Saccharomyces cerevisiaeによる食品ゴミからのエタノール発酵に関する基礎的検討
Fundamental Study of Kitchen Refuse Utilization for Ethanol Fermentation by *Saccharomyces cerevisiae*

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The possibility of generating renewable bio-fuel energy through ethanol fermentation of kitchen refuse by *Saccharomyces cerevisiae* ATCC 26602 was established in this work. The acid-tolerant yeast *S. cerevisiae* ATCC 26602 was selected from among four *S. cerevisiae* strains as the best ethanol fermentation agent for kitchen refuse medium. The optimal kitchen refuse medium for ethanol production had a glucose concentration of only 12% (w/v) and consisted of saccharified kitchen refuse. Additional nitrogen supplementation was not necessary. A maximal ethanol concentration of 59.38 g/L with a 0.50 ethanol yield (Ye) was obtained with the optimal medium composition in flask culture under shaking and without pH control. No significant difference was observed in ethanol production and glucose consumption between sterilized, pasteurized and non-sterilized kitchen refuse medium samples. A final ethanol concentration of 50.22 g/L was obtained using a 20-L bioreactor with a working volume of 12 L under non-sterilized conditions at an agitation rate of 100 rpm at 30°C for 18 h. Therefore, the ethanol fermentation of kitchen refuse under non-sterilized conditions by *S. cerevisiae* ATCC 26602 may offer an economical alternative for bio-fuel production in industrial applications.

**Key words:** ethanol fermentation, kitchen refuse, *Saccharomyces cerevisiae*

1. Introduction

As a clean and renewable energy source, ethanol fuel has been drawing global interest in recent years. One of the reasons is its contribution to greenhouse gas reduction, because ethanol derived from fermentation is considered to be carbon-neutral [1].

The major feedstocks for global ethanol production are sugar cane (Brasil) and corn grain (USA) [2]. A dramatic increase in ethanol production using the current sugar-cane- and corn-based technology may not be sustainable, because the corn and sugar cane grown for ethanol will compete with the limited agricultural land necessary for food and feed production. Therefore, ethanol production from renewable resources, especially organic waste, is expected to be one of the ideal alternatives.

Kitchen refuse has a promising potential as fermentable substrate, because of its rich contents of carbohydrates and other nutrients such as nitrogen sources, minerals and vitamins. Kitakyushu City, which has one million inhabitants, discharges enormous amounts of kitchen refuse, i.e. 0.15 million tons/year. The use of kitchen refuse as a fermentation substrate for value-added products such as lactic acid [4], succinic acid [5], biogas [6], fertilizer [7] and animal feed [8] has been reported.

The more highly rich and complex the fermentation medium, the greater the amount of ethanol generated by *Saccharomyces cerevisiae* in the fermentation process [3]. However, the literature to date does not mention ethanol fermentation of kitchen refuse by *S. cerevisiae*.

So, the main objective of this work was to investigate the feasibility of ethanol fermentation of kitchen refuse by *S. cerevisiae*. Four strains of *S. cerevisiae* were compared and one was selected for its high ethanol production capacity and acid tolerance. The effects of the sugar concentration and other nutrients such as nitrogen sources on optimal yeast growth and ethanol production were deter-
mined using batch cultures grown in flasks under shaking. Furthermore, a kinetic study was carried out and the application of non-aseptic techniques was examined using a 20-L bioreactor.

2. Materials and Methods

2.1 Kitchen refuse preparation

Food waste was provided by the local supermarket (Wakamatsu, Kitakyushu and Fukuoka, Japan). About 70 kg of kitchen refuse was collected in spring in the Kyushu area. Kitchen refuse was treated as medium following steps shown in Fig. 1. We separated it into 3 groups by hand: the carbohydrate group (rice, bread, pasta, noodles, etc.), the protein group (fish, beef, pork, chicken, etc.) and the vegetable/fruit group. Thereafter, the kitchen refuse was again mixed in the following proportions: 43% (w/w) carbohydrates, 19% (w/w) protein and 38% (w/w) vegetables with fruits. The raw food waste material was minced by a food-processing machine (Model MKB C-22, Masuko Sangyo Co., Ltd., Japan) into 0.05-0.5 cm bits and suspended in 200% (w/w) water. Next, the kitchen refuse suspension was saccharified by glucoamylase (20000 NAGASE ENZYMES, Japan) at a concentration of 300 ppm at 50°C for 6 h in a 90-L bioreactor. During the enzymatic reaction, the mixing rate was 100 rpm. After saccharification, the solution was filtrated through a 6×6-mm wire mesh, then frozen and thawed twice. Finally, the suspended solids (SS) were removed by centrifugation at 3,000 rpm for 10 min. A clear saccharified kitchen refuse solution was obtained and used as the substrate for ethanol fermentation in this work.

The glucose concentration of the saccharified kitchen refuse solution reached around 300 g/L, and was adjusted to different concentrations using tap water.

2.2 Microbial strains and culture condition

2.2.1 Yeast inoculum preparation

Saccharomyces cerevisiae ATCC 24860, 26602, 36858 and 36859 were selected because of their acid tolerance and ethanol productivity. ATCC 26602 also shows coagulation property. Four strains were purchased from the American Type Culture Collection (Rockville, MD) and used throughout this study. These yeasts were maintained at 4°C on yeast-malt agar (YMA) slants. The composition of the agar was as follows: yeast extract 3 g/L, malt extract 3 g/L, peptone 5 g/L, glucose 10 g/L and agar 20 g/L. The cultures were sub-cultured every 2 weeks and incubated at 30°C for 36 h.

Fig. 1 Pre-treatment of the ethanol fermentation medium derived from kitchen refuse.
2.2.2 Medium preparation

The culture media listed below were used in this study.

Pre-culture medium

Synthetic pre-culture medium (SPM): glucose 30 g/L, yeast extract 5 g/L and peptone 3 g/L.

Kitchen refuse pre-culture medium (KRP): the KRP was prepared by adjusting the glucose concentration of the saccharified kitchen refuse to 50 g/L, after which 2 g/L yeast extract and 2 g/L peptone were added.

Ethanol production medium

Synthetic ethanol production medium (SEPM): glucose 100 g/L, yeast extract 5 g/L, peptone 3 g/L, KH₂PO₄ 5 g/L, NH₄Cl 1.5 g/L, MgSO₄ 0.7 g/L and KCl 1.7 g/L.

Kitchen refuse–ethanol production medium (KREPM): this medium was only composed of saccharified kitchen refuse with a glucose concentration ranging from 100 to 300 g/L. The effects of nitrogen supplementation on ethanol production was examined by adding 2 g/L yeast extract, 2 g/L peptone, 2 g/L polypeptone, 2 g/L (NH₄)₂SO₄ or 2 g/L urea.

All media were sterilized in an autoclave at 121°C for 15 min and the pH was not adjusted.

2.3 Yeast cultivation

2.3.1 Seed culture

One loop of pure yeast culture was transferred from an agar slant to a 300-mL Erlenmeyer flask sealed with a cotton plug and containing 50 mL of sterilized pre-culture medium. The culture was incubated in a rotary shaker at a shaking rate of 100 rpm at 30°C for 18 h. The yeast obtained was used for subsequent sub-cultures.

2.3.2 Ethanol fermentation

For the flask experiments, 1 mL of the seed culture was transferred to a 300-mL Erlenmeyer flask containing 50 mL of production medium. The yeast was grown for 24 h at 30°C in a rotary shaker at a rotation rate of 100 rpm. Every experiment was performed in triplicate under the same conditions.

Batch culturing was carried out at 30°C under agitation at a rate of 100 rpm in a 20-L jar bioreactor with a working volume of 12 L. The pH of the medium was not controlled. A small amount of air was supplied at a flow rate of 0.10vvm for 10 h to stimulate yeast-cell growth. The external air was sterilized by passing it through a membrane filter (0.2 μm).

2.4 Analysis

The viable cells were counted using YMA. The plates were incubated at 30°C for 48 h and the final colony count was taken as the average of three plates for the dilution containing 30 to 300 colonies per plate. In order to determine the dry weight, the yeast cells were collected by centrifugation at 3,000 rpm for 5 min and washed twice with a 0.85%-solution of NaCl. Then, the yeast cells were dried for 24 h at 105°C and cooled down in a desiccator before being weighed. Ethanol was quantified using a gas chromatograph (Model GC-17A, Shimadzu) equipped with a packed column with BPX capillary columns and a flame ionization detector. The temperature of the injector and detector were 80°C. The oven temperature was maintained at 40°C for 1 min and raised to 130°C at a gradient of 20°C/min. Helium was used as the carrier gas at a flow rate of 5 mL/min. Propanol was used as the internal standard and hydrogen as the flaming gas. The glucose concentration was enzymatically analyzed by the glucose oxidase-peroxidase enzyme (Glucose Test Kit, Toyobo, Japan). The suspended–solid (SS) content was evaluated following the APHA standard method [9]. The pH was checked before and after each fermentation period.

3. Results

3.1 Comparison of ethanol production

The results for ethanol production from kitchen refuse medium and synthetic medium by the four strains of S. cerevisiae is summarized in Table 1. Kitchen refuse medium as a substrate for ethanol production showed the same high efficiency as the synthetic medium. No obvious pH fluctuation was observed during the fermentation. The pH value was maintained at around 5.0 in the synthetic medium and at 4.2 in the kitchen refuse medium. The glucose contained in both media was totally consumed and supported the growth of all four S. cerevisiae strains in all cases. Almost the same dry biomass weights were obtained from the synthetic medium and the kitchen refuse medium. Similar fermentation efficiencies and ethanol productivities were obtained in the synthetic medium and the kitchen refuse medium. The glucose concentrations of 60.61 and 57.52 g/L were obtained from the synthetic medium and the kitchen refuse medium, respectively. Similar ethanol concentrations, 59.52 and 59.42 g/L, were obtained from the synthetic medium by S. cerevisiae ATCC 24860 and 36858, respectively, whereas slightly lower ethanol concentrations, 56.68 and 56.51 g/L, were obtained, respectively, when kitchen refuse used as the medium. The lowest ethanol concentration was 50.45 g/L, produced from kitchen
refuse medium by \textit{S. cerevisiae} ATCC 36859.

### 3.2 Effects of the initial pH on ethanol production from kitchen refuse by \textit{S. cerevisiae}

The effects of the initial pH value of the kitchen refuse medium on ethanol production were examined, and the results are summarized in Table 2. When the initial pH ranged from 3 to 5, there was no influence on the growth of the four ethanol-producing yeast strains. After 24 h, the glucose, at a concentration of about 117 g/L, was completely consumed in all experimental runs. In the case of \textit{S. cerevisiae} ATCC 24860 and 26602, the ethanol concentration was not affected by the initial pH value. Ethanol concentrations of 56.50 and 57.84 g/L were generated by \textit{S. cerevisiae} ATCC 24860 and 26602, respectively. On the other hand, ethanol production by \textit{S. cerevisiae} ATCC 34858 and 34859 seems to have been affected by the initial pH. A slight increase in ethanol concentration was observed when the initial pH raised from 3 to 5. The ethanol concentration increased from 52.06 g/L at pH 3 to 52.71 g/L at pH 4 and to 54.97 g/L at pH 5 in the case of \textit{S. cerevisiae} ATCC 36858. A similar trend, 54.44 g/L at pH 3, 55.26 g/L at pH 4 and 56.76 g/L at pH 5, was observed with ATCC 36859. However, when the ethanol productions by \textit{S. cerevisiae} ATCC 24860, 26602, 34858 and 34859 were compared, \textit{S. cerevisiae} ATCC 26602 was found to be the most suitable yeast strain for ethanol production from kitchen refuse medium. It was selected for further investigation.

### 3.3 Effects of nitrogen sources on ethanol production from kitchen refuse by \textit{S. cerevisiae}

The effects of nitrogen source supplementation to kitchen refuse on ethanol production by \textit{S. cerevisiae} ATCC 26602 were examined and the results are summarized in Table 3. (NH₄)₂SO₄, yeast extract, malt extract, peptone, poly-peptone and urea were applied as nitrogen sources. Additional nitrogen supplementation to the kitchen refuse medium was not required. 

Only the cell biomass was slightly affected by the presence of a nitrogen source. A higher cell biomass was obtained when an organic nitrogen source was added, as compared to an inorganic nitrogen source or no supplementation.

Moreover, when the ethanol yield (\(Y_{p/s}\)) was calculated,

### Table 1 Ethanol fermentation by four strains of \textit{S. cerevisiae}.

<table>
<thead>
<tr>
<th></th>
<th>Synthetic medium</th>
<th>Kitchen refuse medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strains of \textit{Saccharomyces cerevisiae}</td>
<td>Strains of \textit{Saccharomyces cerevisiae}</td>
</tr>
<tr>
<td></td>
<td>ATCC 24860</td>
<td>ATCC 26602</td>
</tr>
<tr>
<td>Final biomass concentration [g/L]</td>
<td>8.14</td>
<td>7.87</td>
</tr>
<tr>
<td>Final ethanol concentration [g/L]</td>
<td>59.5</td>
<td>60.6</td>
</tr>
<tr>
<td>Ethanol productivity [g/L/h]</td>
<td>2.48</td>
<td>2.33</td>
</tr>
<tr>
<td>Ethanol yield (\left[ Y_{p/s} \right]) [g/g]</td>
<td>0.486</td>
<td>0.495</td>
</tr>
<tr>
<td>Sugar consumption [%]</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

The initial glucose concentration of the synthetic medium was 122.53 g/L. The initial glucose concentration of the kitchen refuse medium was 116.67 g/L. The results are the average of three experiments.

\[ Y_{p/s} = \frac{\text{Mass of ethanol formed}}{\text{Mass of glucose consumed}} \]

### Table 2 Comparison of ethanol production from kitchen refuse by \textit{S. cerevisiae} at various adjusted pH values.

<table>
<thead>
<tr>
<th></th>
<th>\textit{S. cerevisiae} ATCC 24860</th>
<th>\textit{S. cerevisiae} ATCC 26602</th>
<th>\textit{S. cerevisiae} ATCC 36858</th>
<th>\textit{S. cerevisiae} ATCC 36859</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial control pH</td>
<td>3 4 5</td>
<td>3 4 5</td>
<td>3 4 5</td>
<td>3 4 5</td>
</tr>
<tr>
<td>Final biomass concentration [g/L]</td>
<td>7.21</td>
<td>8.30</td>
<td>8.44</td>
<td>7.29</td>
</tr>
<tr>
<td>Final ethanol concentration [g/L]</td>
<td>56.4</td>
<td>56.4</td>
<td>56.7</td>
<td>57.7</td>
</tr>
<tr>
<td>Ethanol productivity [g/L/h]</td>
<td>2.35</td>
<td>2.35</td>
<td>2.36</td>
<td>2.41</td>
</tr>
<tr>
<td>Ethanol yield (\left[ Y_{p/s} \right]) [g/g]</td>
<td>0.483</td>
<td>0.483</td>
<td>0.486</td>
<td>0.494</td>
</tr>
<tr>
<td>Sugar consumption [%]</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

The initial glucose concentration of the kitchen refuse medium was 116.71 g/L. The results are the average of three experiments.

\[ Y_{p/s} = \frac{\text{Mass of ethanol formed}}{\text{Mass of glucose consumed}} \]
an average of 0.49 g/g was obtained under each condition. Furthermore, the initial pH and the final pH of the kitchen refuse medium were around both 4.2, which indicates that no contamination occurred during the fermentation.

3.4 Effects of glucose concentration on ethanol production from kitchen refuse by *S. cerevisiae*

The ethanol fermentation performances from various initial glucose concentrations were evaluated, and the results are presented in Table 4. A strong influence of the initial glucose concentration on the sugar consumption was observed. The sugar content was completely consumed within 24 h when the initial glucose concentration was between 100 and 180 g/L. Above that concentration range, *S. cerevisiae* ATCC 26602 showed a decreasing sugar consumption tendency. Increasing the glucose concentration had a pronounced effect on the final ethanol yield. The highest ethanol yield, 0.50 g/g, was obtained from a glucose concentration of 118.20 g/L.

When a higher glucose concentration than the above was supplied, a slight decrease in ethanol yield, which resulted in a sharp reduction of fermentation efficiency, was observed. Moreover, the pH value showed only a slight variation, changing from 4.1 to 4.3 at the beginning of the experiment, and then remaining at that level until the end.

3.5 Effect of asepsis on ethanol production from kitchen refuse by *S. cerevisiae*

To evaluate the effect of asepsis on ethanol production, two types of asepsis technologies were applied. One was sterilization, in which the kitchen refuse medium was autoclaved at 121°C for 15 min. The other was pasteurization, in which the kitchen refuse medium was heated to 63°C for 30 min, followed by quick cooling to about 4°C. Non-sterilized medium was chosen as the control in this study. Figure 2 shows the batch profiles for ethanol fermentation from kitchen refuse by *S. cerevisiae* ATCC 26602. The same patterns of growth, glucose consumption and ethanol production were observed in the sterilized, pasteurized and non-sterilized samples incubated in a 20-L bioreactor. The three kinds of medium showed no significant differences in their initial pH values and fermentation profiles. In the sterilized medium, the pH changed from 4.24 to 4.09, and from 4.14 to 3.98 and 4.22 to 4.04 in the pasteurized medium and the non-sterilized medium, respectively, as a result of fermentation. The sugar was completely consumed within 18 h under all conditions. The ethanol production rates, sugar consumption rates and numbers of viable cells also did not show any significant differences. Under sterilization, the final etha-

### Table 3 Effects of nitrogen sources on ethanol production from saccharified kitchen refuse by *S. cerevisiae* ATCC 26602.

<table>
<thead>
<tr>
<th>Nitrogen sources</th>
<th>(NH₄)₂SO₄</th>
<th>Urea</th>
<th>Yeast extract</th>
<th>Malt extract</th>
<th>Peptone</th>
<th>Polypeptone</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final biomass concentration [g/L]</td>
<td>7.57</td>
<td>7.63</td>
<td>8.09</td>
<td>7.68</td>
<td>7.95</td>
<td>7.87</td>
<td>7.71</td>
</tr>
<tr>
<td>Final ethanol concentration [g/L]</td>
<td>57.8</td>
<td>58.0</td>
<td>58.4</td>
<td>57.9</td>
<td>58.6</td>
<td>57.4</td>
<td>58.6</td>
</tr>
<tr>
<td>Ethanol productivity [g/L/h]</td>
<td>2.41</td>
<td>2.42</td>
<td>2.43</td>
<td>2.41</td>
<td>2.44</td>
<td>2.39</td>
<td>2.44</td>
</tr>
<tr>
<td>Ethanol yield [Yₚ/s] [g/g]</td>
<td>0.488</td>
<td>0.489</td>
<td>0.493</td>
<td>0.488</td>
<td>0.494</td>
<td>0.484</td>
<td>0.494</td>
</tr>
<tr>
<td>Sugar consumption [%]</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

The control culture was grown without supplementing complex nitrogen sources. The initial glucose concentration of the kitchen refuse medium was 118.54 g/L. The results are the average of three experiments.

\[
Y_{p/s} = \frac{\text{Mass of ethanol formed}}{\text{Mass of glucose consumed}}
\]

### Table 4 Effects of glucose concentration on ethanol production from saccharified kitchen refuse by *S. cerevisiae* ATCC 26602.

<table>
<thead>
<tr>
<th>Glucose concentration of saccharified kitchen refuse [g/L]</th>
<th>107</th>
<th>121</th>
<th>144</th>
<th>173</th>
<th>197</th>
<th>247</th>
<th>283</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final biomass concentration [g/L]</td>
<td>7.47</td>
<td>7.83</td>
<td>7.72</td>
<td>7.95</td>
<td>8.12</td>
<td>5.81</td>
<td>1.11</td>
</tr>
<tr>
<td>Final ethanol concentration [g/L]</td>
<td>52.2</td>
<td>59.4</td>
<td>70.3</td>
<td>84.7</td>
<td>81.9</td>
<td>44.1</td>
<td>3.86</td>
</tr>
<tr>
<td>Ethanol yield [Yₚ/s] [g/g]</td>
<td>0.489</td>
<td>0.490</td>
<td>0.487</td>
<td>0.491</td>
<td>0.490</td>
<td>0.230</td>
<td>0.110</td>
</tr>
<tr>
<td>Sugar consumption [%]</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>89.3</td>
<td>77.5</td>
<td>12.4</td>
</tr>
</tbody>
</table>

The results are the average of three experiments.

\[
Y_{p/s} = \frac{\text{Mass of ethanol formed}}{\text{Mass of glucose consumed}}
\]
Fig. 2  Kinetic profile of ethanol fermentation from kitchen refuse by S. cerevisiae ATCC 26602. □Viable cells, ●glucose concentration, △ethanol concentration): (A) Sterilized condition; (B) pasteurized condition; (C) non-sterilized condition

4. Discussion

With the aim of lowering the production cost of ethanol, the feasibility of a less expensive kitchen refuse medium for pre-cultivation and fermentation was investigated. Only glucoamylase was used for saccharification, because main component of carbohydrate group in kitchen refuse are starch. No contamination or microbial growth was observed during saccharification. Lower pH due to lactic acid assimilation during storage and transportation, and higher temperature for saccharification might be the reason for suppression. In the case of pre-cultivation, although a large quantity of yeast inoculum (6.5% v/v) was added to the batch culture, the yeast strains grew well in both SPM and KRPM medium. In KRPM medium, no artificial glucose was used, and the amount of yeast extract and peptone used was less than in SPM medium. Therefore, from the economical viewpoint, the use of KRPM medium in the pre-culture step is more competitive and attractive than the use of conventional SPM medium. And no contamination observed during this fermentation step owing to the microbial tolerance to higher ethanol concentration.

The best yeast strain of the four ATCC strains for highly efficient ethanol production from kitchen refuse was selected. The type of yeast adopted in the fermentation process must be carefully selected to match the type of raw material, since the fermentation efficacy of the yeast depends on various physicochemical parameters of the substrate [10]. Therefore, acid tolerance was one of the most important factors in the selection of the best yeast strain in this work. Lactic acid was found to initially contaminate kitchen refuse during preservation, resulting in a low initial pH, around 3-5, of the saccharified kitchen refuse liquor [11]. We finally selected S. cerevisiae ATCC 26602, because of its high and stable ethanol production performance from acidic saccharified kitchen refuse. In addition, this yeast strain showed self-flocculating characteristics in kitchen refuse medium, a trait favorable to the increase of fermentation productivity.

Several compounds in the form of free amino nitrogen,
such as proteins, amino acids and vitamins, were supplied to improve ethanol tolerance and boost the final ethanol concentration [12, 13]. Normally, in industrial ethanol fermentation, \((\text{NH}_4\text{H})_2\text{SO}_4\), urea and yeast extract are supplemented as stimulative factors of yeast cell accumulation [14]. Conversely, the results shown in Table 3 indicate that it was not necessary to add other nutrients to the saccharified kitchen refuse solution to increase ethanol production. A similar phenomenon was also found when whey powder (CWP) was utilized for ethanol production [15]. The highest sugar consumption and the highest final ethanol concentration (10.5% v/v) were obtained from CWP liquor fermented by \textit{Kluyveromyces marxianus} NRRL-1195 without the addition of any other nutrients, whereas ethanol fermentation from acid-hydrolyzed cellulosic pyrolysatse by \textit{S. cerevisiae} needed supplementary urea as a nitrogen source to achieve high ethanol production [14]. When yeast extract was added to wheat mash containing 350 and 379 g of dissolved solids/L, ethanol productivities of 1.9 and 1.4 g/L/h were obtained [16, 17]. Furthermore, the supplementation of glucose medium with 12 g/L yeast extract, 3 g/L glycine and 20 g/L soya flour achieved a reduction of the fermentation time to 28 h, which means that the ethanol productivity was enhanced by 50% [13]. The complexity or richness of the kitchen refuse medium might have an effect on the ethanol yield. Vigegas et al. (1985) [3] also concluded that complex media support high ethanol production. However, it is difficult to determine the composition of mixed organic wastes such as kitchen refuse because the components vary depending on the season or the source of discharge. Therefore, more detailed studies on the effect of various kitchen refuse components, i.e. lipids and proteins, on ethanol fermentation by \textit{S. cerevisiae} should be carried out. The amount of dissolved solids after fermentation would be another important issue concerning to further application.

As can be seen in Table 4, the ethanol concentration, productivity, theoretical yields and sugar consumption values decreased as the sugar concentration of the kitchen refuse reached above 200 g/L. Identical results were obtained when ethanol fermentation from beet molasses [18] and soy bean pulp hemi cellulose was conducted [19]. Increasing sugar concentration resulted in a reduction of the total ethanol production. This reduction might have several causes, including the production of compounds other than ethanol, such as glycerol, acetic acid and \(	ext{CO}_2\). Also, it is possible that the compounds released in the medium consequently caused microbial stress. In addition, substrate inhibition or high osmotic pressure exerted by the concentrated sugar medium could also have caused slow cell proliferation [20]. These hypotheses are consistent with the biomass data.

The high efficiency and excellence of kitchen refuse as a substrate for ethanol production by \textit{S. cerevisiae} was confirmed in this study. Non-sterilized saccharified kitchen refuse could be directly used for ethanol fermentation by \textit{S. cerevisiae} ATCC 26602, without subsequent contamination or deterioration of ethanol productivity. This may be due to the large amount of yeast inoculum (6.5% v/v) applied to our batch culture. The acidity of the saccharified kitchen refuse medium also prevented contamination. Neelakantam et al. (2005) have reported that lowering the pH of the mash to 4.0 or less is one of the practices routinely followed to control bacteria contamination in fuel ethanol plants.

Extensive research on non-sterilized ethanol fermentation from renewable resources such as sweet sorghum [21], beet molasses [22] and cane molasses [23] has been intensively pursued in view of reducing the ethanol production cost. About 30-40% of the processing energy consumption can be saved by employing non-sterilized fermentation [24]. Kitchen refuse is one of the best substrates for ethanol fermentation, because it is economically feasible and available throughout the year without competing with human food or animal feed.

5. Conclusion

The excellent properties of kitchen refuse as a substrate for ethanol fermentation was shown in this work. No significant difference was observed between the synthetic medium and the kitchen refuse medium. When we compared four ATCC strains of \textit{S. cerevisiae}, ATCC 26602 showed a high ethanol production capacity regardless of the acidity of the saccharified kitchen refuse medium. Nitrogen supplementation of the kitchen refuse medium was not necessary. A maximum ethanol concentration of 59.38 g/L, equivalent to an ethanol yield of 0.50, was obtained from kitchen refuse medium with an initial glucose concentration of 118.20 g/L in flask culturing. Moreover, no pH control or asepsis was necessary for the ethanol fermentation from kitchen refuse medium by \textit{S. cerevisiae} ATCC26602. We also successfully scaled up the experiment from flask culturing to the use of a 20-L bioreactor. An ethanol concentration of 50.22 g/L, equivalent to an ethanol yield of 0.42, was obtained using a 20-L bioreactor without any sterilization.
Acknowledgements

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References


地球温暖化対策と枯渇性化石資源代替の観点から、バイオマス由来の自動車用燃料製造に、近年世界的関心が急増している。ガソリン代替としてのエタノールにせよ、軽油代替のバイオディーゼルにせよ、バイオマス由来の自動車用燃料製造において、持続可能性の観念が重要となる。すなわち、エタノール製造におけるトウモロコシやサトウキビ、またバイオディーゼルにおける油ヤシなど原料作物と食糧との競合、あるいは需要増に応じる土地利用変化による生物多様性の減少などの問題が指摘される。また、発酵原料化が期待される木質系バイオマスについては、前処理に要する投入エネルギー削減、五炭糖の利用に関する技術的課題の克服が必要な状況にある。

著者らは、炭素源やその他栄養源が豊富に含まれる食品廃棄物を発酵原料とする資源化プロセス、すなわちバイオマスプラスチックであるポリ乳酸やポリブチルコハク酸の原料となる有機酸の発酵法による製造を報告してきた。

再生可能エネルギーであるバイオ燃料を、食品廃棄物を原料として、ATCC株のエタノール発酵により製造するプロセスを、ビーカースケールで検討した。ここでは、食品廃棄物を酵素法により糖化し、得られた糖化液を原料とするエタノール発酵に取組んだ。

4種のATCC株による食品廃棄物由来培地からのエタノール産生能の比較から、耐酸性を有するATCC26602株を選択し、食品廃棄物由来の糖化液の糖濃度の影響や、外部からの新たな窒素源添加あるいは培地滅菌の要不要など詳細に検討した。食品廃棄物由来の糖化液を用いるエタノール発酵において、至適グルコース濃度は、12%であった。また、外部からの新たな窒素源の添加は不要であった。至適培地組成でpH調整を行わない振とう培養によるフラスコ試験の結果、エタノール濃度は、59.4 g/L、糖あたりのエタノール収率50%が得られた。食品廃棄物由来の糖化液を発酵培地とする際の滅菌について、滅菌、殺菌、無滅菌を比較したところ、エタノール産生およびグルコース消費に、顕著な差異はみられなかった。フラスコ試験からスケールアップして、20 Lのバイオリアクターを用いた場合（無滅菌、30℃で18時間培養）にも、エタノール濃度は、50.2 g/L、糖あたりのエタノール収率42%が得られた。

無滅菌条件下でも発酵が可能という点は、食品廃棄物からのエタノール発酵の商業生産に大きな展望を拓くものである。今後は、発酵形式の検討や、商業生産に向けたプロセス検討とスケールアップを行う予定である。