Malaysian Journal of Analytical Sciences, Vol 20 No 6 (2016): xxx - xxx
DOI: http://dx.doi.org/10.17576/mjas-2016-2006-zz

MALAYSIAN JOURNAL OF ANALYTICAL SCIENCES
Published by The Malaysian Analytical Sciences Society
ISSN 1394 - 2506

EFFECT OF pH ON FLUX DECLINE DURING FRACTIONATION OF GLUCOSE FROM CELLULOSE HYDROLYSATE THROUGH A POLYSULFONE MEMBRANE

(Kesan pH pada Penurunan Fluks bagi Pemeringkatan Glukosa daripada Hidrolisis Selulosa Melalui Membran Polisulfon)

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Received: 21 October 2015; Accepted: 14 June 2016

Abstract

Concentrating glucose after enzymatic hydrolysis of lignocellulosic biomass is an important step in order to prepare the stream to undergo the fermentation process. However, glucose itself can be inhibitor for the enzymatic hydrolysis, hence in situ glucose removal is recommended. Ultrafiltration represents a promising procedure for isolation of enzymes from hydrolysate and for the removal of glucose. One of the obstacles in successfully utilizing ultrafiltration's membranes has been due to the membrane fouling. The flux behavior of polysulfone (PSF) membrane was studied in concentrating glucose from cellulose hydrolysate during dead end ultrafiltration. Different pH of solutions was used and Kumar's model was applied to analyse the fouling mechanism. 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.

Keywords: enzyme hydrolysis, lignocellulosic biomass, ultrafiltration, polysulfone, fouling

Abstrak

Pemekatan glukosa selepas hidrolisis enzim bagi penghasilan bioetanol daripada biojisim lignoselulosa merupakan langkah yang penting bagi penyediaan untuk proses fermentasi selanjutnya. Penuras ultra menjadi pilihan bagi kaedah yang berpotensi untuk penyekatan enzim dan penyingkiran glukosa daripada larutan hidrolisis. Namun, salah satu rintangan bagi penggunaan maksima bagi penuras ultra adalah kotoran. Kriteria fluks bagi membran polisulfon di kaji dari pemekatan glukosa oleh larutan hidrolisis selulosa di penuras ultra hujung mati. Larutan yang berlainan pH digunakan untuk mengkaji profil fluks dan model Kumar digunakan untuk menganalisis mekanisma kotoran. Kotoran yang minima diperolehi pada larutan yang mempunyai pH yang lebih tinggi daripada nilai titik isoelektrik larutan hidrolisis. Halangan pembentukan kek telah dikenalpasti sebagai mekanisma yang dominan bagi pengurangan fluks.

Kata kunci: hidrolisis enzim, biojisim lignoselulosa, penuras ultra, polisulfon, kotoran

Introduction

Biorenewable products from lignocellulosic biomass has been the focus of attention around the world due to the depletion of petroleum derived product and its advantages of environmental friendly, cheap and widely available [1,2]. The main routes to produce fuels from biomass requires three major steps (1) pretreatment to delignify and

liberates cellulose and hemicelluloses from the matrix, (2) enzymatic hydrolysis to depolymerize carbohydrate polymers to release free sugars, and (3) fermentation [3]. 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 [4,5]. In order to increase the conversion of enzymatic hydrolysis, the removal and concentration of the sugars from the hydrolysate can contribute towards not only in improving 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 [6,7].

Ultrafiltration (UF) membrane process 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 application of ultrafiltration membranes in biorefinery 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 [8]. 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 [9,10].

A number of studies have shown that ultrafiltration is feasible to recover the enzymes and at the same time remove the fermentable sugars. However, limited study has been conducted on fouling characteristics and mechanism of cellulose hydrolysate and the effects of pH. For this study, a hydrophilic type membrane, polysulfone (PSF) with 20kDa 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. [11], which was modified from the model by J. Hermia and J. 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.

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	$\frac{dV}{dt} = Y_3 - X_3 V$

Materials and Methods

Materials

Polysulfone commercial ultrafiltration (UF) membranes were chosen in order to minimize uncertainties with respect to the membrane material. The 20 kDa PSF 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.

Characterization of model cellulose hydrolysate

In this study, a model of cellulose hydrolysate was formed by mixing microcrystalline cellulose powder, glucose and cellulase enzymes. The hydrolysate was analyzed for molecular weight and isoelectric point (IEP). 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. Gel electrophoresis in denaturing conditions was performed in sodium dodecyl sulfate (SDS-PAGE) according to the method by Laemmli (1970) resolving gel consisted of 15% polyacrylamide in Tris-HCl (1.5M, pH 8.8), while stacking gel consisted of 4.5% polyacrylamide in Tris-HCl (1.0 M, pH 6.8). Sample solutions were mixed at 1:2 (v/v) ratios with the sample buffer and heated at 90 °C for 3 minutes before loading. Aliquots of 40 – 42 µL samples were loaded into individual wells and a constant current was passed through the gel for 2 h to obtain separation of the peptides. Protein markers 10 – 260 kDa was used for molecular-weight determination. The gel sheets were stained with Gelcode Blue Safe Protein stain [12]. As for the determination of isoelectric point (IEP) of cellulose hydrolysate, the zeta potential at a given pH was recorded by a zeta potential titration apparatus using Malvern Zetasizer Nano ZS, UK. The IEP of the hydrolysate was determined where the zeta potential was zero at the pH value.

Ultrafiltration process

The 20 kDa polysulfone membrane purchase from Koch was employed throughout the present study. The active membrane surface area was 14.67 cm2. Fresh membranes were soaked in pure water overnight prior to each run to remove the preservative liquids from the manufacturer before 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.

Analytical Methods

Glucose content was analysed by HPLC (Agilent G1311A) equipped with refractive index (RI) detector and Rezex ROA column (300 x 7.80mm). An amout 0.005 N H2SO4 was used as the mobile phase as a flow rate of 0.6 ml/min and the column temperature was maintained at 60 °C. Protein concentration was measured by the Bradford protein assay using bovine serum albumin (BSA) as standard [13].

Result and Discussion

Characterisation of cellulose hydrolysate

Fractionation is controlled by the membrane pore size and the operating parameters. Determination of molecular size of the retained macromolecules is essential as size can be the criterion for separation. UF membranes retain 90% of molecular mass in daltons of a macrosolute, so a specific small molecule can be fractionated into permeate from the hydrolysate. In concentrating glucose via ultrafiltration process, glucose will be allowed to pass through membrane pores while the enzymes will be retained for recycle. Molecular weight of cellulose hydrolysate was analyzed by SDS polyacrylamide gel electrophoresis (Figure 1). From Figure 1 it was shown that the molecular weight of hydrolysates was 70 kDa. Similar result has also been reported by Andersen et al. [14] as 70 kDa calculated with the average cellulase enzyme in MW.

Amino acids in the protein contain both a basic and an acidic group because of the amphoteric character that can be positive or negative charge depending on the pH of the solution. If the amino acids did not migrate in the electrical field, which is it is not positive nor is negative charge, this conditions called as the IEP of the amino acids [15]. The isoelectric point is one of the characteristics parameter for a protein, as different proteins have different value of isoelectric point. IEP has important effects on the properties of cellulose hydrolysate solution in membrane process. By adjusting the pH, a complete separation of various proteins can be obtained. The isoelectric point of cellulose hydrolysate was 3.9.

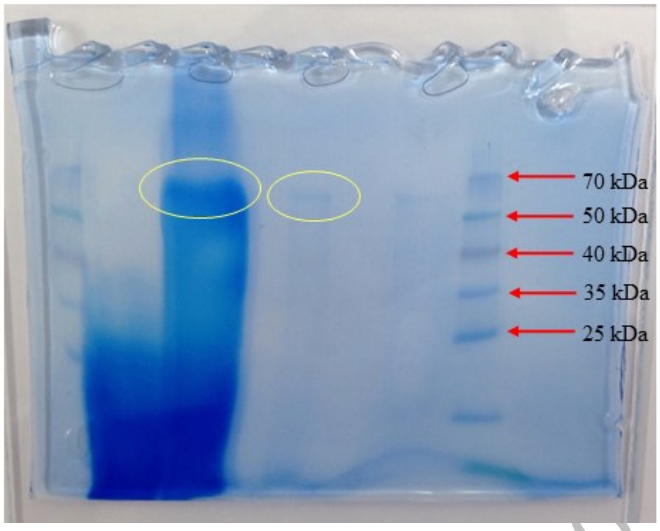

Figure 1. Electrophoresis profile of the cellulose hydrolysis

Effect of pH

The interaction between membrane and solute in different pH was studied in the dead-end stirred ultrafiltration with constant operating 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 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 as compared to high pH value. Similar experiment trends were reported by other researchers [7,16,17].

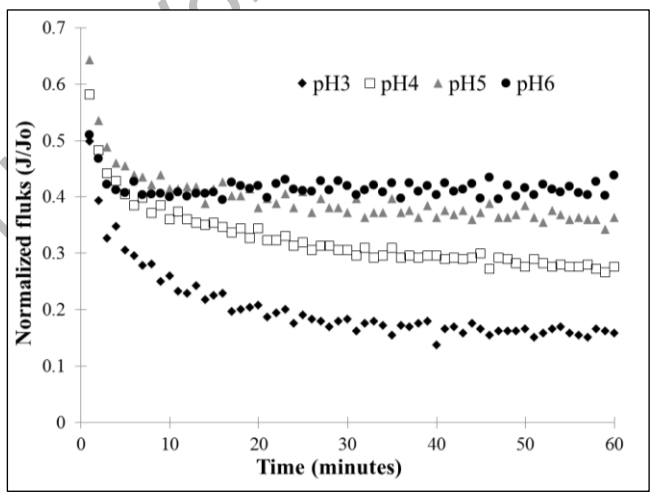

Figure 2. Normalized flux declines of cellulose hydrolysate in different pH of solution

The feasibility of fractionation depends on charge, thus more charged ions are retained better than monovalent ions [18]. In addition, the counterion exchange process between the ion exchanger and the counterions in the external solution has to be considered, specifically proteins of large molecular weight [19]. 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.

All these could explain why the lowest permeate flux was obtained at pH below the hydrolysate's IEP (~pH3). Figure 3 illustrates the observation for pH3. Below the IEP, both the cellulose hydrolysate (positively charged) and PSF membranes (negatively charged) have opposite charges, 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 other molecules [10]. As the pH was raised to pH 5 and pH 6 (away from the IEP), observation shows that the protein solubility increases and lead to much lesser accumulation on the membrane surface.

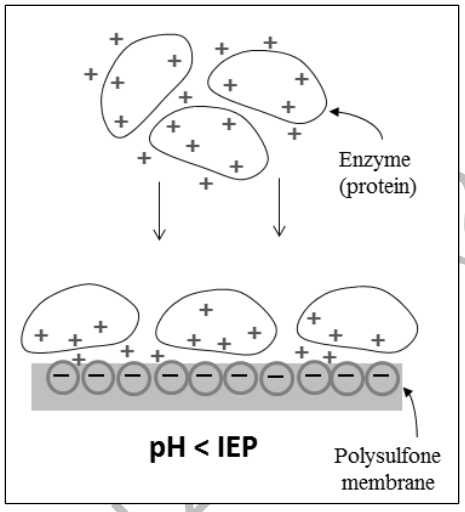

Figure 3. Schematic illustration of possible configurations of cellulose hydrolysate at pH lower than IEP

The membrane blocking resulted from the accumulation on the membrane surface would affected the permeation of glucose in the process. As illustrated in Figure 4, the permeation of glucose at pH 3 is the lowest compared to other pH, indicating that much glucose was unable to pass through the PSF membrane because of the blocking by the protein on the membrane surface, while at pH 5 the permeation of glucose is the highest.

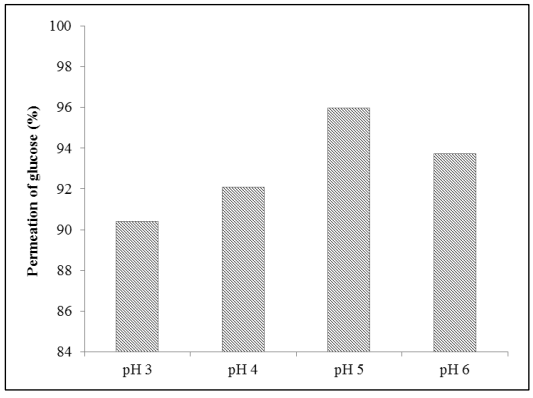

Figure 4. Permeation of glucose in different pH

Fouling mechanism

Kumar's equation was applied to identify the mechanism of fouling during ultrafiltration of cellulose hydrolysate. Y-axis and X-axis according to Table 1 was fitted using Matlab R2012a (Figure 5). The best fit parameter was obtained by minimizing the sum of squared residuals (SSR) that could explain the experimental data rationally (Table 2).

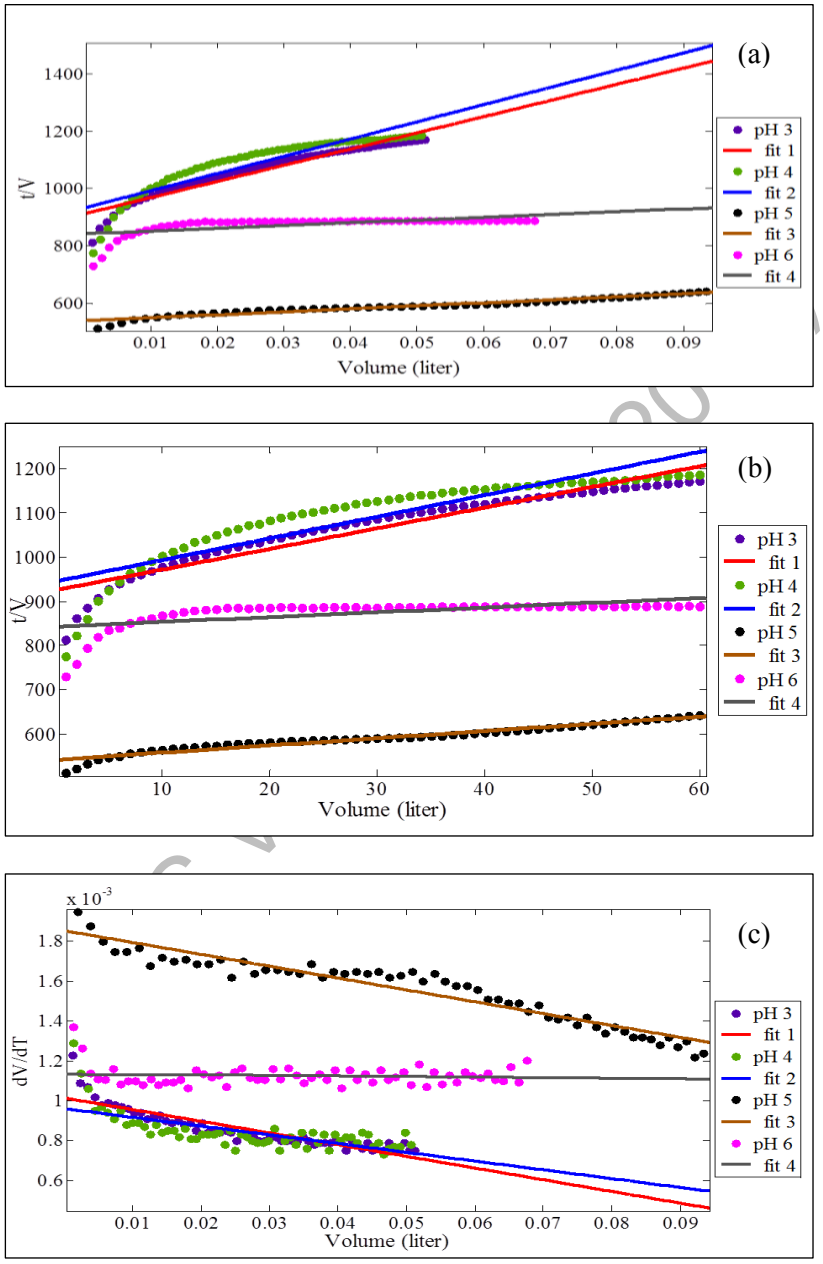

Figure 5. Cake formation model (a), standard pore blocking (b) and complete pore plugging model (c) for cellulose hydrolysate ultrafiltration

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 R2 were higher than others R2 values, which were mostly above 0.9. Thus

this indicated that at pH5 the possibility that 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.

Table 2. The value of R2 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	0.9282	0.9084	0.7968
4	0.8117	0.7937	0.8558
5	0.9449	0.9453	0.9133
6	0.3753	0.3769	0.0107

Similar results have also been observed for glucose permeation in hollow fibre membrane whereby formation of thin layer of cake denoted the membrane blocking [6]. Blocking mechanism for filtration at pH6 was found to be unsuitable with the fitted model, may be due to the less accumulation of protein on membrane surface due the stronger electrostatic repulsion between protein and the PSF membrane.

Conclusion

It has been shown that solution pH has 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 pH, 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.

Acknowledgement

This study was financially supported by the LRGS grant (LRGS/2013/UKM-UKM/PT/03) from Ministry of Higher Education and Universiti Kebangsaan Malaysia.

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