Abstract

In this work, lipase was immobilized on different nano-supports to produce the immobilized biocatalyst using different immobilization techniques (adsorption and covalent linking), which can be used for cost-effective biodiesel production. Different type of nanoparticles (IOMNPs, chitosan coated IOMNPs and mesoporous silica nanoparticles) were chemically synthesized, which can be used as a suitable nano-support. The particle sizes and PDI (Polydispersity Index) of nanoparticles were optimized by changing the chemical concentrations used in synthesis of nanoparticles and by changing ultrasonication rate. The sizes and PDI of all nanoparticles were in the range of 10-100 nm and 0.2, respectively. Then, all the nanoparticles were treated with APTES (3-Aminopropyl)triethoxysilane to obtain the surface amino-functionalized nanoparticles (APTES-IOMNPs, APTES-C-IOMNPs and APTES-MSNs) and followed by immobilization of lipase. Lipase (20U/mL) from the *Bacillus subtilis* was adsorbed and covalently linked on APTES functionalized nanoparticles and APTES functionalized chitosan coated nanoparticles, respectively. The physiochemical properties of acid oil sample were evaluated for maximum yield by assessing the color (yellow grease), pH (5.1), density (0.7588 g/m3) and acid value (16.48 mg KOH/g). Acid oil was pre-treated with heat before transesterification reaction and the effect of temperature and time during heat treatment on acid value were observed. Then, the all immobilized biocatalysts were tested in transesterification reaction under optimized of methanol-oil molar ratio 3:1, with enzyme dose of 5%, and 125 rpm at 37 °C for 72 hours. The maximum fatty acid methyl ester (FAME) conversion were observed by using Lipase-APTES-IOMNPs (using $FeSO_4$), Lipase-APTES-C-IOMNPs and Lipase-APTES-MSNs were 93.1%, 87.1% and 85.4%, respectively. Then these immobilized biocatalysts showed the recyclability to four cycles with up to 60% yield. The biodiesel samples produced using acid oil satisfied the ASTM standard for fuel properties. So, the immobilization of lipase on different supports not only enhance the biodiesel production on large scale, but also makes economical by allowing the reusability of enzyme.