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SEPARATION OF REDUCING SUGARS AND MICROORGANISM FORM HYDROLYSATE BY ULTRAFILTRATION

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Abstract

Hydrolysate in ethanol production process consists of the important material such as reducing sugar, enzyme and microorganism. If we can enhance the concentration of reducing sugar in the hydrolysate, it will be increase the yield of ethanol production. This work aims to study the separation of reducing sugar and microorganism by using membrane technology to increase the concentration of reducing sugar. There are two substrates for preparation of hydrolysate. They are bagasse and Napier grass. The experiments used ultrafiltration membrane at three different flow rate; 13, 18, and 24 ml/min. The results show that the flow rate of 13 ml/min is the best condition for separation of reducing sugar and microorganism in bagasse hydrolysate. The percentage of rejection of reducing sugar, cellulase activity, and microorganism are 31.50%, 26.90%, and 73.68% respectively. When we used this condition for filter Napier grass hydrolysate, the percentage of rejection of reducing sugar, cellulase activity, and microorganism are 63.10%, 43.83%, and 60.61% respectively. These results indicate that the slow flow rate is better than fast flow rate for membrane separation and the type of substrate has an effect on percentage of rejection.

Keywords: Hydrolysate, Bagasse, Reduced sugar, Membrane, Napier grass

Introduction

Thailand 4.0 is the hot issue in every field right now in Thailand because the Thai government needs to improve the country to the sustainability by using the Thailand 4.0 strategy. For chemical engineer, biofuels and biochemicals will be the main focus in 2018 and the next few years. Bioethanol is one of the most interesting biofuel for Thailand. There are a lot of sources for bioethanol production such as rice, corn, sugarcane etc. For sustainability, the use of food crops as a feed stock for production of bioethanol may not be a good choice. Therefore, lignocellulosic materials such as grass, wheat straw, and crop residues are the best alternative to produce bioethanol [1]. For bioethanol fermentation from lignocellulosic biomass, the first step is hydrolysis to convert the biomass or cellulose to reducing sugar and the second step is fermentation to produce bioethanol [2]. The first step is very important because it is the key process to produce the reactant of the bioethanol reaction. If we can produce a lot of reducing sugar, we will get high yield of bioethanol.

The product from hydrolysis process is hydrolysate which consists of many materials. For example, lignocellulosic materials, microorganism, reducing sugar, and enzyme. There are a lot of investigations about hydrolysate as a pretreatment step of bioethanol process. Manasrah et al. reported the use of ultrafiltration membranes to recovery of galactoglucomannan from wood hydrolysate [3]. Hamelinck et al. studied the state of the art of hydrolysis-fermentation technologies of the utilization of lignocellulosic biomass for bioethanol production [2]. Koivula et al. investigated various pretreatment methods of wood hydrolysates and enhanced membrane filtration for hemicellulose recovery from pretreatment process [4, 5]. Siwarasak et al. used Tricoderma reesei RT-P1 for bioethanol production from sweet sorghum fresh stalks [6]. As mentioned above, there are many investigators studied about pretreatment of raw material for bioethanol production but Napier grass which is the potential material is lack of information.

This work aims to investigate the separation of reducing sugar and microorganism in the hydrolysates. The selected technology is ultrafiltration membrane. The main composition of hydrolysate consists of reducing sugar, microorganism, and enzyme. If the concentration of reducing sugar increased, the yield of bioethanol will be increased. Therefore this project used the ultrafiltration membrane to separate the reducing sugar and enzyme. Then the enzyme can recycles into the hydrolysis step and the reducing sugar with high concentration will be used in the fermentation.

Materials and methods

Materials

There are two substrates for production of hydrolysate. The first one is sugarcane bagasse and the second one is Napier grass. The microorganism is Tricoderma reesie RT-P1. The chemical for the experiments and analysis are sodium hydroxide (NaOH), calcium hydrogen phosphate ($CaHPO_4$), magnesium sulfate ($MgSO_4.7H_2O$), urea ($46\%(NH_4)_2SO_4$), sulfuric acid (H_2SO_4), Phosphate (NPK-0-52-34), and distilled water. The ultrafiltration membrane commercial grade (Quix stand, GE Medical Systems (Thailand)) was used in this study. It made from polyethersulphone , molecular weight cut off is 30 kDa, and the area of filtration is 420 cm^2 as shown in Figure 1 [7].

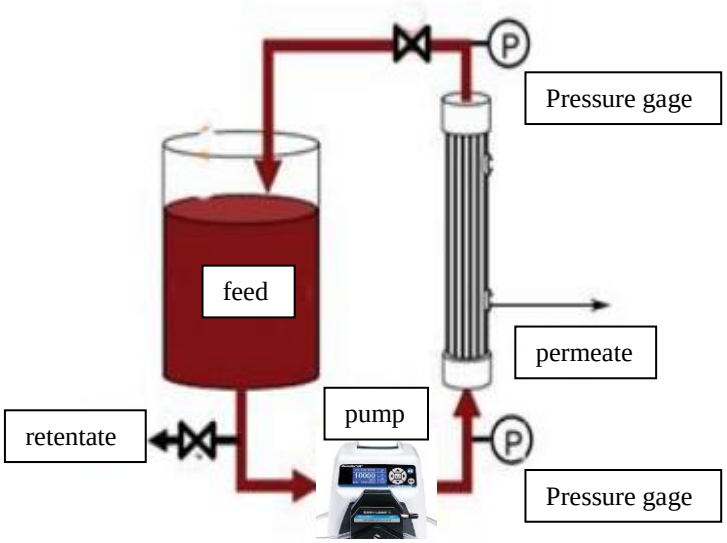

Figure 1 The experimental set up for membrane separation (adopted from Kitsuwan et al. 2015)

Methods

For substrate preparation, two kinds of hydrolysates were used in this study; one from sugarcane bagasse and one from Napier grass. They were both produced by hydrolysis. The sugarcane bagasse was reduced size by physical treatment and dried in the oven at 130 °C until the moisture content lower than 6%. The sugarcane bagasse was mixed with 2 M NaOH solution at the weight ratio of 1:10 for 24 h. The mixture was filtered by cheesecloth and added 2 M NaOH for boiling by water bath at 70 °C for 90 min. The mixture was filtered by cheesecloth again and washed by water until the pH equal to 7. The last step of preparation was dried in the oven at 120 °C. Then the pretreated material was analyzed for cellulose, hemicellulose, and lignin. For microorganism and culture media preparation, the compositions of the culture media are shown in Table 1. Tricoderma reesei RT-P1 was produced by solid state fermentation without sucrose [6].

For hydrolysate preparation, 50 g of pretreated substrate was mixed with 3 plates of microorganism in 1000 mL of culture media. The weight ratio of pretreated substrate and culture media was 1:20 (g:ml) in the 2000 ml

Erlenmeyer flask. The flask was closed by the cotton and stirred at room temperature for 2 days. Then the mixture was filter by vacuum filtration and used centrifuge to separate the solid from the liquid before membrane filtration.

For membrane filtration experiments, 1000 ml of the filtrated hydrolysate was filled in the feed tank. The peristaltic pump was set at the 30 rpm and maintained the pressure drop at 5 psig. The time was recorded when the volume of the feed tank reduced 25 ml. When the feed solution reduced every 100 ml, the samples were collected at retentate and permeate streams for calculation of the rejection and flux of the system. Repeat the experiments at the peristaltic pump speed 60 rpm and 90 rpm. Pure water fluxes were measured before and after the filtration of the hydrolysate.

Table 1 Culture media composition

Composition	Media
$CaHPO_4$ (g)	1
$MgSO_4.7H_2O$ (g)	1
urea (46%$(NH_4)_2SO_4$) (g)	8
Phosphate (NPK-0-52-34) (g)	15
Water (ml)	100
pH	5

Reference: Siwarasak et al. 2012

Results and discussion

Hydrolysate from sugarcane bagasse

Figure 2 shows the relationship between the permeate flux and volume of the feed at different flow rates. The permeate flux is very high at the first stage because the membrane is clean at the beginning so the molecules flow through the membrane easily. The higher flow rate has the higher flux compare to the lower one because the pore is free so the molecules with higher flow rate pass the membrane more than the lower flow rate. After a period of time, the permeate flux reduces significantly because there are some molecules blocked the membrane so the molecules cannot pass the membrane. This phenomenon is quite common in membrane filtration process. It is call membrane fouling [4].

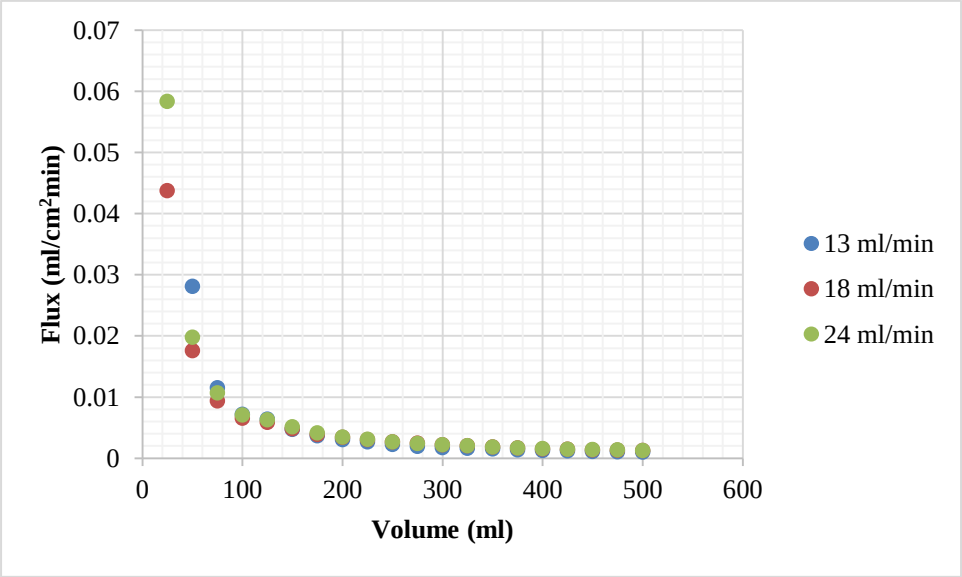

Figure 2 The relationship between flux and volume at various flow rate

The amount of reducing sugar, cellulose activity, and microorganism concentration in the feed, retentate, permeate and percentage of rejection after the separation by ultrafiltration membrane are shown in Table 2. For reducing sugar concentration, the higher one shows in the retentate stream when compare with the permeate stream at every flow rate but the rejection percentage at the flow rate of 13 ml/min shows the lowest value. For cellulase activity and microorganism cell concentration, the results show different trend. At 13 ml/min, there is the highest percentage of rejection. Therefore, the ultrafiltration membrane is suitable for separation of reducing sugar and enzyme cellulase.

Table 2 Reducing sugar concentration, cellulose activity, and microorganism concentration after ultrafiltration at different flow rates

Flow rate (ml/min)	Reducing sugar (g/l)			Rejection (%)
	Feed	Retentate	Permeate	
13	33.05	38.51	26.38	31.50
18	34.20	38.88	25.55	34.28
24	35.29	42.32	26.88	36.48
	Cellulase activity (g/l)			Rejection (%)
	Feed	Retentate	Permeate	
13	17.42	19.18	14.02	26.90
18	17.52	19.37	15.53	19.82
24	22.90	20.25	15.03	25.78
	Microorganism concentration (cell/ml)			Rejection (%)
	Feed	Retentate	Permeate	
13	1.8×10^7	1.4×10^7	3.7×10^6	73.68
18	1.7×10^7	1.3×10^7	3.7×10^6	71.70
24	8.8×10^6	6.3×10^6	2.4×10^6	60.90

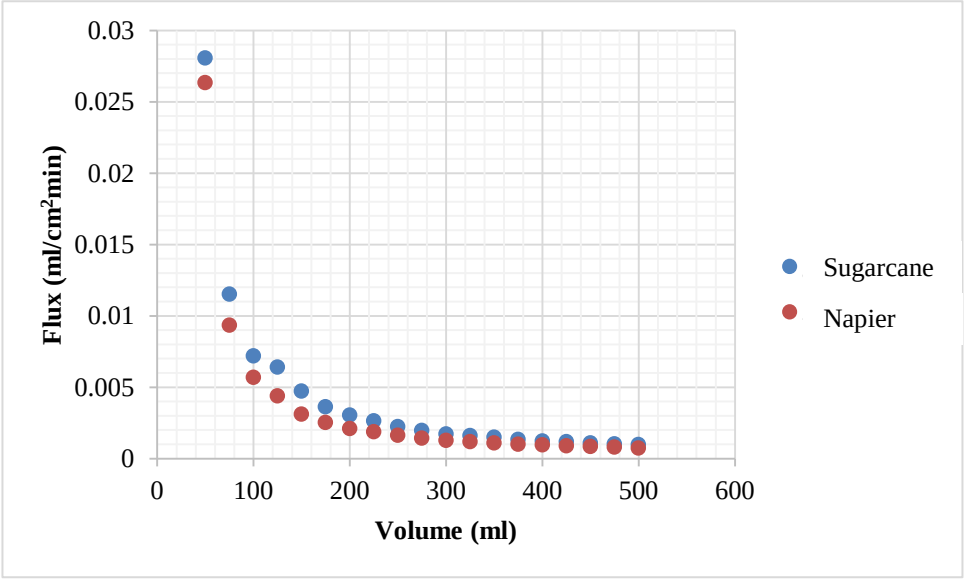

Figure 3 The relationship between flux and volume of sugarcane bagasse and Napeir grass

Hydrolysate from Napier grass

Figure 3 and Table 3 are the results of flux and percentage of rejection of hydrolysate from Napier grass compare with hydrolysate from sugarcane bagasse. As shown in Figure 3, the permeate flux of Napier grass hydrolysate is lower than sugarcane bagasse hydrolysate. The reason for the limitation of the membrane process due to the decrease of transmembrane flux is known as concentration polarization [8]. For separation of reducing sugar and enzyme cellulase in the Napier grass hydrolysate, the results in Table 3 show the different trend because the rejection percentage quite similar between reducing sugar and microorganism concentration. This means the ultrafiltration membrane cannot separate between reducing sugar and microorganism.

Table 3 Rejection percentage of Napier grass hydrolysate at optimal condition compare with sugarcane bagasse hydrolysate

Flow rate (ml/min)	Rejection (%)		
	Reducing sugar (g/l)	Cellulase activity (g/l)	Microorganism concentration (cell/ml)
Napier grass	63.10	43.83	60.61
Sugarcane bagasse	31.05	26.90	73.68

Conclusion

This study was conducted to find possible method to recycle enzyme and enhanced the concentration of reducing sugar. It was found that the suitable condition for separation of reducing sugar is 13 ml/min. The rejection percentages are 31.50%, 26.90%, and 73.68% for reducing sugar, cellulase activity, and microorganism concentration respectively.

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