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Isolation And Screening of High-Lipid-Containing Microalgae from Limestone-Rich Aquatic Bodies in Ariyalur District

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Abstract
The study focuses on the isolation and screening of microalgae from aquatic limestone mine environments, with an aim to identify high-performing candidate strains for applications such as biofuel production, wastewater treatment, and chemical extraction. The isolation process includes methods like serial dilution and the use of culture media to separate diverse microalgal species. Following isolation, the strains were screened based on growth rates, morphometric characteristics, and lipid content. To evaluate the biomass and lipid productivity of the isolates, experiments were conducted under controlled conditions, specifically using artificial lighting on an 8:16 hour light/dark cycle at a temperature of 25±2°C. Lipid quantification was performed gravimetrically, employing hexane as the extraction solvent. Among the tested isolates, Scenedesmus sp. stood out with impressive metrics: it achieved a growth rate of 5.73 g/l, a biomass of 3.56 g/l, and a lipid yield of 44.95% of dry cell weight measured on the 29th day of cultivation.The findings indicate that Scenedesmus sp. is the most promising candidate strain; however, the study also suggests that growth enhancement strategies and genetic engineering techniques could be applied to further optimize its biomass and lipid yields. This reflects a trend in biotechnology aiming to harness microalgae for sustainable energy and resource recovery.

1. INTRODUCTION

The isolation and identification of algal species from their natural environments is a recognized technique in phycology. Each algal species has specific growth requirements, influenced by several physicochemical factors that are critical for their growth and development. Among these factors are temperature, pH, and salinity, which vary from species to species and are crucial for optimizing cultivation conditions. Notably, the use of microalgae as a limestone marker has been documented since the work of Glock Waldo in 1923, highlighting the historical significance of these organisms in geological studies. Recent studies by Judd et al. (2015) and Guschina et al. (2015) further support the importance of understanding these environmental parameters in algal cultivation.

Aquatic microalgae represent a diverse group of photosynthetic microorganisms with distinct ecological needs. Identified as some of the earliest life forms on Earth, they have been found within various terrestrial environments. Globally, approximately fifty thousand species of microalgae exist, including Cyanobacteria along with prokaryotic and eukaryotic types; however, only about thirty thousand of these have been analyzed in detail, particularly within freshwater ecosystems such as lakes, ponds, and rivers (Idenyi JN et al., 2016). These microorganisms are seen as invaluable resources for a variety of biotechnological applications, including but not limited to wastewater treatment (as noted by Craggs, R.J. et al., 1996), biodiesel production (Demirbas, A., &Demirbas, M.F., 2011), and serving as dietary supplements for both humans and animals (Enzing et al., 2014; Spolaore et al., 2006). Recent efforts, supported by significant funding, have been directed towards identifying microalgae species that demonstrate high levels of bioactive metabolites, signifying their potential in various industries (Hu, G.P et al., 2008).

The isolation of microalgae strains demonstrating rapid growth, high intrinsic lipid content, and substantial biomass productivity is essential, as highlighted by various studies (Hannon et al., 2010; Elliott et al., 2012; Abdelaziz et al., 2014). The total lipid content in microalgae, encompassing glycolipids, phospholipids, and neutral lipids, shows considerable variation both across and within distinct microalgae groups (Enzing et al., 2014). Research indicates that omega-3 fatty acids comprise 30% to 40% of the total fatty acids present in several microalgae species, including Nannochloropsis sp. (rich in EPA), Schizochytriumlimacinum (rich in DHA), and Phaeodactylumtricornutum (Poblete-Castro et al., 2012). This characterization of microalgae

underlines their potential in human nutrition, serving as supplements for addressing various physiological disorders, enhancing preventive health strategies, and functioning as a source of sustainable synthetic dietary supplements (Beetul et al., 2016).

Microalgae are a valuable source of bioactive compounds, including polyphenols, carotenoids, polysaccharides, omega-3 fatty acids, and polyunsaturated fatty acids (PUFAs), as highlighted by several studies (Peng, J. et.al., 2011; Haimeur, A. et.al., 2012; Goiris, K et.al., 2012). The lipid content of microalgae can vary significantly, ranging from 20% to 70%. This variability in lipid content and the fatty acid composition is chiefly determined by genetic and phenotypic variables, in addition to environmental factors and cultivation methodologies (Nuzzo, G. et.al., 2013). Consequently, for the purpose of large-scale lipid production from microalgae, it is critical to select species that not only demonstrate competitiveness but are also easy to cultivate and capable of adapting to the specific environmental conditions of the cultivation area. Ariyalur district, known for its significant limestone deposits, primarily consists of the Cretaceous Ariyalur group of rocks, notably the Kallankurichi Formation. These limestone deposits, which vary from cement grade to superior quality, are crucial for cement production in the region (Babu et al., 2014). Characteristically, the limestone exhibits a yellowish-brown color, is fossiliferous, and has a compact structure with thicknesses ranging from 8 to 12 meters in some zones. Major cement companies have secured mining leases in the area, with considerable reserves located in the taluks of Ariyalur and Sendurai (NMET, 2019) Additionally, the limestone found in this district is influenced by coccolithophores—microalgae that engage in photosynthesis to absorb and store carbon dioxide from the atmosphere as calcium carbonate. This biomineralization process allows for the creation of limestone at a quicker rate than traditional geological formations, suggesting its potential as a sustainable alternative in construction. The primary objective of the current study was to assess, separate, and isolate various microalgal samples gathered from multiple locations across the Ariyalur district, further exploring their implications and uses in conjunction with local limestone resources.

2. MATERIALS AND METHODS

2.1 Sampling and Isolation

In this study, a total of 116 samples were gathered from various terrestrial (soil) and aquatic (waterbody) locations within the Ariyalur District (Latitude – 11.1503, Longitude – 79.0685) (**Figure -1, Table 1 to 3).** The samples were collected in five distinct stages, as each stage presents the likelihood of discovering different species of microalgae due to seasonal transitions (from summer to rainy and from rainy to winter), which influence changes in environmental conditions. For the terrestrial samples, approximately 100 g of each sample was collected in polybags and mixed thoroughly. All water samples were collected in blue-capped containers, with each containing around 100 ml of water. All samples were subsequently stored at 4°C in a refrigerator.

2.3 Isolation and Enrichment of microalgae

The isolation of various microalgal species was achieved through established techniques using sterilized Bold Basal medium (BBM). The process involved utilizing enrichment culture methods to obtain unialgal colonies, carried out in a controlled culture room with a 16:8 light and dark cycle, maintaining temperatures around 28±20°C and a pH of 8.0 for optimal growth. Water samples were collected and inoculated into autoclaved BBM in 250 ml conical flasks, which were then incubated for 10-15 days under proper light conditions. Green algal growth was observed, and unialgal cells were isolated using Pasteur pipettes. These cells were subsequently re-cultured in a 100 ml conical flask of sterilized BBM for another incubation of 10-15 days. A solid agar medium for culture maintenance was prepared by dissolving 15-18 g of purified agar in 1 liter of medium, followed by autoclaving. Plates containing this medium were also incubated for 10-15 days. Purification of isolated microalgal strains was accomplished through repeated subculturing, plating, and streaking on suitable solid media while maintaining culture purity through regular microscopic examinations. Discrete colonies were inoculated into fresh medium for further studies during the exponential phase.

2.4 Serial Dilution Method

A loopful enrichment sample was prepared using aquatic and soil samples, where 1 g of soil was added to 10 ml of distilled water to capture various microalgal cells. This mixture was streaked onto Petri dishes containing

agar BBM medium. The dishes were incubated under controlled light and temperature conditions for 15 to 20 days, resulting in the observation of multiple green-colored colonies.

2.5 Micromanipulation

The study focused on isolating microalgal cells from various water and soil samples using solid agar-based medium and Bold Basal broth medium (BBM). After multiple reculture steps on agar plates and subculturing in liquid medium, distinct cell growth was observed after an incubation period of 15-20 days. The microalgal colonies, transferred to 250 ml conical flasks with 100 ml of liquid BBM, showed a color change in the medium to green and some flasks developed web-like growth from different algal genera. To achieve unialgal growth from these mixed colonies, Pasteur pipettes were used in a precise transfer method involving sterilized BBM drops on a glass slide. After isolating unialgal cells, they were transferred into a conical flask with sterilized BBM and, following another 15-20 days of incubation, unialgal cell growth was achieved and subsequently transferred to BG-11 medium.

2.6 Purification by axenization of microalgal isolates

A variety of algal cells, along with certain fungal and bacterial colonies, can develop concurrently. To prevent the proliferation of undesirable microbial species, it is essential to purify the desired algal colonies. The purification process employs the axenization technique, specifically utilizing a triple antibiotic solution. This method is particularly aimed at obtaining axenic strains of microalgal cultures, as noted by Kaushik in 1987. Preparation of a triple antibiotic solution for microalgae involves dissolving 100 mg of penicillin G and 50 mg of streptomycin sulfate in 10 ml of distilled water, followed by adding 10 mg of chloramphenicol to 1 ml of 95% ethanol and mixing this with the antibiotic solution. The final solution is filtered through a 0.20 µm cellulose membrane into a sterilized 20 ml vial, which is then sealed with aluminum foil. For the axenization procedure, 25 µl of this triple antibiotic solution is added to 5 ml of nutrient broth, which is inoculated with 0.5 ml of a homogeneous microalgal suspension. After a 5-hour incubation at 25±1°C, the broth is transferred to a sterilized centrifuge tube and centrifuged at 3500 rpm, discarding the supernatant into a phenol solution. The pellet is washed three times with sterilized distilled water, followed by resuspension in 5 ml of BG-11 medium. The inoculated culture tubes are incubated under a light intensity of 3.5 - 4 klux at 28±2°C for 14 days before a portion is transferred back to nutrient broth and incubated overnight at 28±1°C. The cultures are then evaluated for bacterial contamination against a control; if contamination persists, the axenization process is repeated.

2.7 Growth and maintenance

Microalgal strains from various isolates were cultivated in a modified BG-11 medium at a controlled temperature of 30 ± 2°C and a light intensity of 3.5 klux, following a light:dark cycle of 16:8 hours. The pH was maintained between 8.0 and 8.2 for optimal growth. A solid agar medium was prepared by adding 15-18 g of purified agar per liter of BG-11 medium, which was autoclaved for culture maintenance. After 14 days of incubation, cultures were streaked onto agar plates to preserve pure colonies, which were then inoculated into 250 mL flasks with 100 mL of BG-11 medium and incubated until reaching the exponential growth phase over an additional 14 days. Morphological characterization of isolated strains was performed using authenticated identification keys. Additionally, the purity of the cultures was checked through microscopic observations and by routinely streaking onto solid agar-based BG-11 medium. Discrete colonies were subsequently inoculated into fresh medium for studies in the exponential phase, ensuring minimized morphological and morphometric variations.

2.8 Morphological characterization and morphometry of microalgae

The morphological characterization of isolated microalgae involved examining factors such as cell morphology, cell type, filament type, and the presence of heterocysts and trichomes. The identification of microalgal strains adhered to the keys by Desikachary (1959) and Geitler (1932) for cyanophyceae, and those by Komarek et al. (1983), Hindak F (1988), and Prescott G (1961) for chlorophyceae. These morphological studies were conducted on specimens in the exponential growth phase. Measurements of morphological variables were taken from fresh material using a light microscope (Olympus, model: CX40RF200), and micrographs were obtained with an Olympus (CAMEDIA C-5060 WIDEZOOM) digital compact camera.

2.9 Measurement of Growth Rate and Cell Count in Microalgae

All isolated microalgal strains were cultivated under controlled conditions, with growth rates measured on days 15, 21, and 28 using a hemocytometer. To use the hemocytometer, a thick cover glass is placed over the grids, and a small sample of microalgae is introduced. After a one-minute settling period, the grid is visually

inspected for cell distribution before a direct count is performed under a microscope. The grid comprises nine large squares (1mm²), each divided into 25 medium squares (0.23mm each side), and with further subdivisions into 16 small squares (0.05 mm each side). The average cell count is taken from the central large square, along with counts from the 25 medium squares. A total of six counts taken from each medium square are multiplied by a conversion factor of ×10^4 to determine cell density, reflecting the total number of cells in the central large square.

2.10 Physical and Cultural Management

Environmental parameters, including Light, Temperature, and pH, were meticulously controlled in an airtight incubation cum culture room designed for optimal cultural conditions. The room featured six iron racks, each measuring 4×8 feet and divided into three compartments equipped with seven white tube lights on the ceiling. Individual light intensity was adjustable via switches, with measurements taken using a Lux meter. A timer managed the photoperiod for each compartment. Temperature control was achieved through an air conditioner and heater, with monitoring carried out using a thermometer for each rack. pH levels of the isolation and growth medium were maintained at a specific level using a digital pH meter. Additionally, cultural optimization involved adjusting concentrations of various chemicals in the BG-11 medium, thereby enhancing the manipulation of light, temperature, and pH for improved growth conditions.

2.11 Cultivation of microalgal isolates for biomass production

Microalgal biomass was cultivated using liquid BG-11 medium under controlled cultural and environmental conditions. Twenty 250 ml conical flasks, each with 100 ml of BG-11 medium, were autoclaved. A 1 ml aliquot of homogenized algal suspension was inoculated into each flask in a laminar flow bench setting and incubated for 15, 22, and 29 days. Each isolate was cultivated in triplicate to assess biomass and lipid content, with the mean values for biomass weight (g/l) and lipid percentage calculated. The data underwent statistical analysis, including ANOVA, to evaluate the standard error of the mean. The harvested biomass was dried at 60°C for 48 hours, following the methodology by Storms et al. (2014), and subsequently used for lipid quantification.

2.12 Lipid quantification

Lipid quantification for the algal species was performed using a modified gravimetric technique by Bligh and Dyer, as referenced by Storms et al. (2014). About 40 mg of dry algal biomass was weighed and placed into a mortar prewashed with hexane. The biomass was ground for 5 minutes, and hexane was added to create a slurry that was homogenized. This mixture was then centrifuged for 20 minutes at 10,000 rpm. The supernatant was collected in a pre-weighed metal dish and allowed to evaporate in a fume hood. The mass of the extracted oil was measured gravimetrically post-evaporation and represented as a percentage of the dry cell weight (dcw) of the algal biomass.

3. RESULTS

Among the 116 isolates, a total of twenty microalgal species were identified, including *Aphanothece sp., Phormidium sp., Aulosira sp., Gloecapsa sp., Aulosira sp., Lyngbya sp., Chlorella sp., Chroococcus sp., Nostoc sp., Nostoccalcasie sp., Oscillatoria sp., Microcoleus sp., Chlorella sp., Botryococcus sp., Chlorococcum sp., Oscillatoria sp., Microcoleus sp., Chlamydomonas sp.,* and*Scenedesmus sp.***(Figure-2).** The identified species are categorized into two groups of algal taxa: Chlorophyta (green algae) and Cyanobacteria (blue-green algae). The Cyanobacteria isolates include *Aphanothece sp., Phormidium sp., Aulosira sp., Gloecapsa sp., Aulosira sp., Lyngbya sp., Botryococcus sp., Chroococcus sp., Nostoc sp., Nostoccalcasie sp., Oscillatoria sp.,* and*Microcoleus sp.*, while the Chlorophytes comprise *Chlorella sp.* and *Scenedesmus sp.*, along with *Chlamydomonas sp.*and*Chlorococcum sp.* A variety of parameters were examined to assess algal biomass production and lipid accumulation strategies. The experimentation commenced with the isolation of a potent microalgal strain as a candidate for biofuel. Following the isolation of 20 microalgal strains from cyanobacteria and green algae **(Table -5)**, all isolates were initially purified and cultivated under axenic conditions in liquid BG-11 medium. The first experiment focused on measuring the growth rate of all 20 microalgal strains. The growth rate patterns exhibited irregular yet continuous trends for each strain. A subsequent experiment was conducted to observe the lipid content of each isolate under standard laboratory growth conditions. Based on the observed growth rate patterns and lipid accumulation capabilities, two promising strains were selected. Notably, isolate number 18 (*Scenedesmus* AAFW-18) was further cultivated under various altered growth conditions in the laboratory to evaluate its

lipid accumulation strategy. Lipids were extracted from the two promising strains and purified through trans-esterification, resulting in the production of fatty acid methyl esters (FAME).

Isolation of lipid-producing microalgae from terrestrial and aquatic environments Isolation of lipid-producing microalgae was conducted utilizing well-established isolation techniques. The BBM medium was primarily employed for isolating microalgal strains from 116 terrestrial and aquatic samples. The growth of pre-treated samples was monitored over a duration of 15-20 days. The emergence of a greenish hue on the Petri dishes containing solid medium, as well as in flasks with liquid medium, confirmed the growth and presence of certain microalgae. Following this, these algal growths were repeatedly transferred to fresh medium contained in flasks. Subsequently, enrichment culture techniques, serial dilution methods, streak plate methods, and micromanipulation methods were implemented to achieve unialgal growth **(Table- 4)**. Ultimately, these algal isolateswere transferred to the selective BG-11 medium. A total of 20 microalgal isolates were selected and cultivated in pure culture within the laboratory using liquid BG-11 medium. The purification of microalgae was performed to sustain unialgal colonies, for which a triple antibiotic solution was utilized.

3.1 Characterization of isolates (*Visual characterization*)

All 20 isolates were cultivated in liquid and solid BG-11 medium for 15 days, revealing diverse growth patterns. Isolates 07 and 04 had a yellowish-green coloration on Petri plates, while isolate 14 displayed scattered cells in the flask. Isolates 19, 14, 11, 10, and 06 showed similar visualization, with isolate 06 demonstrating the best growth on the Petri dish. Isolates 18, 17, 15, and 08 exhibited light green coloration in the flask, hinting at their possible identification as green algae. Isolates 20 and 02 formed dot-like colonies on Petri plates and jelly-like structures in flasks. Isolate 13 presented a network of fine filaments in both environments, and isolate 09 revealed a dense green cellular mass in the flask.

3.2 Microscopy of isolates

Microscopy was conducted on 20 isolates at a magnification of 40X using an Olympus light microscope, with micrographs captured via a CAMEDIA digital camera **(Figure -3)**. The examination focused on various characteristics: thallus color, trichome presence, heterocyst existence, cell type (prokaryotic or eukaryotic, transparent or opaque), and cell shapes (including filamentous, oval, flat, rectangular, and circular). Utilizing identification keys from Desikachary (1959) and Geitler (1932), all isolates were classified at the genus level. Additionally, morphometric analysis was performed on 15-day-old cultures using Image Pro+ 4.5 software, revealing distinct shapes for the isolates: isolates 20, 08, 07, and 06 were barrel-shaped; isolate 19 was oblong; isolates 18, 15, and 14 were nearly rectangular; isolates 17, 13, 12, 11, and 09 were cylindrical; isolates 10, 05, 03, and 01 were rectangular; and isolates 04 and 02 were elongated. **(Table -6).**

3.3 Measurement of Growth Rate

The evaluation of the growth rates of various isolates in liquid BG-11 medium was performed using a hemocytometer to obtain direct cell counts from a 1 ml cell suspension under a light microscope. Data were collected in triplicate on the 15th, 22nd, and 29th days. Across all isolates, growth patterns were irregular yet continuous, as detailed in Tables 4 and 7. On the 15th day, isolates 18, 15, 14, 13, 11, 10, 05, 04, 02, and 01 exhibited rapid growth, while the others showed moderate increase. By the 22nd day, isolates 20, 17, and 13 demonstrated significantly higher growth rates compared to the others, who maintained regular growth. By the 29th day, almost all isolates showed marked improvement, with isolates 20, 19, 18, 17, 16, 15, 14, 13, 08, 03, 02, and 01 achieving exceptional growth. The highest growth rates were observed in isolates 01, 20, and 03 on the respective days of measurement, while the lowest rates were recorded for isolates 20, 03, and 15. This study indicates varying growth dynamics among the isolates over the observation period.

3.4. Estimation of Dry Biomass (g/l) and Lipids (%)

All microalgal isolates were cultivated in 250 mL flasks with 100 mL of BG-11 medium under standard growth conditions for 15, 22, and 29 days, totaling 180 flasks in the incubation chamber. Biomass was collected through centrifugation and dried at 60℃, with results detailed in Table 8 and Figure 4. Biomass accumulation ranged among the 20 isolates, with isolate number 18 showing the highest biomass at 1.44±0.15 g/l (15th day), 2.86±0.007 g/l (22nd day), and 3.56±0.0007 g/l (29th day), while the lowest was recorded for isolate number 11 at 0.13±0.09 g/l (15th day), 0.45±0.0007 g/l (22nd day), and 0.66±0.0005 g/l (29th day). Notably, isolate number 14 also showed significant biomass on the 29th day. The increased biomass accumulation may correlate with enhanced lipid content. Lipid extraction followed the Bligh and Dyer (1959) method, revealing a progressive increase in lipid percentage from day 15 to day 29 (refer to Table 9 and Figure 5). Except for isolates 19 (11.16%), 18 (23.73%), 15 (8.23%), and 14 (7.63%), most showed lipid content below 10% by

day 29, with over 10 isolates accumulating less than 10%. However, a linear increase in lipid percentage was consistent across all microalgal isolates throughout the extended incubation periods.

4.DISCUSSION

A total of twenty microalgal species were isolated. Although the Ariyalur district Aquatic water bodies is a diverse range of microalgal species (Tendaupenyu 2012; Mhlanga and Mhlanga 2013), these twenty species were particularly robust, inherently tolerant, and quickly adapted to laboratory conditions, thereby facilitating their isolation. The isolates were classified into two groups of algal taxa: Chlorophyta (including*Scenedesmus sp., Chlamydomonas sp., Chlorococcum sp., Chlorella sp.,* and Botryococcus *sp.)* and C*yanobacteria (comprising Aphanothece sp., Phormidium sp., Aulosira sp., Gloecapsa sp., Lyngbya sp., Chroococcus sp., Nostoc sp., Nostoccalcasie sp., Oscillatoria sp.,* and *Microcoleus sp.*). Cyanobacteria have long been recognized as significant sources of toxins in Lake Chivero and its associated rivers (Mhlanga et al. 2006). This group of algae has also been observed to have limited potential for biodiesel production due to its preference for glycogen and/or polyhydroxyalkanoates as carbon storage molecules rather than lipids (Lynch *et al*. 2015). In Chlorophytes have been extensively researched, and their potential for lipid production has been established (Lynch *et al.* 2015; Kalsum et al. 2018; Li et al. 2020; Khalaji*et al*. 2021). They primarily utilize triacylglycerols (TAGs) as their main carbon storage molecules (Lynch *et al.* 2015). Not only do algae from the Chlorophytagroup excel as lipid producers, but they are also relatively easy to culture (Massimi and Kirkwood 2016). This enhances their viability as feedstock for lipid production. Growth rates play a crucial role in lipid production as they influence the quantity of lipid yield over a specified duration. Algal species exhibiting elevated growth rates facilitate lipid accumulation in the shortest possible timeframe (Massimi and Kirkwood 2016). Furthermore, high growth rates enable microalgae to surpass most potential contaminants (Luangpipat 2013). Consequently, comparative growth assays were conducted under controlled laboratory conditions in this research. The indigenous *Scenedesmus sp*. demonstrated the highest growth rate among all the isolates, achieving an exponential growth rate of 5.73 in 29 the day. A similar finding was reported by Kim *et al.* (2013) when they subjected in Chlorella sp. to autotrophic cultivation. Nevertheless, other studies utilizing the same species indicated significantly higher growth rates than those recorded in this investigation (Duong et al. 2015; Juntila*et al.* 2015). These discrepancies may be attributed to differences in culture conditions and genotypic variations. The native *Scenedesmus sp*. attained a growth rate sufficiently high to exceed that of its foreign counterpart (imported *Scenedesmus sp.*). However, pairwise comparisons utilizing Tukey's HSD test revealed that the difference in growth rates between the two strains is not statistically significant ($p>0.05$). The laboratory conditions to which the microalgae were subjected were unfamiliar to both strains, resulting in no strain demonstrating a technical advantage over the other regarding biomass yield. However, the native *Scenedesmus* isolate displayed a favorable characteristic for lipid production in terms of growth. This finding corroborates the hypothesis proposed by this study, which posits that bioprospecting in a genetically underexplored country may indeed lead to the identification of isolates with competitive growth rates and lipid yields. The native *Scenedesmussp*and imported *Scenedesmus sp*. did not exhibit lag phases indicating their strong adaptability to new environmental conditions (Shen *et al.* 2017). Rapid adaptability is a sought-after biological trait and serves as a primary selection criterion in the cultivation of algal biomass for biofuels, as it influences the feasibility of growing that microalgal species in cost-effective yet extreme environments such as wastewater (Shen *et al.* 2017). Similar studies in *Scenedesmus* sp. ISTGA1 isolated from marble mining site came out to be a potential candidate for CO_2 sequestration, efficiently incorporating excess CO_2 into biomass for subsequent use in biodiesel production. The growth in terms of OD, Chlorophyll a content, biomass productivity and lipid content of the isolate were maximal under excess inorganic carbon supplementation reported RituTripathi et al., 2015

In contrast, Microcystis sp. displayed lag phases, indicating a lack of adaptability to new environmental conditions. Under local geographical, climatic, and ecological conditions, algal strains are less likely to exhibit dominance and high adaptability compared to their native counterparts (Duong 2016). Therefore, the native *Scendesmus sp*. emerges as a more suitable candidate for lipid production. Its prolonged growth phase, in contrast to its foreign counterpart, facilitates greater accumulation of essential biomass. Despite the general perception that chlorophytes are resilient and capable of producing high biomass yields, this was not observed in the isolated *Scenedesmus sp*.in BBM

Technological advancements may be further utilized to enhance the productive capabilities of the isolated strains. Existing technologies, such as genetic engineering, can be applied to improve the biomass and lipid yield of these isolates (Duong 2016). Additionally, the manipulation and optimization of the culture microenvironment can further augment the biomass and lipid yield potential of the isolates. The environmental conditions of the culture significantly affect the types of fatty acids produced, which ultimately influence the quality of the biodiesel (Li *et al.* 2020). Research has shown that lipid yield in cultured algae can increase as nitrogen levels in the culture media are depleted (Tan *et al.* 2016). Nitrogen deprivation triggers a reallocation of photosynthetic ATP towards lipid biosynthesis (Cobos*et al.* 2017). Thus, induced nitrogen starvation may result in optimal lipid yield. Furthermore, spectral intensity is also a contributing factor to enhanced lipid synthesis in algae. Under high spectral intensities, algae tend to produce greater amounts of lipids, a strategy believed to serve as a protective mechanism against harsh environmental conditions (Junying et *al.* 2013). The quality of the spectral light can also stimulate lipid synthesis in microalgae. The activity of the enzymes carbonic anhydrase and ribulose bisphosphate carboxylase/oxygenase (Rubisco) is enhanced by blue light. These enzymes are essential for the microalgal carbon cycle and significantly influence lipid yield (Vadivelloo*et al.* 2015; Sero*et al.* 2020). Consequently, lipid production in algal cells can be optimized by inducing nitrogen starvation while utilizing the optimal spectral quality and intensity after the necessary biomass yield has been achieved.

5. CONCLUSION

The study investigates the isolation and screening of microalgae from limestone mine environments to identify strains suitable for biofuel, wastewater treatment, and chemical extraction. Isolation involved serial dilution and culture media methods, followed by screening for growth rates, morphometric traits, and lipid content under controlled conditions (25±2°C, 8:16 hour light/dark cycle). Scenedesmus sp. emerged as the top candidate, with a growth rate of 5.73 g/l, biomass of 3.56 g/l, and lipid yield of 44.95% dry cell weight on day 29. The study highlights potential growth enhancement strategies and genetic engineering to further optimize yields, aligning with trends in biotechnology for sustainable energy and resource recovery.

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Figure-1 Fresh water Microalgae in Ariyalur district

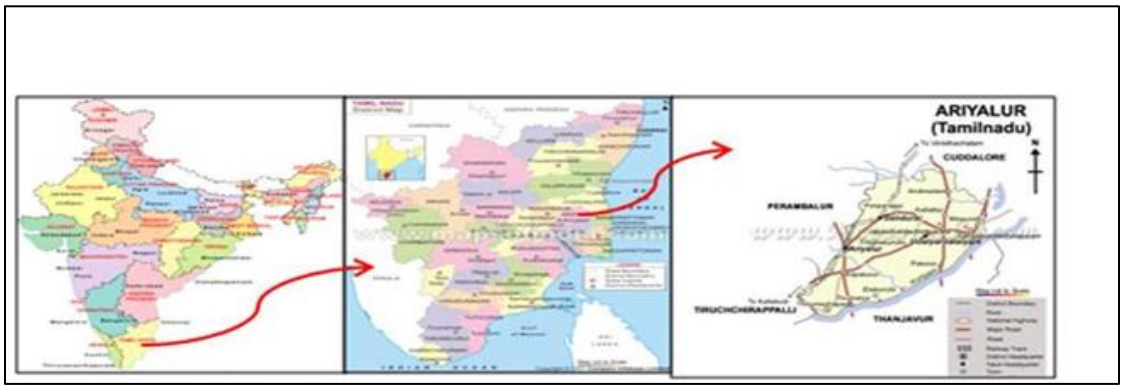

Figure-2

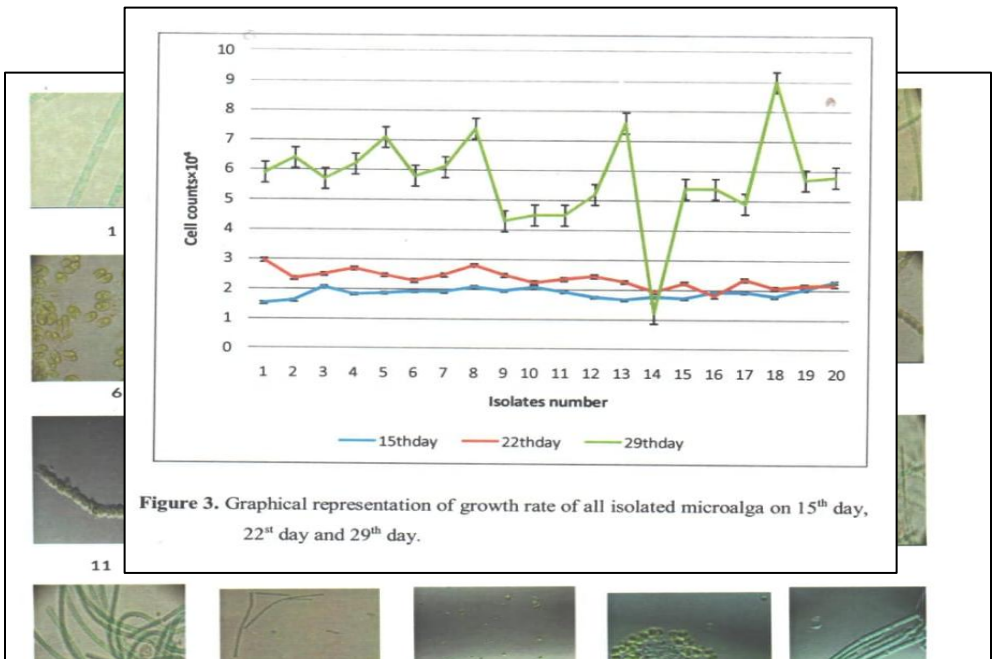

Figure 3. Graphical representation of growth rate of all isolated microalga on 15th day, 22st day and 29th day.

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Table 1. Ariyalur District block wise samples detail.

Sampling Site	LAKE/TANK Ariyalur :block	LAKE/TANK Thirumanur Block	LAKE/TANK Jayakondam Block	LAKE/TANK T.Palur Block	LAKE/TANK Andimadam Block	LAKE/TANK Sendurai Block	Total
Terrestrial	6	14	14	12	8	7	61
Aquatic	13	16	7	5	7	7	55
Total	19	30	21	17	15	14	116

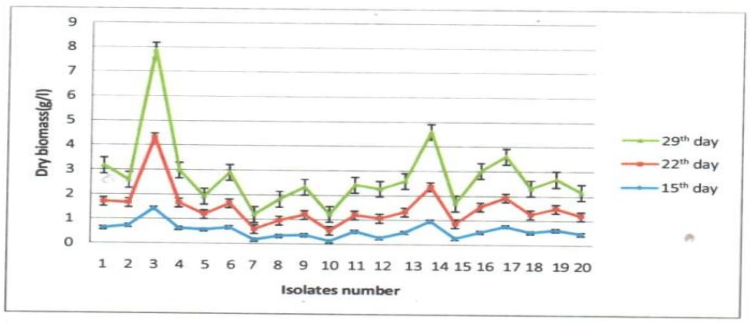

Figure 4. Graphical representation of dry biomass of all isolateson15th day, 22nd day and 29th day.

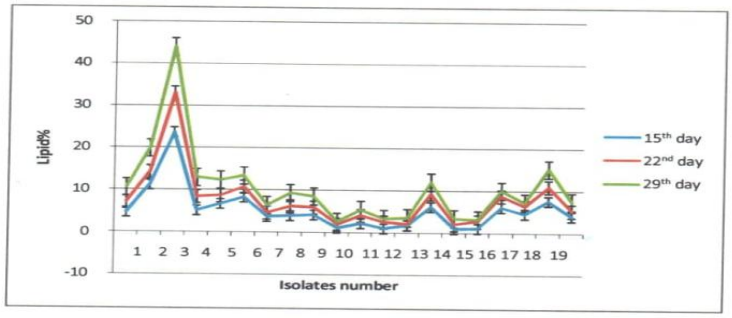

Figure. 5. Graphical representation of lipids % of all isolateson15th day, 22nd day and 29th day.

Table 2.AriyalurDistrict block wise Samples type (on the basis of type of aquatic body and soil habitat).

S.No.	Type of sample	No. of samples
1.	Moist soil samples	41
2.	Fresh water samples	4
3.	Sewage water samples	12
4.	Pond water samples	3
5.	Cropland soil samples	20
6.	Lake water samples	30
7.	Running water samples	3
8.	Shallow water samples	3
Total no. of samples	-	116

Table 3.AriyalurDistrict block wise Codes of samples collected for present study.

Location	Terrestrial site*	Aquatic site*
Ariyalur :Block	AMSS 01,AMSS 02.AMSS 03, AMSS 04, ACLS 05,ACLS 06	AMSW/ 07,AMSW 08,AMSW 09,AMSW 10,ANLW 11,AKLW 12,ASPW 13,AVLW 14,AVLW 15 ,ATLW 16,ARPW 17 ,AAFW 18,AUPW19
Thirumanur Block	TMSS 20|,TMSS 21,TMSS 22,TMSS 23, TMSS 24,TMSS 25,TMSS 26,TMSS 27 TMSS 28,TMSS 29,TCLS 30,TCLS 31 TCLS 32,TCLS 33	TMSW34,TMSW 35, TFWS36,TFWS37,TFWS 38, TSWS 39,TSWS 40,TSWS 41,TPRW 42,TNRW 43,TALW 44,TTLW 45,TMLW 46,TSLW 47,TVLW 48,TVLW 49
JAYANKONDAM Block	JMSS 50,JMSS 51,JMSS 52,JMSS 53,JMSS 54,JMSS 55,JMSS 56,JMSS 57,JMSS 58,JMSS 59,JCLS 60,JCLS 61,JCLS 62,JCLS 63	JMSW 64,JMSW 65,JMLW 66 ,JPLW 67,JPLW 68,JVLW 69,JMSW 70
T.PALUR Block	PMSS 71,PMSS 72,PMSS 73,PMSS 74,PMSS 75,PMSS 76,PMSS 77,PCLS 78,PCLS 79,PCLS 80,PCLS 81,PMSS 82	PSLW 83,PKLW 84,PSLW 85,PPRW 86,PMSW 87
ANDIMADAM BLOCK	AMSS 88,AMSS 89,AMSS 90,AMSS 91,AMSS 92,ACLS 93,ACLS 94,AMSS 95	AALW 96,ASLW 97,AVLW 98,APLW 99,AMSW 100,AKLW 101,AKLW 102
SENDURAI BLOCK	SCLS 103,SCLS104,SCLS105,SCLS106, SMSS 107,SMSS 108,SMSS 109	SSLW 110,SNLW 111,SELW 112,STLW 113,SMSW 114,SNLW 115,SKLW 116

***Abbreviations:**
AMSS:Ariyalur block moist soilsample;AMSW:AriyalurblockMunicipal sewage water; ACLS:AriyalurblockCropland soil samples;ANLW:Ariyalur block Nagamangalam Andi Eri lake water;AKLW:Ariyalur block KallankurichiEri lake water;ASPW:Ariyalur block AriyalurSitheri lake water;AVLW:Ariyalur block VilankudiPeriyaEri lake water;AVLW:Ariyalur block VellurPeriyaEri lake water ;ATLW ;Ariyalur block ThelurPeriyaEri lake water;ARPW:Ariyalur block RayampuramEri Pond water;AAFW:Ariyalur block AgaramPeriyaEri lake water;AUPW:Ariyalur block UppuodaiPeriyaEri Pond water;TMSS:Thirumanur block moist soil sample;TMSW :ThirumanurblockMunicipal sewage water;TFWS:ThirumanurblockFreshwatersample;TSWS:Thirumanurblockshallow water sample;TPRW:Thirumanur block PullambadibCanel running water;TNRW:ThirumanurblockNanthiyarrunningwater;TALW:Thirumanurblock ArasanEri lake water;TTLW:ThirumanurblockThoothurEri lake water;TMLW; ThirumanurblockManodalEri lake water; TSLW ; ThirumanurblockSukkiranEri lake water ;TVLW :ThirumanurblockVettakudiEri lake water;TVLW:ThirumanurblockVannanEri lake water;TCLS:ThirumanurblockCropland soil samples;JMSW ;**Jayakondam**blockMunicipal sewage water;JCLS:**Jayakondam**blockCropland soil samples:**JMSS;Jayakondam** block moist soilsample;JPLW :**Jayakondam** block PonEriErilakewater;JVLW:**Jayakondam** block ValavanEri lake water:JPLW:**Jayakondam** block Pandian Eri lake water;JVLW:**Jayakondam** block VeeramangudiOdaiEri lake

water;PMSW ;**T.Palur**blockMunicipal sewage water;PCLS:**T.Palur**blockCropland soil samples:**PMSS;T.Palur**block moist soilsample;PSLW :**T.Palur** block SripuranthanPeriyaEri lake water;PKLW**:T.Palur** block KaraikurichiKovathattaiEri lake water;PSLW**T.Palur** block SundakudiEri lake water;AMSW ;**Andimadam**blockMunicipal sewage water;ACLS:**Andimadam**blockCropland soil samples:**AMSS;Andimadam** block moist soilsample;AKLW :**Andimadam** block KallankuliEri lake water;AKLW**:Andimadam** block KattathurPeriyaEri lake water;AALW**Andimadam** block AnikuthichanEri lake water;ASLW**Andimadam** block SathanapattuEri lake water;AVLW**Andimadam** block VilanthaiPeriyaEri lake water;APLW**Andimadam** block PeriyaKrihnapuramPeriyaEri lake water;SMSW ;**Sendurai**blockMunicipal sewage water;SCLS:**Sendurai**blockCropland soil samples:**SMSS;Sendurai** block moist soilsample;SNLW :**Sendurai** block NakkampadiPeriyaEri lake water;SKLW**:Sendurai** block KulmurPeriyaEri lake water;SSLW**Sendurai** block SenduraiPeriyaEri lake water;SNLW**Sendurai** block NallanayagapuramEri lake water;SELW**Sendurai** block EachankattuPeriyaEri lake water;STLW**Sendurai** block ThalavaiPeriyaEri lake water;

Table- 4 Effect of liguid/Solid BBM Medium on mono algae colonies of Ariyalur District Aquatic body and soil habitat.

S.No.	Isolate No.	Isolate code	Sampling site	Visual observations recorded from petridish/liquid medium
1	01	JVLW 69	Lake water sample	Bright green colored cellular growth appeared in flask/dark green granules appeared on the petriplate
2	02	TCLS 33	Moist soil sample	Dark green clump of cells appeared in flask/dots of cells appeared on petri plate
3	03	TVLW 49	Lake water sample	Dark green cells were appeared in flask/ petriplate
4	04	JMSW 70	Sewage watersample	Yellowish green color appeared on petriplate
5	05	SMSW 114	Sewage water sample	Dark green color mass of cells appeared in flask/petri plate
6	06	SMSS 108	Moist soil sample	Green color appeared on petri plate
7	07	AWLW 14	Fresh water sample	Yellowish green color appeared on petriplate/scattered group of cells appeared in flask
8	08	**SMSW 114**	Sewage water sample	Light green cellular mass was seen abundantlyin flask/clear greenish layer of cells appearedon petri plate
9	09	**SCLS 106**	Garden soil sample	Dark green dense slippery mass of cells wasappeared in flask/thick layer of dark green cellsappeared on petri plate
10	10	**ACLS 93**	Moist soil sample	Green color appeared on petri plate/in flask
11	11	**PCLS 80**	Crop land soilsample	Green color appeared on petri plate/in flask
12	12	**AUPW 19**	Pond water sample	Dense green cellular mass appeared in flask
13	13	**JMSS 56**	Moist soil sample	Net of fine filaments appeared on petriplate/similarly appeared in flask
14	14	**ARPW 17**	Pond water sample	Green color liquid appeared in flask
15	15	**AMSS 88**	Moist soil sample	Bright Green color homogenous solution inflask/growth on petri plate
16	16	**TSWS 40**	Shallow watersample	Dense green cellular mass appeared in flask
17	17	**AMSW 09**	Sewage watersample	Very light color solution appeared inflask/growth appeared on petri plate

18	18	AAFW 18	Fresh water sample	Light green color liquid appeared in flask
19	19	TFWS 38	Fresh water sample	Green color liquid appeared in flask
20	20	TMSW34	Sewage watersample	Dark green jelly like growth appeared inflask/dots on petri plate

Table -5 Isolatedmicroalgal strains of Aquatic body and soil habitat of Ariyalur district.

S.No	Isolate No.	Generic Name	Division
1	01	*Aphanothece sp.*	Cyanobacteria
2	02	*Phormidium sp.*	Cyanobacteria
3	03	*Aulosira sp.*	Cyanobacteria
4	04	*Lyngbya sp.*	Cyanobacteria
5	05	*Gloecapsa sp.*	Cyanobacteria
6	06	*Aulosira sp.*	Cyanobacteria
7	07	*Lyngbya sp.*	Cyanobacteria
8	08	*Botryococcus sp.*	Green algae
9	09	*Chroococcus sp.*	Cyanobacteria
10	10	*Nostoc sp.*	Cyanobacteria
11	11	*Nostoccalcasie sp.*	Cyanobacteria
12	12	*Ossilatoria sp.*	Cyanobacteria
13	13	*Microcoleus sp.*	Cyanobacteria
14	14	*Chlorella sp.*	Green algae
15	15	*Chlorococcum sp.*	Green algae
16	16	*Ossilatoria sp.*	Cyanobacteria
17	17	*Chlamydomonas sp.*	Green algae
18	18	*Scenedesmus sp. .*	Green algae
19	19	*Chlorella sp.*	Green algae
20	20	*Phormidium sp.*	Cyanobacteria

Table :6 Analysis of 20 Microalgae streams of Aquatic body and soil habitat of Ariyalur district.

Isolate No.	Generic Name	Cell shape	Length(µm)	Breadth(µm)
01	*Aphanothece sp.*	Rectangular	2.39-5.68	2.11-5.58
02	*Phormidium sp.*	Elongated	2.67-5.90	2.53-4.27
03	*Aulosira sp.*	Rectangular	3.85-7.02	3.25-5.04
04	*Lyngbya sp.*	Elongated	2.88-5.75	2.10-4.84
05	*Gloecapsa sp.*	Rectangular	3.85-6.09	2.67-5.04
06	*Aulosira sp.*	Barrel	2.35-4.13	1.8-2.48
07	*Lyngbya sp.*	Barrel	4.27-8.44	4.12-6.84
08	*Botryococcus sp.*	Barrel	4.12-8.74	3.32-6.59
09	*Chroococcus sp.*	Cylindrical	3.08-5.98	2.92-6.28
10	*Nostoc sp.*	Rectangular	3.08-6.67	2.49-7.98
11	*Nostoccalcasie sp.*	Cylindrical	3.12-5.69	2.59-5.55
12	*Ossilatoria sp.*	Cylindrical	3.56-6.56	3.34-6.67
13	*Microcoleus sp.*	Cylindrical	3.89-6.67	2.45-4.78
14	*Chlorella sp.*	Elongated & almost rectangular	3.23-7.59	3.89-4.90
15	*Chlorococcum sp.*	Elongated & almost rectangular	2.96-6.98	2.23-6.01

16	*Ossilatoria sp.*	Barrel	4.10-6.48	5.98-7.67
17	*Chlamydomonas sp.*	Cylindrical	3.10-5.45	2.78-3.61
18	*Scenedesmus sp. .*	Elongated & almost rectangular	4.12-8.74	3.32-6.59
19	*Chlorella sp.*	Oblong	2.88-7.88	2.11-5.75
20	*Phormidium sp.*	Barrel	2.89-5.54	2.10-4.23

Table 7 : Effect of growth and measurement rate at different incubation periods

Cell counts									
Isolate No.	**15th day**			**22nd day**			**29th day**		
	Mean (g/l)±SE	S.D.	C.V. %	Mean (g/l)±SE	S.D.	C.V. %	Mean (g/l)±SE	S.D.	C.V. %
01	2.36±0.02	0.04	1.84	2.17±0.008	0.16	0.07	5.82±0.16	0.032	0.05
02	2.03±0.01	0.01	0.94	2.14±0.11	0.023	0.10	5.73±0.14	0.028	0.04
03	1.77±0.02	0.05	2.95	2.07±0.009	0.019	0.09	9.0±0.018	0.036	0.04
04	1.91±0.02	0.04	2.08	2.34±0.014	0.029	0.12	4.92±0.013	0.027	0.05
05	1.93±0.02	0.04	2.23	1.84±0.01	0.033	0.18	5.14±0.017	0.034	0.06
06	1.54±0.01	0.03	2.07	1.94±0.014	0.029	0.15	5.41±0.012	0.024	0.04
07	1.77±0.009	0.01	0.97	2.24±0.015	0.030	0.13	1.23±0.012	0.024	0.19
08	1.70±0.01	0.02	1.45	1.94±0.014	0.029	0.15	7.63±0.018	0.036	0.04
09	1.77±0.01	0.02	1.13	2.27±0.009	0.019	0.08	5.23±0.017	0.034	0.06
10	1.93±0.01	0.024	1.12	2.44±0.014	0.029	0.12	4.51±0.017	0.034	0.07
11	2.0±0.006	0.01	0.64	2.34±0.014	0.029	0.12	4.50±0.008	0.016	0.03
12	1.95±0.015	0.03	1.53	2.24±0.014	0.029	0.13	4.32±0.016	0.032	0.07
13	2.06±0.006	0.01	0.64	2.46±0.006	0.013	0.05	7.43±0.018	0.036	0.04
14	1.94±0.01	0.03	1.74	2.83±0.018	0.036	0.12	6.13±0.018	0.036	0.05
15	1.94±0.01	0.02	1.50	2.46±0.006	0.013	0.05	5.81±0.007	0.015	0.02
16	1.84±0.01	0.03	1.73	2.27±0.009	0.018	0.07	7.12±0.013	0.027	0.03
17	1.84±0.01	0.03	1.65	2.61±0.09	0.018	0.71	6.22±0.013	0.027	0.04
18	2.16±0.02	0.04	2.25	2.53±0.018	0.036	0.14	5.73±0.017	0.34	0.05
19	1.66±0.02	0.04	2.42	2.36±0.005	0.011	0.04	6.42±0.010	0.021	0.03
20	1.94±0.01	0.03	1.64	2.95±0.015	0.03	0.10	5.93±0.017	0.035	0.05

Table 8 .Estimates of dry biomass (g/l) of 20 micro algal isolates at three incubation periods

Dry biomass (g/l)									
Isolate No.	15th day			22nd day			29th day		
	Mean (g/l)±SE	S.D.	C.V. %	Mean (g/l)±SE	S.D.	C.V. %	Mean (g/l)±SE	S.D.	C.V. %
01	0.46±0.005	0.011	0.24	0.73±0.006	0.013	0.17	0.96±0.005	0.011	0.11
02	0.64±0.009	0.018	0.28	0.86±0.005	0.011	0.13	1.22±0.011	0.022	0.18
03	0.54±0.006	0.013	0.23	0.74±0.005	0.011	0.15	1.07±0.007	0.015	0.14
04	0.76±0.005	0.011	0.14	1.15±0.009	0.019	0.16	1.66±0.006	0.013	0.07
05	0.54±0.007	0.015	0.29	1.03±0.011	0.023	0.23	1.46±0.007	0.015	0.10
06	0.26±0.005	0.011	0.42	0.63±0.012	0.024	0.37	0.85±0.007	0.015	0.18
07	0.96±0.005	0.011	0.11	1.43±0.014	0.028	0.19	2.24±0.007	0.015	0.07
08	0.52±0.012	0.025	0.49	0.84±0.005	0.011	0.13	1.26±0.007	0.015	0.12
09	0.26±0.005	0.011	0.42	0.83±0.011	0.023	0.28	1.22±0.014	0.028	0.23
10	0.55±0.007	0.014	0.26	0.66±0.005	0.01	0.15	1.23±0.009	0.019	0.15
11	0.13±0.009	0.019	1.39	0.45±0.007	0.15	0.35	0.66±0.005	0.011	0.17
12	0.36±0.005	0.011	0.31	0.83±0.011	0.023	0.27	1.13±0.011	0.023	0.20
13	0.34±0.005	0.011	0.32	0.64±0.005	0.011	0.17	0.86±0.005	0.011	0.13
14	0.16±0.005	0.011	0.68	0.44±0.007	0.015	0.35	0.63±0.011	0.023	0.37
15	0.66±0.005	0.011	0.17	0.96±0.005	0.011	0.11	1.27±0.007	0.015	0.12
16	0.56±0.005	0.011	0.20	0.65±0.009	0.019	0.29	0.74±0.007	0.015	0.21
17	0.65±0.009	0.019	0.29	1.05±0.009	0.018	0.17	1.34±0.007	0.015	0.11
18	1.44±0.015	0.030	0.21	2.86±0.007	0.015	0.05	3.56±0.007	0.015	0.04
19	0.75±0.009	0.019	0.25	0.94±0.008	0.016	0.17	0.94±0.007	0.015	0.16
20	0.64±0.005	0.011	0.17	1.07±0.007	0.015	0.14	1.47±0.007	0.015	0.10

Table 9 .Lipid % of isolated microalgae

Isolate No.	15th day			22nd day			29th day		
	Lipid % SE(M) ±	S.D.	C.V. %	Lipid % SE(M) ±	S.D.	C.V. %	Lipid % SE(M) ±	S.D.	C.V. %
01	3.97±0.007	0.015	0.03	5.33±0.014	0.028	0.05	7.52±0.013	0.027	0.03
02	7.63±0.002	0.023	0.03	11.04±0.014	0.029	0.02	15.25±0.009	0.018	0.01
03	4.53±0.011	0.023	0.05	6.34±0.015	0.030	0.04	7.26±0.008	0.017	0.02
04	6.06±0.006	0.012	0.02	8.76±0.007	0.015	0.01	10.06±0.008	0.017	0.01
05	1.06±0.006	0.013	0.12	2.76±0.006	0.013	0.04	3.15±0.010	0.021	0.06
06	1.03±0.012	0.024	0.23	2.05±0.007	0.014	0.07	3.43±0.011	0.023	0.06
07	6.15±0.009	0.019	0.03	9.27±0.009	0.019	0.02	12.06±0.008	0.016	0.01
08	1.83±0.014	0.029	0.16	2.06±0.006	0.013	0.06	3.55±0.007	0.015	0.04
09	1.03±0.011	0.023	0.22	2.53±0.011	0.023	0.09	3.33±0.012	0.025	0.07
10	2.17±0.009	0.019	0.08	4.05±0.010	0.021	0.05	5.45±0.011	0.022	0.04
11	1.05±0.004	0.008	0.07	1.6±0.011	0.023	0.14	2.5±0.010	0.020	0.08
12	4.14±0.007	0.015	0.03	5.94±0.015	0.030	0.05	8.54±0.015	0.031	0.03
13	3.95±0.009	0.018	0.04	6.14±0.015	0.030	0.04	9.33±0.012	0.024	0.02
14	3.66±0.007	0.015	0.04	4.61±0.02	0.052	0.11	6.3±0.010	0.020	0.03
15	8.23±0.011	0.023	0.02	10.76±0.007	0.015	0.01	13.43±0.014	0.028	0.02
16	6.71±0.014	0.029	0.04	8.66±0.006	0.013	0.01	12.43±0.012	0.025	0.02
17	5.13±0.012	0.024	0.04	8.43±0.018	0.036	0.04	12.95±0.007	0.014	0.01
18	24.72±0.014	0.028	0.01	34.03±0.015	0.030	0.09	44.95±0.007	0.015	0.03
19	11.16±0.005	0.01	0.01	14.26±0.007	0.015	0.01	19.87±0.009	0.019	0.09
20	4.64±0.007	0.015	0.03	7.13±0.014	0.028	0.03	10.64±0.007	0.015	0.01