International Journal of
Science and Engineering Investigations vol. 7, issue 83, December 2018
ISSN: 2251-8843

Immobilization Study of Saccharomyces Cerevisiae on Polyurethane Foam for Ethanol Production

Jorge David Alguiar Bellido[1], Fernanda Ribeiro Lemos[2],
Mateus Chaves Almeida de Oliveira[3], Melina Prota[4], Jane S. F. Magalhães[5]
[1]Professor at Chemical Engineering Department of Universidade Federal de Sao Joao del-Rei, Brazil
[2]Master Student at Universidade Federal de Vicosa, Brazil
[3]Master of Science in Engineering by Heriot Watt University, UK
[4,5]Chemical Engineering Bachelors by Universidade Federal de Sao Joao del-Rei, Brazil
([1]jalguiar@gmail.com, [2]nanda.rlemos@gmail.com, [3]mateus.chaves@yahoo.com.br)

Abstract- Fermentation is a biological process that occurs by the action of bacteria or fungi attached or not to solid supports by an immobilization process - ensuring higher cell density and enabling higher production. Several studies were performed using immobilization of *Saccharomyces cerevisiae* for ethanol production, but few of them used polyurethane foam as support (affordable, high porosity, low density, interconnected pores, and low-cost foam). Therefore, the objective of this research is to study the immobilization of *Saccharomyces cerevisiae* on polyurethane foam for the alcoholic fermentation and analyze the influence of the surface area of immobilization in production efficiency, concentration of non-volatile suspended solids and leaching of immobilized cells during the process. For this, an automated reactor was used to process the immobilization and subsequent fermentation. Solid tests, alcohol yield and scanning electron microscopy (SEM) were used to analyze the results, which showed that the ethanol production increases with growth of surface area of polyurethane foam (8.6, 9.0 and 11.0 mL of ethanol per liter of solution for the cultures at 480, 720 and 960 cm^2 of foam, respectively). Additionally, treatment of the support (NaOH solution and high temperature) decreases the leaching of yeast during the process by 46 %. Thus, the ethanol production via fermentation with immobilization of *Saccharomyces cerevisiae* on pretreated polyurethane foam presented a good alternative in comparison of traditional systems - reducing the concentration of non-volatile suspended solids and facilitating the separation of final product.

Keywords- *Fermentation, Immobilization, Polyurethane, Saccharomyces Cerevisiae*

I. INTRODUCTION

Fermentation is a biological process that occurs under action of bacteria or fungi to produce energy and ethanol or lactic acid in the oxygen absence. According to Lima et al.[1], the optimal temperatures for the industrial production of ethanol are between 26 °C and 35 °C. Inoculum concentration must be appropriate to the wort sugars, which can come from different raw materials, and high alcohol levels can compromise the viability of cells [2,3].

Since 1960, several techniques have been developed for the immobilization of microorganisms. Among these techniques is adsorption, which consists in fixing microorganisms on solid supports [4]. The purposes of the immobilization process are to ensure higher cell density allowing higher production rate [5], avoiding highly expensive separation and optimizing the production process [6]. At this stage, the compatibility between the support and the microorganism is fundamental to obtain the desired product [7], as well as easy access to the support, being preferred low cost and affordable materials [8, 9 10].

Kourkoutas et al. [11] studied the immobilization of *Saccharomyces cerevisiae* on apple pieces to produce wine, showing that on this support, the yeast is more resistant to temperature changes. Eiadpum, Limtong and Pisalaphong [12] studied other cases of temperature change. Kostov et al. [13] modeled the immobilization and fermentation process of *Saccharomyces cerevisiae*. Many authors [14 – 25] have studied the immobilization of *Saccharomyces cerevisiae* in inorganic substances as Y-alumina and calcium or sodium alginate. These studies presented good results for the fermentation, such as increased tolerance to inhibition by substrate. However, it was also observed the degradation of the gel, low mechanical strength, and leaching of yeast with loss of productivity [26].

The polyurethane foam is an example of support for this purpose because it presents low cost and good physical characteristics for the immobilization process, such as high porosity, low density and interconnected pores [27 – 30]. The *Saccharomyces cerevisiae* yeast can be immobilized on this support through its cultivation in aerobic medium with high concentration of glucose [31]. Moraes et al. [32] studied this process in different geometric shapes of this foam for alcoholic fermentation. The polyurethane foam in cube shape presented a yield comparable to production without immobilization and fewer quantities of non-volatile suspended solids in the product. The immobilization of *Saccharomyces cerevisiae* for the production of ethanol also presents challenges to be

overcome: leaching of yeast, low mass transfer rate, and low immobilization efficiency [33]. On the other hand, Singh et al., Singha et al., Yu et al. and Jeyaa et al.[34 – 36] showed that pretreatment with sodium hydroxide in concentrations of 0.5 to 1.0 mol L^{-1} helped to increase the affinity of the support with the inoculum, reducing the leaching. In the same direction, a great challenge of the alcohol industry is to reduce the spending in separation processes at end of pipe [37].

Few studies have been developed and others need to be performed to completely evaluate the ethanol production process via fermentation of immobilized *Saccharomyces cerevisiae* on polyurethane. Thus, the objective of this research is to study the immobilization of *Saccharomyces cerevisiae* on polyurethane foam in cubes format for the alcoholic fermentation, analyzing the influence of the surface area of immobilization on the following parameters: production yields, concentration of non-volatile suspended solids and leaching of immobilized cells during the process.

II. Material and Methods

A. Immobilization Process

At the beginning, Saccharomyces cerevisiae (Fleischmann, Petropolis, Brazil) was activated by the contact of 15 g of this yeast with 50 mL of distilled water (10 g L^{-1} of yeast during fermentation) at 30 °C in an incubator (LUCA 223, Lucadema, São Paulo, Brazil) during 10 min. Then, this solution was disposed in a CSTR reactor (UPcontrol, automatic control, Porto Alegre Brazil) with the defined number of polyurethane cubes (Tiete Espumas, São Paulo, Brazil) for immobilization. During this step, 950 mL of solution containing 50 g of glucose (Quimisul®, Joinville, Brazil) was added to the reactor and air was fed through a compressor in a rate of 6 L min^{-1} (Boyu Sc-7500, Ouro Branco Brazil) (1.1 W). This process lasted for 1h under agitation (0.33 g-force).

B. Fermentation Process

After the immobilization, the solution was completely drained from the reactor and 1.5 L of glucose solution 50 g L^{-1} was added, the air supply was interrupted, and the stirring (100 rpm) was kept, initiating the fermentation process. At time zero, the initial sample was taken and 1 mL of this solution was used for the non-volatile suspended solids test (section 2.3). During this step, samples were collected following the time of reaction. Ethanol concentration was calculated with support of a pycnometer (PlenaLab, 25 mL, São Paulo, Brazil), according to Equation 1, in which ρ is the specific mass of the *solution* (first sample collected), the *sample* (collected following the time of reaction), and pure *ethanol*. After 4 hours of reaction, a second 1 mL sample was collected and submitted to non-volatile suspended solids test.

$$\rho_{sample} = \rho_{solution} * (1 - x) + \rho_{ethanol} * x \quad (1)$$

C. Test of solids

Before the experiment, the mass of four sets of five randomly selected cubes was measured. After the experiment, other four random sets of five cubes were selected and disposed in the kiln (Sterilinger, São Paulo, Brazil) for 1 h at 80 °C and their respective masses were measured to verify the efficiency of the immobilization process.

The non-volatile suspended solids test was performed by the disposal of 1 mL of the first and last samples during the fermentation process on watch glasses and its insertion in the kiln (1 h, 105 °C). The concentration of non-volatile suspended solids and leaching of yeast from the foam during the fermentation process were determined. The procedure was repeated with 480, 720 and 960 cm^2 of polyurethane foam external area in cube shapes of 1 cm each side.

D. Pretreatment of the Support

After determining the amount of foam presenting better fermentation conditions according to the previously discussed parameters, a new experiment with the best amount of foam was conducted by the pretreatment of this foam in aqueous solution of sodium hydroxide (1 mol L^{-1} and 2 mol L^{-1}) for 3 min at room temperature and 5 min at 60 °C before the immobilization and fermentation processes (as presented before).

E. Scanning Electron Microscopy (SEM)

For morphological studies in microscopic level, the scanning electron microscopy (SEM) (FEI Inspect S50™, Hillsboro, Oregon) was used to analyze some samples of polyurethane in different stages of the process. For the preparation of samples, polyurethane cubes, after immobilization and after fermentation were disposed in an incubator (33 °C, 2 h). To make SEM readings, the cubes were placed on a metallic surface, attached by two carbon strips, and submitted to low vacuum and tension.

F. Fermentation for 48 hours

The procedure described in items 2.1 and 2.2 were repeated for a period of 48 h to analyze the fermentation by similar time to those addressed in most references. Likewise, the procedure involving pretreatment of the support (section 2.4) was also applied for the 48 h experiment. The analytical methods were the same as described previously (sections 2.2 and 2.3).

G. Free Fermentation and Sterilization

For comparison, the general procedure was performed in a free culture system (non-immobilized).

All equipment and solutions involved in the procedure described in section II were subjected to sterilization with sodium hypochlorite or steam autoclave (121 °C, 20 min) before and after use.

III. Results and Discussion

A. Fermentation Yield

Fig. 1 shows the curves of ethanol production in non-immobilized and immobilized systems for 480, 720 and 960 cm^2 of external surface area of polyurethane foam.

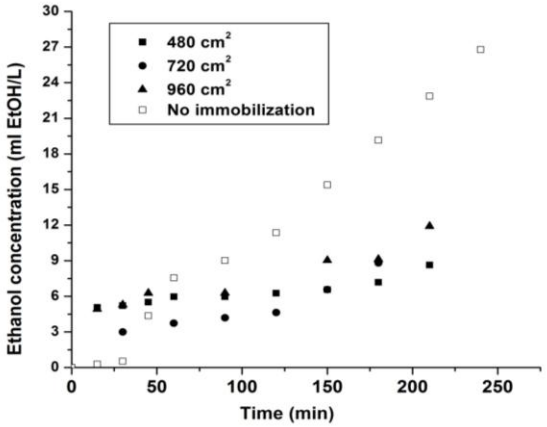


Figure 1. Ethanol production as a function of time for free cultures of Saccharomyces cerevisiae and immobilized on 480, 720 and 960 cm2 of external surface area of polyurethane foam.

By analyzing the curves shown in Fig. 1, it can be verified that during the fermentation process time (4 h), the non-immobilized experiment showed the highest production of ethanol. This result is not consistent with what was observed by Singh et al. [15], who obtained the highest yield for fixed systems. However, the short time for the immobilization (1 h), does not allow the maximum potential support immobilizer to be reached. Consequently, there is less concentration of cells in this procedure than those systems with greater immobilization time or free cells [34]. In this case, the production of ethanol in immobilized systems is muddled by the low cell density. In this study, the immobilization time was the same for all examined immobilized systems, so they can be compared among themselves. It is observed that there is an increase in the ethanol production from immobilized cultures with increased foam external surface area available for immobilization. The experiments presented final concentrations of approximately 8.7, 9.0, and 11.0 mL of ethanol per liter of solution for the immobilizations in 480, 720 and 960 cm^2, respectively.

By using the polyurethane foam, the mass transfer rate between the solution and the interior foam portion interferes with the kinetics of the fermentation process, decreasing the processing capacity of the culture medium in a short fermentation time [32, 33]. Therefore, it is another reason for the low ethanol production rate by the fixed systems under study.

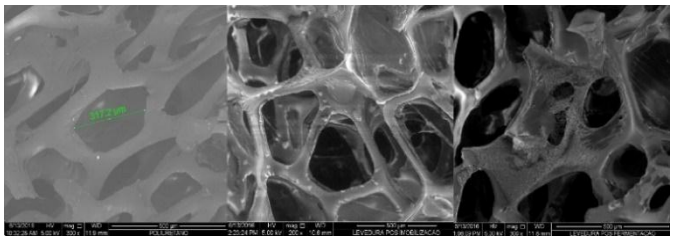
B. Leaching

TABLE I. NON - VOLATILE SUSPENDED SOLIDS CONCENTRATIONS AT THE BEGINNING AND THE END OF THE FERMENTATION PROCESS.

Polyure-thane surface (cm2)	Solids at the beginning of fermentation (g L-1)	Solids at the end of fermentation (g L-1)	Variation during the fermenta-tion (g L-1)	Solids by external area of immobi-lization (mg cm-2)
480	38.8	44.7	5.9	18.44
720	33.6	41.1	7.5	15.63
960	40.3	49.5	9.2	14.38

From the data in Table 1, it can be inferred that in all three experiments using polyurethane foam to immobilize *Saccharomyces cerevisiae*, the process of increasing the mass of suspended solids occurred. This increase happens due to the leaching of part of the cells that were originally immobilized on the support. This result is similar to the observed by Mishara et. al and Singh et. al. [14, 15]. The main cause of leaching is the mechanical shaker. However, it is necessary for the homogenization of temperature and inoculum concentrations of substrate and product of the culture medium [38].

Many studies have been conducted with the immobilization of *Saccharomyces cerevisiae* in calcium alginate [14 – 22]. In this support, the total leached quantity (0.125 g L^{-1}) was lower than that observed in the present study for all tests with polyurethane foam [15]. Therefore, it can be stated that although the affinity of the polyurethane foam toward the yeast is lesser than that observed in calcium alginate, this affinity is enough to allow the immobilization for the alcoholic fermentation, as observed by the result of scanning electron microscopy (SEM) of the polyurethane foam during different stages of the process under study, shown in Fig. 2.



(a) (b) (c)

Figure 2. Scanning electron microscopy of the polyurethane foam: (a) before the immobilization, (b) after immobilization and (c) after fermentation.

The series of scanning electron microscopy images in Fig. 2 shows the structure and porosity of the polyurethane foam, as well as endorses the immobilization process. Fig. 2a shows the morphology of the support raw material – without use or treatments. The surface and structure of the polyurethane are built with many holes and cavities suggesting that this support is porous enough to allow the entry of *Saccharomyces cerevisiae* during the immobilization process. These spaces found in the support provide a favorable environment for the growth of yeasts and allow the transference between the substrate (glucose) and the product (ethanol) produced by the yeast [10]. Analyzing Fig. 2 (b) and (c), it can be clearly noted that some yeasts were firmly adsorbed on the surface of the support, infiltrated in the various pores and then, multiplied. As can be seen by analyzing the white areas in the figures, the amount of yeasts present in Fig. 2 (c) is considerably larger than in Fig. 2 (b).

From the previously presented data, the parameter of non-volatile suspended solids was calculated by a rate with the external area of immobilization, as shown in Table 1, to assist in determining the most productive immobilization assay. The

experiment carried out with *Saccharomyces cerevisiae* immobilized in 960 cm^2 polyurethane external area showed the best results with the least amount of yeast leached by immobilized area.

Other solid tests (Section 2.C), along with the SEM, show the efficiency of immobilization with quantification of the mass of polyurethane foam cubes before procedure (49.2 mg $cube^{-1}$) and after it (90.2 mg $cube^{-1}$). Throughout these tests, there was an increase in mass of the cubes during the procedure demonstrating the retention of yeast in the polyurethane structure during the process.

C. Pretreatment of the Support

As noted by Singh et al. [15], the treatment of sugar cane bagasse as support with NaOH could increase the attractiveness of the support–inoculum for the *Saccharomyces cerevisiae* immobilization [39]. Thus, the procedure with 960 cm^2 polyurethane foam was repeated by adding the chemical treatment stage with NaOH as presented in Section 2.4, prior to the immobilizing step. Table 2 shows the final results in the fermentation for 960 cm^2 of polyurethane foam with two types of pretreatment of the support and without it.

TABLE II. RESULTS OBTAINED FOR AN IMMOBILIZED FERMENTATION IN 960 CM² OF EXTERNAL AREA OF POLYURETHANE FOAM WITH AND WITHOUT PRETREATMENT OF THE SUPPORT

	Concentration of Ethanol (mL of ethanol per L of solution)	Leaching (g L^{-1})	Leaching per surface area of immobilizati-on (mg cm^{-2})
No pretreated foam	11.00	9.2	14.38
Pretreated foam with NaOH at 1 mol L^{-1} and 60 ^oC	10.58	5.0	7.81
Pretreated foam with NaOH at 2 mol L^{-1} and 60 ^oC	11.24	5.2	8.13

From Table 2, it is observed that the pretreatment is very efficient because it inhibited the leaching process, in addition to not interfering with the productivity of alcoholic fermentation – tests show low variation in the production of ethanol. The total leaching system was reduced by approximately 46 % with the use of pretreatment with NaOH 1.0 mol L^{-1} and 60 °C. Another experiment was performed with 2.0 mol L^{-1} sodium hydroxide, with a 44 % reduction in leaching compared to the no pretreatment system, and increase of 4.0 % in the leaching compared with the pretreated system with 1.0 mol L^{-1} NaOH. Consequently, the increase of concentration of NaOH showed no improvement in reducing leaching.

Therefore, it is viable to use the polyurethane foam treated with NaOH (1 mol L^{-1}) and 60 °C as a support for the immobilization of *Saccharomyces cerevisiae* in order to reduce the leaching of the yeast during the fermentation process. At the same time, this pretreatment enables the reuse of immobilized cubes in future production runs with lower losses of cellular material.

Stability fermentation tests. The Fig. 3 shows the obtained results for the fermentation during a 48 hours' period.

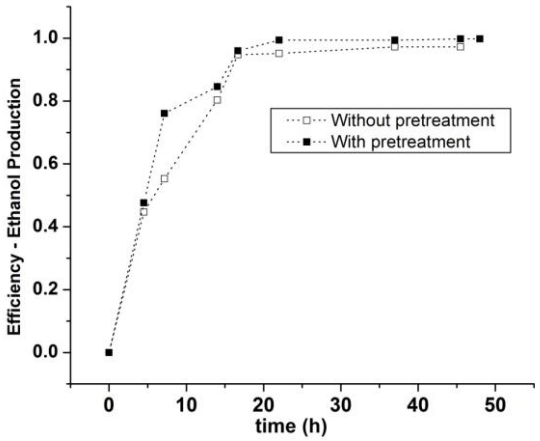


Figure 3. Ethanol production as a function of time for: no pretreatment and with pretreated foam.

The results presented in Fig. 3 show that the yield after 48 h of fermentation in cases with and without pretreatment are close. These results are similar to those obtained by De Bari et al. [24]. Despite the proximity, the process without the pretreatment had a slightly lower yield than the process with pretreatment, 97.26 % and 99.79 %, respectively. It is also noted that the yield after 48 h of the process with pretreatment was very close to the ideal value for the total conversion of glucose into ethanol. Similar results were obtained by other authors [14, 18, 34 – 40].

Duarte et al. [16] observed that after 10 h of fermentation in similar conditions, but supported in calcium alginate, the yield was approximately 61 %. For this study, after 10 h of fermentation, the yield was approximately 73.53 % in the system without pretreatment, and 81.78 % in the pretreated system. These results show that despite the increased leaching in the immobilization of *Saccharomyces cerevisiae* in polyurethane compared to immobilization in alginate of calcium or sodium, there is also an increase in the reaction speed - achieving a yield 34 % greater in the polyurethane foam than in the alginate systems during the first 10 hours of fermentation for the system with pretreated support.

From the analysis of Fig. 3, it is observed that the reaction rate of the pretreated system is greater than that one without pretreatment. Thus, these experiments can be used to estimate the time necessary to reach a yield approximately equal to that achieved by the end of 48 h. The pretreatment system presents a yield of about 99.36 % for a 22 h fermentation. Consequently, this system takes more than twice this time to increase the yield of 0.43 % – demonstrating the possibility to shut the fermentation down after 22 h to obtain savings in the process performance. Likewise, the system without pretreatment presents a yield of 96.19 % after 30 h procedure, taking over 18 h to rise the yield of 1.10 %.

Both processes represented in Fig. 3 showed considerable leaching – greater than that observed for the respective processes lasting only 4 h. However, as discussed in Section 3.3, the process involving pretreatment of the support showed lower leaching. The leaching values were 13.00 g L^{-1} for the case without pretreatment (20.31 mg cm^{-2}) and 8.030 g L^{-1} for the pretreatment process (12.55 mg cm^{-2}). Finally, Table 3 summarizes the discussion regarding the long runs.

TABLE III. RESULTS FOR STABILITY FERMENTATION TESTS IN 48H OF REACTION

	No pretreated foam	NaOH (1 mol L^{-1}) and 60 °C pretreated foam
Ethanol production (g)	37.25	38.22
Yield at 48 h (%)	97.26	99.79
Yield at 10 h (%)	73.53	81.78
Reaction time for yield close to the maximum (h)	30.00	22.00
Leaching (g L^{-1})	13.00	8.030
Leaching per surface area of immobilization (mg cm^{-2})	20.31	12.59

IV. CONCLUSION

The results presented for immobilization of *Saccharomyces cerevisiae* in polyurethane foam in the shape of cubes of 1 cm per side confirm that the system is preferable when compared to the immobilization in calcium alginate performed previously. However, the leaching rate, despite having been reduced by 46 % by pretreatment with NaOH at high temperature (60 °C), is still higher than the showed with this other support. In polyurethane, it was possible to prove the multiplication and growth of yeast in the process, using the scanning electron microscopy. From an economic point of view, using polyurethane as support is more advantageous then other common supports, once it is cheaper, more affordable and can be reused to prevent its disposal in the environment, through the upholstered furniture.

The main function of immobilization for the alcoholic fermentation is to reduce spending on end of pipe separation processes. Thus, experiments have shown that it is possible to reduce the mass of non-volatile solids suspended in ethanol production by non-immobilized system of 60.0 g L^{-1} to 45.0 g L^{-1} (Table2) using immobilization in 960 cm^2 pretreated polyurethane foam with sodium hydroxide at 1.0 mol L^{-1} and 60°C.

It is suggested to perform other tests with larger time of fermentation for the selected system: immobilization in 960 cm^2 varying the time and temperature, since the variation of the sodium hydroxide concentration did not show significant changes in the leaching rate or production system.

ACKNOWLEDGMENT

We acknowledge the company Vallourec Tubular Solutions Brazil (VSB) for the SEM analysis and the CNPq and FAPEMIG agencies for the support.

REFERENCES

[1] U. Lima, E. Aquarone, W. Borzani and W. Schmidell, "Industrial biotechnology: biochemical engineering." São Paulo: Edgard Blücher Ltda, vol. 3, p. 254, 2001.

[2] K. Kim, I. Choi, H. Kim, S. Wi and H. Bae, "Bioehtanol production from the nutriente stress-induced microalga Chlorella vulgaris by enzymatic hydrolysis and immobilized yeast fermentation". Bioresour. Technol., vol.153, pp. 47-54, 2014.

[3] R. Razovski and V. Vucurovic, "Bioethanol production from sugar beet molasses and thick juice using *Saccharomyces cerevisiae* immobilized on maize stem ground tissue". Fuel, vol. 92, n.1, pp.1-8, 2012.

[4] G. Junter and T. Jouenne, "Immobilized viable microbial cells: from the process to the proteome in leader or the cart before the horse". Biotechnol. Adv. New York, vol.22, n.8, pp. 633-658, 2004.

[5] D. Bergmaie, C. P. Champagne and C. Lacroix, "Growth and exopolysaccharide production during free and immobilized cell chemostat culture of *Lactobacillus rhamnosus* RW-9595M". J. Appl. Microbiol., vol.98, n.2, pp. 272-284, 2005.

[6] J. Yu, X. Zhang and T. Tan, "An novel immobilization method of *Saccharomyces cerevisiae* to sorghum bagasse for ethanol production". J. Biotechnol., vol.129, n.3, 415-420, 2007.

[7] M. Petre, G. Zarnea, N. P. Adrian and E. Gheorghiu, "Biodegradation and bioconversion of cellulose wastes bacterial and fangal cells immobilized in radiopolymerized hydrogels". Resour. Conserv. Recycl., vol.27, pp. 309-332, 1999.

[8] P. Ariyajaroenwong, P. Laopaiboon, P. Jaisil and L. Laopaboon, "Repeated-batch ethanol production form sweet sorghum juice by *Saccharomyces cerevisiae* immobilized on sweet sorghum stalks". Energies, vol.5, n.4, pp. 1215-1228, 2012.

[9] L. Laopaiboon and P. Laopaiboon, "Ethanol production from sweet sorghum juice in repeated-batch fermentation by *Saccharomyces cerevisiae* immobilized on corncob". World J. Microbiol. Biotechnol., vol.28, n.2, pp. 559-566, 2012.

[10] V. Vucurovic and R. Razmovski, "Sugar beet pulp as support for *Saccharomyces cerevisiae* immobilization in bioethanol production". Ind. Crops Prod., vol.39, 128-134, 2012.

[11] Y. Kourkoutas, M. Komaittis, A. A. Koutinas and M. Kanellaki, "Wine production using yeast immobilized on apple pieces at low and room temperatures". J. Agric. Food Chem., Easton, vol.49, n.3, pp. 1417-1425, 2001.

[12] A. Eladpum, S. Limtong and M. Pisalaphong, "High-temperautre ethanol fermentation by immobilized coculture of *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*". J. Biosci. Bioeng., vol.114, n.3, pp. 325-329, 2012.

[13] G. Kostov, S. Popova, V. Gochev, P. Hristova, M. Angelov and A. Georgieva, "Modeling of batch alcohol fermentation with free and immobilized yeasts *Saccharomyces cerevisiae* 46 EVD". Biotechnol. & Biotechnol. Equip., vol.26, n.3, 2012.

[14] A. Mishra, A. Sharma, S. Sharma, R. Bagai, A. Mathur, R. Gupta and D. Tulli, "Lignocellulosic ethanol production employing immobilized *Saccharomyces cerevisiae* in packed bed reactor." Renew. Energy, vol.1, pp. 1-7, 2016.

[15] A. Singh, P. Sharma, A. K. Saran, N. Singh and N. R. "Bishnoi. Comparative study on ethanol production from pretreated cane bagasse using immobilizes *Saccharomyces cerevisiae* on various matrices". Renew. Energy, vol.50, pp.488-493, 2013.

[16] J. C. Duarte, A. R. Rodrigues, P. J. S. Moran, G. P. Valença and J. R. Nunhez, "Effect of immobilized cells in calcium alginate beads in alcoholic fermentation". AMB Express, v.3, pp. 31-35, 2013.

[17] C. Galanakis, C. Kordulis, M. Kanellaki, A. Koutinas, A. Bekatorou and A. Lycourghiotis, "Effect of pressure and temperature on alcoholic fermentation by *Saccharomyces cerevisiae* immobilized on Y-alumina pellet". Bioresour. Technol., vol.114, n.1, pp. 492-498, 2012.

[18] F. Ghorbani, H. Younesi, A. Sari and G. Najafpour, "Cane molasses fermentation for continuous ethanol production in an immobilized cells reactor by *Saccharomyces cerevisiae*". Renew. Energy, vol.36, n.2, pp. 503-509, 2011.

[19] S. Behera, S. Kar, R. Mohanty and R. Ray, "Comparative study of bio-ethanol production form mahula (*Madhuca latifólia* L.) flowers by *Saccharomyces cerevisiae* cells immobilized in agar and Ca-alginate matrices". Appl. Energy, vol.87, n.1, pp. 96-100, 2010.

[20] K. Lee, I. Choi, Y. Kim, D. Yang and H. Bae, "Enhanced production of bioethanol and ultrastructural characteristics of reused *Saccharomyces cerevisiae* immobilized calcium alginate beads". Bioresour. Technol., vol.102, n.17, 2011.

[21] V. Ivanova, P. Petrova and J. Hristov, "Application in the ethanol fermentation of immobilized yeast cells in matrix of alginate/magnetic nanoparticles, on chitosan-magnetite microparticles and cellulose-coated magnetic nanoparticles". Int. Rev. Chem. Eng., vol.3, n.2, pp. 289-299, 2011.

[22] L. Jamai, K. Sendide, K. Eittayebi, F. Errachidi, O. Hamdouni-Alami, M. A. Tahri-Joouti, T. Mcdermott and M. Eittayebi, "Physiological difference during fermentation between calcium alginate-immobilized *Candida tropicalis* and *Saccharomyces cerevisiae*". FEMS Microbiol. Letters, vol. 204, n.2, pp. 375-379, 2001.

[23] Y. Chen, Q. Liu, T. Zhou, B. Li, S. Yao, A. Li, J. Wu and H. Ying, "Ethanol production by repeated batch and continuous fermentations by *Saccharomyces cerevisiae* immobilized in a fibrous bed bioreactor". J. Microbiol. Biotechnol., vol.23, n.4, pp. 511-517, 2013.

[24] I, De Bari, P. Canio, D. Cuna, F. Liuzzi, A. Capece and P. Romano, "Bioethanol production from mixed sugars by *Scheffersomyces stipitis* free and immobilized cells, and co-cultures with *Saccharomyces cerevisiae*". New Biotechnol., vol.30, n.6, pp. 591-597, 2013.

[25] S. Nikilic, L. Majovic, D. Pejin, M. Rakin and M. Vukasinovic, "Production of bioethanol from corn meal hydrolyzates by free and immobilized cells of *Saccharomyces cerevisiae* var. *Ellipsoideus*". Biomass and Bioenergy, vol.34, n.10, pp. 1499-1456, 2010.

[26] S. Behera, R. Mohanty and R. Ray, "Ethanol production from mahula (*Madhuca latifólia L.*) flowers with immobilized cells of *Saccharomyces cerevisisae* in Luffa cylindrica L. Sponge discs". Appl. Energy, vol.88, pp. 212-215, 2011.

[27] A. J. Silva, J. S. Hirasawa, M. B. Varesche, E. Foresti and M. Zaiat, "Evaluation of support materials for the immobilization of sulfate-reducing bacteria and methanogenic archaea". Anaerobe, London, vol. 12, n. 2, pp. 93-98, 2006.

[28] M. Fujita, A. Era, M. Ike, S. Soda, N. Miyata and T. Hirao, "Decolorization of heat-treatment liquor of waste sludge by a bioreactor using polyurethane foam-immobilized white rot fungus equipped with an ultramembrane filtration unit". J. Biosci. Bioeng., Osaka, vol.90, n.4, pp. 387-394, 2000.

[29] N. K. Patril, Y. Veeranagouda, M. Vijaykumar, S. Nayak and T. Karegoudar, "Enhanced and potential degradation of o-phthalate by *Bacillus sp.* immobilized cells in alginate and polyurethane". Int. Biodeterior. Biodegrad., Barking, vol.57, n.2, pp. 82-87, 2006.

[30] T. Romaskevic, S. Budriene, K. Pielichowski and J. Pielichowski, "Application of polyurethane based materials for immobilization of enzymes and cells: A Review". Chemija, vol.17, pp. 74-89, 2006.

[31] L. Wang, D. Ridgway, T. Gu and M. Moo-Young, "Bioprocessing strategies to improve heterologous protein production in filamentous fungal fermentations". Biotech. Adv., New York, vol. 23, n. 2, pp.115-129, 2005.

[32] L. Moraes, M. Oliveira and J. Santora, "Immobilizing *Saccharomyces cerevisiae* in polyurethane foam". Univ. Utah: Proj. Lab., 2014.

[33] A. Domínguez, S. R. Couto and A. Sandromán, "Amelioration of ligninolytic enzyme production by *Phanerochaete chrysosporium* in airlift bioreactors". Biotech. Lett., Dordrecht, vol. 23, n. 6, pp. 451-455, 2001.

[34] N. Singh, G. Srivastava, M. Talat, H. Raghubanshi, O. N. Srivastava and A. M. Kayastha, "Cicer *α*-galactosidase immobilization onto functionalized graphene nanosheets using response surface method and its applications". Food Chem, vol.142, pp. 430-438, 2014.

[35] A. Singha, S. Tutejaa, N. Singhb and N. Bishnoi, "Enhanced saccharification of rice straw and hull by microwave alkali pretreatment and lignocellulolytic enzyme production". Bioresour. Technol., vol.102, pp. 1773-1782, 2011.

[36] M. Jeyaa, Y. Zhanga, I. Kima and J. Lee, " Enhanced scarification of alkali-treated rice straw by celulase from *Trameters hirsute* and statistical optimization of hydrolysis conditions by RSM". Bioresour. Technol., vol.100, pp. 5155-5161, 2009.

[37] K. Prasad, S. V. Mohan, Y. V. Bhaskar, S.V. Ramanaiah, V. L. Babu, B. R. Pati and P. N. Sarma, "Laccase production using *Pleurotus ostreatus* 1804 immobilized on PUF cubes in batch and packed bed reactors: influence of culture conditions". J. Clin. Microbiol., Washington, vol. 43, n.3, pp. 301-307, 2005.

[38] R. Liu and F. Shen, "Impacts of main factors on bioethanol fermentation from stalk juice of sweet sorghum by immobilized *Saccharomyces cerevisiae* (CICC 1308)". Bioresour. Technol. vol. 99, pp. 847-854, 2008.

[39] J. Yu, G. Yue, J. Zhong, X. Zhang and T. Tan, "Immobilization of *Saccharomyces cerevisiae* to modified bagasse for ethanol production". Renew. Energy, vol.35, n.6, pp. 1130-1134, 2010.

[40] R. Razmovski and V. Vucurovic, "Ethanol production from sugar beet molasses by *Saccharomyces cerevisiae* entrapped in an alginate-maize stem ground tissue matrix". Enzyme Microb. Technol. vol. 48, pp. 378-385, 2011.