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Lipase Catalysed Transesterfication

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There has been great emphasis on the use of biodiesel due to worldwide scarcity of fossil fuels, increasing crude oil prices and environmental concerns to reduce pollution. Production of biodiesel from biologically derived oils involves transesterification of these triglycerides with short chain alcohols to yield alkyl esters of fatty acids. These can be used in engines which are having compression-ignition (diesel) and are termed as "biodiesel". Transesterification catalysis reaction can be chemical and enzymatic by nature [1]. The disadvantages of chemical synthesis processes (for instance expenditure of high energy, triglycerides transesterification with high free fatty acid content) and downstream processes (like glycerol recovery, withdrawn of water and inorganic salts from the product, and treatment of alkaline waste water) makes the chemical approach more complex and costly [2]. Thus, the need for development of clean, reliable, eco-friendly and benign processes are motivating researchers to use biological systems as possible "biocatalysts". Use of biocatalyst creates the operation environment friendly, however, has certain limitations like elevated cost of enzyme (lipase), less yield, high reaction time, requirement of water as well as organic solvents in the reaction mixture.

Lipase belongs to hydrolases class of enzyme with EC 3.1.1.3. They exhibit hydrolytic and synthetic activity [3]. Utilization of mono, di and tri glycerides and free fatty acids in transesterification reaction, inhibition of low product, efficient activity, less reaction time, reutility of immobilized enzyme, yield in non-aqueous media, temperature and alcohol resistance are most required features of the lipase. Therefore search for an ideal enzyme is required which can catalyze the transesterification reaction with combinations of all these desired characteristics. From soil samples of China, a psychrophilic lipase producing bacterium Pseudomonas fluorescens (strain B68) was screened by Luo et al. [3]. In their study, psychrophilic lipase enzyme (lipB68) immobilization on cellulose fabric was performed for examining the transesterification activity for the production of biodiesel. In their study, 92% yield was obtained at an optimum temperature of 20°C in 12 hours. A lower optimum temperature majorly decreases the energy needed for the process for many geographical places. A study from Zhang et al. [4] has investigated the activity of marine lipase from *Bacillus pumilus* B106 which showed high tolerance to salinity, pH and temperature. Recently, template-based modeling of psychrophilic lipases has been reported by Xu et al. [5].

To decrease the reaction time as much as possible, various strategies have been applied to enhance the yield of enzyme. With the various combinations of sources of lipase, the immobilization media and alcohol varied reaction conditions such as temperature, time duration and agitation speed have to be optimized. Rodrigues et al. [6] used different conditions of temperature and molar ratio of alcohol to show the differential activity of three commercial lipases on varied oil sources viz. soybean, sunflower and rice bran. To analyze the activity of immobilized lipase, Shah et al. [1] optimized the conditions for the transesterification of Jatropha oil with ethyl alcohol using Chromobacterium viscosum and the yield was up to 92%. Encinar et al. [7] reported that the presence of co-solvents facilitates the reaction of transesterification. It is concluded from their studies that high biodiesel yield can be achieved in small reaction times, even at room temperature. Winayanuwattikun et al. [8] reported the use of potential lipolytic microorganism for catalyzing biodiesel production using palm oil as feedstock.

The high cost associated with the enzyme is one of the major disadvantages of using enzyme-based processes. Immobilization of enzymes has usually been used to get derivatives of reusable enzyme. Immobilized enzymes are more stable. Hence cost can be reduced by recycling the biocatalyst. Immobilization has been reported to result in increased activity in case of the biocatalysts in non-aqueous media Shah et al. [1]. Thus, an immobilized form of the enzyme has been utilized by many transesterification processes employing lipases Du et al. [2] Oda et al. [9]. One of the important sources of lipase for biodiesel production is microorganisms like *Mucor miehei, Rhizopus oryzae*, *Candida antarctica,* and *Pseudomonas cepacia.* Several carriers like silica, porous kaolinite particles, celite, SM-10, cellulose fabric have been used for immobilization. In the study of Du et al. [2], silica gel promotes acyl migration in the transesterification reaction when used as the immobilization substrate. But the activity of the enzyme decreases on recycling immobilized enzyme Iso et al., [10]. Factors like desorption, inactivation of substrate or inhibition of product might be the reason for this decrease of enzyme activity Rodrigues et al., [6]. In recent study of Takaya et al. [11], the whole-cell biocatalyst based on a fungus *Aspergillus oryzae* having lipase was employed for biodiesel production. In order to alleviate the price of biodiesel production alternative sources have also been used. One of the excellent choice among them is waste cooking oil. Using KBr impregnated CaO as catalyst, production of biodiesel from waste cooking oil was reported by Mahesh et al. [12].

Farooq et al. [13] used waste chicken bones derived heterogeneous catalysts for biodiesel production by transesterification of waste cooking oil. The review of literature suggests that an efficient lipase enzyme with combination of all desired characteristics has not been reported so far. With the introduction of directed mutation in the active site of lipase, there will be a possibility of enhancement in enantio-selectivity of lipase.

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