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Directing filtration to optimize enzyme immobilization in reactive membranes

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ABSTRACT

In this work, fouling principles in force in ultrafiltration were deployed to understand the role of selected variables—applied pressure (1–3 bar), enzyme concentration (0.05–0.2 g L^{-1}), pH (5–9) and membrane properties—on fouling-induced enzyme immobilization. The immobilization and subsequent enzymatic reaction efficiency were evaluated in terms of enzyme loading, conversion rate and biocatalytic stability. Alcohol dehydrogenase (ADH) was selected as a model enzyme. Lower pressure, higher enzyme concentration and lower pH resulted in higher irreversible fouling resistance and lower permeate flux. High pH during immobilization produced increased permeate flux but declines in conversion rates, likely because of the weak immobilization resulting from strong electrostatic repulsion between enzymes and membrane. The results showed that pore blocking as a fouling mechanism permitted a higher enzyme loading but generated more permeability loss, while cake layer formation increased enzyme stability but resulted in low loading rate. Low pH (near isoelectric point) favored hydrophobic and electrostatic adsorption of enzymes on the membrane, which reduced the enzyme stability. Neutral pH, however, promoted entrapment and hydrogen bonding of enzymes on the membrane, which improved the enzyme stability. This study suggests that a compromise between different fouling/immobilization mechanisms must be found in order to maximize the immobilization performance, both in terms of enzyme loading and also of enzyme activity.

1. Introduction

Membrane fouling is a major limitation in most processes involving membrane bioreactors [1–4], especially in enzymatic membrane reactors (EMR). Fouling reduces the membrane performance in terms of separation efficiency and permeate flux [5,6]. Membrane fouling commonly consists of an external cake/gel layer and/or an internal pore blocking of the membrane, which are caused by complex interactions between membrane and foulants, such as hydrophobic/electrostatic adsorption, particle deposition/aggregation, hydrogen bonding and bio-affinity [7–9]. Various fouling control strategies such as increasing shear rate on the membrane, modifying pH, or applying an external electric field may contribute to prevent fouling formation, but can also exert a negative effect on the enzymes by e.g. accelerating enzyme inactivation. Inversely, immobilization in/on membranes can increase the stability of the enzyme, albeit commonly resulting in permeability loss [10,11]. To this regard, Sen et al. found that the permeability of ultrafiltration (UF) membranes dropped by 19–87% after covalent immobilization of β-galactosidase enzymes on membranes [12]. Giorno et al. reported that after immobilizing fumarase within the spongy layer of capillary membrane by entrapment (0.009–0.052 mg cm^{-2}), the membrane permeability decreased by 43–84% because of the membrane pore blockage [13].

Since enzymes in EMR will inevitably foul the membrane, we hypothesized that controlled fouling could be used deliberately to immobilize enzymes in an efficient manner. At the same time, the activity and stability of the enzymes could be improved by manipulating filtration variables according to fouling formation mechanisms. To this regard, and based on many parallels between membrane fouling mechanisms and enzyme immobilization strategies [1,7,8,10,12,13], a concept of fouling-induced enzyme immobilization was proposed and verified in our previous studies [14]. Albeit fouling is generally considered as a "negative" aspect of membrane filtration; here it is just regarded as a "neutral" phenomenon that enables solutes to be "docked" in/on membrane by simple filtration. In this way, fouling mechanisms can be utilized as "positive" tools for enzyme immobilization. Theoretically, the

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enzyme immobilization process can be consolidated by various fouling mechanisms. However, in order to foster desirable fouling (maintain enzyme activity and stability), (1) optimization of process parameters (e.g. pressure and pH), and (2) selection of suitable operating modes and membranes are necessary. Fouling-induced enzyme immobilization is a simple procedure, and the abundant existing knowledge of membrane fouling, can be used to achieve optimal enzyme immobilization.

A large number of studies about the effects of process parameters and membrane properties on membrane fouling have been reported, especially for protein fouling in ultrafiltration (UF) [2,7,9,15–23]. She et al. found that the fouling rate was higher at higher applied pressures and higher protein concentrations. Fouling was also most severe at the isoelectric point (IEP) of bovine serum albumin (BSA), and the effect of salt on fouling behavior depended on the solution pH [21]. Jones and O'Melia reported that fouling by adsorption was higher at lower pH, and that increasing salt concentrations would decrease adsorption of BSA on a regenerated cellulose UF membrane [17]. Chan and Chen claimed, however, that aggregation and deposition of BSA could be hindered at higher pH and less salt concentration, and that the salt effect might be opposite for different membrane materials and pH [18]. As for the membrane materials, many authors reported that the regenerated cellulose membrane was more resistant to organic fouling than other membrane materials [24,25]. Lim and Mohammad found that there was insignificant fouling under static conditions for cellulose membrane, but severe fouling was observed during the dynamic filtration of gelatin [22]. Using this knowledge, membrane fouling can be well controlled during protein concentration or purification by UF. Although these reports are available as references for fouling-induced enzyme immobilization, there is a scarcity of experience to extend the existing knowledge (mainly for fouling control) to enzyme immobilization, where the purpose is to build a porous, stable and catalytically active enzyme–fouling layer on/in membrane by optimizing process parameters.

The present work was undertaken to examine and optimize the main variables involved in fouling-induced enzyme immobilization, i.e. applied pressure, enzyme concentration, pH and membrane properties on the immobilization and the biocatalytic performance (conversion rate and biocatalytic productivity). Alcohol dehydrogenase (ADH), able to catalyze the conversion of formaldehyde (HCOH) to methanol (CH_3OH) with oxidation of NADH to NAD^+ (the third step of multi-enzymatic catalysis of CO_2 to methanol [26]), was used in this study. In order to provide a better base for circumventing low permeate flux and enzyme leakage/inactivation, the fouling/immobilization mechanisms were modeled using two different membrane fouling models.

2. Materials and methods

2.1. Chemicals and membranes

Alcohol dehydrogenase (ADH, EC 1.1.1.1) from *Saccharomyces cerevisiae*, β-nicotinamide adenine dinucleotide reduced form (NADH) and formaldehyde (37%, w/w) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All enzyme and substrate solutions were prepared using 0.1 M phosphate buffer at different pH. The isoelectric point (IEP) of ADH enzyme is 5.4–5.8 (manufacturer's information). The molecular weights of ADH, NADH and formaldehyde are 141, 0.7 and 0.03 kDa, respectively. Three commercial UF membranes were used, and their main properties are summarized in Table 1 [19,27,28]. The SEM photos of support and skin layers of GR51PP membrane are shown in Fig. 1. Compared with the smooth and dense skin layer (Fig. 1c), the porous support layer is more accessible for enzymes, and fouling layer is easier to be formed in support layer because its fibers (Fig. 1a) can act as fouling "skeleton" or adsorption sites (Fig. 1b). Therefore, in this work, membranes were placed in reversed mode (support layer facing feed). In order to avoid the compression of skin layer, an extra polypropylene support (obtained from the PLTK membrane)

Table 1
Main characteristics of the ultrafiltration membranes used in the study.

Membrane	GR51PP	GR61PP	PLTK
Manufacturer	Alfa Laval	Alfa Laval	Millipore
MWCO (kDa)	50	20	30
Skin material	Polysulphone	Polysulphone	Regenerated cellulose
Support material	Polypropylene	Polypropylene	Polypropylene
Thickness (μm)	300[a]	350[a]	230
Isoelectric point (IEP)	4–5 [28]	5–6 [27]	~3.5 [19]
Permeability ($L\,m^{-2}\,h^{-1}\,bar^{-1}$)[b]	45.2 ± 3.8	52.1 ± 11.3	335.9 ± 7.4

MWCO: molecular weight cut-off.

[a] Own measurements by micrometer.

[b] Own measurements in the "sandwich" configuration at room temperature.

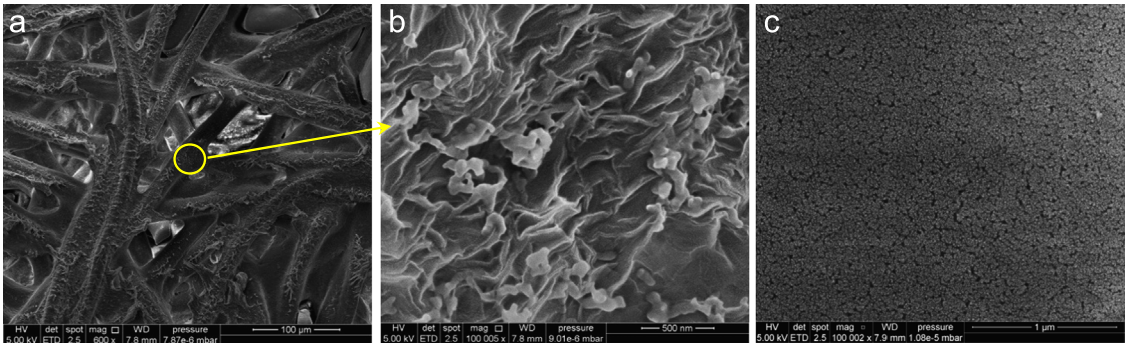

Fig. 1. SEM pictures of (a) support layer × 600, (b) support layer × 100,000 and (c) skin layer × 100,000 of GR51PP membrane.