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Review article

Current status and new developments of biodiesel production using fungal lipases

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HIGHLIGHTS

- This review presents some data on biodiesel production using fungal lipases.
- Whole-cells and solid enzymatic preparation as promising biocatalysts.
- Waste oils, non-edible oils and oleaginous microorganisms as potential feedstocks.
- Some strategies for improving enzymatic biodiesel production.
- Reactor configurations and economic aspects are also presented.

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ABSTRACT

In recent years, the production of renewable fuels such as biodiesel has attracted considerable interest as an alternative to fossil fuels. The biodiesel production via enzymatic processes has considerable advantages over conventional alkali-catalyzed processes. Fungal lipases, which include lipases from filamentous fungi and yeasts, are among the most used biocatalysts in enzymatic synthesis of biodiesel. Different strategies have been studied in order to reduce the biocatalyst cost, and to obtain a more robust biocatalyst with high activity and stability. This paper presents the current status and perspectives for biodiesel production using fungal lipases, and discusses the critical aspects that influence lipase activity and stability such as: choice of raw material and alcohols, type of biocatalyst, use of solvents, and water content. Moreover, the development of whole-cells and solid enzymatic preparations (SEP) obtained by solid-state fermentation, as new promising technologies, the exploration of alternatives low-cost oils as potential feedstocks and the evaluation of possible reactor configurations are also presented. Process considerations and an economic efficacy analysis of industrial enzymatic biodiesel production are also presented.

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1. Introduction

Industrial process currently used for biodiesel production, that uses homogeneous alkaline transesterification of edible oils or animal fat, has some drawbacks such as: difficulty of catalyst recovery, production of large amount of highly alkaline wastewater and requirement of high-quality/high-price raw materials, with low contents of free fatty acids (FFAs) (acidity less than 0.5%) and water, in order to avoid soap formation. These refined oils are relatively expensive, and account for 70% to 80% of overall biodiesel production costs, making it difficult for biodiesel to economically compete with petro-diesel [1–6].

Two-step chemical catalysis, that combines acid catalysis in order to reduce the acidity and further alkaline transesterification, allows the use of low-cost raw materials with high FFA and water content [4,7]. However, despite its insensitivity to FFAs in the feedstock, the acid-catalyzed esterification/transesterification requires complete removal of water from the medium, has relatively slower reaction rate and poor reaction selectivity, is highly energy consuming, and its fluids are difficult to handle, causing problems of corrosion in equipments and waste acid production, with serious environmental impact [5,8,9].

The use of biocatalysts (lipases) for biodiesel production is a promising alternative to circumvent the problems associated with the conventional route. Lipases (glycerol ester hydrolases, E.C. 3.1.1.3) are enzymes that catalyze hydrolysis of esters, particularly long-chain triglycerides (TAGs), producing free fatty acids, di- and monoglycerides, and glycerol. However, in non-aqueous media these enzymes catalyze esterification, acidolysis, aminolysis, alcoholysis, and interesterification reactions with high selectivity, high specificity, and under mild conditions [10,11]. This versatility makes them one of the most employed groups of enzymes in biotechnological applications.

The use of lipases as biocatalysts for biodiesel synthesis requires relatively simple downstream processing steps for the purification of biodiesel and its by-product glycerol. Furthermore, lipases are able to catalyze both esterification and transesterification reactions, which allows the production of biodiesel from cheaper raw materials with high FFA and water content [2]. The comparison of enzymatic technology versus chemical (alkaline and acid) technology for biodiesel production is summarized in several works [6,12,13].

Despite the many advantages, the application of biocatalysts in biodiesel synthesis has some obstacles, such as low biocatalyst productivity (due to low reaction rate and low stability), and the high cost of such enzymes [12,13], which makes this route currently unfeasible for industrial biodiesel production. Many solutions are being studied to overcome these drawbacks, and the main ones are pointed in Fig. 1 and are going to be discussed in this review.

Lipases used in biodiesel production are mainly obtained from fungi (yeasts and filamentous fungi) and many are available in the market, sold by companies such as Novozymes, Amano, Gist Brocades, among others [14,15]. The present review covers the current status and new developments of biodiesel production using fungal lipases.

2. Fungal lipases used for biodiesel production

The most widely used lipase in biodiesel synthesis is the lipase B from the yeast *Candida antarctica* (CALB) [16]. Other yeast lipases include: *Candida rugosa* (CRL), *C. antarctica* A (CALA) and *Candida* sp. 99–125, which has been immobilized on textile and used for biodiesel production in China [16,5,15,12,17]. Lipases obtained from filamentous fungi, such as *Rhizomucor miehei* lipase (RML) [18], *Rhizopus oryzae* lipase (ROL) [19], *Thermomyces lanuginosus* lipase (TLL) [20], *Aspergillus niger* lipase (ANL) [21], and *Penicillium expasum* lipase (PEL) [22] have also been studied. Many of these enzymes are commercially available in immobilized form. Immobilized CALB, RML, and TLL are sold by Novozymes as "Novozym 435" (immobilized on a macroporous acrylic resin), "Lipozyme RM IM" (immobilized on an anionic resin), and "Lipozyme TL IM" (immobilized on a gel of granulated silica), respectively [6,7]. In general, there are four main forms of biocatalysts used in biodiesel synthesis: free and immobilized lipases, and more recently, whole-cells and fermented solids (solid enzymatic preparation-SEP).

2.1. Free lipases

Free lipases consist of liquid formulations that contain stabilizers to prevent enzyme denaturation (e.g. glycerol, sorbitol) and antimicrobials (e.g. benzoate) or powder enzymes, obtained by freeze-drying [7,23]. Free lipases present advantage of easy and low-cost preparation costs. However, although free enzymes are significantly cheaper than immobilized lipases, in many cases they have poor operational stability and can only be used once, as they are inactivated [14]. There are a few reports available on soluble lipase-catalyzed biodiesel production [24,25], including the methanolysis of low cost crude soybean oil using Callera Trans L