ORIGINAL PAPER

Phycoremediation of textile effluent-contaminated water bodies employing microalgae: nutrient sequestration and biomass production studies

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Abstract

Combinatorial process development for rationalized recycling of nutrients employing microalgae may provide realistic solutions to both environment management and energy generation. The present study was performed to investigate nutrient recycling potential of microalgal strains viz. *Anabaena ambigua*, *Chlorella pyrenoidosa* and *Scenedesmus abundans* in terms of biomass productivity and specific growth rate using textile wastewater as a nutrient source at different dilutions (25, 50, 75, 100%). Biomass production kinetics revealed that alga could grow even up to 100% textile wastewater. Comparative phycoremediation potential was evaluated for 25 days employing 75% textile wastewater under batch conditions. The microalgal species were observed to effectively reduce the chloride, nitrate and phosphate concentrations up to 61%, 74.43% and 70.79%, respectively. Maximum chemical oxygen demand reduction efficiency was observed employing *S. abundans* (<85%). Spectral analysis revealed potentiality of applying microalgae for textile wastewater remediation and also provided insight into the possible mechanism involved.

Graphical abstract
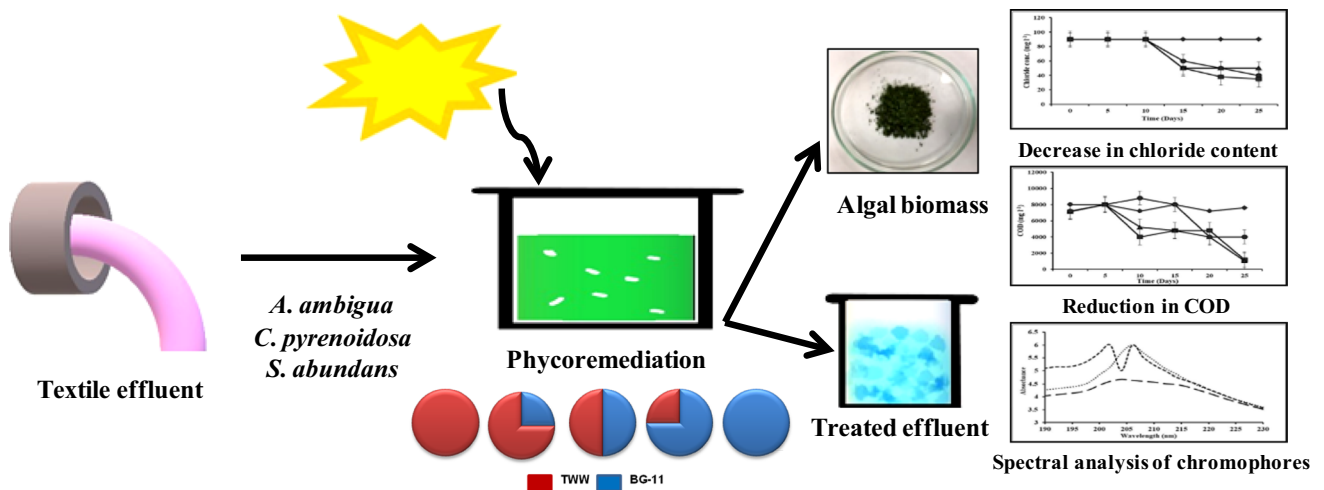
Keywords Textile wastewater · Microalgae · Biomass · Phycoremediation · Environment · Energy

Introduction

Water and energy are two vital resources that preponderate the global economy, but their ample availability is questionable in the upcoming decades due to over exploitation and increasing needs of the ever-growing population. The textile sector is one of the largest and oldest industries in the Indian economy with > 45 million people employed directly and > 20 million people indirectly (Annual Report, 2017–18, Ministry of Textiles). The textile industry contributes to ~ 10% of manufacturing production, 2% of India's GDP and 13% of country's export earnings. The sector is complex in nature using more than 100,000 commercially available dyes and producing approximately 7×10^5 tonnes of effluent annually on a global scale and 13×10^4 tonnes in India only (Annual Report, 2016–17, Ministry of Textiles). Considering the water consumption in various wet processes (viz. scouring, bleaching, dyeing, printing and finishing), the loss of dyes (estimated up to 200,000 tonnes) in the effluent and the volume of discharge, textile industry is considered to be the top level polluter among all other industrial sectors consuming 17–20% of industrial wastewater (Kurade et al. 2015). Textile effluents contain a range of chemicals, i.e., acids, alkalis, dyes, hydrogen peroxide, starch, surfactants dispersing agents and metals along with the predominant presence of dyes that are visible even at very low concentrations (< 1 mg L^{-1} for some dyes). The use of synthetic dyes has been increased drastically in textile and dyeing industries due to their ease and cost-effectiveness in synthesis, a high stability to light, temperature, detergent and microbial attack and variety in colors over natural dyes (El-Kassas and Mohamed 2014). These synthetic dyes due to their complex aromatic structure are more recalcitrant to biodegradation, so when released untreated into water bodies, they pose a serious threat to the health and environment. Dyes have been reported to be carcinogenic and mutagenic and decrease light penetration and photosynthetic activity, thereby limiting the supply of oxygen which in turn inhibits the activity of microorganisms (Ghaly et al. 2014). Hence, the treatment of textile wastewater becomes mandatory before discharging into nearby water bodies as it contains recalcitrant organic, colored, toxicant, surfactant and chlorinated compounds and diverse salts as the major pollutants (Khandare and Govindwar 2015).

The conventional techniques viz. physical and biological adsorption, membrane filtration, oxidation, ozonation are generally used for the remediation of effluents containing dyes. The major drawback with the use of filtration membranes and biological methods is that they involve the phase transfer, generating a large amount of deposited sludge at the bottom of the tanks and have low efficiency in color removal and reduction in organic load (Robinson et al. 2001). Also, the problem in the use of membranes relies on biofilm formation and clogging and therefore further needs for their cleaning and/or replacement. Fungal enzymes in the form of lignin peroxidase, manganese peroxidase, and laccase are suitable for the degradation of dyes in the textile wastewater, and mostly, white rot fungal cultures have shown degradation of azo dyes along with chemical oxygen demand (COD) removal (Prigione et al. 2008). However, this system has intrinsic disadvantages in the form of the longer growth phase, a requirement of nitrogen restrictive environment, and unreliable enzyme production. The major challenge is the stability of the system alone, as after few days bacteria will start growing and the fungi will not be able to degrade the dyes. This might be attributed to the competition between bacteria and slow growing fungi for the availability of nutrients and the production of antibiotic substances or toxins by bacteria that inhibit the fungal growth (Jonstrup et al. 2013). Under anaerobic conditions, through azo reductase enzyme system; many bacteria are capable of degradation of azo dyes, and other water soluble dyes present in textile wastewater (Robinson et al. 2001). In this case, aromatic amines formed are recalcitrant to further degradation. Therefore, the anaerobic bacterial degradation of dyes needs to be coupled with aerobic degradation where complete mineralization of aromatic amines may take place (Anjaneyulu et al. 2005). The major limitation of this coupled system is that the aromatic amines, formed by anaerobic reduction, are unstable under aerobic conditions as they undergo auto-oxidation reactions (Holkar et al. 2016). These results are very far from ideal due to the low biodegradation yields, longer treatment times and generation of sludge (Anjaneyulu et al. 2005). According to the report of Central Pollution Control Board-Government of India (CPCB), 2.65 million per year is the total loss for the recovery of water from the wastewater generated (20.83 m^3 h^{-1}) bearing pollution of total suspended solids 164 mg L^{-1}, total dissolved solids 11358 mg L^{-1}, COD 553 mg L^{-1}, biological oxygen demand (BOD) 220 mg L^{-1} and color 250 Hazen units during processing, i.e., scouring, bleaching and dyeing contamination.

Conventional remediation techniques are expensive, unaffordable by small-scale industries and are not always followed as per the governing standards, resulting in secondary pollution (Khandare and Govindwar 2015). The pollution by industrial sector creates turmoil in the ecosystem, negatively affects the environment and is considered as one of the major factors contributing to the climate change across the globe (Ramanathan and Feng 2009). The concern for global climate change has paved a way for economical bio-based fuel generation by not only utilizing residual resources from agriculture and forestry but also employing algal biomass

(Hernández et al. 2014). Integration of wastewater treatment with algal cultivation may offer an economical and environment savior, solar power-driven resource for sustainable and renewable bioenergy production where algal biomass is produced as a by-product and treated water can be reused for irrigation practices (Leite et al. 2013; Bhatt et al. 2014). Phycoremediation exploits microalgae, macroalgae and cyanobacteria for the removal of nutrients and xenobiotics from wastewater and carbon dioxide from the waste air (Olguín and Sánchez-Galván 2012). Microalgae do not compete with agriculture for their land requirements and also do not compromise food production and supply (Hernández et al. 2014). Water necessities (> 90%) of the algae for growth and biomass production are fulfilled by their capacity to utilize wastewater (Pathak et al. 2015). The algal biomass produced can be further utilized for the production of biofuels through various pathways viz. biogas by anaerobic digestion, bioethanol by fermentation of carbohydrates and bio-crude oil by high-temperature conversions (Park et al. 2011). Additionally, dead algal biomass had also been efficiently employed for the removal of dyes from textile wastewater; for instance, dead *Spirogyra* was employed to remove reactive dye (Synazol) from textile wastewater and concluded that *Spirogyra* has high biosorption efficiency, reusability and stability (Khalaf 2008). *Chlorella vulgaris* can remove 63–69% color from mono-azo dye Tectilon Yellow 2G (Acuner and Dilek 2004).

The present investigation was carried out to evaluate the growth of *Anabaena ambigua, Chlorella pyrenoidosa* and *Scenedesmus abundans* in diluted textile effluents for being utilized as agents of phycoremediation. Textile wastewater was collected in January, 2017 from one of the small-scale block printing textile units located in Sanganer, Jaipur, Rajasthan (India). The remediation potential of algal strains was analyzed and characterized in terms of reduction in COD, BOD along with nitrate, phosphate and chloride content of the effluents. To the best of our knowledge, *A. ambigua* and *S. abundans* are employed for the very first time in the remediation of textile effluent. An extensive literature survey was performed before selecting the strains for remediation studies and the strains selected appeared robust in terms of their biomass production and lipid content, hence selected for the present study.

Materials and methods

Materials

Textile wastewater (TWW) was collected from the block printing, textile units located in the Sanganer (26° 49′ N, 75° 49′ E), Jaipur, Rajasthan (India), and stored at 4 °C till further analysis. These units are small-scale industries mainly

indulging in printing and dyeing, discharge their wastewater without any treatment to the nearby water bodies and soil. Color of the effluent varies according to the dye used. The initial pH of TWW was 4.8 ± 0.1 (Eutech pH 150), and it appeared pink. The sample was having an electrical conductivity (Eutech CON 150) of 2.8 mS and redox potential of 233 ± 0.5 mV (Eutech pH 150).

Microalgae and culture conditions

The microalgae *Chlorella pyrenoidosa* (NCIM No. 2738), *Scenedesmus abundans* (NCIM No. 2897) and cyanobacterium *Anabaena ambigua* (NCIM No. 2785), were procured from the National Collection of Industrial Microorganism (NCIM), Pune, and were maintained as per the recommendation.

A. ambigua and *S. abundans* were maintained in Fog's growth medium: $MgSO_4 \bullet 7H_2O$ (0.2 g L^{-1}), K_2HPO_4 (0.2 g L^{-1}), $CaCl_2 \bullet 2H_2O$ (0.1 g L^{-1}), Fe-EDTA solution (5 ml L^{-1}) and micronutrients H_3BO_3 (286 mg L^{-1}), $MnCl_2 \bullet 4H_2O$ (181.0 mg L^{-1}), $ZnSO_4 \bullet 7H_2O$ (22 mg L^{-1}), $Na_2MoO_4 \bullet 2H_2O$ (39 mg L^{-1}), $CuSO_4 \bullet 5H_2O$ (8 mg L^{-1}) as described by NCIM, Pune. For cultivation of *C. pyrenoidosa*, Fog's growth medium was supplemented with KNO_3 (2%). The initial pH was maintained to 7.2, and the cultures were grown in Erlenmeyer flasks (1 l) with 600 ml culture media, provided with white fluorescent cool light (4*40 W) and photoperiod of 16:8 h at 25 ± 2 °C. Intermittent manual shaking (4–5 times a day) was done to avoid attachment of algal cells on the walls of culture flasks.

Growth of microalgae in TWW

Comparative growth profiles of the microalgal strains were studied employing different concentrations of TWW (25, 50, 75 and 100%) diluted with BG-11 medium ($NaNO_3$ (1.5 g L^{-1}), K_2HPO_4 (0.04 g L^{-1}), $MgSO_4 \bullet 7H_2O$ (0.075 g L^{-1}), $CaCl_2 \bullet 2H_2O$ (0.036 g L^{-1}) Citric acid (0.006 g L^{-1}), ferric ammonium citrate (0.006 g L^{-1}), EDTA (disodium salt) (0.001 g L^{-1}), Na_2CO_3 (0.02 g L^{-1}), and 1 ml of trace element solution from the micronutrient solution containing H_3BO_3 (2.86 g L^{-1}), $MnCl_2.4H_2O$ (1.81 g L^{-1}), $ZnSO_4 \bullet 7H_2O$ (0.22 g L^{-1}), $Na_2MoO_4 \bullet 2H_2O$ (0.39 g L^{-1}), $CuSO_4 \bullet 5H_2O$ (0.079 g L^{-1}), $Co(NO_3)_2$ (0.05 g L^{-1})) (Stanier et al. 1971). The BG-11 growth medium was taken as a control. Autoclaved TWW at various concentrations (25, 50, 75 and 100%) was employed to study the growth of *A. ambigua*, *C. pyrenoidosa*, *S. abundans*, and pH for all the concentrations was adjusted to 6.5 using 0.1 M NaOH buffer. Homogenous algal suspension (10%, v/v) was used for inoculation having an optical density of 2.0 at 680 nm. Growth was monitored every 24th hour (hr) by measuring absorbance at 665 nm and 750 nm (Spectronic 200-Thermo

Scientific, Germany) in terms of total Chlorophyll content (Marker 1980). The cell dry weight was determined on 25th day of remediation experiment. Briefly, an aliquot of the sample (10 ml) was centrifuged (3000×g; 10 min) followed by drying of the pellet in an oven (80 °C, overnight).

The specific growth rate of alga (μ) in the exponential phase of growth was calculated using Eq. (1) (Issarapayup et al. 2009):

$$\mu \left(\text{day}^{-1}\right) = \ln\left(N_2/N_1\right)\left(t_2 - t_1\right) \tag{1}$$

where N_1 and N_2 represent growth of algae (μg mL^{-1}) at time t_1 and t_2.

Biomass productivity is the result of the difference in biomass concentration per unit time between two consecutive sampling times. Estimation of biomass productivity (P, μg mL^{-1} day^{-1}) of alga would provide an efficient growth comparison (Griffiths et al. 2014). Equation (2) was used for productivity measurements.

$$P = \left(N_i - N_o\right)/\left(t_i - t_o\right) \tag{2}$$

where N_i and N_o are the biomass (μg mL^{-1}) at time t_i and t_o, respectively.

Phycoremediation of TWW

Relative remediation potential of microalgal strains was evaluated under batch conditions (25 days) using 75% TWW concentration (diluted with 25% BG-11 medium), selected on the basis of algal growth profiles. Erlenmeyer flasks (1 L) were used as a photobioreactor and were manually shaken 4–5 times a day provided with the intensity of 4 tube-lights (40 W each) for 16 h. The optimum conditions of pH and temperature (6.5; 25±2 °C) were maintained. A control (75% autoclaved TWW) without algal inoculum was used for comparative studies. Aliquots were drawn after fixed time intervals (5 days) and parameters of pollution load viz. BOD (titrimetric method), COD (closed reflux, dichromate titrimetric method), chloride (argentometry method), nitrate (spectrophotometric method) and phosphate (ascorbic acid method) were analyzed in triplicates following the standard protocols (APHA 1998).

Removal efficiency (RE, %) of algal strains employed was calculated by Eq. (3):

$$RE\,(\%) = \left[\left(S_0 - S_f\right)/S_0\right] \times 100 \tag{3}$$

where S_0 and S_f are the nutrient concentrations at the beginning and the end of the experiment, respectively.

In addition, the removal rate (RR, mg L^{-1} day^{-1}) of the analyzed nutrients was calculated using Eq. (4):

$$RR = \left(S_0 - S_i\right)/\left(t_i - t_0\right) \tag{4}$$

where S_i is the nutrient concentration at time t_i.

Spectral profile

Spectral profiles were obtained using a UV–Visible spectrophotometer (Dynamica Halo DB-30). Aliquots were withdrawn at regular intervals (after every 5th day), processed and scanned (190–900 nm) to compare the peaks of major chromophoric groups present in the textile effluent (Urushigawa and Yonezawa 1977; El-Sheekh et al. 2009).

Results and discussion

Growth profile of microalgae in TWW

Various concentrations of TWW (25, 50, 75 and 100%) diluted with appropriate volumes of nutrient medium were used to evaluate the time-course kinetics of microalgal growth by taking the nutrient medium as the control. The observations were recorded at an interval of 24 h by measuring the optical density at 665 nm and at 750 nm and then calculating the total chlorophyll content which is directly proportional to the algal density (Wang et al. 2017). Growth kinetics of microalgae was subsequently characterized in terms of specific growth rate and biomass production (Pathak et al. 2015). The growth curves of microalgal species illustrated all the characteristic growth phases in batch conditions (Fig. 1a–c). Comparative growth profiles of *C. pyrenoidosa* revealed that there was a detectable lag phase up to 72 h of growth. *C. pyrenoidosa* showed maximum growth in the 25% TWW at all the intervals of time. Microalgae showed significantly higher microalgal growth in 75% TWW over the 50% TWW (p value < 0.0001) (Fig. 1a). *S. abundans* had attained the log phase from 24th hour of inoculation in all concentration of TWW and continued to be in the log phase till 288th hour of growth in 75% TWW. Stationary phase was attained after 120 h of growth in 100% TWW (Fig. 1b). In *A. ambigua*, 25% TWW and 50% TWW showed the maximum growth in terms of total chlorophyll content (i.e., 54.98±0.47 μg mL^{-1} and 52.17±0.83 μg mL^{-1} after 240 h of growth, respectively). At all concentrations of TWW, the lag phase was observed till 48 h of growth for *A. ambigua*. The growth of *A. ambigua* was supported by 75% TWW over the 100% TWW as the highest value of chlorophyll content, i.e., 24.39±0.38 μg mL^{-1} was measured after 240 h of growth in 75% TWW over 4.75±0.07 μg mL^{-1} in 100% TWW (Fig. 1c). The higher specific growth rate was achieved in the 75% TWW over the 50% and 100% TWW for all the microalgal cultures. Specific growth rate for *A. ambigua*, *C. pyrenoidosa* and *S. abundans* was 0.127±0.02 day^{-1}, 0.101±0.04 day^{-1} and 0.103±0.01 day^{-1}, respectively, at 10th day in the 75% TWW (Table 1). Maximum biomass productivity for *A. ambigua* (6.44±0.04 mg L^{-1} day^{-1}) and *S. abundans* (6.69±0.17 mg L^{-1} day^{-1}) was
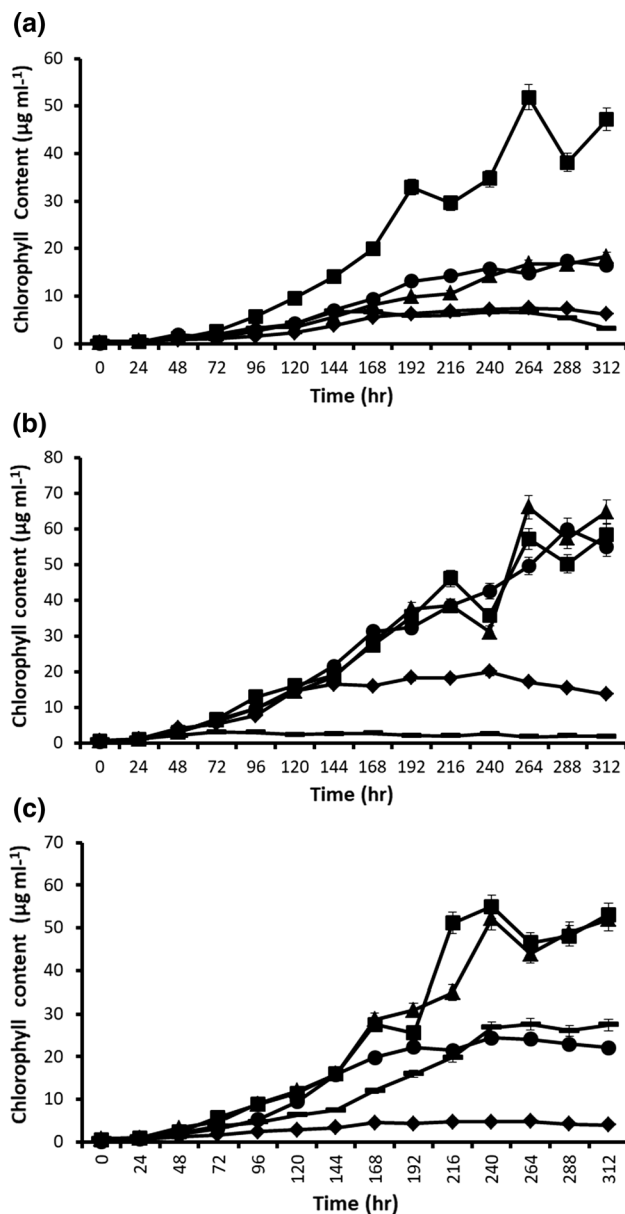
Fig. 1 Growth pattern in terms of total chlorophyll content for *C. pyrenoidosa* (**a**), *S. abundans* (**b**) and *A. ambigua* (**c**) at varying concentrations of textile wastewater (black-and-gray rectangle symbol, control; black square symbol, 25% TWW; filled triangle symbol, 50% TWW; filled circle symbol, 75% TWW; filled diamond symbol, 100% TWW). (Values represent the mean ± SD of three replicates)

observed following 144 h of incubation and for *C. pyrenoidosa* (3.78 ± 0.53 mg L^{-1} day^{-1}) at 168 h in 75% TWW. The total dry biomass produced at the end of 14th day of the experiment was 4.10 g L^{-1} for *A. ambigua*, 4.79 g L^{-1} for *S. abundans* and 3.97 g L^{-1} for *C. pyrenoidosa* in the 75% TWW. For 100% TWW, the biomass productivity of *A. ambigua*, *C. pyrenoidosa* and *S. abundans* was lower than the biomass productivity at 75% TWW toward the end of the experiment. This might be attributed to the fact that

excessive toxicants present in the 100% TWW have inhibited the growth of microalgal species (Ghaly et al. 2014). Although the microalgal growth was more in 25% and 50% TWW, achieving the same required additional use of growth media that can undesirably affect the remediation process economics. Hence, 75% TWW was selected for the further analysis. Similar results supporting the fact that algal species can grow up to 100% TWW but the biomass produced is comparatively lower than 75% TWW were reported by Lim et al., (2010), where they have shown the potential of *Chlorella vulgaris* to grow at 100% TWW, but the biomass yield was lesser. The various nutrients present in the TWW in the form of nitrogen, phosphorous and salts may have supported the growth of microalgae (Rawat et al. 2011). Simultaneously, the presence of toxicants and colored water imposes an adverse effect on the biomass production (Elumalai et al. 2013).

Phycoremediation of textile wastewater

Microalgae cultivation in wastewaters has provided an alternative integrated strategy for bioenergy generation as substantial biomass is produced along with treatment via nutrient removal. The control used for the phycoremediation experiment was autoclaved uninoculated 75% TWW. The reason behind the utilization of autoclaved TWW lies in the fact that unautoclaved TWW can support the growth of various microorganisms (i.e., bacteria and fungi) other than algae. These microbes can compete with algae for nutrition and could also positively/negatively contribute to the remediation process as ample literature is available in support of the remediation efficiency of heterotrophic population. The main objective of the study is to exploit the photoautotrophic nature of the strains utilized for the development of economic remediation process. Hence, autoclaving is required to diminish the heterotrophic microbial population to the negligible levels (Zhou et al. 2012). The characteristics of autoclaved textile effluent collected from Sanganer, Jaipur, are summarized in the Table 2. Efficient and effective phycoremediation of TWW was observed during the sequential operation in terms of COD, nitrate and chloride removal. Electrical conductivity and redox potential were observed to reduce to 2.0 mS and 157.86 ± 1.89 mV at the end of the experiment, respectively.

Chemical oxygen demand

COD removal efficiency exhibited significant variations ranging from 50 to 86%. Among the conditions studied, the maximum COD removal was observed by *S. abundans* (1050 mg L^{-1}) followed by *C. pyrenoidosa* (1200 mg L^{-1}) and *A. ambigua* (4000 mg L^{-1}), taking autoclaved effluent (75%) as the control (COD, 8000 mg L^{-1}) (Fig. 2). At 15th

Table 1 Growth parameters of *A. ambigua*, *C. pyrenoidosa* and *S. abundans* in textile wastewater

Species	*A. ambigua*				
Parameter	μ (day^{-1})				
Treatment	Control	25%TWW	50%TWW	75%TWW	100%TWW
2nd day	0.585±0.02	1.010±0.17	1.181±0.56	0.927±0.17	0.603±0.15
4th day	0.234±0.06	0.427±0.01	0.601±0.04	0.517±0.06	0.368±0.02
6th day	0.173±0.07	0.343±0.02	0.283±0.02	0.521±0.01	0.154±0.09
8th day	0.282±0.06	0.676±0.13	0.075±0.04	0.107±0.18	0.090±0.02
10th day	0.305±0.01	0.029±0.07	0.356±0.24	0.127±0.02	0.004±0.05
Parameter	P (mg L^{-1} day^{-1})				
Treatment	Control	25%TWW	50%TWW	75%TWW	100%TWW
2nd day	0.871±0.02	1.480±0.17	2.340±0.15	1.131±0.09	0.558±0.09
4th day	0.969±0.25	3.065±0.17	4.008±0.49	2.127±0.04	0.751±0.04
6th day	1.204±0.06	4.493±0.27	3.899±0.21	6.443±0.04	0.495±0.36
8th day	3.909±0.17	11.61±3.74	2.189±0.13	2.365±0.09	0.166±0.09
10th day	7.058±0.03	3.878±0.38	4.045±0.16	2.898±0.27	0.015±0.02
Species	*C. pyrenoidosa*				
Parameter	μ (day^{-1})				
Treatment	Control	25%TWW	50%TWW	75%TWW	100%TWW
2nd day	0.649±0.15	0.951±0.50	0.785±0.52	1.701±0.14	0.581±0.14
4th day	0.581±0.09	0.766±0.03	0.536±0.20	0.812±0.49	0.332±0.08
6th day	0.480±0.11	0.383±0.02	0.472±0.01	0.525±0.01	0.521±0.04
8th day	0.032±0.01	0.463±0.05	0.191±0.01	0.338±0.05	0.126±0.03
10th day	0.091±0.02	0.101±0.04	0.296±0.06	0.101±0.04	0.049±0.06
Parameter	P (mg L^{-1} day^{-1})				
Treatment	Control	25%TWW	50%TWW	75%TWW	100%TWW
2nd day	0.448±0.04	0.662±0.21	0.568±0.34	1.600±0.15	0.359±0.08
4th day	1.396±0.25	3.070±0.19	1.387±0.42	1.418±0.59	0.427±0.07
6th day	2.580±0.59	4.472±0.32	2.111±0.01	2.888±0.09	1.548±0.18
8th day	0.193±0.10	12.97±0.15	1.709±0.11	3.784±0.53	0.745±0.01
10th day	0.579±0.14	5.129±0.14	3.649±0.64	1.522±0.05	0.339±0.04
Species	*S. abundans*				
Parameter	μ (day^{-1})				
Treatment	Control	25%TWW	50%TWW	75%TWW	100%TWW
2nd day	0.590±0.09	1.159±0.11	1.150±0.54	1.219±0.17	1.258±0.47
4th day	0.362±0.09	0.647±0.18	0.419±0.01	0.451±0.03	0.361±0.05
6th day	0.094±0.08	0.157±0.02	0.256±0.01	0.368±0.06	0.117±0.01
8th day	0.057±0.01	0.252±0.08	0.281±0.04	0.038±0.01	0.130±0.02
10th day	0.317±0.01	0.495±0.22	0.783±0.05	0.103±0.01	0.092±0.09
Parameter	P (mg L^{-1} day^{-1})				
Treatment	Control	25%TWW	50%TWW	75%TWW	100%TWW
2nd day	0.938±0.19	2.038±0.15	1.991±0.45	2.241±0.28	3.013±0.16
4th day	0.917±0.27	6.145±0.02	3.346±0.14	3.472±0.15	2.323±0.45
6th day	0.239±0.02	2.804±0.82	4.180±0.55	6.693±0.17	1.824±0.22
8th day	0.130±0.01	7.902±2.74	9.278±2.80	1.147±0.79	2.231±0.38
10th day	0.631±0.08	10.80±4.51	7.214±1.87	4.170±0.35	1.731±0.17

μ specific growth rate, *P* biomass productivity

Values represent the mean ± SD of three replicates

Table 2 Characteristics of autoclaved textile effluent used in this study

Parameters	Value
pH	4.80
Biological oxygen demand (BOD) (mg L^{-1})	236.95
Chemical oxygen demand (COD) (mg L^{-1})	8000
Total organic carbon (TOC) (mg L^{-1})	2210
Dissolved organic carbon (DOC) (mg L^{-1})	1756
Dissolved organic nitrogen (DON) (mg L^{-1})	432.06
Dissolved organic phosphate (DOP) (mg L^{-1})	184.75
Total nitrogen (TN) (mg L^{-1})	506.12
Total phosphate (TP) (mg L^{-1})	1262
Nitrate (mg L^{-1})	915.62

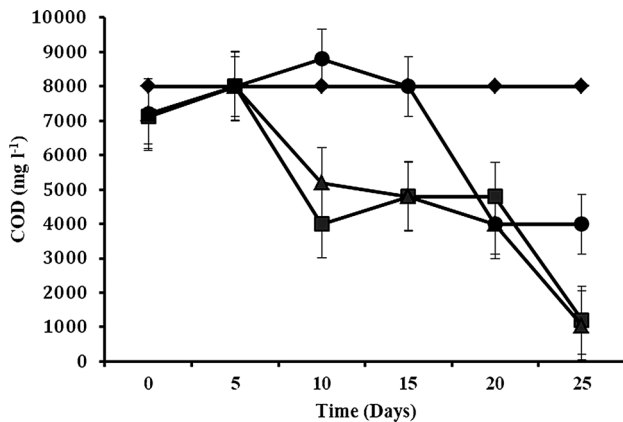All the parameters were analyzed as per the protocols mentioned in APHA (1998)

Fig. 2 Variation in COD at alternate of 5 days in TWW treated with *A. ambigua*, *C. pyrenoidosa* and *S. abundans* (filled diamond symbol, control; black square symbol, *C. pyrenoidosa*; filled triangle symbol, *S. abundans*; filled circle symbol, *A. ambigua*). (Values represent the mean ± SD of three replicates.)

day, the samples containing *C. pyrenoidosa* and *S. abundans* were having an equal concentration of COD (4800 mg L^{-1}). After 15th day of the experiment, *S. abundans* has drastically reduced the COD concentration over *C. pyrenoidosa* and *A. ambigua.* Similarly, the samples remediated by *A. ambigua* and *S. abundans* were showing the same COD concentrations (4000 mg L^{-1}) at 20th day of the experiment. The COD was reduced maximum from 15th to 20th day in the sample remediated by *A. ambigua*, but after this, samples did not show any reduction in COD concentrations. The sample remediated by *S. abundans* showed a gradual decrease in the levels of COD throughout the experiment, whereas *C. pyrenoidosa* showed a major drop from 5th to 10th day and 20th to the 25th day. Removal rate (RR) was maximum for *C. pyrenoidosa* (400 mg L^{-1} day^{-1}) and *S. abundans* (280 mg L^{-1} day^{-1}) at 10th day (Table 3). *C. pyrenoidosa* and *S. abundans* were at par with each other for RR toward the end of the experiment.

COD is indirectly used to measure the presence of organic compounds in the wastewater. Reduction in the amount of COD indicates the efficiency of microalgae to utilize the organic carbon as a substrate for their growth and source of energy (Hu et al. 2012) and hence clarify the water. Ding et al. (2015) have reported that 89.7% of COD was removed by microalgae following 8 days of growth in dairy wastewater. The increase in the concentration of COD in the case of *Chlorella sp.* and *Anabaena sp.* during the initial days of experiment might be due to the release of various soluble organic compounds like exopolysaccharides, dissolved organic matter by microalgae in their surroundings (Marjakangas et al. 2015). Exopolysaccharides (EPS) largely consists of galacturonic acid, ribose, arabinose, xylose, glucose, galactose and rhamnose sugars and are probably released by the microalgae to protect themselves from unfavorable stress conditions. Organic nature of EPS may contribute to the increase in COD levels. Moreover, EPS are involved in cell-to-cell interactions, adhesion and biofilm formation, thereby favoring the survival of microalgae. High concentrations of xenobiotic compounds led to increments in the COD levels, due to their relatively high resistance toward

Table 3 Reduction efficiency (RE) and removal rate (RR) of *A. ambigua, C. pyrenoidosa* and *S. abundans* in textile wastewater

Parameters	*A. ambigua*			*C. pyrenoidosa*			*S. abundans*		
	RE (%)	RR (mgl^{-1} day^{-1})		RE (%)	RR (mgl^{-1} day^{-1})		RE (%)	RR (mgl^{-1} day^{-1})	
	25th day	10th day	25th day	25th day	10th day	25th day	25th day	10th day	25th day
BOD	13.920	1.799	1.319	24.050	2.699	2.279	13.920	2.099	1.319
COD	50.000	0	160	85.000	400	272	86.870	280	278
Chloride	55.550	0	2.199	61.110	0	1.599	44.440	0	1.999
Nitrate	52.950	0	19.390	74.430	9.329	27.260	68.860	2.686	25.220
Phosphate	63.050	0.171	0.796	28.010	0	0.354	70.790	0.699	0.894

Values represent the mean ± SD of three replicates

microbial degradation (Garg and Tripathi 2013). Nian et al. (2007) have also mentioned in their preliminary analysis that textile dye effluents contain high levels of COD. Sivakalai et al. (2013) reported that *Spirulina platensis* had the efficiency to decrease the COD value in textile dyeing effluent. According to their findings, COD concentration was between 1040 and 8010 mg L^{-1} in textile effluent and after remediation by *Spirulina platensis* for 30 days, it was reduced to 101–600 mg L^{-1}.

Biological oxygen demand (BOD)

BOD measures the amount of oxygen required by microorganisms to breakdown the simple substances into decomposable organic matter (Abdel-Raouf et al. 2012). BOD is a critical indicator for the assessment of the health condition of an ecosystem and is also a precise indicator to keep a check on the microalgal growth (Pawlowski et al. 2015). Nearly 24% BOD reduction was observed with *C. pyrenoidosa* followed by 13% with both *S. abundans* and *A. ambigua*. BOD value was 236.95 mg L^{-1} at the start of the experiment and reduced up to 179 mg L^{-1} for *C. pyrenoidosa*, and both *S. abundans* and *A. ambigua* were having 203.96 mg L^{-1} BOD value following 25 days of remediation (Fig. 3). In the case of *C. pyrenoidosa*, the decrease in BOD level was up to 20th day and after that there was no reduction toward the end of the experiment, whereas *A. ambigua* and *S. abundans* showed a gradual decrease in the BOD level from the 15th day to the 25th day. Removal rate was maximum for *C. pyrenoidosa* (2.67 mg L^{-1} day^{-1}) at 10th day followed by *S. abundans* (2.09 mg L^{-1} day^{-1}) and *A. ambigua* (1.79 mg L^{-1} day^{-1}) (Table 3). At 25th day, *A. ambigua* and *S. abundans* were having a similar reduction rate, i.e., 1.32 mg L^{-1} day^{-1}.
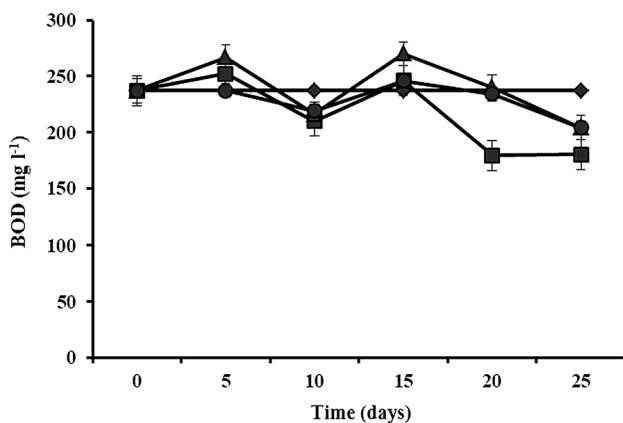

Fig. 3 Variation in BOD at alternate of 5 days in TWW treated with *A. ambigua*, *C. pyrenoidosa* and *S. abundans* (filled diamond symbol, control; filled square symbol, *C. pyrenoidosa*; filled triangle symbol, *S. abundans*; filled circle symbol, *A. ambigua*). (Values represent the mean ± SD of three replicates.)

Dissolved oxygen is consumed by heterotrophs to oxidize organic matter, thereby reducing the BOD of wastewater (Abdel-Raouf et al. 2012). The reasonably lower BOD values (236.95 mg L^{-1}) at the beginning of the experiment might be attributed to the lesser quantities of total and suspended solids in effluents (Alaguprathana and Poonkothai 2015). If the BOD/COD ratio is > 0.3, then the wastewater can be easily treated by biological means; otherwise, it would require a more precise biological system which can sustain more toxic shock to counterpoise these complexities (Mahmoued 2010). The low BOD/COD ratio, i.e., 0.03, observed in the present study supports the comparatively slower degradation rates of TWW components. This might be attributed to the complex and variable composition of TWW in terms of dyes, fixatives, bleaching agents, detergents, heavy metals that constitutes the large non-biodegradable fractions of TWW. Furthermore, the high molecular weight chromophores of TWW may be considered as the major contributors to the increased COD levels and color toxicity due to impermeable nature across cell membrane. This could also correlate with the presence of relatively less microorganisms at the natural textile effluent discharge sites. Colored effluent, toxic compounds and less dissolved oxygen do not support the growth of microbes at natural sites (Oke et al. 2006).

Nitrate and phosphate content

There was a considerable reduction in the concentration of nitrate and phosphate present in the wastewater after the end of phycoremediation experiment. The reduction in the TWW nitrate concentration was 74.43% with *C. pyrenoidosa*, 68.86% with *S. abundans* and 52.95% with *A. ambigua*. The value of nitrate in control (autoclaved effluent) was 915.62 ± 0.39 mg L^{-1} at the beginning of the experiment, and it was reduced to 234.09 ± 2.17 mg L^{-1} for *C. pyrenoidosa*, 285.08 ± 0.54 mg L^{-1} for *S. abundans* and 430.80 ± 1.63 mg L^{-1} for *A. ambigua* at the end of the experiment (Fig. 4a). *A. ambigua* showed a significantly (*p* value, 0.0013 < 0.05) lower reduction rate other two algal species, but there was a steady decrease at all the time intervals. Both *C. pyrenoidosa* and *A. ambigua* showed a prominent decrease in nitrate removal from zero-days to 15th day, and after 15th day, there was a slight decrease till the end. The maximum removal rate was recorded for *C. pyrenoidosa* (45.05 mg L^{-1} day^{-1}) followed by *S. abundans* (43.09 mg L^{-1} day^{-1}) on the 15th day (Table 3).

Nitrogen is required for the synthesis of protein, nucleic acid and phospholipid; therefore, the growth of microalgae is believed to play a major role in nitrogen removal via the processes of uptake, decay and sedimentation (Olguín et al. 2003; Zimmo et al. 2003). Tartte et al. (2010) also recorded the efficiency of *Anabaena variabilis* and *Nostoc muscorum*
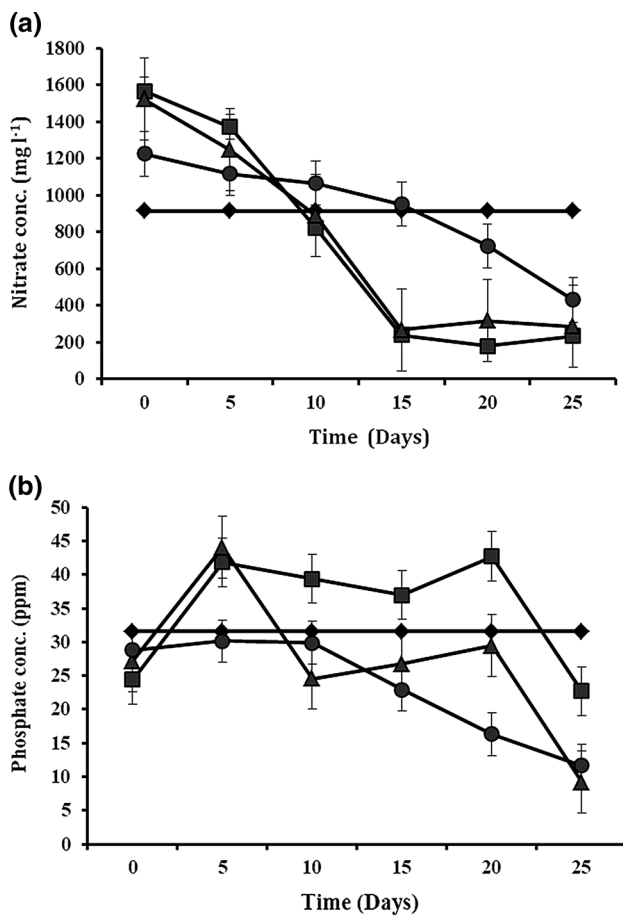
(a)

(b)

Fig. 4 Reduction in nitrate (**a**) and phosphate (**b**) content in TWW treated with *A. ambigua*, *C. pyrenoidosa* and *S. abundans* (filled diamond symbol, control; filled square symbol, *C. pyrenoidosa*; filled triangle symbol, *S. abundans*; filled circle symbol, *A. ambigua*). (Values represent the mean ± SD of three replicates.)
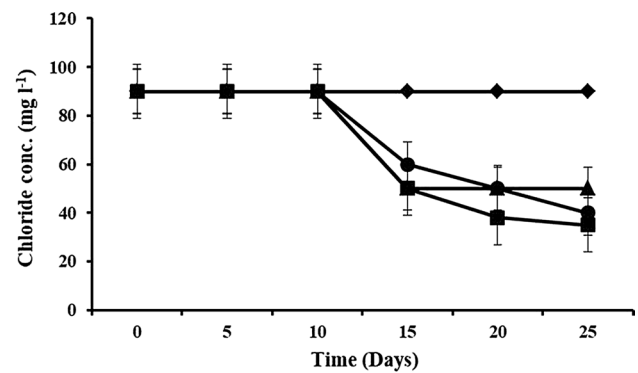

Fig. 5 Chloride content reduction in TWW treated with *A. ambigua*, *C. pyrenoidosa* and *S. abundans* (filled diamond symbol, control; filled square symbol, *C. pyrenoidosa*; filled triangle symbol, *S. abundans*; filled circle symbol, *A. ambigua*). (Values represent the mean ± SD of three replicates.)

phosphate was removed from 100% TWW by using *Chlorella vulgaris*. The phosphate is required for energy transfer and formation of cell membranes and nucleic acids; however, its consumption by microalgae was observed not to be very efficient (Chokshi et al. 2016). *Chlorella pyrenoidosa* was reported to remove 60% nitrate and 87% phosphate from dairy wastewater by absorbing these nutrients for their growth (Kothari et al. 2012). The increase in the algal biomass was a result of a gradual reduction in the nutrient load present in the wastewater (Kothari et al. 2013). *Chlamydomonas polypyrenoideum* was reported to remove 90% nitrate and 70% phosphate from dairy wastewater effluent (Kothari et al. 2013). Dubey et al. (2011) noted a similar trend during phycotreatment of industrial effluents with blue green algae and reported the highest reduction in phosphate using *Nostoc* spp., *Oscillotoria* spp. and *Glococapsa* spp.

Chloride content

Chloride is considered as one of the major inorganic anions present in textile effluents and acts as bleaching agents (Deo and Chinta 2000). 44–61% chloride reduction was achieved with the microalgal species employed in the present study. The initial concentration of chloride in 75% TWW was 89.97 mg L^{-1} (Fig. 5). Till 10th day, no notable reduction in chloride concentration was observed, but it decreased significantly toward the 15th day of the experiment. *A. ambigua* and *S. abundans* were at par with each other at the 20th day, as both were significantly reducing the chloride concentration (49.99 mg L^{-1}) (*p* value, $0.003 < 0.05$). At the end of 25th day, it was 34.99 mg L^{-1} in the sample remediated by *C. pyrenoidosa,* 39.98 mg L^{-1} with *A. ambigua* and 49.97 mg L^{-1} with *S. abundans*. There was no significant removal after 15th day in *S. abundans* (*p* value, $0.011 < 0.05$). *C. pyrenoidosa* showed a slight decline in

for the removal of nitrogenous contaminants from kitchen wastewater (75 and 100% concentration of kitchen wastewater) at the final stage of treatment. Nitrate was significantly reduced up to 80% by using *Chlorella vulgaris* and *Scenedesmus obliquus* grown on TWW.

The phosphate removal efficiency was maximum for *S. abundans* (70.80%), followed by *A. ambigua* (63.05%) and *C. pyrenoidosa* (28.00%) as compared to the control (75% autoclaved effluent). The initial concentration in control was 32 ± 1.21 ppm, which was reduced to 23 ± 1.29 ppm by *C. pyrenoidosa*, 9 ± 0.35 ppm by *S. abundans* and 12 ± 0.09 ppm by *A. ambigua* at the end of the experiment (Fig. 4b). *A. ambigua* showed a gradual reduction in the phosphate concentration at all time intervals, whereas *C. pyrenoidosa* and *S. abundans* showed a prominent decrease from 20th to 25th day. *S. abundans* has a maximum removal rate of 0.89 mg L^{-1} day^{-1}, whereas it was 0.35 mg L^{-1} day^{-1} for *C. pyrenoidosa* and 0.79 mg L^{-1} day^{-1} for *A. ambigua* (Table 3). In a study reported by Lim et al. (2010), 33%

the chloride content from 20th to the 25th day. *A. ambigua* (2.19 mg L^{-1} day^{-1}) showed a maximum removal rate over *C. pyrenoidosa* (1.59 mg L^{-1} day^{-1}) and *S. abundans* (1.99 mg L^{-1} day^{-1}) at the end of the experiment (Table 3).

Excessive amounts of chlorides present in the effluent may harm the agricultural crops, kill microorganisms and bleach the plant cells. Chloride affects the plants in terms of scorched leaf margins, smaller and thicker leaves as well as reduced plant growth (Alaguprathana and Poonkothai 2015). Levin (1960) had postulated that the algal species concentrate chloride ions in their system, a method for using algae to desaline saline water. The physiological reasons are still unknown, and this is confined to the analysis of several species (Levin 1960). The studies of the effect of chloride ion with isolated chloroplast in vitro have indicated that Cl^{-} plays a role of essential co-factor for photosynthesis. This postulate is further supported by the fact that chloride ion is vital for the growth of the plants (Norman 1977).

UV–Visible spectra

The spectral analysis was performed to study the decrease in the chromophores before and after phycoremediation. The main peaks obtained in the 190–230 nm region (Fig. 6) may be assigned to a range of chromophores in the form of 1,3-butadiene ($\lambda_{max} = 217$ nm), vinyl acetylene ($\lambda_{max} = 219$ and 228 nm), acrolein ($\lambda_{max} = 210$ nm), crotonaldehyde ($\lambda_{max} = 218$ nm), acetic acid ($\lambda_{max} = 204$ nm), acetamide ($\lambda max = 220$ nm), ethyl acetate ($\lambda_{max} = 211$ nm), nitromethane ($\lambda_{max} = 201$ nm) ,etc. (Urushigawa and Yonezawa 1977; El-Sheekh et al. 2009). The peaks observed at 200–210 nm in control decreased quantitatively on the 25th day in all the microalgal species. For *A. ambigua*, a new peak was observed at 15th day at the place of a valley at 204 nm (Fig. 6a) and the peak disappeared at the 25th day of the experiment. Similarly for *C. pyrenoidosa*, the peak at 202 nm decreased quantitatively at subsequent intervals of time and the peak at 206 nm also was reduced on the 25th day of the experiment (Fig. 6b). Comparative analysis of *S. abundans* revealed that there were two peaks (at 202 and 206 nm) in control; the peak at 202 nm was disappeared at 15th day of the experiment. The peak at 206 nm was also reduced at 25th day (Fig. 6c). In all microalgal species, a slight increase was observed between 210 and 215 nm at the end of the experiment and it may be attributed to the formation of a new chromophore (Pinheiro et al. 2004). Since TWW is a complex effluent, it contains many compounds in the form of dyes, fixatives, bleaching agents, surfactants, soaps of various metals, etc. So, many peaks were obtained at different wavelengths. Microalgal species may utilize azo compounds as carbon and nitrogen source for their growth. Biodegradation and bioabsorption could be the two possible mechanisms engrossed in
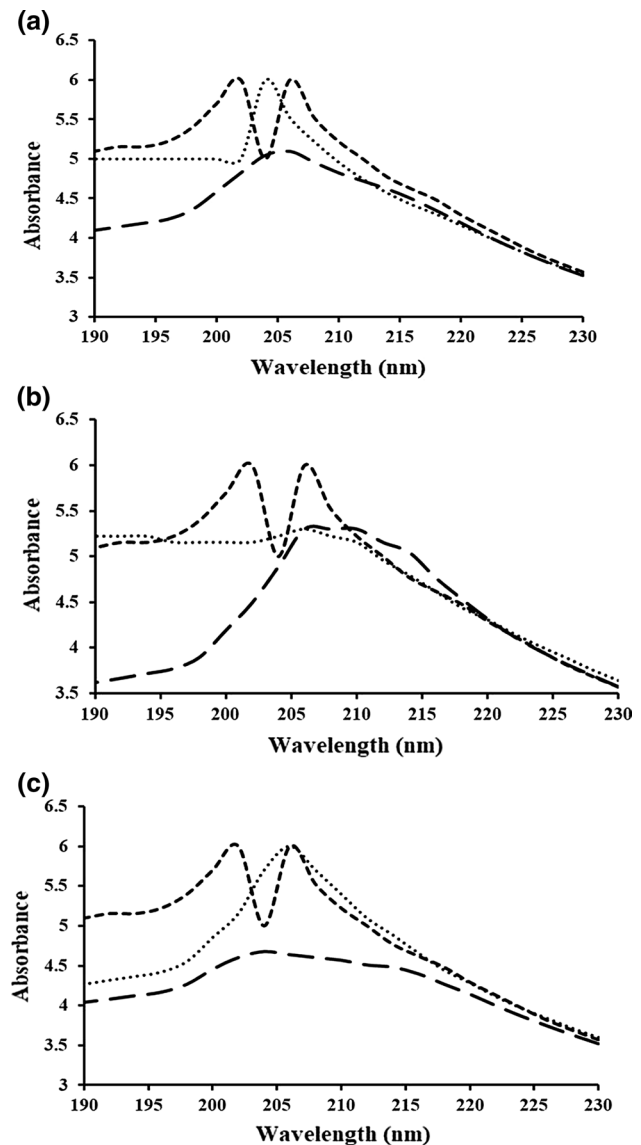
Fig. 6 The UV–visible spectra for TWW treated with **a** *A. ambigua*, **b** *C. pyrenoidosa* and **c** *S. abundans* at control (dash symbol), 15th day (round dot symbol) and 25th day (long dash symbol)

the phycoremediation process. Biodegradation of dyes involved breakage of azo bridge into aromatic amines by the action of azo reductase system and led to the mineralization of native structure. Furthermore, bioabsorption entailed absorption of the dyes by living or dead biomass without affecting the structure (Hernández-Zamora et al. 2015). The appearance and disappearance of various peaks as well as change in their intensity in the spectra obtained in the present study can be correlated with the breakdown and formation of various compounds, and it can be postulated that the strains employed are following biodegradation route for the wastewater remediation.

Conclusion

From the last decade, microalgae have shown its potential to recycle the nutrients within the wastewater sources, and also as a crucial step in the treatment of wastewater. The efficiency of algae for the nutrient removal varies with the type of wastewater and species selected for the remediation (Pittman et al. 2011).

This study showed the possibility of cultivation and simultaneous nutrient removal capacity of *A. ambigua*, *C. pyrenoidosa* and *S. abundans* in the textile wastewater. The notable nutrient removal from the wastewater employing microalgal species suggested the potential of these strains to reduce the pollution load, BOD and COD of wastewater. 75% TWW may be used for the purpose of algal growth and phycoremediation. The microalgal species have efficiently recovered the nitrogen (nitrate) from the wastewater (almost up to 85.06% with *C. pyrenoidosa*). Algal species have significantly reduced the COD of TWW which indicates their capacity of phycoremediation (p value, $0.015 < 0.05$). *S. abundans* showed the optimal removal of phosphate and chloride along with a notable reduction in COD, while *C. pyrenoidosa* showed best results capacity for BOD and nitrate reduction. The TWW may contain many toxic dyes and other harmful contaminants in the form of heavy metals, and these might be affecting the phycoremediation potential of microalgae (Hernández-Zamora et al. 2015). Thus, it can be concluded that *A. ambigua*, *C. pyrenoidosa* and *S. abundans* may be used as a low-cost alternative to assist in the sustainable remediation of textile wastewater and the algal biomass produced can further be used as a fertilizer or for biofuel production (Rawat et al. 2011). Further, real-time monitoring of phycoremediation and scale-up will be required to validate and implement the findings. Additionally, effect of mixed microbial consortium consists of both autotrophic and heterotrophic populations could provide novel insights into development of robust microbiological solutions for large-scale remediation processes. Moreover, techno-economic aspect has to be considered while implementing the algal biomass production coupled to wastewater treatment for bioenergy production in the near future.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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