목질계 당화액을 이용한 숙신산의 생물학적 생산

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Biological conversion of wood hydrolysate to succinic acid by *Mannheimia succiniciproducens* MBEL55E

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Introduction

Succinic acid is dicarboxlic acid produced as an intermediate of the tricarboxlic acid cycle (Gottschalk, 1986) and many anaerobic microorganisms produce succinic acid as the major end-product of their energy matabolism (Zeikus et al., 1999). Although frementative production of succinic acid has several advantages over chemical process, the industrial scale production of succinic acid requires reduction in its production cost (Lee et al., 2000a). Wood hydrolysate has been regarded as one of the most inexpensive carbon source for fermentative production of biochemicals (Lynd et al, 1999).

In this studies, it is evaluated that economical production of succinic acid from wood hydrolysate by *Mannheimia succiniciproducens* MBEL55E. We carried out batch fermentation in the wood hydrolysate based media to investigate the time profile of growth and end product formation.

Materials and Methods

Preparation of wood hydrolysate

Chips (2 × 4 mm) of oak wood were prepared by a chipper designed and built at the Korea Institute of Energy Research. The composition of oak wood was found to be as follows: cellulose (49.3%); hemicellulose (25.9%); Klasson lignin (21.7%). Steam explosion of the oak wood chips was conducted at 215℃ for 6 minutes in an 8-L exploder. Residues obtained after explosion were washed and enzymatically hydrolyzed with Celluclast (Novo Co., Denmark) and Novozyme (Novo Co.) in a reactor having 500 L working volume at 50℃ for 3 days. The concentration of glucose and xylose in the wood hydrolysate was found to be 75 g/L and 20 g/L after these treatments.

Microorganism and growth conditions

Mannheimia succiniciproducens MBEL55E (KCTC 0769BP) is maintained at Department of Chemical and Biomolecular Engineering, KAIST (Taejon, South Korea). Cells were anaerobically grown in sealed anaerobic bottles (100 mL) under CO_2 atmosphere. The medium for seed culture contained per liter: 5 g glucose, 2.5 g polypeptone, 2.5 g yeast extract, 3 g K_2HPO_4, 1 g NaCl, 1 g $(NH_4)_2SO_4$, 0.2 g $CaCl_2{\cdot}2H_2O$, 0.2 g $MgCl_2{\cdot}6H_2O$, and 3 g $MgCO_3$. The medium was heat sterilized (15 min at 121℃) in the anaerobic bottles under

a nitrogen atmosphere. Concentrated H_2SO_4 was then added to the sterile medium to adjust the pH to 6.5. The nitrogen headspace was replaced by CO_2, and $Na_2S{\cdot}9H_2O$ was added to a final concentration of 1 mg/L to ensure anaerobic conditions. After 15 min, the reduced medium was inoculated with 1.5 mL of glycerol stock culture and incubated at 39℃ for 12 h.

1 L of batch cultures were carried out at 39℃ in a jar fermentor (2.5 L, Korea Fermentor Company, Incheon, Korea). The medium for batch culture contained wood hydrolysate containing 20 g or 16 g glucose as a carbon source. 5 g of yeast extract (Difco, Detroit) or 10 g of corn steep liquor were used as a nitrogen source. The pH was controlled at 6.5 using 5 N NaOH and 7 g/L of Na_2CO_3 was added for a high level of CO_2. Foaming was controlled by the addition of Antifoam 289 (Sigma Chemical Co.). The CO_2 gas sparging rate and agitation speed were controlled at 0.25 vvm and 200 rpm, respectively. All chemicals used were of reagent grade and were obtained from either Junsei Chemical Co. (Tokyo, Japan) or Sigma Chemical Co. The gas was scrubbed free of oxygen by passing it through a gas purifier (P.J. Cobert Associates, Inc., St. Louis, MO).

Analytical methods

The concentrations of glucose, succinic acid and acetic acid were measured by high-performance liquid chromatography (Hitachi L-3300 RI monitor, L-4200 UV-VIS detector, D2500 chromato-integrator, Tokyo, Japan) equipped with an ion exchange column (Aminex HPX-87H, 300 mm x 7.8 mm, Hercules, CA) using 0.012 N H_2SO_4 as a mobile phase. Cell growth was monitored by measuring the absorbance at 660 nm (OD_{660}) using a spectrophotometer (Ultrospec3000, Pharmacia Biotech, Sweden). Succinic acid yield was defined as the amount of succinic acid produced from one gram glucose, and was expressed as a percentage.

Results and discussion

Fermentations using wood hydrolysate based medium

Batch fermentation were carried out in the wood hydrolysate based media to investigate the time profile of growth and end-product formation. When the wood hydrolysate based media containing 23 g/L glucose and 5 g/L yeast extract were used, 20 g/L of glucose and 7.4 g/L of xylose were consumed in 28 h of culture. Final succinic acid concentration is 16.26 g/L and a succinic acid yield of 59% and a succinic acid/lactic acid (S/L) ratio of 3.89:1 were obtained at the end of fermentation (Table 1). When wood hydrolysate was used as a carbon source, the fermentation results obtained were similar to those obtained when glucose-based media.

Although wood hydrolysate can be used as an inexpensive carbon source, the use of relatively expensive yeast extract increases the medium cost. Corn steep liquor is a relatively inexpensive nitrogen source and can be used as an alternative to yeast extract for succinic acid prodcution (Lee et al., 2000b). When the wood hydrolysate based media containing 21 g/L glucose and 10 g/L corn steep liquor were used, glucose was completely consumed in 29 h of culture (Figure 1). Final succinic acid concentration is 12.63 g/L and a succinic acid yield of 56% and a S/L ratio of 3.26:1 were obtained at the end of fermentation (Table 1). These results show that corn steep liquor has comparable effectiveness in succinic acid

production.

Fermentations using wood hydrolysate based medium treated with NaOH

The wood hydrolysate based media was treated with NaOH before sterilization with the intention of reducing the formation of solid compounds during sterilization and batch fermentation was carried out. After sterilization, glucose concentration of wood hydrolysate base media decreased from 20 g/L to 16 g/L due to dilution effect of NaOH and chemical reactions.

When the wood hydrolysate based media containing 16 g/L glucose and 5 g/L yeast extract were used, glucose was completely consumed and 5 g/L of xylose was consumed in 12 h of culture (Figure 2). Final succinic acid concentration was 11.53 g/L and a succinic acid yield of 55% and a S/L ratio of 3.25:1 were obtained at the end of fermentation (Table 1). When the pre-treated wood hydrolysate base media was used as a carbon source, the fermentation results (succinic acid yield, productibity, and S/L ratio) obtained were higher than those obtained when the wood hydrolysate base media. When the wood hydrolysate based media containing 13 g/L glucose and 10 g/L corn steep liquor were used, final succinic acid concentration was 9.00 g/L and a succinic acid yield of 46% and a S/L ratio of 1.23:1 were obtained (Table 1). These results show that when corn steep liquor is used as a nitrogen source, we need not the pre-treatment of the wood hydrolysate base media.

Acknowledgements

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Table 1. Batch fermentations (jar fermenter containing 1 L media) of *Mannheimia succiniciproducens* MBEL55E in various media.

	1 L batch culture					
	Glucose 20g/L		WH containing glucose 20 g/L		WH containing glucose 20 g/L (pretreated)	
	YE 5 g/L	CSL 10 g/L	YE 5 g/L	CSL 10 g/L	YE 5 g/L	CSL 10 g/L
Yield (g succinic acid/ g glucose)	0.64	0.58	0.59	0.56	0.55	0.46
Productivity (g/L/h)	1.40	0.75	0.93	0.60	1.15	0.78
Final succinic acid (g/L)	14.08	10.05	16.26	12.63	11.53	9.00
Succinic acid / Lactic acid (g/g)	-	2.56	3.89	3.26	3.25	1.23

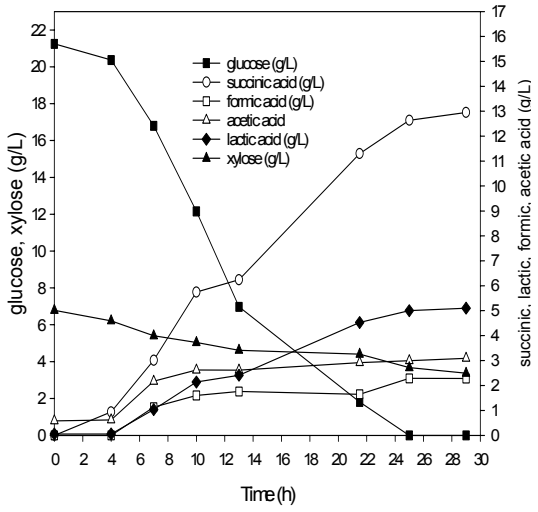

Figure 1. Batch fermentations (jar fermenter containing 1 L media) using wood hydrolysate (WH)- based medium supplemented with corn steep liquor. WH-based medium containing 20 g/L glucose was used for *Mannheimia succiniciproducens* MBEL55E.

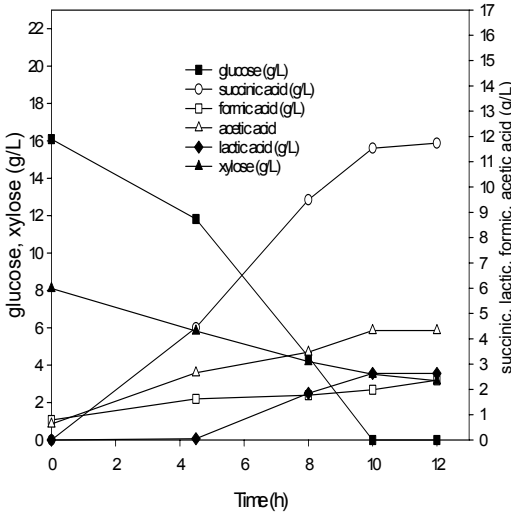

Figure 2. Batch fermentations (jar fermenter containing 1 L media) using wood hydrolysate (WH)-based medium supplemented with yeast extract. WH-based media was treated with NaOH before sterilization. WH-based medium containing 16 g/L glucose was used for *Mannheimia succiniciproducens* MBEL55E.