Continuous ethanol production by fermentation of waste banana peels using flocculating yeast

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Warana Agricultural Goods Processing Co-operative Society (WAGPCOS), a unit producing banana puree at Amrignagar gives out the banana peels along with the foul bananas as a major waste. This waste was tested for its sugar content and was found to contain substantial amount of sugars with hardly any hazardous material for the fermentative microorganisms i.e. yeast. To utilize this waste as a fermentation substrate, extract was prepared from the waste. Highly flocculant yeast Saccharomyces uvarum (NCIM culture 3528) having a high fermentation activity was used for the fermentation. A multi vessel system was prepared for the fermentation of waste extract. The system consisted of a main reactor, a buffer vessel and a separation vessel. The fermentation was carried out under anaerobic conditions with CO₂ bubbling and well maintained pH and temperature. Runs were carried out at different feed rates with continuous yeast cell recycle. It was observed that the waste banana peels are capable of providing enough sugar for the fermentation and hence can be economically utilized for the ethanol production. After the removal of the extract from the waste, the remaining fibrous material can be dried in an easier way and can prove to be a good fodder for the animals.

Instead of going for the conventional ethanol synthesis from petroleum sources, recent trend is concentrated towards the industrial ethanol production from biomass via fermentation. Since the beginning of the last century, the ethanol fermentation producing fuel or feed stock was based on traditional batch process for the manufacture of alcoholic beverages. However, the new techniques like continuous and continuous with cell recycle modes are having advantages in both capital and running costs as compared with the traditional batch process. Various methods that have been studied up till now for continuous fermentation include the mechanical centrifuge or entrapment of the growing cells in polymer gels like κ-carrageenan which returns a concentrated biomass stream to the mixed reactor and permits a relatively clear effluent to be fed to the distillation columns. The most successful technique in this type is that of attached separator (sedimentation vessel) for the flocculation of the microorganisms and recycling of the settled organisms.

WAGPCOS produced puree of about 531 tons of banana last season (1998-99). The waste peels and the waste bananas being thrown out and used as animal fodder if properly dried. Usually it is quite difficult to dry all the waste as due to the sugars present in the waste, it gets contaminated by bacteria and thus forms a foul mass with notorious odour. With the trend of new ages of exploiting every part of the material it was a keen choice of this waste as a source of sugar. The reducing sugar content was checked properly and no hazardous material for yeast was found in the extract obtained from the waste.

For the continuous fermentation of this extract by the yeast effectively and efficiently, it was essential to select a suitable strain of the yeast having a high fermentation activity in addition to good flocculation. Furthermore, a yeast strain having tolerance for high temperature (about 35°C) was desirable. Saccharomyces uvarum (NCIM culture 3528) was capable of satisfying all the needs given above and was obtained from National Chemical Laboratories, Pune.

Experimental Procedure

Organism and culture conditions

Saccharomyces uvarum was maintained at 4°C on nutrient agar slopes. The medium for slopes was MGYP medium i.e. malt extract 0.3%, glucose 1.0%, yeast extract 0.3%, peptone 0.5%. The culture was subcultured every 1½ months. Inocula for the continuous fermentation were prepared in shake flasks containing pre-sterilized 10% MYGP broth containing

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11% glucose at pH 5.5 grown aerobically for 24 h at 30-35°C.

The yeast *S. uvarum* is highly cohesive and resulting flocks quickly sediment in still medium when the pH is between 4 to 7. The yeast settles up to 20% or less height in about 2-2.5 min in 100 mL measuring flask.

Assays—
- The cell count was taken using the Neubaur's cell counting chamber.
- Reducing sugars were determined by using standard DNSA method.
- Ethanol was assayed by distillation of 100 mL of the product sample with 100 mL of distilled water and then measuring the specific gravity of the out coming 100 mL of distillate from the standard charts of specific gravity versus the ethanol concentration.

Medium—The medium for fermentation by the yeast was prepared as the extract of the waste. The peels were crushed and 3 samples were prepared by addition of 100, 200, 300 mL of water and 1N HCl to 1000 g of the banana peels each. The samples were autoclaved at 1.5 kG/cm² pressure for about 25-30 min. The samples were filtered by using cheesecloth and tested for total reducing sugars (TRS). The results are as shown in Table 1.

Fig. 1—Experimental setup

Fig. 2—Effect of dilution rate on productivity and ethanol concentration

The heating increases the hydrolyzing of higher sugars and thus makes available of substantial amount of reducing sugars. For the further increase of sugar % in the feed or the extract simple evaporation was employed to get about 12% of TRS in the feed. Feed for the fermentation was prepared by taking right amount of waste and getting their extract (usually 4-5 L).

Cultivation and Equipment

Inoculum development—A 200 mL medium (10% of the total system volume) as quoted above was prepared and organisms were transferred to it under aseptic conditions. The multiplication of microorganisms was achieved by continuous shaking for about 24 h on a rotary shaker at 30-35°C.

Equipment and continuous operation—A system for a high cell concentration was designed as shown in Fig 1. The system was composed of three vessels, a main reactor or fermenter (1300 mL), a buffer vessel (300 mL) and a separation vessel (450 mL). The inoculum was introduced in the main reactor and addition of feed was started at a controlled flow rate, from which the broth was over flowed into the buffer vessel. The separation vessel accepted the down flow from the bottom of the buffer vessel. The cells coagulated in the settler were recycled into the main reactor at a recycle ratio of 7.0. Withdrawal of the alcohol was done from the top separation vessel. The role of the buffer vessel was to suppress the perturbations in the overflow from the main reactor.
Table 1—TRS testing for the various extracts

<table>
<thead>
<tr>
<th>Sample</th>
<th>Banana peels (g)</th>
<th>Water added* (L)</th>
<th>Acid added (mL)</th>
<th>Extract obtained (mL)</th>
<th>TRS (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1000</td>
<td>100</td>
<td>60</td>
<td>905</td>
<td>101</td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>200</td>
<td>60</td>
<td>1003</td>
<td>91</td>
</tr>
<tr>
<td>3</td>
<td>1000</td>
<td>300</td>
<td>60</td>
<td>1092</td>
<td>83</td>
</tr>
</tbody>
</table>

*In the above testing, addition of at least 100 mL of water was necessary for ease of crushing in the mixer and extraction.

Table 2—Runs and results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Feed rate (F) (m/h)</th>
<th>Dilution rate (D_r) (h)</th>
<th>Residence time (\tau) (h)</th>
<th>Alcohol conc. (P_0) (g/L)</th>
<th>Residual sugar (g/L)</th>
<th>Productivity (D_rP_0) (g/L.h)</th>
<th>Cell conc. (\text{Cell conc.}) (l/mL) (10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>230</td>
<td>0.18</td>
<td>8.91</td>
<td>49.61</td>
<td>58.49</td>
<td>8.928</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>273</td>
<td>0.21</td>
<td>7.50</td>
<td>89.81</td>
<td>11.89</td>
<td>18.56</td>
<td>47.3</td>
</tr>
<tr>
<td>3</td>
<td>310</td>
<td>0.24</td>
<td>6.60</td>
<td>72.53</td>
<td>31.03</td>
<td>17.41</td>
<td>49.8</td>
</tr>
<tr>
<td>4</td>
<td>338</td>
<td>0.26</td>
<td>6.30</td>
<td>61.84</td>
<td>44.41</td>
<td>16.08</td>
<td>48.2</td>
</tr>
</tbody>
</table>

Results and Discussion

The organism growth was little affected by the variation in pH range 4 to 7. Temperature also had little effect on ethanol productivity. The strain of yeast \(S\) \text{uvarum}\ showed high flocculating characteristics and the culture beer above the cell layer, which was taken out as product was almost free from cells and clear. The high flocculating activity of this strain allowed the concentrated cell suspension after treatment in a simple separation vessel or settler.

The cell recycling continuous culture system allowed holding of cells at a high concentration even at higher dilution rate. The several terms used in this continuous operation were

Overall dilution rate \(D\): Dilution rate for reactor \((h)\); \(D_r\): Cell recycle ratio; \(\alpha\): Feed rate of fresh medium \((L/h)\); \(F\): Flow rate of recycling \((L/h)\); \(f\): Volume of \((L)\): Main fermenter: \(V_\text{r}\); Buffer vessel: \(V_b\); Separation vessel: \(V_s\); Amount of TRS \((g/L)\): \(S\); Ethanol concentration \((g/L)\): \(P_0\); Productivity \((g/L.h)\): \(D_rP_0\)

The useful relations relating above variables are as follows: \(D = F / (V_\text{r} + V_b + V_s)\); \(D_r = F / V_\text{r}\); \(\alpha = f / F\)

From the different runs at different dilution rates the following conclusions can be drawn on the results.

Effect of dilution rate on productivity

It is seen from the graph of productivity versus dilution rate (Fig. 2) that, productivity increases initially as the dilution rate is increased up to \(D_r = 0.21 \text{ /h}\). The productivity after this value of \(D_r\) decreases again indicating that this dilution rate \(D_r\) produces a wash out condition when increased above this value. At the smaller dilution rates than the above-mentioned value, the residence time is obviously more and the sugars are consumed to a maximum limit. But as the productivity takes into consideration both the ethanol concentration and the dilution rate, it is low for any dilution rate less than 0.21 /h. At the higher dilution rates, the residence time and the sugar conversion is low with lower alcohol production. Hence, although the product is obtained faster the productivity is low.

At the dilution rate of 0.21 /h both the residence time and the sugar conversion are at the optimum value, so productivity is optimum i.e. the best results are obtained at \(D_r = 0.21 \text{ /h}\), residence time \(7.5\ h\), alcohol concentration of 89.81 g/L. Similar conclusion can be drawn from the graph of dilution rate versus ethanol concentration.

Conclusions

The waste banana peels are capable of providing enough sugar for the fermentation and hence can be economically utilized for the ethanol production. After the removal of the extract from the peels, the remaining fibrous material can be dried in an easier way and can prove a good fodder for the animals.

The flocculating yeast \(S\) \text{uvarum}\ has a very good settling characteristics and gives the product almost
free of the cells which can be directly given to the distillation column. The separation cost is much lower than the traditional systems in case of a simple separation vessel used in the system.

Continuous fermentation with closed loop recycling of yeast cells resulted in reduction of production time, evaporation loss, lesser man power requirement with better control over the fermentation process.

References