



## Review

Using wastewater as a cultivation alternative for microalga *Dunaliella salina*: Potentials and challenges

Gleison de Souza Celente<sup>a,b,c</sup>, Rosana de Cassia de Souza Schneider<sup>a,b</sup>,  
Tiele Medianeira Rizzetti<sup>a,b</sup>, Eduardo Alcayaga Lobo<sup>a</sup>, Yixing Sui<sup>c,\*</sup>

<sup>a</sup> Environmental Technology Post-graduation Program, University of Santa Cruz do Sul, Santa Cruz do Sul, RS, Brazil

<sup>b</sup> Centre of Excellence in Oleochemical and Biotechnological Products and Processes, University of Santa Cruz do Sul, Santa Cruz do Sul, RS, Brazil

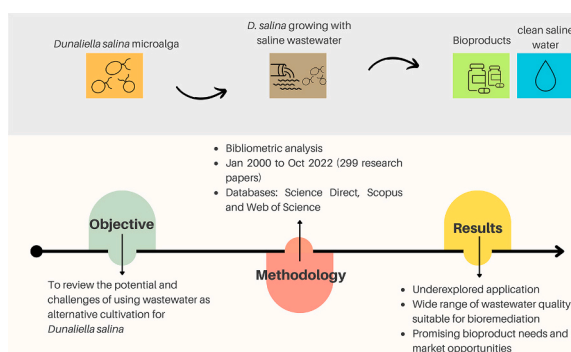
<sup>c</sup> School of Science, Faculty of Engineering and Science, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK



## HIGHLIGHTS

- *Dunaliella salina* is an effective solution for saline wastewater treatment.
- *D. salina* plays dual role: wastewater treatment and bioproduct generation.
- The review proved *D. salina*'s adaptable growth across diverse wastewater sources.
- The review charted a path for future research for sustainable bioproduct yield.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

Editor: Huu Hao Ngo

## Keywords:

*Dunaliella salina*  
Saline wastewater treatment  
Biomass valorization

## ABSTRACT

Untreated or poorly treated wastewater still represents environmental issues world-widely. Wastewater, especially saline wastewater treatment, is still primarily associated with high costs from physical and chemical processes, as high salinity hinders biological treatment. One favourable way is to find the suitable biological pathways and organisms to improve the biological treatment efficiency. In this context, halophilic microorganisms could be strong candidates to address the economics and effectiveness of the saline wastewater treatment process. *Dunaliella salina* is a photoautotrophic microalga that grows in saline environments. It is known for producing marketable bio-compounds such as carotenoids, lipids, and proteins. A biological treatment based on

**Abbreviations:** ATP, Adenosine triphosphate; APX, Ascorbate peroxidase; BCF, Bioconcentration factor; BOD<sub>5</sub>, Biological oxygen demand in five days; CA, Carbonic anhydrase; CAH1, Carbonic anhydrase 1; CAH2, Carbonic anhydrase 2; COD, Chemical oxygen demand; DBP, Di-n-butyl phthalate; DEP, Diethyl phthalate; DMA, Dimethylarsinic acid; DNA, Deoxyribonucleic acid; DOWSF, Diesel-oil water-soluble fraction; EC<sub>50</sub>, Half maximal effective concentration; EPS, Extracellular polymeric substances; Gly, Glycine; GPX, Glutathione peroxidase; GR, Glutathione reductase; GS, Glutamine synthetase; GST, Glutathione S-transferase; LHCII, Light-harvesting chlorophyll-protein complex; NAD(P)H, Nicotinamide adenine dinucleotide phosphate hydrogen; NADP<sup>+</sup>, Nicotinamide adenine dinucleotide phosphate; NTU, Nephelometric turbidity units; PAH, Polycyclic aromatic hydrocarbon; PC, Phytochelatin; POD, Peroxidase; RNA, Ribonucleic acid; ROS, Reactive oxygen species; RuBisCo, Ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, Ribulose-1,5-bisphosphate; SOD, Superoxide dismutase; TDS, Total dissolved soluble; TSS, Total suspended solids;  $\gamma$ -Glu-Cys, Gamma-glutamylcysteine.

\* Corresponding author.

E-mail address: [Y.Sui@greenwich.ac.uk](mailto:Y.Sui@greenwich.ac.uk) (Y. Sui).

<https://doi.org/10.1016/j.scitotenv.2023.168812>

Received 18 August 2023; Received in revised form 3 November 2023; Accepted 21 November 2023

Available online 23 November 2023

0048-9697/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

*D. salina* cultivation offers the opportunity to treat saline wastewater, reducing the threat of possible eutrophication from inappropriate discharge. At the same time, *D. salina* cultivation could yield compounds of industrial relevance to turn saline wastewater treatment into a profitable and sustainable process. Most research on *D. salina* has primarily focused on bioproduct generation, leaving thorough reviews of its application in wastewater treatment inadequate. This paper discusses the future challenges and opportunities of using *D. salina* to treat wastewater from different sources. The main conclusions are (1) *D. salina* effectively recovers some heavy metals (driven by metal binding capacity and exposure time) and nutrients (driven by pH, their bioavailability, and functional groups in the cell); (2) salinity plays a significant role in bioproducts generation, and (3) wastewater can be combined with the generation of bioproducts.

## 1. Introduction

Wastewater treatment still translates into high expenses and tedious tasks for decision-makers due to strict treatment quality standards, high-cost treatment operating systems, and lack of financial support and technical know-how (Dutta et al., 2021). In addition to meeting quality standards, wastewater treatment systems must (i) minimize the resource demand and assist in their valorisation, (ii) recover nutrients (Cobo et al., 2018), (iii) reduce sludge generation (Ding et al. 2021), and (iv) follow social and economic aspects to be sustainable (Padilla-Rivera and Güereca, 2019).

Various activities such as food processing, textile, agriculture-runoff from saline soil zones, aquaculture, fish canning, pharmaceutical, and tannery can generate saline wastewater, resulting in heavy metals, nutrients, organic pollutants, pharmaceuticals, and personal care products, the most commonly found pollutants in saline wastewater (Vo et al., 2020). Nevertheless, saline wastewater treatment still requires high capital costs and energy consumption due to high inorganic salt concentrations (Wen et al., 2018).

Biological system is one of the most cost-effective solutions to treat wastewater (Sukačová et al., 2015). Phototrophic microalgae are microorganisms that use solar energy to consume inorganic nutrients and absorb carbon dioxide (CO<sub>2</sub>) to form proteins, lipids, carbohydrates, pigments, and other molecules through photosynthesis (Ruiz-Martinez et al., 2012). Microalgal-based wastewater treatment systems have been extensively explored over the past five decades (Shankar, 2011) since their inception (Oswald et al., 1953). They can provide water of adequate quality for reuse (de Souza et al., 2019), reduce the acute ecotoxicological (Lin et al., 2007) and genotoxicological potential of wastewater (de Souza et al., 2020), and compete with physical-chemical wastewater treatment processes (Vo et al., 2020). Additionally, microalgal biomass and their high-value extracts present well-established applications in food, feed, healthcare products, nutraceutical, pharmaceutical, and cosmeceutical industries (Mu et al., 2019). A few microalgal species are of particular interests, namely *Arthrospira* sp., *Chlorella* sp. and *Dunaliella* sp., among a few others.

Marine microalgae are widely distributed in lakes and inland seas. *D. salina*, a halophilic member of the phylum Chlorophyta, is a species of increasing interest. So far, research has focused on the ability of *D. salina* to produce compounds of industrial interest, such as carotenoids (Ben-Amotz et al., 2009), lipids (Cho et al., 2016a), glycerol (Zhang et al., 2017), protein (Sui et al., 2019b), and pharmaceutical compounds (Zamani et al., 2019). The potential of *D. salina* is not limited to the generation of bioproducts. It has also been applied to recover nutrients (Amini et al., 2019), heavy metals (Akbarzadeh and Shariati, 2014), and to sequester carbon dioxide (Kim et al., 2017). These features indicate that *D. salina* may be a potential candidate for a cost-effective approach to wastewater treatment. However, there is still a lack of knowledge about biomass cultivation and conversion (Liu and Yildiz, 2018) to assess its economic viability and efficiency. Many researchers have dedicated their studies to the subject in recent decades, and many vital factors have been described. However, most of the data is fragmented, and there has not been a systematic review to identify this microalga's full functionality and use for the phycoremediation of saline wastewater.

In this context, this paper analyses using *D. salina* within 22 years (2000–2022) to treat saline wastewater, reduce its environmental pollution potential and risk, and recover nutrients. The novelty of this research is to (1) discuss using saline wastewater as an alternative cultivation medium to yield valuable microalgal bioproducts, promoting the circular and green economy, and (2) propose a promising approach to turn the costly wastewater treatment into a profitable market.

## 2. Methodology

A bibliometric analysis using VosViewer v. 1.6.15 was carried out to identify the potential use of *D. salina* to treat wastewater and produce bioproducts that could balance the costs associated with conventional wastewater treatment or make it economically beneficial. Keywords “*Dunaliella salina*” and “wastewater”/“effluent” were used to search for article references collected on Science Direct, Web of Science, and Scopus databases from January 2000–October 2022 (Table 1). Duplicates were removed, and a thesaurus file was used to group synonyms. Clusters formed by closely related terms received the same color in the bibliometric map. The size of a term's circle represents its importance (proportional to its number of occurrences), and the thickness of the line connecting two terms represents their relationship. For a specific item, the links and total link strength (adimensional) attributes represent the number of connections an item has and the cumulative strength of these connections with other items. Unfortunately, different species of *Dunaliella* have been incorrectly identified as *D. salina* (for example, *D. tertiolecta*); thus, the bibliometric analysis may include some of these studies.

## 3. Overview of *D. salina* cultivation in wastewater

The bibliometric analysis constructed using the terms: “*Dunaliella salina*” and “wastewater”/“effluent” over the period 2000–2022 shows three clusters (Fig. 1). The Green cluster refers to the ability of *D. salina* to accumulate pigments, represented by the terms “chlorophyll” and “carotenoid,” to deal with stressful conditions. The Red cluster highlights the application of *D. salina* in the energy production context, demonstrated by the terms “energy,” “fuel,” “biofuel,” “biodiesel,” and “biogas.” The Blue cluster demonstrates the primary uses of *D. salina*, such as for food application and wastewater treatment.

*D. salina* application in the food sector is well-known and established, and this is exemplified by the term “food” in the Blue cluster (Fig. 1); however, it is worth mentioning that “wastewater” is equally important as both terms have a similar number of occurrence (29 and 24,

**Table 1**  
Publications sorted by database from January 2000 to October 2022.

Medium	F/2	Conway	Johnson
Cost (USD) Kg <sup>-1</sup> of dry biomass	4.64	16.24	301.62
Components with the highest cost participation	NaNO <sub>3</sub> FeCl <sub>3</sub> .6H <sub>2</sub> O NaH <sub>2</sub> PO <sub>4</sub> .2H <sub>2</sub> O	Cyanocobalamin Na <sub>2</sub> EDTA NaH <sub>2</sub> PO <sub>4</sub> .2H <sub>2</sub> O H <sub>3</sub> BO <sub>3</sub>	KNO <sub>3</sub> MgCl <sub>2</sub> .6H <sub>2</sub> O





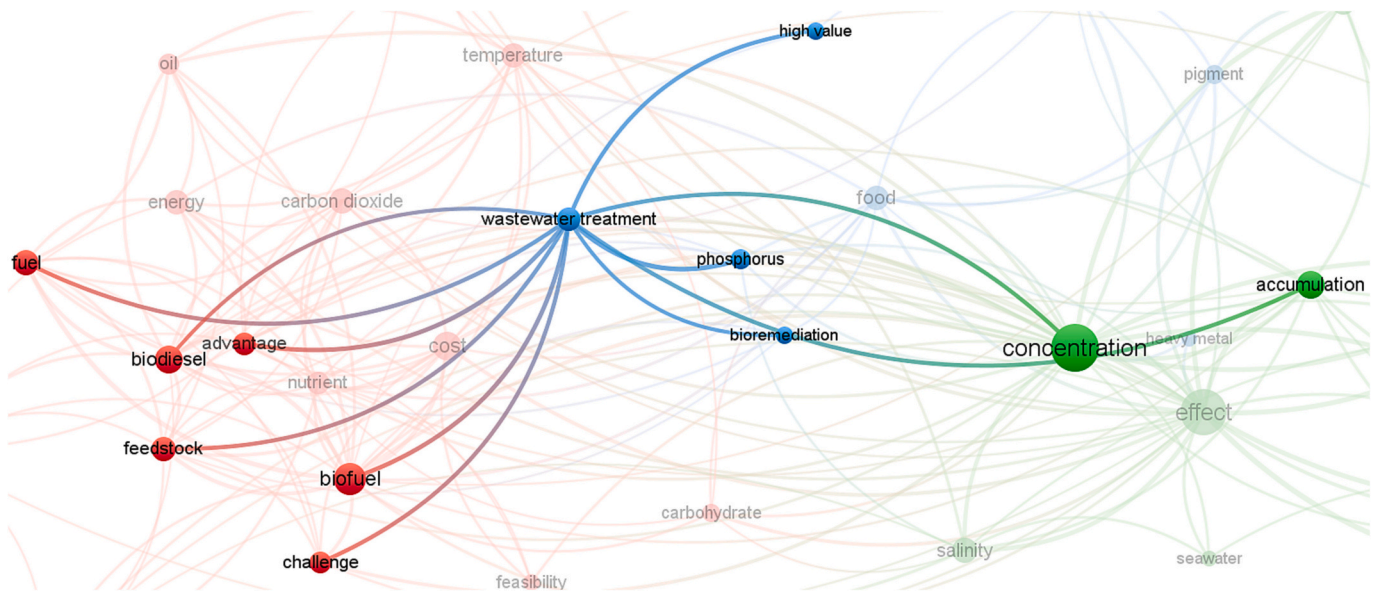


Fig. 2. Bibliometric analysis by VosViewer highlighting the term “wastewater treatment” and its connections. Data corresponds to January 2000–October 2022 in Science Direct, Web of Science, and Scopus databases. The keywords “*Dunaliella salina*” + “wastewater”/“effluent” were used to collect the research articles’ references in November 2022.

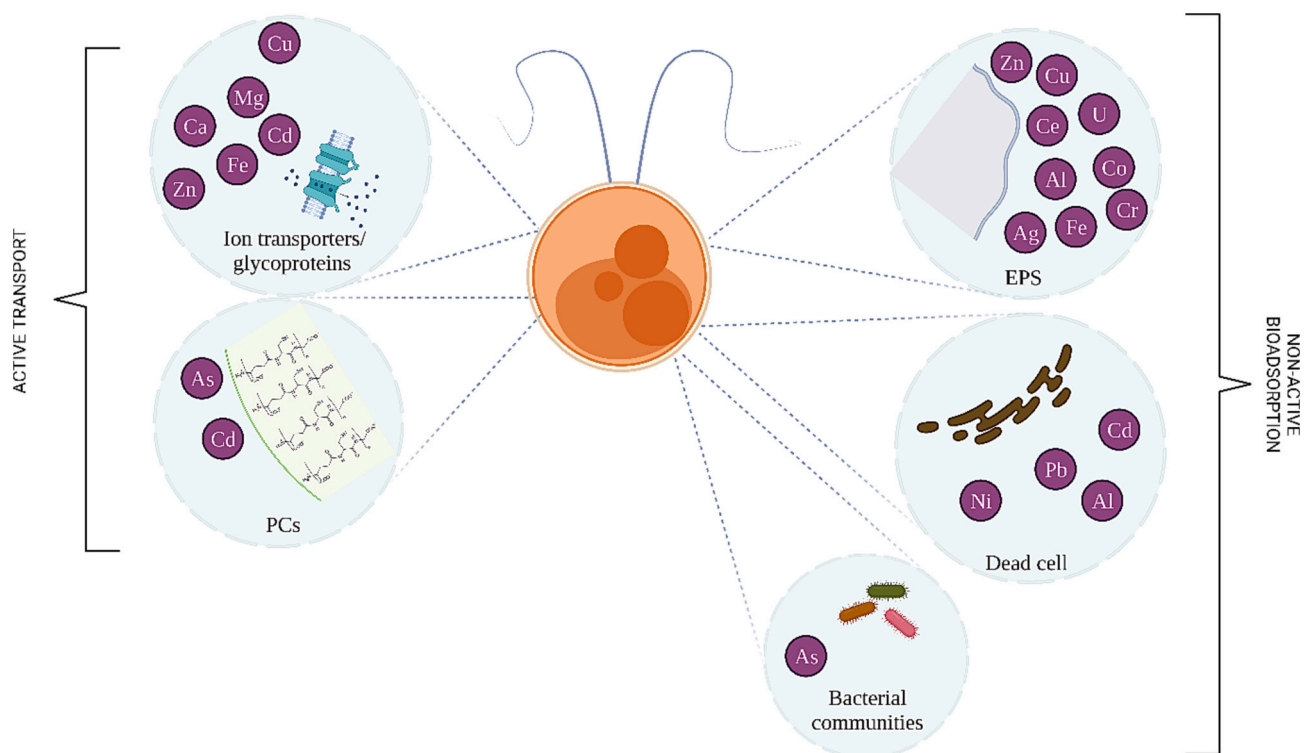


Fig. 3. Potential fate of some metallic elements in the cell of *D. salina*.

metallothioneins (Balzano et al., 2020).

Folgar et al. (2009) described a high value of half the maximum effective concentration for cadmium chloride ( $EC_{50}$ ) ( $48.9 \text{ mg Cd L}^{-1}$  at 96 h of culture); however, the highest percentage of Cd removal was obtained at the concentration of  $5 \text{ mg L}^{-1}$  Cd, where only 11.3 % of the metal was recovered. This result contrasts with Shafik (2008), who found >87 % recovery of  $Cd^{2+}$  by *D. salina* regardless of its initial concentration in the medium ( $5\text{--}15 \text{ mg L}^{-1}$ ), with the cost of decreasing in cell density despite the increase in protein, amino acids,

carbohydrates, and chlorophyll production/activity. Zhu et al. (2019) found similar results: Cd exposure caused levels of chlorophyll *a* and *b* and carotenoid to increase along with antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione S-transferase (GST), and glutathione reductase (GR) and ascorbate peroxidase (APX), and glutathione to decrease. Some of these compounds comprise the ascorbate-glutathione pathway (also called the Asada-Halliwell pathway); others use glutathione as a substrate (Hasanuzzaman et al., 2019). On the other hand, Kaamouh et al. (2022)

reported that  $5 \text{ mg L}^{-1}$  of  $\text{Zn}^{2+}$  or  $\text{Cu}^{2+}$  increased *D. salina* growth and amino acid content: maximal growth rate was  $>0.7$  (10th day) and  $>0.4 \text{ day}^{-1}$  (8th day) for  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$ , respectively.

Cd is transported across the plasma membrane in ionic form by metal ion transporters involved in the uptake of  $\text{Ca}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Zn}^{2+}$ . Similar to the situation in higher plants, exposure is associated with oxidative stress and the formation of a hyperoxidant state. The severity level depends on the metabolic capacity of a given strain to withstand ROS and environmental conditions (Hamidkhani et al., 2021).

Yamaoka et al. (1999) evaluated the ability of the microalga *D. salina* to bioaccumulate As species:  $\text{As}^{5+}$ ,  $\text{As}^{3+}$ , and dimethylarsinic acid (DMA). They showed that growth inhibition followed the order  $\text{As}^{3+} > \text{As}^{5+} > \text{DMA}$  and that the order of accumulation by *D. salina* was  $\text{As}^{5+} \geq \text{As}^{3+} > \text{DMA}$ . They highlighted a positive correlation between glutathione and the absorption of As species. Affinity for  $\text{As}^{5+}$  and  $\text{As}^{3+}$  was reported by Wang et al. (2017). These authors also identified the production of phytochelatin in response to As, especially  $\text{As}^{5+}$ . Ge et al. (2016) reported that 65 proteins appear to be responsible for responding to As stress (Fig. 4), which are up- or down-regulated to deal with toxicity and reduced energy and carbon supply, resulting in an As content of  $19.8 \text{ mg kg}^{-1}$  in *D. salina* after 72 h of exposure to an As concentration of  $11.2 \text{ mg L}^{-1}$  in the medium.

It has been shown that phosphate ( $\text{PO}_4^{3-}$ ) uptake competes with As uptake (Ge et al., 2016; Wang et al., 2017), but interestingly, As accumulation by *D. salina* does not always decrease with increased phosphate concentrations in the medium. This means that other mechanisms, such as allosteric interactions, synthesis of membrane transporters, or involvement of bacterial communities associated with *D. salina* culture, may also play a role and contribute to the overall As uptake. For instance, As efflux from *D. salina* increases with increasing incubation time under  $\text{PO}_4^{3-}$ -limited conditions (Wang et al., 2016).  $\text{As}^{3+}$  oxidation,  $\text{As}^{5+}$  reduction,  $\text{As}^{3+}$  methylation pathways, and symbiotic relationship with other microorganisms also deal with As detoxification (Wang et al., 2013). Wang et al. (2016) identified that the  $\text{As-PO}_4^{3-}$  interaction increases the growth of axenic *D. salina* but did not identify the same effect in non-axenic *D. salina*.  $\text{As}^{5+}$  inhibited the growth of non-axenic *D. salina* after 9-day incubation regardless of  $\text{PO}_4^{3-}$  concentration. *Alteromonas macleodii* on the surface of *D. salina* increased As absorption ( $39.7 \pm 4.0 \text{ mg kg}^{-1}$ ) by the microalga under  $1.12 \text{ mg L}^{-1} \text{ PO}_4^{3-}$ , while the axenic culture was able to absorb  $29.8 \pm 4.1 \text{ mg As kg}^{-1}$ . When cultured under  $0.112 \text{ mg L}^{-1} \text{ PO}_4^{3-}$ , the As assimilation was  $9.3 \pm 1.9 \text{ mg kg}^{-1}$  and  $51.8 \pm 3.0 \text{ mg kg}^{-1}$  for non-axenic and axenic cultures, respectively. This difference is explained by the greater excretion of As in the non-axenic culture.

Extracellular polymeric substances (EPS) show selectivity for heavy metals; for instance, EPSs in *Mucilaginibacter rubeus* exhibit high bioadsorption for  $\text{As}^{3+}$ , copper ( $\text{Cu}^{2+}$ ), and gold ( $\text{Au}^{3+}$ ) but not for  $\text{Zn}^{2+}$ . In contrast, *D. salina* shows greater accumulation of Zn, followed by Cu, cobalt (Co), and Cd (Shafik, 2008), which may be associated with EPS (Kwon et al., 2017). This suggests that EPS composition and metal selectivity differ among different organisms. EPS content is also positively related to salinity. Mishra and Jha (2009) demonstrated the production of  $944 \text{ mg L}^{-1}$  of EPS at the salt concentration of  $294 \text{ g L}^{-1} \text{ NaCl}$ , while at  $29.4 \text{ g L}^{-1} \text{ NaCl}$ , EPS production was only  $56 \text{ mg L}^{-1}$ . As *D. salina* lacks a cell wall, a saline stress response may be associated with EPS production, forming a gel-like polysaccharide layer around the cell to protect it from damage (Mishra and Jha, 2009). Johari et al. (2018) analyzed the influence of salinity on the toxicity of silver nanoparticles and silver nitrate in *D. salina*. Their studies showed that half of the maximum concentration increased with higher salinity. Sendra et al. (2018) found that *D. salina* showed greater tolerance for Ag and cerium nanoparticles than *Chlorella autotrophica*, which has a typical cellulosic cell wall. They suggested that biosorption may have involved EPS in the case of *D. salina*.

Schwarz et al. (2003) showed that a  $175.32 \text{ g L}^{-1} \text{ NaCl}$  concentration resulted in a 5-fold increase in  $\text{Fe}^{3+}$  binding compared to  $58.44 \text{ g L}^{-1} \text{ NaCl}$ . Iron (Fe) is essential for photosynthetic electron transport and other metabolic functions. However, living cells also tightly regulate Fe uptake and storage to minimize the risk of developing a cellular hyperoxidant state via Fenton's chemistry. As  $\text{Fe}^{3+}$ , the usual form taken up by *D. salina*, is also in low availability in aerobic or highly saline aqueous environments (Fisher et al., 1997), an unusual mechanism has evolved to increase  $\text{Fe}^{3+}$  uptake from these environments. This involves not only the usual "housekeeping" iron transport glycoprotein, transferrin but also two other glycoproteins (D-Fox and p130B). Glycoproteins form a complex with transferrin on the outer plasma membrane surface to increase binding and uptake of  $\text{Fe}^{3+}$ ; in doing so, cell proliferation is maintained.

Polysaccharides are polymers that play an essential role in metal binding by *D. salina* (Kaamouh et al., 2022), mainly due to their hydrophobic characteristic resulting from acetyl groups. Uronic acids and sulfates impart a negative charge and acidic property to *D. salina* polysaccharides, exhibiting a high-metal complexing capacity (Mishra et al., 2011). Cecal et al. (2012) described polysaccharides and nucleic acids (another group of polymers) as the main functional groups responsible for uranium (U) removal by *D. salina*. The contribution of polysaccharides to adsorbing lead (Pb), nickel (Ni) (Holan and Volesky, 1994), and Cd (Holan et al., 1993) has been reported for non-living

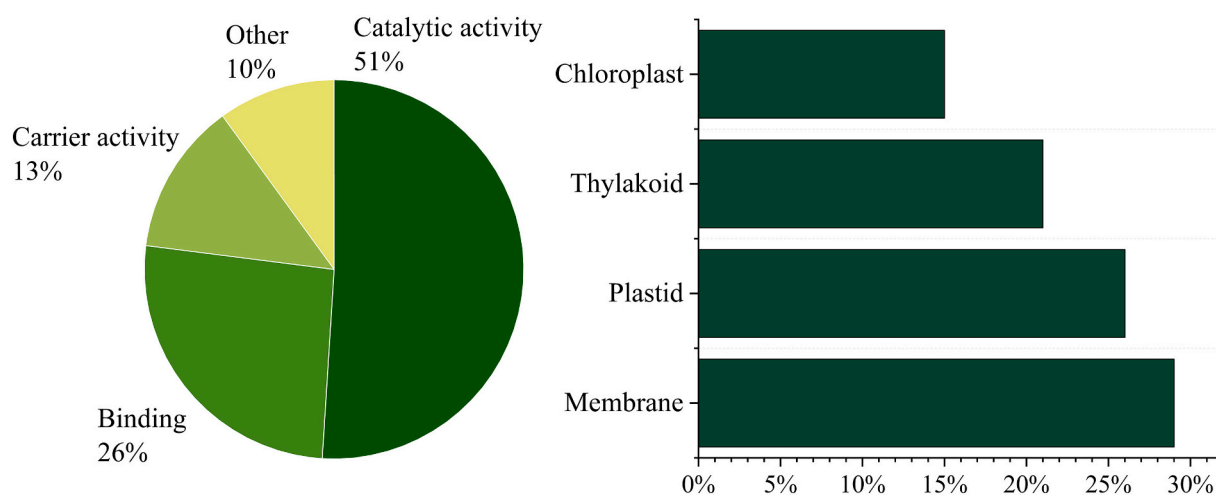


Fig. 4. Distribution of 65 proteins in *D. salina* associated with the stress response to As. Left - distributed according to their function, and right - according to the cellular component. Adapted from Ge et al. (2016).

marine algae biomass and aluminum (Al) for higher plants (Aizawa and Urai, 2020). In *D. salina*, polysaccharides probably represent the binding mechanism for Al, recovered by agglutination and subsequent sedimentation. This feature is also found in other species of *Dunaliella*, although different efficiencies are recorded (Akbarzadeh and Shariati, 2014). Vidyalaxmi et al. (2019) evaluated the potential use of *D. salina* for bioremediation of the heavy metal hexavalent chromium ( $\text{Cr}^{6+}$ ) from saline wastewater. They found that Cr was absorbed by the surface of the microalgal cell; at low ( $5 \text{ mg L}^{-1}$ ) and intermediate ( $15 \text{ mg L}^{-1}$ )  $\text{Cr}^{6+}$  concentrations, *D. salina* showed better removal efficiency compared to higher  $\text{Cr}^{6+}$  concentration ( $25 \text{ mg L}^{-1}$ ) due to possible depletion of binding sites.

Duration of exposure is a crucial factor regarding the bioremediation of heavy metals by *D. salina* (Dineshkumar et al., 2022). Increasing the exposure duration allows more adsorption sites for bioaccumulation (Vidyalaxmi et al., 2019). However, prolonged exposure periods decrease the As uptake, which seems to be maximized in two days, followed by a decrease (Wang et al., 2017). This is likely related to As efflux (Wang et al., 2017). Stimulation by Cd of chlorophyll and carotenoid biosynthesis was favored at Cd concentrations of 0.5 and  $2.5 \text{ mg L}^{-1}$  under 6-h exposure, demonstrating the need to harness solar energy to maintain growth and cope with Cd exposure; however, lipid peroxidation at  $2.5 \text{ mg L}^{-1}$  and  $>96 \text{ h}$  exposure was evident (Zhu et al., 2019). It can be concluded that *D. salina* can deal with Cd with a short exposure period, but more extended periods cause cell death.

Another aspect of the potential of *D. salina* for metal absorption and adsorption is the surface area. Considering an average *D. salina* cell size of  $10.5 \mu\text{m} \times 5.5 \mu\text{m}$ , the surface area is about  $624 \mu\text{m}^2$  with a surface and volume ratio of  $0.47 \mu\text{m}^{-1}$ . This surface area is larger than other species (e.g., *Synechococcus elongatus* (Gallo et al., 2020) and *D. tertiolecta* (Nikookar et al., 2005)). This means that more free sites could interact with metals in the medium.

The fate of heavy metals in *D. salina* directly depends on their affinity and the availability of binding sites in the cell, where polymers, such as polysaccharides and proteins, play a crucial role. Internalization comprises the regulation and triggering of different pathways strongly related to glutathione and its products; however, the sequestration of heavy metals in the cell depends on the exposure time and environmental conditions. Given the right conditions, *D. salina* could bioremediate metal-containing wastewater.

#### 4.2. Macronutrients

Autotrophic  $\text{CO}_2$  fixation is a pathway driven by adenosine triphosphate (ATP) and reducing power, transforming non-biogenic carbon compounds into organic metabolites (Hu et al., 2019). The  $\text{CO}_2$  drainage inside the *D. salina* cell depends directly on its concentration, favoring its reallocation from glycerol to protein and starch under higher concentrations ( $5 \text{ \% v/v}$ ) (Giordano and Bowes, 1997). Generally, stressful conditions retard the carbon fixation by microalgal photosynthesis; however, *D. salina* has its carbonic anhydrase (CA) activity stimulated at increasing salinities, which may also be associated with the  $\text{Na}^+/\text{H}^+$  antiport (Katz et al., 1986). CA is an enzyme that optimizes the inorganic carbon fixation and utilization in high salinities (Fisher et al., 1996). The CAH1 and CAH2 enzymes give *D. salina* the ability to fix inorganic carbon by regulating the  $\text{CO}_2/\text{HCO}_3^-$  equilibrium. CAH1 converts  $\text{HCO}_3^-$  to  $\text{CO}_2$  when the last is in low concentration; CAH2 hydrates  $\text{CO}_2$  when the medium contains significant amounts. This explains the tolerance of *D. salina* to high inorganic carbon concentration (Hou et al., 2016).

The enzyme RuBP carboxylase/oxygenase (RuBisCo) catalyzes the primary  $\text{CO}_2$  reduction reaction and its binding to the acceptor-molecule ribulose-1,5-bisphosphate (RuBP) to form two 3-phosphoglycerate molecules (Andersson and Backlund, 2008). UV-B irradiation induces greater RuBisCo activity in *D. salina*; however, the role of UV-B irradiation in global carbon uptake still needs further studies to cover other mechanisms and compensation pathways (Zhang et al., 2015). The

growth-optimal levels of  $\text{CO}_2$  to support *D. salina* ranges from around 2–6 %. This means that countermeasures must be applied to use *D. salina* to treat high  $\text{CO}_2$  levels ( $> 10 \text{ \% v/v}$ ), such as from flue gas. This includes (1) removing  $\text{O}_2$  from the inlet gas to decrease the  $\text{O}_2:\text{CO}_2$  ratio to promote RuBisCo carboxylase activity and limit oxygenase (Kliphuis et al., 2011); (2) gradually increasing the  $\text{CO}_2$  concentration to acclimatize the microalga; and (3) adjusting  $\text{CO}_2$  dosing to avoid changes in dissolved inorganic carbon and acidification of the medium. By conducting these recommendations, the C fixation can increase about 6.6-fold (Chen and Xu, 2021). Harter et al. (2013) presented a mass balance approach for C and N to quantify the direct emission of greenhouse gases in a culture of *D. salina* supplied with real biodiesel flue gas. Their results proved that *D. salina* can use biodiesel flue gas as a source of N and C; however, the mass balance showed that up to 6 times more  $\text{CO}_2$ -equivalent was emitted, mainly due to anaerobic microbial processes. Therefore, flue gas bioremediation by microalgae, especially in open ponds, must be critically addressed to evaluate and make this treatment viable, and a pre-sterilization may be required.

Although *D. salina* can tolerate high concentrations of dissolved inorganic carbon (Kishi and Toda, 2018), the efficiency in  $\text{CO}_2$  fixation is low ( $< 4 \text{ \%}$ ) compared to other carbon sources such as bicarbonate ( $> 91 \text{ \%}$ ), especially in open ponds, where most of the  $\text{CO}_2$  supply is lost to the atmosphere (Kim et al., 2017). However, this could be mitigated by using immobilized cells instead of free cells, as shown by In-na et al. (2020), who used a 3D-coated loofah sponge as a porous substrate. Their lab-scale results theorized that *D. salina* can capture over  $90 \text{ t CO}_2 \text{ t}^{-1}$  biomass  $\text{yr}^{-1}$ , requiring less water.

Nitrogen is the second most crucial nutrient for microalgae growth, representing  $>10 \text{ \%}$  of its biomass (Becker, 1994). It accumulates mainly in proteins, RNA, DNA, ATP, and some pigments (Geider and Roche, 2002). The pathways involved in nitrogen uptake depend on its form. Nitrate assimilation in eukaryotic microalgae comprises four steps: (1) nitrate transport through the cell cytoplasmic membrane via ATP-dependent permease systems; (2) nitrate reduction to nitrite catalyzed by NAD(P)H-nitrate reductase in the cytosol; (3) nitrite transport into the chloroplast and its reduction to ammonium catalyzed by ferredoxin-nitrite reductase; and (4) ammonium incorporation into the carbon skeleton catalyzed by the ATP-dependent glutamine synthetase (GS)-glutamate synthase cycle (Vega, 2020).

Theoretically, microalgae should prefer to assimilate  $\text{NH}_4^+$  to  $\text{NO}_3^-$ ;  $\text{NH}_4^+$  requires less energy, as demonstrated by the above four assimilation steps (Hellebust and Ahmad, 1989). However, the preferred nitrogen source is species-dependent. Regarding *D. salina*, both nitrogen sources can be used. Giordano and Bowes (1997) cultivated *D. salina* in  $\text{NH}_4^+$  or  $\text{NO}_3^-$ . They demonstrated that  $\text{NH}_4^+$  increased cell size, rate division, and chlorophyll concentration. Giordano (2001) increased the amount of RuBisCo protein by up to 2.5-fold using  $\text{NH}_4^+$  compared to  $\text{NO}_3^-$ ; phosphoenolpyruvate carboxylase activity was also increased, which may enhance light-independent carbon fixation. Nevertheless, Hadizadeh et al. (2020) cultivated *D. salina* ( $\sim 20 \times 10^4 \text{ cell mL}^{-1}$  initial concentration; 14 days) in different stickwater concentrations (0–100 %). They highlighted an increase in cell growth ( $\sim 75$  to  $112.6 \times 10^4 \text{ cell mL}^{-1}$ ), protein (49 % to 59 %) fat (15 % to 16 %), chlorophyll *a* ( $\sim 4.2$  to  $6.5 \mu\text{g L}^{-1}$ ), and chlorophyll *b* ( $\sim 1.6$  to  $3.3 \mu\text{g L}^{-1}$ ) contents at 75 % stickwater ( $242 \text{ mg L}^{-1} \text{ NO}_3^-$  and  $11.13 \text{ mg L}^{-1} \text{ PO}_4^{3-}$ ) compared to control (F/2). Nitrogen availability directly affects chlorophyll production by regulating the NADP+ activity (Chen et al., 2015), affecting carbon assimilation (Chen et al. 2021).

Microalgae favour the assimilation of inorganic orthophosphate ( $\text{PO}_4^{3-}$ ), although they can transform organic phosphorus into inorganic by phosphatase activity. Luxury uptake occurs when inorganic phosphorus is abundant; high-rate and low-affinity transporters are activated under this condition (Su, 2021). It is stored in the cell as phosphate granules used during the scarcity of this nutrient to prolong cell growth (Larsdotter, 2006). Microalgae utilize phosphorus to build genetic material (the most significant P sink in cells), lipids, and ATP (Geider and



Roche, 2002) and as a universal energy currency to power many biosynthetic processes (Dyhrman, 2016). Notably, N and P metabolisms are codependent; thus, their absorption is affected by the bioavailability of each one.

Regardless of some nutrient forms' positive effect on *D. salina* development, the concentration must be considered to avoid stressful conditions, especially if the microalga undergoes sudden changes in the extracellular environment. Racharaks et al. (2015) identified that, when cultivating *D. salina* in municipal anaerobically digested wastewater (10 % v/v) combined with shale gas flowback water, the P concentration (11 mg L<sup>-1</sup>) and N/P ratio of 18 had an inhibitory effect on its growth. Using 100 % stickwater, Hadizadeh et al. (2020) also reported an inhibitory effect on *D. salina* growth, probably due to the high nitrate concentration. Nutrient concentration also determines assimilation, which is not always positively correlated. Roberts et al. (2019) cultivated *D. salina* at a concentration of 4.7 g L<sup>-1</sup> sulfate (SO<sub>4</sub>) and reported a recovery rate of 65 %; when cultivated in a low-SO<sub>4</sub> medium (Jaworski's medium), the removal rate was 6 %. Menke et al. (2012) evaluated the recovery of potassium (K<sup>+</sup>), magnesium (Mg<sup>2+</sup>), and sodium (Na<sup>+</sup>) from hypersaline fertilizer industry wastewater by living *D. salina* cells. They identified that the intracellular content of K<sup>+</sup> and Mg<sup>2+</sup> increased as its extracellular content increased; the same was not identified for Na<sup>+</sup>. This demonstrates that the microalga has transporters to specifically include and exclude ions inside and outside the cell to ensure the functionality of sensitive enzymes.

Considering its nutrients recovery capacity (Table 2) and limitations, many different types of wastewaters could support *D. salina* growth and be suitable for their simultaneous phycoremediation, especially those from food industries (e.g., seafood processing and canning; dairy) and desalination plants. For instance, Shiri et al. (2023) obtained over 80 % NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, and SO<sub>3</sub><sup>2-</sup> removal by combining membrane bioreactor and *D. salina* to treat pre-treated shrimp pond wastewater. These effluents may have high loads of nutrients (Venugopal and Sasidharan, 2021; Vieira Costa et al., 2021), salinity, and pH suitable to grow *D. salina*. However, attention must be paid to avoid inhibiting environments to its growth and nutrient uptake. For example, Amini et al. (2019) reported an efficiency drop in NO<sub>3</sub><sup>-</sup> (from 54 % to 20 %) and PO<sub>4</sub><sup>3-</sup> (82 % to 37 %) removal by *D. salina* biosorbent in real wastewater compared to synthetic nutrient medium with the same nutrient concentrations. High organic content and suspended solids also make nutrient recovery difficult (Amini et al., 2019) due to shading and blocking of functional groups. In addition, some pesticides in agricultural run-off could jeopardize *D. salina* growth (Chen et al., 2007). Salt concentrations >30 g L<sup>-1</sup> should also be avoided, as nutrient uptake capacity may decrease at higher salinity (Liu and Yildiz, 2018). Liu and Yildiz (2018) demonstrated that a higher salt concentration (40 g L<sup>-1</sup>)

hinders NO<sub>3</sub><sup>-</sup> and P removal from tertiary-treated municipal wastewater by *D. salina* compared to lower concentrations (20 and 30 g L<sup>-1</sup>). However, some measures can be applied to wastewater treatment without many restrictions, such as previous aerobic/anaerobic treatment to reduce organic matter and TDS content, pH, and salinity correction using chemicals or mixing with other effluents.

#### 4.3. Organic pollutants

One explanation for the biodegradability of organic carbon is the photoproduction of hydroxyl radicals, its decomposition into bioavailable carbon sources, and the promotion of beta-carotene (Liu et al., 2019). Although this microalga is known for its autotrophic behavior, mixotrophic cultivation of *D. salina* has been documented, and the positive effect on its growth and beta-carotene (Morowvat and Ghasemi, 2016). Liu et al. (2019) treated diesel-oil water-soluble fractions (DOWSFs) with *D. salina* (2–10 × 10<sup>6</sup> cell mL<sup>-1</sup> initial concentration; F/2 medium). They reached a degradation efficiency of up to 60.3 % after nine days of culture. Its density was favored with a higher DOWSF concentration up to 4.0 mg L<sup>-1</sup> (>30 × 10<sup>4</sup> cell mL<sup>-1</sup>); a decrease in the *D. salina* density was noted when DOWSF exceeded this concentration (<20 × 10<sup>4</sup> cell mL<sup>-1</sup>). Phytoplankton has a strong lipophilic ability. Thus, organic compounds can reduce the chlorophyll *a* content by preventing carbon dioxide and nutrient absorption, which can harm cell growth; however, *D. salina* maintains high photosynthetic activity at high concentrations of petroleum hydrocarbon.

Zhang et al. (2022) analyzed the effect of co-culturing *Halomonas mongoliensis* and *D. salina* on phenol degradation under different operation conditions: 5.5–11.5 pH; 120–360 μmol m<sup>-2</sup> s<sup>-1</sup> light intensity, 19–37 °C, 300–600 mg L<sup>-1</sup> phenol concentration, and 8:1–2:1 *D. salina*/*H. mongoliensis* ratio. The co-culture could completely degrade 400 mg L<sup>-1</sup> of phenol within five days at optimal conditions: 2:1 *D. salina*/*H. mongoliensis* ratio, 120 μmol m<sup>-2</sup> s<sup>-1</sup> light intensity, 25 °C, and 7.5 pH. At this condition, *D. salina* grew from 0.4 (initial biomass) to 0.55 g L<sup>-1</sup> while its monoculture almost could not grow. Cho et al. (2016b) cultivated *D. salina* (2 × 10<sup>4</sup> cell mL<sup>-1</sup> initial concentration; 10-day cultivation; F/2-Si medium) in phenol and estimated the EC<sub>50</sub> phenol concentration for *D. salina* to be 155 mg L<sup>-1</sup>. The chlorophyll *a* and *b* and total carotenoid content were also increased at 50 and 100 mg L<sup>-1</sup> phenol. In contrast, the chlorophyll *a/b* ratio decreased. This may indicate that phenol inhibits the ability of *D. salina* to carry out photosynthesis since the chlorophyll *a/b* ratio is strongly related to the light-harvesting chlorophyll-protein complex (LHCII). The degradation of phenol by *D. salina* may be linked to the absorption of dissolved phenol - which could be facilitated by the lack of a cell wall - and its conversion into growth metabolites. Biomass and lipid content increased after

**Table 2**  
Recovery of some nutrients by *D. salina*.

NO <sub>3</sub> <sup>-</sup> (%)	NH <sub>4</sub> <sup>+</sup> (%)	PO <sub>4</sub> <sup>3-</sup> (%)	SO <sub>4</sub> <sup>2-</sup> (%)	Cl <sup>-</sup> (%)	Na <sup>+</sup> (%)	HCO <sub>3</sub> <sup>-</sup> (%)	Cell condition	Medium	References
54.0		82.0					Bioabsorbent	Nutrient stock solution (350 mg L <sup>-1</sup> of NO <sub>3</sub> <sup>-</sup> and PO <sub>4</sub> <sup>3-</sup> ; pH 7)	(Amini et al., 2019)
20.0		37.0						NO <sub>3</sub> <sup>-</sup> and PO <sub>4</sub> <sup>3-</sup> -enriched wastewater (350 mg L <sup>-1</sup> of NO <sub>3</sub> <sup>-</sup> and PO <sub>4</sub> <sup>3-</sup> ; pH 7)	(Amini et al., 2019)
62.0	42.2	64.7					Immobilized	Johnson	(Thakur and Kumar, 1999)
44.0	25.0	34.7					Free	Johnson	(Liu and Yildiz, 2018)
88.0	70.7	45.7*					Free	75 % Pretreated municipal wastewater (0.95 mg L <sup>-1</sup> NH <sub>4</sub> <sup>+</sup> , 40.7 mg L <sup>-1</sup> NO <sub>3</sub> <sup>-</sup> , 3.8 mg L <sup>-1</sup> PO <sub>4</sub> <sup>3-</sup> ) + 25 % Bold basal medium (30 g L <sup>-1</sup> salt, 184 mg L <sup>-1</sup> NO <sub>3</sub> <sup>-</sup> , 163 mg L <sup>-1</sup> PO <sub>4</sub> <sup>3-</sup> )	(Liu and Yildiz, 2018)
	68.0	29.0					Free	Diluted washing water from a sugar mill process (3.2–6.6 mg L <sup>-1</sup> NH <sub>4</sub> <sup>+</sup> , 1.2–3.1 mg L <sup>-1</sup> PO <sub>4</sub> <sup>3-</sup> )	(Putri and Muhaemin, 2010)
			65.0				Free	Modified Jaworski's Medium (4.73 g L <sup>-1</sup> SO <sub>4</sub> <sup>2-</sup> )	(Roberts et al., 2019)
				33.1	29.5	14.3	Free	Saline lake (2.2 g L <sup>-1</sup> Cl <sup>-</sup> , 2.1 g L <sup>-1</sup> Na <sup>+</sup> , and 2.1 g L <sup>-1</sup> HCO <sub>3</sub> <sup>-</sup> )	(Moayed et al., 2019)
96.1		87.5					Free	Desalination brine (summer)	(Ortega Méndez et al., 2012)
98.9		44.2					Free	Desalination brine (winter)	

acclimation to phenol. Increased lipid content within the cell may be conducive to phenol detoxification (Duan et al., 2017).

Ashok et al. (2020) evaluated the bioaccumulation capacity of *D. salina* to recover different concentrations (from 0 to 900  $\mu\text{g L}^{-1}$ ) of  $^{13}\text{C}$ -phenanthrene from the F/2 medium (48-h exposure). The average bioconcentration factor (BCF) in *D. salina* by dry weight was  $2590 \pm 787 \text{ L kg}^{-1}$  ( $\log \text{BCF} = 3.41$ ), with maximum removal of approximately 77 % and  $^{13}\text{C}$ -phenanthrene accumulation of  $1546.69 \pm 366.14 \text{ fg cell}^{-1}$  at 900  $\mu\text{g L}^{-1}$   $^{13}\text{C}$ -phenanthrene. The photosynthetic efficiency ( $F_v/fm$ ) did not significantly differ when the microalga was exposed to 0 up to 30  $\mu\text{g L}^{-1}$   $^{13}\text{C}$ -phenanthrene.

Chi et al. (2019) assessed the ability of *D. salina* to degrade endocrine-disrupting compounds, namely diethyl phthalate (DEP) and di-n-butyl phthalate (DBP). They cultivated *D. salina* ( $1 \times 10^5 \text{ cell mL}^{-1}$ ) on a single-compound medium, i.e., containing only DEP or DBP, or on a mixed medium. After 96-h of exposure, the microalga degraded 91.2 % of DEP and 34.5 % of DBP in the single-compound medium; when *D. salina* was cultivated in the medium with a mix of DEP and DBP, it reached efficiencies of 71.1 % and 21 %, respectively, which were lower than in the single-compound medium. The bioconcentration percentage of DEP and DBP in *D. salina* was 0.17 % (DEP) and 7.1 % (DBP) for the single-compound medium, while for the mixed medium, the bioconcentration percentage was 0.52 % and 9.7 %, respectively. This indicates that, although the compounds competed for the same enzymatic active site, which resulted in lower biodegradability, *D. salina* can bioaccumulate these compounds within the cell.

The activity of cytochrome P<sub>450</sub>, an important enzyme that functions as a catalyzer and binding in many biochemical reactions, is also presented in *D. salina* and has been indicated as a potential mechanism for biodegradation of polycyclic aromatic hydrocarbon (PAH) by *Cyclotella caspia* (Liu et al., 2006), which may be one of the pathways for the degradation of organic compounds. Chan et al. (2006) reported that PAH adsorption occurs in living and dead cells in *Selenastrum capricornutum*; however, only living cells can absorb and degrade it. The process is similar to metal recovery: passive physicochemical adsorption followed by slow absorption in the cell. The same could be expected for *D. salina*, with the difference that the lack of a cell wall should facilitate intracellular accumulation and degradation.

Shiri et al. (2023) combined a membrane bioreactor with *D. salina* to treat pre-treated shrimp pond wastewater (700–900  $\text{mg L}^{-1}$  COD, 560–660  $\text{mg L}^{-1}$ , and 50–70 NTU turbidity). According to their results, *D. salina* allowed the formation of sludge mass in a shorter time, and the treatment unit reached over 90 % COD, BOD<sub>5</sub>, and turbidity removal.

The mixotrophic growth of the *Dunaliella* genus remains a topic of inconclusive debate. Some authors have reported that it not only grows mixotrophically (Wan et al., 2011) but also improves protein productivity (e.g., Kadkhodaei et al. (2015)). These results contradict the widely accepted view that *Dunaliella* is an obligate photoautotrophic genus (Rao, 2019) and that organic carbon can be deleterious to its growth (Kim et al., 2012). We previously demonstrated that *D. tertiolecta* could not assimilate organic carbon and that its growth was attributed to CO<sub>2</sub> assimilation when NaHCO<sub>3</sub> was unavailable (Celente et al., 2022).

**Table 3**

Costs (USD) to produce 1 kg of *D. salina* biomass by using different mediums (F/2, Conway, and Johnson) (Colusse et al., 2020).

Medium	F/2	Conway	Johnson
Cost (USD) Kg <sup>-1</sup> of dry biomass	4.64	16.24	301.62
Components with the highest cost participation	NaNO <sub>3</sub> FeCl <sub>3</sub> .6H <sub>2</sub> O NaH <sub>2</sub> PO <sub>4</sub> .2H <sub>2</sub> O	Cyanocobalamin Na <sub>2</sub> EDTA NaH <sub>2</sub> PO <sub>4</sub> .2H <sub>2</sub> O H <sub>3</sub> BO <sub>3</sub>	KNO <sub>3</sub> MgCl <sub>2</sub> .6H <sub>2</sub> O

## 5. Wastewater treatment and bioproducts generation

The artificial medium is expensive (de Souza Schneider et al., 2018) (Table 3) and can limit the viable yield of bioproducts mainly because of the costs of components such as artificial sea salt (or NaCl) (Colusse et al., 2021), NaNO<sub>3</sub>, cyanocobalamin, and KNO<sub>3</sub> (Colusse et al., 2020). Using wastewater to produce bioproducts can optimize and reduce the costs associated with the culture medium, especially regarding nutrients. Cell density increased by 170 % (at 50 % vinasse) and 160 % (at 75 % vinasse), reaching  $522$  and  $493 \times 10^4 \text{ cell mL}^{-1}$ , respectively, while control (Conway medium) reached about  $300 \times 10^4 \text{ cell mL}^{-1}$ . Chlorophyll *a* ( $8.53 \mu\text{g L}^{-1}$ ), pheophytin *a* ( $3.04 \mu\text{g L}^{-1}$ ), and total carotenoids ( $5 \mu\text{g L}^{-1}$ ) reached the highest content at 75 % treated vinasse, higher than at control (Conway medium): approximately  $3 \mu\text{g L}^{-1}$  chlorophyll *a*,  $1 \mu\text{g L}^{-1}$  pheophytin *a*, and  $3 \mu\text{g L}^{-1}$  total carotenoids. The same authors also reported a drastic reduction (69 %) in medium cost using treated rice vinasse (optimal concentration of 69 %) to cultivate *D. salina* compared to Conway medium (using seawater as a salt source).

In general, the potential use of wastewater to produce and recover microalgal biomass needs four significant steps: wastewater treatment, harvesting, bioproduct purification, and final application (Fig. 5). Different drying methods could be adopted when aiming at different bioproducts and applications. In general, solar drying is the cheapest method, but requires large surface area and longer period; convective drying such as in an oven could offer fixed temperature settings; spray drying could potentially retain more nutrient and high-value compounds; freeze drying is widely used to prevent cell rupture hence retaining the most cell constituents (Muhammad et al., 2020). Nevertheless, from product storage point of view, drying step can be omitted if a wet-route storage should be better. Wet-route storage typically adopts an anaerobic condition and can retain cell constituents (without loss occurring from drying) for 1–4 months, but other factors such as temperature, light and oxygen also affect the product stability during storage (Muhammad et al., 2020). Two different approaches can be taken to produce bioproducts from wastewater. The first comprises the set of a target bioproduct, which will determine the source and quality of wastewater, strain, and purification method. This approach has the final user as the decision-maker, exemplified in Table 4. The second approach has the industry that treats the wastewater as the decision-maker, i.e., the industry assesses its wastewater quality and identifies the potential bioproduct and final user that best suits it. This approach aims to recognize the potential biomass and recovered bioproduct users without requiring unfeasible modifications to the existing treatment plant.

*D. salina* revenue market increased by over 15 % from 2016 (USD 80.95 million) to 2021 (USD 93.49 million); the global sales are expected to reach over USD 120 million and 1500 tons of biomass by 2026, an increase of approximately 30 % compared with 2021 for both parameters. Pharmaceuticals and healthcare products, followed by food additives, represent the largest market share (Fig. 6a) (Maia Research, 2021). This concerns the yield of several natural carotenoids, such as beta-carotene, alpha-carotene, zeaxanthin, lutein, and cryptoxanthin, by this microalga (Meticulous Market Research, 2021). For example, regarding 9-*cis* beta-carotene produced by *D. salina*, revenues can reach more than USD 35 million p.a. (Harvey and Ben-Amotz, 2020). *D. salina* can also generate substantial amounts of glycerol, lipids, and proteins, convenient resources to the increasing demand for these products (Fig. 6b). Monte et al. (2020) assessed the economic performance of a biorefinery to extract protein, carbohydrates, carotenoids, lipids, and glycerol following the method proposed in Fig. 5. They proposed three scenarios based on the raw material (*D. salina* biomass) purchased cost: USD 48.3 (scenario 1), 52.5 (scenario 2), and 56.8 (scenario 3) kg dry biomass (1 euro = 1.05 dollars). Considering the revenue bio-refined product of USD 78.8, they calculated a return of investment of 888, 309, and 278 %, internal rate of return of 99, 39, and 36 %, and pay-back of 1.1, 3.2, and 29.2 years for scenario 1–3, respectively.

Carotenoids, especially beta-carotene, can be obtained in large



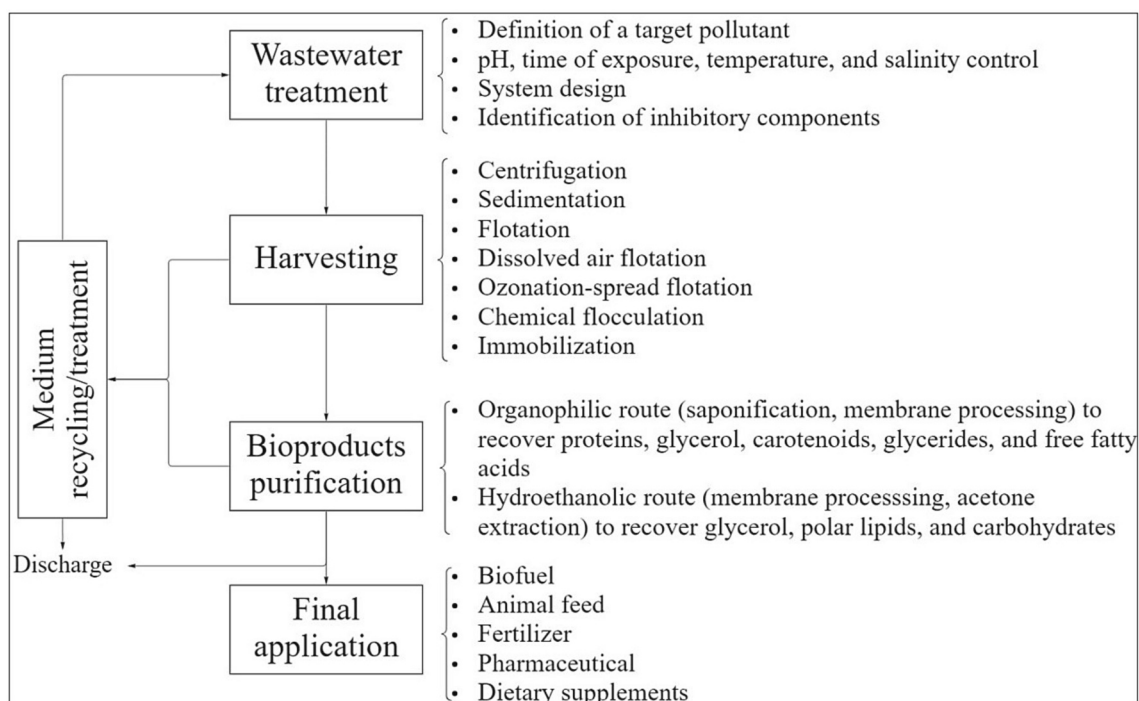


Fig. 5. Wastewater treatment and bioproducts generation scheme. Adapted from Jais et al. (2017) and Monte et al. (2020).

amounts by cultivating *D. salina* in a high-salinity medium. The selling price of carotenoids is estimated at USD 840 kg dry biomass (1 euro = 1.05 dollars) (Monte et al., 2020), offering excellent market potential. Even wastewater with the presence of Cu (Nikookar et al., 2005),  $\text{Fe}_2\text{WO}_6$  (Hassanpour et al., 2020), and petroleum hydrocarbon (Liu et al., 2019) can support and increase the yield of these compounds. Thus, desalination plants, mining, and petroleum companies could benefit from the carotenoid market and earn up to \$40 per cubic meter of medium (Zhu et al., 2018). Hadizadeh et al. (2020) yielded  $3.64 \mu\text{g L}^{-1}$  beta-carotene and 16.3 % lipid content in 75 % (diluted in F/2 medium) stickwater from a fishmeal factory with a high concentration of calcium ( $\text{Ca}^{2+}$ ) ( $2062.50 \text{ mg L}^{-1}$ ),  $\text{K}^+$  ( $1441.50 \text{ mg L}^{-1}$ ),  $\text{NO}_3^-$  ( $242.00 \text{ mg L}^{-1}$ ), and  $\text{PO}_4^{3-}$  ( $11.13 \text{ mg L}^{-1}$ ). Combining these different media with low-irradiation UV-B ( $0.25 \text{ KJ m}^{-2} \text{ d}^{-1}$ ) above 72 h exposure may even increase the yield of carotenoids (Zhang et al., 2015).

Protein production is enhanced by N availability in the medium, and its content within the cell can be on the order of nearly three times at higher N concentrations. Coupling N starvation followed by adding this nutrient in large amounts can trigger luxury uptake and increase the intracellular protein yield (Sui et al., 2019a) and N recovery from the medium. N-rich wastewater can be used as a source of N. It can be recycled to provide N-depleted wastewater for the first step of the cultivation or to promote the yield of essential amino acids, which are increased under N limitation (Sui et al., 2019a).  $\text{NH}_4^+$  as an N source favors the reallocation of C from glycerol and starch to protein and pigments (Giordano, 2001), which can be explored.

Compared to other bioproducts, lipid content in *D. salina* is considered low, explaining its lowest global market share (Fig. 6a); however, *D. salina* can still offer a reliable source of lipid, which can be sold at USD 63 kg dry biomass (1 euro = 1.05 dollars) (Monte et al., 2020). Zhang et al. (2022) constructed an ecosystem model to convert *D. salina* into jet fuel using catalysts hydrothermal liquefaction, significantly increasing bio-oil yield to 49.1 %. They also reported that *D. salina* converted approximately 41 kg of organic carbon in wastewater into 100 kg of dry biomass. Ranjbar et al. (2015) coupled the treatment of saline hydraulic fracturing produced water with lipid generation. Their results showed that *D. salina* can reduce toxic metals in wastewater and be viable for

biodiesel production.

Similarly, Saravanan and Gurumoorthy (2020) used dairy wastewater mixed with artificial saline wastewater to yield lipids to produce biofuel. They reached 54 % (w/w) lipid after 21 cultivation days with four main groups found in biodiesel (Pentadecanoic acid methyl ester, 10-Octadecenoic acid methyl ester, 9-Octadecenoic acid methyl ester, and 9-Hexadecenoic acid). The use of flue gas can also result in increased lipid accumulation, as C is reallocated from carbohydrates to lipids when  $\text{CO}_2$  is highly available (20 % v/v); however, acclimatization and gradual increase in  $\text{CO}_2$  dose must be performed; otherwise, *D. salina* will have its growth and lipid productivity hampered (Chen and Xu, 2021).

Carbohydrate is generally not the primary goal of microalgal biomass, mainly due to its lower value than other bioproducts. However, it still offers a reliable market opportunity. It can be used to produce biofuel and cultivate microorganisms, such as glucose, after hydrothermal liquefaction (Pirwitz et al., 2016). Carbohydrates can be obtained after the recovery of glycerol, lipids, and carotenoids by hydroethanolic and organophilic routes (Monte et al. 2020a) (Fig. 5). The remaining biomass can produce biomass charcoal to generate more CO and  $\text{H}_2$  (Yang et al., 2012).

## 6. Challenges and future perspectives

Currently, *D. salina* examples for wastewater treatment are still scarce, resulting from a lack of information about its potential for phycoremediation. Studies on the use of *D. salina* for wastewater treatment are not comprehensive, and most of them focus on target pollutants or are limited to laboratory scale, with few researchers focusing on the scalability of systems to cultivate *D. salina* (Besson et al., 2019; Díaz et al., 2020). Some problems associated with its use are wastewater components' variability, sustained investment, and unstable biomass production. In this regard, genetic engineering has been getting attention to overcome the limitations of *D. salina* and expand the range of its pollutant targets (Feng et al., 2020). Another approach to overcome unfavorable conditions is to use non-living cells to obtain a reliable pathway to capture nutrients, such as particle and intraparticle

**Table 4**  
Opportunities for bioproduct generation using wastewater as a cultivation medium.

Bioproduct	Potential extraction method	Potential sources	Application
Glycerol	Ultrasound + solvent extraction (ethanol) + nano filtration membrane (Monte et al. 2020)	Desalination plants brine Aquaculture Herbicides and pesticides industry Pharmaceutical industry	Food additive Pharmaceutical and nutraceutical Biodiesel Bioethanol
Lipid	Solvent extraction (Hernández et al., 2014) Supercritical CO <sub>2</sub> extraction (Hernández et al., 2014) Ultrasound + chloroform-methanol extraction (González-Balderas et al., 2020) Ozonation + chloroform-methanol extraction (González-Balderas et al., 2020)	Desalination plants brine Aquaculture Herbicides and pesticides industry Pharmaceutical industry Fossil fuel power plants Thermoelectric power plants Waste incineration	Biodiesel Biogas Biolubricants Pharmaceutical and nutraceutical purposes Food and beverage additives Dietary supplement
Carotenoid	Organic solvent (typically hexane) (Pourkarimi et al., 2020)	Desalination plants brine Aquaculture Herbicides and pesticides industry Pharmaceutical industry Desalination plants brine Mining Chemical industry Fertilizer industry Run-off Mining Chemical industry Petrochemical plants	Pharmaceutical and nutraceutical purposes
Protein	Alkali extraction (defatted biomass) (González-Balderas et al., 2020) Ultrasound + solvent extraction (ethanol) + nanofiltration membrane (Monte et al. 2020)	Desalination plants brine Herbicides and pesticides industry Pharmaceutical industry Petrochemical plants Secondary/tertiary wastewater	Food and animal feed
Beta-carotene	Solvent extraction (Pourkarimi et al., 2020)	Desalination plants brine Herbicides and pesticides industry Pharmaceutical industry Petrochemical plants Secondary/tertiary wastewater	Food and beverage additives Dietary supplements Cosmetics Animal feed
Carbohydrate	Acid extraction (defatted and after protein extraction) (González-Balderas et al., 2020) Ultrasound + Acid extraction Chemical hydrolysis (Karemore and Sen, 2016)	Secondary/tertiary wastewater Mining Chemical industry	Food H <sub>2</sub> generation Biochar

diffusion, ion exchange or chemisorption, electrostatic attraction, and multilayer adsorption (Ratnasari et al., 2022). For example, Amini et al. (2019) used *D. salina* dried biomass as an adsorbent to remove dissolved nitrate (NO<sub>3</sub><sup>-</sup>) and phosphate (PO<sub>4</sub><sup>3-</sup>) from saline water. Ionic interactions with amino-, carboxylic-, hydroxyl-, and carbonyl-groups were indicated by FTIR in binding these nutrients. Similarly, Dineshkumar et al. (2022) identified *D. salina* as a cheap biosorbent alternative

to recover Pb from industrial effluent. They associated the process with alkene, amine, alkyl halide, and alcohol functional groups.

Process optimization to reduce costs is inherent in cleaner technologies. For instance, providing CO<sub>2</sub> to promote microalgal biomass productivity can represent up to 27 % of production (de Godos et al., 2014) and 81 % of total utility costs (Sui et al., 2020). In addition to CO<sub>2</sub> supplementation, atmospheric losses contribute to the footprint and economic feasibility. Caia et al. (2018) obtained a reduction in CO<sub>2</sub> supply of 14 %, 40 %, and 38 % by decreasing the open pond's water level from 0.2 m to 0.1 m, reducing the paddle rotation speed from 21 rpm to 13 rpm, and increasing the pH set point from 7.2 to 8.0, respectively. However, the reduction of CO<sub>2</sub> supply due to the increase in pH came at the expense of a 17 % decrease in biomass productivity. Another viable alternative to reduce the costs associated with CO<sub>2</sub> is the application of flue gas as a carbon source (considering that measures have been taken to offset greenhouse gas emissions) (Harter et al., 2013).

Open ponds are the preferred system for microalgae cultivation; however, due to the gradual increase of salinity by evaporation (Ishika et al., 2019), the use of *D. salina* can generate wastewater rich in glycerol, especially when treating wastewater with higher temperatures (> 35 °C) that promote glycerol leaks, which must be treated before disposal (Oren, 2010). Another issue regarding open ponds is the risk of contamination by other microorganisms that may be pathogenic or compete for nutrients (Racharaks et al., 2015). This can be resolved with disinfection products that *D. salina* can resist (Moreno-Garrido and Canavate 2001).

Biomass harvesting offers another challenge for a more viable treatment system, mainly to generate more bioproducts (Ermiş et al., 2021). Microalgae harvesting contributes to circa 20 to 30 % of the total cost of production. The most common harvesting methods are centrifugation, flotation, sedimentation, and filtration. Energy consumption is a major limiting factor; for instance, centrifugation consumes about 8 kWh m<sup>-3</sup> (Monte et al., 2018). Centrifugation and solvent extract methods account for >90 % of energy consumption for biodiesel production, while ozone-rich microbubble extraction requires about 36 % of energy (Kamaroddin et al., 2020).

Two-step separation is commonly applied to reduce the costs associated with microalgal biomass harvesting, which comprises (1) concentrating the biomass to about 2–7 % of TSS, followed by (2) dewatering to further concentrate the microalgae slurry to 15–25 % of TSS. The first step can be accomplished by coagulation/(bio)floculation, gravity sedimentation, flotation, and electrical-based methods, while the second step can be accomplished by filtration or centrifugation. The appropriate method depends on the microorganism characteristics and target bioproduct (Barros et al., 2015). Monte et al. (2018) proposed two scenarios for harvesting *D. salina*. The first scenario (one-step scenario) used five centrifuges (capacity of 3 m<sup>3</sup> h<sup>-1</sup>; total investment of USD 657 k (1 euro = 1.05 dollars)) while the second scenario (two-step scenario) used a membrane (14 ultrafiltration elements with a capacity of 1.1 m<sup>3</sup> h<sup>-1</sup> each and an investment cost of USD 173 k) to pre-concentrate the biomass and one centrifuge (capacity of 3 m<sup>3</sup> h<sup>-1</sup> and investment cost of USD 137 k). They considered a capacity of processing algae biomass of 112,000 m<sup>3</sup> year<sup>-1</sup>, investment, operational, maintenance, and membrane replacement costs. The one-step scenario had a total cost of USD 1.22 m<sup>-3</sup>, while the two-step scenario had a total cost of USD 0.59 m<sup>-3</sup>, a reduction of 52 % and 45 % in the total cost of ownership and energy.

The small diameter and surface load of microalgae compromise effective harvesting, resulting in costly processes (Brentner et al., 2011). As *D. salina* does not have a cell wall, the costs associated with rupture and extraction of bioproducts are expected to be lower than for cells with a wall (Kamaroddin et al., 2020). The pH can be controlled to optimize the harvesting of *D. salina*, which does not naturally auto-flocculate. Besson and Guiraud (2013) achieved >90 % recovery efficiency by adding sodium hydroxide to increase the pH in a Mg-rich

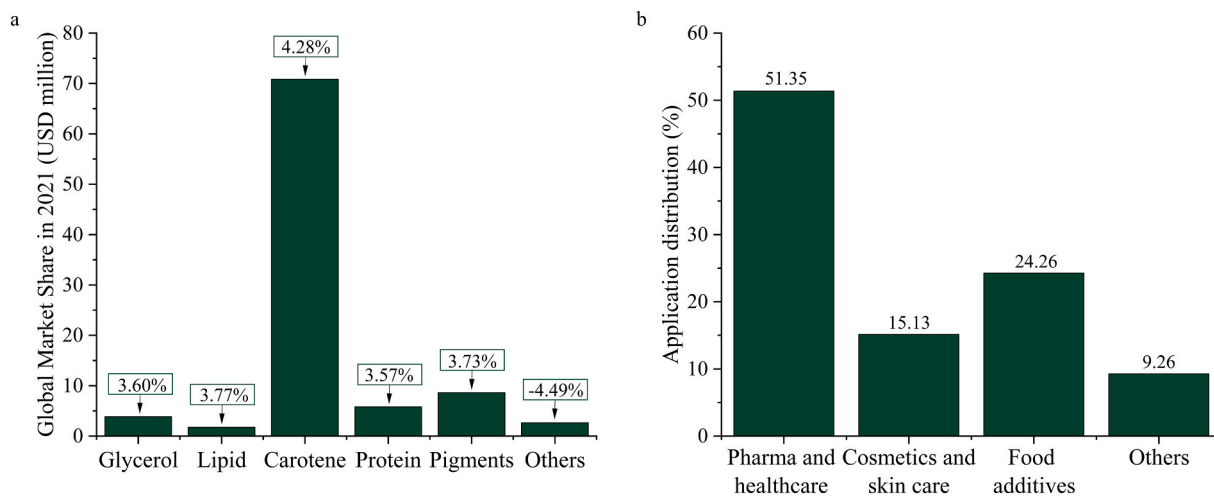


Fig. 6. (a) Market distribution by application. Data from 2020 (Maia Research, 2021) and (b) Global *D. salina* market for glycerol, lipid, carotene, protein, pigments, and others based on 2021. The values in rectangles represent the growth rate (GR) compared to the previous year.

medium. Dissolved air floatation combined with pH modulation is also a promising method for harvesting microalgal biomass from wastewater (Leite et al., 2020). However, when treating high-carbon-containing wastewater, the increase in pH must occur only to recover the biomass, as it impacts the carbon availability in the medium; for instance, most of the inorganic carbon (IC), which can be >99 % in natural brines, is as methanoate ( $\text{HCO}_2^-$ ) and carbonate ion ( $\text{CO}_3^{2-}$ ) at high pH. These IC forms are less available for uptake (Harvey and Ben-Amotz, 2020).

*D. salina* may have phenotypic traits that allow it to survive in extreme conditions, for instance, high temperatures (45 °C) (Gallego-Cartagena et al., 2019). This is useful when treating hot wastewater. However, temperature influences the carbon availability in the medium.  $\text{CO}_2$  solubility in the medium is reduced at higher temperatures, influencing microalgal growth. Considering a temperature below 30 °C, the  $\text{CO}_2$  mass transfer is 5 g g<sup>-1</sup>, while at higher temperatures, the mass transfer is typically >10 g g<sup>-1</sup>. Temperatures above 35 °C are reported to stimulate glycerol leakage from *D. salina* cells. This leak supports the growth of fungi, yeasts, and halophilic Archaea (Harvey and Ben-Amotz, 2020), which participate in wastewater treatment (Lefebvre and Moletta, 2006). This feature can help treat low-carbon wastewater; however, glycerol is a valuable bioproduct of industrial relevance; thus, preventing glycerol leakage helps to recover biomass with more application opportunities.

Some components and their concentration in the wastewater can hamper the production of bioproducts. The herbicide butachlor acetanilide (present in runoffs) affects the activities of the photosynthetic pigment, protein content SOD, and peroxidase (POD). It has an inhibitory effect on *D. salina* growth under high concentrations (Chen et al., 2007). However, some studies demonstrate a biphasic effect on some pesticides by *D. salina* (Chen and Jiang, 2011). This shows that the increase in the concentration of some pesticides triggers the defense system of *D. salina*, such as the yield of carotenoids to face stress. Alkyl ethoxy sulfates, anionic surfactants widely used in household detergents and cleaning products, degrade well under aerobic conditions; however, *D. salina* is highly sensitive to them. Therefore, even small concentrations of alkyl ethoxy sulfates compromise the use of *D. salina* for bioremediation of domestic wastewater (Sibila et al., 2008).

Dissolved oxygen represents a limiting factor concerning the density of *D. salina* (El Gammal et al., 2017), which could be attributed to oxidative stress in this microalga. Likewise, hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) offers oxidative stress to *D. salina*, producing hydroxyl radical ( $\text{HO}^\bullet$ ). This radical is involved in lipid peroxidation. As a defense mechanism, *D. salina* generates APX that removes  $\text{H}_2\text{O}_2$ . However, APX activity in *D. salina* is deficient compared to *D. tertiolecta* (Nikookar et al., 2005).

Gallo et al. (2020) found that mixed cultures (*D. salina* coupled with the cyanobacteria *Synechococcus elongatus*) showed greater tolerance to the oxidative effects produced by  $\text{Cu}^{2+}$ . These findings suggest that, although oxidative radicals may jeopardize the growth of *D. salina*, mixed cultures can overcome this problem.

Therefore, the challenges of cultivating *D. salina* in wastewater involve all stages of production: quality of the medium, culture system, harvesting, and purification. However, technological challenges do not prevent studies from advancing wastewater to cultivate this microalga, improving the cost-benefit ratio since they have already been addressed for many other species (Acién et al., 2017).

## 7. Conclusions

This research aimed to identify the potential use of *D. salina* for wastewater treatment and the production of bioproducts to make both processes more sustainable. Based on the analyzed data, we conclude that the applications of *D. salina* for wastewater treatment are far from exhausted. More research should be carried out, mainly using real wastewater to cover its complexity. The wide range of medium quality that can support the growth of this microalga demonstrates the potential for bioremediating wastewater from different sectors without requiring restrictive adaptations of the treatment unit. It also demonstrates the potential medium source that best fits different bioproducts and market opportunities. In addition, the main conclusions that may help future research on the subject are listed below:

- D. salina* can effectively recover As, Al, Cd, and Cr species;
- A longer exposure time provides more locations for metal binding. However, the recovery of some metals requires that the biomass be harvested periodically with a short period between harvestings;
- Several proteins are regulated to deal with toxic exposure, and the membrane appears to be the main barrier against toxicity;
- Stringent pH values determine nutrient bioavailability and assimilation;
- Salinity plays a significant role in the bioavailability of nutrients in the medium and the production of bioproducts;
- Amino, carboxylic, hydroxy, and carbonyl function groups are fundamental for nutrient binding;
- Wastewater treatment can be combined with the generation of bioproducts, such as lipids, carotenoids, and glycerol, depending on their origin;
- Studies on better harvesting methods should be carried out to ensure the viability of *D. salina* in wastewater treatment;



- i. Target pollutants must be defined, and the treatment parameters must be established to avoid inhibitory effects, especially in wastewater with high heterogeneity of pollutants;
- j. The biological treatment of wastewaters can benefit from using a consortium of microorganisms, as long as it does not affect the quality of the bioproducts generated by *D. salina*.

### 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

No data was used for the research described in the article.

### Acknowledgments

**Funding:** This study was partially supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, by Brazilian National Council for Scientific and Technological Development (CNPq) – protocol 310228/2019-0.

### CRedit authorship contribution statement

**Conceptualization:** Gleison de Souza Celente, Rosana de Cassia de Souza Schneider, and Tiele Medianeira Rizzetti; **Methodology:** Gleison de Souza Celente, Rosana de Cassia de Souza Schneider, and Tiele Medianeira Rizzetti; **Formal analysis and investigation:** Gleison de Souza Celente; **Writing - original draft preparation:** Gleison de Souza Celente; **Writing - review and editing:** Rosana de Cassia de Souza Schneider, Tiele Medianeira Rizzetti, Eduardo Alcayaga Logo, and Yixing Sui; **Funding acquisition:** Yixing Sui; **Supervision:** Rosana de Cassia de Souza Schneider, Tiele Medianeira Rizzetti, and Yixing Sui.

### Funding

This study was partially supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, by the Brazilian National Council for Scientific and Technological Development (CNPq) – protocol 310228/2019-0.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.168812>.

### References

Ación, F.G., Molina, E., Fernández-Sevilla, J.M., Barbosa, M., Gouveia, L., Sepúlveda, C., Bazaes, J., Arbib, Z., 2017. 20 - economics of microalgae production. In: *Microalgae-Based Biofuels and Bioproducts*. C. Gonzalez-Fernandez and R. Muñoz, Woodhead Publishing: 485-503. <https://doi.org/10.1016/B978-0-08-101023-5.00020-0>.

Aizawa, T., Urai, M., 2020. Structural analysis of an aluminum-binding capsular polysaccharide produced by *Acidoecella aluminidurans* strain AL46, an aluminum-tolerant bacterium isolated from plant roots in a highly acidic swamp in actual acid sulfate soil. *Carbohydr. Res.* 498, 108163 <https://doi.org/10.1016/j.carres.2020.108163>.

Akbarzadeh, N., Shariati, M., 2014. Aluminum remediation from medium by *Dunaliella*. *Ecol. Eng.* 67, 76–79. <https://doi.org/10.1016/j.ecoleng.2014.03.014>.

Amini, M., Amini Khoei, Z., Erfanifar, E., 2019. Nitrate (NO<sub>3</sub><sup>-</sup>) and phosphate (PO<sub>4</sub><sup>3-</sup>) removal from aqueous solutions by microalgae *Dunaliella salina*. *Biocatal. Agric. Biotechnol.* 19, 101097 <https://doi.org/10.1016/j.bcab.2019.101097>.

Andersson, I., Backlund, A., 2008. Structure and function of Rubisco. *Plant Physiol. Biochem.* 46 (3), 275–291. <https://doi.org/10.1016/j.plaphy.2008.01.001>.

Ashok, A., Kottuparambil, S., Høj, L., Negri, A.P., Duarte, C.M., Agustí, S., 2020. Accumulation of <sup>13</sup>C-labelled phenanthrene in phytoplankton and transfer to corals resolved using cavity ring-down spectroscopy. *Ecotoxicol. Environ. Saf.* 196, 110511 <https://doi.org/10.1016/j.ecoenv.2020.110511>.

Balzano, S., Sardo, A., Blasio, M., Chahine, T.B., Dell'Anno, F., Sansone, C., Brunet, C., 2020. Microalgal metallothioneins and phytochelatins and their potential use in bioremediation. *Front. Microbiol.* 11, 517.

Barros, A.I., Gonçalves, A.L., Simões, M., Pires, J.C.M., 2015. Harvesting techniques applied to microalgae: a review. *Renew. Sust. Energ. Rev.* 41, 1489–1500.

Becker, E.W., 1994. *Microalgae: Biotechnology and Microbiology*. Cambridge University Press.

Ben-Amotz, A., Polle, E., Rao, D.S., 2009. *The Alga Dunaliella: Biodiversity, Physiology, Genomics and Biotechnology*. Science Publishers, Jersey, Plymouth (555pp).

Besson, A., Guiraud, P., 2013. High-pH-induced flocculation–flotation of the hypersaline microalga *Dunaliella salina*. *Bioresour. Technol.* 147, 464–470. <https://doi.org/10.1016/j.biortech.2013.08.053>.

Besson, A., Formosa-Dague, C., Guiraud, P., 2019. Flocculation-flotation harvesting mechanism of *Dunaliella salina*: from nanoscale interpretation to industrial optimization. *Water Res.* 155, 352–361. <https://doi.org/10.1016/j.watres.2019.02.043>.

Bhuvaneshwari, M., Thiagarajan, V., Nemade, P., Chandrasekaran, N., Mukherjee, A., 2018. Toxicity and trophic transfer of P25 TiO<sub>2</sub> NPs from *Dunaliella salina* to *Artemia salina*: effect of dietary and waterborne exposure. *Environ. Res.* 160, 39–46. <https://doi.org/10.1016/j.envres.2017.09.022>.

Brentner, L.B., Eckelman, M.J., Zimmerman, J.B., 2011. Combinatorial life cycle assessment to inform process design of industrial production of algal biodiesel. *Environ. Sci. Technol.* 45 (16), 7060–7067.

Caia, M., Bernard, O., Bechet, Q., 2018. Optimizing CO<sub>2</sub> transfer in algal open ponds. *Algal Res.-Biomass Biofuels Bioprod.* 35, 530–538. <https://doi.org/10.1016/j.algal.2018.09.009>.

Cecal, A., Humelnic, D., Rudic, V., Cepoi, L., Cojocari, A., 2012. Removal of uranyl ions from UO<sub>2</sub>(NO<sub>3</sub>)<sub>2</sub> solution by means of *Chlorella vulgaris* and *Dunaliella salina* algae. *Cent. Eur. J. Chem.* 10 (5), 1669–1675. <https://doi.org/10.2478/s11532-012-0092-4>.

Celente, G.S., Rizzetti, T.M., Schneider, R.CdS, Harvey, P.J., Sui, Y., 2022. Organic carbon is ineffective in enhancing the growth of *dunaliella*. *Fermentation* 8 (6), 261.

Chan, S.M.N., Luan, T., Wong, M.H., Tam, N.F.Y., 2006. Removal and biodegradation of polycyclic aromatic hydrocarbons by *Selenastrum capricornutum*. *Environ. Toxicol. Chem.* 25 (7), 1772–1779.

Chen, H., Jiang, J.-G., 2011. Toxic effects of chemical pesticides (trichlorfon and dimehyo) on *Dunaliella salina*. *Chemosphere* 84 (5), 664–670.

Chen, Y., Xu, C., 2021. How to narrow the CO<sub>2</sub> gap from growth-optimal to flue gas levels by using microalgae for carbon capture and sustainable biomass production. *J. Clean. Prod.* 280, 124448 <https://doi.org/10.1016/j.jclepro.2020.124448>.

Chen, C.-H., Liu, Z.-Q., Fu, F., Xiang, X.-L., Qin, Y.-S., 2007. Effect of acetanilide herbicides butachlor on physiological and biochemical of microalgae *Dunaliella salina* [J]. *Ecol. Sci.* 1.

Chen, Y., Tang, X., Kapoore, R.V., Xu, C., Vaidyanathan, S., 2015. Influence of nutrient status on the accumulation of biomass and lipid in *Nannochloropsis salina* and *Dunaliella salina*. *Energy Convers. Manag.* 106, 61–72. <https://doi.org/10.1016/j.enconman.2015.09.025>.

Chi, J., Li, Y., Gao, J., 2019. Interaction between three marine microalgae and two phthalate acid esters. *Ecotoxicol. Environ. Saf.* 170, 407–411. <https://doi.org/10.1016/j.ecoenv.2018.12.012>.

Cho, K., Hur, S.-P., Lee, C.-H., Ko, K., Lee, Y.-J., Kim, K.-N., Kim, M.-S., Chung, Y.-H., Kim, D., Oda, T., 2016a. Bioflocculation of the oceanic microalga *Dunaliella salina* by the bloom-forming dinoflagellate *Heterocapsa circularisquama*, and its effect on biodiesel properties of the biomass. *Bioresour. Technol.* 202, 257–261. <https://doi.org/10.1016/j.biortech.2015.12.047>.

Cho, K., Lee, C.H., Ko, K., Lee, Y.J., Kim, K.N., Kim, M.K., Chung, Y.H., Kim, D., Yeo, I.K., Oda, T., 2016b. Use of phenol-induced oxidative stress acclimation to stimulate cell growth and biodiesel production by the oceanic microalga *Dunaliella salina*. *Algal Res.* 17, 61–66. <https://doi.org/10.1016/j.algal.2016.04.023>.

Clemens, S., 2006. Evolution and function of phytochelatin synthases. *J. Plant Physiol.* 163 (3), 319–332. <https://doi.org/10.1016/j.jplph.2005.11.010>.

Cobbett, C.S., 2000. Phytochelatin biosynthesis and function in heavy-metal detoxification. *Curr. Opin. Plant Biol.* 3 (3), 211–216. [https://doi.org/10.1016/S1369-5266\(00\)80067-9](https://doi.org/10.1016/S1369-5266(00)80067-9).

Cobo, S., Dominguez-Ramos, A., Irabien, A., 2018. Minimization of resource consumption and carbon footprint of a circular organic waste valorization system. *ACS Sustain. Chem. Eng.* 6 (3), 3493–3501.

Colusse, G.A., Mendes, C.R.B., Duarte, M.E.R., Carvalho, Jcd, Nosedá, M.D., 2020. Effects of different culture media on physiological features and laboratory scale production cost of *Dunaliella salina*. *Biotechnol. Rep.* 27, e00508 <https://doi.org/10.1016/j.btre.2020.e00508>.

Colusse, G.A., Santos, A.O., Rodrigues, J.M., Barga, M.C., Duarte, M.E.R., de Carvalho, J. C., Nosedá, M.D., 2021. Rice vinasse treatment by immobilized *Synechococcus pevalekii* and its effect on *Dunaliella salina* cultivation. *Bioprocess Biosyst. Eng.* 44 (7), 1477–1490.

Díaz, J.P., Inostroza, C., Ación, F.G., 2020. Scale-up of a Fibonacci-type photobioreactor for the production of *Dunaliella salina*. *Appl. Biochem. Biotechnol.* 1–17.

Dineshkumar, R., Sowndariya, M., Kalaiselvi, S., Israth Rehana, G., Durai Murugan, M., Kavisri, M., 2022. Effective removal of lead (Pb) by natural biosorbent marine microalgae (*Dunaliella salina*) through batch experiment. *Biomass Convers. Biorefr.* <https://doi.org/10.1007/s13399-021-02260-9>.

Ding, A., Zhang, R., Ngo, H.H., He, X., Ma, J., Nan, J., Li, G., 2021. Life cycle assessment of sewage sludge treatment and disposal based on nutrient and energy recovery: a review. *Sci. Total Environ.* 769, 144451.

- Duan, W., Meng, F., Lin, Y., Wang, G., 2017. Toxicological effects of phenol on four marine microalgae. *Environ. Toxicol. Pharmacol.* 52, 170–176. <https://doi.org/10.1016/j.etap.2017.04.006>.
- Dutta, D., Arya, S., Kumar, S., 2021. Industrial wastewater treatment: current trends, bottlenecks, and best practices. *Chemosphere* 285, 131245. <https://doi.org/10.1016/j.chemosphere.2021.131245>.
- Dyhrman, S.T., 2016. In: Borowitzka, M.A., Beardall, J., Raven, J.A. (Eds.), *Nutrients and Their Acquisition: Phosphorus Physiology in Microalgae. The Physiology of Microalgae*. Springer International Publishing, Cham, pp. 155–183. [https://doi.org/10.1007/978-3-319-24945-2\\_8](https://doi.org/10.1007/978-3-319-24945-2_8).
- El Gammal, M.A.M., Nageeb, M., Al-Sabee, S., 2017. Phytoplankton abundance in relation to the quality of the coastal water – Arabian Gulf, Saudi Arabia. *Egypt. J. Aquat. Res.* 43 (4), 275–282. <https://doi.org/10.1016/j.ejar.2017.10.004>.
- Ermis, H., Koyuncu, I., Altinbas, M., 2021. Pilot scale microalgae harvesting by a membrane: cross flow vs. submerged membrane. *Desalin. Water Treat.* 211, 391–398. <https://doi.org/10.5004/dwt.2021.26365>.
- Feng, S., Hu, L., Zhang, Q., Zhang, F., Du, J., Liang, G., Li, A., Song, G., Liu, Y., 2020. CRISPR/Cas technology promotes the various application of *Dunaliella salina* system. *Appl. Microbiol. Biotechnol.* 104 (20), 8621–8630.
- Fisher, M., Gokhman, I., Pick, U., Zamir, A., 1996. A salt-resistant plasma membrane carbonic anhydrase is induced by salt in *Dunaliella salina*. *J. Biol. Chem.* 271 (30), 17718–17723.
- Fisher, M., Gokhman, I., Pick, U., Zamir, A., 1997. A structurally novel transferrin-like protein accumulates in the plasma membrane of the unicellular green alga *Dunaliella salina* grown in high salinities\*. *J. Biol. Chem.* 272 (3), 1565–1570. <https://doi.org/10.1074/jbc.272.3.1565>.
- Folgar, S., Torres, E., Perez-Rama, M., Cid, A., Herrero, C., Abalde, J., 2009. *Dunaliella salina* as marine microalgae highly tolerant to but a poor remover of cadmium. *J. Hazard. Mater.* 165 (1–3), 486–493. <https://doi.org/10.1016/j.jhazmat.2008.10.010>.
- Gallego-Cartagena, E., Castillo-Ramirez, M., Martinez-Burgos, W., 2019. Effect of stressful conditions on the carotenogenic activity of a Colombian strain of *Dunaliella salina*. *Saudi J. Biol. Sci.* 26 (7), 1325–1330. <https://doi.org/10.1016/j.sjbs.2019.07.010>.
- Gallo, M., Morse, D., Hollnagel, H.C., Barros, M.P., 2020. Oxidative stress and toxicology of Cu<sup>2+</sup> based on surface areas in mixed cultures of green alga and cyanobacteria: The pivotal role of H<sub>2</sub>O<sub>2</sub>. *Aquat. Toxicol.* 222 <https://doi.org/10.1016/j.aquatox.2020.105450>.
- Ge, Y., Ning, Z., Wang, Y., Zheng, Y., Zhang, C., Figeys, D., 2016. Quantitative proteomic analysis of *Dunaliella salina* upon acute arsenate exposure. *Chemosphere* 145, 112–118. <https://doi.org/10.1016/j.chemosphere.2015.11.049>.
- Geider, R.J., Roche, J.L., 2002. Redfield revisited: variability of C[ratio]N[ratio]P in marine microalgae and its biochemical basis. *Eur. J. Phycol.* 37 (1), 1–17. <https://doi.org/10.1017/S0967026201003456>.
- Giordano, M., 2001. Interactions between C and N metabolism in *Dunaliella salina* cells cultured at elevated CO<sub>2</sub> and high N concentrations. *J. Plant Physiol.* 158 (5), 577–581.
- Giordano, M., Boves, G., 1997. Gas exchange and C allocation in *Dunaliella salina* cells in response to the N source and CO<sub>2</sub> concentration used for growth. *Plant Physiol.* 115 (3), 1049–1056.
- de Godos, I., Mendoza, J.L., Acien, F.G., Molina, E., Banks, C.J., Heaven, S., Rogalla, F., 2014. Evaluation of carbon dioxide mass transfer in raceway reactors for microalgae culture using flue gases. *Bioresour. Technol.* 153, 307–314. <https://doi.org/10.1016/j.biortech.2013.11.087>.
- Goh, P.S., Wong, K.C., Ismail, A.F., 2022. Membrane technology: a versatile tool for saline wastewater treatment and resource recovery. *Desalination* 521, 24.
- González-Balderas, R.M., Velásquez-Orta, S.B., Valdez-Vázquez, I., Orta Ledesma, M.T., 2020. Intensified recovery of lipids, proteins, and carbohydrates from wastewater-grown microalgae *Desmodesmus* sp. by using ultrasound or ozone. *Ultrason. Sonochem.* 62, 104852.
- Hadzizadeh, Z., Mehrgan, M.S., Shekarabi, S.P.H., 2020. The potential use of stickwater from a kikka fishmeal plant in *Dunaliella salina* cultivation. *Environ. Sci. Pollut. Res.* 27 (2), 2144–2154. <https://doi.org/10.1007/s11356-019-06926-w>.
- Hamidkhani, A., Asgarani, E., Saboora, A., Hejazi, M.A., 2021. Comparison of selenium-induced antioxidant responses and bioaccumulation in two strains of the halotolerant alga *Dunaliella salina*. *Bot. Mar.* 64 (4), 275–287. <https://doi.org/10.1515/bot-2020-0078>.
- Harter, T., Bossier, P., Verreth, J., Bodé, S., Van der Ha, D., Debeer, A.-E., Boon, N., Boeckx, P., Vyverman, W., Nevejan, N., 2013. Carbon and nitrogen mass balance during flue gas treatment with *Dunaliella salina* cultures. *J. Appl. Phycol.* 25 (2), 359–368.
- Harvey, P.J., Ben-Amotz, A., 2020. Towards a sustainable *Dunaliella salina* microalgal biorefinery for 9-cis  $\beta$ -carotene production. *Algal Res.* 50, 102002.
- Hasanuzzaman, M., Bhuyan, M.H.M.B., Anee, T.I., Parvin, K., Nahar, K., Mahmud, J.A., Fujita, M., 2019. Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. *Antioxidants (Basel, Switzerland)* 8 (9), 384. <https://doi.org/10.3390/antiox8090384>.
- Hassanpour, M., Hosseini Tafreshi, S.A., Amiri, O., Hamadani, M., Salavati-Niasari, M., 2020. Toxic effects of Fe<sub>2</sub>WO<sub>6</sub> nanoparticles towards microalgae *Dunaliella salina*: Sonochemical synthesis nanoparticles and investigate its impact on the growth. *Chemosphere* 258, 127348. <https://doi.org/10.1016/j.chemosphere.2020.127348>.
- Hellebust, J.A., Ahmad, I., 1989. Regulation of nitrogen assimilation in green microalgae. *Biol. Oceanogr.* 6 (3–4), 241–255. <https://doi.org/10.1080/01965581.1988.10749529>.
- Hernández, D., Solana, M., Riaño, B., García-González, M.C., Bertuccio, A., 2014. Biofuels from microalgae: lipid extraction and methane production from the residual biomass in a biorefinery approach. *Bioresour. Technol.* 170, 370–378.
- Holan, Z., Volesky, B., 1994. Bioadsorption of lead and nickel by biomass of marine algae. *Biotechnol. Bioeng.* 43 (11), 1001–1009.
- Holan, Z., Volesky, B., Prasetyo, I., 1993. Bioadsorption of cadmium by biomass of marine algae. *Biotechnol. Bioeng.* 41 (8), 819–825.
- Hou, Y., Liu, Z., Zhao, Y., Chen, S., Zheng, Y., Chen, F., 2016. CAH1 and CAH2 as key enzymes required for high bicarbonate tolerance of a novel microalgae *Dunaliella salina* HTBS. *Enzym. Microb. Technol.* 87–88, 17–23. <https://doi.org/10.1016/j.enzmictec.2016.02.010>.
- Hu, G., Li, Y., Ye, C., Liu, L., Chen, X., 2019. Engineering microorganisms for enhanced CO<sub>2</sub> sequestration. *Trends Biotechnol.* 37 (5), 532–547. <https://doi.org/10.1016/j.tibtech.2018.10.008>.
- In-na, P., Umar, A.A., Wallace, A.D., Flickinger, M.C., Caldwell, G.S., Lee, J.G.M., 2020. Loofah-based microalgae and cyanobacteria biocomposites for intensifying carbon dioxide capture. *J. Co<sub>2</sub> Util.* 42, 14. <https://doi.org/10.1016/j.jcou.2020.101348>.
- Ishika, T., Moheimani, N.R., Laird, D.W., Bahri, P.A., 2019. Stepwise culture approach optimizes the biomass productivity of microalgae cultivated using an incremental salinity increase strategy. *Biomass Bioenergy* 127. <https://doi.org/10.1016/j.biombioe.2019.105274>.
- Jais, N.M., Mohamed, R., Al-Gheethi, A., Hashim, M.A., 2017. The dual roles of phycoremediation of wet market wastewater for nutrients and heavy metals removal and microalgae biomass production. *Clean Techn. Environ. Policy* 19 (1), 37–52.
- Johari, S.A., Sarkheil, M., Behzadi Tayemeh, M., Veisi, S., 2018. Influence of salinity on the toxicity of silver nanoparticles (AgNPs) and silver nitrate (AgNO<sub>3</sub>) in halophilic microalgae, *Dunaliella salina*. *Chemosphere* 209, 156–162. <https://doi.org/10.1016/j.chemosphere.2018.06.098>.
- Kaamouh, M., El-Agawany, N., Omar, M.Y., 2022. Environmental toxicological evaluation (in vitro) of copper, zinc and cybutryne on the growth and amino acids content of the marine alga *Dunaliella salina*. *Egypt. J. Aquat. Res.* <https://doi.org/10.1016/j.ejar.2022.07.004>.
- Kadkhodaei, S., Abbasiliasi, S., Shun, T., Masoumi, H.F., Mohamed, M., Movahedi, A., Rahim, R., Ariff, A., 2015. Enhancement of protein production by microalgae *Dunaliella salina* under mixotrophic conditions using response surface methodology. *RSC Adv.* 5 (48), 38141–38151.
- Kalra, R., Gaur, S., Goel, M., 2021. Microalgae bioremediation: a perspective towards wastewater treatment along with industrial carotenoids production. *J. Water Process Eng.* 40, 24.
- Kamaroddin, M.F., Rahaman, A., Gilmour, D.J., Zimmerman, W.B., 2020. Optimization and cost estimation of microalgal lipid extraction using ozone-rich microbubbles for biodiesel production. *Biocatal. Agric. Biotechnol.* 23 <https://doi.org/10.1016/j.bcab.2019.101462>.
- Karemore, A., Sen, R., 2016. Downstream processing of microalgal feedstock for lipid and carbohydrate in a biorefinery concept: a holistic approach for biofuel applications. *RSC Adv.* 6 (35), 29486–29496.
- Katz, A., Kaback, H.R., Avron, M., 1986. Na<sup>+</sup>/H<sup>+</sup> antiporter in isolated plasma membrane vesicles from the halotolerant alga *Dunaliella salina*. *FEBS Lett.* 202 (1), 141–144.
- Kim, W., Park, J.M., Gim, G.H., Jeong, S.-H., Kang, C.M., Kim, D.-J., Kim, S.W., 2012. Optimization of culture conditions and comparison of biomass productivity of three green algae. *Bioprocess Biosyst. Eng.* 35 (1), 19–27.
- Kim, G.-Y., Heo, J., Kim, H.-S., Han, J.-I., 2017. Bicarbonate-based cultivation of *Dunaliella salina* for enhancing carbon utilization efficiency. *Bioresour. Technol.* 227, 72–77. <https://doi.org/10.1016/j.biortech.2017.04.009>.
- Kishi, M., Toda, T., 2018. Carbon fixation properties of three alkalihalophilic microalgal strains under high alkalinity. *J. Appl. Phycol.* 30 (1), 401–410.
- Kliphuis, A.M., Martens, D.E., Janssen, M., Wijffels, R.H., 2011. Effect of O<sub>2</sub>: CO<sub>2</sub> ratio on the primary metabolism of *Chlamydomonas reinhardtii*. *Biotechnol. Bioeng.* 108 (10), 2390–2402.
- Kwon, H.K., Jeon, J.Y., Oh, S.J., 2017. Potential for heavy metal (copper and zinc) removal from contaminated marine sediments using microalgae and light emitting diodes. *Ocean Sci. J.* 52 (1), 57–66. <https://doi.org/10.1007/s12601-017-0001-z>.
- Larsdotter, K., 2006. Wastewater treatment with microalgae—a literature review. *Vatten* 62 (1), 31.
- Lefebvre, O., Moletta, R., 2006. Treatment of organic pollution in industrial saline wastewater: a literature review. *Water Res.* 40 (20), 3671–3682.
- Leite, LdS, dos Santos, P.R., Daniel, L.A., 2020. Microalgae harvesting from wastewater by pH modulation and flotation: assessing and optimizing operational parameters. *J. Environ. Manag.* 254, 109825 <https://doi.org/10.1016/j.jenvman.2019.109825>.
- Li, M., Barbaro, E., Bellini, E., Saba, A., Sanità di Toppi, L., Varotto, C., 2020. Ancestral function of the phytochelatin synthase C-terminal domain in inhibition of heavy metal-mediated enzyme overactivation. *J. Exp. Bot.* 71 (20), 6655–6669. <https://doi.org/10.1093/jxb/eraa386>.
- Lin, L., Chan, G.Y.S., Jiang, B.L., Lan, C.Y., 2007. Use of ammoniacal nitrogen tolerant microalgae in landfill leachate treatment. *Waste Manag.* 27 (10), 1376–1382. <https://doi.org/10.1016/j.wasman.2006.09.001>.
- Liu, Y., Yildiz, I., 2018. The effect of salinity concentration on algal biomass production and nutrient removal from municipal wastewater by *Dunaliella salina*. *Int. J. Energy Res.* 42 (9), 2997–3006. <https://doi.org/10.1002/er.3967>.
- Liu, Y., Luan, T.G., Lu, N.N., Lan, C.Y., 2006. Toxicity of fluoranthene and its biodegradation by *Cyclotella caspia* alga. *J. Integr. Plant Biol.* 48 (2), 169–180.
- Liu, F., Tu, T., Li, S., Cai, M., Huang, X., Zheng, F., 2019. Relationship between plankton-based B-carotene and biodegradable adaptability to petroleum-derived hydrocarbon. *Chemosphere* 237. <https://doi.org/10.1016/j.chemosphere.2019.124430>.
- Maia Research, 2021. *Global Dunaliella Salina Market Research Report, Segment by Major Players, Types, Applications and Regions, 2016–2026*.

- Menke, S., Sennhenn, A., Sachse, J.H., Majewski, E., Huchzermeyer, B., Rath, T., 2012. Screening of microalgae for feasible mass production in industrial hypersaline wastewater using disposable bioreactors. *Clean-Soil Air Water* 40 (12), 1401–1407. <https://doi.org/10.1002/clen.201100402>.
- Meticulous Market Research, 2021. *Dunaliella Salina* Market to Reach \$117.6 Million by 2027 — Exclusive Report Covering Pre and Post COVID-19 Market Analysis and Forecasts by Meticulous Research®. <https://www.globenewswire.com/en/news-release/2021/04/08/2206675/0/en/Dunaliella-Salina-Market-to-Reach-117-6-Million-by-2027-Exclusive-Report-Covering-Pre-and-Post-COVID-19-Market-Analysis-and-Forecasts-by-Meticulous-Research.html> (Accessed September 6th 2021).
- Mishra, A., Jha, B., 2009. Isolation and characterization of extracellular polymeric substances from micro-algae *Dunaliella salina* under salt stress. *Bioresour. Technol.* 100 (13), 3382–3386. <https://doi.org/10.1016/j.biortech.2009.02.006>.
- Mishra, A., Kavita, K., Jha, B., 2011. Characterization of extracellular polymeric substances produced by micro-algae *Dunaliella salina*. *Carbohydr. Polym.* 83 (2), 852–857. <https://doi.org/10.1016/j.carbpol.2010.08.067>.
- Moayed, A., Yargholi, B., Pazira, E., Babazadeh, H., 2019. Investigated of desalination of saline waters by using *Dunaliella Salina* algae and its effect on water ions. *Civil Eng. J.* 5 (11), 2450–2460.
- Monte, J., Sá, M., Galinha, C.F., Costa, L., Hoekstra, H., Brazinha, C., Crespo, J.G., 2018. Harvesting of *Dunaliella salina* by membrane filtration at pilot scale. *Sep. Purif. Technol.* 190, 252–260.
- Monte, J., Ribeiro, C., Parreira, C., Costa, L., Brive, L., Casal, S., Brazinha, C., Crespo, J.G., 2020. Biorefinery of *Dunaliella salina*: sustainable recovery of carotenoids, polar lipids and glycerol. *Bioresour. Technol.* 297 <https://doi.org/10.1016/j.biortech.2019.122509>.
- Morowat, M.H., Ghasemi, Y., 2016. Culture medium optimization for enhanced β-carotene and biomass production by *Dunaliella salina* in mixotrophic culture. *Biocatal. Agric. Biotechnol.* 7, 217–223.
- Mu, N., Mehar, J.G., Mudliar, S.N., Shekh, A.Y., 2019. Recent advances in microalgal bioactives for food, feed, and healthcare products: commercial potential, market space, and sustainability. *Compr. Rev. Food Sci. Food Saf.* 18 (6), 1882–1897.
- Muhammad, G., Alam, M.A., Xiong, W., Lv, Y., 2020. Microalgae biomass production: an overview of dynamic operational methods. In: Xu, J.L., Alam, M., Xu, J.L., Wang, Z. (Eds.), *Microalgae Biotechnology for Food, Health and High Value Products*. Springer, Singapore. [https://doi.org/10.1007/978-981-15-0169-2\\_13](https://doi.org/10.1007/978-981-15-0169-2_13).
- Nikookar, K., Moradshahi, A., Hosseini, L., 2005. Physiological responses of *Dunaliella salina* and *Dunaliella tertiolecta* to copper toxicity. *Biomol. Eng. J.* 22 (4), 141–146.
- Noctor, G., Queval, G., Mhamdi, A., Chaouch, S., Foyer, C.H., 2011. *Glutathione*. In: *The Arabidopsis Book* 9: e0142-e0142. <https://doi.org/10.1199/tab.0142>.
- Oren, A., 2010. Industrial and environmental applications of halophilic microorganisms. *Environ. Technol.* 31 (8–9), 825–834.
- Ortega Méndez, J.A., Mendoza, H., Santiago, D.E., Aridane Rodríguez, F., Gil Lodos, M., Carmona, L., 2012. Reuse of SWRO brine for the production of carotenoids from *Dunaliella salina* and removal of macronutrients. *Desalin. Water Treat.* 49 (1–3), 115–122.
- Oswald, W.J., Gotaas, H., Ludwig, H.F., Lynch, V., 1953. Algae symbiosis in oxidation ponds: III. Photosynthetic oxygenation. In: *Sewage and Industrial Wastes*, pp. 692–705.
- Padilla-Rivera, A., Güereca, L.P., 2019. A proposal metric for sustainability evaluations of wastewater treatment systems (SEWATS). *Ecol. Indic.* 103, 22–33.
- Pirwitz, K., Rihko-Struckmann, L., Sundmacher, K., 2016. Valorization of the aqueous phase obtained from hydrothermally treated *Dunaliella salina* remnant biomass. *Bioresour. Technol.* 219, 64–71. <https://doi.org/10.1016/j.biortech.2016.06.095>.
- Pourkarimi, S., Hallajisani, A., Alizadehdakheel, A., Nouralishahi, A., Golzary, A., 2020. Factors affecting production of beta-carotene from *Dunaliella salina* microalgae. *Biocatal. Agric. Biotechnol.* 29, 101771.
- Putri, W.A.E., Muhaemin, M.M.M., 2010. Phosphorus and ammonium ions removal by using The microalgae *Dunaliella salina*. *J. Penlitian Sains* 13 (3).
- Racharaks, R., Ge, X., Li, Y., 2015. Cultivation of marine microalgae using shale gas flowback water and anaerobic digestion effluent as the cultivation medium. *Bioresour. Technol.* 191, 146–156. <https://doi.org/10.1016/j.biortech.2015.04.065>.
- Ranjbar, S., Quaranta, J., Tehrani, R., Van Aken, B., 2015. *Algae-Based Treatment of Hydraulic Fracturing Produced Water: Metal Removal and Biodiesel Production by the Halophilic Microalgae Dunaliella salina*. *Bioremediation and Sustainable Environmental Technologies*, Third International Symposium on Bioremediation and Sustainable Environmental Technologies.
- Rao, S., 2019. Cultivation, Growth Media, Division Rates and Applications of *Dunaliella* Species. *The Alga Dunaliella, Biodiversity, Physiology, Genomics and Biotechnology*, pp. 44–89.
- Ratnasari, A., Syafiuddin, A., Zaidi, N.S., Hong Kueh, A.B., Hadibarata, T., Prastyo, D.D., Ravikumar, R., Sathishkumar, P., 2022. Bioremediation of micropollutants using living and non-living algae - current perspectives and challenges. *Environ. Pollut.* 292, 118474 <https://doi.org/10.1016/j.envpol.2021.118474>.
- Roberts, K.P., Heaven, S., Banks, C.J., 2019. Semi-continuous anaerobic digestion of the marine micro-algal species *I. galbana* and *D. salina* grown under low and high sulphate conditions. *Algal Res.* 41 <https://doi.org/10.1016/j.algal.2019.101564>.
- Ruiz-Martínez, A., García, N.M., Romero, I., Seco, A., Ferrer, J., 2012. Microalgae cultivation in wastewater: nutrient removal from anaerobic membrane bioreactor effluent. *Bioresour. Technol.* 126, 247–253.
- Saravanan, A., Gurumoorthy, P., 2020. “Biofuel Production from Marine Microalgae Using Dairy Industry Wastewater.” *Journal of Seybold Report* ISSN NO 1533: 9211.
- Schwarz, M., Zamir, A., Pick, U., 2003. Iron-binding properties of TTF, a salt-induced transferrin from the Alga *Dunaliella salina*. *J. Plant Nutr.* 26 (10–11), 2081–2091.
- Sendra, M., Blasco, J., Araújo, C.V.M., 2018. Is the cell wall of marine phytoplankton a protective barrier or a nanoparticle interaction site? Toxicological responses of *Chlorella autotrophica* and *Dunaliella salina* to Ag and CeO<sub>2</sub> nanoparticles. *Ecol. Indic.* 95, 1053–1067. <https://doi.org/10.1016/j.ecolind.2017.08.050>.
- Shafiq, M.A., 2008. Phytoremediation of some heavy metals by *Dunaliella salina*. *Glob. J. Environ. Res.* 2 (1), 01–11.
- Shankar, B., 2011. Low-cost treatment for attenuation of nitrate from groundwater. *J. Eng. Technol. Res.* 3 (1), 16–21.
- Shiri, M., Hashemifard, S.A., Abdi, G., 2023. The potential of microalgae *Dunaliella salina* to treat shrimp pond wastewater in a PAN/GO membrane bioreactor. *Chemosphere* 318, 138001.
- Sibila, M.A., Garrido, M.C., Perales, J.A., Quiroga, J.M., 2008. Ecotoxicity and biodegradability of an alkyl ethoxysulphate surfactant in coastal waters. *Sci. Total Environ.* 394 (2–3), 265–274. <https://doi.org/10.1016/j.scitotenv.2008.01.043>.
- de Souza Schneider, R.C., de Moura Lima, M., Hoeltz, M., de Farias Neves, F., John, D.K., de Azevedo, A., 2018. Life cycle assessment of microalgae production in a raceway pond with alternative culture media. *Algal Res.* 32, 280–292.
- de Souza, Celeste G., Colares, G.S., Machado, E.L., Lobo, E.A., 2019. Algae turf scrubber and vertical constructed wetlands combined system for decentralized secondary wastewater treatment. *Environ. Sci. Pollut. Res.* 26 (10), 9931–9937.
- de Souza, Celeste G., Colares, G.S., da Silva, Araújo P., Machado, E.L., Lobo, E.A., 2020. Acute ecotoxicity and genotoxicity assessment of two wastewater treatment units. *Environ. Sci. Pollut. Res.* 1–8.
- Su, Y., 2021. Revisiting carbon, nitrogen, and phosphorus metabolisms in microalgae for wastewater treatment. *Sci. Total Environ.* 762, 144590.
- Sui, Y., Muys, M., Van de Waal, D.B., D’Adamo, S., Vermeer, P., Fernandes, T.V., Vlaeminck, S.E., 2019a. Enhancement of co-production of nutritional protein and carotenoids in *Dunaliella salina* using a two-phase cultivation assisted by nitrogen level and light intensity. *Bioresour. Technol.* 287 <https://doi.org/10.1016/j.biortech.2019.121398>.
- Sui, Y., Muys, M., Vermeer, P., D’Adamo, S., Vlaeminck, S.E., 2019b. Light regime and growth phase affect the microalgal production of protein quantity and quality with *Dunaliella salina*. *Bioresour. Technol.* 275, 145–152. <https://doi.org/10.1016/j.biortech.2018.12.046>.
- Sui, Y., Jiang, Y., Moretti, M., Vlaeminck, S.E., 2020. Harvesting time and biomass composition affect the economics of microalgae production. *J. Clean. Prod.* 259, 120782 <https://doi.org/10.1016/j.jclepro.2020.120782>.
- Sukačová, K., Trtílek, M., Rataj, T., 2015. Phosphorus removal using a microalgal biofilm in a new biofilm photobioreactor for tertiary wastewater treatment. *Water Res.* 71, 55–63.
- Thakur, A., Kumar, H., 1999. Nitrate, ammonium, and phosphate uptake by the immobilized cells of *Dunaliella salina*. *Bull. Environ. Contam. Toxicol.* 62 (1), 70–78.
- Tsuji, N., Hirayanagi, N., Okada, M., Miyasaka, H., Hirata, K., Zenk, M.H., Miyamoto, K., 2002. Enhancement of tolerance to heavy metals and oxidative stress in *Dunaliella tertiolecta* by Zn-induced phytochelatin synthesis. *Biochem. Biophys. Res. Commun.* 293 (1), 653–659. [https://doi.org/10.1016/S0006-291X\(02\)00265-6](https://doi.org/10.1016/S0006-291X(02)00265-6).
- Tsuji, N., Hirayanagi, N., Iwabe, O., Namba, T., Tagawa, M., Miyamoto, S., Miyasaka, H., Takagi, M., Hirata, K., Miyamoto, K., 2003. Regulation of phytochelatin synthesis by zinc and cadmium in marine green alga, *Dunaliella tertiolecta*. *Phytochemistry* 62 (3), 453–459. [https://doi.org/10.1016/S0031-9422\(02\)00559-9](https://doi.org/10.1016/S0031-9422(02)00559-9).
- Ulrich, K., Jakob, U., 2019. The role of thiols in antioxidant systems. *Free Radic. Biol. Med.* 140, 14–27. <https://doi.org/10.1016/j.freeradbiomed.2019.05.035>.
- Vega, J.M., 2020. Nitrogen and Sulfur Metabolism in Microalgae and Plants: 50 Years of Research. *Progress in Botany* Vol. 81, F. M. Cánovas, U. Lüttge, C. Leuschner and M.-C. Risueño. Cham, Springer International Publishing: 1–40. [https://doi.org/10.1007/124\\_2018\\_26](https://doi.org/10.1007/124_2018_26).
- Venugopal, V., Sasidharan, A., 2021. Seafood industry effluents: environmental hazards, treatment and resource recovery. *J. Environ. Chem. Eng.* 9 (2), 104758 <https://doi.org/10.1016/j.jece.2020.104758>.
- Vidyalaxmi, Kaushik, G., Raza, K., 2019. Potential of novel *Dunaliella salina* from Sambhar salt lake, India, for bioremediation of hexavalent chromium from aqueous effluents: an optimized green approach. *Ecotoxicol. Environ. Saf.* 180, 430–438. <https://doi.org/10.1016/j.ecoenv.2019.05.039>.
- Vieira Costa, J.A., Cruz, C.G., Centeno da Rosa, A.P., 2021. Insights into the technology utilized to cultivate microalgae in dairy effluents. *Biocatal. Agric. Biotechnol.* 35, 102106 <https://doi.org/10.1016/j.cbac.2021.102106>.
- Vo, H.N.P., Ngo, H.H., Guo, W., Chang, S.W., Nguyen, D.D., Chen, Z., Wang, X.C., Chen, R., Zhang, X., 2020. Microalgae for saline wastewater treatment: a critical review. *Crit. Rev. Environ. Sci. Technol.* 50 (12), 1224–1265.
- Wan, M., Liu, P., Xia, J., Rosenberg, J.N., Oyler, G.A., Betenbaugh, M.J., Nie, Z., Qiu, G., 2011. The effect of mixotrophy on microalgal growth, lipid content, and expression levels of three pathway genes in *Chlorella sorokiniana*. *Appl. Microbiol. Biotechnol.* 91 (3), 835–844. <https://doi.org/10.1007/s00253-011-3399-8>.
- Wang, Y., Zhang, C., Wang, S., Shen, L., Ge, Y., 2013. Accumulation and transformation of different arsenic species in nonaxenic *Dunaliella salina*. *Huan jing ke xue Huan jing ke xue* 34 (11), 4257–4265.
- Wang, Y., Zhang, C.H., Lin, M.M., Ge, Y., 2016. A symbiotic bacterium differentially influences arsenate absorption and transformation in *Dunaliella salina* under different phosphate regimes. *J. Hazard. Mater.* 318, 443–451. <https://doi.org/10.1016/j.jhazmat.2016.07.031>.
- Wang, Y., Zhang, C., Zheng, Y., Ge, Y., 2017. Phytochelatin synthesis in *Dunaliella salina* induced by arsenite and arsenate under various phosphate regimes. *Ecotoxicol. Environ. Saf.* 136, 150–160. <https://doi.org/10.1016/j.ecoenv.2016.11.002>.
- Wen, J., Dong, H., Zeng, G., 2018. Application of zeolite in removing salinity/sodicity from wastewater: a review of mechanisms, challenges and opportunities. *J. Clean. Prod.* 197, 1435–1446.
- Yamaoka, Y., Takimura, O., Fuse, H., Murakami, K., 1999. Effect of glutathione on arsenic accumulation by *Dunaliella salina*. *Appl. Organomet. Chem.* 13 (2), 89–94.



- Yang, C., Jia, L., Su, S., Tian, Z., Song, Q., Fang, W., Chen, C., Liu, G., 2012. Utilization of CO<sub>2</sub> and biomass char derived from pyrolysis of *Dunaliella salina*: the effects of steam and catalyst on CO and H<sub>2</sub> gas production. *Bioresour. Technol.* 110, 676–681. <https://doi.org/10.1016/j.biortech.2012.01.124>.
- Zamani, H., Rastegari, B., Varamini, M., 2019. Antioxidant and anti-cancer activity of *Dunaliella salina* extract and oral drug delivery potential via nano-based formulations of gum Arabic coated magnetite nanoparticles. *J. Drug Deliv. Sci. Technol.* 54, 101278 <https://doi.org/10.1016/j.jddst.2019.101278>.
- Zhang, X., Tang, X., Zhou, B., Hu, S., Wang, Y., 2015. Effect of enhanced UV-B radiation on photosynthetic characteristics of marine microalgae *Dunaliella salina* (Chlorophyta, Chlorophyceae). *J. Exp. Mar. Biol. Ecol.* 469, 27–35. <https://doi.org/10.1016/j.jembe.2015.04.002>.
- Zhang, X., Tang, X., Wang, M., Zhang, W., Zhou, B., Wang, Y., 2017. ROS and calcium signaling mediated pathways involved in stress responses of the marine microalgae *Dunaliella salina* to enhanced UV-B radiation. *J. Photochem. Photobiol. B Biol.* 173, 360–367. <https://doi.org/10.1016/j.jphotobiol.2017.05.038>.
- Zhang, X., Shi, Y., Chen, Y., Hu, H., Cheng, F., Li, R., Wu, Y., 2022. Ecosystem simulation and environmental impact analysis of transforming microalgae to produce jet fuel. *J. Clean. Prod.* 333, 130100 <https://doi.org/10.1016/j.jclepro.2021.130100>.
- Zhu, C., Zhai, X., Jia, J., Wang, J., Han, D., Li, Y., Tang, Y., Chi, Z., 2018. Seawater desalination concentrate for cultivation of *Dunaliella salina* with floating photobioreactor to produce  $\beta$ -carotene. *Algal Res.* 35, 319–324. <https://doi.org/10.1016/j.algal.2018.08.035>.
- Zhu, Q.-L., Guo, S.-N., Wen, F., Zhang, X.-L., Wang, C.-C., Si, L.-F., Zheng, J.-L., Liu, J., 2019. Transcriptional and physiological responses of *Dunaliella salina* to cadmium reveals time-dependent turnover of ribosome, photosystem, and ROS-scavenging pathways. *Aquat. Toxicol.* 207, 153–162.