Journal of Biotechnology 164 (2012) 260–265

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

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

Effects of pretreatment on the microbial community and L-lactic acid production in vinasse fermentation

Jianguo Liu[a,b], Qunhui Wang[a,*], Shuang Wang[a], Xiaohong Sun[c], Hongzhi Ma[a], Yukihiro Tushiro[d]

[a] Department of Environmental Engineering, University of Science and Technology Beijing, Beijing 100083, China
[b] College of Energy and Power Engineering, Inner Mongolia University of Technology, Inner Mongolia 010051, China
[c] Beijing Agro-Biotechnology Research Center, Beijing 100081, China
[d] Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

ARTICLE INFO

Article history:
Received 13 July 2012
Received in revised form 6 August 2012
Accepted 10 August 2012
Available online 30 August 2012

Keywords:
Microwave–alkali coupled pretreatment
PCR-DGGE
Microbial community
Lactobacillus casei
L-Lactic acid

ABSTRACT

Microwave–alkali and steam–alkali coupled pretreatments were carried out to improve the yield and optical purity of L-lactic acid produced using vinasse fermentation. *Lactobacillus casei* was inoculated into the system to initiate fermentation. Polymerase chain reaction denaturing gradient gel electrophoresis was used to analyze the microbial community during fermentation with and without the pretreatments. The original bacterial genus in vinasse was essentially inactivated, whereas *L. casei* became the dominant genus after 24 h of fermentation. The system subjected to microwave–alkali coupled pretreatment released more reducing sugars and produced more lactic acid (up to 30.32 g/L), which is twice that without pretreatment. In addition, the proportion of lactic acid in the organic acids also increased. The optical purity of the L-lactic acid produced under the microwave–alkali coupled pretreatment reached 91%, which is 2% higher than that under the steam–alkali coupled pretreatment and 7% higher than that under the control conditions. Therefore, the microwave–alkali coupled pretreatment is an effective method for the highly efficient bioconversion of vinasse into bioenergy.

1. Introduction

Vinasse, a by-product of the liquor brewing process, is produced in large amounts and it has high water content, but it is easily contaminated and it emits a foul smell, which make it harmful to the environment. At present, vinasse is mainly pretreated to disrupt its complex lignocellulosic structure and produce high-value products such as hydrogen, ethanol, methane and lactic acid (Laopaiboon et al., 2010; Zheng et al., 2009; Pattra et al., 2011). Marques and Rivas used recycled paper sludge and corncobs to produce lactic acid using *Lactobacillus rhamnosus* (Marques et al., 2008; Rivas et al., 2004). Shen and Xia (2006) and Vially et al. (2010) used *Lactobacillus delbrueckii* and *Rhizopus oryzae*, respectively, for the fermentation pretreatment of cellulosic waste to manufacture lactic acid. Lactic acid can be classified into L-, D-, and DL-lactic acid, which can be specifically produced through chemical synthesis (Madzingaido et al., 2003). L-Lactic acid has good biocompatibility because of its levorotatory configuration, which can only be obtained through biofermentation. It is widely used in the food, medicine, cosmetics, pesticide, tobacco, leather, and the textile industries, as well as in the production of biodegradable plastics from polylactic acid, the green solvents L-methyl lactate and L-ethyl lactate, and so on.

A previous study on the parameter optimization of vinasse lactic acid fermentation found that the efficiency of lactic acid production is very low without pretreatment (Liu et al., 2010). However, the efficiency is significantly improved after steam–alkali and microwave–alkali coupled pretreatment, which, compared with the non-pretreated group, increase lactic acid production by 94.5% and 192.1%, respectively. However, steam–alkali and microwave–alkali coupled pretreatments destroy the vinasse fiber structure and even kill microorganisms in the vinasse substrates because of the high temperatures they require, which affect the microbial community structure. The changes in the community structure directly affect the target product and the other metabolites of the fermentation system (Karadag and Puhakka, 2010; Lefeber et al., 2011). Moreover, the optical purity of L-lactic acid is more likely to be affected. However, few references have reported the effects of pretreatment on the structure of the microbial community and, ultimately, on the fermentation process and product yield of cellulosic material fermentation.

The traditional method for identifying the microbial community structure is purification, but isolation is necessary prior to purification. This requirement makes the traditional method time-consuming and complex. Furthermore, 85–99% of the original microorganisms cannot be purified. Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) overcomes

* Corresponding author at: Department of Environmental Engineering, University of Science and Technology Beijing, 30 Xueyuan Road, Haidian District, Beijing 100083, China. Tel.: +86 010 6233 2778; fax: +86 010 6233 2778.
E-mail address: wangqh59@sina.com (Q. Wang).

0168-1656/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jbiotec.2012.08.014

the limitations of the traditional method. Recently, PCR-DGGE has widely been used worldwide. Omar and Ampe (2000) used this method to study the dynamic changes in the strains and quantity of microorganisms during the different stages of fermentation. Siragusa et al. (2009) used PCR-DGGE analysis of the structural classification of lactic acid flora and dominant flora in the fermentation of sourdough. Camu et al. (2007) used PCR-DGGE to study the dynamic changes in lactic acid and acetic acid bacteria during the natural fermentation of Ghana beans. These studies help us understand the production of fermentation products and their relationship with the dynamic changes in flora.

This study investigates the changes in the microbial community during fermentation before and after pretreatment and their effects on the yield and optical purity of the resulting L-lactic acid. Thus, this study provides a theoretical basis for the further optimization of vinasse fermentation.

2. Materials and methods

2.1. Materials and equipment

Vinasse was collected from a liquor factory in Beijing. Vinasse is the residue of wine brewing that uses sorghum and rice husk as the raw material and accessory, respectively. In vinasse, fiber, hemicellulose, and lignin account for 27.23%, 21.17%, and 14.95% of the total dry matter, respectively. Hence, fiber accounts for more than half of the total dry matter, whereas the crude protein and the crude fat content are considerably less.

An industrial cellulase enzyme preparation that has 270 U g^{-1} CMC activity was purchased from Beijing Donghua Qiangsheng Biotechnology Co., Ltd. *Lactobacillus casei* was purchased from China Centre of Industrial Culture Collection (CICC). All reagents used for liquid were of chromatography grade. All other reagents were of analytical grade.

2.2. Pretreatment and fermentation of vinasse

In our previous research, the independent use of NaOH [at 0.02, 0.04, 0.06, 0.08, and 0.1 g g^{-1} vinasse (dry weight)], single microwave treatment (at 383, 523, and 700 W), single steam treatment (10 min, 121 °C), and microwave–alkali and steam–alkali coupled pretreatments were investigated. The optimum pretreatment conditions were follows: 0.06 g NaOH g^{-1} of vinasse (dry weight), 523 W of microwave power, and 121 °C steam for 20 min. We tested these optimum pretreatment conditions to examine the products and changes in the microbial community during vinasse fermentation (Liu et al., 2010; Wang et al., 2010).

The steam–alkali coupled pretreatment was performed as follows: samples with 0.06 g NaOH g^{-1} of vinasse (dry weight) were first adjusted to a solid–liquid ratio of 1:2, and then sterilized by autoclaving at 121 °C for 10 min. The samples were allowed to stand at room temperature for 2 h, and then water was added to the samples to compensate for the water lost during pretreatment (Wang et al., 2010).

The microwave–alkali coupled pretreatment was performed as follows: water was added to the samples containing 0.06 g NaOH g^{-1} vinasse (dry weight) to obtain a solid–liquid ratio of 1:2. The samples were placed into an Erlenmeyer flask with a ventilation plastic film and were heated by microwave at 700 W for 8 min. After allowing them to stand at room temperature for 2 h, water was added to the samples to replace the water lost during the pretreatment.

Simultaneous saccharification and fermentation was performed as follows: after the steam–alkali and microwave–alkali coupled pretreatments, cellulase was added to the vinasse at 60.6 U g^{-1} of vinasse (dry weight) and 10% of inoculation size was inoculated into the mixture. The vinasse was then placed in a fermentation bottle and was mixed thoroughly by oscillation for 1 min. Simultaneous saccharification and fermentation was performed at 35 °C and 50 rpm. Samples (1.5 mL) were collected regularly and centrifuged at 4000 × *g* for 5 min. Then, the supernatant liquid was collected to analyze the content of reducing sugar, lactic acid, L-lactic acid, and other organic acids. The entire fermentation process was carried out under anaerobic conditions.

2.3. Analytical methods

(1) DNA extraction and purification

DNA was extracted according to the K-CTAB method proposed by Zhou et al. (1996). The DNA was then purified using a Promega Wizard system (a DNA purification system for small amounts of DNA) after crude extraction. Agarose gel electrophoresis was used to analyze 5 μL of the crude and the purified DNA solution.

(2) PCR amplification

A pair of universal primers was used to amplify the purified DNA. The primers F338-GC (CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG) and R518 (ATT ACC GCG GCT GCT GGC) were used to amplify the purified DNA (EDC-810, Beijing Dongsheng Innovative Biotechnology Co., Ltd.). The PCR reaction system contained 1 μL of the template, 4 μL of dNTP solution (final concentration of 1 pmol μL^{-1}), 1 μL of each primer (10 mmol L^{-1}), 5 μL of 10× PCR buffer ($MgCl_2$ free), 1 μL of DNA polymerase, and sufficient sterile Milli-Q water to reach a final volume of 50 μL. The PCR amplification conditions were as follows: predenaturation for 3 min at 94 °C; denaturation for 60 s at 94 °C; annealing at 57 °C for 90 s; extension at 72 °C for 90 s for 30 cycles; and final extension at 72 °C for 10 min. The sizes of the PCR-amplified products were assessed through electrophoresis on 1% (w/v) agarose gel.

(3) Analysis of the PCR product using denaturing gradient gel electrophoresis (DGGE)

The DGGE of the PCR products was performed using a DCode Universal Mutation System (Bio-Rad Laboratories, Hercules, CA, USA). The PCR products were introduced into 8% (w/v) polyacrylamide gel with a 35–60% denaturant gradient. The electrophoresis was performed at a constant voltage of 100 V and at 60 °C for 6 h. After electrophoresis, the gels were silver stained and observed using a gel imaging analysis system (Gel Doc 2000, Bio-Rad, USA) and photographed.

2.4. Analysis method

The 16S rDNA was used in the bacterial community analysis to select the target band from the DGGE profiles. Through gel extraction, PCR re-amplification, and DGGE identification as a single band, 20 μL of the PCR amplification products were collected and submitted for sequencing of the different 16S rDNA sequences. The Sequin for win32 (NCBI, USA) disposable sequencing results were submitted to the genetic database at GenBank (http://www.ncbi.nlm.nih.gov/BLAST/). Then, sequence homology information was obtained by comparing the sequence with the database sequences using the BLAST program from GenBank.

Sugar, organic acids, and L/D-lactic acid were measured through liquid chromatography using a C18 amino acid column and a chiral column. Among them, L-lactic acid was determined using a chiral column (Model) (4.6 mm × 250 mm) and an ultraviolet absorption detector. Shimadzu Prominence was determined using an HPLC System. Copper sulphate (2 mmol L^{-1}) was used as the mobile phase at a flow rate of 1.0 mL min^{-1} and the detection was performed at 254 nm. The injection volume was 5 μL for both