Studies on efficient acetone-butanol-ethanol fermentation from lignocellulosic biomass

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論文題名　：Studies on efficient acetone-butanol-ethanol fermentation from lignocellulosic biomass（リグノセルロース系バイオマスからの効率的なアセトン-ブタノール-エタノール発酵に関する研究）

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論　文　内　容　の　要　旨

n the butanol production from acetone-butanol-ethanol (ABE) fermentation in terms of high cost of substrate,

Recently, butanol has attracted interesting attention as an alternative biofuel. However, there are some drawbacks i low butanol concentration and productivity, and product inhibition. To decrease substrate cost, alternatives derived from lignocellulose-based feedstock can be efficiently used for ABE fermentation. In addition, improvement of continuous fermentation process can also overcome some of above drawbacks. Therefore, the objective of this study is to establish a highly productive fermentation system and to achieve efficient utilization of lignocellulose-derived feedstocks for butanol production.

Continuous culture with high cell density is an advanced technique to avoid culture instability, strain degeneration, cell wash out and metabolic oscillations. Because xylose is the second most abundant sugar in lignocellulosic hydrolysate after glucose, a continuous butanol production system by cell recycling *Clostridium saccharoperbutylacetonicum* N1-4 was established to examine the characteristics of butanol fermentation from xylose. Compared with conventional continuous culture, a dry cell weight of 17.4 g L^{-1} was obtained by cell recycling, which led to a 2-fold increase in xylose consumption, butanol concentration and productivity. Finally, the maximum butanol productivity of 3.32 g L^{-1} h^{-1} was obtained at a dilution rate of 0.78 h^{-1}.

Lignocellulosic hydrolysate has complex composition beside sugars, so the efficient ABE fermentation from eucalyptus was investigated. Firstly, feasibility of ABE fermentation from eucalyptus hydrolysate without nutrients supplementation was researched. Increasing solid concentration of steam-exploded eucalyptus from 6.7% (w-dry matter v^{-1}) to 25% led to an increment of initial glucose concentration in the hydrolysate. However, ABE production from obtained hydrolysate decreased when solid concentration was over 10%. Additionally, although cellulase loading of 35 FPU g^{-1} (dry eucalyptus) was optimized as the minimum dosage for the efficient glucose conversion from cellulose, ABE fermentation was feasible only if the cellulase loading was over 35 FPU g^{-1}. The maximum ABE of 12.3 g L^{-1} was obtained when solid concentration was 10% with cellulase loading of 175 FPU g^{-1}.

Subsequently, the strategies for the improvement of ABE fermentation from eucalyptus hydrolysate were developed. The capability of eucalyptus hydrolysate for ABE fermentation was found to be improved by diluting the hydrolysate, which decreased the inhibition by substrate and fermentation inhibitors (5-hydroxymethyl furfural and furfural), but incomplete glucose utilization was observed. In order to enhance glucose consumption, TY medium was supplemented to eucalyptus hydrolysate (solid concentration of 10%; cellulase loading was 175 FPU g^{-1}) without dilution. After TY medium supplementation, glucose consumption and ABE production were enhanced by 34.9% and 30.6% respectively. It also elucidated that nitrogen source in the hydrolysate is one of the most important parameters to improve cell growth and ABE

fermentation. In addition, when eucalyptus hydrolysate of minimum cellulase loading of 35 FPU g^{-1} was supplemented with TY medium, ABE concentration of 15.9 g L^{-1} was achieved with the complete sugar consumption.

Finally, in order to achieve high ABE production with low cellulase loading (35 FPU g^{-1}) and minimum nutrients supplementation, nutrients in TY medium including three kinds of nitrogen source were optimized by statistical experimental designs. $FeSO_4 \cdot 7H_2O$, tryptone, and yeast extract were screened by Plackett-Burman design as the significant components. Moreover, the optimized medium composition (15.3 mg L^{-1} $FeSO_4 \cdot 7H_2O$; 7.64 g L^{-1} tryptone; 3.04 g L^{-1} yeast extract) for the maximum ABE production (17.0 g L^{-1}) was predicted by Box-Behnken design. Compared with TY medium, Glucose consumption and ABE production rate were accelerated by supplementing the optimized medium. Finally, high ABE production of 16.9 g L^{-1} with lower utilization of cellulase, complete sugar consumption, and minimum nutrients supplementation was achieved by statistical experimental designs.

In conclusion, a highly productive continuous culture system for butanol production using xylose was established, and high ABE production makes eucalyptus a potential feedstock for butanol production.