Biological conversion of biomass to succinic acid

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Abstract

Batch cultivations of *Anaerobiospirillum succiniciproducens* have been systematically studied for the economical production of succinic acid from wood hydrolysate with corn steep liquor (CSL) as a nitrogen source. CSL was found to be an alternative complex nitrogen source for *A. succiniciproducens* when glucose and wood hydrolysate were used as carbon sources. Compared with polypeptone and/or yeast extract, CSL had similar effects on fermentation performance such as succinic acid yield and a ratio of succinic acid to acetic acid in the fermentation of wood hydrolysate as well as glucose. This means that succinic acid can be produced more economically from wood hydrolysate and CSL than relatively expensive carbon and nitrogen sources. Besides its low cost, the alternative medium served as a green technology for succinic acid production because it gives a net-zero effect on global warming.

Introduction

Currently, succinic acid receives a great deal of attention as a green feedstock because of its multiple functionalities: succinic acid can be used for the manufacture of synthetic resins and biodegradable polymers and as an intermediate for chemical synthesis[1)]. To date, succinic acid has mostly been produced by chemical processes. In the past few years, however, fermentative production of succinic acid from glucose by anaerobic bacteria has attracted great interest[2)]. Also, succinic acid fermentation has been recently regarded as a novel and green technology because CO_2, which is one of the greenhouse gases, is incorporated into succinic acid during the anaerobic fermentation of sugars. As a succinic acid-producing microorganism, an anaerobic bacterium *Anaerobiospirillum succiniciproducens* has been used because it can produce a significant amount of succinic acid from sugars[3-5)]. Although fermentative production of succinic acid has several advantages over chemical process-based one, the commercialization of the fermentative succinic acid should have an economical competition over the counterpart. One way to reduce the cost of the fermentation process is to use renewable carbons such as agricultural, diary waste products and other cellulosic biomass. Although these materials must be refined into their component sugar molecules before used as carbon sources, they are relatively inexpensive and abundant renewable material and CO_2 gas-capturer, which thus give a net-zero effect on global warming. Therefore, the use of the inexpensive substrates can make the fermentation process for succinic acid more competitive and environmentally friendly.

In this study, we report on the feasibility of an economical production of succinic acid from wood hydrolysate by *A. succiniciproducens*. We also attempted to find an effective method for the utilization of wood hydrolysate with corn steep liquor.

Materials and methods

Organism and growth conditions. *Anaerobiospirillum succiniciproducens* (ATCC 29305) was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in sealed anaerobic bottles containing 100 mL minimal salts medium1 (AnS1) containing 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 contains per liter, 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. The medium was heat sterilized (15 min at 121°C) in anaerobic bottle with nitrogen headspace. To the sterile medium, concentrated H_2SO_4 was added to adjust the pH to 6.5 ± 1. The nitrogen headspace was replaced by CO_2, and $Na_2S{\cdot}9H_2O$ (final concentration: 1 mg/L) was added to ensure strict anaerobic condition. After 15 min, the reduced medium was inoculated with 2.5 ml glycerol stock culture and incubated at 39 °C for 24 h. For the flask experiments, cells were grown at 39 °C in sealed anaerobic bottles containing 100 mL of a minimal salts medium2 (AnS2) containing per liter: 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 $MgCO_3$. To the AnS2 medium, glucose or wood hydrolysate and complex nitrogen sources [yeast extract, polypeptone, or cone steep liquor (CSL)] were added at the different concentrations. Batch cultures were carried out at 39 °C in a jar fermenter (2.5 L, Korea Fermenter Company, Incheon, Korea) containing 1 L of AnS2 containing glucose or wood hydrolysate as a carbon source supplemented with the complex nitrogen sources (polypeptone, yeast extract, or CSL). The pH was controlled at 6.5 using 2 M Na_2CO_3. Foam was controlled by adding Antifoam 289 (Sigma Chemical Co.). 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), Difco company or Sigma Chemical Co. Gas was scrubbed free of oxygen by passing through a gas purifier (P.J. Cobert Associates, Inc., St. Louis, MO).

Preparation of Wood hydrolysate. Chips of oak wood of size 2 X 4 mm were made by a chipper designed and built in our laboratory as the initial feed material. The composition of oak wood is as follows: cellulose (49.3%); hemicellulose (25.9%); Klasson lignin (21.7%). Steam explosion of the oak wood chips was conducted at 215 °C for 3 minutes in an 8-L exploder. The sugar yields obtained after steam explosion are 88% for glucan and 0.2% for xylose-maltose-galactose (XMG). However, the concentration of the individual sugars (xylose, maltose, galactose) was not detected. Five kg (dry weight) of residues obtained after explosion was washed and enzymatically hydrolyzed with Celluclast (Novo Co., Denmark) and Novozyme (Novo Co., Denmark) in a reactor with 30 L working volume (Korea Fermentor Co., Korea) at 50 °C for 3days. The enzyme loading was 20 IU/g-residue. The concentration of glucose in the wood hydrolysate was measured to be 28 g/L. Although other sugars were detected in the wood hydrolysate, they were not fermentable sugars.

Analytical methods. The concentrations of glucose, succinic acid and acetic acid were measured by high-performance liquid chromatography (Hitachi L-3300 RI monitor, 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.

Results and discussion

Batch cultivation of *A. succiniciproducens* has been systematically studied for the production of succinic acid from wood hydrolysate. It was possible to convert wood hydrolysate into succinic acid with a high efficiency. Corn steep liquor (CSL) contains a lot of nutrients to microorganisms so that some microorganisms can grow on the medium supplemented with CSL as a sole nitrogen source. *A. succiniciproducens* can grow the CSL-containing medium supplemented with glucose as a carbon source (Fig. 1). In addition, CSL have similar effects on fermentation performances such as succinic acid yield and a ratio of succinic acid to acetic acid compared with polypeptone and/or yeast extract when glucose was used (Table 1). It was known that wood hydrolysate contains fermentable sugar and nitrogen source so that nonfastidious microorganism such as yeast can grow on the only wood hydrolysate-containing medium. However, this was not true for *A. succiniciproducens*, which did not grow in a medium containing wood hydrolysate without additional complex nitrogen sources. However, when the wood hydrolysate-containing media were supplemented with yeast extract and/or polypeptone, *A. succiniciproducens* could grow on the wood hydrolysate-containing medium (Fig.2). For the economical production of succinic acid, CSL was examined if it can be an alternative nitrogen source to relatively expensive yeast extract and/or polypeptone in the fermentation of wood hydrolysate. Fermentation of wood hydrolysate with CSL showed the similar fermentation performance to that with yeast extract and polypeptone (Fig. 3). In conclusion, succinic acid can be produced more economically from wood hydrolysate and CSL with a green technology.

Acknowledgment

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References

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Table 1. Fermentation performances with yeast extract and/or polypeptone and corn steep liquor

	Glucose (20 g/L)									Glucose (10 g/L)				Glucose (20 g/L)				
	PP^a (g/L)			YE^b (g/L)			PP + YE (g/L)			CSL^c (g/L)				CSL (g/L)				
	2.5	5	7	2.5	5	7	2.5+2.5	5+2.5	5+5	7.5	10	15	30	5	7.5	10	15	30
Initial glucose (g/L)	20.4	19	19.4	20	19.7	20	20.1	19.3	20	9.8	10	10.3	10.2	19.5	19.5	20.0	20	20.4
Final glucose (g/L)	3.9	2	0	1.9	0.8	0	0	0	0	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	17.9	18.2	8.7	8.8	9.0	9.2	13.5	16	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	4.3	2.2	2.3	2.4	2.4	3.3	3.8	4.5	4.4	3.8
Yield (%, g/g)	87	87	89	90	90	90	90	91	91	89	88	87	88	87	86	89	88	88
S/A (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	4.0:1	3.8:1	3.8:1	4.0:1	4.1:1	4.0:1	4.0:1	4.0:1	3.8:1

a. Polypeptone b. Yeast extract c. Corn Steep Liquor d. Ratio of succinic acid to acetic acid

Fig. 1. Fermentation of glucose with CSL.

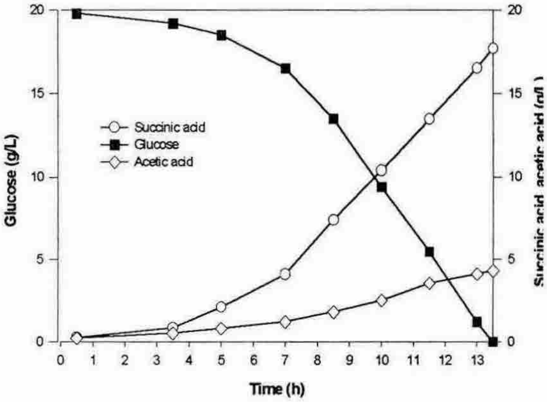

Fig. 2. Fermentation of wood hydrolysate with polypeptone and yeast extract

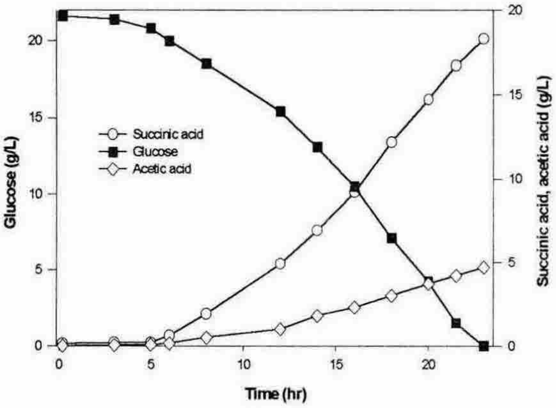

Fig. 3. Fermentation of wood hydrolysate with CSL.