Algal Research 74 (2023) 103184

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Increased production of extracellular polysaccharides in *Arthrospira platensis* as a protective response against saxitoxin: Implications to outdoor mass production

Zakaria A. Mohamed [a,*], Rehab O. Elnour [b], Saad Alamri [c], Mohamed Hashem [c]

[a] Department of Botany and Microbiology, Faculty of Science, Sohag University, Sohag 82524, Egypt
[b] Biology Department, Faculty of Sciences and Arts, Dahran Al-Janoub, King Khalid University, Abha, Saudi Arabia
[c] King Khalid University, College of Science, Department of Biology, Abha 61413, Saudi Arabia

ARTICLE INFO

Keywords:
Arthrospira platensis
Cyanotoxins
Adsorption
Outdoor ponds
Mass production

ABSTRACT

Cyanobacteria of both toxic and nontoxic species frequently coexist and thrive in the same habitat. However, little is known about how nontoxic species can mitigate the negative impacts of the toxins produced by toxic species. The current study examined the potential adaptive response of *Arthrospira platensis* to saxitoxin (STX) in batch cultures. This cyanobacterium was exposed to various STX concentrations for seven days, and growth and antioxidative biomarkers were observed. During the first two days of incubation, the growth (i.e., cell density and chlorophyll-a) dramatically decreased in STX-treated cultures compared to control. Glutathione-S-transferase (GST), superoxide dismutase (SOD), and lipid peroxidation levels increased along with this decline in growth in STX-treated cultures. These parameters were restored to control values on day 3 of incubation and remained at levels that were relatively close to those of control cultures during the remaining period of experiment. The changes in these parameters were associated with the amount of extracellular polysaccharides (EPS) released into the media of STX-treated cultures, demonstrating the role of this EPS in protecting *A. platensis* cells from this toxin. The results of isotherm experiments confirmed the ability of the released EPS to adsorb STX onto their surfaces, with adsorption capacity ($k = 2.9$) comparable with that of activated carbon ($k = 3.6$). This suggests that *A. platensis* used EPS to build a defense mechanism to limit STX uptake into its cells. This EPS can give *A. platensis* the advantage of growing in natural lake water in outdoor systems for mass production without experiencing any negative effects from STX. Additionally, EPS could be used in drinking water and wastewater treatment facilities as an ecofriendly adsorbent for saxitoxin and other positively charged cyanotoxins.

1. Introduction

Arthrospira platensis (formerly *Spirulina platensis*) is filamentous cyanobacterium, which grows enormously under strong sunlight, high temperature and highly alkaline conditions [1]. *A. platensis* attracts the attention of researchers and food regulatory authorities such as European Food Safety Agency (EFSA) and the Food and Drug Administration (FDA) [2], due to its nutritional and medicinal features [3]. More specifically, *Arthrospira/Spirulina* biomass is used as a nutritional supplement for animals and humans, as it is rich in protein, carbohydrates, minerals, essential fatty acids, and vitamins [2]. Because of its high component content of phycocyanin pigment and polysaccharides, *Arthrospira* biomass is utilized as functional food to fight or prevent cancer [4]. *Arthrospira* biomass has been produced in large commercial scale [1] as well as in small ponds and pots for local consumption [5]. However, high costs of chemical ingredients of culturing medium (e.g., Zarrouk medium) make it unsuitable effective for economic effective mass production [6]. This has necessitated the search for lower cost nutrient sources for *Arthrospira* biomass production such as lake water and agro-industrial wastes [7]. Nevertheless, one of the major challenges facing the use of unprocessed natural or wastewater for the cultivation of *A. platensis* is the presence of xenobiotics (e.g., cyanotoxins) in these waters, which may have negative effects on *A. platensis* growth or contaminates the commercial biomass product.

Several studies have reported that microalgae and cyanobacteria including *A. platensis* can develop its own protective mechanisms to

* Corresponding author at: Department of Botany, Faculty of Science, Sohag University, Sohag 82524, Egypt.
E-mail address: zakaria.attia@science.sohag.edu.eg (Z.A. Mohamed).

https://doi.org/10.1016/j.algal.2023.103184
Received 6 February 2023; Received in revised form 24 June 2023; Accepted 24 June 2023
Available online 1 July 2023

avoid the toxicity of such hazardous xenobiotics [8,9]. Among these protective substances, the negatively charged extracellular polysaccharides (EPS) were found to be released by cyanobacteria to bind positively charged heavy metals, preventing their uptake and toxicity to the cells [10,11].

While growing *A. platensis* in lake water in the laboratory, our research team observed that a culture of *A. platensis* showed slimy texture of culture medium at the bottom of the culture vessel. This slimy substance was preliminarily identified as extracellular polysaccharides. The ELISA test revealed the presence of saxitoxin in medium of *A. platensis* culture. We, therefore, hypothesized that *A. platensis* cells released EPS into the medium as an adaptive mechanism against the toxicity of saxitoxin. This hypothesis was tested by studying the effect of different concentrations of saxitoxin on the growth and antioxidant system of *A. platensis*. The study also evaluated the mechanism by which the released EPS could protect *A. platensis* against saxitoxin.

2. Materials and methods

2.1. Experimental organism

Arthrospira platensis- C1G was obtained from the Culture Collection of LEGE at *CIIMAR* (Interdisciplinary Centre of Marine and Environmental Research (Porto, Portugal). The cyanobacterial strain was grown axenically in Zarrouk liquid medium adjusted at pH 9 [12]. The cultures were aerated with sterile (0.22 μm) bubbling air and CO_2 (1 %) at a flow-rate of 25 mL min^{-1} and incubated at temperature 30 °C with an illumination (white light) of 100 μmol photons m^{-2} s^{-1} produced by neon tubes in 14:10 h light/dark cycle. At the late exponential growth phase (10 days), the cultures were centrifuged (6000 xg,15 min, 4 °C) and the pellets were washed twice with sterilized medium and resuspended in fresh medium for use in the saxitoxin treatment experiments.

2.2. Exposure of A. platensis to saxitoxin

To investigate the potential toxicity of saxitoxin on *A. platensis*, the exponentially growing cells were inoculated into 500 ml flasks containing 200 mL Zarrouk liquid medium (pH 9) to give an initial optical density 0.078 (at 560 nm) corresponding to biomass concentration of 8.21 mg L dry weight. Thereafter, different initial concentrations of saxitoxin (0.5, 1, 5, 10, 20, and 50 μg L^{-1}) were added to cyanobacterial cultures. These concentrations were selected based on the range (1–26 μg L^{-1}) of environmentally relevant concentrations of extracellular STX [13,14]. Cyanobacterial cultures without STX were used as positive controls. Culture medium containing STX but without cyanobacterial cells served as negative control. Both control and treated cultures were incubated under the same growth conditions outlined above for 7 days. Both treatment group and control were made in triplicate. At regular intervals, an aliquot (10 mL) was aseptically withdrawn from each culture suspension for growth measurements and determinations of lipid peroxidation, antioxidant enzyme activities and extracellular polysaccharide concentrations. At each sampling time, the cultures were examined for the bacterial contamination under epifluorescence microscope after staining with 4′,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich Co., U.S.A.) [15].

2.3. Measurement of A. platensis growth

A. platensis growth was monitored daily in both control and STX-treated cultures by measuring the optical density at 560 nm in 2 mL of agal suspension [16]. Biomass values were determined using OD_{560} results as described by Xue et al. [17] and Önem et al. [18]. At the same time, chlorophyll- a (Chl-a) content was estimated by methanol extraction of cyanobacterial cells (2 mL of agal suspension) filtered on Whatman GF/C glass fiber filter. The absorbance of the centrifuged supernatant (6000 ×*g*, 10 min) was measure spectrophotometri-cally, and Chl-a content was calculated according to the equation described in Porra [19] and expressed as mg g^{-1} dry biomass.

2.4. Determination of antioxidant enzyme activity and lipid peroxidation

A. platensis cells were harvested from control and treated cultures by centrifugation (6000 xg, 10 min, 4 °C) and the cell pellets were washed with sterilized distilled water and centrifuged again. The pellets were then ground with ice-cold sodium phosphate buffer (0.1 M, pH 7) in a porcelain mortar. The extracts were centrifuged (6000 xg, 10 min, 4 °C) and the supernatants were kept at −20 °C until use. Concentration of total protein was determined in the extracts based on the method of Bradford [20].

Superoxide dismutase (SOD) activity was evaluated by monitoring the inhibition of nitro blue tetrazolium (NBT) according to the method of Beyer and Fridovich [21]. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 2 μM riboflavin, 0.1 mM EDTA and 1 ml of the enzyme extract. The reaction mixture was exposed to light (80 μmol m^{-2} s^{-1}) for 20 min. At the same time, two portions of reaction mixture without enzyme extract were prepared; one of them was kept in light and used as reference for calculating absorbance for one unit SOD, while another part was kept in darkness and used as a blank. The reaction was quenched by switching off the light and absorbance was read at 560 nm. The amount of enzyme that caused a 50 % decrease in NTB reduction is referred as one unit of SOD activity (expressed as unit mg^{-1} protein).

Glutathione-*S*-transferase (GST) Enzyme activity was determined according to Habig and Jakoby [22] by measuring the decrease in absorbance at 340 nm due to conjugation of reduced glutathione (GSH) to 1-chloro-2,4-dinitrobenzene (CDNB). The enzyme amount that transforms 1 mM of CDNB per min was referred as one GST unit using a molar extinction coefficient of 9.6 mM^{-1} cm^{-1}.

Lipid peroxidation was assayed by determining malondialdehyde (MDA) concentrations according to the method described by Heath and Packer [23]. 2 mL of reaction solution (thiobarbituric acid (0.5 %) in 10 % trichloroacetic acid) were mixed with 2 mL phosphate buffer cyanobacterial extract, and the mixture was heated in boiling bath for 15 min. After cooling, the mixture was centrifuged (6000 xg, 10 min) and the absorbance of the supernatant was measured spectrophotometerically at 532 and 600 nm. The value of absorbance at 600 nm (non-specific was subtracted from the reading recorded at 532 nm. The MDA concentration was then calculated using its extinction coefficient of 157 mM^{-1} cm^{-1}.

2.5. Determination of released exopolysaccharides

Extracellular polysaccharides (EPS, also known as released polysaccharides), which are secreted into the extracellular matrix or loosely associated with the cell surface and frequently form a slime layer (biofilm), were the focus of our study rather than capsular polysaccharides (CPS), which are tightly and covalently linked to the cell surface and create a capsule around the cell [24,25]. Extracellular polysaccharides released from *A. platensis* cells into the culture medium under saxitoxin stress was recovered with the method described by Parikh and Madamwar [26]. Briefly, an aliquot of the culture medium was centrifuged (6000 xg, 20 min) and the resulting supernatant was filtered through a Whatman No.2 filter paper. EPS in the filtered supernatant was precipitated with 3 volumes of 95 % (*v*/v) ice-cooled ethanol for 12 h at 4 °C.The precipitated EPS was recovered by centrifugation (10,000 xg,15 min) and the supernatant was discarded. Total carbohydrates of EPS were determined as reduced sugars analyzed by the phenol– sulfuric acid method [27]. The sulfate content of EPS was determined after hydrolysis in N-hydrochloric acid 1 M at 105 °C for 5 h by the $BaCl_2$ gelatin method described by Mousavian et al. [28]. The hydrolyzed EPS was incubated with the reagents (3 % trichloroacetic acid and barium chloride-gelatin) for 20 min at room temperature. The intensity of the

color generated was measured spectrophotometrically at 360 nm. The amount of sulfate was calculated from calibration curve using K_2SO_4 as a standard. The uronic acid content of EPS was determined according to the method of Mojica et al. [29]. Briefly, 1.2 ml of sodium tetraborate solution (0.0125 M) in concentrated sulfuric acid and 20 μL of 4 M sulfamic acid were added to 200 μl of precipitated EPS and the mixture was boiled for 5 min in water bath. The solution was ice-cooled for 3 min and centrifuged (5000 xg, 5 min). Then, 20 μl hydroxyphenol solution (0.15 % *v*/v in 0.5 % NaOH) was added to the supernatant and the absorbance of color generated after 4 min. was read at 520 nm. The uronic acid concentration is calculated using a calibration curve prepared from various concentrations (0–5 μg μl^{-1}) of D-glucuronic acid as a standard.

2.6. Detection of saxitoxin uptake into A. platensis cells

To test the STX uptake and accumulation in *A. platensis cells,* the collected biomass of toxin-treated cells was washed with distilled water and centrifuged (5000 xg, 10 min) to remove both EPS released into the medium as well as those loosely attached to cell surfaces, and STX adsorbed to them. The pellets were resuspended in 0.5 M acetic acid and lysed bead beating [30]. The mixture was then centrifuged (5000 xg, 10 min) to remove cell debris, and the supernatant was diluted with ELISA buffer and used directly in ELISA test. ELISA assay was carried out using an Abraxis Saxitoxin ELISA kit (Abraxis LLC, United States) according to the manufacturer's instructions. The detection limit was 0.015 μg L^{-1}.

2.7. Testing the capability of polysaccharides for STX adsorption

To test the capability of extracellular polysaccharides released from *Arthrospira* cells, isotherm experiments were conducted in laboratory using the final EPS recovered from culture medium (in Section 2.5). The experiments were carried out in 100 ml-glass bottles, each containing 50 ml Zarrouk's medium (pH 9) and supplemented with different concentrations of STX (0.1,1,10,50 and100 mg mL^{-1}) and EPS at a concentration of 300 mg L^{-1}. Two controls were used; one contained STX solution without EPS, and another contained EPS solution with no STX. The bottles were incubated in a thermostatically controlled shaker (200 rpm) at 30 °C for 12 h.

The Freundlich isotherm equation was used to fit the adsorption isotherm data.

$$qe = KCe^{1/n}$$

where qe = STX concentration adsorbed onto EPS (μg g^{-1}), Ce = STX concentration remaining in bulk solution (μg L^{-1}) at equilibrium, K is the adsorption capacity of EPS for STX, and n is the affinity of EPS to adsorb STX. The data were plotted on logarithmic scale and the regression isotherm coefficients were calculated.

To evaluate the firmness of STX adsorption onto EPS (i.e., reversible or irreversible binding?), the pellets of EPS with adsorbed STX collected from adsorption experiment were suspended in Zarrouk's medium (pH 9) and shaken at 30 °C for 1 h. The solution was centrifuged (6000 ×*g*,15 min), and the amount of STX potentially released back into the washing solution was determined in the supernatant by ELISA.

2.8. Statistical analysis

Statistical variance analysis of the data with three replicates between the control and treated samples were analyzed by One-Way ANOVA followed by a multiple comparison using Tukey-test ($P < 0.05$). Correlations between STX concentrations in *A. platensis* cells and EPS concentrations released into the medium were measured using Spearman rank correlation coefficients using the Excel data analysis tool.

3. Results

3.1. Effect of saxitoxin on A. platensis growth

As shown in Figs. 1 and 2, the addition of different concentrations of STX exhibited an inhibitory effect on the growth of *A. platensis.* The cell density and dry weight of STX-treated cells of *A. platensis* decreased sharply during the first 2 days of incubation period compared to control ($P < 0.05$). On the third day, these growth parameters of treated cultures increased to levels with no significant difference with that of control culture of this cyanobacterium (Figs. 1 and 2).

Similarly, chlorophyll-a content of STX-treated cells of *A. platensis* showed significant decrease ($P < 0.05$) compared to control during the first 2 days of the experiment (Fig. 3). Thereafter, this pigment increased to values close to that of control ($P > 0.05$).

3.2. Saxitoxin uptake into A. platensis cells

The analysis of STX in *A. platensis* cells revealed the presence of this toxin in the cells at varying concentrations increasing with the initial STX concentrations during the first two days of incubation (Table 1), indicating the uptake of this toxins into *A. platensis* cells. After that, the SXT content of the cells decreased gradually until vanished by the day 5 of incubation. This decrease in STX content of the cells correlated ($r = -0.8$) with the quantity of EPS released from the cells into the culture medium (Table 1 and Fig. 4), indicating that EPS have a potential role in preventing STX diffusion into *A. platensis* cells.

3.3. Effect of saxitoxin on extracellular polysaccharides

The results shown in Fig. 3 revealed an increase in EPS released into the medium of STX-treated cultures of *A. platensis* compared to control culture. The amount of these EPS varied significantly ($P < 0.05$) with increasing STX concentration in the medium (Fig. 4).

Higher concentrations of STX (10-50μgL^{-1}) promoted the release of greater polysaccharides into the culture medium in comparison to lower toxin concentrations (0.5-5 μg L^{-1}). Additionally, EPS released into the medium of STX-treated cultures also increased along the incubation period. The chemical analysis showed that these EPS contained high amounts of sulfate (24.3 %) and uronic acid (15.1 %) (Table 2).

3.4. Effect of saxitoxin on lipid peroxidation and antioxidant enzymes

MDA content (as indicator of lipid peroxidation) of STX-treated cells of *A. platensis* increased significantly during the first 2 days of the incubation period compared to control (Fig. 5). The lipid peroxidation in this cyanobacterium was STX concentration-dependent, where the highest MDA content was obtained at the highest STX concentration used in our experiments (50 μg L^{-1}) and the lowest was recorded at the lowest STX concentration (0.5 μg L^{-1}). On the third day of the experiment, MDA concentrations showed a significant decrease in STX-treated cultures of *A. platensis* to levels close to that of control cultures (Fig. 5) and remained at these low levels all over the incubation period. Interestingly, the decrease in MDA contents corelated with the increase of EPS concentration released into the medium ($r = -0.8$). Similar to lipid peroxidation, the activity of the antioxidant enzymes, GST and SOD of *A. platensis* increased in a response to STX, and that increase was toxin concentration-dependent during the first 2 days of experiment (Figs. 6, 7). On the third day and over the course of the experiment, the activities of these enzymes decreased and were restored to that of control culture ($P > 0.05$).

The decrease in GST and SOD activities occurred after 2 days of incubation and associated remarkably ($r = -0.9$) with EPS concentrations released into the medium of STX-treated cultures of *A. platensis.*

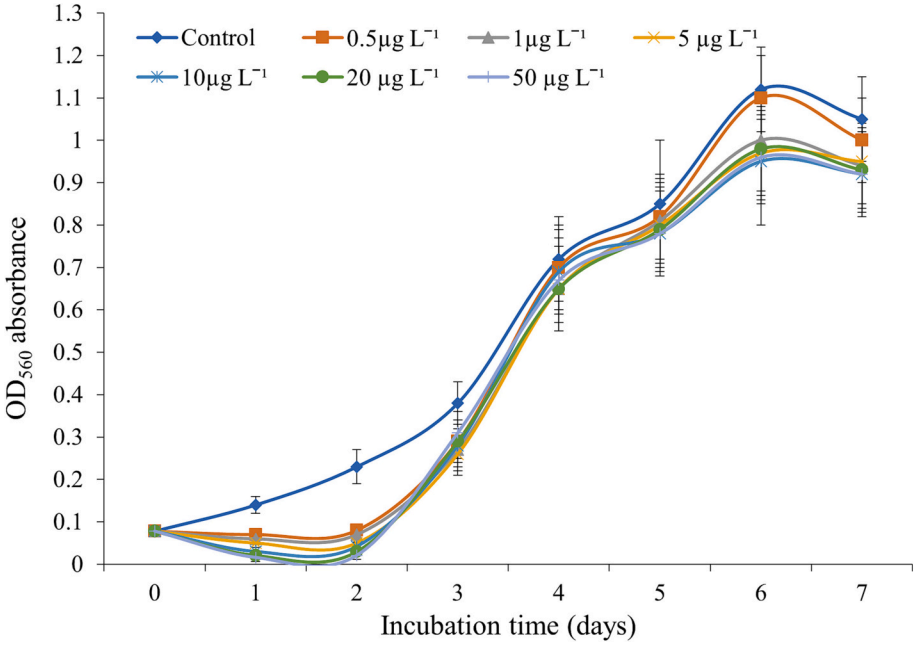

Fig. 1. Changes in biomass (as OD_{560} absorbance) of *A. platensis* exposed to different concentrations of saxitoxin. Data are the means ±SD of three replicates.

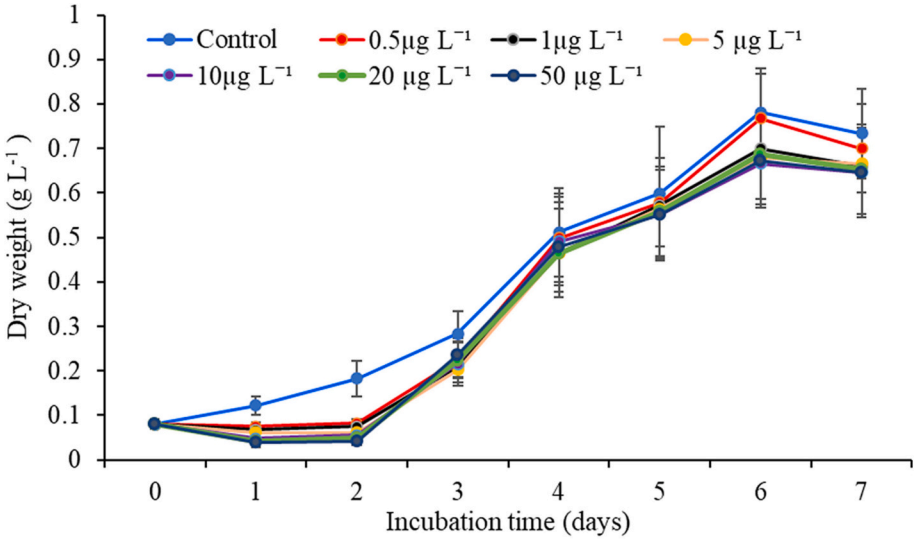

Fig. 2. Changes in biomass (as dry weight, g L^{-1}) of *A. platensis* exposed to different concentrations of saxitoxin. Data are the means ±SD of three replicates.

3.5. Adsorption of STX by Arthrospira EPS

The results of adsorption isotherm experiment revealed a significant decrease ($P < 0.05$) in STX concentrations in the solution containing EPS compared to control solution not-containing EPS, indicating the ability of these polysaccharides to bind STX onto their surfaces (Fig. 8). This adsorption efficiency varied significantly (P<0.05) with the initial STX concentrations (Fig. 7). The highest adsorption percentage of STX by EPS (99.9 %) was obtained at initial STX concentration of 1 µg L^{-1}, while the lowest (70 %) was obtained at initial STX concentration of 100 µg L^{-1} (Fig. 8).

The results also showed that sorption isotherm of STX by EPS was well fitted to the Freundlich model (Fig. 9). These EPS had higher adsorption capacity (i.e., high k value) and intensity (i.e., low 1/n value) for this toxin (Table 3).

Additionally, the toxin release assay demonstrated the firmness of STX to EPS, where no STX was detected in the washing solution of EPS-STX complex (Data are not shown).

4. Discussion

Both toxic and nontoxic species of cyanobacteria commonly coexist and flourish in the same habitat [31,32]. However, the nontoxic species seem to develop some strategies to confront the toxic effects of toxins produced by toxic species. The results of present study showed that *A. platenis* responded to the presence of STX by inducing the EPS production and its releasing into the medium to hinder the toxin uptake into the cells.

During the first two days of incubation with STX, and cell density and chl-a content of *A. paltensis* were affected and significantly decreased,

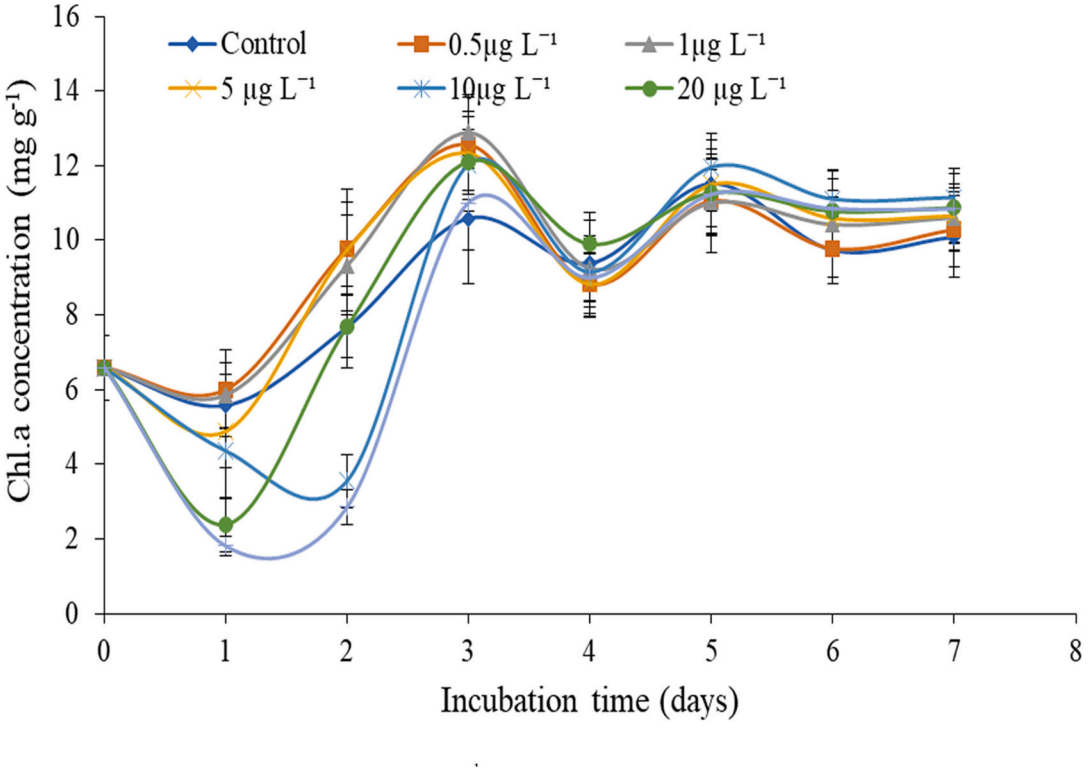

Fig. 3. Changes in chlorophyll-a content (mg g^{-1} dry biomass) of *A. platensis* cells exposed to different concentrations of saxitoxin. Data are the means ±SD of three replicates.

Table 1
Changes in SXT concentrations (μg g^{-1}) in *A. platensis* cells exposed to different concentrations of STX for 7 days.

Incubation time (days)	STX concentrations in the cells (μg g^{-1})					
	Initial STX concentrations (μg L^{-1})					
	0.5	1	5	10	20	50
0	ND	ND	ND	ND	ND	ND
1	0.55 ± 0.1	1.3 ± 0.2	5.36 ± 0.4	9.34 ± 1.1	24.2 ± 3.2	56.4 ± 4.5
2	0.63 ± 0.15	1.5 ± 0.3	6.73 ± 0.8	10.9 ± 2.2	26.7 ± 3.9	67.8 ± 5.6
3	0.23 ± 0.07	0.9 ± 0.1	3.7 ± 0.5	5.6 ± 0.8	14.6 ± 2.1	32.5 ± 4.7
4	0.11 ± 0.02	0.24 ± 0.06	1.13 ± 0.2	2.11 ± 0.06	4.15 ± 0.7	8.2 ± 1.3
5	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND	ND

ND = STX is nondebatable at the detection limit of ELISA.

but they increased again after the third day and were restored to those of control cultures. The decrease in cell density and chl-a content of *A. paltensis* during the first two days of incubation may be due to the free radicals generated in the cells by STX, which they are well known to reduce the cell proliferation [33] and pigment degradation [34]. Our results supported this suggestion as the decrease in the cell density and chl-a content was concomitant with the increase in the parameters of antioxidant system (e.g., lipid peroxidation, GST, SOD) in STX-treated cells of *A. platensis*.

This reflects that exposure to STX caused *A. platensis* cells to experience oxidative stress.

However, in our study, an increase in the cell density and chl-a content accompanying with a decrease in MDA content and antioxidant enzyme activities, occurred after 3 days of incubation in STX-treated cultures and restored to the values of control cultures. Such negative effects of STX on growth parameters and antioxidative system of *A. platensis* indicate the uptake of this toxin into the cells. Such negative effects of STX on growth parameters and antioxidative system of *A. platensis* indicate the uptake of this toxin into the cells. As STX is hydrophilic and has small molecular mass (299 Da) [35], it can cross the outer membrane of Gram-negative bacteria) e.g., Cyanobacteria) by passive diffusion through pores composed of 'porin' proteins contained in this membrane [36]. When STX enters the cyanobacterial cell, it can act as an inducer activating the expression of eps genes, encoding the glycosyltransferase enzyme involved in EPS biosynthesis, leading to an increase in EPS production. This is similar to other environmental stresses that induce EPS synthesis genes in bacteria [37].

The results of present study also revealed a strong correlation between the reduction in ROS production and the increase in EPS released into the medium of toxin-treated cultures. This indicates the involvement of these EPS in trapping ROS produced under STX stress. However, the rise in EPS amount released into the medium of STX-treated cultures was correlated with the steady decline in STX concentrations in *A. platensis* cells until they became undetectable at day 5 of incubation. This demonstrates that EPS restricted the STX uptake into *A. platensis* cells, ameliorating the stress caused by STX on the cells. These findings therefore support earlier research showing that EPS has the ability to bind toxic substances and prevent their diffusion into the cells [9,11,38].

In the present study, the results of Freundlich adsorption isotherm parameters (i.e., adsorption capacity (k) and the affinity of the adsorbent,1/n) are highly correlated, indicating that STX adsorption on EPS is favorable. The isotherm parameter values (k = 2.89, and$1/n$ = 0.76) obtained in our study for adsorption of STX onto EPS, can be compared with those (3.6, 0.39, respectively) reported for STX adsorption by activated carbon [39].

Additionally, the removal efficiency of STX at initial concentration of 100 μg L^{-1} by EPS in our study (70 %) was higher than that achieved by powdered activated carbon (57 %) at initial STX concentration of 1.6 μg

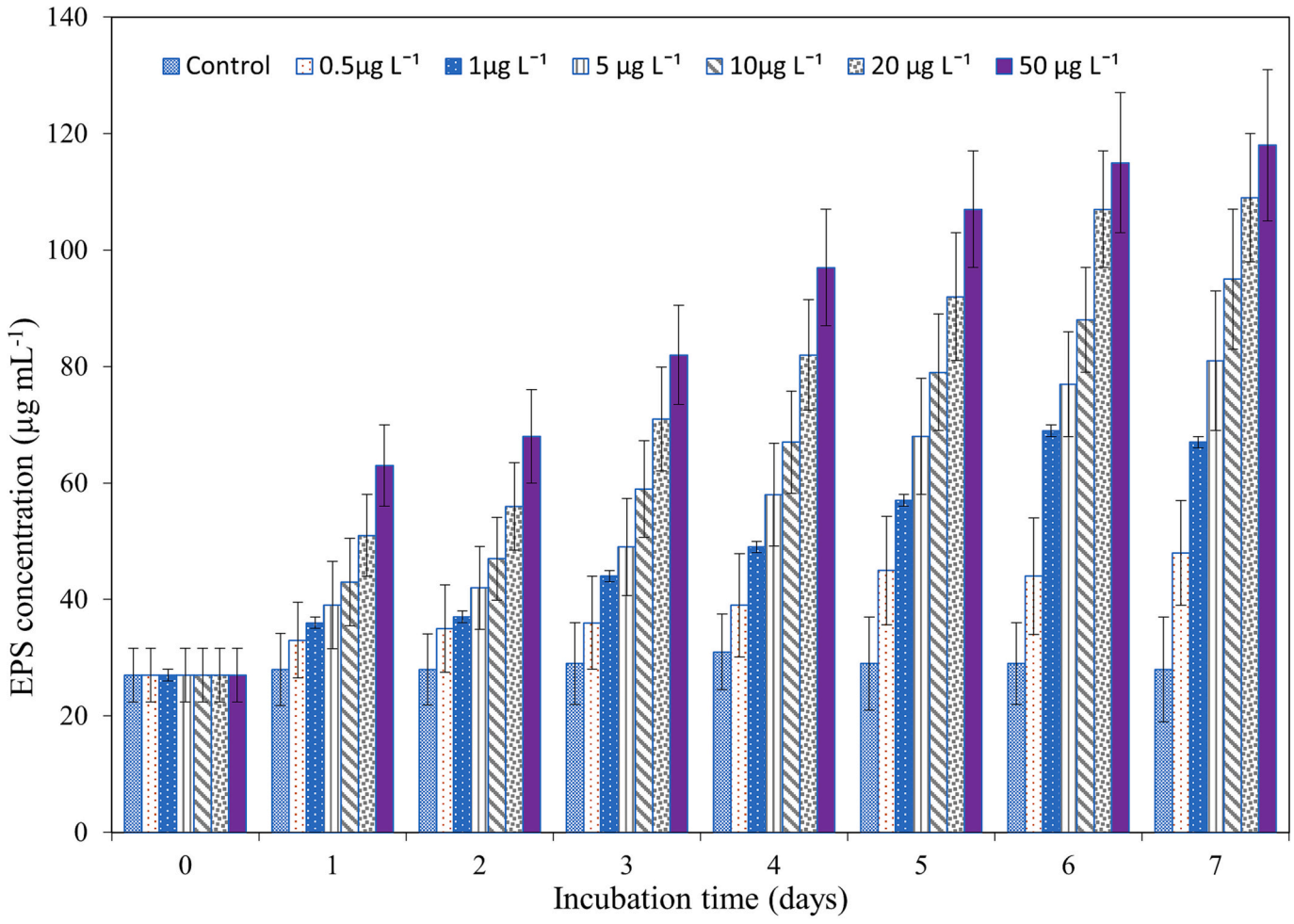

Fig. 4. Changes in concentrations of extracellular polysaccharides (EPS, µg mL^{-1}) released into the medium of *A. platensis* cultures exposed to different concentrations of saxitoxin. Data are the means ±SD of three replicates.

Table 2
Uronic acid, sulfate, carbohydrate and protein contents of EPS released into the medium of *A. platensis* cultures under STX stress conditions.

	Carbohydrate	Protein	Uronic acid	Sulfate
%/EPS	53.2 ± 2.3	7.4 ± 0.8	24.3 ± 1.2	15.1 ± 0.7

L^{-1} [40]. The 70 % removal of STX by EPS indicates that each one gram of EPS could remove 233 µg STX from water, compared to removal efficiency of 250 µg STX achieved by one gram of powdered activated carbon [39].

The adsorption of STX by EPS may be due to the electrostatic interaction of negatively charged groups (sulfate and uronic acid) present on the surface of EPS with the positively charged amine groups of STX [39]. Our results support this suggestion, as EPS released in STX-treated cultures contained high amounts of sulfate and uronic acid (Table 2). Other studies also reported that uronic acids and/or sulfate esters confer a negative charge to polysaccharides, providing them distinctive feature for binding cations particularly heavy metals [41]. Recently, Olan et al. [42] also demonstrated the sorption of STX onto the polysaccharide carrageenan with high sulfate content. In comparison, the maximum STX adsorption capacity of our EPS released by *A. platensis* (125.7 µg g^{-1}) is much higher than the STX adsorption capacity (1.3 µg g^{-1}) of carrageenan [42]. Moreover, the binding of STX to EPS was very stable, as none of STX adsorbed onto EPS released into the washing solution. This trait opens the possibility of using these polysaccharides as chelating agents for bioremediation purposes and removing saxitoxin and other positively charged cyanotoxins in drinking water treatment plants.

5. Conclusions

The present study provided evidence that *A. platensis* growing in STX-treated cultures synthesized and released higher amounts of EPS than in control cultures to avoid STX toxicity.

Interestingly, this cyanobacterium survived the toxin stress simultaneously with the release of EPS into the medium and recovered its growth and antioxidative parameters to control levels, with no STX identified in the cells. This indicates that *A. platensis* employed EPS to create a defense mechanism to restrict STX uptake into its cells. The k and n values obtained from our isotherm experiments confirmed the ability of EPS released by *A. platensis* to adsorb STX onto their surfaces, with adsorption capacity comparable with that of activated carbon. Owing to such binding ability and their biodegradability and nontoxicity [43,44], these EPS could be recommended as ecofriendly adsorbent for saxitoxin and other positively charged cyanotoxins in drinking and wastewater water treatment plants. However, further studies should be undertaken to investigate the capability of these EPS for removing cyanotoxins under in situ conditions (i.e., water treatment plants). Moreover, the induction of EPS by *A. platensis* in the presence of STX could increase the safety of growing this cyanobacterium for mass

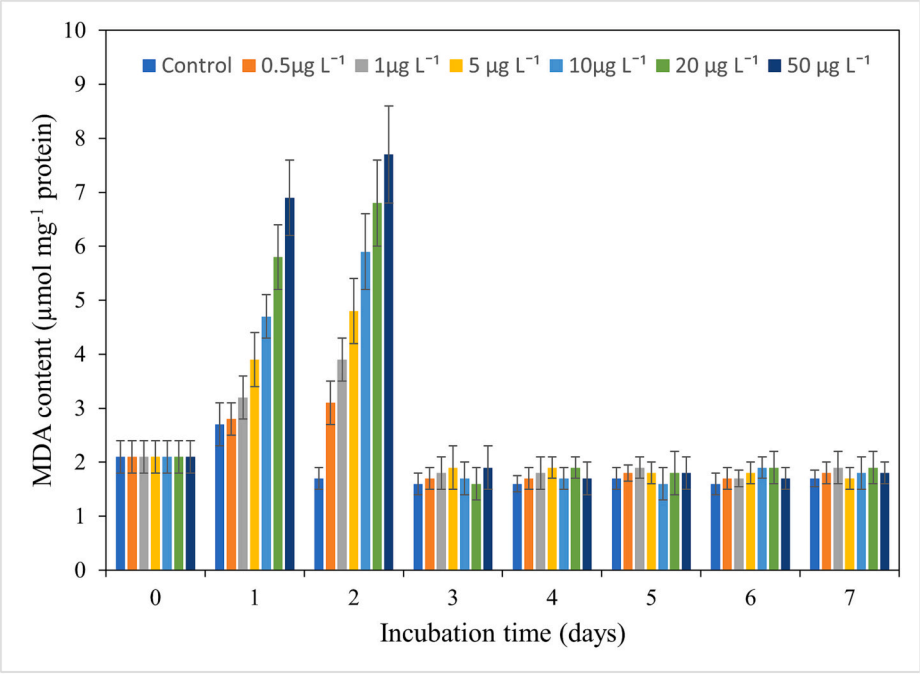

Fig. 5. Changes in Malondialdehyde content (MDA, µmol mg^{-1} protein) of *A. platensis* cells exposed to different concentrations of saxitoxin. Data are the means ±SD of three replicates.

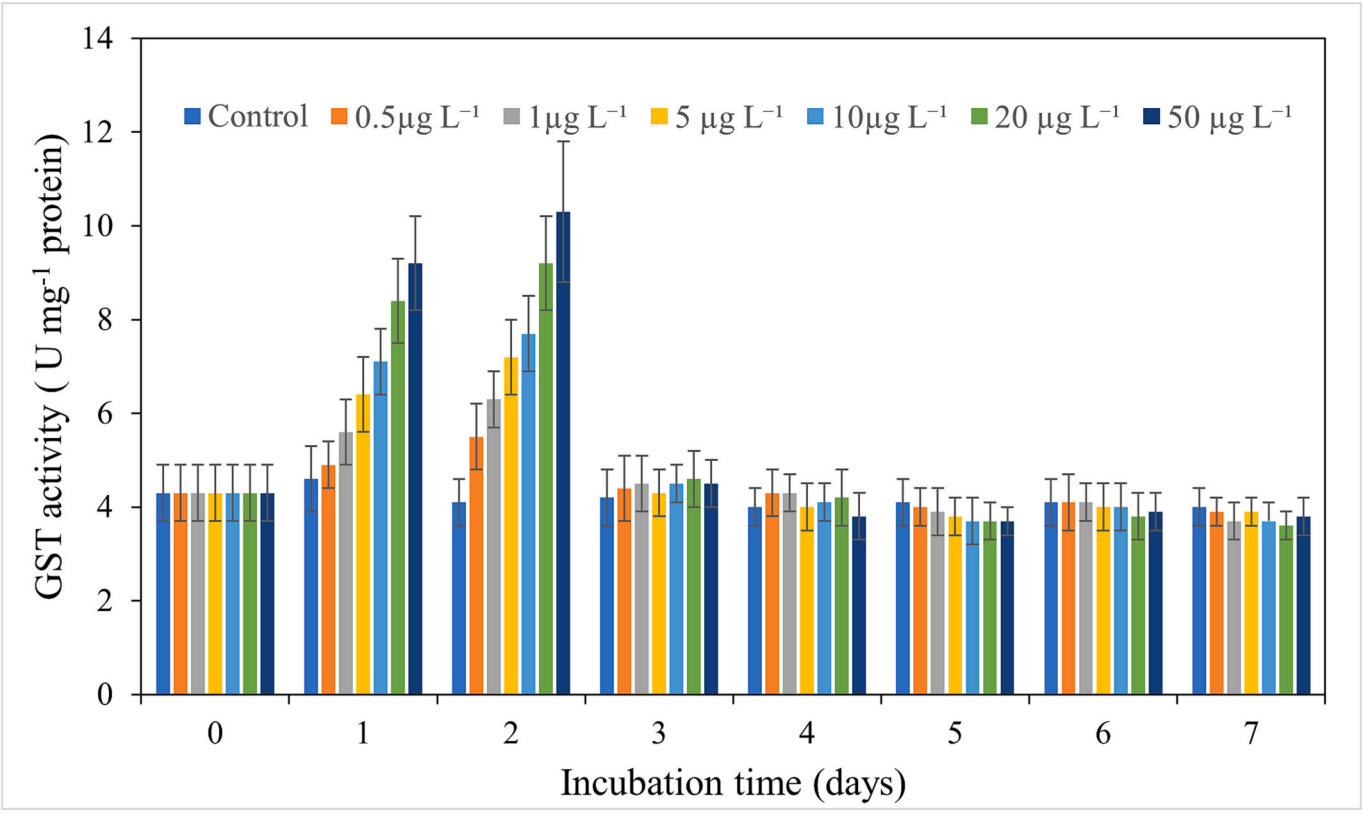

Fig. 6. Changes in GST activity (U mg^{-1} protein) of *A. platensis* cells exposed to different concentrations of saxitoxin. Data are the means ±SD of three replicates.

production in outdoor lakes and ponds possibly containing toxic cyanobacteria. Being secreted extracellularly and loosely attached to cell surfaces, these EPS with adsorbed STX can be easily extracted and separated from the cells to yield harvested biomass free of cyanotoxins. *A. platensis* can also be utilized as a source of EPS for biotechnological uses.

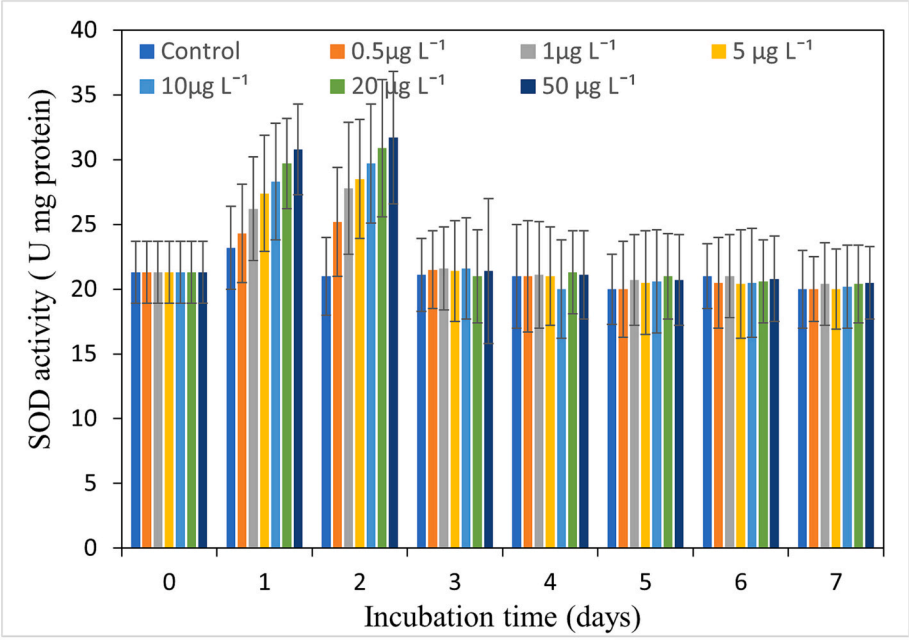

Fig. 7. Changes in SOD activity (U mg^{-1} protein) of *A. platensis* cells exposed to different concentrations of saxitoxin. Data are the means ±SD of three replicates.

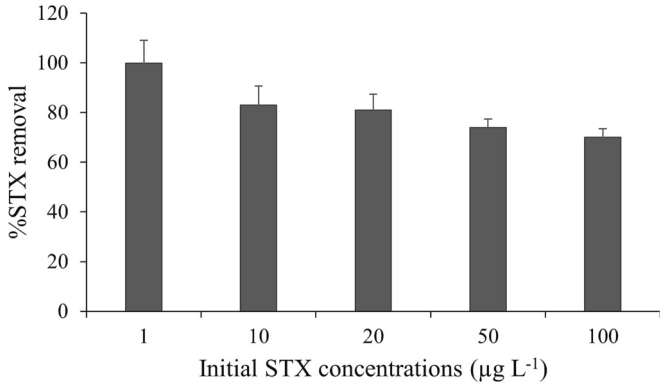

Fig. 8. Saxitoxin removal percentages by EPS released from *A. platensis* cells. Data are the means ±SD of three replicates.

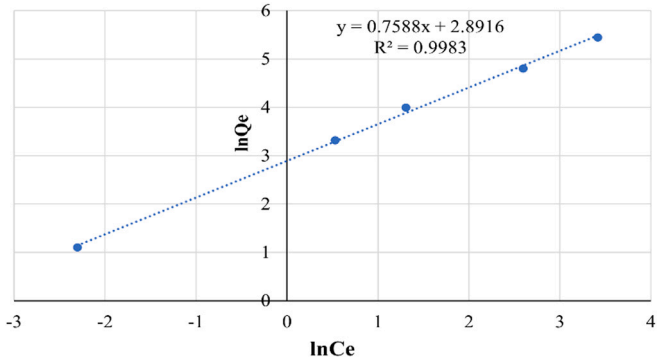

Fig. 9. Freundlich isotherm modeling for adsorption of STX on EPS released by *A. platensis* cells. Data are presented as mean from triplicate runs.

Table 3
Freundlich adsorption parameters for STX by EPS released by *A. platensis* cells. Ce, initial STX concentration, Qe, amount of STX adsorbed on EPS, K and n, adsorption coefficients indicating adsorption capacity and intensity, respectively.

C_e (µg L^{-1})	Q_e (µg g^{-1})	K(µg g^{-1})(L/µg)$^{1/n}$	1/n
1	3.3	2.89	0.76
10	32.3		
20	61		
50	125.3		
100	125. 7		

Each value is the mean of three readings.

CRediT authorship contribution statement

Yasser Mostafa, Saad Alamri, Mohamed Hashem and Sulaiman Alrumman cultured the cyanobacterial strain and monitored its growth during batch experiments and are responsible for project administration and funding acquisition. Zakaria Mohamed designed the experiments of this study and was a major contributor in writing the manuscript. All authors participated in collection, analysis, and interpretation of the data, read and approved the final manuscript.

Funding

The authors extend their appreciation to the Deanship of Scientific Research, King Khalid University for funding this work through research groups program under grant number R.G.P. 1/155/44, was used in the design experiments and collection, statistical analysis, data interpretation and writing the manuscript.

Ethical approval

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgements

The authors would like to thank the Deanship of Scientific Research, King Khalid University for funding this work through research groups program under grant number R.G.P. 1/155/44. The authors extend their appreciation to the Interdisciplinary Centre of Marine and Environmental Research (Porto, Portugal) for generously gifting the *Arthrospira platensis* strain.

References

[1] M.A.B. Habib, M. Parvin, T.C. Huntington, M.R. Hasan, A review on culture, production and use of spirulina as food for humans and feeds for domestic animals and fish, FAO Fisheries and Aquaculture Circular No 1034 (2008) 33. http://www.fao.org/docrep/014/i0424e/i0424e00.htm.

[2] J.L. García, M. Vicente, B. Galán, Microalgae, old sustainable food and fashion nutraceuticals, Microb.Biotechnol. 10 (2017) 1017–1024, https://doi.org/10.1111/1751-7915.12800.

[3] A.S. Gad, Y.A. Khadrawy, A.A. El-Nekeety, S.R. Mohamed, N.S. Hassan, M. A. Abdel-Wahhab, Antioxidant activity and hepatoprotective effects of whey protein and *Spirulina* in rats, Nutrition 27 (2011) 582–589, https://doi.org/10.1016/j.nut.2010.04.002.

[4] S. Khandual, E.O.L. Sanchez, H.E. Andrews, J.D.P. de la Rosa, Phycocyanin content and nutritional profile of *arthrospira platensis* from mexico: efficient extraction process and stability evaluation of phycocyanin, BMC Chemistry 15 (2021) 24, https://doi.org/10.1186/s13065-021-00746-1.

[5] R.R. Siva Kiran, G.M. Madhu, S.V., Satyanarayana, *Spirulina* in combating protein energy malnutrition (PEM) and protein energy wasting (PEW)- a review, J. Nutr. Res. 1 (2015) 62–79.

[6] D.P. Mulokozi, M.S.P. Mtolera, A.J. Mmochi, Spirulina (*Arthrospira fusiformis*) as a potential protein source in practical diets for fry mariculture of Rufiji tilapia (*Oreochromis urolepis urolepis*), WIO Journal of Marine Science 18 (2019)57–67. https://doi.org/https://doi.org/10.4314/wiojms.v18i2.6.

[7] G. Markou, L. Wang, J. Ye, A. Unc, Using agroindustrial wastes for the cultivation of microalgae and duckweeds: contamination risks and biomass safety concerns, Biotechnol. Adv. 36 (2018) 1238–1254, https://doi.org/10.1016/j.biotechadv.2018.04.003.

[8] Z.A. Mohamed, Polysaccharides as a protective response against microcystin-induced oxidative stress in *Chlorella vulgaris* and *Scenedesmus quadricauda* and their possible significance in the aquatic ecosystem, Ecotoxicol. 17 (2008) 504–516, https://doi.org/10.1007/s10646-008-0204-2.

[9] S. Naveed, C. Li, X. Lu, S. Chen, B. Yin, C. Zhang, Y. Ge, Microalgal extracellular polymeric substances and their interactions with metal(loid)s: a review, Crit. Rev. Environ. Sci. Technol. 49 (2019) 1769–1802, https://doi.org/10.1080/10643389.2019.1583052.

[10] J. Bender, P. Phillips, Microbial mats for multiple applications in aquaculture and bioremediation, Bioresour. Technol. 94 (2004) 229–238, https://doi.org/10.1016/j.biortech.2003.12.016.

[11] C.S. Freire-Nordi, A.A.H. Vieira, O.R. Nascimento, The metal binding capacity of *Anabaena spiroides* extracellular polysaccharide: an EPR study, Process Biochem. 40 (2005) 2215–2224, https://doi.org/10.1016/j.procbio.2004.09.003.

[12] C. Zarrouk, Contribution à létude d'une Cyanophycée, influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de Spirulina maxima (Setch et Gardner) Geitler, Ph.D. Thesis, University of Paris, France, 1966.

[13] M. Munoz, S. Cirés, Z.M. de Pedro, J.Á. Colina, Y. Velásquez-Figueroa, J. Carmona-Jiménez, A. Caro-Borrero, A. Salazar, M.C. Santa MaríaFuster, D. Contreras, E. Perona, A. Quesada, J.A. Casas, Overview of toxic cyanobacteria and cyanotoxins in Ibero-American freshwaters: challenges for risk management and opportunities for removal by advanced technologies, Sci. Total Environ. 761 (2021), https://doi.org/10.1016/j.scitotenv.2020.143197, 143197.

[14] J. Li, K.M. Persson, Quick detection method for paralytic shellfish toxins (PSTs) monitoring in freshwater - a review, Chemosphere 265 (2021)128591. https://doi.org/https://doi.org/10.1016/j.chemosphere.2020.128591.

[15] B.H. Pyle, S.C. Broadway, G.A. McFeters, A rapid, direct method for enumerating respiring enterohaemorrhagic *Escherichia coli* 0157:H7 in water, Appl. Environ. Microbiol. 61 (1995) 2614–2619, https://doi.org/10.1128/aem.61.7.2614-2619.1995.

[16] H. Tunca, A. Doğru, F. Köçkar, B. Önem, T.O. Sevindik, Evaluation of Azadirachtin on *Arthrospira plantensis* Gomont growth parameters and antioxidant enzymes, Annales de Limnologie – intern, J. Limnol. 56 (2020) 8, doi:10.1051/limn/2020008 357, doi:10.1051/limn/2020008.

[17] S. Xue, Z. Su, W. Conga, Growth of *Spirulina platensis* enhanced under intermittent illumination, J. Biotechnol. 151 (2001) 271–277, https://doi.org/10.1016/j.jbiotec.2010.12.012.

[18] B. Önem, A. Doğru, T. Ongun Sevindik, H. Tunca, Preliminary study on the effects of heavy metals on the growth and some antioxidant enzymes in *Arthrospira platensis*-M2 strain, Phycol. Res. 66 (2018) 23–30, doi: 10.1111/pre.12202. doi: 10.1111/pre.12202.

[19] R.J. Porra, The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b, Photosynthesis Res. 73 (2002) 149–156, https://doi.org/10.1023/A:1020470224740.

[20] M.M. Bradford, A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254, https://doi.org/10.1016/0003-2697(76)90527-3.

[21] W.F. Beyer Jr., I. Fridovich, Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions, Anal. Biochem. 161 (1987) 559–566, https://doi.org/10.1016/0003-2697(87)90489-1.

[22] W.H. Habig, W.B. Jakoby, Assay for differentiation of glutathione S-transferases, Methods Enzymol. 77 (1981) 398–405, https://doi.org/10.1016/S0076-6879(81)77053-8.

[23] R.L. Health, L. Packer, Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation, Arch. Biochem. Biophys. 125 (1968) 189–198, https://doi.org/10.1016/0003-9861(68)90654-1.

[24] H.L. Tytgat, S. Lebeer, The sweet tooth of bacteria: common themes in bacterial glycoconjugates, Microbiol. Mo.l Biol. R 78 (2014) 372–417, https://doi.org/10.1128/MMBR.00007-14.

[25] A.A. Zeidan, V.K. Poulsen, T. Janzen, P. Buldo, P.M.F. Derkx, G. Øregaard, A. R. Neves, Polysaccharide production by lactic acid bacteria: from genes to industrial applications, FEMS Microbiol. Rev. 41 (2017) S168–S200, https://doi.org/10.1093/femsre/fux017.

[26] A. Parikh, D. Madamwar, Partial characterization of extracellular polysaccharides from cyanobacteria, Bioresour. Technol. 97 (2006) 1822–1827, https://doi.org/10.1016/j.algal.2019.101655.

[27] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for the determination of sugar and related substances, Anal. Chem. 28 (1956) 350–356, https://doi.org/10.1021/ac60111a017.

[28] Z. Mousavian, M. Safavi, F. Azizmohseni, M. Hadizadeh, S. Mirdamadi, Characterization, antioxidant and anticoagulant properties of exopolysaccharide from marine microalgae, AMB Expr. 12 (2022), https://doi.org/10.1186/s13568-022-01365-2.

[29] K. Mojica, D. Elsey, M.J. Cooney, Quantitative analysis of biofilm EPS uronic acid content, J. Microbiol. Methods 71 (2007) 61–65, https://doi.org/10.1016/j.mimet.2007.07.010.

[30] A.P. Negri, G.J. Jones, S.I. Blackburn, Y. Oshima, H. Onodera, Effect of culture and bloom development and of sample storage on paralytic shellfish poisons in the cyanobacterium *Anabaena circinalis*, J. Phycol. 33 (1997) 26–35, https://doi.org/10.1111/j.0022-3646.1997.00026.x.

[31] Z.A. Mohamed, Harmful cyanobacteria and their cyanotoxins in Egyptian fresh waters – state of knowledge and research needs, Afr. J. Aquat. Sci. 41 (2016) 361–368, https://doi.org/10.2989/16085914.2016.1219313.

[32] T. Papadimitriou, K. Kormas, E. Vardaka, Cyanotoxin contamination in commercial Spirulina food supplements, J. Consum. Prot. Food. Saf. 16 (2021) 227–235, https://doi.org/10.1007/s00003-021-01324-2.

[33] Z. Hu, Y. Liu, D. Li, A. Dauta, Growth and antioxidant system of the cyanobacterium *Synechococcus elongates* in response to microcystin-RR, Hydrobiol. 534 (2005) 23–39, https://doi.org/10.1007/s10750-004-1319-y.

[34] N. Nagalakshmi, M.N.V. Prasad, Copper-induced oxidative stress in *Scenedesmus bijugatus*: protective role of free radical scavengers, Bull. Environ. Contam. Toxicol. 61 (1998) 623–628, https://doi.org/10.1007/s001289900806.

[35] M. Wiese, P.M. D'Agostino, T.K. Mihali, M.C. Moffitt, B.A. Neilan, Neurotoxic alkaloids: Saxitoxin and its analogs, Mar. Drugs 8 (2010) 2185–2211, https://doi.org/10.3390/md8072185.

[36] M.C. Wiener, P.S. Horanyi, How hydrophobic molecules traverse the outer membranes of gram-negative bacteria, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 10929–10930, https://doi.org/10.1073/pnas.1106927108.

[37] P.T. Nguyen, T.T. Nguyen, D.C. Bui, P.T. Hong, Q.K. Hoang, H.T. Nguyen, Exopolysaccharide production by lactic acid bacteria: the manipulation of environmental stresses for industrial applications, AIMS Microbiol. 17 (2020) 451–469, https://doi.org/10.3934/microbiol.2020027.

[38] D. Cruz, V. Vasconcelos, G. Pierre, P. Michaud, C. Delattre, Exopolysaccharides from cyanobacteria: strategies for bioprocess development, Appl. Sci. 10 (2020) 3763, https://doi.org/10.3390/app10113763.

[39] H. Shi, J. Ding, T. Timmons, C. Adams, pH effects on the adsorption of saxitoxin by powdered activated carbon, Harmful Algae 19 (2012) 61–67, https://doi.org/10.1016/j.hal.2012.05.008.

[40] J. Rorar, L.D. Garcia, T. Cutright, Removal of saxitoxin and anatoxin-a by PAC in the presence and absence of microcystin-LR and/or cyanobacterial cells, J. Environ. Sci. 128 (2023) 161–170, https://doi.org/10.1016/j.jes.2022.08.015.

[41] R. De Philippis, G. Colica, E. Micheletti, Exopolysaccharide-producing cyanobacteria in heavy metal removal from water: molecular basis and practical applicability of the biosorption process, Appl. Microbiol. Biotechnol. 92 (2011) 697–708, https://doi.org/10.1007/s00253-011-3601-z.

[42] D.E.B. Olano, L.A. Salvador-Reyes, M.N.E. Montaño, R.V. Azanza, Sorption of paralytic shellfish toxins (PSTs) in algal polysaccharide gels, Algal Res. 45 (2020), 101655, https://doi.org/10.1016/j.algal.2019.101655.

[43] M. Angelaalincy, N. Senthilkumar, R. Karpagam, G.G. Kumar, B. Ashokkumar, P. Varalakshmi, Enhanced extracellular polysaccharide production and self-sustainable electricity generation for PAMFCs by *Scenedesmus* sp. SB1, ACS Omega 2 (2017) 3754–3765, https://doi.org/10.1021/acsomega.7b00326.

[44] W. Zhao, Y. Huang, M. Wang, C. Pan, X. Li, Y. Peng, B. Li, Post-endogenous denitrification and phosphorus removal in an alternating anaerobic/oxic/anoxic

(AOA) system treating low carbon/nitrogen (C/N) domestic wastewater, Chem. Eng. (2018) 450–458, https://doi.org/10.1016/j.cej.2018.01.096. J339.