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Butanol production from hemicellulosic hydrolysate of corn fiber by a *Clostridium beijerinckii* mutant with high inhibitor-tolerance

Ting Guo [a,b,1], Ai-yong He [a,1], Teng-fei Du [a,1], Da-wei Zhu [a], Da-feng Liang [b], Min Jiang [a,*], Ping Wei [a], Ping-kai Ouyang [a]

[a] *State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing University of Technology, Nanjing 211816, PR China*
[b] *Guangzhou Sugarcane Industry Research Institute, Guangzhou 510316, PR China*

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ABSTRACT

A *Clostridium beijerinckii* mutant RT66 with considerable inhibitor-tolerance obtained by continuous culture was used for butanol production from non-detoxified hemicellulosic hydrolysate of corn fiber treated with dilute sulfuric acid (SAHHC). In fed-batch fermentation, 1.8 L of diluted SAHHC containing 10 g/L of reducing sugar was provided during the acidogenic phase and 0.2 L of concentrated SAHHC containing 300 g/L of reducing sugar was provided during the solventogenic phase. The mutant produced a total amount of solvents of 12.9 g/L, which consisted of 3.1 g/L of acetone, 9.3 g/L of butanol and 0.5 g/L of ethanol. A solvent yield of 0.35 g/g sugar and a productivity of 0.18 g/L h in 72 h were achieved. The remarkable inhibitor-tolerance of *C. beijerinckii* RT66 demonstrates that this may be an excellent strain for butanol production from ligocellulosic materials.

1. Introduction

Fossil fuel depletion and global warming have spurred efforts to replace petroleum with renewable resources such as lignocellulosic biomass as feedstock. Fuels and chemicals can be produced from biomass by biorefineries, which are facilities that automate the biomass conversion processes (Adsul et al., 2011). Lignocellulosic materials are the most abundant renewable resources on the planet, and have great potential as biofuel fermentation substrates (Koukiekolo et al., 2005). Corn fiber is a major byproduct of the corn milling process and contains 60–70% carbohydrates, of which 30% is hemicelluloses, the second most abundant polysaccharides in nature.

Due to diminishing oil resources and increasing prices, various bioconversion programs have been initiated to produce biofuels as a partial replacement for fossil fuels and have gained significant attention in many countries (Dürre, 2007). Butanol is a 4-carbon primary alcohol that has many superior properties as an alternative fuel compared to ethanol (Dürre, 2007; Jin et al., 2011; Swana et al., 2011). Butanol is one of the metabolic products of acetone/butanol/ethanol (ABE) fermentation by solventogenic clostridia (Jones and Woods, 1986). ABE fermentation has major disadvantages, especially the high cost of the substrates and recovery. Seeking potential renewable biomass as a substrate for butanol production is an attractive proposition for introducing an economically competitive biological process (Ni and Sun, 2009; García et al., 2011).

Dilute acid pretreatment of hemicelluloses has been seen as a preferred method, results in a highly digestible substrate for further enzymatic cellulose hydrolysis with reasonably high sugar yields (Hu et al., 2010). However, a range of toxic compounds, including weak acids, furan derivatives and phenolic compounds, are generated during hydrolysis of lignocellulosic materials (Eva and Bärbel, 2000), therefore, no microorganism can efficiently produce butanol from lignocellulosic biomass (Weber et al., 2010). The inhibitor detoxification is difficult and increases production costs. Improving the inhibitor-tolerance of microorganisms is a promising method for industrial-scale fermentation. The total soluble phenolic compounds (TPC) in sulfuric acid-treated corn fiber hydrolysate (SACFH) are potent inhibitors of ABE production by *Clostridium beijerinckii* BA101. Acetates, furfural and hydroxymethylfurfural (HMF) do not inhibit growth or ABE production by *C. beijerinckii* BA101. When *C. beijerinckii* BA101 was used to produce butanol from SACFH, however, cell growth and butanol production were inhibited, whereas 9.3 g/L ABE was achieved only after detoxification with XAD-4 resin (Ezeji et al., 2007; Qureshi et al., 2008).

In our laboratory, the highly inhibitor-tolerant, butanol-producing mutant strain *C. beijerinckii* IB4; was screened by low-energy ion implantation and used for butanol fermentation from a

* Corresponding author. Tel./fax: +86 25 58139927.
E-mail address: bioengine@njut.edu.cn (M. Jiang).
[1] These authors contributed equally to this work.

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non-detoxified hemicellulosic hydrolysate of corn fiber treated with dilute sulfuric acid (SAHHC). When non-detoxified SAHHC was used as a substrate for butanol production, 9.5 g/L of ABE and 6.8 g/L of butanol were produced, with an ABE yield of 0.34 g/g sugars in 72 h (Guo et al., 2012). However, only 2.3 g/L of butanol was obtained by *C. beijerinckii* IB4, when the TPC concentration in SAHHC-based medium was increased to 1.7 g/L (including 33.4 g/L of reducing sugar).

The aim of this study was to produce a *C. beijerinckii* mutant with greater inhibitor tolerance that could efficiently produce butanol from SAHHC via a continuous selection technique to offer an economically competitive biological process for butanol production from corn fiber.

Table 1
The major composition of SAHHC and concentrated SAHHC after sterilization at 121 °C for 15 min.[a]

	SAHHC (g/L)	Concentrated SAHHC (g/L)
Total sugar	55.2 ± 1.6	300 ± 5.6
Xylose	44.6 ± 0.9	244.6 ± 3.9
Glucose	4.7 ± 0.4	25.6 ± 1.4
Arabinose	3.32 ± 0.3	18.9 ± 1.3
TPC	2.77 ± 0.48	15.2 ± 1.48
HMF	0.38 ± 0.04	2.08 ± 0.14
Furfural	0.66 ± 0.11	3.6 ± 0.81

[a] SAHHC, hemicellulosic hydrolysate of corn fiber treated with dilute sulfuric acid; TPC, total soluble phenolic compounds; HMF, hydroxymethylfurfural.

2. Methods

2.1. Chemicals and gas

The phenolic compounds vanillic aldehyde and tannin were purchased from Sigma Chemicals. Other chemicals were of reagent grade and were obtained from either Sinochem or Fluka Chemical. Corn steep liquor was from Anhui BBCA Biochemical Co. and N_2 was from the Nanjing Special Gases Factory (Nanjing, PR China).

2.2. Preparation of SAHHC

Corn fiber with a moisture content of 5.6% (w/w) was obtained from Shandong Zhengde Foods Ltd., China. The corn fiber were ground with a commercial plant grinder and passed through 20–40 mesh screens before mixing with 3.6% (w/w) sulfuric acid at a ratio of 1:5 (w/w). The suspension was hydrolyzed in an autoclave at 121 °C for 2.5 h. The raw hydrolysate was adjusted to pH 6.0 with solid $Ca(OH)_2$ at 50 °C and then filtered through filter paper to remove any solid material. The raw SAHHC was concentrated 5.4-fold using a rotary evaporation instrument at 60 °C. After treatment at 121 °C for 15 min, the major composition of SAHHC and concentrated SAHHC were shown in Table 1.

The raw SAHHC (containing 2.77 g of TPC and 55.2 g/L of reducing sugar) was diluted to required TPC concentrations, which are as follows: TPC 1.4 (containing 30.3 g/L of reducing sugar), TPC 1.7 (containing 33.4 g/L of reducing sugar), TPC 1.9 (containing 36.4 g/L of reducing sugar), TPC 2.1 (containing 40.3 g/L of reducing sugar) and TPC 2.3 (containing 45.2 g/L of reducing sugar).

2.3. Microorganism and culture conditions

C. beijerinckii IB4 (a mutant derived from *C. beijerinckii* NCIMB 8052) was cultivated at 37 °C for 14 h in yeast extract/peptone/starch (YPS) medium (3.0 g yeast extract, 5.0 g peptone, 10.0 g soluble starch, 2.0 g ammonium acetate, 2.0 g NaCl, 3.0 g $MgSO_4{\cdot}7H_2O$, 1.0 g KH_2PO_4, 1.0 g K_2HPO_4 and 0.1 g $FeSO_4{\cdot}7H_2O$ per liter, adjusted to pH 6.0 with 1 mol/L NaOH) (Guo et al., 2012), purged with nitrogen gas to remove dissolved oxygen. This culture (weight of cells was 1.8 g/L) was used for continuous selection culture and transferred to the butanol production medium (10% (v/v) inoculation). The selection agar plates had YPS medium containing 0.02 g/L of resazurin and SAHHC (containing 1.4 g/L of TPC and 30.3 g/L reducing sugar).

2.4. Continuous cultures

Fig. 1 illustrates the continuous culture system (Ye et al., 2010). Continuous culture system was a 500-mL glass test tube with various tube fittings and an operating volume of 200 mL. Agitation resulted from sterile N_2 being pumped into the bottom of the vessel. The overflow was forced out of the vessel by the air pressure. No

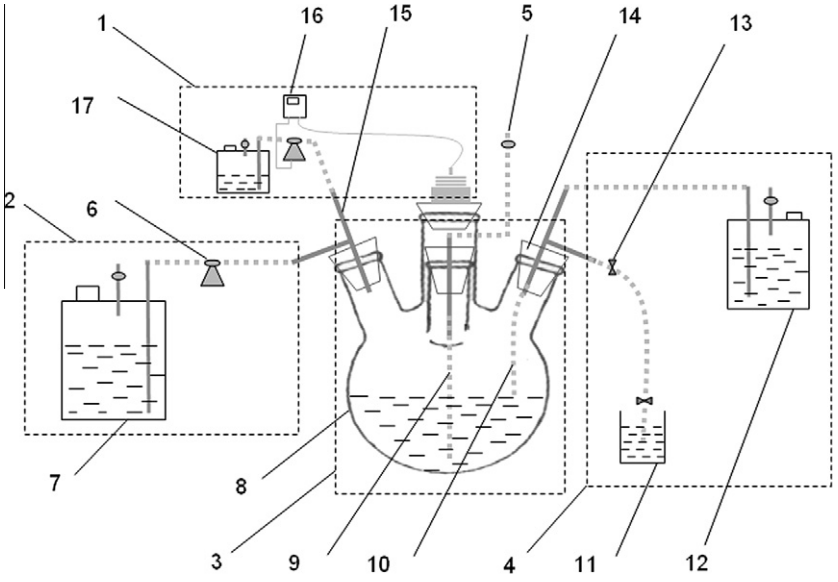

Fig. 1. The continuous culture system. 1-pH control system, 2-fed-batch system, 3-reaction system, 4-discharging and sampling system, 5-inlet gas system, 6-pump, 7-feeding medium bottle, 8-reactor, 9-gas inlet pipe, 10-liquid control pipe, 11-lye sealing bottle, 12-waste liquid bottle, 13-globe valve, 14-rubber spigot, 15-glass T-tubes, 16-pH/mV meter, and 17-acid and alkali bottles.