

Chlorella sp. can flourish when properly ventilated and supplied with 40% of CO₂¹¹. Microalgae are beneficial in cutting back the hazardous amount of carbon dioxide in the air, but they produce substantially more oil than energy crops typically seen as a source of biodiesel. To create a cost-effective way to manufacture biofuels from algae, a nutrient solution must be developed, an energy-saving harvesting technique must be implemented, and an efficient method for extracting lipids must be discovered.

Chlorella sp. is a green, unicellular freshwater algae with rapid growth and a simple cultivation process. The lipid content of *Chlorella* sp. is approximately 24-38% by weight of dry biomass. A commonly used and successful method for acquiring oil for biodiesel production is the solvent extraction procedure. This is because almost all of the oil is recovered, leaving merely 0.5-0.7% of oil in the feedstock. Furthermore, the operating costs of this method are relatively low compared to the extraction with supercritical fluids¹²⁻¹³. *Chlorella vulgaris* has a strong cell wall. To improve the quality of the oil, ultrasonic-aided oil extraction was implemented to increase the extraction of algal oil, a straightforward and successful process that takes place under low thermal heat conditions and protects the molecular composition of the oil that was taken out¹⁴. This technique is controlled by a range of factors like power, temperature, duration, and amount of solvent.

This research work investigated the extraction of algal oil from *Chlorella* sp. The ultimate objective of the study was to effectively extract algal oil from biomass. The proportions of solvent to biomass, temperature of extraction, duration of extraction, and type of solvent were optimized to increase the highest achievable algal oil yield. GC-MS analysis was used to study the presence of the fatty acids in the extracted algal oil.

Experimental Section

Materials used

The microalgae biomass of *Chlorella* sp. was purchased from True Life Healthcare, Porur, Chennai India. Analytical grade solvents like chloroform, hexane, isopropanol, petroleum ether and dichloromethane were procured from Santosh Scientific Pvt. Ltd. of Ambattur, Chennai. All chemicals used for the extraction process were

analytical grade (99% purity) and were used without further purification for the experiments.

Extraction of algal oil from *Chlorella* sp.

The solvent extraction methods were commonly used because of their high lipid recovery rate, it is therefore essential to maximize the effectiveness of the solvent extraction process¹⁵. Initially, algal oil was extracted from *Chlorella* sp. using homogeneous solvents such as chloroform, hexane, isopropanol, petroleum ether, and dichloromethane. In this study, extraction began with a homogeneous solvent and was then followed by a heterogeneous solvent after comparing oil yield percentage. Compared to homogeneous solvent extraction, heterogeneous solvent extraction yields a higher lipid recovery. Therefore, mixed solvents of chloroform and isopropanol at different ratios ranging from 1:5 to 5:1 were studied for the algal oil extraction from *Chlorella* sp.

A Soxhlet apparatus was used for algal oil extraction process. The 300 mL cylindrical Soxhlet extractor was placed at the top of the round bottom flask with the extractant inside. The upper part of the extractor was connected to a condenser, which was equipped with a water inlet and outlet for cooling. The solvent was heated to create a vapour, which then ascends the arm of the distillation apparatus and enters the chamber holding the thimble packed with algal biomass. This method allows for more complete extraction of samples in less time than many conventional methods and the main advantage is the recovery of the solvent for reuse¹⁶⁻¹⁷.

Optimization of algal oil extraction from *Chlorella* sp.

The 10 g of algal biomass was added to 100 mL of solvent and was sealed with aluminium foil to prevent solvent vapourization. The ultrasonication time was varied between 5-20 min with a constant frequency of 20 kHz and electric power of 250 V AC in an ultrasonic bath. The beaker containing the mixture of algal biomass and solvent was submerged in the ultrasonic bath, and the device was turned on. Following the ultrasonication process, the sample was decanted into a sterile centrifuge vial and the vial was spun at 3500 rpm for 20 min duration. The pellet was used for the extraction of algal oil after the supernatant was collected¹⁸. A Soxhlet apparatus was used for further extraction experiments. Different parameters were optimized in order to get the highest possible yield of algal oil from the process. The

parameters such as algal biomass to solvent ratio (1:3 to 1:6), process time (30-150 min), temperature (30-80°C) and ultrasonication time (5-30 min) were studied and optimized. The extract was stored and the average yield of the algal oil was determined by applying the Eq. (1).

$$\text{Yield (\%)} = \frac{\text{Weight of extracted Algal oil}}{\text{Weight of algal biomass}} \times 100\% \dots(1)$$

Kinetics and activation energy of algal oil extraction from *Chlorella* sp.

A detailed knowledge of kinetics is essential for understanding oil extraction. The purpose of this study is to determine kinetics and activation energy. The activation energy is the minimum amount of energy required for the extraction process to occur. The obtained experimental results provided valuable insights into the dynamics of oil extraction¹⁸⁻²⁰. The rate of algal oil extraction was studied using Eq. (2).

$$\frac{dY}{dt} = kY^n \dots(2)$$

where, Y represents the oil yield in percentage, k represents rate constant, t represents extraction process time, and n represents extraction order.

The Arrhenius equation shown in Eq. (3) was used to calculate the activation energy necessary for algal oil extraction.

$$k = Ae^{-\left(\frac{E_a}{RT}\right)} \dots(3)$$

where, A represents Arrhenius constant, E_a represents activation energy in kJ mol^{-1} , R represents universal gas constant in $\text{kJ mol}^{-1}\text{K}^{-1}$, T represents temperature in Kelvin.

Results and discussion

Effect of different solvents on algal oil extraction from *Chlorella* sp.

The effective oil extraction from algal biomass depends on the choice of a solvent system. To increase the oil yield, the properties should include good extraction and low viscosity. The cell walls of the algal biomass's cell walls are broken by the high polarity, which also extracts lipids from the microalgal cells¹⁹.

Chloroform, hexane, isopropanol, petroleum ether, and dichloromethane were the solvents used in a single solvent system. The experiments were conducted with the following conditions²¹: temperature of about 45°C, time duration of 1 h, solvent to biomass ratio of 3: 1. The algal oil yields were found to be of 10.8, 4.6, 9.1, 1.8, and 2.6% from the solvents Chloroform, hexane, isopropanol, petroleum ether, and dichloromethane, respectively. As shown in Fig. 1 (a), the oil yields from chloroform and isopropanol were found to be high. Consequently, the two solvents were used in heterogeneous solvent extraction in different ratios (1:5, 4:2, 3:3, 2:4 and 5:1) with above experimental conditions and oil yield percentages were found as 9.7%, 10.8%, 12.3%, 11.4% and 10.2% using the volume ratios 1:5, 4:2, 3:3, 2:4 and 5:1, respectively. As depicted in Fig. 1 (b), a maximum algal oil yield of 12.3% was achieved with the volume ratio of 3:3. Therefore, this solvent ratio was used for further extraction processes.

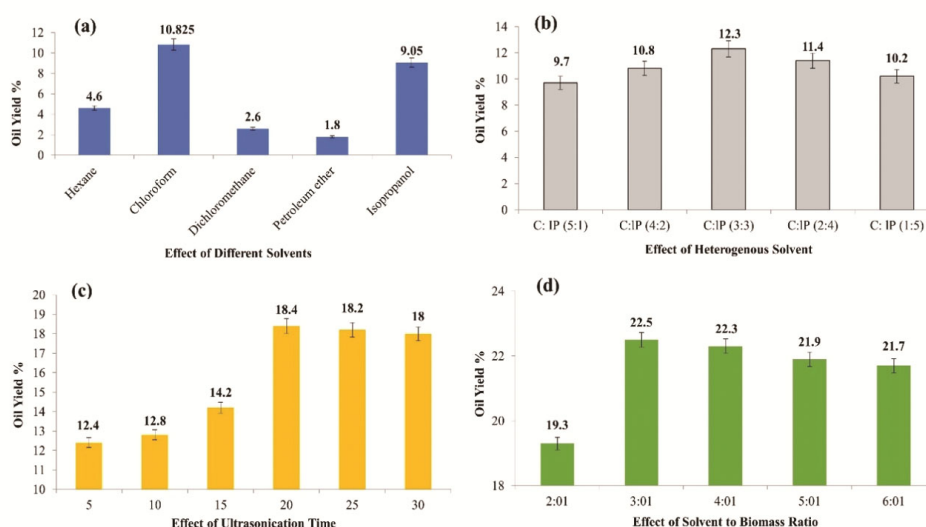


Fig. 1 — Effect of (a) different solvents, (b) heterogenous solvent ratio, (c) ultrasonication time and (d) solvent to Biomass ratio on algal oil yield from *Chlorella* biomass

Effect of ultrasonication time on extraction of algal oil from *Chlorella* sp.

The efficiency of ultrasonic assisted extraction in algal oil extraction was investigated at various time of ultrasonication at optimized solvent ratio and reaction time of 5, 10, 15, 20, 25 and 30 min. According to Fig. 1(c), highest algal oil yield of 18.4 % were observed at 20 min. Due to the ultrasonic wave ability to break down the *Chlorella* sp. cell wall, the ultrasonic assisted extraction produces a significant amount of algal oil. This shows that the ultrasonication pretreatment of the algal biomass will contribute greatly to effective extraction of algal oil from the biomass.

Effect of solvent to biomass ratio on extraction of algal oil from *Chlorella* sp.

The solvent to biomass ratio plays a major role in the extraction process. Thus, it was essential to ensure that this ratio remains within acceptable limits throughout the entire extraction process. In the current study, the effect of solvent to biomass ratio on oil extraction varied in a range from 2:1 to 6:1 was studied by keeping other parameters such as optimized co-solvent ratio and ultrasonication time as constant. When the ratio of solvent to biomass was increased from 2:1 to 6:1, the oil yield increased from 19.3 to 22.5%, as shown in Fig. 2 (a). The algal oil yield of the algal biomass did not improve further when the solvent-to-mass ratio of 3:1 was increased. Therefore, the 3:1 solvent to biomass ratio was used to extract algal oil from the biomass for further experiments.

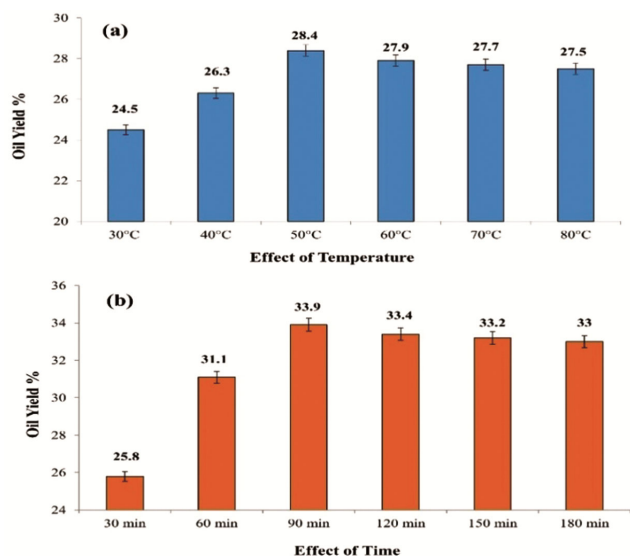


Fig. 2 — Effect of (a) temperature and (b) time on algal oil yield from *Chlorella* biomass

Effect of time and temperature on algal oil extraction from *Chlorella* sp.

The effect of the temperature on the algal oil extraction was studied in the range of 30-80°C. As the temperature rises from 30 to 50°C, the algal oil yield increases from 24.5% to 28.4% shown in Fig. 2 (b). Maximum algal oil yield of 28.4% was achieved at 50°C. The length of the extraction process has a significant impact on the amount of oil that can be extracted from algal biomass. As shown in Fig. 2 (c), the effect of extraction time on algal oil extraction was varied from 30 min to 150 min. The findings show that an algal oil yield of 33.9% was obtained at 90 min, which was found to be the maximum yield. A 3:1 solvent to biomass ratio and a temperature of 50°C were used for the extraction. Even though the time was extended to 180 min, the amount of algal oil extracted remained constant after 90 min. At 90 min of extraction time, the oil extraction yield for *Chlorella* biomass was found to be of 33.9%.

Kinetics and activation energy of algal oil extraction from *Chlorella* sp.

Different time intervals (30, 60 and 90 min) and temperature intervals (30, 40, and 50°C) were used to study the kinetics of the oil extraction process. An illustration of the relationship between $\ln [Y]$ and $\ln (dY/dt)$ was studied at various temperatures and times. The algal oil extraction from *Chlorella* sp. was fitted to first order kinetics because the extraction process depended on the extraction time and temperature. The graph between $\ln K$ and $1/T$ shown in Fig. 3 shows that the activation energy of the extraction process was determined to be $E_a = 10.049 \text{ kJ mol}^{-1}$.

Characterization of extracted algal oil using GC-MS analysis

By using a GC-MS (Model 8890, Agilent) with a 2 mm direct injector and a 15 m column of Alltech EC-5, the fatty acid composition of the extracted algal

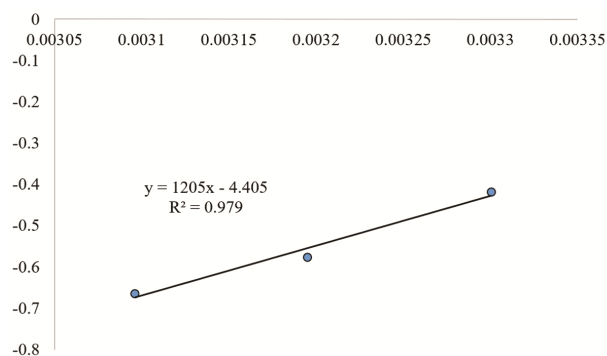


Fig. 3 — Determination of activation energy for algal oil extraction from *Chlorella* biomass

Fig. 4 — GC-MS chromatogram of extracted algal oil from *Chlorella* biomass

oil was examined. The obtained chromatogram of the algal oil was shown in Fig. 4. The presence of fatty acids plays a major role in the production of fatty acid methyl ester. The presence of essential fatty acids thus provides an effective and economical way to produce methyl esters. As observed in the chromatogram, two distinct peaks with a retention time of 10.386 and 16.698 min was found which corresponds to the existence of octadecanoic acid as the predominant compound.

Conclusion

The biomass of *Chlorella* sp. was used to extract the algal oil using the solvent extraction method along with ultrasonication. As a result of one factor optimization, a binary solvent (chloroform: isopropanol) at a 3:1 ratio was found to be the suitable solvent for algal oil extraction from *Chlorella* biomass. The optimized conditions for maximum algal oil extraction was found to be with extraction time of 90 min and the temperature of 50°C under ultrasonic wave influence for 20 minutes. It was observed that 33.9% of the algal oil yields would be achieved under these optimized conditions. Therefore, the presence of the fatty acids from the extracted *chlorella* oil was found to be the potential source for FAME production based on the GC-MS results. This would majorly help in the sustainable production of biodiesel in future years.

References

- 1 Ranjith K R, Hanumantha R P & Arumugam M, *Front Energy Res*, 2 (2015) 61.
- 2 Asif M & Muneer T, *Renew Sust Energy Rev*, 11 (2007) 1388.
- 3 Ahmad S, Kothari R, Pathak V V, Tyagi V V, Pandey A K & Sari A, *Biomass Convers Bior*, 5 (2021) 15.
- 4 Kumar D & Singh B, *Biomass Bioenerg*, 131 (2019) 105398.
- 5 Santos-Ballardo D U, Rossi S, Reyes-Moreno C & Valdez-Ortiz A, *Rev Environ Sci Biotechnol*, 15 (2016) 243.
- 6 Ahmad A L, Yasin N M, Derek C J C & Lim J K, *Renew Sust Energy Rev*, 1 (2011) 584.
- 7 Hein L & Leemans R, *Ambio*, 41 (2012) 341.
- 8 Robak K & Balcersek M, *Food Sci Biotechnol*, 56 (2018) 174.
- 9 Mata T M, Martins A A & Caetano N S, *Renew Sust Energy Rev*, 14 (2010) 217.
- 10 Eloka-Eboka A C & Inambao F L, *Appl Energy*, 195 (2017) 1100.
- 11 Weyer K M, Bush D R, Darzins A & Willson B D, *Bioenergy Res*, 3 (2010) 204.
- 12 Rawat I, Kumar R R, Mutanda T & Bux F, *Appl Energy*, 103 (2013) 444.
- 13 Ahmad A L, Yasin N M, Derek C J C & Lim J K, *Environ Technol*, 35 (2014) 2244.
- 14 Příbyl P, Cepák V & Zachleder V, *Appl Microbiol Biotechnol*, 94 (2012) 549.
- 15 Abdullah N, Amran N A & Yasin N H M, *Malays J Anal Sci*, 21 (2017) 735.
- 16 López-Bascón M A & De Castro M L, *Elsevier*, 1 (2020) 327.
- 17 Veeranan T, Kasirajaan R, Gurunathan B & Sahadevan R, *Renew Energy*, 127 (2018) 64.
- 18 Naveenkumar R & Baskar G, *Ind Crops Prod*, 162 (2021) 113273.
- 19 Baskar G, Naveenkumar R, Mohanapriya N & Aiswarya R, *Ind Crops Prod*, 124 (2018) 954.
- 20 Naveen kumar R & Baskar G, *Energy Sources A: Recovery Util Environ Eff*, 44 (2022) 2497.
- 21 Georgiopoulou I, Tzima S, Pappa G D, Louli V, Voutsas E & Magoulas K, *Molecules*, 27 (2022) 29.