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Techno-economic evaluation of conditioning with sodium sulfite for bioethanol production from softwood

Adnan Cavka [a], Carlos Martín [a,*], Björn Alriksson [b], Marlene Mörtsell [c], Leif J. Jönsson [a]

[a] Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden
[b] SP Processum AB, SE-891 22 Örnsköldsvik, Sweden
[c] SEKAB E-Technology AB, SE-891 26 Örnsköldsvik, Sweden

HIGHLIGHTS

- Inhibition problems can be alleviated *in situ* in bioreactors using reducing agents.
- Conditioning of pretreated spruce with sodium sulfite was evaluated.
- Reductions of yeast load or enzyme load compensate for cost of sodium sulfite.
- Estimation of required reduction levels: yeast, ⩾0.68 g/L; enzyme, ⩾1 FPU/g WIS.

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ABSTRACT

Conditioning with reducing agents allows alleviation of inhibition of biocatalytic processes by toxic by-products generated during biomass pretreatment, without necessitating the introduction of a separate process step. In this work, conditioning of steam-pretreated spruce with sodium sulfite made it possible to lower the yeast and enzyme dosages in simultaneous saccharification and fermentation (SSF) to 1 g/L and 5 FPU/g WIS, respectively. Techno-economic evaluation indicates that the cost of sodium sulfite can be offset by benefits resulting from a reduction of either the yeast load by 0.68 g/L or the enzyme load by 1 FPU/g WIS. As those thresholds were surpassed, inclusion of conditioning can be justified. Another potential benefit results from shortening the SSF time, which would allow reducing the bioreactor volume and result in capital savings. Sodium sulfite conditioning emerges as an opportunity to lower the financial uncertainty and compensate the overall investment risk for commercializing a softwood-to-ethanol process.

1. Introduction

Energy security and environmental concerns favor energy carriers from renewables, such as plant biomass, compared to the utilization of fossil resources, such as oil. Large-scale utilization of sugarcane-based first generation bioethanol as a transportation fuel started in 1975 in Brazil (Goldemberg et al., 2004), which remained the world leader until 2005, when the United States became the largest ethanol producer using corn starch as the main feedstock. Currently USA and Brazil produce, respectively, around 50 and 26 billion liters annually, and they provide around 87% of the world's fuel ethanol market (REN21, 2013; McMillan et al., 2014). Cellulosic ethanol produced from lignocellulosic biomass does not affect the food sector and can serve as a useful complement to ethanol from cane sucrose and corn starch (Ho et al., 2014). A lignocellulose-to-ethanol biorefining process also has potential to generate other products including energy carriers based on lignin and on digestion of parts of hemicelluloses to biogas.

In lignocellulose-to-ethanol processes, cellulose is hydrolyzed with either acids or enzymes, and the released sugars are converted to ethanol by a fermenting microorganism, usually the yeast *Saccharomyces cerevisiae*. These two steps can be performed either separately as a separate hydrolysis and fermentation (SHF) or combined in a simultaneous saccharification and fermentation (SSF) (Öhgren et al., 2007). If the hydrolysis is to be performed enzymatically it should be preceded by a pretreatment step that should ensure the reactivity of cellulose towards cellulolytic enzymes (Chandra et al., 2007; Hu and Ragauskas, 2012; Galbe and Zacchi, 2012; Behera et al., 2014).

* Corresponding author. Tel.: +46 90 7866099.
E-mail address: carlos.martin@chem.umu.se (C. Martín).

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During acid pretreatment carbohydrates and lignin are partially degraded leading to formation of by-products, some of which have an inhibitory effect on fermenting microorganisms and cellulolytic enzymes (Pienkos and Zhang, 2009; Jönsson et al., 2013). Recalcitrant forms of biomass, such as softwood, require harsh pretreament conditions that increase problems with inhibitors. Recycling of process water would also lead to increasing problems with inhibitors. Furthermore, the trend towards using high solids concentrations to gain higher ethanol titre (Kristensen et al., 2009) also results in higher inhibitor concentrations.

Detoxification by different chemical, biological and physical means, also known as conditioning, is one strategy for minimizing inhibition problems (Pienkos and Zhang, 2009; Jönsson et al., 2013). Results achieved so far suggest that potent detoxification methods give good results also for strongly inhibitory lignocellulosic hydrolysates, while other measures, such as using more resistant microbial strains, tend to have a more limited effect (Jönsson et al., 2013). This becomes obvious in studies where the fermentation of inhibitory lignocellulosic hydrolysates is benchmarked against reference fermentations without inhibitors.

A common weakness of most detoxification methods is the requirement of an additional process step, which adversely affects the process cost. This is true for example with regard to treatment with alkali, which is otherwise known as a very potent detoxification method (Jönsson et al., 2013). That drawback is overcome by detoxification with reducing agents, such as sodium dithionite, sodium sulfite and sodium borohydride, an approach that was recently developed by our group (Alriksson et al., 2011; Cavka et al., 2011; Cavka and Jönsson, 2013). Since conditioning with reducing agents can be performed at commonly used fermentation pH and temperature in the presence of microorganisms and enzymes, and since the reaction with the inhibitors is rapid, no separate process step is required. Additionally, this novel detoxification method neither results in sugar degradation nor in formation of precipitates, and it can be applied *ad hoc* if inhibition signs are observed during the fermentation (Cavka, 2013).

The most studied inhibitors of fermenting microorganisms include aliphatic carboxylic acids (such as acetic acid, formic acid, and levulinic acid), furan aldehydes [such as furfural and 5-hydroxymethylfurfural (HMF)], and phenolic compounds (for example coniferyl aldehyde and ferulic acid) (Jönsson et al., 2013). Sulfur oxyanions, such as sulfite and dithionite, sulfonate inhibitors which renders them less reactive, charged at process-relevant pH values, and highly hydrophilic (Cavka et al., 2011). Sodium borohydride reduces inhibitors, which become less reactive but not as hydrophilic as the corresponding sulfonated substances as no charge is introduced (Cavka and Jönsson, 2013). Previous results indicate that sulfur oxyanions are effective against inhibitors of both microbes and enzymes, while sodium borohydride is effective against inhibitors of microbes, but not inhibitors of enzymes (Cavka and Jönsson, 2013). Neither sulfur oxyanions nor sodium borohydride react with sugars (Alriksson et al., 2011; Cavka and Jönsson, 2013), and therefore inhibitory effects of sugars on cellulolytic enzymes are not affected by treatments with these substances. Inhibitory effects of sugars on cellulolytic enzymes can instead be decreased by using SSF (Öhgren et al., 2007) or by using enzymes that are less susceptible to sugar inhibition.

The current work was aimed to clarify the economic feasibility of sodium sulfite conditioning of spruce slurries prior to SSF for ethanol production. Sodium sulfite was used for conditioning as it has a favorable effect on both microbial and enzymatic conversion and as it is an industrial chemical that is well suited for process up-scaling. Selected experimental options were tested in order to demonstrate the importance of conditioning of the slurries for running SSF at lower yeast and enzyme loads. Based on the experimental results, a techno-economic evaluation was performed to elucidate whether the resulting economic benefits can offset the cost of the addition of sodium sulfite.

2. Methods

2.1. Raw material and pretreatment

Debarked wood chips of Norway spruce (*Picea abies*) were pretreated thermo-chemically by SEKAB E-Technology in the Biorefinery Demonstration Plant (BDP) in Örnsköldsvik, Sweden. The pretreatment was performed in a 30-L reactor, loaded to approximately 50% during operation. Spruce wood chips were treated in continuous mode at an overpressure of 20 bar (corresponding to 210 °C). Sulfur dioxide was added at a rate of 1.2 kg/h, which corresponds to approximately 1% of spruce dry weight (DW). The pretreatment lasted 7 min, and finished with a sudden release of pressure. The resulting slurry had a water-insoluble solids (WIS) content of around 18.5% and its pH was around 1.5. The slurry was cooled directly after pretreatment and stored at 4 °C until further use.

2.2. Detoxification

Prior to detoxification, 1.4 kg of the pretreated slurry was diluted with Milli-Q water to a WIS content of 12.5% in a 4-L plastic container, and its pH was adjusted to 5.5 with 10 M sodium hydroxide. Then sodium sulfite powder was added to the diluted slurry for reaching a concentration of 12.5 mM. The suspension was mixed manually and allowed to react for 10 min at room temperature (20 °C).

2.3. Simultaneous saccharification and fermentation (SSF) at lab scale

The effect of detoxification with reducing agents on fermentation parameters was investigated in a set of SSF experiments using 250 mL Erlenmeyer flasks filled with 100 g of spruce slurry, either detoxified or non-detoxified, with a pH of 5.5 and a WIS content of 12.5%. Since it has previously been shown that nutrient supplementation is not required for SSF of pretreated Norway spruce (Alriksson et al., 2011), no extra nutrients were added in order to simplify the subsequent techno-economic evaluation. Freeze-dried yeast (*S. cerevisiae* Ethanol Red, Fermentis Ltd., Marcq-en-Baroeul, France) and a state-of-the-art preparation of cellulolytic enzymes from a leading enzyme manufacturer were added directly to the fermentation flasks according to the experimental design (see Section 2.4), and the SSF was run in batch mode of operation. The flasks were sealed with Parafilm (Pechiney Plastic Packaging Company, Chicago, IL, USA) to prevent evaporation losses, and they were incubated for 96 h at 35 °C and 120 rpm in an orbital shaker (Ecotron, Infors AG, Bottmingen, Switzerland). Samples for sugar and ethanol analysis were withdrawn at 0, 24, 48, 60, 84 and 96 h of fermentation. The 48-h ethanol concentrations were used for calculating the volumetric productivity (Q) and the specific productivities on basis of either initial yeast inoculum (qx) or enzyme dosage (qz).

2.4. Experimental design

Two series of SSF experiments were performed. In the first series, the yeast concentration was varied between 1 and 2 g/L, and the enzyme load between 5 and 15 FPU/g WIS, while sodium sulfite was either added (12.5 mM) or not added (Table 1). Using the Modde 8.0 statistical software (Umetrics, Umeå, Sweden), a second series of experiments was performed for further evaluation of the yeast concentration, which was varied between 0.5 and