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Enzymatic Hydrolysis of Oil Palm Empty Fruit Bunch using Membrane Reactor

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Enzymatic hydrolysis of oil palm empty fruit bunch (EFB) was performed in a membrane reactor system. The reactor was incorporated with polyethersulfone (PES) ultrafiltration membrane to increase the cellulose conversion and reduce enzyme dosage. Parameters such as enzyme and substrate loading, transmembrane pressure (TMP) and cross-flow velocity (CFV) were investigated on the filtration flux behaviour and retention characteristics during filtration process. The cellulase enzyme was completely retained as the rejection was above 98 % for all cases and complete transmission of sugar molecules was achieved when polyethersulfone membranes with molecular weight cut-off (MWCO) of 10 kDa was used. Results on hydrodynamic parameters show that the permeate flux increased with increasing TMP and CFV. However, the enzyme and reducing sugar rejection remain fairly constant. The concentration polarisation (up to 75 % of total resistances) was the main factor that contributed to the flux decline followed by cake layer and membrane pores resistances. Cellulase was tested for its reusability up to 216 h in enzymatic membrane reactor. Productivity increased remarkably from 0.003 to 0.01 g reducing sugars/FPU enzyme in batch reactor and enzymatic membrane reactor. These results demonstrate the potential of using coupled enzymatic reactor and membrane separations for the production of reducing sugars and enzyme recovery in EFB hydrolysis.

1. Introduction

Palm oil industry is one of the top industries in Malaysia. However, processes in palm oil industry produces large amount of biomass waste in forms of empty fruit bunch (EFB), oil palm fronds (OPF) and oil palm trunks (OPT) and this creates an environmental problem. Currently, there is a considerable interest in the conversion of oil palm lignocellulosic biomass to the production of fuels and renewable chemicals. To convert the biomass into sugars, the lignocellulosic biomass is initially pre-treated to break up the plant structure (Huang et al., 2011). The cellulosic and the hemicellulosic portions are then hydrolysed by cellulase enzymes or dilute acids into sugar, which is then converted to other chemicals. Compared to acid hydrolysis, enzymatic hydrolysis has numerous advantages such as highly selective glucose yield, mild reaction condition, and less environmental impact.

Cellulase enzymes catalyse the hydrolysis of cellulose to products such as cellobiose and glucose. Cellulase is a multicomponent enzyme mixture that consists of exo-cellobiohydrolases (CBH), endoglucanase (EG) and β-glucosidases (Jorgensen et al., 2007). Cellulase work synergistically to hydrolyse cellulose to glucose. CBH generally act on the ends of the glucose polymers while EG act at random locations on the cellulose. These enzymes hydrolyse cellulose to smaller oligosaccharides, primarily cellobiose. Cellobiose is hydrolysed to glucose by β-glucosidases. The factors affecting enzymatic hydrolysis of cellulose include substrates, cellulase activity, reaction conditions and strong product inhibition (Zhang et al., 2012). The slow reaction rate by product inhibition is the major obstacle in achieving an economically viable process. However, the reaction rate can be substantially increased by removing the sugar products. Both cellubiose and glucose cause end-product inhibition.

An approach to reduce inhibition by glucose and cellobiose is to remove the products from hydrolysis by applying membrane to the reaction system. A membrane reactor contains an ultrafiltration membrane which

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retains the high molecular weight components like substrate and enzymes, while allowing lower molecular weight components such as sugars, to pass through the membrane.
Enzymatic hydrolysis of lignocelluloses biomass in membrane reactor has been studied by using various feedstocks such as crystalline cellulose (Malmali et al., 2014), rice straw (Yang et al., 2006), and corn stalk (Yang et al., 2009). Studies have shown that membrane reactor give a higher productivity compared to conventional batch reaction. Yang et al. (2008) reported that the cellulose enzymatic hydrolysis rate in a membrane reactor increased four times compared with that obtained in a batch reactor and also enzyme activity is not greatly altered when they are used in membrane reactors. By taking advantage of the size differences between the enzymes, substrate and the products of the reaction, the membrane can be used to separate the enzyme and substrate from the reaction mixture and recycle it back to the main reaction vessel for further reaction. For product-inhibition reaction like hydrolysis of cellulose, sugars can be permeated out from reactor thus increasing the conversion of cellulose and yield of sugar.
Several publications on EFB enzymatic hydrolysis focus on pre-treatment process (Bouza et al., 2016) and optimising the ratio of cellulase and β-glucosidase enzyme in the reactor (Noratiqah et al., 2013). Significant advances have been made in the bioconversion of EFB, studies in recovery and reusability of enzyme have not being pursued. All of the studies in EFB hydrolysis deal with reaction in batch reactor. Parameter on EFB hydrolysis in an enzymatic membrane reactor such as enzyme and substrate loading, cross-flow velocity and transmembrane pressure are not studied. The effect of those parameters on filtration resistances like membrane, membrane pores, cake layer and concentration polarisation resistance were also not investigated for EFB hydrolysis in an enzymatic membrane reactor. The aim of this study is to investigate the effect of process variables on the performance of enzymatic hydrolysis of EFB using membrane reactor. An ultrafiltration membrane with molecular weight cut-off (MWCO) of 10 kDa was used to recover and reuse of cellulase enzymes during enzymatic hydrolysis and at the same time continuously removing the products (sugars).

2. Experimental

2.1 Raw Materials

The lignocelluloses biomass used in this study was oil palm empty fruit bunch (EFB). It was collected from Malaysian Palm Oil Berhad (MPOB) located in Selangor, Malaysia. After the sampling, the EFB was air dried in an oven at 60 °C to avoid fungus growth, then followed by grinding process (300 – 500 μm). The grounded EFB biomass were then stored in an oven at 50 °C until further use. After that, the EFB was pre-treated using hot water and NaOH following the method from Hamzah et al. (2011).
The cellulase enzyme was purchased commercially from Novozyme Malaysia Sdn Bhd. It was a commercially available cellulolytic complex known as Celluclast 1.5 L and its activity was 46.25 FPU/mL, which was determined by the filter paper method (FPU) according to Mandels and Andreotti (1976).

2.2 Enzymatic Membrane Reactor

Membrane reactor consisted of a reactor, a 500 mL flask connected to the cross-flow membrane separator (see Figure 1). Commercial ultrafiltration PES membrane with 10 kDa MWCO was used. Due to its low protein adsorption PES was used.

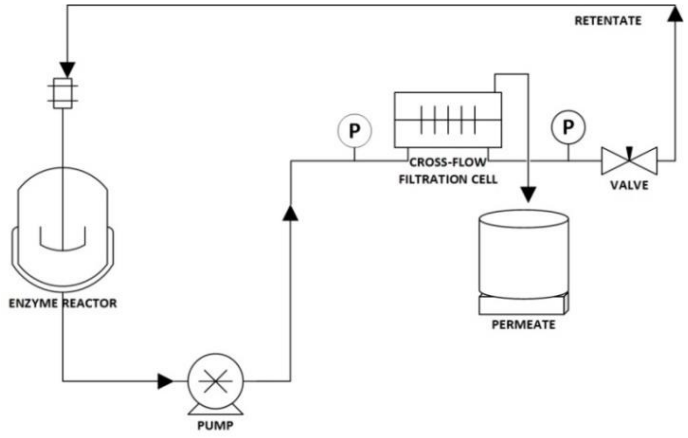

Figure 1: Schematic diagram of the enzyme membrane reactor

A weighed amount of treated EFB was placed into the reactor. Then, 50 mmol L^{-1} of citrate buffer and cellulase were added into the flask to a final volume (200 mL). The reactor was kept at 50 °C, which is the optimal catalytic temperature for cellulase. The reaction was conducted at a constant pH 5.0, maintained by citrate buffer. An initial hydrolysis period of 24 h was conducted in the reactor. Then, separation process was started and 100 mL of permeate was collected. The retentate was recycled back into the reactor. The sample taken was put into boiling water to deactivate the enzyme activity before it was analysed. The reducing sugar concentration obtained in the permeate flow was measured by using dinitrosalicylicacid (DNS) method and the enzyme concentration was determined using Bradford's assay.

2.3 Membrane Performance

The important parameters that measure the performance of a membrane process are flux and rejection. Flux (J) is expressed in Eq(1):

$$\mathrm{J\ (L\ h^{-1}\ m^{-2})} = \frac{\mathrm{V_P} \times 3{,}600\ (\mathrm{s\ h^{-1}})}{1{,}000\ (\mathrm{mL/L})\ \mathrm{x\ A.}\ \Delta \mathrm{t}} \quad (1)$$

where V_P (mL) is the volume of the permeate, A (m^2) is the effective membrane area, Δt (s) is the time elapsed since the first drop of permeate.
Rejection, R_i, is defined as in Eq(2):

$$\mathrm{R_i} = \left(1 - \frac{\mathrm{C_{Pi}}}{\mathrm{C_F}}\right) \times 100\ \% \quad (2)$$

where C_{pi} is the concentration of solute i in permeate and C_F is concentration of i in feed. In this study, low rejection of reducing sugar (RS) and high rejection of enzymes, R_E, are desired. All experimental runs were carried out in triplicate and mean values were reported.
According to the basic filtration equation (Mulder, 1996), the total filtration resistance, R_T, can be calculated quantitatively using Eq(3):

$$\mathrm{R_T} = \mathrm{TMP}/\eta.\mathrm{J} \quad (3)$$

where TMP is the transmembrane pressure, J is the filtration flux and η is the dynamic viscosity of the permeate. It is assumed that the total filtration resistance is the sum of the intrinsic resistance of the membrane (R_m), the cake resistance from the cake layer formed on the membrane surface (R_c), the concentration polarisation (R_{cp}), and the fouling resistance caused by pore blocking (R_b) (Juang et al., 2008), such that R_T is defined as in Eq(4):

$$\mathrm{R_T} = \mathrm{R_m} + \mathrm{R_c} + \mathrm{R_{cp}} + \mathrm{R_b} \quad (4)$$

The pure water flux was first measured to find R_m in the filtration experiments. R_m is always present and characterised by the pore shape and size and thickness of the membrane. Then, the substrate suspension was filtered, and the filtration flux was recorded. After recording the filtration flux, the substrate suspension was replaced with distilled water under the same operating conditions, and the resistance obtained in this step is the sum of R_m, R_b, and R_c. The pure water flux after removing the cake layer was measured to determine the fouling resistance due to pore blocking. This cake layer was removed from the membrane surface using a suitable brush before the substrate hydrolysis reaction feed was switched to pure water. These resistances can be calculated using Eq(5) (Juang et al., 2008) until Eq(8) (Zhong et al., 2011):

$$\mathrm{R_m} = \mathrm{TMP}/\eta.\mathrm{J_{w0}} \quad (5)$$

$$\mathrm{R_b} = \mathrm{TMP}/\eta.\mathrm{J_{w1}} - \mathrm{R_m} \quad (6)$$

$$\mathrm{R_c} = \mathrm{TMP}/\eta.\mathrm{J_{w2}} - \mathrm{R_m} - \mathrm{R_b} \quad (7)$$

$$\mathrm{R_{cp}} = \mathrm{TMP}/\eta.\mathrm{J_s} - \mathrm{R_m} - \mathrm{R_b} - \mathrm{R_c} \quad (8)$$

where J_s is the flux of the substrate suspension at steady state, J_{w0} is the initial water flux, and J_{w2} and J_{w1} are the final water flux before and after removing the cake layer.
Productivity is defined as grams of reducing sugars produced over activity of cellulase enzyme used for the hydrolysis reaction as seen in Eq(9):

$$\mathrm{Productivity\ (g\ FPU^{-1})} = \mathrm{RS\ (g)/Enzyme\ used\ (FPU)} \quad (9)$$

3. Result

Parameters such as transmembrane pressure, cross flow velocity, enzyme and substrate loading were varied to study the effects of physicochemical, hydrodynamics, and operation conditions on the EFB hydrolysis and rejections for both cellulases and sugars. For all cases, the reactor was kept at pH 5.0 and a temperature of 50 °C in citrate buffer. Table 1 shows the rejection of cellulase and reducing sugar for different enzyme loading. Results show that the enzyme was completely retained as the rejection was above 98 %, for all cases. This is expected due to the large difference in molecular weight (MW) of the cellulase enzyme mixture (endoglucanase (52 kDa), exoglucanase (62 kDa), and β-glucosidase (76 kDa)) and MWCO of 10 kDa. A SDS page of feed, permeate and retentate shows the molecular weight distribution of the solutions (Figure 2). The result revealed that the molecular weight distribution was unaltered in the feed and retentate, which means that the enzyme was stable during ultrafiltration. No band (i.e. enzyme) was observed in the permeate, confirming the rejection results. The reducing sugars rejection was below 5 % in all enzyme loadings, indicating that reducing sugars permeate easily through the membrane. Reducing sugar permeated easily through the membrane due to its low MW (180 Da). Flux decreased from 14.3 L/m^2.h to 6.9 L/m^2.h as the enzyme loading increased from 23.13 FPU/g to 115.63 FPU/g at fixed substrate loading. The reason of the decreased flux could be caused by the concentration polarisation due to increased enzyme concentration.

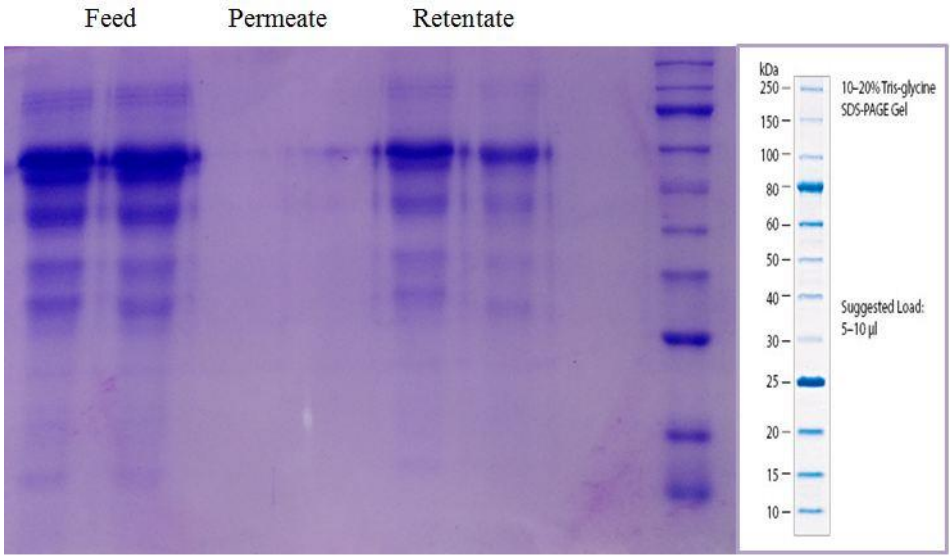

Figure 2: SDS page of cellulase enzyme in the feed, permeate, and retentate after filtration with 10 kDa PES membrane

Table 1: Rejection of cellulase and reducing sugar for different enzyme loading at fixed substrate loading

Enzyme loading (FPU/g)	Cellulase Rejection (%)	Reducing sugar Rejection (%)
23.13	98.50 ± 0.59	2.75 ± 2.67
69.38	99.18 ± 0.79	4.14 ± 1.31
115.63	99.25 ± 0.68	2.66 ± 0.42

When enzyme loading was fixed at 115.63 FPU/g, increasing substrate concentration from 5 g/L to 20 g/L resulted in the flux decrease from 9.03 L/m^2.h to 6.94 L/m^2.h. As the substrate concentration increased, more cellulose was available for hydrolysis and higher reducing sugars concentration in the hydrolysate was achieved. Increasing substrate loading have no effect towards the rejection of cellulase and reducing sugar. The hydrolysis reaction was optimum when enzyme and substrate loading were 115.63 FPU/g and 20 g/L. Further increase in the concentrations caused in the decrease in sugar concentrations, which was probably due to product inhibition.

Results on hydrodynamic parameters show that the permeate flux increased with increasing TMP and CFV. The enzyme and reducing sugar rejection remain fairly constant, which are 99 % and 2 %, for all cases of different TMPs and CFVs. Resistance-in-Series-Model was used to identify the responsible hydraulic resistances. Results show that the concentration polarisation (up to 75 % of total resistances) was the main

factor that contributed to the rate and extent of flux decline in the enzymatic membrane reactor, followed by cake layer and membrane's pores resistances. As the cellulose was degraded, it causes the pore to be blocked and later accumulated to form cake layer.

Under the determined optimal conditions, cellulase was tested for its reusability to up to 216 h. The enzyme was added into the reactor at the beginning of hydrolysis and no further addition of enzyme was done. To determine the reusability of the enzyme, EFB was added three times with substrate concentration of 20 g/L at 36 h intervals. Filtration process was done every 24 h of operation to recycle the cellulase back into the reactor. Figure 3 shows the reducing sugar concentration in the permeate for 216 h of operation in enzymatic membrane reactor (fed-batch operation). It can be seen that the cellulase in the fed batch operation was still active after 216 h of operation because it combined the product removal and enzyme retention together so that the rate of reducing sugars production in the fed-batch reactor was kept at a high value after the substrate-feeding step. The final substrate conversion in batch seemed to be limited by product inhibition as the products were not removed. Productivity increased remarkably from 0.003 g to 0.01 g of reducing sugar/FPU enzyme in batch reactor and enzymatic membrane reactor because the enzyme was reused. The enzyme activity decreased with time as evident from the concentration of the reducing sugar. The decreased activity could be to enzyme deactivation to shear stress, unproductive adsorption on substrate, and increased lignin concentration (Andric et al., 2010).

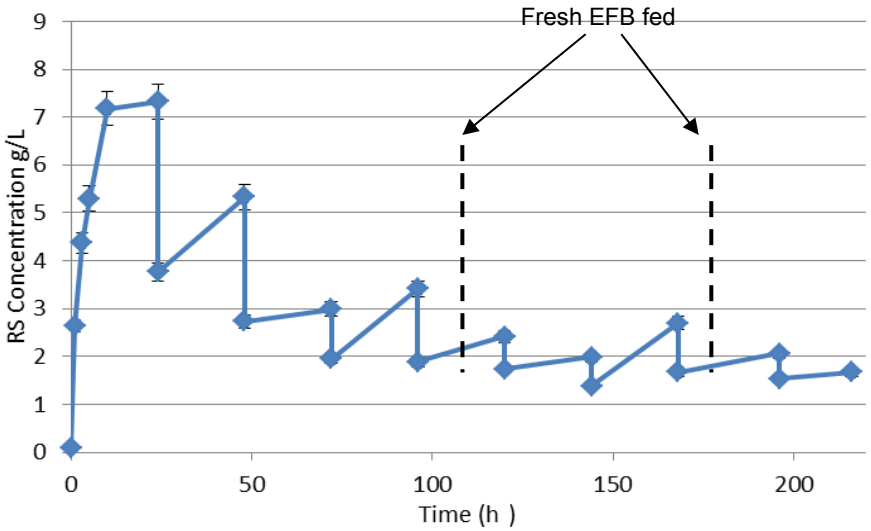

Figure 3: Reducing sugar concentration at permeate for 216 h of operation in enzymatic membrane reactor (fed-batch operation). (2 bar, 50 °C, 20 g/L, 115.63 FPU/g substrate)

4. Conclusions

The enzymatic hydrolysis of pre-treated EFB in a membrane reactor was investigated. Ultrafiltration PES membrane with 10 kDa MWCO was utilised, and high rejection of cellulases (98 - 99 %) and almost total transmission of sugar were achieved. The rejection of enzyme and reducing sugar were both independent of enzyme and substrate loading, transmembrane pressure and cross-flow velocity. Concentration polarisation in cross-flow enzymatic membrane reactor was observed to dominate the total resistances, affecting the filtration flux. Resistances such as cake layer and membrane pore resistance were also contributing to the flux decline of the filtration due to membrane fouling. Productivity of the enzymatic cross-flow membrane reactor increased remarkably when compared with the batch reactor. Results obtained clearly demonstrate the potential of using enzymatic membrane reactor for the production of sugars from EFB.

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