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MICROENCAPSULATION OF YEAST CELLS IN THE CALCIUM ALGINATE MEMBRANE

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SUMMARY

Cells of *Saccharomyces cerevisiae* (ATCC 24858) were encapsulated in the calcium alginate membrane and cultured. Swelling of the capsule was prevented by adding 0.2 g $CaCl_2$ to 1 L growth medium. The dry cell concentration based on the inner volume of the capsule reached 309 g/L, which was much higher than could be obtained by cell entrapment. All the cells remained inside the capsule during the cultivation. The flux of CO_2 through the capsule membrane increased approximately twice by adding a nonionic surfactant to the $CaCl_2$ solution during the step of capsule formation.

INTRODUCTION

In fermentation processes cell immobilization is considered as a way of increasing bioreactor productivity opposed to cell recycling (Tyagi and Ghose 1982; Lee and Chang 1987). Cells can be attached on the surface of porous polyurethane or Cellite particles (van Wezel 1967; Reuveny *et al.* 1983), entrapped in the matrix of alginate, chitosan or collagen (Kierstan and Bucke 1977; Cheetham *et al.* 1979; Tanaka *et al.* 1984), and immobilized in the hollow fiber matrix (Knazek *et al.* 1972).

Microencapsulation of enzymes was pioneered by Chang (1972) and later the technique was successfully applied to the animal cell culture by Lim and Sun (1980). Nigam *et al.* (1988) developed one-step microencapsulation method with calcium aliginate, which was much simpler than the Lim's three-step method.

Even though the conventional cell entrapment method is simple in procedure, it has a limitation in increasing biomass per unit volume of the matrix. Adding too much biomass weakens the strength of gel matrices. Also very often live cells leak out from the matrices and grow in a medium as free cells. To avoid these problems, we used microencapsulation method to achieve high yeast concentration in the matrix and to remain all the cells inside the capsule. This process can be applied in various ways. The capsules can be used in a fermentor as active immobilized cells provided the capsules do not swell for a longer period. The cells containing useful intracellular enzymes can also be grown in a high concentration to use only the enzyme activity. The latter will significantly simplify enzyme immobilization method currently in use.

MATERIALS AND METHODS

Organism and Media

The strain used was *Saccharomyces cerevisiae* (ATCC 24858). The medium for the cell growth was (per litre) : glucose, 20 g; yeast extract, 3 g; Bactopeptone, 5 g; malt extract, 3 g. The medium for the ethanol production was (per litre) : glucose, 100 g; yeast extract, 8.5 g; $CaCl_2$, 0.06 g.

Microencapsulation

Cells (30 ml) grown for 8 h on the growth medium were harvested and suspended in a 1.0 % $CaCl_2$ solution (50 ml). Xanthan gum was added into the $CaCl_2$ solution to form spherical capsules. Non-toxic, non-ionic surfactant, $Nonoxynol_{95}$ [polyethyleneglycols mono (nonyl-phenyl) ether] was also used to improve the permeability of the membrane capsule wall. The cell suspension was dropped by a syringe dispenser (Hamilton, MA, USA) into a 0.5 % Na-alginate solution stirred by a magnetic bar. The volume of a drop was approximately 6 μl. The schematic diagram of the experimental apparatus is shown in Figure 1. The capsules formed in a Na-alginate solution were washed for 10 min with distilled water and shrunk for 10 min in a 0.1 M HEPES, pH 7.4 buffer solution. The cell-seeded capsules were cultured at 37℃ in the growth medium.

To determine the dry cell concentration, the capsules were dissolved in a 0.1 M citric acid - 0.2 M Na_2HPO_4, pH 5 buffer solution and centrifuged for 20 min at 1740 g. The cells were dried for 24 h at 100 ℃.

Entrapment

Centrifuged cells from a 3 ml broth solution were inoculated in a litre of a Na-alginate solution. The cells were immobilized by dropping a 0.6 % Na-alginate solution into a 1.0 % $CaCl_2$ solution.

Analytical methods

Glucose concentrations were determined spectrophotometrically at 450 nm using peroxidase glucose oxidase method. Ethanol concentrations were determined by gas chromatography.

RESULTS AND DISCUSSION

Optimum Conditions for Making Spherical Capsules

The capsules should be made without tail since the capsules with tails tend to leak cells. The capsules of the latter form were made when drops of $CaCl_2$ solution sat on a stationary Na-alginate solution. The major factors influencing spherical capsules were the rate of calcium alginate membrane formation and the force balance on the surface of a $CaCl_2$ drop, which were related to the shear stress generated by the revolution rate of the alginate solution. Figure 2 shows that the yield of spherical capsules changed with the depth of the cavity developed by the revolution of the medium. It was 80 % at 0.8 cm of the cavity depth and 50 % at 1.4 cm. It was necessary to add a small amount of xanthan gum to increase the viscosity of $CaCl_2$ solution. The weight % of $CaCl_2$ and

Na-alginate were 1.0 and 0.6, respectively. The optimum conditions to get 100 % yield of spherical capsules were that the cavity depth was 1.0 cm in the reactor of 7.5 cm in diameter and 3.0 cm in height, and the amount of the xanthan gum was 10 % of the total $CaCl_2$ by weight.

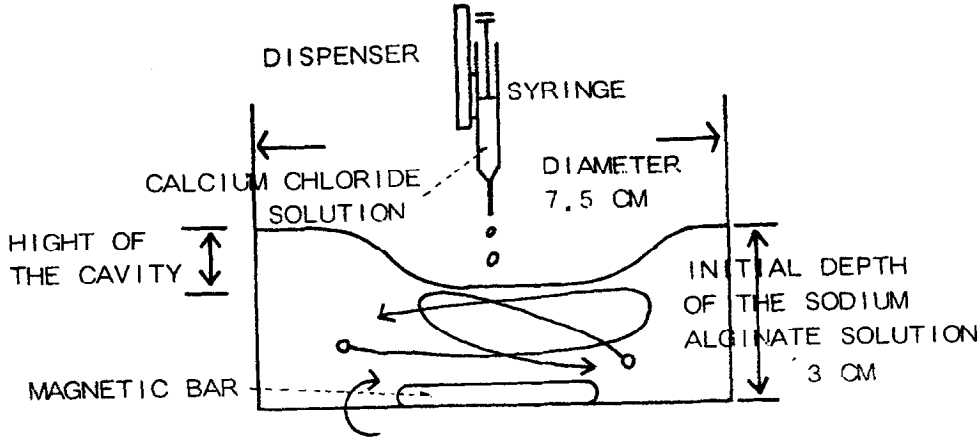

Figure 1. Schematic diagram of capsule making system.

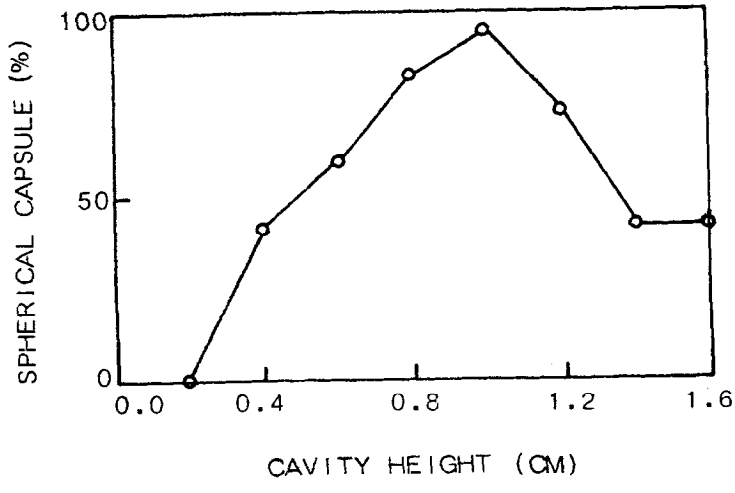

Figure 2. Yield of spherical capsule according to the height of cavity in the reactor.

Addition of $CaCl_2$ to the Growth Medium

Yeast cells immobilized in the capsules were cultured in the growth medium not containing $CaCl_2$. During the culture the capusles swelled to twice its initial size and the wall became thicker from 0.2 mm to 1.5 mm. It seems that the calcium ions in the membrane were dissociated from the membrane and diffused into the growth medium. Adding 0.2 g $CaCl_2$ to a litre of the growth medium kept the capsule from swelling. Figure 3 compares the swollen and unswollen capsules. The dry weight of the yeast cells in a capsule cultured for 10 h was 1.5 mg. The dry cell concentration was 309 g/L based on the inner space and 253 g/L based on the total bead volume. The maximum dry cell concentration in the whole cell enzyme immobilization in a polymer matrix is known to be usually less than 50 g/L (Klein and Wagner 1979). Therefore, the microencapsulation is considered to be a good way of increasing biomass in gel matrix without losing them in the medium.

Addition of Surfactant During the Capsule Formation

The capsules started to produce ethanol when they were placed in the production medium. However, the capsules began to rupture because of the vigorous CO_2 formation. To avoid this problem, 0.5 g surfactant polyethyleneglycols mono(nonyl-phenyl) ether was added to a litre of the $CaCl_2$ solution during the step of capsule formation. The capsules with the surfactant did not burst open when the culture was carried out at a temperature lower than 37 ℃. The surfactant-free capsules half packed with yeast cells became swollen in 30 min of ethanol production. The rate of ethanol production per capsule during the first hour was 110 μmol/h. The capsule expanded from 2.3 mm to 3.3 mm in diameter. The maximum flux of CO_2 through the swollen capsule was estimated to be 8.7 $\mu l/(cm^2 \cdot h)$ at 34 ℃. The capsules with the surfactant did not expand during 5 h of the culture in the production medium and maintained a comparable ethanol production rate of 1.08×10^{-4} mol/h to that of the surfactant-free capsules. The diameter of these capsules remained at 2.3 mm and the CO_2 flux was 15 μl, almost twice that of the surfactant-free capsules.

Microencapsulation vs. Entrapment

We carried out the entrapment of yeast cells in the calcium alginate beads for comparison with the current encapsulation. The initial diameter of the beads was 2.0 mm, a little smaller than that of the capsules. It increased to 2.5 mm after 20 h of the culture in the growth medium. The addition of 0.02 % of $CaCl_2$ also prevented the beads from swelling (Figure 4). The comparisons with the microencapsulation were made in Table 1. The cells detached from the beads grew in the medium and amounted to 650 % of the cells in the beads after 14 h of the culture, while all the cells in the microcapsules stayed inside the beads.

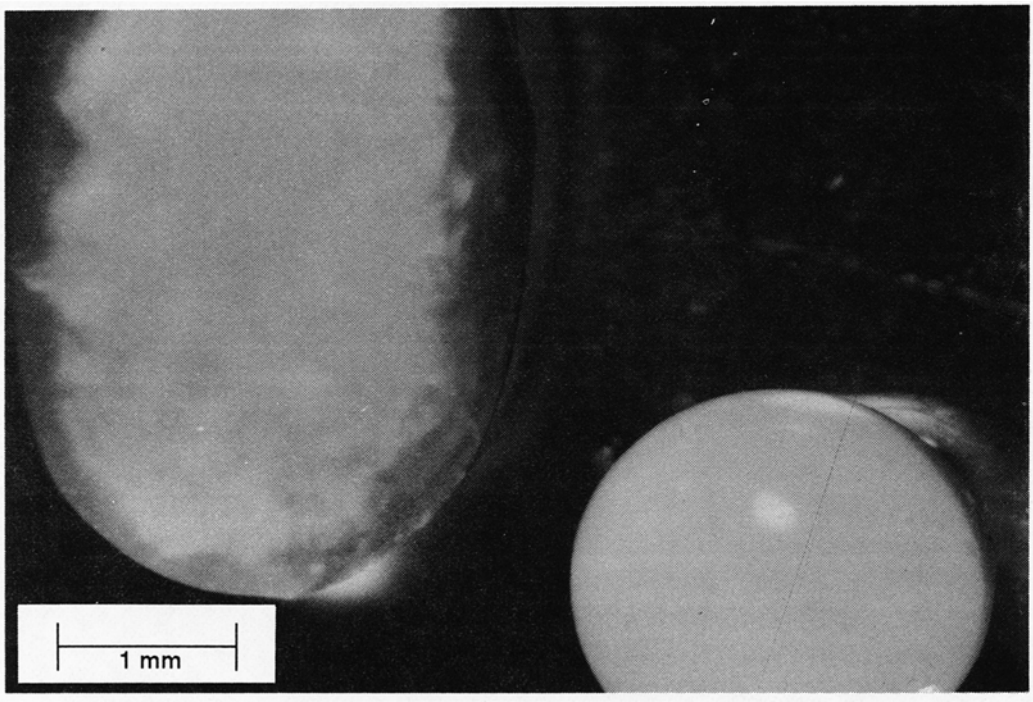

Figure 3. The state of encapsulated yeast cells after ethanol production, which were grown in the conventional medium (left) and new growth medium containing $CaCl_2$ (right), respectively for 10 h. (x 27)

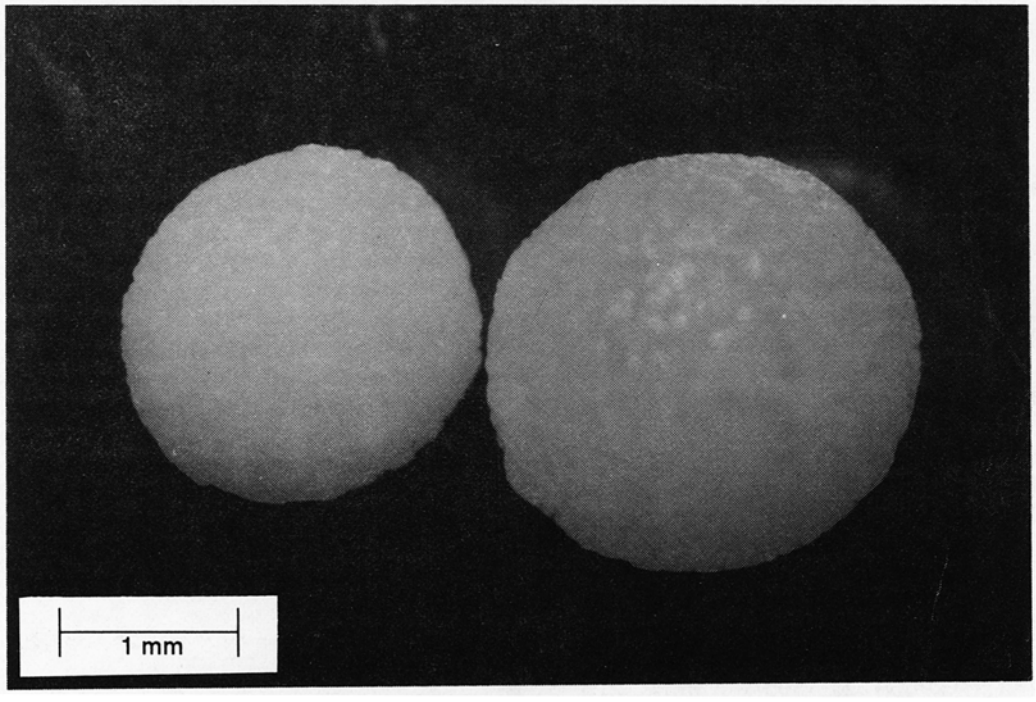

Figure 4. Yeast cells immobilized inside the beads for 22 h in the conventional medium (right) and new growth medium containing $CaCl_2$ (left). (x 27)

Table 1. Comparison between microencapsulation and entrapment

Specification \ Method	Microencapsulation	Entrapment
Bead size (mm)	2.3 (2.1)[1]	2.0 → 2.5
Bead cell conc. (g/L)	1.73 → 253 (309)[1]	1.73 → 146
Cell leakage test[2]		
in beads (g/L)	0.9	0.72
in solution (g/L)	0	4.68

[1]Values based on the inner space.
[2]30 beads were suspended in a 50 ml solution and incubated.

CONCLUSIONS

1. Addition of $CaCl_2$, surfactant and xanthan gum could keep capsules from distorting or rupturing during the culture.
2. Yeast cells can be grown in a high cell concentration of 253 g/L based on the total capsule volume or 309 g/L based on the inner core volume of the capsule.
3. One hundred % of the cells can be contained inside the capsule, while in cell entrapment more cells grew in the medium than in the matrix.

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