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Impact of Three Different Immobilization Techniques on Batch Fermentation Performance and Substrate Inhibition

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ABSTRACT

This paper is focused on comparing three different immobilized yeast fermentation performances and impact of substrate inhibition on the process. The yeast used for this study was a brewing strain of *Saccharomyces cerevisiae.* The immobilization techniques used were: entrapment and capsulation method in alginate and immobilization in gelatin structure. Objective of this study was to make a comparison in terms of specific growth rate of microorganisms and kinetic constants. Fermentations were performed also in different substrate concentrations to study substrate inhibition effect. Fermentations were carried out on beer wort in constant conditions. As a result we conclude that the capsulated yeast fermentation was almost similar to the free cell process. Substrate inhibition effect was stronger on high concentration substrate fermentation but was noticed a reduced effect of substrate inhibition on immobilized yeast fermentation process compared to free cell performance, as a result of a reduced diffusion effect due to support matrix.
Key words: Fermentation rate, Immobilized yeast, Entrapment, Capsulation, Gel immobilization, Substrate inhibition effect

INTRODUCTION

Immobilization in biotechnology is the technique used for the physical or chemical fixation of cells, organelles, enzymes, or other proteins into a solid matrix or retained by a membrane, in order to increase some fermentation characteristics of continuous fermentation. Therefore it is expected that the microenvironment surrounding the immobilized cells is not necessarily the same experienced by their free-cell counterparts. All kind of immobilizations have found wide applications not only in the field of biotechnology but also in pharmaceutical, environmental, food and biosensor industries (Gorecka and Jastrzebska, 2011). By immobilization is obtained the maximum yeast concentration which can lead to higher productivity and fermentation rate and lower cost therefore reducing expenses, tolerance or protection of cells from substrate or product inhibition and an easy separation of the yeast cells from the liquid phase (Banik, 2015). The aim of this study is to find the immobilized fermentation that is closer to free cell parallel and sugar concentration effect on process performance. These comparisons are made in terms of substrate consumption rate, fermentation kinetic coefficient and fermentation performance.

Cell immobilization

Were used three different immobilization techniques:

1. Entrapment immobilization
2. Capsulation immobilization
3. Immobilization in gelatin

These techniques are based on inclusion of the cells in a porous matrix which allows the mass transfer of nutrients and metabolites preventing cells from diffusing into the surrounding medium (Kourkoutas, 2004).

Mathematical models

Kinetic constants to evaluate the fermen

tation process were calculated based on the mathematical model for the growth of microorganisms know as *Monod model (eq:1.2 (1))*and the linearization equation of *Lineweaver- Burk (eq:1.2(2))* (Xhangolli, Malollari, 2009)*:*

$$\mu = \mu_{max} \frac{s}{K_s + s} \qquad (eq:1.2\ (1))$$

$$\frac{1}{\mu} = \frac{K_S}{\mu_{max}} \frac{1}{s} + \frac{1}{\mu_{max}} \qquad (eq:\ 1.2(2))$$

s –substrate concentration (g/l)
Ks – Half velocity constant, the value of s when $\mu/\mu_{max} = 0.5$
μ -the specific growth rate (h^{-1})
μ_{max} - maximum growth rate

Specific growth rate, μ, is calculated as a ratio of substrate concentration (sugar) and time needed to consume the respective amount of substrate.

Effect of substrate inhibition

Specific growth rate can be influenced by different inhibitors as pH, temperature, product and substrate. This study is focused on substrate inhibition which is a very important factor for microorganism growth. Any deviation from normal correlation of specific growth rate and substrate concentration, show an influence the substrate has over cell growth, therefore over the fermentation process (Xhangolli, Malollari, 2009). The mathematical model that applies best to this situation is the *Andrews* equation:

$$\mu = \frac{\mu_{max} s}{K_s + s + K_i s^2}$$

K_i -the inhibition constant and K_s, μ_{max}, S and μ are as mentioned above in section 1.2.

MATERIALS AND METHODS

Yeast

For this this study was used a brewing strain of *Saccharomyces cerevisiae* from brewery "*Stefani & Co*" in Albania. The beer wort used as a fermentation medium was provides by the same brewery, already homogenized and sterilized and ready to use for inoculation and fermentation process. The yeast was cultivated and from microscopic observation the yeast had this characteristics: cells of second generation, with no contamination from other microorganisms and 86% vitality.

Entrapment immobilization

Entrapment consists in mixing the yeast with a 6% solution of sodium alginate and pour out this mixture drop by drop in a 0.1M solution of calcium chloride $CaCl_2$. The beads (Figure 1) obtained are left in a solution of $CaCl_2$ for 30 minutes in order to increase their stability. Before inoculation the beads are washed 3 times with distillated water to remove the remaining cells not entrapped or excess calcium ions (Duarte, 2013).

Capsulation immobilization

The purpose of this technique is to encapsulate the yeast in calcium alginate gel, but is the reverse technique of entrapment immobilization. A 1.3% calcium chloride $CaCl_2$ and 1.3% of carboxymethylcellulose solution and a 0.6% solution of sodium alginate are prepared. Yeast cells are mixed with the solution of calcium chloride and carboxymethylcellulose and then poured drop by drop in the Na-alginate solution in continues stirring. The beads obtained (Figure 1) are washed 3 times with sterilized and distillated water than stored in 1,3% $CaCl_2$ solution for 30 minutes. Entrapment immobilization is the reverse technique and consists in mixing the yeast with a 6% solution of sodium alginate and pour out this mixture drop by drop in a 0.1M solution of calcium chloride $CaCl_2$. The beads obtained are left in a solution of

$CaCl_2$ for 30 minutes in order to increase their stability. Before inoculation the beads are washed 3 times with distillated water to remove the remaining cells not capsulated or excess calcium ions (Duarte, 2013).

Gel immobilization

A 10% solution of gelatin is prepared, which provides a solid structure for the yeast. 20% formaldehyde solution is added to increase the stability of the gel prepared. The mixture is poured in a sterilized plane surface with a short height depending on the size of the "beads" (Figure 1). After storing in the fridge till the gel is solid, we cut the gel in cubes so the size and volume is the same with the beads prepared with the other two methods of immobilization. Before inoculation they are washed 3 times with distilled water (Xhangolli, L).

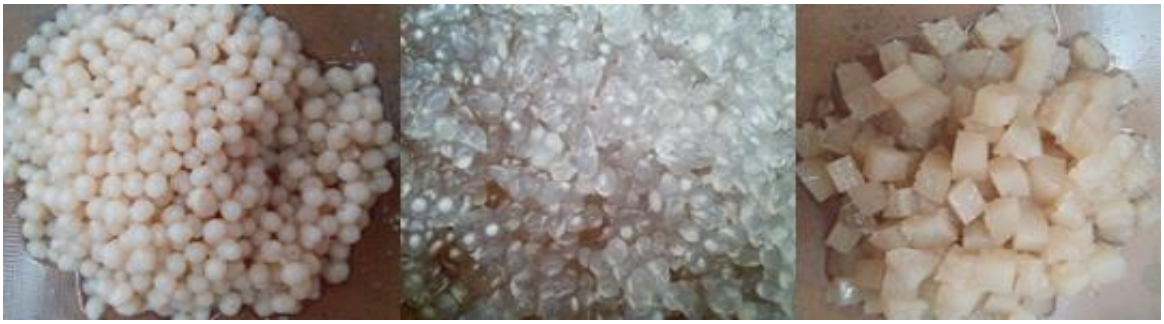

Figure 1: Entrapment immobilization(L), capsulation immobilization (C), gel immobilization (R)

Batch fermentations

Figure 2: Fermentation process

Fermentations were carried out in 250 ml volume, in 12, 16 and 20°Bx substrate concentration, to survey the substrate inhibition effect. All inoculated with immobilized yeast as described in section above and respective batch inoculated with free cells.

RESULTS AND DISCUSSION

Comparison of immobilized and free cell fermentation

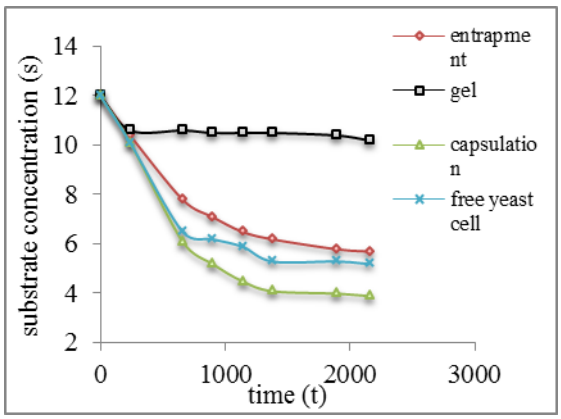

Figure 3: correlation of s~ t for batch fermentation starting with 12°Bx substrate concentration

With the same yeast culture we have performed three batch fermentations in total (Figure 3, 4 and 5). We noticed that the structure of the entrapment immobilized yeast was more stable than the capsulated ones. Regarding to the structure stability of gel, was noticed that the beads were dissolved in the medium, releasing the yeast

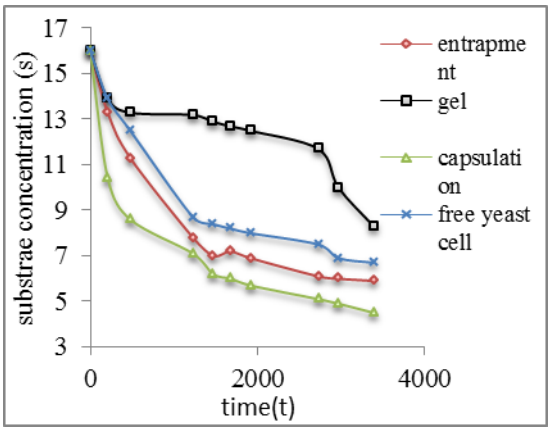

Figure 4: correlation of s~ t for batch fermentation starting with 16°Bx substrate concentration

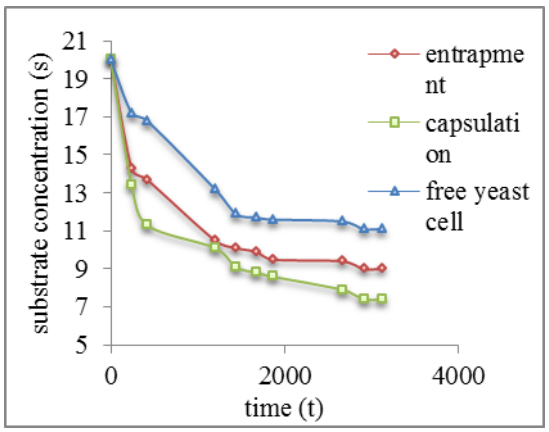

Figure 5: correlation of s~ t for batch fermentation starting with 20°Bx substrate concentration

free and continuing the fermentation as a traditional process. Comparing the entrapment and the capsulated fermentations, there are not many notable differences, but is important to point out that fermentation starts sooner when the medium is inoculated with capsulated beads. This is attributed to the thinner layer of alginate between the yeast and the substrate needed for the process to start. The kinetic constants generated from the linearization of correlation of specific growth rate and the sugar concentration, show that in optimal conditions of fermentation (12°Bx), the maximum specific growth rate of capsulated yeast is approachable to the free cells (Table1 and 2).

Table 1: Maximum growth rate for three batch fermentations.

μ_{max} (1/min)	12°Bx	16°Bx	20°Bx
Entrapment	0.0235	0.0163	0.00545
Gel	1.9×10^{-4}	5.2×10^{-3}	-
Capsulation	0.028	0.0108	0.00805
Free yeast cell	0.0292	0.01209	0.00574

Table 2: Half velocity constant for three batch fermentations.

Ks	12 °Bx	16°Bx	20°Bx
Entrapment	47.293	34.545	22.999
Gel	12.24	16.315	-
Capsulation	21.287	19.642	21.752
Free yeast cell	45.739	34.911	30.179

Substrate inhibition in fermentation performance

The substrate inhibition is a known phenomenon in high substrate concentration , conditioning the cell growth and impact the fermentation performance. Immobilization increases tolerance to high sugar concentration. Both entrapment and

capsulated yeast finished fermentation in 5-6 ^{o} Bx, comparing to free cells that stopped fermentation at 7 ^{o}Bx(Figure 3, 4 and 5) due to substrate inhibition effect. If the sugar concentration in the medium increases to 20 ^{o}Bx, the fermentation has a slow start and reach the end in higher sugar concentration. The capsulated immobilized yeast is the fastest to adopt in the environment conditions (Figure6). Referring to the kinetic constants (Table 1and 2) the substrate inhibition factor has a big influence reducing the specific growth rate, which means that the cells have difficulties in adopting and growing in this environment.

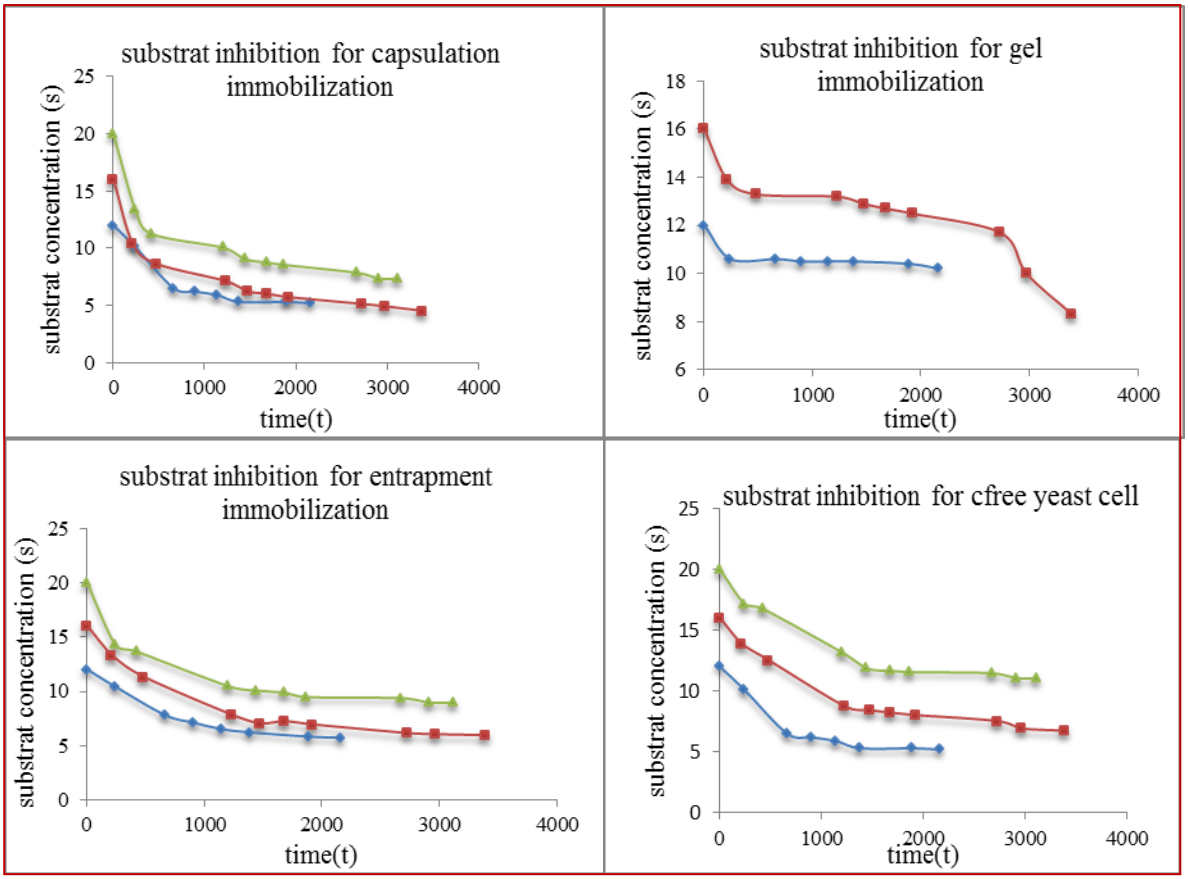

Figure 6: Substrate inhibition effect for immobilized and free cell fermentation blue-12^{o}Bx, red-16^{o}Bx and green-20^{o}Bx

CONCLUSION

- The gel immobilization is not a recommended technique, because of the low stability of the matrix
- In terms of fermentation performance, capsulated immobilized yeast fermentation process was similar to the free cell one.
- The entrapment and capsulated immobilized beads can be reused up to 6 fermentation cycles.
- Immobilization techniques increased tolerance to high substrate concentration.
- The immobilized support can protect the yeast cells from physicochemical factors.

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