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THE USE OF A NEW TECHNIQUE TO IMMOBILIZE YEAST CELLS IN ALGINATE CAPSULES

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ABSTRACT

A new technique for the encapsulation of yeast cells by elimination of any thickening agent has been evaluated. The proposed procedure is based on the application of a concentrated suspension of cells which already has the sufficient viscosity to obtain spherical capsules with a semipermeable membrane. Measurements have been conducted which show that, for suspensions with yeast cell concentrations higher than 20% dry weight, apparent viscosity depends not only on the yeast concentration, but also on shear rate. The influence of sodium alginate and calcium chloride concentrations on membrane thickness has also been studied.

Keywords: immobilization, encapsulation, viscosity, alginate capsules

INTRODUCTION

Immobilized whole cells offer several important advantages over suspended cells: enhanced productivity, ability to reuse the biocatalyst, reduced risk of contamination, and simpler isolation of product [1]. Various techniques have been developed for the immobilization of whole cells. Among these, calcium alginate gel entrapment has been widely used because gelation can be easily performed under mild conditions. In whole cell immobilization, high cell loading is required to obtain high activity of the biocatalyst. The maximum cell loading in the alginate gel is limited to only 25% by volume because of poor mechanical strength. Cell leakage during fermentation processes is another drawback of the conventional entrapment method.

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Encapsulation is regarded by some researchers [2,3,4] as one of the most promising methods for cell retention, which have been shown to overcome these disadvantages arising from entrapment in the gel. In this method, cells are confined in a thin, semipermeable membrane. The cell concentration in the capsule can be higher than in the gel-core beads due to a better availability of space. Compared with entrapment, encapsulation has the advantage of having a lower resistance to diffusion. Encapsulation can be carried out by using either a natural or a synthetic polymer. The encapsulation of cells in alginate capsules is one of the most widespread methods [5,6]. Liquid core alginate capsules can be produced by adding a cell-suspended solution of calcium chloride dropwise into a sodium alginate solution with agitation. In this method, the calcium chloride solution contains a thickening agents such as xanthan [7,8], carboxymethylcelulose [9], starch [10], and polyethylene glycol [3] to prevent deformations caused by the shear stress arising from agitation in the alginate solution. Usually, a two-steps procedure is used. In the first step, capsules are produced and in the second step capsules are incubated in a growth medium to proliferate the cells. The technology has successfully been applied in both enzymatic biotransformations [7,11,12] and fermentation processes [1,6,9].

It is the aim of the present work to characterize a novel technique for the encapsulation of yeast cells by elimination of any thickening agent. This technique is based on the application of a concentrated suspension of cells which already has the sufficient viscosity to obtain spherical capsules with a semipermeable membrane.

MATERIALS AND METHODS

Materials

Low viscosity sodium alginate from *Macrocystis pyrifera* was purchased from Sigma-Aldrich. A reagent grade calcium chloride was provided by POCh. Fresh baker's yeast was purchased on the local market in the form of pressed blocks.

Methods

A Reotest-2 rotational viscometer was used for determination of the rheological behavior of the yeast cell suspension under steady state shear conditions. The yeast cells were encapsulated in alginate capsules via an extrusion process followed by interfacial gelation (Fig. 1). The cell paste (8, 10, 12, 14, and 16 g wet weight) was suspended in a 3 ml calcium chloride solution of a different concentration (2, 4, and 8% w/v) by using a magnetic stirrer. A two milliliter volume of the above suspension was extracted into a syringe and extruded through an injection needle (1.2 mm external diameter) on the surface of a sodium alginate solution (0.5, 1.0, and 1.5 %w/v). The distance between the needle tip and the sodium alginate solution was fixed at 15 cm to provide a spherical droplet. The sodium alginate solution was stirred during the gelation process to

prevent the capsules from sticking together. After the appropriate time of membrane formation (5 – 30 min) the capsules were recovered through a wire mesh and rinsed with distilled water to wash out any excess of sodium alginate from around the capsules. To reinforce the alginate membrane, the capsules were resuspended in a 1% w/v calcium chloride solution with stirring for 20 min. The external diameter of the capsules and the alginate membrane thickness were measured under microscope using a scale on a lens. All the above procedures were carried out at room temperatures. Formulation data for the preparation of the alginate capsules are presented in Table 1.

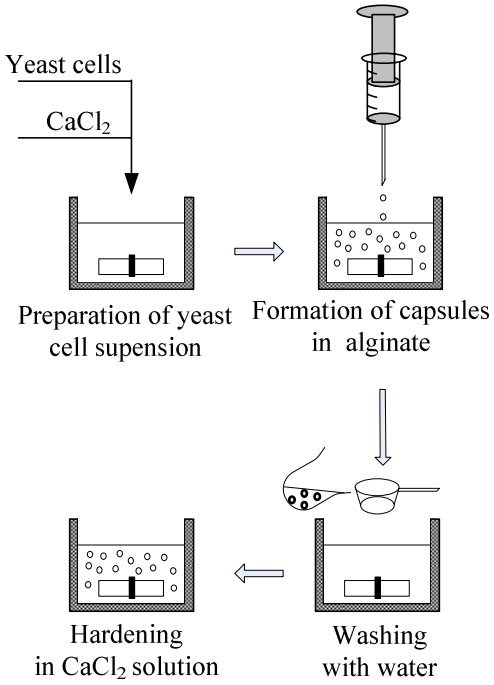

Fig. 1. Schematic presentation of the yeast cells capsule formation system

Table 1. Formulation data for the preparation of alginate capsules

Code	Weight of wet yeast cells (g)	Calcium chloride concentration (%)	Sodium alginate concentration (%)
C1	8	4	1.0
C2	10	4	1.0
C3	12	2	1.0
C4	12	4	1.0
C5	12	8	1.0
C6	14	4	0.5
C7	14	4	1.0
C8	14	4	1.5
C9	16	4	1.0

RESULTS AND DISCUSSION

Viscosity of yeast suspension

The knowledge of the viscosity of a yeast suspension is essential for the design of the encapsulation process. Unfortunately, most experimental data have been published on the viscosity of diluted yeast suspensions. In the rheology of diluted yeast cells suspensions, the relationship linking viscosity with cell concentrations is the Einstein equation:

$$\eta_r = \eta/\eta_w = 1 + 2.5\ \varepsilon \quad (1)$$

where:

η_r – relative viscosity of suspension,
η_w – viscosity of continuous medium,
ε – volumetric fraction of cells.

For higher volume fractions of the cells, the second degree polynomial function has successfully been used to describe relative viscosity. The nonlinear term in the equation takes into account the interaction of cells at higher concentrations. Other types of empirical equations were also used [13]. All these equations were verified experimentally for volumetric fractions ε lower than 0.15. Newtonian behaviour was reported for yeast concentrations up to 150 kg/L.Our measurements show that for suspensions with yeast cell concentrations higher than 20% dry weight apparent viscosity depends not only on the yeast concentration, but also on shear rate. The obtained results indicate that a 20% dry weight suspension of yeast cells has the sufficient apparent viscosity for resisting the shear stress during agitation of a sodium alginate solution.

Preparation of liquid-core alginate-membrane capsules

If the appropriate viscosity of yeast cell suspension is ensured, spherical capsules can be produced quite easily, using a simple apparatus (Fig. 1). Gelation started just at the moment when the cationic solution containing calcium ions was dropped into anionic alginate and a membrane was formed around the liquid core with the yeast suspension. Diffusion of calcium ions resulted in the progressive build-up of a calcium alginate layer and an increase of membrane thickness. Figure 2 shows the growth of a membrane thickness with gelation time. The membrane grew rapidly during the first 5 min and then leveled off. No significant increase in the membrane thickness was observed after 15 min, indicating a complete utilization of calcium ions for the cross-linked alginate.

The membrane thickness increased with a concentration of calcium chloride (Fig. 3). For increasing calcium chloride concentrations, the mass of calcium ions per unit volume inside the liquid core increases. Thus, it is reasonable that membrane thickness is controlled by the availability of calcium ions. The influence of sodium alginate concentration on the membrane thickness was also

studied. No significant changes of the membrane thickness were observed for alginate concentrations between 0.5 and 1.5 %. The capsule diameters for all used formulations were in the range 3.3 – 3.6 mm. The core diameter, obtained from the external diameter and the membrane thickness, was always of the same order of magnitude (3.2 ± 0.1 mm).

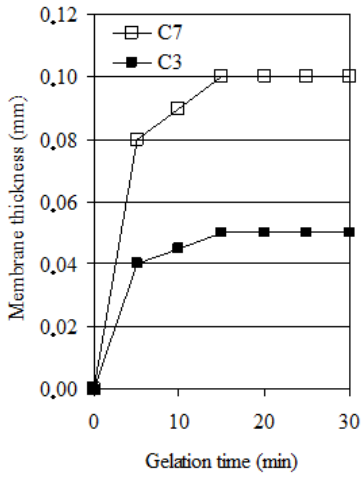

Fig. 2. Effect of gelation time on membrane thickness for systems C3 and C7

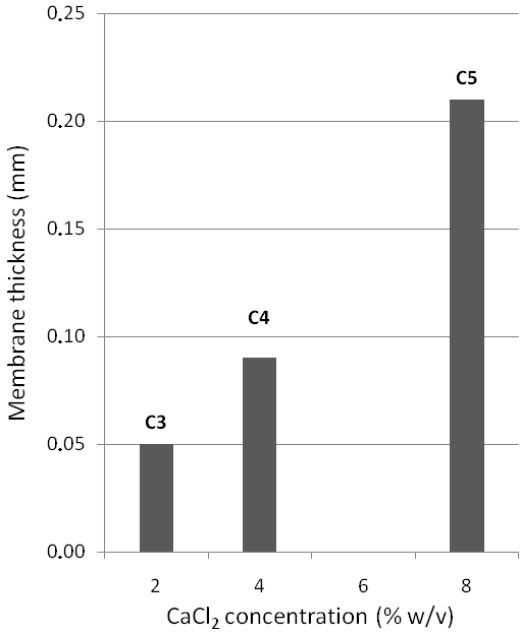

Fig. 3. Effect of calcium chloride concentration on membrane thickness after 15 min of gelation

CONCLUSIONS

A yeast cell suspension in calcium chloride solution without any thickener can be efficiently immobilized in liquid-core alginate capsules. The yeast cell concentration is a key parameter for obtaining spherical capsules. The membrane thickness can be controlled by modulation of the preparation conditions including calcium chloride concentration and gelation time.

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