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INFLUENCE OF THE MEDIUM ON THE ALCOHOLIC FERMENTATION PERFORMANCE OF TWO DIFFERENT IMMOBILIZATION YEAST TECHNIQUES COMPARED TO FREE YEAST CELL FERMENTATION

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Abstract

The idea of immobilization or known also as microencapsulation was first introduced in 1964 and the aim was transplanted cells protection. Cell immobilization in alcoholic fermentation is a rapidly expanding research area because of its attractive technical and economic advantages compared to the conventional free cell system. In this study we analyze two different immobilization techniques of beer and bread yeast in alginate beads.

Yeast was immobilized with two different methods of immobilization, entrapment and capsulation in alginate and inoculated in two different mediums. Objective was to compare immobilized fermentation rate related to free yeast cells process and how the medium influence the fermentation process. Mediums used were beer wort and sugar solution. Comparison was made in terms of substrate consumption rate, fermentation kinetic coefficients and optimum fermentation medium. A continuous fermentation process was developed.

There were no notable differences between two methods of immobilization in the same fermentation medium. Differences are shown between immobilized and free yeast cell fermentation rate and also between same immobilized fermentation developed in different mediums. Immobilized yeast fermentation results very productive in continuous fermentation compared to free yeast cell fermentation, making this an approachable technique. There is a notable difference in free and immobilized yeast fermentation in beer wort compared to sugar solution, developed by beer yeast, this because of the more complex substrate in the first case that is especial in normal metabolic growth activity.

Comparing two immobilized yeast cell techniques we prefer entrapment technique because we take more uniform, consistent and smaller in diameter beads compared to capsulation, resulting in a higher wort diffusion rate.

***Key words**: Yeast, Immobilized cells, Entrapment, Capsulation, Free cell, Fermentation rate.*

1. Introduction

Immobilization is a general term used to describe a biocatalyst, as enzymes, cellular organelles, animal or plant cells, entrapped or attached in a matrix (Gorecka and Jastrzebska [1]) and it was first introduced in the early 1964 to protect the transplanted cells. Fixation into a solid matrix increases the stability of the biocatalyst and makes possible their repeated and continued use [4]. There has been a great interest in application of immobilized cells in food industry and biomedical sciences because of the possibility to reuse the immobilized cells in a high-scale process which reduces the production cost. Focusing on cell immobilization in alcoholic fermentation which is a rapidly expanding research area because of the technical and economic advantages compared to the conventional free cell system (Margaritis and Merchant [2]), we are going to talk about how the fermentation environment and the immobilized technique used affect the fermentation process. During fermentation influentional parameters can adversly influence the specific growth rate and inhibition can be caused either by product or substrate concentration. The viability of the yeast popullation, it's specific growth rate of fermentation and the sugar uptake rate are directly related to the medium conditions (Najafpour *et al*., [3]). Kinetics of fermentation with immobilized yeast is also influenced by permeability of the capsule, this is also known as the "skin effect"

(Holcberg and Margalith [5]). The permeability in the matrix membrane is changed by making this layer thinner or increasing the concentration of substrate. In this case is very important to choose the right immobilization support. The fermentation continuity and the influence of the fermentation environment on the yeast cell growth can be determined also by the maximum specific growth rate. The aim is to maintain the greatest viability and metabolic activity of the cells, allowing the process to be carried out with high efficiency for the longest time (Berlowska *et al.*, [6]). The overall objective of this study is to provide and introduction two techniques of immobilization, to compare capsulated and entrapped immobilized beer wort fermentation rate to free yeast cells process in quantitative terms and how the fermentation environment influence the alcoholic fermentation performance.

2. Materials Methods

2.1 Yeast strain

A commercial strain of *Saccharomyces cerevisiae* for bread fermentation and a brewing strain of *Saccharomyces cerevisiae* from brewery "Birra Stela" in Albania were used for the fermentation. The species are the same but different strains. The strains were already cultivated and before immobilization the yeast was inoculated in YPD to see the cell concentration. Characteristics of yeast from microscopic observation are: cells of prime generation in good budding phase with no contamination from other microorganisms and vitality of 83%.

2.2 Fermentation medium

The fermentation process was developed simultaneously in two different mediums. The yeast was inoculated in beer wort from the brewery, sterilized and homogenized and ready to use for inoculation and fermentation process and also in a solution containing cane sugar suspended in sterilized distillated water. Both of the fermentation started at a concentration of 18 °Brix for beer wort, and 15 °Brix for cane sugar parallel process.

2.3 Immobilization

Yeast immobilization was carried out by two different techniques, capsulation and entrapment. The purpose of these techniques is to encapsulate the yeast in a calcium alginate gel, but they are performed in reverse. For the capsulation immobilization is prepared, a 1.3% calcium chloride $CaCl_2$ and 1.3% of carboxymethylcellulose solution and a 0.6% solution of sodium alginate. 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 are washed 3 times with sterilized and distillated water than stored in 1.3% $CaCl_2$ solution for 30 minutes (Rrathone *et al.*, [8]). 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 entrapped or excess calcium ions (Duarte *et al.*, [7]).

2.4 Batch fermentation in beer wort

Fermentations in beer wort were followed simultaneously, one with traditional fermentation where free yeast cells were inoculated and two others inoculated with immobilized yeast in two different methods described above. In each Erlenmeyer flask 500 mL of fermentation medium were added and inoculated respectively 10 g of non-immobilized yeast, 12 g of entrapped yeast and 15 g of capsulated yeast, to make sure that the cell biomass is the same in each batch. Fermentation was followed by measuring the sugar concentration every 3 hours, using suitable refractometer. The accuracy of this data can be effected by the ethanol produced during the fermentation (Holcberg and Margalith [5]).

2.5 Batch fermentation in sugar solution

For each fermentation using beer wort as a medium, a parallel fermentation was designed using the same conditions, but in cane sugar solution as fermentation medium.

3. Results and Discussions

3.1 Visual Appearance

In both fermentation mediums the visual appearance perception is the same. Compared to free cell fermentation, the immobilized cell controls seemed to be less active referring to foam formed on the surface of the must and gas bubbles were seen to be leaving the beads. The foam phenomenon can be a result of the beads material that may change the viscosity and surface tension of the must (Holcberg and Margalith [5]). As shown in Figure 1 also the free cell fermentation batch is very turbid and the color of must changes very quickly.

Figure 1. Foam collar and gas bubbles on the surface of must

3.2 Effect of the fermentation medium

Fermentations by free cell and immobilized yeast were performed by a commercial and a brewery strains of *Sacharomyces*, in two different mediums.

Giving that the medium is complex in nutrients, makes it easier for the yeast to overtake the optimum metabolic activity reaching the end of fermentation in lower sugar concentration, nearly 9 °Bx. As expected (Figure 2), the free suspended yeast fermentation reaches faster the higher alcohol level and finishes the fermentation sooner than the immobilized controls in both mediums.

The two controls with immobilized yeast in both mediums have a very similar trend of sugar content decrease but the entrapped immobilized yeast fermentation is more similar to the free yeast cell control. As between the yeast cells and the medium there is a barrier of alginate, fermentation needs more time to start compared to the free cell fermentation control and the sugar content decreases gradually until it reaches the end of the process. The influence of the can also be observed by the maximum cell specific growth rate μ_{max}, which is higher in must medium. This constant is calculated by Lineweaver-Burk linearization of Mihael-Menten equation (Xhangolli and Malollari [9]) and Table 1 and Figure 3 show a comparison in terms of maximum specific growth rate.

Table 1. Specific growth rate μ_{max} for commercial yeast strain in different types of fermentation

Yeast	Sugar solution	Beer wort
free yeast cells	0.056 1/min	0.779 1/min
immobilized enrapped yeast	0.026 1/min	0.124 1/min
immobilized capsulated yeast	0.0346 1/min	0.156 1/min

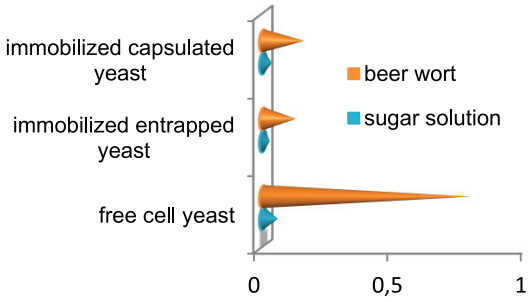

Figure 3. Chart of maximum specific growth rate (μ_{ma}) for free cell fermentation, entrapment and capsulation immobilization fermentation in cane sugar medium for commercial strain

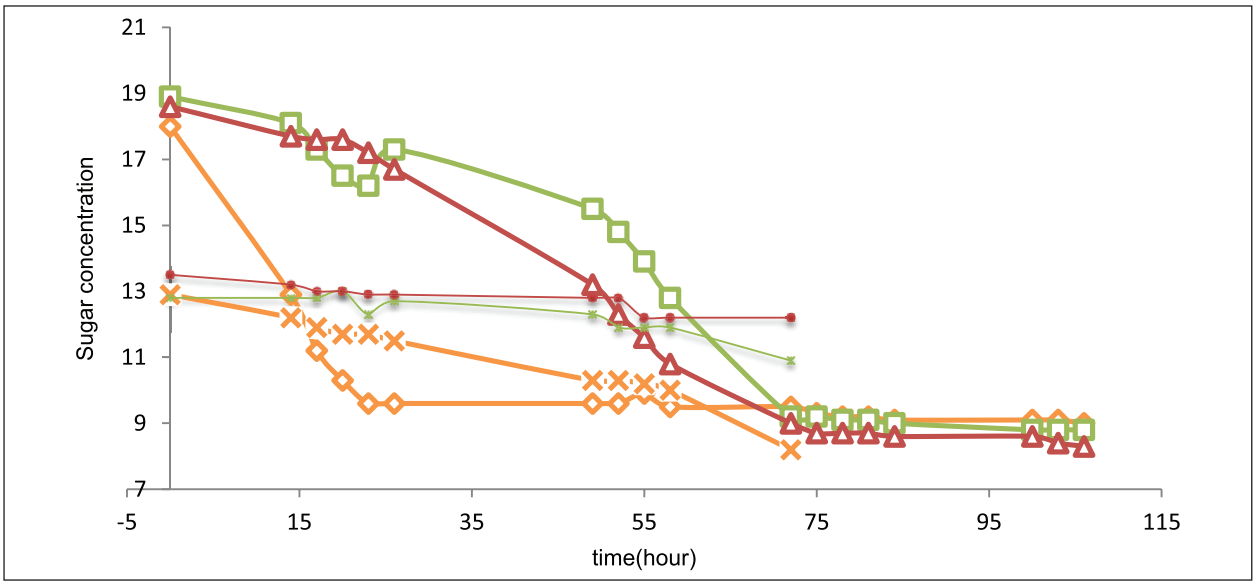

Figure 2. Correlation of sugar concentration and time of commercial *Saccharomyces cerevisiae* in beer wort and in cane sugar solution fermentation environment, free yeast fermentation (orange), entrapped immobilized yeast (green), capsulated immobilized yeast (red) in beer wort and sugar solution medium

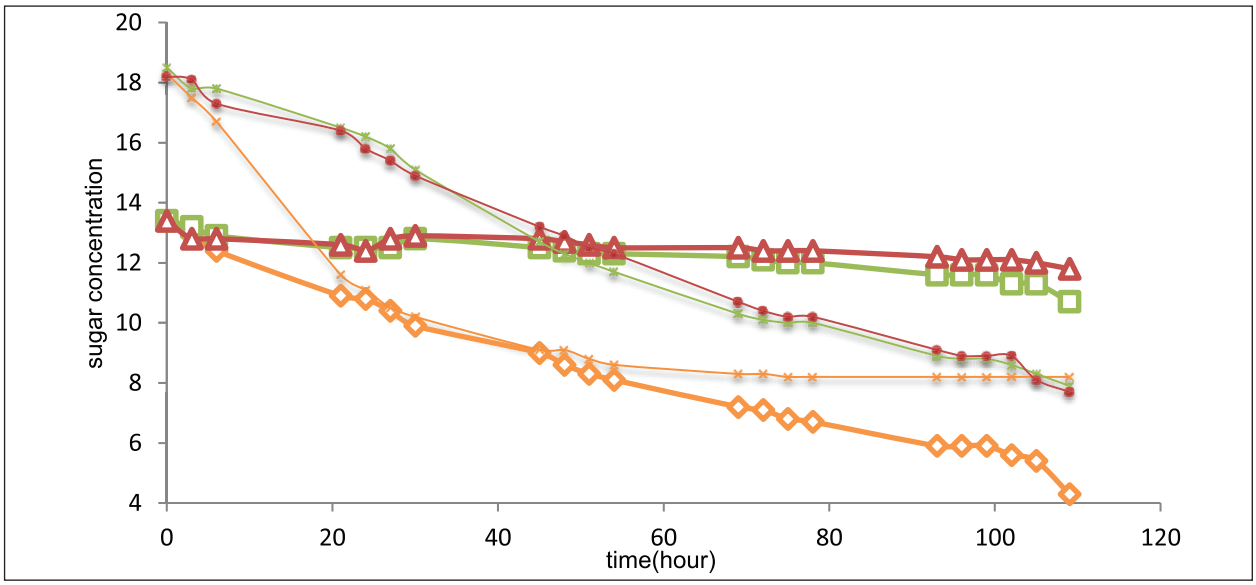

Figure 4. Correlation of sugar concentration and time of brewing strain of *Saccharomyces cerevisiae*, free yeast fermentation (blue), entrapped immobilized yeast (green), capsulated immobilized yeast (red) in beer wort and sugar solution medium

Referring to the fermentation process by brewing yeast strain of *Saccharomyces cerevisiae* illustrated in Figure 4, we can say that the result is the same as described above.

Cane sugar fermentation medium is not the optimum environment to perform the fermentation process because of the insufficiently of nutrient complex, not helping the biomass to grow and develop stopping the fermentation in high sugar concentration. Giving that this strain is characteristic for beer fermentation, unlike the commercial strain, and referring back to the maximum cell specific growth rate μ_{max} shown in Table 2 and Figure 5, it is clear that yeast cells are easily adapted to the environment, the fermentation process has a continuous decrease, resulting in lower sugar concentration compared to cane sugar fermentation control where the yeast after 30 hour of fermentation seems to have stopped the sugar consumption. The similarity between the two fermentations with immobilized yeast are present in both fermentation cases.

Table 2. Specific growth rate μ_{max} for brewing yeast strain in different types of fermentation

Yeast	Sugar solution	Beer wort
free yeast cells	0.038 1/min	0.113 1/min
immobilized entrapped yeast	0.038 1/min	0.141 1/min
immobilized capsulated yeast	0.147 1/min	0.512 1/min

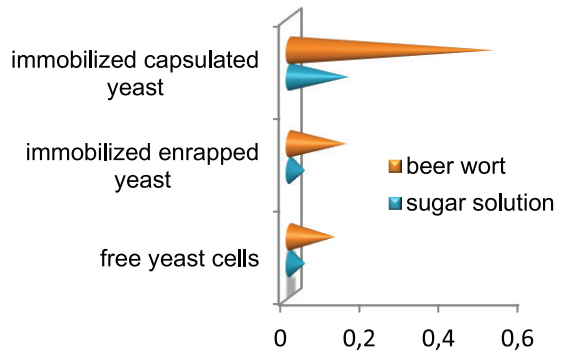

Figure 5. Chart of maximum specific growth rate (μ_{ma}) for free cell fermentation, entrapment and capsulation immobilization fermentation in beer wort medium for brewing strain

4. Conclusions

- Fermentation environment has to be complex to provide nutrients necessary for optimal growth of cells resulting in positive outcome from the fermentation process. But the medium depends on the yeast strain used. Immobilized yeast cells need more time to adapt to the fermentation process but if the environment is suitable they reach the end of fermentation at the same sugar level as free yeast cells.

- Since the two immobilization techniques don't show big differences and reach a considerable low sugar level nearly 7 - 10 hours later than free cells, change of the thickness of the matrix surrounding the bead should be considered, making it easier for the nutrients to enter the bead and the fermentation product to exit the bead support.

- Differences noted between a simple and a more complex fermentation environment are noted, the next step of the study should be focused on how immobilization effect the fermentation process with inhibitors.

- Immobilized techniques should be considered as they seem to play an important role in fermentation process and may revolutionize the way that industry of beverages operate and also how this techniques can be applied in industrial scale and how they affect the organoleptic and other chemical-physical properties.

5. References

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