International Journal of Research and Review
www.gkpublication.in E-ISSN: 2349-9788; P-ISSN: 2454-2237

Review Article

Enzyme Immobilization: Research and Studies

Sunil J. Kulkarni

Datta Meghe College of Engineering, Airoli, Navi Mumbai, Maharashtra, India.

Received: 09/06/2016 *Revised: 16/07/2016* *Accepted: 16/07/2016*

ABSTRACT

The synthesis of many chemicals needs high temperature and pressure. Many chemicals like ethanol, amino acids, citric acids, nitrates, nitrites, fine chemicals, essential compounds can be synthesized by biological pathways. Enzyme catalyzed reactions provide huge boost to the efforts concentrated on cost effective and environment friendly technology. Immobilization of enzyme has advantages such as high thermal and operational stability, improved pH stability. Various investigators have carried out investigations to study effect of immobilization on enzyme activities. The current review summarizes research and studies on enzyme immobilization.

Key words: Enzyme catalyzed reactions, entrapment, covalent bonding, stability, activity, and reuse.

INTRODUCTION

The synthesis of various compounds from raw materials needs proper selection of process and process conditions. The chemical reaction in many cases needs high temperature and pressure. Also lower selectivity and yield are cause of concern in some cases. Also dealing with side products also becomes nasty problem in some cases. It is always desired to carry out a reaction at moderate temperature, low pressure, high yield and selectivity, high conversion. The activation energy for a reaction can be reduced and the reaction can be made to happen at lower temperature by using catalysis. The enzymes are catalysts used in biochemical reactions. The enzyme catalyzed reactions are specific in nature and yield highly specific reactions. The enzymes are labeled by putting ‘ase’ with the reaction. Like amylase (amyl ‘ase’), phosphatase etc. The production of various chemicals and compounds by enzyme catalyzed reaction provides alternative route to some reactions. [1-5] Many compounds such as ethanol, starch, amino acids, citric acid and many other pharmaceutical and fine chemicals, intermediates are synthesized by using enzyme catalyzed reactions. [6-10] The stability of these enzymes at various temperatures, pH and thermal conditions is many times cause of concern. The immobilization of enzymes solves this problem almost completely. Various physical and chemical methods can be used for immobilization. Various investigators have carried out research on effect of immobilization on enzyme activity. The current review summarizes research and studies on enzyme immobilization.

RESEARCH AND STUDIES ON ENZYME IMMOBILIZATION

Tavano et.al carried out studies on the immobilization of sweet potato amylase. [11] They established a simplified procedure for the preparation of immobilized beta-amylase. They used non-purified extract from fresh sweet potato tubers. They observed that immobilization decreased enzyme activity to around 15%. According them, beta-amylase from sweet potato is a promising element for study. The advantage being its readily available and cheap source, its stability and immobilization possibility. Also they reiterated that it produced very stable derivatives, with both improved temperature and pH stability by immobilization.

Singh et.al carried out studies on activity and stability of immobilized alpha-amylase. [12] They used alpha-amylase produced by Bacillus acidocaldarius for their studies. They discussed different immobilization methods such as entrapment, physical adsorption, covalent and ionic bonding. They used different carriers for immobilization. According to these studies, thermal and pH stabilities of immobilized enzymes were higher compared to free enzyme. They also observed that immobilized amylase on glass beads (covalent binding) and cation exchange resin (ionic binding) had the highest immobilization yield. It was found that immobilized enzyme on glass beads had half life period of 83 minutes while half life period of immobilized enzyme on cation exchange resin was 61 minutes. The enzymes could be used 7-8 times with substantial retention of activity.

Cigil et.al used modified polyimide material for immobilization of alpha amylase. [13] They used pyromellitic dianhydride (PMDA) and 4, 4'-oxydianline (4, 4'-ODA) in the solution of N, N dimethylformamide (DMF) for covalent immobilization on modified polyamide. They found that immobilized enzyme exhibited better stability then free enzymes. They also observed that the immobilization shifts the optimum pH by 2.5 towards acidic region. Singh used different matrices for immobilization of alpha amylase. [14] In her investigation she used 7 different carriers namely agar-agarose, wood fiber, wood chips, gelatin, wood powder, calcium alginate and butanol sodium alginate. She used immobilization techniques such as simple adsorption, adsorption followed by cross-linking, combined adsorption and gel entrapment. Also she studied factors such as the enzyme activity, stability at various temperature and pH, immobilization efficiency and treatment of carriers. She observed that sodium alginate method of immobilization yielded best results followed by Agar-agarose. The optimum pH shifted to lower values for immobilized enzymes than free one. For immobilized enzymes optimum reaction temperature shifted to higher values by 15-20 ^oC.

Sharmin et.al carried out immobilization on glass surface. [15] They used immobilized enzymes in development of biosensors. They immobilized alkaline phosphatase by covalent technique. According to these studies best conditions for enzyme activity were a 10% concentration of coupling reagent, pH 9.0 and 2 gram of silica. They observed that the covalently bonded enzymes can be reused 4 times without loss in activity after wash.

Romo-Sanchez et.al investigated immobilization of commercial cellulase and xylanase. [16] According to them, it is desired to have enzyme which is highly stable and economically viable in terms of reusability. They used chemical methods such as adsorption, reticulation, and crosslinking-adsorption for immobilization. In their research, the reusability of enzymes was the most striking finding, according to them. They observed that immobilized cellulase using glutaraldehyde exhibited 64% activity retention after 19 cycles. Zhang et.al immobilized lipase using alginate hydrogel beads. [17] They carried out enzymatic evaluation in hydrolysis of p-nitrophenol butyrate. They studied and discussed enzyme behavior with respect to temperature, pH, embedding and lyophilized time. They observed that activity of the enzyme-alginate composite was maintained without marked deactivation up to 6 repeated cycles.

In their review, Abdelmajeed et.al discussed immobilization technology for enhancing bio-products industry. [18] According to this review, immobilized enzymes have found their place in the sub-disciplines such as biomedicine, pharmacology, cosmetology, food and agricultural sciences. In medical field immobilized enzymes find application in drug delivery systems, diagnosis and treatment of diseases, as well as in sensors for the management of weight and diabetes. For production of organic and inorganic compounds immobilization can be employed. Also textile and food industry can utilize immobilized enzyme technology for better production. Dey et.al carried out an investigation on immobilization of a-amylase. [19] They produced the enzyme by using bacillus circulans GRS 313. They immobilized enzyme by entrapment on calcium alginate beads. They observed that the enzyme activity was affected by bead size. The optimum size was 2 mm. They observed that, even after seventh use, the immobilized enzyme showed 85 percent of its initial activity.

Nawani et.al studied the effect of process parameters on immobilization of enzyme. [20] They carried out studies on immobilization and stability of a lipase from thermophilic bacillus sp. In their research they observed that enzyme showed enhancement in activity in presence of benzene or hexane (30% v/v each). They used different solid supports for immobilization. They found that the immobilized/cross linked enzyme was more thermostable. Alftren and Hobley, in their investigation used magnetic particles as support for immobilization. [21] They carried out studies on laboratory and pilot scale with cellulases for hydrolysis of lignocelluloses. They found that highest immobilized enzyme activities were obtained using magnetic particles activated with cyanuric chloride. The method employed by them enabled simultaneous immobilization and purification. Papain Immobilization was studied by Ding et.al [22] They carried out immobilization on macroporous polymers carrier. Hydrazine hydrate was used for aminating macroporous polymer carrier. Glutaraldehyde cross-linking or diazo-coupling was used for immobilization of papain. They observed that activity recovery of immobilized papain can reach 60.1%. They found that immobilization brought about remarkable stability, better reusability and environmental adaptability than free papain.

Zucca and Sanjust investigated inorganic materials as support for immobilization. [23] They chose the materials which meet the criteria like complete insolubility in water, reasonable mechanical strength and chemical resistance. They found that materials like silica, silicates, borosilicates and aluminosilicates, alumina, titania, and other oxides were suitable for the purpose. According to them, operators should carefully evaluate every feature of both supports and functionalization/ activation methods.

Viet et.al carried out studies on immobilization of cellulase enzyme in calcium alginate gel. [24] They used entrapment method for immobilization. Maximum efficiency of immobilization for 3 mm bead diameter and 30 minutes time was 83.645%. The optimum pH for both free and immobilized enzyme was 4.5. These studies indicated that immobilized enzyme can stand higher acidity and temperature than free enzyme. Also they observed that even after 5 recycles, 69.2 percent activity was exhibited by enzyme.

CONCLUSION

Many compounds such as ethanol, starch, amino acids, citric acid and many other pharmaceutical and fine chemicals and intermediates are synthesized by using enzyme catalyzed reactions. The stability of these enzymes at various temperatures, pH and thermal conditions is many times cause of concern. The immobilization of enzymes solves this problem almost completely.

Various physical and chemical methods can be used for immobilization. In most of the cases immobilization decreases optimum pH for reactions by 2-3 pH units. Also it was observed in many investigations that even after 5-8 times reuse; enzymes exhibit high (68-85 percent) activity. It can be concluded that immobilization bring about remarkable stability, better reusability and environmental adaptability than free enzyme.

REFERENCES

1. Ajay Kumar Singh, Sanat Rath, Yashab Kumar, Harison Masih, Jyotsna K. Peter, Jane C. Benjamin, Pradeep Kumar Singh, Dipuraj, Pankaj Singh, "Bio-Ethanol Production From Banana Peel By Simultaneous Saccharification And Fermentation Process Using Cocultures Aspergillums Niger And Saccharomyces Cerevisiae", Int. J. Curr. Microbiol. App. Sci, 2014, 3(5), 84-96.
2. Patanjali Varanasi, Priyanka Singh, Manfred Auer, Paul D Adams, Blake A Simmons, Seema Singh, "Survey Of Renewable Chemicals Produced From Lignocellulosic Biomass During Ionic Liquid Pretreatment", Biotechnology For Biofuels, 2013, 6(14), 2013,1-9.
3. Sunil J. Kulkarni, "Use of Biotechnology for Synthesis Of Various Products From Different Feedstocks -A Review", International Journal Of Advanced Research In Bio-Technology, 2014, 2(2), 01-03.
4. Tracy M. Carole, Joan Pellegrino,And Mark D. Paster, "Opportunities In The Industrial Biobased Products Industry", Applied Biochemistry And Biotechnology, 2004, 113–116, 871-888.
5. Sunil Jayant Kulkarni, "Research on Biocatalysts: A Review", International Journal of Research, 2014, 2(5), 784-788.
6. Veena Ramachandran, Nisha Pujari, Tanmay Matey, Sunil Kulkarni, "Enzymatic Hydrolysis for Glucose-A Review", International Journal of Science, Engineering and Technology Research, 2013, 2(10), 1937-1942.
7. Veena Ramachandran, Nisha Pujari, Tanmay Matey, Sunil Kulkarni, "Enzymatic Hydrolysis of Cassava using wheat seedlings", International Journal of Science, Engineering and Technology Research, 2014, 3(5), 1216-1219.
8. Sunil J. Kulkarni, Nilesh L. Shinde, Ajaygiri K. Goswami, "A Review on Ethanol Production from Agricultural Waste Raw Material",Internation Journal on Scientific Reseach in Sciencce, Engineering and Technology, 2015, 1(4), 231-233.
9. Sunil Jayant Kulkarni, "Production of Citric Acid: A Review on Research and Studies", International Journal of Advanced Research Foundation, 2015, 2(11),17-20.
10. Sunil Jayant Kulkarni, "Research and Studies on Vinegar Production-A Review", Int.Journal on Scientific Research In Science And Tech., 2015,1(5),146-148.
11. Enzymeolga Luis Tavanoa, Roberto Fernandez-Lafuente, Antonio José Goulart, Rubens Monti, "Optimization Of The Immobilization Of Sweet Potato Amylase Using glutaraldehyde-Agarose Support; Characterization Of The Immobilized Enzymeolga", Process Biochemistry,2013, 48,1054–1058.
12. Pushpendra Singh, Paras Gupta, Ravindra Singh And Rajesh Sharma, "Activity And Stability Of Immobilized Alpha-Amylase Produced By Bacillus Acidocaldarius", Int. J. Of Pharm. & Life Sci., 2012, 3(12), 2247-2253.
13. Aslı Beyler-Çigil, Emrah Çakmakç, Özkan Danış, Serap Demir, Memet Vezir Kahraman, "Alpha-Amylase Immobilization On Modified Polyimide Material", Chemical Engineering Transactions, 2013, 32, 1687-1692.
14. Sunita Singh, "A Comparitive Study on Immobilization of Alpha Amylase Enzyme on Different Matrices", International Journal of Plant, Animal and Environmental Sciences, 2014, 4(3), 192-197.
15. F. Sharmin, S. K. Rakshit, H.P.W. Jayasuriya, F. Sharmin, S. K. Rakshit, H.P.W. Jayasuriya, "Enzyme Immobilization on Glass Surface for the Development of Phosphate Detection Biosensor", Agricultural Engineering International: The CIGR Ejournal, 2007, 9, 1-15.
16. Sheila Romo-Sanchez, Conrado Camacho, Héctor L. Ramirez, María Arévalo-Villena, "Immobilization Of Commercial Cellulase And Xylanase

By Different Methods Using Two Polymeric Supports", Advances In Bioscience And Biotechnology, 2014, 5, 517-526.
17. Shuang Zhang, Wenting Shang, Xiaoxi Yang, Shujuan Zhang, Xiaogang Zhang, Jiawei Chen, "Immobilization of Lipase using Alginate Hydrogel Beads and Enzymatic Evaluation in Hydrolysis of p-Nitrophenol Butyrate", Bull. Korean Chem. Soc., 2013, 34(9), 2741-2746.
18. Nadia A. Abdelmajeed, Olfat A. Khelil And Enas Nabil Danial, "Immobilization Technology For Enhancing Bio-Products Industry", African Journal Of Biotechnology, 2012, 11(71), 13528-13539.
19. Gargi Dey, Bhupinder Singh and Rintu Banerjee, "Immobilization of A-Amylase Produced By Bacillus Circulans GRS 313", Brazilian Archives of Biology and Technology, 2003, 46(2), 167-176.
20. Neerupma Nawani, Rajvinder Singh, Jagdeep Kaur, "Immobilization and Stability Studies of A Lipase from Thermophilic Bacillus Sp: The Effect of Process Parameters on Immobilization of Enzyme", Electronic Journal of Biotechnology, 2006, 9(5), 559-565.
21. Alftrén, Johan; Hobley, Timothy John, "Immobilization of Cellulases on Magnetic Particles to Enable Enzyme Recycling during Hydrolysis of Lignocelluloses", National Food Institute, Technical University of Denmark. 2014,1,1-132.
22. Liang Ding, Zihua Yao, Tong Li, Qiang Yue, Jia Chai, "Study On Papain Immobilization On A Macroporous Polymer Carrier", Turk J Chem., 2003, 27, 627- 637.
23. Paolo Zucca, Enrico Sanjust, "Inorganic Materials as Supports for Covalent Enzyme Immobilization: Methods and Mechanisms", Molecules, 2014, 19, 14139-14194.
24. Tran Quoc Viet, Nguyen Phuoc Minh, Dong Thi Anh Dao, "Immobilization of Cellulase Enzyme in Calcium Alginate Gel and Its Immobilized Stability", American Journal of Research Communication, 2013, 1(12), 254-267.

How to cite this article: Kulkarni SJ. Enzyme immobilization: research and studies. Int J Res Rev. 2016; 3(7):31-35.

****