SHORT REPORT

Dry biodetoxification of acid pretreated wheat straw for cellulosic ethanol fermentation

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Abstract

Background: The efficient removal of toxic inhibitors from pretreated lignocellulose biomass is crucially important for consequent cellulosic ethanol fermentation. *A. resinae* ZN1 biodetoxifies all toxic inhibitors at the neutral pH of 4–6, and the neutralization of acid catalyst in the pretreated lignocellulose is required. However, aqueous alkaline solutions such as sodium hydroxide solution and calcium hydroxide slurry are used which generate several difficulties.

Results: In this study, a dry biodetoxification method was investigated using dry calcium carbonate ($CaCO_3$) powder as an acid-neutralizing reagent to avoid the use of an aqueous alkaline solution. Dry biodetoxification provides a mild and stable pH without the generation of phenolic compounds. The acid pretreated and dry biodetoxified wheat straw was used as the feedstock of ethanol fermentation and the same performance with the wet biodetoxification using aqueous $Ca(OH)_2$ slurry. The 72 g/L or 9.1% (v/v) of ethanol produced from wheat straw was very close to that of ethanol from corn starch.

Conclusions: Dry biodetoxification provided a practical method to simplify the process of conventional wet biodetoxification to reduce the time, cost and labor.

Keywords: Biodetoxification, Acid pretreatment, Ethanol, Calcium carbonate ($CaCO_3$) powder, Lignocellulose

Introduction

Pretreatment is an essential step to overcome the recalcitrance of lignocellulosic biomass to facilitate enzymatic hydrolysis. Pretreatment of lignocellulosic biomass generates various toxic inhibitors such as furfural, 5-hydroxymethylfurfural (HMF), acetic acid and phenolic aldehydes, which required to be removed before bioconversion of enzymatic hydrolysis and fermentation process (Klinke et al. 2014; Jing et al. 2009; Palmqvist and Hahn-Hagerdal 2000; Dong and Bao 2010). A kerosene fungus *Amorphotheca resinae* ZN1 was isolated for complete removal of inhibitors from acid pretreated lignocellulose biomass in our previous studies (Zhang et al. 2010). The biodetoxified biomass has been applied to produce ethanol (Liu et al. 2018), chiral lactic acid (Qiu et al. 2017,

2018), glutamic acid (Wen et al. 2018), citric acid (Zhou et al. 2017), and gluconic acid (Zhang et al. 2016).

Amorphotheca resinae ZN1 grows and metabolizes at the neutral pH range of 4–6, thus the acid catalyst in the pretreated biomass has to be neutralized before biodetoxification (Zhang et al. 2010). Generally, aqueous calcium hydroxide slurry was used to neutralize the acid to pH 5 before biodetoxification. However, the use of calcium oxide (CaO) and calcium hydroxide slurry and mixing of small portion of them with a large amount of solid biomass generate several difficulties including: (1) the reduced solid contents of lignocellulosic biomass; (2) incomplete mixing between the solid particles and liquid slurry; (3) generation of phenolic compounds; (4) expensive and unsafe to handle. Furthermore, a high amount of carbon dioxide is generated and the high energy consumption is required during calcium oxide (CaO) manufacture. Here, we proposed a complete dry biodetoxification using inert calcium carbonate ($CaCO_3$) powder to neutralize the acid catalyst in lignocellulose biomass to avoid the use of the aqueous alkaline solution.

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In the dry biodetoxification, pH changes slowly and complete mixing could be realized during the mild neutralization period. $CaCO_3$ neutralized acid pretreated biomass can be kept in an open environment because of its stable nature and convenient onsite biodetoxification can be performed after pretreatment. This study tested the reaction balance of the biodetoxification, metabolism, and neutralization, as well as cellulosic ethanol fermentation performance.

Methods

Lignocellulosic biomass and reagents

Wheat straw was obtained from Nanyang, Henan, China, in the year 2018. The collected wheat straw was washed, dried and milled using a hammer crusher to pass through the 10 mm (diameter) apertures, and stored in plastic bags at room temperature in dry condition. The raw wheat straw contained 34.59% of cellulose, 24.26% of xylan, 18% of lignin, and 9.6% of ash by weight percentage measured according to NREL protocols (Sluiter et al. 2008, 2012).

Cellulase enzyme Cellic CTec 2.0 was purchased from Novozymes (China), Beijing, China. Yeast extract and peptone were purchased from Oxoid, Basingstoke, Hampshire, UK. $CaCO_3$ and $Ca(OH)_2$ were purchased from Shanghai Titanchem Co. Other chemicals and reagents included glucose, xylose, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, NaOH, and H_2SO_4 were purchased from Lingfeng Chemical Reagent Co.

Strains and media

Biodetoxification fungus *Amorphotheca resinae* ZN1 was isolated in our previous studies and stored in China General Microorganism Collection Center (CGMCC), Beijing, China. The potato dextrose agar (PDA) medium for *A. resinae* ZN1 included 200 g/L potato extract juice, 20 g/L glucose, and 20 g/L agar.

Ethanol fermentation strain *Saccharomyces cerevisiae* XH7 was kindly provided by Prof. XM Bao from Shandong University, Jinan, Shandong, China (Li et al. 2015). The medium used for *S. cerevisiae* XH7 included: (i) medium for strain activation; YPD included, 20 g/L glucose, 20 g/L peptone and 10 g/L yeast extract; (ii) for seed culture, 5% (w/w) of biodetoxified wheat straw, 2 g/L KH_2PO_4, 2 g/L $(NH_4)_2SO_4$, 1 g/L $MgSO_4$ and 10 g/L yeast extract; (iii) for adaptation, 10% (w/w) of biodetoxified wheat straw, 2 g/L KH_2PO_4, 2 g/L $(NH_4)_2SO_4$, 1 g/L $MgSO_4$ and 10 g/L yeast extract; (iv) for SSCF, 2 g/L KH_2PO_4, 2 g/L $(NH_4)_2SO_4$, 1 g/L $MgSO_4$ and 10 g/L yeast extract.

Dry acid pretreatment, dry biodetoxification and ethanol fermentation

Dry acid pretreated wheat straw was prepared according to Zhang et al. (2011) and He et al. (2014). The pretreated wheat straw contained 26.7 mg/g DM of glucose and 153.3 mg/g DM of xylose, 3.14 mg/g DM of furfural, 1.46 mg/g DM of HMF, 12.52 mg/g DM of acetic acid, 0.43 mg/g DM of vanillin, 0.51 mg/g DM of syringaldehyde and 0.36 mg/g DM of 4-HBA, determined according to NREL protocols (Sluiter et al. 2008, 2012).

The dry acid pretreated wheat straw was neutralized to pH 5.5 with dry $CaCO_3$ powder and 20% of $Ca(OH)_2$ slurry, respectively, and disk milled to remove the long cellulosic fibers. Then, inoculated with 10% (v/v) solid seed of *A. resinae* ZN1 for biodetoxification at ambient temperature for 60 h. Ethanol fermentation was performed in 5 L bioreactor. The biodetoxified wheat straw was pre-hydrolyzed into liquid hydrolysate slurry at 50 °C, pH 5 for 12 h. Then, the simultaneous saccharification and co-fermentation (SSCF) was performed by inoculating shortly adapted fermentation yeast *Saccharomyces cerevisiae* XH7 into the hydrolysate at 10% (v/v) in the same bioreactor. The nutrients addition included 2 g/L of KH_2PO_4, 2 g/L of $(NH_4)_2SO_4$, 1 g/L of $MgSO_4$ and 10 g/L of yeast extract. The SSCF was carried out at 30 °C, pH 5.5 for 120 h.

Analysis

Glucose, xylose, ethanol, and inhibitors such as acetic acid furfural and HMF were analyzed using HPLC (LC-20AD, refractive index detector RID-10A, Shimadzu, Japan) with Bio-Rad Aminex HPX-87H column at 65 °C. The mobile phase was 5 mM H_2SO_4 at the flow rate of 0.6 mL/min. Syringaldehyde, vanillin, and hydroxybenzaldehyde (4-HBA) were analyzed using the reversed-phase HPLC (LC-20AT, UV/VIS detector SPD-20A, Shimadzu, Japan) with a YMC-Pack ODS-A column at ambient temperature (Liu et al. 2018). The mobile phase was 100% acetonitrile with 0.1% formic acid at the flow rate of 1.0 mL/min. The yeast cell viability during the simultaneous saccharification and co-fermentation (SSCF) was assayed on the YPD medium Petri dish by counting colony-forming units (CFU), when the 100 μL of the 10^5 diluted fermentation broth sample withdrawn after every 12 h was stretched and cultured at 30 °C for 48 h (Gu et al. 2015).

Results and discussion

Dry neutralization of acid catalyst using calcium carbonate and the biodetoxification assay

Dry biodetoxification of acid pretreated wheat straw for cellulosic ethanol fermentation was investigated using $CaCO_3$ powder as a pH neutralizing agent. Figure 1 shows the biodetoxification profiles of the major inhibitors and sugar consumption using dry neutralization by $CaCO_3$ powder. Furfural, HMF, acetic acid, and the three phenolic aldehydes were degraded quickly by *A.*
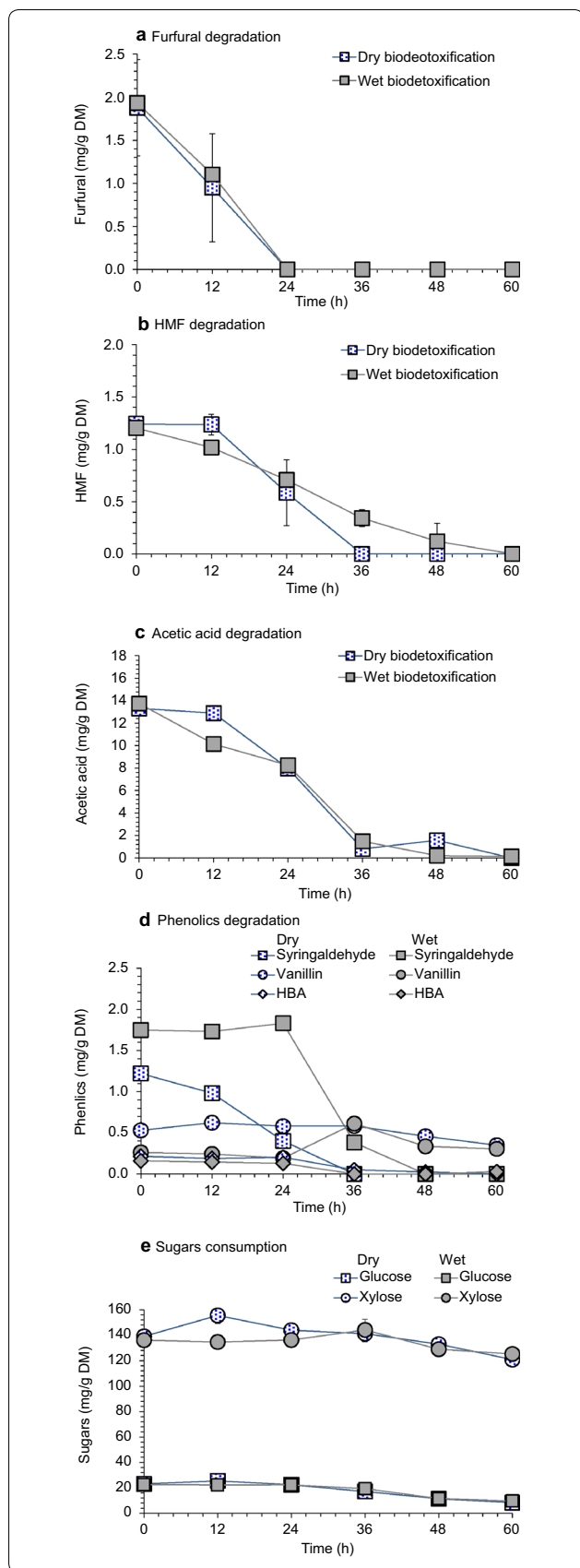
Fig. 1 Inhibitors and sugars consumption profiles in dry biodetoxification using $CaCO_3$ powder and wet biodetoxification using $Ca(OH)_2$ slurry. Biodetoxification was performed at ambient temperature. **a** Furfural degradation; **b** HMF degradation; **c** acetic acid degradation; **d** phenolics (syringaldehyde, vanillin and HBA) degradation and **e** sugar (glucose and xylose) consumption. Conditions: 10% solid seed of *A. resinae* ZN1, biodetoxified for 60 h

resinae ZN1 (Fig. 1a–d). The fermentable sugars including glucose and xylose maintained approximately constant before and after the biodetoxification (Fig. 1e). The results indicate that the biodetoxification of the inhibitors in the acid pretreated wheat straw was fast and complete with the use of both $CaCO_3$ powder and aqueous $Ca(OH)_2$ slurry and the fermentable sugars were almost untouched. This study proves that dry biodetoxification is a simple and an efficient method to remove all inhibitors compares to conventional wet biodetoxification within 24–48 h (Zhang et al. 2010), that is enough for performing the consequent ethanol production by the SSCF process.

Evaluation of ethanol fermentation using dry biodetoxified wheat straw

The ethanol production through simultaneous saccharification and co-fermentation (SSCF) of the acid pretreated and dry biodetoxified wheat straw was investigated using *S. cerevisiae* XH7. Figure 2 shows the ethanol fermentation at 30% (w/w) solids loading using dry and wet biodetoxified feedstocks. The results show that glucose was quickly consumed after inoculation of *S. cerevisiae* XH7 and converted into ethanol and xylose was consumed continuously till the end of fermentation by *S. cerevisiae* XH7 in both (dry and wet) biodetoxified wheat straw (Fig. 2a, b). The 72 g/L or 9.1% (v/v) of ethanol was obtained from dry biodetoxified wheat straw, very close to that of ethanol from corn starch (Liu and Bao 2017). This may be economically viable to produce in large scale.

Amorphotheca resinae ZN1 is a biodetoxification strain and used on the pretreated wheat straw to remove the inhibitors. After the biodetoxification, wheat straw was enzymatically hydrolyzed into fermentable sugars such as glucose and xylose. The fermentation microorganism *S. cerevisiae* XH7 was used to convert the glucose and xylose sugars to ethanol. The aerobic fungus *Amorphotheca resinae* ZN1 was no long alive because of the anaerobic condition in hydrolysis and ethanol fermentation.

The dry biodetoxification method performs excellent cellulosic ethanol production and provides mild and stable pH with no reduction of fermentable sugars and solid
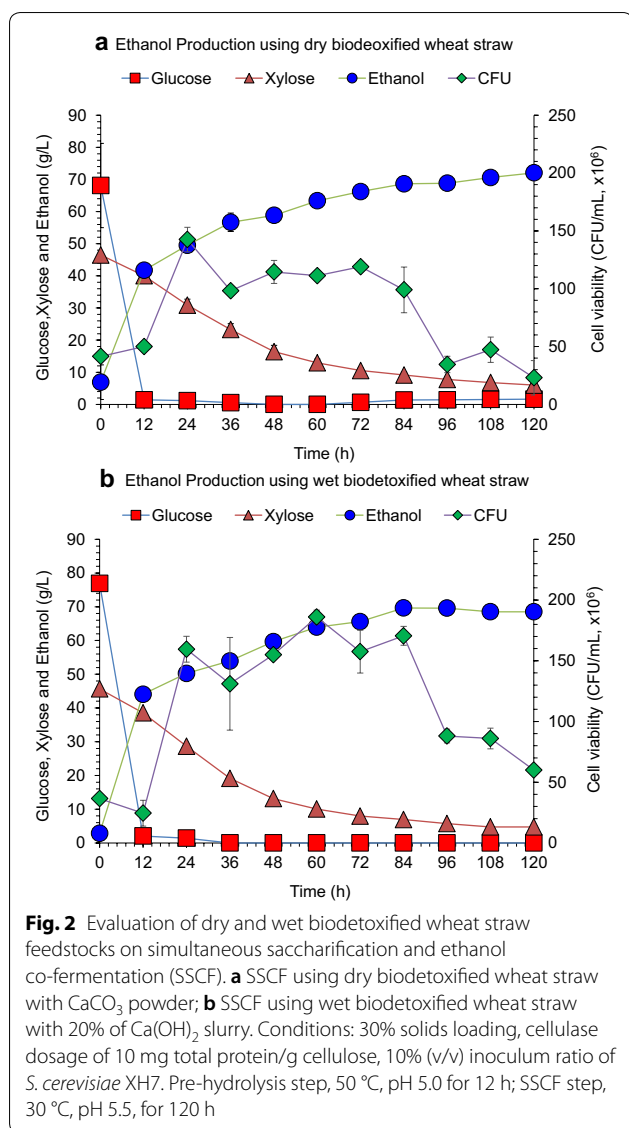
Fig. 2 Evaluation of dry and wet biodetoxified wheat straw feedstocks on simultaneous saccharification and ethanol co-fermentation (SSCF). **a** SSCF using dry biodetoxified wheat straw with $CaCO_3$ powder; **b** SSCF using wet biodetoxified wheat straw with 20% of $Ca(OH)_2$ slurry. Conditions: 30% solids loading, cellulase dosage of 10 mg total protein/g cellulose, 10% (v/v) inoculum ratio of *S. cerevisiae* XH7. Pre-hydrolysis step, 50 °C, pH 5.0 for 12 h; SSCF step, 30 °C, pH 5.5, for 120 h

contents and without the generation of phenolic compounds. Dry biodetoxification is a practical method to improve biodetoxification efficiency for cellulosic ethanol production from acid pretreated lignocellulose feedstock.

Conclusion

Dry biodetoxification in this study realized complete mixing of solid–solid particles without loss of solid content and generation of phenolic derivatives which is more safe and costly effective than that of conventional wet biodetoxification. Consequently, use of dry biodetoxified biomass achieved high cellulosic ethanol production [72 g/L or 9.1% (v/v)] and all advantages of this method make it possible for further industrial applications.

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