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Enhanced Biodiesel Production from *Oedogonium sp.* and *Saccharomyces cerevisiae* Using Modified Bligh and Dyer Lipid Extraction Method

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Abstract

The production of alternate renewable, clean fuel sources like biodiesel is being explored in this present study using green algae (Oedogonium sp.) and yeast (Saccharomyces cerevisiae) isolated from mining sites. The study aims to use the newly modified Bligh and Dyer method for extracting lipids from Oedogonium sp. and S. cerevisiae to produce biodiesel. The modified method increased the lipid content to 3.951% for Oedogonium sp and 2.621% for S. cerevisiae compared to Soxhlet apparatus and conventional methods. The biodiesel produced from the lipids is analyzed as per IS1448 standards and is compatible with ASTMD 6751 biodiesel standards. The modified Bligh and Dyer lipid extraction is one of the most efficient lipid extraction methodologies that will contribute to the research in biodiesel production.

Keywords: Baker's Yeast, Biodiesel, Bligh and Dyer, Lipids, *Oedogonium* sp, Transesterification

1.0 Introduction

Biomass is known for its renewable nature and ability to absorb CO_2 from the atmosphere through photosynthesis and has received attention as an alternative energy source in the recent decade. Algae, both macro and micro, typically have a higher photosynthetic efficiency than other types of biomass. Proteins, carbohydrates, lipids, and nucleic acids make up most algae. Fatty acids can be up to 40% of algae's dry biomass, and the lipid quantities vary depending on the type of algae. *Oedogonium* is a filamentous, unbranched free-living green algae that can be used to make biodiesel. The characteristic rings can easily recognize at the ends of *Oedogonium sp.* alga cells. Adegoke *et al.* studied biodiesel production from *Oedogonium capillare* by extracting lipids using n-hexane[1]. The filamentous green algae extracted from mining industries are helpful in adsorption of trace metals from waste waters[2].

The Soxhlet method is frequently used for lipid extraction from biomass. The method involves lipid extraction using hexane, chloroform, and methanol, like organic solvents. The main disadvantage of the Soxhlet method is its long duration and the requirements for large amounts of solvents. This solvent disposal is a costly process and also causes harm to the environment. The Bligh and Dyer method was developed in 1959 and is the most popular for lipid extraction from biological samples[3]. The process mainly consists of a two-step extraction method. The sample is mixed with methanol, chloroform, and water in the first step, and after phase separation, the lipids in the chloroform phase are measured in the second

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step. The newly modified Bligh and Dyer method is used for lipid extraction from eleven micro algae isolated from Egypt's collected soil and water samples[4]. Hac Isa *et al.* suggested modification of the Bligh and Dyer method by incorporating enzymatic hydrolysis in the first step for enhanced lipid removal from microalgae *Schizochytrium sp*[5]. *S. cerevisiae* is one of the yeast species used in wine making and baking for many centuries. The approximate lipid composition in dry biomass of Baker's yeast is 4.4%[7]. The lipids can be extracted and converted to biodiesel. Singh *et al.* suggested a solvent extraction method (Bligh and Dyer) for yeast lipid extraction[8]. Ghosh *et al.* published a newly modified method for enhanced lipid production for green algae[4].

The current study aims to use the newly modified Bligh and Dyer method for enhanced lipid removal from *Oedogonium sp.* and *S. cerevisiae*[4]. The lipid extracted from these species is transesterified using NaOH as a catalyst. IS1448 standards are used for testing biodiesel, and the results are compared with ASTMD 6751 biodiesel standards

2.0 Materials and Methods

2.1 Sample Collection

Oedogonium sp. algal culture was carefully recovered from wastewaters at Kolar gold mining fields, Karnataka, India. The isolated culture was cultivated on Hill and Machlis media (Table 1)[9], a specified medium developed with inorganic salts and vitamin B12 that is used for the growth of green alga (*Oedogonium sp.*)[4]. The organisms were sub-cultured every two weeks. The *S. cerevisiae* (Baker's yeast) was extracted from GKVK farms at the University of Agricultural Sciences in Bangalore, Karnataka, India.

2.2 Lipid Extraction by Soxhlet Method

Sixty grams of green algae (*Oedogonium sp.*) sample is taken for producing lipids using the Soxhlet apparatus. About 12 grams of dry biomass of *Oedogonium sp.* is added to the Soxhlet extraction tube. The tube is dried in the oven for 1 hour at 105^0 C and refrigerated for 30 minutes. The Soxhlet flask can take 100 ml of chloroform. The Soxhlet apparatus is run overnight for 20 hours at a rate of 2 to 3 drops per second condensate rate. The chloroform is distilled out and the residual lipid is weighted. The method is repeated 5 times until enough lipid is extracted from the green algae[4].

Table 1. Hill and Machlis media for green algae growth[9]

Compounds	Macro nutrients (millimole/ liter)	Trace Elements	Micro nutrients (gram /100ml)
$CaCl_2.2H_2O$	0.5	$Na_2B_4O_7.10H_2O$	4.770
$MgSO_4.7H_2O$	0.5	$CuSO_4.5H_2O$	0.250
KNO_3	1.0	$CoCl_2.6H_20$	0.238
KH_2PO_4	2.5	$MnCl_2.4H_2O$	0.198
Vitamin B_{12}	$2.5x10^{-6}$ / liter	$ZnSO_4.7H_2O$	0.287
		$(NH_4)_6Mo_7O_{24}$ $4H_2O$	0.177
		$FeSO_4.7H_2O$	0.278
		$N(CH_2COOH)_3$	1.910

2.3 Bligh and Dyer Method

Two grams dry weight of algal sample *Oedogonium sp.* is taken with 20 ml methanol and 20 ml chloroform in a mortar and pestle and homogenized for 10 min. Again 20 ml of chloroform and 20 ml of water are added and homogenized. A glass funnel is used with 150 mm whatman (Dassel, Germany) filter paper for the separation of the solvent. The residual solution is kept in a separating funnel; after 15 minutes, two layers are visible. The bottom layer was transferred from the separating funnel into the original flask, and 20ml of chloroform was then added. The sample was once more filtered, and the solvent was then removed. Using a volumetric pipette, the top layer (the chloroform phase) was transferred to a tared tray for evaporation. Under an infrared lamp, the solvent evaporated between 55°C and 60°C. After the tray had cooled down, the lipid in the flask was collected and weighed. The process is repeated 30 times to extract lipids for biodiesel production.

2.4 Newly Modified Bligh and Dyer Method

A 2.5gram dry weight of the sample is taken with 40 ml methanol and 20 ml chloroform in a mortar and pestle. The sample is homogenized for 10 min, and 20 ml chloroform and 20 ml water are added. The sample is ultrasonicated for 10 min, and the process is repeated 24 times until 60 grams of dry weight is processed[4]. The same procedure is repeated for *S. cerevisiae* until enough lipid is extracted for biodiesel production.

The *S. cerevisiae* sample from the homogenizer is added to the centrifugal tubes. The centrifugal pump is run for 30 minutes at 10,000 rpm. The three layers are

obtained as shown in the below diagram. The top layer is the water-methanol layer, and the bottom layer is the chloroform layer and the middle layer is solid biomass. The layers are carefully separated and maintained at high temperatures (105°C) to remove methanol, excess water and chloroform. The pure lipid is removed and stored for biodiesel production (Figure 1).

2.5 Biodiesel Extraction

The biodiesel extraction from lipids is done using the method adopted by Adegoke *et al.* The lipid samples are mixed with 100 ml of methanol and 6.25 ml of H_2SO_4 for transesterification. The temperature is maintained at 60°C for 90 minutes in a water bath[1]. The product is left for 12 hours in a separating funnel to remove glycerine as a byproduct. The biodiesel is washed with 5% volume/weight water until it becomes clean. The biodiesel is cooled to room temperature and kept under a running fan for 6 hours.

3.0 Results and Discussions

3.1 Growth of Green Algae

The optimal growth parameters for the *Oedogonium sp* are a temperature 20-30 °C temperature, a pH of 6.5 and a light intensity of 1200 lux (shaded sunlight). The experiments are conducted in open area ponds for two months, where the outside Bengaluru temperature ranges from 18°C to 35°C. The specific growth rate of the *Oedogonium sp.* is found to be 0.0678 grams dry weight per day per litre.

3.2 Lipid Extraction and Biodiesel Production

The Soxhlet apparatus accommodates 12 gms of *Oedogonium sp*. as shown in the below diagram (Figure 1). The experiment is conducted for 18 hrs for lipid extraction from green algae, and for each run, 0.32 gms of lipid is obtained. The procedure is repeated until 60 grams of *Oedogonium sp*. is processed. 15 grams of wet *Oedogonium sp.* gives 1.802 grams of dry biomass when dried for 24 hours at 60°C in a hot air oven under standard conditions. The Soxhlet method gives approximately 1.6 grams of lipids, the Bligh and Dyer method gives 1.702 grams, and the newly modified Bligh and Dyer method gives 2.371 grams. The results are tabulated in Table 2.



(a) (b)

Figure 1. *S. cerevisiae* sample **(a)** before centrifugation **(b)** after centrifugation.

The lipid extracted from green algae *Oedogonium sp.* is used for biodiesel production. About 2.371 grams of lipid is transesterified using methanol and NaOH for biodiesel production. 1.617 grams of biodiesel is obtained after transesterification resulting in 68.2% extraction efficiency. The *S. cerevisiae* (Baker's yeast) is used to extract lipids, followed by biodiesel production through transesterification. 150g of the locally available Baker's yeast (*S. cerevisiae*) is used for the study. A paste with a yellowish-brown hue is produced during the process. The yellowish-brown hue paste is centrifuged at 10000 rpm for 30 minutes. Three distinct layers of the methanol, chloroform, and residual phases are produced after centrifugation (Figure 1). The chloroform layer is removed, and the bottom layer, which contains pelletized yeast, is reprocessed. The procedure is repeated until enough lipid content is obtained for biodiesel transesterification. 3.94 grams of lipid is extracted from 150 grams of yeast, giving 2.62% extraction efficiency. The biodiesel produced after the transesterification process is 2.78 grams with 70.55% biodiesel extraction efficiency.

3.3 Biodiesel Characteristics

The biodiesel is analyzed as per IS 1448 standards, and the results are compared with ASTM D6751 biodiesel standards. A significant amount of biodiesel is produced by extracting lipids using the newly modified Bligh and Dyer method from *Oedogonium sp* and *S. cerevisiae.* The fuel properties listed in the Table 3 shows the effectiveness of the process.

Table 2. Comparison of lipids extracted from *Oedogonium sp*. by Soxhlet method, Bligh and Dyer method and the newly modified Bligh and Dyer method

Method	Dry Weight (g)	Lipids (g)	Lipid (%)
Soxhlet method	60	1.6	2.667
Bligh and Dyer method	60	1.702	2.83
Newly modified Bligh and Dyer method	60	2.371	3.951

Table 3. Fuel properties for biodiesel produced from *Oedogonium sp* and *S. cerevisiae*

Fuel Properties	Biodiesel Properties		Test Protocol	Std. Biodiesel Properties (ASTM D6751)	ASTM Standards
	Oedogonium Sp	*S. cerevisiae*			
Calorific value (MJ/kg)	38.2	40.1	IS: 1448 (P 6)	37 to 42.5	ASTM D613
Flash point (°C)	105	108	IS: 1448 (P 66)	130	ASTM D2500
Cloud point (°C)	-3 to 4	-3 to 4	IS: 1448 (P 6)	-3 to 12	ASTM D287
Density at 15°C	0.89	0.86	IS: 1448 (P 32)	870 – 900	ASTM D445

Manjunath *et al.* also reported similar properties for biodiesel extracted from sludge palm oil[10]. The ASTM and IS standards are adopted by Manjunath *et al.*[10].

4.0 Conclusion

The lipids extracted from *Oedogonium sp.* (green alga) and *S. cerevisiae* (yeast) using the newly modified Bligh and Dyer method is found to be effective when compared with the Soxhlet method, and conventional Bligh and Dyer method. About 1.12% increase in lipid extraction is observed from the conventional Bligh and Dyer method to the ultrasonication-added Bligh and Dyer method. Further lipid extraction from *S. cerevisiae* (yeast) is done using the modified Bligh and Dyer method. About 2.66% of lipids are extracted from *S. cerevisiae* (Bayer's Yeast). Rattray *et al.* reported 2.9 to 9.0% (weight or yeast dry weight) of total extractable lipids from *S. cerevisiae* cells[11-13]. 70.55% biodiesel extraction efficiency is achieved for lipids extracted from *S. cerevisiae*, and 68.2% is achieved for lipids extracted from *Oedogonium sp.* The biodiesel properties listed in the Table 3 are found to be compatible with ASTM standards.

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