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Optimization of the Growth and Performance of Several Cynobacteria Species in a Pilot Scale Raceway Pond for CO_2 Bio-Sequestration

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ABSTRACT

Due to the limited availability of fresh water and the high cost of land for plant culture, microalgae cultivation has attracted significant attention in recent years and has been shown to be the best option for CO_2 bio-sequestration. Bio-sequestration of CO_2 through algae bioreactors has been hailed as one of the most promising and ecologically benign methods available. This research study was taken up to alleviate certain limitations associated with the technology such as low CO_2 sequestration efficiency and low biomass yields. In this study three distinct cyanobacterial strains, *Chlorella* sp., *Synechococcus* sp., and *Spirulina* sp., were tested in 10 litre raceway ponds for their capacity for CO_2 bioconversion and high biomass production under various CO_2 concentrations at different EC. The highest growth rate of all tested cyanobacterial strains was observed during the first 4 days of cultivation under CO_2 5% to 10%. Additionally, all these cyanobacterial strains were explored for their bioremediation capabilities. The results showed that the *Chlorella* sp., *Synechococcus* sp., and *Spirulina* sp. were able to remove COD of the wastewater by 56%, 48% and 77% respectively and the BOD removal efficiency was 48%, 30% and 52% respectively. The primary results indicated that the *Spirulina* sp. was to be the best cynobacteria studied in terms of biomass production, CO2 bioconversion, and bioremediation capacities. Therefore, the *Spirulina* sp. was further scaled up in 1500 litre raceway pond for CO_2 bio-sequestration and biomass production. The biomass collected was utilised to extract biomolecules such as protein, carbohydrate and lipids.

Keywords : CO_2 bio-sequestration, Cyanobacteria, Spirulina sp., Raceway Pond, Biosorption

I. INTRODUCTION

All countries are dealing with a variety of problems right now, but none is more perplexing than global warming (Lowe et al 2009). The fundamental cause of climate change was the generation of greenhouse gases (GHGs) by humans (Solomon et al., 2007). Consequently, in order to manage the emissions of

these gases, it is vital to design technology that is both environmentally benign and cost effective. Carbon dioxide (CO_2) is the primary greenhouse gas which is the main reason for the climate change; carbon capture and storage (CCS) systems are critical components of GHGs mitigation strategies (Cao and Caldeira, 2010). As a result, various mechanisms and protocols, particularly in the case of large source emissions, are being used to reduce greenhouse gas emissions resulting from anthropological activities. Because of their potential to reduce greenhouse gas emissions, CCS technologies are included in these initiatives (Gough, 2008). A physical fixation strategy and a chemical adsorption strategy have both been proposed as ways to slow the increase in CO_2 concentration. Aside from that, biological mitigation as a climate change mitigation strategy has received a great deal of attention in recent years due to the generation of secondary pollutants from these strategies.

The biofixation of CO_2 achieved by microalgae and cyanobacteria has received a great deal of interest across the globe because of its high efficiency in CO_2 removal (Ono and Cuello, 2006; Chang and Yang, 2003; Fan et al., 2007). A wide range of applications for the microalgal biomass that is produced, including the production of biofuels and medical supplements, can be made with this material(Arata et al., 2013). The microalgae *Spirulina platensis* has previously been identified as a most popular microalgae strain that has been shown to be effective in the biofixation of carbon dioxide (Carvalho et al., 2004; Zeng et al., 2012; Soletto et al., 2008). Furthermore, *Spirulina* is widely regarded as one of the most important sources of commercially viable renewable feedstock for the production of single cell protein and other high-value metabolites, the most notable of which is phycocyanin, which is found in high concentrations in the microalgae (Leema et al., 2010). *Spirulina* is also widely regarded as a potential source of single cell protein and other high-value metabolites for human consumption. This natural food is commonly used to combat malnutrition because of its high nutritional content, which is comprised of high-quality proteins, carbohydrates, fats, vitamins, minerals, pigments, and antioxidants, as well as its high nutritional content. Therefore, *Spirulina* cultivation has expanded to rural areas in a number of developed and developing nations.

When it comes to photosynthesis, algae and green plants have developed a mechanism known as the carbon concentration mechanism (CCM). Sequestration studies, on the other hand, are preferred because CCM enhances algae growth rates (Mehar et al., 2019). The ability of cyanobacteria and microalgae to utilize dissolved inorganic carbon (DIC) has recently been the subject of numerous studies. They can change their affinity for DIC (HCO_3 and CO_2), depending on the concentration of DIC in their environment, and there is an active mechanism that allows CO_2 to be concentrated inside the cell (Ghoshal et al., 2002; Riebesell et al., 2007). Climate and environmental factors such as light, temperature, nutrient status, and salinity all have an impact on photosynthesis and productivity of biomass. Biomass measurement or growth rate evaluations are required in order to determine the potential of a microalgal system for directly removing CO_2. It was previously reported that the efficiencies of *Spirulina* biomass in raceway pits are affected by the above mentioned climate and environmental factors. The pH of a solution is important for cell development, CO_2 utilization, and contamination prevention. Unwanted changes in the pH of the cultivation medium disrupt the balance between gaseous CO_2 in the medium and other DIC, reduce the availability of nutrients, and impair the photosynthetic and metabolic processes of microalgae, among other things. *Spirulina* growth is accelerated by the alkaline pH, which results in increased mass transfer of CO_2 and high HCO_3 ions in the water. Despite this, it is well known that high CO_2 levels prevent the accumulation of

phycobiliprotein and other pigments in the body, such as phycocyanine.

The majority of studies, however, have concentrated on culturing microalgae in fresh water, but considering the wide variation in water quality around the globe, this paper studied the efficiency of various cynobacteria species on CO_2 sequestration. The pH of the culture medium is not controlled in the usual method of microalgae production, which is a disadvantage. In other circumstances, the pH is maintained through the addition of acid (HCl) or alkali (NaOH), which not only results in an increase in chemical waste but also has the potential to damage the cells themselves. As a result, in this investigation, we attempted to overcome the bottlenecks associated with the use of chemical carbon species by supplementing the culture with gaseous CO_2, at the same time optimizing the ideal pH.

II. MATERIAL AND METHODS

2.1 Collection of microalgae strain, culture media, and seed culture development for pilot scale raceway pond

The cynobacteria species used in this study were obtained from the National Collection of Industrial Microorganisms (NCIM), in Pune, Maharastra, India. Using a modified Zarrouk's medium, all tested cynobacteria species such as *Chlorella* sp., *Synechococcus* sp. and *Spirulina* sp. were inoculated into a 2L Erlenmeyer flask containing 1L autoclaved media and incubated at room temperature under cool white fluorescent lighting with a light intensity of 27 E m^{-2} $s^{-1}$and a photoperiod of 12:12 h light:dark. Zarrouk's medium (ZM) is the name given to the modified nutritional medium used in the current investigation for the sake of simplicity and data representation in the results. The modified ZM medium was composed of $NaHCO_3$ (10 g), K_2HPO_4 (0.5 g), $NaNO_3$ (2.5 g), K_2SO_4 (1.0 g), NaCl (1.0 g), $MgSO_4.7H_2O$ (0.2 g), $CaCl_2.2H_2O$ (0.04 g), $FeSO_4.7H_2O$ (0.01 g), Na_2EDTA (0.08 g), H_3BO_3 (2.8 g), $MnCl_2.4H_2O$ (1.8 g), $ZnSO_4.7H_2O$ (0.2 g), $CuSO_4.5H_2O$ (0.074 g), and MoO_3 (0.015 g). Chemicals, reagents, metal ions, and medium components were acquired from Himedia, Mumbai, India, and were of analytical grade or higher purity available. Moreover, following the exponential growth face of the microalgae and prior to the establishment of the stationary growth phase, the seed culture for experiments was grown consecutively by scaling the culture from 1 L to 4 L. Further, the cultures were propagated in carboys in order to scale up to a volume of 20 L at ambient conditions. Additionally, the culture was acclimatized with the addition of pure CO2 (99.99 %) as the sole source of carbon and scaled up to 360 L using the seed culture development open raceway ponds available at the pilot scale Spirulina cultivation facility, where it was successfully grown to maturity. The optical density (O.D.) of *Spirulina* at 560 nm was measured on a regular basis to track its development.

2.2. Design, construction, and operation of a micro algae growing system in a pilot scale open raceway pond

Primary screening of all the tested cunobacteria species were PVC chamber having 28 cm height, 88 cm length and 64 cm width containing a total volume of 10 L in laboratory conditions. Moreover the screeing of the best cynobacterial species there cultivation was carried out in a 1500L open raceway pond having a working volume of 1500 L having 700 cm height, 300 cm length and 400 cm width. In the designed pond data logging for pH, temperature, light, dissolved oxygen (DO), and agitation was automatic. Most of the *Spirulina* cultivators record all these measurements manually which is very laborious and time consuming. The pH, dissolved oxygen (DO), and temperature sensors were immersed in the culture. The key feature of this system is the ability to adjust the pH of the culture medium by the addition of CO_2

at varying concentrations and flow rates to the medium. An additional CO_2 supply unit, which purges the CO_2 based gas on pH set points, an air mixing system to regulate CO_2 concentration, and the management of gas flow rate were included in the system.

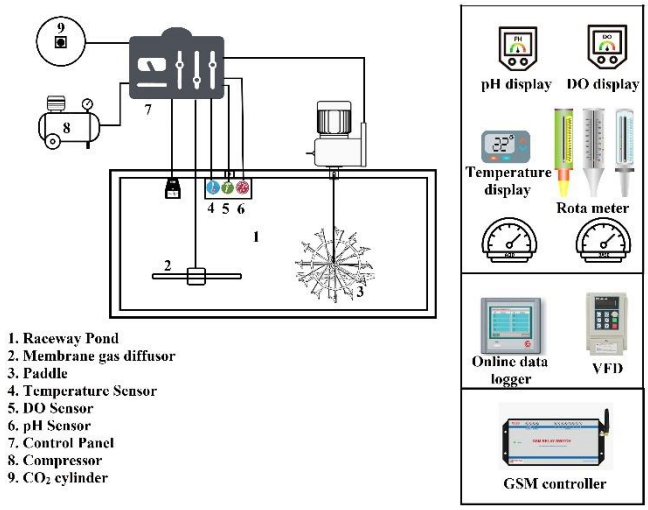


Figure 1: A graphical representation of the 1500 litre open raceway pond, including the control panel and CO_2 feeding system.

2.3 Performance of *Spirulina* sp. at varied pH levels in in a 1500L open raceway pond

Effect of various pH on the overall treatment performance of the *Spirulina* was determined. The study was conducted in the ambient temperature of the month March to July, 2019. The culture medium used in this study is ZM except $NaHCO_3$ which was replaced by CO_2 as a sole source of carbon. The municipal water supplied for the domestic use was used for the preparation of the culture growth medium to reduce the overall all treatment cost. The acclimatized seed culture prepare in the ambient condition in a small outdoor pond was used as a inoculum. The culture containing optical density of 0.2 at 560 nm was used as an innoclum in the working volume of the pond (1500 L). Using pure food grade gaseous CO2, we were able to regulate and maintain the pH of the culture at the desired level (either pH 7.5, 8.5 or 9.5).

2.4 Cell proliferation, quantification of biomass, and determination of ash content

Cell growth of the *Spirulina* sp. was determined by calculating the optical density of the culture at 560 nm by using UV–vis spectrophotometer (Shimadzu, UV-1800). Calculation was done by plotting the standard curve prepared by diluting the Spirulina culture of varying OD verses concentration of cells (mg L^{-1}). Moreover the biomass content was determined by using dry weight procedure. Briefly fixed and same volume of culture suspension was filtered through a pre-weighed glass fibre filters (0.45 µm), and dried in a hot air oven for 12 h at 60 °C. Further the biomass was gravimetrically calculated based on the change in filters weight, and the dry weight (mg L^{-1}). Whereas, approximately 3 g of lyophilized Spirulina biomass was dried and corrected for the amount of moisture present in the sample using an automated moisture analyser (Sartorious, MA160) to determine the ash content. Additionally, the total ash content was determined by incinerating the moisture analyzer sample for 6–8 hours at 525 °C in a muffle furnace.

2.5. Harvesting, lyophilisation, and storage of biomass

The filtration process was used for the harvesting of the culture after the completion of the a pilot scale raceway pond. Primarily 1mm sieve was used to remove the debris and external products from the culture. After that the pre-filtered microalgae culture was filtered through a 100 µm mesh size nylon cloth to obtain a wet biomass. Further to remove the salt from the collected biomass they were washed with 1 N HCl. Further, for 24 h the harvested biomass was freeze-dried using a vacuumed lyophilizer. The dried and powdered culture biomass was also kept at 4°C before being used in the subsequent analysis.

2.6 Determination of productivity of biomass, proteins, carbohydrates, lipids, and phycocyanine

2.6.1 Biomass productivity

The biomass productivity of the microalgae was determined by measuring the change in the biomass by following the below mentioned formula:

$$P = B_t - B_0 / (t - t_0)$$

The starting biomass concentration at time t_0 is represented by B_0, whereas the biomass concentration at any time t is represented by B_t.

2.6.2 Estimation of total protein

Total nitrogen content was estimated using the protein analyser (Thermo Flash 2000) on the lyophilized biomass (30 mg). The following formula was used to calculate the biomass's total protein content:

Total protein (%) = Total nitrogen (%) × 6.25

2.6.3 Estimation of total carbohydrate

The phenol sulfuric acid technique using glucose as a reference was used to assess the total carbohydrate content. Dried biomass (w/v) was dissolved in boiling water for 20 minutes and digested with 5 ml of 2.5 M hydrochloric acid (HCl). To eliminate effervescence and get final quantification data, the hydrolyzed biomass was chilled, neutralised with anhydrous Na_2CO_3, and diluted (10 ml). The developed colour was measured at 490 nm after adding 1 ml of 5% phenol and 5 ml of 96% H_2SO_4 to the diluted solution (0.2–1.0 ml).

2.6.4. Estimation of total lipids

The lyophilized cynobacteria biomass (100 mg) was dissolved in a 10:10:9 mixture of chloroform, methanol, and water. The samples were then vortexed and shaken overnight before being centrifuged at 5000 g for 5 minutes. Further, the bottommost layer of chloroform containing the lipids was removed using a pre-sterile 0.2 m syringe filter in a pre-weighed glass vial (W1). The chloroform was then evaporated, and the glass vials were re-weighed (W2). The difference in weight of the glass vials (W3 = W2 W1) was used to compute the % lipid content when the quantity of biomass utilized for extraction was taken into account.

2.6.5. Estimation of phycocyanin

To quantify crude phycocyanin (Cp), 20 mg of lyophilised biomass was mixed with 20 ml of 100 mM phosphate buffer (w/v) at pH 7. It was then well blended and refrigerated overnight at 4 °C. The sample was centrifuged the next day, and the absorbance of a supernatant was measured at 615 nm and 652 nm. The phycocyanin extract was quantified using Bennett and Bogorad's equation, which is as follows:

$$Cp = A_{615} - (0.474 \times A_{652}) / 5.34$$

Where; Cp = Crude phycocyanin concentration (g L^{-1}); A_{615}=Absorbance of the extract at 615 nm; and A_{652}=Absorbance of the extract at 652 nm.

2.7. Elemental composition (C, H, N, S and O content) analysis of biomass

With the use of a CHNSO analyser (ELIII, Vario, Germany), the lyophilised biomass was weighed in the range of 5–10 mg and examined for the measurement of the carbon (C), hydrogen (H), nitrogen (N), and sulphur (S) contents, among other things.

2.8. Analysis of carbon dioxide (CO2) bio-fixation rate

The following equation was used to compute the rate of inorganic carbon (IC) use (as equivalent CO2 bio-fixation) RCO_2 (g L^{-1} d^{-1}).

$$R_{CO2} = C_c P (M_{CO2}/M_c)$$

Where C_c represents the carbon content (% dry cell weight) of cynobacteria biomass and P represents biomass production. M_c and M_{CO2} where, the molecular weights of CO_2 and carbon, respectively.

2.9. Determination of alkalinity, NO_3^- and PO_4^{2-}

Representative samples of culture media were collected and centrifuged at 5000 rpm for 10 minutes, biomass was discarded and supernatant was used to analyze the concentrations of alkalinity, NO_3^- and PO_4^{2-}. The alkalinity was measured by following the titrimetric method by following the procedure described by Cheng et al. (2018). It was necessary to monitor the alkalinity of the culture medium on a daily basis in order to calculate the concentration of dissolved inorganic carbon ($HCO3^-$ and CO_3^-). The concentrations of NO_3^- and PO_4^- in the cynobacteria species were measured on a daily basis during the cultivation period. The amount of NO_3^- -N in the water was estimated by measuring the absorbance at 220 nm and 275 nm using an ultraviolet–visible spectrometer. The Vanadomolybdophosphoric acid colorimetric technique was used to quantify the content of PO_4^{2-}-P in the sample.

2.10 Characterization of wastewater

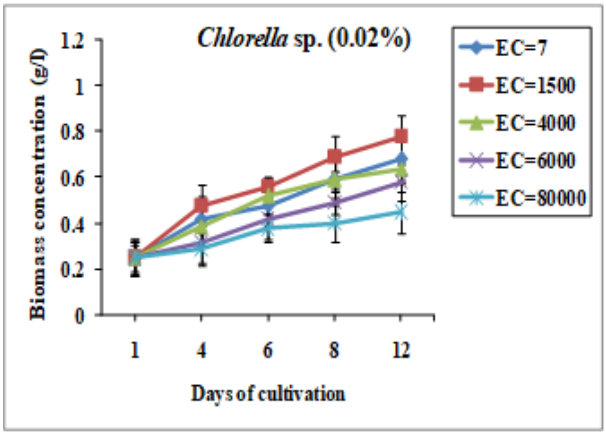
Chemical oxygen demand (COD), biochemical oxygen demand (BOD), total solids (TS), pH, phenols, total dissolved solids (TDS), sulphate, nitrate, alkalinity, acidity, total hardness, and the presence of heavy metals were used to characterise the wastewater. The pH value was determined using a digital pH metre (ANALAB, India). BOD was determined using a DO metre (HACH, DO 6). The COD value was determined using a HACH DRB200 thermoreactor and a HACH DR6000 spectrophotometer. Metals were detected using a Perkin Elmer Optima 3300 RL ICP-OES (Inductive Coupled Plasma-Optical Emission Spectrometer). All other parameters were determined in accordance with APHA 2005.

III. RESULTS AND DISCUSSION

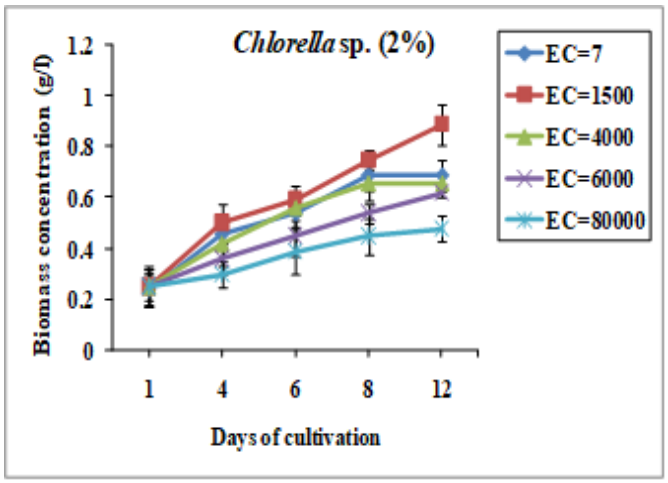
3.1 Screening of cynobacteria species for CO_2 bio-sequestration

Initially in this study, three distinct cynobacteria species, including *Spirulina* sp., *Chlorella* sp., and *Synechococcus* sp., were primarily tested for their capacity to bio-sequester CO_2 in 10 litre raceway ponds. The biomass productivity, growth rate, and CO_2 sequestration rate of each cynobacteria species were determined at varying levels of CO_2 and EC. The effect of various CO_2 concentrations (0.02%, 2%, 5%, and 10%) on the growth of each tested cynobacteria species is depicted in Fig.1. It has been widely documented in the published literature that microalgae are more effective at carbon sequestration, although each examined cynobacteria species is efficient at growing at elevated CO_2 concentrations. Additionally, it was previously reported that when CO_2 concentrations were increased, microalgae cells grew more efficiently, and their organelles containing chlorophyll a and b made them very effective at photosynthetic activities that result in the conversion of CO_2 to O_2 (Singh and Singh, 2014). As shown in the Fig. 1 all three tested cynobacteria species were efficient grown in the CO_2 concentration of 5 and 10%. Similarly, Shabani et al. (2016) observed that *Spirulina platensis* and *Chlorella vulgaris* grew at their highest rates at CO_2 concentrations ranging from 0.03 to 10%. In another study Tang et al. (2011) observed the highest growth rate of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* at CO_2 concentrations ranging from 5% to 30%. Upendar et al. (2017) evaluated the effacement of *Synechococcus* sp. NIT18 for CO_2 sequestration and also reported a high biomass of the tested cynobacteria at elevated CO_2 concentrations (5-20%).

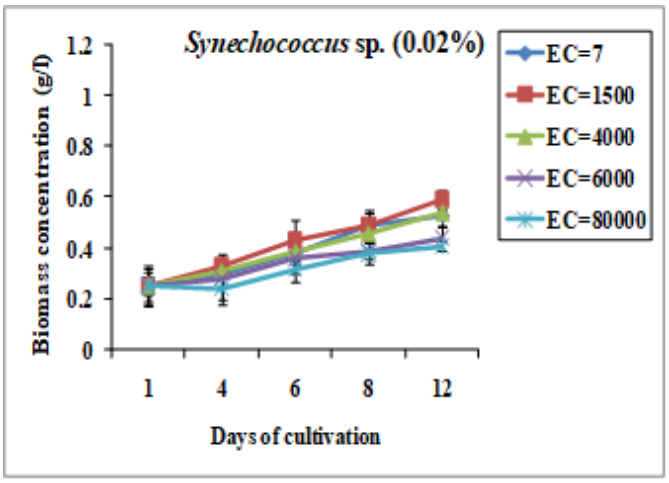
(a)



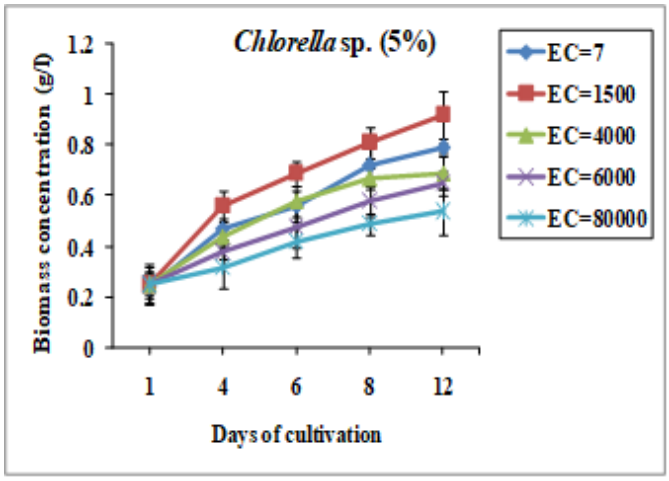
(b)


Chlorella sp. (2%)
Biomass concentration (g/l)
Days of cultivation
EC=7
EC=1500
EC=4000
EC=6000
EC=80000

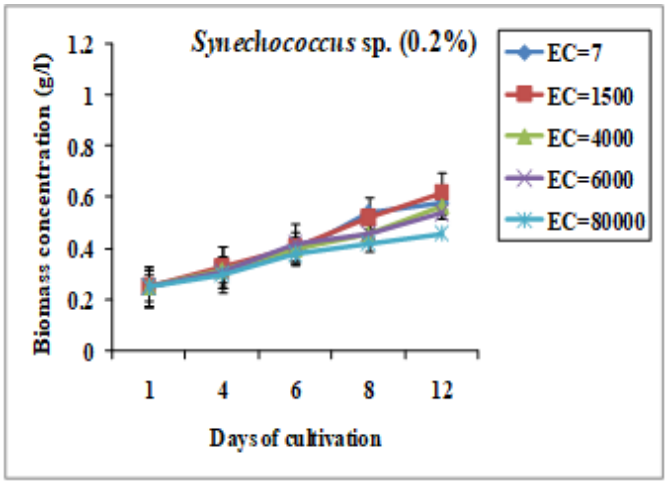
(e)


Synechococcus sp. (0.02%)
Biomass concentration (g/l)
Days of cultivation
EC=7
EC=1500
EC=4000
EC=6000
EC=80000

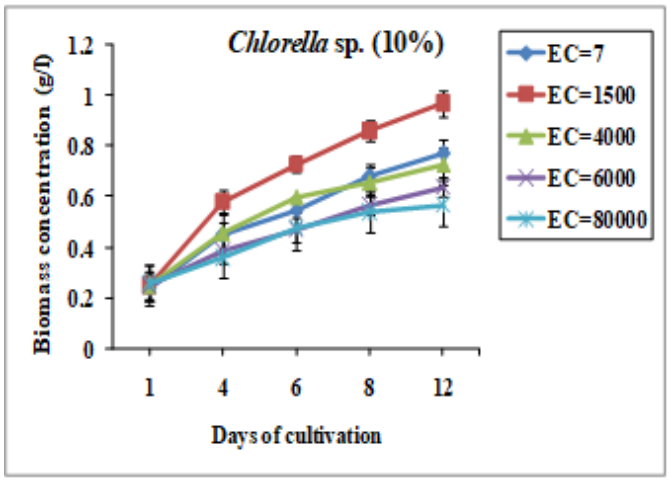
(c)


Chlorella sp. (5%)
Biomass concentration (g/l)
Days of cultivation
EC=7
EC=1500
EC=4000
EC=6000
EC=80000

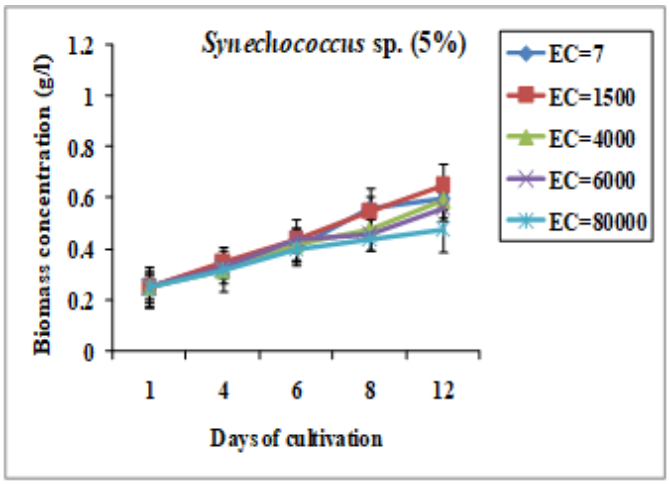
(f)


Synechococcus sp. (0.2%)
Biomass concentration (g/l)
Days of cultivation
EC=7
EC=1500
EC=4000
EC=6000
EC=80000

(d)


Chlorella sp. (10%)
Biomass concentration (g/l)
Days of cultivation
EC=7
EC=1500
EC=4000
EC=6000
EC=80000

(g)


Synechococcus sp. (5%)
Biomass concentration (g/l)
Days of cultivation
EC=7
EC=1500
EC=4000
EC=6000
EC=80000

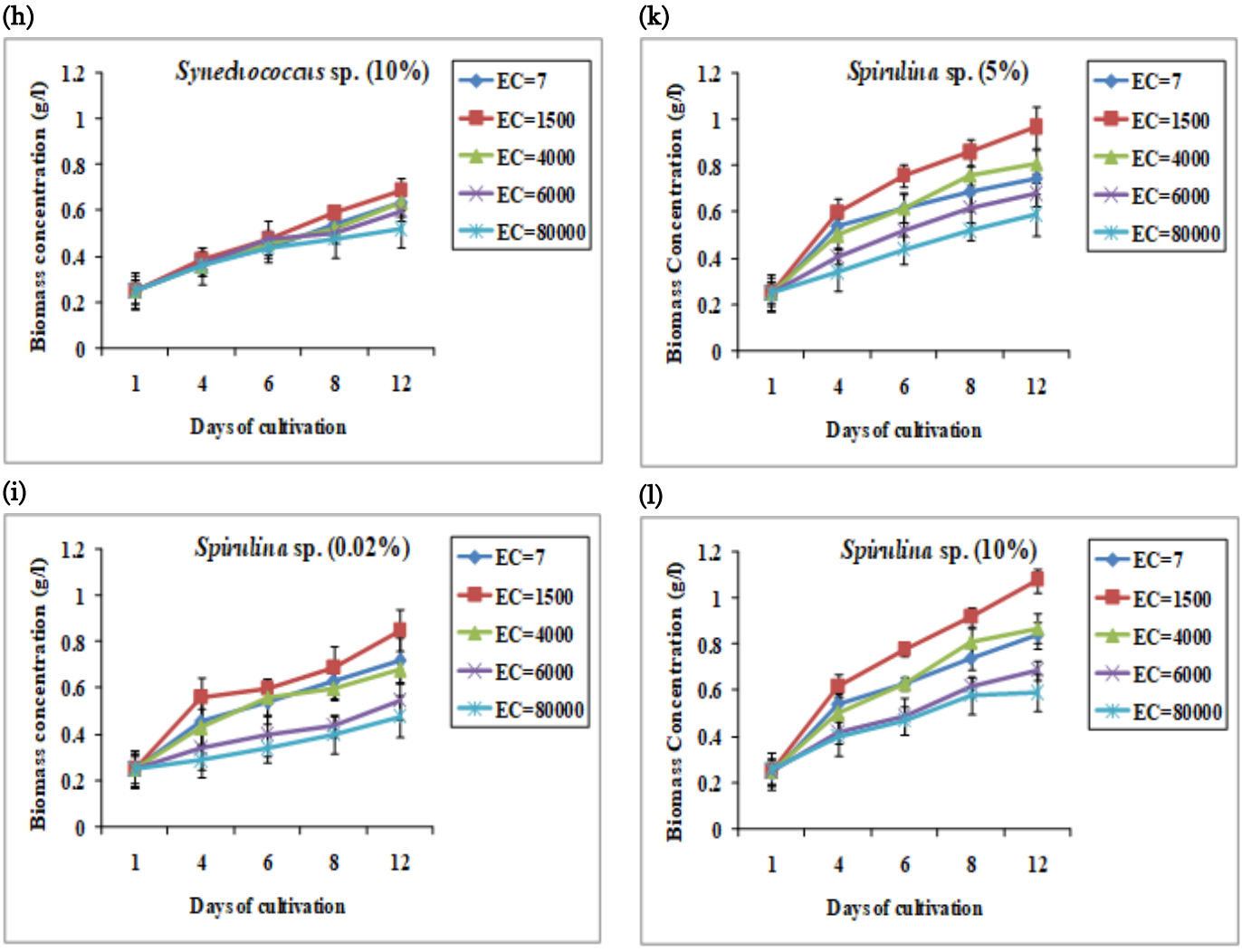


Figure 2 : Biomass production of Chlorella sp., Synechococcus sp. and Spirulina sp. under various CO2 concentration and EC in 10 litre PVC raceway ponds.

As illustrated in Fig. 1, a significant increase in the growth rate of all tested cynobacteria species was observed after the initial four days of cultivation at EC 1500, moreover after that time period of cultivation the growth rate was increased subsequently. *Chlorella* sp. produced 0.78, 0.89, 0.92, and 0.97 g/l of biomass in the presence of CO_2 concentrations of 0.02, 2, 5, and 10%, respectively, in four different experiments. Whereas *Synechococcus* sp. showed 0.59, 0.62, 0.65 and 0.69 g/l of biomass production in the presence of CO_2 concentrations of 0.02, 2, 5, and 10%, respectively. While as compared to *Chlorella* sp. and *Synechococcus* sp., *Spirulina* sp. showed highest biomass production of 0.85, 0.92, 0.97,

and 1.08 g/l in the presence of CO_2 concentrations of 0.02, 2, 5, and 10%, respectively. This indicated that among all tested cynobacteria species *Spirulina* sp. was the best cynobacteria for the growth and CO_2 bio-sequestration which was further used in large scale raceway pond.

3.2 Wastewater treatment efficiency of screened cynobacteria species

The removal of COD, BOD, and other wastewater parameters such as alkalinity, acidity, and metal ions is considered to be the primary indicator of a microrganism's treatment efficiency. Following the results shown in Table 1, all three of the examined cynobacteria species are effective in the treatment of wastewater collected from the discharge point of the CETP treated effluent into the sabarmati river.

Parameters (mg/L)	Wastewater	*Chlorella* sp. treatment	*Synechococcus* sp. treatment	*Spirulina* sp. teratment
COD	810±11	356±15	417±12	185±17
BOD	431±8	221±7	298±18	204±3
TSS	46±4	28±4	32±6	17±2
TDS	54±6	30±4	38±2	18±1
Phenols	68±14	38±6	43±5	23±4
Sulphate	63±3	27±4	38±7	15±2
Nitrate	21±3	10±1	17±3	N.D.
Alkalinity	13±4	6±2	9±1	N.D.
Acidity	62±9	18±6	41±4	N.D.
Total hardness	38±3	27±5	23±3	14±3
Iron	6.32	N.D.	2.84	N.D.
Copper	4.86	N.D.	1.42	N.D.
Zinc	5.6	N.D.	2.18	N.D.
Barium	3.6	N.D.	N.D.	N.D.
Chromium (VI)	44.6	11.8	24.6	4.9
Cobalt	32.4	24.3	28.8	18.4

N.D.= Not detected

Table 1: Characterization of treated and untreated wastewater by tested cynobacteria species.

The BOD_5:COD ratio is the primary indicator of a water's biodegradable nature. It was previously reported that BOD_5:COD ratios less than 0.1 were considered non-biodegradable, while BOD_5:COD ratios greater than 0.5 were considered biodegradable (Azbar et al., 2004; Han et al., 2015). The BOD_5:COD ratio of the wastewater used for cynobacterial screening was 0.53, indicating that the water is readily biodegradable. After treatment of wastewater with *Chlorella* sp., *Synechococcus* sp., and *Spirulina* sp., the BOD5: COD ratio increased to 0.62, 0.71, and 1.10, respectively, indicating that all of these tested cyanobacteria species are not only treating the wastewater but also simultaneously increasing the BOD_5: COD ratio of the water, resulting in complete mineralization of the organic compounds present in the wastewater after its discharge into natural ecosystem. Moreover, *Chlorella* sp., *Synechococcus* sp., and *Spirulina* sp., were also found to be efficient in the COD removal of the wastewater. The *Chlorella* sp. showed 56% COD removal, *Synechococcus* sp. showed 48% COD removal and *Spirulina* sp. showed the maxium COD removal of 77%. Additionally the BOD removal was found tobe 48%, 30% and 52% by *Chlorella* sp., *Synechococcus* sp., and *Spirulina* sp. respectively.

3.3 Effect of temperature on the growth of *Chlorella* sp., *Synechococcus* sp., and *Spirulina* sp.

After 10 days of cultivation, the effect of temperature was determined at optimum EC and 10% CO_2 concentration. According to the results, all of the tested cynobacteria species grew efficiently at temperatures ranging from 30 to 35 ˚C.

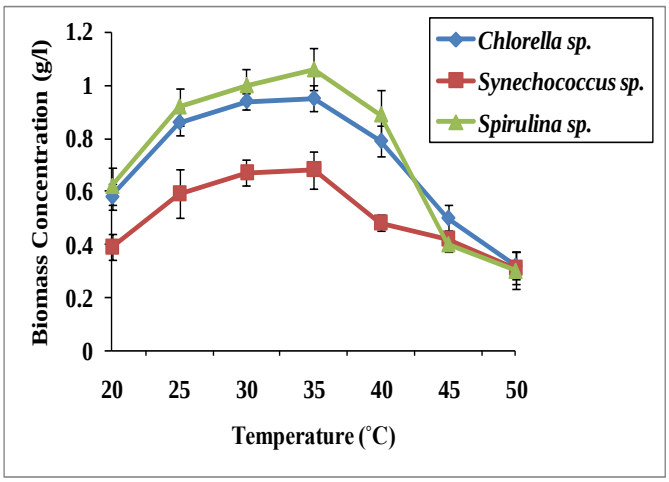


Figure 3: Biomass production of *Chlorella* sp., *Synechococcus* sp. and *Spirulina* sp. at various temperatures.

Chlorella sp. produced the maximum biomass (0.95 g/l) at 35°C, while *Synechococcus* sp. produced the maximum biomass (0.68 g/l) at 35°C. Additionally, when compared to *Chlorella* sp. and *Synechococcus* sp., *Spirulina* sp. showed the maximum biomass production (1.06 g/l) at 35°C. These findings suggest that *Spirulina* sp. can be used for bio-sequestration at ambient temperature. Previously similar results were observed by Oliveira et al. (1999) authors observed the maximum growth of S. maxima and S. platensis between the temperature 25 to 35 ˚C.

3.4 Effect of pH variations induced by CO_2 feeding on the production of *Spirulina* sp. biomass in a 1500 litre raceway pond

It has previously been shown in the literature that the pH of the culture medium has a significant impact on the growth and cell behaviour of microalgae throughout the cultivation period (Mehar et al., 2019). The scarcity of knowledge on the intake and secretion of chemical species has clouded our understanding of the stoichiometry of algae's photosynthetic growth (Scherholz et al., 2013). As a result, it is difficult to attain high Spirulina growth rates, which would increase biomass productivity and economic viability of commercial systems such as open raceway ponds. .

As shown in Table 2, Spirulina sp. was capable of growing at all four pH values tested, including pH 7, 8, 9, and 10. The production of biomass was determined to be between 46 and 73 mg/l/d.

Table 2: Biomass production (mg/l/d) of *Spirulina* sp. in 1500 litre raceway pond, carbon content (%) and CO_2 bio-fixation rate (g/l/d) after 10 days of cultivation

Cultivation conditions	Biomass production (mg/l/d)	Carbon content (%)	CO_2 bio-fixation rate (g/l/d)
pH 7 ± 0.1 (+CO_2)	46 ± 2.85	41 ± 0.25	0.08 ± 0.02
pH 8 ± 0.1 (+CO_2)	68 ± 3.75	52 ± 0.41	0.13 ± 0.03
pH 9 ± 0.1 (+CO_2)	59 ± 4.52	50 ± 0.63	0.11 ± 0.04
pH 10 ± 0.1 (+CO_2)	48 ± 4.21	48 ± 0.79	0.12 ± 0.02
ZM (-CO_2; No pH control)	73 ± 3.66	51 ± 0.82	0.14 ± 0.01
ZM (+CO_2; No pH control)	67 ± 2.28	46 ± 0.47	0.10 ± 0.02

Furthermore, the biomass production of Spirulina sp. at different CO_2 levels and pH levels was compared to the ZM medium with and without CO_2 supplementation and pH control. The highest biomass production was recorded at pH 8 (68 3.75 mg/l/d), which was 20 mg/l/d more than pH 10 but less by 5 mg/l/d in ZM. Variable biomass productivities at various pH levels may be related to the fact that variations in medium pH are known to affect the ionisation of nutritional molecules and chemical

species, potentially limiting their intracellular availability. Moreover, the high CO_2 bio-sequestration rate was observed at pH 8.

3.5 Determination of Protein, carbohydrate, lipid, phycocyanin content of *Spirulina* sp. biomass

Figure 4 depicts the protein, carbohydrate, lipid, and phycocyanin composition of Spirulina sp. biomass.

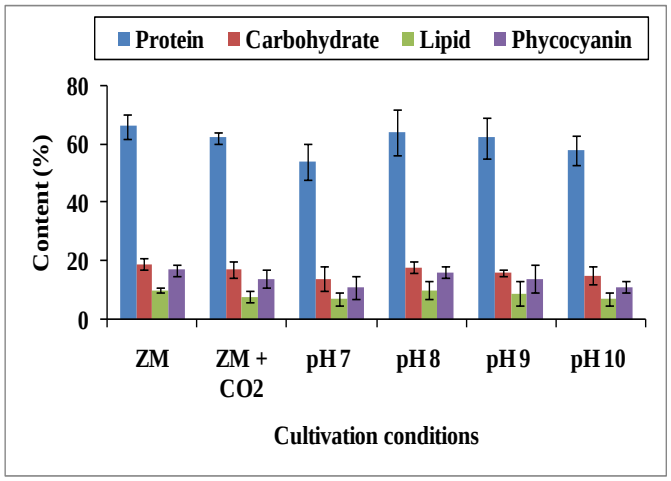


Figure 4: Protein, lipid, carbohydrate, and phycocyanin concentrations under varied culture conditions.

It has previously been observed that environmental conditions, as well as pH change, have a significant impact on the generation of microalgae biomass. In order to adapt to the environmental circumstances, microalgae store their chemical energy in the form of carbohydrates, lipids, and proteins. Moreover Spirulina was reported to contain 40–70% high quality protein, including all necessary amino acids. Spirulina sp. biomass had a total protein content of 54–66 % under all cultivation conditions. The highest protein content (66 %) was observed under standard culture conditions in ZM medium which was very close to the total protein content observed at pH 8 (64). Among all tested pH conditions Spirulina sp. showed the maximum total protein concentration at pH 8. At different pH levels, a similar pattern was seen in the increase of total carbohydrate-like protein in biomass. The total carbohydrate content varied between 14–19%, with a maximum of 18% at pH 8 and 16 % at pH 9. This was almost identical to the carbohydrate concentration seen in the presence of ZM medium (19%). The total lipid content was comparable at pH 8 and under conventional culture conditions (with ZM). Additionally, it was revealed that the other three pH conditions accumulated 7% (pH 7), 9% (pH 9) and 7% (pH 10) of lipid. Because of the presence of gamma-linoleic acid in Spirulina's lipids, these lipids have been shown to have high nutraceutical value. Most significantly, Spirulina is extensively farmed for the production of C-phycocyanin (C-PC), a pigment that plays a critical role as a light harvesting pigment, exhibits a variety of bioactive properties, and has a significant economic value in the nutraceutical and pharmaceutical industries. The total C-PC content at various pH conditions had varied between 11–17%, with a maximum of 16% at pH 8 and 14 % at pH 9. This was almost identical to the carbohydrate concentration seen in the presence of ZM medium (17%).

IV. CONCLUSIONS

In areas with insufficient fresh water resources, such as dry and semi-arid regions, where plant growth is likewise constrained by a lack of available water, growing algae is one of the best proposed options. In comparison to *Chlorella* sp. and *Synechococcus* sp., *Spirulina* sp. was shown to be more effective in producing biomass and sequestering CO_2. Additionally, Spirulina sp. was shown to have a greater capacity for bioremediation than *Chlorella* sp. and *Synechococcus* sp. The maximum biomass production of *Spirulina* sp. was observed at 35-37 ˚C, suggesting that the tested cynobacterial species were suitable for use at ambient temperature in open environment. Specifically, the objective of this study is to better understand the behaviour and capabilities of tested cynobacterial cultures at varied pH levels that are maintained by CO_2 feeding. Using an open raceway pond with a capacity of 1500 Litre, we

successfully designed, manufactured, installed, and operated a pilot scale system for Spirulina cultivation at ambient conditions.

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