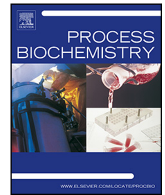
Process Biochemistry 49 (2014) 1941–1949

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio



A novel strategy for sequential co-culture of *Clostridium thermocellum* and *Clostridium beijerinckii* to produce solvents from alkali extracted corn cobs

Zhiqiang Wen, Mianbin Wu, Yijun Lin [1], Lirong Yang, Jianping Lin *, Peilin Cen

Key Laboratory of Biomass Chemical Engineering of Ministry of Education, Department of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, China

ARTICLE INFO

Article history:
Received 22 March 2014
Received in revised form 17 June 2014
Accepted 10 July 2014
Available online 1 August 2014

Keywords:
ABE fermentation
Consolidated bioprocessing
Sequential co-culture
Lignocellulose
Sugars accumulation
Optimization

ABSTRACT

Increasing concern on fossil fuels depletion and global environmental deterioration has recently renewed interest in butanol production from lignocellulose by acetone–butanol–ethanol (ABE) fermentation. Unfortunately, there was barely microorganism possessing the compound ability of lignocellulose decomposition and solvents production natively. Comparing with complicated genetic and metabolic engineering strategy to engineer native cellulolytic or solventogenic bacteria, mixed culture is a convenient and feasible approach for ABE fermentation by consolidated bioprocessing (CBP) from lignocellulose. But few communities of microorganisms succeeded in producing butanol of high titer or productivity without adding butyrate. Here a novel strategy for sequential co-culture of *Clostridium thermocellum* ATCC 27405 and *Clostridium beijerinckii* NCIMB 8052 was proposed to produce solvents efficiently in one pot reaction with alkali extracted corn cobs (AECC), a low-cost renewable feedstock, as the sole carbon source. In this strategy, soluble sugars accumulation by *C. thermocellum* hydrolyzing AECC was considered to be paramount for the CBP and was promoted considerably by contrast with previous co-culture studies. Under the combinatorial optimal culture parameters for sugars accumulation and ABE production, the CBP decomposed 88.9 g L^{-1} of AECC and manufactured ABE 19.9 g L^{-1} (acetone 3.96, butanol 10.9 and ethanol 5.04 g L^{-1}) in 200 h without feeding butyrate.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Increasing concerns about dizzily rising gasoline prices and deteriorating global environment have recently focused attention to alternative automobile fuels, especially biofuels such as butanol and ethanol. As the main product of acetone–butanol–ethanol (ABE) fermentation, butanol is traditionally produced from corn, cassava, molasses or other food crops [1]. At the same time, these starch or sugar based materials are also the main stuffs food and feed industries. The utilization of them for biofuel industries in large tonnage scale would lead to the shortage and prices rise for food and feed. For instance, the percentage of US corn production used for bioethanol production rose from 5% around in 2000 to more than 40% in 2011 and 2012, in the mean time, the corn price raised from roughly USD 2 per bushel to average USD 6–7 per bushel [2]. In order to guarantee the sustainability of biofuel industry, new economical technology for biofuel production from non-grain materials should be developed.

As the most abundant renewable recourses on earth, lignocellulosic biomass provides one of the thorough solutions for sustainable development of ABE fermentation [1]. In most cases, biological or chemical breakdown of lignocellulosic materials was required for subsequent ABE production [3–7], in respect that not all solventogenic clostridia have evolved to utilize lignocellulose as a carbon source directly. The problem is that the cost of cellulase used to hydrolyze lignocellulose has made these efforts above uneconomical [8].

It has been proposed that consolidated bioprocessing (CBP) was an efficient and economical method for biofuel production from low-price renewable feedstocks, since it combines cellulase production, cellulose hydrolysis and fermentation in one pot, which dramatically reduces the cost of enzymes production, equipments and operation [9]. In CBP, a single organism or community of

Abbreviations: AEC, Calkali extracted deshelled corn cobs; ABE, acetone–butanol–ethanol; CBP, consolidated bioprocessing; CCR, carbon catabolite repression; YE, yeast extract.

* Corresponding author. Tel.: +86 0571 87951220; fax: +86 0571 87951220.
E-mail address: linjp@zju.edu.cn (J. Lin).

[1] Present address: Third Institute of Oceanography, State Oceanic Administration, Xiamen 361005, China.

http://dx.doi.org/10.1016/j.procbio.2014.07.009
1359-5113/© 2014 Elsevier Ltd. All rights reserved.

organisms must be developed to utilize lignocellulose rapidly and produce biofuels with high yield and titer.

Although cellulase (or cellulosome) genes have been cloned into *Clostridium beijerinckii* or *Clostridium acetobutylicum* to produce butanol from cellulose in a single step, the level of expressed heterologous cellulase (or cellulosome) was rather low [10–13]. Attempts to introduce the (iso)butanol biosynthetic pathway into cellulolytic bacteria did not come up to expectations either [14,15]. Original or artificial mixed culture of cellulolytic microorganisms and solventogenic bacteria is another approach for ABE fermentation by CBP [16–19]. By comparison with natural community of microorganism, artificial symbiosis was easier to regulate and more stable. Examples of these artificial symbioses include the co-culture of solventogenic *C. acetobutylicum* and cellulolytic *Clostridium cellulolyticum* or *Clostridium thermocellum* [17,18]. However, the communities of organisms failed to produce solvents, except adding butyric acid to induce the solventogenic phase of *C. acetobutylicum*. In view of the phenomenon, *Clostridium saccharoperbutylacetonicum* strain N1-4, whose induction mechanism of butanol production differs somewhat from that of other butanol-producing clostridia, was chosen as the partner for *C. thermocellum* [19]. This bacteria pair succeeded in manufacturing 7.9 $g\,L^{-1}$ of butanol from 40 $g\,L^{-1}$ of Avicel cellulose in 11 days, but offered a rather low ABE productivity of 0.0375 $g\,L^{-1}\,h^{-1}$.

In fact, for ABE fermentation by *C. acetobutylicum* or *C. beijerinckii*, only acids were produced when the concentration of carbon source was not high enough in the medium [20,21]. We observed that only very small amount of sugars accumulated during saccharification in all artificial systems above, which explained why ABE fermentation by *C. acetobutylicum* or *C. beijerinckii* ceased in the phase of acidogenesis. As a consequence, it was of a top priority to provide sufficient carbon source by cellulolytic microorganisms for the solventogenic microbe to grow well and to switch from acidogenesis to solventogenesis. Besides, various components in crude lignocelluloses made CBP using lignocelluloses more complicated than pure cellulose [22]. It was very indispensable to study the CBP based on real lignocellulosic biomass.

To refrain from producing acids only, a novel co-culture strategy was developed in the present study to produce solvents efficiently without feeding butyrate in one pot reaction with alkali extracted corn cobs (AECC), a low-cost renewable feedstock, as the sole carbon source. In this strategy, soluble sugars accumulation by *C. thermocellum* ATCC27405 decomposing AECC was considered to be paramount for the CBP and was promoted considerably by contrast with previous co-culture studies [17–19]. It is known that *C. thermocellum* ATCC27405 is one of the most efficient cellulose-degrading bacteria [23]. Meanwhile, *C. beijerinckii* NCIMB8052 was selected as the production strain for ABE fermentation in the symbiotic system. Since *C. beijerinckii* possesses a relatively relaxed carbon catabolite repression (CCR) mechanism [22,24], research on lignocellulosic biomass-based ABE production by *C. beijerinckii* has recently intensified [3–6,25].

The present work involved ABE fermentation using AECC, a low-cost and abundant agricultural residue after a simple pretreatment, as the raw material by sequential co-culture of *C. thermocellum* and *C. beijerinckii*. The optimization of some important parameters on AECC degradation and ABE production was performed.

2. Materials and methods

2.1. Alkali extracted corn cobs (AECC)

The corn cobs were cut into pieces approximately 1 cm in length, then ground into powders to pass through a series of standard sieve from 8 to 100 mesh. Alkali extracted fractions were prepared by autoclaving 6% (w/v) corn cobs at 121 °C for 20 min with 1% (w/v) NaOH, followed by neutralizing with 1% (w/v) H_2SO_4. These fractions were thoroughly washed with distilled water and dried at 80 °C for 24 h [26].

The component of AECC was determined using a raw fiber extractor-FIWE 3 (Velp Scientifica/Goodwill (HK) Technology Ltd., Hong Kong, China) [27]. The cellulose, hemicellulose and lignin contents of the AECC were 69.8, 27.4 and 1.47% (w/w), respectively, compared to 44.9, 33.2 and 14.5% before alkali extraction.

2.2. Strain and medium

C. thermocellum ATCC27405 was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany). The stock culture was maintained in 25% glycerol and frozen at −80 °C.

C. beijerinckii NCIMB 8052 was kindly provided by Professor Sheng Yang, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences (CAS). The stock culture was maintained in 25% glycerol and frozen at −80 °C. *C. beijerinckii* was anaerobically pre-cultured in a TGY medium (1 L TGY medium containing 5 g of tryptone, 3 g of yeast extract, 2 g of glucose, 0.25 g of L-cysteine HCl and 0.001 g of resazurin; pH 6.5). It was incubated under static conditions at 37 °C for 12–16 h i.e. until the log phase was reached.

C. thermocellum were anaerobically pre-cultured in 200 mL of medium with AECC as the sole carbon source under static conditions at 60 °C for 24 h before inoculation. The medium was slightly modified by comparison to that described before [28]. One liter of medium (pH 7.5) contained 75.0 g of AECC, 2.1 g of NH_4Cl, 2.9 g of $K_2HPO_4{\cdot}3H_2O$, 1.5 g of KH_2PO_4, 1.0 g of $MgCl_2{\cdot}6H_2O$, 0.15 g of $CaCl_2{\cdot}2H_2O$, 6 g of yeast extract, 1.0 g of L-cysteine HCl, 0.001 g of resazurin and 20 mL of a trace metal solution [29]. NH_4Cl was filter-sterilized using a 0.22 μm pore size filter, and the other solutions were autoclaved at 121 °C for 20 min, followed by cooling to room temperature under 100% N_2. The medium was also used for a single culture of *C. beijerinckii* NCIMB 8052 and *C. thermocellum* ATCC 27045.

2.3. ABE fermentation from AECC

A sequential co-culture of *C. thermocellum* and *C. beijerinckii* was carried out in a 5 L bioreactor (Biotech-5BGH, Baoxing Bioengineering Equipment Co. Ltd., Shanghai, China) with a 2 L working volume with pH control if necessary by the automatic addition of 5 N NaOH. Agitation was kept constant at 100 rpm. *C. thermocellum* was incubated for 1–5 days at 60 °C before *C. beijerinckii* was inoculated, and then the temperature fell to 37 °C. Samples were taken at regular intervals for the analysis of biomass, substrate and products concentration.

The optimization of sugars accumulation was performed by varying the culture conditions of *C. thermocellum* in 500 mL shaken flasks with a 400 mL working volume in a reequipped 6-channel refrigerated pH-controlled feed shaker (SHpH6 shaker incubator, Shanghai Guoqiang Bioengineering Equipment Co. Ltd., Shanghai, China). Optimization of ABE production by *C. beijerinckii* was assessed in the broth resulting from the incubation of *C. thermocellum* for 96 h with pH 7.0 controlled. Samples were taken at the end of fermentation for analysis and comparison.

The inoculum size of *C. thermocellum* in all experiments was 10% (v/v) unless otherwise indicated.

2.4. Real-time PCR

Total RNA was isolated from freshly collected culture samples using trizol (Invitrogen, CA, USA) following the manufacturer's

Download English Version:

https://daneshyari.com/en/article/34454

Download Persian Version:

https://daneshyari.com/article/34454

Daneshyari.com