Journal of Applied Phycology (2022) 34:2941–2954
https://doi.org/10.1007/s10811-022-02847-3

Effect of nitrogen source and nickel concentration on green microalga *Botryococcus braunii* growth and its remediation potential

M. M. Areco[1,2] · L. Rojas[3] · D. G. Noseda[2,4] · V. Passucci[1,2] · N. Rotella[3] · G. Curutchet[1,2]

Received: 10 March 2022 / Revised and accepted: 9 September 2022 / Published online: 29 September 2022

Abstract

The effects of NO_3^- and/or NH_4^+, and nickel on the growth and photosynthesis-respiration metabolism of *Botryococcus braunii* were analyzed. NO_3^-, NH_4^+ and Ni(II) removal capacity are described in terms of metabolic and non-metabolic processes. Results demonstrate that *B. braunii* can live in a pH range from 3 to 9. The total productivity (P) and the productivity of the growth phase (P_v) are higher (≈ 58 and 61 mg L^{-1} day^{-1}, respectively) when the medium contains NH_4^+, than when it contains NO_3^- (≈ 45 and 51 mg L^{-1} day^{-1}, respectively). NH_4^+ consumption results in a decrease of the pH of the medium from 7 to 3. *Botryococcus braunii* reverse the acidic conditions of the medium when NO_3^- is metabolized (pH from 5 to 8–8.5). Ni(II)-specific removal is mainly due to adsorption and increases along with pH and initial metal concentration. The Hill model best describes the adsorption experimental data. The stoichiometric correlations between H^+ desorption and nickel adsorption were 1:5, 1:3 and 1:2 for pH values of 5, 6 and 7, respectively. The present work is a new contribution on the biotechnological potential of *B. braunii* to live and grow at different pH and to remove NO_3^-, NH_4^+, and Ni(II) by metabolic and non-metabolic pathways.

Keywords *Botryococcus braunii* · Chlorophyta · Ni(II) · NO_3^- · NH_4^+ · Remediation · Metabolism

Introduction

The discharge of wastes into natural water bodies, for example effluents containing heavy metals and/or high concentrations of non-toxic compounds such as nitrogen, have been affecting water quality and transforming, damaging, or even destructing aquatic ecosystems (Renuka et al. 2013; Gonçalves et al. 2017).

Nitrogen in natural waters can be found mainly as NH_4^+, NO_2^-, NO_3^-, other nitrogen oxides and organic forms. One of the main problems related to high nitrogen concentrations in water bodies is eutrophication, causing the degradation of freshwater ecosystems and posing a public health risk for the nearby populations, since eutrophication may cause algal blooms (Cai et al. 2013; Dsikowitzky et al. 2015; Kheriji et al. 2015).

Many industrial applications such as milling, mining, and surface finishing industries use different heavy metals in their production processes (Malik 2004). Among them, Nickel is considered an essential micronutrient, since it participates in different biological processes in trace amounts, but at higher concentrations it can cause several problems in nature and health (Hussain et al. 2013). This metal is present in nature and may reach soils and natural water bodies through the alteration of the parent rock, but the main problem regarding nickel contamination are the numerous anthropogenic activities that lead to nickel accumulation (Küpper and Kroneck 2007; Helaoui et al. 2020). The environmental chemistry of nickel is mainly associated to Ni(II), which, like many heavy metals, is poorly soluble in aqueous matrices at neutral pH. Its concentration in water depends on the pH, being soluble at pH < 8, insoluble as $Ni(OH)_2$ (between other oxides and

✉ G. Curutchet
gcurut@gmail.com

1 Instituto de Investigación e Ingeniería Ambiental (IIIA), CONICET- UNSAM, 25 de Mayo y Francia, CP 1650 Buenos Aires, San Martín, Argentina

2 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290, CP 1033 Buenos Aires, Argentina

3 Instituto de Investigación e Ingeniería Ambiental (3Ia), UNSAM, 25 de Mayo y Francia, CP 1650 Buenos Aires, San Martín, Argentina

4 Instituto de Investigaciones Biotecnológicas (IIBIO), UNSAM, 25 de Mayo y Francia, CP 1650 San Martín, Buenos Aires, Argentina

hydroxides) from pH 8 to 10, and soluble as $Ni(OH)_4^{-2}$ and other anions at very alkaline pH (Ciesielczyk et al. 2013). In water and sewage nickel can be attached to suspended matter or as insoluble compounds, mainly oxides and hydroxides. The particles may be transported or sink in the sediments, where they become truly "chemical time bombs" (Ter Meulen 1993; Tufo et al. 2018).

Dissolution, precipitation, and sorption processes are the main factors responsible for heavy metal mobility in the environment and the fate of nickel in contaminated effluents and its impact on environment is closely related to them. Knowledge about microorganisms capable of adsorbing nickel or modify ionic aqueous equilibria leading to nickel precipitation are of great importance to understand these processes and design potential remediation techniques. In this context, many studies in the literature report on the removal of nickel from wastewater produced by metal plating industries, where metals are concentrated in the effluents (Moersidik et al. 2020; Ates and Basak 2021; Pérez Jiménez et al. 2021). Adsorption is a widely studied technique for the removal of heavy metals from wastewater (Long et al. 2018; Ates and Basak 2021; Pérez Jiménez et al. 2021).

These contamination trends clearly evidence the need for effective treatment methods before discharging contaminants into natural bodies. Many of these metals are expensive, then their recovery from effluents and wastes, is of economic importance. In recent years, the development of new technologies for the removal of contaminants from natural waters have been studied (Gomes et al. 2016; Jais et al. 2017; Zhang et al. 2018). Microalgae may offer a new approach for the treatment of wastewater from a variety of industries, agriculture and the food industry and also for municipal wastewaters. Microalgae assimilate carbon (C), nitrogen (N) and phosphorus (P) (Morales-Amaral et al. 2015; Cheng et al. 2017) and, have the capacity to remove heavy metals (Areco et al. 2018; Daneshvar et al. 2019; Urrutia et al. 2019; Mariam et al. 2021) and some toxic organic compounds (Matamoros et al. 2015; Sutherland and Ralph 2019; Xie et al. 2019). Furthermore, microalgae may be cultivated in wastewaters under proper conditions, avoiding the use of huge amounts of clean water, reducing the costs implied in biomass cultivation.

Botryococcus braunii is a green planktonic freshwater microalga that has a worldwide distribution. It is known for its capacity to generates hydrocarbons in the outer layers of the cell wall (Maxwell et al. 1968), but since its growth is slow the use of this alga as a natural source of hydrocarbons is still considered to be quite difficult (Nakamura et al. 2017). Nevertheless, this extensively studied alga may be considered for other purposes such as remediation rather than just for hydrocarbon production and other biotechnological potentials of this algae have been partially explored, for example its heavy metals removal capacity. The capacity of *B. braunii* to remove zinc and copper from solution through metabolic mechanisms has been also studied (Areco et al. 2013, 2018), since microalgae metabolize inorganic carbon which affects the inorganic carbon equilibrium and therefore, the pH of the medium (Decostere et al. 2013). On the other hand, little has been published regarding the capacity of *B. braunii* to remediate nutrients such as nitrogen from wastewaters. Cheng et al. (2017) have compared the ammonium and total phosphorus removal efficiency of *B. braunii*, *Chlorella vulgaris* and *Desmodesmus* sp. from piggery wastewater, while Massa et al. (2017) studied ammonium removal from liquid digestate. *Botryococcus braunii* also has been studied as a tertiary treatment for livestock wastewater, where ammonium (Kim and Kim 2017) and/or nitrate were the nitrogen sources (Órpez et al. 2009; Drexler et al. 2013). Thus, *B. braunii* may be used in bioremediation processes of waters containing high concentrations of nitrate and ammonium, and heavy metals, but further studies need to be conducted to evaluate its application for the remediation of effluents or natural waters.

Even though the ability of *B. braunii* to remove nutrients has been studied (Mennaa et al. 2015; Kim and Kim 2017; Rinna et al. 2017), little has been published regarding its preference for different nitrogen sources, or about its capacity to remove nickel from solution while reverting the acid conditions of the effluent. Since *B. braunii* has been widely studied for its capacity to produce hydrocarbons, exploring new biotechnological approaches may lead to the development of a variety of biotechnological processes where the concept of circular economy may be applied.

The present work aims to determine the effects of different nitrogen sources (NO_3^- and/or NH_4^+) and nickel concentrations on the growth and the photosynthesis-respiration metabolism of *B. braunii*. Its capacity to fluctuate the pH of the medium, depending on the nitrogen source used, and to remove nickel from wastewater by metabolic processes and by its adsorption capacity (non-metabolic removal) is also studied.

Materials and methods

Metal solution and metal quantification

Stock nickel solutions were made with $NiCl_2.6H_2O$ and distilled water. The concentration of the solutions ranged from 0.035 to 9.5 mM at a pH of 6.0 ± 0.3. Ni(II) concentrations in solution were measured with atomic absorption spectroscopy (AAS) (Rice et al. 2017).

Algal strain and stock culture conditions

Botryococcus braunii (strain MB3N Stock B 2441) was originally obtained from the Algal culture Center of the University

of Texas. Stock cultures were kept in liquid and solid Bold Basal Medium (BBM, Medium composition—see Table in Online Resources 1) and were sub-cultured every 3 or 4 weeks.

Experimental design

All liquid cultures were kept at 25 ± 1 °C, 130 rpm, and under light:dark cycles (16:8 h and 30 µmol photons m^{-2} s^{-1}), unless otherwise indicated.

Biomass estimation and specific growth rate

The growth of the biomass was determined by turbidimetry; measuring optical density at 680 nm (OD_{680nm}); cell counting (Neubauer chamber) and dry weight that was measured gravimetrically (centrifugation at 10,000 × *g* for 5 min) or after filtration on a cellulose acetate filter (GF/C 0.45 µm, Whatman), that was rinsed with distilled water and dried (60 °C) till constant weight.

To establish the correlation between cell counting and biomass dry weight (mg), 100 mL of BBM was added to each of 13 Erlenmeyer flasks containing a sample of the original *B. braunii* inoculum (25%). Samples were incubated as described in "experimental design" section. Samples were taken at different time intervals (0, 7, 10, 14, 21, 25, 28, 35, 43, 53, 67, 97 and 112 days), and cell counts and dry weight were measured. Correlation analysis between alga growth measured gravimetrically and by cell counting demonstrated a linear correlation (R = 0.998). The equation obtained was:

$$Cell\ number = 3 \times 10^8 * dry\ weight - 4 \times 10^7 \quad (1)$$

The biomass volumetric productivity (P, mg L^{-1} day^{-1}) was calculated (Choi and Yu 2015) using Eq. (2):

$$P = \frac{(X_1 - X_0)}{(t_1 - t_0)} \quad (2)$$

where X_0 and X_1 are the initial and final biomass concentration (mg L^{-1}), and t_0 and t_1 (day) are the initial and final cultivation times, respectively.

Effect of nitrogen source and concentration on the biomass growth

Cultures were grown with different NO_3^- ($NaNO_3$) and NH_4^+ ($(NH_4)_2SO_4$) proportions (0% of NO_3^- and 100% of NH_4^+; 0% of NH_4^+ and 100% of NO_3^-; and 50% NO_3^-/NH_4^+) and with different nitrate concentrations (NO_3^- = 0.7–7 mM).

To analyze the growth of *B. braunii* under low nitrogen concentrations respect to the control (BBM Medium) experiments were carried out in a 7-L stirred-tank bioreactor (BioFlo 110, New Brunswick Scientific; USA), interfaced with Biocommand Bioprocessing software (New Brunswick Scientific) for the control of parameters and the obtention of the data (see bioreactor diagram in Online Resources 2). Experiments were performed in batch mode using modified BBM (NO_3^- concentration = 0.7 mM), and under light:dark cycles (16:8 h and 30 µmol photons m^{-2} s^{-1}). 400 mL of a concentrated culture of *B. braunii* in late growth phase (4×10^8 cells mL^{-1}) was used to inoculate the bioreactor with 4 L of medium. Both temperature and pH were not adjusted throughout the culture. Agitation was maintained (75 rpm) to prevent the settling of microalgae. Filter-sterilized (0.22 µm) air was supplied at a constant flow (0.25 vvm). The pH was measured in situ using a pH electrode (Mettler-Toledo GmbH, Germany) and the dissolved oxygen level (dO_2, % saturation) was determined with a polarographic probe (InPro6110/320, Mettler-Toledo).

The nitrate concentration in solution was determined by the cadmium reduction method (Rice et al. 2017) and ammonium concentrations were determined by a colorimetric method (Wiener Lab, Argentina).

Effect of nickel on biomass growth

Cultures were exposed over 50 ± 1 days to several nickel concentrations (0, 0.035, 0.05, 0.085, 0.17 and 0.25 mM). Cultures were kept at 25 ± 1 °C, 130 rpm, and under light:dark cycles (16:8 h and 30 µmol photons m^{-2} s^{-1}).

In all the experiments described in the present section, aliquots (3 mL) were taken at different time periods to evaluate the pH variation, growth ($OD_{680\ nm}$, cell counting and/or dry weight), biomass productivity (Eq. 2) and final NO_3^-, NH_4^+or Ni(II) concentration in solution.

Remediation of nitrogen and nickel

Nitrogen removal All the experiments were conducted with *B. braunii* cultivated in Erlenmeyer flasks containing BBM and incubated in batch mode at pH 5.00 ± 0.25, with the corresponding variation of NO_3^- and/or NH_4^+ concentrations.

Nickel toxicity and removal All the experiments were made in batch mode using 1 g L^{-1} of living or dead biomass.

Nickel removal efficiency by *B. braunii*. Live biomass Experiments were conducted as depicted in Areco et al. (2018) in BBM medium, using different nickel initial concentrations (0, 0.035, 0.05, 0.085, 0.17 and 0.3 mM). Samples were incubated for 50 ± 1 days at initial pH of 5.0 ± 0.3, and were kept at 25 ± 1 °C, 130 rpm, and under light:dark cycles (16:8 h and 30 µmol photons m^{-2} s^{-1}).

Nickel biosorption experiments. Dead biomass For sorption experiments the biomass was suspended in solutions with different Ni(II) concentrations (between 0.035 and 9.4 mM) at pH 6 ± 0.3, to avoid metal precipitation. Algal biomass was separated from metal solutions by centrifugation (443 RCF for 10 min) and filtration (0.45 µm). Then the final metal concentrations in solutions were measured and metal uptake (q) was determined (Eq. 3) (Davis et al. 2000).

$$q = \frac{(C_i - C_{eq}) * V}{m} \quad (3)$$

where C_i and C_{eq} are the initial and equilibrium metal concentration (mM), respectively; V the volume of the solution (L) and m is the dry weight of the algae (g).

The net sorption capacity of *B. braunii* was determined as depicted by Areco et al. (2018) using 0.150 ± 0.05 g L^{-1} of protonated dead biomass. The initial Ni(II) concentration used was 1.7 ± 0.1 mM, at initial pH of 4, 5, 6 or 7 that were adjusted using NaOH (0.058 ± 0.001 M). Experiments were carried out by triplicate for each pH value.

Control experiments were conducted in the absence of the adsorbent (microalgae) and a second control experiment was performed with biomass and without nickel.

Photosynthesis and respiration activity measurement. Respirometric studies

In order to study the effect of two different nitrogen sources (NO_3^- or NH_4^+) and/or different nickel concentrations (0, 0.085 and 0.17 mM) on the respiration and photosynthesis metabolism of *B. braunii*, experiments were conducted at pH 5.0 ± 0.3 as in Areco et al. (2018). The gas concentrations (CO_2 and O_2) were measured over 26 h with a Micro-Oxymax gas analyzer (Columbus Instruments). Estimations of gas consumption or production rates were made following time course of gas composition.

Surface characterization: SEM–EDS and Zeta potential analysis

Dehydrated microalgae (60 °C till constant weight) with no Ni(II) (control) or with Ni(II) (1 mM) adsorbed, were visualized by a field emission gun scanning electron microscope (FEI, Quanta 250 ESEM) with combined energy dispersive RX-ray analyzer (EDS) Thermo Scientific UltraDry model (voltage of 15.0 kV.). The metal distribution and the elemental composition of the surface of the microalga (mapping), before and after metal adsorption, were determined using Thermo Scientific Pathfinder X-ray microanalysis software.

The electrical charge on the surface of *B. braunii* at different pH values (4.4 to 10.5) were determined using a Zeta Potential Analyzer (ZetaPlus. Brookhaven Instruments Corporation). Determinations were made using an electric field of 16 V cm^{-1}, a current of 15 mA, at 25 °C and at constant ionic strength (10^{-3} M KCl). pH of the different microalgae suspensions (1 g L^{-1}) was adjusted using HCl or KOH. The resulting zeta potential values at each pH are the mean value of 21 replicates.

Statistical analysis

Results are the mean values from three replicate experiments, unless otherwise indicated. One-factor ANOVA was performed (GraphPadPrism 7 software package) for the statistical analysis of the results obtained (growth of *B. braunii* exposed to different Ni(II), NO_3^- and/or NH_4^+ concentrations); Kolmogorov-Smirnovf and Barlett test were used to verified normality and homoscedacity assumptions; means were compared using Tukey test.

Results

Botryococcus braunii growth and culture conditions

The growth of *B. braunii* in BBM (control conditions, see Online Resources 1) reached a plateau after 35 days (see Online Resources 3). The final biomass concentration obtained was 1580 ± 50 mg L^{-1} and the biomass volumetric productivity (P) (Eq. 2) obtained was 45 ± 2 mg L^{-1} day^{-1}. The specific growth rate (µ) was not calculated since the growth of *B. braunii* was not exponential but linear, probably due to a limitation of CO_2 transfer rate during the growth stage. In these conditions the growth rate (*rx*) remains constant and equal to the productivity in the growth phase (P_v) and the specific growth rate (µ) decreases throughout the culture (rx = µ X). The productivity of the linear growth phase (P_v) obtained (Eq. (2)) was 47 ± 2 mg L^{-1} day^{-1}. Since the lag phases of the cultures were short, the total productivities and the productivities in the growth phase obtained were similar in all cases.

Effect of nitrogen concentration on biomass growth, photosynthesis, and respiration

Botryococcus braunii was cultivated in BBM under 0.7–7 mM NO_3^- to determine the biomass growth, changes in the pH of the media, the rate of nitrogen consumption, and total N consumption in different time intervals (Fig. 1a, b, c and d, respectively (NO_3^-)) as well as the rates of photosynthesis and respiration (Figure in Online Resources 4).

There were significant differences ($n = 3$, $p < 0.01$) in the productivities (P and P_v) of the cultures exposed to initial NO_3^- concentrations higher than 1.25 mM compared to those exposed to NO_3^- concentrations lower than 1.25 mM

(Table 1). Even though nitrogen was totally consumed in the first 12 days when NO_3^- initial concentrations were 0.7 and 1.25 mM, the algae kept on growing till the stationary phase was reached after 30 days (Fig. 1a and c). When NO_3^- initial concentrations were higher than 1.25 mM, the stationary phase was reached before total nitrogen consumption. In all cases studied, growth, culture pH and nitrogen consumption show an inflection on day 12 (Fig. 1a, b and c).

Three different cases were observed: Presence of nitrogen with active growth or in stationary phase (cases 1 and 2, respectively); or nitrogen depletion (case 3). In the first case, (systems 0.7 and 1.25 mM till day 12; and systems 3.5, 5 and 7 mM till days 25–30) (Fig. 1d light grey columns) the ratio of NO_3^- consumption increased proportionally to initial NO_3^- concentrations, from 0.4 to 0.8 mmol N g biomass^{-1}. The uptake rate remained between 0.04—0.07 mmol N day^{-1} (Table in Online Resources 5). The growth of all cultures stopped after 25 days (±), probably due to the inhibition caused by an increase in the medium pH till values ≈ 8.5. When maximum growth was reached, all

Table 1 Biomass total productivity (P) after 120 days, and productivity in the growth phase (P_v) obtained for *B. braunii* cultivated in BBM varying the initial NO_3^- concentration in the culture media (0.7, 1.25, 3.5, 5 and 7 mM)

N-NO_3 Initial Concentration (mM)	Total Productivity (mg L^{-1} day^{-1})	Productivity in growth phase (P_v) (mg L^{-1} day^{-1})
0.7	42 ± 3	43 ± 3
1.25	45 ± 2	47 ± 2
3.5	52 ± 4	57 ± 4
5	50 ± 5	52 ± 5
7	53 ± 4	56 ± 4

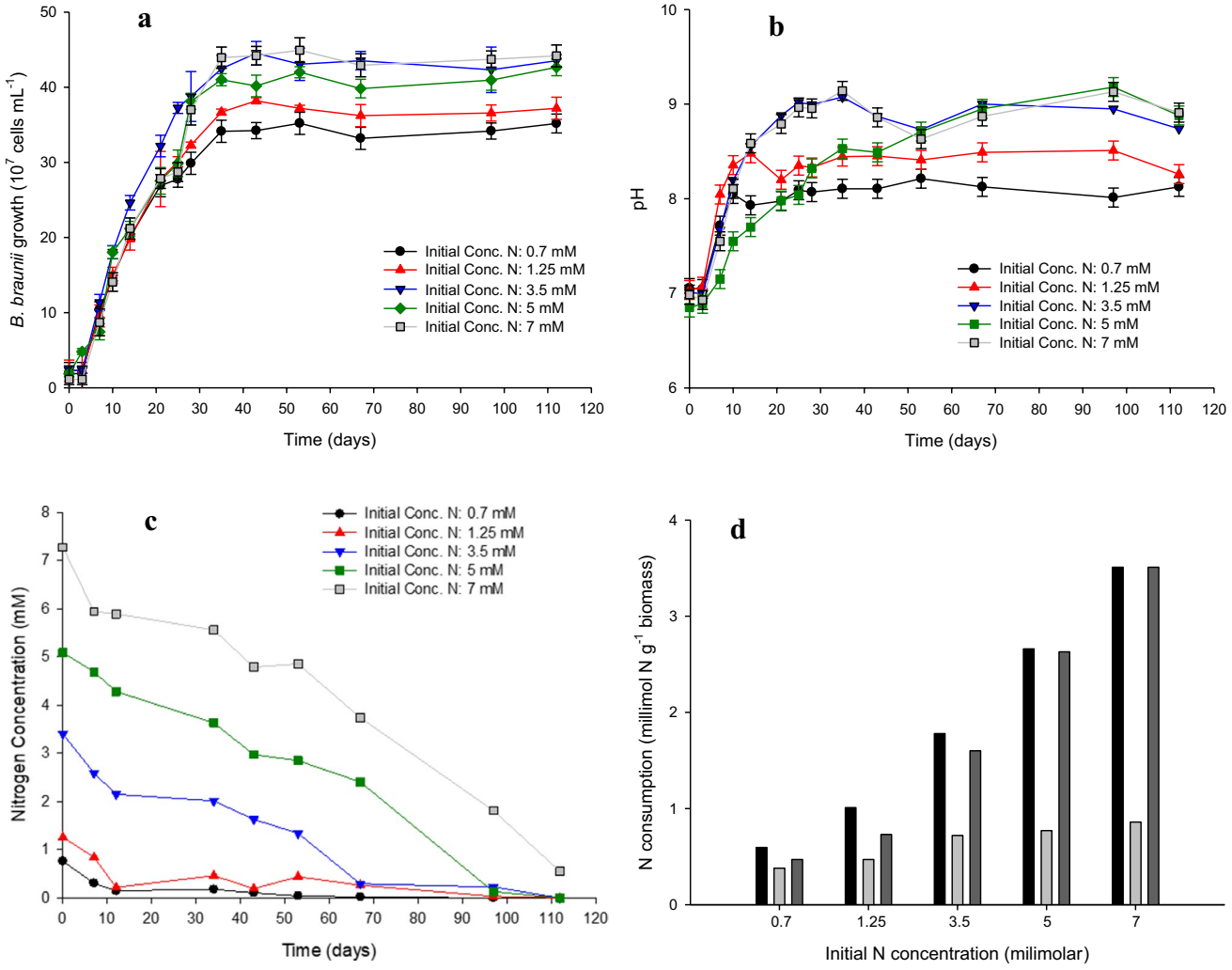

Fig. 1 *Botryococcus braunii* cultivated in BBM varying the initial nitrate concentration in the culture media (0.7, 1.25, 3.5, 5 and 7 mM): **a**: growth; **b**: pH; **c**: nitrogen (NO_3^-) consumption and **d**: total N consumption over 112 days (black bars), N consumption till maximal growth (light-grey bars), N consumption over 100 days (dark-grey bars). Error bars represent the mean standard deviation ($n = 3$)

cultures presented similar ratio of NO_3^- uptake to generated biomass. From this point on the culture that still had nitrogen (case 2) continued its metabolization. The uptake rate and the stoichiometric ratio of nitrogen to biomass uptake were proportional to initial nitrate concentration (Fig. 1d dark grey columns). This result is important when non-growing living biomass is used for nutrient bioremediation.

In cultures with lower nitrogen concentrations (0.7 and 1.25 mM NO_3^-), all nitrogen is consumed in the first 12 days. Nevertheless, the cells continued to grow for ≈ 17 more days, and stopped when the medium pH reached values between 8—8.5 (Fig. 1a and b).

The respiration and photosynthesis rates of *B. braunii*, regarding O_2 production/consumption and CO_2 consumption/production (mg g^{-1} h^{-1}), when cultivated with (black) or without nitrogen (grey) where measured (Figure in Online Resources 4). Results show that there is a basal CO_2 consumption even when there is no NO_3^- in the medium. The physiological activity of microalgae affects the pH of the medium, Fig. 1b shows a continuous increase in pH of the solution till values around 8—8.5, in the first 10 days. Figure 2a and b show the dO_2 and the pH daily fluctuations, respectively, due to photosynthesis and respiration in an open system. Figure 2c shows NO_3^- consumption and algal growth over time. Figure 2b shows an increase in pH from ≈ 6 to ≈ 7.8 in the first 12 days.

Effect of nitrogen source on biomass growth, photosynthesis, and respiration

The growth of *B. braunii* after 35 days was higher when the N source was a mixture of equal proportions of NO_3^- and NH_4^+, and the lowest growth was observed when NO_3^- was the only N source. The productivities (P_v and P) obtained for *B. braunii* exposed to different N sources were higher (≈ 61 mg L^{-1} day^{-1} and 58 mg L^{-1} day^{-1}, respectively) when the culture media contained NH_4^+, than the productivities (P_v and P) obtained when the N source was only NO_3^- (≈ 51 mg L^{-1} day^{-1} and 45 mg L^{-1} day^{-1}, respectively). These results demonstrate that NH_4^+ at the concentrations and pH range studied was not toxic for *B. braunii* (Fig. 3a). NH_4^+ consumption resulted in pH decrease from 7 to 3 (Fig. 3b). In this pH range, the growth of this alga was not affected (Fig. 3a).

When the biomass was grown in culture media containing equal amounts of NO_3^- and NH_4^+, *B. braunii* first consumed NH_4^+ (favored nitrogen source due to its reduced state and energetically favorable assimilation, as previously discussed) and then NO_3^- (Fig. 3c). When NH_4^+ was totally consumed the biomass started to consume NO_3^- (Fig. 3c and d), resulting in an increase in the medium pH (Fig. 3d and Eq. 6).

The knowledge of nitrogen uptake by *B. braunii* from different nitrogen sources and its associated pH changes are of great importance for the design of bioremediation processes of nutrients and heavy metals.

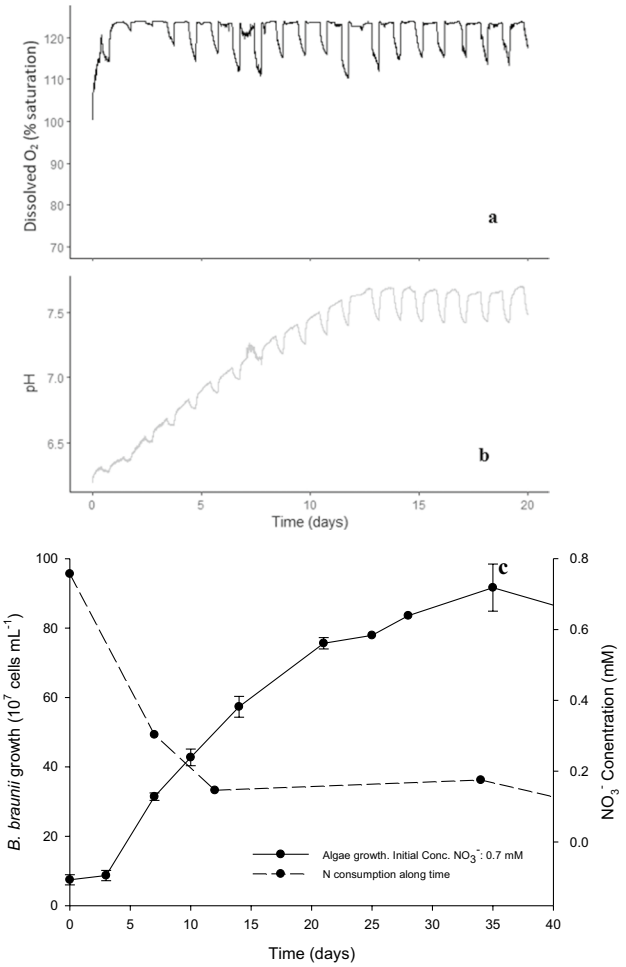

Fig. 2 Variations over time of **a**: dO_2 (% saturation) **b**: pH and **c**: growth and nitrate consumption; due to *B. braunii* photosynthetic and respiration metabolism. Mean and standard deviation ($n = 3$)

When the metabolism of *B. braunii* was analyzed in terms of photosynthesis and respiration, it was found that, even though the growth of the algae was not affected when NH_4^+ was used as the nitrogen source (Fig. 3a), the rate of CO_2 uptake during the light cycles (photosynthesis) was considerably affected, since total CO_2 fixation decreases about 70%. Furthermore, there was an increased (≈ 65%) in O_2 respiratory uptake (with respect to the control) when *B. braunii* was grown with NH_4^+ as the nitrogen source (Table in Online Resources 6).

Nitrogen remediation by *B. braunii*

As shown above, *B. braunii* can grow under a wide range of nitrate concentrations, which are metabolically used by the biomass, reducing nitrates concentrations in solution along

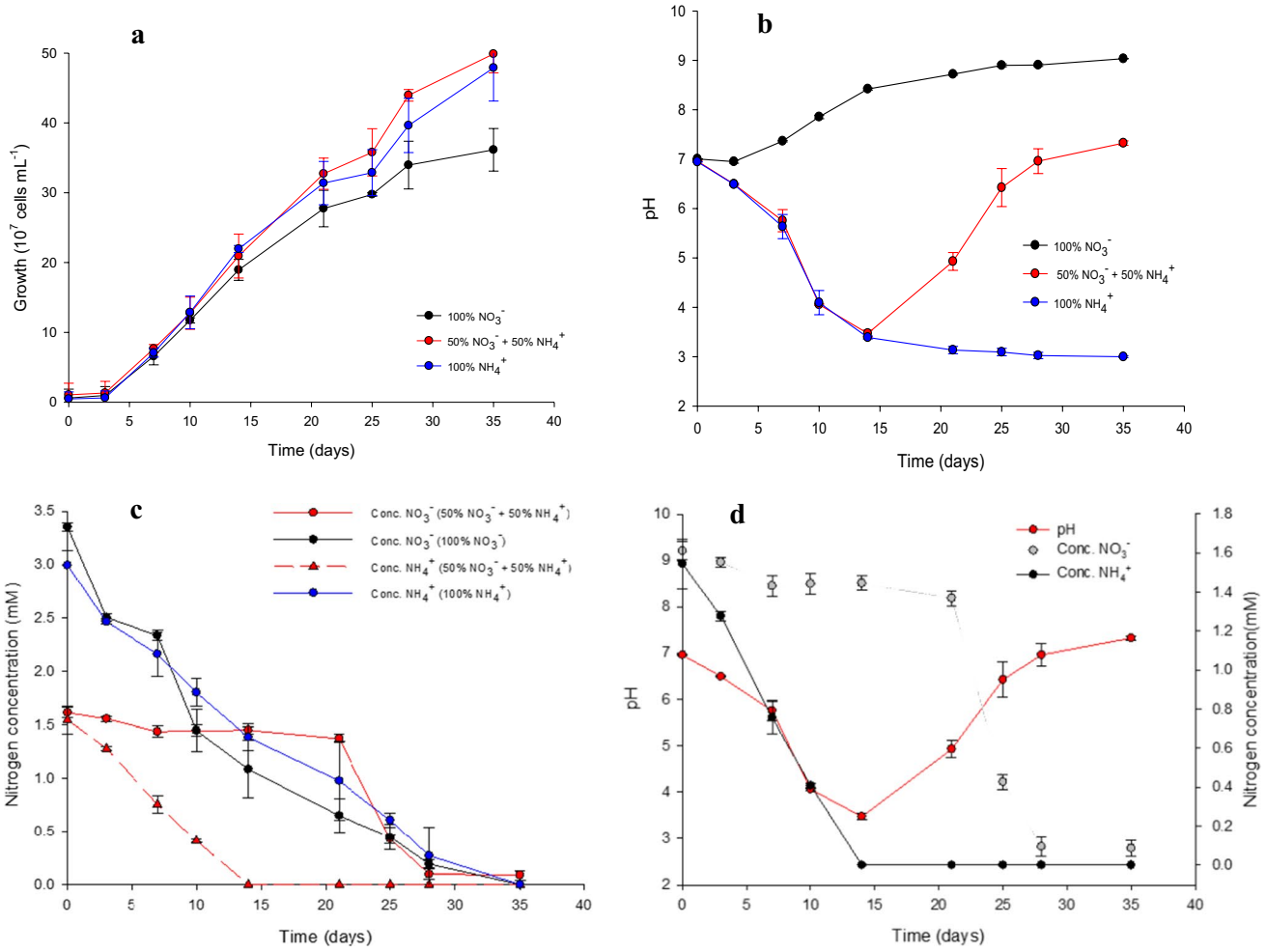

Fig. 3 *Botryococcus braunii* **a**: growth; **b**: culture media pH over time, exposed to different nitrogen source (NO_3^- and/or NH_4^+); **c**: Nitrogen (NO_3^- and/or NH_4^+) consumption by *B. braunii* with different nitrogen sources NO_3^-, NH_4^+ or NO_3^- and NH_4^+; and **d**: pH variation associated with the uptake of NH_4^+ or NO_3^- by *B. braunii* in a culture medium where the nitrogen source was 50% NO_3^- and 50% NH_4^+. Mean and standard deviation ($n = 3$)

time. Figure 3c shows the consumption of variable nitrogen sources (NO_3^-, NH_4^+, or NO_3^- and NH_4^+) over time.

Nickel toxicity and removal

Figure 4a shows the effect of variable Ni(II) concentrations (0 or control, 0.035, 0.05, 0.085, 0.17 and 0.25 mM) added to BBM, on the growth of *B. braunii* over 50 days. Nickel exposure at concentrations higher than 0.05 mM significantly affected (ANOVA ($n = 3$, $p < 0.001$)) the growth of *B. braunii*.

The respiration and photosynthetic rates (in terms of O_2 consumption (▲) and CO_2 assimilation (●) (mg g^{-1} h^{-1}) of *B. braunii* decrease when Ni(II) concentration in solution increases (Fig. 4b). The net balance between the rates of CO_2 production (respiration) and consumption (photosynthesis) in the presence of nickel, agree with the growth curves obtained at the same conditions. Results indicate that the metabolism of *B. braunii* is affected when nickel is present in the medium (Fig. 4b). Nevertheless, *B. braunii* can live under high Ni(II) concentrations (up to 0.05 mM).

Nickel removal efficiency by *B. braunii*. Live biomass

Figure 4c shows total Ni(II) (mM) removed by *B. braunii* (1 g L^{-1}) and final pH after 50 days of being exposed to different Ni(II) concentrations. The metal removal capacity of B. *braunii* increases with initial metal concentrations (mmol metal removed by 1 g of biomass). Furthermore, the increase in the metal concentration in the medium decreases the growth of *B. braunii* (Fig. 4a) leading to a smaller increase on the final pH of the medium.

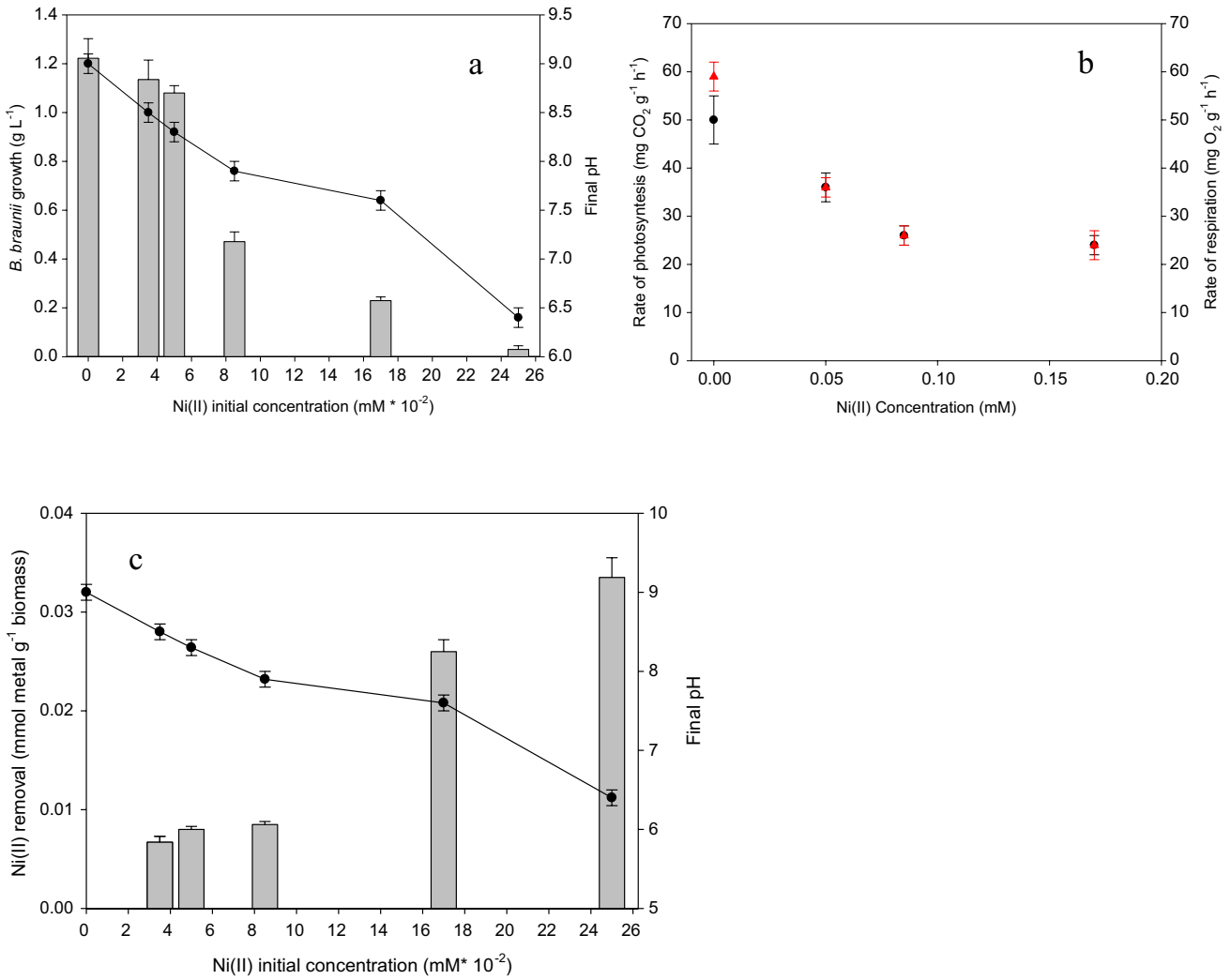

Fig. 4 **a**: Maximal growth (grey bars) of *B. braunii* and maximum culture pH reached (●) after 50 days of being exposed to different Ni(II) concentrations: control or 0, 0.035, 0.05, 0.085, 0.17 and 0.25 mM. Initial pH = 5.0; **b**: the effect of different Ni(II) concentrations (0, 0.05, 0.085 and 0.17 mM) on *B. braunii* respiration and net photosynthesis rate in terms of O_2 consumption (▲) and CO_2 assimilation (•) (mg g^{-1} h^{-1}); and **c**: Ni(II) removal (mmol g biomass^{-1}, grey bars) by *B. braunii* live biomass and maximum pH of the media (●) as a function of metal initial concentration (0, 0.035, 0.05, 0.085, 0.17 and 0.25 mM). Mean and standard deviation ($n = 3$)

Nickel biosorption experiments. Dead biomass

The adsorption capacity of *Botryococcus braunii* Ni(II) adsorption capacity of *B. braunii* dead biomass was studied in batch mode (130 rpm), at room temperature and at pH 6 ± 0.25 to identify the extent of non-metabolic processes in the metal removal capacity of this microalga. The quantity of Ni(II) adsorbed increased rapidly during the first hour and then reached a plateau (data not shown).

Figure 5a represents the nickel adsorption isotherm by *B. braunii*. The Hill isotherm model (Eq. (1) in Online Resources 7) (Foo and Hameed 2010) was applied and it fitted the experimental data ($R^2 = 0.998$). The highest Ni(II) adsorption capacity (q_{SH}) obtained for *B. braunii* when the Hill model was applied to experimental data was 1.23 ± 0.04 mmol g^{-1} and the sorption at very low initial metal concentrations was $q_0 = 0.08 \pm 0.03$ mmol g^{-1}.

When adsorption was analyzed at low Ni(II) concentrations (Figure in Online Resources 8) the adsorption capacity (q) is very low respect to concentrations higher than 3—4 mM. This may be interpreted as a distribution of few binding sites of high affinity (available at low Ni(II) concentrations) and many binding sites of low affinity, that are available for Ni(II) concentrations higher than 3–4 mM.

To estimate the quantity of protons released from the surface of the microalga when metal adsorption takes place, pH was kept constant (4, 5, 6 or 7) by the addition of NaOH (0.058 ± 0.001 M). The amount of proton released from the

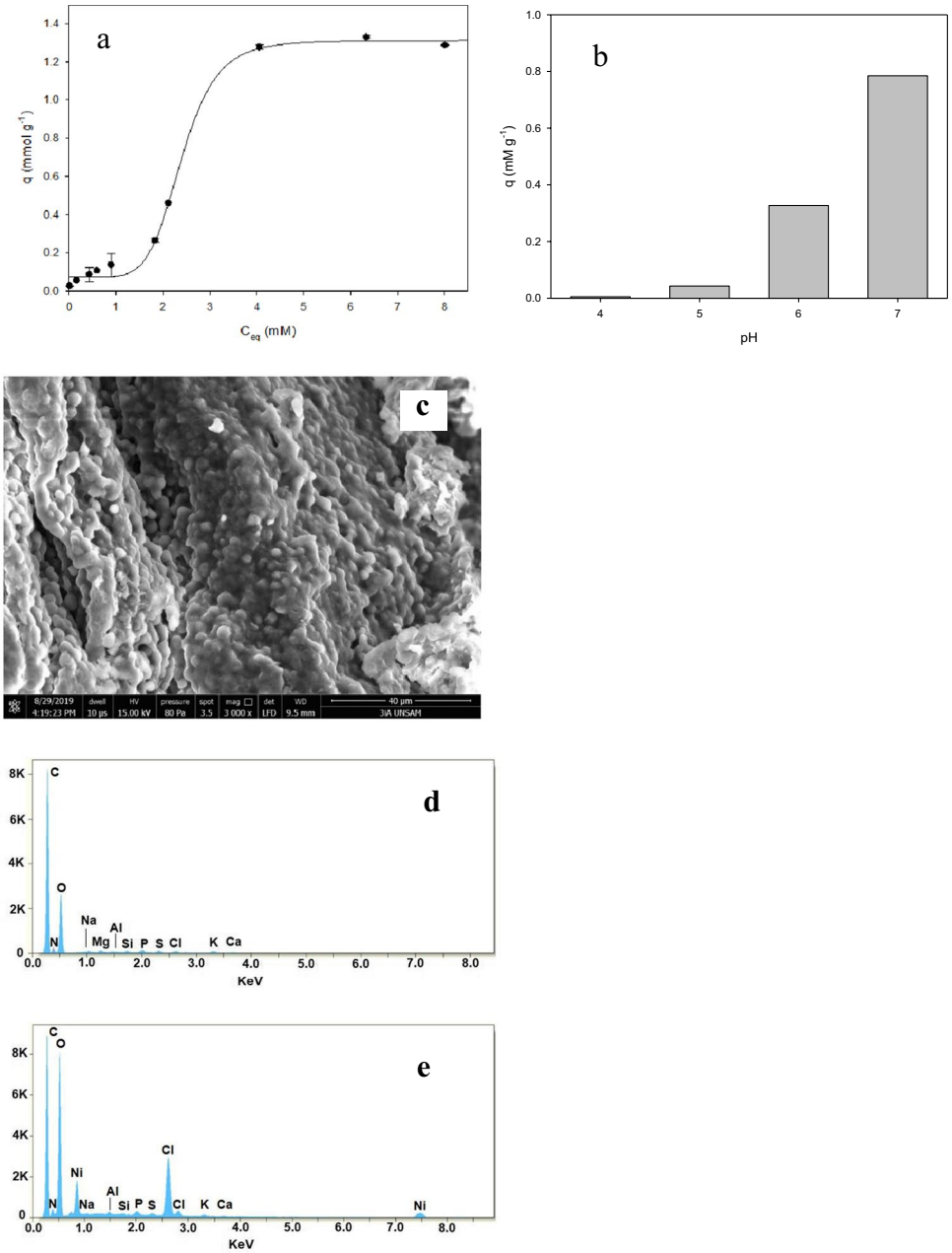

Fig. 5 **a**: Ni(II) adsorption isotherm by *B. braunii* dead biomass, at pH 5.5. Data shown are the mean values from two replicates experiments. Solid lines were calculated using Hill model (R^2: 0.998); **b**: Ni(II) adsorption capacities (q) by *B. braunii* dead biomass obtained at different pH values (4, 5, 6 and 7); **c**: Scanning Electron Microscopy (SEM) of *B. braunii* at 3000X and 15.0 kV where the structure of the surface of *B. braunii* within a matrix of polysaccharide can be seen.; and *B. braunii* EDS images: (**d**) before treatment and (**e**) after Ni(II) removal experiment

surface is proportional to the base added to the solution. At pH 4 there is no net adsorption on the biomass (Fig. 5b). At pH 5, 6 and 7 there is a stoichiometric correlation between nickel uptake and H^+ release by protonated biomass. This correlation changes with pH (Fig. 5b), being 1:5, 1:3 and 1:2 for pH values of 5, 6 and 7, respectively. These results assume that there is a process of ion-exchange between protons and nickel on the surface of the biomass.

Zeta potential results showed that the net charge of the surface is negative in all pH range studied (Figure in Online Resources 9). When zinc adsorption by *B. braunii* was previously studied (Areco et al. 2018), it was shown that the stoichiometric relationship between H^+ released from the surface and the Zn(II) uptake by protonated biomass was 1:1. In the present work the main nickel ion in solution at this pH range is Ni^{2+}, and even though ion-exchange is the main binding mechanism implicated in the biosorption of Ni(II) by *B. braunii*, it is not the only one, since there are other physical and chemical binding mechanism(s) implicated in the process (Davis et al. 2003).

Surface characterization. SEM–EDS

The surface of *B. braunii* was analyzed by scanning electron microscopy (SEM) (Fig. 5c), where the structure of the

surface of *B. braunii* within a matrix of polysaccharide can be seen. In order to verify Ni(II) adsorption, energy-dispersive X-ray spectroscopic (EDS) (Fig. 5d and e) and the metal surface recovery images (Fig. a and b in Online Resources 10) of *B. braunii* were conducted before and after nickel (initial metal concentration of 1 mM) adsorption. EDS show that there is no metal present on the surface of *B. braunii* in control experiments (Fig. 5d and Fig. a in Online Resources 10). After nickel adsorption experiments, the presence of the metal on the surface of the alga was demonstrated (Fig. 5e and Fig. b in Online Resources 10) and the relative amount of metal on the surface of *B. braunii* was ≈ 8%, relative to the total surface composition. These results are consistent with those previously presented in the present paper, where Ni(II) adsorption plays a key role in metal removal.

Discussion

The preset study shows a low *B. braunii* biomass productivity when it was cultivated with different nitrogen sources and concentrations. This slow growth and the linear kinetic observed suggest a limiting gas transfer rate (kinetic limitation) (Posten 2009; Le Gouic et al. 2021). Some authors have studied the effect of nitrogen source on the growth and hydrocarbon accumulation (Zhila et al. 2005; Cheng et al. 2014; Ruangsomboon 2015), others have studied the influence of nitrogen source and photoperiod on the synthesis of exopolysaccharides (Lupi et al. 1994; Wijihastuti et al. 2017) and Podder and Majumder (2017) studied the toxicity and bioremediation of arsenic by *B. braunii*. However, the extent of nickel or nitrogen remediation were not extensively studied, nor the physicochemical changes in the surrounding media due to nitrogen source in the culture media and how these changes affect the remediation processes. Since *B. braunii* has been widely studied for its capacity to produce hydrocarbons, exploring new biotechnological approaches may lead to the development of a variety of algae-based processes where the concept of circular economy may be applied.

The most common inorganic N sources assimilated by photosynthetic organisms are NO_3^-, NO_2^-, and NH_4^+ (Raven and Giordano 2016). The availability and concentrations of these N sources, which in general are small and change depending on environments, usually limit productivity and growth. (Sanz-Luque et al. 2015).

Our results show that there were significant differences ($p < 0.01$) on the productivities (P and P_v) of the cultures exposed to higher NO_3^- initials concentrations (Table 1). In all cases studied the cultures continue to grow for more than 25 days, even when nitrogen was totally consumed in the first 12 days. Then, if the culture grew (measuring cell number) under nitrogen starvation (when initial NO_3^- concentrations were 0.7 and 1.25 mM), there must have been a decrease in the cell size or a change on cellular composition. When nitrogen (NO_3^-) in the media were totally consumed (Fig. 1c) the algae may have reacted to the lack of nitrogen by the degradation of previously accumulated molecules containing nitrogen, such as glutamate (Sanz-Luque et al. 2015). This would explain why the algae kept on growing (Fig. 1a) when nitrogen (NO_3^-) was consumed at day 12 (Fig. 1c), even though there was a reduction on the photosynthesis rate when there was a lack of nitrogen (Fig. 1 Online Resources 4).

During the light periods (photosynthesis), CO_2 consumption resulted in a consequent rise in the pH (Eq. 4). When CO_2 concentration in solution is close to zero, the CO_2 transfer rate reaches a maximum, causing the kinetic limitation in growth that has already been mentioned. (Decostere et al. 2013; Le Gouic et al. 2021). During the dark periods (respiration) the intake of dissolved oxygen and simultaneous CO_2 release shifted the H_2CO_3/HCO_3^- equilibrium to the right (Eq. 5) (Decostere et al. 2013), resulting in a decrease of the pH during the dark cycles. This behavior is clearly seen in Fig. 2a and b.

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ and\ K_1 = \left[HCO_3^-\right].\frac{\left[H^+\right]}{\left[CO_2\right]} = 10^{-pKa1} \quad (4)$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ and\ K_2 = \frac{\left[CO_3^{2-}\right]}{\left[HCO_3^-\right]}.\left[H^+\right] = 10^{-pKa2} \quad (5)$$

When NO_3^- was the only nitrogen source in the medium an increase in pH was observed. This increase in pH is due to the metabolism of NO_3^- uptake that produces OH^- as Eq. (6) shows:

$$NO_3^- + 5.7CO_2 + 5.4H_2O \rightarrow C_{5.7}H_{9.8}O_{2.3}N + 8.25O_2 + OH^- \quad (6)$$

When nitrate was totally consumed (Fig. 2c), the pH remained constant with fluctuations associated with CO_2 uptake or release due to the light:dark cycles.

Results demonstrate that NH_4^+ at the concentrations and pH range studied in the present work was not toxic for *B. braunii* and also demonstrate the ability of this microalgae to live in acid environments, since the use of NH_4^+ as the nitrogen source (mainly on low-buffered media) results in the decrease of the pH of the media till values of ≈ 3 (Eq. 7) (Barsanti and Gualtieri 2014). The low pH in the system affects the NH_4^+ / NH_3 equilibrium, decreasing the concentration of NH_3, which is most toxic for microalgae. These results are different from others cited in the literature, where the use of NH_4^+ as N source decrease the algal biomass, with an irreversible toxicity in the late exponential growth (Lupi et al. 1994; Cheng et al. 2014).

$$NH_4^+ + 5.7CO_2 + 3.2H_2O \rightarrow C_{5.7}H_{9.8}O_{2.3}N + 5.7O_2 + H^+ \quad (7)$$

Knowledge and prediction of pH changes associated with the uptake of different nitrogen sources by *B. braunii* is very

important to design potential effluent treatment processes in which, they can be also used for the removal of heavy metals or will need post-process pH adjustment. Our results also demonstrate that *B. braunii* first consumed NH_4^+ and then NO_3^-. The interaction between NO_3^- and NH_4^+ uptake indicates that NH_4^+ itself inhibits NO_3^- uptake, maybe because NH_4^+ assimilation caused the feedback, inhibition and repression of the enzymes responsible for NO_3^- reduction (Stewart and Markello 1974; Sanz-Luque et al. 2015).

Microalgae biomass productivity is closely related with photosynthesis, and the photosynthesis- respiration metabolism can be affected by the presence of NO_3^- or NH_4^+. In the present study it was found that even though the growth of the algae was not affected when NH_4^+ was used as the nitrogen source (Fig. 3a), the rate of CO_2 uptake during the light cycles (photosynthesis) was considerably affected, decreasing about 70%. As depicted in Eqs. 4 and 5 the dissolution of gaseous CO_2 into water induces principally three carbon species: CO_2, HCO_3^-, and CO_3^{2-}. The concentration of total inorganic carbon and each component depends on the pH of the solution (Borowitzka 2016). CO_2 transfer rate from the atmosphere to the culture is a kinetic limiting factor for photosynthesis rate and it is regulated mainly by CO_2 solubility. Both temperature and pH influence solubility and the rate of hydration of CO_2. Increasing pH enhances the rate of CO_2 transfer into liquid phase (Borowitzka 2016). In the present work, when NH_4^+ was the only nitrogen source, the pH of the medium decreased till values bellow pH = 3 (Fig. 3d), then CO_2 transfer rate should have dropped, decreasing the total CO_2 uptake rate by the microalgae. Results also show an increased (≈ 65%) in O_2 respiratory uptake (respect to the control—BBM) when *B. braunii* was grown in media with NH_4^+ as the nitrogen source (Table Online Resources 5). These results agree with those obtained by Ohmori et al. (1984), where total CO_2 fixation decreased about 60% and respiratory uptake of O_2 increased by about 70% when *B. braunii* was cultivated with NH_4^+ as the nitrogen source.

Even though the metabolism of *B. braunii* is affected when nickel is present in the medium (Fig. 4b), *B. braunii* can live under high Ni(II) concentrations (up to 0.05 mM), and this is an advantage over other microalgae susceptible to low Ni(II) concentrations such as *Chlorella vulgaris* and *Chlorella* sp. (Santos et al. 2019; Macoustra et al. 2020), *Scenedesmus quadricauda* (Strejckova et al. 2019), *Phaeocystis antarctica* and *Cryothecomonas armigera* (Koppel et al. 2018), among others, making it suitable to be used in some industrial wastewater treatment.

Nickel is generally found at very low concentration levels (ppb) in the environment. In water and sewage, nickel can be dissolved or attached to suspended matter in the water. The concentration of nickel in river and lake water is very low, with the average being generally less than 10 ppb (smaller than the concentration used in the present work) (Küpper and Kroneck 2007). Nevertheless, many studies are reported in literature on the removal of nickel from wastewater produced by metal plating industries, where metals are concentrated in the effluents (Adeli et al. 2017; Ates and Basak 2021; Pérez Jiménez et al. 2021). In this sense, adsorption is a widely studied technique for the removal of heavy metals from wastewater (Long et al. 2018; Pérez Jiménez et al. 2021).

The main processes for the removal of different heavy metals by *B. braunii* are different. While Zn(II) removal is mainly due to metabolic processes (Areco et al. 2018), Ni(II) removal is mainly due to adsorption processes.

In this study the nickel adsorption capacity of *B. braunii* pretreated biomass at pH values of 4, 5, 6 and 7 were analyzed. When pH of the solution is higher than pKa of the functional groups present on the surface of the biomass, there are more deprotonated binding sites on the cell wall of the algae (Trinelli et al. 2013), which favors the uptake of cations present in solution and leads to a decrease in the pH of the medium. When zinc adsorption by *B. braunii* was previously studied (Areco et al. 2018), it was shown that the stoichiometric relationship between H^+ released from the surface and the Zn(II) uptake by protonated biomass was 1:1. In the present work the main nickel ion in solution at this pH range is Ni^{2+}, and even though ion-exchange is the main binding mechanism implicated in the biosorption of Ni(II) by *B. braunii*, it is not the only one, since there are other physical and chemical binding mechanism(s) implicated in the process (Davis et al. 2003).

Conclusions

The results obtained in the present work are a part of a series of experiments that lead to a full comprehension of the mechanisms involved in the ability of *B. braunii* to remove heavy metals and nutrients from contaminated environments. In previous works, we demonstrated its capacity to remove Zn(II) and Cu(II). The present work shows its capability to grow in a wide range of pH, its ability to use NO_3^- and/or NH_4^+ as nitrogen sources as well as to remediate them. The results also show that *B. braunii* was able to live and grow in media containing Ni(II), even when respiration and photosynthesis are affected by the metal. This demonstrates the feasibility of using *B. braunii* for the remediation of effluents containing variable Ni(II) concentrations, by means of metabolic (precipitation) and mainly non-metabolic (adsorption) approaches. EDS proved the existence of Ni(II) ions on the surface of the biomass after being exposed to the metal. *Botryococcus braunii* also can metabolically remove NO_3^-, NH_4^+ from aqueous solutions and that the pH of the medium varies depending on the nitrogen source: it became acid when the algae consumed NH_4^+; and basic when it consumes NO_3^-.

These results, along with those previously obtained by us are a novel approach to the potential of *B. braunii* to be used

in the remediation process of effluents containing a wide range of NO_3^-, NH_4^+, and heavy metals such as Cu(II), Zn(II) and Ni(II) and an advance on the study of this alga. Further studies need to be pursued in order to establish the applicability of this new technology in real effluents. For example elucidating the mechanism of adsorption and the extent of the exopolysaccharides on the adsorption of heavy metals, such as Ni(II) is a key finding to understand the physicochemical processes involved. Furthermore, including the main *B. braunii* biotechnological features proposed in the literature within the context of circular economy may lead to the development of a new microalgae-based technology, where the production of hydrocarbons (energy), lipids and other metabolites may be produced while remediating acid effluent loaded with variable concentrations of different pollutants.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10811-022-02847-3.

Acknowledgements The authors acknowledge Universidad Nacional de San Martin, CONICET and FONCyT for their financial support. And Andres Alfonso for his contributions to the present manuscript.

Author contribution All authors contributed to the present article. Dra. Areco and Dr. Curutchet contributed to the study conception, design and the analysis of the results. Material preparation and data collection were performed by Loreta Rojas, Victoria Passucci, Dr. Diego Noseda, Dr. Maria Mar Areco and to a lesser extent Nicolás Rotella. The first draft was written by Dr. Maria Mar Areco and all authors commented on previous versions of the manuscript. Final version was evaluated by Dr. Gustavo Curutchet. All authors have read and approved the final manuscript.

Funding This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (PIP 11220170100770CO); Fondo para la Investigación Científica y Tecnológica (FONCyT) (PICT 2017-2787; PICT 2016–2940; and PICT 2014–2386); and Universidad Nacional de San Martín (UNSAM Investiga. 25 años de Universidad 2018).

Data availability https://doi.org/10.17632/n9s9zgd79z.1

Declarations

Competing interests The authors declare that they have no competing interests.

References

Adeli M, Yamini Y, Faraji M (2017) Removal of copper, nickel and zinc by sodium dodecyl sulphate coated magnetite nanoparticles from water and wastewater samples. Arab J Chem 10:S514–S521

Areco MM, Cainzos V, Curutchet G (2013) Copper removal by *Botryococcus braunii* biomass with associated production of hydrocarbons. Adv Mat Res 825:528–531

Areco MM, Haug E, Curutchet G (2018) Studies on bioremediation of Zn and acid waters using *Botryococcus braunii*. J Environ Chem Eng 6:3849–3859

Ates N, Basak A (2021) Selective removal of aluminum, nickel and chromium ions by polymeric resins and natural zeolite from anodic plating wastewater. Int J Environ Health Res 31:102–119

Barsanti L, Gualtieri P (2014) Algae: anatomy, biochemistry, and biotechnology. CRC Press, Boca Raton

Borowitzka MA (2016) Algal physiology and large-scale outdoor cultures of microalgae. In: Borowitzka MA, Beardall J, Raven JA (eds) The physiology of microalgae. Springer, Dordrecht, pp 601–652

Cai T, Park SY, Li Y (2013) Nutrient recovery from wastewater streams by microalgae: Status and prospects. Renew Sust Energy Rev 19:360–369

Cheng H, Liu Q, Zhao G, Yu S, Yu S (2017) The comparison of three common microalgae for treating piggery wastewater. Desalination Water Treat 98:59–65

Cheng P, Wang J, Liu T (2014) Effects of nitrogen source and nitrogen supply model on the growth and hydrocarbon accumulation of immobilized biofilm cultivation of *B. braunii*. Bioresour Technol 166:527–533

Choi H-J, Yu S-W (2015) Influence of crude glycerol on the biomass and lipid content of microalgae. Biotechnol Biotech Equip 29:506–513

Ciesielczyk F, Bartczak P, Wieszczycka K, Siwińska-Stefańska K, Nowacka M, Jesionowski T (2013) Adsorption of Ni(II) from model solutions using co-precipitated inorganic oxides. Adsorption 19:423–434

da Conceição Gomes MA, Hauser-Davis RA, de Souza AN, Vitória AP (2016) Metal phytoremediation: General strategies, genetically modified plants and applications in metal nanoparticle contamination. Ecotoxicol Environ Saf 134:133–147

Daneshvar E, Zarrinmehr MJ, Kousha M, Hashtjin AM, Saratale GD, Maiti A, Vithanage M, Bhatnagar A (2019) Hexavalent chromium removal from water by microalgal-based materials: Adsorption, desorption and recovery studies. Bioresour Technol 293:122064

Davis TA, Volesky B, Vieira R (2000) *Sargassum* seaweed as biosorbent for heavy metals. Water Res 34:4270–4278

Davis TA, Volesky B, Mucci A (2003) A review of the biochemistry of heavy metal biosorption by brown algae. Water Res 37:4311–4330

Decostere B, Janssens N, Alvarado A, Maere T, Goethals P, Van Hulle SWH, Nopens I (2013) A combined respirometer-titrimeter for the determination of microalgae kinetics: Experimental data collection and modelling. Chem Eng J 222:85–93

Drexler ILC, Bekaan S, Eskandari Y, Yeh D (2013) Feasibility assessment of cultivating native and non-native algae species at different stages of a municipal treatment plant. Proc Water Environ Fed 2013:5402–5413

Dsikowitzky L, Botalova O, Illgut S, Bosowski S, Schwarzbauer J (2015) Identification of characteristic organic contaminants in wastewaters from modern paper production sites and subsequent tracing in a river. J Hazard Mater 300:254–262

Foo KY, Hameed BH (2010) Insights into the modeling of adsorption isotherm systems. Chem Eng J 156:2–10

Gonçalves AL, Pires JCM, Simões M (2017) A review on the use of microalgal consortia for wastewater treatment. Algal Res 24:403–415

Helaoui S, Boughattas I, Hattab S, Mkhinini M, Alphonse V, Livet A, Bousserhine N, Banni M (2020) Physiological, biochemical and transcriptomic responses of *Medicago sativa* to nickel exposure. Chemosphere 249:126121

Hussain MB, Ali S, Azam A, Hina S, Farooq MA, Ali B, Bharwana SA, Gill MB (2013) Morphological, physiological and biochemical responses of plants to nickel stress: A review. Afr J Agric Res 8:1596–1602

Jais NM, Mohamed R, Al-Gheethi AA, Hashim MKA (2017) The dual roles of phycoremediation of wet market wastewater for nutrients and heavy metals removal and microalgae biomass production. Clean Technol Environ Policy 19:37–52

Kheriji J, Tabassi D, Hamrouni B (2015) Removal of Cd (II) ions from aqueous solution and industrial effluent using reverse osmosis and nanofiltration membranes. Water Sci Technol 72:1206–1216

Kim J-Y, Kim H-W (2017) Photoautotrophic microalgae screening for tertiary treatment of livestock wastewater and bioresource recovery. Water (Basel) 9:192

Koppel DJ, Adams MS, King CK, Jolley DF (2018) Chronic toxicity of an environmentally relevant and equitoxic ratio of five metals to two Antarctic marine microalgae shows complex mixture interactivity. Environ Pollut 242:1319–1330

Küpper H, Kroneck PMH (2007) Nickel in the environment and its role in the metabolism of plants and cyanobacteria. In: Sigel A, Sigen H, Sigel RKO (eds) Nickel and Its Surprising Impact in Nature, vol 2. John Wiley & Sons, Chichester, pp 31–62

Le Gouic B, Marec H, Pruvost J, Cornet JF (2021) Investigation of growth limitation by CO_2 mass transfer and inorganic carbon source for the microalga *Chlorella vulgaris* in a dedicated photobioreactor. Chem Eng Sci 233:116388

Long J, Huang X, Fan X, Peng Y, Xia J (2018) Effective adsorption of nickel (II) with *Ulva lactuca* dried biomass: isotherms, kinetics and mechanisms. Water Sci Technol 78:156–164

Lupi FM, Fernandes HML, Tomé MM, Sá-Correia I, Novais JM (1994) Influence of nitrogen source and photoperiod on exopolysaccharide synthesis by the microalga *Botryococcus braunii* UC 58. Enzyme Microb Technol 16:546–550

Macoustra GK, Jolley DF, Stauber JL, Koppel DJ, Holland A (2020) Speciation of nickel and its toxicity to *Chlorella* sp. in the presence of three distinct dissolved organic matter (DOM). Chemosphere 273:128454

Malik A (2004) Metal bioremediation through growing cells. Environ Int 30:261–278

Mariam I, Kareya MS, Rehmanji M, Nesamma AA, Jutur PP (2021) Channeling of carbon flux towards carotenogenesis in *Botryococcus braunii*: A media engineering perspective. Front Microbiol 12:693106

Massa M, Buono S, Langellotti AL, Castaldo L, Martello A, Paduano A, Sacchi R, Fogliano V (2017) Evaluation of anaerobic digestates from different feedstocks as growth media for *Tetradesmus obliquus, Botryococcus braunii*, *Phaeodactylum tricornutum* and *Arthrospira maxima*. Nat Biotechnol 36:8–16

Matamoros V, Gutiérrez R, Ferrer I, García J, Bayona JM (2015) Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: a pilot-scale study. J Hazard Mater 288:34–42

Maxwell JR, Douglas AG, Eglinton G, McCormick A (1968) The botryococcenes—hydrocarbons of novel structure from the alga *Botryococcus braunii*, Kützing. Phytochemistry 7:2157–2171

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

Moersidik SS, Nugroho R, Handayani M, Kamilawatia MAP (2020) Optimization and reaction kinetics on the removal of nickel and COD from wastewater from electroplating industry using Electrocoagulation and advanced oxidation processes. Heliyon 6:e03319

Morales-Amaral MdM, Gómez-Serrano C, Acién FG, Fernández-Sevilla JM, Molina-Grima E (2015) Outdoor production of *Scenedesmus* sp. in thin-layer and raceway reactors using centrate from anaerobic digestion as the sole nutrient source. Algal Res 12:99–108

Nakamura H, Shiozaki T, Gonda N, Furuya K, Matsunaga S, Okada S (2017) Utilization of ammonium by the hydrocarbon-producing microalga, *Botryococcus braunii* Showa. Algal Res 25:445–451

Ohmori M, Wolf FR, Bassham JA (1984) *Botryococcus braunii* carbon/nitrogen metabolism as affected by ammonia addition. Arch Microbiol 140:101–106

Órpez R, Martínez ME, Hodaifa G, El Yousfi F, Jbari N, Sánchez S (2009) Growth of the microalga *Botryococcus braunii* in secondarily treated sewage. Desalination 246:625–630

Pérez Jiménez VA, Hernández-Montoya V, Ramírez-Montoya LA, Castillo-Borja F, Tovar-Gómez R, Montes-Morán MA (2021) Adsorption of impurities from nickel-plating baths using commercial sorbents to reduce wastewater discharges. J Environ Manage 284:112024

Podder MS, Majumder CB (2017) Toxicity and bioremediation of As(III) and As(V) in the green microalgae *Botryococcus braunii:* A laboratory study. Int J Phytoremed 19:157–173

Posten C (2009) Design principles of photo-bioreactors for cultivation of microalgae. Eng Life Sci 9:165–177

Raven JA, Giordano M (2016) Combined nitrogen. In: Borowitzka MA, Beardall J, Raven JA (eds) The physiology of microalgae. Springer, Dordrecht, pp 143–154

Renuka N, Sood A, Ratha SK, Prasanna R, Ahluwalia AS (2013) Evaluation of microalgal consortia for treatment of primary treated sewage effluent and biomass production. J Appl Phycol 25:1529–1537

Rice EW, Baird RB, Eaton AD (2017) Standard methods for the examination of water and wastewater. In: Standard methods for the examination of water and wastewater, 23rd edn. American Public Works Association/Water Environment Federation

Rinna F, Buono S, Cabanelas ITD, Nascimento IA, Sansone G, Barone CMA (2017) Wastewater treatment by microalgae can generate high quality biodiesel feedstock. J Water Process Eng 18:144–149

Ruangsomboon S (2015) Effects of different media and nitrogen sources and levels on growth and lipid of green microalga *Botryococcus braunii* KMITL and its biodiesel properties based on fatty acid composition. Bioresour Technol 191:377–384

Santos FM, Mazur LP, Mayer DA, Vilar VJP, Pires JCM (2019) Inhibition effect of zinc, cadmium, and nickel ions in microalgal growth and nutrient uptake from water: an experimental approach. Chem Eng J 366:385–367

Sanz-Luque E, Chamizo-Ampudia A, Llamas A, Galvan A, Fernandez E (2015) Understanding nitrate assimilation and its regulation in microalgae. Front Plant Sci 6:899

Stewart KM, Markello SJ (1974) Seasonal variations in concentrations of nitrate and total phosphorus, and calculated nutrient loading for six lakes in western New York. Hydrobiologia 44:61–89

Strejckova A, Dvorak M, Klejdus B, Krystofova O, Hedbavny J, Adam V, Huska D (2019) The strong reaction of simple phenolic acids during oxidative stress caused by nickel, cadmium and copper in the microalga *Scenedesmus quadricauda*. Nat Biotech 48:66–75

Sutherland DL, Ralph PJ (2019) Microalgal bioremediation of emerging contaminants-Opportunities and challenges. Water Res 164:114921

Ter Meulen GRB (1993) Delayed effects of chemicals in soils and sediments: "Chemical time bombs." In: Eijsackers HJP, Hamers T (eds) Integrated Soil and Sediment Research: A Basis for Proper Protection Project: Chemical Time Bombs. Springer, Dordrecht, pp 63–66

Trinelli MA, Areco MM, Dos Santos Afonso M (2013) Co-biosorption of copper and glyphosate by *Ulva lactuca*. Colloids Surf B 105:251–258

Tufo AE, Porzionato NF, Curutchet G (2018) Effects of pollution and bioleaching process on the mineral composition and texture of contaminated sediments of the Reconquista River, Argentina. Env Sci Pollut Res 25:21368–21384

Urrutia C, Yañez-Mansilla E, Jeison D (2019) Bioremoval of heavy metals from metal mine tailings water using microalgae biomass. Algal Res 43:101659

Wijihastuti RS, Moheimani NR, Bahri PA, Cosgrove JJ, Watanabe MM (2017) Growth and photosynthetic activity of *Botryococcus braunii* biofilms. J Appl Phycol 29:1123–1134

Xie P, Ho S-H, Peng J, Xu XJ, Chen C, Zhang ZF, Lee DJ, Ren NQ (2019) Dual purpose microalgae-based biorefinery for treating pharmaceuticals and personal care products (PPCPs) residues and biodiesel production. Sci Total Environ 688:253–261

Zhang Q, Yu Z, Zhu L, Ye T, Zuo J, Li X, Xiao B, Jin S (2018) Vertical-algal-biofilm enhanced raceway pond for cost-effective wastewater treatment and value-added products production. Water Res 139:144–157

Zhila NO, Kalacheva GS, Volova TG (2005) Effect of nitrogen limitation on the growth and lipid composition of the green alga *Botryococcus braunii* Kütz IPPAS H-252. Russ J Plant Physiol 52:311–319