Bioresource Technology 136 (2013) 368–376

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Detoxification of lignocellulosic hydrolysates using sodium borohydride

Adnan Cavka [a,b], Leif J. Jönsson [a,*]

[a] Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden
[b] Industrial Graduate School, Umeå University, SE-901 87 Umeå, Sweden

HIGHLIGHTS

- Inhibitors in lignocellulosic hydrolysates prevent efficient bioconversion.
- A new method for detoxification of lignocellulosic hydrolysates is presented.
- Sodium borohydride treatment detoxifies hydrolysates by reduction of inhibitors.
- No extra process step required: can be performed as chemical in situ detoxification.
- Indicates difference between inhibition of microbes and of enzymes.

ARTICLE INFO

Article history:
Received 7 January 2013
Received in revised form 1 March 2013
Accepted 4 March 2013
Available online 14 March 2013

Keywords:
Lignocellulose
Sodium borohydride
Detoxification
Cellulosic ethanol
Inhibitors

ABSTRACT

Addition of sodium borohydride to a lignocellulose hydrolysate of Norway spruce affected the fermentability when cellulosic ethanol was produced using *Saccharomyces cerevisiae*. Treatment of the hydrolysate with borohydride improved the ethanol yield on consumed sugar from 0.09 to 0.31 g/g, the balanced ethanol yield from 0.02 to 0.30 g/g, and the ethanol productivity from 0.05 to 0.57 g/(L × h). Treatment of a sugarcane bagasse hydrolysate gave similar results, and the experiments indicate that sodium borohydride is suitable for chemical in situ detoxification. The model inhibitors coniferyl aldehyde, *p*-benzoquinone, 2,6-dimethoxybenzoquinone, and furfural were efficiently reduced by treatment with sodium borohydride, even under mild reaction conditions (20 °C and pH 6.0). While addition of sodium dithionite to pretreatment liquid from spruce improved enzymatic hydrolysis of cellulose, addition of sodium borohydride did not. This result indicates that the strong hydrophilicity resulting from sulfonation of inhibitors by dithionite treatment was particularly important for alleviating enzyme inhibition.

1. Introduction

Dwindling oil supplies combined with increased demand suggest the need for alternative feedstocks for production of fuels, chemicals, and materials such as plastics. Replacing oil and other fossil resources with sustainable and renewable lignocellulosic raw materials is therefore an exciting opportunity (Ragauskas et al., 2006; Lynd et al., 2008; Sims et al., 2010). Lignocellulosic raw materials such as wood residues and sugarcane bagasse are attractive as feedstock, since they are plentiful and relatively inexpensive.

Lignocellulose, which consists mainly of polymers such as lignin, cellulose and hemicellulose, is a recalcitrant material that offers a challenging problem when it comes to conversion to fermentable sugars. Thermochemical pretreatment, which involves high temperatures and use of acids, alkali or other chemicals, is usually required to make the raw material accessible to hydrolytic enzymes such as cellulases and hemicellulases. Severe conditions used during pretreatment usually lead to partial breakdown of lignin and hemicellulose-derived sugars, and result in the formation of unwanted by-products that in sufficiently high concentrations inhibit both fermenting microorganisms and cellulose-degrading enzymes. Fermentation inhibitors include many different compounds that can be categorized into a few groups, such as aromatic (mostly phenolic) compounds, furan aldehydes, and aliphatic acids (Larsson et al., 1999). Phenolic compounds can also inhibit enzymatic hydrolysis of cellulose (Ximenes et al., 2010). Lignocellulosic hydrolysates contain varying concentrations of inhibitory compounds depending on the composition of the raw material used in the process, and the severity and type of pretreatment used. There are several ways to counteract problems with fermentation inhibitors. The use of resistant fermenting microbes or chemical or biological treatments for detoxification of slurries and hydrolysates have been investigated.

Detoxification, which involves different types of treatments of the hydrolysates have been shown to dramatically improve the fermentability of strongly inhibitory lignocellulosic hydrolysates

* Corresponding author. Tel.: +46 90 7866811; fax: +46 90 7867655.
E-mail address: leif.jonsson@chem.umu.se (L.J. Jönsson).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.03.014

(Alriksson et al., 2006, 2011). The main objection that has been raised against detoxification is the need for an additional process step that would make the bioalcohol process more costly (Hamelinck et al., 2005). Alriksson et al. (2011) showed that this objection does not necessarily hold true, since it was discovered that treatment with reducing agents, including dithionite and hydrogen sulfite, can greatly improve the fermentability of lignocellulose hydrolysates when added directly to the fermentation vessel in the presence of the fermenting microorganism, *Saccharomyces cerevisiae*. Cavka et al. (2011) later showed that detoxification with sulfur oxyanions, such as sulfite and dithionite, results in sulfonation of fermentation inhibitors, a mechanism that also converts them to highly hydrophilic charged molecules. Treatment of lignocellulosic hydrolysates with reduced sulfur compounds also has positive effects on *Escherichia coli* (Nieves et al., 2011).

In this study we have investigated the effects of sodium borohydride on lignocellulosic hydrolysates and we have also used mass spectrometry (MS) to study the effects on selected model inhibitors. Furthermore, the effects were also compared to those of sodium dithionite and sodium hydrogen sulfite, which previously were shown to be potent agents of detoxification (Alriksson et al., 2011). As treatment of inhibiting compounds with sodium borohydride by necessity will generate other products than the sulfonated compounds that were identified after treatment with sulfite or dithionite, it is of mechanistic interest to compare the efficiency of sodium borohydride with that of the sulfur oxyanions. Furthermore, as is also the case with sulfite and dithionite, sodium borohydride is an industrial chemical that can be considered for large-scale processes (Rittmeyer and Wietelmann, 2002). In addition, there is a connection between sodium borohydride and dithionite, since sodium borohydride is used in the production of dithionite. Thus, both scientific and technical reasons motivate the study of the effects of sodium borohydride on lignocellulosic hydrolysates.

2. Methods

2.1. Pretreatment and hydrolysis of lignocellulosic raw materials

The hydrolysates used in this study were produced from sugarcane bagasse or from chipped wood of Norwegian spruce (*Picea abies*). The raw materials were first pretreated thermochemically and the resulting slurries were then converted by enzymatic hydrolysis. The liquid fractions obtained after removal of the lignin-rich solid residues remaining after pretreatment and enzymatic hydrolysis are referred to as hydrolysates. The hydrolysates thus contain sugars derived from both hemicellulose and cellulose.

The pretreatment of bagasse and spruce was performed by SEKAB E-Technology in the Swedish biorefinery demonstration plant (Örnsköldsvik, Sweden). The bagasse was pretreated in continuous mode in a 30-L reactor, which was filled approx. to 50% during operation. The pressure was 14 bar (188 °C), and the bagasse was impregnated with SO_2 (0.3 kg SO_2/h, which corresponds to around 0.6% SO_2/kg of sugarcane bagasse (DW, dry weight)). The residence time in the reactor was 10 min, and the resulting pH was 2.1. Unbarked spruce wood chips were treated in a continuous mode in the same reactor, but at a pressure of 18 bar (204 °C). There was an addition of 1.2–1.3 kg SO_2/h, which corresponds to 1% SO_2/kg of spruce wood chips (DW). The residence time in the reactor was 7–8 min, and the resulting pH was 1.4–1.5. After pretreatment, the spruce and bagasse slurries were cooled and stored at 4 °C until further use.

The pH of the bagasse slurry was adjusted to 5.3 with a 5 M solution of sodium hydroxide. The dry-matter content of the bagasse slurry was 18.3%. Six 2-L shake flasks were filled with 950 g of bagasse slurry. The pH of the spruce slurry was also adjusted to 5.3 with a 5 M solution of sodium hydroxide. Four 2-L shake flasks were each filled with 950 g of spruce slurry. The dry-matter content of the spruce slurry was 12.1%.

Commercially available preparations of cellulase and cellobiase were added to the slurries. The cellulase preparation, which was from *Trichoderma reesei* ATCC 26921, had a stated activity of 700 endoglucanase units (EGU)/g (Sigma–Aldrich, Steinheim, Germany) and the loading was 319 EGU/g of solids (DW). The cellobiase preparation, Novozyme 188, had a stated activity of 250 cellobiase units (CBU)/g (Sigma–Aldrich) and the loading was 23 CBU/g of solids (DW). After addition of enzymes, the slurries were incubated with shaking (Kuhner Lab-Therm LT-X, A. Kühner AG, Birsfelden, Switzerland) at 45 °C and 110 rpm for 72 h.

After hydrolysis, the slurries were centrifuged (Allegra X-22R, Beckman Coulter, Brea, CA, USA) at 4500*g* for 10 min at a temperature of 4 °C. The pH of the liquid fractions, the hydrolysates, was adjusted to pH 2.0 with a 12 M solution of HCl. The hydrolysates were stored at −80 °C until further use.

The monosaccharide content of the bagasse hydrolysate was: 85.3 g/L glucose, 18.8 g/L xylose, 3.4 g/L mannose, 1.4 g/L arabinose, and 0.7 g/L galactose. The bagasse hydrolysate contained 7.7 g/L acetic acid, 4.5 g/L furfural, and 0.7 g/L HMF. The monosaccharide content of the spruce hydrolysate was 84.4 g/L glucose, 13.7 g/L mannose, 8.0 g/L xylose, 2.0 g/L galactose, and 1.9 g/L arabinose. The spruce hydrolysate contained 4.3 g/L acetic acid, 2.0 g/L furfural, and 1.7 g/L HMF.

2.2. Treatment of hydrolysates

The treatment of the lignocellulosic hydrolysates was performed in a similar way as the treatments performed with sulfur-containing reducing agents in previous studies (Alriksson et al., 2011; Cavka et al., 2011). Prior to the treatments, the pH was adjusted to 6.0 with a 5 M solution of sodium hydroxide. The treatment of the hydrolysates was performed in 30 mL glass vessels equipped with magnetic stirrer bars and placed on a magnetic stirrer plate (IKA-Werke, Staufen, Germany) at room temperature (20 °C). Sodium borohydride (fine granular for synthesis, $\geqslant$98%, Sigma–Aldrich) was added as a powder directly to each of the vessels in different concentrations and allowed to react during 20 min. All treatments and experiments were performed in duplicates.

2.3. Concentration experiments

Experiments with different additions of sodium borohydride to lignocellulosic hydrolysates were performed in order to investigate if the sodium borohydride had any positive effect on the fermentablity of these lignocellulosic hydrolysates. Twenty-four and a half milliliter of hydrolysate were transferred to 30-mL glass vessels with magnetic stirring, two drops of anti-foam were added to counteract surface tension, and the sodium borohydride was then added directly to the vessels. The concentrations of sodium borohydride that were used were based on the total amount of HMF and furfural in each of the hydrolysates, and set to correspond to concentrations ranging from 0.1 to 1 furan aldehyde equivalents. For the experiment with bagasse hydrolysate the concentrations studied were 7, 15, 23, 31, 39, 47, and 55 mM. For the spruce hydrolysate, the concentrations were 4, 10, 16, 22, 28, 34, and 40 mM.

2.4. Effect on S. cerevisiae and time of addition

Experiments with glucose in 50 mM sodium citrate buffer, pH 6.0, were performed to investigate the effects, positive or negative,