Abstract

The principal objective of this work is immobilization of lipase and application of the immobilized lipase for hydrolysis and transesterification of sunflower oil. Lipase was isolated from the culture supernatant of *Pseudomonas aeruginosa* PG01. A high activity (4.1±0.05U/ml) in the supernatant was detected when sunflower oil (2g/l) was used as the sole substrate. A significant production of lipase in burnt oil was observed, which was about 75% of the activity obtained when sunflower oil was used as the substrate. The extracellular lipase was successfully fractionated from the culture supernatant by ammonium-sulfate fractionation method, which was further purified by DEAE-sepharose anion exchange chromatography. The specific activity of the purified lipase was increased to 2.59 fold. The molecular weight of the DEAE sepharose filtered purified *Pseudomonas* lipase was estimated by SDS-PAGE was found to be 55 kDa. The optimum temperature and pH of the isolated lipase was 30°C and 7, respectively. The $t_{½}$ of the lipase at 4°C and pH 7 was nearly 23 months. The stability of the powdered lipase diminished steadily with the increasing temperature from 20 °C to 80 °C with $t_{½}$ of 62 days and 87 min at 20°C and 80°C, respectively. We report silk mat, a known biodegradable and renewable biomaterial, as support for the chemical immobilization of lipase. The chemically immobilized lipase was successfully used for the hydrolysis of sunflower oil for the production of fatty acids. This lipase catalyzed hydrolysis was found to occur effectively when the substrate sunflower oil was used as emulsion, mediated by either a chemical surface active agent, Triton X100, or a biological surface active agent, such as rhamnolipid. Further, we demonstrated that the rhamnolipid was a better surface-active agent than the Triton X100 for the hydrolysis, as evident from the 25.9% higher lipase activity realized by using emulsion created by biosurfactant over Triton X100. Although, the efficiency of the immobilized lipase was nearly 12% less than the free lipase on the biosurfactant-mediated

hydrolysis of sunflower oil, the immobilized lipase could be reused even up to the third reaction cycle in this biosurfactant-mediated conversion. Hence this appears as potential industrial biocatalytic process for hydrolysis of vegetable oil. The production of methyl esters of fatty acids (biodiesel) from sunflower oil using immobilized lipase catalyzed transesterification reaction in a fabricated packed bed bio-reactor was investigated. Silk-cocoon was identified as novel and efficient support matrix for the lipase immobilization to carry out the reaction. The lipase was chemically immobilized on degummed and desericinated silk-cocoon by glutaraldehyde cross linking to a lipase loading density of 738.74U (or 0.22 g) /g silk-cocoon. The K_m of the immobilized lipase was 257.26 μM, which is nearly 47 % less than the K_m (451.26 μM) of the free lipase. Low K_m value of the immobilized lipase is attributed to the hydrophobic nature of the matrix that facilitated the substrates diffusion to the enzyme surface. A packed bed bio-reactor was successfully fabricated with the immobilized lipase for transesterification of sunflower oil for the production of biodiesel. The biodiesel yield of 81.62 % was obtained at 48 hours reaction time, 6:1 methanol: oil ratio (v/v) and 30°C. Whereas maximum biodiesel yield of 89% was obtained at 37°C under similar conditions. The conversion yield followed a third order polynomial (R^2= 0.95). The immobilized lipase showed high operational stability at 30°C. The substrate conversion was only marginally decreased till third cycle (each of 48 hours duration) of the reaction since less than even 5 % of the original activity was decreased in each of the second and third cycle. The findings demonstrated the potential of the silk-cocoon as lipase immobilization matrix for industrial production of biodiesel.