Process Biochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

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

Butyric acid production from oilseed rape straw by *Clostridium tyrobutyricum* immobilized in a fibrous bed bioreactor

Jin Huang[a,b,*], Han Zhu[a], Wan Tang[a], Pu Wang[a], Shang-Tian Yang[b]

[a] College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou, 310014, China
[b] William G. Lowrie Department of Chemical and Biomolecular Engineering, The Ohio State University, 151 West Woodruff Avenue, Columbus, OH 43210, USA

ARTICLE INFO

Article history:
Received 26 May 2016
Received in revised form 22 July 2016
Accepted 16 August 2016
Available online xxx

Keywords:
Clostridium tyrobutyricum
Oilseed rape straw
Immobilized cells
Butyric acid

ABSTRACT

Butyric acid is an important specialty chemical with wide industrial applications, while economical production via feasible large-scale fermentation requires low-cost substrate and an efficient process. In this study, hydrolyzed oilseed rape (OSR) straw was used as an alternative carbon source for immobilized-cell fermentation of *Clostridium tyrobutyricum* to produce butyric acid. Compared to the free-cell fermentation, higher butyrate yield (0.43 g/g vs. 0.37 g/g), productivity (2.46 g/L/h vs. 0.93 g/L/h) and concentration (13.6 g/L vs. 11.6 g/L) were obtained with immobilized cells of *C. tyrobutyricum* in repeated-batch fermentation. Furthermore, fed-batch fermentation using pretreated OSR straw hydrolysate resulted in a high butyric acid concentration of 50.2 g/L, with yield of 0.38 g/g. Finally, the repeated batch fermentation was carried out, which achieved high butyrate yield and volumetric productivity during long-term operation. With desirable characteristics such as economic viability and competitiveness, as well as being environmentally friendly, the fermentation developed in this work should provide an efficient process for industrial production of butyric acid.

1. Introduction

Butyric acid is a four-carbon short chain fatty acid normally produced via oxidation of butyraldehyde, derived from the oxosynthesis or hydroformylation of propylene [1]. As an important chemical, butyric acid is widely applied in chemical, food, and pharmaceutical industries, such as uses in the manufacture of cellulose acetate butyrate plastics [2] and treatments of hemoglobinopathies, cancer and gastrointestinal diseases [3]. However, current industrial production of butyric acid is dominated by petroleum-based chemical synthesis. Considering environmental pollution, as well as increasing concerns for human safety related to petroleum derived products, "naturally" derived butyric acid via microbial fermentation becomes an attractive alternative. Owing to their great biosynthetic potential, several Clostridial species are preferred for industrial bio-production of butyric acid, and the most promising one is *Clostridium tyrobutyricum*, which is capable of producing butyric acid with high selectivity and can tolerate high concentrations of butyrate and acetate [4,5]. On the other hand, the fibrous-bed bioreactor (FBB) using immobilized cells is a method to achieve efficient production of many bio-based products including acetic acid, butyric acid and propionic acid [6], which presents higher productivity, product yield and final product concentration than those of free-cell fermentation. In addition, the immobilization of microbial cells has some beneficial characteristics, including facilitation of continuous fermentation, preventing cell loss, etc.

Concerns about exhausting oil reserves and environmental pollution, as well as global warming and climate change caused by greenhouse gas emissions in the past few decades, current research and development in industrial biotechnology is focusing on biorefineries to produce bio-fuels (including ethanol and butanol) and bio-based products (such as plastics, fibers and organic acids) from non-grain or non-sugar feedstocks (e.g., biomass feedstock) [7–11]. Typically, agricultural residues and wastes including rice straw, wheat straw, wood, byproduct from the corn milling process (corn fiber) and waste paper were applied as biomass feedstocks, which are composed of polysaccharides containing five and six carbon sugars. Although butyrate producing strains (such as *C. tyrobutyricum*, *C. butyricum*, *C. thermobutyricum*) have an advantage to utilize both hexose and pentose sugars released from agricultural residues [1,2], these feedstocks normally require hydrolysis using alkali or acid pretreatment and enzymes prior to use. Sugars derived from lignocellulosic biomass allow valuable products to be pro-

* Corresponding author.
E-mail address: huangjin979@zjut.edu.cn (J. Huang).

http://dx.doi.org/10.1016/j.procbio.2016.08.019
1359-5113/

duced from abundant, cheap and renewable resources, and their pretreatments would reduce enzyme consumption and decrease undesirable byproducts such as furfural and 5-hydroxymethyl furfural (HMF).

Oilseed rape (OSR) has been widely cultivated and spans most regions of China for its high economic value of rapeseed, but the OSR straw is primarily burned and returned to fields in rural agriculture. The production of OSR straw in China has reached ~4.02 million tons annually and is steadily increasing [12]. Incineration of OSR straw not only wastes resources, but also acts as a serious source of pollution to the environment. As a widely available non-grain raw material, OSR straw contains about 34.7% (w/w) glucan, 19.4% (w/w) xylan and 8.3% (w/w) other sugars (including arabinose, galactose and mannose) [13]. It could be effectively applied after hydrolysis as carbon sources for microbial production of bioethanol and biogas [13–15]. OSR straw is a type of lignocellulosic feedstock; its physiochemical, structural and cell wall properties have hindered the accessibility of target cellulose. Pre-treatment of OSR straw could alter the structure of lignin and hemicelluloses, and disrupt the crystalline structure of cellulose, thus enhancing the extraction of glucose from biomass. Many pretreatment techniques have been explored on OSR straw, including microwave, wet oxidation, hydrothermal, dilute acid, alkali and autocatalytic popping [16]. Among these methods, alkaline pre-treatment, which mainly removes lignin and acetyl and uronic substitutes on the hemicellulosic portion of biomass and causes the swell of biomass via solvation and saphonication effects, can greatly enhance the accessibility of cellulose to enzymatic hydrolysis and increase sugars production.

As the first trial to use OSR straw for value-added products, an efficient process for butyric acid production was developed in this study. Sugars were extracted from lignocellulose in OSR straw via alkaline pre-treatment and enzymatic hydrolysis. Then, OSR straw hydrolysates containing xylose and glucose were applied in repeated-batch and fed-batch fermentations to evaluate the feasibility and efficiency of butyric acid production via *C. tyrobutyricum* immobilized in a fibrous-bed bioreactor. Finally, efficient production of butyric acid from OSR straw hydrolysate was demonstrated using the fermentation process developed in this work.

2. Materials and methods

2.1. Cultures and medium

A mutant strain of *C. tyrobutyricum* ZJU8235 with inactivated *ack* gene (provided by Professor Zhinan Xu, Zhejiang University) that produced a high yield of butyric acid was used in this study [17,18]. Unless otherwise noted, the cultivation medium contained 30 g/L glucose, 5 g/L yeast extract (BBI), 5 g/L peptone (BBI), 3 g/L $(NH_4)_2SO_4$, 1.5 g/L K_2HPO_4, 0.6 g/L $MgSO_4{\cdot}7H_2O$, 0.03 g/L $FeSO_4{\cdot}H_2O$, and sterilized at 121 °C for 20 min.

2.2. Pretreatment and hydrolysis of oilseed rape straw

The OSR straw was obtained from the field (Jinhua, Zhejiang, China) during the harvest season in June 2015. After alkaline pretreatment (NaOH solution for 40 min at 121 °C) of OSR straw, the pH of solid fraction was controlled at 4.8 via addition of 4 M hydrochloric acid. Then, glucose was extracted by enzymatic hydrolysis using cellulase from *Trichoderma reesei* (Hunan Hong Ying Biotech Co. LTD) and β-glucosidase from *Aspergillus niger* (Jiangsu Ruiyang Biotech Co. LTD) for 24 h at 50 °C and 200 rpm. To optimize the hydrolysis process, three-level orthogonal tests have been designed to study the effects of OSR straw biomass (5, 10, 15%) and NaOH concentration (0.5, 0.63, 0.75 M) during the alkaline pre-treatment, as well as the biomass (5, 10, 15%), cellulase and β-glucosidase loading (70, 105, 140 U/g and 25, 37.5, 50 U/g, respectively) during enzymatic hydrolysis. Glass beads were added to enhance mass transfer during enzymatic hydrolysis. The OSR straw hydrolysate was filtered and stored at 4 °C for future use, its concentrated syrup was prepared by vacuum evaporation at 50 °C until the concentration of reducing sugars reached ~400 g/L.

2.3. Fermentation studies with fibrous-bed bioreactor

The fermentation system consisted of a 5 L stirred-tank fermentor and a 0.5-L fibrous-bed bioreactor (FBB) connected by a recirculation loop. The fermentation was operated at 37 °C and 150 rpm, and the pH was controlled at 6.0 by automatic addition of 2 M NaOH. The FBB was made of a glass column packed with spiral wound cotton towel (organic; 185 mm × 300 mm; 5 mm in thickness; with >95% porosity) [19]. Briefly, 100 mL of cell suspension in serum bottle was inoculated to the fermentor and cultivated until the cell density reached 6.0 (OD_{600}). Cell immobilization was then carried out by circulating the fermentation broth through the fibrous bed at a pumping rate of 0.25 mL/min. After approximately 48 h of continuous circulation, most of the cells were immobilized and the cell density in the broth became constant. The medium circulation rate was then increased to 100 mL/min to start the fermentation. For fed-batch fermentation, pulse feeding of concentrated carbon source was initiated whenever the reducing sugars were almost exhausted in the broth. Samples were taken at regular interval for the analysis of biomass, substrate, and product concentrations. The butyrate yield was calculated from the sum of butyrate obtained in batch, repeated-batch or fed-batch fermentation divided by the total glucose consumed in the medium.

2.4. Analytical methods

Cell density was analyzed by measuring optical absorbance at a wavelength of 600 nm (OD_{600}) on a spectrophotometer (SMP500-15135-DFXU). Concentrations of butyric acid and acetic acid were determined by gas chromatography with flame ionization detector and Stabilwax column (Restek 10624, USA) (30 m × 0.32 mm × 0.25 μm, d_f = 0.25) [20]. The concentrations of sugars were measured by using HPLC-ELSD with Bio-Rad Aminex HPX-87H column (40 °C oven temperature, 50 °C detector temperature, 350 kPa carrier gas pressure and 0. 25 mM sulfuric acid as mobile phase at a flow rate of 0.6 mL/min). The retention times of glucose, xylose, galactose and mannose were 9.027 min, 9.674 min, 9.620 min and 9.602 min. The concentrations of furfural and HMF were measured by HPLC with PDA (photo-diode array) detector set at 275 nm and 284 nm, respectively [21].

3. Results and discussion

3.1. The hydrolysis of OSR straw

Under optimal hydrolysis conditions, the highest glucose yield of 52.8% (g glucose/g OSR straw) was obtained (Supplemental Table S1) and the sugar composition of OSR hydrolysate was determined as: 50 g/L glucose and 6.78 g/L other sugars including xylose, galactose and mannose. According to a previous report [13], the concentration of xylose, which can be utilized by *C. tyrobutyricum* for butyric acid production [17], did not exceed 3.62 g/L in OSR hydrolysate. The concentrations of furfural and HMF were 0.011 mg/mL and 0.003 mg/mL, respectively. The SEM images monitoring the entire hydrolysis process demonstrated that the OSR straw was almost completely digested (Supplemental Fig. S2).

Please cite this article in press as: J. Huang, et al., Butyric acid production from oilseed rape straw by *Clostridium tyrobutyricum* immobilized in a fibrous bed bioreactor, Process Biochem (2016), http://dx.doi.org/10.1016/j.procbio.2016.08.019