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A comparison between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using steam-pretreated corn stover

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Abstract

Two different process configurations, simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF), were compared, at 8% water-insoluble solids (WIS), regarding ethanol production from steam-pretreated corn stover. The enzymatic loading in these experiments was 10 FPU/g WIS and the yeast concentration in SSF was 1 g/L (dry weight) of a *Saccharomyces cerevisiae* strain. When the whole slurry from the pretreatment stage was used as it was, diluted to 8% WIS with water and pH adjusted, SSF gave a 13% higher overall ethanol yield than SHF (72.4% versus 59.1% of the theoretical). The impact of the inhibitory compounds in the liquid fraction of the pretreated slurry was shown to affect SSF and SHF in different ways. The overall ethanol yield (based on the untreated raw material) decreased when SSF was run in absence on inhibitors compared to SSF with inhibitors present. On the contrary, the presence of inhibitors decreased the overall ethanol yield in the case of SHF. However, the SHF yield achieves in the absence of inhibitors was still lower than the SSF yield achieves with inhibitors present.

Keywords: Steam pretreatment; SSF; SHF; Ethanol production; SO_2; Corn stover; Ethanol concentration

1. Introduction

Ethanol, produced from sugar, starch and lignocellulosic biomass, is a liquid bio-fuel with the potential to replace some of the liquid fossil fuels used in transportation. Bio-ethanol, produced from corn grain (starch) and sugar cane (sucrose) is currently the most common renewable fuel [1] and the introduction of ethanol on to the fuel market has been facilitated by the positive effects of low-blend ethanol–petrol mixtures [2]. However, it is clear that the large-scale use of bio-ethanol will require lignocellulosic biomass to be used as raw material [3,1]. Furthermore, current data suggest that only lignocellulosic ethanol (ethanol made from lignocellulosic biomass) offers large reductions in greenhouse gas emissions compared with fossil fuels [3]. Bio-fuels also provide the opportunity for non-oil-producing countries to be self-sufficient in fuel.

Bio-ethanol can be produced from any biomass, thus access to raw material is virtually unlimited. For example, agricultural by-products (straw, sugar cane bagasse, stover) provide a readily available, vast source of cheap biomass [4]. However, the production of ethanol from lignocellulosic raw materials is more difficult than from sugar or starch. Lignocellulosic materials consist primarily of three components, namely cellulose, hemicellulose and lignin, of which the first two can be hydrolysed to monomeric sugars [5], which can then be fermented to ethanol using a hexose- and pentose-fermenting organism. The hydrolysis of lignocellulose to monomeric sugars can be achieved in many different ways. One thoroughly investigated method is to first treat the material using steam pretreatment [6], with or without a catalyst. Several studies have been carried out on steam explosion as a method of pretreating corn stover, the raw material used in this study, using dilute sulphuric acid or SO_2 as a catalyst, with the aim of solubilizing hemicellulosic sugars, and rendering the remaining cellulose accessible to subsequent enzymatic hydrolysis [7–11].

After pretreatment, enzymatic hydrolysis is used to convert the residual cellulose and hemicellulose into monomeric sugars.

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The sugars are then fermented to ethanol using yeast. When enzymatic hydrolysis and fermentation are performed sequentially, it is referred to as separate hydrolysis and fermentation (SHF). However, the two process steps can be performed simultaneously, i.e. simultaneous saccharification and fermentation (SSF). This was first done by Takagi et al. in 1977 [12]. SSF was shown to be superior to separate hydrolysis and fermentation when the whole slurry from steam pretreatment of softwood was used [13]. Combining the two process steps also results in a lower capital cost, and the fact that the ethanol concentration is higher during SSF than SHF reduces the risk of contamination [14]. However, mixing the lignin residues with the yeast (as in SSF) makes yeast recirculation very difficult. In addition, the temperature optima for the yeast and the enzymes used differ, which means that the conditions used in SSF cannot be optimal for both the enzymes and the yeast.

Pretreatment hydrolysate has an inhibitory effect on cellulose conversion in the enzymatic step [15,16] but this can be overcome by fermentation of the pretreatment hydrolysate prior to enzymatic hydrolysis [17]. SSF may thus exhibit lower inhibition of the enzymes due to the concomitant fermentation. The pretreatment hydrolysate also has an inhibitory effect on the yeast, however, at low concentrations some of the inhibitors can actually have a positive effect on the ethanol productivity and the ethanol yield by stressing the yeast [18].

In this study, bench-scale (5 L) SSF and SHF at 8% water insoluble solids (WIS) were compared using steam-pretreated corn stover. A WIS content of 8% is high enough to obtain reasonable high ethanol concentrations, but low enough to ensure that complete cell death is avoided. Both the whole slurry (with all the inhibitors present) and washed slurry were used in order to distinguish between inhibition due to by-products formed in the steam pretreatment stage and sugar-inhibition of the enzymes. The enzymes used were commercial enzyme mixtures from Novozymes A/S, Denmark, and the yeast was a *Saccharomyces cerevisiae* strain cultivated in the steam-pretreatment hydrolysate and thus adapted to the harsh environment of SSF.

2. Materials and methods

2.1. Raw material

All the investigations were performed using corn stover from North America, kindly supplied by NREL, Golden, Colorado, USA. After collection, the corn stover was chopped, air-dried and then stored at room temperature. The sugar and lignin contents of the raw material were determined according to NREL [19].

2.2. Experimental set-up

After impregnation with SO_2, the stover was steam-pretreated at 190 °C for 5 min. These conditions have previously been determined to be optimal for SO_2-impregnated, steam pretreatment of corn stover [11]. The resulting slurry was then analysed and used in SSF and enzymatic hydrolysis (EH). The WIS content was adjusted with water, after which enzymes or enzymes and yeast were added. The initial WIS concentration in all the experiments was 8%.

An overview of the experimental set-up is shown schematically in Fig. 1.

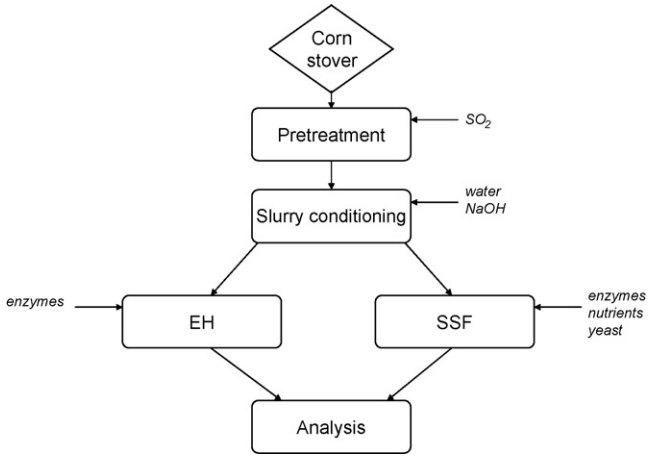

Fig. 1. The experimental procedure.

2.3. Steam pretreatment

The water content in the corn stover was determined and the corn stover was impregnated with 3% SO_2 (w/w, based on the dry weight). Steam pretreatment was carried out in 50 g batches in a 2-L steam gun (Stake Tech II batch reactor, Stake Tech-Norvall, Ontario, Canada). Several batches were pretreated, after which the material was collected for analysis and stored at −20 °C before use. Solid fractions, generated by pretreatment, were analysed in the same way as the raw material, and the liquid fraction was analysed with respect to monomeric and oligomeric sugars, acetic acid, 5-hydroxy methyl furfural (HMF) and furfural.

2.4. SSF and EH

The SSF and EH experiments were performed in a 15-L bioreactor with a working weight of 5 kg (Applikon, Schiedam, The Netherlands). The slurry from the pretreatment stage was either used as it was or was thoroughly washed with tap water. The pH was then adjusted to 5.0 with concentrated NaOH and the WIS concentration was adjusted by the addition of tap water. In one series of experiments, additional glucose and xylose were added to the washed and conditioned slurry to adjust the sugar concentrations in the liquid fraction to the same level as in the experiment with the whole slurry. Sugar addition was based on the sugar content in the liquid fraction of the pretreated slurry.

A commercial cellulase mixture, cellulase NS 50013 (69.5 Filter Paper Unit (FPU)/mL), supplemented with the β-glucosidase preparation, beta-glucosidase NS 50010, both from Novozymes A/S, Bagsværd, Denmark, was used. The enzymatic activity in the experiments was 10 FPU/g WIS and the β-glucosidase supplementation constituted 25% of the volume of cellulase added. The commercial xylanase mixture, Multifect xylanases, (Genencor International, Rochester, NY, USA), was also added in one series of experiments. The dosage of xylanases was based on the protein content in the xylanase mixture (43 g/mL) and equalled 0.006 g xylanase protein/g WIS, which was equivalent to 20% of the protein content in the cellulase added.

A spent sulphite liquor-adapted strain of *S. cerevisiae*, (Tembec I) provided by Tembec Ltd. (Témiscaming, QU, Canada), was used at a concentration of 1 g/L (dry yeast). This yeast ferments glucose but not xylose. The yeast was purified and then cultivated on the liquid obtained after pretreatment of corn stover to adapt it to the conditions used in SSF (see Section 2.5 below).

Nutrients were added in the SSF experiments so that the concentrations in the fermentor were 0.5 g/L $(NH_4)_2HPO_4$, 0.025 g/L $MgSO_4{\cdot}7H_2O$ and 1.0 g/L yeast extract. The temperature in the fermenters was kept at 35 °C during SSF and 45 °C during EH, and all experiments were run for 120 h with the pH being maintained at 5.0 by manual addition of a 50% NaOH. Samples were withdrawn after 0, 2, 4, 8, 24, 28, 32, 48, 72, 96 and 120 h, and analysed regarding ethanol, sugars, acetic acid, lactic acid and sugar degradation products.

The dry matter of the liquid, DM_{liquid}, and of the whole slurry, DM_{slurry}, were measured by drying a sample of the liquid fraction and of the slurry,