Stability Studies of Immobilized Saccharomyces Cerevisiae in Calcium Alginate and Carrageenan Beads

Rahmath Abdulla, Warda Abdul Ajak, Siti Hajar & Eryati Derman
Faculty of Science and Natural Resources
Universiti Malaysia Sabah
Sabah, Malaysia
rahmahabdulla@gmail.com

Abstract—Currently the resources for fossil fuels are depleting together with increase in fuel prices. This has urged the need for cheaper alternative fuels especially biofuels. The production of the most common liquid biofuel which is bioethanol using immobilized yeast cells is an approach taken to increase its demand in the world's market. There are various methods for the immobilization of yeast cells; however before they can be applied in the industry the stability of the immobilization technology must be investigated. This research aims to study the stabilities of immobilized S. cerevisiae in calcium alginate and carrageenan beads for bioethanol production. The S. cerevisiae was immobilized in calcium alginate and carrageenan beads using entrapment method. Next, screening for the optimal concentration of sodium alginate and semi refined carrageenan matrices were determined by employing fermentation and bioethanol quantification using GC-MS. Concentrations of 2% (w/v) calcium alginate and 2% (w/v) semi refined carrageenan beads were identified to produce the highest bioethanol yield which were 0.286 g/mL and 0.065 g/mL respectively. The two beads were then chosen to be tested in various stability studies with respect to bioethanol production such as storage stability, reusability, pH, thermaland permeability test. It was found out that a concentration of 2% (w/v) calcium alginate beads were more stable as immobilization matrix for S. cerevisiae as compared to 2% (w/v) semi refined carrageenan.

***Index Terms*—Immobilization, Stability, Saccharomyces, Alginate, Carrageenan**

I. INTRODUCTION

Immobilization is the process of adhering biocatalysts (whole cells or isolated enzymes) to a solid support which can be an organic or inorganic material such as derivatized cellulose or glass, ceramics, metallic oxides and a membrane [1]. Immobilization systems are used to confine the biocatalysts into a defined space so that their bio-activities can be retained and reutilized continuously or repeatedly over a long period of time [2]; [3]. The choice of proper immobilization method and the support materials are the key parameters in cell immobilization [4]. Immobilization influence on the fermentation performance and the parameters affecting the immobilization are important for efficient bioethanol production and in achieving acceptable product quality [5].

Immobilization of whole cells in natural polymers such as alginate and carrageenan beads has long been studied in developing immobilization yeast cell system for bioethanol production [6]. Immobilization of whole cell systems and enzymes is of major importance in the improvement of the stability, activity and reusability of these biocatalysts [7]. Immobilization process employed alginate and carrageenan is more environmental friendly and less costly as it can be simply extracted from the local seaweeds [8]. Despite the associated problems of using alginate and carrageenan for yeast cell immobilization system, the encapsulation in calcium alginate and carrageenan are still being commonly used in studies with ethanol fermentation due to their requirement for mild conditions and the simplicity of the used procedure [7]; [9].

Immobilization of *S. cerevisiae* was found to be one of the most useful and novel method for ethanol production [10].*S. cerevisiae* is the number one microorganisms used in bioethanol production as it is inexpensive, easily available and capable in undergo alcoholic fermentation and for fermented food applications [11]. Immobilized *S. cerevisiae* also can be used to test the polymers suitability as carriers for cell immobilization for ethanol production.

Objective of this research is to study the stabilities of immobilized S. cerevisiae in natural polymers such as calcium alginate and carrageenan for bioethanol production. Therefore, screening is done to determine the optimal concentration of alginate and carrageenan matrices by employing fermentation process. The effects of various stability studies with respect to bioethanol production such as storage stability, reusability, pH and thermal stability, permeability test on the immobilized S. cereviseae were investigated. Finally, the performances of calcium alginate and carrageenan beads as support materials to the immobilization of yeast cells for bioethanol production were compared.

II. MATERIALS AND METHODS

A. Materials

Saccharomyces cerevisiae(Baker's yeast) was used throughout the experiment. The purified culture was obtained by isolating it on YPD agar medium [12]. Subculturing was

IJHM

This is an open access article under the CC BY-SA 4.0 license (https://creativecommons.org/licenses/by-sa/4.0/)

done in every 2 months. All other chemicals and reagents used were of analytical grade and obtained commercially.

B. Immobilization of of S. cerevisiae on calcium alginate beads

Immobilization of *S. cerevisiae* was done by modifying the method of Rakin*et al*. [13]. Sodium alginate concentrations of 2%, 3% and 4% (w/v) and calcium chloride solution were prepared [14]. Three different types of beads were prepared by droplet addition of the polymer ± cell suspension to 6% calcium chloride solution using plastic syringe equipped with blunt needle [13]. The beads were allowed to harden for 30 minutes and then washed with distilled water and kept at 4 ℃ in both 0.2 % (w/v) yeast glucose solutions [2].

C. Immobilization of of S. cerevisiae on carrageenan beads

Immobilization of *S. cerevisiae* on carrageenan beads was done by modifying the method of Rakin*et al.* [13].Three different semi refined carrageenan concentrations of 1%, 2% and 3% (w/v) were prepared [13]. Carrageenan beads were formed by dripping the carrageenan solutions into 2% KCl solution using syringe equipped with needle. The beads were allowed to harden for 20 minutes and then washed with distilled water and kept at 4 ℃ in both 0.2 % (w/v) yeast and glucose solution [2]; [15].

D. Determination of optimal concentration of alginate and carrageenan solutions

Screening for the optimal concentration of sodium alginate and semi refined carrageenan matrices were determined by employing fermentation and bioethanol quantification using GC-MS. *S. cerevisiae* cells were immobilized with three different concentrations of sodium alginate (2%, 3% and 4%) and carrageenan (1%, 2% and 3%) [14]. Fermentation medium were prepared and its pH were maintained at 4 and placed in an orbital shaker for 24 h at 30°C and 200 rpm [16]; [17].Optimization of Bioethanol production process

E. Determination of storage and reusability studies

The storage stability of immobilized *S. cerevisiae* in calcium alginate and carrageenan beads were done by storing in citrate-phosphate buffer (0.05 M, pH 5), 20 mMTris/HCl buffer (pH 7.5) and sterile saline water at 4 ℃ for 10 days [18]; [19]. The stability of the calcium alginate and carrageenan beads were tested every 2 days, via invertase assay and the amount of glucose was tested via DNSA method. The immobilized *S. cerevisiae* in calcium alginate and carrageenan beads were repeatedly used twelve times. The operational stability of immobilized *S. cerevisiae* in calcium alginate and carrageenan beads of optimal concentration was investigated via invertase assay and the amount of glucose was determined using DNSA method [20].

F. Determination of Membrane Permeability

The membrane permeability of immobilized yeast in calcium alginate and carrageenan beads was done by modifying the method from Namthabad&Chinta [20]. The diffusion of glucose from the exterior solution into the beads was measured by the disappearance of the glucose. Estimation of glucose disappearance was calculated using standard curve obtained by DNSA method. 1 g of immobilized yeast from each calcium alginate and carrageenan beads was placed into 2 mg/mL of glucose solution in acetate buffer of pH 4 at 30℃.

G. Determination of pH and thermal stability

The immobilized *S. cerevisiae* in calcium alginate and carrageenan beads were exposed to Tris-HCl buffer with different pH values of 2, 3, 4, 5 and 6 for 1 h at 4 ℃[21]. The calcium alginate and carrageenan beads (1 g) were suspended in acetate buffer at pH 4, and then 20 mL of preheated sucrose medium (60 mg/mL) was added. The thermal stability of immobilized *S. cerevisiae* in calcium alginate and carrageenan beads were done by incubating them at different temperature ranging from 25-50 ℃ for 1 h [21]. The activities of the cells were assayed using invertase assay [22].

H. Invertase Assay

Immobilized *S. cerevisiae* in calcium alginate and carrageenan beads were added to 20 mL of sucrose solution (60 mg/mL) in acetate buffer (pH 4) and incubated for 15 minutes at 30 ˚C. The reducing sugars produced by sucrose hydrolysis were measured by spectrophotometric method using 3,5-dinitrosalycylic acid reagent.

III. RESULTS AND DISCUSSION

A. Immobilized yeast characterization

The isolated colonies grown on YPD agar medium was propagated in a defined liquid medium for 2 days and the yeast suspension was obtained via centrifugation. The yeast suspension was then used in immobilization via encapsulation method using alginate and carrageenan as carriers. The method produced spherical beads as shown in Figure 1, with the yeast cells entrapped within the beads.

The encapsulation method had successfully immobilized the *S. cerevisiae* cells in calcium alginate and carrageenan beads. The beads were spherical with average diameter of 3 mm and 4 mm for calcium alginate and carrageenan beads respectively. Beads with smaller diameter are preferred so that mass transfer can be minimized [23]. Alginate is a suitable cell entrapment matrix as it is less expensive, non-toxic, has good mechanical properties and is reversible [24]. Studies reported by Ellaiah*et al*. found that alginate is a good matrix for the cell immobilization for the lipase production [25]. Immobilization of *S. cerevisiae* Type II on PVA-alginate.

This is an open access article under the CC BY-SA 4.0 license (https://creativecommons.org/licenses/by-sa/4.0/)

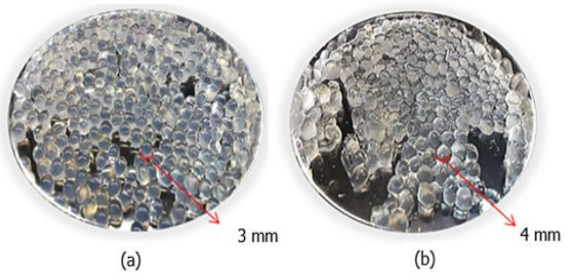


Fig. 1. Immobilization of *S. cerevisiae* cells using entrapment method (a) Immobilized in calcium alginate support (b) immobilized in carrageenan support.

B. Optimal concentration of sodium alginate and carrageenan solutions

Determination of the optimum concentration is important as it influence the efficiency of the immobilization process [26]. *S. cerevisiae*cells were immobilized with three different concentrations of sodium alginate and carrageenan respectively as shown in Table 1 and Table 2. The six different beads were fermented and the analysis of ethanol on the product of fermentation was done using GC-MS. From the table, *S. cerevisiae* cells immobilized using 2% (w/v) sodium alginate and 2% (w/v) carrageenan concentrations produced the highest amount of bioethanol which was 0.286 g/mL and 0.065 g/mL respectively.

Hence, the two concentrations, 2% (w/v) were chosen to be the optimum concentrations of sodium alginate and carrageenan solutions. In literature, it is reported that the 2% (w/v) concentration of alginate in beads was a suitable concentration for the alginate beads activity for ethanol production [27]. Higher concentration may have more rigid and stable beads for immobilization but it will cause diffusion problems [13]. Moreover, high concentration gels were hard to work with as it can cause clogging the needle. Gels were fragile at lower concentration, therefore enzyme can bound loosely to the gel [26].

From the table, *S. cerevisiae* cells immobilized using sodium alginate produced higher amount of ethanol which is 0.286 g/mL compared to carrageenan which only produced 0.065 g/mL ethanol. According to Ryu and Lee, ethanol productions by cells immobilized with alginate were much better compared to the cells immobilized with carrageenan [28].

From the table, *S. cerevisiae* cells immobilized using sodium alginate produced higher amount of ethanol which is 0.286 g/mL compared to carrageenan which only produced 0.065 g/mL ethanol. According to Ryu and Lee, ethanol productions by cells immobilized with alginate were much better compared to the cells immobilized with carrageenan [28].

Table I: Bioethanol concentration and yield obtained by GC-MS analysis from fermentation using immobilized S. cerevisiae cells in different concentration of sodium alginate solution.

Sodium alginate conc. (w/v)	Bioethanol conc. (g/mL)	Bioethanol yield (%)
2%	0.2857	23.8
3%	0.1820	15.2
4%	0.0302	2.5

Table II: Bioethanol concentration and yield obtained by GC-MS analysis from fermentation using immobilized S. cerevisiae cells in different concentration of semi refined carrageenan solution.

Carrageenan conc. (w/v)	Bioethanol conc. (g/mL)	Bioethanol yield (%)
1%	0.0513	4.3
2%	0.0646	5.4
3%	0.0307	2.6

C. Storage Stability

Catalytic activity of the enzymes are easily lost and denatured, hence careful handling and storage is important [29]. Storage stability is essential for various applications of biocatalysts [30]. Figure 2 and 3 shows the storage stability of immobilized *S. cerevisiae* cells of alginate and carrageenan for a period of ten days. Both immobilized *S. cerevisiae* cells were stored in three different buffers namely Tris-HCl (T-HCl) buffer (20 mM, pH 7.5), citrate-phosphate (CP) buffer (0.05 M, pH 5) and saline water (SW) (0.9% NaCl) at 4˚C.

It was observed that the alginate beads stored in Tris-HCl buffer and carrageenan beads stored in citrate-phosphate buffer showed relatively better activity compared to that stored in the other two buffers. The carrageenan beads stored in saline water disintegrated after 4 days of storage, meanwhile both carrageenan beads stored in Tris-HCl and citrate-phosphate buffers disintegrated after 6 days of storage. It can be seen that the calcium alginate beads were able to maintain their integrity throughout the 10 days of storage in all three buffers without loss of any activity.

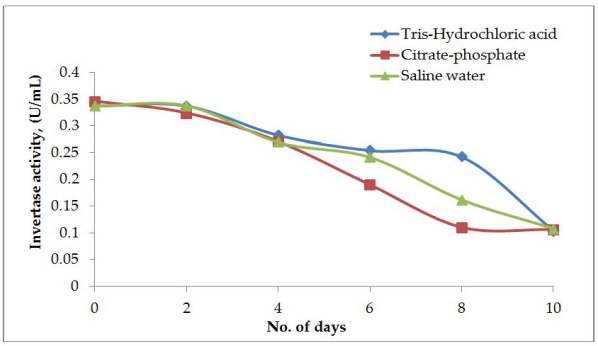


Fig. 2. Storage stability of immobilized S. cerevisiae cells in 2% (w/v) calcium alginate stored in three different buffers.

This is an open access article under the CC BY-SA 4.0 license (https://creativecommons.org/licenses/by-sa/4.0/)

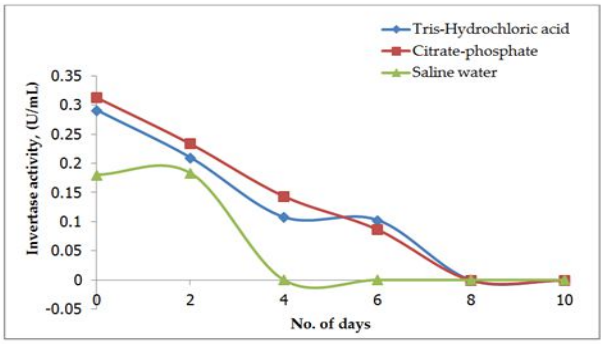


Fig. 3. Storage stability of immobilized S. cerevisiae cells in 2% (w/v) carrageenan stored in three different buffers.

D. Reusability.

Efficient design of an immobilized enzyme is affected by its reusability [8]. Reusability is the most significant benefits if using immobilized enzymes over the native enzymes [31]. The invertase activity of immobilized S. cerevisiae cells in alginate and carrageenan beads were measured under the same storage conditions for over a period of twelve reuses is shown in Figure 4.

The carrageenan beads started to disintegrate at the fifth cycle, therefore the amount of glucose was unable to be determined. However, the calcium alginate beads lasted throughout the twelve cycles and its activity decreased steadily throughout the test. The carrageenan beads are more fragile, therefore it could not withstand to be use repeatedly compared to the alginate beads [26]. Comparing the operational stability of both beads, 2% (w/v) calcium alginate beads were more stable with higher activity shown making it more suitable to be reused repeatedly.

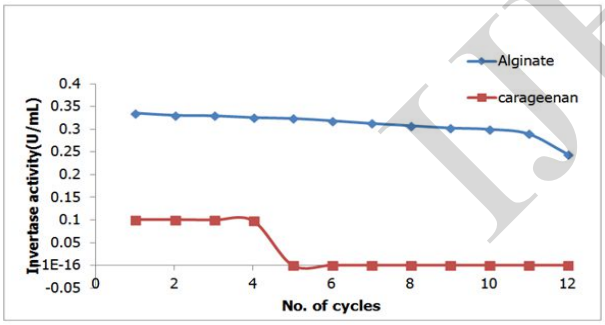


Fig. 4. Reusability tests of immobilized S. cerevisiae cells in 2% (w/v) calcium alginate and 2%

E. pH Stability.

The effects of pH on activity of immobilized yeasts are shown in Figure 5. Both immobilized yeasts in calcium alginate as well as carrageenan followed typical bell shaped curve for pH. Optimum pH for both types of immobilized beads was 4. However, immobilized yeasts in calcium alginate showed higher activity as compared to its carrageenan counterpart. According to Mahmood, a drop in the activity at certain pH range depicts that the immobilization faces some limitations [18]. Breakage of ionic bonds which hold the tertiary structure of the enzyme can happen due to a change in pH [8].Changes in pH also can cause denaturation and alter the structure of enzyme [29].

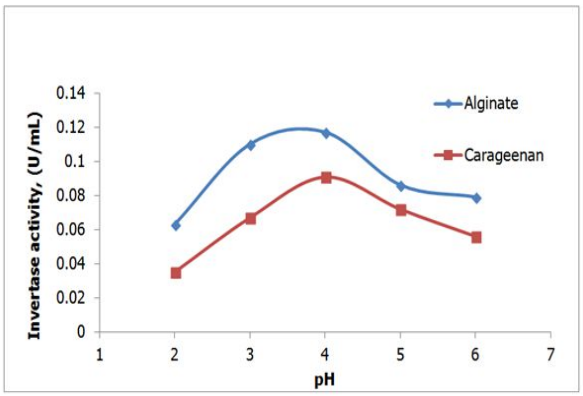


Fig. 5. The effect of pH towards the activity of immobilized S. cerevisiae cells immobilized in 2% (w/v) calcium alginate beads and 2% (w/v) carrageenan beads.

F. Thermal stability

Thermal stability of immobilized yeasts on calcium alginate and carrageenan beads (25-50˚C) is depicted in Figure 3. The optimum temperature for both types of immobilized yeasts was at 30 ˚C. This shows that higher activation energy is needed for the immobilization process [8]. Regardless of the type of matrix used, the immobilization of the *S. cerevisiae* is usually showed a maximum ethanol production at 30˚C [28].

Immobilized beads of carrageenan disintegrated at higher temperatures making calcium alginate beads to be more stable. Alginate beads have higher cell activity and were able to maintain their matrices integrity in the temperature range tested. Carrageenan beads however disintegrated after 35˚C, hence indicating that the carrageenan beads are very poor in strength and stability. This is because, when carrageenan solution is warm, the chain exists as random coils. However, on cooling double helices formed and produced cross linked gel. Therefore, cross linking in carrageenan beads is thermally reversible, where at high temperature the cross links disintegrated. After the optimum temperature, the activity of the enzyme is decreased rapidly as its structure was altered and the catalytic properties were reduced and destroyed eventually [29].

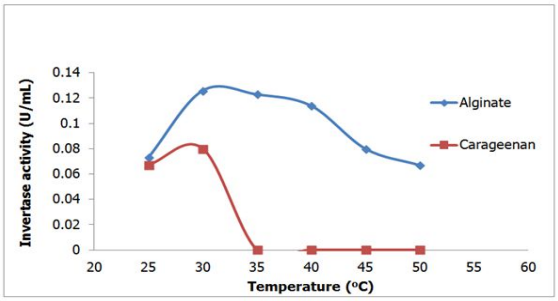


Fig. 6. The effect of temperature towards the activity of immobilizedS. cerevisiae cells immobilized in 2% (w/v) calcium alginate beads and 2% (w/v) carrageenan beads.

IJHM

This is an open access article under the CC BY-SA 4.0 license (https://creativecommons.org/licenses/by-sa/4.0/)

G. Membrane Permeability

The diffusivity of glucose over a period of 180 minutes in calcium alginate and carrageenan beads is shown in Figure 7. Both beads showed steady rate of glucose diffusion into the beads, however there was large difference in amount of glucose diffused into the beads. Higher amount of glucose was able to diffuse into 2% (w/v) calcium alginate beads compared to 2% (w/v) carrageenan beads. Decrease in the diffusion of the substrate into the beads leads to the decrease of the substrate conversion into product [26].

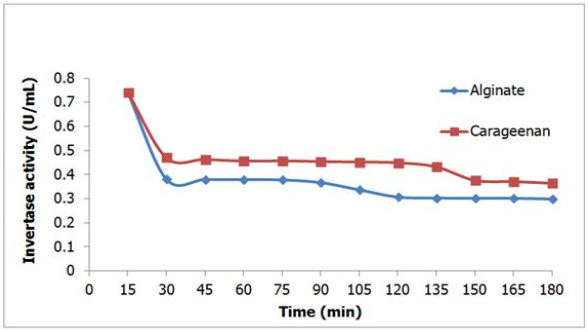


Fig. 7. Fig. &. Diffusivity of glucose over time in 2% (w/v) calcium alginate and 2% (w/v) carrageenan beads.

IV. CONCLUSION

In short, a comparison on stability of immobilized S. cerevisae in calcium alginate and carrageenan beads was carried out for bioethanol production. From the studies, it was found that 2% (w/v) was the optimum concentration of sodium alginate and carrageenan which gave the highest porosity. After comparison of the performance of calcium alginate and carrageenan matrices for stabilities studies of immobilization of S. cerevisiae, it can be concluded that calcium alginate served as a better immobilization platform.

ACKNOWLEDGMENT

This work was supported by the Fundamental Research Grant Scheme (FRG0366-SG- 1/2014).

REFERENCES

[1] T. Johannes, M. R. Simurdiak, and H. Zhao, "Biocatalysis," In Encyclopedia. Chem. Process.,pp. 101-110, 2006.

[2] F.Ghorbani, H.Younesi, A.Esmaeili Sari, and G.Najafpour, "Cane molasses fermentation for continuous ethanol production in an immobilized cells reactor by *Saccharomyces cerevisiae*," Renew.Energy., vol.36(2), pp. 503-509, 2011. URL – https://doi.org/10.1016/j.renene.2010.07.016

[3] K. R. Jegannathan, S.Abang, D. Poncelet, E. S. Chan, and P. Ravindra, "Production ofbiodiesel using immobilized lipase – A critical review," Critic. Reviews inBiotechnol., vol. 28(4), pp. 253–64, 2008. URL – https://doi.org/10.1080/07388550802428392

[4] M. M. Elnashar, M. A. Yassin, and T.Kahil, "Novel thermally and mechanically stable hydrogel for enzyme immobilization of penicillin G acylase via covalent technique,"J. Appl. Polym. Sci., vol.109(6), pp. 4105-4111,2008. URL - http://dx.doi.org/10.1002/app.28379

[5] P. J. Verbelen, D. P. De Schutter, F. Delvaux, K. J.Verstrepen, and F. R. Delvaux, "Immobilized yeast cell systems for continuous fermentation applications,"Biotechnol.Letters.,vol. 28(19), pp.1515-1525, 2006. URL - https://link.springer.com/article/10.1007%2Fs10529-006-9132-5?LI=true

[6] H. Y. Wang, and D. J. Hettwer, "Cell immobilization in k-carrageenan with tricalcium phosphate,"Biotechnol. Bioeng.,vol. 24(8), pp. 1827-1838, 1982. URL - http://dx.doi.org/10.1002/bit.260240809

[7] J.Necas, and L. Bartosikova, "Carrageenan: a review," Vet. Medic., vol. 58(4), pp. 187-205,2013. URL - http://vri.cz/docs/vetmed/58-4-187.pdf

[8] R. Abdulla, and P.Ravindra, "Characterization of cross-linked *Burkholderiacepacia* lipase in alginate and k-carrageenan hybrid matrix,"J. Taiwan Inst. Chem. Eng.,vol 44(4),pp. 545-551, 2013. URL -https://doi.org/10.1016/j.jtice.2013.01.003

[9] C. M.Montealegre, E. R.Dionisio, L. V.Sumera, J. R. T. Adolacion, and R. L. De Leon, "A comparison between the performance of *S. cerevisiae* cells immobilized in Nata de Coco biocellulose and calcium alginate during continuous bioethanol production,"Inter. J. Chem. Eng. Appl., vol.3(4), pp. 237-24,2012. URL – http://www.ijcea.org/show-38-524-1.html

[10] J. Yu, X. Zhang, and T. Tan, "An novel immobilization method of *Sacharomycescerevisiae* to sorghum bagasse for ethanol production," J. Biotechnol, vol. 129(3), pp. 415-420, 2007. URL -https://doi.org/10.1016/j.jbiotec.2007.01.039

[11] S. Plessas, A.Bekatorou, A. A.Koutinas, M. Soupioni, I. M. Banat, and R. Marchant, "Use of *Saccharomyces cerevisiae* cells immobilized on orange peel as biocatalyst for alcoholic fermentation," Bioresour. Technol, vol. 98(4), pp. 860-865, 2007. URL - https://doi.org/10.1016/j.biortech.2006.03.014

[12] 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,"arXiv preprint arXiv:1105.0619, 2011.

[13] M. Rakin, L.Mojović, S.Nikolić, M.Vukasinovic, and V. Nedovic, "Bioethanol production by immobilized *Sacharomycescerevisiae* var. *ellipsoideus* cells,"African J. Biotechnol., vol.8(3), pp. 464-471, 2009. URL - https://www.ajol.info/index.php/ajb/article/viewFile/59844/48118

[14] I. Călinescu, P.Chipurici, A.Trifan,and C.Bădoiu, "Immobilisation of *Saccharomyces cerevisiase* for the production of bioethanol,"University Politechnica of Bucharest, Scientific Bulletin., vol. 74(1), pp. 33-40, 2012.URL – https://www.scientificbulletin.upb.ro/rev_docs_arhiva/full2e3_876162.pdf

[15] M.Vassileva, B.Eichler-Lobermann, A. Reyes, and N.Vassilev, "Animal bones char solubilization by gel-entrapped Yarrowialipolytica on glycerol-based media,"Sci. World J.Vol2012, Article ID 907413, pp. 1-5,2012. URL - http://dx.doi.org/10.1100/2012/907143

[16] J. C.Duarte, J. A. R. Rodrigues, P. J. Moran, G. P. Valença, and J. R. Nunhez, "Effect of immobilized cells in calcium alginate beads in alcoholic fermentation," inAMB Express., vol.3(1), pp. 31,2013. URL -https://doi.org/10.1186/2191-0855-3-31

[17] N. D. Mai andT. T. T. Dong, "Use of Immobilized Yeast Cell in Alcohol Fermentation from Molasses," Science & Technology Department, vol. 11(9). URL – http://www.vnulib.edu.vn:8000/dspace/bitstream/123456789/3141/3/sedev0908-11.pdf

[18] W. A. Mahmood, "Hydrolysis of Sucrose by Immobilized *Saccharomyces Cerevisiae* Invertase," Mesopotamia J. Agric., vol. 38(1), pp. 10-10,2010.URL –

This is an open access article under the CC BY-SA 4.0 license (https://creativecommons.org/licenses/by-sa/4.0/)

https://iasj.net/iasj?func=fulltext&aId=27739
[19] J. Maritz, H. M. Krieg, C. A.Yeates, A. L. Botes, and J. C. Breytenbach, "Calcium alginate entrapment of the yeast *Rhodosporidiumtoruloides* for the kinetic resolution of 1, 2-epoxyoctane,"Biotechnol Letters., vol.25(20), pp. 1775-1781, 2003. URL –
[20] S. Namthabad, and R.Chinta, "Robust encapsulation of yeast for bioethanol production. MSc Thesis, University of Borås, School of Engineering, Sweden, 2014. URL - http://bada.hb.se/bitstream/2320/13194/1/Namthabad%20and%20Chinta.pdf
[21] M. Karra-Châabouni, I.Bouaziz, S.Boufi, A. M. Botelho do Rego, and Y.Gargouri, "Physical immobilization of *Rhizopusoryzae* lipase onto cellulose substrate: Activity and stability studies.Colloids and Surfaces B," Biointer.,vol. 66(2), pp. 168-177, 2008. URL –
[22] K. Szymańska, W.Pudło, J.Mrowiec-Białoń, A.Czardybon, J. Kocurek, and A. B. Jarzębski, "Immobilization of invertase on silica monoliths with hierarchical pore structure to obtain continuous flow enzymatic microreactors of high performance,"Microporous and Mesoporous Mat.,vol. 170, pp. 75-82, 2013. URL - https://doi.org/10.1016/j.micromeso.2012.11.037
[23] S. Nikolić, L.Mojović, M.Rakin, and D.Pejin, "Bioethanol production from corn meal by simultaneous enzymatic saccharification and fermentation with immobilized cells of *Saccharomyces cerevisiae* var. *ellipsoideus*," Fuel.,vol. 88(9), pp. 1602-1607,2009. URL - https://doi.org/10.1016/j.fuel.2008.12.019
[24] C. Vogelsang, R. H. Wijffels, and K. Østgaard, "Rheological properties and mechanical stability of new gel-entrapment system applied in bioreactors," Biotechnol. Bioeng., vol. 70(3), pp. 247–253, 2000. URL - http://onlinelibrary.wiley.com/doi/10.1002/1097-0290(20001105)70:3%3C247::AID-BIT1%3E3.0.CO;2-%23/full
[25] P.Ellaiah, T.Prabhakar, B.Ramakrishma, A. T. Taleb, and K.Adinarayana, "Production of lipase by immobilized cells of *Aspergillusniger*," Process Biochem., vol. 39, pp. 525-528,2004. URL - https://doi.org/10.1016/S0032-9592(01)00340-5
[26] R. Mahajan, V. K. Gupta, and J. Sharma, "Comparison and Suitability of Gel Matrix for Entrapping Higher Content of Enzymes for Commercial Applications," Indian J. Pharm. Sci., vol. 72(2), pp. 223-228, 2010. URL - https://dx.doi.org/10.4103%2F0250-474X.65010
[27] G.Najafpour, H.Younesi, and K. S. K. Ismail, "Ethanolfermentation in an immobilized cell reactor using *Saccharomyces cereviae*," Bioresour. Technol, vol. 92(3), pp. 251-260,2004. URL- https://doi.org/10.1016/j.biortech.2003.09.009
[28] S. R. Ryu, and K. H. Lee, "Comparison of Immoblization matrix for ethanol fermentation by *Zymomonasmobilis* and *Saccharomyces cerevisiae*," J. Microb. Biotechnol., vol. 7(6), pp. 438-440,1997. URL - http://www.jmb.or.kr/journal/download.php?Filedir=../submission/Journal/007/&num=3111
[29] H. B. Yildiz, M.Kamaci, H.Azak, O.Secgin, and O. Suer, "A comparative study: Immobilization of yeast cells and invertase inpoly(ethyleneoxide) electrodes," J. Mol. Cat. B: Enzym., vol. 91, pp. 52-58,2013. URL - https://doi.org/10.1016/j.molcatb.2013.02.009
[30] Y.Hu, S.Tang,L.Jiang, B.Zou, J. Yang, and H. Huang, "Immobilization of *Burkholderiacepacia* lipase on functionalized ionic liquids modified mesoporous silica SBA-15.," Process Biochem., vol. 47(12), pp. 2291–2299, 2012. URL - https://doi.org/10.1016/j.procbio.2012.09.007 https://doi.org/10.1016/j.colsurfb.2008.06.010
[31] D. A. Butterfield, "Biofunctional Membranes," New York: Springer Science and Business Media, 2013,pp. 137.

IJHM

This is an open access article under the CC BY-SA 4.0 license (https://creativecommons.org/licenses/by-sa/4.0/)