Utilisation of fruits waste for citric acid production by solid state fermentation

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Abstract

A solid state fermentation method was used to utilise pineapple, mixed fruit and maosmi waste as substrates for citric acid production using \textit{Aspergillus niger} DS 1. Experiments were carried out in the presence and absence of methanol at different moisture levels. In the absence of methanol the maximum citric acid was obtained at 60\% moisture level whereas in the presence of methanol the maximum citric acid was obtained at 70\% moisture level. The stimulating effect of methanol was less at lower moisture level. The inhibitory effect of metal ions was also not observed and maximum citric acid yield of 51.4, 46.5 and 50\% (based on sugar consumed) was obtained from pineapple, mixed fruit and maosmi residues, respectively.

\textit{Keywords}: Solid state fermentation; Citric acid; Fruits waste; \textit{Aspergillus niger}

1. Introduction

Citric acid is a commercially valuable product, widely used in the food, pharmaceutical and beverage industries as an acidifying and flavour-enhancing agent. It is produced commercially by submerged fermentation of sucrose or molasses based medium \cite{1-3}. Recently, there have been an increasing number of reports on the use of solid state fermentation (SSF) processes as an alternative to submerged fermentation \cite{4-6}. This is because of lower energy requirements, higher product yields little risk of bacterial contamination, less wastewater generation and environmental concerns regarding the disposal of solid waste \cite{8,9}. Certain metal ions (Fe\textsuperscript{+++}, Mn\textsuperscript{+++}, Zn\textsuperscript{++} etc.) are known to be inhibitory to citric acid production by \textit{Aspergillus niger} in submerged fermentation, even at very low concentration \cite{1}. SSF gives high citric acid yield without inhibition related to presence of metals at high concentration \cite{10}. Shankaranand and Lonsane, \cite{11} even reported that addition of minerals into the production media to a certain level enhanced citric acid production. Therefore SSF is certainly a good way of utilising nutrient rich solid waste as a substrate.

Fruits waste (pineapple, mixed fruits and maosmi residue) produced in huge amount in India, is either used as an animal feed or disposed to the soil. Since fruit waste is rich in carbohydrate and other nutrients, it can serve as a substrate for citric acid production using SSF. In recent years, considerable interest has been developed in using agricultural wastes, including apple pomace \cite{4-7}, grape pomace, \cite{12}, kiwi fruit peel \cite{13} carob pod \cite{14}, kumanara \cite{9}, okara \cite{15} as substrates for citric acid production. The objective of this work was to evaluate the suitability of pineapple, mixed fruits and maosmi residues as a substrate for citric acid production by SSF.

2. Material and methods

2.1. Organism

\textit{A. niger} DS 1, isolated from rotten lemon was used in the present study. It was maintained on potato dextrose agar slants and stored at 4 \degree C and subcultured after every 2 weeks interval.
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<thead>
<tr>
<th>Waste</th>
<th>70% Moisture</th>
<th>Yield (%)</th>
<th>Sugar consumed (%)</th>
<th>60% Moisture</th>
<th>Yield (%)</th>
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<td></td>
<td>Citric acid (g/100 g)</td>
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<td>Citric acid (g/100 g)</td>
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<td>Pineapple</td>
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<td>54.2</td>
<td>99.9</td>
<td>9.7</td>
<td>46.8</td>
<td>100</td>
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<tr>
<td>Mixed fruit</td>
<td>10.7</td>
<td>46.5</td>
<td>98.2</td>
<td>9.7</td>
<td>43.0</td>
<td>97.5</td>
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<tr>
<td>Maosmi</td>
<td>14.6</td>
<td>50.0</td>
<td>92.7</td>
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<tr>
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<td>28.7</td>
<td>99.0</td>
<td>7.1</td>
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<tr>
<td>Maosmi</td>
<td>10.1</td>
<td>33.2</td>
<td>95.6</td>
<td>9.2</td>
<td>30.4</td>
<td>95.3</td>
</tr>
</tbody>
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### 2.2 Media preparation and SSF

Three different fruits wastes (pineapple, mixed fruit and maosmi residues) were procured from a juice extracting shop of the local market and the material from one single batch was used in all the studies in order to minimise any possible interference due to variation in composition of residues.

Fruit waste was dried in an oven at 60 °C for 2 days, ground and screened to collect the particles of the size between 1.2 and 1.6 mm. Five gram grounded fruit waste was taken in 250 ml conical flask and moistened with distilled water to desired moisture level (varying from 50 to 80%). Since maosmi waste contained less sugar it was moistened with sucrose solution to increase the sugar level to 31.8 g/100g of dry solid (previously optimised). The flasks containing medium were sterilized at 121 °C for 1 h to provide proper cooking to the substrate and to increase its amenability for microbe. After sterilisation, flasks containing media were allowed to cool to room temperature and inoculated with 1 ml of spores suspension (2 x 10⁷ spores/ml) of A. niger DS 1 followed by proper mixing. The spore suspension was prepared in sterilised distilled water and Tween 80 solution by scratching a slant of A. niger DS 1. The concentration of spores was adjusted to 2 x 10⁷ spores/ml and used in each experiment. After inoculation flasks were incubated at 30 °C in a humidity controlled incubator. Methanol (4% v/v) was added to the medium before inoculation and after sterilisation of the medium. One flask was harvested every day and the entire content of the flask was taken as sample for the estimation of citric acid and residual sugar.

### 2.3 Extraction and analytical methods

Fermented material of the flask was dried in an oven at 50 °C and extracted by the addition of 100 ml of distilled water. The mixture was agitated on a rotary shaker for 2 h and then filtered through Whatman filter paper no. 1. The supernatant was used for the estimation of total residual sugar and citric acid. Total sugar was determined by the phenol sulphuric acid method of Dubois et al. [16] and citric acid was estimated by the acetic anhydride and pyridine method of Marier and Boulet [17].

### 2.4 Biomass estimation

Since there is no direct method for the estimation of biomass in SSF, biomass was estimated on the basis of loss of organic matter or dry solid during the course of fermentation. A known amount of substrate (accurately weighed) was taken, moistened with a known amount of moisture and inoculated with a known volume of spore suspension. In such a way a number of flasks were prepared and fermentation was carried out as described previously. Every day, one flask was harvested and the culture dried in an oven at 50 °C (to constant weight) and weighed. The daily loss of weight was calculated by subtracting the daily dry (day 1–9) fermented material of the flask from the initial dry weight of the material of the flask. The loss of organic matter or dry weight is presumably due to the loss of CO₂. Therefore the rate of respiration can be calculated by calculating the loss of organic matter, which gives an estimate of growth (data not shown in paper).
3. Results and discussion

The pineapple, mixed fruit and maosmi residues, contained 20.85, 23.4 and 10.2 g sugar/100g of dry solid, respectively. These wastes were used as substrates for citric acid production. The results are given in Tables 1 and 2. Since the requirement for moisture depends upon the characteristic of the substrate in SSF, the experiments on pineapple waste were conducted at different moisture levels (50, 60, 70 and 80%) in the absence and presence of methanol and the results are shown in Figs. 1 and 2. The maximum rate of citric acid production was observed on the 4th, 5th and 6th days of fermentation and the maximum con-
Fig. 3. Citric acid production from mixed fruit waste at 60 and 70% Moisture Level in the presence and absence of methanol. Symbols m50, m60, m70 and m80 are representing citric acid production and symbols ms50, ms60, ms70 and ms80, are representing sugar consumption at 50, 60, 70 and 80% moisture level, respectively.

Fig. 4. Citric acid production in SSF using maosmi residue. Symbols M and Ms are representing citric acid production and residual sugar at 60 and 70% moisture level in the presence (60p and 70p) and absence (60a and 70a) of methanol.

centration of citric was obtained after 8 days of fermentation. About 99% sugar was consumed at the end of the 8th day of fermentation. In the absence of methanol, the maximum citric acid was produced at 60% moisture level whereas in the presence of methanol maximum citric acid was produced at 70% moisture level. The requirement of moisture increased in the presence of methanol, which may be due to the dehydrating action of methanol. The rate of citric acid production was increased in the presence of methanol whereas rate of sugar consumption was decreased. Rate of sugar consumption was also lower when fermentation was carried out at a lower moisture level. The maximum sugar consumption was observed at 70% moisture level. At 80% moisture level both citric acid production and sugar consumption was less. This
may be due to reduced porosity and poor heat and mass transfer.

Since changes in citric acid production were observed only on 60 and 70% moisture levels the experiments on mixed fruit waste were carried out only at these moisture levels in the presence and absence of methanol and the results are shown in Fig. 3. Fermentation was carried out for 8 days. Similar stimulatory effects of methanol, as in case of pineapple waste, were observed in this case. In the absence of methanol, citric acid production was favoured at 60% moisture level but sugar consumption was comparatively lower than 70% whereas in the presence of methanol both citric acid production and sugar consumption was favoured at 70% moisture level.

Since maosmi residue contained less sugar, it was supplemented with sucrose. The maximum citric acid concentration 14.6 g/100g of dry solid with 50% (based on sugar consumed) yield was obtained after 9 days of fermentation (Fig. 4). At the end of fermentation about 92.7% sugar was consumed. In maosmi residue, the maximum citric acid production was obtained at 70% moisture level in the presence and absence of methanol (Fig. 4) and this indicated that the requirement of moisture and methanol for maximum citric acid production depends on the characteristics of solid substrate.

Hang and Woodams [4,5] reported 88 and 60 (based on sugar consumed) yields of citric acid from apple pomace and grape pomace. Hang et al. [13] produced 100 g citric acid/kg of kiwi fruit peel with yield of 60% by A. niger NRRL 567 in the presence of 2% methanol. Roukas [14] obtained 55% citric acid yield from carob pod using A. niger ATCC 9142. Khare et al. [15] obtained 52.8% citric acid yield from okara (soy residue) using A. niger NRRL 330. Lu et al. [9] obtained 54% citric acid yield from kumara using A. niger Yang no. 2. The increase in citric acid yield with methanol is a general phenomenon and is commonly used in citric acid production [1]. Hang and Woodams [4-6] reported similar stimulatory effects of methanol on citric acid production by SSF using A. niger. The mechanism of increase in citric acid yield in the presence of methanol is not known. Maddox et al. [18] reported that methanol increased the permeability of cells to citrate. Hamissa [19] and Dasgupta et al. [20] have reported that moderate concentrations (1–3%) of methanol decreased the iron and manganese uptake by the fungus and doubled the citric acid yield.

4. Conclusion

SSF of pineapple, mixed fruit and maosmi waste by A. niger DS 1 in the presence of 4% methanol yielded 54.2, 46.5 and 50% (based on sugar consumed) citric acid. From the results of the present study, it may be concluded that fruit processing solid residues can serve as a substrate for the production of some value added product using SSF. The use of fruit waste for the production of citric acid may have the combined benefit of utilising a low-grade waste while producing a commercially valuable product.

References