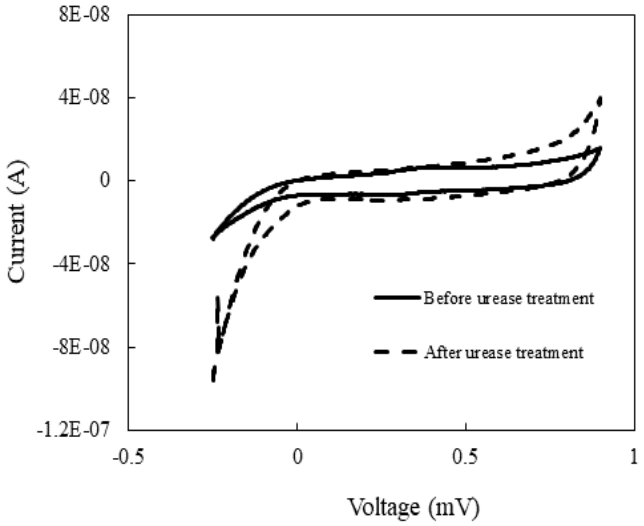

Fig. 1. Current before and after urease treatment for 16.7 μM urea

For the purpose of quantification, a standard curve was derived by measuring the amplitude of current change according to the concentration of bicarbonate (Fig. 2). Since this curve

was expected to agree with the form of the theoretical expression representing the ion concentration and the potential difference - the Nernst equation because the current of CV was linearly proportional to the potential difference. In addition, it was recognized that the CV measurement was properly performed as the predictions was consistent with the experimental results. The amount of the urea converted to bicarbonate could be calculated using the derived standard curve.

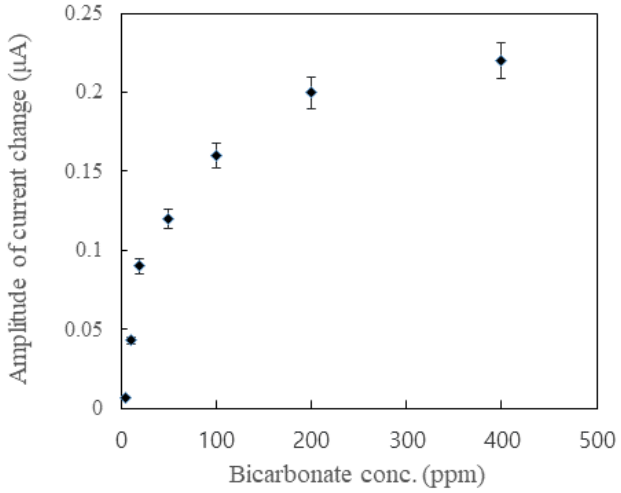

Fig. 2. Standard curve between current and bicarbonate concentration

The conversion of urea to bicarbonate anion over time was analyzed. After about 1 hour of reaction time, most of the urea was converted, and the conversion was saturated after 1 hour and 30 minutes (Fig. 3).

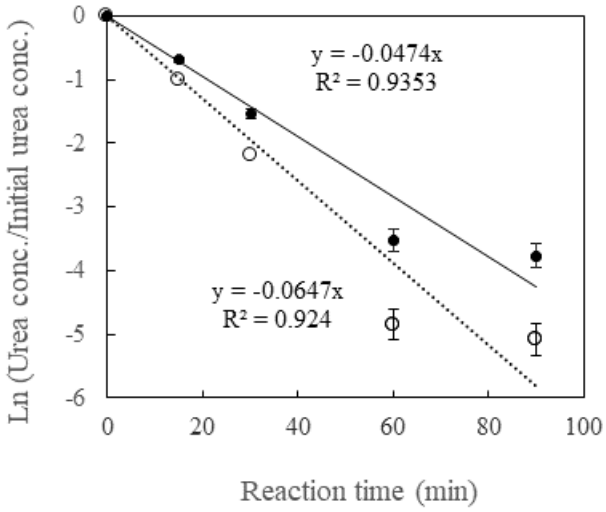

Fig. 3. Plot of first-order equation for urea concentration over time. Solid line is for urease on particle, and dot line is for polymer-inserted urease on particle

For the analysis of the reaction rate by enzymes, the first-order reaction of urea concentration was considered. The result of fitting with the first-order reaction was suitable for representing the characteristics of the reaction with a rate constant of 0.0474 min^{-1} and a determination coefficient of 0.9353 [10,11].

After the polymer was inserted between the urease and the microparticle, the characteristics of the reaction became faster. As shown in Fig. 3, the rate constant with the polymer insertion was 0.0647 min^{-1} with a determination coefficient of 0.924. The increase in the rate constant indicated that the polymer insertion facilitated the conversion, which was interpreted as the more frequent collisions between the urea and the urease more frequently due to the insertion because PEG was highly soluble in aqueous solution.

4. CONCLUSION

Urease covalently immobilized on the magnetic particles facilitated the conversion from urea to bicarbonate. The polymer insertion between the urease and the particle enhanced the conversion. The results of this study seem to be useful in designing a reactor for recycling wastewater.

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