Biodiesel production from microalgae

Abstract

Algal biodiesel represents a promising renewable alternative for reducing greenhouse gas emissions and dependence on fossil fuels due to its exceptionally high oil productivity. In this study, seven algal strains were isolated from diverse natural habitats, of which three microalgae (MG-1, MG-2, and MG-3) were selected for oil extraction using the hexane–ether method. Oil yield was higher at boiling temperature for all strains, with yields of 31 ml, 23 ml, and 16 ml from 2.5 g biomass of MG-1, MG-2, and MG-3, respectively.

Microscopic identification revealed MG-1, MG-2, and MG-3 as *Cyanobacteria spp.MG1 Phormidium* spp., and *Scenedesmus* spp. Followed by molecular identification of most efficient one, the sequence was submitted to GenBank under accession number *Cyanobacteria spp.MG1* PP702711. Biodiesel was produced via alkali-catalyzed transesterification, yielding 4 ml biodiesel from 5 ml oil of *Cyanobacteria spp.MG1* which showed the highest oil and biodiesel productivity among the tested strains. FTIR analysis confirmed the chemical composition of the biodiesel, and partial purification was achieved using a charcoal column. Biodiesel characterization indicated a cetane number of 54, demonstrating that algal biodiesel, particularly from *Cyanobacteria spp.MG1* is an efficient and eco-friendly alternative to petroleum diesel.

Keywords: Microalgae, Algal biodiesel, Transesterification, Renewable biofuels, FTIR analysis

INTRODUCTION

The global demand for energy is increasing continuously due to rapid industrialization and population growth. One of the major drawbacks of petroleum-based fuels is the severe atmospheric pollution caused by their combustion. Petroleum diesel combustion is a significant source of greenhouse gas (GHG) emissions and also releases other harmful air pollutants, including nitrogen oxides (NOx), sulfur dioxide (SO_2), carbon monoxide (CO), particulate matter, and volatile organic compounds.

Algae are considered one of the most promising feedstocks for biodiesel production due to their exceptionally high oil yield. Algal biomass can produce up to 250 times more oil per acre than conventional crops such as soybeans and 7–31 times more oil than palm oil. In fact, biodiesel derived from algae may be one of the few viable options capable of meeting current automotive fuel demands. Among algae, microalgae are particularly advantageous because they contain higher lipid content than macroalgae and can be grown rapidly and efficiently. Although the concept of using microalgae as a fuel source is not new, it has gained renewed attention due to the escalating prices of petroleum fuels and growing concerns about global warming associated with fossil fuel consumption. However, limited literature is available on biodiesel production from macroalgal species such as *Oedogonium* and *Spirogyra*. Therefore, the present study was undertaken to investigate optimal transesterification conditions, biodiesel yield, and key physical properties—including ester yield, glycerin formation, and sediment content—of biodiesel produced from algae (A.B.M. Sharif *et al*., 2008).

Biodiesel is defined as mono-alkyl esters of long-chain fatty acids derived from vegetable oils, animal fats, or waste cooking oil. It is renewable, non-toxic, biodegradable, non-flammable, and environmentally friendly. Compared to conventional petroleum diesel, biodiesel offers several advantages, including higher flash point, improved cetane number, and reduced exhaust emissions. However, biodiesel production from edible crops has raised concerns regarding food-versus-fuel competition. In contrast, algae can be cultivated on non-arable land with minimal freshwater requirements and do not compete with food crops. Moreover, algal biomass productivity is several times higher than that of terrestrial plants (Veeramuthu *et al.*, 2014).

The increasing global demand for biofuels highlights the urgent need to identify highly productive, non-food biomass sources for sustainable fuel production. The U.S. Department of Energy–supported Aquatic Species Program (1978–1996) demonstrated the potential of microalgae as a renewable and sustainable feedstock for biodiesel production (Keesoo *et al.*, 2014).

Algae also play a crucial role in the global carbon cycle by capturing atmospheric CO_2 and converting solar energy into chemical energy through photosynthesis. *Chlorella vulgaris* has been

widely recognized as a promising biodiesel feedstock due to its rapid growth and ease of cultivation. However, under normal growth conditions, its lipid content is approximately 20% of dry biomass, which may not meet industrial-scale requirements (Lenka *et al*., 2015).

Microalgae can assimilate CO_2 from various sources, including atmospheric CO_2, dissolved carbonates, and industrial flue gases. Biomass energy accounts for approximately 10.4% of total global energy consumption, and nearly 77.4% of renewable energy originates from biomass sources. Algal biomass is considered one of the most efficient bioenergy options due to its adaptability to diverse climatic conditions. Raceway pond systems are regarded as a techno-economically feasible approach for large-scale algal cultivation compared to photobioreactors (Neda *et al*., 2019).

Microalgae are regarded as an ideal biodiesel feedstock because they are aquatic, non-edible, genetically modifiable, and fast-growing, with productivity 3–35 times higher than terrestrial plants in terms of energy content. Additionally, their cultivation requires less water than conventional crops (Vandana *et al.,* 2015). Lipid content in microalgae generally ranges between 20–50%, making them suitable for biodiesel production. Research has primarily focused on eukaryotic microalgae such as *Botryococcus braunii*, *Chlorella spp*., *Chlamydomonas reinhardtii*, and *Nannochloropsis spp*., due to their high lipid accumulation. Cyanobacteria, although prokaryotic, are also gaining attention for biodiesel production owing to their rapid growth rates and lipid productivity (Keshini *et al*., 2014).

The efficiency of biodiesel production depends on both feedstock quality and cultivation conditions. While replacing petroleum diesel with biodiesel offers environmental benefits, certain challenges remain, including lower energy output and potential increases in NO_2 emissions. Nevertheless, microalgae-derived biodiesel has gained significant interest due to higher biomass yield, superior photosynthetic efficiency, and faster growth compared to traditional energy crops (Marium *et al*., 2020). On average, biodiesel yields from microalgae are 10–20 times higher than those obtained from oilseed crops (Lusia *et al*., 2008).

Hence, the objective of the present study is to produce renewable, non-toxic, cost-effective, and eco-friendly biodiesel from microalgal biomass using an alkali-catalyzed transesterification process.

MATERIAL AND METHODS

1.1 Sample Collection

Samples were collected from different natural resources such as Lonar Lake, Farm pond, Salim Ali Lake and local marshy (Drywall) places brought to laboratory processing.

1.2 Isolation of Microalgae from Natural Resources

Microalgae were isolated using the colony-picking method. BG-11 medium (pH 7.2) was inoculated with small algal colonies obtained from collected natural samples (designated MG-1, MG-2, and MG-3) using a sterile inoculation loop. The cultures were incubated under natural sunlight conditions until visible algal growth was observed. Incubation was carried out under light intensity of approximately 1500 lux with a 16:8 h light–dark cycle, and alternatively at 3000 lux under the

same photoperiod at 28 °C for 14 days. After growth on Petri plates, individual colonies were repeatedly sub-cultured onto freshly prepared BG-11 medium until pure single strains were obtained (Naila *et al.*, 2020; Krishna moorthy *et al.*, 2020; Bhaskar, 2021; Keesoo *et al*., 2014).

1.3 Cultivation of Microalgae

Open ponds are the oldest and simplest systems for mass cultivation of microalgae. In present work, MG-1, MG-2 Algae were cultivated by the open pond method by using plastic tray of near about 1foot depth under natural condition, and daily observed the algal growth. In this system, the shallow pond is commonly with about 1foot deep; algae are cultured dependent circumstances same to the natural environment. (Baig *et al.* 2022; Lenka *et al*., 2015).

1.4 Evaluation of Micro algal Growth Potential by Cell Dry Weight Measurement

Algal biomass was harvested and washed twice with distilled water, followed by drying in an oven at 60 °C for 48 h. Based on dry biomass productivity, promising microalgal strains were selected for further studies. (Zehra *et al.*, 2022).

1.5 Identification of Efficient Microalgae

Cultivated algal cultures were initially identified based on morphological characteristics such as color and growth appearance in open-pond cultivation (Fig. 5). This preliminary classification was used only to differentiate isolates at a basic level. Detailed morphological identification was carried out based on cultural and microscopic characteristics. A drop of the water sample was placed on a clean glass slide and examined under an inverted microscope at 10× and 40× magnifications. Algal cell characteristics—including color, basal body, cell arrangement and pattern, length and width of vegetative cells, sheath width, type and position of spores, presence or absence of hormogonia and akinetes, nature of cell type, coil and helical shape, presence or absence of gas vacuoles, and pigment color—were observed. Images were captured using a microscope-mounted camera following the method described by Krishna Moorthy *et al*., (2020). The most efficient biodiesel-producing algal isolate was further identified by 16S rRNA gene sequencing using the Sanger sequencing method at Progenome Life Science, Chh. Sambhajinagar, and the obtained sequence was submitted to GenBank under the accession number PP702711.

1.6 Algal Oil Extraction

Algal oil extraction was performed with minor modifications following Indumathi *et al.* (2014), Harvind *et al.*, 2014. And A.B.M. Sharif *et al*. (2008). Dried algal biomass (MG-1, MG-2, and MG-3) was incubated at 80 °C for 20 min to remove residual moisture and ground thoroughly using a mortar and pestle. The powdered biomass was mixed with hexane and ether (20 mL each) in clean, dry bottles and heated in a water bath for 10 min. After cooling, the mixture was transferred to a separating funnel and allowed to settle for 24 h. The oil layer was then separated and collected.

1.7 Biodiesel Production

Biodiesel was produced through an alkali-catalyzed transesterification process. Extracted algal oil was heated to 45 °C in a water bath. Sodium methoxide was prepared by dissolving 0.25 g NaOH pellets in 24 mL anhydrous methanol. The methoxide solution was added to the warm oil in a 3:1 ratio (methoxide: oil) and stirred vigorously for 90 min at 37 °C. The reaction mixture was transferred to a separating funnel and allowed to settle for 24 h. The upper biodiesel layer was

separated, while the lower glycerol and soap layer was removed. Biodiesel yield was measured and recorded (Indumathi *et al*., 2014).

1.8 Characterization of Biodiesel

The produced biodiesel was characterized using various physicochemical analyses, including flame test, pH, density, specific gravity, cetane number, aniline point, API gravity, diesel index, and FTIR analysis (Indumathi *et al.*, 2014).

1.8.1: Flame Test: 250 µL of biodiesel was ignited in a Petri plate, and the burning duration was recorded.

1.8.2: Oil Extraction Efficiency (wt. %) = Mass of oil extracted (grams) / the total mass of dried algae ×100

1.8.3: Density: Calculated as mass per unit volume of biodiesel.

1.8.4: Specific Gravity: Ratio of biodiesel density to water density.

1.8.5: Aniline Point:

Determined following standard procedures to assess aromatic content. Take 1 mL each of dry aniline and algal biodiesel in a clean test tube and position the thermometer so that its immersion mark is at the liquid level without touching the tube walls. Stir the mixture rapidly while avoiding air bubbles. If the mixture is immiscible at room temperature, heat at a rate of 1–3 °C/min until complete miscibility is achieved. Then allow the mixture to cool and record the temperature at which uniform turbidity first appears; this temperature is noted as the aniline point

1.8.6: API Gravity: Calculated using the formula: = (141.5/Density of biodiesel) –131.5

1.8.7: Diesel Index: ={Aniline Point (^{0}F)*^{0}API}/100

1.8.8: Cetane Number: Cetane number = Density*Diesel Index+ 10.

1.8.9: FTIR Analysis

FTIR spectroscopy was performed to identify functional groups and assess the chemical composition of algal biodiesel in comparison with petroleum diesel. FTIR analysis was conducted at the Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Chh. Sambhajinagar.

1.9 Partial Purification of Biodiesel

The crude biodiesel obtained through alkali-catalyzed transesterification was further purified by column chromatography using activated charcoal as the stationary phase. The purified eluent was collected and used as final biodiesel (Kulkarni *et al.,* 2019).

2. RESULTS and DISCUSSION

2.1 Collection of Microalgal samples:

In the present study, seven microalgal samples (Fig. 1) were collected from diverse natural habitats, including Lonar Lake, farm ponds, Salim Ali Lake, and local marshy (drywall) areas. The samples were transported to the laboratory for further isolation and identification of microalgae. Similar approaches for algal sampling have been reported by Baig *et al.,* (2022) from Chasma Achusin, Quetta (Pakistan), and by Hossain *et al.,* (2008), who collected algal samples from the Phycology Laboratory, University of Malaya, Malaysia.

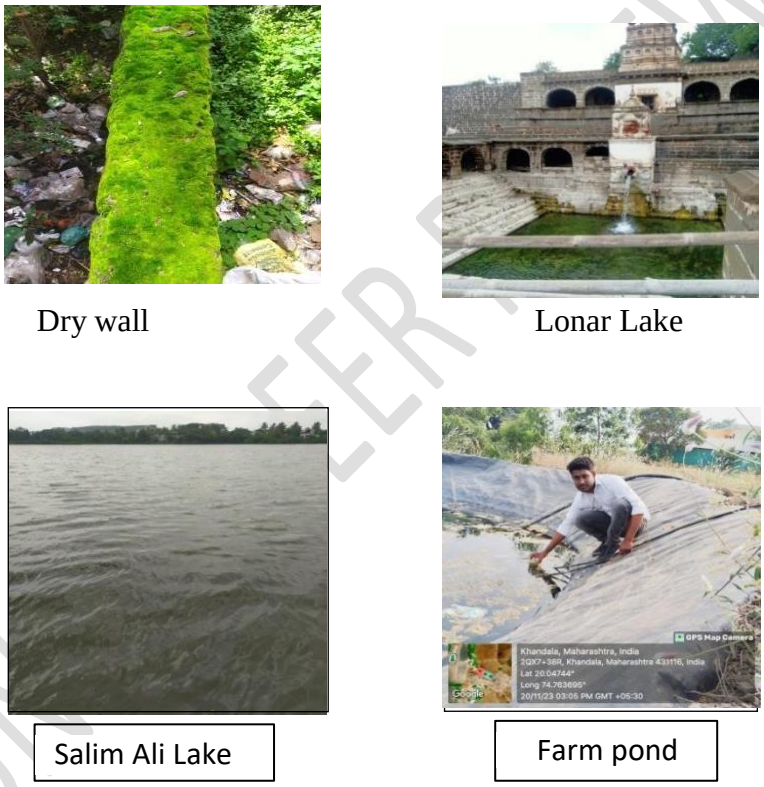


Fig. 1: Microalgal samples collected from different marshy locations

2.2 Isolation of Microalgae

Out of the seven collected samples, three microalgal isolates—designated MG-1, MG-2, and MG-3—were successfully isolated using the colony-picking method (Fig. 2). Isolation was performed after incubation under a light intensity of 1500 lux with a 16:8 h light–dark

photoperiod for 14 days. The isolated strains were further cultivated to evaluate their suitability for oil extraction and biodiesel production. Comparable isolation strategies were reported by Ghani *et al.* (2020) while Dr. Uday Bhaskar (2020) in his work isolated four algae (SP1, SP2, SP3&SP4) in Growth Chamber.

Fig. 2: Isolated microalgae in a growth chamber

2.3 Cultivation of Microalgae

The isolated microalgae (MG-1, MG-2, and MG-3) were cultivated using an open pond system. Profuse algal growth was observed after 15 days of incubation under natural light conditions (Fig. 3). Similar cultivation methods have been employed by Baig *et al.* (2022) and Lenka *et al*. (2015), while Mahya *et al.* (2013) reported alternative systems such as photobioreactors, heterotrophic cultures, and algae turf scrubbers.

Day1 (MG1) **Day8 (MG1)** **Day15 (MG1)**

Day1 (MG1) **Day8 (MG1)**

Day15(MG2)

Fig. 3: Day-wise growth of cultivated microalgae

2.4 Evaluation of Micro algal Growth Potential by Cell Dry Weight Measurement

After 15 days of cultivation, algal biomass was harvested, washed twice with distilled water, and dried at 60 °C for 48 h (Fig. 4). Dry biomass estimation revealed that MG-2 exhibited the highest biomass yield (20.8 g), followed by MG-3 (9.36 g) and MG-1 (7.45 g) (Table 1). All isolates were selected for further analysis. Similar biomass productivity assessments were reported by Zehra *et al* ., (2022).

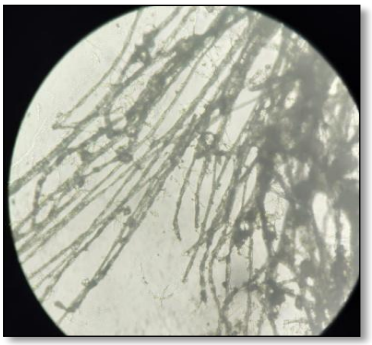
Fig. 4: Dried algal biomass in powdered form

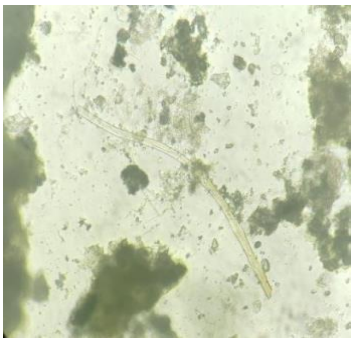
Table 1. Dry biomass yield of isolated microalgae

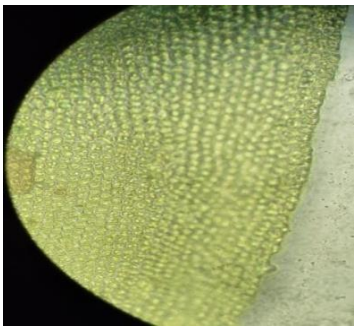
Sample	Dry weight
MG-1	7.45g
MG-2	20.8g
MG-3	9.36g

2.5 Identification of Efficient Microalgae:

Preliminary identification of MG-1, MG-2, and MG-3 was performed based on morphological characteristics such as color, growth pattern, and microscopic features observed under an inverted microscope. Based on cellular morphology—including cell size, sheath structure, pigmentation, presence of akinetes, gas vacuoles, and filament structure—the isolates were identified as *Cynobacteria spp*. (MG-1), *Phormidium spp.* (MG-2), and *Scenedesmus spp.* (MG-3) (Fig. 5). Similar identification methods were reported by Ghani *et al.* (2020), Keesoo *et al.* (2014), and Zehra *et al*. (2022). The most efficient isolate (MG-1) was further confirmed by 16S rRNA gene sequencing. Genomic DNA was extracted using a Nucleospin Microbial DNA Kit and amplified using specific primers. Sequencing was performed using the Sanger method, and the consensus sequence was analyzed using BLAST against the NCBI GenBank database. MG-1 showed 99.17% similarity with an uncultured cyanobacterial clone, and the sequence was submitted to GenBank under accession number *Cyanobacteria spp.MG1* PP702711.







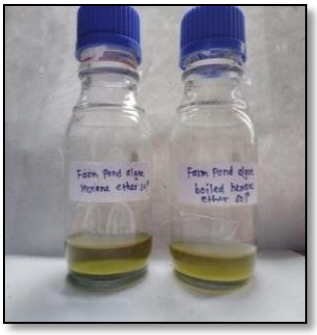
MG-1 (*Cynobacteria spp.)* **MG-2 (*Phormidium spp.)*** **MG-3(*Scenedesmus spp.)***

Fig. 5: Inverted microscopic images of isolated microalgae

2.6 Algal Oil Extraction

Oil extraction was carried out from all three isolates using a hexane–ether solvent mixture under both room temperature and boiling conditions (Fig. 7). Higher oil yields were observed under boiling conditions for *Cyanobacteria spp.*MG1 PP702711 and *Scenedesmus* spp., whereas oil extraction from *Phormidium* spp. was unaffected by temperature (Table 2). The hexane–ether solvent system yielded higher oil recovery compared to acetone or hexane alone. To the best of our knowledge, this study is the first to report algal oil extraction using a hexane–ether mixture under boiling conditions.

Microalgae +Hexane & Ether solution **Oil separation**



Extracted micro algal oil

Fig. 6: Algal oil extraction using hexane–ether solvent

Table 2. Oil yield from different microalgae

Sr. No.	Algae Name	Amount of Algae (in gram)	Oil extracted(in ml)	
			Room Temp.	Boiled
1)	*Cyanobacteria spp.MG1* PP702711	**2.5**	**20**	**23**
2)	*Phormidium spp.*	2.5	21	16
3)	*Scenedesmus spp***.**	2.5	14	31

2.7 Biodiesel Production

Biodiesel was produced from the extracted oils of all three microalgae using an alkali-catalyzed transesterification process (Fig. 8). Among the isolates *Cyanobacteria spp.MG1* PP702711 yielded the highest biodiesel output and was selected for further characterization. From 5 mL of algal oil, up to 4 mL of biodiesel was obtained, demonstrating the efficiency and cost-effectiveness of the process (Table 3). Similar methodologies were reported by Sharif *et al.* (2008), Indumathi *et al.* (2014), and Baig *et al.* (2022).

Fig.No.7: Trans-esterificationofMG-1, MG-2, MG-3.

Cyanobacteria spp.MG1 ***Phormidium spp.*** ***Scenedesmus spp.***

Fig.8: Biodiesel production from microalgae

TableNo.3: Amount of biodiesel produced different sample

Sr. No.	Microalgae name	Amount of algal oil (in ml)		Amount of Biodiesel(in ml)	
		Room temp. solution	Boiled solution	Room temp. solution	Boiled solution
1	*Cyanobacteria spp.MG1* PP702711	5	14	4	5
2	*Phormidium spp.*	5	14	-	7
3	*Scenedesmus spp.*	5	14	1.4	0.58

2.8 Characterization of Biodiesel

The produced biodiesel was evaluated for physicochemical properties including flame test, density, specific gravity, pH, and cetane number (Table 4). *Oscillatoria* spp. biodiesel exhibited the highest cetane number (54), indicating superior ignition quality. Density values ranged from 0.67 to 0.77 kg/L, consistent with reported biodiesel standards.

Cyanobacteria spp.MG1 ***Phormidium spp.*** ***Scenedesmus spp.***

Fig. 9: Flame test of algal biodiesel

TableNo.4: Characterization of Biodiesel

Sr. No.	Biodiesel obtained from microalgae		pH	Specific gravity	Density /L	Cetanenumber
1	*Cyanobacteria spp.MG1* PP702711	RT	6	0.611	0.68	44
		Boiled	6	0.772	0.77	54
2	*Phormidium spp.*	Boiled	6	0.672	0.67	49
3	*Scenedesmus spp.*	RT	5	0.722	0.72	29
		Boiled	6	0.732	0.73	36

2.9 FTIR Analysis

FTIR spectroscopy confirmed that the functional groups present in algal biodiesel closely resembled those of petroleum diesel (Fig 10). Characteristic absorption peaks corresponding to alkanes, amines, nitro groups, and alkenes were observed, validating the chemical compatibility of algal biodiesel as a diesel substitute. Similar observations were reported by Rahman *et al.* (2017).

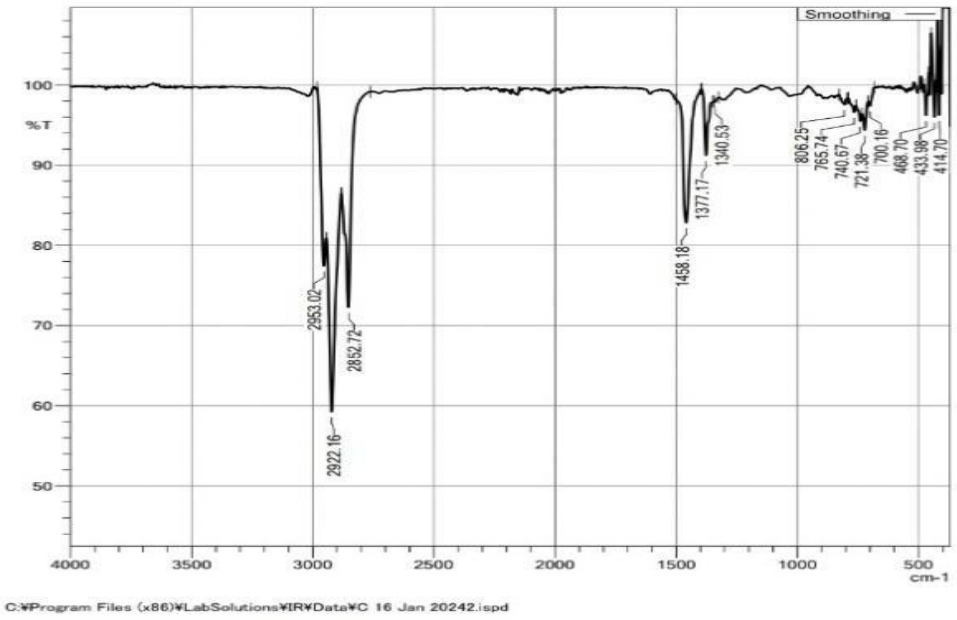


Fig No. 10: FTIR Analysis of Produced Biodiesel

2.10 Partial Purification of Biodiesel

Crude biodiesel was partially purified using column chromatography with activated charcoal. Post-purification, the biodiesel showed reduced impurities, improved clarity, and increased ignition time, confirming enhanced fuel quality (Table 5). Similar purification strategies were reported by Kulkarni *et al.* (2019).

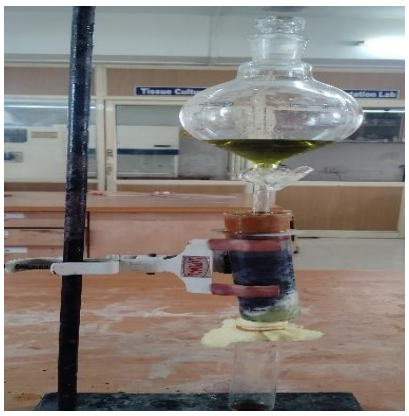


Fig. No.11: Partial purification of Biodiesel column chromatography

Fig. No. 12: Purified & unpurified Biodiesel

Table No.5: Effect of Purification

Unpurified Biodiesel		**Purified Biodiesel**	
Amt. of Biodiesel	**Ignition Time**	**Amt. of Biodiesel**	**Ignition Time**
100µl	**7 sec.**	**100 µl**	**15 sec.**

CONCLUSIONS

Seven microalgal strains were successfully isolated from diverse natural sources, including Lonar Lake, a farm pond, marshy dry walls, Panchakki, Salim Ali Lake, Deogiri Fort, and a dry wall sample from Salim Ali Lake. Among these, the most promising isolates—MG-1, MG-2, and MG-3—were identified under an inverted microscope as *Cyanobacteria spp.MG1* PP702711 *Phormidium spp*., and *Scenedesmus spp*., respectively.

Oil extraction using the hexane–ether method at boiling temperature resulted in higher lipid recovery from all three microalgal species. The extracted oils were efficiently converted into biodiesel via an alkali-catalyzed transesterification process. The produced microalgal biodiesel was characterized using key fuel parameters, including cetane number, aniline point, density, diesel index, and FTIR analysis, which collectively confirmed its suitability as a diesel fuel.

Among the evaluated strains, *Cyanobacteria spp.MG1* PP702711 demonstrated the highest oil yield and biodiesel production, followed by *Phormidium* spp. and *Scenedesmus* spp. Partial purification of biodiesel was successfully achieved using activated charcoal. The high cetane number (54) obtained indicates that algal-based biodiesel is an eco-friendly, cost-effective, and promising alternative to conventional petroleum diesel.

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