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Fouling-induced enzyme immobilization for membrane reactors

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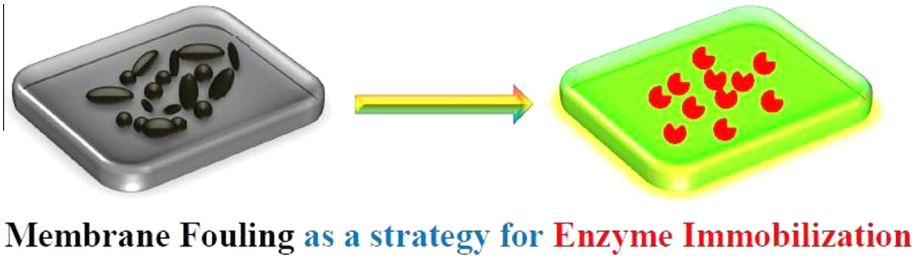
HIGHLIGHTS

- Fouling-induced enzyme immobilization in membranes is proposed.
- Two membrane orientations for enzyme immobilization are evaluated.
- Alcohol dehydrogenase and glutamate dehydrogenase are used to verify this concept.
- The enzymes immobilized in the membrane support layer retain most of their activity.
- The catalytically active membranes have high efficiency and recycling stability.

GRAPHICAL ABSTRACT



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ABSTRACT

A simple enzyme immobilization method accomplished by promoting membrane fouling formation is proposed. The immobilization method is based on adsorption and entrapment of the enzymes in/on the membrane. To evaluate the concept, two membrane orientations, skin layer facing feed (normal mode) and support layer facing feed (reverse mode), were used to immobilize alcohol dehydrogenase (ADH, EC 1.1.1.1) and glutamate dehydrogenase (GDH, EC 1.4.1.3), respectively. The nature of the fouling in each mode was determined by filtration fouling models. The permeate flux was larger in the normal mode, but the reverse mode allowed for higher enzyme loading and stability, and irreversible fouling (i.e. pore blocking) developed more readily in the support structure than in the skin layer. Compared with an enzymatic membrane reactor (EMR) with free enzymes, the novel EMR with enzymes immobilized in membrane support improved the enzyme reusability (especially for ADH), and reduced the product inhibition (especially for GDH).

1. Introduction

Enzyme biocatalysis is positioned as a green and sustainable technology (Wohlgemuth, 2010) but achievement of satisfactory bioconversion efficiencies and effective use of the enzymes may require special measures such as re-cycling or immobilization for successful industrial application (Garcia-Galan et al., 2011). Re-use of enzymes can be accomplished by using enzymatic membrane reactors (EMR), in which the enzymes are retained and separated from the end products via a selective membrane (Andrić et al., 2010; Giorno and Drioli, 2000; Jochems et al., 2011; Rios et al., 2004). In addition, membrane technology is gaining momentum for separation of biorefinery products resulting from enzyme catalyzed conversions (Pinelo et al., 2009; Rios et al., 2004). In EMR, the enzyme molecules may be free in solution in the reactor, i.e. retained from the product by the membrane, or immobilized in the porous structure or on the surface of the membrane (Cabrera-Padilla et al., 2009; Jochems et al., 2011; Yurekli and Alsoy Altinkaya, 2011; Zhang et al., 2010). Integration of enzyme immobilization with membrane technology enables continuous operation, facile product purification, and prevents

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Table 1
Similarities between membrane fouling and enzyme immobilization mechanisms (Guo et al., 2012; Sassolas et al., 2012).

Fouling types	Behavior description	Immobilization mechanisms
Adsorption fouling	Particles are adsorbed in/on membrane by hydrophobic and electrostatic interactions	Adsorption
Pore blocking	Particles are entrapped or embed in membrane pores	Entrapment
Membrane surface modification by foulants	Particles are combined with membrane by chemical binding between their functional groups	Covalent coupling
Combined fouling or inorganic–organic fouling or "cake" layer	Particles are combined with membrane by chemical cross-linker (acting as "bridge" between particles and membrane or among particles)	Cross-linking
Biofouling	Microorganisms or bioactive particles grow or adhere on membrane	Affinity

product inhibition (Andrić et al., 2010; Giorno and Drioli, 2000; Jochems et al., 2011; Rios et al., 2004). Membrane fouling is, however, commonly inevitable and detrimental to membrane performance. It is well known that physical adsorption, pore blocking, gel or cake formation, and biofouling are the main fouling types for membrane filtration (Guo et al., 2012). Fouling is particularly problematic during protein fractionation by ultrafiltration (UF), causing dramatic flux decline and reduction in membrane selectivity (Ghosh, 2002; Huisman et al., 2000; Luo et al., 2011).

On the other hand, deliberate promotion of fouling might be used as a strategy for immobilization of enzymes in membranes, since membrane fouling and enzyme immobilization mechanisms share a number of features (Table 1). For instance, entrapment of enzymes in the membrane pores can be considered as membrane fouling, i.e. pore blocking, as well as an enzyme immobilization entrapment mechanism. Based on such similarities (Table 1), membrane fouling with enzymes can be regarded as a multifunctional immobilization strategy based on adsorption and entrapment (Beier et al., 2007; Mazzuca et al., 2006). If a functional reagent is added into the enzyme solution, the enzymes may be covalently bound to the membrane by cross-linking, a mechanism which has been coined as "combined fouling" (Contreras et al., 2009) (Table 1). Giorno and co-workers (Giorno et al., 2001, 2006) immobilized enzymes within an asymmetric capillary membrane by crossflow filtration for a biphasic membrane bioreactor. The enzyme activity did not decay after entrapment in the sponge layer of the capillary membrane, implying that enzymes could be immobilized within the membrane by a simple filtration.

The present study was undertaken to assess the concept of fouling-induced enzyme immobilization, and notably to examine the possible significance of the membrane orientation for the enzyme immobilization and the subsequent operational performance of the EMR. This is the first attempt to explain enzyme immobilization process from a different angle, i.e. fouling formation. In order to keep the process simple and low-cost, a common commercial membrane material – polysulphone/polypropylene was used. The conversion from formaldehyde to methanol catalyzed by alcohol dehydrogenase (ADH, EC 1.1.1.1) and cofactor (NADH), which is the third step of multi-enzymatic catalysis of carbon dioxide (CO_2) to methanol (Obert and Dave, 1999), and the regeneration of cofactor (NADH) by glutamate dehydrogenase (GDH, EC 1.4.1.3) and glutamic acid (El-Zahab et al., 2008), were used as examples for assessing this new concept. In this sense, this study can be seen as a preliminary stage of investigating CO_2 conversion using enzymatic catalysis and membrane technology. Compared to other immobilization methods, immobilization by fouling formation in a membrane is preferred in this case because it can be used to allow a gaseous substrate (e.g. CO_2) to be forced to pass the enzyme multiple times, offers opportunities for substrate/product separation directly, and also enables coimmobilization of several enzymes by the synergistic effect of multi-fouling mechanisms.

2. Membrane fouling models

As illustrated in Fig. 1, enzyme molecules are carried to the membrane surface by convective transport, where the solvent passes through the membrane and the retained enzymes cause a local concentration increase. Meanwhile, the rejected particles, i.e. in this case the enzymes, cannot readily diffuse back into the bulk solution, and as a result a concentration polarization (CP) layer and subsequently an irreversible fouling layer will form in/on the membrane (Fig. 1). Presumably, due to the support layer being a porous protection layer, the support layer is envisaged to function as a shelter within which the shear rate becomes negligible, which results in the production of a thick CP layer, in turn

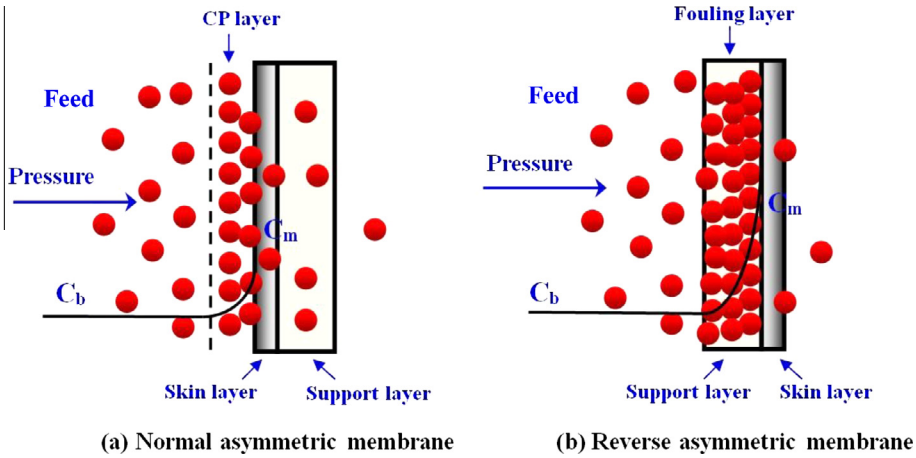

Fig. 1. Schematic diagram of two membrane orientations for enzyme immobilization (C_b: feed concentration in bulk; C_m: solute concentration on membrane skin layer; CP: concentration polarization).