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Enzymatic biodiesel production: An overview of potential feedstocks and process development

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HIGHLIGHTS

- ▶ Enzymatic conversion of potential feedstocks into biodiesel.
- ▶ Process development expands the viability of enzymatic biodiesel production.
- ▶ A high potentiality of enzymes for a wide range of feedstock specifications.
- ▶ Various reactor systems and cost evaluation are also presented.

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ABSTRACT

The increased global demand for biofuels has prompted the search for alternatives to edible oils for biodiesel production. Given the abundance and cost, waste and nonedible oils have been investigated as potential feedstocks. A recent research interest is the conversion of such feedstocks into biodiesel via enzymatic processes, which have considerable advantages over conventional alkali-catalyzed processes. To expand the viability of enzymatic biodiesel production, considerable effort has been directed toward process development in terms of biodiesel productivity, application to wide ranges of contents of water and fatty acids, adding value to glycerol byproducts, and bioreactor design. A cost evaluation suggested that, with the current enzyme prices, the cost of catalysts alone is not competitive against that of alkalis. However, it can also be expected that further process optimization will lead to a reduced cost in enzyme preparation as well as in downstream processes.

1. Introduction

Biodiesel, which refers to fatty acid alkyl esters, has attracted considerable attention as an alternative fuel for diesel engines. Owing to its environmental advantages and the increase in petroleum price, a rapid increase in biodiesel production is observed. The global biodiesel production currently reaches about 6 billion liters/year and represents 10% of the entire biofuel production (Nogueira, 2011). Biodiesel is mainly produced by transesterification (i.e., methanolysis) of edible oils such as those from rapeseed, soybean, sunflower, and palm, thus leading to conflict with food supply. According to an estimation in 2007, about 7% of global edible oils were used for biodiesel production (Balat, 2011). More oil supply will be necessary to meet the growing demand for biodiesel production, where particular attention should be paid to various feedstocks as a potential alternative to edible oils.

The method of producing biodiesel is a technological area that challenges researchers to develop more efficient and environmentally benign processes. Although a homogeneous alkali-catalysis method has been conventionally and widely applied to biodiesel production, this method requires complicated downstream processes including the removal of inorganic salts from the product, the recovery of salt-containing glycerol, and the treatment of alkaline wastewater (Fukuda et al., 2001). To prevent the generation of soap, the conventional process also requires rigorous feedstock specifications such as low contents of water and free fatty acids. Owing to these drawbacks, growing attention has been paid to the development of an alternative method using heterogeneous catalysts (Helwani et al., 2009).

An enzymatic method using lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) can provide a solution to the aforementioned problems as it allows easy recovery of biodiesel and glycerol (Fukuda et al., 2001). Attention should also be paid to the versatile lipase activity, which facilitates the simultaneous catalysis of triglycerides and fatty acids by transesterification and esterification (Véras et al., 2011). Moreover, the enzymatic method can be operated at relatively low temperatures and atmospheric pressure, thus reducing energy consumption. During the research studies of enzymatic biodiesel production, researchers have faced a difficult

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problem regarding the low tolerance of lipase toward acyl acceptors (i.e., methanol). A pioneering work by Shimada et al. (1999) showed that over 96% conversion in a solvent-free reaction system can be achieved by employing a stepwise process using less than one molar equivalent of methanol to oils. They further showed that adding more than 1.5 M equivalents of methanol (insoluble in oil) leads to an irreversible lipase inactivation and that, in a three-stepwise process using one molar equivalent of methanol, the immobilized *Candida antarctica* lipase can be reused for more than 50 cycles of batch methanolysis in a laboratory vessel without a significant loss of lipase activity.

Following the above work, lipase-catalyzed methanolysis has been studied extensively using various lipase sources, leading to the elucidation of many useful reaction parameters (Antczak et al., 2009). A previous study showed the inhibition of lipase activity by the byproduct glycerol, which is more likely due to mass transfer limitation in the immobilized lipase (Watanabe et al., 2000). To overcome this obstacle, a cosolvent reaction system in which both oil and glycerol are dissolved has also been proposed (Du et al., 2008). The production cost of immobilized lipase greatly influences the economic viability of enzymatic biodiesel production, thus prompting researchers to find an alternative method of preparing the enzyme (Fukuda et al., 2008; Zhang et al., 2012). These research studies provide an important basis of realizing the potential of enzymatic biodiesel production.

The cost of raw materials currently constitutes a large percentage of the total production cost of biodiesel (Balat, 2011; Haas et al., 2006), which indicates that selecting the appropriate feedstock is of considerable importance for ensuring the economic feasibility of the process. Using unrefined oils including waste and nonedible oils is a better alternative. In this review, the first part covers the potential feedstocks for biodiesel production. Of conceivable interest based on the aforementioned backgrounds is the possibility of applying an enzymatic method to the production of biodiesel from various feedstocks. Therefore, this review mainly presents the current status of research on the enzymatic biodiesel production from various feedstocks. To integrate these attempts with the scaling up of the process, research on bioreactor design and cost evaluation is also presented.

2. Potential feedstocks

Selecting the appropriate biodiesel feedstock depends on the national conditions in a production area. Apart from edible oils, the potential feedstocks for biodiesel production are classified into three categories: waste oils, nonedible plant oils, and oleaginous microorganisms (Table 1).

2.1. Waste oils

Given the abundance and cost of raw materials, one candidate is waste oils including waste cooking oil, grease, and soapstock. Other byproducts can also be new feedstocks: tall oil (a byproduct of the pulp and paper industry) and tobacco seed oil (byproduct of tobacco leaf production). Waste cooking oils are generated from vegetable oils fried at high temperatures. This process causes chemical reactions such as hydrolysis, polymerization, and oxidation (Lam et al., 2010). The component of waste oils from different origins varies from 0.4 to 55% in moisture content and from 1.3 to 193 mg-KOH/g-oil in acid value. Although process optimization is necessary for each case, the abundance of waste oils makes them attractive as feedstocks. The total amount of waste cooking oil in Europe, North America, and Asia reaches 16.6 million tons in a year, which can numerically satisfy the total oil demand for biodiesel production (Azócar et al., 2010b).

2.2. Nonedible plant oils

Nonedible oils obtained from jatropha, karanja, mahua, polanga, rubber, soapnut, and castor are less expensive than edible oils and potentially available for producing biodiesel. The selection of these oils would depend on the estimated availability of oil seeds in each country. In particular, *Jatropha curcas* oil is considered one of the promising feedstocks for biodiesel production. The jatropha tree can grow on waste land with minimum water and fertilizer demand, and the presence of toxic compounds such as phorbol esters limits the applications of the extracted oils. The high oil contents in the jatropha seed, ranging from 30% to 50% based on seed weight, also show promise for biodiesel production (Azócar et al., 2010b). However, the high contents of water and fatty acids lead to difficulties in the direct application of the conventional alkali-catalysis method alone. Regarding fuel properties, the oxidation stability of biodiesel from *J. curcas* oil is close to those from soybean and rapeseed oils, thus facilitating the blending of jatropha and palm biodiesels to achieve a better low-temperature property with improved oxidation stability (Sarin et al., 2007).

2.3. Oleaginous microorganisms

The extensive acreage required for sufficient production of oilseed crops hampers the broader use of biodiesel. Because some microorganisms are capable of accumulating oils intracellularly with a high yield, the use of oleaginous microorganisms as an alternative oil supplier has received considerable attention. This approach does not need arable land and possibly enables oil production from abundant carbon sources without competition with food production. The main microorganisms are microalgae, bacteria, yeast, and fungi.

Microalgae can utilize carbon dioxide and sunlight for oil accumulation under the environmental conditions specific to different species such as *Botryococcus*, *Chlorella*, *Cylindrotheca*, *Nitzschia*, and *Schizochytrium*. Under optimal growth conditions, heterotrophic microalgae synthesize medium- and long-chain fatty acids.

Table 1
Potential alternatives to edible oil as raw materials for biodiesel production.

Source	Oil seed content (% dry weight)	Main lipids	Features
Waste oil	Not applicable	Triglycerides and fatty acids	High acidity, high abundance, and low cost
Non-edible plant oil	28–48	Triglycerides and fatty acids rich in unsaturated fats	High oil content and adaptation to wide agroclimatic conditions
Oleaginous microorganism			
Microalgae	15–77	Triglycerides, fatty acids, and hydrocarbons	High lipid accumulation level and conversion of carbon dioxide into oils
Bacteria	18–70	Fatty acids and wax esters	High growth rate and easy culture method
Yeast	25–72	Triglycerides and fatty acids	High growth rate and easy culture method
Fungi	25–86	Fatty acids and unusual lipids	High growth rate and easy culture method

Source: Ahmad et al. (2011), Azócar et al. (2010b), Balat (2011), and Meng et al. (2009).

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