PRODUCT REMOVAL STRATEGY AND FOULING MECHANISMS FOR CELLULOSE HYDROLYSIS IN AN ENZYMATIC MEMBRANE REACTOR

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ABSTRACT

Enzymatic cellulose hydrolysis from lignocellulose biomass has been extensively studied as the product from the hydrolysis can be used to convert into renewable biochemical such as bioethanol. Cellulose hydrolysis were traditionally carried out in a batch reactor. However, cellulose hydrolysis in batch reactor leads to product inhibition which results in low yield of glucose. Kinetic study of cellulose hydrolysis in batch reactor was performed, and showed that cellulase was inhibited by glucose and cellobiose in a competitive manner, with K_i of 2.58 g/L and 2.24 g/L respectively. Therefore, it is necessary to separate glucose from the hydrolysis reactor in order to minimize product inhibition. In this study, enzymatic membrane reactor (EMR) was used to reduce the amount of enzyme used and to prevent product inhibition. The filtration technique used was ultrafiltration (UF) in a crossflow mode. Before performing cellulose hydrolysis in an EMR, a membrane screening was done to select a suitable membrane to be used in the EMR. Results had shown that HFK-131 membrane is the most suitable membrane as it has the lowest contact angle and the highest permeability. Cellulose hydrolysis was then carried out in an EMR with different substrate concentrations (5 g/L to 20 g/L) and different product removal strategies in order to study their effect on the product yield, membrane performance, and fouling mechanisms. The PES membrane showed almost 95% and above rejection of cellulase as the cellulase molecular weight (MW) was larger than molecular weight cut off (MWCO) of the membrane. Hermia's pore blocking model was applied to determine the predominant fouling mechanism of the membrane filtration. From the results, intermittent product removal at 24 hours interval was better as the cellulose conversion could achieve more than 80% and the membrane flux decline is less severe than the product removal at 4 hours interval. For the effect of substrate concentrations, the cellulose conversion decreased from 88.48% to 61.43% with increasing substrate concentration. The flux also declined from 23.92 $L/m^2.h$ to 15.15 $L/m^2.h$ as the substrate concentrations were increased resulting in more cellulose to be deposited on the membrane surface, and leads to a more severe membrane fouling. It was also observed that the cake layer model was the predominant fouling mechanisms at 5 g/L and 10 g/L of substrate concentration, whereas 20 g/L has a combination of complete pore blocking and cake layer model. This result was further proved by SEM images, where the fouled membrane at 20 g/L appeared to have the most fouling layer on the membrane surface. Besides that, the membrane surface roughness increased with increasing substrate concentration, with the highest at 38.50 nm at 20 g/L. Results demonstrate the potential of using EMR for the production of reducing sugars and enzyme recovery in cellulose hydrolysis. With known fouling mechanism of cellulose hydrolysis in EMR, further improvement of the EMR operation at high substrate concentration could be done to minimize fouling.

ABSTRAK

Hidrolisis selulosa enzimatik dari biomas lignoselulosa telah dikaji secara mendalam kerana produk dari hidrolisis boleh digunakan untuk menukar kepada biokimia yang boleh diperbaharui seperti bioethanol. Hidrolisis selulosa secara tradisinya dijalankan dalam reaktor kelompok. Namun, hidrolisis selulosa dalam reaktor kelompok mengakibatkan pelumpuhan produk dan mengurangkan hasil glukosa. Kajian kinetik hidrolisis selulosa dalam reaktor kelompok telah dijalankan, dan keputusan menunjukkan activiti enzim selulase telah dilumpuh oleh glukosa dan selobiosa dalam keadaan berdaya saing, dengan Ki 2.58 g/L and 2.24 g/L masing-masing. Oleh itu, glukosa perlu diasingkan dari reaktor hidrolisis supaya pelumpuhan produk boleh diminimakan. Dalam kajian ini, enzim membran reaktor (EMR) digunakan untuk mengurangkan kegunaan enzim dan mencegah pelumpuhan produk. Teknik penapisan yang digunakan ialah penapisan ultra dalam keadaan aliran silang. Sebelum menjalankan hidrolisis selulosa dalam EMR, pemilihan membran telah dilakukan supaya membran yang sesuai boleh dipilih untuk digunakan dalam EMR. Keputusan telah menunjukkan polietersulfon (PES) 10 kDa adalah membran yang paling sesuai kerana ia mempunyai sudut sentuhan yang paling rendah dan ketelapan air yang paling tinggi. Hidrolisis selulosa dijalankan dalam EMR dengan mengunakan pemekatan substrat (5 g/L to 20 g/L) dan strategi pemisahan produk yang berbeza untuk mengkaji kesan-kesan terhadap hasil produk, prestasi membran, dan mekanisme kotoran membran. Membran PES menunjukkan hampir 95% dan ke atas penolakan selulase kerana selulase berat molekul (MW) lebih berat dari nilai potongan berat molekul (MWCO) membran. Model menyekat pori Hermia telah digunakan untuk menentukan mekanisme kotoran yang utama dalam membran penapisan. Separasi produk terputus-putus dalam 24 jam jarak waktu adalah lebih baik kerana konversi selulosa telah mencapai lebih dari 80% dan keturunan fluks membran adalah kurang serius apabila dibandingkan dengan separasi produk dalam 4 jam jarak waktu. Konversi selulosa telah menurun dari 88.48% kepada 61.43% dengan pemekatan substrat yang meningkat. Fluks membran juga telah menurun dari 23.92 L/m2.h ke 15.15 L/m2.h apabila pemekatan substrat meningkat. Hal ini telah mengakibatkan pengumpulan selulosa yang banyak atas permukaan membran dan kotoran membran yang serius. Mekanisme kotoran yang utama ialah model lapisan kek dalam pemekatan substrat 5 g/L dan 10 g/L, manakala 20 g/L mempunyai kombinasi model penyekatan pori lengkap dan model lapisan kek. Keputusan ini telah dibuktikan dengan gambar SEM, di mana membran yang kotor mempunyai lapisan kotoran yang paling banyak atas permukaan membran. Selain itu, kekasaran permukaan membran bertambah dengan pemekatan substrat yang meningkat. 38.50 nm merupakan kekasaran permukaan yang paling tinggi di 20g/L. Keputusan menunjukkan potensi kegunaan EMR untuk penhasilan gula penurun dan pemulihan enzim dalam hidrolisis selulosa. Dengan mengetahui mekanisme kotoran hidrolisis selulosa dalam EMR, penambahbaikan EMR yang selanjutnya boleh dijalankan untuk meminimakan kotoran membran dalam operasi EMR yang menggunakan pemekatan substrat yang tinggi.

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LIST OF ABBREVIATIONS

AFM	-	Atomic Force Microscopy
ATR-FTIR	-	Atteunuated Total Reflection-Fourier Transform Infrared Spectroscopy
ATWS	-	Acid Treated Wheat Straw
BSA	-	Bovine Serum Albumin
CMC	-	Carboxymethyl Cellulose
DNS	-	Dinitrosalicyclic Acid
EI complex	-	Enzyme-Inhibitor Complex
ES complex	-	Enzyme-Substrate Complex
E/S ratio	-	Enzyme to substrate ratio
EMR	-	Enzymatic Membrane Reactor
FPU	-	Filter Paper Units
FTIR	-	Fourier Transform Infrared Spectroscopy
HMF	-	Hydroxymethylfurfural
IF	-	Irreversible fouling
KB_r	-	Potassium bromide
MM	-	Michaelis-Menten
MWCO	-	Molecular Weight Cut Off
NaOH	-	Sodium hydroxide
PA	-	Polyamide
PES	-	Polyethersulfone
PS	-	Polysulfone
PVDF	-	Polyvinylidene difluoride
RC	-	Regenerated cellulose
RS	-	Reducing sugar
RMS	-	Root mean squared
SEM	-	Scanning electron microscopy
TMP	-	Transmembrane pressure
UF	-	Ultrafiltration

LIST OF SYMBOLS

C_B	-	Concentration in the bulk
C_P	-	Concentration of solute in the permeate
$C_{permeate}$	-	Concentration of solute in permeate
C_{feed}	-	Concentration of solute in feed
[C]	-	Cellulose concentration
[E]	-	Cellulase concentration
[EC]	-	Cellulose-cellulase complex
[G]	-	Glucose
J	-	Permeate flux
J_0	-	Initial permeate flux
k	-	Fouling constant
k_b	-	Complete pore blocking model constant
k_c	-	Cake filtration model constant
k_i	-	Intermediate pore blocking model constant
k_s	-	Standard pore blocking model constant
K_2	-	Rate constant for the production of glucose
K_i	-	Inhibition constant
K_M	-	Michaelis-Menten constant
K_s	-	Dissociation constant for [EC] complex formation
L_p	-	Pure water permeability
L_{pb}	-	Pure water permeability before ultrafiltration
L_{pa}	-	Pure water permeability after ultrafiltration
n	-	Discrete constants for different type of fouling mechanisms
R_m	-	Membrane resistance
R_i	-	Rejection coefficient
r_p	-	Membrane pore size
t	-	Filtration time
v	-	Rate of product formation
V_{max}	-	Maximum rate of reaction
V	-	Permeate volume

Δx	-	Membrane thickness
τ	-	Tortuosity
ε	-	Porosity

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cellulose hydrolysis from lignocellulosic biomass has been extensively studied as the product from the hydrolysis can be used to produce renewable energy, which is carbon neutral and environmentally friendly. Cellulose can be found abundantly in natural residual lignocellulosic material such as wheat/rice straw, palm empty fruit bunches or sugar bagasse and it has been widely used in lignocelluloses biorefinery (Lynd *et al.*, 2008; Zhang, 2009; Rashid *et al.*, 2013). These low-cost lignocellulosic materials can be converted into fermentable sugars, which reduces the waste disposal costs and concomitantly meets the growing demand for energy (Lynd *et al.*, 2002; Walker and Wilson, 1991; Gan *et al.*, 2003). The sugars can further be converted to fuels and chemicals like ethanol, organic acids and biodegradable plastics (Walker and Wilson, 1991; Lynd and Zhang, 2004). The conversion of waste cellulosic residues to bio-ethanol involves delignification of cellulose, depolymerization of carbohydrate polymers to free sugars via enzymes and fermentation of these sugars to produce ethanol (Cheung and Anderson, 1997)

There are two usually used methods to transform cellulose into reducing sugar, which are acid hydrolysis and enzymatic hydrolysis. Acid hydrolysis uses dilute acid or concentrated acid to reduce cellulose into reducing sugar. Although it is one of the most commonly used approaches, the yield from acid hydrolysis is low and there is a higher chance of producing inhibited product such as hydroxymethylfurfural from acid hydrolysis (Carvalho *et al.*, 2013). Therefore, enzymatic hydrolysis is often preferable as it is carried out in a milder condition and has less impact on the environment. The enzymatic hydrolysis is carried out using an enzyme known as cellulase, where it consists of a mixture of endoglucanases, exoglucanases as well as β-glucosidases (Ghazali *et al.*, 2017). These three enzymes work synergistically to convert cellulose

into glucose (Sofia and Rodrigues, 2014). The endoglucanases and exoglucanases reduce cellulose into cellobiose, then the cellobiose will be hydrolyzed into glucose by β-glucosidases.

Cellulose hydrolysis is traditionally carried out in a classical batch reactor at a maintained temperature and pH, where the substrate, enzyme, and the product stay in the same reactor and the product will be collected at the end of the process (Nguyenhuynh *et al.*, 2017a). Batch hydrolysis only allows the enzyme to be used once and needs new enzymes for a new batch of hydrolysis. Therefore, there are some disadvantages of using the batch reactor, which are low productivity, high operating costs (due to the addition of enzyme for each batch), and loss of catalytic activity due to enzyme inactivation (Rios *et al.*, 2004). Product inhibition is also one of the major problems in batch hydrolysis, where the rate of reducing sugar is affected by inhibitors such as glucose and cellobiose. Other than product inhibition, another drawback of batch cellulose hydrolysis is the enzyme wastage, as the enzyme will be replaced for each hydrolysis process, despite the enzyme still possess some catalytic activity.

The alternative approach to replace the batch reactor is the enzymatic membrane reactor (EMR). In recent years, EMR has caught researchers' interest for its potential ability to prevent product inhibition and to increase the product yield (Ghazali *et al.*, 2017; Andrić *et al.*, 2010; Zain *et al.*, 2017). Some studies concluded that higher conversion of cellulose into glucose can be achieved by removing glucose using membrane reactors (Gavlighi *et al.*, 2013; Gan *et al.*, 2002). For cellulose hydrolysis, the membrane reactor consists of a reactor for enzymatic reaction and a membrane separation unit (Nguyenhuynh *et al.*, 2017a). After the hydrolysis is completed, the enzyme will be retained in the membrane while the reducing sugar will permeate through the membrane. The main purpose in enzymatic membrane reactor is to make sure that more than 90% of the enzymes are being rejected while carrying out separation process, and at the same time, maintaining full enzymatic activity inside the hydrolysis reactor.

For the cellulose hydrolysis in an EMR, ultrafiltration (UF) is used as the separation process to retain cellulase as well as removing the reducing sugar produced

from the enzymatic reaction. The membrane used in UF is chosen based on the enzyme molecular weight. UF membranes can retain large molecules with a molecular weight ranging from 10 to 100 kDa (Nguyenhuynh *et al.*, 2017a), and it is being widely applied in biological products separation, in particular protein. The following studies also used UF membrane to separate cellulase from the reducing sugars (Rad *et al.*, 2017; Amirilargani *et al.*, 2012). The cellulase (macromolecules) in the liquid phase can be retained by UF. Reducing sugar such as glucose and cellobiose are smaller than the pore size of UF membrane, therefore they can pass through the UF membrane easily and enters permeate.

1.2 Problem Statement

One of the main drawbacks of cellulose hydrolysis in the batch reactor is product inhibition that lowers the product yield. Glucose and cellobiose, the reducing sugars produced from cellulose hydrolysis, has been reported to be the inhibitors of the hydrolysis process and reduce the rate of cellulose hydrolysis. This is because of the presence of glucose and cellobiose inhibit the enzyme β-glucosidase in the cellulase complex system. Therefore, it is crucial to separate these two reducing sugar from these products to prevent inhibition. Besides product inhibition, enzyme wastage is also another problem which makes the batch process expensive due to high enzyme cost. A significant amount of the cellulase remains active in the batch reactor. The cellulase used after hydrolysis process will not be reused and will be treated as waste although the enzyme still possesses some enzymatic activity after hydrolysis. To overcome this problem, recovery of cellulase is one of the best strategies to reduce enzyme cost as the cellulase can be reused and not wasted (Gomes *et al.*, 2015; Tian *et al.*, 2015; Haven *et al.*, 2015). Cellulase recycling can be done in a membrane reactor. The retained cellulase in the membrane is recycled back to the reactor to perform the hydrolysis reaction continuously. Recovery and reuse of enzyme can also achieve the zero-release of water in the enzyme treatment stage (Wang *et al.*, 2016). Therefore, product inhibition and cellulase waste can be minimized by having an ideal EMR system.

However, membrane fouling remains a major obstacle hindering the practical application of EMR. Membrane fouling causes permeate flux decline, as well as deposition of fouling layer (Jiang, 2007). Membrane fouling limits the use of membrane separation, leads to membrane resistance, decrease the efficiency of the product separation process, and increase the operation and energy cost of EMR. Although membrane fouling has been well reported, the underlying mechanism remains incompletely understood due to the diversity of operational conditions, membrane materials, and configurations used in different studies in EMR (Ozgun *et al.*, 2013). Therefore, it is important to study the membrane fouling mechanisms and fouling layer formation in EMR (Meng *et al.*, 2007; Herrera-Robledo *et al.*, 2010). It is crucial to identify the pore-blocking mechanisms that occur during membrane filtration of cellulose hydrolysate to select a proper cleaning strategy (Choi *et al.*, 2005).

1.3 Objectives

(a) To determine the kinetics of enzymatic cellulose hydrolysis in a batch reactor.
(b) To evaluate the effect of product removal strategy on the reducing sugar yield in an EMR.
(c) To determine the membrane fouling mechanism of cellulose hydrolysis in a membrane reactor.

1.4 Research Scope

This research is done to perform cellulose hydrolysis in an EMR by using crossflow ultrafiltration with a UF membrane. Before performing cellulose hydrolysis in an EMR, the batch hydrolysis was performed to study the kinetics of the process. The hydrolysis process used cellulase as an enzyme and microcrystalline cellulose as a substrate. Inhibitors such as glucose and cellobiose with a concentration of 5 g/L and 10 g/L was added into the batch reactor and react together with substrate and enzyme. Type of inhibition and kinetic parameters of cellulose hydrolysis was studied.

The UF membranes from different companies were used to screen for its pure water permeability and contact angle. The membrane was chosen based on the highest pure water permeability and the lowest contact angle. Two product removal time was used in this research, which are product removal time at 4 hours interval and 24 hours interval. Cellulose conversion and flux were analyzed for these two product removal time. The effect of substrate concentration used in EMR on the product yield, flux, and fouling mechanism was also been evaluated. The substrate concentration varies from 5 g/L to 20 g/L for cellulose hydrolysis in an EMR. Besides that, the rejection of reducing sugar and cellulase enzyme was studied throughout the research.

Fouling mechanism of the hydrolysis process was analyzed using Hermia's model and the flux data recorded was used to fit the model. Reducing sugar concentration obtained in the permeate flow was measured using dinitrosalicylicacid (DNS) method. Bradford assay was used to determine the percentage of cellulase enzyme rejected back into the reactor.

1.5 Significance of the Study

This study was significant to reduce enzyme cost by reusing enzyme during cellulose hydrolysis in an EMR. Moreover, this research provided a platform to understand better in cellulose hydrolysis kinetics of cellulase from *Trichoderma reesei* to increase the understanding of the enzymatic reaction. Furthermore, cellulose hydrolysis in an EMR has not been investigated for its effect on different product removal time and substrate concentration on fouling mechanism and product yield. Therefore, it is important to study the membrane fouling mechanism so that a suitable cleaning approach could be selected with known fouling mechanism.

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