Immobilization of Alcohol Dehydrogenase in membrane: Fouling mechanism at different enzyme concentration

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Abstract. Fouling induced enzyme immobilization on polymer membrane with controlled variables can retain enzyme stability and performance. In this study, the effect of enzyme concentration towards the fouling-induced enzyme immobilization was investigated. Alcohol dehydrogenase (ADH) was used as model enzyme to be immobilized on poly(ether) sulfone (PES) membrane at different concentration (0.05, 0.10 and 0.20 g/L). The transmembrane pressure and pH were fixed at 2 bar and pH 7 respectively during the process takes place. The result shows that higher enzyme concentration gives higher loading efficiency with 83.95%. Hermia’s model predicted that standard and intermediate blocking dominating the fouling mechanism at all enzyme concentration tested.

1. Introduction
Enzymes have big potential as catalyst in various practical applications. However, enzyme encounters limitations in terms of chemical and physical stability hence make it a greatest obstacle for enzymatic catalysis application in processes with robust condition [1]. Therefore, in order to overcome this problem, immobilization of enzyme on solid carrier such as membrane has been seen as the best option. Immobilization of enzyme on membrane provides advantages as there is combination of reaction and separation in one single unit process [2].

The immobilization process is achieved by dead-end filtration of enzyme solution. The filtration process would cause flux declination of the membrane indicates that the enzymes has fouled the membrane. Generally, membrane fouling is most serious problem which affecting systems performance resulting to frequent membrane cleaning and also necessary to replace membrane which eventually increases operation cost [3]. However, it is suggested that controlled fouling could be utilized in order to immobilize the enzyme on the membrane hence improving the stability and performance of the enzyme itself [4]. A concept of fouling-induced enzyme immobilization was proposed and verified in the previous study by Luo et.al, based on the correspondence findings between membrane fouling mechanism and enzyme immobilization strategies [3].

Though fouling commonly regarded as the negative aspect in the membrane filtration process, but here it is considered as a “neutral” phenomenon where the enzyme would be “docked” in/on membrane by simple filtration. Hence, fouling mechanism can be regarded as positive tools for enzyme immobilization. Desirable fouling which could retain enzyme stability and performance could be achieved by optimized variables. One of variables that could be taken into consideration is the concentration of the enzyme in the feed solution. Zhao et.al., concludes that increasing concentration in the feed solution will eventually increases the rate of membrane fouling [4]. Study from Liao et.al.,
microalgal biomass concentration (MBC) gives impact on membrane fouling and total resistance. High MBC results in faster cake layer formation which contributes to membrane fouling potential [5].

This study was conducted to analyse the dominating fouling mechanism on enzyme concentration of 0.05, 0.10 and 0.20 g/L. A simple dead-end filtration technique was applied to immobilize the Alcohol dehydrogenase (ADH, EC 1.1.1.1) on the support layer of the commercial polyethersulfone (PES) membrane. The fibrous structure of support layer of the polymer membrane were utilized as the “skeleton” for enzyme immobilization. Hermia model were fitted with the experimental data during the immobilization process takes place to estimate the fouling model with the highest fitting accuracy.

2. Filtration blocking model
Filtration blocking model consist of four different mechanism – complete blocking, standard blocking, intermediate blocking and cake filtration [6]. Hermia’s model was selected to quantify the flux decline in the ultrafiltration membrane for selected enzyme concentration in the feed solution as it is the most comprehensive model describing dead-end filtration in batch system [7][8]. It is applied to describe the deposition of particles on filter media or membrane as schematically illustrated in table 1.

| n = 2 (complete blocking) | Filtration blocking equations | Schematic diagram |
|---------------------------|------------------------------|------------------|
| ln J_p = ln J_o - K_c t   |                              |                  |

| n = 1.5 (standard blocking) | Filtration blocking equations | Schematic diagram |
|-----------------------------|------------------------------|------------------|
| 1 - J_p^{0.5} = 1 - J_o^{0.5} + K_s t |                           |                  |

| n = 1 (intermediate blocking) | Filtration blocking equations | Schematic diagram |
|-------------------------------|------------------------------|------------------|
| 1 / J_p = 1 / J_o + K_i t    |                              |                  |

| n = 0 (cake layer) | Filtration blocking equations | Schematic diagram |
|--------------------|------------------------------|------------------|
| 1 / J_p^2 = 1 / J_o^2 + K_cl t |                       |                  |

The law of filtration for dead-end filtration at constant pressure can be written as:

\[
\frac{d^2 t}{dV^2} = K \left( \frac{dt}{dV} \right)^n
\]

where t is the filtration time (s), V is the permeate volume ($m^3$), K is the constant and n can have different values depending on different type of fouling. J_o is the permeate flux at t = 0 while K_o, K_c, K_s and K_cl are the constant for different models. The experimental data were fitted in these linear model and the
linear regression coefficient was compared to describe the most possible fouling mechanism at different enzyme concentration (table 1).

3. Methodology

3.1. Materials
Alcohol dehydrogenase (ADH, EC 1.1.1.1, 300 U/mg) from Saccharomyces cerevisae, β-nicotinamide adenine dinucleotide reduced form (NADH) and formaldehyde (37%) were purchased from Sigma-Aldrich. 0.1M Tris-HCl buffer at pH 7 was used to prepare all the enzyme and substrate solutions in this study. Characteristics of commercially available UF membrane used were summarized in Table 2.

Table 2. Main characteristics of UF membrane.

| Membrane | Manufacturer         | MWCO (kDa) | Skin material          | Thickness (mm) | Permeability (L/m.h.bar) |
|----------|----------------------|------------|------------------------|----------------|--------------------------|
| MK-PES   | Synder Filtration    | 30         | Poly(ether)sulfone (PES)| 0.17*          | 71.65*                   |

*Own measurement

3.2. Experimental set-up and procedure
The experimental procedure was adopted from Luo et.al, with some modification [3]. The dead-end filtration was carried out in a stirred cell (Amicon 8050, Millipore, USA) with effective surface area of the membrane disc of 13.4 cm². The membrane was placed in the membrane holder by ‘sandwich’ mode – own support layer facing feed with additional polymer support beneath the skin layer (Figure 1). Nitrogen gas was supplied into the cell in order to ensure a constant transmembrane pressure during the experiment. In order to determine the permeate flux, the permeate was collected at 4 mL interval in precision cylinder.

New membrane was used for each experiment. Virgin PES membrane was soaked in 0.1M NaOH to activate the membranes’ pores [9], then the membrane was cleaned under pressure with ultrapure water to remove the preservative layers according to the manufacturer instruction. Initial water permeability of the membrane in sandwich mode was measured at 1 bar with ultrapure water. Experimental procedures were done in three duplicates.

![Figure 1. Schematic diagram of the process.](image-url)
3.3 Enzyme immobilization and catalytic reaction

30 mL of ADH enzyme solution with different concentrations (0.05, 0.10 and 0.20 g/L) at pH 7 was fed into the cell for subsequent enzyme immobilization process. The pressure was kept constant at 2 bar until 28 mL of permeate collected (an aliquot of 4 mL each time). After immobilization process, the membrane was rinsed with 5 mL of buffer for 3 times without applying pressure. The enzyme loading after immobilization was calculated by mass balance from the total amount of final retentate and rinsing residual. The concentration of enzyme immobilized was measured as protein concentration by following Bradford’s protein assay using UV/VIS spectrophotometer (Perkin Elmer, Germany).

A 30 ml substrate mixture consisted of 100 mM formaldehyde and 100 µM NADH was poured into the Amicon stirred cell which already equipped with immobilized ADH on the membrane. Immediately, a pressure of 2 bar was purged into the cell and the permeate was collected every 4 mL.

3.4 Calculated parameter

The immobilization efficiency was expressed as enzyme loading rate (%) by the following formula:

\[
\text{Loading rate (\%)} = \frac{m_i}{m_t} \times 100
\]

where;

- \( m_i \) = amount of immobilized enzyme, mg
- \( m_t \) = amount of enzymes in the feed solution, mg

The membrane fouling testing was used to analyse the fouling mechanism. Flux recovery rate (FRR), total fouling ratio (\( R_t \)), reversible fouling ratio (\( R_r \)) and irreversible fouling ratio (\( R_{ir} \)) was calculated as follows:

\[
FRR (\%) = \frac{J_{w2}}{J_{w1}} \times 100
\]

\[
R_t = \left(1 - \frac{J_p}{J_{w1}}\right)
\]

\[
R_r = \left(\frac{J_{w2} - J_p}{J_{w1}}\right)
\]

\[
R_{ir} = \left(\frac{J_{w2} - J_{w1}}{J_{w1}}\right) \times 100
\]

where;

- \( J_p \) = permeate flux, L/m².h.bar
- \( J_{w1} \) = initial pure water flux, L/m².h.bar
- \( J_{w2} \) = final pure water flux, L/m².h.bar
4. Result and discussion

4.1 Membrane permeability

The permeate flux of bare membrane and after enzyme immobilization is shown in Figure 2. Generally, the permeate flux after the immobilization of ADH enzyme decreases from the initial permeate flux as the ADH enzyme would foul the membrane. Previous study from Luo et al. and Marpani et al. also shows the same trend in permeate flux declination when the membrane was subjected to dead-end filtration of enzyme solution [1-2]. Cao et al. reported that such declination of the permeate flux during the immobilization process occurs as a results of pore blocking or deposition of enzyme aggregates caused by the formation of the fouling layer [11]. From the figure 2, it can be seen that the flux after immobilization with 0.10 g/L ADH enzyme is higher than 0.05 and 0.20 g/L ADH enzyme. Generally, increasing enzyme concentration would increase the tendency of the pore blockage formed on the membrane. However, low feed concentration would likely to cause the enzymes particles to diffuse through the membrane pores rather than forming the fouling layer hence restricting the flow of the water through the membrane pores giving the lower flux after immobilization. As for higher enzyme concentration (0.20 g/L), the tendency of the enzyme particles to agglomerate on the surface of the membrane due to oversaturation in the feed solution, cause the crowding on the surface rather than be flushed through the membranes’ pores resulting in lower flux obtained [12-13].

![Figure 2. Permeate flux of bare PES membrane and after enzyme immobilization at different concentrations.](image)

4.2 Enzyme loading

Enzyme loading in/on polymer membranes at different enzyme feed concentration was shown in the Table 3. After the dead-end filtration process to immobilize the enzyme, it is found that the amount of enzyme successfully immobilized at 0.20 g/L concentration is highest on PES membrane. This happened as expected where the higher amount of enzyme supplied in the feed would give higher enzyme loading. Generally, with higher foulant concentration (enzymes) it would accelerates the fouling formation in the membrane filtration and also increase the probabilities for foulants deposition/aggregation on the membrane or pore blockage [3].
Table 3. Enzyme loading at different concentrations.

| Enzyme concentration (g/L) | Amount of enzyme (mg) | Enzyme loading (%) |
|---------------------------|----------------------|--------------------|
|                           | Feed     | Permeate | Retentate | Washing residue |                           |
| 0.05                      | 1.5      | 0.6782   | 0.0338    | 0.0367          | 50.09                      |
| 0.10                      | 3.0      | 0.5659   | 0.0509    | 0.1343          | 74.96                      |
| 0.20                      | 6.0      | 0.8803   | 0.0420    | 0.0409          | 83.95                      |

4.3 Fouling mechanism

Fouling in the membrane can occur at different parameters including interactions between foulant and the membrane surface [14]. In this study, it is expected that with higher enzyme concentration fed to the filtration system will directly increase the fouling rate. However, flux recovery ratio (FRR) calculated for all the biocatalytic membranes indicates that biocatalytic membrane with 0.20 g/L enzyme feed have the highest FRR when compared to 0.05 and 0.10 g/L enzyme feed as shown in Figure 3.

Figure 3. Flux recovery ratio for biocatalytic membrane.

The irreversible fouling ratio existed for all conditions but the lowest was at enzyme feed of 0.20 g/L. The lower irreversible fouling ratio indicates low tendency for pores clogging in the membrane thus, foulants attached can be easily removed by membrane surface cleaning [14]. This also explained the higher FRR achieve for enzyme feed of 0.20 g/L.

Figure 4 shows the comparison of the coefficient of regression of Hermia’s model fitting based on the experimental data according to the model at different enzyme concentration. In this study, enzyme solution was filtered through the membrane in the dead-end filtration cell by batch mode operation where the permeate was continuously collected which results in increase feed concentration and volume reduction as filtration process going.

It was found that standard and intermediate blocking was dominating the fouling mechanism at different enzyme concentration as shown in Table 4. The standard blocking was considered as the smaller particle size than the pore size would deposits on the pore wall thus gradually fouled the pore as the filtration progress. As in the intermediate blocking, it was assumed that the rate of pore blocking is proportional to the number of open pores [6]. As shown in Table 1, intermediate blocking occurs with monolayer fouling and pore blocking where in this study it might be happening between skin and support surface with additional layer of enzyme would accumulate on this monolayer aggregates by weaker force of attraction (i.e., hydrogen bonding). Therefore, it is coherent with the higher enzyme loading.
achieve with increasing enzyme feed concentration. After subsequent washing operation, the enzyme that accumulates on the monolayer aggregates would washed away since they are attracted by weak force hence confirmed the higher FRR obtained for higher enzyme feed concentration.

Figure 4. Linear fitting of experimental permeate flux at different enzyme concentration according to Hermia’s fouling model; (a) complete blocking model; (b) standard blocking model; (c) intermediate blocking model and (d) cake layer model.

Table 4. Summary of the values of $R^2$ from the model fitting.

| Enzyme concentration (g/L) | Complete blocking | Standard blocking | Intermediate blocking | Cake layer |
|----------------------------|-------------------|-------------------|----------------------|------------|
| 0.05                       | 0.9962            | **0.9990**        | 1.000                | 0.9963     |
| 0.10                       | 0.9880            | 0.9970            | 1.000                | 0.9891     |
| 0.20                       | 0.9929            | **0.9983**        | 1.000                | 0.9935     |

5. Conclusion
Declination of flux shows that the enzyme has fouled the membrane with higher enzyme loading obtained at the higher enzyme concentration. The fouling mechanisms was investigated according to Hermia’s model and experimental data fitting gives that standard and intermediate blocking dominates the fouling mechanism at all enzyme concentration tested hence the concept of fouling-induced immobilization could be utilised as a stable enzyme immobilization method.

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