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How lipase technology contributes to evolution of biodiesel production using multiple feedstocks

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Given the increasing interest in alternative processes for producing biodiesel, we focused on the latest screening of lipases and bioprocess design using multiple feedstocks. The implementation of lipase technology to the biodiesel industry is in the early stages. However, current research has made phenomenal advances in generating lipase variants and in engineering biodiesel production. The structural insights into lipase stability, together with primary screening, have opened up opportunities for acquiring lipase variants that are highly tolerant under industrially relevant conditions. The versatility of lipases is promising for process intensification, where time-consuming and costly steps can possibly be avoided. To judiciously overcome uncertainties in the biodiesel industry, further research on technology development integrated with supply chain models is necessary.

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Introduction

Biodiesel, a mixture of fatty acid alkyl esters, has globally attracted considerable attention as an alternative fuel obtained from renewable sources. Various edible and nonedible oils can be used as possible feedstocks for biodiesel production. Although traditional vegetable oils such as rapeseed oil, soybean oil, and palm oil currently dominate the global biodiesel market, the food versus fuel debate has raised concerns about use of food crops for biofuels, promoting the development of other multiple feedstocks including energy crops, waste oils, and microbial oils for next-generation biofuels [1]. In particular, the high lipid titers and carbon-source independent nature of several microbes recently highlight the potential of these oleaginous organisms as a platform for efficient oleochemical production [2,3]. Because there still remains the issues of limited oil supply and cost reduction, researchers have searched for alternatives to conventional biodiesel production. Therefore, the improvement of biodiesel production using multiple feedstocks is one of the significant topics worldwide.

Despite the numerous scientific publications and patents, most of the biodiesel production technologies practically applied are based on the homogeneous alkali-catalysis method. This classical method requires rigorous feedstock specifications (e.g. low contents of water and free fatty acids) and complicated downstream processes including the removal of inorganic salts from the product, the recovery of salt-containing glycerol, and the treatment of alkaline wastewater [4]. Nonetheless, the overall economic efficiency of the conventional process has been the source of industrial legitimacy, whereas technologies using oils with high amounts of free fatty acids are improved by introducing an additional esterification step.

The use of lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) in biodiesel production has been reported as early as in 1990 by Mittelbach [1]. The general attractive features of enzymatic biodiesel production are as follows: Firstly, easy recovery of biodiesel and glycerol; secondly, versatile lipase activity for the simultaneous catalysis of triglycerides and fatty acids; and finally, the use of relatively low temperatures and atmospheric pressure, thus reducing energy consumption [1,4,5]. Owing to its potential, extensive studies of enzymatic biodiesel production have been conducted over the past two decades, some of which are dealing with the major challenges in adapting the lipase-catalyzed process to industrial conditions.

Of conceivable interest in this field is the possible ways for the latest technology advances to improve the biodiesel production that is expanding the volume of requirements in terms of technical, logistic, economical, and public considerations. In this review, therefore, we mainly focus on the latest screening of lipases and the versatile production of biodiesel from multiple feedstocks. In this paper, we also cover the industry trends and supply chain management, focusing on the aspects that are important in our opinion.

Current industry trends

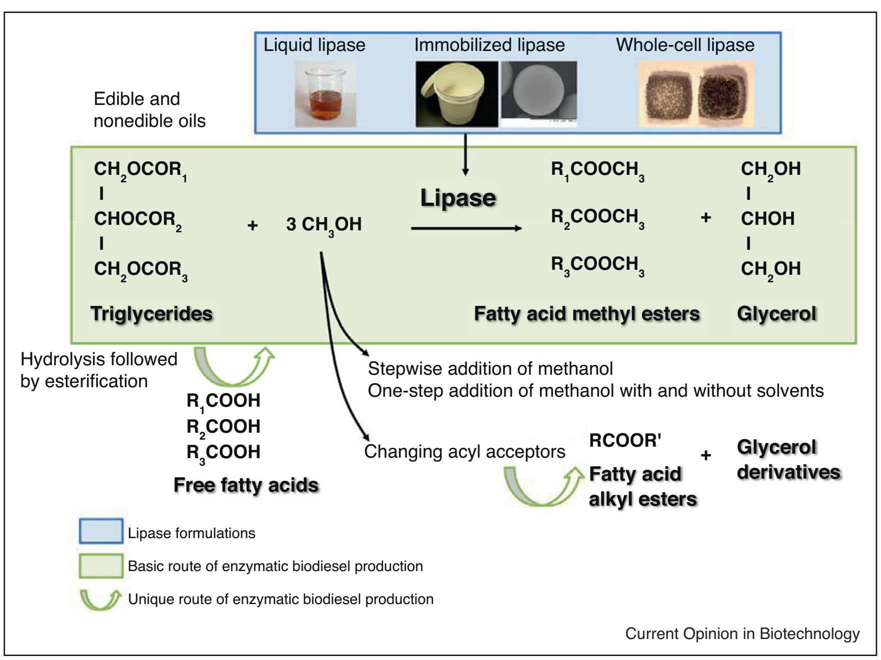
Since the pioneering studies by Shimada *et al.* [6] and Kaieda *et al.* [7] showed that the immobilized and liquid formulations of lipase can be used for plant oil methanolysis in a solvent-free system (Figure 1), numerous articles on the process configuration using these enzymes have been published [8,9•]. With the launching of new enzyme technologies, strategic partnerships between enzyme suppliers and biodiesel refiners were established. Several US biofuel companies including Piedmont Biofuels, Blue Sun Biodiesel, and Viesel Fuel have announced their works in collaboration with the global enzyme producer, Novozymes. For full-scale commercialization, some issues seem to remain; however, by leveraging the flexibility of a lipase for putative feedstocks with any free fatty acid content, efforts have been directed towards a pilot plant operation using a stirred tank reactor [10]. Although a considerable number of studies suggest the use of immobilized lipases [5], a liquid-lipase formulation has become mainstream for this group because the production cost of immobilized lipases greatly affects the economic viability of enzymatic biodiesel production. On the other hand, Tsinghua University in China has developed a novel process with an improved lifetime of immobilized lipases, leading to the first commercial facilities with a capacity of 20 000 tons/year [11]. In Japan, recycling waste cooking oil as biodiesel has locally been established. By using this kind of feedstock, Bio-energy, a spin off from Kobe University and Kansai Chemical Engineering, has developed continuous-mode operation using a packed-bed reactor with a capacity of 200 L/day, which is the first implementation of lipase technology to meet the Japanese biodiesel regulations and specifications (B5 blends) [12].

Lipase sources

During the research studies of enzymatic biodiesel production, researchers have faced substantial difficulties in overcoming the constraints associated with enzyme robustness. Although lipases are ubiquitous in most living organisms (microbial lipases), their availability and ease of production have made them potential biocatalysts for industrial applications. Nonetheless, the use of native lipases and conventional methods of purification from microbial cultures has a number of disadvantages such as low yield and low stability of lipases. To meet the specific requirements of enzymatic biodiesel production, molecular techniques for the production of recombinant heterologous lipases in a host have been developed. The approaches also include directed evolution and rational design to improve the thermostability and solvent tolerance of lipases, as reviewed previously [13•,14•].

Despite the large number of microbial lipases identified, there exists several factors hampering their implementation because some of the microbial lipases require chaperones and post-translational modifications specific to the host. In fact, a highly methanol-tolerant lipase from

Figure 1



Overview of lipase-catalyzed biodiesel production.

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