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Research Article

Biochemical Characterization of *Chlorella Sorokiniana* towards Wastewater Treatment

Rajesh M. Jorgewad[1], Namrata Swami[1], Mudita Kadam[1], Shweta Patil[1], Basvaraj Palabhanvi[1] and Laxmikant H. Kamble[2]

[1]Department of Biotechnology Engineering,
KIT's College of Engineering, Kolhapur 416234 Maharashtra, India.
2. School of Life Sciences, S. R. T. M. University Nanded 431606, India
rajeshgudmalwar001@gmail.com Phone No. +919923957189

[*]**Corresponding author:** Email: rajeshgudmalwar001@gmail.com Tel. +919923957189

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ABSTRACT

Today, wastewater disposal is a critical issue worldwide. Also, the continuous need for energy and food is focusing us to search the feasibility of wastewater recycling and resource recovery. To overcome this problem, microalgae (*Chlorella sorokiniana* and *Scendesmus abundance*) cultivation having different application plays an important role for example wastewater treatment, biofuel production, feed and chemical, pharmaceutical. Using different carbon sources (glucose, starch, cellulose), protein, nitrate and biomass estimation were carried out and also effect of heavy metals were studied. Maximum growth was observed 1.5%glucose concentration under dark condition. In case of *Chlorella sorokiniana* increase in biomass under Artificial light was observed whereas *Scendesmus abundance* grows slowly under artificial light. Hence, *Chlorella sorokiniana* was selected for further studies under artificial light. *Chlorella sorokiniana* grown in waste water has shown better results.

This research work concluded that the selected microalgae strain that is *Chlorella sorokiniana* were studied with different carbon sources such as glucose, starch, cellulose and varying nitrate concentration and micro nutrient concentration. Also studied changes in biomass yield, microalgae cell constituents like protein and nitrate utilization kinetics for strain under natural and artificial light.

Keywords: - *Chlorella sorokiniana*, microalgae, wastewater, *Scendesmus abundance*

I INTRODUCTION

The present study illustrates the efficiency of microalgae based treatment system. Treatment of wastewater with Microalgae based system have the ability to remove nutrients (Nitrogen, Phosphorus and other nutrients), heavy metals, toxic substances (both organic and inorganic), BOD, COD and other impurities present in the wastewater by using the sunlight, CO_2, and impurities like nutrients present in the wastewater. The microalgae is having the ability to fix the excess carbon dioxide present in the environment and releases the oxygen which will somehow help in reduce global warming. According to the various study, the nutrients removal efficiency of microalgae based wastewater treatment system is very high as it removes 78-99% of Nitrogen and Phosphorus. The treatment system also succeeds to remove

40-65% of COD, BOD and other impurities present in wastewater. The microalgae treatment system is economical, green, and environmental friendly option of wastewater treatment [1]. Understanding of the factors that affect algae growth is essential. The growth rate of algae and cyanobacteria is influenced by physical, chemical and biological factors. Physical factors include light and temperature, chemical factors includes availability of nutrients and carbon dioxide, and biological factors are e.g. composition between species, grazing by animals and virus infections while the operational factors includes bioreactor design, mixing and dilution rate. Algae are a large and diverse group of simple organisms. Algae can be classified into two main groups as macroalgae and microalgae. Macroalgae are the large (in the size of inches and greater), Macroalgae are multicellular mostly growing in pond. These large multicellular algae are called seaweed. On the other hand, microalgae, are tiny (in the size of micrometers) unicellular algae that normally grow in suspension within water body[2].Cloudiness within a pond appears due to microalgae. Both types of algae grow extremely quickly compared to terrestrial plants .By considering this fact that it grow quickly makes them a promising crop for the human use[3-4]. Furthermore, there are two basic types of cells in algae, prokaryotic and eukaryotic. Prokaryotic lack membrane bounded organelles (such as plastids, mitochondria, nuclei, golgi bodies, and flagella) while the eukaryotic have the membrane bound organelles [5]. Microalgae recognize as one of the oldest living organism and called as thallophytes (plants lacking roots, stems and leaves). They have chlorophyll as their primary photosynthetic pigment and lack a sterile covering of cells around the reproductive cell [6]. Some of the most biotechnologically relevant microalgae are the green microalgae (chlorophyte) *chlorella sp., Haematococcus pluvialis, Dunaliella salina* and the *cynobacteria spirulina* species which are widely studied by many researchers also commercialized and used, mainly as nutritional supplement for human and as animal feed additives.

II MATERIALS AND METHODS

*Chlororella sorokiniana*as is a fresh water green microalgae. Their cells division rate is quite fast and divides into new cells every 17.24hrs. *Chlorella sorokiniana* is used to improve its biofuel production efficiency. This strain can grown photo- autotrophically in BG-11 medium in flasks at 25°C, 70 µmol photons m^{-2} s^{-1}, with a photoperiod of 16:8 h light: dark condition and also best grow in 35- 40°C.

Scenedesmus abundans, genus of about 70 species of colonial green algae (family Scenedesmaceae) a common component of freshwater plankton. *Scenedesmus* species are used experimentally to study pollution and photosynthesis and are a potential source of biodiesel. In sewage purification processes, the algae provide oxygen for the bacterial breakdown of organic matter and thereby help to destroy other harmful substances. *Scenedesmus* species are nonmotile and usually consist of 4, 8, 16, or 32 cells arranged in a row. Some species are spiny or feature bristles. Reproduction is by nonmotile spores called autospores.

Pure culture of *Chlorella sorokiniana* and *Scendesmus abundance* were obtained from the NCIM, Pune and maintained on BG-11 agar plates. These strains were grown photoautotrophically in BG-11 medium in flasks at 25°C, 70 µmol photons m^{-2} s^{-1}, with a photoperiod of 16:8 h light: dark. Irradiance was provided by warm-white fluorescent lamps. For physiological measurements, cultures were harvested during the logarithmic growth phase (about 1-3 ± 10^7 cells ml^{-1}). *Chlorella sorokiniana* is a single-cellular algae that typically ranges in size from 3 to 4 µm [7], though cells as small as 2 µm and as large as 4.5 µm have been reported [8-9].

Maximum growth rates for *Chlorella sorokiniana* vary based on the growth medium and conditions. In controlled growth media under phototrophic conditions, the algae concentration can double in as little as four to six hours.

Under mixotrophic conditions, which involve combining multiple energy sources, the addition of glucose to the medium can support a doubling

in algae concentration in no more than three hours. *Chlorella sorokiniana* grows best between 35 and 40˚C [10].

BG11 Media Composition

Sr. No.	Chemical	Quantity (gm/l)
1	Sodium nitrate	1.5
2	Potassium phosphate	0.0004
3	Magnesium sulphate heptahydrate	0.075
4	Calcium chloride	0.027
5	Sodium carbonate	0.02
6	Citric acid	0.006
	Microelements	
7	Ammonium ferric citrate	0.006
8	EDTA Na2Mg	0.001
9	Manganese chloride	1.810
10	Zinc sulphite	0.222
11	Sodium molybdate	0.390
12	Copper sulphate	0.079
13	Cobalt nitrate	0.0494
14	Boric acid	2.830
15	pH -	7.4

Various carbon sources

1. Glucose 1.5%
   Bg11+ 1.5 g for 100 ml
2. Cellulose 1.5%
   Bg11+ 1.5 g for 100 ml
3. Starch 1.5%
   Bg11+1.5 g for 100 ml

Wastewater Media

Sr. no	Wastewater	Distilled water	Total volume
1	200	0	200
2	100	100	200
3	150	50	200
4	175	25	200

Nitrate estimation for *Chlorella Sorokiniana* was performed by taking 1.0 ml of cell culture was centrifuged at 10,000 rpm for 5 minutes to separate the pellets. Supernatant was collected and used for analysis. 100 µl of sample was added in 0.4 ml of reagent A. Sample was incubated at 25^0C for 20 minutes. After that 9.5 ml of reagent B was added to the contents. Reaction mixture was cool to room temperature and OD at 410 nm was measured. Nitrate content in medium was calculated using the correlation equation.

C. Sorokiniana pigments were extracted with methanol (99.9%) during 30 min in the dark at 45ºC.Absorption spectrum was collected in the range 400–750 nm on a spectrophotometer. Chlorophyll-a (CChl-a), chlorophyll-b (CChl-b) and photoprotective carotenoids (CPPC) concentrations were determined according to the equations of Ritchie (2006) for chlorophylls and Strickland and Parsons (1968) for carotenoids[11-12].

C. Sorokiniana pigments were estimated by spectrophotometrically by wavelength scan of absorbance from 375 to 750 nm. Multiply values by volume of acetone extract and divide by the volume filtered (all in milliliters). The results are in milligrams per liter. Note that each OD value is corrected for the absorbance at 750 nm (e.g. $OD_{664} = Abs_{664} - Abs_{750}$). Absorbencies at 480, 652 and 665 nm were corrected from turbidity by subtracting absorbencies at 750 nm. Pigment content was obtained by dividing biomass concentration.

Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) of Wastewater:

Prior to completing the COD test, a series of known standards were prepared using KHP (potassium hydrogen phthalate). Most wastewater samples were fall in the high range, so standards of 100, 250, 500 and 1000 mg/L ware typically prepared. COD standards can also be purchased. For BOD test, a wastewater samples were brought to be in the pH range of 6.5 - 7.5 by using acid or base. Samples were observed under microscope for adequate microbiological population. Specialized 300 ml BOD bottles were allowed to full filling with no air space and provide an airtight seals were used. The bottles were filled with the sample to be tested or dilution with distilled water and various amounts of the wastewater sample were added to reflect different dilutions. One bottle was kept filled only with dilution water as a control or blank. A DO meter was used to measure the initial dissolved oxygen concentration (mg/L) in each bottle, which which was close to 8.0 mg/l. Each bottle was

placed into a dark incubator at 20°C for five days. After five days (± 3 hours) the DO meter was used again to measure a final dissolved oxygen concentration (mg/l), were reduced to 4.0 mg/l. The final DO reading was then subtracted from the initial DO reading and the result was BOD concentration (mg/l). Some wastewater sample required dilution, the BOD concentration reading was multiplied by the dilution factor. Protein Estimation of *Chlorella Sorokiniana* was measured by Folin – Lawry method [13].

III RESULTS AND DISCUSSION

Selected green microalgae strain *Chlorella sorokiniana* were studied for biomass yield, protein and nitrate utilization kinetics. Also, pigment quantification procedures were performed using methanol, but due to procedural inefficiency the results were not valedictory.

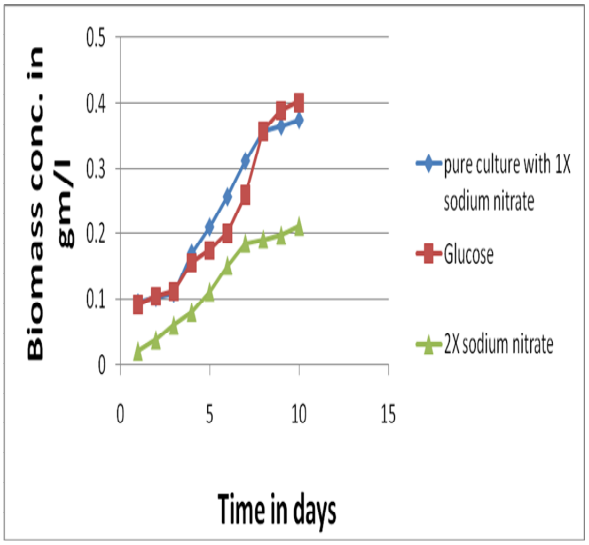

Figure 1: Growth kinetics of *Chlorella sorokiniana* with glucose and 2X sodium nitrate addition

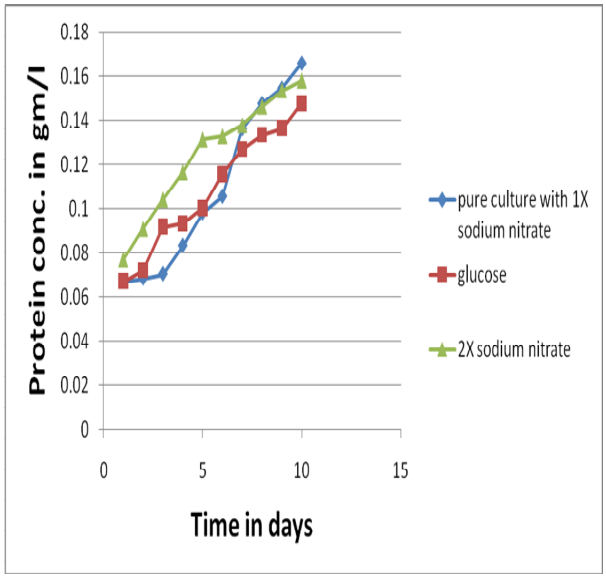

Figure 2: Protein kinetics of *Chlorella sorokiniana* with addition of glucose and 2X sodium nitrate

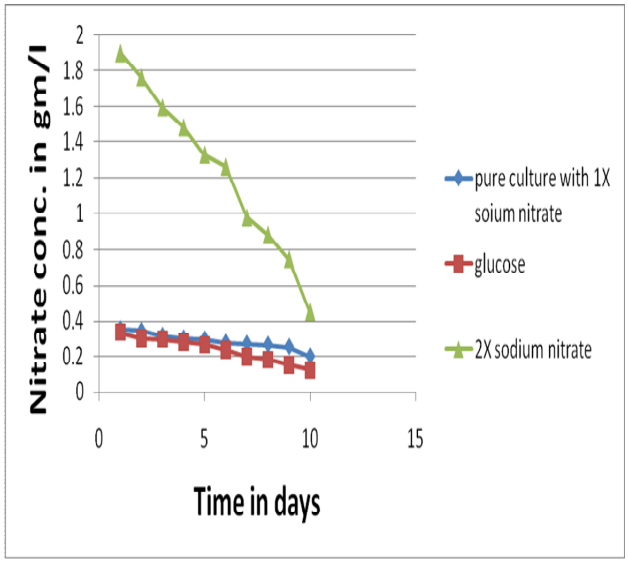

Figure 3: Nitrate uptake kinetics of *Chlorella sorokiniana* with glucose and 2X sodium nitrate

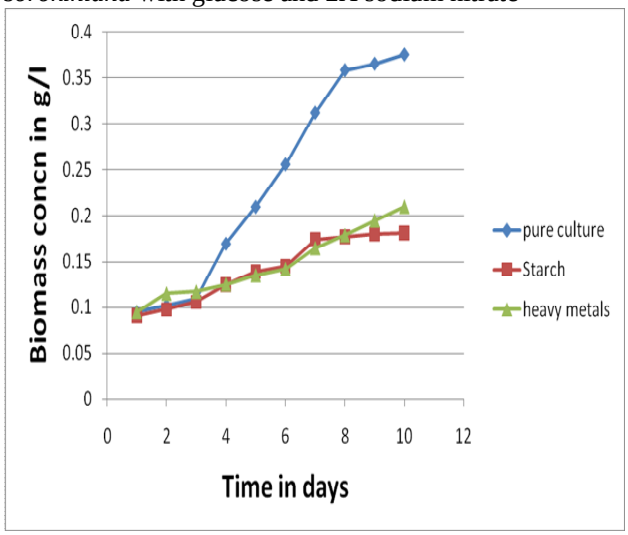

Figure 4: Growth kinetics of *Chlorella sorokiniana* with starch and addition of heavy metals (cadmium0.1%)

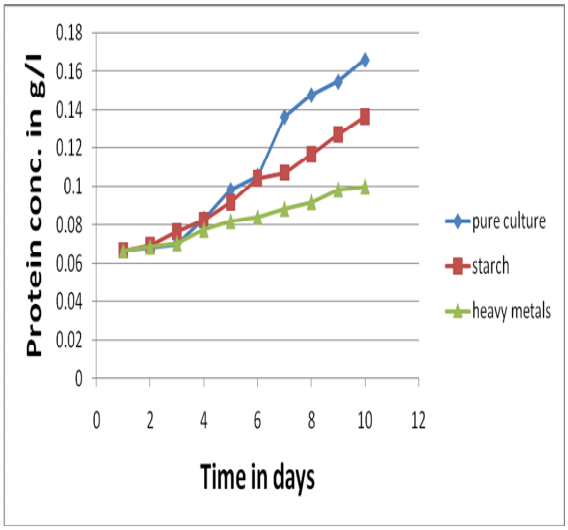

Figure 5: Protein kinetics of *Chlorella sorokiniana* with addition of starch and heavy metals (cadmium0.1%)

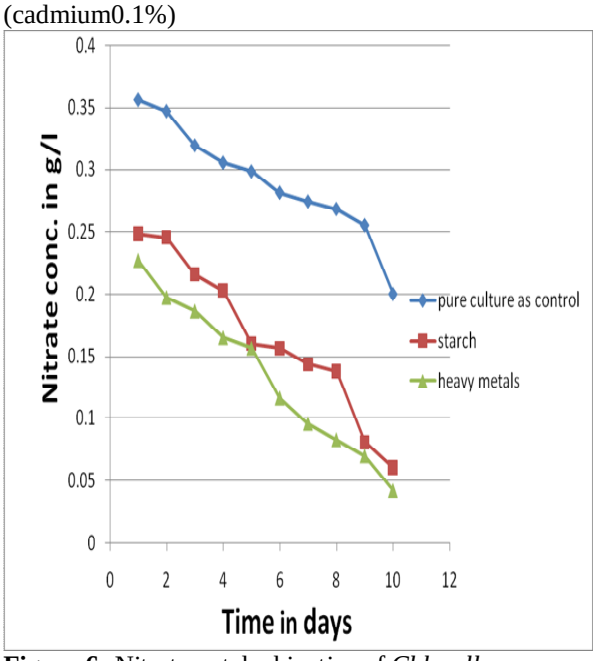

Figure 6: Nitrate uptake kinetics of *Chlorella sorokiniana* with addition of starch and heavy metals (cadmium0.1%)

Here, it shows that *chlorella sorokiniana* flourishes well in presence of glucose. However the graph remains steady when heavy metals and starch are added to medium. Hence, this indicates that *chlorella sorokiniana* could be used for wastewater treatment in industries for nutrient recovery where nitrate is recovered efficiently.

Growth curve of *Chlorella sorokiniana* was studied in presence of natural light as well as artificial light.

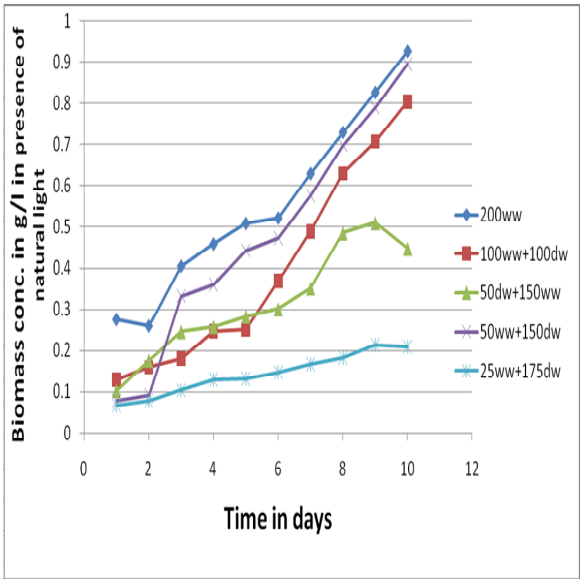

Figure 7: Growth kinetics of *Chlorella sorokiniana* with different dilutions in presence of natural light

ww= wastewater **and dw=** distilled water

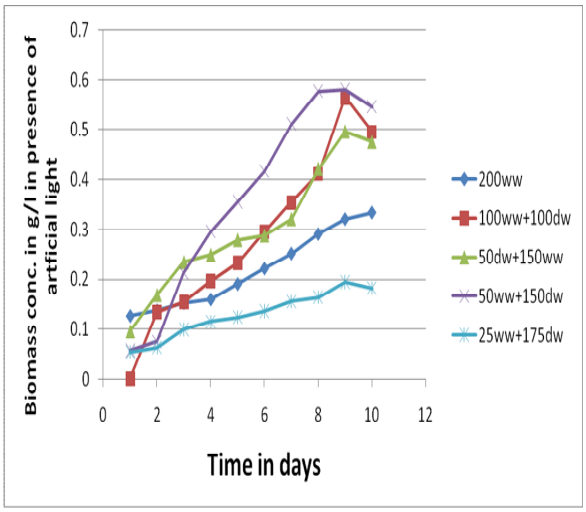

Figure 8: Growth kinetics of *Chlorella sorokiniana* with different dilutions in presence of artificial light

IV DISCUSSION

Chlorella sorokiniana works better in wastewater and the carbon source is better as glucose which would be used for dairy water treatment. *C. sorokiniana* grows vigorously in the presence of natural light in comparison to artificial light. *C. sorokiniana* is very compatible to the natural environment and can easily adapt to sewage wastewater so, it could be used commercially for nutrient recovery purpose. *Chlorella sorokiniana* flourishes in presence of glucose as carbon source. Pigment extraction from microalgae remains in recent research trend. A microalga possesses more potential in wastewater treatment and also possesses high

capacity for nutrient recovery in industry. Depleting reserves and souring prices of petroleum and oil have daunting effect on the economy of many developed and developing nations and forced researchers, government and federal agencies to opt for development of alternate fuels. In recent years, there is renewed interest in the field of algal biofuel production owing to its ability to grow in non-agricultural waste land and municipal wastewater/agricultural runoff water. Microalgae are employed in agriculture as biofertilizers and soil conditioners. The majorities of cyanobacteria are capable of fixing atmospheric nitrogen and are effectively used as biofertilizers. These algal pigments have potential as natural colorants for use in food, cosmetics and pharmaceuticals, particularly as substitutes for synthetic dyes. Algal biodiesel production includes five major processes i.e., cultivation, harvesting, drying, cell disruption, and oil extraction (lipid extraction) and transesterification of extracting lipids from Sustainability microalgae.

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