Bioresource Technology 203 (2016) 173–180

Contents lists available at ScienceDirect

Bioresource Technology

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

Impacts of lignocellulose-derived inhibitors on L-lactic acid fermentation by *Rhizopus oryzae*

Li Zhang [a,b], Xin Li [a], Qiang Yong [a], Shang-Tian Yang [b,*], Jia Ouyang [a], Shiyuan Yu [a]

[a] College of Chemical Engineering, Nanjing Forestry University, 159 Longpan Road, Nanjing 210037, PR China
[b] William G. Lowrie Department of Chemical and Biomolecular Engineering, The Ohio State University, 151 West Woodruff Avenue, Columbus, OH 43210, USA

HIGHLIGHTS

- Inhibitors of *R. oryzae* in corn cob and corn stover hydrolysates were identified.
- For sugar degradation products, furfural and HMF were more toxic than weak acids.
- For phenolics from lignin, syringaldehyde and trans-cinnamic acid were most toxic.
- They strongly inhibited lactate dehydrogenase, but not alcohol dehydrogenase.
- They thus shifted the metabolic flux from lactic acid to ethanol biosynthesis.

ARTICLE INFO

Article history:
Received 6 November 2015
Received in revised form 28 November 2015
Accepted 8 December 2015
Available online 17 December 2015

Keywords:
Rhizopus oryzae
Inhibitors
Lactic acid
Lactate dehydrogenase
Alcohol dehydrogenase

ABSTRACT

Inhibitors generated in the pretreatment and hydrolysis of corn stover and corn cob were identified. In general, they inhibited cell growth, lactate dehydrogenase, and lactic acid production but with less or no adverse effect on alcohol dehydrogenase and ethanol production in batch fermentation by *Rhizopus oryzae*. Furfural and 5-hydroxymethyl furfural (HMF) were highly toxic at 0.5–1 g L^{-1}, while formic and acetic acids at less than 4 g L^{-1} and levulinic acid at 10 g L^{-1} were not toxic. Among the phenolic compounds at 1 g L^{-1}, trans-cinnamic acid and syringaldehyde had the highest toxicity while syringic, ferulic and *p*-coumaric acids were not toxic. Although these inhibitors were present at concentrations much lower than their separately identified toxic levels, lactic acid fermentation with the hydrolysates showed much inferior performance compared to the control without inhibitor, suggesting synergistic or compounded effects of the lignocellulose-degraded compounds on inhibiting lactic acid fermentation.
© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Considerable attention to synthetic plastic pollution and ecological interests are driving the development of technologies for the production of biodegradable plastic poly-lactic acid (PLA) using L-lactic acid as the monomer (Thongchul, 2013; Wang et al., 2015). *Rhizopus oryzae* can ferment sugars, starch and lignocellulosic biomass into pure L-(+)-lactic acid, which can be metabolized safely as food or feed additives, whereas bacterial fermentation and chemical synthesis usually yield mixtures of L-(+) and D-(−) forms (Juodeikiene et al., 2015; Kuo et al., 2015). Moreover, *Rhizopus* species can grow on cost-effective media and separation is more economical than recovery from bacterial fermentation broth (Zhang et al., 2015). In addition, *R. oryzae* can utilize sugars present in lignocellulosic biomass hydrolysates to produce L-lactic acid in a separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF) process (Saito et al., 2012; Wang et al., 2015; Zhang et al., 2015).

However, most of the lignocellulosic biomass applied to produce L-lactic acid must be pretreated due to the complex structure of lignocellulose (Wang et al., 2015; Zhang et al., 2015). A range of lignocellulose-degradation products (also called inhibitors) are inevitably generated during pretreatment (Jönsson and Martín, 2016) and they may inhibit subsequent enzymatic hydrolysis (Ximenes et al., 2010, 2011) and fermentation (Kim et al., 2013; Xiros and Olsson, 2014). These inhibitors are generally classified into three major groups: weak acids (including formic acid, acetic acid, and levulinic acid), furans (mainly 5-hydroxymethylfurfural (HMF) and furfural), and phenolic compounds (Almeida et al., 2007; Jönsson and Martín, 2016). Acetic acid is formed by the de-acetylation of hemicellulose. Furfural and HMF are from pentose and hexose dehydration, respectively. Subsequent degradation of furfural and HMF generates formic acid and

* Corresponding author. Tel.: +1 614 2926611; fax: +1 614 2923769.
E-mail address: yang.15@osu.edu (S.-T. Yang).

http://dx.doi.org/10.1016/j.biortech.2015.12.014
0960-8524/© 2015 Elsevier Ltd. All rights reserved.

levulinic acid, respectively. Various phenolic compounds are formed from lignin breakdown (Almeida et al., 2007). However, little is known about the tolerance of *R. oryzae* to these degradation products and their effects on L-lactic acid production.

R. oryzae converts glucose into cell biomass and L-lactic acid and ethanol as major metabolic products (Thongchul, 2013). Several key enzymes, including pyruvate decarboxylase (PDC), pyruvate carboxylase (PC), alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH), are required for production of these metabolites. The dissimilation of pyruvate is a key metabolic step in the balance between respiratory and fermentative metabolisms. The ATP and cofactors NADH produced in the TCA cycle facilitate the conversion of pyruvate to L-lactic acid by LDH (Thitiprasert et al., 2011). Meanwhile, pyruvate is decarboxylated to acetaldehyde by PDC followed by the oxidation of acetaldehyde to ethanol by ADH. LDH and ADH thus play key roles in driving the carbon metabolic flux from pyruvate toward either L-lactic acid or ethanol, and their activities would determine final product yields and the ratio of lactic acid to ethanol (Büyükkileci et al., 2006; Chotisubha-Anandha et al., 2011). Although the general properties and roles of LDH and ADH in the production of L-lactic acid and ethanol by *R. oryzae* is well understood, little is known about their activities as affected by the inhibitors present in the lignocellulosic biomass hydrolysate.

The aims of this study were to characterize the degradation products from corn stover and corn cob, the most abundant agricultural residues in China, and understand their inhibition effects on L-lactic acid production by *R. oryzae*. We focused on the effects of five carbohydrate-degradation products (formic acid, acetic acid, levulinic acid, furfural, and HMF) and ten lignin-degradation products (3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid, 4-hydroxybenzaldehyde, vanillin, *p*-coumaric acid, syringaldehyde, ferulic acid, and trans-cinnamic acid) found in the hydrolysates of corn stover and corn cob. These compounds were added at different concentrations in the synthetic medium containing glucose and xylose as substrates to study their inhibition effects on LDH and ADH activities, cell growth, and L-lactic acid and ethanol production by *R. oryzae*. The data obtained in this study can fill the information gap needed to guide the optimization of pretreatment and detoxification processes, facilitating the development of an economical process for L-lactic acid production by *R. oryzae* from lignocellulosic biomass such as corn cob and corn stover.

2. Methods

2.1. Enzymatic hydrolysis of corn cob and stover

The alkali-pretreated corn cob (ACC) (provided by Jiangsu Kangwei Biologic Co., Ltd.) was obtained by stewing milled corn cob in a 12-m^3 alkali extraction tank containing 7% (w/v) NaOH at 85–90 °C for 1 h. After removing the liquid, which had a high content of hemicellulose and was used for xylo-oligosaccharides production (Zhang et al., 2015), by vacuum filtration, the solid fraction (after drying) was stored in plastic bags at 4 °C until use. The ACC contained (dry weight basis) 55.8% cellulose, 21.4% hemicellulose and 10.7% lignin. Acid-catalyzed steam-exploded corn stover (ASCS), also obtained from Jiangsu Kangwei Biologic Co., Ltd., was prepared in a 3-L reactor with 1.29% (w/v) sulfuric acid at 0.8 MPa (gauge pressure) and 175 °C for 5 min (Zhu et al., 2014). After removing the liquid and drying, the solid fraction was stored in plastic bags at 4 °C until use. The ASCS contained (dry weight basis) 37.3% cellulose, 16.3% hemicellulose and 28.0% lignin.

Enzymatic hydrolysis of ACC and ASCS was performed using Cellic® CTec2 (Novozymes), a cellulase complex consisting of cellulases, β-glucosidases and hemicellulase, to degrade cellulose and hemicellulose to fermentable sugars. Unless otherwise noted, the hydrolysis was carried out in 250-mL Erlenmeyer flasks with 10% (w/v) solid loading and an enzyme dosage of 0.06 g g^{-1} biomass at pH 5.0–5.5, 50 °C, and 150 rpm. All experiments were duplicated and the average values are reported. Before use as a substrate in fermentation, the enzymatic hydrolysate was centrifuged and filtered to remove solid residues. ACC and ASCS hydrolysates were then concentrated in a rotary evaporator (BÜCHI R-200, BÜCHI Shanghai Trading LLC) at 70 °C and 160 mbar until the glucose concentration reached approximately 60 g L^{-1}.

2.2. Culture and media

R. oryzae NLX-M-1, obtained from the Institute of Biochemical Engineering, Nanjing Forestry University, Nanjing, China, was first grown on potato-dextrose agar plates at 30 °C for 3–5 days to generate spores. The spores were suspended in sterile water and the spore concentration was determined by counting the spores on a hemocytometer under a microscope. The seed culture medium consisted of (g L^{-1}): 50 glucose, 3 $(NH_4)_2SO_4$, 0.75 $MgSO_4{\cdot}7H_2O$, 0.20 $ZnSO_4{\cdot}7H_2O$, 0.30 KH_2PO_4 and 0.005 $FeSO_4{\cdot}7H_2O$. All media were sterilized by autoclaving at 121 °C, 15 psig for 30 min. The seed cultures were prepared in 250-mL Erlenmeyer flasks, each containing 50 mL preculture medium, inoculated with a spore suspension to 10^6 spores mL^{-1}, and incubated at 30 °C for 12 h in a rotary shaker agitated at 170 rpm.

2.3. Fermentation studies

Mixed sugars and enzymatic hydrolysates of ACC and ASCS were used to study cell growth, L-lactic acid and ethanol production, and key enzyme activities in batch fermentation. The 12-h seed-culture at an inoculation size of 5% (v/v) was introduced into a 250-mL Erlenmeyer flask containing 100 mL fresh medium. The synthetic medium contained (g L^{-1}): 1.5 $(NH_4)_2SO_4$, 0.38 $MgSO_4{\cdot}7H_2O$, 0.10 $ZnSO_4{\cdot}7H_2O$, 0.15 KH_2PO_4, and 60 glucose and 15 xylose as carbon source. For the enzymatic hydrolysates, they were supplemented with minerals with the same composition as in the synthetic medium. For good cell growth and L-lactic acid production, the pH was maintained at ~6.0 with $CaCO_3$ at 30 g L^{-1} unless otherwise noted. For the inhibition studies, inhibitors found in ACC and ASCS hydrolysates were added into the synthetic medium at desirable concentrations. After autoclaving the medium at 121 °C for 30 min, the fermentation was performed at 35 °C in a rotary shaker at 170 rpm for 2–3 days until all sugars were depleted or L-lactic acid production had ceased. All fermentation studies were repeated 2–3 times and average values are reported.

2.4. Preparation of cell extract

Fungal cells cultivated in fermentation medium were centrifuged (5 min at 3000 rpm) and the wet pellets were stored at −80 °C until use. Harvested pellets were washed with 0.1 M phosphate buffer (13.93 g K_2HPO_4 and 2.72 g KH_2PO_4 dissolved in 1000 mL distilled water, pH 7.4) to remove residual medium components. Then, cells were resuspended in 0.1 M phosphate buffer and disrupted by sonication (pulse on, 3 s; pulse off, 5 s; pulse 40%) on ice for 10 min using SCIENTZ JY 92-IIN sonicator (Ningbo, China) and centrifuged at 6000 *g* for 15 min at 4 °C. The supernatant (crude extract) was collected for analysis of the activities of LDH and ADH in both forward (f) and backward (b) directions.

Download English Version:

https://daneshyari.com/en/article/7072442

Download Persian Version:

https://daneshyari.com/article/7072442

Daneshyari.com