BIOMASS AND BIOENERGY 76 (2015) 24–30

Available online at www.sciencedirect.com

ScienceDirect

http://www.elsevier.com/locate/biombioe

Feasibility of reed for biobutanol production hydrolyzed by crude cellulase

Youshuang Zhu [a,1], Fengxue Xin [b,*,1], Yunkang Chang [a], Ying Zhao [a], Wong Weichong [b]

[a] Department of Biological Science, Jining Medical University, 272067, China
[b] Department of Civil and Environmental Engineering, National University of Singapore, Singapore 117576, Singapore

ARTICLE INFO

Article history:
Received 30 April 2014
Received in revised form
1 November 2014
Accepted 10 February 2015
Available online 23 March 2015

Keywords:
Cellulase
Organosolv pretreatment
Reed
Solid state fermentation
Biobutanol

ABSTRACT

The aim of this study was to efficiently utilize reed for both cellulase and biobutanol production. The unprocessed cellulase blend produced under solid-state fermentation using reed as the substrate showed a similar reducing sugar yield using Whatman filter paper to the commercial enzyme blend (38.61%). Organosolv pretreatment method could efficiently reduce hemicellulose (29.3%–14.6%) and lignin (17.2%–14.1%) content and increase cellulose content (42.5%–62.3%) from reed. Enzymatic hydrolysis of organosolv-pretreated reed using the crude cellulase with enzyme loading of 25 FPU/g reed, 20% solid content at 50 °C and pH 5.5 resulted in a reed hydrolysate containing 40.01 g/L glucose and 3.55 g/L xylose after 72 h. Fermentation of the hydrolysate medium by *Clostridium acetobutylicum* produced 9.07 and 14.24 g/L of biobutanol and ABE with yield of 0.21 g/g and 0.33 g/g, respectively. This study proved that crude cellulase complex produced under solid state fermentation and organsolv pretreatment can efficiently provide reed hydrolysate that can be converted to biobutanol without any commercial cellulase usage.

1. Introduction

Sustainable alternative transportation fuels are in high demand and are of interest as second-generation biofuels [1]. One such biofuel is biobutanol produced from non-food biomass. Biobutanol is one of the main metabolic products of acetone/butanol/ethanol (ABE) fermentation by solventogenic clostridia. Biobutanol has demonstrated its superiority to bioethanol in terms of energy density, engine compatibility and safety, and has become the center of research as the next generation biofuel since 2005 [2].

Raw material has been the most cost-intensive part of ABE fermentation [3]. The cost of fermentation substrates contributes to over 60% of the total production expenditures, so it is crucially important, from a process economics perspective, to identify inexpensive biomass feedstock that can be fermented by *Clostridium* species [3]. A number of low-cost fermentation substrates have previously been evaluated. Reed (member of order Poales), an abundant and inexpensive lignocellulosic biomass in Asia and Europe is yet to be

* *Corresponding author*. Department of Civil and Environmental Engineering, National University of Singapore, T-Lab 07-03N, 5A Engineering Drive 1, Singapore 117576, Singapore. Tel.: +65 6516 8482; fax: +65 6774 4202.
E-mail address: xinfengxue@gmail.com (F. Xin).
[1] These authors contributed equally to this work.
http://dx.doi.org/10.1016/j.biombioe.2015.02.013
0961-9534/

investigated in terms of their potential for biochemical conversion by *Clostridium* spp. to biobutanol and other solvents. Around 70 million tons of reed are obtained annually, in which Asia and Europe have the highest reed output [1,3]. The planting areas for reed in China are more than 0.67 million hectares, which provide a reed output of more than 3 million tons. Reed has been used as raw material in the papermaking industry for years because of its high cellulose content and good fiber properties. Given its superior properties, reed may be considered a good alternative raw material for biobutanol production.

The cellulosic biobutanol production from lignocellulose consists of three main steps such as pretreatment, enzymatic hydrolysis of cellulose, and biobutanol fermentation. Production of fermentable sugars from lignocellulose, which involves pretreatment and saccharification, is the bottleneck in the cellulosic biofuel production [4]. Various methods have been developed to improve biomass fractionation by using pretreatment processes, such as dilute acid, alkaline, aqueous ammonia, steam explosion, wet oxidation, phosphoric acid-acetone, organosolv and liquid hot water pretreatment methods [5–8]. Among them, acid or alkali pretreatment is considered the most mature technology for biofuel production from lignocellulose. However, when using acids or alkali at severe conditions involving a high temperature or a long time duration of pretreatment, the generation of substances inhibitory to fermentative microorganisms, such as carboxylic acids, sugar degradation products, phenolic compounds, and inorganic salts, is not avoidable [6]. Liquid hot water could efficiently increase the percentage of cellulose content of reed biomass; however, it would be achieved at the expense of increased thermal energy cost [7]. Organosolv pretreatment is effective in removing the recalcitrance of lignocellulose, including woody and non-woody biomass, for enzymatic cellulose saccharification [6,8]. It has been studied on various woody biomass (like bamboo, horticultural waste et al.) and non-woodly substrates (like rice straw, corn stover et al.) substrates in hemicelluloses/lignin degradation and cellulose crystallinity reduction, especially under mild conditions. It is therefore an effective pretreatment method for biomass biological conversion to fuels and chemicals [5–7].

Another dependent factor to the production economics of biobutanol is the cost of cellulases. Efforts towards cost reduction have been directed at increasing enzyme production by finding hyperactive microbial strains, efficient fermentation techniques and recovery systems. The use of solid state fermentation using cheap biomass resources as substrate can further improve production economics [8].

In order to achieve biobutanol production from reed with low cost demand, i.e. pretreatment with low-energy input and high removal of hemicellulose and lignin and no commercial cellulase purchase et al., a bioprocess was designed and tested to ascertain the suitability of reed as a solid substrate for both biobutanol and cellulase production. Solid state fermentation using the substrate of reed was first set up to achieve cellulase production (step1); then organosolv treatment under mild conditions was carried out to efficiently remove hemicellulose and lignin of reed (step 2); and lastly after enzymatic hydrolysis using the crude cellulase complex (step 1) and treated reed (step 2), the enzymatic hydrolysate was further fermented by solventogenic *Clostridium* species for biobutanol production. To the best of our knowledge, this is the first attempt of generating biobutanol from this specific type of waste using such an integrated process.

2. Material and methods

2.1. Materials

Reed used in this study was friendly provided by Yingkou Papermaking Mill, Liaoning Province, China. The one-year old reed was harvested in fall and air-dried at room temperature in our laboratory. The reed was mechanically milled with a lab mill (Ultra Centrifugal Mill ZM 200, Retsch GmbH, Germany) and sieved through standard mesh sieves (200–500 μm) to obtain the powder of 200–500 μm particle sizes. The commercial *Trichoderma reesei* cellulase (Celluclast 1.5 L) with FPase activity of 187 IU/mL and β-glucosidase activity of 32 IU/mL was provided by Novozymes China.

2.2. Microorganisms and culture conditions

T. reesei RUT-C30 ATCC 56765 and *Clostridium acetobutylicum* ATCC824 were obtained from ATCC (American Type Culture Collection). *T. reesei* RUT-C30 was routinely maintained and sporulated on potato dextrose agar plate for 2 weeks till spore crop was developed. Four milliliters of sterile 0.05% Tween 80 solution was added to the plate and swirled to gently release the spores. Approximately, 1 mL of the spore suspension consisting of 10^6–10^7 spores was used as the inoculum [9].

Fermentation studies were conducted in 125 mL screw capped bottles containing 100 mL of medium. Prior to autoclaving the enzymatic hydrolysate containing 40.01 g L^{-1} of glucose and 3.55 g L^{-1} xylose, the pH was adjusted to 6.5 using 2 M NaOH. The medium containing carbon source and yeast extract (3 g L^{-1}; Sigma, USA) was sterilized at 121 °C for 15 min. On cooling to 35 °C under oxygen-free nitrogen atmosphere (in an anaerobic chamber), filter-sterilized P2 stock solutions [(buffer: KH_2PO_4, 50 g L^{-1}; K_2HPO_4, 50 g L^{-1}; ammonium acetate, 220 g L^{-1}), (vitamin: para-amino-benzoic acid, 0.1 g L^{-1}; thiamin, 0.1 g L^{-1}; biotin, 0.001 g L^{-1}), and (mineral: $MgSO_4 \cdot 7H_2O$, 20 g L^{-1}; $MnSO_4 \cdot H_2O$, 1 g L^{-1}; $FeSO_4 \cdot 7H_2O$, 1 g L^{-1}; NaCl, 1 g L^{-1})] were added (1 mL each), followed by inoculation with highly active cells of *C. acetobutylicum* ATCC 824 (5 mL cell suspension in 100 mL medium).. The synthetic medium containing the same amount of sugar (40.01 g L^{-1} of glucose and 3.55 g L^{-1} xylose) with the same composition and initial pH was used as the control medium.

2.3. Enzyme production and enzymatic assays

Solid state fermentation was carried out according to our previous work [9]. The culture temperature was kept at 30 ± 1 °C for 7 days, and the initial pH was 5.0. Filter paper (FPase) and endo-glucanase (CMCase) activities were measured according to IUPAC recommendations [10]. FPase and CMCase activities were determined by measuring the reducing sugars produced from Whatman no.1 filter paper (grade 3, catalogue number 1003 150, Whatman international,