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 EC₅₀ > 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 ± 0.04% and 52.19 ± 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 (Osi-banjo 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...
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 (EC₅₀) 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. EC₅₀ 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, total alkalinity, 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 (K₂HPO₄·3H₂O) 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), nitrate-N (2.93 mg L⁻¹), nitrite-N (1.95 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 CaCO₃ 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 high copper concentration (0.149 mg L⁻¹) and low...
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](image)

**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 (EC50) 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                          | EC50 of the raw effluent | EC50 of 1.0 g sod. alginate treated effluent | EC50 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 ≤ 0.05

| Parameter      | Unit       | Raw sample                        | Sodium alginate treatments (g L−1) | Sodium alginate treatments (g L−1) | Sodium alginate treatments (g L−1) | Sodium alginate treatments (g L−1) |
|----------------|------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
|                |            |                                   | 0.25                              | 0.5                                | 1.0                               | LSD 0.05                          |
| pH             | Unit       | 9.79 ± 0.01a                      | 9.05 ± 0.02b (7.6%)               | 8.86 ± 0.03c (9.4%)               | 8.01 ± 0.02d (18.2%)              | 0.042                             |
| Ammonium-N     | mg L−1     | 185.76 ± 0.14a                    | 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 ± 0.006 c (5.5%)            | 0.26 ± 0.003 d (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            |            | 232.5a                            | 160.49 b                          | 137.28 c                          | 109.07 d                         | 7.95                              |
| Total alkalinity | mg CaCO3 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                             |

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 CaCO3 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 ≤ 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 ≤ 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
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 EC₅₀ of 53.6% and 83.7%, respectively. *P. subcapitata* growth was slightly increased with an EC₅₀ value 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 EC₅₀ was typically > 100% for both test algae, whereas the *P. subcapitata* EC₅₀ 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).
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 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\(_2\)HPO\(_4\) 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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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.

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