



Bioremediation and Biomass Production with the Green Microalga *Chlorococcum humicola* and Textile Mill Effluent (TE)

Dharitri Borah¹ · Bervin Kennedy¹ · Subramanian Gopalakrishnan¹ · Arutselvan Chithonirai¹ · Thajuddin Nooruddin¹

Received: 4 March 2019/Revised: 17 May 2019/Accepted: 30 May 2019/Published online: 18 June 2019
© The National Academy of Sciences, India 2019

Abstract The study aimed at possible bioremediation of textile mill effluent (TE) and simultaneous production of useful biomass. The green, unicellular microalga, *Chlorococcum humicola* was grown in TE. Even in undiluted effluent, this alga reduced several important effluent parameters from 9 to 94% in 6 days and produced increased biomass with improved cell contents as compared to control. Strikingly, NO₃ and NO₂–nitrogen went below detectable limits within 3 days. In a comparative growth experiment, the growth rate of the alga was maximum in 10% dilution of effluent with supplementation of 0.15% NaNO₃. This alga produced extracellular H₂O₂, even in dark which was limited by nitrogen. TE-grown cells accumulated 32% more lipids and 25% more starch as compared to control. Further, two allelopathic extracellular free fatty acids, namely palmitic and linoleic acids, were detected.

Keywords Microalgae · Textile mill effluent · Bioremediation · Biomass · Hydrogen peroxide · Biochemical parameter

Significance Statement The microalga in undiluted textile mill effluent produced powerful oxidant H₂O₂ that could oxidize recalcitrant pigments and dyes; accumulated 25% more starch and 32% lipids that would be useful to utilize the biomass for bio-energy production.

✉ Thajuddin Nooruddin
nthaju2002@yahoo.com

¹ Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli, Tamil Nadu 620024, India

Introduction

Abating pollution by bioremediation using microalgae and cyanobacteria is called phycoremediation. It uses processes such as biosorption, uptake, bioaccumulation and biodegradation. Many toxic compounds like phenolics, pesticides, antibiotics, recalcitrant chemicals, pigments, lignin and melanoidin have all been reported to be detoxified and degraded by Cyanobacteria [1]. Textile mill effluent (TE) is a harsh environment for living organisms due to its adverse physico-chemical parameters. Textile industry is a large water-consuming industry, discharging huge volumes of effluent containing lot of dye residues due to poor uptake of colour by fabrics. Unlike other ionic dyes, dispersed dyes cannot be easily removed by available flocculants due to their non-ionic nature. The effluent is dark, oxygen depleted and has toxic levels of metals and chlorine. Hence, it cannot support life except some native microbes adapted to harsh environments. Bioremediation of such an effluent requires a tough organism that can not only survive but also degrade and utilize the contents for its growth. Therefore, this study was carried out as the biomass of *Chlorococcum humicola* cultivated in TE produces different value-added biochemicals [2].

Material and Methods

Textile mill effluent (TE) was collected from a Textile industry in Karur, Tamil Nadu, India.

Microorganism and Culture Conditions

Microalga *C. humicola* NRMCF-0134 was obtained from the culture collection of the National Repository of

Microalgae and Cyanobacteria—Freshwater (NRMC-F)—at Bharathidasan University, Tiruchirappalli, India. The culture was maintained in Chu10 medium [3] under a light intensity 2000–3000 lx (14: 10 h, light: dark) at a temperature of 25 ± 2 °C. Experiments were carried out outside the laboratory under open conditions in 500-mL conical flasks with the 250 mL working volume of medium (standard medium/TE) for 6 days with an initial inoculum of $1.52\text{--}1.81 \times 10^7$ cells/mL, manual shaking thrice daily and with natural light under a roof to avoid direct sunlight and photobleaching. The temperatures ranged from 26 to 36 °C (average 30.4 ± 3.21 °C), and the average light intensity was $145 \mu\text{mol}/\text{m}^2/\text{s}$ (± 37.45). A negative control without microalga was also maintained. The nutrient levels were estimated at 3-day intervals.

Characterization of TE

Physico-chemical analyses of TE for both untreated and treated with microalgae were performed following standard protocols [4].

Estimation of Colour and Nutrient Removal

TE was scanned in the region of 400–800 nm in a UV–Vis spectrophotometer (Agilent Cary 60 spectrophotometer) before and after biomass growth. To identify the preferred absorption maximum (λ_{max}) for colour removal studies, the absorption spectrum was obtained by (1) scanning of TE without centrifugation, (2) centrifugation of TE and scanning the settled dye dissolved in 90% acetone [5] and (3) centrifugation of TE and scanning the settled dye dissolved in distilled water. The peak at 590 nm was the highest for TE and dye in distilled water. Hence, absorbance at 590 nm was chosen as λ_{max} to study colour removal from the effluent following standard equation [6].

Biomass Production and Growth Characterization

To assess biomass production efficiency of growth media, the microalga was cultivated in different standard media, viz. Chu10 [3], BG11 [7] and MN [8] and TE [100% TE; TE (90%) + NaNO₃ (0.15%) and TE (90%) + Urea (0.04%)]. MN medium was adjusted to 10 ppt to imitate the salinity of TE. Biomass was estimated by cell count (cells/mL). The yield coefficient, growth rate and doubling time were determined by standard formulae [9–11]. Biomass production in TE was also monitored with the adjusted pH 5–11.

Determination of Dye Removal

Removal of dye residues in TE was determined by Fourier transformed infrared spectroscopy (FTIR) functional group

profiling within the wave numbers $400\text{--}4000 \text{ cm}^{-1}$ on PerkinElmer Spectrum 2 (PerkinElmer, USA) [6].

Biochemical Analyses of the Biomass

Chlorophyll *a* was estimated using 80% methanol [12] and total carotenoid content (TCC) using 85% acetone [13]. Total carbohydrate and starch were quantified by Anthrone method [14, 15] and soluble protein by Lowry's method [16]. Extraction of lipid and identification of fatty acids were done by standard procedures [17, 18]. The Fatty acid Methyl Esters (FAME) were analysed by gas chromatography–mass spectrometry (GCMS-QP2010 Ultra, Column: Rtx-5MS). The column oven temperature was 140 °C, injection temperature 280 °C and carrier gas helium.

Extraction of Metabolites

Extraction of metabolites was performed following standard procedure [6].

Hydrogen Peroxide (H₂O₂) Estimation

Extracellular hydrogen peroxide (H₂O₂) was estimated following standard procedure [19]. Diurnal variation of H₂O₂ production was measured at 5-h interval for 35 h, and the effect of major nutrients was studied either with sodium nitrate (NaNO₃) or with di-potassium hydrogen phosphate (K₂HPO₄) in *C. humicola* grown Chu 10 medium.

Visualization of Microalgal Lipids and Starch

Lipid droplets were visualized by Nile red staining [20] under a Laser Scanning Confocal Microscope Workstation, Model LSM 710 (Carl Zeiss Microscopy GMBH, Germany). Starch was stained by Lugol's iodine.

Results and Discussion

Remediation of TE

Colour reduction was studied by observing the changes in the absorption spectrum in visible light. No sharp peak was present in the effluent absorption spectrum (Fig. 1a) due to partial solubility of the dyes. Adding acetone (90%) increased dye solubility by improved intermolecular solute–solvent interaction [5] and resulted in sharp peaks in the absorption spectrum. The blue-shift in the peak at ~ 790 nm for acetone is due to the difference in extinction coefficient of acetone: water solution and dye only in water, confirming the presence of dye molecules like quinone, azo and polymethine. *C. humicola* removed > 80%

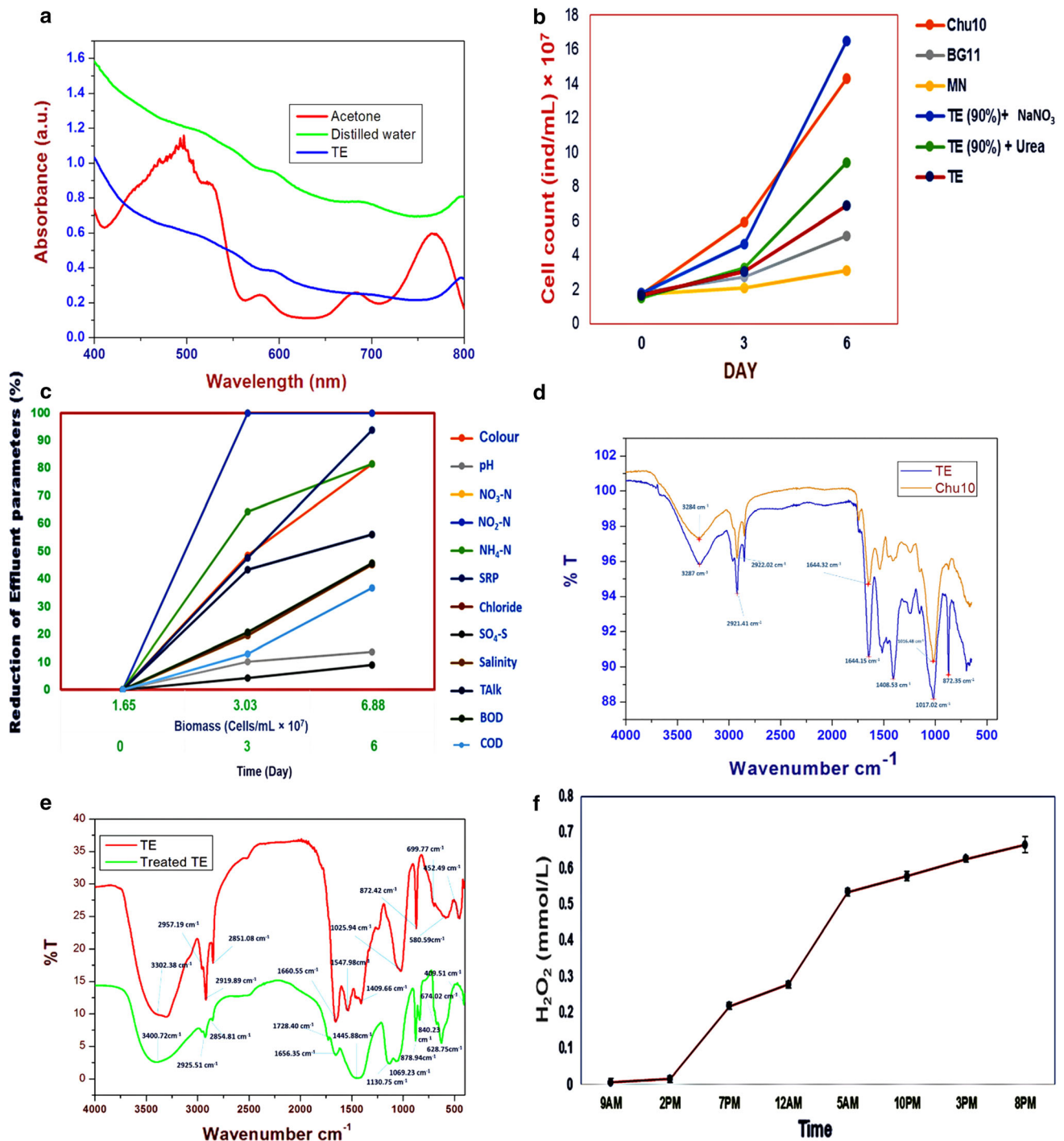


Fig. 1 a Absorption spectrum of TE dye in different solvents, b biomass growth in different media, c the relation between the reduction in effluent parameters and biomass production, d FTIR

spectrum of microalgal cells with and without adsorbed dye, e FTIR spectrum of TE and degraded product of treated TE, f time course study on H₂O₂ production. % T % Transmittance

of colour as well as NH₄-N and over 90% of SRP in 6 days (Table 1). The highly alkaline pH of untreated TE (pH 11.4) was reduced to pH 9.87 on day 6. Both NO₃-N (0.29 mg/L) and NO₂-N (0.15 mg/L) were totally removed before day 3. Microalgae utilize large amounts of nitrogen

and phosphorus for their growth and remove heavy metals. In 6 days, SRP was reduced up to 94%, followed by NH₄-N (81%), total alkalinity (56%), BOD (46%), salinity (45%), COD (37%), pH (14%) and SO₄-S (9%). Elemental composition (ppm) in TE was found to be in the order: Na

Table 1 Physicochemical parameters of TE (100%) before and after cultivation of *C. humicola* (\pm SD, $n = 3$)

Parameters	TE (before treatment)	TE (after treatment)			
		Day 3	Reduction (%)	Day 6	Reduction (%)
Colour (590 nm)	Pink (Abs = 0.629)	0.323	48.62	0.117	81.63
pH	11.4 (\pm 0.12)	10.26 (\pm 0.02)	10.23	9.87 (\pm 0.02)	13.70
NO ₃ -N (mg/L)	0.29 (\pm 0.04)	ND	ND	ND	ND
NO ₂ -N (mg/L)	0.15 (\pm 0.02)	ND	ND	ND	ND
NH ₄ -N (mg/L)	65.82 (\pm 0.29)	23.40 (\pm 5.01)	64.45	12.19 (\pm 1.67)	81.48
SRP (mg/L)	76.08 (\pm 0.30)	39.76 (\pm 0.69)	47.73	4.67 (\pm 0.21)	93.86
Chloride (mg/L)	5599.84 (\pm 100)	4499.87 (\pm 173.20)	19.64	3066.58 (\pm 115.47)	45.23
SO ₄ -S (mg/L)	33.85 (\pm 0.33)	32.42 (\pm 1.25)	4.22	30.81 (\pm 1.17)	8.98
Salinity (ppt)	10.14 (\pm 0.18)	8.15 (\pm 0.31)	19.63	5.56 (\pm 0.21)	45.17
Total Alkalinity (mg/L)	6133.33 (\pm 305.51)	3466.67 (\pm 230.94)	43.48	2666.67 (\pm 115.47)	56.21
BOD (mg/L)	1600 (\pm 200)	1266.67 (\pm 115.47)	20.83	867 (\pm 110)	45.81
COD (mg/L)	5600 (\pm 200)	4866.67 (\pm 115.47)	13.09	3533.33 (\pm 305.05)	36.90

(16.28 \pm 0.004) > K (4.71 \pm 0.08) > Fe (3.22 \pm 0.003) > Mg (2.38 \pm 0.03) > Co (0.51 \pm 0.02) > Cd (0.09 \pm 0.01) > Cu (0.02 \pm 0.001) > Cr (0.01 \pm 0.001). The highest reduction of Fe (74.47%) followed by Co (44%), Na (38%) and Cd (17%) was observed. Heavy metal reduction might be due to both adsorptions to binding groups (OH⁻, SH⁻, COO⁻) at the cell surface and absorption by cytoplasm and vacuoles. TE also has a native microflora, which might also help limited removal of nutrients and facilitate algal absorption. Thus, it is the innate adaptive and competitive capability of an organism that makes it to successfully colonize such a tough environment through complex biological interactions. Self-degradation of TE without the microalga was not evident.

TE has nitrogen sources, viz. NO₃-N, NO₂-N, NH₄-N and organic-N. In TE (100%), after 3 days, the major source of assimilable nitrogen was NH₄-N (Table 1). This confirms the ability of the organism to survive in NO₃-N and NO₂-N limited conditions utilizing NH₄-N as its nitrogen source. The microalga could also have utilized dissolved organic nitrogen (DON) from metabolites or enzymes of microbial origin [21]. Usually, when both NH₄-N and NO₃-N are present, NO₃-N uptake begins only after NH₄-N is fully exhausted [22]. However, in *C. humicola* non-ammonical forms of nitrogen could be utilized even when NH₄-N is present. There was an increase in the growth rate of the cells grown in TE (90%) + Urea (0.04%) as compared to cells grown in TE (100%) on day 6 (Fig. 1b), due to the additional availability of urea as nitrogen source.

In TE (100%), a nearly twofold increase in cell biomass not only reduced several parameters but almost eliminated NO₃-N and NO₂-N even on day 3 (Fig. 1c). Probably in

TE (100%), cell size increased due to high nutrient load in the effluent and cells' high intrinsic metabolic capability aided by easy diffusion of nutrients due to its initial small size. Increase in size of cells in TE (100%) decreased the cell count/mL. The internal nutrient pool, NH₄-N and NO₃-N interaction, N: C ratio, and the cells' biovolume which is nearly equal to the cells' carbon content, are important for such regulation [22].

Changes in Functional Groups

In FTIR spectra, band intensity for all the functional groups increased for the algal biomass with the adsorbed dye in treated TE (Fig. 1d). The appearance of two new sharp bands at 1408.53 cm⁻¹ and 872.35 cm⁻¹ is due to C-H bending and the aromatic C-H band (meta) on day 6. Increase in band intensities of the functional groups indicates surface adsorption of the dye molecules to the cells grown in TE (Fig. 1d). The presence of different functional groups in both untreated TE and treated TE is due to different vibrational frequencies of the molecules. The degraded products in treated effluent showed more functional groups as compared to untreated TE with a blue-shift (Fig. 1e, Table 2). C=O stretch for carboxylic acid (fatty acid) observed in treated TE at 1728.40 cm⁻¹ was similar to the result in GC-MS analysis. A strong stretch for C=O was observed at 1660.55 cm⁻¹ in TE, the intensity of which decreased and shifted to 1656.35 cm⁻¹ on treatment. The band seen at 1547.98 cm⁻¹ for amide (NH) disappeared in treated TE while the band at 1409.66 cm⁻¹ for an alkane C-H bending was shifted and widened at 1445.88 cm⁻¹ on treatment. The ester C-O group band position at 1025.94 cm⁻¹ of TE was not only shifted but

Table 2 FTIR band position in TE and treated TE

Band position (cm ⁻¹)	TE	Treated TE
3302.32	Present	Shifted to 3400.72 cm ⁻¹
2957.19	Present	Not significant
2919.89	Present	Shifted to 2925.51 cm ⁻¹
2851.08	Present	Shifted to 2854.81 cm ⁻¹
1728.40	Absent	Present
1660.55	Present	Shifted to 1656.35 cm ⁻¹
1547.98	Present	Absent
1409.66	Present	Shifted to 1445.88 cm ⁻¹
1025.94	Present	Multiple bands at 1130.75 cm ⁻¹ and 1069.23 cm ⁻¹
872.42	Present	Two stretches at 878.94 cm ⁻¹ and 840.23 cm ⁻¹
699.77	Present	Shifted to 674.02 cm ⁻¹
580.59	Present	Shifted to 628.75 cm ⁻¹
452.49	Present	Shifted to 409.51 cm ⁻¹

also split into multiple bands on treatment due to the formation of products of different molecular weights. Instead of the strong stretch observed for aromatic C–H at 872.42 cm⁻¹ in TE, there were two stretches at 878.94 cm⁻¹ and 840.23 cm⁻¹ in treated TE. Overall, the peaks seen in untreated TE shifted to higher wavelengths upon treatment. This is clearly the indication of the reduced mass of the dye molecules and decreased bond length due to biodegradation of molecules.

H₂O₂ Production and Dye Degradation

Chlorococcum humicola produced extracellular H₂O₂, both in light and in dark. Time course study on H₂O₂ production (35 h) revealed increased accumulation with time, not limited by light (Fig. 1f). Thus, its production is not directly connected to photosynthesis and depended on cell number, growth phase and temperature [23]. The reduction in colour of TE had essentially been brought about by the powerful oxidant H₂O₂ as reported in the degradation of the recalcitrant pigment melanoidin by the cyanobacterium *Oscillatoria boryana* [21]. H₂O₂ production is also considered a self-defence mechanism against competitors and abiotic stress. Ammonium in TE could react with extracellular H₂O₂ and bleach the dye. Apart from photosynthesis, the additional liberation of oxygen due to decomposition of H₂O₂ in the highly alkaline condition of TE contributed to decreasing BOD and increasing dissolved oxygen (DO) (Table 1). At high pH in TE, Fe might react with H₂O₂ to form insoluble iron hydroxide, which enhanced peroxide decomposition. Iron hydroxide might be re-dissolved, and the metal ion be released. This

increases the catalytic activity and bleaching of dye. The active agent in alkaline condition could also be perhydroxyl radical (HO₂) rather than hydroxyl radical (HO) [24]. The importance and role of H₂O₂ in the bioremediation of TE cannot be overemphasized, and the ability of *C. humicola* to produce H₂O₂ has been demonstrated in this study.

Effect of Nutrients on H₂O₂ Production

Chlorococcum humicola in 100% TE generated H₂O₂, which on day 3, was 0.12 mmol/L (Fig. 2a). H₂O₂ which could not be detected in untreated TE indicated the inability of native microbes to produce H₂O₂. In the Chu10 medium, there was a substantial reduction in H₂O₂ production in the absence of NO₃-N with no significant difference in cell count (Fig. 2a, b). Extracellular hydrogen peroxide production is reported to be regulated by the redox enzymes on the microalgal cell surfaces [25]. Deficiency of nitrogen might have impaired the functioning of cell surface enzymes and reduced the H₂O₂ production. It was also observed that amino acids, viz. glutamate and glutamine, were responsible for enhancement of H₂O₂ production. This indicated that nitrogen assimilation affected H₂O₂ production. However, phosphate starvation did not significantly affect H₂O₂ production (Fig. 2a). Intracellular inorganic nitrogen (IIN) also serves as a nitrogen reserve in a cell. The cells during the cultivation in TE might have accumulated a large IIN pool, which might have helped H₂O₂ production initially. Then, decline of IIN (N-limitation) reduced H₂O₂ production. Microalgae with small cell volumes accumulated large quantities of IIN, which helped cell division for a limited period. In TE, cells were large with many storage products like starch and lipids (Figs. 2c, 4).

Effect of pH on Biomass Production

Biomass production was the highest at pH 8 and the least at pH 5 (Fig. 2d). In general, cytosolic pH is maintained at 6.5–7.5, and the retardation of growth at very low and very high pH range (pH > 8) might be due to internal acidification and alkalization of cells. This affected the growth and photosynthesis of microalgae.

Characterization of Biomass Production

TE (90%) + NaNO₃ (0.15%) combination was better (16.5 × 10⁷ cells/mL) than Chu10 on day 6 (14.3 × 10⁷ cells/mL). For TE, biomass yield was 0.87 × 10⁷ cells/mL/day. The biomass yield and growth rate were found to be in the order of TE (90%) + NaNO₃ (0.15%) > Chu 10 > TE (90%) + Urea (0.04%) > TE

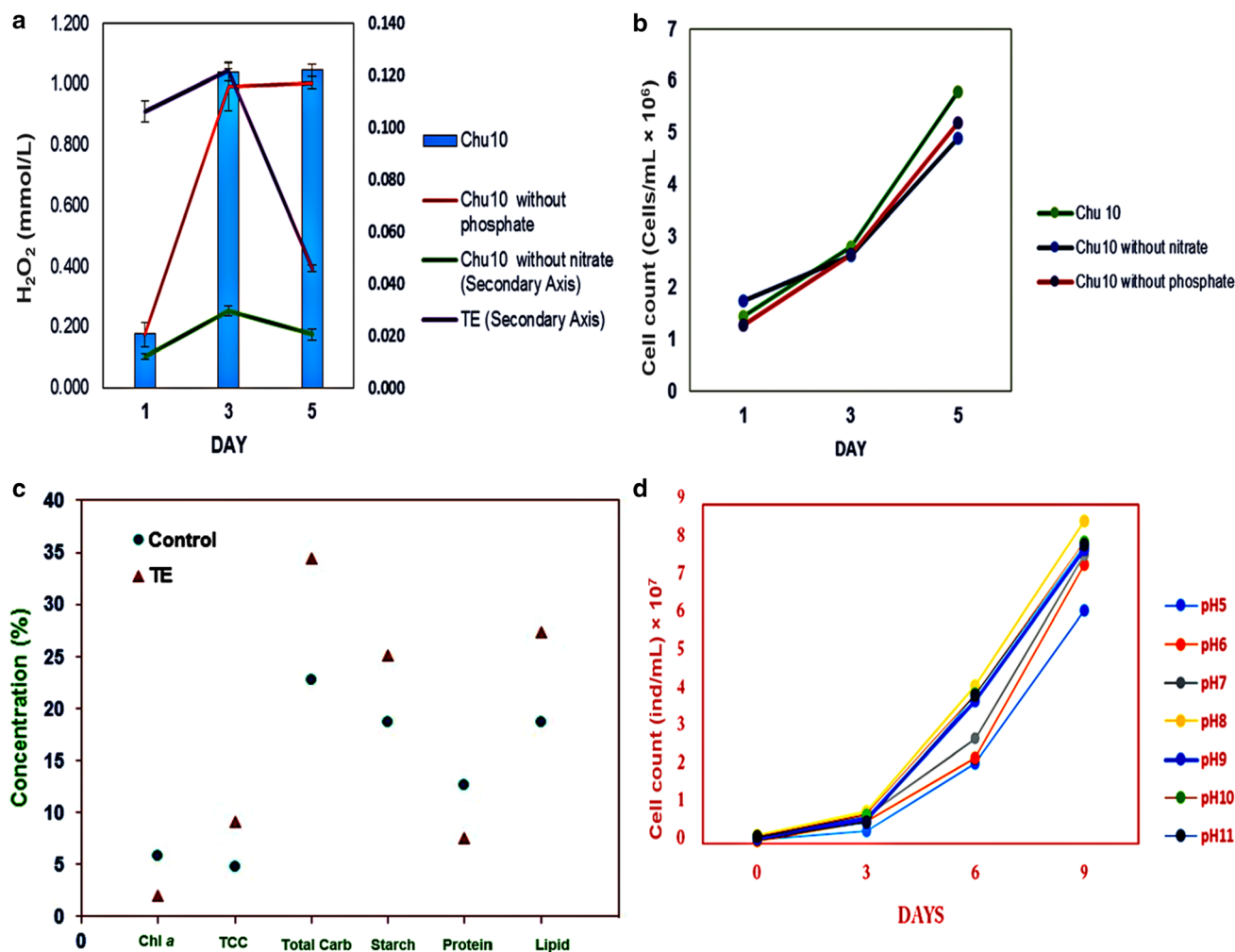


Fig. 2 a Effect of nutrients on H_2O_2 production, b effect of nutrients on cell growth, c biochemical parameters of *C. humicola*, d growth of alga in different pHs. Vertical bars are \pm SD ($n = 3$)

Table 3 Growth characteristics of *C. humicola* in different cultivation media

Cultivation media	Biomass yield (cells/mL/day) $\times 10^7$	Growth rate (/day)	Doubling time (days)
Chu 10	2.10	0.36	1.95
BG11	0.55	0.17	3.98
MN	0.23	0.10	6.89
TE (90%) + NaNO_3 (0.15%)	2.46	0.37	1.85
TE (90%) + Urea (0.04%)	1.31	0.30	2.28
TE (100%)	0.87	0.24	2.91

(100%) > BG11 > MN (Table 3). Interestingly, cell count was 2.41-fold higher in Chu 10 as compared to TE, while a twofold increase in cell volume was observed in the cells cultivated in TE (Fig. 3).

Size range of TE-grown cells was more (2.5 to 15 μm) as compared to standard media (2.5 to 6 μm) (Fig. 3). Cell wall was smooth and thick in TE-grown cells. A single pyrenoid and zoospores are frequent in Chu 10, while aplanospores are common in both Chu 10 and TE (Fig. 4a). Under stress in TE, the zoospores could have lost their motility and developed into large cells with thick cell wall, which were also capable of releasing new cells at a slow rate.

Biochemical Characterization of TE-Grown Microalgae

Physiological response of the microalgal cells to the nutritional stress of TE could be understood by biochemical analyses which could also identify the possible biotechnological applications. Chlorophyll *a* content was more than double in cells grown in Chu 10 medium (5.8 mg/g) as compared to those in TE (2.06 mg/g). Total

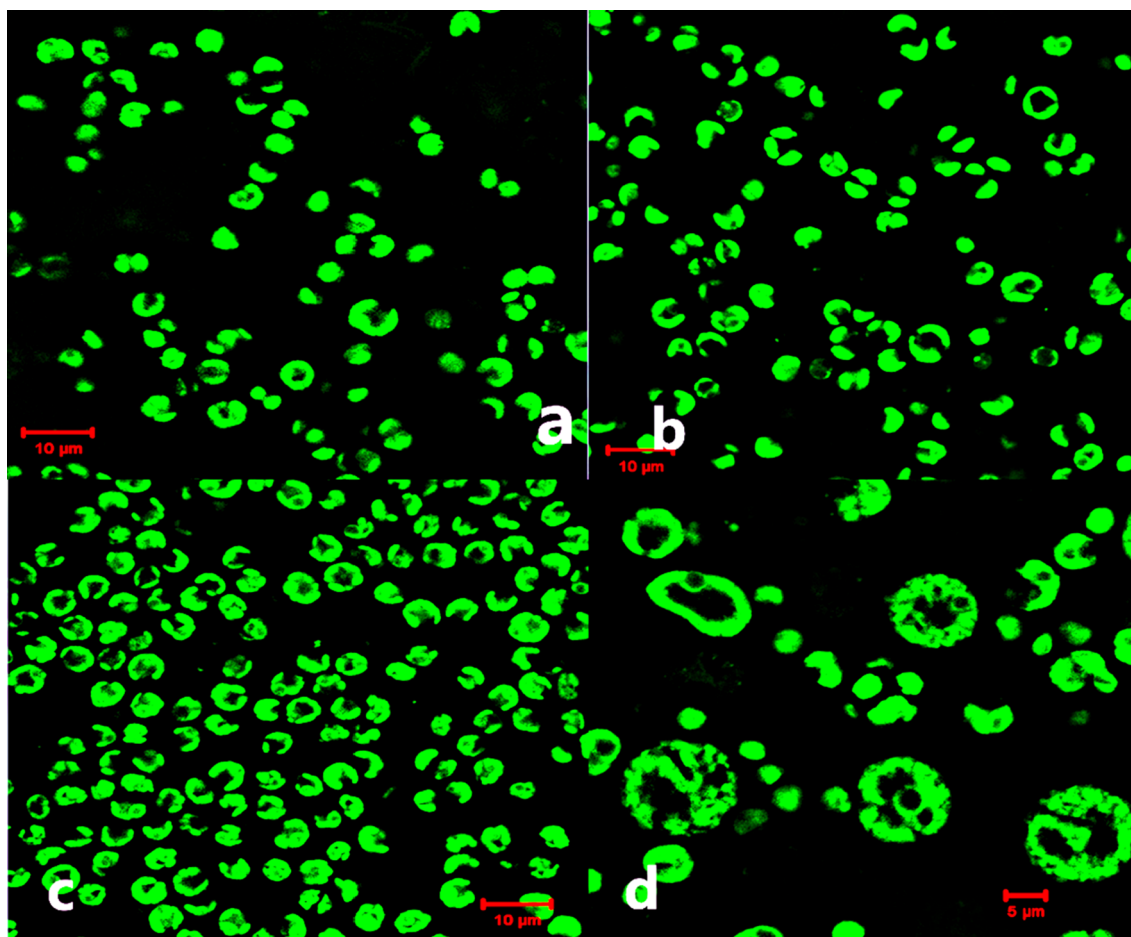


Fig. 3 Morphological variations in *C. humicola* in **a** Chu10, **b** BG11, **c** MN and **d** TE medium

Carotenoid Content (TCC) was higher in TE cultivated cells (9.06 mg/g) than Chu 10 (Fig. 2c). Total carotenoid content (TCC) protects the organism from stress. Higher TCC in TE-grown cells revealed its defence against photo-oxidative damage and prevention of lipid peroxidation under stressful environment of TE. Over time, when nutrients get depleted, carbohydrates accumulated in cells, whereas protein content decreased, due to depletion of nitrogen (Fig. 2c). Total carbohydrates were about 34% higher in TE-grown cells with a 25% increase in starch alone (Figs. 2c, 4a, b). Soluble protein was 40% higher in Chu 10-grown cells as compared to TE-grown cells (Fig. 2c). *C. humicola* cells accumulated 27.4% lipids in TE, whereas it was only 18.75% lipids for cells grown in Chu10 (Figs. 2c, 4c, d). Recently, similar stress-induced increase in lipids and starch under nitrogen limitation was reported in *Chlorococcum* sp. TISTR 8583 [26]. Fatty acid profile of the lipids of *C. humicola* revealed 65% of saturated fatty acids. Total fatty acids included saturated ones like hexadecanoic acid (63.28%) and nonanoic acid

(1.67%); mono unsaturated fatty acids (MUFA) hexadecenoic acid (2.30%) and 11-octadecenoic acid (5.53%); polyunsaturated fatty acids (PUFA) 9,12-octadecadienoic acid (Linoleic acid, 10.45%) and 9,12,15-octadecatrienoic acid (α -linolenic acid, 16.76%). Higher lipid content with a good proportion of saturated fatty acids increases the value of biomass as it is preferred for quality biodiesel production. Greater accumulation of starch in the TE-stressed cells indicates that *C. humicola* can also be used to produce starch-based biofuels like bioethanol and biobutanol.

Allelopathic Metabolites

One of the arsenals useful to overcome microbial competition is the release of allelopathic substances into the medium. Two allelopathic free fatty acids, viz. hexadecanoic acid (17.79%) and 9,12-octadecadienoic acid (82.21%), were detected in *C. humicola* treated TE (100%) and not in untreated TE (Table 4). Allelopathic free fatty

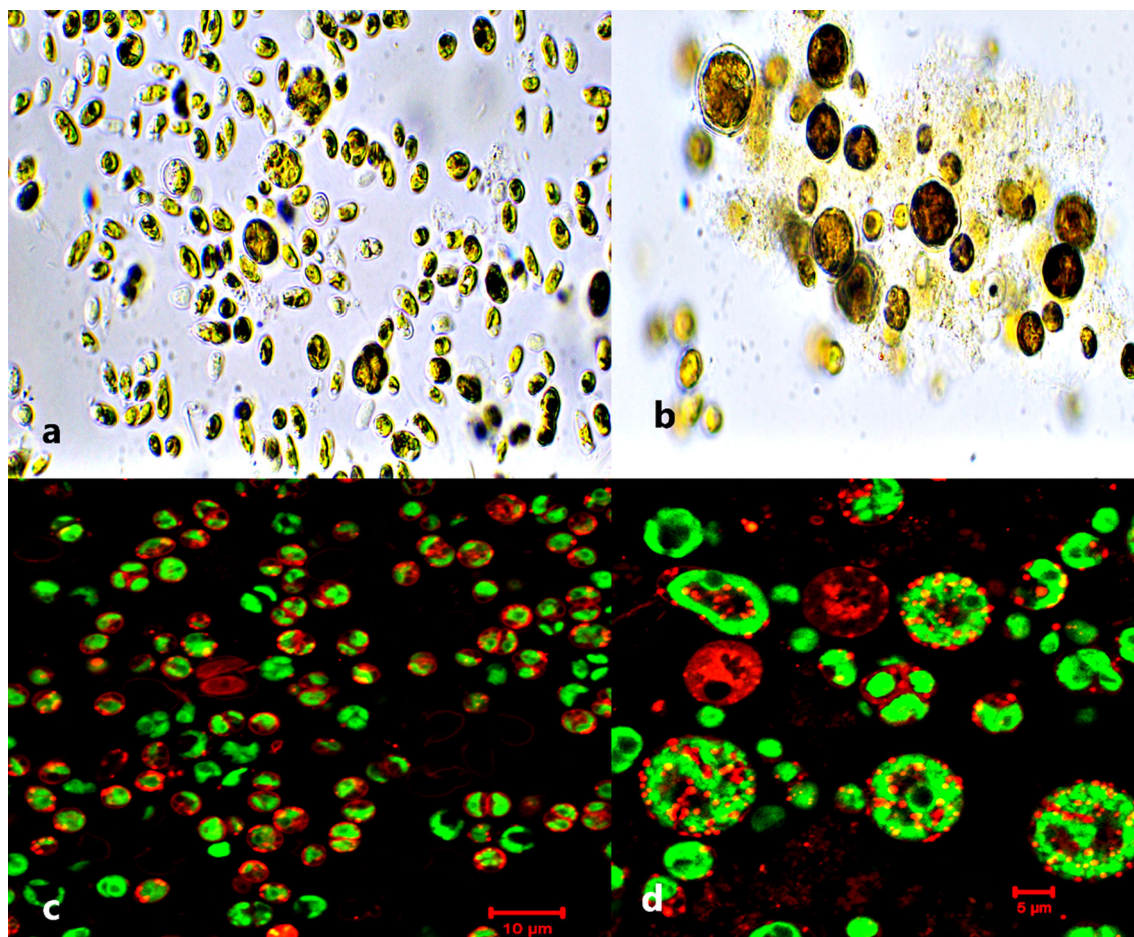


Fig. 4 Starch accumulation in the cells in **a** Chu 10, **b** TE; lipid accumulation in the cells **c** Chu 10 and **d** TE

Table 4 Fatty acid composition of the lipids in cells and metabolites

Fatty acids		Cells (%)	Metabolites (%)
Nonanoic acid (9:0)	SFA	1.67	ND
Hexadecanoic acid (16:0)		63.28	17.79
Hexadecenoic acid (16:1)	MUFA	2.30	ND
11-Octadecenoic acid (18:1)		5.53	ND
9,12-Octadecadienoic acid (18:2)	PUFA	10.45	82.21
9,12,15-Octadecatrienoic acid (18:3)		16.76	ND

acid excretion is influenced by the phase of growth, population density, nutrient stress and carbon assimilation rate.

Conclusion

The present study has established that bioremediation and production of useful biomass are feasible because of: (1) the ability of *C. humicola* to adapt to undiluted TE; (2)

increased growth; (3) increased cell storage products, namely lipids and starch; (4) production of H_2O_2 to degrade dye residues; (5) ability to remove pollutants and (6) production of allelopathic fatty acids for competitive growth. Apart from the waived cost of nutrients, phycoremediation also eliminates the requirement of water for biomass production and results in pollution abatement, qualifying this to be an economical and green treatment technology fit for scale-up.

Acknowledgements The authors acknowledge the Department of Science and Technology-Promotion of University Research and Scientific Excellence (DST-PURSE) programme (SR/FT/LS-113/2009); Advanced Instrumentation Research Facility (AIRF) at Jawaharlal Nehru University, New Delhi, India; and Department of Biotechnology (DBT), Government of India project on National Repository for Microalgae and Cyanobacteria—Freshwater (BT/PR7005/PBD26/357).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest to publish this manuscript.

References

- Subramanian G, Uma L (1996) Cyanobacteria in pollution control. *J Sci Ind Res* 55:685–692
- Sivasubramanian V (2015) Phycoremediation and business prospects. In: Prasad MNV (ed) *Bioremediation and bioeconomy*, 1st edn. Elsevier, Amsterdam, pp 421–456
- Chu SP (1942) The influence of the mineral composition of the medium on the growth of planktonic algae: part I. *Methods and culture media*. *J Ecol* 30:284–325
- Thajuddin N (1991) Marine cyanobacteria of the southern east coast of India—Survey and Ecobiological studies. Ph.D. thesis, Bharathidasan University, Tiruchirappalli
- Arslan-Alaton I (2004) Homogenous photocatalytic degradation of a disperse dye and its dye bath analogue by silica dodecatungstic acid. *Dyes Pigm* 60:167–176
- Olukanni OD, Osuntoki AA, Awotula AO, Kalyani DC, Gbenle GO, Govindwar SP (2013) Decolorization of dyehouse effluent and biodegradation of congo red by *Bacillus thuringiensis* RUN1. *J Microbiol Biotechnol* 23:843–849
- Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignment, strain histories and properties of pure cultures of cyanobacteria. *J Gen Microbiol* 111:1–61
- Rippka R (1988) Isolation and purification of cyanobacteria. In: Packer L, Glazer AN (eds) *Methods in enzymology*. Academic Press, San Diego, pp 3–27
- Yadav V, Prappulla SG, Jha A, Poonia A (2011) A novel exopolysaccharide from probiotic *Lactobacillus fermentum* CFR 2195: production, purification and characterization. *Biotechnol Bioinf Bioeng* 1:415–421
- Zhu L, Wang Z, Shu Q, Takala J, Hiltunen E, Feng P, Yuan Z (2013) Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Res* 47:4294–4302
- Luo L, He H, Yang C, Wen S, Zeng G, Wu M, Zhou Z, Lou W (2016) Nutrient removal and lipid production by *Coelastrella* sp. in anaerobically and aerobically treated swine wastewater. *Bioresour Technol* 216:135–141
- McKinney G (1941) Absorption of light by chlorophyll solutions. *J Biol Chem* 140:315–322
- Jensen A (1978) Chlorophylls and carotenoids. In: Hellebust A, Cragie JS (eds) *Handbook of phycological methods*. Cambridge University Press, London, pp 59–70
- Spiro RG (1966) Analysis of sugars found in glycoproteins. *Methods Enzymol* 8:3–26
- Brányiková I, Maršálková B, Doucha J, Brányik T, Bišová K, Zachleder V, Vítová M (2011) Microalgae—novel highly efficient starch producers. *Biotechnol Bioeng* 108:766–776
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275
- Folch J, Lees M, Sloan-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem* 226:497–509
- Miller L, Berger T (1985) Bacteria identification by gas chromatography of whole cell fatty acids. Hewlett Packard, Gas Chromatography, Application Note, Hewlett-Packard, Avondale, Pennsylvania
- Graf E, Penniston JT (1980) Method for determination of hydrogen peroxide, with its application illustrated by glucose assay. *Clin Chem* 26:658–660
- Hu Z, Patten T, Pelton R, Cranston ED (2015) Synergistic stabilization of emulsions and emulsion gels with water soluble polymers and cellulose nanocrystals. *ACS Sustain Chem Eng* 3:1023–1031
- Kalavathi DF, Uma L, Subramanian G (2001) Degradation and metabolization of the pigment—melanoidin in distillery effluent by the marine cyanobacterium *Oscillatoria boryana* BDU 92181. *Enzyme Microb Technol* 29:246–251
- Flynn KJ, Fasham MJR, Hipkin CR (1997) Modelling the interactions between ammonium and nitrate uptake in marine phytoplankton. *Philos Trans R Soc Lond* 352:1625–1645
- Twiner MJ, Trick CG (2000) Possible physiological mechanisms for production of hydrogen peroxide by the ichthyotoxic flagellate *Heterosigma akashiwo*. *J Plankton Res* 22:1961–1975
- Dannacher J, Schlenker W (1996) The mechanism of hydrogen peroxide bleaching. *Text Chem Color* 28:24
- Palenik B, Zafriou OC, Morel FMM (1987) Hydrogen peroxide production by a marine phytoplankton. *Limnol Oceanogr* 32:1365–1369
- Rehman ZU, Anal AK (2019) Enhanced lipid and starch productivity of microalga (*Chlorococcum* sp. TISTR 8583) with nitrogen limitation following effective pretreatments for biofuel production. *Biotechnol Rep* 21:e00298

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.