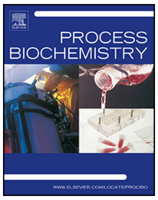
Process Biochemistry 51 (2016) 1538–1543

Contents lists available at ScienceDirect

Process Biochemistry

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



Biobutanol production from sugarcane bagasse hydrolysate generated with the assistance of gamma-valerolactone

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ARTICLE INFO

Article history:
Received 30 March 2016
Received in revised form 6 June 2016
Accepted 11 June 2016
Available online 14 June 2016

Keywords:
Gamma-valerolactone
Sugarcane bagasse
Lignin removal
Butanol production

ABSTRACT

Production of butanol from renewable resources is an attractive approach for introducing an economically competitive process. In the present study, sugarcane bagasse was hydrolyzed with the assistance of gamma-valerolactone (GVL). After phase separation, direct hydrolysis of sugarcane bagasse resulted in 24.08 g/L total sugars. Removal of lignin significantly increased the total sugar concentration to 248.41 g/L with a high yield of 0.87 g/g. Due to the low level of inhibitors in hydrolysate, the obtained hydrolysate was used as substrate for biobutanol production. Batch fermentation with 41 g/L sugars produced a high ABE concentration of 14.26 g/L, including 4.1 g/L acetone, 9.3 g/L butanol and 0.86 g/L ethanol. The fermentation with 61 g/L sugars greatly inhibited cell growth and solvent production due to the high concentration of NaCl and GVL. This study demonstrates that sugarcane bagasse hydrolyzed in GVL/water solution could be an alternative substrate for the low-cost production of biobutanol.

1. Introduction

Exploring sustainable resource for the production of fuels is being driven by various environmental concerns resulting from the rapid consumption of petroleum-derived feed stocks [2]. Acetone–butanol–ethanol (ABE) fermentation is considered as a way to upgrade renewable resources into high value-added products such as butanol, acetone, and acetoin [14,30]. As a superior biofuel, butanol has received increasing interest because it offers several advantages over ethanol for gasoline–alcohol blending, including high energy content, low miscibility with water, and low volatility [4,29]. Commercial fermentative production of butanol has been applied more than 100 years; however, it is currently more costly than production from propylene due to the high cost of substrates [13].Using lignocellulosic material as substrate for butanol production is an attractive approach for introducing an economically competitive biological process [7]. Numerous efforts have been made to produce butanol from lignocellulosic material, such as wheat straw [21], corn stover [17], and barley straw [22]. Hydrolysis of lignocellulosic material by concentrated mineral acid could release most sugars at high sugar yield [24]. However, high concentration of toxic compounds could be generated, including weak acids, furan derivatives and phenolic compounds, which could strongly inhibit cell growth and decrease solvent production [10]. On the other hand, the recovery of the concentrated acid is critical to the economics of the hydrolysis process [25].

Recently, the use of polar aprotic solvents has been shown to be beneficial in the chemical conversion of lignocellulosic biomass with low concentration of H_2SO_4 (5 mM). The polar aprotic solvent of GVL is a green solvent, which can be produced from biomass-derived levulinic acid. With the assistance of GVL, conversion of lignocellulosic biomass to levulinic acid (LA) and hydroxymethylfurfural (HMF) could be achieved and this process displayed significant improvements in catalytic activity and selectivity [1,5]. When water was used as a reactant in hydrolysis reaction (20 wt% water/80 wt% GVL), lignocellulosic material could be hydrolyzed into corresponding sugars [16]. Furthermore, GVL can be recycled in hydrolysis process, indicating that GVL is a promising solvent for biomass processing.

Lignin consists of 15–30% by weight of lignocellulosic biomass and tightly cross-linked with the other components, which hinders the hydrolysis process. Lignin was reported to dissolve in GVL at a high amount [15,16]. Thus, the dissolved lignin in GVL/water solution would decrease the dissolving capacity of sugars and resulted

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http://dx.doi.org/10.1016/j.procbio.2016.06.013

in negative effects on the reuse of GVL. Besides, lignin remains as a kind of byproduct which can hardly be degraded to fermentable carbohydrates and hitherto no evidence has shown any organism can directly use lignin as carbon or energy source [31]. Therefore, lignin should be removed first in order for successful exploitation of cellulose and hemicelluloses in the GVL/water solution.

In order to reduce our dependence on petroleum, efficient hydrolysis of lignocellulosic biomass is vital. Thus, the main objective of present study is placed on the conversion of sugarcane bagasse into fermentable sugars by using GVL/water solution, which then could be further upgraded into the superior biofuel butanol. Microwave-alkali pretreatment was conducted to remove lignin content for improving the hydrolysis process. Inhibitors in sugarcane bagasse hydrolysate including weak acids, furan derivatives and phenolic compounds were first detected. Moreover, the viability of the obtained hydrolysate as feedstock for butanol fermentation was also determined.

2. Methods

2.1. Raw material

Sugarcane bagasse was from Guangzhou Sugarcane Industry Research Institute (Guangzhou, China). It was thoroughly washed and then dried in an oven at 60 °C before the experiments. The sugarcane bagasse used in this study contains 48% cellulose, 25% hemicellulose, 22% lignin and 3% ash.

2.2. Microorganism and culture conditions

Clostridium acetobutylicum XY16 was screened by our laboratory and stored in China Center for Type Culture Collection (CCTCC No. M 2010011). It was maintained as a cell suspension at −80 °C and subsequently cultured in a 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 $FeSO_4{\cdot}7H_2O$, adjusted to pH 6.0 with 1 mol/L HCl) [8]. The cells of *C. acetobutylicum* XY16 were cultured at 37 °C. A seed inoculum of 3 ml from an overnight 10 ml YPS culture was added to 27 ml fresh YPS medium for anaerobic cell growth. After incubating for 8 h, a 10% (v/v) inoculum was used to start the anaerobic ABE fermentation with modified P2 medium (per liter: K_2HPO_4 0.50 g, KH_2PO_4 0.50 g, CH_3COONH_4 2.2 g, $MgSO_4{\cdot}7H_2O$ 0.20 g, $MnSO_4{\cdot}H_2O$ 0.01 g, $FeSO_4{\cdot}7H_2O$ 0.01 g, NaCl 0.01 g, corn steep liquor 1 g) [20]. The modified P2 medium was used as production medium for butanol fermentation. The initial pH was adjusted to 6.0 with 1 M HCl. Oxygen-free nitrogen was used to maintain anaerobic condition during seed culture and ABE fermentation. All the chemicals were of reagent grade, except for corn steep liquor, and unless otherwise stated, all chemicals used in this study were purchased from either Sinochem (Shanghai, China) or Fluka Chemical (Buchs, Switzerland). Corn steep liquor (main components: Water 84.41%, Solid material, 10.75%; Protein, 2.31%; Total sugar 2.07%, Total nitrogen, 0.37%; Amino acids, 0.041%; Biotin and microelement, 0.049%) was purchased from Anhui BBCA Biochemical Co. (Bengbu, China). Nitrogen was from Nanjing Special Gases Factory (Nanjing, China).

2.3. Pretreatment methods

Microwave-alkali pretreatment of sugarcane bagasse was carried out by using 1% NaOH as pretreatment reagent. Sugarcane bagasse with 5 wt% loading was added to this alkaline solution in a stoppered flask and subjected to microwave pretreatment at 400 W as described by Binod et al. [3]. Microwave pretreatment was applied with the time range from 2 to 10 min. After pretreatment, biomass was thoroughly washed with deionized water until the pH of effluent was similar to that of deionized water and then dried at 60 °C. The dried solid residue was used for the analysis of its composition.

2.4. Hydrolysis of sugarcane bagasse

Hydrolysis of sugarcane bagasse with the assistance of GVL was performed as described by Luterbacher with some modification [15]. It was carried out in a 200 ml autoclave with 100 ml working volume. The vessel and head of autoclave were dried overnight at 60 °C to remove residual water prior to start each reaction. GVL was purchased from Sigma Aldrich with the purity of 98%. Sugarcane bagasse along with GVL/water solution (80 wt% GVL, 20 wt% water and 5 mM or 50 mM H_2SO_4) was sealed in the autoclave. The autoclave was then progressively heated to 216.8 °C using a 10 min ramp for every 10°C (homogeneous reactor JXF, China). The temperature was allowed to equilibrate at 216.8 °C for 3 min. The obtained hydrolysate was cooled in an ice water bath and filtered with a 0.2 mm syringe filter.

2.5. Separation of hydrolysate

After hydrolysis, aqueous phase can be separated along with the carbohydrates from GVL/water solution by addition of NaCl or liquid CO_2 extraction and the carbohydrates will be concentrated to about five-fold by removal of GVL which consists of 80 wt% of the initial GVL/water solution. Liquid CO_2 extraction has the drawback of complex operation and high equipment cost, besides it will also cause loss of sugar component [15]. Thus, to simplify the separation process, addition of NaCl was used. 12 wt% sodium chloride (NaCl) was added to the hydrolysate to create a separate aqueous phase (NaCl content is given as mass fraction of the salt and water mixture). The aqueous phase was collected using a syringe and needle to analyze its compositions.

2.6. ABE fermentation

To investigate the effects of NaCl and GVL remained in hydrolysate on the cell growth of butanol producing strain *C. acetobutylicum* XY16, different dosages of NaCl and GVL were added to the culture medium and cells were cultured in 100 ml anaerobic bottles. Oxygen-free nitrogen was bubbled through the medium to remove oxygen after sampling. The separate hydrolysate was set to 393 K for 60 min in an oil bath to hydrolyze any oligomers to glucose and xylose, and then the above sugar solution was used as substrate for ABE fermentation. Batch fermentation by *C. acetobutylicum* XY16 was conducted in a 500 ml glass fermentor (300 ml working volume). The sugar solution generated by GVL/water solution was diluted by concentrated P2 medium to the final fermentable sugar concentration of 41 g/L and 61 g/L. Appropriate amount of antifoam was added into the bioreactor at the beginning of fermentation to control foaming. The fermentation temperature was maintained at 37 °C. pH condition was set as described in our previous work [9]. Samples were taken at regular intervals and centrifuged at 8000 rpm for 10 min. Supernatants were used for the analysis of ABE solvents, acids, and sugars.

2.7. Analytical methods

After hydrolysate at 119.8 °C for 60 min, the sugars were analyzed by high performance liquid chromatography (HPLC, Chromeleon server monitor, P680 pump, Dionex, USA) equipped with a refractive index detector (RI101, Shodex, USA) and an ion exchange chromatographic column (Aminex HPX-87H,