Ethanol Production from Biomass - Optimization of Simultaneous Saccharification and Fermentation with Respect to Stirring and Heating

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ABSTRACT

The purpose of this study was to optimize ethanol production in a small glass reactor. The ethanol was produced through simultaneous saccharification and fermentation (SSF) of steam pretreated spruce. Ordinary commercial yeast was used with a concentration of 5 g/L. The method of pretreatment was steam-explosion with SO_2 as catalyst. The pretreatment liberates cellulose, giving the enzymes better access to the cellulose. The optimization involved modifying heating and stirring. The reactor was provided with a glass jacket allowing it to be heated with a water bath instead of a hot plate. Different stirring speeds were tried and a new stirrer was designed. The new design was also incorporated as a stirring parameter along with the old one. Different concentrations of pretreated material, 7% & 10% water insoluble solids (WIS) were used. The more material used in SSF the more ethanol is potentially produced, but both enzymes and yeast are susceptible to inhibitors present in the pretreated material. Best result was achieved with the jacketed reactor with 7% WIS and slow stirring with the old stirrer; yielding an ethanol end concentration of 29.9 g/L and a yield of 61.2 %. The yield in question was the amount of ethanol produced from the pretreated material as % of the theoretical possible based on the hexose content in the raw material. The commercial yeast was found unable to cope with the inhibitory effects experienced with 10% WIS. The enzymes, on the other hand, retained most of their activity. Even with 7% WIS reproducibility was poor. However, to draw a conclusion with statistical certainty more experiments are needed.

INTRODUCTION

Ethanol has been a part of human culture since the dawn of time, but it was not until late nineteenth century that ethanol was first used as a fuel source. Ethanol’s role as a fuel source, however, was not long lived and was quickly abandoned in favor of petroleum based fuels. The oil crisis in the nineteen seventies ignited a new interest in ethanol and its use a fuel source.

It is generally believed that the carbon dioxide generated from human activities being released into the atmosphere is the main culprit in global warming. If bioethanol is used as fuel the net emission of carbon dioxide is zero due to its role in the photosynthesis. Here, the emitted carbon dioxide resulting from combustion is utilized in the photosynthesis of the replanted autotrophs from which the ethanol is produced. Also, the relatively short time span involved in generating new raw material for producing bio-ethanol makes it a renewable resource. As always though, it is the economic reality that determines when a transition or even a shift in emphasis on fuel selection is due.

Today almost all bioethanol, i.e. ethanol produced from renewable resources, is produced in Brazil, USA and Canada. The vast majority of the bioethanol is used in the transport sector as an oxygenated fuel additive. The raw material used is sugar-based or starch-based [1]. In Brazil sugar canes are used, from which the sugar is simply extracted and then fermented. When using starch based raw material it first has to be saccharified before being fermented. The hydrolysis can be achieved with acids or enzymes or a combination of both. Cellulose based materials, like softwood, are even harder to hydrolyze than the starch based equivalent. The reason for trying to produce ethanol from lignocellulosic material is the vast quantities of potential raw material in places with that type of wooded areas. This is where the incentive to create a commercially viable process for producing ethanol from softwood stems from.

Objective

The aim in this study was to optimize ethanol production in a 1.5L glass reactor. The ethanol was produced through simultaneous saccharification and fermentation (SSF) of pretreated spruce, and the variables used to optimize the process are stirring and heating. Optimizing the ethanol production means achieving the highest ethanol concentration possible and increasing reproducibility. The end result in this study was to explore the suitability of using the 1.5L glass reactor for SSF experiments.

MATERIAL & METHODS

Experimental Design

The objective in this study was to improve the SSF process taking place in a 1.5L glass reactor. Improvement means generating a higher end concentration of ethanol. The two parameters used to improve the SSF were heating and stirring. The reproducibility was also investigated. It was believed that a more even heating and better stirring would improve on reproducibility as well. By jacketing the reactor a water bath could be used. The effects of stirring were investigated by using two different stirrers and three different stirring speeds (300 RPM, 500 RPM and 700 RPM). Calibration revealed the
actual stirring speeds to be 320 RPM, 520 RPM and 720 RPM.

Taking into account the available space left in the reactors, for improving on the original stirrer a new stirrer was designed. The possibility of using different amounts of WIS was also investigated. Previous studies have shown that 5% WIS is about as high as an SSF process can handle with reliable results [2]. It is of interest to be able to use as high WIS content as possible because it would mean achieving a higher ethanol concentration.

When the WIS content exceeds a certain percentage the inhibitory effects on the yeast is too high, preventing the ethanol production. The enzymes also experience inhibition but not to the same extent. A WIS content of 7% and 10% were tried. The amount of pretreated material used in each run was based on the concentration of water insoluble substances (WIS). The WIS content of the pretreated material was determined by taking a specific amount of pretreated material and then washing it with distilled water, to remove water-soluble substances, and then drying it followed by placing it in an exsiccator before weighing it. The WIS content was found to be 16.8% (weight).

The results were evaluated by comparing yield based on glucose and end concentration of ethanol. The exact combination of experimental parameters for each type of reactor is shown in Table 1.

**Raw Material**
The raw material used in this study was spruce (Picea abies) with the bark removed. It was obtained from Degeberga, Sweden.

**Pretreatment**
Production of bioethanol from spruce is done by pretreating the spruce and then hydrolyzing it and finally fermenting it. The pretreatment is necessary because the cellulose, whose glucose building blocks are used in the fermentation, is well shielded by a matrix built up by the hemicellulose and lignin.

The spruce was therefore chipped to a size of 2-10 mm. To determine the dry matter (DM) content the chipped spruce was placed overnight in an oven at 105°C and then placed in an exsiccator before being weighed. The chipped material had a DM content of 44.4%.

The chipped material was impregnated with SO\textsubscript{2} with a dosage of 3 % (weight SO\textsubscript{2} / weight moisture). The impregnation was done by sealing the chipped wood together with the gaseous SO\textsubscript{2} in a plastic bag for 20 minutes.

To determine the actual amount of SO\textsubscript{2} that had been absorbed in the impregnation step the material in the plastic bag was thoroughly ventilated and weighed. The actual amount of absorbed SO\textsubscript{2} turned out to be 2.4%. The material was then treated with saturated steam at 215°C for 5 minutes in a steam explosion unit and finally stored in sealed plastic containers at 4°C. The steam pretreatment equipment has been described previously [3].

**Simultaneous Saccharification and Fermentation**
After the pretreatment the glucose molecules are still imprisoned in long chains of cellulose and hemicellulose and therefore not readily available for fermentation. This is why hydrolysis is necessary.

Simultaneous saccharification and fermentation (SSF) is a method for producing ethanol that utilizes enzymatic bond breaking and parallel to the enzymatic activity the yeast are fermenting the sugar, i.e. simultaneously.

**Experimental Procedure**
SSF was performed in the 1.5L reactor previously described. The reactor was weighed while empty and at the end of each experimental run, to be able to determine the dilution effects caused by the pH adjustment (evaporation effects are neglected). Both the jacketed and the original reactors had the same reaction volume of 1.5L and were made of glass. The reactor was autoclaved (121°C) for 40 minutes along with all its accessories except the pH meter and thermometer.

A starting batch size of 750g was a suitable volume for the 1.5L reactor. A nutrient solution was prepared, to create better conditions for the yeast. 100mL of the water (distilled) added to reach the batch size of 750g is used as a solvent for the nutrient. The ingredients and concentrations of the nutrient solution were 0.5 g/L (NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4}, 0.025 g/L MgSO\textsubscript{4}·7H\textsubscript{2}O, 1 g/L yeast extract.

The nutrient solution was also autoclaved for 40 min (121°C). The concentrations refer to the total batch size of 750g. When dealing with liquid concentration (g/L), as in the case of the nutrient solution, the total batch was assumed to have the

<table>
<thead>
<tr>
<th>Table 1. Experimental parameters.</th>
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<tr>
<td><strong>Unjacketed reactor</strong></td>
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<tr>
<td>7% WIS; new stirrer; medium stirring speed</td>
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<tr>
<td>10% WIS; old stirrer; medium stirring speed</td>
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<tr>
<td>10% WIS; new stirrer; high stirring speed</td>
</tr>
<tr>
<td><strong>Jacketed reactor</strong></td>
</tr>
<tr>
<td>7% WIS; new stirrer; low, medium &amp; high stirring speed</td>
</tr>
<tr>
<td>10% WIS; old stirrer; medium &amp; high stirring speed</td>
</tr>
<tr>
<td>10% WIS; new stirrer; high stirring speed</td>
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</table>
same density as water to simplify the necessary calculations.

The yeast used for fermentation was ordinary baker’s yeast, *Saccharomyces cerevisiae* (Jästbolaget AB, Rotebro, Sweden). The yeast concentration was set at 5 g/L and it refers to the amount of dry material (DM) commercial yeast in the total batch size. The DM content of the yeast was determined by drying a yeast sample in an oven at 105°C and then placing it in an exsiccator before being weighed. The DM content was 30%, resulting in the amount of yeast added being 12.5g.

The pretreated material was placed in the reactor along with the nutrient solution and all of the water (distilled) needed to achieve the final batch size of 750g except the water used to dissolve the yeast. Dissolving the yeast facilitates the adding of the yeast, which had a tendency to crumble. The content of the reactor was continuously being stirred at this point.

The yeast and the enzymes were added simultaneously, but not until after the pH had been adjusted and temperature had reached 37°C. The pH meter was calibrated before each run and then the automatic controller was set to raise the pH to approximately pH 5 and maintaining that pH level throughout the experiment, by adding a 10% (weight) NaOH solution. When the reactor had reached 37°C and pH 5 the yeast was added along with the enzymes. The dosage of enzymes was 15 FPU / g WIS, resulting in addition of 12.1g cellulase and 2.7g β-glucosidase. The commercial cellulase and β-glucosidase used are Celluclast 1.5L (Novozymes, Bagsvaerd, Denmark) and Novozyme 188 (Novozymes, Bagsvaerd, Denmark).

Although the reactor should not be airtight it should be fairly tightly sealed to prevent access of excessive amounts of air. In the presence of oxygen the yeast metabolism shifts from fermentation to cell respiration, but an outlet for the generated CO₂ was needed.

The process was monitored extra carefully during the first couple of hours when the risk of excessive foam generation due to the CO₂ being formed was greatest. To combat this problem a small volume (2 mL) of an anti emulsion chemical was added. The product used is called Antifoam R Emulsion (Dow Corning, Michigan, USA).

Samples were taken at approximately 0 h, 2 h, 4 h, 6 h, 8, 10, 24 h, 28 h, 32 h, 48 h, 52 h and 72 h; meaning that each experiment has a total running time of approximately 72 hours. The samples were centrifuged and filtered through micro filters and then stored for later HPLC analysis.

### Analysis

Two HPLC machines (Shimadzu, Kyoto, Japan) were used for analyses of the liquid samples. One was fitted with a Aminex HPX-87P column (Bio-Rad, Hercules, CA, USA) and the other with a Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA). They were calibrated to be able to detect the following compounds: Cellobiose, xylose, galactose, arabinose, mannos, lactic acid, glycerol, hydroxymethylfurfural (HMF), acetic acid and furfural.

Samples from the spruce are also analyzed with HPLC both in its raw form and its pretreated form. A sample from the liquid fraction of the pretreated material was also analyzed. The object of the spruce analysis was to determine the following compounds: Glucose, xylose, galactose, arabinose, and mannos.

Both the raw spruce and the pretreated spruce were analyzed with a standardized method from National Renewable Energy Laboratory (NREL) that determines thee structural carbohydrates and lignin in biomass [4].

### RESULTS AND DISCUSSION

#### Raw Material

Table 2 shows the composition of the raw material. These values are susceptible to variation caused by genetic and environmental variability of the raw material. The composition of the pretreated spruce values vary with pretreatment method and conditions.
Experimental Evaluation

To evaluate the results of this study the production of ethanol was used. The ethanol yield from the pretreated material is presented as well but the primary means of evaluation is the end concentration of ethanol. The ethanol is presumed to be produced exclusively from the glucose and mannose. The ethanol concentration readings are a result of the HPLC analysis.

However, to be able to discuss an ethanol yield the initial total amount of available glucose and mannose has to be determined, as well as the total amount of produced ethanol. The amount of glucose is determined by using the results presented in Table 2. Determining the total amount of produced ethanol is complicated by the fact that the end DM (dry matter) content of the bio slurry was not measured. To sidestep this problem the volume needed to calculate the total amount of produced ethanol is defined as the original batch minus the WIS content.

A successful run meant that all the glucose and mannose freed by the enzymes were converted to ethanol, see Figure 1. Both the original run and the duplicate run with 7 % WIS and medium stirring went well and yielded 28.9 g/L and 29.6 g/L of ethanol respectively after 72 h.

Figure 1. Ethanol and glucose concentration with medium stirring and the old stirrer.

An unsuccessful run meant that the yeast failed to produce ethanol due to inhibition, which most often did not affect enzymes, see Figure 2. In the run with 10% WIS and medium stirring the ethanol production was almost zero both for the original run and the duplicate run after 72 h.

Figure 2. Ethanol and glucose concentration with medium stirring and the old stirrer.

Discussion and Future Work

In Table 3 the ethanol end concentrations from the experiments presented in section 4.3 are presented, along with the ethanol yield based on glucose and mannose. The ethanol concentrations have been averaged whenever possible.

Looking at the results it is hard to tell whether or not the SSF performance was improved as a result of the modifications to the heating and stirring. The jacketed version did actually achieve better results than the original, but only marginally so. In fact, a larger fraction of the experiments performed with the jacketed failed than with the original.

One possible interpretation of this is that the modifications done to the original reactor had a detrimental effect on SSF performance, although it is more likely that the failure can be blamed on the fickle nature of bakers’ yeast.

More experiments are needed to draw a final conclusion with statistical certainty. In retrospect it is evident that too much effort was put into investigating the 10% WIS scenario. Instead these resources should have been used to investigate the 7% cases more thoroughly or to investigate an even lower WIS percentage. SSF with 5% WIS would have been of interest.

If the frequency of successful runs were higher with 5% it would indicate that bakers’ yeast cannot handle 7% in WIS in general and the failure experienced with 7% WIS in this study was not due to chance. It is also possible to cultivate the yeast on the pretreated material and letting it adapt to the inhibitors. The use of antibiotics to stop infections is also a possibility.
Table 3. Summary of the end ethanol concentration and yield for the conducted experiments. The presented results are averages when possible. The ethanol yield refers to ethanol produced from the pretreated material.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>End Ethanol Concentration (g/L)</th>
<th>Ethanol Yield (%)</th>
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<tbody>
<tr>
<td>Unjacketed reactor, 7% WIS, old stirrer, slow stirring</td>
<td>29.1</td>
<td>60.1</td>
</tr>
<tr>
<td>Unjacketed reactor, 7% WIS, old stirrer, medium stirring</td>
<td>29.2</td>
<td>60.0</td>
</tr>
<tr>
<td>Unjacketed reactor, 7% WIS, old stirrer, fast stirring</td>
<td>27.9</td>
<td>57.1</td>
</tr>
<tr>
<td>Unjacketed reactor, 7% WIS, new stirrer, medium stirring</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unjacketed reactor, 10% WIS, old stirrer, medium stirring</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unjacketed reactor, 10% WIS, new stirrer, fast stirring</td>
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<td>-</td>
</tr>
<tr>
<td>Jacketed reactor, 7% WIS, old stirrer, slow stirring</td>
<td>29.9</td>
<td>61.2</td>
</tr>
<tr>
<td>Jacketed reactor, 7% WIS, old stirrer, medium stirring</td>
<td>26.7</td>
<td>54.7</td>
</tr>
<tr>
<td>Jacketed reactor, 7% WIS, old stirrer, fast stirring</td>
<td>28.6</td>
<td>58.5</td>
</tr>
<tr>
<td>Jacketed reactor, 7% WIS, new stirrer, slow stirring</td>
<td>-</td>
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<tr>
<td>Jacketed reactor, 7% WIS, new stirrer, medium stirring</td>
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<td>Jacketed reactor, 10% WIS, new stirrer, fast stirring</td>
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References


