

Effect of tetramethylammonium-hydroxide on urea conversion via enzymes immobilized on magnetic microparticles

Jin-Won Park

Department of Chemical and Biomolecular Engineering, College of Energy and Biotechnology, Seoul National University of Science and Technology
232 Gongneung-ro, Nowon-gu, Seoul, 01811, Republic of Korea
Corresponding Author: Jin-Won Park

ABSTRACT:

The effect of TMAH(tetramethylammonium-hydroxide) on the conversion of urea to bicarbonate by the immobilized enzyme was investigated. Urease was covalently immobilized on the magnetic particles, and it facilitated the conversion of urea to bicarbonate in the absence of TMAH. The conversion did not change until the concentration of TMAH reached 10 ppm, but decreased by less than half at 20 ppm. The results of this study seem to be useful in deriving the conditions for recycling wastewater generated in the semiconductor process.

KEYWORDS: Urea conversion, Enzyme immobilization, Magnetic particles, Tetramethylammoniumhydroxide

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I. INTRODUCTION

Ultrapure water is critically used 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 microstructure manufacturing requires a lot of water to rinse the structure, it has been considered to add the used-water into newly-supplied-water highly possibly including urea. In the used-water, tetramethylammonium-hydroxide (TMAH) is usually dissolved because TMAH is a common etchant for microstructure manufacturing. Therefore, it is aimed to investigate the effect of TMAH on the urea conversion via enzymes immobilized on magnetic microparticles.

II. EXPERIMENTS

Enzyme immobilization

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. 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 (7).

Cyclic-voltammetry

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. Under the concentration of TMAH from zero to 10 ppm, the conversion of 200 ppm urea was measured.

III. RESULTS

Current without TMAH

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.

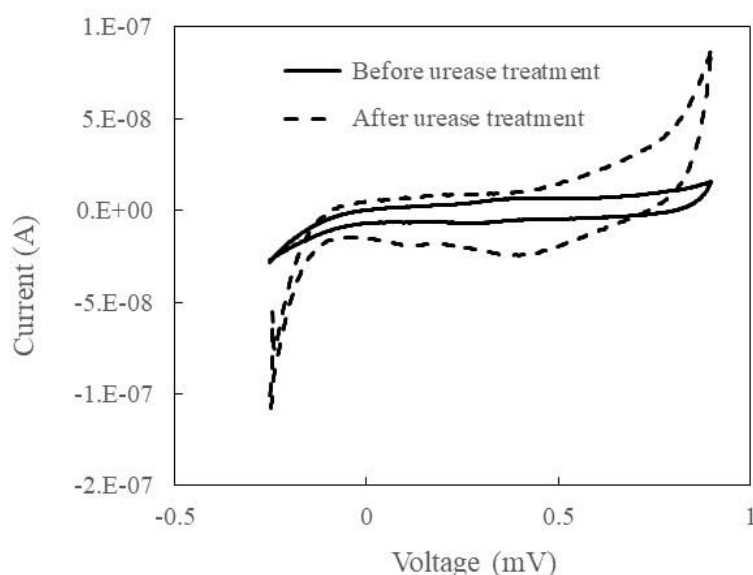


Fig.1. Current before and after urease treatment for 200 ppm urea

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.

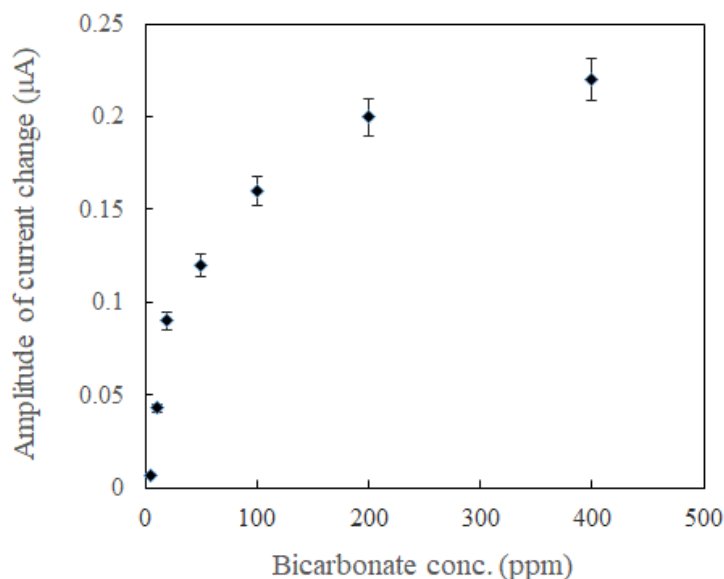


Fig.2. Standard curve between current and bicarbonate concentration

Current in the presence of TMAH

In order to find out the effect of TMAH, the current was measured in the solution including TMAH only. Then, the solution including both TMAH and urea

was characterized with CV. Fig. 3. suggests that the changes in the current were observed under the solution condition – 200 ppm urea, 10 ppm TMAH, and 200 ppm urea + 10 ppm TMAH, respectively.

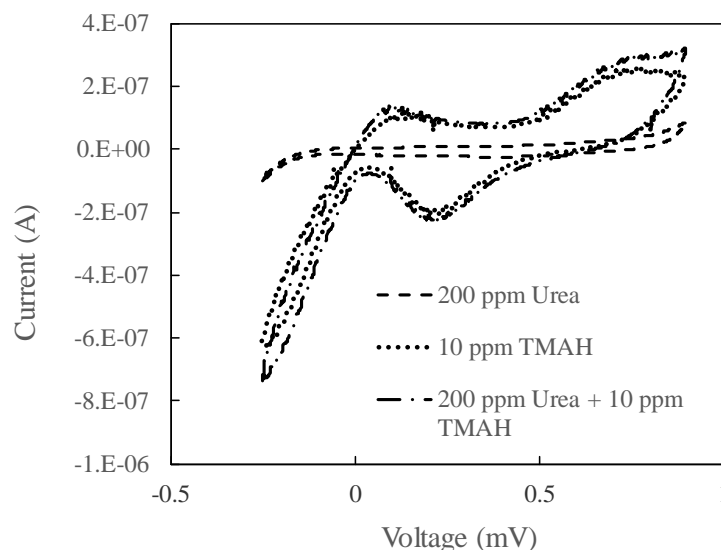


Fig.3. Current at 200 ppm urea, 10 ppm TMAH, and 200 ppm urea + 10 ppm TMAH after urease treatment

Since TMAH is a strong base, its dissociation occurs extremely frequently. This frequent dissociation caused the fluctuation of the current wave and the increase in the current-change amplitude. As shown in Fig. 3, the amplitude of 10 ppm TMAH is much greater than that of 200 ppm urea, although the urea concentration was 20 times higher than TMAH. After the urea was added to the

TMAH solution, the amplitude became larger than that of the TMAH solution before the addition.

The change in the amplitude was monitored with respect to the TMAH concentration. From zero to 20 ppm of TMAH, the amplitude was increased as expected. When the urea was added, the amplitude was also increased. However, the change in the amplitude according to the urea

addition was different between zero to 10 ppm and 15 to 20 ppm of TMAH. The change was identical

at zero to 10 ppm, while it became lower at 15 and 20 ppm. The data were summarized in Table 1.

Table 1. Current at each concentration of TMAH and 200 ppm urea addition

	TMAH (ppm)							
	0.0	0.5	1.0	2.0	5.0	10.0	15.0	20.0
Amplitude of current change ($\times 10^{-7}$ A)	0.4	1.7	3.3	4.8	6.9	8.5	9.4	10.1
	TMAH (ppm) + 200 ppm Urea							
	0.0	0.5	1.0	2.0	5.0	10.0	15.0	20.0
Amplitude of current change ($\times 10^{-7}$ A)	1.9	3.2	4.8	6.3	8.4	10.0	10.6	10.8

At zero to 10 ppm, the amplitude of current change induced by the urea addition was around 1.5×10^{-7} A. At 15 and 20 ppm of TMAH, the amplitude by the urea addition was 1.2×10^{-7} and 0.7×10^{-7} A, respectively. This decrease in the amplitude may be explained by two hypotheses. One is the enzyme denaturation caused by the increase in TMAH concentration, and the other is the inhibition of bicarbonate generation according to the Le Chatelier's Principle. The way to prove these hypotheses was to collect the urease immobilized on the magnetic-microparticles using magnetic field and to reuse them in the urea solution without TMAH. When the ureases were reused, the amplitude of current change was back to 1.9×10^{-7} A. Therefore, it has been found that the immobilized urease was little denatured up to 20 ppm of TMAH.

Below 10 ppm, the amplitude of current change induced by the urea addition was constant. In other words, the urea conversion was constant at zero to 10 ppm of TMAH. This constancy was believed because the ion movement was generated by the Brownian motion rather than the concentration gradient. As the TMAH concentration became higher, the concentration gradient became more dominant and the ion movement was restricted. The restriction of the movement was led to the more crowded ions and the urea conversion was inhibited.

IV. CONCLUSION

In this study, the effect of TMAH (tetramethylammonium-hydroxide) on the conversion of urea to bicarbonate by the immobilized enzyme was investigated. Urease was covalently immobilized on the magnetic particles, and it facilitated the conversion of urea to bicarbonate in the absence and the presence of TMAH. The conversion was monitored at zero to 20 ppm of TMAH, and the amplitude of the current change was found at each condition. The urea conversion was constant at zero to 10 ppm of

TMAH, and decreased at 15 and 20 ppm of TMAH. The decrease was interpreted due to the Le Chatelier's Principle because the reuse of the urease without TMAH showed returned to 1.9×10^{-7} A. The results of this study seem to be useful in deriving the conditions for recycling wastewater generated in the semiconductor process.

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