Effect of Silver Nanoparticles on the Synthesis of Algal Lipids

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Abstract

In light of the increasing depletion of fossil fuel reserves throughout the world, excessive pollution from greenhouse gases, and a gradual increase in carbon dioxide (CO_2) content in the atmosphere as a result of many anthropogenic interventions that have significantly altered the global carbon cycle, renewable energy sources have major future potential. Due to the ability to alter their lipid processes in response to environmental changes, microalgae can be utilized as a replacement since they are versatile enough to thrive in a range of environments and serve as a source of bioenergy. It is also an appealing medium for absorbing the extra CO_2 existing in the atmosphere. Water samples with visible algal colonies were collected from different sources in India and isolated on BG11 medium. To boost the lipid yield of the strains, silver nanoparticles prepared from ginger (*Zingiber officinale*) extracts were added after the specific strains of microalgae had their biomass production assessed. According to morphological analyses, all the isolates were spherical, green in colour, unicellular in structure, and had a range of cell sizes. The highest lipid concentration was identified in the microalgal isolate JUMAC-7 whereas the lowest was found in JUMAC-4 according to research on how silver nanoparticles triggered lipid synthesis. Therefore, the inclusion of silver nanoparticles opens a new paradigm for efficient lipid production and consequent quality biodiesel production.

1 INTRODUCTION

With the rapid decline in fossil fuel reserves across the globe in addition to the excessive greenhouse gas-induced pollution and the resultant global warming, renewable energy

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resources have significant potential for generations to come. The relationship between sustainable development and renewable energy is one of utmost importance (Chen et al., 2022). From the advent of the industrial revolution, fossil fuels have been the most sought-after source of energy for various reasons such as large availability, high efficiency, ease of purification, etc., but the overexploitation of these resources has brought us now to a state of severe shortage let alone have reserves for the coming generations (Singh and Singh, 2012). Biodiesels are considered a better alternative to petroleum-based fuels as they are biodegradable and non-toxic in nature. They consist of mono-alkyl esters of long-chain fatty acids derived from renewable feedstocks like vegetable oil or animal stocks by the transesterification processes (Ogino and Amoah, 2019).

Microalgae, a renewable bioresource has the potential to take over the current industrial production of organic compounds and pharmaceuticals by replacing fossil-based conventional production (Mehariya *et al.,* 2021). The most abundant source of a variety of naturally occurring biomolecules with added value and bioactive substances is microalgal biomass, which is why it is regarded as the most promising feedstock for the food and pharmaceutical industries. The pharmaceutical and nutraceutical industries greatly use microalgae-based bioactive substances such as carotenoids, peptide molecules, phycocyanins, polyphenols, and polyunsaturated fatty acids (PUFAs) as functional components (Dhandayuthapani *et al.,* 2021).

With a diverse range of photosynthetic microorganisms, microalgae can fix CO_2 from the atmosphere and create biomass more quickly and effectively than terrestrial plants. They are a promising feedstock for use in various industrial applications including food, feed, nutraceuticals, medicines, and biofuels (Junior *et al.,* 2020). Microalgae have numerous benefits over conventional crops for the production of biofuels, including rapid growth and productivity, the possibility for high lipid or carbohydrate contents, and the capacity to thrive in seawater, saline irrigation water, or wastewater. However, having a sizable biomass production capacity is crucial to manufacturing biofuels from microalgae (Branco-Vieira *et al.,* 2020).

Algae are found in practically all terrestrial habitats and have potential uses in agriculture as biofertilizers and soil conditioners to increase soil fertility and plant productivity. There are substances known as microalgal stimulants that can promote plant growth. These substances function similarly to phytohormones, support enzymatic and antifungal activities, and aid in plant production of proteins and amino acids (Braun and Colla, 2022). Additionally, algal development lessens soil erosion by controlling water movement. They also play a part in agricultural wastewater, soil reclamation, soil fertility, microbiological crust formation, and the biocontrol of agricultural pests (Abdel-Raouf *et al.,* 2012). Microalgae are known to be the pri-

mary photosynthesizers on Earth and are capable of producing various significant pigments such as xanthophylls, astaxanthin, chlorophylls, and phycobiliproteins. The formation of pigment in microalgae is influenced by several variables which include the availability of nutrients, salinity, pH, temperature, light wavelength, and light intensity (Nwoba *et al.,* 2019).

The term "nanoparticles" refers to particles with a diameter between 1 and 100 nm. They have significantly increased in popularity in recent years (Singh *et al*., 2021). The high surface-to-volume ratio of nanoparticles is the main factor contributing to their effectiveness. Particles' chemical, physical, and biological characteristics significantly alter at the nanoscale scale. The main factor allowing nanoparticles to be used in a range of industries is their increased capabilities brought about by their small size (Zhang et al., 2016). Although many different metals can be used to create nanoparticles, silver nanoparticles hold the top spot in nanotechnology due to their versatility. Silver nanoparticles (AgNPs) are helpful for a range of applications, including catalysis, imaging, electronics, and optoelectronics, due to their increased properties, which include high electrical, optical, and thermal conductivity. In addition, AgNPs are seen to have enhanced innate physical, chemical, and biological capabilities among other metal nanoparticles, which greatly increases their value (Vishwanath and Negi, 2021). Microalgae have been demonstrated to create gold, cadmium, and platinum ions in addition to silver nanoparticles (Brayner *et al*., 2006; Jacob *et al*., 2021). Since AgNPs feature antifungal, antibacterial, anti-neoplastic, wound healing, and conductivity capabilities, they stand out among the many metal nanoparticles. As a result, these nanoparticles are increasingly useful in biological, sensing, imaging, and drug delivery applications (Sharma *et al*., 2015; Stabryla *et al*., 2021).

2 MATERIALS AND METHODS

2.1 Collection of wastewater samples

According to Table 1, wastewater containing visible algal colonies was gathered from several sources in India (Abou-Shanab *et al*., 2022). The table contains correct information about the locations, pH, and temperature of the samples that were gathered. The acquired material was securely delivered to the lab and kept there at 4 °C for storage. While collecting the samples, appropriate safety precautions (an apron, gloves, and masks) were maintained.

2.2 Algal growth medium

Algae were grown using BG 11 (HiMedia) in this investigation.

TABLE 1

Isolates of microalgae obtained from differentsources of wastewater in India

S. No.	Location	Temperature (oC)	pH
1	Pushkar lake, Rajasthan	21	6.9
2	Kishangarh, Rajasthan	21	8
3	Budha Pushkar lake, Rajasthan	21	7.1
4	Karadyakere, Jakkanahalli	28	5.58
5	Somanahalli, Karnataka	28	6.09
6	Hirikere lake, Nagamangala, Karnataka	19	7.5
7	Kukkanahallikere, Mysore, Karnataka	19	6.5
8	Gadwal, Telangana	29	8.5

2.3 Morphological characterization of microalgal isolates

The algal isolates were identified at the microscopic level using botanical methods after being visually described (Kaur *et al*., 2019; John *et al*., 2003). On a glass slide, an algal suspension drop was and placed, covered with a coverslip, and the cells were examined using a light microscope (10X and 40X). It was noted how the cells were shaped.

2.4 Synthesis of silver nanoparticles

2.4.1 *Preparation of ginger extracts*

In the current investigation, ginger (*Zingiber officinale*) extract was used. The components were procured from the Bangalore market and cleaned by rinsing them in sterile distilled water. These brand-new, spotless materials were finely chopped and ground in a mortar and pestle (20 g of the sample in 100 ml of distilled water). Whatman No. 1 filter paper was used to thoroughly filter the resulting infusion (Baki and Anderson, 1973).

2.4.2 *Synthesis of silver nanoparticles*

The solution was made by combining 10 ml of a pure extract with 90 ml of 1 mM silver nitrate aqueous solution. Tests were conducted with the appropriate controls at room temperature (27 $\pm$ 2 °C) and at 60 °C (Baki and Anderson, 1973).

2.5 Lipid Extraction

With a few minor adjustments, total algal lipids were extracted in accordance with Folch et al. (1957). A 20-milliliter sample of microalgal culture was centrifuged for five minutes at 10,000 rpm. Then, 1mL of ice-cold 0.2N perchloric acid was applied to the pelleted algal cultures. This pellet was put on the glass Petri plate and left there for 24 hours at 60 °C. The biomass was pulverized into powder form after being dried. The powder was placed in a centrifuge tube with a ten-milliliter solution of chloroform and methanol (2:1) and centrifuged at 10,000 rpm for five minutes. A thin layer of lipid with a dark orange colour was allowed to dry out in the test tube with the supernatant.

2.6 Lipid estimation

Microalgal lipid samples were made in chloroform and then placed in a boiling water bath for 45 minutes with the addition of 2 ml of dichromate solution (2.5 g of K_2CrO_7 in 1L of conc. H_2SO_4). At room temperature, the mixture was chilled. A 100 mL dilution of the combination made from 1 mL was used, and the absorbance was measured at 350 nm. The values were presented on a standard graph using palmitic acid and were represented in mg/mL (Narasimha *et al*., 2011).

A control for each of the eight samples was also evaluated without the incorporation of silver nanoparticles for comparison.

3 RESULT AND DISCUSSION

3.1 Isolation of microalgae

Microalgae-containing water samples were taken from several lakes in and around Bangalore (Karnataka), then cultured in the BG11 medium. The isolates JUMAC-1, JUMAC-2, and JUMAC-3 were taken from several lakes in Bangalore and given the right amount of sunshine to develop. To get appropriate microalgae cultures, more samples were considered and allowed to develop. All of the samples were labeled with the numbers JUMAC-1, JUMAC-2, JUMAC-3, JUMAC-5, JUMAC-6, JUMAC-7, and JUMAC-8 after being collected. Using the streak plate method, samples were extracted and purified in Petri plates containing BG 11 medium that had been 2 % agar added. The growth rates of the samples were then measured. These isolates flourished in the laboratory at typical temperatures (25–30 °C), phototrophic conditions, and daylight illumination. Each individual colony was chosen, subcultured, and their purity was preserved as seen in **Fig. 1**.

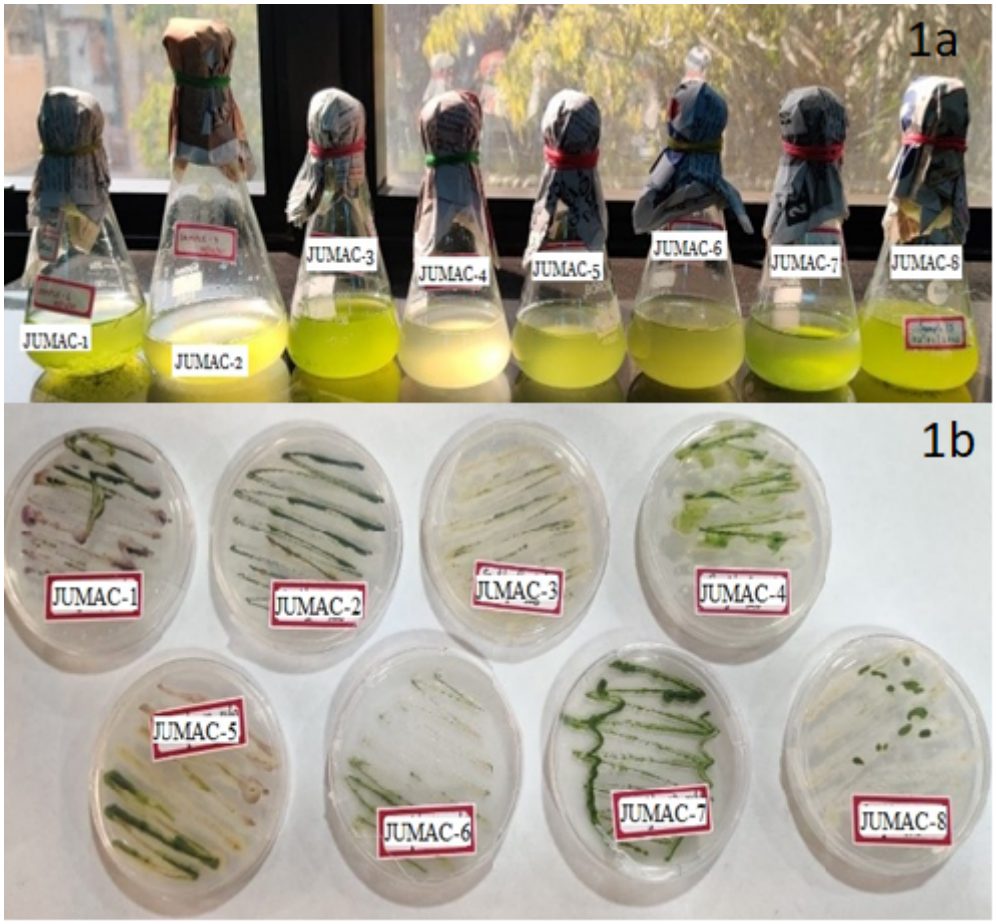

FIGURE 1

(1a) Algal samples in suspension culture of BG11 medium (1b): First subculture of algal samples in Petri-plates with BG-11 medium

Other media, such as Bold's Basal Medium (BBM) can be employed according to several research (Karthikeyan *et al.*, 2019; Chiellini *et al.*, 2020). Algae demonstrated positive growth on the plates made from several water samples and pre-inoculated with liquid TAP medium.

3.2 Morphological characterization and identification of microalgal isolates

The morphology of microalgal isolates was characterized using light microscopy at magnifications of 10x and 40x based on the colour of the colonies, cell shape, and cell structure (unicellular or multicellular). Numerous typical green microalgae and cyanobacteria were present in the isolates. All of the isolates were found to be spherical, green in colour, unicellular in structure, and of varying cell sizes. These microalgal isolates were provisionally recognized as species belonging to the Chlorophyceae class based on morphological characterization (**Fig. 2**).

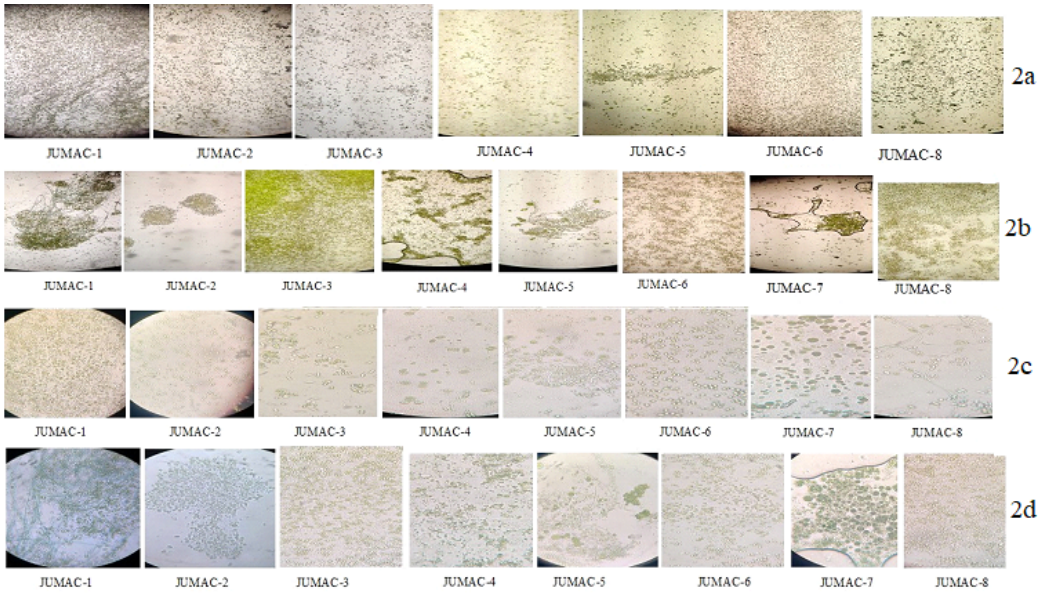

FIGURE 2

2(a): Algal cultures in Petri-plate under 10x magnification
2(b): Suspension culture of algal samples under 10x magnification
2(c): Algal plates under 40x magnification
2(d): Algal suspension cultures under 40x magnification

According to research (Lee et al., 2014), species of *Chlorella, Chlorococcum, Cosmarium (unicellular), Coelastrum, Ankistrodesmus* (colonial), *Cladophora, Hydrodictyon, Nostoc*, and *Anabaena* are the predominant strains of algae found in freshwaters (filamentous).

3.3 Silver nanoparticles synthesis

Zingiber officinale extract was used to create silver nanoparticles. The colour of the reaction fluid changed from golden yellow to dark brown with ginger extracts when the algal cultures were incubated with the silver nanoparticles, as shown in **Fig. 3**, demonstrating the production of silver nanoparticles (AgNPs). The excited surface plasmon resonance (SPR) of the AgNPs is responsible for the colour shift, and the size and concentration of the NPs affect the colour depth. By using colorimetric measurement at a wavelength of 640 nm, the creation of AgNPs was further investigated. The obtained readings revealed a small steepening in the value after every third day. The graph indicated that there was a consistent growth in the OD readings for 3 days but slightly gets reduced on subsequent days.

To have a precise estimation of the nanoparticle production as shown in **Fig. 4**, the data was classified as having a duration of 5 days. Algae are recognized for their potential to hyper-

FIGURE 3

(3a): Algal samples on thefirst day of inoculation with nanoparticles (3b): Algal samples after subsequent days of adding nanoparticles

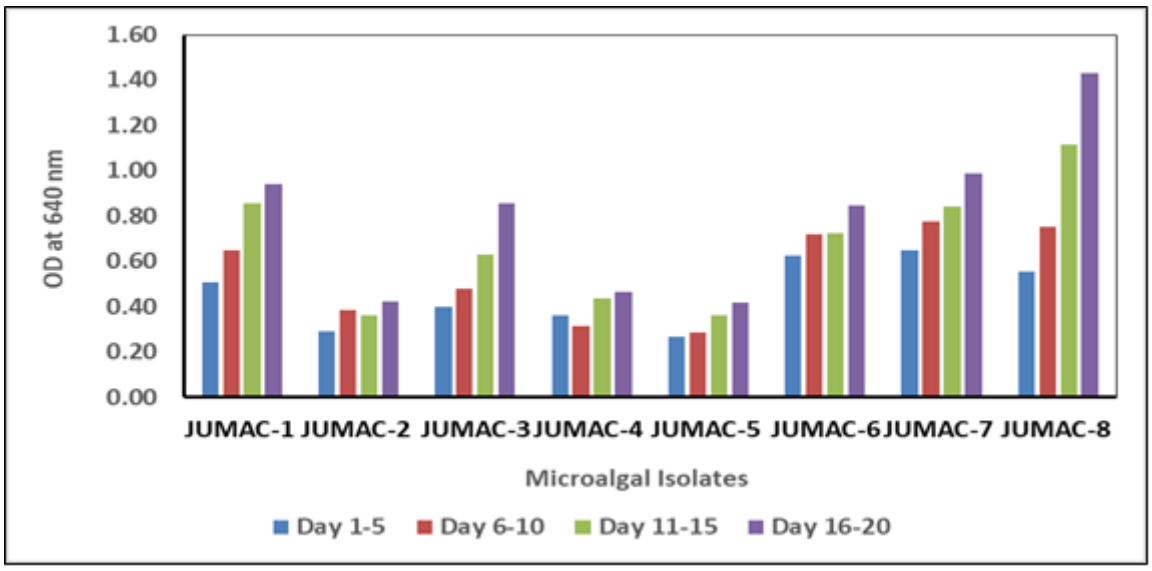

FIGURE 4

AverageOD of samples in 5 days intervals

accumulate heavy metal ions and remodel into more pliable geometries (Fawcett *et al*., 2017). Algae have thus been suggested as a model organism for producing bio-nanomaterials.

3.4 Total lipid content

The total amount of lipid content in microalgae is directly proportional to the making of biofuel. So far, several techniques have been published for the quantification of microalgal lipids, principally including the traditional gravimetric approach utilizing extraction solvents, nile red lipid visualization method, SPV, and TLC (Chen *et al*., 2018). Various organic solvents or combinations of different solvents have been suggested to selectively a complicated combination of organic molecules that can be processed to obtain lipids. The Folch method (Folch et al., 1957) employs the use of chloroform-methanol (2:1 by volume) for the extraction of lipids from endogenous cells. Lipid extraction and partitioning are accomplished concurrently in the Bligh and Dyer technique (1959), wherein proteins are precipitated in the interface of two liquid phases.

According to the current investigation results, the microalgal isolate JUMAC-7 had the highest amount of lipid content, whereas JUMAC-4 had the lowest amount. It is common practice to conduct colorimetric analysis of isolated lipids using the acid-dichromate technique. It is well known that dichromate is employed in the quantitative analysis of lipids. It was found that the solution colour changes from brown to blue. The control strain of JUMAC-7 also showed highest lipid yield and over all lipid yield of all the control strains were found to be lesser than the AgNP incorporated ones proving that AgNP has increased the lipid yield of the strains shown in **Fig. 5.**

4 CONCLUSION

Microalgae have become a potentially viable alternative to fossil fuels. These microbes fall within the broad category of photosynthetic microbes. They transform CO_2 into a range of beneficial substances, including biofuels, meals, feed, and medications. The productivity of microalgae is 20–40 times that of oil crops. Depending on the microalgae, lipids can make up as much as 80 % of their dry biomass. The development of sustainable biofuels from microalgae could thus be an important source in the future. Because it is environmentally beneficial, biodiesel is recognized as an alternative renewable energy source. Microalgae biodiesel is environmentally beneficial, non-corrosive, carbon-neutral, and biodegradable.

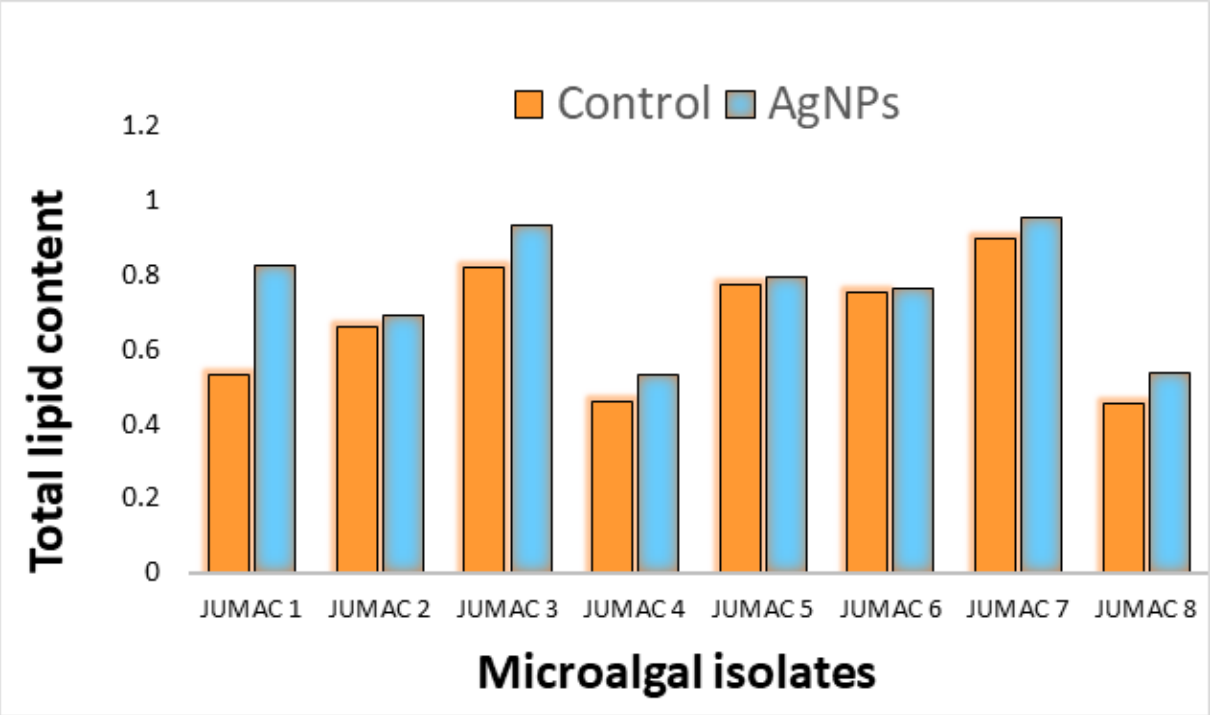

FIGURE 5

Comparisonof the lipid yield with and without the incorporation of AgNPs.

While there are many algae strains available for researching growth rates with the potential to produce biofuel, the best strain depends on the environment. The selection of powerful strains is a continual process in algal research because algal strains isolated from nearby natural habitats are thought to be the best for large-scale growth since they are best adapted to the local environment. The current study found that JUMAC-1, JUMAC-7, and JUMAC-8 grew the fastest out of the eight microalgal isolates studied. The best cycle for growth was the 12:12h light: dark cycle.

Overall, the freshwater lake isolate JUMAC-7 from Bangalore had a higher growth rate, biomass, and lipid content. JUMAC-7 appears to be a potential option for additional research into methods to maximize the production of biomass and biodiesel.

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