

## Comparative study of bioethanol production from mahula (*Madhuca latifolia* L.) flowers by immobilized cells of *Saccharomyces cerevisiae* and *Zymomonas mobilis* in calcium alginate beads

Shuvashish Behera<sup>1</sup>, Ramesh C Ray<sup>2</sup> and Rama C Mohanty<sup>1\*</sup>

<sup>1</sup>Department of Botany, Utkal University, Bhubaneswar 751 004, India

<sup>2</sup>Central Tuber Crops Research Institute (CTCRI) (Regional Centre), Bhubaneswar 751 019, India

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This study presents ethanol production from mahula flowers in submerged fermentation (SmF) using immobilized cells of *Saccharomyces cerevisiae* (CTCRI strain) and *Zymomonas mobilis* (MTCC 92) in calcium alginate as beads. Maximum ethanol concentrations were 154.5 and 134.55 g kg<sup>-1</sup> flowers using immobilized cells of *S. cerevisiae* and *Z. mobilis*, respectively. Immobilized cells of *S. cerevisiae* in calcium alginate beads were more effective (14.83% more yield) for ethanol production than immobilized cells of *Z. mobilis*.

**Keywords:** Bioethanol, Fermentation, *Madhuca latifolia*, *Saccharomyces cerevisiae*, *Zymomonas mobilis*

### Introduction

Ethanol production by fermentation has received special attention to solve world energy crisis<sup>1</sup>. Agricultural wastes and forest based materials (cassava bagasse, sugarcane bagasse, apple pomace, peels, skins and stones of different fruits, mahula flower, etc.) are considered most promising alternative for ethanol production by submerged (SmF) fermentation.

Mahula (*Madhuca latifolia* L.) tree is found in abundance in tropical rain forests of Asian and Australian continents<sup>2</sup>. Its flowers are a rich source of fermentable sugars, which can be potentially converted into bioethanol. Yeast, *Saccharomyces cerevisiae*, has been used as major ethanol producing microorganism. *Zymomonas mobilis*, gram negative anaerobic bacterium, is another suitable organism for ethanol production<sup>3</sup>. Yeast (*S. cerevisiae*) has been used for ethanol production from mahula flowers using submerged (SmF)<sup>2,4</sup> and solid state fermentation (SSF)<sup>5</sup>.

Immobilization of whole microbial cells and their application in bioprocessing has been of interest for 30 years<sup>6</sup>. Immobilization of whole cells for ethanol production offers several advantages<sup>7</sup> (ease to separate cell mass from bulk liquid for possible reuse, facilitates

continuous operation over a prolonged period, enhances reactor productivity, ensures higher efficiency of catalysis). One of the most suitable carriers for cell immobilization is entrapment in calcium alginate as bead<sup>8,9</sup> due to simple and cost effective<sup>10</sup> technique. Sodium alginate, precursor of calcium alginate and a non-toxic chemical, is most suitable as an immobilization matrix for entrapping biomolecules and microorganisms<sup>8</sup>. This technique has been used extensively in fermentation industries for producing amino acids<sup>11</sup>, enzymes<sup>12</sup>, organic acids<sup>13</sup> and ethanol<sup>4,14</sup>.

This study compares production of ethanol from mahula flowers in SmF by using immobilized cells of *S. cerevisiae* and *Z. mobilis* in calcium alginate as beads.

### Materials and Methods

#### Mahula Flowers

Fresh mahula flowers were collected from forests of Keonjhar District of Orissa, India, during March-April 2007. At Microbiology Lab, CTCRI, flowers were washed in tap water and sun-dried for 7 days to reduce moisture content to 16-18.6%. Sun-dried flowers mixed thoroughly before use. Flowers (pH 4.5-4.8) had following composition (dry wt basis): moisture, 24.00-25.85; starch, 0.94-0.95; total sugar (glucose, fructose, sucrose and maltose), 36-38; crude protein, 6-7; crude fiber, 10.0-12.5; total ash, 1.6-2.0; and undetermined solids, 10.6-13.7%.

\* Author for correspondence  
E-mail: rc\_mohanty@sify.com

### Microorganisms and Culture Conditions

*Z. mobilis* MTCC 92 was procured from IMT, Chandigarh. *Z. mobilis* and *S. cerevisiae* (CTCRI strain) were earlier used for ethanol fermentation<sup>7,8</sup> maintained on *Z. mobilis* specific medium (ZSM) [glucose, 100; yeast extract, 2; urea, 1;  $\text{KH}_2\text{PO}_4$ , 1;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5; and agar, 15 g l<sup>-1</sup>; pH 6.5]. Yeast (*S. cerevisiae*) was maintained on Malt extract-Yeast extract-Glucose-Peptone (MYGP) medium [malt extract, 3; yeast extract, 5; peptone, 5; glucose, 20; agar, 15 g l<sup>-1</sup>; pH 5.5]. Both cultures were stored at 4 ± 0.5°C for further use.

### Inoculum Preparation for Immobilized Cells

For preparation of starter cultures, 100 ml of respective growth media (ZSM for *Z. mobilis* and MYGP for *S. cerevisiae*) were taken in Erlenmeyer flasks (250 ml each), sterilized at 121°C for 20 min and inoculated with a loopful of cultures. Cell (*Z. mobilis* or *S. cerevisiae*) suspension (equiv 10%) was added to sodium alginate solution (4% w/v) in a 1:1 (vol) ratio and mixed thoroughly. Cell-alginate mixture was then cast into beads by dropping from a hypodermic syringe into cold sterile 0.1 M  $\text{CaCl}_2$  solution. These beads (diam, 3.0 mm) were hardened by keeping in dilute (0.1 M)  $\text{CaCl}_2$  solution for 24 h at 4°C with gentle agitation<sup>15</sup>. Finally, beads were washed with sterile distilled water to remove excess  $\text{Ca}^{2+}$  ions and untrapped cells before being used for fermentation. To obtain a high cell density, gel beads containing immobilized cells were immersed in their respective growth medium (ZSM or MYGP) for 24 h at 30°C.

### Fermentation Medium

Mahula flowers were grinded (flower: water, 1: 5) in a mixer-grinder (TTK Prestige Ltd., Bangalore, India) to make slurry. Then, slurry was cooked by steaming at 80-100°C for 60-80 min. After cooling,  $(\text{NH}_4)_2\text{SO}_4$  was added to slurry as nitrogen source (rate, 1 g l<sup>-1</sup>) and pH was adjusted to 5.5 for fermentation by yeast and 6.5 for fermentation by *Z. mobilis*. Then, two sets of mahula slurries were separately inoculated with immobilized cells of either *Z. mobilis* or *S. cerevisiae* cells. These two sets (n=3) of flasks were incubated for 96 h at room temperature (RT) (30±2°C).

### Analytical Methods

Fermented broths (in triplicate) were removed at 24 h intervals and contents were analyzed for total sugar and ethanol. By measuring specific gravity of distillate using reported method<sup>16</sup>, ethanol content of fermented

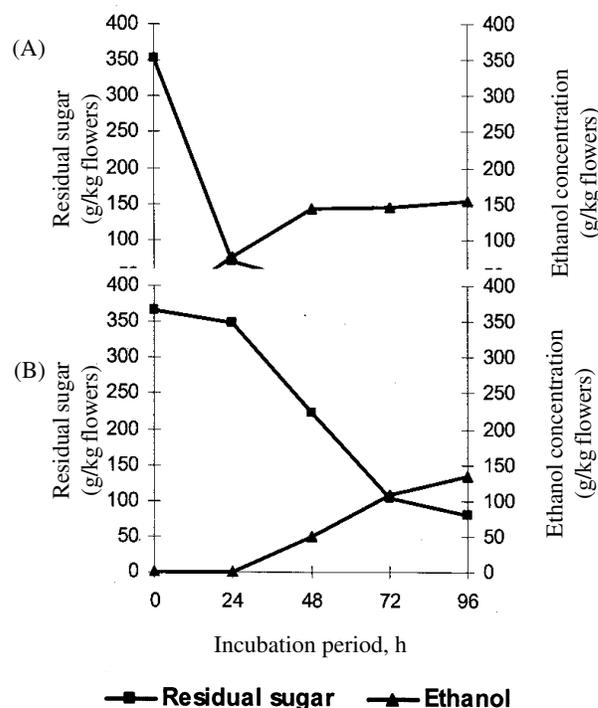


Fig. 1— Comparison of ethanol production between immobilized cells of: A) *S. cerevisiae*; and B) *Z. mobilis*

broth was determined. Total sugar was assayed by Anthrone method<sup>17</sup>. pH was measured using a pH meter (Systronics, Ahmadabad, India) fitted with a glass electrode. Fermentation kinetics was calculated using reported formulae<sup>18</sup>.

### Results and Discussion

Mahula flowers (100 g) after cleaning were blended with water (1:5) to dilute bulkiness of mash before steaming and subsequent SmF by Ca-alginate entrapped immobilized cells of yeast, *S. cerevisiae* and bacterium, *Z. mobilis*. Ethanol production started after 24 h (*S. cerevisiae*) and 48 h (*Z. mobilis*) and maximum ethanol productions were achieved at 96 h of fermentation.

Comparing of ethanol production and sugar utilization from mahula flowers by both immobilized strains in SmF (Fig. 1), sugar utilization capacity of immobilized cells of *S. cerevisiae* was found faster than that of immobilized cells of *Z. mobilis*. In both cases, ethanol production increased concomitant with increase in sugar utilization. In case of *S. cerevisiae*, concentration of residual sugars fell rapidly and consistently during 24 h of fermentation, after which decline was gradual up to 96 h (Fig. 1A). However, residual sugar decreased gradually in case of *Z. mobilis* (Fig. 1B). At 24 h of fermenta-

Table 1—Growth and fermentation kinetics of immobilized cells of *S. cerevisiae* and *Z. mobilis* on mahula flower

	Immobilized cells of <i>S. cerevisiae</i>	Immobilized cells of <i>Z. mobilis</i>
Final ethanol ( $P$ , g l <sup>-1</sup> )	25.75	22.425
Final biomass concentration ( $X$ , g l <sup>-1</sup> )	4.28	1.57
Specific growth rate ( $\mu$ , h <sup>-1</sup> )	0.098 <sup>a</sup>	0.079 <sup>b</sup>
Cell yield ( $Yx/s$ , g g <sup>-1</sup> )	0.08	0.033
Ethanol yield ( $Yp/s$ , g g <sup>-1</sup> )	0.483	0.473
Volumetric substrate uptake ( $Qs$ , g l <sup>-1</sup> h <sup>-1</sup> )	0.554	0.493
Volumetric product productivity ( $Qp$ , g l <sup>-1</sup> h <sup>-1</sup> )	0.268	0.233
Conversion rate into ethanol, %	96.75	94.64

tion, immobilized cells of *S. cerevisiae* produced ethanol (75.6 g kg<sup>-1</sup> flowers), whereas no ethanol was produced by *Z. mobilis* strain during that period. Finally, after 96 h of fermentation, maximum ethanol concentrations were obtained with immobilized cells of *S. cerevisiae* (154.5 g kg<sup>-1</sup> flowers) and *Z. mobilis* (134.55 g kg<sup>-1</sup> flowers) grown in mahula flower slurry. Superiority and efficacy of *S. cerevisiae* over *Z. mobilis* entrapped in propylene or plastic composite supports are also reported during production from molasses<sup>19,20</sup>.

Immobilized cells of *S. cerevisiae* in calcium alginate beads were more effective (14.83% more yield) for ethanol production than *Z. mobilis* cells (Table 1). Final biomass concentration, cell yield, ethanol yield, volumetric substrate uptake, volumetric product productivity and final sugar to ethanol conversion rate of immobilized cells of *S. cerevisiae* in SmF were 172.61, 142.42, 2.11, 12.37, 15.02 and 2.23%, respectively, higher than those of *Z. mobilis*. Thus immobilized cells of *S. cerevisiae* were more efficient ethanol producer than *Z. mobilis* cells as also reported for molasses using *Saccharomyces* than *Zymomonas* for ethanol fermentation<sup>20</sup>.

## Conclusions

Ethanol production from mahula flowers by immobilized cells of *S. cerevisiae* in calcium alginate gel beads was more (14.83%) than that of *Z. mobilis*. Final values [biomass concentration ( $X$ ), 172.61; cell yield ( $Yx/s$ ), 142.42; ethanol yield ( $Yp/s$ ), 2.11; volumetric substrate uptake ( $Qs$ ), 12.37; and volumetric product productivity ( $Qp$ ), 15.02%] of immobilized cells of *S. cerevisiae* in SmF were higher than respective values of *Z. mobilis* after 96 h of fermentation.

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