

Biocatalyst Synthesis in a Microfluidics Flow System for Esterification of Phytosterol Ester

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Extended Abstract

The production of effective solid biocatalyst through immobilizing the enzymes on a carrier is one of the important applications in catalysis. Nanoparticles are widely used as the support for enzymes where the properties (morphology and pore size) of the solid surface and the particle's size play an important role in deciding the enzymes immobilization yield on the solid which directly affecting the catalytic performance of the produced biocatalyst. Thus, synthesizing nanoparticles with controlled size and shape (narrow particle size distribution) is an important target that large numbers of research works were aiming for. Besides that, the immobilization conditions including the time and the immobilization environment are crucial for obtaining an effective biocatalyst without causing irreversible damage to enzymes. Conventional mixing methods used in nanoparticle synthesis are one of the most effective factors in determining the quality of the nanoparticles themselves. The mass transfer area between the reactants in the conventional production methods is controlled by the mixer design and operation and that will control the reaction time which will affect the produced nanoparticle's quality. A high-precision mixing method is believed to enhance the mass transfer area and nanoparticles quality and provide an excellent platform for enzyme immobilization as well. Microfluidic technology provides a new platform in nanomaterials synthesis due to the precise handling of fluid within the microfluidic devices. Among that, the emulsion method (droplet generation) in microfluidic demonstrated various benefits including effective mixing, larger surface area, and reduction in contamination possibilities as the reactants are encapsulated in segmented droplets. The highly efficient mixing in microfluidic devices also creates a new path in immobilizing lipase on the solid carrier to produce biocatalyst. This present work aims to introduce and investigate the effect of high-precision mixing provided using microfluidics technology on silica nanoparticles synthesis and lipase immobilization for the production of nano-sized catalysts for the esterification reaction. In this work, a polydimethylsiloxane microreactor was first designed and fabricated using the direct writing method. Silica nanoparticles were synthesized in the microreactor adapting sol-gel method by varying nonionic Sorbitan Monooleate surfactant concentrations (1 – 5 vol/vol%), and residence time, and the results were compared with nanoparticles produced from a bench-scale system. The synthesized silica nanoparticles were characterized using Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray Analysis (EDX), and X-ray diffraction (XRD), and nitrogen physisorption analysis. The nanoparticles were then immobilized with *Rhizomucor miehei* lipase in both batch-scale and microreactor system methods. The buffer solution was collected periodically and brought for lipase activity assay analysis. The biocatalysts were characterized using Fourier-transform infrared spectroscopy (FTIR). The catalytic performance of the synthesized biocatalysts was investigated and validated through the esterification of phytosterol and quantitative analysis was conducted using gas chromatography. From the results, the silica nanoparticles produced from the microflow system were smaller in size with higher monodispersity and perfect spherical configuration compared to the irregular shapes of silica nanoparticles synthesized in bulk. The calcined silica nanoparticles produced in the bench-scale

and microflow system had a mean size of 1.9 μm and 480 nm, respectively when observed using SEM. The characterization results confirmed high-quality silica nanoparticles were synthesized with smaller size, higher monodispersity, and larger pore size and volume. The experimental results showed that the lipase had a higher loading capacity on silica nanoparticles synthesized in a microflow system with an immobilization yield of about 90%. The present work indicates that the mixing method is not the most significant parameter in lipase immobilization compared to the properties of the carriers (morphology and size) themselves. The FTIR spectrum indicated that lipase was successfully immobilized on the silica nanoparticles via physical adsorption where the immobilized lipase showed all the bands demonstrated by silica nanoparticles and free lipase. The immobilized lipase showed a high degree of esterification confirming the high reaction rate and product yield. The immobilized lipase demonstrated a maximum degree of esterification of 97.3% under reaction conditions of 2.5 mg/ml catalyst loading; molar ratio of β -sitosterol to sunflower seed oil: 1:2; 150 rpm; 50 $^{\circ}\text{C}$; 4 hours. This present work showed the feasibility of microreactors in synthesizing smaller and uniform nanoparticles to be applicable in different fields such as reactions and drug delivery.

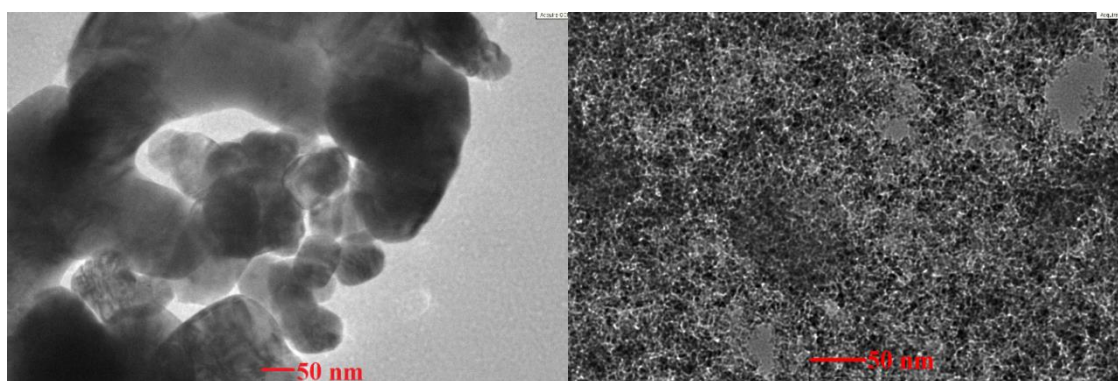


Figure 1: TEM images of silica nanoparticles produced from (a) batch and (b) microreactor system.

Keywords: Microfluidics; Silica Nanoparticles; Sol-gel; Lipase; Nanocatalyst; Esterification

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