Jin-Won Park. International Journal of Engineering Research and Applications
www.ijera.com
ISSN: 2248-9622, Vol. 15, Issue 11, November 2025, pp 35-37

RESEARCH ARTICLE OPEN ACCESS

Influence of Membrane Pore Size on Immobilized Urease Activity

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, Republic of Korea, 01811

ABSTRACT
Membranes with different size of pores were used respectively as a ureases immobilization substrate. The reduction in the pore size lead to the uneven distribution of materials as the enzyme faciliated the urea conversion. The unevenness eventually caused the inhibition of the more conversion of urea to bicarbonate. The results of this study are expected to be useful in desiging a reactor for purification system to remove urea impurity.
Keywords **–** Membrane pore size, Urease immobilization, Urea conversion

Date of Submission: 01-11-2025 Date of acceptance: 08-11-2025

I. INTRODUCTION

Ultrapure water is essential to high-tech industries and acquired through more than several steps such as filtration, degassing, oxidation, and adsorption [1]. Urea is a unique molecule with high water-solubility, low molecular-weight, no volatility, little corrosiveness, and chemical stability [2]. This uniqueness makes urea good as an excellent material to melt ice, but makes it weak as a difficult impurity to be removed from water.

The current method for the removal is UV irradiation to generate radical to react with the urea. Only UV irradiation is insufficient for the removal at low urea concentration, and either persulfate or hypochlorous-acid is added to the reaction [3,4]. However the addition caused the second contamination, and the irradiation caused the enormous power consumption.

Since urease is able to convert urea into bicarbonate, the enzyme is considered as an alternative [5]. The enzyme immobilization lead to the increase in the conversion efficiency. Although, porous membranes were used as a support for the immobilization. the local urea concentration in the pores may be critical because the conversion is reversible. This study aims to investigate the changes in urease kinetics induced by immobilization pore sizes.

II. MATERIALS AND METHODS

50 U Urease solution of 20 mM MES covered the was added to cover the whole surface of each membrane with 20, 100, and 200 nm pore diameter for 3 hours, after the membranes were treated with 2.5% (v/v) ethyl(dimethylaminopropyl) carbodi- imide/N-hydroxysuccinimide (EDC/NHS) for 45 minutes. The immobilization was confirmed using X-ray photoelectron spectroscopy (XPS), and the immobilized amount was by subtracting the unbound from the injected concentrations identified using Bradford reagent. The amount was estimated to be 1.0 μM and 28 ng-protein/μm^2 [6].

Since the urea became ionic through the facilitation by the urease, the urea conversion was measured using cyclic voltammetry (CV) composed of composed of Ag/AgCl reference electrode, a Pt wire counter electrode, and a glassy carbon working electrode that were immersed in a desired solution contained in a Pyrex glass cell. 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.

III. RESULTS AND DISCUSSION

Using scanning electron microscope, the pore sizes were confirmed as shown in Fig. 1 (next page). After the confirmation, each step for the urease immobilization was performed followed by the characterization of the membrane using XPS. The results from the characterization were

summarized in Table 1 (next page). It was found that the carbon ratio after the EDC/NHS treatment and the sulfur ratio after the urease treatment increased. The change in ratio indicates that each step was performed successfully because the carbon and the sulfur originated from EDC/NHS and urease, respectively, in the initial alumina membranes without both elements.

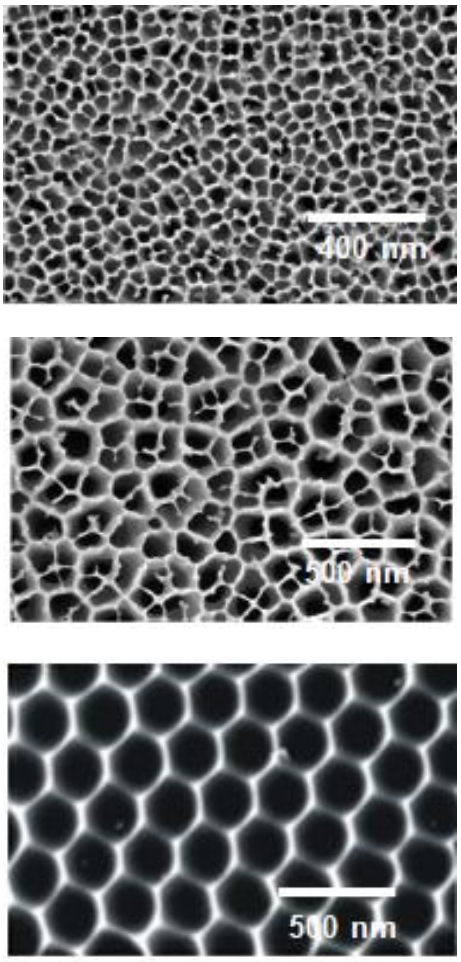

Fig. 1. Membrane structure, 20 nm pore (top), 100 nm (middle), and 200 nm (bottom).

Table 1. X-ray photoelectron spectroscopy results after each step.

	Bare alumina membrane	EDC/NHS	Urease
C 1s	0.1%	23.7%	25.7%
N 1s	0.1%	6.1%	6.6%
O 1s	55.3%	40.2%	42.3%
Al 2p	44.5%	30.5%	25.1%
S 2p			0.3%

Since the urease was found to induce the urea conversion previously, this study was for the change in the conversion with respect to the pore size of the surface where the urease was immobilized [5]. For all size, the conversion rate was linearly proportional to the urea concentration. The relation between the rate and the concentration was shown in Fig. 2. For the experimental data of Fig. 2, the least square fitting was performed and the determination coefficients were estimated into over 0.9. Therefore, the fitting equation reasonably suggested the physical behavior of the conversion in each pore size. The fitting corresponded to the first-order reaction of the urea concentration with 0.0525 min^{-1} for 200 nm, 0.0474 min^{-1} for 100 nm, and 0.0327 min^{-1} for 20 nm as the rate constants [7,8].

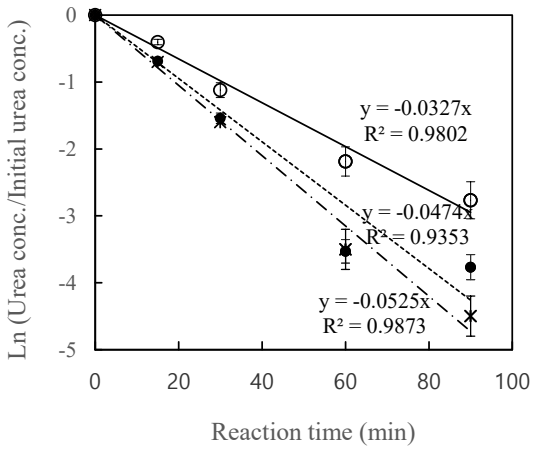

Fig. 2. Plot of first-order equation for urea concentration over time, solid line for 20 nm pore, dot-dash line for 100 nm pore, and dot line for 200 nm pore.

It was found that the reduction in the pore size lead to the decrease in the rate constant. This result seemed from the limitation of urea mass transfer, which was caused by the less urea-gradient due to the short supply in the smaller pore. The shortage was ambiguous for 100 and 200 nm pore because the size difference was relatively less prior to 100 minutes, but became clearer with the longer reaction time. This is consistent with the reciprocal relation between the Ln(Urea conc./Initial urea conc.) and the reaction time.

IV. CONCLUSION

The smaller the pores of the surface where the urease was immobilized were used, the slower the conversion of urea occurred. The change in the conversion seems to be induced by the less gradient of urea in the pore. The difference became more obvious with the longer reaction time.

Acknowledgements

This study was supported by the Research Program funded by Seoul National University of Science and Technology.

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