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Fermentative Production of Succinic Acid from Glucose and Corn Steep Liquor by *Anaerobiospirillum succiniciproducens*

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Abstract *Anaerobiospirillum succiniciproducens* requires expensive complex nitrogen sources such as yeast extract and polypeptone for its growth and succinic acid production. It was found that *A. succiniciproducens* was able to grow in a minimal medium containing glucose when supplemented with corn steep liquor (CSL) as the sole complex nitrogen source. The concentration of CSL had a significant effect on the glucose consumption by *A. succiniciproducens*. When 10-15 g/L of CSL was supplemented, cells were grown to an OD_{660} of 3.5 and produced 17.8 g/L succinic acid with 20 g/L glucose. These results are similar to those obtained by supplementing yeast extract and polypeptone, thereby suggesting that succinic acid can be produced more economically using glucose and CSL.

Keywords: succinic acid, corn steep liquor, fermentation, *Anaerobiospirillum succiniciproducens*

Recently, succinic acid has received a great deal of attention as a green feedstock for the manufacture of synthetic resins, biodegradable polymers, and chemical intermediates [1,2]. To date, succinic acid has mostly been produced by chemical processes. However, in the past few years, the fermentative production of succinic acid from glucose by anaerobic bacteria has attracted great interest [3]. The fermentative production of succinic acid can be regarded as a green technology not only because renewable substrates are used for its production, but also because CO_2, a greenhouse gas, is incorporated into succinic acid during the fermentation. The anaerobic bacterium *Anaerobiospirillum succiniciproducens* has most often been employed because it can produce a significant amount of succinic acid from glucose [4,5].

Although the fermentative production of succinic acid has several advantages over chemical processes, commercial scale production of fermentative succinic acid still requires a reduction in the production cost. *A. succiniciproducens* requires yeast extract and polypeptone for its growth and the production of succinic acid [5]. One possible way to reduce the cost of the fermentation process is to use cheap complex nitrogen sources instead of expensive yeast extract and polypeptone. Accordingly, this article reports on the economical production of succinic acid by *A. succiniciproducens* from glucose using inexpensive corn steep liquor as the complex nitrogen source.

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Anaerobiospirillum succiniciproducens (ATCC 29305) was obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were grown in sealed anaerobic bottles containing 100 mL of minimal salts medium 1 (AnS1) plus 5 g/L glucose, 2.5 g/L polypeptone, and 2.5 g/L yeast extract with CO_2 as the gas phase. The AnS1 medium contained 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 1 g Na_2CO_3 per liter. The medium was heat sterilized (15 min at 121°C) in the anaerobic bottles with a nitrogen headspace. 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 strict anaerobic conditions. After 15 min, the reduced medium was inoculated with 2.5 mL of a glycerol stock culture and incubated at 39°C for 24 h.

For the flask experiments, exponentially growing cultures, washed anaerobically with a glucose-deficient medium, were used to inoculate sealed anaerobic bottles containing 100 mL of minimal salts medium 2 (AnS2). The AnS2 medium contained 3 g K_2HPO_4, 1 g NaCl, 5 g $(NH_4)_2SO_4$, 0.2 g $CaCl_2 \cdot 2H_2O$, 0.4 g $MgCl_2 \cdot 6H_2O$, 5 mg $FeSO_4 \cdot 7H_2O$, and 10 or 20 g Na_2CO_3 per liter. Glucose and complex nitrogen sources [yeast extract, polypeptone, or corn steep liquor (CSL)] were then added to the AnS2 medium at varying concentrations as indicated in the Results and Discussion section. The cells were grown anaerobically at 39°C.

Batch cultures were carried out at 39°C in a jar fermentor (2.5 L, Korea Fermentor Company, Inchon, Korea) containing 1 L of the AnS2 medium containing glucose as the carbon source supplemented with complex nitrogen sources [polypeptone (Difco Laboratories,

Table 1. Comparison of fermentation performances between yeast extract and/or polypeptone and corn steep liquor

	Glucose (20 g/L)													
	PP [a] (g/L)			YE [b] (g/L)			PP + YE (g/L)			CSL [c] (g/L)				
	2.5	5	7	2.5	5	7	2.5+2.5	5+2.5	5+5	5	7.5	10	15	30
Initial Glucose Concentration (g/L)	20.4	19	19.4	20	19.7	20	20.1	19.3	20	19.5	19.5	20.2	20	20.4
Final Glucose Concentration (g/L)	3.9	2	0	1.9	0.6	0	0	0	0	4	2	0	0	3
Succinic Acid (g/L)	14.3	14.8	17.2	16.3	17.1	18.1	18	17.3	18.2	13.5	15	18	17.6	15.4
Acetic Acid (g/L)	3.6	3.5	4.3	3.9	4.2	4.4	4.3	4.2	4.4	3.3	3.8	4.5	4.4	3.8
Succinic Acid Yield (%, g/g)	87	87	89	90	90	90	90	91	91	87	86	89	88	89
S/A[d] (g/g)	4.0:1	4.2:1.	4.0:1	4.2:1	4.1:1	4.1:1	4.2:1	4.1:1	4.1:1	4.1:1	4.0:1	4.0:1	4.0:1	3.9:1

[a] Polypeptone, [b] Yeast extract, [c] Corn steep liquor, [d] Ratio of succinic acid to acetic acid

Detroit, MI, USA), yeast extract (Difco Laboratories), or CSL (Sigma Chemical Co., St. Louis, MO, USA)] as in the Results and Discussion section. The pH was controlled at 6.5 using 2 M Na_2CO_3. Foaming was controlled by adding 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 Junsei Chemical Co. (Tokyo, Japan), Difco Labora-tories (Detroit, MI, USA) or Sigma Chemical Co. Gas was scrubbed free of oxygen by being passed through a gas purifier (P. J. Cobert Associates, Inc., St. Louis, MO, USA).

The concentrations of glucose, succinic acid, and acetic acid were measured by a high-performance liquid chromatograph (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 × 7.8 mm, Hercules, CA, USA) using 0.012 N H_2SO_4 as the mobile phase. The cell growth was monitored by measuring the absorbance at 660 nm (OD_{660}) using a spectrophotometer (Ultrospec 3000, Pharmacia Biotech, Sweden). The succinic acid yield was defined as the amount of succinic acid produced from one gram of glucose consumed, and was expressed as a percentage.

The medium cost is a key aspect in the fermentative production of bulk biochemicals and biofuels. Therefore, for the purpose of developing a cost-effective fermentation medium, several industrially available nutrient sources have been previously evaluated for their effectiveness in the production of ethanol [6] and lactic acid [7], and corn steep liquor (CSL) would appear to be one of the cheapest complex nitrogen sources available [8]. Although CSL contains various nutritional components, certain fastidious bacteria cannot grow on a medium containing only CSL due to a deficiency of essential components. *A. succiniciproducens* is also known to be a fastidious bacterium [9]. Until now, the combination of yeast extract and polypeptone has been used to support the growth of *A. succiniciproducens* and efficient production of succinic acid [5]. However, for the economical production of succinic acid from glucose, the feasibility of CSL as an alternative nitrogen source for the cultivation of *A. succiniciproducens* was examined. As shown in Table 1, the fermentation results including the succinic acid concentration, succinic acid yield, and ratio of succinic acid to acetic acid (S/A) obtained with CSL were similar to those obtained with yeast extract and polypeptone. Succinic acid yields of 86-89% and S/A ratios of 3.9:1-4.1:1 were obtained when using CSL as the complex nitrogen source. Accordingly, these results suggest that CSL can be used as a good alternative nitrogen source for *A. succiniciproducens* when glucose is used as a carbon source.

The glucose consumption by *A. succiniciproducens* was found to be dependent on the concentration of CSL because the glucose was not completely consumed when the concentration of CSL was below 7.5 g/L. In addition, excessive amounts of CSL (30 g/L) had an adverse effect on the glucose consumption and succinic acid production (Table 1). This result suggests that the concentration of CSL should be optimized for the complete consumption of glucose. Therefore, a batch culture of *A. succiniciproducens* was carried out in a medium containing an optimal concentration of CSL (10 g/L). The glucose was completely consumed after 13.5 h and the cells grew to an OD_{660} of 3.5 (Fig. 1). The final succinic acid concentration obtained was 17.8 g/L. The succinic acid yield of 89% and S/A ratio of 4.0:1 obtained at the end of the fermentation are similar

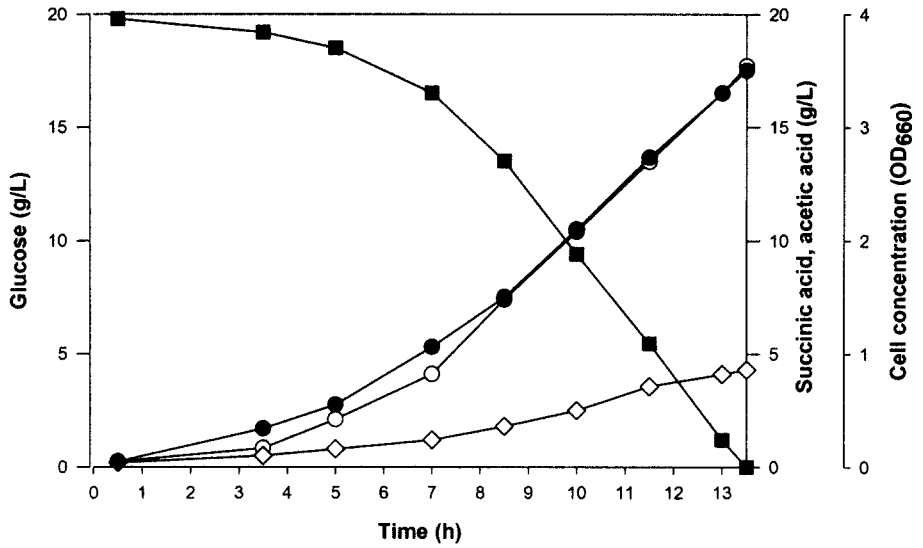

Fig. 1. Batch fermentation using a glucose-based medium supplemented with corn steep liquor (CSL). Glucose-based medium containing 20 g/L of glucose and 10 g/L of CSL was used for *A. succiniciproducens*. Symbols are cell concentration (●), glucose (■), succinic acid (○), and acetic acid (◇).

to those obtained in a glucose-based medium supplemented with yeast extract and polypeptone [4]. Therefore, the utilization of CSL can make the fermentation process for succinic acid production more economical.

In conclusion, this study shows that a low cost medium consisting of glucose and corn steep liquor can be used as an alternative to the expensive yeast extract and polypeptone in the fermentative production of succinic acid.

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