Potential of Lipase Immobilized Polyethersulfone (PES) Membrane in Biphasic Reactor for Oil Hydrolysis

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Abstract*—This study aimed to enhance the filtration process by incorporating enzyme to the membrane support system. In this study, an enzymatic membrane was applied to investigate the use of enzyme from bacteria origin that was able to enhance the separation of oil-water interfaces and as well as the effectiveness of the enzyme immobilization membrane. Lipase from Aspergillus niger origin is used to be immobilized onto the Polysetherulfone membrane. A membrane reactor with biphasic system was configured at laboratory scale in order to possess an organic-aqueous system with lipase immobilized membrane. The performance of the reactor was investigated with the olive oil hydrolysis in order to study the effect of the operating variables. The results found that the degree of hydrolysis (%) was the highest at 6-hour and with initial lipase concentration of 3 mg/ml which resulted in 0.11 mg/cm^2 amount of lipase immobilized.

***Keywords*— Aspergillus niger lipase, hydrolysis, olive oil.**

I. INTRODUCTION

The fast-ever growing oil industry up until this century has arisen many concerns especially the effects towards the environment in various degrees. Hydrocarbons pollution is a very serious issue in the environment and represents 70% of environmental pollutions. This is the reason why the researchers have taken an alternative to go deeper into the refinement of biological treatment since there are potential affordable alternatives. A lot of studies have proven that large number of enzymes from bacteria; fungi and plants have been reported to be involved in the biodegradation of toxic organic pollutants. There are many methods and new technologies that have been developed and still in research and development (R&D) stage towards finding the most economical, effective and efficient solutions to this problem. However, most of the methods found can be very effective at reducing wide range of contaminants but at the same time have several consequences. These methods are complex, uneconomical and lack public acceptance. The associated deficiencies in these methods have focused efforts towards harnessing microbial enzyme as a suitable alternative. The purposes of this study are to study the effect of Lipase concentration immobilized on the membrane upon the oil hydrolysis and to study the effect of time course of the oil hydrolysis.

II. METHODOLOGY

A. Materials

Aspergillus niger lipase (EC 3.1.1.3) (Sigma –Aldrich, USA), Polyethersulfone (PES) membrane (courtesy of postgraduate student), glutaraldehyde (R&M Chemicals), sulphuric acid (R&M Chemicals), acetone (Systerm-ChemAR) were used for lipase immobilization on membrane. Albumin fraction V (Merck-Chemicals), Bradford Reagent (R&M Chemicals) was used for estimation of protein. Isooctane (R&M Chemicals), buffer solution (Systerm-ChemAR) and olive oil were procured.

B. Methods

i. Lipase immobilization and amount of lipase membranes estimation

PES membranes with polymer concentration of 1.0 M were used for the lipase immobilization. Glutaraldehyde solution with concentration of 25% w/w was prepared and the pH was altered to 3 by adding 0.1M H_2SO_4. The membrane was then immersed in the 20 mL of the prepared glutaraldehyde solution at room temperature. It was left for an overnight with gentle shaking and washed thoroughly with distilled water and acetone to eliminate any roughly adhered chemicals on the membrane. The process of covalent immobilization of lipase molecules onto the surface of membrane was carried out by immersing the membrane in 2 mg/mL lipase solution prepared in phosphate buffer (pH 7.0). The solution was then incubated at 4 °C. It was then left for 3 hours with gentle shaking and washed with phosphate buffer together with distilled water.

The lipase immobilized membranes were kept in buffer solution in order to provide an ideal condition for the lipase to remain its lipase activity.

For determination of the amount of lipase immobilized on the membrane, the initial and final lipase concentration after immobilization was calculated. Bradford Protein Assay was used to determine lipase concentration in final lipase solution with calibration curve and Albumin fraction V was used as standard protein.

ii. Hydrolytic experiment by using the membrane reactor

The membrane reactor was made of glass and designed like a cube with a holed-centered glass partition in the middle that divided the cube into two separate volumes in order to separate between the aqueous and organic phases. The hole was made in order to attach the lipase immobilized membrane. Hence, those two phases were parted by enzymatic membrane and was a combination of bioreactor and membrane separation stage. Figure 1

is the representation of the experimental set-up of the enzymatic membrane reactor.

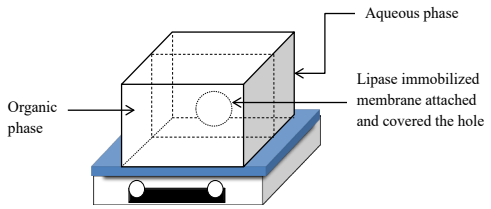

Figure 1: Set-up of enzymatic membrane reactor.

The membrane was treated as catalyst provision for improved/better selective interaction with the reactant as well as for selective elimination of product(s). This helped the reaction and the concurrent separation at the membrane.

Since the hydrolytic experiment was influenced by diffusive manner, the enzymatic reaction measurement was performed by filling 100 ml of olive oil solution into one side of the membrane reactor and 100 ml of 0.1 M phosphate buffer solution at pH 7.0 on another side and carried out at room temperature.

iii. Lipase activity measurement and degree of hydrolysis determination (Percentage conversion).

The lipase activity was assayed based on the olive oil hydrolysis by lipase and the free fatty acid produced was titrated with NaOH. The amount of free fatty acids in the sample can be determined by the equivalent amount of alkali consumed. Hence, the enzyme activity can be obtained. The lipase activity was denoted as lipase unit (LU), where one LU is the amount of enzyme releases one µ mol of fatty acid per one minute at room temperature (37 °C). ((Fadıloğlu & Söylemez, 1998)(Minovska, Winkelhausen, & Kuzmanova, 2005)).

In order to determine the degree of hydrolysis, 0.5 ml of sample was taken from the organic phase at various time intervals. The concentration of free fatty acid was calculated by using titration method. 0.01 N of sodium hydroxide was used to titrate with the sample and phenolphthalein was used as an indicator. Degree of hydrolysis, *X* is determined as shown below (Serri, Kamarudin, & Rahaman, 2008).

$$X\% = \frac{(ml\ NaOH\ used)\ (molarity\ of\ NaOH)\ (average\ molecular\ weight\ of\ fatty\ acid)}{10\ (weight\ of\ sample)} \quad (1)$$

C. Factors that affecting the hydrolysis

There were many influencing factors for hydrolysis such as the oil nature, type of solvents, time of reaction, pH of the aqueous phase and concentration of the substrate. The focuses on this study are the time of reaction as well as the enzyme concentration. The other factors were kept constant based on the optimum results from some research findings which included olive oil for nature of oil, isooctane as the type of solvents, pH 7 of the aqueous phase and 0.05 M of substrate concentration.

i. Time of reaction

The relationship between the time taken of operation of the membrane reactor and its effects to the hydrolysis was studied. It was operated at various time intervals (2, 4, 6 hours). (Shweta Gupta, 2016) (Wang, Wang, & Xu, 2006).

ii. Enzyme concentration

The effect of the enzyme loading is studied in the range of 2.0 mg/ml – 3.0 mg/ml for the performance of the hydrolytic reaction. Preferably, the concentration of the enzyme used is not exceeded more than the range since it might retard the immobilization effect (Shweta Gupta, 2016)

III. RESULTS AND DISCUSSION

A. Immobilization of lipase

i Immobilization of lipase on PES membrane

Simple micrographs (500x magnification of 2.0 megapixels) of immobilized *Aspergillus. niger* lipase onto PES membrane were represented in Figure 2 and 3. These were the visual evidences of lipase immobilization onto the surface of the membrane. There were visible spots (whitish spots) of lipase throughout the membrane surface in Figure 3 while there were no visible spots on the membrane before immobilization (Figure 2).

Figure 2: Simple micrograph of PES membrane before lipase immobilization at concentration of 2 mg/ml.

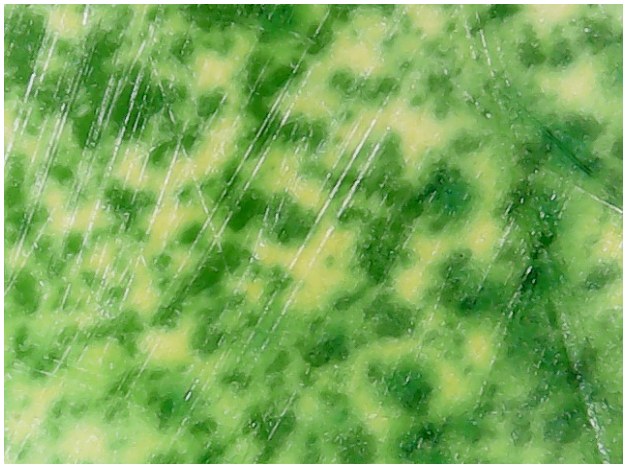

Figure 3: Simple micrograph of PES membrane with lipase of 2 mg/ml.

ii. Field Emission Scanning Electron Microscopy (FESEM) analysis

FESEM analysis of immobilized *A. niger* lipase were depicted in Figure 4 and it was seen that there were small spots of lipase compared to in Figure 5 which was the membrane before immobilization. It was seen that there were no whitish spots on the membrane before immobilization (S. Gupta, Singh, & Bhattacharya, 2010; Shweta Gupta, 2016).

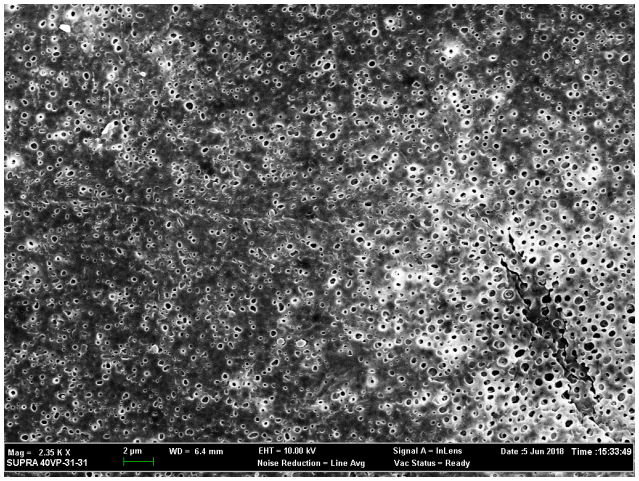

Figure 4: FESEM of PES membrane before lipase immobilization.

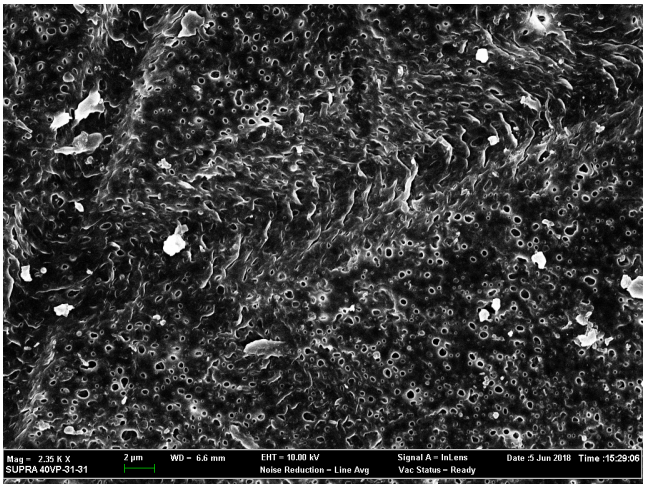

Figure 5: FESEM of PES membrane after 2 mg/ml of lipase immobilization.

ii. Lipase concentration

The variation of lipase concentrations (2, 2.5, 3mg/ml) were implemented in order to analyse the highest lipase loading onto the membrane. Gupta (2016) stated that chances of immobilization can be enhanced as the lipase concentration increased and the optimum lipase concentration in her study was discovered to be 2.5 mg/ml and the immobilized lipase was 2.1 mg/cm^2.Beyond this concentration, the final amount of immobilized lipase dropped significantly. This was probably because of viscosity increment of the lipase solution that had proven the hindering effect on immobilization (Shweta Gupta, 2016).

The determination of immobilized lipase was done by using Bradford Protein Assay to measure the concentration of lipase in the final solution with a calibration curve that was established previously. The immobilized lipase amount was calculated by determine the difference in concentration between initial and final solutions.

The results for the amount of lipase immobilized were shown in Table 1. From the results, it can be said that as the initial lipase concentration increased, the lipase immobilization onto the membrane was also increased. At 2.5 mg/ml, the immobilized lipase was 0.08 mg/cm^2 while at 3 mg/ml; it increased to 0.11 mg/ cm^2. Since this was the opposite from the research finding, there were probably some limitations that contributed to this situation such as the uneven thickness of the PES membrane which it was configured at laboratory scale.

Table 1: Lipase immobilization on membranes

Initial lipase concentration (mg/ml)	Amount of lipase immobilized (mg/cm^2)
2	0.08
2.5	0.09
3	0.11

Hydrolysis of oil

i Enzymatic membrane reactor

The membrane reactor with attached lipase onto the surface of the membrane is a favourable methodology to many enzyme-catalysed processes specifically for lipase. The two-phase membrane reactor was designed and constructed at laboratory scale. This membrane reactor is an attractive approach for hydrolysis of various model compounds since diffusion process in two-phase system is applied. Since oil and fats possess restricted solubility in water, lipase is recognized to act on oil-water interface and this membrane reactor is ideal for such cases since lipase immobilized membrane creates the boundary between those two phases (Shweta Gupta, 2016).

The hydrolysis occurred in the organic phase and a few factors that contributes to the process includes organic phase nature, time of reaction, concentration of substrate, and as well as the pH. However, in this study, the nature of substrate concentration of substrate and pH were kept constant by using olive oil, at 0.5 M and pH 7.0 respectively based on the optimum results from the research findings (Shweta Gupta, 2016).

ii Time course for hydrolysis

The hydrolytic experiments were conducted at various operated time (2, 4, 6 hours) at 37 °C for each lipase concentration (2, 2.5, 3mg/ml). From the results obtained, it can be seen that the degree of hydrolysis showed an increasing trend as the time was up to 6 hours. This situation was similar with the findings from Goswami, Basu, & De, 2013; Gupta, 2016; Yigitoglu & Temocin (2010) where the degree of hydrolysis increased as the time period increased.

Based on the graph in Figure 6, it was studied that the hydrolysis percentage increased as the time period increased. This trend was seen on the effect of the concentration as well. Degree of hydrolysis increased steadily as the concentration of immobilized lipase increased. The maximum percentage conversion occurred after the reaction was performed for 6 hours. This finding is consistent with that obtained by Goswami et al. (2013) which proposed that the immobilized lipase on the membrane catalyses the hydrolysis well.

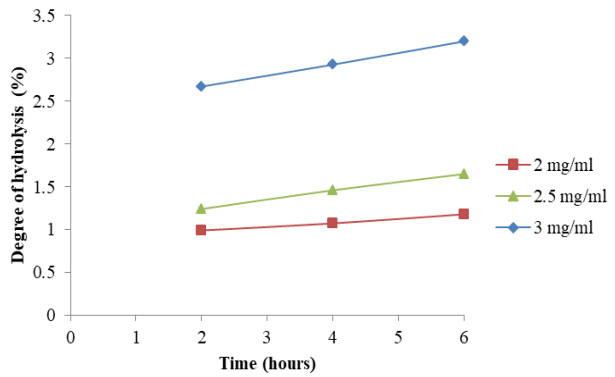

Figure 6: Degree of hydrolysis at various lipase concentrations (2, 2.5, 3 mg/ml) at different time interval.

IV. Conclusion

Incorporating the membrane reactor with lipase is one of the highly promising methods to numerous lipase-catalysed processes. This enzymatic membrane reactor is designed and constructed at laboratory scale and acts as an attractive approach for hydrolysis of various model compounds since diffusion process in two-phase system is applied. Some of the influencing factors for oil hydrolysis were studied specifically the time of reaction and enzyme concentration. The results found that the optimum initial lipase concentration for loading was 3 mg/ml which resulted in 0.11 mg/cm^2 lipase immobilized. This was in contrary with the research finding that was stated by Shweta Gupta (2016) that the optimum initial lipase concentration was 2.5 mg/ml. There were probably some limitations that contributed to this situation such as uneven thickness of the PES membrane which was configured at laboratory scale. Degree of hydrolysis (%) was the highest at 6-hour and with initial lipase concentration of 3 mg/ml which resulted in 0.11 mg/cm^2 lipase immobilized.

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