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Cellulose hydrolysis in an enzymatic membrane reactor: fouling mechanism

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Abstract. Enzymatic Membrane Reactor (EMR) was applied to overcome drawback from batch cellulose hydrolysis such as inability of reusing the enzyme and product inhibition which leads to low product yield. However, the major problem of EMR is membrane fouling. Therefore, the membrane fouling mechanisms in EMR was investigated using Hermia's pore blocking model. In this study, fed batch cellulose hydrolysis in EMR was carried out for 72 hours. The hydrolysate was filtered using polyethersulfone (PES) membrane at 24 hours interval and the flux of permeate was recorded. The cellulose hydrolysis has successfully converted more than 80% of the substrate into reducing sugar in EMR, which is approximately 20% more in batch hydrolysis. The product inhibition was minimized when there was glucose removal in EMR. The flux was analysed and the result showed that the membrane fouling was controlled by cake filtration mechanism. The larger size of substrate and enzyme than the membrane pore size blocked the membrane pore and eventually cause the development of cake layer.

1. **Introduction**

Lignocellulosic biomass such as sugarcane baggase, corn stover and paper sludge has been known as fossil fuels replacement in producing bioenergy by transforming cellulose from the biomass into fermentable sugar for bioethanol production [1, 2]. Acid hydrolysis and enzymatic hydrolysis are two common methods to reduce cellulose into sugar [3]. Acid hydrolysis has disadvantages such as low yield, high operating cost, and high chance in producing inhibited product such as hydroxymethylfurfural (HMF) [3]. Therefore, enzymatic hydrolysis is more preferable as it is carried out in a milder condition and has less impact towards the environment [4]. However, the reducing sugar produced such as glucose and cellobiose inhibits the enzymatic activity substantially because it will only be collected at the end of the hydrolysis process [5, 6]. Hence, the rate of hydrolysis slows down significantly. To minimize product inhibition, sugar removal from the hydrolysate by ultrafiltration (UF) can help to improve the hydrolysis yield and recover the enzymes for the next batch of hydrolysis [7, 8]. Consequently, the cost of the enzymatic hydrolysis will be decreased as the enzyme usage is decreased.

The UF process and hydrolysis process can be integrated into one whole unit, which known as enzymatic membrane reactor (EMR) [7]. EMR consists of a membrane separation unit and a hydrolysis reactor. Studies have shown that the cellulose conversion is higher using EMR than using a batch reactor. There is an increase by six folds of the total glucose under fed batch hydrolysis in an EMR [9]. Besides that, there was also a study which reported that the hydrolysis rate increased four times compared with that obtained in a batch reactor [10].

Despite the advantages of EMR, it has one key limitation which is membrane fouling which causes the permeate flux to decrease over time in UF [7, 11, 12]. Macromolecules such as cellulose and enzymes will deposited on the surface, causing blockage of the membrane pore, which will slowly forms a gel layer and later attribute to fouling [13]. Membrane fouling limits the use of membrane separation, leads to membrane resistance, decrease efficiency of product separation process and increase the operation and energy cost of EMR [7]. Although membrane fouling has been well reported, the underlying mechanism remain partially understood. Therefore, the identification of fouling mechanisms is important so that a proper selection of fouling control strategy can be applied.

2. Experimental

2.1. Materials

Microcrystalline cellulose (99% purity) purchased from R&M Chemicals was used in this study. The cellulase from *Trichoderma reesei* was bought from Sigma Aldrich and its activity was 71.15 FPU/ml, which was determined by using filter paper assay (FPU) [14].

2.2. Enzymatic Membrane Reactor (EMR)

EMR comprised of a hydrolysis reactor (100 ml Scott bottle) connected to a Sterlitech CF042A crossflow cell (as shown in Figure 1). Flat sheet ultrafiltration polyethersulfone (PES) membrane with 10 kDa MWCO obtained from Sterlitech was used. The PES membrane was cut according to the template which has a membrane active area of 42 cm^2. Peristaltic pump was used to recirculate the hydrolysate.

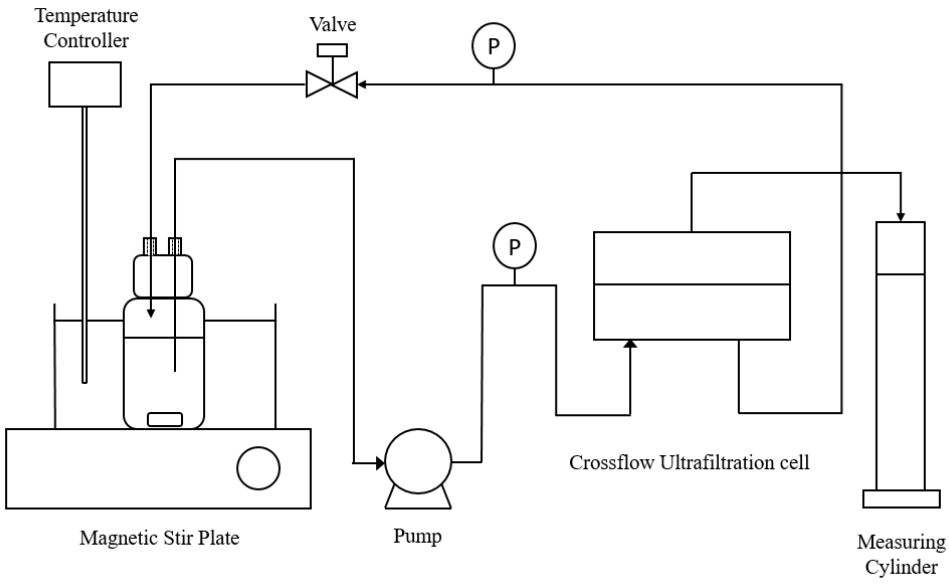

Figure 1. Configuration of Enzymatic Membrane Reactor.

10 g/L of microcrystalline cellulose was placed into the reactor. Citrate buffer with the concentration of 50 mM and cellulase (1.36g/L) were then added into the Scott bottle to a final volume of 100 ml. The hydrolysis was allowed to carry out at a controlled temperature of 50 ℃ and pH of 5.0 for 24 hours. After that, the ultrafiltration process started at a constant pressure of 2 bar by filtering 50 ml of the hydrolysate. Permeate was collected in the measuring cylinder and the retentate was recycled back into the reactor. 50 ml of citrate buffer and 10g/L of fresh substrate were then added into the hydrolysis

reactor and the reaction continued for another 24 hours. The permeate sample was placed in 80 ℃ water bath to deactivate the enzymatic activity and centrifuged at 13000 rpm for 5 minutes. The concentration of reducing sugar obtained in permeate was analysed by using dinitrosalicyclic acid (DNS) method [15] and the enzyme concentration was measured using Bradford's assay [16]. The procedure was repeated at 48th and 72th hour.

2.3. Membrane Performance

The membrane performance is determined by permeate flux and rejection. Flux (J) is expressed as [12]:

$$J\ (L\ h^{-1}\ m^{-2}) = \frac{V_p \times 3600\ (s\ h^{-1})}{1000\frac{mL}{L} \times A \times \Delta T} \quad (1)$$

where V_p (ml) is the permeate volume, A (m^2) is the effective membrane area, Δt (s) is the time elapsed since the first drop of permeate. Higher permeate flux represents higher membrane performance.

Rejection or retention coefficient is defined as [12]:

$$R = (1- \frac{C_{permeate}}{C_{Feed}}) \times 100\% \quad (2)$$

where $C_{permeate}$ is the concentration of solute in permeate and C_{feed} is the concentration in feed. In general, a good membrane indicates high rejection of cellulase and low rejection of the sugar.

2.4. Pore Blocking Model

There are four kinds of pore blocking models that used to describe the blocking phenomenon in crossflow ultrafiltration, which are complete pore blocking model, intermediate pore blocking model, standard pore blocking model and cake layer model.

The general equation of Hermia's pore blocking model is shown as [17]:

$$\frac{d^2t}{dv^2} = k(\frac{dt}{dv})^n \quad (3)$$

Where t is the filtration time, V stands for volume of permeate, k is the constant and n represents discrete constants for different fouling type: complete pore blocking (n=2), standard pore blocking (n=1.5), intermediate pore blocking (n=1) and lastly cake layer model (n=0)

Complete pore blocking model assumes that the molecules completely block membrane entrance and only takes place over the surface of the membrane. For n =2, the linearized form of Equation 3 is expressed as [17- 19]:

$$\ln(J^{-1}) = \ln(J_0{}^{-1}) + k_b t \quad (4)$$

The next model is standard pore blocking. This model considers that the molecules can easily pass through the membrane pore and accumulate over the pore walls. This is because the molecules in permeate are smaller than membrane pore size. Therefore, pore blocking occurs inside the pores of the membrane. For n=1.5, the linearized form is:

$$J^{-0.5} = J_0{}^{-0.5} + k_s t \quad (5)$$

Intermediate pore blocking happens when the particles in permeate and the membrane pores have the similar size, and hence block the entrance of the membrane pore. However, it is not a complete blockage, not all molecules can seal a separate membrane pore. For n=1, the linearized form is:

$$J^{-1} = J_0{}^{-1} + k_i t \quad (6)$$

Cake layer model happens when particles are bigger than membrane pore and will accumulate on membrane surface and thus results in the development of a 'cake'. For n=0, the linearized form for this model is:

$$J^{-2} = J_0{}^{-2} + k_c t \quad (7)$$

In Equation 4-7, J represents the permeate flux (L h^{-1} m^{-2}), t is the filtration time (min), J_0 is the initial permeate flux and k_b, k_s, k_i and k_c are the constants of the model. The most possible fouling mechanism can be determined by fitting experimental data using these linear models and comparing their regression coefficients (R^2) [19, 20].

3. Results and Discussion

3.1. Intermittent Separation of Product

Cellulose hydrolysis was carried out with intermittent reducing sugar removal followed by the addition of fresh cellulose and the replenishment of buffer. The hydrolysis reactor was initially operated in a batch mode for 24 hours, then followed by a discharge of 50% of the hydrolysate through ultrafiltration. The reducing sugar concentration was seen to be increased with time at the first 24 hours until it reached 5.48 g/L. A drastic drop of reducing sugar concentration could then be observed at every 24 hours interval as this was resulted from the replenishing of citrate buffer. After the feeding of fresh cellulose, the same increasing trend of the reducing sugar concentration was noticed, and reached a concentration of 5.93g/L and 6.45 g/L at 48th hour and 72th hour respectively. However, the reaction rate at 48th hour and 72th hour was not as high as at 24th hour, probably caused by enzymatic activity loss due to shear deactivation and increasing binding of the enzyme molecules at the non-hydrolysable region of the solid substrate [11].

The cellulose conversion can be clearly seen to be greater than batch reaction. Batch reaction achieved 63.26% of cellulose conversion while the final conversion of 82.14% at the end of the reaction for EMR. This can be explained that EMR has successfully reduced product inhibition and therefore cellulase has more active site for the substrate to bind with it. The higher conversion in EMR was also reported by other researchers. Gan et al. reported that the cellulose conversion in batch reactor is 35% as compared to EMR which is 53% [11]. Besides that, Henley et al. also showed that there was 90% of conversion in EMR while only 40% of conversion achieved in batch reactor [21].

3.2. Membrane Flux and Rejection

The flux behaviour of PES membrane was observed for three filtrations at 24 hours interval and the flux profile was presented in figure 2. In the three filtrations, the flux loss was quick at the first 15 minutes then followed by a steady decrease in flux. The gradual flux decline was caused by the cellulase adsorption and deposition of cellulose on the membrane surface which slowly form the cake layer.

The number of filtration and the increasing concentration of substrate also affect the membrane flux. It was observed that the membrane flux declined with time from initially 27.24 L/m^2.hr at the first filtration to 18.72 L/m^2.hr at the third filtration. Besides that, the pure water permeability of the PES membrane also decreased 65% before filtration and after the 3rd filtration, as shown in Figure 3. In Qi et al. study, the pure water permeability decreased 43.7% after ultrafiltration of steam exploded wheat straw hydrolysate [22]. Besides that, 62.5% of water permeability loss was reported by Sueb et al. after filtering xylan hydrolysate [23]. Both flux and permeability reduction were due to membrane fouling. The feeding of the cellulose to the hydrolysis reactor increased the amount of cellulose, which caused more cellulose to be accumulated on the membrane surface and therefore decreased in flux.

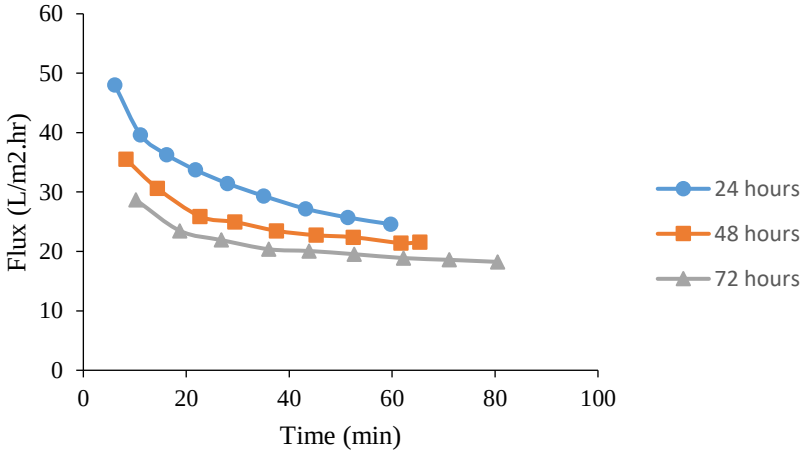

Figure 2. Flux profile at 24th, 48th, and 72th hour

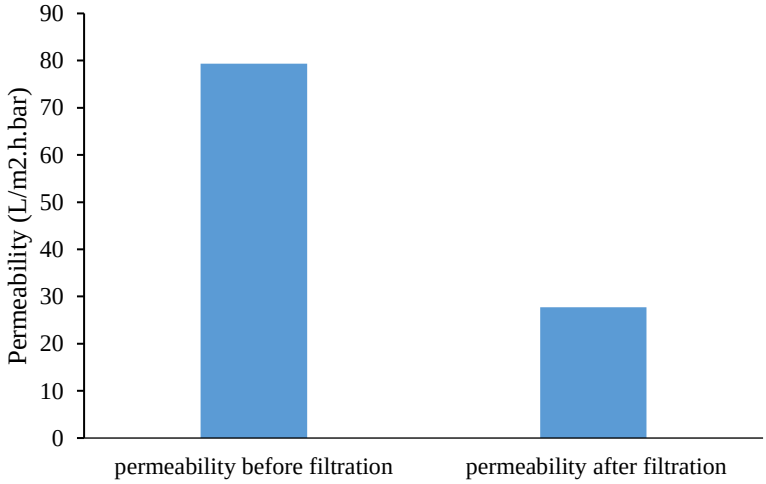

Figure 3. Permeability of 10 kDa PES membrane before and after filtration.

In terms of rejection, the reducing sugar rejection was 2.8%. This is because smaller size of the reducing sugar than the membrane pore size (180 Da) allowed it to permeate easily through the membrane. Result also showed that cellulase was almost completely retained as the rejection was 98.68%. This is due to the larger size of the cellulase enzyme mixture than the membrane pore size (endoglucanase 46 kDa, beta-glucosidase 75.3 kDa, cellobiohydrolase 52.2 kDa) [12]. From the rejection result, it can be concluded that the PES 10 kDa membrane is suitable for cellulase recovery.

3.3. Fouling Mechanism

Pore blocking model was applied to identify the fouling mechanism during ultrafiltration of cellulose hydrolysate. The flux data was fitted using Matlab 2013a (figure 4). Table 1 showed all the corresponding R^2 to indicate the best fitting model.

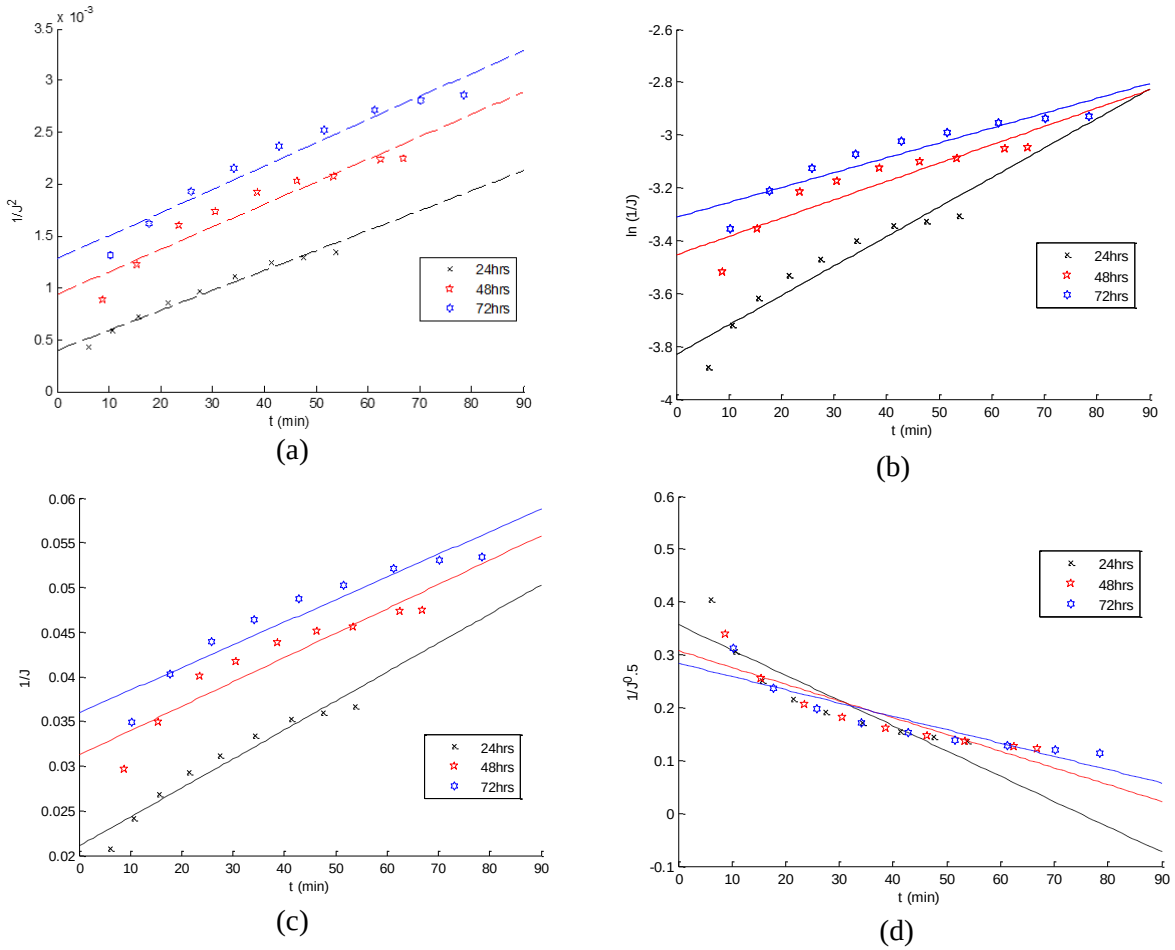

Figure 4. Linear fitting of pore blocking model (a) cake layer model (b) complete pore blocking (c) intermediate pore blocking (d) standard pore blocking.

It was observed from the figure 4 that the fitting of the experimental data to the all four types of fouling mechanisms, the best fitting was for cake layer model, as the values of R^2 were higher than other values, which were above 0.9. This indicated that cake layer model is the dominant fouling mechanism. This is most probably because it was difficult for the cellulase and cellulose to enter into the membrane pores [20].

Table 1. Regression coefficient (R^2) for different pore blocking model

Time (hr)	Complete Pore Blocking	Cake Filtration	Intermediate Pore Blocking	Standard Pore Blocking
24	0.802	0.970	0.842	0.814
48	0.828	0.909	0.872	0.832
72	0.868	0.950	0.906	0.831

The size of the cellulose and cellulase were substantially bigger than PES 10 kDa pore size, and they deposited on the membrane surface without sealing the pores. This leads to the cake layer formation which impeded the molecules entrance into the membrane pore and hindered the molecules to pass through the membrane, and gradually caused flux decline [19]. In Zain et al. study, the model which had the highest R^2 (0.997) was cake layer model for cellulose hydrolysis in an EMR at pH 5, where it dominated the fouling mechanisms when compared to other pore blocking model [20]. The differences

in Zain et al. (2016) study was that they investigated the effect of pH on the fouling mechanisms for cellulose hydrolysis, instead of investigating the effect of replenishing fresh cellulose on fouling mechanisms of cellulose hydrolysis.

4. **Conclusion**

The fouling mechanism of enzymatic cellulose hydrolysis was investigated. The study focused on the effect of replenishing fresh cellulose on fouling mechanisms of cellulose hydrolysis in an EMR. PES 10 kDa membrane was controlled by cake layer model due to larger size of the foulant such as cellulose and cellulase. Besides that, cellulose hydrolysis in an EMR was proved to have better cellulose conversion as compared to batch reaction. Therefore, membrane technology has achieved a promising result to minimize product inhibition. PES 10 kDa membrane was alsoable to reject 98.68% of cellulase, again proved its ability to retain the enzyme for the next batch of hydrolysis reaction. Despite its good performance in enzyme retention, PES 10 kDa membrane showed its fouling behaviour as the flux declined at the second and third filtration. Therefore, a proper membrane cleaning should be proposed to remove the cake layer from the membrane for further use.

Acknowledgements

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