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Sodium borohydride removes aldehyde inhibitors for enhancing biohydrogen fermentation

Richen Lin [a], Jun Cheng [a,*], Lingkan Ding [a], Wenlu Song [a,b], Junhu Zhou [a], Kefa Cen [a]

[a] State Key Laboratory of Clean Energy Utilization, Zhejiang University, Hangzhou 310027, China
[b] Department of Life Science and Engineering, Jining University, Jining 273155, China

HIGHLIGHTS

- $NaBH_4$ detoxification was performed for effective removal of aldehyde inhibitors.
- Detoxification efficiencies of furan aldehydes were higher than those of phenolics.
- $NaBH_4$ provided reducing power for inhibitors reduction and enhanced H_2 production.
- Addition of 30 mM $NaBH_4$ resulted in 99.3% recovery of hydrogen yield.

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ABSTRACT

To enhance biohydrogen production from glucose and xylose in the presence of aldehyde inhibitors, reducing agent (i.e., sodium borohydride) was *in situ* added for effective detoxification. The detoxification efficiencies of furfural (96.7%) and 5-hydroxymethylfurfural (5-HMF, 91.7%) with 30 mM $NaBH_4$ were much higher than those of vanillin (77.3%) and syringaldehyde (69.3%). Biohydrogen fermentation was completely inhibited without detoxification, probably because of the consumption of nicotinamide adenine dinucleotide (NADH) by inhibitors reduction ($R–CHO + 2NADH \rightarrow R–CH_2OH + 2NAD^+$). Addition of 30 mM $NaBH_4$ provided the reducing power necessary for inhibitors reduction ($4R–CHO + NaBH_4 + 2H_2O \rightarrow 4R–CH_2OH + NaBO_2$). The recovered reducing power in fermentation resulted in 99.3% recovery of the hydrogen yield and 64.6% recovery of peak production rate. Metabolite production and carbon conversion after detoxification significantly increased to 63.7 mM and 81.9%, respectively.

1. Introduction

The extensive utilisation of non-renewable fossil fuels (e.g., coal and petroleum) has led to severe environmental pollution and energy crisis, both of which emphasise the significance of renewable biofuel production (Caspeta et al., 2013; Sims et al., 2010). Hydrogen has attracted worldwide attention because of its high energy density and clean combustion product. Fermentative hydrogen production from lignocellulosic biomass offers the advantages of energy-saving, longer-term sustainability and favourable carbon balances (Cheng et al., 2011; Guwy et al., 2011; Kothari et al., 2012). Effective pretreatments (e.g., acid hydrolysis, steam explosion and hot water treatment) of lignocellulosic biomass are generally required prior to fermentation (Haghighi Mood et al., 2013; Lin et al., 2015). Besides large amounts of fermentable sugars (e.g., glucose and xylose) generated from cellulose and hemicellulose, pretreatment of lignocellulosic biomass generates inhibitory compounds that interfere with microbial growth and poses a significant challenge for efficient biohydrogen fermentation. Typical lignocellulosic-derived inhibitory compounds include furan aldehydes [e.g., furfural and 5-hydroxymethylfurfural (5-HMF)] and phenolic aldehydes (e.g., vanillin and syringaldehyde), which are recognised as strong inhibitors for microbial fermentation (Behera et al., 2014; Mills et al., 2009).

The development of detoxification strategies that can decrease the aldehydes concentration in hydrolysates and lessen inhibitory effects on microbes is significant for efficient biofuel production. Different detoxification approaches, which are categorized as physical, chemical, and biological methods, have been proposed to convert inhibitory compounds into inactive forms or lower the concentrations of these compounds (Palmqvist and Hahn-Hägerdal, 2000; Parawira and Tekere, 2010). Vacuum evaporation is a physical method used to decrease the contents of volatile inhibitors (e.g., furfural and vanillin) in the hydrolysate. However, this method also

* Corresponding author. Tel.: +86 571 87952889; fax: +86 571 87951616.
E-mail address: juncheng@zju.edu.cn (J. Cheng).

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increases the concentration of non-volatile inhibitors (e.g., lignin derivatives) and consequently inhibitory effects. Chemical approaches including calcium hydroxide overliming, ion exchange resins, activated charcoal and electrochemical method have been studied for detoxification. Detoxification using NH_4OH is optimised at pH 9.0 and 60 °C. The removal efficiencies of furfural, 5-HMF and phenols in spruce hydrolysate exceed 60% (Alriksson et al., 2006). Up to 90% of the furan aldehydes and 8.9% the fermentable sugars are removed from starch hydrolysate using activated carbon, and the subsequent biohydrogen production is enhanced by 70% (Lee et al., 2011). The electrochemical method has been applied to remove phenolic compounds with 71% of the total phenolics removed in rice straw hydrolysate (Lee et al., 2015).

However, the aforementioned chemical detoxification methods require a separate process step, strict detoxification conditions (e.g., time period, pH and temperature), and inevitably lead to the loss of fermentable sugars and increase of production costs. Biological detoxification also results in the loss of sugars and the need for prolonged incubation time with the detoxifying microbes (Parawira and Tekere, 2010). A recent focus in chemical detoxification involves the possibility of performing the detoxification and fermentation in the same bioreactor (i.e., *in situ* detoxification) using reducing agents. *In situ* addition of reducing agent (dithionite, sulfite and borohydride) under mild reaction conditions to hydrolysates of spruce wood or sugarcane bagasse dramatically enhances bioethanol fermentation. The treatment can be performed at a temperature and pH suitable for bioethanol fermentation. Results show that adjusting the pH or temperature to achieve the desired detoxification effect is not required (Alriksson et al., 2011). The detoxification with reducing agent ($Na_2S_2O_4$) effectively improves the fermentability of pretreated spruce, which contains aldehyde inhibitors (e.g., furfural and 5-HMF) (Xiros and Olsson, 2014). Inhibitory hydrolysates of spruce wood and sugarcane bagasse are treated with the reducing agents (i.e., dithionite and sulfite) to achieve improved fermentability (Alriksson et al., 2011). Addition of 10 mM dithionite gives the best results on ethanol fermentation. Treatment of the Norway spruce hydrolysate with sodium borohydride ($NaBH_4$) improves the ethanol yield from 0.02 to 0.30 g/g using *Saccharomyces cerevisiae* (Cavka and Jonsson, 2013). $NaBH_4$ can be added in high concentrations (up to about 50 mM) to the fermentation without affecting the fermenting microorganism negatively. This result suggests borohydride is suitable for chemical *in situ* detoxification.

Although reducing agents are effective to enhance bioethanol fermentation, studies on their effects on aldehyde inhibitors removal, fermentable sugars degradation and subsequent biohydrogen fermentation remain limited. In the present study, $NaBH_4$ was selected as a reducing agent for inhibitors detoxification. $NaBH_4$ is an industrial chemical that can be considered for large-scale processes (Rittmeyer and Wietelmann, 2002), and is the most frequently used hydride in modern organic chemistry. It is a mild and inexpensive reagent for applications in a wide range of reduction processes (Periasamy and Thirumalaikumar, 2000). The effects of different additions of $NaBH_4$ on aldehyde inhibitors detoxification, glucose and xylose consumption, subsequent biohydrogen fermentation and metabolite distribution are comprehensively investigated. The inhibitory effect of aldehydes on biohydrogen fermentation and principle of $NaBH_4$ detoxification are also elucidated.

2. Methods

2.1. Inoculum

Mixed hydrogen-producing bacteria (HPB) were isolated and enriched from anaerobic digestion sludge sampled from a biogas plant in Zhejiang Province, China. The sludge was pre-treated in an autoclave at 100 °C for 30 min to suppress the non-spore-forming hydrogen consumers (e.g., methanogenic microorganisms), then was acclimated three times to activate spore-forming HPB. The dominant species in HPB was *Clostridium butyricum* (Cheng et al., 2010).

2.2. Detoxification of fermentative inhibitors by $NaBH_4$

The detoxification experiments, as shown in Table 1, were performed in glass bottles. Each bottle was added with 225 mL feedstock solution (1.5 g of glucose and 1 g of xylose) and fermentative inhibitors (15 mM furfural, 15 mM 5-HMF, 15 mM vanillin and 15 mM syringaldehyde). Then $NaBH_4$ (0, 15, 30 or 45 mM) was directly added into each bottle, and the reaction was allowed to proceed for 20 min at 35 °C to determine the detoxification effect. Solutions before and after detoxification were sampled to analyse concentration changes of glucose, xylose, furfural, 5-HMF, vanillin and syringaldehyde.

2.3. Biohydrogen fermentation

The detoxified and un-detoxified solutions (225 mL) were subjected to biohydrogen fermentation to investigate the detoxification effect of $NaBH_4$ on fermentability. Table 1 shows the experimental design of biohydrogen fermentation. Control experiment was conducted using 1.5 g of glucose and 1 g of xylose as feedstock supplemented with 0.5 g of yeast extract as the nitrogen source. The initial pH was adjusted to 6.0 ± 0.1 by using 6 M HCl and 6 M NaOH solution. The bottles were inoculated with 25 mL of HPB, sealed with rubber stoppers, purged with nitrogen gas for 10 min, and maintained at 35 ± 1.0 °C for biohydrogen fermentation. All experiments were performed in duplicate.

2.4. Analytical methods

The concentrations of glucose, xylose, furfural, 5-HMF, vanillin and syringaldehyde were determined using a high-performance liquid chromatograph (HPLC; Agilent 1200, USA) equipped with an Aminex HPX-87H column (Bio-Rad, USA). Five mM H_2SO_4 was used as the mobile phase at 65 °C and a flow rate of 0.5 mL/min. Glucose and xylose were analysed using a refractive index detector. Furfural, 5-HMF, vanillin and syringaldehyde were analysed using an ultraviolet detector at 278 nm.

Hydrogen and carbon dioxide concentrations were analysed on a gas chromatography system (GC; Agilent 7820A, USA) equipped with a thermal conductivity detector and a 5 A column (Φ 3 mm × 3 m; Agilent, USA). The temperatures of injection port and thermal conductivity detector were 200 and 300 °C, respectively. The initial column temperature was set at 65 °C for 1 min, increased to 145 °C at a heating rate of 25 °C/min, and then held at 145 °C for 3 min. Argon gas was used as the carrier gas at a flow

Table 1
Experimental design of $NaBH_4$ detoxification and biohydrogen fermentation.

No.	Detoxification process		Biohydrogen fermentation
	Aldehyde inhibitors[a] (mM)	$NaBH_4$ (mM)	Glucose + xylose (g/L)
1	0	0	6 + 4
2	60	0	6 + 4
3	60	15	6 + 4
4	60	30	6 + 4
5	60	45	6 + 4

[a] Aldehyde inhibitors = furfural (15 mM) + 5-HMF (15 mM) + vanillin (15 mM) + syringaldehyde (15 mM).