Note

A Modified Process for Soy Sauce Fermentation by Immobilized yeasts

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In a previous paper we described a soy sauce fermentation process using a two-stage bioreactor with cylindrial filamentous ceramics with fixed yeasts (Zygosaccharomyces rouxii and Candida versatilis). With this process, fermentation time was reduced to 8 days in continuous fermentation for a long period of operation. Since preparation and handling of cylindrial filamentous ceramics are rather cumbersome for a pilot scale production of soy sauce, a more convenient ceramic bead would be desirable for the bioreactor.

To solve such difficulties, we used ceramic beads instead of cylindrial filamentous ceramics in the two-stage bioreactor, on which cells of Z. rouxii and C. versatilis were fixed. The bioreactor with ceramic beads reduced the fermentation time for soy sauce fermentation to 6 days without any differences in soy sauce quality, while that with cylindrial filamentous ceramics needs 8 days. A continuous beer brewing system with yeast immobilized on a granular ceramic carrier was reported. We describe in this paper soy sauce fermentation with a plant consisting of a two-stage bioreactor with immobilized yeasts on ceramic beads. The same two yeast strains, Z. rouxii IFO 1877 and C. versatilis G001, were used as in our previous report. The culture media, feed solution for soy sauce fermentation, and composition of seed and main mashes were the same as those described in our previous report. The reactors were made of glass 4.5 cm in diameter and 20 cm in height. The feed solution was pumped in from the conical shaped bottom of the bioreactors. Sizes and dimensions of the ceramic beads manufactured by Noritake Co., Ltd., Japan. are shown in Table I. The total working volume of the bioreactor was 200 ml and the ceramic beads occupied 70% of the reactor volume. After being filled with the ceramic beads, bioreactors were autoclaved at 120°C for 15 min. Immobilization of yeast cells on the ceramic beads were done as follows: cells of both of the yeast strains were separately cultivated in 200 ml of the main culture medium, harvested by centrifugation, and suspended in 200 ml of feed solution to give a cell concentration of about $5 \times 10^8$ per ml. Peristaltic pumps were used to circulate the cell suspension at a flow rate of 60 ml/hr 12 hr for immobilization of the yeast cells. In a continuous process, the rate of feed solution was maintained at 66 ml per day and the total retention time was about 6 days. All the reactor systems used were installed with a ceramic filter (Tokyo Rikakikai Co., Ltd., Tokyo) between reactors I and II to avoid mixing free cells from reactor I to II. Measurement of reducing sugar, ethanol, total nitrogen, formol nitrogen, total acidity, NaCl, and aroma components, and preparation of samples for scanning electron microscope and observation of immobilized yeast cells by an electron microscope were done as described previously. Viable cells were stained with modified methylene blue solution and counted using a haemacytometer at $400 \times$ magnification. Ceramic beads fixed with yeast cells were crushed using glass rod in 10 ml of sterilized water in test tube for viable cell counting.

Scanning electron micrographs of soy sauce yeasts, Z. rouxii and C. versatilis, immobilized on ceramic beads, are shown in Fig. 1. Obviously, both the yeast cells were absorbed on surface and in the pores of ceramic carrier. Viable cells immobilized on ceramic beads in the reactors I and II, at the beginning of operation for soy sauce fermentation were about $1 \times 10^8$ cells per bead (about 0.064 cm$^3$). After 3 months of operation, viable cell counts of immobilized yeasts were scored by taking sample beads from top, bottom, and middle of both reactors. Viable cell counts on beads from reactors remained constant with about $1 \times 10^8$ cells per bead.

The results of the continuous operation by reactor I with immobilized Z. rouxii cells are shown in Fig. 2. Production profiles of ethanol, 2-PE, and main aroma components, gave the same good results as compared to the previous

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Diameter (mm)</th>
<th>Ingredients (w%)</th>
<th>Distribution of pore size ($\mu$m)</th>
<th>Average pore size ($\mu$m)</th>
<th>Porosity (v%)</th>
<th>Surface area (cm$^2$/g)</th>
<th>Water absorption capacity (w%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bead ceramics</td>
<td>4-6</td>
<td>Al$_2$O$_3$ : SiO$_2$ 90 : 10</td>
<td>10-500</td>
<td>80-90</td>
<td>52-55</td>
<td>300</td>
<td>30</td>
</tr>
</tbody>
</table>
Fig. 1. Scanning Electron Micrographs of Yeasts Adsorbed on Ceramic Beads.
(a) Z. rouxii IFO 1877 (Reactor I); (b) C. versatilis G001 (Reactor II).

Fig. 2. Changes in Ethanol and Aroma Components by Reactor I in Continuous Fermentation Using Ceramic Beads.
Aroma components: □, ethyl acetate; ●, n-propyl alcohol; △, iso-butyl alcohol; ▲, iso-amyl alcohol.

Fig. 3. Product concentration profiles of 2-PE, 4-EG, and aroma components during the continuous fermentation in reactor II with immobilized C. versatilis are shown. Results obtained from reactor II showed that good products can be obtained from ceramic beads as well as the cylindrical filamentous ceramics which was used previously.

Comparison of soy sauce produced by the bioreactor with cylindrical filamentous ceramics and ceramic beads...
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Fig. 3. Changes in Ethanol and Aroma Components by Reactor II in Continuous Fermentation Using Ceramic Beads.

Aroma components: [ ], ethyl acetate; [ ], n-propyl alcohol; [ ], iso-butyl alcohol; [ ], iso-amyl alcohol.

Table II. Comparison of Aroma Components of Soy Sauce Produced by the Bioreactor with Conventional Sample

<table>
<thead>
<tr>
<th>Aroma components&lt;sup&gt;a&lt;/sup&gt;</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>X</th>
</tr>
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<tr>
<td>Conventional method</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>(A)*</td>
<td>2.29</td>
<td>5.3</td>
<td>1.4</td>
<td>17.7</td>
<td>25.0</td>
<td>5.0</td>
<td>13.3</td>
<td>4.9</td>
<td>3.5</td>
<td>6.5</td>
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<tr>
<td>Immobilized method</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A)**</td>
<td>2.50</td>
<td>20.0</td>
<td>2.2</td>
<td>15.0</td>
<td>10.0</td>
<td>5.0</td>
<td>2.3</td>
<td>1.7</td>
<td>4.0</td>
<td>3.3</td>
</tr>
<tr>
<td>(B)**</td>
<td>2.21</td>
<td>16.0</td>
<td>2.4</td>
<td>14.7</td>
<td>10.5</td>
<td>4.9</td>
<td>1.2</td>
<td>1.5</td>
<td>4.3</td>
<td>3.2</td>
</tr>
<tr>
<td>(C)**</td>
<td>2.40</td>
<td>21.6</td>
<td>0.5</td>
<td>63.2</td>
<td>35.3</td>
<td>--</td>
<td>8.3</td>
<td></td>
<td>5.2</td>
<td>4.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> I, ethanol (v/v%); II, 2-PE (mg/l); III, 4-EG; IV, iso-amyl alcohol; V, iso-butyl alcohol; VI, n-propyl alcohol; VII, ethyl acetate; VIII, ethyl lactate; IX, furfuralcohol; X, n-butyl alcohol.

* Commercial product. ** Our data. *** Filamentous ceramics. **** Data from Osaki et al. 2)

with the commercial one are summarized in Table II. It was shown that the major constituents and aroma components of soy sauce produced by bioreactor system were the same as the conventional sample and also those in the data of Osaki et al. 3) and Hamada et al. 4) Furthermore the time required for fermentation was shortened to 6 days, 2 days shorter than the process with cylindrical filamentous ceramics.

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References