SELECTION OF NATURAL MATERIALS FOR *Saccharomyces cerevisiae* IMMOLIZATION

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ABSTRACT

The interest in cell immobilization for alcoholic beverage production has increased in the last decades due to the several advantages that this process presents, including reduced risk of contamination, biocatalyst recycling, rapid product separation and others. Moreover immobilized cells systems have been considered as a promising alternative to improve the performance of biotechnological processes, since in these systems, immobilized cells completely maintain their biological functions with increased stability that may often lead to increased cell productivity. However, the correct selection of immobilization carrier is essential to design an effective system to each particular purpose. The objective of the present study was to find a cheap and abundant natural material of food grade purity suitable for immobilization of *Saccharomyces cerevisiae* yeast, which is usually used in fermentation processes for wine production. Four different carriers were evaluated, namely grape seeds, grape skin, grape stems and corn cobs.

MATERIALS AND METHODS

The support materials were prepared by washing with distilled water and drying at 60 °C until constant mass. A commercial *Saccharomyces cerevisiae* was the yeast strain used in the experiments. For inoculum preparation, the yeast was cultivated in YPD medium in static conditions at 30°C for 24h. Fermentation runs were performed in semi-synthetic medium with the following composition (% w/v): glucose 12, yeast extract 0.4, (NH$_4$)$_2$SO$_4$ 0.1, KH$_2$PO$_4$ 0.1, and MgSO$_4$ 0.5. The assays were carried out in 500 ml Erlenmeyer flasks containing 200 ml of medium and 2 g of the material carrier. The flasks were statically incubated at 30 °C for 24 h. For comparison, assays under the same conditions described above were also performed without support addition. Fermentations were carried out in duplicate, and samples were taken periodically for estimation of biomass, glucose consumption and ethanol production. Immobilized cells concentration was determined at the fermentation end.

RESULTS AND DISCUSSION

All the fermentation runs with immobilized cells lasted after 24 h, half of the necessary time for total glucose consumption in medium containing free cells. The four supports showed no differences in terms of produced ethanol and ethanol productivity ($Q_p$) (Table 1).

Table 1: Multiple comparison analysis (Tukey’s test) with 95 % of confidence for the concentration of immobilized cells ($C_{\text{biom}}$) and ethanol ($C_{\text{eth}}$), ethanol yield ($Y_{\text{P/S}}$) and productivity ($Q_p$)

<table>
<thead>
<tr>
<th>Carrier</th>
<th>$C_{\text{biom}}$/ (mg/g)</th>
<th>$C_{\text{eth}}$/ (g/L)</th>
<th>$Y_{\text{P/S}}$/ (g/g)</th>
<th>$Q_p$/ [g/(L/h)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>corn</td>
<td>22.20 $^b$</td>
<td>53.48 $^a$</td>
<td>0.51 $^b$</td>
<td>3.35 $^a$</td>
</tr>
<tr>
<td>stems</td>
<td>4.08 $^a$</td>
<td>53.89 $^a$</td>
<td>0.44 $^a$</td>
<td>3.37 $^a$</td>
</tr>
<tr>
<td>skin</td>
<td>25.10 $^b$</td>
<td>54.46 $^b$</td>
<td>0.49 $^b$</td>
<td>3.41 $^b$</td>
</tr>
<tr>
<td>seeds</td>
<td>1.68 $^a$</td>
<td>54.05 $^a$</td>
<td>0.51 $^b$</td>
<td>3.38 $^a$</td>
</tr>
</tbody>
</table>

Corn cobs and grape skins were the best material carriers for *S. cerevisiae* immobilization (Figure 1), since they immobilized the highest amount of cells (22.2 ± 0.9 mg/g and 25.1± 10.8 mg/g, respectively) and fermentation with these immobilized cells gave elevated ethanol yield ($Y_{\text{P/S}} = 0.51$ and 0.49 g/g, respectively) and productivity ($Q_p = 3.35$ and 3.41 g/(Lh), respectively). On Figure 2 are demonstrated images of the immobilized supports. These results are of great interest since the material did not require any pre-treatment to be used as immobilization carrier.
Figure 1: Ethanol production, glucose consumption and concentration of free biomass in function of time.

Figure 2: Photographs (SEM) of supports with immobilized cells A - corn cobs, B - grape skins, C - grape stems, D - grape seeds.