USE OF IMMOBILIZED YEAST CELL IN ALCOHOL FERMENTATION FROM MOLASSES

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ABSTRACT: *This research focuses on the current status of alcoholic fermentation including biomass resource, various concentration of alginate for Saccharomyces cerevisiae immobilization and finding out the optimal alginate concentration that is the most alcoholic fermentation, cell immobilized yield, comparing alcoholic fermentative yield of free and immobilized S.cerevisiae which experimental conditions are the analogy of free and immobilized S.cerevisiae such as optimal pH, optimal temperature, molasses concentration, storage stability, re-use and the batch fermentation of immobilized S.cerevisiae.*

The results attained in this experiment indicate that the selected S.cerevisiae and entrapping method for immobilization present high and stable. Immobilized cell yield is 99.65%, fermentative yield is 68.68%, storage stability is 35 days that Alco3.0% (yeast cells were immobilized by alginate solution 3% w/w that is optimal concentration) was compared by free yeast, and re-use is 6 times for 18 days.

Key words: *immobilized yeast, free yeast, entrapping method, alginate.*

1.INTRODUCTION

The various methods are going to use cell immobilization that employing the use of immobilized yeast cells selected by entrapping method. The carrier materials of yeast immobilization include collagen, chitosan/chitin, alginate, k-carrageenan etc entrapping method. Of these, the calcium alginate is preferred because of its high fermentable activity, simple manner of preparation, and stability. [4]

Alginates are linear unbranched polymers of polysaccharides family containing two uronic acid polymers but they are soluble in water. The first, that is D-mannuronic acid (M) and the second, that is L-glucuronic (G) which is able to cross-link with multivalent cations such as Ca^{2+}, Ba^{2+} etc and is made by bio-composite but non-solution. [9]

Saccharomyces cerevisiae is yeast, single-celled fungi, which multiply by budding or in some case by division; it is very interesting that is alcohol fermentation. *S.serevisiae* has thick – walled, oval cell, around 10 μm long by 5 μm wide. [3]

Molasses are thick and dark-colored syrup which contains about 50 wt% sugar and about 50 wt% of organic and inorganic compounds, including water. It is the most widely used sugar for alcoholic fermentation. [10]

2.MATERIALS AND METHODS

2.1.Materials

S.cerevisiae of Cat Tuong Co, Ltd Vietnam, sodium alginate of Hai Chau Co, Ltd China and chemicals used in this study were supplied by Chinese company.

2.2.Methods

2.2.1.Determination of molasses total sugar colorimetric methods with phenol [1]

Equation 1 is used to calculate total sugar of molasses, which added fermentative medium of free and immobilized yeast.

Total sugar (%) = $(X*10^n*10^{-6} / m)*100\%$ (1)

Where

X (μg) is the total sugar of sample to calculate calibration of saccharose concentration (0.1% w/v) with spectrophotometer (λ =490nm).

10^n (g/μg) is dilution factor.

10^6 are μg which was exchanged for gram.

m (g) is sample of weight.

2.2.2.Measurement of cell biomass concentration [6] [7]

Thomas' cell counting chamber

Equation 2 is used to count the numbers of cell in biomass

$N = [((a/b)*400) / 0.1]*10^3*10^n$ (2)

Where

N is the amount of cells in the sample.

a is the numbers of cell in 5 large squares.

b is 80 (16 small squares multiplies 5 large squares).

400 is the amount of small squares.

0.1 is volume of the 400 small squares.

10^3 are mm^3 which was exchanged for milliliter.

10^n is dilution factor.

Optical density

Equation 3 is used to determine OD value of cell biomass.

$N' = X*10^n$ (3)

Where

N' is the amount of cells in the sample.

X is the total cells of sample that was calculated standard curve of various *S.cerevisiae* biomass at λ = 600nm.

10^n is dilution factor.

Both cell counting method and optical density method will be used to make the standard curve of cell biomass.

2.2.3.Determination of alcoholic content of fermentative solutions by Mohr salt [2]

Equation 4 is used to calculate alcoholic content that was made by fermentative process.

Alcohol (g) = $[(a – b)*12.5] / a$ (4)

Where

a is the number of Mohr salt milliliters of blank sample.

b is the number of Mohr salt milliliters of test sample.

12.5 represents index.

2.2.4.Immobilized S.cerevisiae by entrapping method [5; 8]

One gram of cell was mixed with 99g sodium alginate, which is various concentration of sodium alginate. Yeast–alginate mixture was dropped into CaCl2 0,2M solution by pump. The beads were allowed to harden for 45 min and then were washed sterile water that is pH = 4 and were incubated overnight at 4^0C.

2.2.5.Determination of alcoholic content and fermentative medium yield that was used by free and immobilized yeast. [8; 11]

Including of effect factors are sugar concentration, pH, amount of cells, temperature, and fermentable time.

Alcoholic yield of free yeast was compared by immobilized yeast in fermentative process.

3.RESULTS AND DISCUSSION

3.1.Determination of molasses total sugar

Molasses was diluted two times (ML2) then ML2 was diluted 104 times to test solution (TSs). Result from this experiment

Table 3.1.The OD value of TSs

	Blank (ml)	Test (ml)			Test Average
Value of OD	0.157	0.448	0.441	0.450	0.446
ΔOD	0.289				

Slope 'a' of saccharose 0.1% standard curve is 0.01. The amount of the TSs is 28.9 μg.

% Yield of ML2 is 24.42% and % yield of molasses is 48.84%.

ML2 was diluted by various solutions that are 10, 11, 12, 13, 14 and 15%. These solution will be used the next experiments.

3.2.Standard curve of yeast concentration and relation between the numbers of yeasty cells and the optical density

Various concentration of yeasty biomass was determined by the value OD at λ = 600nm and was exchanged by lgx.

y = ax + b that a is 1.5381 and b is 6.0895. Using the slope and intercept function of Microsoft Excel to determine a and b, and RSQ function determines R2 = 0.9866.

Note: The test samples were diluted by 10^3.

Table 3.2 and figure 3.1 show the relation between the numbers of yeast and optical density.

Table 3.2.Relation between the OD value (λ = 600nm) and lgx

Test sample	1	2	3	4	5
OD value (λ = 600nm)	0.149	0.225	0.339	0.455	0.524
The number of cells (x)	195.10^4	280.10^4	450.10^4	605.10^4	755.10^4
lgx	6.2900	6.4472	6.6532	6.7818	6.8779

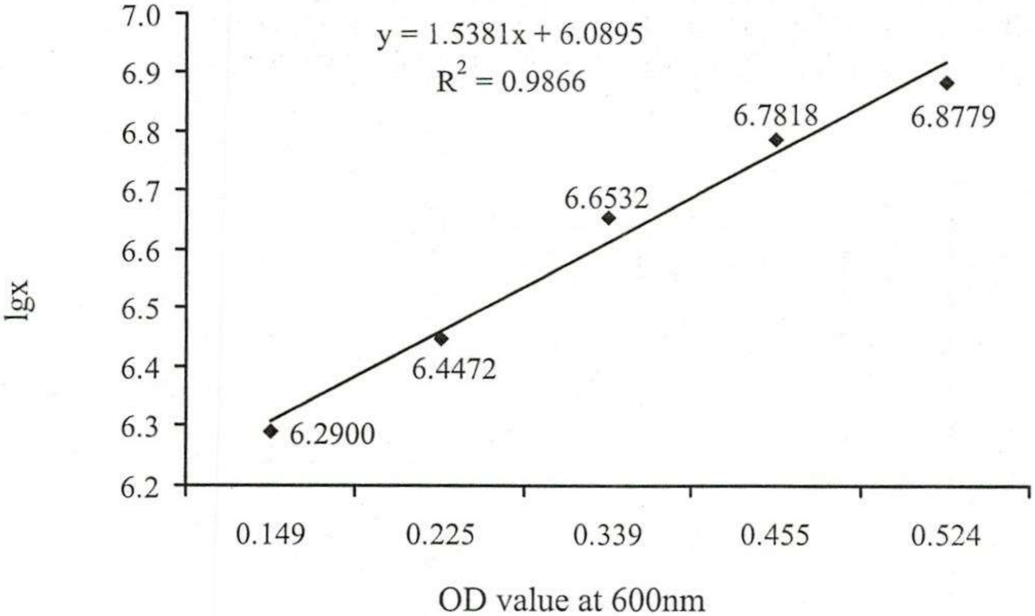

Figure 3.1. Relation between the OD value (λ = 600nm) and lgx

Determination of:

0.5 g yeasty biomass is OD = 0.164 and lgx = 6.3417, such that number of yeasty cells is 22.10^8 cells.

1.0 g yeasty biomass is OD = 0.427 and lgx = 6.7463, such that number of yeasty cells is 54.10^8 cells.

3.3.Determination of optimal concentration of alginate solution on yeasty cell immobilization

In this work, one-gram biomass will be mixed with various alginate concentrations (Alco) which include 1.5, 2.0, 2.5, 3.0 and 3.5%. 100 ml of these mixture solutions were made various beads.

Table 3.3 shows the number of cells which were immobilized and immobilized yield.

Table 3.3. Relation between the alginate concentrations and immobilized yield

Alginate concentration (%)	1.5	2.0	2.5	3.0	3.5
Yeast biomass (g)	1	1	1	1	1
Amount of immobilized yeast cell (g)	0.9905	0.9920	0.9943	0.996	0.9970
Immobilized yield (%)	99.05	99.20	99.43	99.65	99.70
Total weight of beads (g)	35.3291	37.0715	38.5216	40.5325	41.8729

Immobilized yield increases when alginate concentration also increases. Immobilized yield of bead 3.5% that is highest but it isn't sure that alcoholic fermentative yield is highest.

Figure 3.2 shows fermentative yield of various beads. In this work, experimental conditions of various beads which are analogy of fermentative medium, including pH = 4, sugar concentration = 12%, temperature = 30^0C, fermentative time = 72^h, amount of cells in various beads which is analogy (the beads have 1 g biomass or # 56.10^8 cells), re-use = 3. The above experimental conditions bases oneself on the optimal conditional fermentation of free yeast.

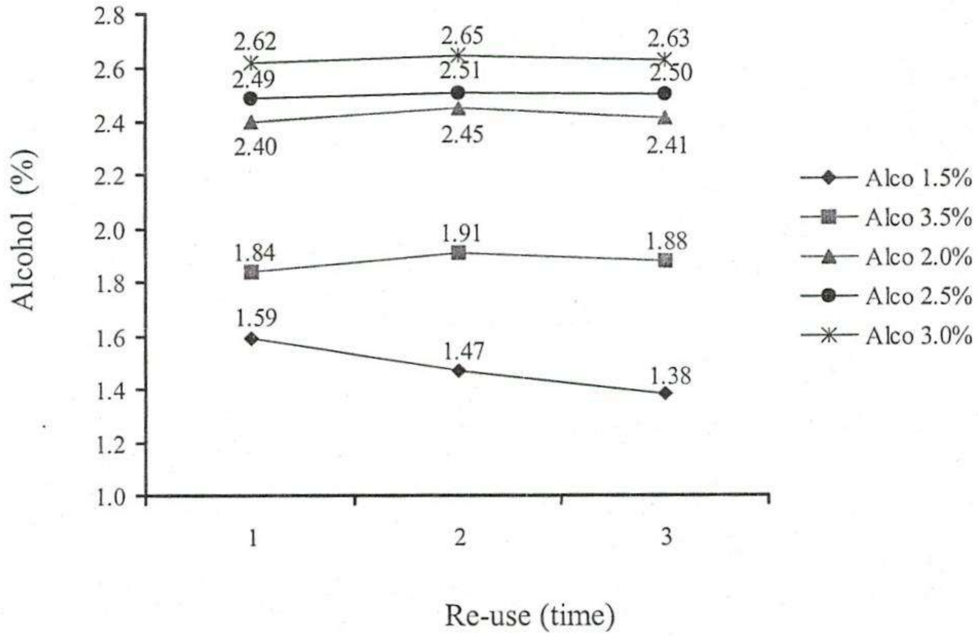

Figure 3.2. Relation between the various beads and alcoholic fermentative yield

Alco1.5% has fermentative yield of alcohol that is lowest because it is very soft and disintegrating in fermentative process, this cause for yeast cells which can be released the beads. Alco2.0%, Alco2.5% and Alco3.0% which have fermentative yield of alcohol that are similar but Alco2% and Alco2.5% which both are softer and more disintegrative than Alco3.0%. The evolution of carbon dioxide causes an internal mechanical loading on the beads, which led to disintegration of most of these calcium alginate beads.

Alcoholic fermentative yield of Alco3.0% is highest because matrix system of bead is fit and highest diffusion efficiency. Alcoholic fermentative yield of Alco3.5% is 1.88 ± 0.035% and lower than Alco3.0% that is 0.75% because matrix system of Alco3.5% is thicker and harder than Alco3.0%, this cause for the lower diffusion efficiency of the Alco3.5%.

Alco3.0% was selected the next experiments.

3.4.Determination of optimal sugar concentration of fermentative medium

The conditions of this experiment include medium volume = 100 ml, pH = 4.5, yeasty biomass = 0.5 g, fermentative temperature = 30^0C, fermentative time = 72^h and sugar concentration is various from 10 – 14%. The condition of fermentative process for free and Alco3.0% yeast are similar.

Figure 3.3 shows optimal sugar concentration of free yeast and Alco3.0% that is different. Optimal sugar concentration of free yeast that is 12% and alcoholic mass (Fr) that is 4.38 g or 4.38% when Alco3.0% is 11% and alcoholic mass (Im) that is 2.88 g or 2.88%.

Im/Fr is 65.75% and this is fermentative yield of Alco3.0% which compares with free yeast.

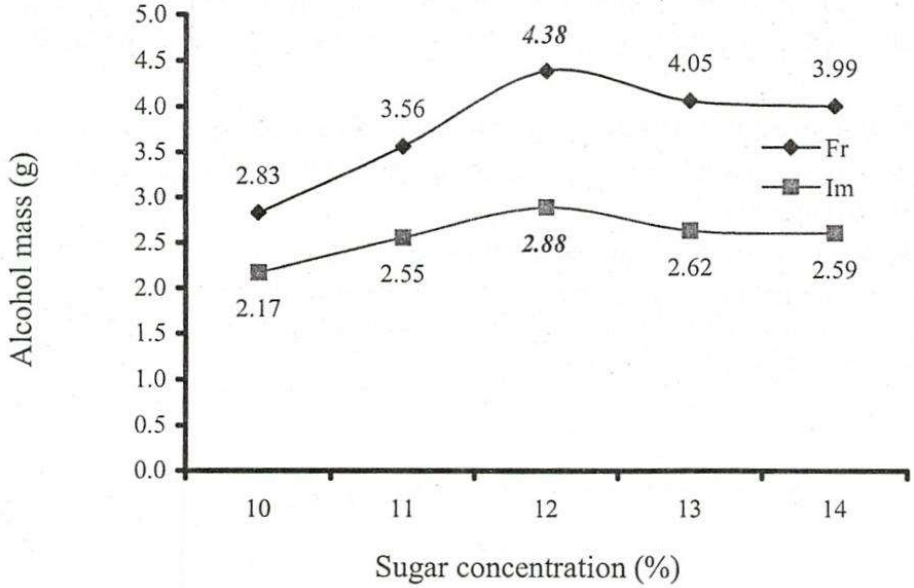

Figure 3.3. Alcoholic mass of free yeast and Alco3.0%

Fermentative opt pH and opt t^0 of free yeast and Alco3.0% are similar that are pH = 4 and $t^0 = 30^0C$.

3.5.Determination of optimal fermentable time of free yeast and Alco3.0%

This experimental conditions are as similar as section 3.4 but fermentable time is going to vary from $12 - 96^h$ that unit is 12^h. Each of 12^h is going to test alcoholic mass per time.

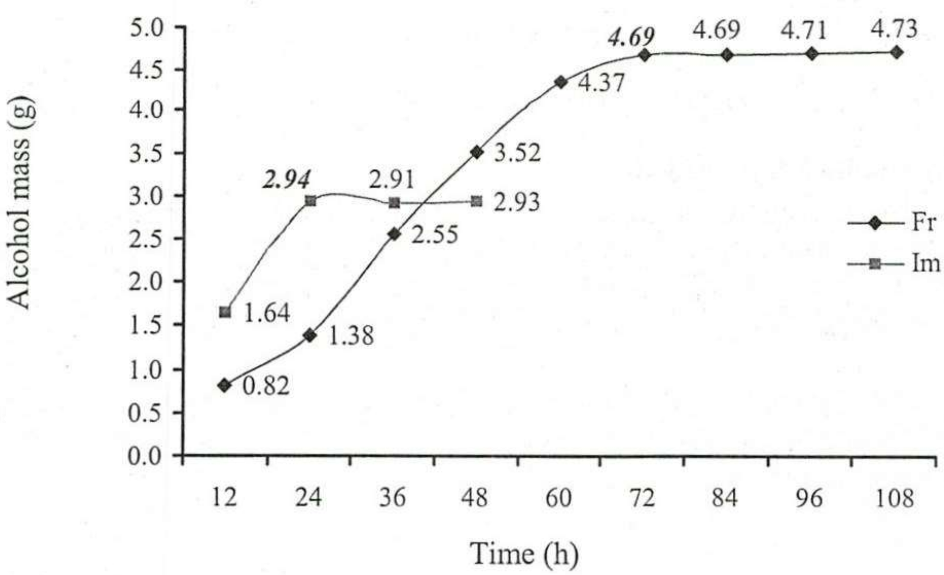

Figure 3.4. Optimal fermentable time of free yeast and Alco3.0%

Figure 3.4 shows optimal fermentable time of free yeast and Alco3.0% that are different. Optimal fermentable time of free yeast is 72^h but Alco3.0% is 24^h. Fermentative yield is 62.68%.

3.6.Determination of re-use Alco3.0% in batch process for alcoholic fermentation

This experiment bases oneself on section 3.5 and in order to examine hard features of Alco3.0% when it carried out at this work.

Figure 3.5 shows the results for the alcoholic mass of Alco3.0% that is 2.60 ± 0.07 g, Alco3.0% is able for re-use of 6 times, but time 7 is alcoholic mass which is less half-life (half-life was expressed by alcoholic mass). Alcoholic mass average of 6 times is 2.60 ± 0.07 g or 2.60% and alcoholic mass of time 7 is 1.25 g.

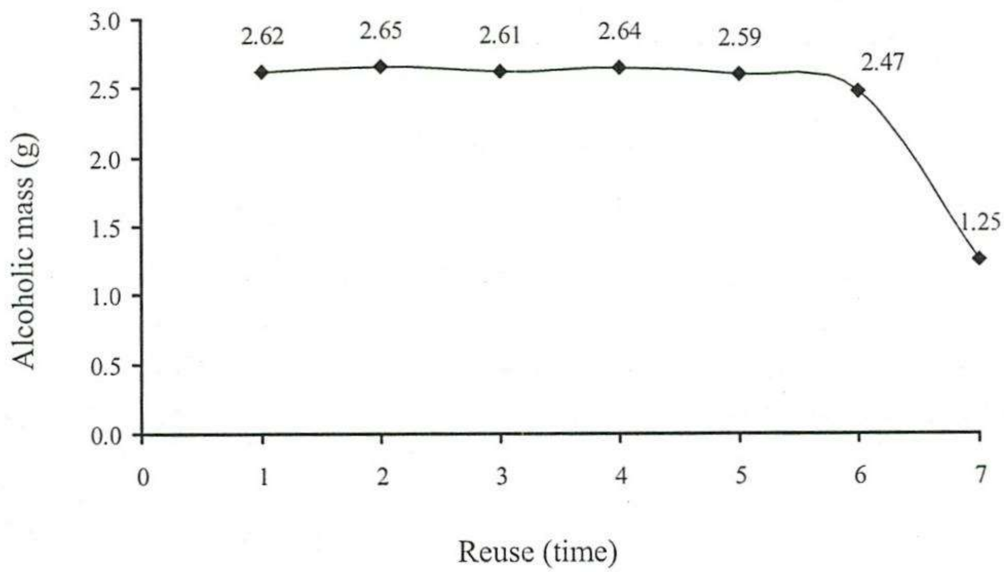

Figure 3.5. The reuse result of Alco 3%

3.7.Storage stability of Alco3.0%

In general, the fermentative activities of free yeast aren't stable during storage and base oneself on technological staff's information of Cat Tuong Co, Ltd, their fermentative activity only are stable on 10 days when was stored at 4^0C. This experiment, Alco3.0% is going to store at 4^0C and examined alcoholic mass which fermentative process was carried out by 7 days per time.

Figure 3.6 shows stability of Alco3.0% is 35 days when stores at 4^0C, the fermentative activity was expressed by alcoholic mass (g) and was stable during fermentation.

Collective result is going to show table 3.4

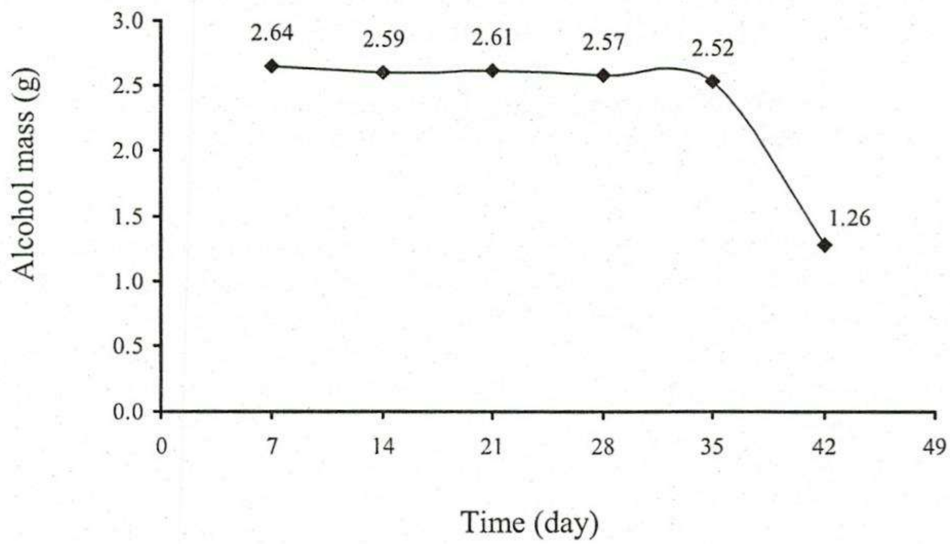

Figure 3.6. Stability of Alco3.0% store at 4^0C

Table 3.4. Comparison on effect free and immobilized yeast (Alco3.0%) fermentation

Factors	Unit	Free yeast	Immobilized yeast
Volume of fermentative medium (FV) is 100ml			
pH of FV		4	4
Temperature of FV	0C	30	30
Concentration of total sugar	% (w/w)	12	11
Mass of consumed sugar	g	9.2	6.18
Mass of cell	g	1.0	0.5
Time of fermentation	h	72	24
Re-use	time	0	6
Storage of stability at 4^0C	day	10	35
Mass of alcohol	g	4.69	2.96
Fermentative yield	w/v %	100	68.68

4.CONCLUSION

The results attained in this research indicate that the selected S.cerevisiae and entrapping method for immobilization present high and stable, immobilized cell yield is 99.65%, fermentative yield is 68.68%, storage stability is 35 days that Alco3.0% was compared with free yeast. And re-use is 6 times for 18 days.

The experiment is self-supporting for finance so that the results have limited. We suggest that should be study adding to complete and application in alcohol fermentation in large scale.

Adding studies will include batch and continuous alcoholic fermentative process which is pilot.

SỬ DỤNG TẾ BÀO MẦM MEN CỐ ĐỊNH TRONG QUÁ TRÌNH LÊN MEN ALCOHOL TỪ RỈ ĐƯỜNG MÍA

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TÓM TẮT: *Thí nghiệm sẽ tập trung vào các yếu tố ảnh hưởng trong quá trình lên men alcohol của tế bào nấm men cố định bao gồm sinh khối tế bào, các nồng độ alginate và xác định nồng độ alginate tối ưu trong qúa trình cố định tế bào bằng phương pháp nhốt, xác định hiệu suất cố định tế bào và so sánh hiệu suất lên men của tế bào cố định với tế bào tự do gồm các yếu tố như pH, nhiệt độ, nồng độ rỉ đường, thời gian lưu trữ, tái sử dụng và ứng dụng lên men alcohol bán liên tục.*

Một số kết quả thu nhận trong thí nghiệm đã thể hiện được việc chọn S.cerevisiae và phương pháp bẫy với hiệu suất cao và tính ổn định, hiệu suất cố định tế bào là 99,65%, hiệu suất lên men là 68,68%, thời gian lưu trữ là 35 ngày đối với Alco3.0% (tế bào nấm men được cố định trong dung dịch alginate 3% là tối ưu) được so sánh với tế bào tự do. Alco3% được tái sử dụng 6 lần trong suốt 18 ngày liên tục.

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