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Biopolymer treatment of ammonium-rich industrial effluents for the mass cultivation of microalgae

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Abstract

Although wastewater reutilization for microalgae culturing can meet the dual goals of wastewater treatment and biomass production, some effluents with high contaminant concentrations are toxic to microalgae, necessitating pretreatment protocols to lower the toxicity before bioremediation. The present study aimed to bioremediate the industrial effluents of El Delta Co. for Fertilizers and Chemical Industries (Mansoura, Egypt), using sodium alginate as a pretreatment to enable reuse as a growth medium for microalgae culturing. Various water quality parameters signified the inferior state of the effluent with an ammonia-N concentration of 185.76 mg L^{-1}. Toxicity investigations of the raw industrial effluents revealed toxicity to *Chlorella sorokiniana*, *Scenedesmus vacuolatus* and *Pseudokirchneriella subcapitata.* Effluent bioremediation was adopted using different concentrations of the biopolymer sodium alginate, and 1.0 g L^{-1} sodium alginate resulted in the highest removal of both ammonia-N and heavy metals. *Chlorella sorokiniana* and *S. vacuolatus* successfully grew in the 1.0 g L^{-1} alginate-treated effluent. *Chlorella sorokiniana* removed 87.8% of the ammonia-N, 75% of the copper, and 100% of the phosphorus. *Scenedesmus vacuolatus* consumed 85.7% of the ammonia-N, 66.7% of the copper, and 100% of the phosphorus. Adjusting the N:P mass ratio to 9.9 resulted in high tolerance of *C. sorokiniana* and *S. vacuolatus* to the effluent toxicity, with an $EC_{50} > 100\%$. The 1.0 g L^{-1} sodium alginate-treated effluent stimulated *C. sorokiniana* and *S. vacuolatus* growth relative to the control. Additionally, *C. sorokiniana* and *S. vacuolatus* had the highest biomass production and protein content, reaching 1.42 and 0.74 g L^{-1} and $57.04 \pm 0.04\%$ and $52.19 \pm 0.02\%$, respectively, in the treated effluent. Therefore, it was concluded that this bioremediation approach using the 1.0 g L^{-1} alginate pretreatment followed by microalgal cultivation (*C. sorokiniana* and *S. vacuolatus*) successfully treated the industrial effluent, representing a promising protocol for bioremediation practices.

Keywords Bioremediation · Water quality · Sodium alginate · *Chlorella sorokiniana* · *Scenedesmus vacuolatus* · Industrial effluents

Introduction

Water quantity and water quality are among the major global problems facing humanity, particularly due to the limited availability of freshwater resources which has caused severe water scarcity in some regions (Schwarzenbach et al. 2010; Mohie El Din and Moussa 2016). In Egypt water demand is continually increasing due to the growing population and deterioration of the Nile River caused by anthropogenic inputs over several decades (Abd el-Lateef et al. 2011; Ali et al. 2014). In a worldwide freshwater survey, Egypt was listed among the ten countries that will run out of water by 2025 (Osman et al. 2016).

Parallel to the water deficit, wastewater production is rising due to increasing agricultural, urban, and industrial activities, causing extensive water pollution (Yadav 2019). Industrial wastewater is a serious issue in developing populated countries, including Egypt, particularly affecting natural water bodies near industrial areas. Several types of pollutants are generated by different industries. For example, the industry of fertilizers generates wastewater containing high quantities of heavy metals and phosphorus and nitrogen-rich compounds that induce eutrophication and negatively impact aquatic life and human health (Osibanjo et al. 2011; Refaay et al. 2021a). Thus, wastewater management offers the hope of slowing, perhaps even halting, the loss of usable water by producing an effluent that

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may be directly reused or returned to the water cycle with minimal environmental damage (Lofano and Brown 2010).

Characterizing the physical, chemical, and biological composition of wastewater is critical to improving and tailoring treatment techniques. In this regard, microalgae are considered a useful biological indicator of water quality because they are susceptible to the impacts of pollutants and heavy metal contaminants in wastewater (Parmar et al. 2016). Microalgae genera frequently used in toxicity bioassays include *Chlamydomonas*, *Chlorella*, *Scenedesmus*, and *Selenastrum*. (Ruiz-Marin et al. 2010; Li et al. 2011; Mennaa et al. 2015; Xu et al. 2016; Yamagishi et al. 2017). In short, evaluating the toxicity of different pollutants in the ecosystem using microalgae is highly efficient because some microalgae can also remediate water (Ray et al. 2021). Microalgae have shown high potential for the removal of inorganic phosphorus and nitrogen from wastewater (Aslan and Kapdan 2006; Park et al. 2010; Posadas et al. 2015).

Despite the ability of microalgae to grow and tolerate toxic pollutants in various wastewaters, elevated concentrations of ammonium, phosphorus, and heavy metals may inhibit microalgae growth (Kumar et al. 2015; Das et al. 2018; Li et al. 2019). Thus, a pretreatment would be necessary to reduce the concentration of such pollutants to a tolerable level, enabling microalgae to grow efficiently (Huang et al. 2018). Common techniques for wastewater treatment include precipitation, adsorption, ion exchange, flocculation, and electrochemical methods. Adsorption separation technology is regarded as a promising option for the remediation of wastewater due to the simple design and operation and time-efficiency of adsorption methods (Hua et al. 2014; Gisi et al. 2016).

Among the various types of adsorbents that have been used to remove contaminants from wastewater, hydrogels are promising due to several unique properties, such as their tunable structure, elasticity, high porosity, swelling ability, fast sorption rate, and reusability (Gombotz and Wee 2012; Makhado et al. 2020). In particular, sodium alginate has been widely used to prepare safe, nontoxic, biodegradable, and eco-friendly hydrogel. Additionally, the carboxyl groups of sodium alginate can serve as an active site for adsorbing metal ions from wastewater. Thus, sodium alginate gel is a superior adsorbent for ammonium, phosphorus, and heavy metals from wastewater (Zouboulis and Katsoyiannis 2002; Wan et al. 2014).

The objectives of the current investigation are to assess the quality and toxicity of the industrial effluents from El Delta Co. for Fertilizers and Chemical Industries (EFCI), bioremediate the wastewater using sodium alginate, and evaluate the suitability of the biologically treated wastewater for producing *Chlorella sorokiniana* and *Scenedesmus vacuolatus*.

Materials and methods

The study area

The investigated wastewater receives alkaline ammonia-rich industrial effluents from EFCI, located about 2 km north of Mansoura City, Egypt (31° 04′ 20.1" N, 31° 23′ 57.5" E).

Wastewater sampling and characterization

Sample collection, handling, and processing were conducted according to Peltier and Weber (1985). Wastewater samples were filtered through a GF/C glass microfiber filter (47 mm) and stored at 4 °C in the dark until analysis. The following physicochemical parameters were investigated according to the methods described in APHA (2005): water temperature, pH, biological oxygen demand (BOD), dissolved reactive phosphorus (DRP), ammonia-N, total alkalinity, nitrate–N, nitrite-N, total dissolved phosphorous (TDP), chemical oxygen demand (COD), dissolved oxygen (DO) and heavy metals (Fe, Zn, Cu, Pb, Ni, Cd, and Mn).

Microalgal isolates and culture conditions

The isolates (*Chlorella sorokiniana* and *Scenedesmus vacuolatus*) used in this study were obtained from the culture collection of the phycology laboratory of the Faculty of Science, Mansoura University, Egypt. Axenic cultures from each isolate were identified and deposited in GenBank under the accession numbers (MZ348902 and MZ348903). Cultures were maintained in modified *Navicula* nutrient medium (Starr 1978) at 25 °C under constant illumination (50 µmol photons m^{-2} s^{-1}).

Wastewater toxicity assessment

Wastewater toxicity was evaluated using an algal growth inhibition assay according to the International Organization for Standardization (ISO 2005) protocol. The standard test alga *Pseudokirchneriella subcapitata* and, in parallel, *C. sorokiniana* and *S. vacuolatus* were used to assess the effluent toxicity. A serial dilution technique was used to prepare nine concentrations of the test effluent. Three culture flask replicates were used for each effluent concentration and algal species. About 10 mL of algal nutrient solution medium (Miller and Greene 1978) was added to each flask, except flask (1). Next, 1.0 mL aliquots of 5-day old cultures of *P. subcapitata*, *C. sorokiniana*, and *S. vacuolatus* with cell densities of 5,000 cells mL^{-1} were separately inoculated into the test flasks. The flasks were incubated for 5 days

on a shaker at 20 °C under constant illumination (50 µmol photons m^{-2} s^{-1}). The direct cell count was used to assess toxicity.

The half-maximal effective concentration (EC_{50}) expresses the minimum effluent concentrations that inhibit algal growth by 50% compared with the control culture. The toxicity response parameter data were plotted as the relative percentage of its control against the corresponding effluent concentration. EC_{50} was calculated using the straight-line graphical interpolation method (Walsh et al. 1987).

Biopolymer-based industrial effluent treatment

The bioremediation protocol was performed using three doses of powdered sodium alginate (0.25, 0.5, and 1.0 g per 1.0 L of effluent). After stirring for 5 min, 250 mL of 0.1-M calcium chloride was added with continuous stirring. The mixture was then incubated at room temperature overnight (12 h) to allow for complete precipitation of the calcium alginate. After 12 h, the supernatants were carefully collected to analyze the ammonia-N, nitrate–N, nitrite-N, dissolved reactive phosphorus, total dissolved phosphorus, total alkalinity, and copper contents.

Investigation of *C. sorokiniana* and *S. vacuolatus* growth in the sodium alginate-treated effluent

The partially treated industrial effluents (200 mL) were used as a nutrient medium for *C. sorokiniana* and *S. vacuolatus* growth in 500 mL conical flasks. The treated effluent was inoculated with 20 mL of 5-day-old culture and incubated for 7 days at 25 °C under constant illumination (50 µmol photons m^{-2} s^{-1}). A parallel set of control cultures for each test alga was prepared in *Navicula* nutrient medium and cultured under the same growth conditions. At the end of the incubation period, cultures were kept in the dark and allowed to stand overnight for autoflocculation. The clear filtrate was separated by centrifugation (2,688 × *g* for 10 min) to determine the residual ammonium-N, nitrate–N, nitrite-N, dissolved reactive phosphorus, total dissolved phosphorus, and copper contents.

N:P mass ratio optimization

The 1.0 g sodium alginate treatment induced the highest efficiency of nutrient removal from the effluent, but the N:P mass ratio calculation revealed a condition of P-limitation. Thus, the N:P mass ratio was adjusted to 9.9 using P-stock ($K_2HPO_4.3H_2O$) modified *Navicula* nutrient medium solution to promote *C. sorokiniana* and *S. vacuolatus* growth (Miller and Greene 1978; Li et al. 2011; Wang et al. 2012).

Small-scale mass cultivation experiment for growing the test algae in sodium alginate-treated effluent with and without N:P mass ratio adjustment

The small-scale mass cultivation experiment was conducted using three 10-L plastic jars. *C. sorokiniana* and *S. vacuolatus* were separately cultivated in 1.0 g L^{-1} sodium alginate-treated effluent with and without N:P mass ratio adjustment, using modified *Navicula* nutrient medium as a control. Each jar was inoculated with 800 mL of 5-day-old cultures of *C. sorokiniana* and *S. vacuolatus* and incubated for 7 days at 25 °C under constant illumination (50 µmol photons m^{-2} s^{-1}). At the end of the incubation period, the culture jars were allowed to stand overnight in the dark to reach complete sedimentation. Then, cells were collected by centrifugation (2,688 × *g* for 5 min). The algal biomass was oven-dried (60° C) and the protein, carbohydrate, and lipid contents were determined.

Biochemical analysis of microalgae biomass

Crude protein was analyzed using the Bradford (1976) method, with modifications described by Stoscheck (1990). The total carbohydrate content was estimated according to Hedge et al. (1962). Lipids were extracted via the Soxhlet solvent extraction method (Sadasivam and Manickam 1996) and gravimetrically measured.

Statistical analysis

The values for each measurement represent the mean of three replicates ± SD (standard deviation). Multiple means were compared by analysis of variance (one-way ANOVA and post-hoc tests) using SPSS v. 20 for Windows 10.

Results

Effluent characteristics

The mean values of the investigated physicochemical parameters of the wastewater are presented in Table 1. The wastewater was alkaline with a pH of 9.79. The effluent contained high amounts of ammonium-N (185.76 mg L^{-1}), nitrite-N (2.93 mg L^{-1}), and nitrate–N (1.95 mg L^{-1}) and low quantities of TDP (0.511 mg L^{-1}) and DRP (0.31 mg L^{-1}). The N:P mass ratio (232.4) revealed a P-limitation condition in the effluent. Total alkalinity was high, with a mean value of 1,998.02 mg $CaCO_3$ L^{-1}. BOD (18.05 mg L^{-1}) and COD (18.9 mg L^{-1}) values were found to be higher than the DO value (4.97 mg L^{-1}). For the heavy metals, the effluent had a high copper concentration (0.149 mg L^{-1}) and low

Table 1 Summary statistics of the mean value of some physical and chemical wastewater parameters concentrations investigated at Eldelta for Fertilizers and Chemical industries (EFCI) company

Parameter	Unit	Values
pH		9.79 ± 0.01
Water temperature	°C	17.97 ± 0.06
Ammonia-N	mg L^{-1}	185.76 ± 0.14
Nitrite-N	mg L^{-1}	2.93 ± 0.09
Nitrate–N	mg L^{-1}	1.95 ± 0.03
DRP	mg L^{-1}	0.31 ± 0.009
TDP	mg L^{-1}	0.511 ± 0 .02
N:P weight ratio		232.4
Total alkalinity	mg $CaCO_3$ L^{-1}	1998.02 ± 0.03
DO	mg L^{-1}	4.97 ± 0.06
BOD	mg L^{-1}	18.05 ± 0.02
COD	mg L^{-1}	18.9 ± 0.09
Cu	mg L^{-1}	0.339 ± 0.002
Mn	mg L^{-1}	0.125 ± 0.002
Cd	mg L^{-1}	0.031 ± 0.001
Pb	mg L^{-1}	0.175 ± 0.02
Ni	mg L^{-1}	0.142 ± 0.02
Fe	mg L^{-1}	0.149 ± 0.005
Zn	mg L^{-1}	0.150 ± 0.002

manganese concentration (0.125 mg L^{-1}), as illustrated in Table 1.

Toxicity assessment of raw industrial wastewater

The dose–response curves (Fig. 1) illustrate the growth patterns of *C. sorokiniana*, *S. vacuolatus,* and *P. subcapitata* in response to different concentrations of the raw effluent. The results presented in Table 2 and Fig. 1A show the high toxicity of all effluent doses on the growth of *P. subcapitata* (EC_{50} = 0.061%), *S. vacuolatus* (EC_{50} = 16.8%), and *C. sorokiniana* (EC_{50} = 25.9%).

Effluent characteristics after treatment with sodium alginate

The data presented in Table 3 demonstrate the significant ($p \leq 0.05$) removal of nutrients after sodium alginate treatments (0.25, 0.5, and 1.0 g L^{-1}). Treatment with 1.0 g L^{-1} of sodium alginate induced the highest nutrient bioremoval of the major nutrients present in the effluent: ammonium-N concentration declined from 185.76 to 70.22 mg L^{-1} (62.2% removal), nitrate–N concentration declined from 1.95 to 1.01 mg L^{-1} (48.2% removal), and nitrite-N concentration declined from 2.93 to 1.85 mg L^{-1} (36.9% removal). Similarly, DRP and TDP concentrations were reduced from 0.31 to 0.26 mg L^{-1} (16.1% removal)

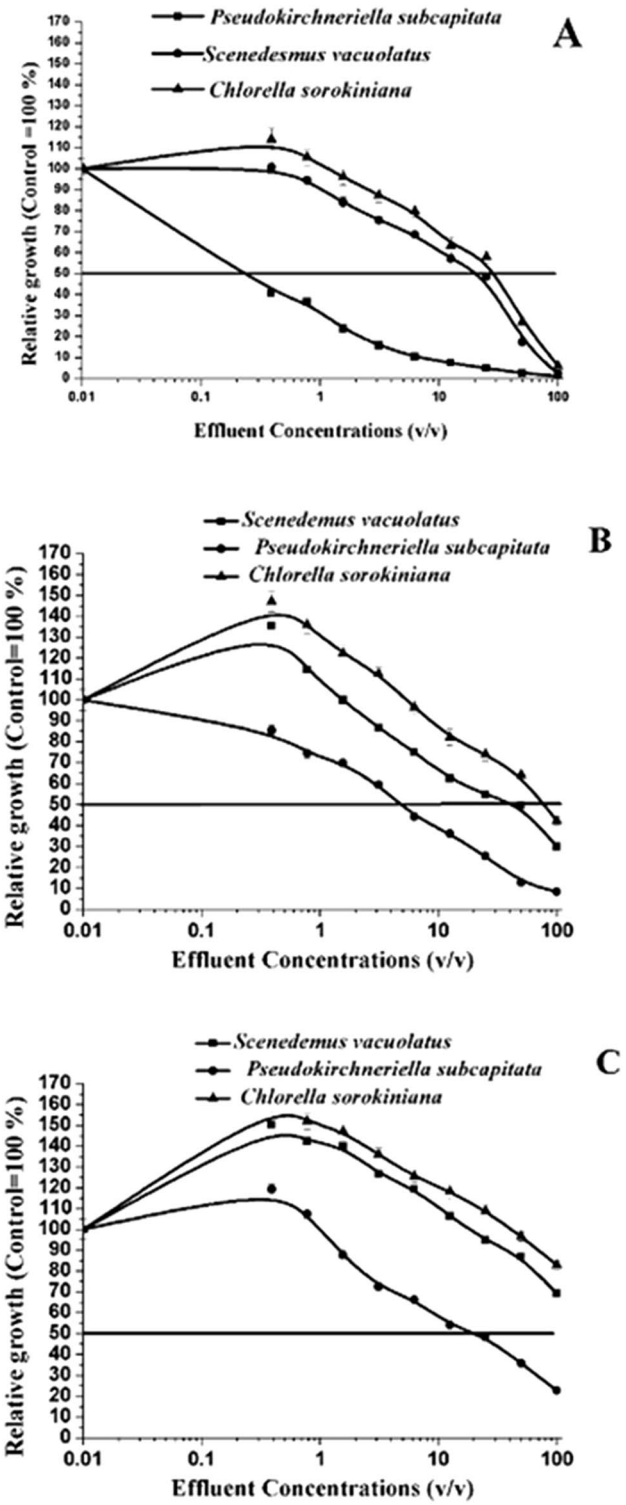

Fig. 1 Dose–response curves of test algae *Chlorella sorokiniana*, *Scenedesmus vacuolatus* and *Pseudokirchneriella subcapitata* grown in different concentrations of the raw effluent (**A**), in different concentrations of 1.0 g sodium alginate-treated effluent (**B**) and in 1.0 g sodium alginate-treated effluent with N:P mass ratio adjustment (**C**)

Table 2 Toxicity (EC_{50}) of raw effluent, 1.0 g sodium alginate treated effluent, and 1.0 g sodium alginate-treated effluent after N:P adjustment on growth of *Pseudokirchneriella subcapitata, Chlorella sorokiniana* and *Scenedesmus vacuolatus*

Test algae	EC_{50} of the raw effluent	EC_{50} of 1.0 g sod. alginate treated effluent	EC_{50} after N:P adjustment
Pseudokirchneriella subcapitata	0.061%	5.6%	11.5%
Chlorella sorokiniana	25.9%	83.7%	> 100%
Scenedesmus vacuolatus	16.8%	53.6%	> 100%

Table 3 The chemical wastewater parameters concentrations of raw effluent samples before and after sodium alginate treatment. Data represents mean ± SD, $n = 3$. Different letters indicate significant differences at $p \leq 0.05$

Parameter	Unit	Raw sample	Sodium alginate treatments (g L^{-1})			
			0.25	0.5	1.0	LSD 0.05
pH	Unit	9.79 ± 0.01[a]	9.05 ± 0.02[b] (7.6%)*	8.86 ± 0.03[c] (9.4%)	8.01 ± 0.02[d] (18.2%)	0.042
Ammonium-N	mg L^{-1}	185.76 ± 0.14 [a]	119.84 ± 0.01 [b] (35.5%)	97.63 ± 0.02 [c] (47%)	70.22 ± 0.006 [d] (62.2%)	0.153
Nitrite-N	mg L^{-1}	2.93 ± 0.09 [a]	2.35 ± 0.01 [b] (19.8%)	2.13 ± 0.02 [c] (27.3%)	1.85 ± 0.02 [d] (36.9%)	0.089
Nitrate–N	mg L^{-1}	1.95 ± 0.03 [a]	1.55 ± 0.02 [b] (20.5%)	1.28 ± 0.02 [c] (34.4%)	1.01 ± 0.02 [d] (48.2%)	0.048
DRP	mg L^{-1}	0.31 ± 0.009 [a]	0.296 ± 0.006 [b] (4.5%)	0.293 ± .006 [b] (5.5%)	0.26 ± 0.003 [c] (16.1%)	0.441
TDP	mg L^{-1}	0.51 ± 0.02 [a]	0.475 ± 0.006 [b] (6.8%)	0.443 ± 0.006 [c] (13.1%)	0.41 ± 0.006 [d] (19.6%)	0.023
N:P		232.5 [a]	160.49 [b]	137.28 [c]	109.07 d	7.95
Total alkalinity	mg $CaCO_3$ L^{-1}	1998.02 ± 0.03 [a]	1569.06 ± 0.02 [b] (21.5%)	1254.23 ± 0.02 [c] (37.2%)	986.1 ± 0.01 [d] (50.6%)	0.032
Copper	mg L^{-1}	0.339 ± 0.002 [a]	0.28 ± 0.01 [b] (17.4%)	0.18 ± 0.01 [c] (46.9%)	0.12 ± 0.02 [d] (64.6%)	0.025

* % bioremoval

and 0.51 to 0.41 mg L^{-1} (19.6% removal), respectively. Total alkalinity was reduced from 1,998.02 to 986.1 mg $CaCO_3$ L^{-1} (50.6% removal), and copper was reduced from 0.339 to 0.12 mg L^{-1} (64.6% removal).

Investigation of *C. sorokiniana* and *S. vacuolatus* growth in sodium alginate-treated effluent

The dry biomass production of the algae grown in sodium alginate-treated effluent is shown in Table 4. *C. sorokiniana* and *S. vacuolatus* biomass production was significantly higher ($p \leq 0.05$) in 1.0 g L^{-1} sodium alginate-treated effluent at 0.362 and 0.164 g L^{-1}, respectively, whereas no growth was observed in the 0.25 and 0.5 g L^{-1} sodium alginate-treated effluents for both test algae.

Table 4 *Chlorella sorokiniana* and *Scenedesmus vacuolatus* biomass (dry wt. g L^{-1}) grown on different alginate-treated effluents. Data represents mean ± SD, $n = 3$. Different letters indicate significant differences at $p \leq 0.05$

Treatments	Dry weight biomass (g L^{-1})	
	Test algae	
	Chlorella sorokiniana	*Scenedesmus vacuolatus*
Control*	0.451 ± 0.012 g [a]	0.242 ± 0.011 g [a]
0.25 g	No growth [b]	No growth [b]
0.5 g	No growth [b]	No growth [b]
1.0 g	0.362 ± 0.018 g [c]	0.164 ± 0.005 g [c]
LSD 0.05	0.066	0.009

* Modified *Navicula* medium

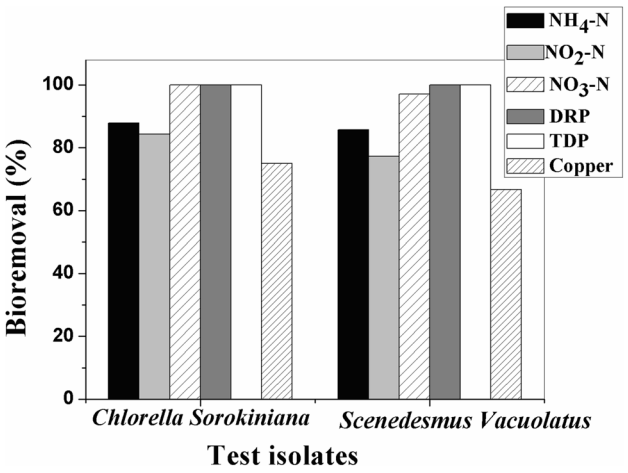

Fig. 2 Bioremoval (%) of total soluble inorganic nitrogen (NH_4-N, NO_3-N and NO_2-N), phosphorus and copper after 7 days growth of *Chlorella sorokiniana* and *Scenedesmus vacuolatus*

Bioremoval potentiality of *C. sorokiniana* and *S. vacuolatus* for sodium alginate-treated effluent chemical components

Figure 2 illustrates the decline in ammonium-N content in 1.0 g L^{-1} sodium alginate-treated effluent, from an initial concentration of 70.22 mg L^{-1} to 8.54 mg L^{-1} and 10.03 mg L^{-1} with the test algae *C. sorokiniana* and *S. vacuolatus*, respectively. Generally, the bioremoval percentage of the total soluble inorganic nitrogen (ammonium-N, nitrite-N, and nitrate–N) fluctuated between 77.3 and 100%. Furthermore, phosphorus was completely removed in both *C. sorokiniana* and *S. vacuolatus* cultures, and the copper concentration was reduced by 75% and 66.7%, respectively.

Toxicity assessment of 1.0 g L^{-1} sodium alginate-treated effluent

The results illustrated in Table 2 and Fig. 1B show that 1.0 g L^{-1} sodium alginate-treated effluent stimulated the growth of both *S. vacuolatus* and *C. sorokiniana*, with an EC_{50} of 53.6% and 83.7%, respectively, *P. subcapitata* growth was slightly increased with an EC_{50} of 5.6%.

Toxicity assessment after N:P mass ratio adjustment of 1.0 g L^{-1} sodium alginate-treated effluent

The (9.9 w/w) N:P mass ratio adjustment of sodium alginate-treated effluent resulted in the growth stimulation of *S. vacuolatus* and *C. sorokiniana* as the EC_{50} was typically $^{>}$ 100% for both test algae, whereas the *P. subcapitata* EC_{50} value was 11.5% (Table 2; Fig. 1C).

Growth responses of *C. sorokiniana* and *S. vacuolatus* grown in 1.0 g L^{-1} sodium alginate-treated effluent with and without N:P mass ratio adjustment

The 1.0 g L^{-1} sodium alginate-treated effluent with an adjusted N:P mass ratio generated the maximum protein content in *C. sorokiniana* (57.04 ± 0.04%), whereas the lowest content (54.79 ± 0.01%) was recorded in the control culture. However, *C. sorokiniana* also exhibited the highest lipid content (16.01 ± 0.01%) and total carbohydrate content (16.03 ± 0.04%) in the control culture (Fig. 3A).

The highest *S. vacuolatus* protein content (52.19 ± 0.02%) was recorded in the 1.0 g L^{-1} sodium alginate-treated effluent with an adjusted N:P ratio. The highest total lipid content (12.16 ± 0.03%) and total carbohydrate content (10.03 ± 0.01%) were observed in the control culture (Fig. 3B).

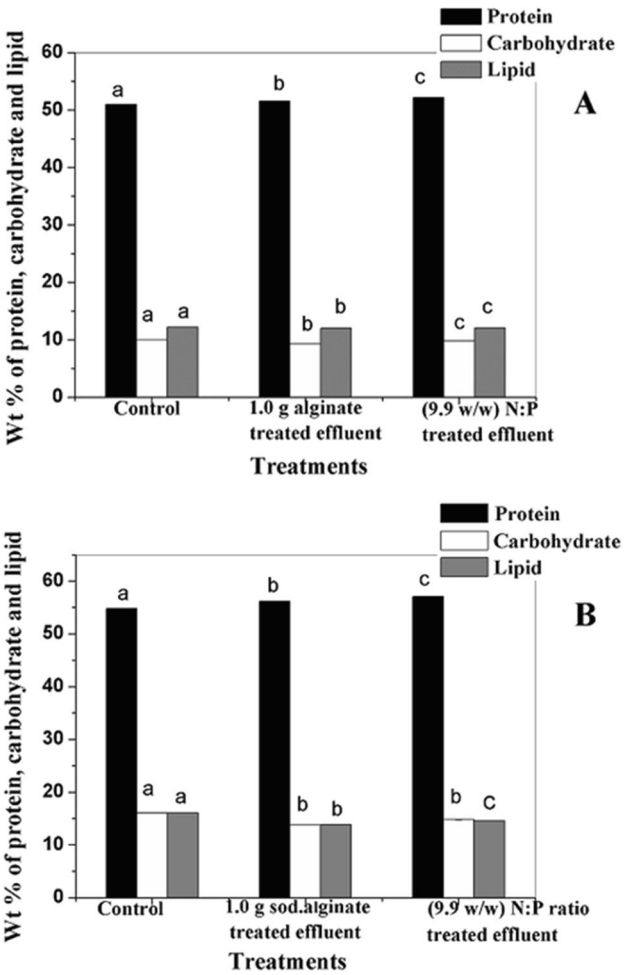

Fig. 3 Mean variations in wt % of total protein, total carbohydrate, and total lipid contents of *Chlorella sorokiniana* (**A**) and *Scenedesmus vacuolatus* (**B**) grown in 1.0 g sodium alginate-treated effluent with and without N:P mass ratio adjustment

The highest dry weight biomass for *C. sorokiniana* (1.42 g L^{-1}) and *S. vacuolatus* (0.74 g L^{-1}) were recorded in the 1.0 g L^{-1} sodium alginate effluent with an adjusted N:P mass ratio (Fig. 4).

Discussion

Bioremediation and wastewater recycling have become necessary for human life, as water is the most precious natural resource. Furthermore, the continuous discharging of wastewater to ecosystems without appropriate treatment has created severe environmental and health hazards (Osibanjo et al. 2011). Therefore, developing methods for the sustainable treatment and reuse of wastewater is a significant global challenge (Li et al. 2011; Ji et al. 2013).

The physicochemical analysis (Table 1) revealed that EFCI effluents were highly rich in inorganic nitrogen (ammonium-N) and copper, which reflects the grossly polluted conditions of the effluent. The toxicity of ammonium-N to aquatic organisms is strongly pH-dependent; in natural aquatic habitats with high alkalinity and pH values above 8, most ammonium-N forms unionized ammonia, which is highly toxic to aquatic communities (Leta et al. 2003; Seyoum et al. 2003; Shiwanand and Tripathi 2013). Similar results were also reported by El-Sheekh et al. (2005) Abdel-Hamid et al. (2017) and Refaay et al. (2021a) in the same study area. However, it is difficult to predict the toxicity of wastewater from physical and chemical analysis; thus, algal bioassay toxicity assessments were performed to assess the toxicity of the investigated effluent.

Toxicity assessment using algal bioassays (Table 2 and Fig. 1A) revealed the toxic effects of the effluent on

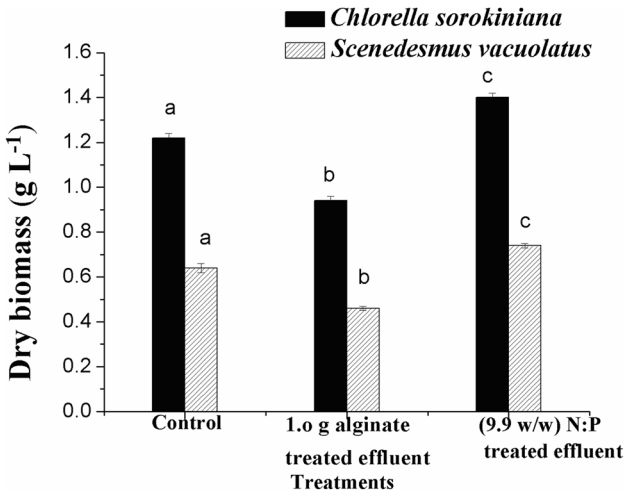

Fig. 4 Mean variation of dry biomass (g L^{-1}) of *Chlorella sorokiniana* and *Scenedesmus vacuolatus* grown in 1.0 g sodium alginate-treated effluent with and without N:P mass ratio adjustment

the three test algae (*P. subcapitata*, *C. sorokiniana*, and *S. vacuolatus*); the low EC_{50} values indicated the high toxicity of the tested sample (Walsh et al. 1987). The marked growth inhibition of *C. sorokiniana* and *S. vacuolatus* may be attributed to the high concentration of ammonium-N and copper. In this context, Levy et al. (2008) and Kondzior and Butarewicz (2018) reported that copper was the most toxic element, affecting the growth rate and photosynthesis of microalgae via the reduction of chlorophyll *a* and *b*, total carotenoids, and starch granules. In addition, copper exposure alters metabolism as well as chloroplast ultrastructure and increase intra-thylakoid space in microalgae (Kropat et al. 2015; Yong et al 2021).

Khanh et al. (2013), Markou et al. (2016), and Li et al. (2019) suggested high ammonia concentrations induce an uncoupling effect on electron transport in photosystems I and II by breaking down the proton gradient required for photophosphorylation and affecting the oxygen evolution complexes, delaying microalgae growth. The current findings are consistent with those of Park et al. (2010), Posadas et al. (2017), and Li et al. (2019), who reported the marked growth inhibition of *C. sorokiniana* and *Scenedesmus* sp. at concentrations of ammonium higher than 100 ppm.

Therefore, the sodium alginate pretreatment process was necessary to minimize the concentrations of toxic ammonia-N and heavy metals, particularly copper, in the effluent to support the growth of *C. sorokiniana* and *S. vacuolatus* in this study. Several studies have reported using natural polymers, such as sodium alginate, as efficient and eco-friendly biosorbents for environmental pollutants (Zouboulis and Katsoyiannis 2002; Hussain et al. 2007; Durai and Rajasimman 2011; Wan et al. 2014). Our results indicated the excellent capability of 1.0 g L^{-1} sodium alginate treatment to remove toxic pollutants from the investigated effluent (Table 3). The removal capacity may be ascribed to the alginate structure which is rich in carboxyl groups, favoring the biosorption of inorganic impurities and heavy metals and is suggested to be superior to other techniques for wastewater treatment (Tsekova et al. 2010; Molina and Quiroga 2012). Moreover, alginate can form stable biodegradable gels in the presence of divalent cations, such as Ca^{2+}. Hydrogen ions displace calcium ions on the carboxylic acid groups of the adjacent chains, forming a calcium alginate polymeric matrix characterized by excellent pollutant biosorption capability via passive adsorption between metal ions and the binding sites on the molecular structure (Alluri et al. 2007; Singh et al. 2012; Tiwari and Kathane 2013). The present results are in harmony with those obtained by (Shukr 2005; El-Tayieb et al. 2013).

The current experimental outcomes suggest the possibility of using this partially treated effluent as the sole N source for the mass production of microalgae that are capable of high biomass production in nutrient-rich media, like *C.*

sorokiniana and *S. vacuolatus* (Refaay et al., 2021b; Spain et al. 2021). The 1.0 g L^{-1} sodium alginate-treated effluent (ammonia-N 70.22 mg L^{-1}) was found to stimulate the growth of both *C. sorokiniana* and *S. vacuolatus* relative to control. Similar results were also reported by Wang et al. (2010a, 2010b) and Khanh et al. (2013), who observed algal growth at ammonium-N concentrations from 43 to 100 mg L^{-1}. Consequently, the present results (Fig. 2) confirmed the bioremediating potential of *C. sorokiniana* and *S. vacuolatus* on the partially treated effluent, enabling its usage as a commercial nutrient medium supporting growth production.

In this regard, numerous studies have indicated that microalgae possess high capabilities for the bioremoval of inorganic pollutants, particularly nitrogen, phosphorus, and copper, from wastewaters through assimilatory uptake into the cell (Hoffmann 1998; Aslan and Kapdan 2006; Garcia et al. 2006; Park et al. 2010; Posadas et al. 2015).

Moreover, Mandal et al. (2018), Pritchard et al. (2015) and Lachmann et al. (2019) indicated that ammonium is the preferable nitrogen form for microalgae because of the low metabolic cost to reduce it to organic matter, whereas microalgae must consume energy and produce enzymes (i.e., nitrate reductase and nitrite reductase) for nitrate/nitrite reduction. Furthermore, microalgae have apparent differences in their copper removal abilities, which mainly depend on the structure and type of algae cells as well as the quantities of related groups (Zeraatkar et al. 2016). Previous studies have shown that the early absorption of copper could be due to copper adsorption to the outer cell components of microalgae, such as polysaccharides, mucilage, and cell walls. Additionally, the copper absorption process in algae may be due to rapid non-metabolic-dependent adsorption followed by a slow metabolic-dependent uptake process (Kaplan 2013; Anu et al. 2016).

The high tolerance of both *C. sorokiniana* and *S. vacuolatus* to the treated effluent toxicity (Fig. 2B) and their marked growth stimulation at low effluent concentration levels were the pillars upon which these microalgae were selected for the bioremediation process of the partially treated wastewater. In this regard, the inorganic nutrient bioremoval efficiencies of microalgae are determined by various factors, such as the initial concentration of nutrients and the mass ratio of N/P (De-Bashan et al. 2002; Aslan and Kapdan 2006; Zhen-Feng et al. 2011; Kim et al. 2016). Miller and Greene (1978), Li et al. (2011), and Wang et al. (2012) reported that the optimal N:P ratio for freshwater microalgae (i.e., *Chlorella* sp. and *Scenedesmus* sp.) cultivation was 5–10. Therefore, the N:P mass ratio of the treated effluent was adjusted to 9.9 to optimize the growth of *C. sorokiniana* and *S. vacuolatus*.

However, Dominguez-Bocanegra et al. (2004), Richmond (2004), and Borowitzka (2005) documented that the media type affected the growth and composition of microalgae in addition to the mass ratios of different components. The present results revealed an increase in dry biomass without a marked alteration in biomass composition (lipid, protein, and carbohydrate contents) of both *C. sorokiniana* and *S. vacuolatus* when grown in the treated effluent after N:P mass ratio adjustment, which may have contributed to the improvements in supplying phospholipids, genetic materials, and energy for cell division as reported by Wu et al. (2015) and Meza et al. (2015).

In terms of the cost of chemicals used in the treatment protocol of this study, according to the estimates of international prices from different suppliers, the average price of sodium alginate is 2 US$ kg^{-1}, and the price for K_2HPO_4 is 0.12 US$ kg^{-1}. Thus, the cost per 1 m^3 (effluent) is 2.00456 US$ m^{-3}. The mixing could be performed using the infrastructure of the wastewater treatment tanks. This emphasizes the merits of such a treatment strategy, not only as a low-cost alternative but also as a simple technology that can be easily incorporated into current practices to treat industrial effluents for the economic production of microalgae biomass.

Conclusions

It can be concluded that the treatment of ammonia-N rich industrial effluents with powdered sodium alginate polymer (1.0 g L^{-1}) followed by microalgae (*C. sorokiniana* and *S. vacuolatus*) production, using this treated effluent as the sole nitrogen source, succeeded as an effective and simple protocol for bioremediating such industrial effluents and generating considerable microalgae biomass. This bioremediation strategy is a simple and cost-effective approach, and the treated effluent can be considered a commercial medium for microalgae growth.

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Author contributions D.A.R. prepared the manuscript. D.A.R. and D.M.M conducted the experiments. M.H.H, M.I.A, S.A.S. and D.A.R. designed the study. All authors have read and approved the final manuscript.

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Data availability The authors confirm that all the data are available within the article.

Declarations

Conflicts of Interest The authors declare no conflict of interest.

References

Abd el-Lateef EM, Hall JE, Farrag MA, Farrag AA (2011) Agro-economic studies on wastewater reuse in developing marginal areas in West Delta, Egypt. Int J Water Resour Arid Environ 1:110-115

Abdel-Hamid M, EL-Amier Y, Abdel-Aal E, EL-Far G (2017) Water quality assessment of El-Salam Canal (Egypt) based on physico-chemical characteristics in addition to hydrophytes and their epiphytic Algae. Int J Ecol Dev Res 3:28-43

Ali E, Shabaan-Dessouki S, Soliman A, El Shenawy A (2014) Characterization of chemical water quality in the Nile River, Egypt. Int J Pure App Biosci 2:35–53

Alluri HK, Ronda SR, Settalluri VS, Bondili JS, Suryanarayana V, Venkateshwar P (2007) Biosorption: An eco-friendly alternative for heavy metal removal. Afr J Biotechnol 6:2924–2931

Anu PR, Bijoy Nandan S, Jayachandran PR, Don Xavier ND (2016) Toxicity effects of copper on the marine diatom, *Chaetoceros calcitrans*. Reg Stud Mar Sci 8:498–504

Aslan S, Kapdan IK (2006) Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecol Eng 28:64–70

Borowitzka MA (2005) Culturing microalgae in outdoor ponds. In: Anderson RA (ed) Algal Culturing techniques. Elsevier Academic Press, London, pp 205–218

Bradford M (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Das P, AbdulQuadir M, Thaher M, Khan S, Chaudhary AK, Alghasal G, Al-Jabri HMSJ (2018) Microalgal bioremediation of petroleum-derived low salinity and low pH produced water. J Appl Phycol 31:435–444

De-Bashan LE, Moreno M, Hernandez JP, Bashan Y (2002) Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae *Chlorella vulgaris* coimmobilized in alginate beads with the microalgae growth-promoting bacterium *Azospirillum brasilense*. Water Res 36:2941–2948

Dominguez-Bocanegra A, Legarreta IG, Jeronimo FM, Campocosio AT (2004) Influence of environmental and nutritional factors in the production of astaxanthin from *Haematococcus pluvialis*. Bioresour Technol 92:209–214

Durai G, Rajasimman M (2011) Biological treatment of tannery wastewater-a review. J Environ Sci Technol 4:1–17

El-Sheekh MM, El-Shouny WA, Osman ME, El-Gammal EW (2005) Growth and heavy metals removal efficiency of *Nostoc muscorum* and *Anabaena subcylindrica* in sewage and industrial wastewater effluents. Environ Toxicol Pharmacol 19:357–365

El-Tayieb MM, El-Shafei MM, Mahmoud MS (2013) The Role of Alginate as Polymeric Material in Treatment of Tannery Wastewater. Int J Sci Technol 2:218–242

Federation WE, Association A (2005) Standard methods for the examination of water and wastewater. American Public Health Association (APHA), Washington, p 21

Garcia J, Green B, Lundquist T, Mujeriego R, Hernández-Marine M, Oswald W (2006) Long term diurnal variations in contaminant removal in high rate ponds treating urban wastewater. Bioresour Technol 97:1709–1715

Gisi DS, Lofrano G, Grassi M, Notarnicola M (2016) Characteristics and adsorption capacities of low-cost sorbents for wastewater treatment: A review. Sustain Mater Technol 9:10–40

Gombotz WR, Wee SF (2012) Protein release from alginate matrices. Adv Drug Deliv Rev 64:194–205

Hedge J, Hofreiter B, Whistler R (1962) Carbohydrate chemistry. Academic Press, New York

Hoffmann JP (1998) Wastewater treatment with suspended and non-suspended algae. J Phycol 34:757–763

Hua YMX, Pan DD, Ding et al. (2014) Adsorption mechanism of Cr (VI) and phosphate coexisting system by tetraethylenetetramine functionalized nano-polymer materials. Sci Sin Chim 44:1776–1787

Huang J, Kankanamge NR, Chow C, Welsh DT, Li T, Teasdale PR (2018) Removing ammonium from water and wastewater using cost-effective adsorbents: A review. J Environ Sci 63:174–197

Hussain S, Aziz HA, Isa MH, Adlan MN, Asaari FA (2007) Physico-chemical method for ammonia removal from synthetic wastewater using limestone and GAC in batch and column studies. Bioresour Technol 98:874–880

ISO (2005) Water quality – freshwater algal growth inhibition test with unicellular green algae. Geneva, Switzerland

Ji MK, Kim HC, Sapireddy VR, Yun HS, Abou-Shanab RA, Choi J, Lee W, Timmes TC, Jeon BH (2013) Simultaneous nutrient removal and lipid production from pretreated piggery wastewater by *Chlorella vulgaris* YSW-04. Appl Microbiol Biotechnol 97:2701–2710

Kaplan D (2013) Absorption and adsorption of heavy metals by microalgae. In: Richmond A, Hu Q (Eds) Handbook of Microalgal Culture: Applied Phycology and Biotechnology, 2nd ed. John Wiley & Sons Ltd, Oxford, pp 602–611

Khanh N, Kitaya Y, Xiao L, Endo R, Shibuya T (2013) Selection of microalgae suitable for culturing with digestate from methane fermentation. Environ Technol 34:2039–2045

Kim HC, Choi WJ, Chae AN, Park J, Kim HJ, Song KG (2016) Evaluating integrated strategies for robust treatment of high saline piggery wastewater. Water Res 89:222–231

Kondzior P, Butarewicz AE (2018) Effect of heavy metals (Cu and Zn) on the content of photosynthetic pigments in the cells of algae *Chlorella vulgaris*. J Ecol Eng 19:18–28

Kropat J, Gallaher SD, Urzica EI, Nakamoto SS, Strenkert D, Tottey S, Mason AZ, Merchant SS (2015) Copper economy in *Chlamydomonas*: Prioritized allocation and reallocation of copper to respiration vs. photosynthesis. Proc Natl Acad Sci 112:2644–2651

Kumar SK, Dahms HU, Won EJ, Lee JS, Shin KH (2015) Microalgae—A promising tool for heavy metal remediation. Ecotoxicol Environ Saf 113:329–352

Lachmann SC, Mettler-Altmann T, Wacker A, Spijkerman E (2019) Nitrate or ammonium: Influences of nitrogen source on the physiology of a green alga. Ecol Evol 9:1070–1082

Leta S, Assefa F, Dalhammar G (2003) Characterization of tannery wastewater and assessment of downstream pollution profiles along Modjo River in Ethiopia. Ethiop J Biol Sci 2:157–168

Levy JL, Angel BM, Stauber JL, Poon WL, Simpson SL, Cheng SH, Jolley DF (2008) Uptake and internalisation of copper by three marine microalgae: Comparison of copper- sensitive and copper-tolerant species. Aquat Toxicol 89:82–93

Li Y, Chen YF, Chen P, Min M, Zhou W, Martinez B, Zhu J, Ruan R (2011) Characterization of a microalga Chlorella sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresour Technol 102:5138–5144

Li X, Li W, Zhai J, Wei H, Wang Q (2019) Effect of ammonium nitrogen on microalgal growth, biochemical composition and photosynthetic performance in mixotrophic cultivation. Bioresour Technol 273:368–376

Lofano G, Brown J (2010) Wastewater management through the ages: A history of mankind. Sci Total Environ 408:5254–5264

Makhado E, Pandey S, Modibane KD, Kang M, Hato MJ (2020) Sequestration of methylene blue dye using sodium alginate poly(acrylic acid) @ ZnO hydrogel nanocomposite: kinetic, isotherm, and thermodynamic investigations. Int J Biol Macromol 162:60–73

Mandal S, Shurin JB, Efroymson RA, Mathews TJ (2018) Functional divergence in nitrogen uptake rates explains diversity–productivity relationship in microalgal communities. Ecosphere 9:e02228

Markou G, Depraetere O, Muylaert K (2016) Effect of ammonia on the photosynthetic activity of *Arthrospira* and *Chlorella*: A study on chlorophyll fluorescence and electron transport. Algal Res 16:449–457

Mennaa FZ, Arbib Z, Perales JA (2015) Urban wastewater treatment by seven species of microalgae and an algal bloom: biomass production, N and P removal kinetics and harvestability. Water Res 83:42–51

Miller WE, Greene JC (1978) The *Selenastrum capricornutum* algal assay bottle test: Experimental design, application and data interpretion protocol. EPA-600/9–78–018, U. S. Environmental Protection Agency, Corvallis, OR

Mohie el din MO, Moussa AM (2016) Water management in Egypt for facing the future challenges. J Adv Res 7:403-412

Molina ME, Quiroga AJ (2012) Alginates: Production, Types and Applications. Nova Science Publishers, NY

Osibanjo O, Daso AP, Gbadebo AM (2011) The impact of industries on surface water quality of River Ona and River Alaro in Oluyole Industrial Estate, Ibadan, Nigeria. Afr J Biotechnol 10:696–702

Osman R, Ferrari E, Mcdonald S (2016) Water scarcity and irrigation efficiency in Egypt. Water Econ Policy 2:1650009

Park J, Jin HF, Lim BR, Park K, Lee K (2010) Ammonia removal from anaerobic digestion effluent of livestock waste using green alga *Scenedesmus* sp. Bioresour Technol 101:8649–8657

Parmar TK, Rawtani D, Agrawal YK (2016) Bioindicators: the natural indicator of environmental pollution. Front Life Sci 9:110–118

Peltier WH, Weber CI (1985). Methods for measuring the acute toxicity of effluents to freshwater and marine organisms.3rd ed. US EPA, Cincinnati, OH. EPA/600/4–85/013

Posadas E, Delmar- Morales M, Gomez C, Acien FG, Muñoz R (2015) Influence of pH and CO_2 source on the performance of microalgae-based secondary domestic wastewater treatment in outdoors pilot raceways. Chem Eng J 265:239–248

Posadas E, Alcántra C, Garcia-Encina P, Gouveia L, Guieysse B, Norvill Z, Acien F, Markou G, Congestri R, Koreiviene J (2017) Microalgae cultivation in wastewater. In: Gonzalez-Fernandez C, Muñoz R (eds) Microalgae-based biofuels and bioproducts. Woodhead Publishing, London, pp 67–91

Pritchard DW, Hurd CL, Beardall J, Hepburn CD (2015) Restricted use of nitrate and a strong preference for ammonium reflects the nitrogen ecophysiology of a light-limited red alga. J Phycol 51:277–287

Ray GJ, Santhakumaran P, Kookal S (2021) Phytoplankton communities of eutrophic freshwater bodies (Kerala, India) in relation to the physicochemical water quality parameters. Environ Dev Sustain 23:259–290

Refaay DA, Mohammad DM, Shaaban SA, Abdel-Hamid MI, Hussein MH (2021a) Evaluation of the toxicity induced by industrial effluent of El-Delta for fertilizers and chemical industries company. Egypt J Aquat Biol Fish 25:859–874

Refaay DA, El-Marzoki EM, Abdel-Hamid MI, Haroun SA (2021b) Effect of foliar application with *Chlorella vulgaris Tetradesmus dimorphus*, and *Arthrospira platensis* as Biostimulants for common bean. J Appl Phycol 33:3807–3814

Richmond A (2004) Biological principles of mass cultivation. In: Richmond A (ed) Handbook of Microalgal Cultures. Biotechnology and Applied Phycology. Blackwell Science, Oxford, pp 125–177

Ruiz-Marin A, Mendoza-Espinosa LG, Stephenson T (2010) Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresour Technol 101:58–64

Sadasivam S, Manickam A (1996) Biochemical Methods for Agricultural Sciences, New Age International (P) Ltd., New Delhi. pp1–97

Schwarzenbch RP, Egli T, Hofstetter TB, Vongunten U, Wehrli B (2010) Global water pollution and human health. Annu Rev Environ Resour 35:109–136

Seyoum L, Fassil A, Daihammer G (2003) Characterization of tannery wastewater and assessment of downstream pollution profiles along Modjo River in Ethiopia. Ethiop J Bio Sci 2:157–168

Shiwanand A, Tripathi G (2013) A review on ammonia toxicity in fish. Asia Pac J Life Sci 7:193

Shukr BA (2005) Biotechnological studies on alginate productivity of some Egyptian brown algae. M.Sc Thesis, Mansoura University, Egypt 110 p

Singh L, Pavankumar AR, Lakshmanan R, Rajarao GK (2012) Effective removal of Cu^{2+} ions from aqueous medium using alginate as biosorbent. Ecol Eng 38:119–124

Spain O, Plöhn M, Funk C (2021) The cell wall of green microalgae and its role in heavy metal removal. Physiol Plant 173:526–535

Starr RC (1978) The culture collection of algae at the University of Texas at Austin. J Phycol 14:47–100

Stoscheck CM (1990) Quantitation of protein. Meth Enzymol 182:50–68

Tiwari A, Kathane P (2013) Superparamagnetic PVA-alginate microspheres as adsorbent for Cu^{2+} ions removal from aqueous systems. Int Res J Environ Sci 2:44–53

Tsekova K, Todorova D, Ganev S (2010) Removal of heavy metals from industrial wastewater by free and immobilized cells of *Aspergillus niger*. Int Biodeterior Biodegrad 64:447–451

Walsh GE, Dean CH, Mclaughlin LL (1987) Comparison of the EC50s of algal toxicity tests calculated by four methods. Environ Toxicol Chem 6:767–770

Wan Y, Chen X, Xiong G, Guo R, Luo H (2014) Synthesis and characterization of three-dimensional porous graphene oxide/sodium alginate scaffolds with enhanced mechanical properties. Mater Express 4:429–434

Wang L, Li Y, Chen P, Min M, Chen Y, Zhu J, Ruan RR (2010a) Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella* sp. Bioresour Technol 101:2623–2628

Wang L, Min M, Li Y, Chen P, Chen Y, Liu W, Y, Ruan R, (2010b) Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. Appl Biochem Biotechnol 162:1174–1186

Wang H, Xiong H, Hui Z, Zeng X (2012) Mixotrophic cultivation of *Chlorella pyrenoidosa* with diluted primary piggery wastewater to produce lipids. Bioresour Technol 104:215–220

Xu M, Xu S, Bernards M, Hu Z (2016) Evaluation of high density algal cultivation for secondary wastewater polishing. Water Environ Res 88:47–53

Yadav S (2019) Pryagaraj or river city suffering from a water pollution and solution for safe a river to it by plants. Int J Sci Dev Res 4:268–278

Yamagishi T, Yamaguchi H, Suzuki S, Horie Y, Tatarazako N (2017) Cell reproductive patterns in the green alga *Pseudokirchneriella subcapitata* (= *Selenastrum capricornutum*) and their variations

under exposure to the typical toxicants potassium dichromate and 3, 5-DCP. PLoS ONE 12:e0171259
Yong W-K, Sim K-S, Poong S-W, Wei D, Phang S-M, Lim P-E (2021) Interactive effects of warming and copper toxicity on a tropical freshwater green microalga *Chloromonas augustae* (Chlorophyceae). J Appl Phycol 33:67–77
Zeraatkar AK, Ahmadzadeh H, Talebi AF, Moheimani NR, McHenry MP (2016) Potential use of algae for heavy metal bioremediation, a critical review. J Environ Manage 181:817–831
Zhen-Feng S, Xin L, Hong-Ying H, Yin-Hu W, Tsutomu N (2011) Culture of *Scenedesmus* sp. LX1 in the modified effluent of a wastewater treatment plant of an electric factory by photo-membrane bioreactor. Bioresour Technol 102:7627–7632
Zouboulis AI, Katsoyiannis IA (2002) Arsenic removal using iron oxide loaded alginate beads. Ind Eng Chem Res 41:6149–6155