Microencapsulation of the yeast by a sol-gel method

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Introduction

Cells and enzymes are immobilized on or in supports in different methods such as absorption, covalent linkage, entrapment, cross linking and microencapsulation. (G. Bickerstaff 1997) To protect microorganisms against process stresses, control the exit rate of microorganisms from bioreactors, controlled release and etc.

Biologic materials are microencapsulated in two major methods: extrusion and emulsification (M. Goosen 1993) A dispersed colloidal precursor in a vegetable oil has been used in this work 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. A simple process of Sol-Gel is shown in Figure (1). (S. Watton 2002)

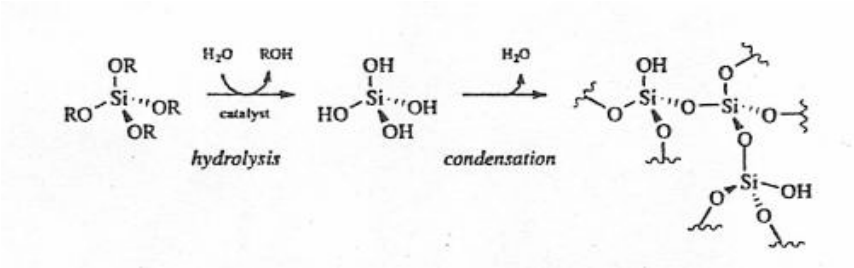

Figure1: A basic Sol-Gel process

Antiprogestrone was immobilized in silica-poly (3-amino propylsioxane) monolith using a Sol-Gel process. (I. Gill 2000) Genetically modified maroxella spp. Cells were immobilized using a Sol-Gel method to diagnose organophosphates. Studies showed 5% decrease in bioactivity of cells in compare with 30% for free cells. (D.Yu 2005) Cells were suspended in sodium alginate solution and dropped in to CaCl2 to produce microcapsules. (S. Bahatia 2005) Microencapsulation of cells as a biocatalyst was done using a multi-loop bioreactor in line with a coaxial extrusion. (M. Bucko 2005) Also, catalytic antibody was immobilized in organo substituted SiO2 by a Sol-Gel method. (K. Kato 2005)

Material and methods

Specification of materials which used in this experience is as follows :
TMOS MERCK 98 % ; Saccharomyces Serevisiae, S.I. Lesaffre ; Yeast Extract Bioconnection ; D(+)-Glucose-Monohydrate MERCK ; Liquid vegetable oil Behshar Industries (mixture of soybean, sun flower and canola oil) ; HCL MERCK 36 % ; H_2SO_4 MERCK 98 % ; n-Hexane MERCK 97 %

And the main instruments are as follows :
Magnetic mixer IKA® LABORTECHNIK USA ; Digital scale METTLER College 1399 Swiss ; PH-meter HORIBA F-12 Japan ; SEM LEO 440i England ; Centrifuge Sigma laboratory 3K30 Germany

Production of microcapsules
10 gr TMOS is mixed with 150 µl of HCL (0.01 M). 2.5 gr dry 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 at 600 rpm. Then the emulsion is stirred with a magnetic mixer at room temperature and 600 rpm for 30 min.

During the process gel particles containing microcapsules appeared. Mixture is poured on a paper filter to separate liquid phase consisting of oil and water from solid phase. Then microcapsules remaining on the filter were washed with n-Hexane twice to remove oil completely. Finally the remained liquids are separated by centrifuge and moved to a sterilized plastic plate and kept in a refrigerator at 4 º C.

Measuring the bioactivity
As we know, non-aero fermentation of S.C. is like equation (1) :

$$C_6H_{12}O_6 \longrightarrow 2\,C_2H_5OH + 2CO_2 \qquad (1)$$

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

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

Microcapsules which produced at different rates and free yeast which went through the same process as microcapsules but without TMOS (as a blank test) are added to culture. The erlen consisting of microcapsule/free yeast is fitted with an elastomer cap and sealed with Para film. The erlen is connected with a plastic pipe to the erlen of sulfuric acid as an absorbent of CO_2. The erlen of microcapsule/free yeast is placed on a digital scale, zeroed and the decrease of the weight is written during 3 hours.

Results and Discussion

Bioactivity of free yeast and microencapsulated yeast manufactured were compared. This work was repeated in days 1, 14, 21 & 28 after microencapsulation. (Fig. 2)

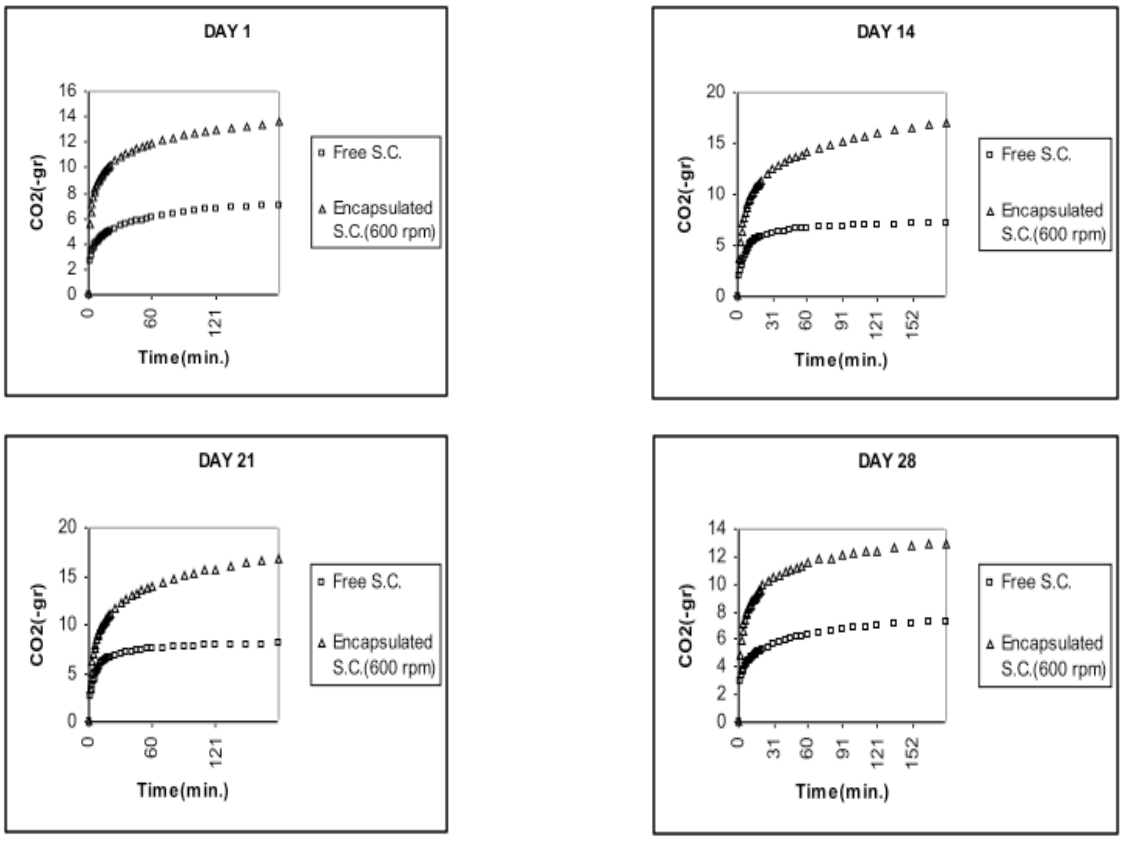

Figure 2: CO2 release from free S.C. and microencapsulated S.C. manufactured in 600 in days 1,14,21,28

Conclusions

As shown in fig. (2) In all graphs, rate of weight decrease of erlen consisting of microcapsules is high and then the slope of the graphs decreases. Meaning that CO_2 leakage from culture was decreased; in other words the rate of bioactivity was decreased. 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.

Comparison of this work with the same works shows better results. Nassif (2002) immobilized E.Coli in TMOS and viability of bacteria was increased in to 65% and 40% after 2 and 4 respectively.

In an 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 minute 200.

But in this work, the immobilized yeast has had viability till 4 weeks. Also, the maximum activity was occurred in the first 20 minutes.

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