



Sustainable biodiesel production from oleaginous yeasts utilizing hydrolysates of various non-edible lignocellulosic biomasses



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ABSTRACT

Biodiesel, as one of the best alternative fuels, has a number of advantages over petro diesel, such as originating from a renewable and domestic feedstock which reduces the net production cost of biodiesel. In the recent years, biodiesel has received increasing interest due to energy crisis worldwide along with exhausting reserves and the shortage of oil supplies. The major problem behind the use of vegetable oil for biodiesel production is sustainability because it directly competes with human food. To combat this problem, the other renewable sources have been developed as microbial oils have similarity to vegetable oils and extensively used for biodiesel production. Oleaginous yeasts have recently been suggested as microscopic biofactories and alternative lipid producer to vegetable oil for a more sustainable biodiesel industry. It is a potential novel technology where non-edible lignocellulosic biomasses are exploited as raw materials for biodiesel production from oleaginous yeasts which drop net greenhouse gas emissions by substituting the practice of fossil fuels and would convey benefits to rural economies and national energy security. The usage of oleaginous yeasts have many advantages over other renewable sources like faster growth rate, shorter life cycle, easier scale-up, with no effects from the season and climate variation, and can serve as the excellent oil accumulating renewable feedstocks which are non-competitive to food resources and do not require arable land. Non-edible lignocellulosic biomass, consists of three different types of natural polymers, namely cellulose, hemicellulose, and lignin, is the most abundant renewable bioresource in the biosphere. The production of fermentable sugars from hydrolysates of various non-edible lignocellulosic biomass, either by physical, chemical or enzymatic hydrolysis has been utilized as feedstock in bioethanol or biodiesel production, extensively. During hydrolysis generation of non-carbohydrate compounds, such as 5-hydroxymethylfurfural (HMF), furfural acetic acid and phenolic compounds have various effects on the growth of microorganisms, their metabolism, as well as on final products, presenting a key challenge in the biological conversion of biomasses.

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1. Introduction

The availability of petroleum-based resources (fossil fuels) continues to decline with increasing demand for energy worldwide [1]. In order to diminish dependence on fossil fuels, many countries have received extensive interest in biomass-based bio-fuels, which are usually derived from renewable and domestic feedstocks. In India and other developing countries, direct combustion of lignocellulosic biomasses (LB) is routinely used for heat generation and cooking purposes. Direct combustion of LB has led to many problems such as environmental pollution and low energy efficiency. In order to substitute the direct combustion of LB, it can be converted into high-quality bio-products and produced energy by other means. A lot of lignocellulosic biomasses is obtained from forest woody feedstocks, agricultural residues, herbaceous and municipal solid wastes and non-edible energy crops [2]. Sugarcane bagasse, sugar cane husk, wheat and rice straws and corn stover are most promising plant residues derived LB which are used for energy generation in U.S., Asia and Europe [3–5]. Lignocellulosic biomasses are mainly composed of three different polymers such as cellulose, hemicellulose, and lignin. These polymers are toughly intertwined and associated with non-covalent bonds and covalent cross-linkages [6]. Cell walls of plants have lignocellulose as a primary building block in which cellulose plays a major structural role. Celluloses are organized as microfibrils that are surrounded by hemicellulose and lignin (Fig. 3). The clubhouse is the repeat unit of cellulose containing D-glucose subunits linked to each other by β -(1,4)-glycosidic bonds [7]. A suitable pretreatment is required to break the lignocellulosic biomasses to obtain cellulose units and further treated with enzyme cocktails or sulfuric acid to generate sugars [8]. The treated cellulose releases mono and oligosaccharides composed of pentose and hexose sugars that are also known as hydrolysates. These hydrolysates are utilized by oleaginous microorganisms as potential carbon sources for lipid synthesis. From last decade, various researches have been performed to improve the efficiency of hydrolysis. The efficiency is determined by presence of high concentration of monosaccharides in hydrolysates. However, the hydrolysis of LB is restricted by the production of toxic compounds such as furfural (pentose sugars by-product), hydroxyl methyl furfural (HMF; hexose sugar by-product), phenolic acid (lignin by-product) and acetate (deacetylation product of hemicellulose). These by-products show the various inhibitory effect on the

fermentation process of lipid production and decline the yield of final products [9,10].

Biodiesel is renewable, non-hazardous, biodegradable, sustainable, nonflammable, eco-friendly and free from sulfur and aromatic contents. It has higher cetane number and higher flash point than conventional diesel. Its inherent lubricity increases the engine efficiency, so no need to add extra lubricant for engines [11]. It emits almost 78% less net carbon dioxide on a lifecycle basis compared to conventional diesel fuel. These properties of biodiesel make it an ideal fuel for polluted metro cities and play a major role in the aspect of climatic change as it is climatic neutral [12–16]. Biodiesel is mainly produced by a transesterification reaction with vegetable oils (edible or non-edible), waste cooking oils and animal fats [17]. Due to global food securities, oil derived from food sources cannot fulfill the requirement for biodiesel production in large scale. Therefore, it is necessary to search sustainable feedstocks for biodiesel production such as non-edible lignocellulosic energy crops [18]. However, direct utilization of these substrates to produce biodiesel involve high production cost. Out of which 75% of the total cost is spent on raw materials which are the major obstacle for its large scale production and widespread application [19]. The use of animal fats, waste cooking oils, and oils from non-food crops as feedstocks are a good choice to reduce the production cost [20,21]. Moreover, this strategy also cannot fulfill the requirement of renewable fuels for current energy demand. Therefore, researchers are looking for novel oil resources to produce biodiesel. Among various resources, lipid produced by microorganisms, involving yeasts, bacteria, molds and algae known as single cell oil (SCOs) are considered as promising feedstock for biodiesel production due to its similarity with vegetable oils in fatty acid compositions [22–25]. These microorganisms can utilize organic carbon to synthesize oils in their cellular compartment. Moreover, the productivity of many microorganisms is reported more than the oil producing crops. It has been reported that only a minor population of yeasts accumulate more than 25% of lipids [26,27]. The genera of yeasts which considered as oleaginous are *Rhodospiridium*, *Rhodotorula*, *Yarrowia*, *Cryptococcus*, *Candida*, *Lipomyces* and *Trichosporon*. Among these oleaginous yeast genera, *Rhodospiridium* is found to produce the highest amount of lipid in its cellular compartment [28].

The advantage of oleaginous yeast culture is the independence of weather conditions that is more prone in the case of plants. It takes hardly 5–9 days to achieve lipid accumulation (stationary

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