Clarification of Glucose from Cellulose Hydrolysate by Ultrafiltration with Polyethersulfone Membrane

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Abstract

Ultrafiltration was used to clarify glucose from cellulose hydrolysate using polyethersulfone (PES) membrane. The flux behavior of PES membrane was studied in concentrating glucose from cellulose hydrolysate during dead end ultrafiltration in different pH of solutions and Kumar's model was applied to analyse the fouling mechanism. The permeation of glucose achieved more than 93% for all the different pH solution. The permeate flux decreased over time as a result of membrane fouling. The minimum fouling was obtained at pH solution above the IEP due to protein-protein and membrane-protein repulsions alleviating aggregation and fouling. Cake formation blocking was identified as the dominant mechanism for flux decline.

Key words: glucose, enzyme hydrolysis, lignocellulosic biomass, ultrafiltration, polyethersulfone

1. Introduction

Pretreatment and enzymatic hydrolysis are a prerequisite step for subsequent microbial fermentation from cellulose biomass in producing useful bioproducts and biofuels.The streams resulted from the pretreatment and enzymatic hydrolysis contains dilute sugars, along with excess components such as enzymes and biomass. The sugars released from hydrolysis may inhibit enzyme significantly, hence retarding the rate of enzymatic degradation [1-2]. By removing and concentrating the sugars from the hydrolysate can contribute towards not only in improving the conversion of the hydrolysis but also in recovering the used enzymes. This can help to decrease the cost of enzymatic hydrolysis by reducing the consumption of enzyme [3-4].

Ultrafiltration (UF) membrane process which have pore sizes ranging from 0.005 to 0.1 μm, an intermediate between nanofiltration and microfiltration, is a pressure-driven process used for removing solutes, such as oils, particulate matters, bacteria, suspended solids, large macromolecules, and proteins. In biorefinery, UF play an essential role as an integrated process widens the scope of possible operating strategies, such as product removal and enzyme recycling to reduce the enzyme costs and enhance the hydrolysis [5]. The application of ultrafiltration membranes provided an opportunity for retaining the free enzyme from discharge with the effluent after hydrolysis while at the same time separating the glucose for the subsequent fermentation process. The main drawback have been noted with the reduction of permeate flux over time in ultrafiltration, caused by the accumulation of feed component in the membrane porous structure, chemical interaction between solutes and membrane materials on the membrane surface. These macromolecules especially the polysaccharides caused the decrease in the permeate flow through the membrane which can be attributed to the fouling of its surface where the non-permeating solutes tending to form a gel layer [6-7].

In this study, the glucose from cellulose hydrolysate was clarified by a hydrophilic type membrane, polyethersulfone (PES) with 20 kDa molecular weight cut off (MWCO) was employed in a dead-end UF filtration mode. The influence of solution's pH and permeate flux was studied and the mechanism of fouling was modeled using Kumar's model.

Membrane blocking model was adopted by Kumar et al., [8], which modified from the model by Hermia and Granger. Table 1 shows the linearized equation of the model. For the cake formation model, cake was formed when the particles larger than the average pore size accumulated on the membrane surface. For standard pore blocking, the particles in the fluids entered the pores and adhered to the innerpore walls, thus the adhesion of particles to the walls decreased the available pore diameter and increased the resistance of the membrane. For complete pore plugging model, the particles plugged individual pores and the flow diverted to other pores that plugged successively.

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Table 1: Blocking filtration model

Type of blockage	Characteristic equation
Cake formation model	$\frac{t}{V}=X_1 V+Y_1$
Standard pore blocking	$\frac{t}{V}=X_2 V+Y_2$
Complete pore plugging model	$\frac{dV}{dt}=Y_3 -X_3 V$

2. Materials and Methods

2.1 Materials

Polyethersulfone commercial ultrafiltration (UF) membranes were chosen in order to minimize uncertainties with respect to the membrane material. The 20 kDa PES membrane was purchased from Koch. The high purity cellulose was used to prepare the feed solution and was supplied by Sigma Aldrich. The analytical grade glucose, citric acid, tri-sodium citrate were purchased from Nacalai Japan and used as foulant model in the feed solution.

2.2 Characterization of model cellulose hydrolysate

A model of cellulose hydrolysate was formed by mixing microcrystalline cellulose powder, glucose and cellulase enzymes. A 6% (w/w) microcrystalline cellulose, 33.3 g/l glucose and 8FPU cellulase were added in reverse osmosis water and mixed well for 30 minutes. The hydrolysate was analyzed for molecular weight (MW) by SDS polyacrylamide gel electrophoresis and isoelectric point (IEP) recorded by a zeta potential titration apparatus using Malvern Zetasizer Nano ZS, UK where the zeta potential was zero at the pH value. Table 2 shows the main physicochemical properties of cellulose hydrolysate used in the experiment.

Table 2 : Principle characteristics of the cellulose hydrolysate

Properties	Value
pH at room temperature	4.8
MW distribution from SDS Page	70kDa
Isoelectric point (IEP)	*3.9*

2.3 Ultrafiltration process

The 20 kDa PES membrane purchased from Koch was employed throughout the present study. The active membrane surface area was 14.67 cm^2. Fresh membranes were soaked in pure water overnight prior to each run to remove the preservative liquids from the manufacturer before it can be used. Experiments were performed in the dead end stirred cell (Sterlitech HP4750). The stirred cell is equipped with single blade stirrer and rotates at 500 rpm to minimize the layer formation of high solution concentration in the adjacent areas at membrane surface and to prevent the formation of a series vortex in the cell. The operation pressure in the system was maintained by nitrogen gas. The feed solution was conducted under constant pressure at 2 bar. Permeate flux was calculated based on the mass of permeate collected on the balance for about 60 minutes.

2.4 Analytical methods

Glucose content was analysed by HPLC (Agilent G1311A) equipped with refractive index (RI) detector and Rezex ROA column (300x7.80 mm). 0.005 N H_2SO_4 was used as the mobile phase as a flow rate of 0.6 ml/min and the column temperature was maintained at 60 °C. Enzyme concentration was measured by the Bradford protein assay using bovine serum albumin (BSA) as standard [9].

3. Results and Discussion

3.1 Concentrating glucose by UF

The permeability and selectivity of the membrane separation are mainly influenced by membrane pore size and the effective size of the components of the feed. Molecules with larger size than the largest membrane pore will be completely rejected while molecules of smaller size can pass through the barrier. UF membranes retain 90% of molecular mass in daltons of a macrosolute, so a specific small molecule can be fractionated into permeate from the hydrolyzate. The specification of a commercial UF membrane is not the pore size, but mostly the cut off value that is the molar mass.

Fractionation of cellulose hydrolysate by 20 kDa PES membrane in four different pH solutions were carried out and the respective results on permeation of glucose and rejection of enzyme are compared in Figure 1. In concentrating glucose via ultrafiltration process, glucose which has molecular weight cut off of 180 Da will be allowed to pass through membrane pores while the enzymes with 70 kDa of MWCO will be retained for recycle. As can be seen, the permeation of glucose is good for all the solution with more than 93% of glucose completely transmitted through the membrane. The highest glucose concentration was obtained in the pH solution of 5. In contrast, the majority of enzyme (more than 85%) was rejected for all the solution and at pH5 solution retained most of the enzyme.

In previous investigation, the rejection of enzyme and glucose were nearly more than 70% and 63% respectively, for different types and MWCO of UF and NF membranes [2,4,10].

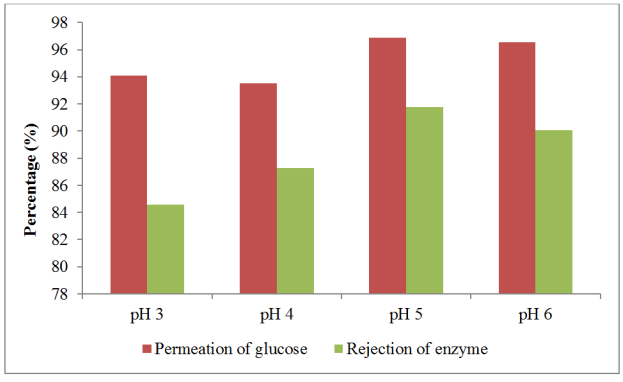

Figure 1 : Permeation of glucose and rejection of enzyme of UF permeate

The UF membranes with the performance of high rejection of enzyme but low rejection of glucose, high flux and high antifouling gives the advantages for the separation performances. Since the mechanisms are based on pore flow and size exclusion, the polymer material itself does not have direct influence on flux and selectivity in UF. The solution flow drags suspended particles and macrosolutes to the surface of the membrane where they are rejected due to their excessive size relative to the membrane pores. This simple process concentrates particles or macromolecules in the upstream nonpermeate stream and produces essentially pure low molecular weight permeate downstream. In the UF stage, the enzyme in the hydrolysate suspension that was recovered in the retentate could be used for subsequent round of hydrolysis of cellulosic substrate. Glucose that completely passed through the UF membrane could be collected in the permeate and ready to use for fermentation [11].

3.2 Effect of pH

During permeation, solutions are exposed to different processing conditions such as interaction with the membrane and the membrane pores' surface. The interaction between membrane-solute-solute was studied in the dead-end stirred ultrafiltration with constant transmembrane pressure at 2 bar. Figure 2 shows the fouling behaviour of cellulose hydrolysate for several pHs illustrated in terms of permeate flux relative to initial water flux (J/Jo). Initially the fouling behaviour for all solution can be seen as rapid flux decline for the first 10 minutes and followed by gradual flux decline. It is noted that the permeate flux was responsive to the solution pH, thus low pH value exhibited severe flux decline compare to high pH value. Similar experiment trends were reported by other researchers [4, 13-14].

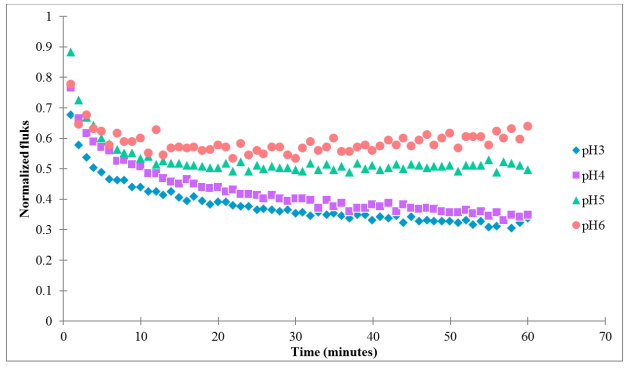

Figure 2 : Normalized flux declines of cellulose hydrolyzate in different pH of solution

The pH of the solution to be filtered can change both the charge of the membrane and the charge of some of the molecules to be filtered. The counterion exchange process between the ion exchanger and the counterions in the external solution has to be considered, specifically enzyme (protein) of large molecular weight. Protein molecule in solution has positive as well as negative charges distributed on its surface by changing the pH of the solution [15]. The net charge of the macromolecular protein due to pH-based interactions of various constituent groups is positive if the pH is less than the isoelectric point; the net charge is negative if the solution pH is more than the IEP. Thus as long as the solution pH is different from the IEP, the protein surface has some net positive or net negative charges. As shown in Figure 2, higher permeate flux observed at pH6 and the lowest at pH3. The fouling that happened in these cases could explain in Figure 3 why the lowest permeate flux obtains at pH below the hydrolyzate's IEP (~pH3). Below the IEP, both the cellulose hydrolyzate (positively charged) and PES membranes (negatively charged) have opposite charge, thus attraction forces are dominant over repulsion forces and affect the initial flux decline rate at the initial fouling stage. At pH of solution near to IEP (~pH4), the solubility of a protein built out of amino acids is minimum and has tendency to form aggregates with othe molecules [7]. As the pH moves away from the IEP, raising the pH to 5 and 6, the protein solubility increases and the leading to much lesser accumulation on the membrane surface.

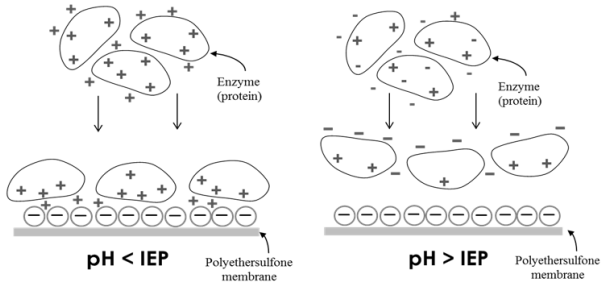

Figure 3 : Schematic illustration of possible configurations of cellulose hydrolyzate at pH lower and higher than IEP

3.3 Fouling mechanism

Kumar's equation was applied to identify the mechanism of fouling during ultrafiltration of cellulose hydrolyzate. Y-axis and X-axis according to Table 1 was fitted using Matlab R2012a (Figure 4). The best fit parameter was obtained by minimizing the sum of squared residuals (SSR) that could explain the experimental data rationally (Table 3).

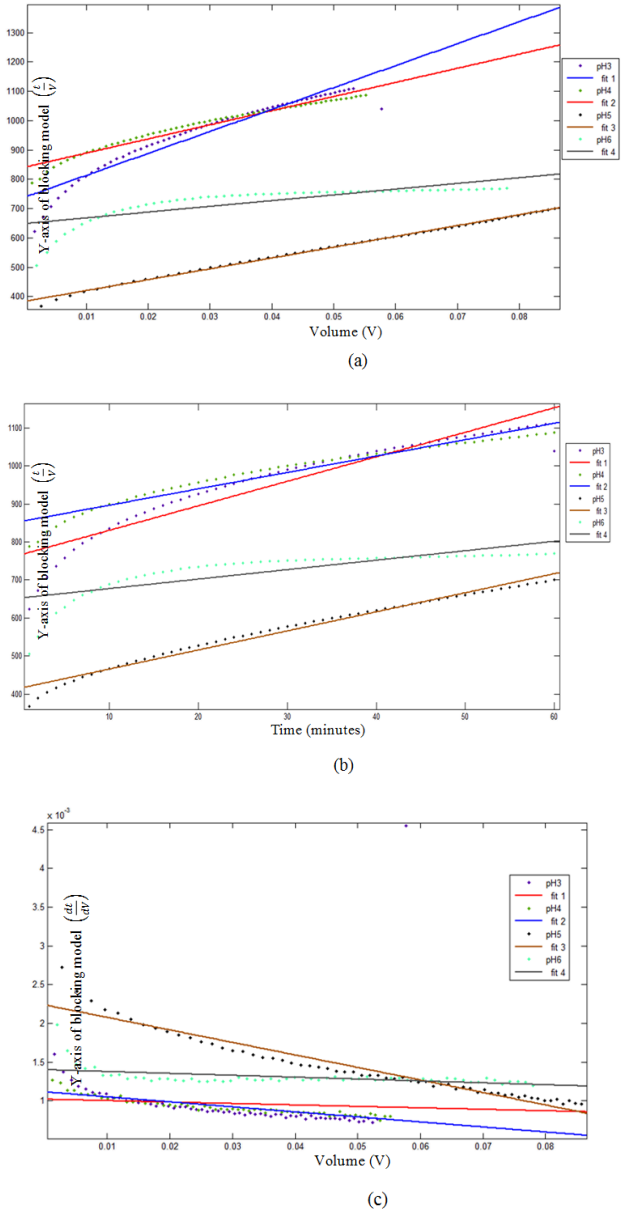

Figure 4 : Cake formation model (a), standard pore blocking (b) and complete pore plugging model (c) for cellulose hydrolyzate ultrafiltration

Table 3 : The value of R^2 obtained from the experimental data in the study of the effect of pH solution upon membrane fouling

pH	Cake formation mode	Standard pore blocking	Complete pore plugging model
3	0.92	0.896	0.003
4	0.957	0.939	0.866
5	0.997	0.979	0.93
6	0.626	0.619	*0.244*

It may be observed from the figure that the fitting of experimental data to the all three types of blocking, the best fitting occurred in pH5, as the values of R^2 were higher than others R^2 values, which were mostly above 0.9. Thus this indicated that at pH5 the possibility of the flux decline could be controlled by the combination of the three blocking model. Solution at pH3 were fouled mainly by cake formation, which could explain the electrostatic attraction between particles of protein and the fresh membrane that lead to the formation of cake layer impeding the entrance of molecules into the membrane pore and keeps them back on the cake layer. Blocking mechanism for filtration at pH6 was found to be unsuitable with the fitted model, which may be due to less accumulation of protein on membrane surface due the stronger electrostatic repulsion between protein and the PES membrane.

4. Conclusion

This work demonstrated the clarification of glucose from cellulose hydrolysate and the behavior of flux decline and fouling of UF by PES membrane. The permeation of glucose was achieved to more than 93% and the recovery of enzyme succeeded to 85%. It has been shown that solution pH have significant effect on the extent of cellulose hydrolysate fouling in membrane process. At higher solution pH (pH 5 and 6), the samples showed minimum membrane flux decline due to protein-protein and membrane-protein repulsions alleviate aggregation and fouling. The permeate flux decline profiles of all the solution were compared to the Kumar's model. These results showed that the fouling at pH5 was predominantly contributed by three types of blocking. Under all the solution pHs, it was observed that the values of R^2 of cake formation model were always greater than those obtained from other fouling mechanisms. This indicates that the cake formation model dominated in fouling studies, and followed by standard pore blocking and complete pore plugging model.

5. Acknowledgements

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