Note

An Improved CARV Process for Bioethanol Production from a Mixture of Sugar Beet Mash and Potato Mash

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Received October 14, 2010; Accepted December 11, 2010; Online Publication, March 7, 2011 [doi:10.1271/bbb.100744]

A mixed mash of sugar beet roots and potato tubers with a sugar concentration of 23.7% w/v was used as a feedstock for bioethanol production. Enzymatic digestion successfully reduced the viscosity of the mixture, enabling subsequent heat pretreatment for liquefaction/sterilization. An energy-consuming thick juice preparation from sugar beet for concentration and sterilization was omitted in this new process.

Key words: ethanol production; sugar beet mash; potato mash; conversion after reduction of viscosity (CARV); simultaneous saccharification and fermentation (SSF)

Bioresources to be used for bioethanol production depend on the production region. Bioethanol producers should have a great interest in crops grown in their own region as raw material of bioethanol because of its cheaper price and its convenience of collection, transportation, and storage.

Sugar beet (Beta vulgaris L.) is one of the most important basic crops in Hokkaido, Japan, because it is essential to the crop-rotation system of upland farming, consisting of sugar beet, beans, potato, and wheat.1,2 Sugar beet roots are very bulky and relatively expensive to transport. Once harvested, the main sugar, sucrose, quickly deteriorates. Therefore, the processing plants for sugar beet–bioethanol production should be located in the vicinity.

Ogbonna et al.3 have reported that raw sugar beet juice is an optimal substrate for ethanol production, requiring neither pH adjustment nor nitrogen source supplement. Another attractive benefit of sugar beet juice is that it requires less energy input than starchy feedstocks, which require liquefaction/saccharification. On the other hand, sugar beet juice has a low sugar content for direct bioethanol production as well as low tolerance against microbial contamination. Hence thick juice preparation with the aid of heat energy is thought to be indispensable for concentration and sterilization. Also, hot water is used during the extraction of sugar beet juice, resulting in dilution of the sugar juice, producing thin juice.

In this study, we focused on the use of potato (Solanum tuberosum L.) mash (PM) for adjustment of the sugar concentration of sugar beet mash (SBM) in order to omit the energy-consuming thick-juice preparation for sugar concentration. Sugar beet and potato are regarded as the most appropriate set of crops in Hokkaido in terms of both local and seasonal availability. SBM was used instead of sugar beet juice, in order not to dilute the sugar concentration by sugar extraction with hot water.

Recently, we developed a pretreatment method, the CARV (conversion after reduction of viscosity) process, for PM for bioethanol production under conditions of very high gravity (VHG).4 Enzymatic viscosity reduction of PM makes possible easy liquefaction without the addition of water. The alkali-aided enzymatic viscosity reduction process (Alkali-CARV) was also established in order to reduce the viscosity of SBM enough to perform efficient fermentation.5 However, the concentration of ethanol from SBM was at most 7–8% (v/v), and the process without heat treatment of SBM requires a bacterial control agent such as 100 ppm (final concentration) sodium metabisulfate.

In this study, we developed and briefly evaluated an improved CARV process using a mixture of SBM and PM for bioethanol production by simultaneous saccharification and fermentation (SSF). In the process, the sugar concentration of the mixture can be controlled so as to avoid both the stressful VHG conditions of PM conversion and the low ethanol concentration of SBM conversion. Also, the mixture can be sterilized during the liquefaction of starch from PM, yielding a process without the need of bacterial control agents during the fermentation process.

Sugar beet (cv. Hokkai 87) roots and potato (cv. Konafubuki) tubers were collected from the National Agricultural Research Center for the Hokkaido Region of Japan. Roots and the tubers were cut and ground by a procedure previously described.4,5 The two materials were mixed at a ratio of 1:1 w/w for comparative studies of the viscosity property and the capacity to produce ethanol between single and mixed materials.

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Abbreviations: AGU, novo glucoamylase unit; CARV, conversion after reduction of viscosity; FBG, fungal β-glucanase unit; FPU, filter paper unit; KNU, novo α-amylase unit; MIX, an equal quantity of the two mashes; PGU, polygalacturonase unit; PM, potato mash; SBM, sugar beet mash; SSF, simultaneous saccharification and fermentation; VHG, very high gravity
Component analysis was done to evaluate the potential of a mixture of sugar beet and potato mashes as feedstock for ethanol production. Table 1 summarizes the components of sugar beet mash (SBM), potato mash (PM), and the 1:1 w/w mixture of both mashes (MIX). The values were comparable to those in our previous studies.4–6 Sucrose from SBM and starch from PM were the main components of the mixture, comprising 28.4 and 46.6% w/w of dry matter respectively. The amount of fermentable sugars (soluble sugars and starch) available in the mixture was 23.7% w/w of wet matter. If complete conversion of fermentable sugars to ethanol is accomplished, 1 kg of the mixture would yield approximately 121 g (153 mL) of ethanol.

We also investigated the viscosity reduction patterns of SBM, PM, and MIX by enzyme addition (Fig. 1). The enzymatic viscosity reduction of the mashes was measured with a Rapid-Visco Analyzer (RVA, Newport Scientific, Warriewood, Australia). A cell-wall degrading enzyme mixture (5 mL) including Pectinex Ultra SP-L (Novozyme A/S, Bagsvaerd, Denmark, 14.4 PGU g⁻¹ of mash), Celluclast 1.5L (Novozymes A/S, 0.33 KNU g⁻¹ of mash), and 14.4 PGU, Celluclast 1.5L, 0.13 FPU, and Viscozyme L 0.07 FBG respectively.

After enzymatic viscosity reduction of the mixture, liquefaction and ethanol fermentation of the resulting mash were performed. Three hundred g of sugar beet and potato mash were weighed into a 2L jar fermentor (Labo-Controller MDL-8G, B.E. Marubishi, Tokyo), mixed with the cell-wall degrading enzymes (Novozyme A/S, Pectinex Ultra SP-L 14.4 PGU, Celluclast 1.5L 0.13 FPU, and Viscozyme L 0.07 FBG respectively). A cell-wall degrading enzyme mixture (5 mL) including Pectinex Ultra SP-L 14.4 PGU, Celluclast 1.5L 0.13 FPU, and Viscozyme L 0.07 FBG respectively.

Fig. 1. Viscosity Reduction Patterns of Sugar Beet Mash (SBM, 15.5% w/v), Potato Mash (PM, 21.1% w/v), and the 1:1 w/w Mixture (MIX, 18.5% w/v) under Enzyme Addition. The enzyme dosages per g of mash were Pectinex Ultra SP-L 14.4 PGU, Celluclast 1.5L 0.13 FPU, and Viscozyme L 0.07 FBG respectively.

Table 1. Components of the Sugar Beet Mash (SBM), the Potato Mash (PM), and the 1:1 w/w Mixture of the Two Mashes (MIX)  

<table>
<thead>
<tr>
<th>Components</th>
<th>SBM</th>
<th>PM</th>
<th>MIX</th>
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</thead>
<tbody>
<tr>
<td>Soluble sugars</td>
<td></td>
<td></td>
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<tr>
<td>Sucrose</td>
<td>70.5 ± 1.59</td>
<td>0.2 ± 0.02</td>
<td>28.4 ± 0.69</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.5 ± 0.00</td>
<td>1.5 ± 0.01</td>
<td>1.1 ± 0.01</td>
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<tr>
<td>Fructose</td>
<td>0.3 ± 0.00</td>
<td>1.5 ± 0.01</td>
<td>1.0 ± 0.01</td>
</tr>
<tr>
<td>Starch</td>
<td>1.0 ± 0.27</td>
<td>77.4 ± 1.37</td>
<td>46.6 ± 0.10</td>
</tr>
<tr>
<td>Cellulose</td>
<td>2.0 ± 0.04</td>
<td>1.8 ± 0.01</td>
<td>1.9 ± 0.02</td>
</tr>
<tr>
<td>Lignin</td>
<td>7.1 ± 0.31</td>
<td>2.1 ± 0.08</td>
<td>2.8 ± 0.26</td>
</tr>
<tr>
<td>Ash</td>
<td>2.7 ± 0.10</td>
<td>4.0 ± 0.30</td>
<td>3.2 ± 0.20</td>
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</tbody>
</table>

*Initial pH levels of SBM, PM, and MIX were 6.7, 5.6, and 6.0 respectively.

The water contents (% w/w wet matter) of SBM, PM, and MIX were 76.7, 68.4, and 72.2 respectively.

*Mean ± standard deviation of triplicate determinations.
gradually produced, and attained maximum concentrations over 48 h of fermentation. Transient accumulation of residual dextrins and glucose occurred during the initial 6 h due to a rapid starch hydrolysis rate. The residual dextrins were 0.6% w/v after 48 h of fermentation, indicating that the starch was almost completely degraded and converted to ethanol. In addition, the concentration of fructose slowly decreased during fermentation. The low consumption rate of fructose was most probably due to the different affinity for sugars of yeast strain. A discrepancy between glucose and fructose utilization during fermentation by wine yeast strains was reported by Berthels et al. It is considered that the utilization of fructose was critically important at the end of fermentation in this study. The resulting ethanol yield was 14.2% v/v (92.4% conversion rate) of the theoretical yield based on the available glucose and fructose. These results suggest that the improved CARV-SSF can be successfully applied in ethanol production using the mixture of SBM and PM as feedstock.

In summary, we found that ethanol was effectively produced from a mixture of sugar beet and potato mash by improved CARV-SSF processes. Oda and Nakamura recently proposed an excellent ethanol-production system by the use of yeast (Kluyveromyces marxianus) for efficient fermentation of a mixture of sugar beet molasses and cheese whey, the two main byproducts of the food industry in Hokkaido. In contrast, the new CARV-process uses the raw agricultural products in Hokkaido as feedstocks for bioethanol directly, without producing a thick juice. Also, a minimum addition of the water in the process should save costs in wastewater treatment, as well as costs in purchasing equipment. Thus the process would provide a new system of simple and efficient bioethanol production in the Hokkaido Region as well as other sugar-beet-growing areas of the world.

Acknowledgment

This work was supported by a grant from the Ministry of Agriculture, Forestry, and Fisheries of Japan (Rural Biomass Research Project, BEC-BA240).

References