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Isolation, Identification and biomass productivity analysis of microalga *Scenedesmus rubescens* from DTU Lake

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Abstract
Due to the increasing demand of energy by leaps and bounds, it is requisite to identify the local solution for the generation of energy to mitigate the future demand. Microalgae are the most abundant and rapidly growing photosynthetic microorganisms capable of sequestering CO_2 from the environment and water to produce biomass in comparison to other energy crops. Herewith, we have focussed on the isolation and characterization of a potential microalgae strain from the local water bodies under natural environmental conditions which provides an edge to commercialization of microalgae biofuel production. Microalgae sample obtained from Delhi Technological University's lake was used. Light microscope, Scanning electron microscope, Fourier transform infrared spectroscopy and 28S rDNA analysis were performed for identification of microalgae. With the help of these potential identification tools, microalga Scenedesmus rubescens was identified. After the identification, effect of various abiotic factors such as light (intensity, color and period), pH of medium, shaking period and temperature on algal growth rate and biomass productivity was studied. Scenedesmus rubescens provided maximum biomass productivity in white light of 36W intensity under illumination of 16 hours. pH 8, 24 hours shaking period and 26±2ºC temperature were also found to be best for this microalga.

Keywords: Microalgae, *Scenedesmus rubescens*, FTIR, SEM, 28S rDNA, Biomass productivity.

Introduction

Microalgae utilises sunlight and fix CO_2 during photosynthesis and produces biomass more efficiently and rapidly than terrestrial plants. They have been considered for biomass to energy production, based on their fast growth rate, biomass productivity and compatibility for the various kinds of biofuels. They accumulate large amounts of storage lipids primarily in the form of triacylglycerols (TAG). The fatty acid profile is important in selecting microalgae as a feedstock for biodiesel production. An algae-based bio-refinery could potentially integrate several different conversion technologies to produce biofuels including biodiesel, aviation fuel (commercial and military), ethanol, and methane, as well as valuable co-products including oils, protein, and carbohydrates, etc[1,2]. Wastewater from industrial and domestic sources is also a potential source for microalgal biomass production which can be utilized in the production of biofuels. Due to fast industrial growth, water and air pollution level has increased tremendously and it is causing environmental degradation. So, it is required to treat wastewater and various emissions before discharging them into the environment [3.] Microalgae are known to its nutrient removal efficiency from the industrial effluents and wastewater.[4, 5.] As fossil fuels are depleting at very fast rate, we need to explore newer eco-friendly and economically sustainable sources of energy to meet the global demand.
The isolation of microalgae from local water bodies like lakes or ponds is a useful strategy for obtaining potential species tolerant to the conditions prevalent in the area, such an organism tending to have the ability to grow without applying any extra effort with having good biomass productivity. High lipid or carbohydrate producing microalgae are the desired ones for the production of biodiesel and bioethanol respectively. Analysis of the bulk biochemical composition of the microalgae is essential for the understanding of biological processes happening in the cell and abiotic factors affecting the accumulation of various metabolites within the cell. As per required biofuel production, it is desired to know the biomolecules composition of the cell for determination of the optimum use of biomass for biofuel production. The lipid-rich microalgae are suitable feedstock for biodiesel production while carbohydrate-rich microalgae are best sources for bioethanol production. Several factors either biotic or abiotic influences microalgal growth. Abiotic factors include the intensity and amount of light, the temperature, salinity and pH of the medium and nutrient concentrations etc [6-9].
Bacteria, cyanobacteria, fungi, competition by other microalgae and predation by other aquatic organisms come under biotic factors which limit the algal growth. Other operational factors like dilution and shear rate, the process and frequency of harvesting, depth of the tank etc. also influence the microalgal growth rate and thus the biomass productivity. The microalgal biomass and the biochemical composition's amount may be influenced and improved by manipulating the surrounding environment [10, 11].

To optimise the biomass productivity, the influence of various abiotic factors such as light intensity, light colour, light period, pH of medium, shaking period and temperature on algal growth rate has been studied in the present study. In these experiments, only one factor at a time was variable while all other conditions were kept constant.

The culture conditions which were controlled for the algal growth were light, temperature, pH and constant mixing or aeration. Isolated *Scenedesmus rubescens* will be investigated for biofuel production, wastewater treatment, heavy metal removal from waste water and green synthesis of Nanoparticle in the next study.

Materials and Methods

Sample collection and isolation of microalgae

For microalgae isolation, the freshwater sample, collected from Delhi Technological University's lake (DTU Lake) was first filtered for the removal of solid and undesired particles and, then inoculated in BG-11 Medium. For growth, this preparation was incubated at 27ºC in an artificial light chamber under illumination with a white fluorescent light at 300µmol/m^2 s^{-1} on a photoperiod of 16L: 8D for one week. After proper growth of microalgae was observed in the culture flask, the culture was spread onto BG11 Agar plate, and kept at the same growth conditions. Colonies were sub-cultured repeatedly into the same medium under same growth conditions until a culture dominated by the pure strain was developed. The sub-cultured individual colonies were picked and transferred into the BG-11 medium and scaled up successfully. Thus isolated and established pure culture was obtained.

The isolated microalga was cultivated in the BG11 medium at similar conditions for 24days in a working volume of 200 ml in Erlenmeyer flasks. Flasks were agitated twice or thrice a day manually to prevent any deposition at the base of the flask and to ensure proper mixing. Cultivation lasted for up to 24 days with samples being taken on a period of 24 hours and analysed on spectroscopy as well as by calculating dry cell mass of the sample. The dry cell weight of the microalgal culture was estimated by taking 50ml of culture and centrifuging it at 3000 rpm for 10 minutes and hence separated biomass was dried in an oven for the time till the complete dry condition is achieved. After a substantial period of growth, 100ml of the sample was taken and biomass was harvested and preserved for the identification purpose.

Growth Medium:

For growth of the microalgal isolate, BG11 (ATCC medium 616) media was used. The composition of media consists (gram/litre):Sodium nitrate ($NaNO_3$),1.5; ($K_2HPO_4.3H_2O$) 0.04;Magnesium Sulphate ($MgSO_4.7H_2O$),0.075; Calcium Chloride ($CaCl_2.2H_2O$),0.036; Citric Acid,0.006; Ferric Ammonium Citrate,0.006; EDTA (disodium magnesium salt),0.001;Na_2CO3,0.01; and one millilitre of **A5** Solution which contains (gram/litre):H_3BO_3,2.86; $MnCl_2.4H_2O$,1.81; $ZnSO_4.7H_2O$,0.222; $Na_2MoO_4.2H_2O$,0.39; $CuSO_4.5H_2O$,0.079; $Co(NO_3)_2.6H_2O$,0.0494. The pH of medium was kept 8.

Identification of isolated microalgae

Morphological Identification

Isolated microalgae species was examined under light microscope and scanning electron microscope (SEM) for cell morphology. SEM was performed for the absolute morphological determination of the microalgae isolate. Scanning Electron Microscope provides the absolute morphological details of the material and this property is utilised for the characterization of the microalgae. FTIR spectroscopy was also performed for the biochemical composition characterization[12].The biomolecules composition *i.e.* lipid, carbohydrate, protein, chlorophylls etc. of algal biomass is usually investigated and analysed by the conventional chemical extraction method followed by gravimetric determination and spectroscopy[13]. For rapid determination of the isolated microalgal sample for its biochemical composition, use of FTIR is a highly advanced technique and powerful analytical tool[13, 14]. FTIR is a non-destructive and rapid method, which helps in identification of the vibrational structure of materials[15]. The IR spectrum of the microalgal sample was recorded on FTIR at room temperature under region 4000-400 cm^{-1}. Analysis of composition, shape and intensity of absorption at specific wave numbers reveals information about functional groups and molecular structure that can then be used for identification [13, 16].

Molecular Identification

28S rDNA molecular examination was performed for identification of the isolated microalgae (Eurofins Genomics India Pvt. Ltd. Bangalore, India). DNA was isolated from the culture by using Nucleospin DNA extraction kit. Its quality was evaluated on 2.0% Agarose gel, a single band of high-molecular weight DNA has been observed. Fragment of 28S rDNA gene was isolated and amplified by Merk PCR amplification kit. A single discrete PCR amplicon band of 800 bp was observed when resolved on Agarose. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 28SF (5ACCCGCTGAATTTAAGCATA3) and 28SR (5CCTTGGTCCGTGTTTCAAGA3) primers using BDT v3.1 Cycle

sequencing kit on ABI 3730xl Genetic Analyzer (3730xl ABI-96 Capillary machine). Consensus sequence of 28S rDNA gene was generated from forward and reverse sequence data using aligner software.

Biomass productivity Analysis in different culture conditions

Light intensity

The isolated microalga was cultivated under six different light intensity regime in BG11 medium having pH 8 and light period of 16L: 8D for 24 days and growth was monitored by spectrophotometer. Reading was noted at a period of two and three days and plotted to derive a growth curve on the basis of absorbance and time.

Light colour

Isolated microalga was inoculated in BG11 medium having pH 8 and growth was monitored for 24 days. Three different colours Red, Blue and White colour light was given to the flask by wrapping flask with red and blue coloured transparent polyethylene sheet onto the flask. One flask was left unwrapped and kept directly under the white light.

Light period

Three different light periods i.e. 24L: 0D, 16L: 8D and 12L: 12D were given to the microalgal culture separately and growth was monitored. BG11 medium with pH 8 was used as a growth medium. The experiment was done for 24 days and growth pattern was plotted.

pH

Microalgal biomass productivity was calculated under different pH regimes. pH ranges from 6 to 11 were used under white light with a light period of 16L: 8D at room temperature for the growth of microalgae. BG11 medium was prepared and pH was set and autoclaved before the inoculation.

Temperature

Three different temperature regimes 18°C (±2), 26°C (±2) and 30°C (±2) were provided to the microalgae culture and allowed to grow under white light with a light period of 16L: 8D at room temperature for the growth of microalgae.

Shaking period

Three different shaking periods were provided to the microalgae culture under white light with a light period of 24h at room temperature for the growth of microalgae. Microalgae culture flasks were kept on a shaker for agitation purpose. One flask was kept on continuous shaking for the entire growth period. Second flask was given an 18 hours shaking on daily basis. Third flask was manually shaked for twice or thrice a day.

Results and Discussion

Freshwater microalga was isolated and its morphology was determined by Light microscope, SEM and FTIR. Characteristics and morphological features of the isolate have demonstrated its close similarity with microalgae *Scenedesmus sp*. The microalgae isolate was finally identified as *Scenedesmus rubescens* based on 28s rDNA molecular identification.

Isolation and identification of microalgae

Light Microscope Studies

Inverted light microscope (Magnus) determined that cells are spherical in shape. The colour of colony is green and it can be easily perceived by the Figure-1.

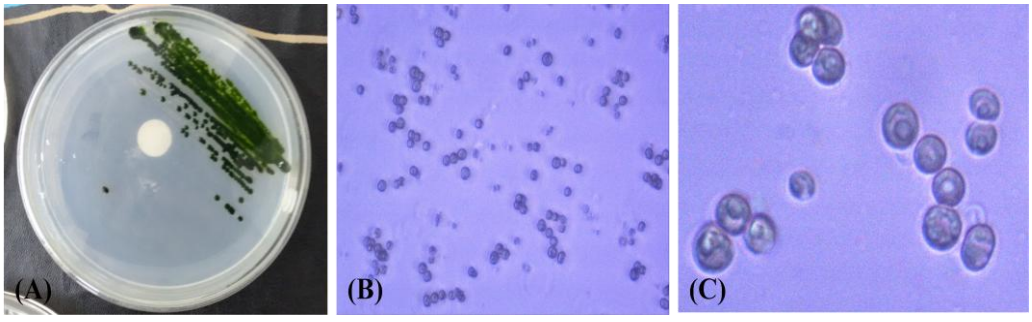
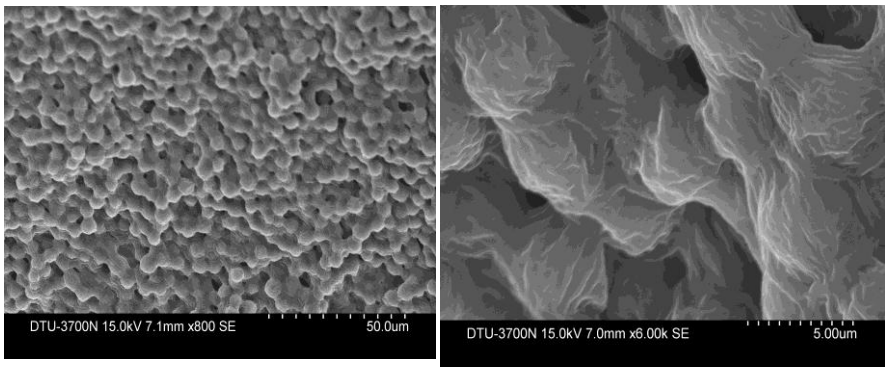


Figure-1: Isolated colonies of microalga *Scenedesmus rubescens* in agar plate (a) and under inverted light microscope (20X) (b) and 40X (C) enriched with BG-11 media. The isolated colonies were further used to establish pure culture, morphological, molecular and biomass productivity analysis.

Scanning Electron Microscope Studies:

In examining the physical structures of microalgae, SEM provides good visual images of possible microalgal structure and morphology. It scans solid samples with a beam of electrons, which interacts with the atoms that make up the sample to produce signals. These signals contain topographic information about the samples[2]. The average cell size was in the range of 5.6-5.8µm (Figure 2).



(a) **(b)**

Figure-2: Morphological determination of microalga *Scenedesmus rubescens* by scanning electron microscope (S-3700) (a) depict the resolution of microalga at 800 SE and (b) at 6000 SE. With the help of these resolutions average size of 5.8 microns has been confirmed. DTU: Delhi Technological University; 3700N

Fourier Transform Infrared Spectroscopy Studies

FTIR provides the metabolic fingerprinting of the material by discriminating and identifying the various functional groups present in it. The composition and structure of molecules and functional groups can be determined by analysing the position/width and infrared light absorption of the sample. Biomolecules such as lipid, carbohydrate and protein etc. has their own characteristics IR spectra, which is rich in molecular composition and functional aspects and which allow identification and quantification based upon absorbance For microalgae sample, the sample is analyzed in 4000-400 cm^{-1} wave number range with a resolution of 4 cm^{-1}[13, 15-17]. Following FTIR chromatogram (Figure-3) and table (Table-1) shows the presence of various functional groups attributed the protein; lipid and carbohydrate present in the *Scenedesmus rubescens* (Figure 3)**.**

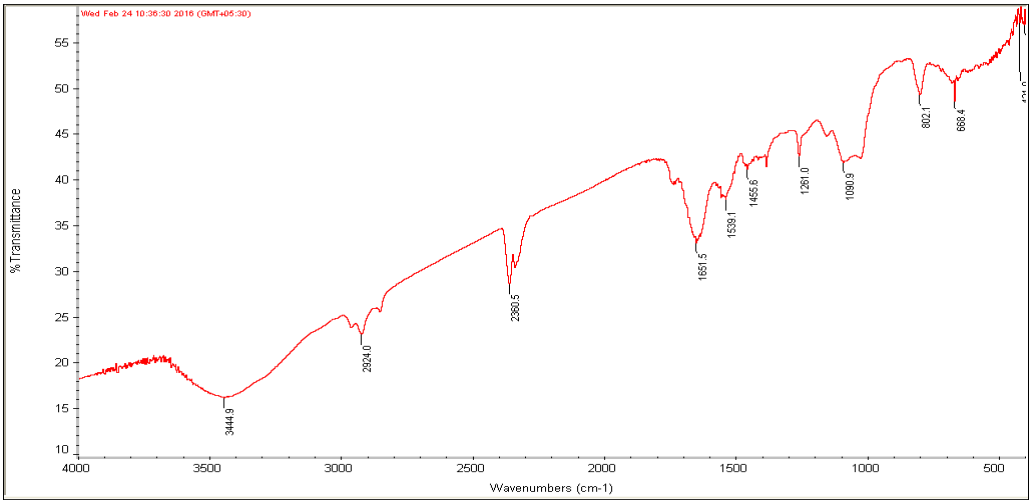


Figure-3: FTIR Spectra (4000-400 cm^{-1}) of *Scenedesmus rubescens.* Characteristic functional groups contributing to the formation of % transmittance bands at specific wave numbers.

Table-1: Tentative assignment of bands found in FTIR spectra of *Scenedesmus rubescens.* [12, 14, 18]

S. No.	Typical band assigned to lipid and carbohydrate	Wave number range cm $^{-1}$	Main peak cm^{-1} *(Scenedesmus rubescens)*
1	Water V(O-H) stretching Protein V(N-H) stretching	3029-3639	3444.9
2	Lipid –carbohydrate mainly Vas (CH2) and Vs(CH2) stretching	2809-3012	2924
3	Protein amide I band mainly V(C=O) stretching	1583-1709	1651.5
4	Protein amide II band mainly σ(NH)bending V(C-N) stretching	1481-1585	1539.1
5	Protein σas (CH2) and σ s(CH3) bending of methyl lipid as (CH2) bending of methyl	1425-1477	1455.6
6	Nucleic acid (other phosphate containing compounds) Vas> P=0 stretching of phosphodiesters	1191-1356	1261.0
7	Carbohydrate V (-O-C) of polysaccharides. Nucleic acid (other phosphate containing compounds) Vas> P=0 stretching of phosphodiesters	1072-1099	1090.9
8	Carbohydrate V(C-O-C) of polysaccharides	980-1072	1026

Molecular Identification studies:

The 28S rDNA gene sequence was used to carry out basic Local Alignment Search Tool (BLAST) with the nrdatabase of NCBI genebank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4 (Table 2).

Table-2: Sequences producing significant alignments. Nearest relatives (Accession Number, % Identicity) observed in GenBank when BLAST was performed with microalgae consensus sequence. Microalgae displayed the maximum identity with *Scenedesmus rubescens*, JX289896.1

Description	Max score	Total score	Query cover	E value	Ident	Accession
Scenedesmus rubescens isolate EP1H8 28S ribosomal RNA gene, partial sequence	778	778	99%	0	94%	JX289896.1
Acutodesmus obliquus partial 28S rRNA gene, strain KGE18	773	773	99%	0	93%	HE965014.1
Asterarcys sp. MS3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	761	761	99%	0	93%	KM893430.1
Scenedesmus obliquus partial 28S rRNA gene, isolate YSL009	761	761	99%	0	93%	FR751197.1
Scenedesmus obliquus partial 28S rRNA gene, isolate YSL012	761	761	99%	0	93%	FR751168.1
Scenedesmus obliquus partial 28S rRNA gene, isolate YSR007	761	761	99%	0	93%	FR751165.1
Scenedesmus obliquus isolate YSW14 large subunit ribosomal RNA gene, partial sequence	761	761	99%	0	93%	HM103381.1
Scenedesmus obliquus partial 28S rRNA gene, isolate YSR016	758	758	99%	0	93%	FR751169.1
Scenedesmus obliquus isolate YSL02 28S ribosomal RNA gene, partial sequence	758	758	99%	0	93%	GU732415.1
Acutodesmus obliquus partial 28S rRNA gene, strain KGE31	756	756	99%	0	93%	HF536545.1

Biomass Productivity Analysis

Microalgal growth depends upon the various environmental factors mainly light, pH, temperature, and availability of nutrients as they not only influence the photosynthetic activity of the microalgae but also affects the biochemical composition, cellular metabolism and morphology. Other factors such as shaking and species richness are also important factors influencing microalgal growth and metabolism. As a whole, these environmental factors significantly affect the CO2 fixation and photosynthesis, thus resulting into alteration of carbon fixation and biochemical composition [19-25]. The isolated microalgal sample was inoculated in BG11 medium and incubated at room temperature under a photoperiod of 16L: 8D for growth. Microalgal growth was monitored on a regular basis on spectrophotometer by observing absorbance at 670nm. A growth curve was plotted for a period of 24 days between absorbance and number of days. Figure-5 shows the absorbance noted on various days of culture. This isolated sample was then used as inoculums in the various experiments.

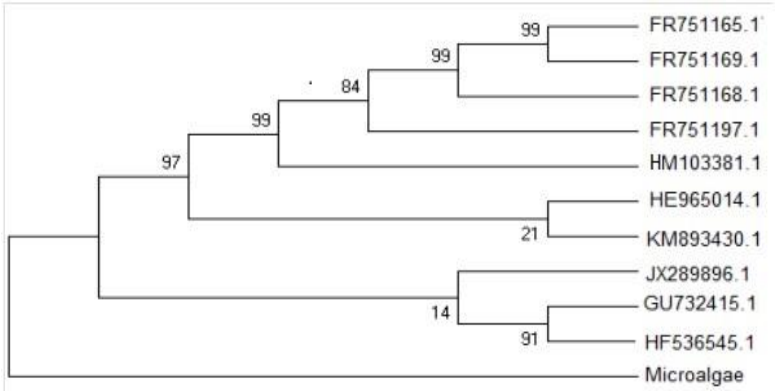


Figure-4: The evolutionary history was inferred using the Neighbour-Joining method. The phylogenetic tree was constructed using MEGA 4

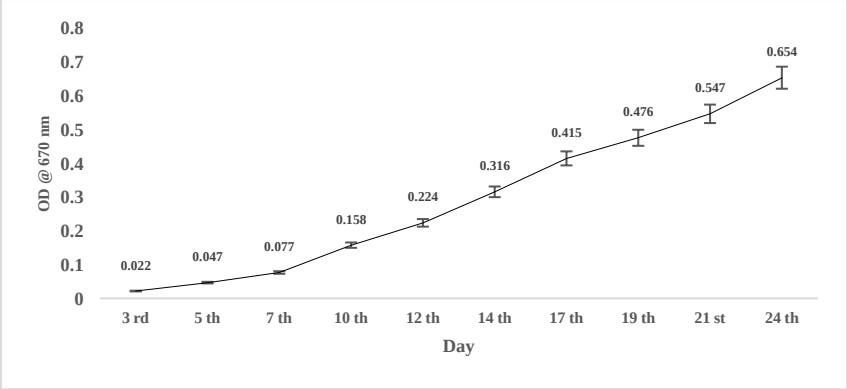


Figure 5: Growth curve of isolated microalga *Scenedesmus rubescens* for a period of 24 days. OD: optical density; 670 nm.

Proper light intensity is a requisite condition for the photosynthesis reaction [33] as it affects the algal growth through its impact on photosynthesis reaction. Growth rate is maximum at optimum intensity and the rate decreases as the intensity increases or decreases in comparison to optimum condition [34]. At light intensity more than the optimum, photo-inhibition reaction occurs and thus reduction in light utilization efficiency [24, 36-38]. This efficiency can be enhanced by allowing the culture to remain in longer dark period while using high light intensities. During this prolonged dark period photosynthesis system works efficiently by optimally utilising captured photons and converting this light energy into chemical energy by avoiding photo-inhibition effects [37]. This happens because high light intensity disrupts the chloroplast lamellae [38] and inactivation of enzymes involved in carbon dioxide fixation [39]. However, algal growth rate under increasing light intensities depends upon the types of algal species as well as other culture conditions like temperature and pH etc. [34].

The growth was allowed in six different light intensities 10, 15, 25, 36, 60 and 100 Watt in BG11 medium having pH 8 and light period of 16L: 8D for 24 days and growth was monitored by spectrophotometer at 670nm. All the intensities were provided by yellow light bulb except one, 15watt. For 15watt light intensity, a white fluorescent light was used. Initially a high growth rate was noticed in the flask maintained at 100 W intensity conditions. Later on, the growth rate was found high in the flask maintained at 36 W. Colour of light has a very significant effect on the biomass productivity. The fact established well by higher growth observed in flask with the 15 W flasks in this experiment. Reading was noted at a period of two or three days and plotted to derive a growth curve on the basis of absorbance and time (Figure-6). Biomass productivity was recorded on spectrophotometer at 670 nm when allowed to grow under three different light colours white, blue and red light. During first two weeks, high growth rate was observed in white light condition, after that a significant change was observed in algal growth. Blue and red light both conditions showed the comparable growth rate to the white in third week onwards of microalgae culture. In an experiment done on light intensity, it was found that 15 W white fluorescent lights gives high biomass productivity in comparison to yellow light with having intensity 100 watt. The maximum biomass productivity was achieved in white light condition (1.56 mg/ml) followed by red (1.37mg/ml) and blue light (0.83mg/ml) (Figure-7).

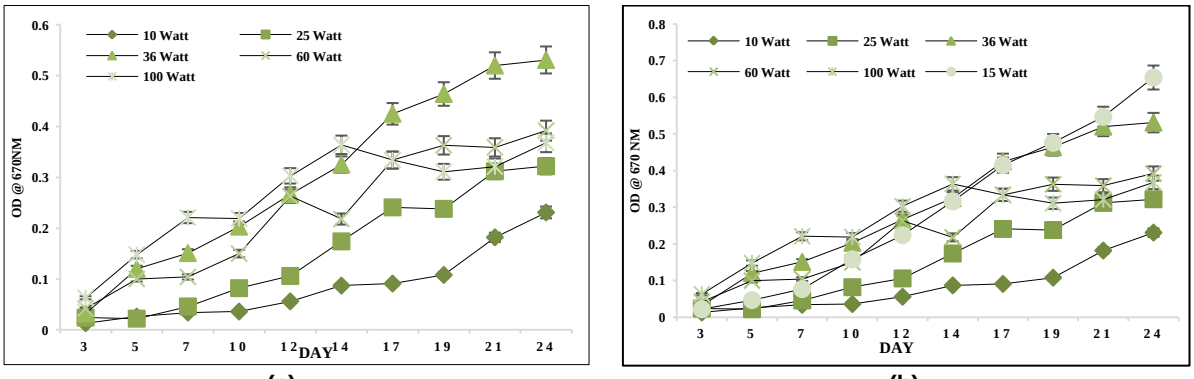
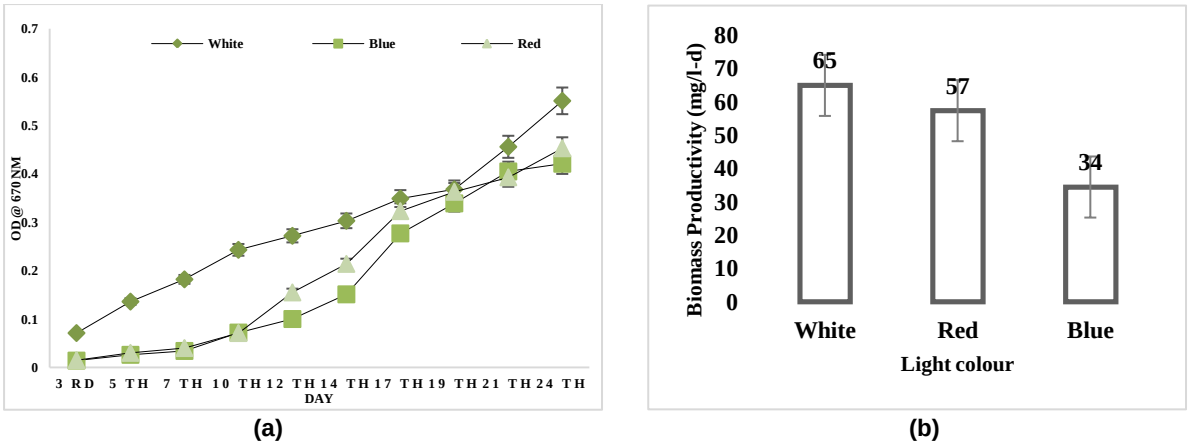


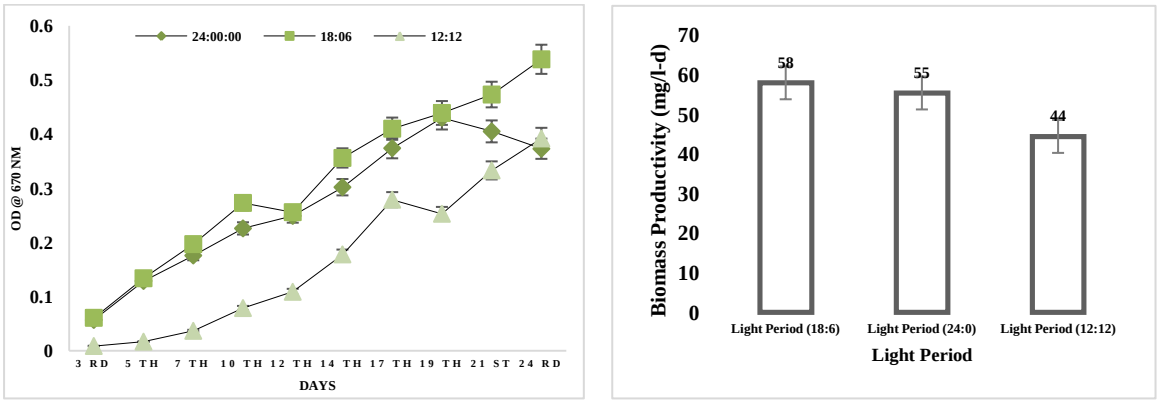
Figure 6: Effect of light intensities on biomass growth and productivity of microalga. (a) Compares the biomass productivity of all intensities of yellow colour light where 36 W provided maximum productivity while (b) compares the productivity with 15 W fluorescent lights. The biomass productivity of 15 W fluorescent lights is better than 36 W yellow lights. OD: optical density; 670 nm.



(a) (b)

Figure 7: Effect of light colour on biomass growth and biomass productivity of microalga. (a) Compares the growth of microalga in three different light colour and white colour provides maximum growth. (b) Also supports the fact that white colour light is suitable for microalga as it provides maximum biomass productivity. OD: optical density; 670 nm.

Light period is important mainly because of dark reactions as well as CO_2 fixation. Studies showed that dark phase is beneficial for microalgae growth because certain enzymes of pentose cycle which is used in photosynthesis and carbon dioxide fixation are active during dark phase and inactive during light phase[40]. Colours of light for providing illumination to microalgae culture has substantial effect on growth rate due to the wavelength spectral energy of various light colours. Wu and Merchuk [36] studied the effect of light and dark periods on the microalgal growth and found that with increasing photon flux density, specific growth increases up to a certain value and then it decreases with increased photon flux density. When cultured under three different photoperiods, microalgal growth rate was observed highest in a photoperiod of 16 L: 8D hour's dark with biomass productivity of 1.39mg/ml at the end of 24 days of cultivation. When grown under 24 hours' light supply the microalgal culture shown the biomass productivity of 1.33mg/ml while under 12 hours' photoperiod the biomass productivity was 1.07 mg/ml (Figure-8).

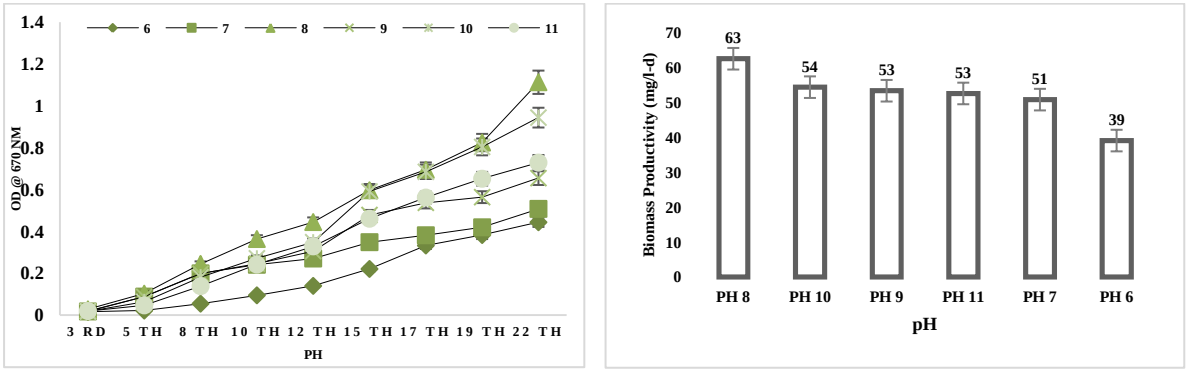


(a) (b)

Figure 8: Effect of light period (light: dark) on biomass growth and biomass productivity of microalga. (a) represents a comparison of growth in three different light periods and 18L:06D shows maximum growth. (b) 18L:06D also provided maximum biomass productivity. OD: optical density; 670 nm.

pH is one of the important factors for algal growth as it can affect the activity of different enzymes. Studies on the effect of pH on microalgae demonstrate that different algal species have a different level of tolerance to pH. The pH of the media has also the significant effect on the morphology of the algal cells. It influences the carbon availability, metabolism and thus the biochemical composition [26]. The pH determines the solubility and accessibility of CO_2 and essential nutrients, and because it can have a significant impact on algal metabolism [6, 27]. The pH of the culture media rises significantly during cultivation due to the uptake of inorganic carbon. Highest microalgal biomass productivity was observed at a pH 8 with biomass productivity of 1.50mg/ml among six

different media pH, 6 (0.94 mg/ml), 7(1.22 mg/ml), 8(1.50 mg/ml), 9(1.28 mg/ml), 10 (1.31 mg/ml) and 11(1.26 mg/ml) when tested in BG-11 for highest growth rate and biomass productivity (Figure-9).

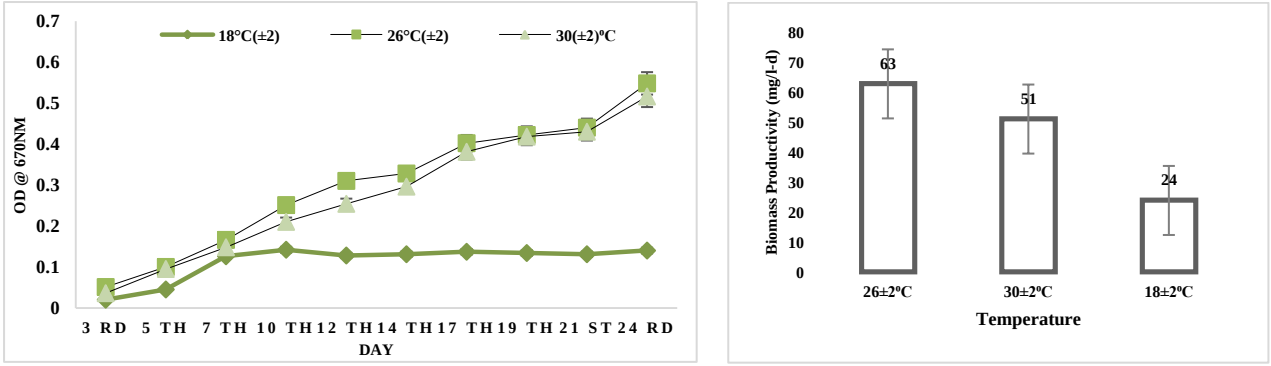


(a) **(b)**

Figure 9: Effect of pH on biomass growth and biomass productivity of microalga. (a) & (b) both represent maximum growth and biomass productivity at pH 8. Alga favours alkaline pH for its growth and biomass productivity. OD: optical density; 670 nm.

Photo-inhibition also depends upon the temperature of the culture medium. Low temperature enhances the light inhibition and thus affects the nutrients uptake rate as well as decrease the fluidity in the cell membrane. Temperature beyond the optimum affects the protein synthesis and thus results into reduction of growth rate [29]. It influences the CO_2 assimilation and O_2 evolving activity of the PS II system of photo-synthesis. It has been reported that it influences microalgal growth rate, cell morphology, various biomolecules composition and nutrient compositions. Various studies based on temperature dependent photo-inhibition suggest that at low temperature, electron transport decreases at a particular photon flux due to decreased rate of CO_2 assimilation. Low temperature causes inhibition of reactive oxygen species and therefore resulting in reduced photo-inhibition by protecting photo system II of photosynthesis system[30]. Morris et al [19] reported that protein synthesis rate increases at lower temperature with marine microalgae *Phaeodactylum tricornutum* [31]. Very similar to this, Rhee and Gotham[32] also reported a significant increment in protein concentration in Scenedesmus sp. at lower temperature.

Out of three different temperature regimes 18°C(±2), 26°C(±2), and 30°C(±2), the maximum biomass yield (1.51 mg/ml) was observed in the flask maintained at 26°C(±2). Microalgal culture has shown very less growth at a temperature or below 18°C (±2) with biomass productivity (0.57 mg/ml), while there was very less difference in growth was observed at 30°C(±2) (1.22 mg/ml), when to compare to high biomass yielding temperature regime (figure-10).

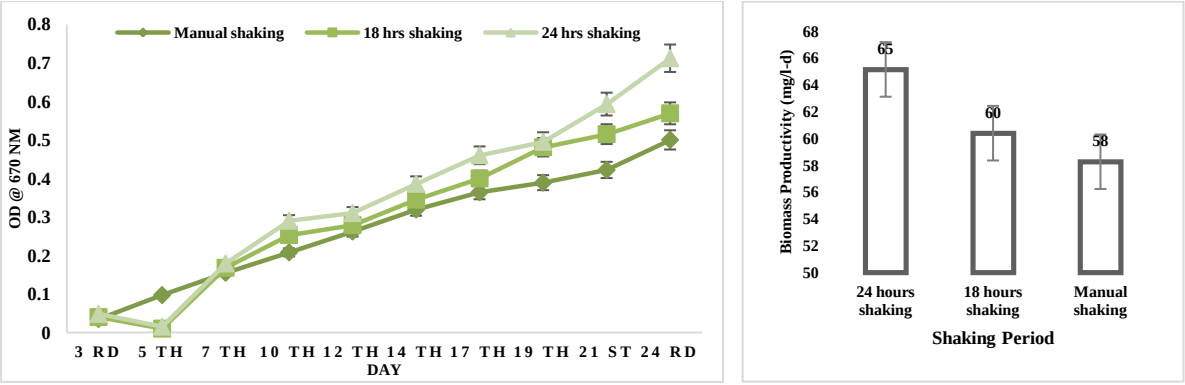


(a) **(b)**

Figure 10: Effect of temperature on biomass growth and productivity of microalgae. (a) And (b) both represent that maximum growth and productivity was found at temperature 26°C (±2). Growth was almost same at 30°C (±2) which represents that this microalgae can be grown within a range of temperature from 26-30°C (±2). OD: optical density; 670 nm.

In high dense culture, self-shading occur which results in decrease in light absorption capacity of photosynthetic antenna. Thus shaking is essential for mixing of nutrients and to prevent the settling of biomass. Agitation or shaking provides proper mixing which plays an important role in the increasing of microalgal biomass productivity [27].

In this experiment, out of three shaking conditions 24hours on shaking, 18 hours a day on shaking and manual shaking, 24 hours on shaking gave the highest biomass productivity 1.56 mg/ml while 18 hours shaking and manual shaking resulted in to 1.45 mg/ml and 1.40mg/ml biomass productivity respectively (figure-11).



(a) **(b)**

Figure 11: Effect of shaking period on biomass growth and productivity of microalga. (a) Represents comparative growth of microalga in different shaking period while (b) provides information of biomass productivity. Shaking of 24 hours is found best for both growth as well as biomass productivity of microalga. OD: optical density; 670 nm.

Conclusion

Based on morphology and molecular examination, microalgae isolated were identified as *Scenedesmus rubescens.* Effects of various culture conditions on green microalgae isolate *Scenedesmus rubescens* has been studied and thus concluded that the maximum growth rate and biomass productivity can be achieved by providing light having intensity, color and photoperiods of 36W, White and 16 hours respectively. Alkaline pH especially pH 8 supports the maximum growth rate and thus maximum biomass productivity. The optimum growth rate was observed in the 24h shaking period and 26 (±2) ºC temperatures. In future, effect of these culture conditions on biochemical composition of microalga will be studied to finalise the best culture condition for obtaining compatible feedstock for biofuels. Additionally, the microalga will be tested for its nutrient and heavy metal removal capacity from wastewater, biofuel production and nanoparticles synthesis.

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