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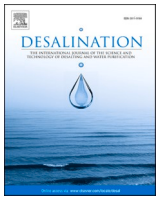

Biodesalination of saline aquaculture wastewater with simultaneous nutrient removal and biomass production using the microalgae *Arthrospira* and *Dunaliella* in a circular economy approach

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HIGHLIGHTS

- *A. platensis* and *D. salina* were cultivated in saline aquaculture wastewater.
- EC was reduced by up to 45 %.
- Biosorption and bioaccumulation likely contributed to biodesalination.
- Cultivation of *A. platensis* was scaled up without loss of performance.

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ABSTRACT

Freshwater scarcity is escalating due to factors such as climate change, droughts, increased demand, population growth, and poor water management practices. A promising sustainable solution to this challenge is biodesalination using microalgae. We demonstrate that *Arthrospira platensis* and *Dunaliella salina* can thrive in saline aquaculture wastewater, reducing both its salinity and the concentration of nutrients. The salinity removal ability was quantified through measurements of electrical conductivity (EC) and ICP-OES, revealing reductions in EC by up to 45 % (from 31.5 to 17.2 ms/cm) for *A. platensis* and 35 % (from 31.5 to 20.5 ms/cm) for *D. salina.* FESEM indicated the formation of a salt layer on the surface of both microalgae, suggesting biosorption and bioaccumulation as likely mechanisms. FTIR spectroscopy analysis demonstrated the binding of functional groups within the cell wall of *A. platensis* and the cell membrane of *D. salina* with the ions present in the medium. Scaling up the cultivation of *A. platensis* in a photobioreactor under non-sterile conditions validated the processes' potential for industrial-scale biodesalination. Although the treated water did not reach the standards for irrigation or potable use, this approach provides a preliminary desalination step, reducing burden on subsequent treatments and simultaneously providing nutrient removal and biomass production.

1. Introduction

There are many arid and semiarid regions in the world suffering from both low and inappropriately timed rainfall events [1,2]. In these regions, groundwater is a significant resource for agricultural and urban-industrial development. However, groundwater quality is deteriorating due to changes in precipitation patterns and increased evapotranspiration, along with excessive well drilling and overuse of water resources. Consequently, there is a notable increase in the concentration of total dissolved solids (TDS) in groundwater, leading to the significant issue of groundwater salinity in many urban areas [2–4]. The establishment of industries that can use saline groundwater is essential for further development in such areas. Notably, fish farming has emerged as a widespread industry that capitalizes on saline groundwater [5]. Although this industry creates added value and has an important role in the food supply chain, the issue of freshwater scarcity remains. To foster sustainable development in light of these challenges [6], it is necessary to both manage water resources effectively and adopt a circular economy approach, aiming to maximize the yield of products from every amount of water used. Additional processes introduced should have a

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low environmental footprint and should not require a high level of technology.

Desalination processes, usually of a physicochemical nature, such as reverse and forward osmosis, electrodialysis, multistage flash distillation, vapor compression distillation, and multiple effect distillation, are resource-intensive. As such, there is growing interest to augment them with biobased desalination processes that are easier to set up and operate, ensuring efficiency, durability, cost-effectiveness, and environmental sustainability. A current trend is the development of biodesalination processes that utilize living microorganisms [7,8]. Among these microorganisms, microalgae and cyanobacteria are particularly promising due to their autotrophic growth and their ability to fix carbon dioxide, positioning them as ideal for more environmentally friendly desalination approaches [9]. These approaches leverage biosorption and bioaccumulation processes. Biosorption is defined as the binding of ions to the cell surface, while bioaccumulation is the intracellular uptake of the ions and molecules [10]. From these two processes, biosorption contributes most to the biodesalination process and is a simple and cost-effective method to provide fresh water from saline and brackish sources while minimizing the environmental impact [8].

The desalination capability of several microalgae species has been studied, although not in aquaculture wastewater. This includes *Chlorella vulgaris* [11], *Dunaliella salina* [12], and species from the genera *Chlorella*, *Chlorococcum*, *Desmodesmus*, *Scenedesmus*, and *Monoraphidium* [13]. Notable recent successes include the biodesalination of a by-product of crude oil extraction using an indigenous algal consortium [14], the use of immobilized cells of *Chlamydomonas reinhardtii* to desalinate seawater [15], or the use of halophilic strains of *Scenedesmus* sp. and *Chlorella vulgaris* on a pilot scale [16]. A life cycle assessment of such algae-based desalination systems has confirmed a lower environmental footprint when compared to conventional desalination processes [17]. In particular, absorption was found to be the prevailing microalgae desalination mechanism (studied using *Scenedesmus obliquus*), which can be accurately predicted with a Langmuir isotherm adsorption model [18].

Previous studies on microalgae cultivation in saline aquaculture wastewater have focused solely on nutrient removal and biomass production. This includes utilizing *Chlorella minutissima* for treating wastewater from salmon aquaculture [19], *Neochloris* sp. for tilapia aquaculture [20], and *Scenedesmus obliquus* for shrimp (*Penaeus vannamei*) farming [21]. So far, research into bioremediation and biodesalination of saline aquaculture effluent has not been conducted.

This study focuses on cultivating microalgae in salmon aquaculture effluent to obtain estimates on 1) biomass productivity, 2) biodesalination efficiency, and 3) nutrient removal efficiency. The species selected for this research were *Arthrospira platensis* and *Dunaliella salina*. *D. salina* is a green microalgae that thrives in hyper-saline environments with salt concentrations above 300 g l^{-1} [22]. It lacks a cell wall and the biosorption occurs on the surface of the cell membrane. It is a key biological source of beta-carotene, with applications in the food, feed, and cosmetics industries [23]. *A. platensis* is a cyanobacterium containing high-value nutrients like proteins, polyunsaturated fatty acids, and vitamins, and is an established food supplement. Although *A. platensis* has not been reported in marine habitats, it is able to grow in fresh, brackish, or even seawater environments [24]. These two species were chosen for this study due to the established [25–27] and patented [28,29] technology for their production processes. Several companies produce biomass of these species on a large scale and products are available on the market (e.g., Earthrise Nutritionals (www.earthrise.com), Plankton Australia Pty Limited (www.planktonaustralia.com). Therefore, a biodesalination approach with these species has an additional economic advantage, as valuable products may be obtained.

This study's innovations include 1) investigating the desalination ability of *A. platensis*, 2) examining the desalination of actual saline aquaculture effluent, and 3) exploring the simultaneous biodesalination, nutrient removal, and biomass production of two microalgae species with commercial potential. To the best of our knowledge, no other study has reported on the desalination ability of *A. platensis* and there is no published work on biodesalination of saline aquaculture wastewater. Although nutrient removal and biomass production from aquaculture wastewater have been studied, their concurrent study with biodesalination is lacking.

The results pave the way toward cost-effective production of valuable biomass alongside the biodesalination and remediation of saline aquaculture wastewater. This, in turn, could facilitate the subsequent efficacy of physicochemical desalination processes. Taken together, leveraging microalgae may provide the necessary technology to connect necessary water treatment steps with biomass production within a circular economy framework.

2. Materials and methods

2.1. Strains and media

Dunaliella salina (INACC 0602) and *Arthrospira platensis* (INACC 2224) were obtained from the Iranian National Algae Culture Collection (INACC), Tehran, Iran. Zarrouk's medium [30,31] with an initial pH of 9 was used for the preculture of *A. platensis*. f/2 medium [32,33] with an initial pH of 7.5 was used for the preculture of *D. salina*. Cultures were maintained in their respective medium in an incubator (Jal Tajhiz, JTSL40, Karaj, Iran) under a PAR irradiance of 75 μmol m^{-2} s^{-1} provided by fluorescent lamps with a light: dark cycle of 16:8 h at a temperature of 28 ± 2 °C for *A. platensis* and 26 ± 2 °C for *D. salina*.

2.2. Experimental design, setup, and conditions

All cultivations were carried out in raw aquaculture wastewater in a non-sterile environment, and cultivation conditions were identical to those stated above if not mentioned otherwise. Two distinct aquaculture wastewater samples, AWW1 and AWW2, were sourced from two salmon trout rearing ponds, located at Ezhiyeh, Isfahan province (32.4378° N, 52.3787° E) and Kafran, Isfahan province (32.4275° N, 52.4745° E), with no pretreatment or addition of nutrients.

Twelve experimental cultivations were carried out in aerated (0.8 l/min) 1-l shake flasks containing 800 ml aquaculture wastewater (Table 1). Cultivations were either carried out in AWW1 or AWW2 and with either *A. platensis* or *D. salina*, resulting in four different treatment combinations. In experiments 1 to 4, the cells were washed twice prior to inoculation to prevent a carry-over of residual nutrients from the preculture. To this end, 200 ml of the preculture was centrifuged at 2500

Table 1
List of all experiments that were carried out in this study. The experiments differ by the cultivation vessel used, method of inoculation, microalgae species, and wastewater used as medium.

Exp. No.	Vessel	Inoculum	Species	Aquaculture wastewater
1	Shake flask	200 ml of washed preculture	*A. platensis*	AWW1
2				AWW2
3			*D. salina*	AWW1
4				AWW2
5		200 ml of preculture	*A. platensis*	AWW1
6				AWW2
7			*D. salina*	AWW1
8				AWW2
9		200 ml of preculture	*A. platensis*	Recycled medium from exp. 5 (AWW1)
10				Recycled medium from exp. 6 (AWW2)
11			*D. salina*	Recycled medium from exp. 7 (AWW1)
12				Recycled medium from exp. 8 (AWW2)
13	PBR	200 ml of preculture	*A. platensis*	AWW2

rpm for 20 min, the supernatant was discarded, and the cells were resuspended in distilled water. Experiments 5 to 8 were carried out identically, but the washing step was omitted. All eight cultivations lasted ten days. At the end of cultivations 5 to 8, the microalgae were removed by centrifugation, and the pH of the used wastewater was adjusted to either 9 (for the cultivation of *A. platensis*) or 7.5 (for the cultivation of *D. salina*) and reinoculated with the same microalgae species (experiments 9 to 12). These cultivations in recycled supernatant lasted for another seven days. All twelve cultivations were replicated three times.

Experiment 13 investigated the potential of scaling-up the biodesalination process using the most efficient culture from the previous twelve experiments. This culture, *A. platensis* grown in AWW2, was used to inoculate a photobioreactor (PBR). The PBR setup consisted of a glass vessel (working volume of 2700 ml) equipped with an aeration system (2 l/min) and a condenser (Fig. 1). The vessel was placed in an electric heating mantle, which maintained the temperature at 28 ± 0.2 °C. A condenser extracted the moisture from the outlet air and, thus, eliminated the effects of evaporation on the ion concentrations of the medium. The PBR was illuminated with a fluorescent lamp, located above the vessel in a fixed position. This scale-up experiment was also replicated three times.

2.3. Growth measurement

Growth was monitored every second day by measuring optical density at 680 nm (Shimadzu 240, Spectrophotometer, UV/Vis, Kyoto, Japon). At the same time, the morphology of the cells was controlled using an optical microscope (Hund, H600 Wilo-Prax, Wetzlar, Germany).

The biomass produced throughout the experiment was measured by gravimetric determination of the dry weight at the end of each experiment. 50 ml of the culture was centrifuged at 3228 *g* for 15 min and the supernatant was discarded. The pellet was washed with deionized water. This was repeated three times. The pellet was transferred to a preweighed crucible and dried at 60 °C for 24 h. After reaching room temperature in a desiccator, the crucible was weighed again, and the difference was used to calculate the dry weight.

2.4. Desalination properties and analytical methods

Desalination efficacy was gauged by electrical conductivity (EC) measurements of the medium using an EC meter (PrismaTech, model BPTC-500, Iran). Additional analyses of TDS, chemical oxygen demand (COD), total hardness, and concentrations of chloride, nitrate, sulfate, and phosphate in the culture medium were conducted according to standard methods for the examination of water and wastewater [34].

The common approach to determining the desalination extent is analyzing the concentration of ions in raw and treated wastewater [14–16,35]. Hence, the concentration of different ions (Ca^{2+}, Na^{+}, K^{+}, and Mg^{2+}) in the aquaculture wastewater, in the supernatant obtained at the end of experiments 5 to 8, and in a white precipitate that formed during experiments 6 and 13, were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES, Varian Vista-PRO, Australia).

In order to provide evidence for biosorption (one of the mechanisms of biodesalination), advanced analytical techniques such as FTIR, FE-SEM, EDS, and XPS are commonly employed [36,37]. In this study, FTIR, FE-SEM, and EDS analyses were conducted to confirm that biosorption had occurred.

To verify the biosorption of ions on the cell surface, cells were analyzed using Fourier-transform infrared spectroscopy (FTIR; Rayleigh, model WQF-510A, China), performing 32 scans per minute. The spectra were recorded in the range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}.

The adherence of salt particles on the cell surface of *A. platensis* and *D. salina* was inspected using a field emission scanning electron microscope (FE-SEM, Quanta 450, FEG, USA), run at 20 and 25 kV on dried samples coated with a thin layer of gold.

For the FE-SEM and FTIR analyses, the biomass samples of *A. platensis* were prepared to exclude precipitated salts. This involved allowing the precipitates in the culture from experiment 6 to settle for two hours, while the microalgal cells remained suspended. This was followed by the decantation and centrifugation (at 3228 *g* for 15 min) of the supernatant. The resulting pellet was washed three times with distilled water before drying at 60 °C for 24 h. The dried biomass was then used for the FE-SEM and FTIR analysis. Biomass samples of *D. salina* from experiment 8 followed the same preparation procedure, except for the settling and decanting step, which was unnecessary due to the absence of precipitates during cultivation.

2.5. Statistical analysis

All experiments and analyses were performed three times. The balanced experimental design allowed for full-factorial analyses of variance on growth and EC reduction in experiments 1 to 12, including the factors microalgae species (2 levels: *A. platensis*, *D. salina*), wastewater source (2 levels: AWW1, AWW2), and treatment variations (3

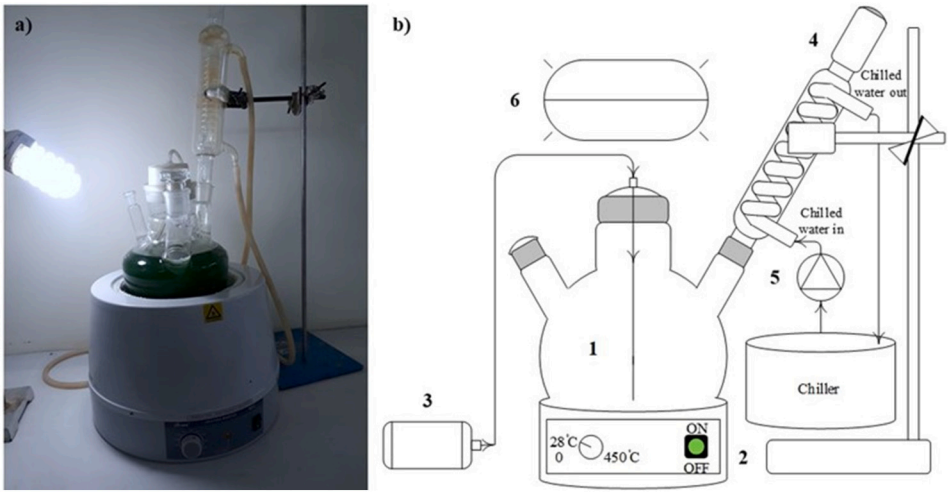

Fig. 1. a) Actual setup of the photobioreactor, b) Schematic of the photobioreactor consisting of 1) glass vessel, 2) electric heating mantle, 3) aeration system, 4) reflux condenser, 5) chilled water flow in the condenser, and 6) lighting system.

levels: inoculum washed/medium not recycled (experiments 1 to 4), inoculum not washed/medium not recycled (experiments 5 to 8), inoculum not washed/medium recycled (experiments 9 to 12), as well as their interactions. A Spearman rank correlation was applied to the average values from experiments 1 to 12 to investigate the relationship between growth and EC reduction. All statistical analyses were performed using R software, version 4.3.1.

3. Results and discussion

3.1. Cell growth and biodesalination

The microalgae demonstrated successful growth in all experiments (Fig. 2). The identity of both species was confirmed at the end of all cultivations through light microscopy (typical pictures with characteristic shapes are shown in Fig. 3). A significant difference in biomass yield

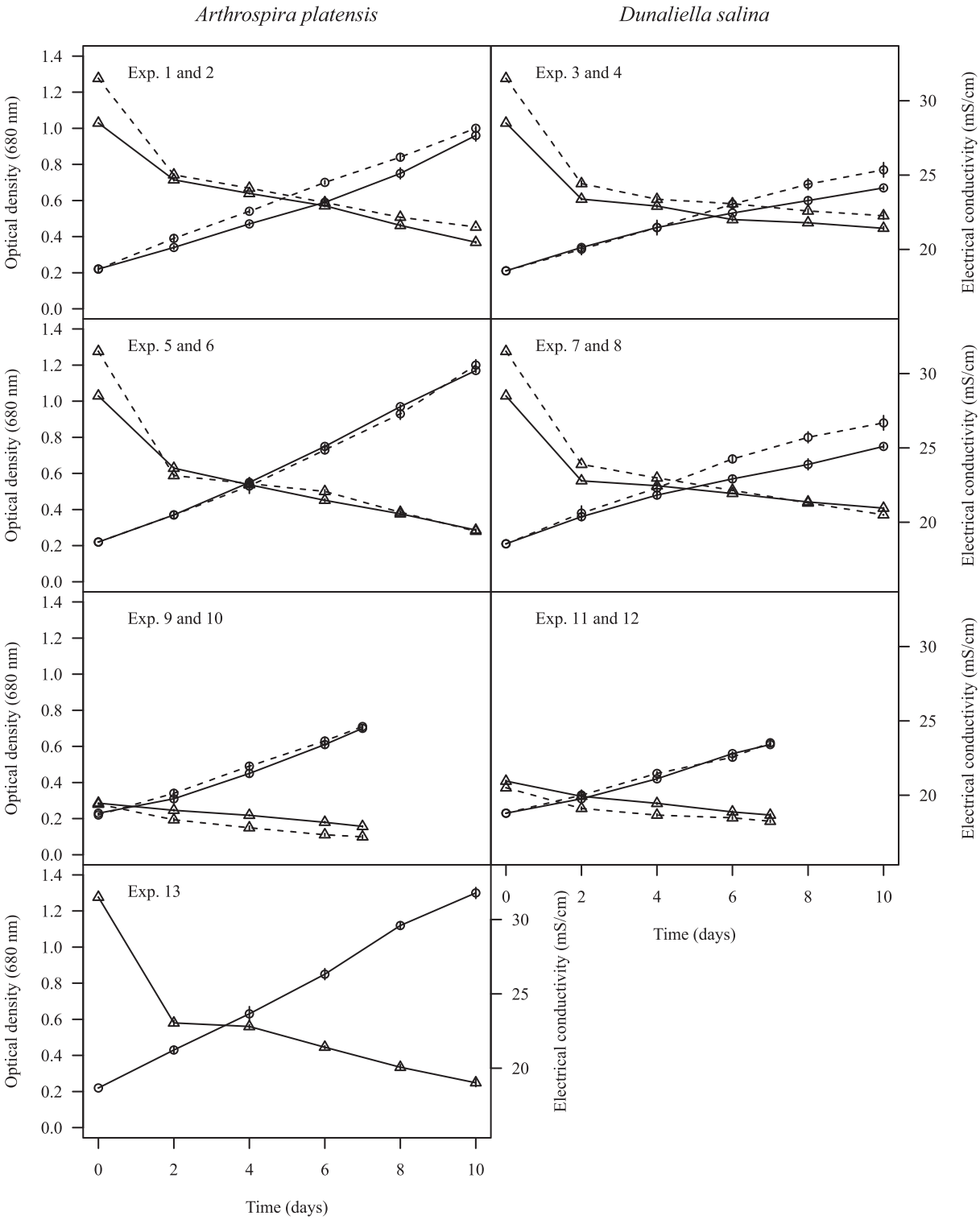

Fig. 2. Changes in optical density (circles) and electrical conductivity (triangles) during the cultivation of *A. platensis* and *D. salina* in aquaculture wastewater (AWW1: solid lines, AWW2: dashed lines) experiments 1–13 (see Table 1 for details). Data shows the mean of three replicates, error bars show the standard deviation.

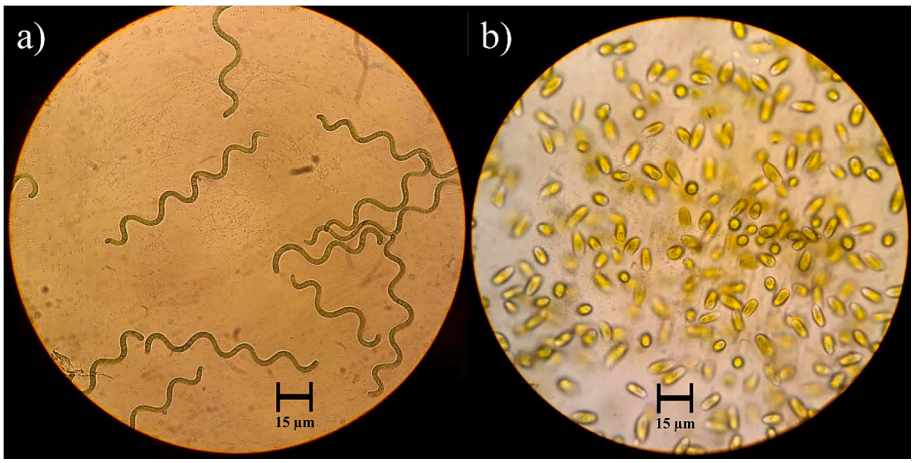

Fig. 3. Microscope images (400× magnification) of a) *A. platensis* (experiment 6), and b) *D. salina* (experiment 8).

was observed between the two species, with *A. platensis* consistently outperforming *D. salina* in terms of biomass production (Fig. 4; $F_{1,24} = 560.4.2$, $p < 0.001$). Both species demonstrated superior growth in AWW2 (Fig. 4; $F_{1,24} = 148.0$, $p < 0.001$), possibly due to its higher nutrient concentration, specifically organic carbon (measured by COD) and phosphorous (Table 2), which improved either autotrophic or even mixotrophic growth [38].

The effect of available nutrients was also observed when inocula were washed or when the medium was recycled (Fig. 4; $F_{2,31} = 1470.2$, $p < 0.001$). Washing the cells before inoculation in wastewater reduced their growth, likely because the residual nutrients from the preculture had been removed. Using recycled medium also reduced their growth, again, likely due to nutrient depletion.

EC decreased continuously throughout all experiments (Fig. 2), with a strong correlation observed with microalgal growth (Spearman's $\rho = 0.91$, $df = 11$, $p < 0.001$). Therefore, all effects observed corresponded to those found in the analysis of the growth data. EC was reduced more by

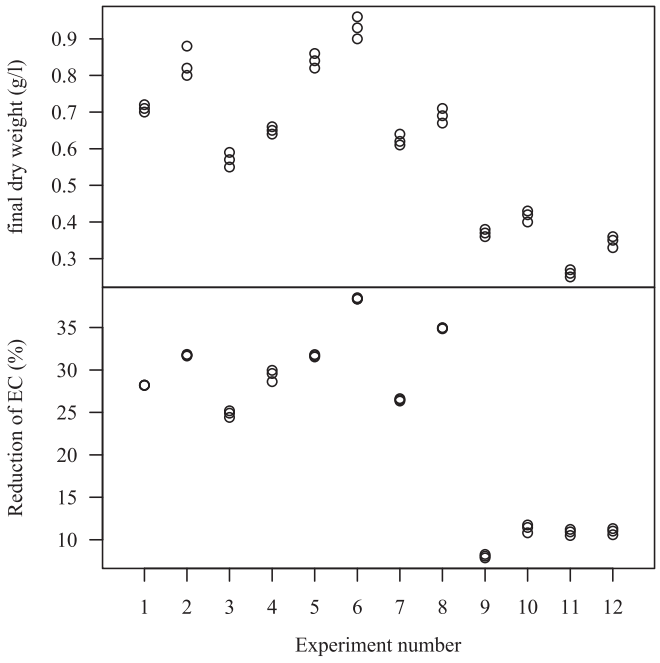

Fig. 4. Biomass dry weight (upper panel) of and percentage by which EC was reduced (lower panel) by either *A. platensis* or *D. salina* at the end of experiments 1–12 (see Table 1 for details). All cultivations were replicated three times.

A. platensis than by *D. salina* (Fig. 4; $F_{1,24} = 351.7$, $p < 0.001$) and more in AWW2 than in AWW1 (Fig. 4; $F_{1,24} = 1750.6$, $p < 0.001$). Furthermore, the availability of nutrients affected the decline in EC (Fig. 4; $F_{2,24} = 16{,}987.6$, $p < 0.001$). Pre-washing the cells prior to inoculation resulted in a less substantial reduction in EC, and the use of recycled medium significantly limited the subsequent EC decline.

Notably, the most pronounced EC reductions occurred in the first two days of cultivation (Fig. 2). This initial sharp decline did not coincide with a growth spurt, and, thus, may be due to biosorption, i.e., the physical adherence of ions to the algal surface as a key early mechanism. The cell walls of *A. platensis* and *D. salina* are composed of functional groups such as phosphate, hydroxyl, carboxyl, and amino groups, which are known for nutrient adsorption. Properties such as the large surface area and strong binding capacity of *A. platensis* and *D. salina* can also increase their potential for biosorption [39,40]. The consistent decrease in EC following the initial phase, although at a lower rate, suggests that bioaccumulation, including physiological and biochemical intracellular changes, cell growth, and nutrient removal [18,41,42] became the prevailing mechanism. Throughout the experiments, as *A. platensis* and *D. salina* grew, there was a corresponding decrease in EC. This pattern – first a rapid decrease followed by a sustained reduction in EC – was previously observed, when an indigenous microalgae consortium was used for saline wastewater desalination [14].

Repeating the microalgae cultivation in recycled wastewater (experiments 9 to 12) considerably improved the overall decline in EC. In total, EC was reduced by 37.2 % and 45.4 % when *A. platensis* was repeatedly cultivated in AWW1 and AWW2, respectively. EC was reduced by 34.5 % and 42.1 % when *D. salina* was repeatedly cultivated in AWW1 and AWW2, respectively.

Experiments 1 to 12 demonstrated the ability of both *A. platensis* and *D. salina* to perform biodesalination on a smaller scale. Notably, the most significant decline in EC was observed when *A. platensis* was cultivated in AWW2 (experiment 6). This successful outcome led to scaling up and repeating the experiment in a larger PBR, which yielded results (Fig. 2g) that were very similar in terms of final optical density (1.2 and 1.3 in experiments 6 and 13, respectively), final dry weight (0.93 g l^{-1} and 0.98 g l^{-1}), and EC reduction (38 % and 40 %). These findings suggest that the biodesalination efficiency observed on a small scale can also be achieved in a larger system. Such scalability can potentially streamline and reduce the costs of subsequent desalination steps while concurrently yielding valuable biomass containing protein, carbohydrates, and fatty acids [43–45].

Detailed measurements of selected ions and further variables associated with salinity provided further evidence for the salinity reduction achieved through the cultivation process (Table 2). It is important to note that while EC is indicative of the presence of ions, TDS includes

Table 2
Analysis of aquaculture wastewater before (AWW1 and AWW2) and after cultivation of *A. platensis* (Ap) and *D. salina* (Ds). Data shown are mean ± SD of three replicates.

Parameter	AWW1	AWW2	Exp. 5 (AWW1, Ap)	Exp. 6 (AWW2, Ap)	Exp. 7 (AWW1, Ds)	Exp. 8 (AWW2, Ds)
Ca^{2+} (mg/l)	34.46 ± 0.97	583 ± 1.1	1.72 ± 0.06	15.36 ± 0.64	12.65 ± 0.32	18.46 ± 0.74
Mg^{2+} (mg/l)	1032 ± 3	710 ± 3	306 ± 1	53.20 ± 1	549 ± 2	453 ± 1
Na^{+} (mg/l)	10,005 ± 36	11,017 ± 49	6314 ± 27	7303 ± 28	4662 ± 21	6257 ± 24
Cl^{-} (mg/l)	9926 ± 41	11,255.77 ± 56	8200 ± 36	999.42 ± 14	6544.07 ± 30	5241.65 ± 21
K^{+} (mg/l)	48.75 ± 0.19	51 ± 0.26	46.42 ± 0.23	35.69 ± 0.12	39.19 ± 0.16	42.94 ± 0.14
PO_4^{3-} (mg/l)	0.23 ± 0.01	0.57 ± 0.06	0	0	0	0
SO_4^{2-} (mg/l)	2135.11 ± 13.8	2001.20 ± 13.6	953.40 ± 8.9	1516.11 ± 12.4	1141.40 ± doi:10.2	1044.25 ± 9.1
Total hardness (mg/l)	4847 ± 19	6408 ± 25	2858.57 ± 13	4837 ± 16	3125 ± 15	6000 ± 23
Total nitrogen (mg/l)	6.68 ± 0.29	5.97 ± 0.2	1.56 ± 0.08	1.54 ± 0.15	1.28 ± 0.09	1.42 ± 0.1
COD (mg/l)	198.10 ± 1.5	268.66 ± 2.3	61.33 ± 0.69	99.16 ± 1.02	103 ± 1.4	184.01 ± 1.12
TDS (mg/l)	18,000 ± 100	21,000 ± 150	13,000 ± 50	14,000 ± 50	14,500 ± 100	15,000 ± 100
EC (ms/cm)	28.50 ± 0.19	31.50 ± 0.24	19.47 ± 0.15	19.40 ± 0.2	20.95 ± 0.3	20.50 ± 0.21
pH	8.30 ± 0.2	8.13 ± 0.3	9.40 ± 0.1	9.30 ± 0.4	8.41 ± 0.1	8.44 ± 0.4

both the presence of ions and dissolved organic material [46,47]. As microalgae absorb and/or consume both ions and dissolved organic material during growth, a reduction in these values before and after microalgal cultivation is expected [48].

The overall decrease in various chemical parameters (Table 2) confirmed the observed reduction in EC (Fig. 4). Along with the EC reduction in experiment 6 (38 %), TDS was reduced by 33 % (from 21,000 mg/l to 14,000 mg/l), total hardness was reduced by 25 % (from 6408 to 4837 mg/l), and the concentration of all ions measured decreased as well. Notably, both species completely removed phosphate, and approximately 75 % of total nitrogen from the medium. There were differences in ion removal efficiency between the two species, which could be attributed to differences in their elementary compositions [49,50]. For example, *A. platensis* removed 91 % of chloride and 33 % of sodium (Table 2; experiment 6), while *D. salina* removed 53 % of chloride and 43 % of sodium (Table 2; experiment 8).

Although biodesalination of aquaculture wastewater was successfully achieved, as evidenced by substantial decreases in EC and other values (Table 2), permissible levels for irrigation were not reached (2 ms/cm for EC and 50 mg/l for COD (https://www.doe.ir/)). This is a repetition of limitations found in previous studies [13,41,49] and may necessitate the employment of an additional treatment step. However, the biodesalination pretreatment described here reduces the burden on subsequent desalination methods, which in turn, consume less energy and have a lower environmental impact. Therefore, it is recommended to integrate this biodesalination process as a preliminary treatment before applying more energy-intensive physicochemical desalination methods.

Another salt removal mechanism that was observed was the formation of white sediments during the cultivation of *A. platensis* in wastewater (experiments 5, 6 (Fig. 5a), and 13 (Fig. 5b)). It appears that, in addition to biosorption and bioaccumulation, precipitation of ions in the culture medium of *A. platensis* was another biodesalination mechanism. The precipitation might be the result of the pH rise during the cultivation of *A. platensis* (e.g., from 8.13 to 9.30 in experiment 6; Table 2). The shift in pH can affect the solubility and precipitation of calcium hydroxide, as per the following equilibrium equation:

$$Ca(OH)_2\,(s) \rightleftharpoons Ca^{2+}\,(aq) + 2OH^{-}\,(aq)$$

An increase in hydroxyl ions (due to the metabolic activity of *A. platensis* in experiments 5, 6, and 13) causes a shift in the equilibrium, leading to the precipitation of Ca^{2+} ions as $Ca(OH)_2$ [51]. A similar explanation also applies to magnesium and potassium hydroxide. Elemental analysis of the sediment supported this, identifying a composition of 36 % calcium, sodium, magnesium, and potassium. Thus, *A. platensis* removes ions not only by biosorption and bioaccumulation

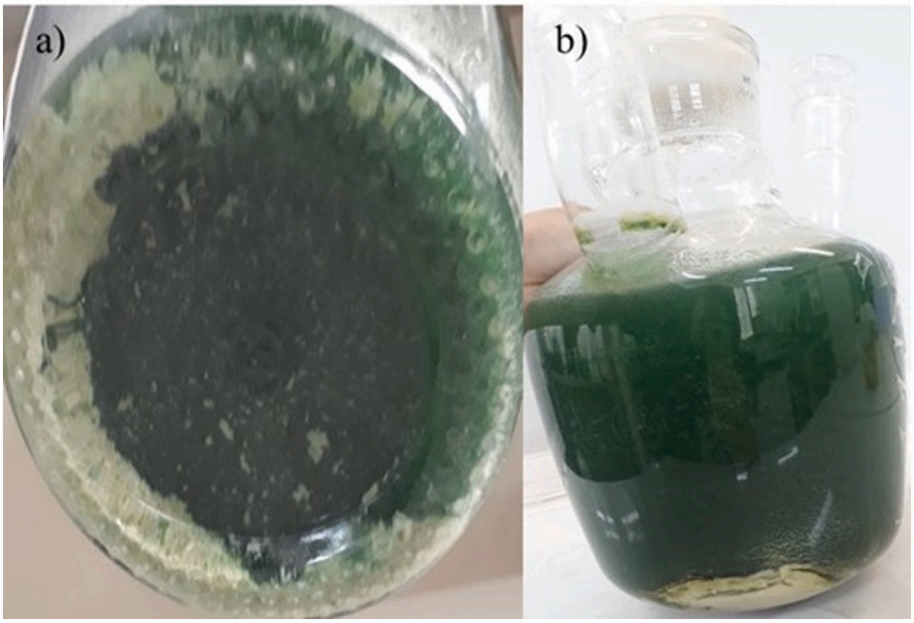

Fig. 5. White sediments that were formed during the cultivation of *A. platensis* in AWW2 in a) experiment 6 and b) experiment 13.

but also through sediment formation (due to the pH increase), which provides an additional advantage for the biodesalination of wastewater.

3.2. Structural features of salt biosorption and bioaccumulation

3.2.1. FE-SEM analysis

Biosorption of salt particles on the surface of the biomass of *A. platensis* and *D. salina* was confirmed by comparison of the FE-SEM images of the microalgal biomass before and after biodesalination (Fig. 6). A comparison of the topographical structures before and after desalination showed evidence of sediments that were acquired during the cultivation both in *A. platensis* (Fig. 6a and Fig. 6b, respectively) and *D. salina* (Fig. 6c and Fig. 6d). In addition, EDS (Energy-dispersive X-ray spectroscopy) analysis confirmed the presence of a low amount of Na^{+} and Mg^{2+} before biodesalination in both species (Fig. 6a and Fig. 6c) and adsorption of various ions such as Mn^{2+}, Ca^{2+}, Fe^{2+}, and additional Mg^{2+} and Na^{+} on the surface of *A. platensis* after biodesalination (Fig. 6b), and adsorption of ions such as Ca^{2+}, K^{+}, and additional Mg^{2+} and Na^{+} on the surface of *D. salina* after biodesalination (Fig. 6d). In addition, the EDS data quantifying biomass ion content (Table A.1) highlighted substantial differences before and after desalination. For instance, the concentration of Ca^{2+} in the biomass of *A. platensis* after biodesalination was 36.31 %, while it was not detectable before.

The EDS data confirms the biosorption ability of both microalgae and their ability to bind light metal ions such as Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} to the cell surfaces' acidic functional groups during the experiments. However, in the case of *A. platensis*, ion exchange, as one of the working mechanisms in biosorption, led to the release of some of the light metal ions, while Mn^{2+} and Fe^{2+} were absorbed on the cell surface [52,53]. *Spirulina* sp. showed the ability to replace naturally bound light metal ions such as Na^{+}, K^{+}, and Ca^{2+} with Co^{2+}, Cu^{2+}, Mn^{2+}, and Zn^{2+} [54]. FE-SEM images and EDS results of a study on the biodesalination ability

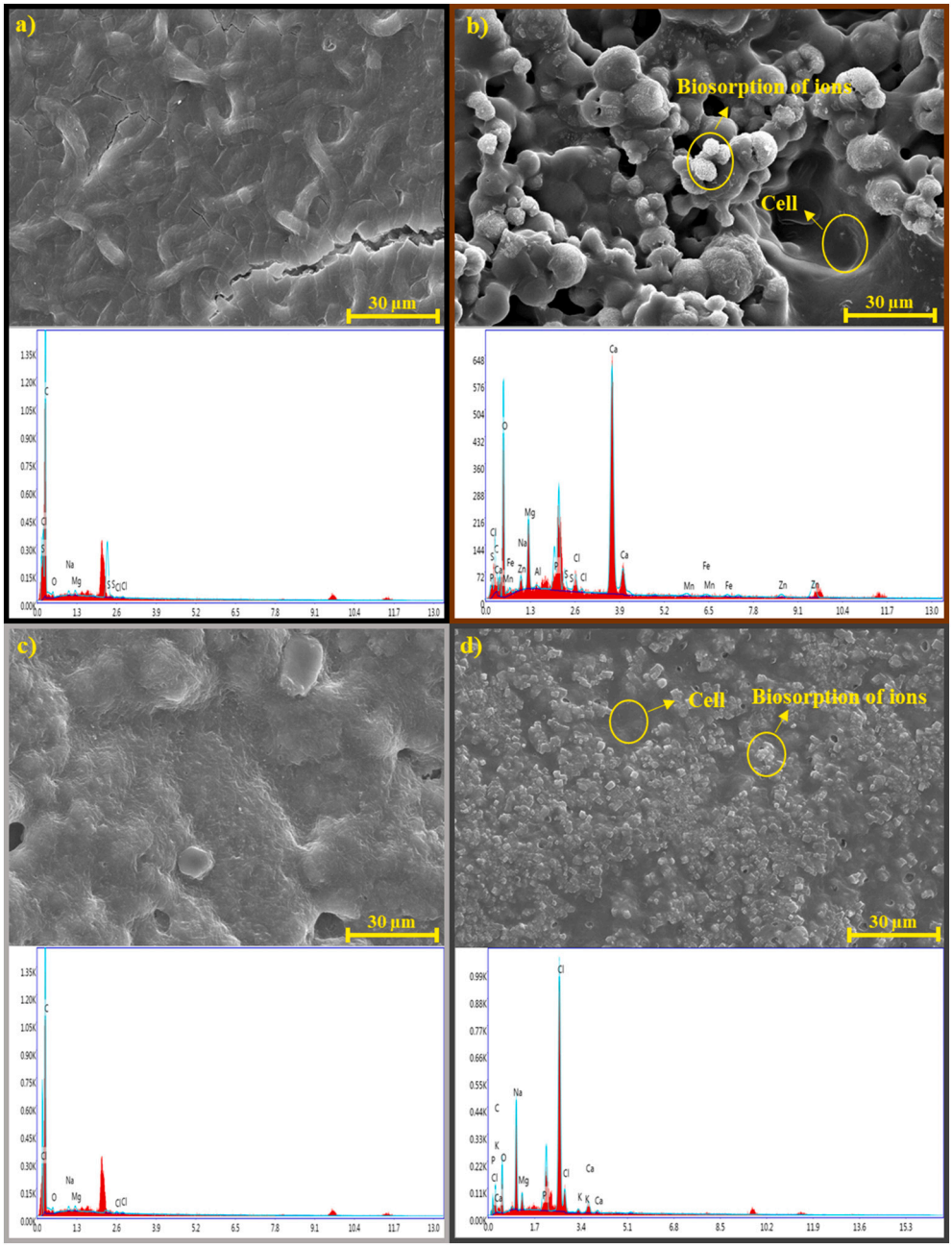

Fig. 6. FE-SEM and EDS images of dried biomass of *A. platensis* a) before and b) after biodesalination and of *D. salina* c) before and d) after biodesalination.

of the cyanobacterium *Phormidium keutzingianum* also confirm the results of this study [55].

3.2.2. FTIR analysis

FTIR spectroscopy was employed (Fig. 7) to confirm the biosorption of ions to the functional groups of the cell walls of the microalgae *A. platensis* and *D. salina*.

A. platensis has a cell wall made up of multilayers of glucan and peptidoglycan polymers, formed with molecule chains composed of sugars and/or amino acids. The outside of the cell wall is covered with a layer of acidic polysaccharides [56] that play an important role in enhancing biosorption [57]. Contrary to *A. platensis*, *D. salina* lacks a cell wall and the cell is enclosed by an elastic plasma membrane [58]. Hence, the cell walls of *A. platensis* and the plasma membrane of *D. salina* consist of protein, fatty acids, and polysaccharides with different functional groups such as carboxyl (e.g., fatty acid and amino acids), hydroxyl (e.g., polysaccharides, proteins), amine (e.g., proteins), etc., that possess positive and negative charges capable of absorbing ions from the culture medium such as Cl^-, Na^+, Mg^{2+}, Ca^{2+}, etc.

The FTIR results showed a change in the transmittance values after biodesalination, suggesting ion adsorption by microalgae [59]. Results also demonstrated the broadening of peak values at 3409 cm^{-1} when comparing the initial biomass of *A. platensis* and *D. salina* to the biomass obtained after the cultivation in experiments 5 to 8. This broadening of the peak is typically indicative of NH or OH stretching [18,60], suggesting that negatively charged functional groups within the biomass interacted with positively charged ions in the culture medium, such as Na^+. Additionally, the positively charged protein structural components represented by amide I and amide II bands, with the corresponding wave numbers of 1626 cm^{-1} and 1544 cm^{-1}, respectively, absorbed negative ions such as Cl^- [18,60,61]. The lipids showed the corresponding peaks at 2922 cm^{-1}, with functional groups of symmetric CH_3 stretching or asymmetric CH_2 stretching [55,62], and 619 cm^{-1} with functional groups of CH stretching [62], which participated in the absorption of salt ions.

The above-mentioned functional groups and their respective wavenumbers were in the outer envelope of both microalgae, i.e., *A. platensis* and *D. salina*. However, the functional groups of the polysaccharide structure of the *A. platensis* cell wall, which include glycosidic linkages [63], CH_2 stretching [64], and anti-symmetric stretching of the C–O–C bridge [61], demonstrate interactions with salt ions within the culture medium, as evidenced by the corresponding peaks at 1024 cm^{-1}, 1051 cm^{-1}, and 1153 cm^{-1} (Fig. 7).

4. Conclusion

The results of this study established that the microalgae *A. platensis* and *D. salina* can be cultivated in saline aquaculture wastewater to remove nutrients and produce biomass while concurrently reducing salinity. Over a 10-day cultivation period in aquaculture wastewater, these microalgae reduced EC (ca. 30 to 38 %), effectively lowering COD, TDS, and extracting nutrients and ions such as nitrogen, phosphate, Ca^{2+}, Mg^{2+}, Na^+, K^+, and Cl^-. Moreover, our findings reveal that *A. platensis*, which can reduce EC by up to 45 %, exhibits a higher biodesalination capability than *D. salina*, especially when leveraging recycled cultivation medium. While the desalination method used here did not reach thresholds for irrigation or potable water standards, it offers a significant preliminary treatment option that can reduce the burden on subsequent, more energy-intensive, physicochemical desalination methods. The approach employed here can be carried out in a non-sterile environment, paving the way for scalable applications and potential commercial viability. Importantly, the study highlights the potential of employing a circular economy approach to wastewater treatment, where water is not only purified but also transformed into a source of commercially valuable by-products. The adoption of such biotechnological strategies aligns with sustainable development goals by offering an environmentally friendly solution that contributes to the conservation of freshwater resources and provides a blueprint for future research and industrial application.

CRediT authorship contribution statement

Marzieh Mirzaei: Writing – review & editing, Visualization, Validation, Resources, Methodology, Investigation. **Mohammadhadi Jazini:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Ghazaleh Aminiershad:** Writing – review & editing, Writing – original draft, Visualization, Investigation. **Dominik Refardt:** Writing – review & editing, Visualization, Supervision, Software, Funding acquisition, Formal analysis.

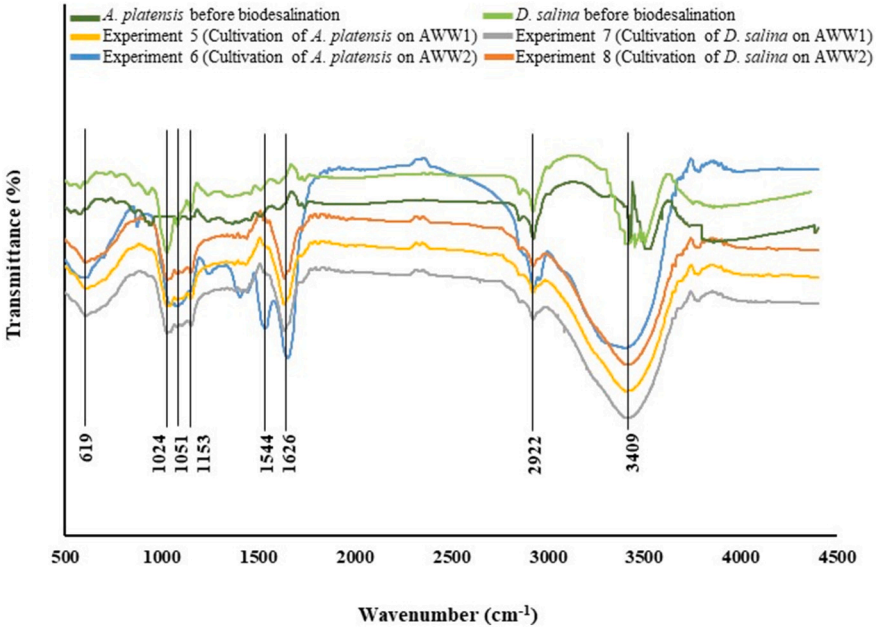

Fig. 7. FTIR spectra of dried microalgal biomass (*A. platensis* and *D. salina*) before and after biodesalination in different experiments.

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

Data will be made available on request.

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Appendix A

Table A.1
EDX analysis showing the elemental composition (% of total weight) of the biomass of *A. platensis* (Exp. 6) and *D. salina* (Exp. 8) biomass before and after desalination.

Element	Exp. 6		Exp. 8	
	before	after	before	after
C	89.08	8.56	93.38	22.26
O	1.74	38.22	3.58	19.38
Na	0.59	1.24	1.35	16.85
Mg	0.45	5.66	1.08	2.32
Al		0.28		
P		3.25		2.09
S	7.98	1.56		
Cl	0.16	1.60	0.61	34.00
K				0.65
Ca		36.31		2.45
Mn		0.45		
Fe		0.53		
Zn		2.34		

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