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Biodiesel Production from Filament Fungi

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ABSTRACT

The lack regarding fossil-based fuels, as well as the harm that traditional energy sources do to the environment, has prompted new research into sustainable energy sources that are sustainable. Biofuel is dedicated to taking the lead globally in creating and applying renewable energy sources. Alternative fuel produced from renewable biological resources is biodiesel. Additionally, microbial sources, such as fungi, bacteria, in addition to algae, could be used to make biodiesel. Under conditions of metabolic stress, fungi can store intracellular lipids exceeding 70% of their biomass. Less research has been done on fungal lipids as a biodiesel source. Therefore, for such an investigation, diesel fuel derived from fungal lipids has been used.

Keywords- Biodiesel, Fungal lipids, Renewable energy, Biofuel, Microbial lipids, Sustainable fuel, Fungi, Alternative energy source.

I. INTRODUCTION

The requirement for energy is rising due to the world's population expansion and industrialization. The inevitable and slow depletion regarding the planet's fossil fuels is one of the biggest problems facing humanity. One of the main causes of environmental pollution and global warming is the extravagant burning of such energy sources for heating of transportation fuels. At a time when energy consumption is skyrocketing, the globe is dealing with diminishing liquid fuel supplies [1]. The supply of fossil fuels may run out by the end of the century in the case when consumption keeps on its current pace. Transport fuels that are generated from petroleum, considered to be scarce as well as contributing to global warming, must be replaced by renewable biofuels. One possible renewable fuel is biodiesel. The only renewable biofuel that has the capacity to totally replace transport fuels generated from petroleum without negatively impacting the supply of food, as well as other crop goods, is biodiesel made from microalgae [2]. Transport fuels, which are renewable as well as carbon neutral, are important for economic, in addition to environmental, sustainability. [3] Using microbial systems to produce biodiesel, even if this method hasn't been used in industry yet. The capacity of microorganisms to thrive on a nearly limitless range of food sources could be crucial in helping mankind overcome its present energy dilemma. Interest in finding solutions for lowering the high price of biodiesel, particularly the loss regarding raw materials, is growing [4]. Furthermore, microbes could be engineered to use a variety of carbon sources, like waste or agricultural byproducts, as feedstock for the synthesis of oils. Throughout times of metabolic stress, a large number of yeasts, molds, and algae show the ability to accumulate intracellular lipids exceeding 70% of their biomass [5]. Yet, there is a lack of knowledge on using microbial sources for producing lipids. For this reason, the current research has been conducted to maximize the synthesis of lipids from oleaginous fungi and to utilize microbes for the manufacture of biofuel.

II. METHODS AND MATERIALS

Samples Collection, Fungal Isolation, and Identification of Oleaginous Fungi

For isolating oleaginous fungi for lipid production with the use of a serial dilution approach. Seven soil samples were obtained from various sites near the Al-Dora refinery in Baghdad at a depth of 5–7 cm, using a metal spatula sterilized with 70% alcohol before each use. The samples were placed in sterile polythene bags, sealed, and promptly transported to the laboratory for mycological analysis. For each sample, 10 g of soil was transferred into a 250 mL conical flask containing 100 mL of sterile distilled water. The mixture was agitated on an electric shaker to produce a uniform suspension, followed by preparation of serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}). Subsequently, 1 mL of the 10^{-3} dilution was inoculated into Petri dishes containing potato dextrose agar (PDA; OXOID, England) and incubated at 27 ± 2°C for five days. After colony development, distinct fungal colonies were isolated and subcultured to obtain pure cultures. Oleaginous fungal isolates were identified based on colony morphology and microscopic characteristics, in accordance with [8].

Screening for lipid-producing oleaginous fungi

The technique described by [11] has been used for screening of lipid-producing oleaginous fungi. Five oleaginous fungi (*Verticillium tricorpus*, *Penicillium raistrickii, Rhizomucor miehei, Mucor circinelloides*, and *Trichoderma reesei*) were used for this test. After being harvested, the culture was examined for biomass (gl-1) and lipids.

Extraction of fungal lipids

Based on lipid production testing, the *Penicillium raistrickii* isolate was selected for the extraction of lipids. Bligh and Tyer technique [12] was used for extracting fungal lipids from dried mycelia. With regard to *P. raistrickii*, the effects of various growth conditions (temperature and pH) as well as nutritional factors (nitrogen and carbon sources) have been examined. The next composition was examined in relation to the lipids as well as biomass production through fungal isolate PR using various carbon sources: pH 5.4, distilled water 1000 ml, 5.0 g/l of yeast extract, and glucose 30.0 (0.16 M). Broth's glucose has been substituted with various carbon sources individually. [13] Fungal isolate of (1g) was employed as mycelial suspension, and various carbon sources, like fructose, glucose, lactose, in addition to sucrose, have been added at concentration of 0.16 M. Cultural conditions: broth of 50 ml dispensed into conical flasks of 250 ml, then sanitized, and then incubated in incubator shaker for seven days at a temperature of 30 °C at 200 rpm. Whatman No. 1 filter paper was used for filtering fungal mycelium. The filtered fungal mycelium's liquids as well as biomass content, have been examined. Their previous research indicated that the optimum source with regard to lipid synthesis was glucose. For determining the optimum level for maximizing the formation of lipids, glucose has been thus taken at various concentrations. Production of lipids and consumption of glucose by fungal isolate *P. raistrickii*. It was investigated how quickly the glucose source was depleted and how lipids were formed in the fungal isolate *P. raistrickii*. The 250 ml Erlenmeyer flask has been filled with screening broth, which had 0.6 M glucose content, and then sterilized. For seven days, *P. raistrickii* (1g) has been inoculated as a mycelial suspension as well as incubated at 220 rpm in the environmental shaker. Flasks have been removed as well as filtered with the use of Whatman No. 1 filter paper following incubation. Furthermore, the amount regarding glucose in filtrates and the amount of biomass and lipids in mycelia were examined. The technique of [13] was used to measure the amount of glucose in the culture filter. After centrifuging the culture filtrate at 6000 rpm and at a temperature of 30 Celsius, supernatant of 0.2 ml was pipetted into a testing tube. After adding 1ml of the alkaline copper tartarate reagent, the mixture has been heated to a boiling water bath for ten minutes. One milliliter of the arsenomolybdate reagent (see below) has been added once it has cooled. Following 10 minutes, the colorimeter's orange-red absorption has been measured at 620 nm. The standard curve was created using the standard's absorbance value. The amount of glucose present was determined by plotting the samples' absorbance values. A variety of nitrogen sources, including ammonium chloride, yeast extract, also ammonium sulphate, were used to investigate the fungal isolate's (*P. raistrickii*) capacity. Various nitrogen sources, like ammonium chloride, yeast extract, also ammonium sulphate, were used for replacing the broth's yeast extract individually. Yeast extract 10.0 g, glucose 0.6 M, temperature 30 °C, and pH 6.5 were the nutrients and growth factors used for large-scale lipid production through a fungal isolate (*P. raistrickii*) in a bioreactor. [14]. In a 5L bioreactor (Lark Innovative Technologies, India) with pH electrodes, disc impeller, and oxygen, fermentation was conducted using the optimal fermentation medium. Additionally, the equipment tracked the vessel level, agitation speed, temperature, pumping rates, gas purging flow rate, in addition to antifoam addition. During the experiment, temperature as well as pH level remained unchanged [15]. Rhodotorula glutinis has been inoculated into fermentation broth at 10% level (28x10 cfu/ml) during a course of 24 hours. Following inoculation, fermentation took place for 5 days. Following 5 days, biomass has been extracted using centrifugation, and the amount of lipids that yeast strains produced was calculated. GC-MS was used to further investigate the lipids for fatty acid profile. Gas Chromatography-Mass Spectrometer (GC-MS) analysis of fatty acid methyl esters [16]. The physicochemical characteristics of fungal strains have been examined to determine their suitability for use as biodiesel. The next characteristics were examined using American Standards for Testing of Materials

(ASTM) (2003). Physical as well as fuel characteristics such as viscosity, density, iodine and acid values, gross heat of combustion, cloud and pure points, and volatility were assessed for bio-diesel esters.

III. RESULTS AND DISCUSSION

Samples Collection, Fungal Isolation, and Identification of Oleaginous Fungi

A total of fungal isolates were isolated and diagnosed depending on their morphological features, microscopic features, and colony morphology in Petri plates. Oleaginous fungal isolates were selected, comprising five genera (*Verticillium, Penicillium, Rhizomucor, Mucor,* and *Trichoderma*).

Identification of five genus oleaginous fungi by PCR and gene sequence

Screening for lipid-producing oleaginous fungi

Production of lipids by fungi isolates

PR sample exhibits high production of lipids as well as biomass (28% & 10 g/l) among the five isolates under study (Fig.1). Thus, PR sample has been chosen for more research. *Penicillium* (PR) uses carbon sources like glucose or cellulose (via cellulolytic enzymes) to produce acetyl-CoA, which is the building block for fatty acid synthesis. This mycelial lipid accumulation behavior was similar to those reported for *Penicillium camemberti*, *Penicillium frequentans*, *Penicillium lilacinum*, and *Penicillium roqueforti* which ranged from 5% to 22%, and to the behavior of other filamentous ascomycetes fungi such as *A. oryzae* A-4, which ranged from 15% to 18% (Abraham and Srinivasan, 1984; Hamid et al., 1987; Lomascolo et al., 1994; Hiu et al., 2010)

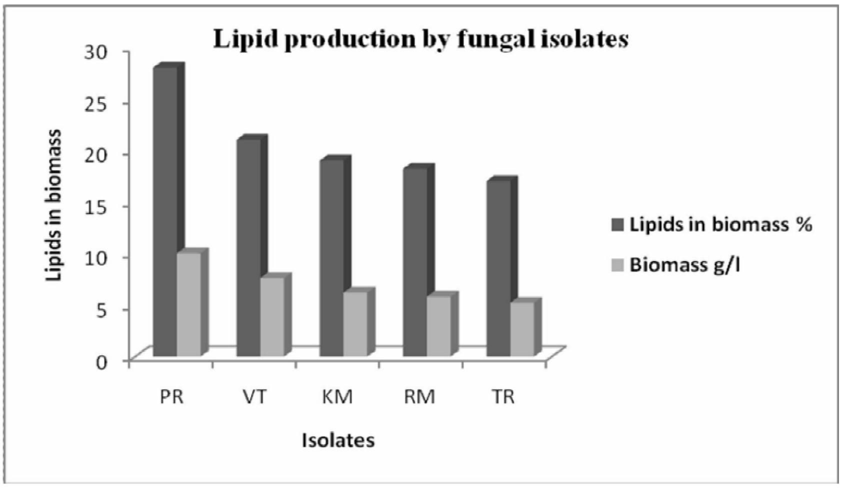

Fig1. Production of lipids by fungal isolates.

Temperature effect on the production of lipids by fungal isolate

Fungus has been cultivated at various temperature ranges (20, 25, 30, 35, and 40 Celsius) for maximizing the amount of lipids produced via fungal isolate (PR). Fungus accumulated the least quantity of lipids (14.3%) at 40°C and the most at 30°C (43.2%) (Fig. 2). According to [17], the current work showed that 30 °C has been the optimal temperature for fungal isolate (PR) in terms of biomass and lipid synthesis.

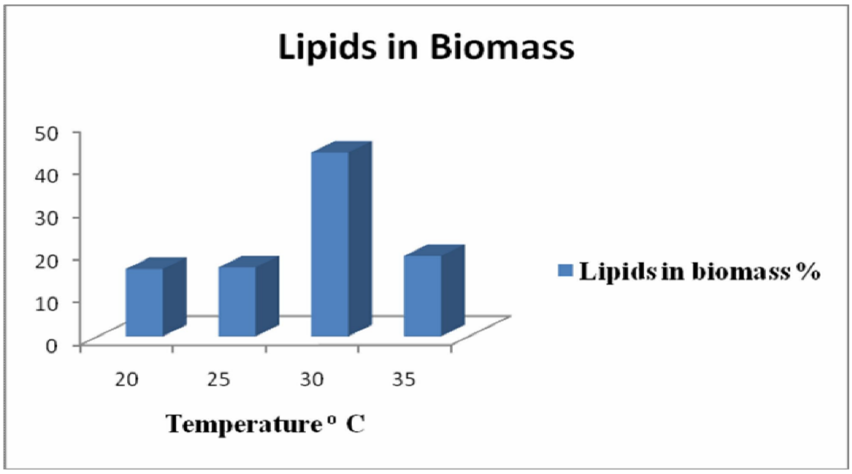

Fig2 Temperature on the production of lipids by fungal isolate (PR)

pH effect on lipids production by fungal isolate (PR)

pH impact on the production of lipids was examined using fungal isolate (PR) at various values of pH, ranging from 5.5, 6.5, 7.5 and 8.5. At a pH of 6.5, the fungus produced the highest amount of lipids (43.5%) as well as biomass (10.7gl-1). At pH 7.5, the lowest levels of lipids (10.20%) and bio-mass (5.60%) were detected, while at pH 8, neither growth nor lipid accumulation were detected. According to [18] [19], pH of 6.0 has been ideal for the formation of biomass and lipids, as well as a high proportion of polyunsaturated fatty acids (PUFAs).

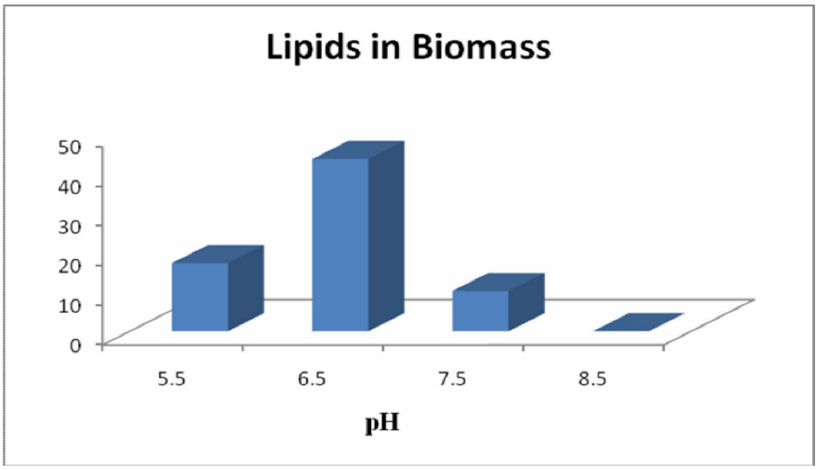

Fig.3 pH on lipids production by fungal isolate (PR)

Physico-chemical properties of fungal lipids

For usage as biodiesel, the physico-chemical characteristics of the lipids generated by the fermenter have been examined and data were recorded. ASTM standards were put to comparison with the acquired values. Physical characteristics included a viscosity of 54.81 cSt at 40°C, a specific gravity of 0.92 g/cc, flash 218°C, 32.05 Kcal/kg calorific value, 4°C cloud, 230°C fire point, 7°C pour point. Chemical characteristics of lipids included Free Fatty Acids (FFA) of 14.55% and an acid value of 28.2. In comparison to the standard (1.9 6.0 cSt and < 0.8), the fungal lipids' viscosity (54.81cSt) and acid value (28.20) have been both greater. However, other characteristics, such as carbon residue as well as flash point, fell within the acceptable range. Fungal lipids' flash and fire point values are within acceptable bounds. Engine blockage results from fungal isolate's higher than normal viscosity, FFA, and acid value, which must be decreased by transesterification. Through using GC-MS, retention durations of fatty acid profiles (palmitic 33.4min), stearic (34.34), as well as oleic acid (34.6%) have been determined.

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