ISSN 1996-0700

Asian Journal of Biotechnology

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Asian Journal of Biotechnology 2 (2): 127-132, 2010
ISSN 1996-0700

Microencapsulation of *Saccharomyces cerevisiae* using a Novel Sol-Gel Method and Investigate on its Bioactivity

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Abstract: Living *Saccharomyces cerevisiae* (S.c.) was microencapsulated with a Sol-Gel method using Tetra Methyl Ortho Silicate (TMOS) as a precursor so that it saved its bioactivity. A W/O emulsification technique was used. A vegetable oil was the organic phase for microencapsulation. Bioactivity of immobilized yeast in microcapsules was investigated, measuring the amount of CO_2 released from culture as a result of fermentation. Production of CO_2 by encapsulated yeast increased about 150% more than free yeast. Repetition of the tests has proved that microcapsules were saved their bioactivity during a month. On the other hand, particle size was decreased from 175 to 110 μm by increasing the mixer rate from 600 to 1200 rpm during gelation and smaller particles showed more bioactivity up to 30%.

Key words: *Saccharomyces cerevisiae*, Sol-Gel, microencapsulation, bioactivity

INTRODUCTION

Cells and enzymes are immobilized on or in supports in different methods such as absorption, covalent linkage, entrapment, cross linking and microencapsulation (Bickerstaff, 1997) in order to protect microorganisms against process stresses, controlled release of metabolites, facility of bioseparation etc.

Biologic materials are microencapsulated using two major methods: extrusion and emulsification (Goosen, 1993). A dispersed colloidal precursor in a vegetable oil has been used in this study and Sol-Gel was the process which selected to encapsulate the yeast because poly-condensation and gelation occur in normal temperature in which biologic materials are alive. Overall reaction of silica gel production can be written as shown in Fig. 1 (Watton *et al.*, 2002).

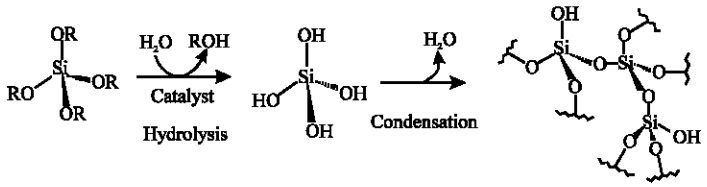

Fig. 1: A basic Sol-Gel process

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Sol-Gel process was developed in order to immobilize proteins and whole-cells. Immobilized antiprogestrone in silica poly (3-amino propylsioxane) monolith using a Sol-Gel process (Gill and Ballesteros, 2000). Yu *et al.* (2005) immobilized genetically modified *Moraxella* sp. Cells by a Sol-Gel method to diagnose organophosphates. Studies showed 5% decrease in bioactivity of cells compared to 30% for free cells. Surita *et al.* (2005) suspended cells in sodium alginate solution and dropped them in to $CaCl_2$ to produce microcapsules. Microencapsulation of cells as a biocatalyst was done by Bucko *et al.* (2005) using a multi-loop bioreactor in line with a coaxial extrusion. Also, Kato *et al.* (2005) immobilized catalytic antibody in organo substituted SiO_2 using a Sol-Gel method. Muller *et al.* (2007) encapsulated *E. coli* in silica after transformation with silicatein-α gene. Guan *et al.* (2008) encapsulated living phichia pastoris in silica after transformation with lysozime gene.

MATERIALS AND METHODS

The study was carried out at the Biotechnology and Microbiology Laboratories of Department of Chemical Engineering, Islamic Azad University, Sciences and Research Branch, during 2007-2009.

Specification of materials used in this experience is as follows:

Chemicals

TMOS, D(+)-Glucose-Monohydrate, HCL, H_2SO_4 and n-Hexane were purchased from MERCK, Liquid vegetable oil from Behshar Industries (Mixture of soybean, sun flower and canola oil) and yeast extract from Bioconnection.

Yeast: Commercial *Saccharomyces cerevisiae* (SAF®)

Production of Microcapsules

Ten grams TMOS is mixed with 150 μL of HCL (0.01 M). 2.5 g commercial yeast is suspended in 10 cc distilled water. Then two mixtures are mixed. The water phase mixture is emulsified in 80 cc vegetable oil and stirred in an ice bath for 15 min. Then the emulsion is stirred with a magnetic mixer at room temperature at 600 rpm for 30 min. This process is repeated at 1200 rpm again.

During the gelation microcapsules appeared. Mixture was filtered to separate liquid phase consisting of oil and water from the solid phase. Then microcapsules remaining on the filter were washed with n-Hexane twice to remove the oil completely. Finally, the remaining liquids are separated by centrifuge and moved to a sterilized plastic plate and kept in a refrigerator at 4°C. Morphology of the microcapsules were considered using SEM. (Fig. 2a, b).

Measuring the Bioactivity

As we know, non-aero fermentation of S.c. is like Eq. 1:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2\ CO_2 \quad (1)$$

So, bioactivity of microorganism can be measured by the amount of CO_2 produced.

Culture is prepared by mixing 2.5 g of yeast extract with 10 g of D (+) glucose monohydrate, solved in distilled water and reached to 500 cc.

Microcapsules produced at different rates and free yeast which went though the same process as microcapsules but without TMOS (as a blank test) are added to culture. The erlen

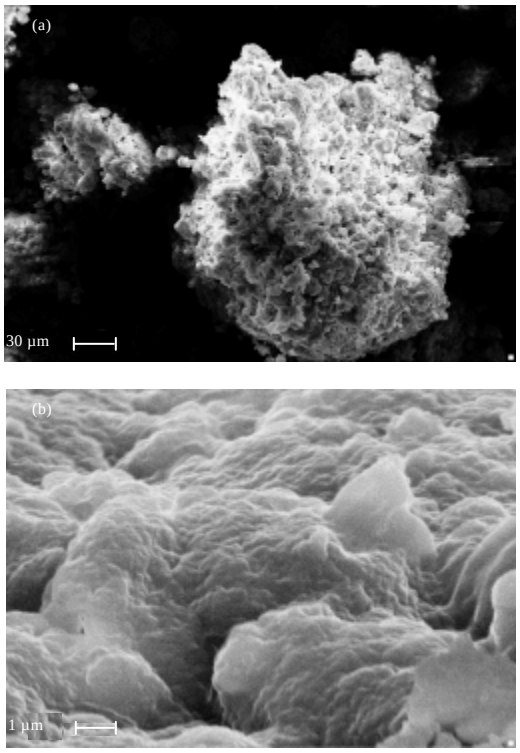

Fig. 2: (a, b) Surface of the microcapsules captured using a SEM shows micro-porous network which leads to mass transfer. Scale-bare represent 1 µm

holding microcapsule/free yeast is fitted with an elastomeric cap and sealed with Para film. The erlen is connected with a plastic pipe to the erlen of sulfuric acid. The erlen containing microcapsule/free yeast is placed on a digital scale, zeroed and the decrease of the weight is recorded during 3 h.

Determination of Particle Size

To determine the particle sizes, dry sieving has been used (Behin, 2004). So, first microcapsules should be dried. To dry, microcapsules are put in an incubator (24 h, 37°C) then (96 h, 45°C). Then dried particles are separated using a shaker (1.5 mm g^{-1}, 3 min). After separation, remaining microcapsules on each sieve were weighed (Lee *et al*., 2001).

RESULTS AND DISCUSSION

Bioactivity of free yeast and microencapsulated yeast manufactured at 600 and 1200 rpm were compared. This study was repeated in days 1, 14, 21 and 28 after microencapsulation (Fig. 3a-d). In the day 1, total CO_2 released from free S.c. captured in 3 h was reached to 6 g. This amount was around 14 g for encapsulated yeast. In the days 14, 21 and 28 there was no change for free yeast but was increased for encapsulated cells up to 16 (600) and 17 g (1200 rpm). In 28th day the total amount of released CO_2 from bigger particles was decreased to 12 g but it had no changes for smaller particles.

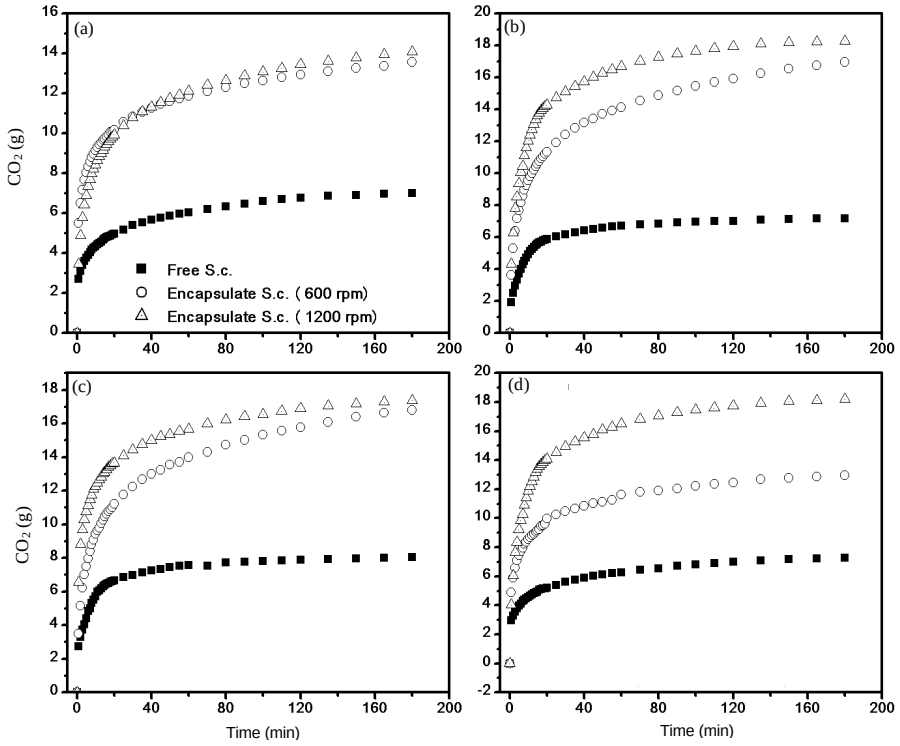

Fig. 3: CO_2 release from free S.c. and microencapsulated S.c. manufactured at 600 and 1200 rpm, (a) 1 day, (b) 14 day, (c) 21 day and 28 day

Percentage of increase or decrease of bioactivity was calculated according to Eq. 2 and graphed in Fig. 4.

$$\text{Percentage of bioactivity} = \frac{CO_2 \text{ released from microcapsule} - CO_2 \text{ released from free yeast}}{CO_2 \text{ released from free yeast}} \times 100 \quad (2)$$

As shown in Fig. 3 in all graphs, the rate of weight decrease of the erlen containing microcapsules is high at first and then the slope of the graphs decreases. Meaning that CO_2 leakage from the culture decreased; in other words the rate of bioactivity decreased that is similar to same work. Also, in all tests, bioactivity of microcapsules is higher than free yeast which went through the similar process.

So, it is clear that bioactivity increases with microencapsulation using our method. Microcapsules manufactured at 1200 rpm with a mean diameter of 110 μm had more bioactivity than microcapsules manufactured at 600 rpm with a mean diameter of 175 μm.

Percentage of changes in bioactivity is shown in Fig. 4a and b. It shows that bioactivity increased up to 140% at 600 rpm and 170% at 1200 rpm in the maximum points.

Morphology of the surface of microcapsules is shown in Fig. 2. In compare with free yeast, Formation of (a) microcapsules can be seen in image and (b) micro pores can be showed with more zoom to the surface.

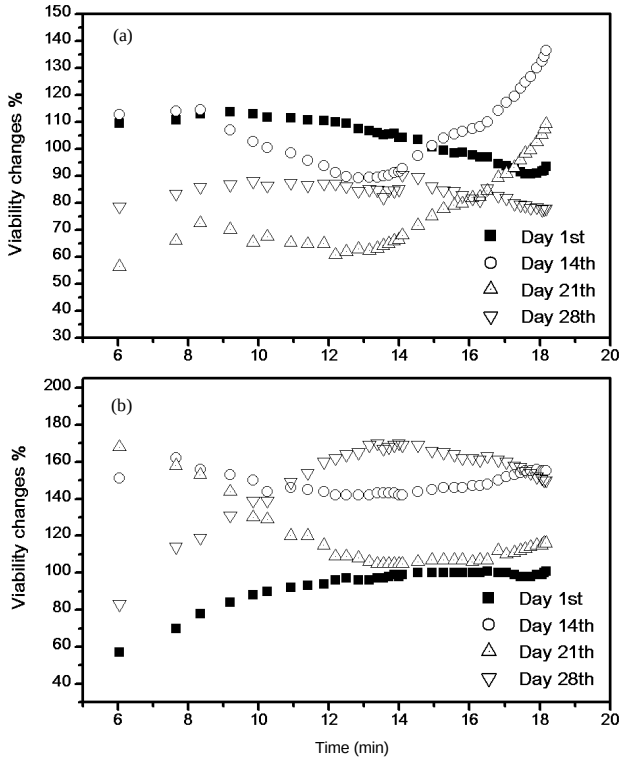

Fig. 4: Percentage of viability change for microcapsules manufactured at (a) 600 and (b) 1200 rpm vs. free yeast

Comparison of this study with the same study shows better results. Nassif *et al.* (2002) immobilized *E. coli* in TMOS and viability of bacteria increased up to 65 and 40% after 2 and 4 weeks, respectively means 25% decrease in two weeks. But in the current experience, viability remains constant for smaller microcapsules in the same time.

In another experience, Pope (1995) immobilized S.c. in TMOS gel. In the day after the first day, they had no activity and the maximum bioactivity took place around 200 min, but in this study, the immobilized yeast has had viability during 4 weeks. Also, the maximum activity was occurred in the first 20 min.

ACKNOWLEDGMENTS

This study is prepared as the result of the MSc thesis has been done in the Faculty of Technology and Engineering of Science and Research Branch of Islamic Azad University. I would like to acknowledge the contributions of technicians of microbiology, organic chemistry and SEM labs and Mrs. Hosna Borhani and Mr. Arash Moghaddam to the development of the study.

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