

Microwave radiation for butanol production biology essay

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UniversityKeywords : Butanol/ Corncobs/ Fermentation/ Microwave Radiation/
Pretreatment

ABSTRACT

Microwave-based chemical pretreatment of corncobs was investigated to produce butanol. Corncobs were pretreated via two-stage pretreatment with NaOH 2 % at 120 °C for 30 min and a solid-to-liquid ratio (SLR) of 67: 1 followed by the second stage pretreatment of H₂SO₄ 1 % at 156 °C for 16 min and SLR of 106: 1 under microwave radiation. The second stage was optimized by using response surface methodology (RSM). Pretreated corncobs were subjected to enzymatic hydrolysis to produce a reducing sugar. The objective of this work was to study the effect of fermentation techniques and time on the Acetone-Butanol-Ethanol (ABE) yield. It was found that overliming and dilution enhance the fermentation of sugars derived by enzymatic hydrolysis. *Clostridium beijerinckii* TISTR1461 can produce ABE yield of 41 % and productivity of 17. 56 g/l•h.

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INTRODUCTION

In the last few years, The high prices of crude oil and increasing concerns over greenhouse effect have renewed the interests in butanol production as an alternative liquid fuel. Butanol can be produced via Acetone-Butanol-

Ethanol or ABE fermentation. The substrate is a significant consideration affecting the butanol production cost (Qureshi and Blaschek, 2000). Lignocellulosic biomass has great potential renewable source as a substrate due to abundant and low cost to produce biobutanol by *Clostridium beijerinckii*. Corncobs are one of agricultural waste in Thailand and it is a dairy industry by-product and can be used as a substrate to produce biobutanol. However, the complex crystalline structure of lignocellulosic biomass is difficult to hydrolyze into reducing sugar. Therefore, pretreatment is required to alter the structure of lignocellulosic biomass to make cellulose more accessible to the enzyme and increase total sugar that used for biobutanol production. Microwave is an alternative method to improve the pretreatment efficiency due to its high heating efficiency, easy operation and easily to combine with chemical reaction. Therefore, the combination of microwave heating and chemical methods was proposed to pretreat lignocellulosic biomass, and improved total sugar in the following enzymatic hydrolysis (Zhu et al., 2006). Since inhibitors from pretreatment process can be problematic for ABE fermentation, the removal of inhibitors from hydrolysates is necessary to enhance microbial growth and fermentation efficiency. The objective of this work was to investigate the optimal condition of second stage of two-stage pretreatment of corncobs by using response surface methodology and study the effect of fermentation techniques and fermentation time on the ABE yield.

EXPERIMENTAL

First Pretreatment of Corn Cobs by Microwave/NaOH in first stage Dried corncobs was pretreated with NaOH 2 % (w/v) using a solid-to-liquid ratio (SLR) 67: 1, g of corncobs: L of solution and then transferred to a microwave oven to treat corncobs at 100 °C for 30 min. After this process was completed, the solid residues were thoroughly washed with tap water to neutral pH and dried in the oven at 105 °C for 24 h (Chen et al., 2011).

Pretreatment of Corncobs by Microwave/H₂SO₄ in second stage The solid residues from the first stage pretreatment were pretreated with H₂SO₄ 1 % using different SLR (25: 1 to 125: 1). Then, it was transferred to a microwave oven in the temperature range of 80 °C to 160 °C for 5 to 25 min. C.

Enzymatic Hydrolysis The mixture from two-stage pretreatment was adjusted to pH 4.8 by NaOH, then Celluclast 160 µl/g pretreated corncobs (cellulase; Sigma Chemicals, 52 FPU) was added. After that the mixture was shaken at 50 °C for 60 h in the incubator shaker and the solid residues were separated. The liquid fraction was collected for sugar analysis. D. Sugars

Analysis Glucose, xylose, and arabinose were determined using an HPLC system equipped with a refractive index detector (Model 6040 XR, Spectra-Physics, USA). An HPLC column (Aminex HPX-87H column, Bio-Rad Lab, USA) was used with 0.005 M sulfuric acid solution as a mobile phase. The flow rate was controlled at 0.6 ml/min and the column temperature was 60 °C. E.

Overliming Process The hydrolysate from enzymatic hydrolysis was adjusted to pH 10 with Ca(OH)₂ and was added Na₂SO₃ 1 g/l. After that the mixture was heated at 90 °C for 30 min, and was cooled to room temperature. The mixture was filtered to separate solid residues out. F. Medium Preparation To

prepare the Difco™ Cooked meat medium (CMM). The mixture of CMM pellet 0.875 g, glucose 0.12 g, and distilled water 6 ml was sterilized at 121 °C for 15 min and cooled to room temperature. After that, one loop of cell spores was put into the prepared solution and heat shock at 80 °C for 2 min. The CMM culture solution was kept in 37 °C and waited for cells activation within 30 h. G. Inoculum Development Yeast extract 100 µl, 0.5 of active growing cells from CMM solution, buffer (KH₂PO₄, K₂HPO₄, and CH₃COONH₄), mineral (MgSO₄•7H₂O, MnSO₄•H₂O, FeSO₄•7H₂O, NaCl), and vitamins (para-amino-benzoic acid, thiamin, biotin) was added in 9.3 ml of hydrolysate. H. Acetone-Butanol-Ethanol (ABE) Fermentation The liquid fraction which was taken from enzymatic hydrolysis or overliming was adjusted to pH 6.6 by H₂SO₄ 98 % and 10 ml of hydrolysate was ferment by using *Clostridium beijerinckii* TISTR1461 at 37 °C for 0 to 72 h. Acetone-Butanol-Ethanol Analysis Acetone, Butanol, and ethanol were measured by a gas chromatograph (Series Perichrome) equipped with a flame ionization detector using Innowax column length 30 m. The flowrate of N₂ was 45 ml/min, detector temperature 240 °C, injection temperature 240 °C, column temperature 170 °C, and volume injection 0.5 µl. J. Acetone-Butanol-Ethanol Fermentation Techniques There are 7 fermentation experiments using 10 ml hydrolysate as a substrate at 37 °C, as shown in Table 1. Table 2 ABE fermentation techniques

Description
C2-stage pretreatment without dilution and overliming
CO2-stage pretreatment + Overliming
D22-stage pretreatment + Diluted 2 times
D2O2-stage pretreatment + Overliming + Diluted 2 times
D42-stage pretreatment +

Diluted 4 times D4O2-stage pretreatment + Overliming + Diluted 4 times W Microwave/NaOH followed by water pretreatment

RESULTS AND DISCUSSION

A. Optimization of the Glucose Concentration using Response Surface Methodology (RSM) RSM with a central composite design (CCD) was conducted to examine the effect of temperature, time, and SLR of second stage pretreatment on glucose concentration. The three-dimensional response surface for the concentration of glucose are shown in Figure 1. The optimal condition of second stage pretreatment for glucose concentration were determined at 156 °C, 16 min, and 106 SLR. The maximum glucose concentration from the confirmation experiment was 48.58 g/l. The polynomial equation describes the glucose concentration of second stage pretreatment (Y1) as a function of temperature, time, and SLR is shown in the equation below: $Y1 = 39.05 + 2.9625x1 - 0.2131x2 + 6.43x3 - 1.2292x1^2 - 1.9723x2^2 - 3.2792x3^2 + 0.93x1x2 + x1x3 - 0.5075x2x3$ (A)

(B) (C) Figure 1 Response surface for glucose concentration: effects of temperature and time (A), temperature and SLR (B), and time and SLR (C). B.

Effect of Fermentation Techniques on Acetone-Butanol-Ethanol (ABE)

yield After two-stage pretreatment and enzymatic hydrolysis, the liquid fraction was sent to the fermentation step to produce Acetone-Butanol-Ethanol (ABE) using *Clostridium beijerinckii*. The result showed that ABE concentration increased rapidly until 48 h of fermentation time before slightly decreased until to 72 h. The highest ABE concentration was obtained at 48 h, as shown in Figure 2. Figure 2 The effect of fermentation time on

ABE concentration. In addition, the results revealed that the overlimed hydrolysate conditioned gave ABE yield higher than non overlimed hydrolysate, as shown in Figure 3. Because the inhibitors was eliminated such as ferulic, and p-coumaric acids that are inhibit microorganism by damaging the hydrophobic sites of the bacteria cells because ferulic and coumaric acids are phenolic acids and phenolic compounds that affect membrane permeability (Heipieper et al., 1994). As a result, overliming treatment can significantly improve the ABE concentration. Therefore, The removal of inhibitors prior to fermentation is essential for successful ABE fermentation. Furthermore, the higher initial sugar concentrations cannot be consumed due to butanol toxicity (Qureshi and Maddox, 2005). Therefore, the dilution is the necessary step to increase ABE yield in fermentation. The results also showed that, the dilution hydrolysate gave higher ABE yield than that of no dilution. And the 4 times dilution gave the highest ABE yield. In addition, the ABE yield from D4O technique was higher than that P2 medium batch fermentation that contained 0. 35 ABE yield. It implied that pretreated corncobs can be used as a carbon source in ABE fermentation. Moreover, it can reduce the cost of the ABE process by using corncobs instead of food crops such as corn, cassava and sugarcane that have a high price and caused food price to go up due to the high demand of food crops. The optimal ABE fermentation condition was obtained with the diluted 4 times hydrolysate combined with overliming (D4O) techniques at 48 h that gave the highest ABE yield of 0. 41 and productivity of 17. 56 g/l•h. It can be concluded that overliming and dilution enhanced the ABE yield. ABE yield was calculated as total ABE produced divided by the total sugar utilized and

productivity was calculated as total ABE produced divided by fermentation time. Figure 3 The effect of fermentation techniques on ABE yield at 37 °C at 48 h.

CONCLUSIONS

The results implied that the two-stage pretreatment combined with microwave radiation on corncobs was effective method to enhance enzymatic hydrolysis accessibility by removing lignin and hemicellulose. The optimum conditions were found at 2 % of NaOH at 100 °C for 30 min, and SLR 67 in first stage followed by 1 % of H₂SO₄ at 156 °C for 16 min, and SLR 106 . lignin and hemicellulose. And the highest glucose concentration can reach up to 48. 58 g/l. For the ABE fermentation process, the optimum technique that could be used was the diluted 4 times hydrolysate combined with overliming (D4O) technique at 37 °C for 48 h. That gave the highest ABE yield of 0. 41, productivity of 17. 56 g/l•h. It can conclude that the dilution and overliming process can reduce fermentation inhibitors, increase cell growth and improve ABE yield.

ACKNOWLEDGEMENTS

The authors are thankful to the National Research University Project of CHE and the Ratchadaphiseksomphot Endowment Fund (EN269B-55) and Center of Excellence on Petrochemical, and Materials Technology, Chulalongkorn University, Thailand. The authors would also like to thank the B&C Pulaski Corporation Limited for supplying corncobs. Their contribution to this thesis work is highly appreciated.