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A novel anaerobic co-culture system for bio-hydrogen production from sugarcane bagasse

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HIGHLIGHTS

- A novel co-culture system was developed for hydrogen production from SCB.
- A new model was established for the evaluation of SCB-pretreatment efficiency.
- Synergism of *T. aotearoense* and *C. thermocellum* for hydrogen production was found.

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ABSTRACT

A novel co-culture of *Clostridium thermocellum* and *Thermoanaerobacterium aotearoense* with pretreated sugarcane bagasse (SCB) under mild alkali conditions for bio-hydrogen production was established, exhibiting a cost-effective and synergetic advantage in bio-hydrogen production over monoculture of *C. thermocellum* or *T. aotearoense* with untreated SCB. The optimized pretreatment conditions were established to be 3% NaOH, and a liquid to solid ratio of 25:1 at 80 °C for 3 h. A final hydrogen production of 50.05 ± 1.51 mmol/L was achieved with 40 g/L pretreated SCB at 55 °C. The established co-culture system provides a novel consolidated bio-processing strategy for bioconversion of SCB to bio-hydrogen.
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1. Introduction

Energy has become a key consideration in discussions of sustainable development today, due to growing concerns over the environmental impact and the inevitable depletion of fossil fuels, and thus the development of alternative energy sources has become a matter of urgency. Lignocellulosic biomass and crop wastes have been considered as potential sustainable feed-stocks for energy production. Sugarcane bagasse (hereafter SCB), a fibrous residue of sugarcane, is one of the largest cellulosic agro-industrial by-products, and approximately 100 million tons of dry SCB are estimated to be produced globally every year (Zhu et al., 2012). SCB is widely studied in the field of bioenergy, though currently the production of sugarcane bioethanol in Brazil is largely based on the fermentation of sugar juice and molasses in autonomous distilleries (Stefano et al., 2012). SCB is rich in cellulose (approximately 50%) and hemicellulose (approximately 25%), and the low content of ash in SCB can significantly facilitate the bioconversion process by fermentation when compared with other crop residues with a higher ash content, such as rice straw and wheat straw. More than 70% of SCB consists of hydrolysable carbohydrates that can yield fermentable sugars for the production of value-added bioproducts (Stefano et al., 2012). Usually, the complex structure of lignocellulosic material is a major obstacle to its conversion into bioenergy, leading to biomass recalcitrance, nonproductive binding and inactivation of enzymes (Feng et al., 2010; Qing and Yang, 2010; Eriksson et al., 2002a,b). Another inhibitor of cellulase is hemicellulose which is composed of pentose (Qing and Yang, 2010). Generally, altering or removing the lignin and hemicellulose can increase their porosity to make them accessible to the enzymes, and thus improve the hydrolysis of the cellulose (Sun and Cheng, 2002). A variety of pretreatment techniques, including physical (comminution, hydrothermolysis), chemical (acid, alkali, solvents, ozone), physico-chemical (steam explosion, ammonia fiber explosion) and biological pretreatment, have been developed to enhance the accessibility of enzymes to cellulosic fibers (Mosier et al., 2005). A large fraction of the hemicellulose, yet very little lignin can be removed from biomass using low and neutral pH pretreatment technologies for lignocellulose. Mild alkali pretreatment has an advantage in removing lignin, and alkaline pretreatment is more effective on agricultural residues and herbaceous crops than on wood materials (Wyman, 1996). Besides, mild alkali pretreatment can lead the complete conversion of cellulose I

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to cellulose II, an easier degradable form with antiparallel chain structure in sodium hydroxide solution (Xu et al., 2012). There is no denying that optimizing the alkali pretreatment conditions can improve the bioconversion of lignocellulosic material by fermentation. Due to the complex structure of lignocellulose, consolidated bio-processing (hereafter CBP) strategy is usually used to improve enzyme production, cellulose and hemicellulose degradation, and bioenergy production. Also, a combination of cellulose- and hemicellulose-hydrolyzing strains can be developed as a co-culture system to enhance bioconversion of lignocellulosic material.

Sugarcane has been widely studied as a biofuel crop in the last decade, yielding hydrated bioethanol and anhydrous bioethanol (gasoline additive) by fermentation and distillation of sugarcane juice and molasses (Hartemink, 2008). Sugarcane bioethanol production has been considered as a mature technology on a commercial scale. However, to date, no information is available on the feasibility of bio-hydrogen production using suitably pretreated SCB. In most previous studies, lime pretreatment was employed in the process of monoculture fermentation, which could result in high cost and low production efficiency. *Clostridium thermocellum* can degrade cellulose into cellobiose and cellodextrins, which can be further fermented into ethanol, acetic acid, lactic acid, hydrogen, and carbon dioxide (Lamed and Zeikus, 1980). Unlike *C. thermocellum* that could partially degrade hemicellulose into xylose without utilizing the product, *Thermoanaerobacterium aotearoense* can yield bio-hydrogen efficiently with xylose as substrate due to its pentose-utilizing ability, in addition to the secreted hemicellulose hydrolases (Li and Zhu, 2011).

To fully utilize SCB as an agriculture residue for bio-hydrogen production, the co-application of an optimal pretreatment and fermentation method is required. The aims of the current study were to (1) investigate the effect of sodium hydroxide and ammonia water–hydrogen peroxide on the main components of SCB; (2) investigate the advantages of co-culture system of *C. thermocellum* and *T. aotearoense* in SCB degradation and bio-hydrogen production; (3) optimize the pretreatment methods and conditions for the bioconversion of SCB into hydrogen; and (4) investigate the effects of the temperature, substrate concentration, and incubation sequence on the fermentative hydrogen production in CBP.

2. Methods

2.1. Material

The SCB was a gift from the Guangzhou Sugarcane Industry Research Institute (Guangdong Province, China). Before analysis, the SCB was washed with distilled water until neutrality, and then dried at 50 °C for 24 h. After that, the SCB was ground, and sieved through a 100-mesh (0.15 mm) sieve, and only such treated particles were used for the fermentation experiments. The SCB composition was analyzed by the AOAC standard as follows: 35.94 ± 0.32% glucan, 19.22 ± 0.58% xylan, 21.34 ± 0.06% Klason lignin, and 3.09 ± 0.06% acid soluble lignin, apart from some other components such as Arabian, moisture, ash, and benzene (Zhu et al., 2012).

2.2. Pretreatment of substrate

In order to obtain a cost-effective pretreatment strategy, the raw SCB was pretreated in the following two methods: (1) the SCB was pretreated with NH_4OH–H_2O_2 solution (51%) as described by Zhu et al. (2012) and (2) the SCB was soaked in sodium hydroxide solution (1%) for 6 h at 150 rpm, followed by an elution with distilled water until neutrality, drying at 50 °C for 24 h, grinding, and sieving as described in Section 2.1. Based on the preliminary study, 20 g/L concentration was chosen as the initial substrate concentration.

2.3. Microorganism strains, media and inoculum preparation

C. thermocellum ATCC 27405 was a gift of Professor Lynd (Dartmouth College, USA). *T. aotearoense* SCUT27 was isolated from a geothermal spring in the south of China, and its lactate dehydrogenase (*ldh*) gene was successfully disrupted via homologous recombination using the constructed vector based on pBLUESCRIPT II SK(+) (Genetimes Technology, Inc, Shanghai, China) (Cai et al., 2010; Li and Zhu, 2011). *C. thermocellum* ATCC 27405 was repeatedly transferred as the seed (over 10 generations continuously) for about 96 h at 55 °C with rotary shaking at 150 rpm (C24KC refrigerated incubator shaker, Edison, New Jersey, United States). *T. aotearoense* SCUT27 was cultured in the same condition until $OD_{600} \sim 0.8$ (Thermo Fisher Scientific GENESYS 10, Bremen, Germany), and then transferred to a new liquid seed medium of optical density ~1.0. Both *C. thermocellum* ATCC 27405 and *T. aotearoense* SCUT27 were grown in serum bottles with MTC Medium (Özkan et al., 2004). For *C. thermocellum* ATCC 27405 and *T. aotearoense* SCUT27, 3 g/L cellulose and 5 g/L xylose were added, respectively, into the MTC Medium as the carbon source. All the serum bottles were sealed with butyl rubber stopper and aluminum seals, and then each bottle was purged and gassed with 100% nitrogen 3 times.

2.4. Batch fermentation of SCB

Batch fermentation of SCB was performed with 120 mL serum bottles to investigate the bio-hydrogen production. Three different sets of triplicate serum bottles (120 mL) containing 20 g/L milling-, ammonia hydrogen peroxide- or sodium hydroxide-pretreated SCB with a work volume of 50 mL. Subsequently, the crimp-sealed serum bottles were purged with 100% nitrogen, and then autoclaved at 115 °C for 20 min. *C. thermocellum* was cultured individually or co-cultured with *T. aotearoense* at 55 °C. All cultures were inoculated with an inoculation volume of 10% (v/v) from freshly prepared cultures at exponential growth phase in serum bottles, with 3 g/L microcrystalline cellulose for *C. thermocellum* and 5 g/L xylose for *T. aotearoense*. For the mono-culture, the seeds of *C. thermocellum* and *T. aotearoense* were injected into the bottles respectively using disposable syringes. For the co-culture, the seeds of *C. thermocellum* and *T. aotearoense* were injected into the bottles at a ratio of 1:1 (v/v, the same as below), with the inoculum size being 10% (v/v, the same as below) for both the mono- and co-culture processes. The fermentation was incubated at 55 °C with rotary shaking at 150 rpm for 168 h. The volume and composition of biogas were analyzed by the gas chromatograph, and only carbon dioxide and hydrogen were detected from gas products. The liquor samples were collected for the analysis of the reducing sugars released, ethanol and the organic acids with high performance liquid chromatography (HPLC) as described below.

2.5. Analyses

2.5.1. Analysis of SCB components

The cellulose, hemicellulose and lignin contents of SCB and solid fraction remaining after pretreatment were measured using a two-step acid hydrolysis method to fractionate the biomass into forms that are more easily quantified by laboratory analytical procedures (LAP) from the National Renewable Energy Laboratory (NREL, 2008).

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