



Production of Biofuels by Micro-, Lab-, Pilot- and Industrial Scale Based Biocatalytic Processes

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The need for the production of biofuels from various renewable sources is becoming increasingly interesting especially as the availability and accessibility of fossil fuels is significantly declining. Biodegradability, low pollution emissions and non-toxicity of raw materials are some properties making biogas, biodiesel and bioethanol more environmentally friendly fuels. Solid-state fermentation could be a suitable technology for the production of value-added products by utilization of the renewable waste materials, which makes it also economically feasible. So far, this technology was used for production of enzymes, organic acids, mushrooms, flavour and aroma compounds, pigments, polysaccharides, hormones, human food and animal feed. Different type of bioreactors have been developed and successfully used for solid-state fermentation of broad range of substrates and in production of value-added products. Solid-state fermentation will be demonstrated as part of anaerobic degradation on lab-, pilot- and industrial-scale of several waste materials such as brewer's spent grain, whey and cow manure, and corn silage and cow manure, respectively. The application of different microreactor systems in intensification of the biodiesel production process is widely studied. However, previous studies of the application of microreactor technology in the production of biodiesel were limited to the use of chemical catalysts. Mild reaction conditions, absence of by-products, reusability, simple separation and purification of the resulting biodiesel as well as lower energy consumption are some of the many advantages that make the enzyme lipase – a biocatalyst – a better choice than traditional chemical catalysts in the process of biodiesel production. Different microreactor systems utilising a commercially available lipase and a lipase produced by solid-state fermentation were used for transesterification of fresh and waste cooking oil while biodiesel was separated using integrated micro separation unit. Selected examples are clear demonstration of environmentally friendly and economic technologies used for efficient production of biofuels on micro-, lab-, pilot- and industrial scale. Compared to the conventional chemical process, lipase-mediated biodiesel production has well-recognized advantages, such as mild reaction conditions, easy product recovery, and environmental friendliness, and thus shows great prospect, especially when low-quality oils with high free fatty acid content are used as the feedstock. However, only a few plants employ the enzymatic process for biodiesel production, and the biggest challenge for the enzymatic process is the high cost of lipase, which is used as the catalyst. Research on developing new enzymes with a low price and high catalytic activity and stability has been one of the most important investigations to reduce enzyme cost. Compared to the conventional alkali process, the biggest advantage of the enzymatic process is that it can efficiently use cheap and low-quality feedstocks with high free fatty acid content, which will significantly reduce the total production cost. Thus, expanding low-quality raw materials for the enzymatic process has great potential. Another major challenge for the enzymatic process is to produce a final biodiesel product meeting standards [10–12]. Reactor design plays an important role in promoting esterification and transesterification reaction equilibria to realize the thorough conversion of oil feedstocks. This review introduces the progress achieved in the enzymatic biodiesel production process, mainly focusing on reducing enzyme cost, expanding low-quality raw. Lipase is widely used as a biocatalyst to catalyze hydrolysis and esterification and transesterification reactions. Although biodiesel preparation mediated by lipase has been extensively investigated, lipase cost is still relatively high compared to chemical catalysts, especially for immobilized lipase. Significant research has been conducted to reduce enzyme cost, such as exploring new lipase production from various microorganisms, lipase modification, and investigating improvements on lipase preparation. Lipase has been successfully modified with enhanced catalytic activity and tolerance to both methanol and high temperatures through the use of advanced biotechnologies. The performance of lipase, including catalytic activity and stability, is improved to a great extent by protein engineering and various enzyme immobilization approaches. For immobilized lipase, a smaller-sized carrier can permit better exposure to the activesites but also causes problems in separating the lipase from the reaction mixture. Using magnetic materials as lipase support helps to quickly separate the lipase by introducing external magnets with minimal mass loss. Furthermore, porous materials including silica, organic polymer, and carbon materials can be coated on the magnetic nanoparticles to improve chemical stabilities and also inhibit their aggregation. Xie et al. immobilized lipase on core-shell structured Fe₃O₄@MIL-100(Fe) (MIL, Materials of Institute Lavoisier) composites for enzymatic biodiesel production. Through amide linkages, the Fe₃O₄magnetite coated with porous MIL-100(Fe) metal-organic framework (MOF) material was adopted to immobilize lipase from *Candida rugosa*, and then the core-shell structured Fe₃O₄@MIL-100(Fe) composites were synthesized. An immobilization efficiency of 83.1% was achieved with an enzymatic activity recovery of 63.5%. Using an external magnetic field, the immobilized lipase could be facily separated from the system. A biodiesel conversion yield of 92.3% was obtained, and 83.6% of the initial activity was displayed after being reused for five batches. Although many technologies have been developed for lipase immobilization at the lab scale, only a few are industrialized due to the high cost of the immobilization process. The main challenge is the high cost of the carrier and the low efficiency of recovering proteins and retaining the enzymatic activity during the immobilization process. As the most widely reported immobilized lipase, Novozym 435 is sold at over \$1000/kg.

An enzymatic biodiesel production line with a capacity of 20,000 t/y was built in China in 2006, in which a combination of different immobilized lipases was used as the catalyst with used cooking oil as the main feedstock. Later, another lipase-mediated biodiesel production line was built with a capacity of 10,000 t/y, in which lipase from *Candida sp.* 99–125 was immobilized on textile membranes. The lipase cost was estimated to be only about \$32/t biodiesel (200 CNY/t biodiesel). In recent years, more immobilized lipase products specifically designed for biodiesel production have been developed, significantly reducing the price of lipase to about \$150/kg, which makes the use of enzymatic processes promising in practical production.

Bottom Note: This work is partly presented at [EuroSciCon Joint Event on Biotechnology, Stem Cell and Molecular Diagnostics](#) April 16-17, 2018 Amsterdam, Netherlands.