THINK INDIA Journal

ISSN:0971-1260
Vol-22-Issue-16-August-2019

Development of a Reusable and Sustainable Biocatalyst By Immobilization of Catalase Enzyme Ontobiowastehen Egg-Shellpowder

Zeba Tabassum[1], Anand Mohan[1], Madhuri Girdhar[1*],
[1]School of Bio Engineering and Bio Sciences, Lovely Professional University, Phagwara (Punjab)
*Corressponding Author: Dr. Madhuri Girdhar, madhurigirdhar007@gmail.com

Abstract

This study aims to achieve immobilization of catalase enzyme onto the egg shell powder through adsorption method. Catalytic activity of catalase immobilized on egg shell matrix has been investigated by means of UV- spectrophotometer. Optimum pH and temperature of both soluble and bound catalase were observed. Under selected experimental conditions stability, reusability, temperature and pH sensitivity of native and immobilized catalase has been compared. Visual and FTIR results indicated that catalase successfully immobilized on egg shell powder. Reusability studies showed that immobilized catalase can be reused 15 times with slight loss of initial activity. Furthermore, immobilized catalase system exhibits improved thermal and pH stability. Result obtained from experiments showed that this immobilized catalase can have applications in various industries i.e. dairy and food, textile, bioremediation, pharmaceutical industries etc. for its potential to increase economical benefits and product high yield. Hen egg shell can be a good and cost effective option for the adsorption of catalase and othercatalysts.

Key words: Immobilization, Catalase, Matrix, Egg shell, Adsorption

1. INTRODUCTION

Catalase is an oxidoreductase enzyme. It is one of the main antioxidant of the defense system in human body [1]. The sole function of catalase is to break down hydrogen peroxide, still it has diverse use as catalyst in industries like dairy, food, textile, pharmaceutical, cosmetic production and also in making of biosensors. Catalase is abundantly present in nearly all living organisms, microorganisms, fruits and vegetables. In mammals, it is present in the peroxisomes for oxidation of many biomolecules [2]. Sumner and Dounce extracted catalase from bovine liver and prepared catalase crystal [3].

Applications of catalase enzyme in industries are diverse. From approval of FDA in food to remove bactericide hydrogen peroxide, to the production of variety of foods, for example sourdough, mayonnaise etc. [4]. Catalase is used in cold pasteurization to develop flavor in some dairy products like swiss cheese. It is also used to check milk quality [5]. In beverage industry,catalase used in beer, wine and vinegar bottling [6]. Use of catalase in packaging industry is evident like food wrappers and medicinal packaging for long shelf life. Catalase is a natural antioxidant, used in antioxidant drugs. Catalase can be used to protect fruit purees from browning [7]. It is successfully used in bioremediation of industrial effluents[8].

Manufacturing enzymes with high specificity, low impurities, stable structure and function is a very tough

ISSN:0971-1260
Vol-22-Issue-16-August-2019

task and costly procedure [9]. Although applications of catalase enzyme are being developed in full swing, native enzymes have limitations in maintaining activities during catalytic processes suchas limitations related to pH and temperature sensitivity, product purification load, lacking reusability, decreased operational and storage stability [10].

Currently, various researches are going on which can improve and stabilize catalyst. One such method is immobilization of enzyme. First immobilization of enzyme appeared in the 1960s. Since then, it is popular among researchers, widely used method in industry till 1976. Immobilization is a technique, which is designed to restrict the free movement of an enzyme. Immobilization means fixation. Fixing of enzyme on the surface of any support and prevent to mix it with product or other things. Research shows a huge similarities between immobilized and free enzyme and also that the immobilized enzyme is more stable and can be reused several times [11]. On the other hand, immobilized enzymes generally have improved thermal and pH stability, recovery and reusability, high purity, and product yields in their industrial applications [12]. It was found that free enzyme has ten times lesser stability and lower yield than immobilized catalyst[13].

Catalase enzyme already been immobilized on various matrices and was investigated by researchers or being used in industries. For example chitosan which are attached with catalase by covalent binding method [14, 12], microcapsule throughencapsulation[15,16],aluminabycovalentbindingandadsorption[17,18,19],silicathroughencapsulation [20, 21, 22], walnut through adsorption [23, 24], bentonite clay through adsorption [25,26].

Stabilization of enzymes is one crucial problem in bio-catalysis, because dissociation of subunits can inactivate the enzymes. Immobilization of enzyme can be a good solution in this case. Main aim of this study is to develop a reusable and sustainable biocatalyst by immobilizing catalase on to agro waste i.e. hen egg shell. So, characteristics of catalase enzyme can be improved and it can reduce cost of production in its industrial applications.

2. MATERIALS ANDMETHODS

2.1 Materials

Bovine liver catalase enzyme purchased from HiMedia. egg shell procured from waste of local poultry firm, Hydrogen peroxide, Potassium phosphate dibasic, Potassium phosphate monobasic, Sodium Citrate dihydrate, Citric Acid, Sodium Acetate (anhydrous), Acetic acid, Sodium bicarbonate, Sodium carbonate, Copper sulphate pentahydrate, Potasssium sodium tartrate tetrahydrate, Folin&Ciocalteu's phenol, Acetone, BSA, Sodium Dodecyl Sulphonate, Potassium hydroxide, Sodium hydroxide, and hydrochloric acid were procured from standard chemical company.

2.2 Preparation of hen egg-shell matrix for catalaseimmobilization

Egg shell is a waste product, available in abundance. For the purpose of experiment, fresh eggs were purchased from local grocery store. A few egg shells were broken into small pieces and boiled in water bath in 0.1% SDS for 15 minutes. The washing of shells were done several times to remove SDS by using

ISSN:0971-1260
Vol-22-Issue-16-August-2019

distilled water. Eggshells were again with wash with acetone, so that water can be removed. By drying egg shells in hot air oven for 4 hour at 60^{o}C, acetone was removed. After that crushing of egg shells were done with mortar and pestle and filter to generate uniform size. Similar type of procedures have been used by researchers [18, 27].

2.3 Immobilization of catalase throughadsorption

Egg shell powder of 0.25g mixed with 4ml of potassium phosphate buffer pH 7. The solution was stirred for an hour and kept at 4ºC for an 1 hour. Then the solution is centrifuged at 2000 rpm for 2 minutes. The supernatant formed was removed and egg shell pallet was taken in 15ml centrifuge tube. Further 5ml catalase solution and 5 ml potassium phosphate buffer pH 7 was mixed with egg shell pallet. The tube was kept on gel rocker for an hour for continuous stirring. Followed by 2 hour of incubation in refrigerator at 4ºC. Again the solution is centrifuged at 2000 rpm for 2 minutes. The supernatant formed was removed and egg shell pellet washed several times with fresh buffer to remove unbound catalase.

2.4 Protein estimation

Amount of protein in enzyme solution was determined by lowry's method. A calibration curve was constructed by using bovine serum albumin (BSA) was as a standard.

2.5 Free and immobilizedcatalase enzyme activity

Activity assay of free and immobilized catalase were analyzed at room temperature spectrophotometrically by measuring the absorbance decrease at 240 nm. Continuous absorbance decrease were noted with the gap of 15 seconds. Absorbance decrease occurs due to decomposition of hydrogen peroxide by the catalase. In this case greater decrease in absorbance indicate higher rate of activity. Activity assay were carried out over the pH ranging from 3 to 10 and temperature range from 25 to 75ºC to determine influence of pH and temperature. This procedure of activity assay was also performed by S.Alkan*et al.*,G.Arabaci and A.Usluoglu[12,23].

2.6 Influence oftemperature

Enzymes are temperature sensitive, denatures in high temp. To determine influence of different temperature on free and egg-shell matrix immobilized catalase enzyme, activity of enzyme was found in potassium phosphate buffer pH 7.0 and temperature of 25 - 85°C.

2.7 Influence ofpH

Enzymes are pH sensitive, work most efficiently at their optimum pH. In other pH their activity varies. In highly acidic or highly alkaline pH, they can stop working. The influence of pH was determined on the free and bound catalase, several buffers (0.05M) were used, from pH 3 to 10 in which both type of enzymes are employed to degrade 80mM hydrogen peroxide. Different buffers used that were Acetate buffer (pH 4 & 5), Citrate buffer (pH 3), potassium phosphate buffer (pH 8, 7, & 6), and Carbonate-bicarbonate buffer (pH 9 & 10).

2.8 Storagestability

ISSN:0971-1260
Vol-22-Issue-16-August-2019

Activity of free and immobilized catalase was investigated at 25ºC for 2 weeks and at 4ºC for 1 month in 50 mM potassium phosphate buffer (pH 7).

2.9 Reusability of immobilized catalase

Under standard assay conditions, by washing with fresh potassium phosphate buffer at pH of 7, the priorly immobilized enzyme which was immobilized was again.

3. RESULTS ANDDISCUSSION

3.1 Protein estimation ofcatalase

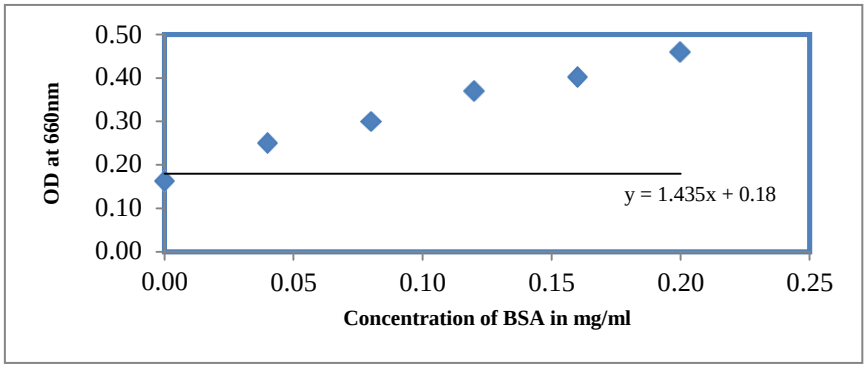

Figure 1. Standard curve for protein estimation

Slope equation for the standard curve is y = 1.435x + 0.18. Absorbance for 0.5ml catalase at 660 nm was 0.343. Then according to the standard curve, protein concentration in catalase is derived as 0.22mg/ml.

3.2 Suitable incubation time forimmobilization

How much incubation time is suitable for catalase immobilization on hen egg shell matrix? To find out the answer to this question, solution containing catalase and egg shell matrix equally divided in 4 parts and kept for incubation for 1 hour, 2 hour, 3 hour, & more than 3 hour (overnight) respectively. It was observed that the solution which was incubated for two hours showed best activity. Drop in the activity were observed in solutions that were kept for incubation period of 3 hours and overnight. Actual reason behind this, is not clear. Though Salleh S. *et al.* in 2006 conducted same kind of experiment and demonstrated in their paper that may be this is due to multi layered adsorption[27].

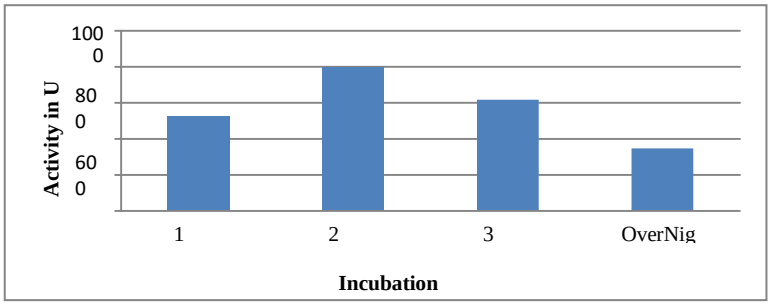

Figure 2. Activity of immobilized catalase with respect to incubation time

ISSN:0971-1260
Vol-22-Issue-16-August-2019

3.3 Effect of temperature on catalyticactivity

The results showed that the temperature for optimum activity was observed in 35°C. It analysis that immobilization method did not show any effect on the enzyme activity. Native and immobilized catalase on natural and modified carbon obtained from walnut had optimumpHat30°C[23].It was analysed that optimumtemperatureobserved at 25°Cforfree catalaseand40°Cforchitosan.

immobilized catalase [12].

Soluble catalase in this case shows itself as very temperature sensitive and denatured after 45°C, as shown in the graph below. Whereas, hen egg-shell immobilized catalase improved its thermal stability immensely, about 2 fold better than the free one. Immobilized catalase worked in higher temperature at 75°C also.

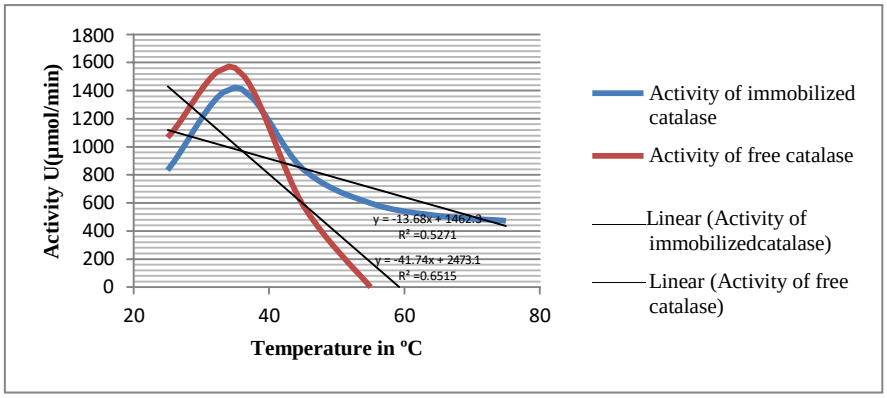

Figure 3. Activity of free and immobilized catalase at different temperatures.

3.4 Effect of pH on catalytic activity ofcatalase

The optimum pH values for both free and immobilized catalse were obtained as 7. At optimum pH, free catalase was showing higher activity compared to immobilized catalase. However egg shell immobilized catalase showed comparatively consistent activity in different pH ranges. Results also indicated the most appropriate results were observed in mobilized egg shell catalase and free catalase. Catalase also helped to increase activity and reduces chance of desorption. In pH 3 soluble catalase was inactive. Free catalase showed sensitivity in acidic pH.

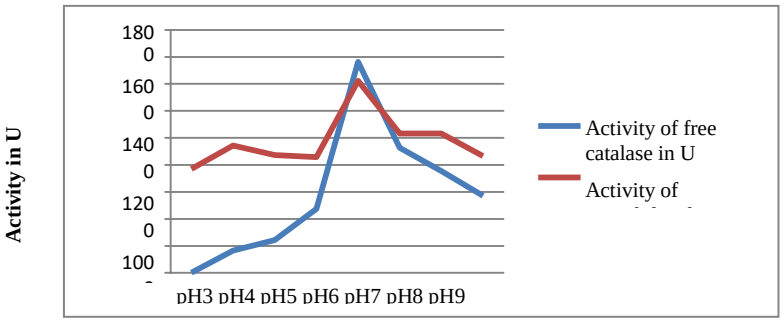

Figure 4. Activity of free and immobilized catalase with respect to different pH.

ISSN:0971-1260
Vol-22-Issue-16-August-2019

3.5 Operationalstability

To check operational stability, native and immobilized catalase both were freshly prepared, checked their activity and then kept for 2 weeks at room temperature. After 2 weeks, their activity checked again and compared with the initial one. Result obtained shows that free catalase looses 92% of its initial activity, whereas immobilized catalase still left with 63% of its initial activity.

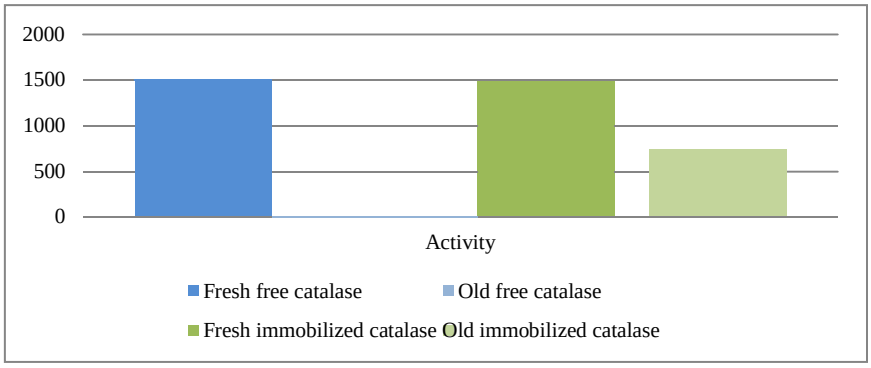

Figure 5. Stability check in room temp.

3.6 Storagestability

To check storage capacity, both type of catalase freshly made, activity checked, followed by kept for a month at 4ºC. After one month there activity checked again and compared with the initial one. Result obtained states that egg shell immobilized catalase retain its activity for longer time whereas free catalase looses activity very rapidly.

Figure 6. Storage stability (one month).

3.7 Reuse of the immobilized catalase

Freshly immobilized catalase on egg shell matrix employed to decompose hydrogen peroxide into oxygen and water. After every use egg shell matrix was washed with fresh buffer. The immobilized catalase on egg shell matrix was found to be active till 15 times of re-use.

ISSN:0971-1260
Vol-22-Issue-16-August-2019

4. CONCLUSIONS

There were various types of supports that are already been used to immobilize catalase, as per literature review, some of those are very useful, some have totally failed. Development of a cost effective and suitable support at the same time, using waste material instead of synthetic commercial matrix for immobilization, is an important aspect in industrial processes where enzymes are used to catalysis. In this studies, catalase enzyme successfully immobilized on hen egg-shell through adsorption to develop reusable and sustainable biocatalyst. Primary result of this paper confirms immobilization of catalase onto eggshell powder through adsorption. FTIR analysis confirmed the intermolecular bonding. Bound catalase was active till 15 time reuse whereas free catalase was not reusable. Reusability is a great option, for cheap production rate. After 8 to 10 use, it started to lose its initial activity very rapidly and after 15 times it completely stopped working. Literature review demonstrated that in most of the cases of catalase immobilization, adsorption method was used. In this experiment also, catalase immobilization through adsorption became successful, suitable to attach catalase on egg shell powder. Advantage is that even the support can be reused after being inactivation of old catalyst, new one can be reloaded. In optimum conditions, free catalase was having activity of 1562 U, whereas after immobilization of that catalase on egg-shell matrix it reduces its activity to 1422 U. That means, after immobilization catalase retained 91% of its native activity, not 100%. Immobilized catalase gained resistance against thermal denaturation, more stability to pH changes, able to work efficiently in a larger range. Though both catalyst shows optimum pH at 7 and optimum temperature at 35°C. Also improve characteristics like it became more stable during operational and storage. After immobilization, catalase can be more useful in degradation of hydrogen peroxide in various industrial applications. Finding new materials for catalase immobilization represents a goal for the researchers. Research should also be focused to overcome the limitations related to immobilization techniques, to improve all round application of immobilizedcatalase.

REFERENCES

[1] S. Tamara,“The searching of active catalase producers among the microscopic fungi,”AnaleleUniversitatii din Oradea, FasciculaBiologie, vol. 2, pp.164-167, 2011.

[2] B. Singh, B.S. Kauldhar,and M. Puri M, “Microbial Enzyme Technology in Food Applications,” New Delhi : CRCPress, 2016.

[3] H. Tauber, and E.L. Petit, “Convenient Method For Preparing Crystalline Catalase From Cow Liver,”J. Biol. Chem, vol. 195, pp.703-706, 1952.

[4] N. Lonkar, and Fraaije M.W, “Catalases as biocatalysts in technical applications: current,”Appl.Microbiol.Biotechnol, vol. 99, pp.3351-3357, 2015.

[5] M. A. Quresi, K. A. Khan, P. Ahmed, and S. Urpit,“Enzymes used in dairyindustries,” Int. J. Appl. Res, vol. 1, pp. 523-527, 2015.

ISSN:0971-1260
Vol-22-Issue-16-August-2019

[6] J. Rocker, M. Schmitt, L. Pasch, K. Ebart, and M. Grossmann,"The use of glucose oxidase and catalase for the enzymatic reduction of the potential ethanol content in wine,"Food Chem, vol. 210, pp. 660- 670, 2016.

[7] M. Castellari, L. Matricardi, G. Arfelli, G. Carpi and S. Galassi, "Effects of high hydrostatic pressure processing and of glucose oxidase-catalase addition on the color stability and sensorial score of grape juice,"Food Sci. Technol. Int, vol. 6, pp.17-23, 2000.

[8] J. Kaushal, S. Mehandia, G. Singh, A. Raina,and S.K. Arya, "Catalase enzyme: Application in bioremediation and food industry,"Biocat. Agri. Biotechnol, vol. 16, pp. 192-199, 2018.

[9] R. O. Cristovao, S. C. Silvério,A. P. Tavares, A. I. Brígida, andJ. M. Loureiro, "Green coconut fiber: a novel carrier for the immobilization of commercial laccase by covalent attachment for textile dyes decolourization,"World J. Microbiol.and Bio,vol. 28, pp. 2827-38, 2012.

[10] H. H. Patel, and A. R. Kharat , "Comparative study of free enzyme and immobilized enzyme on BSA digestion,"Int. J. Pharmaceutical Sci. and Res,pp. 5015-5023, 2012.

[11] E. Kessi, and J. L. Arias, "Using Natural Waste Material as a Matrix for the Immobilization of Enzymes: Chicken Eggshell Membrane Powder for β-Galactosidase Immobilization,"Appl.Biochem.Biotechnol, vol. 187, pp.101-115, 2018.

[12] A.Usluoglu, G. Arabaci, "Catalytic Properties and Immobilization Studies of Catalase from *Malvasylvestris L*," J. Chemistry-Hindawi, vol. 39, pp.6, 2013.

[13] A. G. Cunha, L. G. Fernández, J. V. Bevilaqua, J. Destain, L.M. Paiva, D. M. Freire, L. R. Fernández, and J. M. Guisán. Immobilization of Yarrowialipolytica lipase--a comparison of stability of physical adsorption and covalent attachment techniques,"Appl. Biochem. Biotechnol, vol. 146, pp.49-56, 2008.

[14] G. Singh, S. K. Arya, J. Kaushal, Seema,"Immobilization of catalase onto chitosan and chitosan–bentonite complex: A comparative study," Biotechnology Reports, 2018.

[15] J. Shi, X. Zhang, S. Zhang, X. Wang, Z. Jiang, "Incorporating MobileNanospheres in the Lumen of Hybrid Microcapsules for Enhanced Enzymatic Activity,"ACS Applied Material & Interfaces, vol. 63, pp. 10433-10436, 2013.

[16] X. Wang , Z. Jiang , J. Shi , Y. Liang, C. Zhang, andH. Wu, " Metal–organiccoordination-enabled layer-by-layer self-assembly to prepare hybridtmicrocapsules for efficient enzyme immobilization,"ACS Appl. Mater. Interfaces, vol. 16, pp.3476-3483, 2012.

[17] A. Paar, T. Tzanov, M. Gudelj M., G. M. Gübitz, P. A Cavaco, and S. A. Costa S.A, "Immobilization of catalases from Bacillus SF on alumina for the treatment of textile bleaching effluents,"Enzyme and Microbial Technology, vol. 28, pp.815-819, 2001.

[18] U. Chatterjee, A. Kumar, and G. S. Girdhar, "Goat Liver Catalase Immobilized on Various Solid

ISSN:0971-1260
Vol-22-Issue-16-August-2019

Supports. Journal of Fermentation and Bioengineering, vol. 70, pp.429-430, 1990.

[19] A. M. Eberhardt,V. I. Pedroni, M. A. Volpe, and M. L. Ferreira, "Immobilization of catalase from Aspergillusniger on inorganic and biopolymeric supports for H_2O_2 decomposition,"Applied Catalysis B Environmental, vol. 47, pp.153-163, 2004.

[20] J. M. Bolivar, V. Gascon, C. Marquez-Alvarez, R. M. Blanco, B. Nidetzky, "Oriented Coimmobilization of Oxidase and Catalase on Tailor-Made Ordered Mesoporous Silica," Langmuir: the ACS Journal of surfaces and colloids,vol. 33, pp. 5065-5076, 2017.

[21] T. Itoh, R. Ishii, S. Matsuura, S. Hamakawa, T. Hanaoka , T. Tsunoda, J. Mizuguchi, andF. Mizukami, "Catalase encapsulated in mesoporous silica and its performance,"Biochemical Engineering Journal, vol. 132, pp.167-173, 2009.

[22] Y. Wang and F. Caruso, "Mesoporous silica spheres as supports for enzyme immobilization and encapsulation," Chemistry of Materials, vol. 17, pp.953-961, 2005.

[23] S. Alkan, A. Gür, M. Ertan, A.Savran A, "Immobilization of catalase via adsorption into natural and modified active carbon obtained from walnut in various methods,"African Journal of Biotechnology,vol. 23, pp. 2631-2635, 2009.

[24] P. G. Pifferi, V. Bonora, G. Spagna, andM. Tramontini M, "Immobilization of Catalase on Macromolecular Supports Activated with acid dyes," Process Biochemistry, vol. 31, pp.29-38, 1993.

[25] O. Arsalan, H. Ceylan, and S. Alkan , "Bentonite-supported catalase," Journal of the Serbian Chemical Society, vol. 70, pp. 721-726, 2005.

[26] S. Cengiz, L. Cavas, K, " Yurdakoc, Bentonite and sepiolite as supporting media: Immobilization of *catalase,"*Applied clay science, vol. 65-66, pp.114-120, 2012.

[27] S. Salleh , N A Serri, S, Hena, andH. A. Tajarudin, "Preliminary studies on immobilization of lipase using chicken eggshell," IOPPublishing, 2016.