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Application of Chitosan Flakes as a Support Material for Immobilizing Lipase to Improvise Its Efficiency in Biodiesel Production

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Abstract

Enzymatic mode of biodiesel production has an edge over chemical transesterification method mainly because it is an ecofriendly process. Lipase from microbial sources are used for biodiesel production, is not cost effective. To overcome this disadvantage efforts are taken to increase the reuse of enzyme by immobilizing the enzyme on suitable support material. This study deals with preparation and use of chitosan as a support material for lipase immobilisation.
Keywords: Biodiesel production, Lipase, Immobilisation, chitosan.

Introduction

The global market for biodiesel is poised for explosive growth in the next ten years. Presently biodiesel is produced through chemical transesterification. If 20 percent of the diesel consumption is to be replaced with biodiesel, then 13.38 MMT of biodiesel is to be produced. During the production of such huge quantities of biodiesel the bye-products likely to be formed during the process is 1.07 MMT of glycerol, 0.57 MMT of Soaps and alkali, 1.4 MMT of Methanol, 26.2 MMT of wash water.

Such limitations on the chemical transesterification can be overcome by switching over to bio-transformations using enzyme as a catalyst. Since it is an ecofriendly alternative to the conventional process, it is quite welcome (Iso et al., 2001; Shimada et al., 2002; Pereira et al., 2003; Noureddini and Larsen. 2004 and Al-Zuhair, 2007).

Lipase as Catalyst for Biodiesel Production

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3 (Holmiquist., 1998) being hydrophobic, act upon carboxylic acid esters such as glyceride lipids, at the interface between an aqueous and oil phase (Benjamin and Pandey, 2001; Blanco et al., 2007 and Pereira et al., 2003).

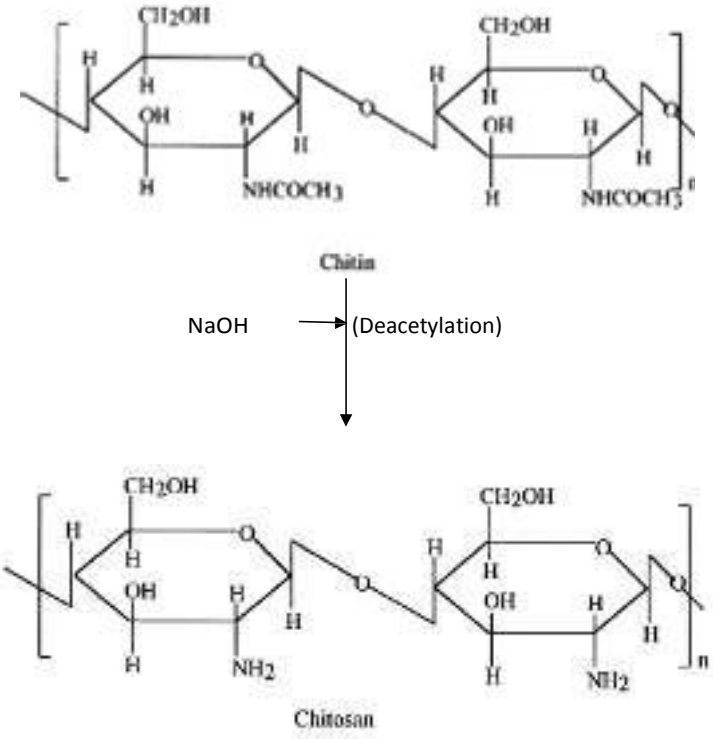
A major drawback of using free enzymes is that they cannot be reused as the enzyme is easily affected by the components of the reaction medium. Also, it is comparatively difficult to

remove the enzyme from the medium for reuse purpose. This problem could be addressed by immobilization

Chitosan as a Support Material for Lipase

To increase the effectiveness of enzyme usage for biodiesel production through immobilizing the enzyme on a suitable support material (in the lab scale), chitosan was tried in this study. Chitin is easily obtained from crab or shrimp shells which are converted to chitosan by deacetylation process. Chitin production is associated with food industries such as shrimp canning.

Fig.15 The structure of chitin and chitosan (Adopted from Ravikumar et al., 2000).



Most of the studies focusing on chitosan have been performed on chitosan flakes, since they are easier to manage than gelled materials. (Quignard et al., 2000). The main limiting parameter with flaked material will be the size of chitosan particles causing d iffusion restrictions. The size of sorbent and catalyst particles should not exceed 100 to 150 mm to avoid significant diminutions in kinetic rates. The size and low porosity of chitosan flakes is an important limiting parameter while using it as a support material for lipase immobilization. The contact time required to reach equilibrium usually lengthens with increasing size of chitosan flakes. The resistance to intra particle diffusion depends on the porosity and pore size of the chitosan flakes and this controls the overall kinetics for the adsorption of the enzyme (Guibal, 2005).

Preparation of Chitosan Flakes

Chitosan flakes were prepared according to the methodology of Shepherd et al. (1997), Kurita et al. (2004) and Yen et al. (2007).

Shells of tiger prawn collected from fish stall were washed thoroughly in running water to relieve it from unnecessary particles. The washed cells were then soaked in 3 percent sodium hydroxide and the mixture was heated for 90 minutes at 60°C to remove the proteins adhered in the shell if any. The alkali was then drained, and the shells were washed repeatedly in the running water. It was then heated with 5 percent hydrochloric acid for 1 h to remove the minerals. It was then washed with water and bleached with chlorine solution.

The bleached product was then soaked in 40 percent sodium hydroxide and heated at 60°C for 3-4 h. The excess alkali was removed by repeated washing and sun dried for 2-3 days to get the flakes.

Chitosan flakes obtained from shrimp shells after processing in the lab scale

Through chemical treatment of shrimp shells , a recovery of 3.6 percent of chitosan in the form of flakes was only possible in the present study. Five trials were carried out and the final weight of the finished product was assessed. From the initial weight of sample taken 35.6 percent product was recovered after deproteinisation process (alkali treatment), 18.4 percent product was obtained after demineralization process (HCl treatment) and 4.76 percent product was obtained after bleaching process which lead to the decolourization. After washing the excess acid with water 0.103 percent product was recovered in the form of flakes which was stored in the refrigerator and used for further studies on immobilization of lipase.

Brzeski (1982) reported a yield of 14 percent chitosan from krill and 18.6 percent from prawn waste (Alimuniar and Zainuddin, 1992). Depending upon the degree of deacetylation the weight of product formed differs (Madhavan, 1992 and Majeti, 2000).

Immobilisation of Lipase on Chitosan Flakes

Lipase immobilization is performed in order to increase the efficiency and economy (Telefoncu et al., 1990; Balcao et al., 1996; Ivanov et al., 1997; Pancreac'h et al., 1997; Chiou and Wu, 2004; Vaidya et al., 2008; Dizge et al., 2009 and Cabral et al., 2009). Many researchers contended that immobilized enzymes have great advantage over the native enzyme mainly on improved stability and easy enzyme removal from the reaction medium (Cetinus and Oztop, 2000).

Among the many methods reported for immobilization (Gandhi et al., 1996, Belleza et al., 2003 and Bryjak et al., 1997) the adsorption shows to be an easy and inexpensive method (Alloue et al., 2008). This immobilization technique in fact involves merely dispersal of enzyme over a large surface of the support (Yang and Wu, 2001). Hence adsorption of lipase on chitosan was preferred for catalyzing the transesterification reaction. A constant weight of lipase in solution was immobilized on chitosan flakes. After immobilization, the protein left in the solution was tested. This gave the measure of lipase immobilized on to the support. A constant weight of lipase in solution was immobilized on different forms of chitosan (same quantity of different chitosan forms were used for the study). After immobilization, the protein left in the solution was tested. This gave the measure of lipase immobilized on to the support.

Result

Chitosan in the form of flakes showed maximum adsorption (97 percent) of lipase which was proved by protein assay following Lowry's method. Thus chitosan flakes is a suitable material for lipase immobilization to be used for biodiesel production.

Scope for Further Study

Different forms of chitosan can be prepared and used as support material for immobilizing lipase and the best form can be identified to be used for biodiesel production.

References

- Al-Zuhair, S. 2007. Production of biodiesel: Possibilities and challenges, Biofuels, Bioprod. Bioref., 1: 57-66.
- Blanco, R.M., Terreros, P., Munoz, N. and E. Serra, 2007. Ethanol improves lipase immobilization on a hydrophobic support, Journal of molecular catalysis B: Enzymatic, 47: 13-20.
- Benjamin, S. and A. Pandey, 2001. Isolation and characterization of three distinct forms of lipases from Candida rugosa produced in solid state fermentation, Brazilian Archives of Biology and Technology, 44: 213-221.
- Belleza, F., Cipiciani, A. and U. Costantino, 2003. Esterase acticity of biocompoites constituted by lipases adsorbed on layered Zirconium phosphates and phophonates: Selective adsorption of different enzyme isoforms, Journal of molecular catalysis B:Enzymatic, 26(12): 47 – 56.
- Bryjak, J., Bachmann, K., Pawlo, B., Maliszewka, I., Trochimezuk, A. and M.N. Kolarz, 1997. Immobilization of lipase and penicillin acylase on hydrophobic acrylic carriers, Chemical Engineering Journal, 249 -256.
- Cabral,P., Fonecab, M.M.R., and S.F.Dias, 2009. Synthesis of ethyl butyrate in organic media catalyzed by Candida rugosa lipase immobilized in polyurethane foams: A Kinetic study, Biochem. Eng.J., 43:327-332.
- Cetinus, S.A. and H.N. Oztop, 2003. Immobilization of catalase into chemically crosslinked chitosan beads, Enzyme and Microbial Technology, 32 : 889-894
- Chiou, S.H. and W.T. Wu, 2004. Immobilization of Candida rugosa lipase on chitosan with activation of the hydroxyl groups, Biomaterials, 25: 197-204.
- Dizge.N., Aydiner, C., Imer, D, Y., Bayramoglu, M. Tanrievan and B.Keskinler, 2009. Biodiesel production from Sunflower, Soybean and waste cooking oils by

transeterification using lipase immobilized onto a novel microporous polymer. Bioresource Technology, 100:1983-1991.

- Gandhi, N.N., Vijayalakhmi, V.V., Sawant, B. and J.B. Johi., 1996. Immobilization of Mucor meihei lipase on ion exchange reins. Chemical Engineering Journal, 61:149-156.
- Guibal, E., 2005. Heterogeneous catalysis on chitosan-based materials: a review, Prog Polym., Sci. 30: 71–109.
- Iso, M., Chen, B., Eguchi, M., Kudo, T. and S. Shreshta, 2001. Production of biodiesel fuel from trilgycerides and alcohol using immobilized lipase. Journal of Molecular catalysis B: Enzymatic, 16: 53-58.
- Madhavan, P., 1992.(Ed.), Chitin, Chitosan and their Novel Applications, Science Lecture Series, CIFT, Kochi, p. 1.
- Majeti, N.V. and M.N.V. Ravi Kumar, 2000. A review of chitin and chitosan applications, Reactive & Functional Polymers, 46:1–27
- Noureddini, H. and G.Larsen, 2004. Final report: Engineering design of stable immobilized enzymes for the hydrolysis and transesterification of triglycerides. Chemical and Biomolecular Engineering Departmental papers, University of Nebraska-Lincon., http: // digitalcommons.unl.edu/.
- Pancreac'h, G. and C.J. Baratti, 1997. Activity of Pseudomonas cepacia lipase in organic media is greatly enhanced after immobilization on a polypropylene support, Appl. Microbiol. Biotechnol., 47: 630-635.
- Pereira, E.B., Zanin, G.M. and H.F. Castro, 2003. Immobilization and catalytic properties of lipase on chitosan for hydrolysis and esterification reactions, Brazilian Journal of Chemical Engineering, 20(4): 343-355.
- Shepherd, R., Reader, S. and A. Falshaw. 1997. Chitosan functional properties.
- Glycolconjugate Journal, 14: 535-542.
- Quignard F, Choplin A. and A.Domard, 2000. Chitosan: a natural polymeric support of catalysis for the synthesis of fine chemicals. Langmuir, 16:9106–9.
- Shimada, Y., Watanabe, Y., Sugihara, A. and Y. Tominaga, 2002. Enzymatic alcoholysis of lipases on macroporous polypropylene toward biodiesel synthesis, Journal of molecular catalysis B: Enzymatic, 17:133-142.
- Telefoncu, A., Dinckaya, E. and Vorlop, K.D., 1990. Preparation and characterization of pancreatic lipase immobilized in eudragit matrix, Applied Biochemistry and Biotechnology, 26:311-317.
- Vaidya, B.K., Ingavele, G.C., Ponrathnam, S., Kulkarni,B.D. and N.Nene, 2008. Immobilization of Candida rugosa lipase on Poly (allyl glycidyl ether–co-ethylene glycol dimethacrylate) macroporous polymer particles, Bioresource Technology, 99:3623-3629.
- Yang, A. and Wu, R, 2001. Mechanical properties and interfacial interaction of a novel bioabsorbable chitin fiber reinforced poly (-caprolactone) composite. J. Mater. Sci. Lett., 20: 977–9
- Yen, P., Xu, Z. and Wu, J., Innocent, C. and P. Seta, 2006. Nanofibrous poly (acrylonitrile-co-maleic acid) membranes functionalized with gelatin and chitosan for lipase immobilization, Biomaterials, 27 : 4169-4176.