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Catalytic performances of chemically immobilized urease under static and dynamic conditions: A comparative study

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ABSTRACT

Immobilized urease has been used for direct removal of urea from aqueous solution and as biological sensing material in the preparation of urea biosensors. The former application is carried out under dynamic condition using ultrafiltration membrane either in tubular form or in flat sheet, while the latter is used in static condition. In this study, the performance of chemically immobilized urease on poly(acrylonitrile-co-sodium methallyl sulfonate) ultrafiltration membrane was determined under both static and dynamic conditions. Results reveal that the immobilization enhanced the thermal and storage stabilities of urease. The hydraulic permeability of urea solution was not influenced by the addition of enzyme layer. The maximum reaction rate measured under pressure in the ultrafiltration unit was found higher compared to the rate observed just under mixing without any pressure applied. The highest urea conversion was found at the lowest transmembrane pressure and the urea concentration in the feed solution. The catalytic activity of the membrane was completely preserved at the end of 450 min of filtration.

1. Introduction

The removal of urea from aqueous solutions is an important problem in various industries ranging from chemical, biomedical to food industries. To prevent water pollution, urea content of fertilizer waste water effluents should be reduced to acceptable limits set by Environmental Protection Agency. Urea present in the alcoholic beverages needs to be eliminated to prevent formation of carcinogenic compounds [1]. During a typical hemodialysis operation, 100–300 L dialysate solution is used to remove toxic compounds, mainly urea, from blood. To reduce the cost of the treatment, regeneration of dialysate solution by removing urea is necessary [1]. For long term human space flights, recycling of wastewater which includes urea coming mainly from human urine is crucial. Commonly used approaches for the removal of urea are nonenzymatic urea hydrolysis which requires high temperatures and pressures and biological conversion of urea nitrogen to dinitrogen which suffers from instabilities of microbial bed. Hence, both methods have high operating costs [2]. Adsorption is not considered as an alternative removal method since urea does not show high affinity to common adsorbents [3]. Urea rejection by reverse osmosis membranes is also not efficient [4,5]. An attractive, alternative removal method is based on the hydrolysis of urea by urease immobilized on a suitable support material. Urease is an enzyme widely occurring in nature and its presence has been detected in numerous organisms, including plants, bacteria, algae, fungi and invertebrates, and also in soils as a soil enzyme. Although urease has different protein structures, it catalyzes the hydrolysis of urea. Urea is quite stable in aqueous solutions with a half life of 3.6 years at 38 °C [6]. Urease catalyzes hydrolysis of urea at a rate 10^{14} times faster than the uncatalysed one. Ureases play a prominent role in the overall nitrogen metabolism in nature. The active site of urease comprises dinickel centers which is responsible to decompose urea into final products carbonic acid and ammonia. The functional and practical properties of ureases with their important applications including medical, analytical, environmental and engineering approaches have been extensively reviewed in literature [7].

Numerous techniques have been developed for immobilizing enzymes onto micro or nano beads, hollow fibers, membranes and capsules. Among these techniques, enzyme immobilization within membrane pores or onto membrane surface has gained growing interest due to controllable transport of reaction substrates and products through the membrane. This is particularly important when the product acts as an inhibitor. Synthetic membranes have been preferred as enzyme carriers because of their low cost, easy surface modification, resistance to biodegradation and thermal and chemical stabilities. A detailed review of the studies over the last two decades on the immobilization of urease has been published by Krajewska [1]. In our work, polyacrylonitrile (PAN) based flat sheet membrane was used as the support since reactive groups can easily be generated on the surface through hydrolization reac-

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Nomenclature

$D_{i,\infty}$	Brownian diffusion coefficient of solute, m^2/s, 1.48×10^{-9}
$D_{eff,i}$	effective diffusion coefficient of urea through ith layer, m^2/s, 1.09×10^{-9} for membrane and 6.55×10^{-10} for enzyme layers.
$K_{i,D}$	hindrance factor for diffusion of component i, dimensionless, 0.96
K_m	substrate concentration at which the observed reaction rate is half of V_{max}, $kmol/m^3$
K_0	overall mass transfer coefficient, m/s, 2.71×10^{-6}
k_s, k_p	mass transfer coefficients for the feed and permeate sides, m/s, 1.04×10^{-5} for feed and 3.97×10^{-6} for permeate sides
k_0	adsorption rate constant, min^{-1}
L_{enz}	thickness of enzymatic layer, m, 50×10^{-9}
L_{mem}	thickness of membrane layer, m, 25×10^{-6}
M_w	molecular weight of water, kg/kmol, 18.02
r	radius of the stirred cell, m, 0.011
Re	Reynolds number, dimensionless, 728
S	substrate concentration, $kmol/m^3$
Sc	Schmidt number, dimensionless, 606
T	temperature, K
V	reaction rate, $kmol/m^2$ s
V_{max}	maximum reaction rate possible if every enzyme molecule is saturated with substrate, $kmol/m^2$ s

Greek symbols

α	a constant which appears in Eq. (11), dimensionless, 0.23
δ_p	thickness of boundary layer, m, 3.73×10^{-4}
ε_i	porosity of ith layer, dimensionless, 0.8 for membrane and 0.48 for enzyme
Φ_i	partition coefficient of component i, dimensionless, 0.96
φ	association factor, dimensionless
λ_i	effective solute to pore size ratio, dimensionless
μ_w	dynamic viscosity of water, kg/m s, 8.94×10^{-4}
ν	kinematic viscosity of water, m^2/s, 8.97×10^{-7}
ν_A	molar volume of urea at its normal boiling point temperature, $m^3/kmol$, 0.058
ρ_i	density of component i, kg/m^3, 1170 for membrane and 997 for water
Γ_{max}	maximum surface adsorbed amount, $\mu g/cm^2$
ω	stirring speed, rev/s

tion. Lin et al. [8] and Yang et al. [9] immobilized urease covalently onto outer surface of polyacrylonitrile (PAN) hollow fiber membrane using gluteraldehyde as a crosslinking agent. Authors have reported that the stability of immobilized urease to pH was higher than those of native counterpart and the former retained 86% of its initial activity after reusing 15 times. Furthermore, removal rate of urea from a dialyzer which consists of urease immobilized membrane was measured 2 times faster than the rate in a regular dialyzer. Godjevargova and Gabrovska [10] immobilized urease covalently onto poly(acrylonitrile-methylmethacrylate-sodium vinylsulfonate) membranes subjected to seven different chemical modifications. The influences of modifications on pH optimum, water flow through the membrane, reaction rates, thermal inactivation and storage stabilities were investigated. Same group also immobilized urease onto PAN-chitosan composite membranes by first depositing chitosan onto PAN and then attaching urease covalently onto chitosan layer [11]. It has been found that the presence of amino groups facilitated the immobilization of urease onto the composite membrane. It is noted that in all of these studies glutaraldehyde was used as a cross linking agent, however, glutaraldehyde and its derivatives induce cytotoxic reactions during desorption [12]. In this study, we have used N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) with N-hydroxysuccinimide (NHS) coupling agent as zero length crosslinker which is reported to be non-cytotoxic in *in vitro* [13] and biocompatible in animal studies [14,15]. Urease in immobilized form are also used as biological sensing material for the fabrication of many types of urea sensors including amperometric [16,17], potentiometric [18,19] and optical [20,21]. Those applications require characterization of urease immobilized membranes under static and dynamic conditions. In view of this fact, in this work, we focused on urease (URE) immobilization onto PAN based ultrafiltration membrane covalently using EDC/NHS crosslinking agents and characterized these membranes in terms of optimum pH, temperature, kinetic parameters and storage stabilities under static conditions and in terms of transport properties and catalytic efficiencies under dynamic conditions.

2. Materials and methods

2.1. Materials

PAN based commercial flat sheet ultrafiltration membrane (AN69) with a molecular weight cut-off value of 30 kDa supplied by Gambro, Hospal, France was used as a support material. It is produced by the copolymerization of acrylonitrile and sodium methallyl sulfonate. The sulfonate groups makes AN69 negatively charged membrane. We have measured the wet thickness of the membrane as 25 μm and its pore radius from SEM images as 20 ± 6 nm which was reported in reference [22]. The porosity of AN69 flat sheet ultrafiltration membrane was reported as 0.8 by Langsdorf and Zydney and we have used this value in the calculations [23]. Jack bean urease type III (EC 3.5.1.5 and U1500-20KU, 40 U/mg solid) and Bradford reagent (B 6916) for the determination of protein amount were purchased from Sigma–Aldrich. EDC and NHS coupling agent were obtained from Sigma and Fluka, respectively. Urea and phosphate buffer were purchased from Fluka. Phenol (Rectabur >99%), sodium nitroprusside dihydrate (Fluka), sodium hydroxide anhydrous pellets (Carlo Erba >97%), sodium hypochlorite (Riedel, 6–14% Cl active), sodium phosphate buffer solutions (NaH_2PO_4, Na_2HPO_4) and acetic acid (Fluka) were purchased from different companies. All aqueous solutions were prepared with milli-Q water (>18 MΩ cm).

2.2. Methods

2.2.1. Fabrication of surface modified AN69 membrane

The surface of $6 \times 6\ cm^2$ AN69 membrane was hydrolyzed in 100 mL of aqueous solution containing 1 M NaOH. The hydrolysis reaction took place on a shaker (150 rpm) at 50 °C for 20 min. The membrane was then successively rinsed with water and then with 0.01 M phosphate buffer solution at pH 5.5. During rinsing, the yellowish red color of hydrolyzed AN69 membrane was turned into white. As a result of chemical modification, cyanide (–CN) groups on the membrane surface were partially converted into carboxylic (–COOH) and amide ($-CONH_2$) groups and was designated as AN69-A. Activation of the carboxylic groups was performed with EDC/NHS coupling agent. For this purpose, $3 \times 3\ cm^2$ of AN69-A membrane was immersed in 10 mL 0.01 M phosphate buffer solution at pH 5.5 containing predetermined amount of EDC/NHS and the solution was stirred at a rate of 100 rpm. In all activation steps, the