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Effect of pH on membrane fouling during alcohol dehydrogenase immobilization in PES membrane

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Abstract

Fouling-induced enzyme immobilization is a technique to immobilize enzyme by positively manipulating the knowledge of membrane fouling. In this study, Alcohol dehydrogenase (ADH) (EC 1.1.1.1) was immobilized in the support layer of ultrafiltration PES membrane at different solution pH (acid, neutral and alkaline). ADH catalyses formaldehyde (CHOH) to methanol (CH_3OH) and simultaneously oxidised nicotinamide adenine dinucleotide (NADH) to NAD^+. The initial feed amount of enzyme is 3.0 mg. The objective of the study aims at the effect of different pH of feed solution during enzyme immobilization, in terms of permeate flux, observed rejection, enzyme loading and fouling mechanism. The results showed that, pH 5 holds the highest enzyme loading which is 65% while pH 7 holds the lowest at 52% out of 3.0 mg as the initial enzyme feed. The permeate flux for each pH decreased with increasing cumulative permeate volume. The observed rejection is inversely correlated with the pH where increase in pH will cause a lower observed rejection. The fouling model predicted that irreversible fouling occurs during enzyme immobilization at pH 7 with standard blocking mechanism while reversible fouling occurs at pH 5 and 9 with intermediate and complete blocking, respectively.

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1.0 Introduction

Enzymatic catalysis is regarded as sustainable green technology. The enzyme is usually immobilized in order to enhance the biocatalytic efficiency (productivity). Immobilization of enzymes on/in membrane is beneficial as desired compound separation can be achieved concurrently with the biocatalytic reactions in enzyme membrane reactors (EMRs) (Luo et al., 2014a; Marpani et al., 2015). Many researchers have focused their research on how enzyme properties like stability, activity or selectivity can be improved by immobilization (An et al., 2015; Gao et al., 2014; Mateo et al., 2007). Immobilization of enzymes in or on membranes can be accomplished via adsorption, covalent bonding, cross-linking or entrapment. Normally, enzyme reusability can be attained by using enzymatic membrane reactor in which enzymes are retained in membrane and separated from product solution (Luo et al., 2014a). Generally, the amount of immobilized protein can be determined by mass balance between the initial solution (feed) and the solutions after immobilization process which is either retentate or permeate (Mazzuca et al., 2006).

Membrane fouling is an inevitable phenomenon occurs in every membrane filtration processes. It was reported that fouling was more severe at the isoelectric point of bovine serum albumin (BSA), where no effect on interaction force between protein molecules (She et al., 2009). At isoelectric point, the net charge on the protein is zero which is no net electrostatic repulsion between or within the macromolecules (Jones & O'Melia, 2000). The insensitivity of the stable flux on the ionic strength is also consistent with the lack of electrostatic repulsive force at pH of isoelectric point. Fouling by adsorption was higher at lower pH values, and increasing salt concentration can reduce electrostatic repulsion between the positively charged protein molecules on regenerated cellulose

membranes. However, Chan & Chen (2001) reported that, formation of aggregation and deposition of BSA can be hindered or reduced at higher pH values which is above the pH of isoelectric point. Therefore, the effect of salt on fouling performance depends on the solution pH. The existence of salts can adjust electrostatic protein-protein interactions and produce the effect of shielding charge and dampening out of intermolecular protein interactions (Chan & Chen, 2001; She et al., 2009).

The membrane fouling commonly compromises the performance of the membrane in terms of separation efficiency and permeate flux (Ismail et al., 2020; Marpani et al., 2019). The main types of fouling membranes are physical adsorption, pore blocking of membrane, gel/cake formation and biofouling which are caused by complex interactions between membranes and foulants such as hydrophobic or electrostatic adsorption, particle deposition or aggregation, hydrogen bonding and bio-affinity (Guo et al., 2012; Sassolas et al., 2012). Nevertheless, we can apply the knowledge of membrane fouling and benefitted the biocatalysis in terms of enzyme-substrate contact time.

Since membrane fouling and enzyme immobilization share several characteristics, we can conclude that, deliberate promotion of fouling might be used as strategy for immobilization of enzymes in membranes. For instance, entrapment of enzymes in membrane pores can be measured as membrane fouling i.e., pore blocking as well as immobilization entrapment mechanism. At the same time, according to the fouling formation theory, activity and stability of enzymes can be improved by manipulating filtration variables. The concept of fouling-induced enzyme immobilization is a simple procedure and abundant existing knowledge of membrane fouling can be used in order to achieve immobilized enzyme in an efficient manner.

The purposes of the present study are to evaluate the effect of pH value on enzyme loading rate in membrane and to examine the effect of pH on permeate flux, observed rejection, enzyme loading and fouling mechanisms. Alcohol dehydrogenase (ADH) has been selected as the model enzyme. This is because ADH is capable to catalyse the conversion of formaldehyde (HCOH) to methanol (CH_3OH) with oxidation of NADH to NAD^+, the third step of multi-enzymatic catalysis of carbon dioxide (CO_2) to methanol (CH_3OH).

2.0 Methodology

2.1 Material

All chemicals used in the experiments were purchased from Sigma-Aldrich (St. Louis, MO, USA) which include alcohol dehydrogenase from *Saccharomyces cerevisiae*, β-nicotinamide dinucleotide (NADH), formaldehyde (37% w/w), sodium acetate trihydrate, acetic acid, dipotassium phosphate (K_2HPO_4), monopotassium phosphate (KH_2PO_4), Trizma-base and hydrochloric acid. Enzyme and substrate solution were prepared with different buffer solution, 0.1 M Acetate buffer at pH 5, 0.1 M tris-HCl at pH 7 and 9. The molecular weight of formaldehyde, NADH and ADH were 0.03 kDa, 0.7 and 141 kDa respectively. The ultrafiltration membrane used is made of poly(ether)sulfone (PES) (skin) and polypropylene (support), 30 kDa molecular weight cut off, with 13.4 cm^2 of effective area (Synder, CA, USA).

2.2 Experimental setup and procedure

Membrane was soaked in ultrapure water for two minutes and followed by 50% ethanol solution for two minutes to remove the protective layer of the membrane based on manufacturer's instructions. The membrane support layer was facing feed. An extra polypropylene support was placed below the skin layer to avoid compression. The ultrafiltration experiments were carried out in a 50 ml stirred cell (Amicon 8050, Milipore, USA) with fixed stirring speed of 100 rpm. Nitrogen gas pressure of 1 bar was purged in to measure water permeability of membrane. Water permeability was measured by filtration of pure water continuously for 10 minutes. A pressure of 2 bar was set during enzyme immobilization and reaction. Permeate was collected in a 10 ml cylinder tube to monitor the permeate flux. All the experiments were performed at room temperature (25°C).

2.3 Enzyme immobilization

Thirty millilitres of ADH enzyme solution at a concentration of 0.1 g/l and pH 7 was poured into the cell. Enzyme immobilization was carried out at 1, 2 and 3 bars. A precision cylinder was used to collect 4 ml of the permeate that will be used for further analysis. . The cylinder was replaced manually every 4 ml of permeate until 28 ml permeate was collected. The fouled membrane was rinsed 3 times at the end of the filtration

with 5 ml of buffer each time. No pressure was applied at this stage. Lastly, the fouled membrane was pressure-filtered by buffer (pH 7) at 2 bar and the permeate was collected for mass balance analysis.

2.4 Enzyme reaction

The enzyme reaction of 30 ml of substrate mixture (134 µM HCOH + 100 µM NADH) at pH 7 was fed into the Amicon stirred cell which was prior immobilized with ADH. Permeates were collected for every 4 ml and analysed immediately. Absorbance at 340 nm was recorded to monitor NADH concentration during reaction.

2.5 Calculated parameters

Observed rejection of enzyme was calculated as:

$$R_{obs}(\%) = \left(1 - \frac{C_p}{C_o}\right) \times 100 \quad (1)$$

where, C_p (concentration of enzyme in permeate) and C_o (concentration of enzyme in the feed) during immobilization.

The amount of enzyme immobilized can be estimated from the following equation:

$$m_i = m_t - C_p V_p - C_r V_r - C_w V_w \quad (2)$$

The enzyme immobilization efficiency of in the membrane is expressed as loading percentage:

$$Enzyme\ loading\ (\%) = \frac{m_i}{m_t} \times 100 \quad (3)$$

where m_i is amount of immobilized enzyme, m_t is the amount of enzyme in the feed, C_p, C_r and C_w are the concentration of enzyme in the permeate, retentate and rinsing residual, and pressure-driven washing, respectively. V_p, V_r and V_w are the volume obtained from the permeate, retentate and rinsing residual, and pressure-driven washing, respectively.

3.0 Results and discussion

3.1 Effect of different pH on membrane permeability and enzyme immobilization

Permeate flux during enzyme immobilization at different solution pH is shown in Fig. 1. Pristine PES membrane recorded a water permeability of 91.5 L/m^2·h·bar. Membrane permeability decreases with time (cumulative permeate volume). The average membrane permeability is decreasing when the solution pH is lower (acid). The trend is the same for all solution pH. Fig. 2 shows the observed rejections as a function of permeate flux at different pH. Observed rejection on membrane is an index of solutes retainment by the membrane whether the solutes show lower solubility in water or the solutes diffusion occurs at a low pace through the membrane. The index is indicated by 100% for completely permeable membrane, while 0% indicates completely impermeable membrane.

During earlier filtration process of enzyme, the flux decline is rapid for the first 10 ml of permeate (Fig. 1). At the same time, the membrane showed to be completely permeable with more than 80% observed rejection (Fig. 2). After some time, the flux decline is becoming gradual (Fig. 1). At this stage, the membrane

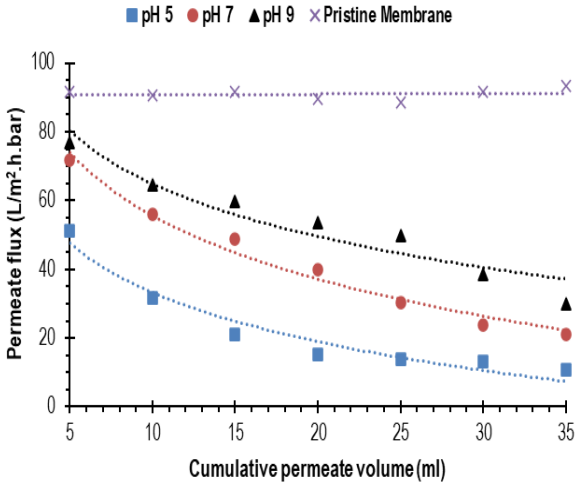

Fig. 1: Permeate flux trend during filtration of enzyme in the support layer of membrane at different pH

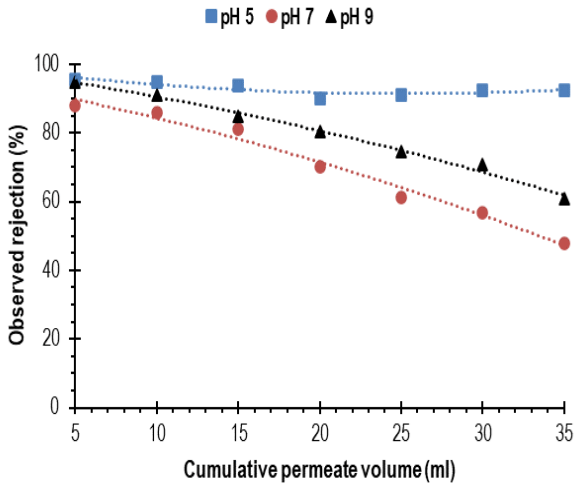

Fig. 2: Membrane observed rejection during filtration of enzyme in the support layer of membrane at different pH

become semi-permeable because the observed rejection is at 50% at the end of filtration process for pH 7 and 60% for pH 9. The observed rejection is stable above 90% throughout filtration process at pH 5. The results show that the membrane was responsive towards different solution pH with severe flux decline observed at the lowest pH (pH 5).

When the pH of solution is neutral, the lowest enzyme loading was recorded which is 52.2% from 3.0 mg in the feed solution (Table 1). The highest enzyme loading is observed at pH 5 with 65.1%. There are two mechanisms by which enzyme could anchor on the membrane. It is either fouling by electrostatic charge and hydrophobic interaction or retention by electrostatic repulsion and steric hindrance (Luo et al., 2014b; Schafer & Semiao, 2013). It could also be a synergistic mechanism of the two mechanisms. It was suggested that at pH 5, the enzyme is anchored on the membrane by electrostatic charge and hydrophobic interaction, while at pH 9, enzyme retention by electrostatic repulsion and steric hindrance dominated (Luo et al., 2014b).

3.2 Effect of different pH on membrane fouling

Membrane fouling degree can be differentiated during enzyme immobilization at different solution pH. The discussion on the effect of different pH on membrane fouling is strongly related to the isoelectric point (pI). pI is the pH of a solution at which the net charge of a protein becomes zero. In other words, the net charge of macromolecular protein is positive if the surrounding pH is less than the pI, while the net charge is negative if the pH is more than the pI. Hence, at any time when the pH of solution is different from pI, the protein surfaces could possess net positive or negative charges. pI for PES membrane is at pH range of 4 to 5 while pI for ADH enzymes is around pH 5.4 to 5.8 (Nyström & Zhu, 1997). Generally, when the filtrating solution is acid (pH 5), the membrane was almost neutral. At this pH, enzyme is positively charged. Fouling is prominent because there is significant hydrophobic adsorption between enzymes and the membrane (Hadidi & Zydney, 2014; Voorthuizen et al., 2001). This explains the highest loading of enzyme at 65.1% at pH 5, compared to pH 7 and pH 9.

In this study, fouling mechanism is described by fitting in permeate flux data during enzyme immobilization at different solution pH into Hermia model. This model is selected considering that it is the most complete model describing dead-end filtration in batch system (Ismail et al., 2019). According to this model, there are mainly four fouling mechanisms, which are described as complete blocking, intermediate blocking, standard blocking, and cake layer formation. Complete blocking is interpreted when the size of ADH enzymes (foulant) is the same with the pore size of the membrane and the foulant deposited on the pores completely blocked the flow passage.

This type of fouling mechanism will result in reduced number of ‘free’ pores. In intermediate blocking, a single foulant could form aggregates with other particles and create multi-layers on the membrane surface which subsequently lead to increase in cake thickness. Standard blocking occurs when the foulant deposited on the internal pore wall, hence reducing the free pore volume. The most severe fouling mechanism is the cake layer formation, whereby all the foulants deposited on top of each foulants which previously had blocked the pores. The type of fouling is determined by the highest degree of model fitness (R^2) when fitting in permeate flux data during enzyme immobilization at different solution pH.

At pH 5, intermediate blocking dominates. At this pH, the surface of the membrane obtained positive net charge because the solution pH is lower than the isoelectric point of the membrane. The feed solution is also positively charged at pH 5. Since the same charges occurred on the surface of the membrane, the enzyme molecules will be repelled away from the surface of the membrane. Nevertheless, during pressure-driven filtration, the enzyme molecules will be ‘forced’ to dock on the surface of the membrane. This allows more enzyme molecules to deposit on the previously accumulated ADH on the membrane pores.

At pH 9, complete blocking dominates. The membrane surface obtained negative net charge because the solution pH is higher than the isoelectric point of the membrane. Different charges interaction on the surface of the membrane causes the enzyme molecules attracted on the surface of the membrane and completely blocked the pores. Only 62.1% is immobilized on the surface of the membrane, there are ‘free’ pores on the membrane compared to intermediate blocking, and responsible for a higher permeate flux from the rest. At pH 7, standard blocking dominates. For this type of fouling, enzyme molecules adsorbed inside the membrane pores and caused reduction in pore diameter (Kirschner et al., 2019).

Table 1: Enzyme loading on the membrane at different pH

Solution pH	Amount of enzyme (mg)				Enzyme loading
	Feed	Permeate	Retentate	Washing Residue	(%)
5	3.0	0.201	0.415	0.430	65.1
7	3.0	0.773	0.201	0.461	52.2
9	3.0	0.624	0.155	0.357	62.1

Table 2: R^2 values calculated from Hermia's model data fitting

pH	Standard blocking	Intermediate blocking	Complete blocking	Cake layer
5	0.9864	**0.9911**	0.9607	0.948
7	**0.9842**	0.978	0.98	0.9434
9	0.9744	0.954	**0.9858**	0.8949

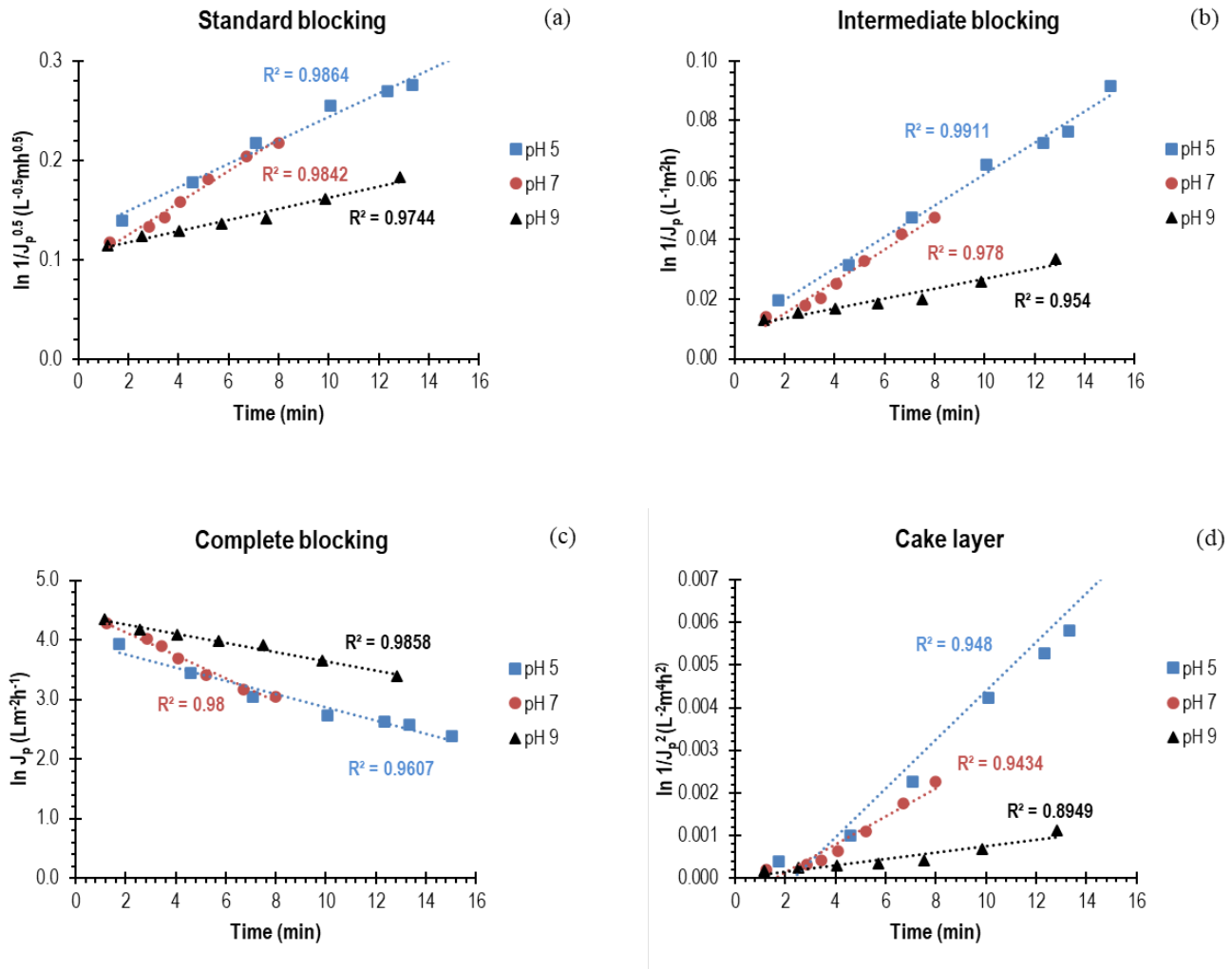

Fig. 3: Linear fitting results of experimental permeate flux during immobilization at different pH according to fouling model by Hermia

4.0 Conclusions

The effect of solution pH 5, 7 and 9 during filtration of ADH enzyme in an attempt to immobilize enzyme in the PES membrane support was investigated upon membrane permeability, enzyme loading and fouling mechanisms. The highest enzyme loading was recorded when immobilization procedure is conducted at pH 5 and the lowest enzyme loading is recorded at pH 7. The docking mechanism of enzymes on the membrane is due to electrostatic charge and hydrophobic interaction at low pH, while electrostatic repulsion and steric hindrance mechanisms dominated enzyme retention at high pH. It was observed that, reversible fouling mechanism occurs at pH 5 (intermediate blocking) and pH 9 (complete blocking). Meanwhile, irreversible fouling indicated by standard blocking at pH 7 could indicate the most suitable

fouling mechanisms to dock the ADH enzyme on the membrane surface. The enzyme is 'protected' inside the membrane pores and could lead to enzyme stability in longer run.

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