Production of butanol (a biofuel) from agricultural residues: Part II – Use of corn stover and switchgrass hydrolysates

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Abstract
Acetone butanol ethanol (ABE) was produced from hydrolysed corn stover and switchgrass using Clostridium beijerinckii P260. A control experiment using glucose resulted in the production of 21.06 g L⁻¹ total ABE. In this experiment an ABE yield and productivity of 0.41 and 0.31 g L⁻¹ h⁻¹ was achieved, respectively. Fermentation of untreated corn stover hydrolysate (CSH) exhibited no growth and no ABE production; however, upon dilution with water (two fold) and wheat straw hydrolysate (WSH, ratio 1:1), 16.00 and 18.04 g L⁻¹ ABE was produced, respectively. These experiments resulted in ABE productivity of 0.17–0.21 g L⁻¹ h⁻¹. Inhibitors present in CSH were removed by treating the hydrolysate with Ca(OH)₂ (overliming). The culture was able to produce 26.27 g L⁻¹ ABE after inhibitor removal. Untreated switchgrass hydrolysate (SGH) was poorly fermented and the culture did not produce more than 1.48 g L⁻¹ ABE which was improved to 14.61 g L⁻¹. It is suggested that biomass pretreatment methods that do not generate inhibitors be investigated. Alternately, cultures resistant to inhibitors and able to produce butanol at high concentrations may be another approach to improve the current process.

1. Introduction
Recent increases in fuel price have challenged all nations across the world to develop their own biofuels from renewable resources such as lignocellulosic crops. However, availability of renewable agricultural biomass is geographically specific such as corn in the United States and sugarcane in Brazil. It should be noted that use of corn in the United States appears not to be cost effective, as there are challenges like food and feed Vs fuel. As corn demand for converting to fuel ethanol increased during the last year (2008), corn prices rose to high levels (256.28 $ tonne⁻¹) [1] thus making it difficult or cost
ineffective to use this substrate for fuel ethanol production. As a result of increase in corn prices, various laboratories across the nation have initiated biofuel production program from wheat straw, barley straw, corn stover, and energy crops such as switchgrass, reed canary grass, and alfalfa. Wheat straw (26.46 $ tonne\(^{-1}\)), barley straw (28.66 $ tonne\(^{-1}\)), pea straw (48.50 $ tonne\(^{-1}\)), corn stover (55.12 $ tonne\(^{-1}\)), grass hay (55.12 $ tonne\(^{-1}\)), and switchgrass (66.14 $ tonne\(^{-1}\)) can be purchased at much lower prices than corn [1]. In our recent work we have demonstrated that wheat straw is a good substrate for butanol production [2,3]. This feedstock and biofuel (butanol) has numerous attractive properties [4,5].

It is indicated that one single crop residue/biomass would not be able to meet the biofuel demand. Hence, focus should be placed on all types of biomass listed in the first paragraph of this section. With this view, we attempted to expand the use of different agricultural substrates for butanol production. In the preceding paper, use of barley straw was investigated [6]. The objective of the present investigation was to use corn stover to produce butanol, as this lignocellulosic material is abundant in the mid-western region of the United States. Another substrate that we investigated was switchgrass, a biomass feedstock promoted as a perennial energy crop. It has been found that both of these substrates require additional treatments to promote fermentation. The hydrolysates of corn stover and switchgrass were prepared employing dilute sulfuric acid pretreatments followed by enzymatic hydrolysis.

2. Materials and methods

2.1. Strain and inoculum development

Clostridium beijerinckii P260 was a generous gift from Professor David Jones, University of Otago (Dunedin, New Zealand). Details of culture propagation have been given in the preceding article [6].

2.2. Corn stover hydrolysate (CSH)

Corn stover (Pioneer 32B83 variety: leaves, stems, and cobs) was obtained from a local farmer (Forest: Geo-coordinates N40.48, W88.93, Elevation 310 m; Illinois, USA) and stored dry at room temperature until needed. The corn was harvested in September/October 2007. Corn stover (stalks, cobs, and leaves) of the maize plants left in field after corn (grains) harvest. 86 g of milled (1.27 mm sieve screen) corn stover was pretreated with dilute [1% (v/v)] H\(_2\)SO\(_4\) and hydrolysed as described for barley straw hydrolysis and WSH [2,6]. To remove inhibitors, CSH and SGH were treated with lime [Ca(OH)\(_2\); known as overliming] as reported for BSH in the previous article [6].

2.3. Switchgrass hydrolysate (SGH)

Switchgrass (Cave-in-Rock variety) was obtained from an established stand located at Mead (Geo-coordinates N41.140, W-96.483, and Elevation 348.6 m), Nebraska (USA) [7]. All field plots were fertilized for high productivity under local soil conditions. Plant materials were harvested (August 2007) at 10 cm stubble height. After harvest the biomass was air dried on a greenhouse bench. The dried switchgrass was milled using a hammer mill (1.27 mm sieve screen) and stored at room temperature until needed. The milled switchgrass was pretreated with dilute [1% (v/v)] H\(_2\)SO\(_4\) and hydrolysed as described for barley straw hydrolysis and WSH [2,6]. To remove inhibitors, CSH and SGH were treated with lime [Ca(OH)\(_2\); known as overliming] as reported for BSH in the previous article [6].

2.4. Fermentation

Fermentation studies were performed as described for BSH in the preceding paper [6]. Prior to fermentation, pH of various media contained in bottles was adjusted to 6.5 using 400 g L\(^{-1}\) NaOH or diluted (10×) H\(_2\)SO\(_4\). Following pH adjustment, the bottles were kept in an anaerobic jar for 48–72 h for anaerobiosis. After this period, the bottles were inoculated with highly motile culture (7 mL cell culture in 100 mL medium) followed by transferring the bottles to anaerobic environment. Where applicable, the hydrolysate fermentation medium was supplemented with filter sterilized glucose solution (from 400 g L\(^{-1}\)) to raise total sugar level to 60 g L\(^{-1}\). This was done to keep the total sugar level approximately 60 g L\(^{-1}\) in all the experiments.

2.5. Analyses

Samples were analyzed for cell mass, sugars, and solvents as described in previous reports [2,6]. The results presented here are an average of duplicate experiments and have an error range of ±5–8%.

3. Results and discussion

In order to compare results obtained in this investigation, a control batch fermentation was operated to produce butanol using glucose as a substrate and C. beijerinckii P260. These results are given in the preceding paper [6]. The control experiment resulted in the production of 21.06 g L\(^{-1}\) total ABE. During the fermentation, an ABE yield and productivity of 0.41 and 0.31 g L\(^{-1}\) h\(^{-1}\) were obtained, respectively. The fermentation was initiated with 58.3 g L\(^{-1}\) glucose in the medium.

CSH was prepared and subjected to fermentation. In the undiluted/untreated CSH medium, the culture did not show any growth and/or fermentation. To promote fermentation of CSH, similar treatment approaches were applied as described for BSH reported in the previous article [6]. The first approach was to dilute the hydrolysate with distilled water followed by supplementing with sterile glucose solution to raise the total sugar level to approximately 60 g L\(^{-1}\). The culture exhibited a long lag phase of approximately 40 h before it initiated.
accumulation of a significant amount of ABE (Fig. 1A). After 96 h of fermentation (at that time fermentation stopped) 16.00 g L⁻¹ ABE (acetone 4.7, butanol 10.4, and ethanol 0.9 g L⁻¹) was produced (Fig. 1A). This resulted in a productivity of 0.17 g L⁻¹ h⁻¹. In the beginning of the fermentation, 6.55 g L⁻¹ acetic acid was present which was reduced to 4.01 g L⁻¹ during the course of fermentation suggesting that the culture was able to utilize some acetic acid. At the end of fermentation, butyric acid concentration was 0.16 g L⁻¹.

The culture used 37.3 g L⁻¹ sugars and cell concentration. This experiment resulted in a yield of 0.43. In the next experiment, CSH and WSH were mixed in a ratio of 1:1 and fermented. The culture fermented this mixture at a greater rate than CSH and water and produced 18.04 g L⁻¹ ABE (Fig. 2A) in 84 h, resulting in a productivity of 0.21 g L⁻¹ h⁻¹. The individual levels of solvents were acetone 5.10, butanol 12.30, and ethanol 0.64 g L⁻¹. Acid levels were 4.36 (acetic) and 0.35 (butyric) g L⁻¹. Initial sugar levels were glucose 38.7, xylose 15.0, arabinose 3.9, and galactose 1.7 g L⁻¹ (Fig. 2B). The residual sugar levels were glucose 14.7 and xylose 3.1 g L⁻¹. The culture used 41.5 g L⁻¹ sugars to produce 18.04 g L⁻¹ ABE, thus resulting in a yield of 0.43. In this experiment, a maximum cell concentration of 0.80 g L⁻¹ was obtained (Fig. 2B). This cell concentration is much lower than obtained in the above experiment (1.89 g L⁻¹) where CSH was diluted 2 fold with water.

Overliming of CSH was applied to reduce inhibitor toxicity. Fermentation stopped at 85 h and during this time, acetone 8.00, butanol 14.50, and ethanol 3.77 g L⁻¹ were produced to give a total of 26.27 g L⁻¹ ABE (Fig. 3A). It should be noted that untreated CSH was not fermented at all. This experiment resulted in a solvent productivity of 0.31 g L⁻¹ h⁻¹. It was observed that the culture started gassing within 3 h of inoculation. In this system, a maximum cell concentration of 0.77 g L⁻¹ was measured (Fig. 3B). In the medium, an initial sugar level of 60.3 g L⁻¹ (glucose 32.6, xylose 22.8, arabinose 3.6, and galactose 1.3 g L⁻¹) was present. During the fermentation, 59.8 g L⁻¹ sugars were used, leaving behind 0.5 g L⁻¹ unused xylose. This resulted in an ABE yield of 0.44. This yield is higher than the control fermentation of glucose and the possible reasons behind this are use of less sugars for cell growth and use of acetic acid that was present in the fermentation medium. At 0 time, 11.1 g L⁻¹ acetic acid was present which was reduced to 5.69 g L⁻¹. It should be noted that the fermentation could be possible due to the removal of inhibitors as a result of overliming. In the four experiments [control, CSH diluted with water, CSH mixed with WSH, and lime treated (LT) CSH], ABE concentrations of 21.06, 16.00, 18.04, and 26.27 g L⁻¹, respectively, were obtained. Levels of ABE in the treated CSH were higher than the control.

Measuring cell concentration in the fermentation broth is another way of evaluating the system. In the control reactor,
a cell concentration of 2.66 g L$^{-1}$ was obtained. In the three systems where CSH was fermented after treatments, reduced cell growth was observed, suggesting that the culture experienced growth inhibition. Corn stover hydrolysate plus water, CSH plus WSH, and lime treated CSH resulted in maximum cell concentrations of 1.89, 0.80, and 0.77 g L$^{-1}$, respectively. As described in our preceding paper, specific productivities for the four systems are compared. The control experiment resulted in a specific productivity of 0.12 h$^{-1}$, while CSH + WSH resulted in a specific productivity (g ABE/g cell h) of 0.27 h$^{-1}$. Lime treated CSH resulted in a specific productivity of 0.40 h$^{-1}$ which is much higher than the control experiment. In a report, Parekh et al. [8] used CSH to produce ABE using Clostridium acetobutylicum P262. The corn stover was pre-treated with SO$_2$. In their report, they achieved an ABE yield of 0.33 (calculated value) which is lower than that achieved in the present studies.

For fermentation of SGH, this substrate was initially fermented without dilution or mixing with WSH. Fermentation was weak and in 72 h fermentation 1.48 g L$^{-1}$ ABE (acetone 0.41, butanol 0.97, and ethanol 0.10 g L$^{-1}$) was produced. Total initial sugar concentration was 60.0 g L$^{-1}$ (glucose 33.2, xylose 20.4, arabinose 3.2, and galactose 3.2 g L$^{-1}$) and residual sugar level following fermentation was 42.1 g L$^{-1}$ (glucose 24.1, xylose 16.2, and arabinose 1.8 g L$^{-1}$). In subsequent fermentations, SGH was diluted two fold by adding water and sugar level was raised to 58.9 g L$^{-1}$ (glucose 45.5, xylose 10.2, arabinose 1.6, and galactose 1.6 g L$^{-1}$) by supplementing with glucose solution. The culture produced 14.61 g L$^{-1}$ ABE (Fig. 4A) in 84 h resulting in a productivity of 0.17 g L$^{-1}$ h$^{-1}$. The levels of acetone, butanol, and ethanol were 4.35, 9.55, and 0.71 g L$^{-1}$, respectively. The individual levels of residual sugars were glucose 19.6, xylose 0.4, and galactose 1.4 g L$^{-1}$ (Fig. 4B).

In this system, a maximum cell concentration of 1.6 g L$^{-1}$ was obtained (Fig. 4B). This fermentation resulted in a solvent yield of 0.39.

For the next fermentation, a 1:1 mixture of SGH and WSH was used. The culture did not produce more than 8.91 g L$^{-1}$ ABE (Table 1) in 96 h of fermentation resulting in a productivity of 0.09 g L$^{-1}$ h$^{-1}$. The reason for low ABE and low productivity of these SGH plus WSH cultures is not apparent. Further, when SGH was treated with lime, and attempts were made to produce ABE, a cell concentration of only 0.20 g L$^{-1}$ was measured suggesting that inhibitors were still present in the medium. We are attempting to treat lime treated SGH with XAD (trade name) resin to remove any residual inhibitors followed by fermentation [9].

In the present studies, we have demonstrated that as a result of inhibition, the culture (strain P260) was not able to grow and produce ABE in undiluted/untreated CSH (and SGH; showed poor growth and fermentation). By diluting these hydrolysates we were able to reduce inhibitory effects and produce solvents. Lime treated CSH resulted in the production of 26.27 g L$^{-1}$ ABE which is superior than the control experiment where glucose was used as a substrate. Results from BSH [6] and CSH fermentations suggest that overliming proved to be a good technique to detoxify the hydrolysates. However,
fermentation of SGH was poor after overliming. It should be noted that lime treatment of hydrolysates resulted in the reduction of sugar levels by 1–4% due to dilution [6].

In our previous paper, we were able to identify three chemicals that were present in WSH, BSH, CSH, and SGH [6]. These chemicals were acetic acid, furfural, and hydroxymethyl furfural (HMF). The concentration range of acetic acid was 6.43–10.10 g L\(^{-1}\), while levels of furfural (0.04–0.64 g L\(^{-1}\)) and HMF (0.12–0.52 g L\(^{-1}\)) were much lower (than acetic acid).

One of the objectives behind using these agricultural residues (wheat straw, barley straw, corn stover and switchgrass) has been to use low cost substrates for this fermentation and bring it closer to commercialization. This fermentation appears to have a potential to be an economically viable process as it was 3–4 decades ago [10]. In order to make it a viable process, various laboratories around the world have made significant technological progress [11–16]. In addition to the use of agricultural residues, cutting edge technologies such as application of high productivity reactors, and cost-efficient product recovery technologies are also being investigated in our laboratory. In the present studies, we have been able to overcome fermentation inhibitor [9,17,18] problems associated with CSH and SGH (partially) fermentation. Furthermore, it is our aim to identify the problems associated with SGH fermentation as it was difficult to ferment this substrate even after overliming. To reduce the cost of butanol production we intend to integrate fermentation with high productivity reactors and energy efficient product recovery technologies.

### 4. Conclusions

A control experiment resulted in the production of 21.06 g L\(^{-1}\) total ABE from glucose using C. beijerinckii P260. In this experiment, an ABE yield and productivity of 0.41 and 0.31 g L\(^{-1}\) h\(^{-1}\) was achieved. We were able to produce solvents from overlimed CSH and the culture accumulated 26.27 g L\(^{-1}\) (ABE productivity 0.31 g L\(^{-1}\) h\(^{-1}\), and yield 0.44) ABE. Overlimed SGH did not show significant cell growth, resulting in poor fermentation. The maximum ABE concentration that was produced from SGH was 14.61 g L\(^{-1}\) (productivity 0.17 g L\(^{-1}\) h\(^{-1}\)) when diluted two fold with water. It is concluded that other pretreatment approaches may be used to pretreat cellulosic biomass that do not generate fermentation inhibitors. Alternately cultures that can metabolize or tolerate inhibitors and still produce ABE in high concentrations should be developed.

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### References


### Table 1 – Production of ABE from switchgrass hydrolysate (SGH) in batch reactor using C. beijerinckii P260.

<table>
<thead>
<tr>
<th>Products and fermentation parameters</th>
<th>SGH mixed with WSH(^a)</th>
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<tbody>
<tr>
<td>Acetone [g L(^{-1})]</td>
<td>2.45</td>
</tr>
<tr>
<td>Butanol [g L(^{-1})]</td>
<td>5.79</td>
</tr>
<tr>
<td>Ethanol [g L(^{-1})]</td>
<td>0.67</td>
</tr>
<tr>
<td>Total ABE [g L(^{-1})]</td>
<td>8.91</td>
</tr>
<tr>
<td>Sugar used [g L(^{-1})]</td>
<td>24.1</td>
</tr>
<tr>
<td>Productivity [g L(^{-1}) h(^{-1})]</td>
<td>0.09</td>
</tr>
<tr>
<td>Yield [-]</td>
<td>0.37</td>
</tr>
</tbody>
</table>

\(^a\) Supplemented with glucose solution to raise initial sugar level to 60 g L\(^{-1}\).


