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A comparative study of free and immobilized brewing yeast fermentation performance based on kinetic parameters

Luljeta Pinguli*, Rozana Troja, Ilirjan Malollari; Vilma Gurazi and Terkida Vaso

University of Tirana, Department of Industrial Chemistry, Faculty of Natural Sciences, Bulervardi Zogu I, 1001, Tirana, Albania
**Corresponding author:* luljeta.pinguli@fshn.edu.al

Abstract

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The study was focused on comparing two different immobilized brewing yeast *Saccharomyces carlsbergensis* fermentations with traditional free cell fermentation in experimental scale. The immobilization techniques used were: entrapment and capsulation method in alginate support. The objective was to choose the most suitable immobilization technique that protects the yeast cells providing a good fermentation performance compared to free yeast cell fermentation.

Kinetic parameters investigation of free and immobilized *Saccharomyces carlsbergensis* was done based on growth kinetics, ethanol productivity and substrate consumption (glucose) using computer simulation for different kinetic models. Entrapment and capsulation immobilization techniques are applicable, effective and of economic benefit. These techniques protected the morphology of cells, and supported cells growth and budding. In normal fermentation conditions entrapment immobilization is similar with free yeast cell fermentation. In inhibitory condition both immobilized methods are more effective than free yeast cell traditional fermentation. Better results give capsulation immobilization method. These results are supported also by kinetic parameter investigation.

Keywords: Yeast immobilization; entrapment; capsulation; fermentation; kinetic parameters; modeling

Introduction

Yeast immobilization techniques are used to improve fermentation performance through physico-chemical fixation of cells in different solid matrix, protecting the cell from the surrounding medium. Choosing the right immobilization technique is very important, in order to protect cell activity, without changing the morphology and physiology of the cells (Kourkoutas et al., 2004; Gorecka & Jastrzebska, 2011). Immobilization technique is a means to address maximum yeast concentration. Once the maximum yeast concentration is achieved, a higher productivity and fermentation rate could be obtained and expenses reduced (Banik, 2005). Cells are surrounded by a biocompatible matrix with a specific permeability, which allows small sized molecules, such as nutrients and oxygen to enter the beads and toxic metabolites to exit the matrix, giving the cells the ability to protect themselves and create an optimal growth environment. Cell immobilization in alcoholic fermentation is a rapidly expanding research area because of its technical and economic advantages compared to the conventional free cell system (Margaritis & Merchant 1984; Stewart & Russell, 1986). This is mainly due to the numerous advantages that cell immobilization offers including enhanced fermentation productivity, feasibility of continuous processing, cell stability and lower costs of recovery, recycling and downstream processing (Kourkoutas et al., 2003).

For the heterogeneous systems, not only the value of the biochemical reaction rate is affected, but also the kinetic model is modified compared to the ideal models describing

the substrate consumption or product formation. For these reasons, the kinetic parameters of the biochemical reactions with immobilized cells differ from those of homogeneous environments. However, immobilized cells still have limited industrial application. The process of immobilization changes not only the environment, but also the physiological and morphological characteristic of cells, and the catalytic activity of enzymes. Therefore the fermentation conditions (kinetics) of the free yeast fermentation and of the immobilized cell process are different (Kostov et al., 2012).

Kinetics of fermentation with immobilized yeast is also affected by permeability of the capsule. Applying the right immobilization technique is very important for the cells as their activity, morphology and physiology ought to be preserved, and a high cell concentration retained in the immobilized bead. Kinetic of glucose consumption, ethanol production and effect of substrate and product inhibition were study based on batch fermentations performance. Specific growth rate is usually affected by the presence of inhibition components in the bioreactor. Any deviation from normal correlation of specific growth rate and substrate concentration, show the substrate impact on cell growth, therefore over the fermentation process (Gurazi et al., 2016). However there is no model universal structure that could perfectly suit glucose fermentation by all possible kinds of strains since each particular strain has its specifics that require an individual approach to kinetics modeling (Duarte et al., 2013; Snoep et al., 1999).

Materials and Methods

There were used two different immobilization techniques both in the same support which was calcium alginate, but they differ in the manner that they trapped yeast cells. 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 (Figure 1) 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 min (Rrathone et al., 2013).

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 min 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., 2013).

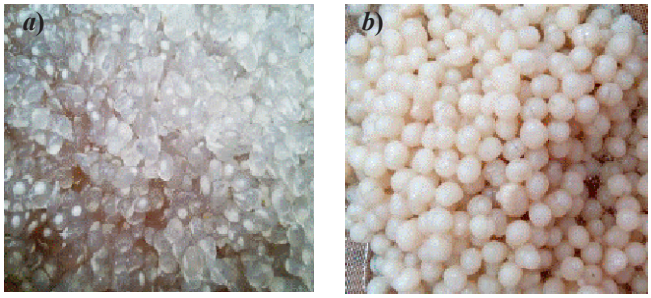


Fig. 1. Immobilized yeast: *a* – capsulation immobilization, *b* – entrapment immobilization

Fermentation

Periodic fermentation was carried out, in 250 ml volume in 12° Plato beer wort substrate. Yeast strain was used in free form and immobilized form in alginate support in beads with a diameter around 4–5 mm. yeast. There were used two different cell density fermentations, 8% (v/v) and 15% (v/v) inoculums. Measurement techniques used were Analitica EBC and Microbiologica EBC from European Brewery Convention (Virkajarvi et al., 2000).

Kinetic parameters evaluation

We chose simplified mass balance mathematical models that reflect only the kinetic rates of the main process reactions: biomass growth, ethanol production and substrate consumption for biomass and product formation (Aiba et al., 2000). The fermentation process kinetics was described with the ordinary differential equation (Di Serio et al., 2001):

$$\frac{dX}{dt} = \mu X$$

$$\frac{dP}{dt} = qX, \qquad (1)$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\frac{dX}{dt} = -\frac{1}{Y_{P/S}}\frac{dP}{dt}$$

where X was biomass concentration, P is ethanol concentration, S substrate concentration, $Y_{X/S}$ and $Y_{P/S}$ were yield coefficients, μ and q were specific growth and product accumulation rates.

The main kinetic parameters are:

– Maximal specific growth rate μ_{max},

– Monod constant, K_s value is the concentration of substrate when μ is equivalent to half of μ_{max}

Inhibition constant K_i, presents how potent an inhibitor is, it is the concentration required to produce half maximum inhibition.

In this research are used these models:

– Monod $$\mu = \mu_{max} \frac{s}{s + K_s} \quad (2a)$$

– Contois $$\mu = \mu_{max} \frac{s}{K_s \times x + s} \quad (2b)$$

– Teisser $$\mu = \mu_{max}(1 - e^{-s/k_s}) \quad (2c)$$

– Substrate inhibition $$\mu = \mu_{max} \frac{s}{K_s + s} e^{-s/k_s} \quad (2d)$$

– Product inhibition (Aiba) $$\mu = \mu_{max} \frac{s}{s + K_s} \exp(-K_i * p) \quad (2e)$$

Results and Discussion

Figures 2 and 3 represent fermentation performance in terms of substrate consumption and ethanol production. Increasing cell density in immobilized beads results in a similar fermentation with free cells. Better results represent entrapment method due to better contact cell-substrate.

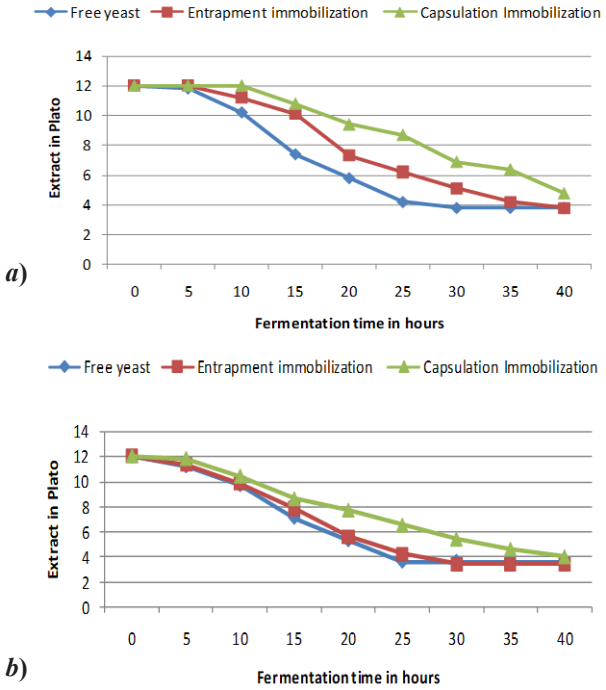


Fig. 2. Extract consumption for capsulated, entrapment immobilized and free yeast fermentation; *a*) 8% (v/v); *b*) 15% (v/v) inoculums

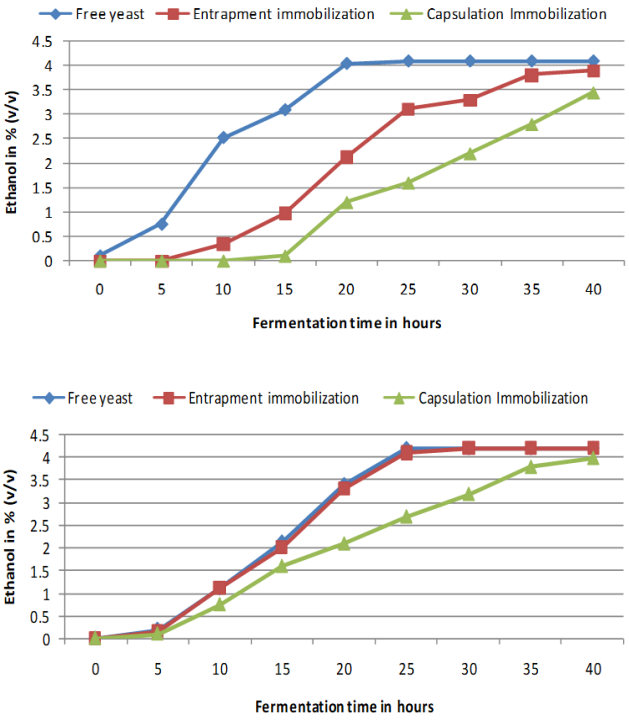


Fig. 3. Fermentation performance in terms of ethanol production for *a*) 8% inoculumn; *b*) 15% inoculumn

Yeast cell vitality is higher for immobilized yeasts compared to free yeasts. Comparing entrapment method with capsulation method better results referring yeast vitality we have with entrapment immobilization method. There is only a slightly difference but is evident during all the batches. Perhaps this phenomenon is related with support structure. The structure of alginate support with entrapment method batch after batches is loosed, bead diameter is increased with 1–2 mm and a part of yeast passed from the alginate bead to suspension. This makes the fermentation similar to free cell fermentation but in the same time refreshes yeasts entrapped in the structure of alginate. On the other hand capsulated structure is stronger and cell "loose" is smaller. For this reason fermentation performance is lower compared to free cell. Higher vitality of immobilized yeast compared to free cell is related also with better maintenance and management of yeast batch after batch. Immobilized cells handle more easily, washed more easily and inoculation process is easier and safer. Contaminants also, were lower at immobilized batches (Table 1 and Table 2).

There is a difference referring number of cells in alginate support for two different methods (Table 3). Number of cells at entrapment beads remain in the same level almost all the time because this structure loose a considerable amount of

Table 1. Cell vitality for the different fermentation batches (8% (v/v) inoculumn). Vitality of yeast used for immobilization and free cell fermentation in the first batch 94%

	Yeast Vitality in %				
	After Ist Batch	After IInd Batch	After III^{d} Batch	After IVth Batch	After Vth Batch
Entrapment immobilization yeast fermentation	87.8%	89.4%	93.6%	93%	92,6%
Capsulation immobilization yeast fermentation	93%	92%	87%	86%	85.7%
Free cell fermentation	75.1%	88.6%	89.4%	83%	78.72%

Table 2. Number of cells inside the alginate support batch after batches compared to the number of yeast cells in the beginning of fermentation. Number of cells is counted with hemocytometer after beads are soluted in Na_2CO_3 solution

	Unused beads	After Ist Batch	After IInd Batch	After III^{d} Batch	After IVth Batch	After Vth Batch
Cell number/ml Entrapment method	3 x 10^7	6 x 10^7	14 x 10^7	9 x 10^7	3.4 x 10^7	4 x 10^7
Cell number/ml Capsulation method	3 x 10^7	20 x 10^7	24 x 10^7	27 x 10^7	31 x 10^7	30 x 10^7

Table 3. Number of yeast cell/ml inside alginate structure and number of cells released in sunspension in fermentation medium for a tipical fermentation. (III batch; 12^0 Plato wort, 8% (v/v) inoculumn)

	Fermentation Time in hours	Entrapment immobilization	Capsulation immobilization
Cell/ml in beads	0	14x10^7	20 x10^7
	12	25x10^7	36x10^7
	24	20 x10^7	44x10^7
	36	12x10^7	32x10^7
Cell/ml in the medium	0	0	0
	12	13x10^2	12
	24	6.6x10^5	3x10^3
	36	9.6x10^6	5x10^4

yeast in the medium. A capsulated bead retains better yeasts inside of their structure and looses only a small amount of yeast in the medium. Yeast forms after the first batch were lengthy.

Morphological characteristics of yeast cells in immobilized beads

Compared to free yeast fermentation a similar cell growth it is noted also for entrapment and capsulated immobilized yeast. Cells were very good developed and multilateral or unilateral budding and pseudomycelium formation was shown. Cell counting performed by Thomas camera showed that cell vitality increases after fermentation in immobilized beads, compared to free cell suspension.

Further investigation was done based also in kinetic parameters. Kinetic parameters were determined based on well known linear equation derived from Monod Model.

To evaluate fermentation performance based on kinetic constant i_{max} and Ks, we have used three linearization methods:

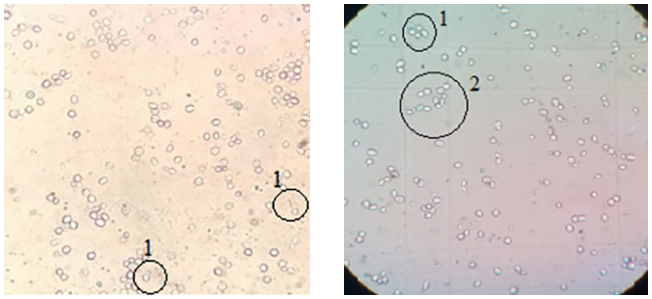
$$\text{Lineweaver – Burk} \qquad \frac{1}{\mu} = \frac{K_s}{\mu_{max}}\frac{1}{s} + \frac{1}{\mu_{max}} \tag{3a}$$

$$\text{Hans Woolf} \qquad \frac{s}{\mu} = \frac{1}{\mu_{max}}s + \frac{K_s}{\mu_{max}} \tag{3b}$$

$$\text{Eadie Hofslee} \qquad \mu = -K_s\frac{\mu}{s} + \mu_{max} \tag{3c}$$

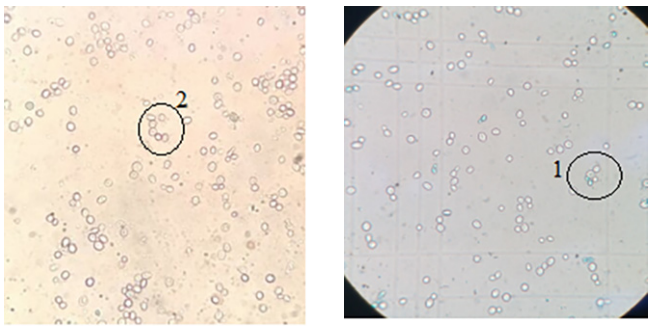
The highest correlation coefficient was chosen, and K_i was determined by the mathematical method trial and error (Figures 4, 5, 6 and 7; Table 4).

The maximum specific growth rate for entrapment yeast cells is almost the same compared to free yeast cell as high is the cell yeast density used in normal fermentation medium and smaller the bead size is. During fermentation, the diameter of immobilized beads increased and structure released more cells in the medium. In the present investigation, they were used in a very good yeast condition, up to six batches (Figure 8).



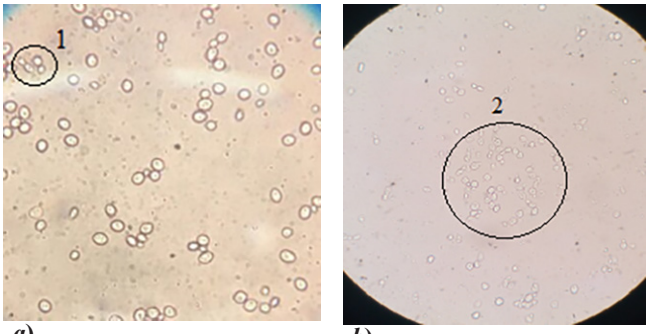
a) *b*)

Fig. 4. Yeast immobilized with entrapment technique, before (*A*) and after fermentation (*B*)



a) *b*)

Fig. 5. Yeasts immobilized with capsulation technique before (*A*) and after fermentation (*B*)



a) *b*)

Fig. 6. Free yeast before (*A*) and after fermentation (*B*)

Table 4. Kinetic constants, i_{max} and K_s for immobilized yeast with entrapment and capsulation method compared to free cell fermentation

		Free cell	Entrapment (4mm)	Capsulation (4mm)
12^0 Plato fermentation	K_S (^{0}P)	10.2	7.1	14.3
	μ_{MAX} (1/h)	0.48	0.4	0.11

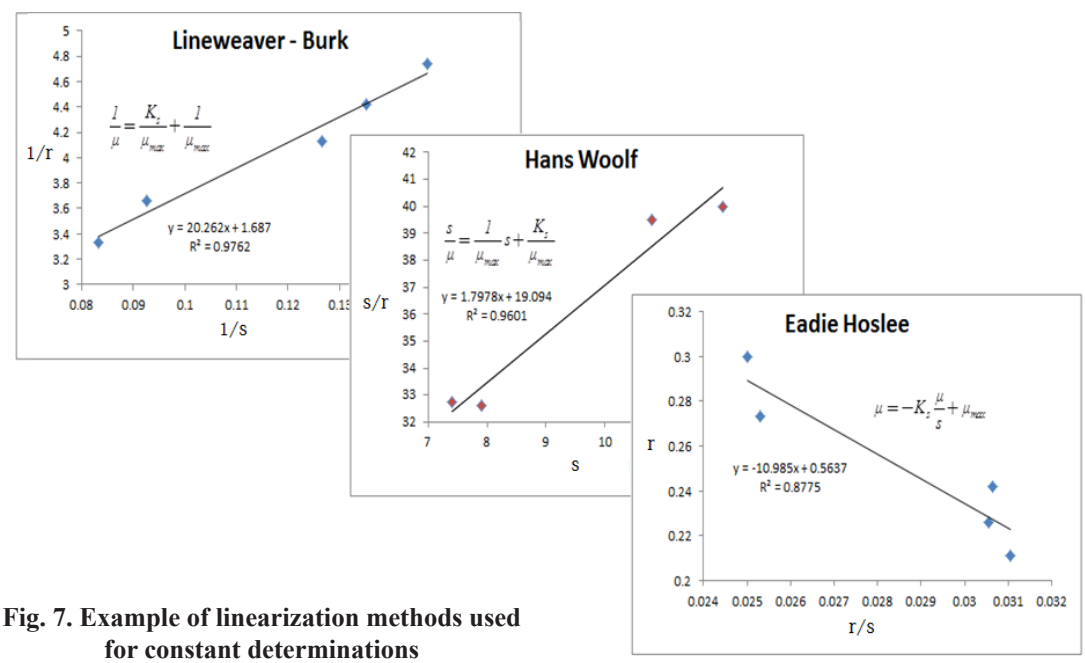
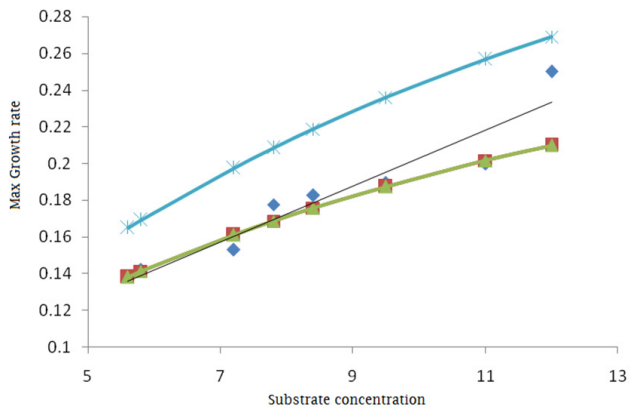
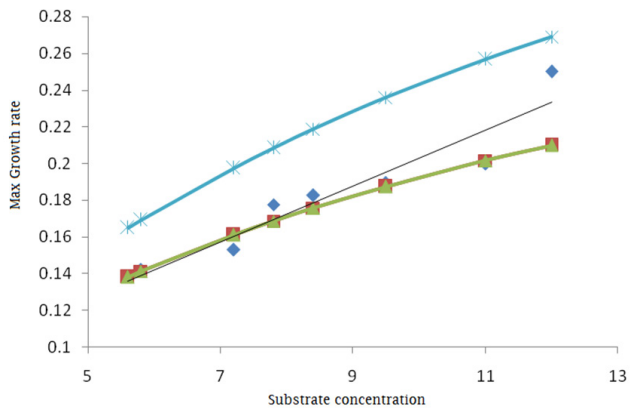


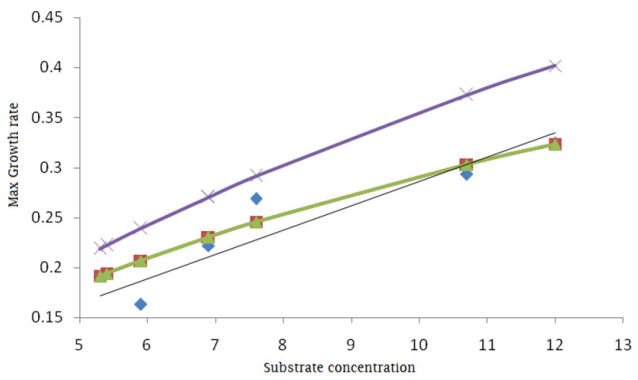
Fig. 7. Example of linearization methods used for constant determinations



a)



b)



c)

Fig. 8. Comparison of mathematical models for specific growth rate in the cases of capsulated immobilized (*a*) cells entrapment immobilized cells (*b*), (c) free cells. Case study for high cell density 15% (v/v)

Conclusions

This study investigated free and immobilized in tow different techniques in alginate of *Saccharomyces carlsbergensis* based on different kinetic parameters, kinetic models, growth curves and fermentation performance. Entrapment and capsulation immobilization techniques are applicable, effective and of economic benefit. We recommend to use the entrapment immobilization technique because the beads are easier to obtain, more uniform, stable and smaller in size which allows a better mass transfer through polymer support. Immobilized yeast is easier to handle than the free cells. In addition, it could be reused both in batch and continuous processes. Entrapment and capsulated immobilization techniques protected the morphology of cells, and supported cells growth and budding. Even with slightly small differences, the entrapment immobilization technique is better compared to capsulation technique.

Capsulation method in normal fermentation condition is less effective than entrapment and free yeast cell fermentation, but is more effective in inhibitory conditions referring our further studies that are not part of this paper. Impact of substrate and product inhibition decreased due to the immobilization techniques. These effective methods of immobilization produces the same alcohol levels, but different beverages in characteristics compared to each other and to free cell fermentations. This study is considered more suitable in continues fermentations where the vitality, high cell load and metabolic activity is needed and this is next step of our research. 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.

References

Aiba, S., Shoda M, Nagatani, M. (2000). Kinetic of product inhibition in alcohol fermentation. *Biotechnology Bioengineering, 67,* 671-90. https://www.ncbi.nlm.nih.gov/pubmed/10699849

Branik, T., Vicente, A. A., Dostalek, P. & Teixeira, J. A. (2005). Continuous beer fermentation using immobilized yeast cell bioreactor systems. *American Chemical Society and American Institute of Chemical Engineering, 21,* 653-663. http://onlinelibrary.wiley.com/doi/10.1021/bp050012u/abstract

Di Serio, M., Tesser, R. & Santacesaria, E. (2001). A kinetic and mass transfer model to simulate the growth of baker's yeast in industrial bioreactors. *Chem. Eng. J., 82,* 347–354. http://www.ingentaconnect.com/content/tandf/bsp/2002/00000013/00000007/art00004

Duarte, J. C., Rodrigues, J. A. R., Moran, P. J. S., Valenca, G. P. & Nunhez, J. R. (2013). Effect of immobilized cells in calcium alginate beads in alcoholic fermentation. *AMB Express 3,* 31.

Gorecka, E. & Jastrzebska, M. (2011). Immobilization techniques and biopolymer carriers. *Biotechnology and Food Science*, *75(1),* 65-86. http://yadda.icm.edu.pl/yadda/element/bwmeta1.element.baztech-article-LOD7-0032-0048

Gurazi, V., Xhagolli, L., Troja, R. & Hereni, S. (2016). Influence of the medium on the alcoholic fermentation performance of two different immobilization yeast techniques compared to free yeast cell fermentation. *Journal of Hygienic Engineering and Design*, *17.* ISSN 1857-8489

Kostov, G., Popova, S., Gochev, V., Koprinkova-Hristova, P., Angelov, M. & Georgieva, A. (2012). Modeling of batch alcohol fermentation with free and immobilized yeasts *Saccharomyces cerevisiae* 46 EVD. *Biotechnology & Biotechnological Equipment*, *26(3),* 3021-3030.

Kourkoutas, Y., Bekatorou, A., Banat, I. M., Marchant, R. & Koutinas, A. A. (2003). Immobilization technologies and support materials suitable in alcoholic beverages production: a review. *Elsevier, Food Microbiology, 21,* 377-397.

Kourkoutas,Y., Bekatorou, A., Banat, I. M., Marchant, R. & Koutinas, A. A. (2004). Immobilization technologies and support materials suitable in alcoholic beverages production**.** A review, *Elsevier, Food Microbiology*, *21,* 377-397. http://www.sciencedirect.com/science/article/pii/S0740002003001072

Margaritis, A. & Merchant, F. J. A. (1984). Advances in ethanol production using immobilized cell systems. *CRC Critical Reviews in Biotechnology*, *1(4),* 339–393.

Rrathone, S., Desai, P. M., Liew, C. V., Chan, L. W. & Heng, P. W. S. (2013). Microencapsulation of microbial cells. *Journal of Food Engineering, 116(2),* 369-381. http://www.sciencedirect.com/science/article/pii/S0260877412006103

Snoep, J. L., Mendes, P. & Westerhoff, H. V. (1999). Teaching metabolic control analysis and kinetic modeling towards a portable teaching module. *The Biochemical Society, 21,* 25- 28. https://www.researchgate.net/publication/237814494_Teaching_Metabolic_Control_Analysis_and_kinetic_modelling_Towards_a_portable_teaching_module

Stewart, G. G. & Russell, I. (1986). One hundred years of yeast research and development in the brewing industry. *Journal of the Institute of Brewing, 92*, 537–558.

Terkida, V., Xhagolli, L. & Malollari, I. (2017). Kinetic modeling of immobilized Yeast Batch Fermentation. *International Journal of Engineering Research & Sciences*, *3(6),* 49-55. ISSN 2395-6992*,* 2017/IJOER-JUN-2017-7. DOI: 10.25125

Virkajarvi, I., Pohjala N. (2000). Primary fermentation with immobilized yeast: some effects of carries materials on the flavour of the beer. *Journal of Institute of Brewing*, *106(5),*311-318.

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