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Culturing *Arthrospira maxima* in mining wastewater: Pilot-scale culturing and biomass valorisation into C-phycocyanin and crude lipid extract

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ABSTRACT

Microalgae cultivation using wastewater as culture media offers a sustainable method for bioremediation that becomes a novel cost-effective tool when coupled to added-value products biorefinery. In this perspective, this work highlights the potential of *Arthrospira maxima* culturing as a new technique for highly sulphated wastewater (3,125 ± 340 mg L^{-1}) bioremediation at industrial scale. The influence of sulphate content on microalgal growth was evaluated and, simultaneously, sulphate bioremediation rate was measured. Accordingly, microalgal biomass was separated from culture media for diluted sulphate content measurement through a turbidimetric procedure. The results indicate that *A. maxima* growth was not hindered when cultured in sulphated wastewater and significantly decreased the sulphate content within the first 6 days (50% of the initial sulphate content). The obtained biomass was valorised through the sequential extraction of C-phycocyanin, by a freeze-thaw method, and crude lipid extract, through a solvent extraction method. Wastewater-cultured microalgae presented a higher phycocyanin production when compared to control group biomass, being the production yield (mg/g) 18.159 ± 0.017 and 38.748 ± 0.072, respectively. Crude lipid extraction assays showed that lipid content in *A. maxima* was not modified when exposed to high concentrations of sulphates. Accordingly, no significant difference was observed between control and experimental group lipidic extracts, achieving both groups close to a 7% of extraction yield. The results obtained confirm the hypothesis of using *A. maxima* industrial cultures for wastewater remediation along with the production of phycocyanin and crude lipid extract, which could be further valorised.

1. Introduction

From the beginning of the Industrial Revolution, our society has been producing an increasing amount of waste, exceeding the natural resilience capacity of our planet. This derives from the economic model used, based on a linear criterion that is grounded on the unsustainable extraction of natural resources and irresponsible waste management (Korhonen et al., 2018). This linear paradigm has produced the irreversible degradation of the environment, especially regarding biodiversity and water sources, which have been damaged through reckless resource extraction activities (Bressanelli et al., 2019). Facing this situation, Pearce and Turner (1990) proposed the model of circular economy,

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which turns the "resources-products-pollutions" mode into "resources-products-regenerated resources" mode, converting wastes from the value chain into new products. This novel economic system is focused on the achievement of the optimum production through reusing wastes and the minimisation of emitted pollution (Wu et al., 2014).

Mining sector is one of the most water-intensive ones and is largely responsible in terms of wastewater discharge, especially due to mineral-processing activities (Velenturf et al., 2019). Mineral-processing facilities use technology that can be categorised into dry and wet technologies. Of these, ore processing is a wet technology and more widespread. The water needed for this type of procedure is determined by the enormous amount of material being processed and the solid density (Chelgani et al., 2019). Thus, large amounts of wastewater are commonly destined into residual process waste streams and their potential as a source of raw materials is missed (Northey et al., 2016).

During the last years, the scientific community has placed considerable attention on the use of microalgae as a novel method for wastewater bioremediation, particularly when applied to domestic and industrial wastewater (Sutherland and Ralph, 2019; Maurya et al., 2022; Yuvraj, 2022). These photosynthetic microorganisms can capture contaminant compounds and turn them into added-value products such as pigments, antioxidants and lipids. Furthermore, microalgae can absorb excess nutrients from industrial wastewater, along with CO_2 sequestration, acting as environmental purifiers (Hemalatha et al., 2019; Marin-Batista et al., 2019). Recent studies have focused on microalgae biorefinery for biofuel production, which can become a feasible alternative to traditional fossil fuels. Nevertheless, the only way to become this technology into a cost-effective option is through an integrated system for simultaneous biorefinery of diverse compounds. Moreover, microalgae can utilise pollutants present in wastewater as nutrients to promote the production of their own biomass (Singh et al., 2021). This ability allows to use them as a bioremediation system for emerging pollutants while being a rich source of bioproducts (Kumar et al., 2020; Nie et al., 2020). Specifically, microalgae from genus *Arthrospira* are a rich source of proteins, lipids and carbohydrates that can be used in human and animal diets (Mitra and Mishra, 2019). These cyanobacteria produce high quantities of pigments with significant applications in the pharmaceutical sector. *Arthrospira* sp. is a major producer of phycobiliproteins such as C-phycocyanin, which has found application in human health as an antioxidant and anti-inflammatory compound (Nur et al., 2019). This pigment has also a strong presence on the market and is expected to reach a global market value of $232 million by the end of 2025 (Silva et al., 2020). Thus, *Arthrospira* cultivation has the potential to make bioremediation culture systems economically feasible, along with providing potential organisms for economic model transition to a circular system.

Based on these considerations, this study aimed to evaluate the culture of *A. maxima* as a novel tool for sulphate remediation from aqueous wastes, as well as its potential to become emerging pollutants into valuable bioproducts. Accordingly, highly-sulphated mining wastewater (3,125 $\pm$ 340 mg L^{-1}) was taken as culture medium for *A. maxima* culturing at an industrial scale. The selected microalgae grew with optimum results in presence of wastewater and showed to remove up to 50% of initial sulphates within 6 days. We believe that this is the first work focused on microalgal sulphate remediation that achieves such a removal rate at an industrial scale. Furthermore, the generated biomass was then utilised for a sequential extraction of phycocyanin and a crude lipid extract, that could be further valorised into novel biofuels. The results obtained showed that *A. maxima* biorefinery potential was not hindered when cultured in mining wastewater. Moreover, presence of highly-sulphated wastewater has shown to be a method to improve phycocyanin production yield, as well as purity, on *A. maxima* To the best of our knowledge, this study is the first attempt to couple microalgae wastewater remediation to diverse compounds extraction from the same biomass sample, specially *Arthrospira* genre.

2. Materials and methods

2.1. Description of the wastewater

The wastewater was generated by mining operations in Spain. Mineral extraction methods produce highly sulphated wastewater, which was sampled from a discharge pipe. Wastewater was sampled by demand and placed in inert plastic containers. Due to the large quantities needed, wastewater was not exposed to physical processes such as filtering or autoclaving. According to the unavailability to transport samples on ice, large volumes were taken by demand to avoid any storage. No turbidity of solid particles was observed.

Chemical profile of the targeted wastewater was evaluated, through inductively coupled plasma mass spectrometry, using an 8900 triple quadrupole ICP-MS (Agilent, United States) (Giner Martínez-Sierra et al., 2015), in order to measure the concentration of diverse pollutants. All analysis followed the ISO 15587-1:2002 requirements. Nevertheless, the only compound that was found to be above the detection limits was the sulphate, which was focused as the main pollutant for this study. Sulphate was measured following the protocol described by Colon et al. (2008). Untreated wastewater contained 3,000 $\pm$ 350 mg L^{-1} sulphate before algal cultivation.

Sulphate measurements were done after adding the culture medium (described in Section 2.2), which contained a modest addition of sulphur through the presence of $FeSO_4$, $ZnSO_4$ and $CuSO_4$ in trace amounts. Based on the measurements taken, wastewater conditioned through the addition of culture medium had a total sulphate concentration of 3,125 $\pm$ 340 mg L^{-1}. Moreover, by the end of experimentation, a total of 1 m^3 of wastewater was taken for experimental trials.

Fig. 1. Culture systems used for large volumes (left: PBRs, right: raceways).

2.2. Algae strain and laboratory culture conditions

The *Arthrospira maxima* strain was provided by the CSIC (Spanish National Research Council) and stored at the facilities of Neoalgae Micro Seaweed Products (Gijón, Spain). Stock cultures were poured into 200 mL borosilicate flasks and grown with the addition of 150 mL of the modified Zarrouk's Medium, as described elsewhere (Blanco-Vieites et al., 2022). This culture medium is composed by (g/L): $NaHCO_3$(18), $NaNO_3$ (2.5), K_2HPO_4 (0.5), 2(KNO_3) (1), NaCl (1), $CaCl_2$ (0.04), Na_2 EDTA (0.08), $Mg(NO_3)_2$ (0.2), $FeSO_4$ (0.01) and a micronutrient solution. This solution is added by 1 mL/L of culture and is composed by (g/L): H_3BO_3 (2.86), $MnCl_2$ (1.81), $ZnSO_4$ (0.22), $CuSO_4$ (0.079) and $(NH_4)_6Mo_7O_{24}$ (1.00). The cyanobacteria strains had to undergo an acclimatisation period before being used in the experiments. Therefore, to standardise the growth curve, the cultures were grown and exposed to renewals 10 times by harvesting 20% of the culture and adding fresh culture medium. To this point, *A. maxima* achieve the stationary growth phase within 10 days of culturing. The laboratory conditions were determined following the recommendations of the Food and Agriculture Organization of the United Nations (FAO, 2021). The temperature and photoperiod were constant at 25 °C and 16:8 h^{-1} (light/darkness), respectively, with a photon flux of 100 μmol s^{-1} m^{-2}. The cultures were homogenised through Marina 200 Air Pumps (Marina, China) at a flow rate of 100 L h^{-1}.

All reagents used were of analytical grade and purchased from Labbox Labware (Barcelona, Spain), with the exception of: NaCl, which was provided by Agrupasal (Asturias, Spain), as well as $NaHCO_3$ and $NaNO_3$, which were purchased from Vadequímica (Barcelona, Spain).

2.3. Up-scaling of the systems and harvesting

Cultures were grown up to 2 L under laboratory conditions (Section 2.1). Afterwards, experimental and control cultures were scaled up to 20-L plastic bottles (Nalgene, United States) and placed inside a greenhouse facility in Gijón, Spain (43.52326797371128, −5.701862389558017). Parameters such as photoperiod, light intensity and temperature were not controlled and dependent on the meteorology of the north of Spain during autumn of 2021. Temperature is a limiting factor for *Arthrospira* growth, as stated by previous studies such as Oliveira et al. (1999) and Saeid and Chojnacka (2016). On this basis, the temperature was recorded on a continuous basis inside the greenhouse. The average temperature was measured at 28.7 °C, which was considered within the optimum temperature range.

Once the control and experimental cultures had reached the late exponential phase, 20 L-bottles were used as inoculum for semi-industrial culture systems. For this part of the study, bubble column closed photobioreactors, PBRs (Aqualgae, Spain), were chosen as they are resistant to contamination with undesired microorganisms and allow a high biomass yield (Angela et al., 2022). The PBRs consisted of vertical acrylic columns, 3 m high and 30 cm wide, with aeration injected from the base of the column through an air blower Bonora IE3 (Motori Bonora S. p. A., Italy). This part of the investigation aimed to produce high-quality cultures as inoculum for industrial algal biomass open-production systems (Neoalgae Micro Seaweed Products, Spain) (Fig. 1).

Further steps of the scaling-up process were performed in a raceway pond during the 2021 summer season. This pond was 3 m long, 2 m wide and 0.3 m deep, with a volume of 600 L. The system was also equipped with a five-blade paddle

wheel to homogenise the culture and facilitate metabolic activities related to pollutant removal (Lage et al., 2021). The raceway was placed in a greenhouse at the Neoalgae Micro Seaweed Products R&D facilities at the University of Oviedo, Spain (43.355748880355605, −5.871960729476839).

After 12 days of culturing in open raceway systems, cultures were harvested by centrifugation in an industrial centrifuge STC 3-06-107 (Gea Westfalia Separators, Germany). This way, algal biomass was collected for further valorisation through the extraction of value-added compounds. Samples of the separated effluent were taken for sulphate content analysis and to determine the removal rates of *A. maxima* in open industrial culture systems.

2.4. Culture growth quantification

The microalgae population was quantified through absorbance analysis and cultures purity, in terms of absence of undesired microalgal species contamination, was verified under taxonomic criteria under an optical microscope (Bioblue BB.1153-PLi). Thus, samples were taken daily (approx. at 12 pm) along experimental culture phases, collecting a sampling volume of 50 mL. Aliquots were taken under sterile conditions and analysed in triplicate. Cell viability of the microalgae was estimated through spectrophotometric analysis with a spectrophotometer (BioChrome Libra S11). Optical density (OD) was measured at 680 nm to minimise errors in biomass quantification, following Michael et al. (2019). Wastewater with Zarrouk's culture medium (Zarrouk, 1966) was used as blank before measuring samples.

2.5. Sulphate remediation analysis

Sulphate remediation was determined during industrial-scale culturing in open systems (Section 2.3), and samples were taken as described in Section 2.4. Samples were centrifuged at 4000 rpm for 5 min at 5 °C to separate algal cells from the supernatant (Mohammadi et al., 2019). A sample from the original wastewater was also centrifuged to evaluate the influence of this process on the diluted sulphates originally present in wastewater, which did not produce any measurable changes. Subsequently, the supernatant samples were applied to measure the sulphate ion concentration according to Garcia and Schultz (2016) and Thangiah (2019). Sulphate ion presence was quantified through a turbidimetric procedure based on sulphate precipitation with barium chloride and the OD measurement at 420 nm. The sulphate removal rate was estimated as a percentage, using Eq. (1) (Mohammadi et al., 2019):

$$\%\ Removal = \frac{(C_i - C_f)}{C_i} \tag{1}$$

where C_i and C_f correspond to the initial and final sulphate concentrations (mg L^{-1}), respectively. Measurements of sulphate concentration were replicated three times, and removal data are shown as averages.

2.6. Pigment extraction

Once finished the bioremediation assays, cultures were harvested and the obtained algal biomass was freeze-dried through lyophilisation for 36 h, using an CoolSafe Touch 110–4 Freeze dryer (Scanvac, Denmark). Afterwards, *A. maxima* biomass was subjected to two-phase extraction to obtain two types of high-added value products: C-phycocyanin and carotenoids.

For C-phycocyanin extraction, freeze-thawing was selected because of its simplicity, high extraction yield and the absence of processes that could damage other molecules of interest for further extraction (Chittapun et al., 2020). Briefly, *A. maxima* biomass (3.5 g) was suspended in distilled water (43 mL), and samples were subjected to a freeze-thawing sequence with three freeze/thaw cycle rounds. These samples were frozen to −40 °C and later thawed overnight at 6 °C under dark conditions for the extraction of phycocyanin. Once defrosted for the 3rd time, samples were centrifuged at 4000 rpm and 5 °C for 15 min, and the supernatant was filtered through filter paper with determined grammage (60 g/cm^2). Samples were then measured using a spectrophotometer Cary 60 UV–Vis (Agilent Technologies, United States) at absorbance spectra of 652, 620 and 280 nm for phycocyanin concentration, purity and yield of phycocyanin, respectively, per gram of extracted biomass. These measurements were then used in Eqs. (2), (3) and (4) (Moraes et al., 2011; Nur et al., 2019; Gorgich et al., 2020):

$$Concentration\ (C_p) = \frac{(Abs_{620} - 0.474 Abs_{652})}{5.34} \tag{2}$$

$$Purity = \frac{Abs_{620}}{Abs_{280}} \tag{3}$$

$$Yield\left(\frac{mg_p}{mg_b}\right) = \frac{C_p \cdot V}{m_b} \tag{4}$$

where Abs_{620}, Abs_{652} and Abs_{280} are, respectively, absorbance at 620, 652 and 280 nm; these values were used in Eqs. (2) and (3) for phycocyanin concentration (C_p) and purity evaluation. To determine the amount of extracted phycocyanin per g of biomass, distilled water volume (V), C_p and dry weight of extracted biomass (mg $_b$) were considered in Eq. (4).

2.7. Crude lipid extract

After C-phycocyanin extraction, residual biomass was lyophilised and exposed to organic solvent extraction to obtain an oily extract that could be further valorised. Extraction was performed by modification of the standard method described by Luengo et al. (2014). Thus, 3.5 g was suspended on 70 mL of a mixture of hexane: ethanol (50:50) and stirred for 2 h at room temperature. Subsequently, samples were centrifugated at 4000 rpm and 19 °C for 15 min to extract separate the residual biomass. The supernatant solvent mixture was then recovered via rotary evaporation for 1 h at 50 °C. After extraction, the oily extract was collected in a glass, and the lipid extraction efficiency was evaluated as a percentage of the initial biomass weight, using Eq. (5):

$$Lipid\ extraction\ (\%) = \left[\frac{(A-B)}{C}\right] \cdot 100 \tag{5}$$

where A is the weight of the evaporation flask with the solvent mixture, B corresponds to the final weight of the flask after evaporation of the solvent used and C is the quantity of biomass used for extraction (2 g in this study).

2.8. Statistical analysis

Experiments were conducted in triplicate, and the results are shown as averages, with the exception of high-volume raceway ponds, which were investigated in duplicate due to the limited facilities. Significance of the data obtained was analysed using one-way analysis of variance (ANOVA) and Tukey's test. Differences were considered significant at $p < 0.05$. PAST Statistical Analysis Software was utilised for statistical analysis and figures design.

3. Results and discussion

3.1. Microalgal growth during scaling up

The growth of *A. maxima* in highly sulphated wastewater was assessed in different culture systems along the scaling-up process, which were keep under constant controlled conditions as described in Section 2.2. Accordingly, it was assumed that those controlled parameters had no influence on fluctuations of growth monitoring measurements. As seen in Fig. 2, 2-L cultures under controlled conditions showed a standard growth curve, which reached the stationary phase by the 12th day. Control group samples multiplied by 3.22 the initial absorbance measurements, reaching a maximum OD_{680} of 1.499. Experimental group samples presented a maximum growth 17.7% below that reached by control samples by the end of the experiment. These results are closely related to what was achieved during the 20-L scaling-up trials, which were conducted in a greenhouse under uncontrolled conditions. Moreover, control group samples reached maximum OD_{680} measurements at 1.439, which is slightly superior to the maximum absorbance obtained for control group samples during controlled condition trials. On the other hand, experimental trials showed a reduction in cellular density yield when compared to control group samples. As well as in controlled condition trials, during PBR culture trials, the maximum cell density was significantly lower when compared to control data (8.4% lower; $p < 0.05$).

The results presented in Fig. 2 show the influence of uncontrolled environmental conditions on the growth of *A. maxima*. Fig. 2(a) shows the standard growth curve in both experimental and control groups, wastewater medium, and unpolluted water, whereas Fig. 2(b) presents a much shorter exponential growth phase, which changed on day 6 of the experiment and turned into a large stationary phase that lasted until day 12. This part of the study shows how *A. maxima* can grow in highly sulphated wastewater without a drastic reduction growth. Nevertheless, Mirhosseini et al. (2021) observed that increasing concentrations of ammonium sulphate hindered biomass generation in this species. Our results strongly disagree with their outcomes and demonstrate how *A. maxima* can grow with optimum results in highly sulphated environments (up to 3,125 $\pm$ 340 mg L^{-1}), even under uncontrolled conditions. Belkin and Boussiba (1991) observed the toxic potential of ammonia in *Arthrospira* cultures, demonstrating the inability of these cyanobacteria to grow in the presence of ammonia. Based on the above results, highly sulphated wastewater seems to slightly hinder *A. maxima* growth, albeit without representing a risk for survival.

When scaled up to PBR (Fig. 2c), based on the OD_{680} values, both groups were initially similar ($p < 0.05$). Both groups showed a clear exponential phase, which was lowered by the 4th day because of the climatic conditions. The impacts of climate variability on microalgal growth have been analysed by Yong et al. (2016), supporting our findings regarding the curbing of the exponential growth phase. In our study, the growth of *A. maxima* was limited when exposed to high concentrations of sulphates. Nevertheless, the PBR growth results were closely related to those obtained by Souza da Silva de et al. (2022), who focused on *A. maxima* culturing optimisation. On this basis, the data obtained during culture trials in this experiment confirm that the growth yield observed during the PBR experiments is the optimum growth yield. This leads us to consider that highly sulphated wastewater media do not delay the evolution of the growth curve in *A. maxima* but prevent this cyanobacterium from achieving the maximum growth yield.

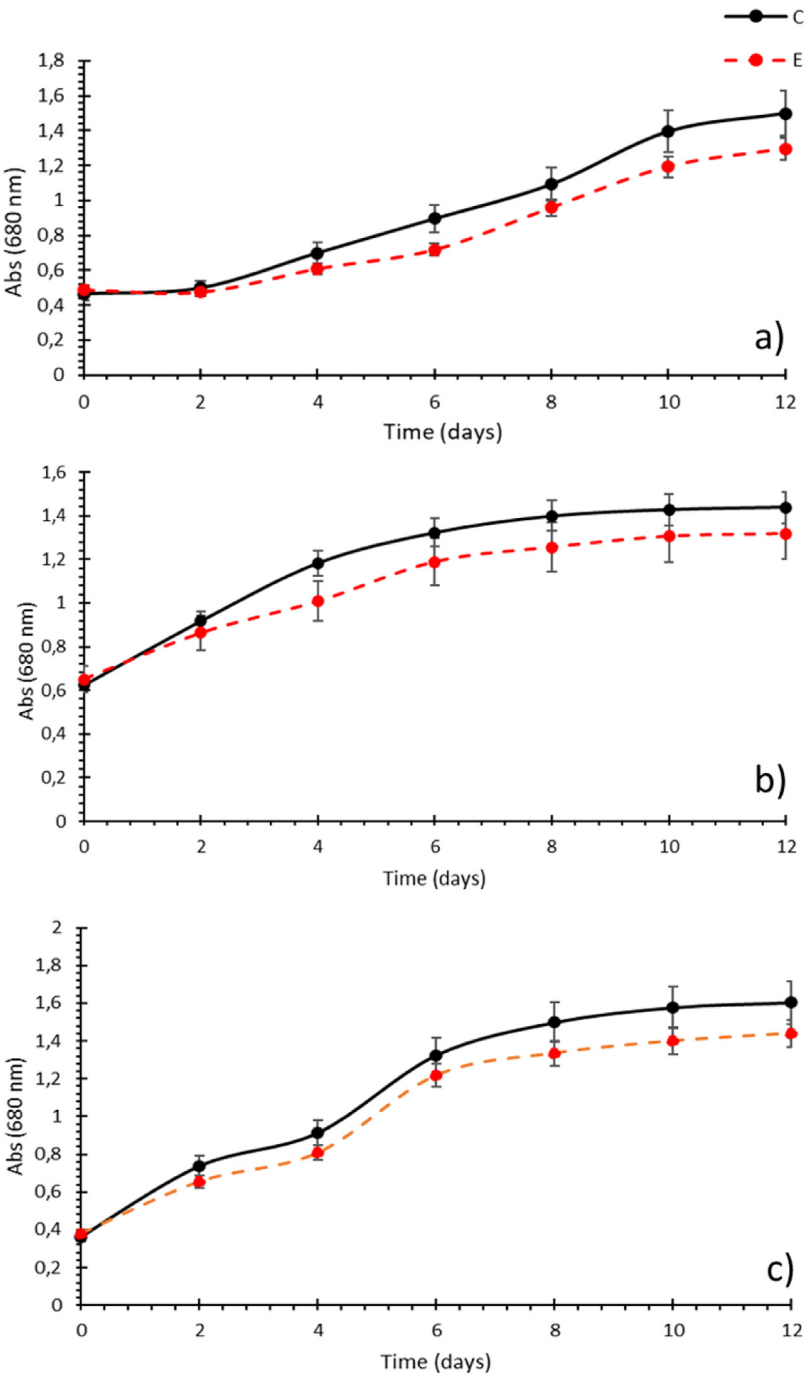

Fig. 2. Growth data of *A. maxima* in the 2-L laboratory trials with controlled conditions (a), 20-L scaling-up trials under sunlight conditions in a greenhouse (b) and PBR cultures (c).

3.2. Raceway assay: growth and sulphate reduction

After the PBR trials, cultures were scaled up to open raceways. In these systems, *A. maxima* was cultured in 100% of wastewater and modified Zarrouk's medium, based on the optimum growth results obtained during previous stages of experimentation. The growth rate and the spectrophotometric measurements of sulphate concentration are shown in Fig. 3.

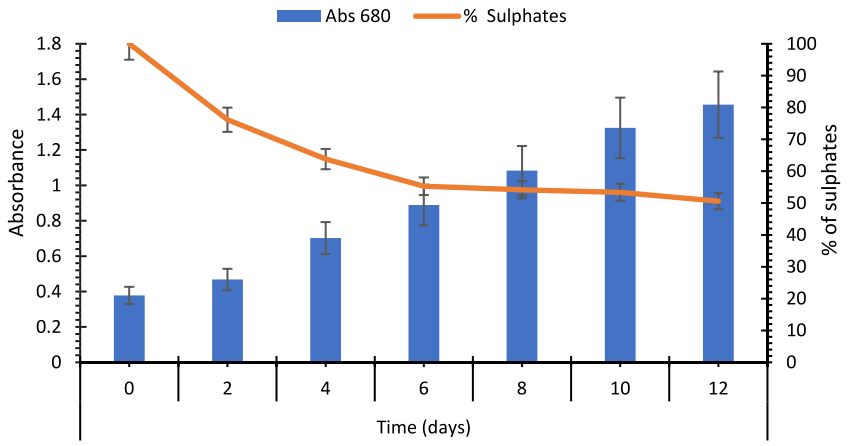

Fig. 3. Diagram showing the relationship between sulphate concentration and *A. maxima* growth.

The growth results obtained during this section of the study show how *A. maxima* can achieve optimum cellular density when cultured in highly sulphated wastewater. Microalgae bioremediation of domestic wastewater, rich in nitrates and phosphates, is widely known and has been reviewed by several authors such as Pham et al. (2022) and Rezania et al. (2021). Nevertheless, to the best of our knowledge, mining wastewater bioremediation through *Arthrospira* culturing is a less studied field. Previous studies have analysed the potential of *Arthrospira* sp. culturing for biomass production with the use of polluted water as culture medium, but the growth yield achieved was clearly lower than that obtained during our study (Ortiz-Villota et al., 2018). Hadiyanto et al. (2014) experimented with palm oil mill effluent as culture medium for *Arthrospira* sp., with good results in terms of biomass production. Our findings support the results of Mera et al. (2014), who analysed the influence of sulphate concentration on cadmium tolerance in *Chlamydomonas moewusii*. According to their findings, sulphate supplementation in culture media increases the synthesis of chelating agents, which protect microalgae from inorganic compound toxicity.

Along with absorbance measurements, Fig. 3 shows the sulphate measurements obtained during this phase of the study. Initial measurements were intensely decreased during the first 48 h of experimentation, being reduced by almost 25% of the initial concentration. Nevertheless, the sulphate reduction rate slowed down from day 2 to day 6 of the experiment, with an average reduction of 5.22% day^{-1}. Our assay shows that sulphate up-taking achieved a stationary phase from day 6 (Fig. 3). From day 6 onward, sulphate measurements were reduced by 0.78% day^{-1}. A sulphate removal stationary phase was also seen by Mohammadi et al. (2018), who analysed the influences of five species of microalgae on sulphate concentrations. Algal biomass generation continued increasing even though sulphate uptake was clearly reduced. This finding is supported by Gastoldi et al. (2021), who observed that a reduced activity of ATP sulfurylase did not limit cyanobacterial growth.

By the end of the experiment, *A. maxima* cultured with optimised nutritional medium had removed up to 52.28% of the initial total sulphates within 12 days. According to Fernandes et al. (2020), sulphur is a crucial element in the downstream cellular mechanisms involved in fatty acid and amino acid synthesis, among others. Thus, sulphate assimilation during the first stages of the culturing process can be attributed to a high anabolic rate. A previous study focusing on microalgae culturing for sulphate removal has achieved a maximum elimination of 37.29% (Mohammadi et al., 2019). To the best of our knowledge, the removal rate obtained in this study is among the highest ever achieved through the use of microalgae cultures. Moreover, Blanco-Vieites et al. (2022) achieved a maximum sulphate removal of 73% by culturing *A. maxima* with the same culture medium as that used in this study. Nevertheless, we determined whether the results achieved during laboratory-scale trials are representative for the industrial application of microalgae bioremediation systems. Mera et al. (2014) studied the effects of elevated concentrations of sulphate on *Chlamydomonas* survival, a eukaryotic microalga, and found that an excessive amount of SO_4^- ions in solution elevate ionic strength, which leads to cellular damage. Our findings suggest that sulphate bioremediation through microalgal cultures is feasible with the wastewater selected.

Previous works have focused on the usage of organic biomass as a novel bioremediation filter for heavy metals bioremediation, nevertheless, sulphate bioremediation through microalgae biotechnology is still a field under study (Banerjee et al., 2017; Mukherjee et al., 2017). On the contrary, sulphate-reducing bacteria have been widely used for sulphate removal in acid mine drainage, as reviewed by Ayangbenro et al. (2018). Furthermore, Zhang and Wang (2014) experimented with sulphate removal through bacteria cultured in a bioreactor for 35 days, achieving removal rates between 50% and 79%. Although the removal rate was higher when compared with our outcomes, we strongly believe that residence time is a crucial factor in bioremediation systems and needs to be further optimised. On the other hand, Ben Ali et al. (2020) studied the influence of wood chips and leaf compost on total sulphate content, achieving a maximum removal of almost 40% of the initial sulphate concentration within the first 48 h of experimentation. Although the removal

Table 1
Phycocyanin extraction results.

Group	Concentration (mg/mL)	Purity	Yield (mg/g)
Control group	0.015 ± 0.001	0.280 ± 0.032	18.159 ± 0.017
Experimental group	0.031 ± 0.006	0.425 ± 0.004	38.748 ± 0.072

rate seems to be clearly higher than that achieved during our study, microalgae can be further valorised into value-added products, converting AMD into a valuable residue.

Currently, industrial systems for sulphate removal include expensive procedures that usually lead to secondary pollution, which does not fit the circular economy paradigm (Hosseini et al., 2019). Accordingly, Han et al. (2021) developed a novel method for sulphate remediation with this technology and achieved a 50% of sulphate reduction in 2 h. Their sulphate removal rate is higher than what was achieved during our study, which leads us to infer that the optimisation of this process is still necessary. Based on the above, we believe that microalgae sulphate bioremediation might yield lower removal rates, but the possibility of its biomass valorisation represents a fortress for including it into novel production systems.

3.3. Pigments extraction

Several techniques can be used for phycocyanin extraction, such as mixing and homogenisation, bead milling, high-pressure homogenisation, high-pressure processing, ultrasound, microwaves and pulsed electric fields, among others (Pez Jaeschke et al., 2021). Nevertheless, the extraction of phycobiliproteins through freeze-thawing cycles, using distilled water used as solvent, is one of the most suitable techniques for C-phycocyanin extraction. This technique is widely used for cyanobacterial cell disruption because of its simplicity: the cellular volume increases when intracellular fluid freezes into crystals, which is followed by cell contraction during thawing. The absence of factors that affect phycobiliprotein stability, such as high temperatures and pH variation, prevent molecular degradation (Selig et al., 2018). In this study, the potential of *A. maxima* as a valuable bioremediatory was analysed. Phycocyanin extraction from lyophilised biomass was performed to verify the potential of this organism to synthetise added-value compounds when cultured in mining wastewater.

In this assay, distilled water was used for phycocyanin extraction through freeze-thawing, obtaining 43 mL of a phycocyanin-rich extract. Absorbance quantification trials showed that the group with the modified culture medium, group "C", presented lower OD_{620} and OD_{280} measurements. On the contrary, cultures grown in the presence of wastewater (group "E" in Table 1) presented absorbance estimations of up to 122% and 43%, OD_{620} and OD_{280}, respectively, higher when compared to the control group.

Evaluation of the results obtained revealed that the experimental cultures presented higher yields in terms of phycocyanin concentration. Accordingly, extraction trials showed how each g of control *A. maxima* biomass produced up to 18.159 ± 0.017 mg of phycocyanin. On the other hand, the wastewater-cultured group presented 38.748 ± 0.072 mg of phycocyanin per g of dry biomass. On this basis, statistical analysis showed that the difference in phycocyanin production among experimental group was significant at $p = 0.009$. Considering that control group cultures were grown in sulphate-deprived media, the absence of sulphur can result in metabolic deficiencies, such as a lack of phycocyanin (Fernandes et al., 2020).

On the other hand, experimental cultures were grown in highly sulphated wastewater, and the lack of sulphate did neither result in metabolic stress nor impede phycocyanin synthesis. Gorgich et al. (2020) investigated phycocyanin extraction methods and obtained lower rates of purity and yield during the first extraction phase. Nevertheless, the optimised the extraction of sulphur, resulting in the production of higher-quality phycocyanin when compared to our study (up to 45.94 mg/g). Tavanandi et al. (2018) also optimised phycocyanin extraction, obtaining a yield of 74.51 mg/g and a purity of 0.56, higher than those obtained in our study. Nevertheless, we consider that optimisation of the extraction process can be coupled to the outcomes of this study to achieve a maximum phycocyanin production from wastewater-cultured microalgae.

Table 1 shows the phycocyanin purity, which is related to the economic value of the obtained extract. According to the results presented above, experimental cultures presented a higher phycocyanin purity, with up to 84% higher when compared to control group extracts ($p = 0.001$). The outcomes of phycocyanin purity evaluation showed that experimental cultures grown in highly sulphated wastewater presented a phycocyanin purity of 0.43 ± 0.02. Our results suggest that the over-exposition of *A. maxima* to sulphur (sulphates in our case) could be a novel technique for improving phycocyanin synthesis in this cyanobacterium, and further studies are needed to verify if this process is feasible. This result is below the outcomes obtained by Silva et al. (2020), who experimented with phycocyanin extraction from a cyanobacterium of the genus *Synechocystis*. Nevertheless, the simplicity of *Arthrospira* sp. culturing and the larger biomass productivity could compensate the lower phycocyanin synthesis at an industrial scale.

As stated by Tan et al. (2020), low-purity phycocyanin extracts (value below 0.7) are considered as food-grade chemicals and can cost up to 180 USD per kg. On this basis, our results suggest that the use of cyanobacteria for highly sulphated wastewater bioremediation represents a feasible novel way for including industry wastes into the circular

economy paradigm. To the best of our knowledge, this is the first attempt of microalgal bioremediation of this type of wastewater at an industrial scale with further valorisation of the obtained biomass. The obtained phycocyanin extract could be directly commercialised, notwithstanding that purity can be enhanced through purification processes such as ultrafiltration, precipitation and ion exchange chromatography, among others (Figueira et al., 2018). Nevertheless, further studies are needed to analyse the purification potential of this phycocyanin extract and to determine whether its purity can be enhanced.

3.4. Crude lipid extract

As stated by Patnaik and Mallick (2021), the traditional use of fossil fuels is responsible for producing vast amounts of harmful substances that pollute the environment and contribute to global warming, making it necessary to obtain renewable and eco-friendly sources of combustibles. On this basis, microalgae culturing for biodiesel production offers several advantages such as the absence of negative impacts on arable land. Microalgae are widely considered as a promising resource for biodiesel production; however, the key metabolic targets for metabolic engineering are still under study (Xue et al., 2021). Moreover, previous works have already stated that using nutrient-rich wastewater as culture media for microalgae culturing can reduce the overall costs for biofuels production, which has been proven to be the bottleneck for this technology (Wang et al., 2020; Chai et al., 2021). Accordingly, for this work it was decided to reuse the biomass utilised for phycocyanin extraction in order to obtain a lipidic extract, that can be further processed into biofuels. Subsequently to phycocyanin extraction, residual biomass was lyophilised and exposed to organic solvent extraction, using a hexane/ethanol mixture at room temperature for 120 min. Hexane is one of the best solvent choices due to its cost effectiveness, synergic effect with other organic solvents and high selectivity for neutral lipid fractions, which can be further converted into biodiesel (Shin et al., 2018). Microalgae are a wide group of photosynthetic microorganisms and are considered the best carbon capture platforms on Earth, transforming and accumulating carbon into organic molecules such as lipids (Prasad et al., 2021). Moreover, their ability to turn pollutants from wastewater into added-value compounds is believed to play a key-role into the optimisation of the biodiesel synthesis into a cost-effective technology (Anerao et al., 2022). Based on these considerations, for this work, crude lipid yield was used as a final index to evaluate extraction efficiency and yield, bearing in mind that the final purpose of the extraction is to valorise the residual biomass from previous steps.

In this study, crude lipid yield was considered the main index for lipid extraction evaluation. Fig. 4 shows the extraction yield of the solvent, expressed in percentage of crude lipid weight per gram of dry biomass. Accordingly, control groups showed a higher concentration of the lipid fraction, which was up to 0.56% higher when compared to the wastewater-cultured microalgae extract. The extraction yield results obtained during this study are similar to those obtained by Hoshino et al. (2017), who investigated the extraction potential of *Arthrospira* sp. biomass with different organic solvents such as hexane. Nonetheless, the same study obtained higher yields of extracted lipids through liquefied dimethyl ether extraction, which, in our opinion, should be considered for further studies. As stated by Sun et al. (2018), microalgal lipid accumulation is highly dependent on biological factors such as the specific strain and on environmental factors. On this basis, as stated by Mondal et al. (2017), controlling environmental conditions such as duration of the photoperiod, or light intensity, could be a simple way to enhance the production of lipids in determined microalgal strains. On this basis, the results of lipids concentration obtained during our study could be optimised through the modification of culture conditions, which will be analysed in future works.

Statistical analysis of the obtained data showed that there was no significant difference between control and experimental groups ($p = 0.8629$), being both groups close to an 8% of lipid fraction regarding to dried biomass The lipid accumulation percentage obtained in this study is similar to the one obtained by Diniz et al. (2017), which we consider a good result that can be further optimised. Moreover, Hernández et al. (2022) demonstrated that different sources of stress, such as exposure to toxic compounds, might affect to the biomolecules recovery efficiency. This type of interactions must be taken into account when using microalgae as a circular economy approach for wastewater harnessing (Hernández et al., 2022). Nonetheless, the lipid yield obtained is lower than the ones obtained by Nagappan and Kumar (2021), who experimented with eukaryotic strains that were cultured in wastewater and exposed to a nitrogen depletion stress. Accordingly, their results show how lipid fraction can be widely improved by the modification of the chemical profile of the culture medium used, specially being a nitrogen-deficient-medium. On this basis, Salas-Montantes et al. (2018) analysed the potential of sulphur deprivation conditions as a stressor for lipid accumulation. Nevertheless, we cannot confirm that the presence of highly sulphated culture media influences the lipid concentration of *A. maxima*. Nevertheless. However, to the best of our knowledge, there are no previous studies on the influence of sulphur excess on the biochemical profile on microalgae, and this issue deserves further attention. Additionally, apart from the lipid synthesis, the extraction methods need to be further optimised in order to achieve a maximum of extraction rate through novel physicochemical extraction methods. On this basis, according to the findings of Cercado et al. (2018), the crude lipidic extract obtained during our study could be further processed. On this basis, the lipidic profile of the obtained extract should be analysed in order to evaluate its potential to be applied for biodiesel production (Purba et al., 2022). Accordingly, we strongly believe that lipid production and extraction from microalgal biomass should be improved through the implementation of novel extraction technologies and culture methods in order to boost the economic viability of biofuels production. However, the overwhelming investments needed for microalgal biodiesel production is the main bottleneck for the development of

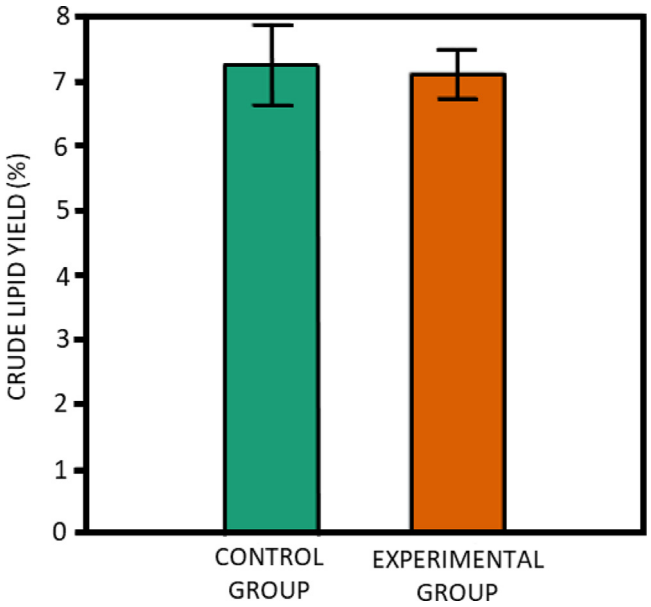

Fig. 4. Comparison between extraction yields of control and experimental groups.

this technology. The US Department of energy has estimated, theoretically, a production cost of 1.77 US dollars per litter of microalgal biodiesel if produced at an industrial scale (U.S. DOE, 2008). Accordingly, the studies of Rawat et al. (2013) have already analysed the reasons behind the technoeconomic unfeasibility of microalgal biodiesel production, which focused on the costly upstream and downstream processes. Nonetheless, their work stated that the use of wastewater as a source of culture media can reduce greatly the overall costs. Moreover, Zhu (2015) also proposed a novel framework for microalgal biodiesel production based on the production of multiple valuable compounds from microalgal biomass. Based on the above, our work support the idea that a mixed bioremediatory and biorefinery approach for high-value products and biodiesel production could avoid the economic difficulties seen in this type of technology.

4. Conclusions

The effects of high concentrations of sulphate on the growth of the microalgae *A. maxima* and its capacity for wastewater bioremediation (sulphate removal) were studied to provide information on the feasibility of this technology at industrial scale. The results of the tolerance assay exhibited promising outcomes, showing that elevated concentrations of sulphates, up to 3,000 $\pm$ 350 mg L^{-1}, do not hinder the growth yield of *A. maxima* when cultured in industrial-size raceway open ponds. In addition, the sulphate analysis trials exhibited optimum outcomes in terms of sulphate removal, with up to 52.28% of removal within the first 6 days. To the best of our knowledge, our study brings encouraging results for the bioremediation field since there are no previous studies that have achieved such a reduction in sulphates by using microalgae bioremediation.

The obtained microalgae biomass was subsequently exposed to a two-sequence valorisation through the extraction of C-phycocyanin and a crude lipid extract that can be further transformed into biodiesel. Phycocyanin extraction trials showed that larger amounts of sulphates positively influence its concentration in *A. maxima* cells, achieving up to 1.46 times higher (mg mL^{-1}). Furthermore, purity and yield (mg mg^{-1}) were also significantly affected in wastewater-cultured microalgae. Accordingly, phycocyanin yield was observed to be increased up to 1.13 times higher, when compared to control group samples. Regarding purity analysis, the results obtained clearly show how sulphate supplementation through highly-sulphated wastewater produces an increasement of phycocyanin purity in *A. maxima*. Purity assays showed that experimental biomass phycocyanin achieved purity levels up to 50% higher. Interestingly, we found no significant difference between control and experimental groups in terms of crude lipid extraction yield, which will be further optimised in future works. We believe that this is the first work that shows the positive influence of sulphate supplementation on phycocyanin synthesis on *A. maxima*. These findings need to be deepened, especially if they could be extrapolated to industrial phycocyanin production systems, in pursuance of optimise economic and quality values.

According to the available references, this is the first approach of microalgal bioremediation systems, for wastewater treatment, coupled to a double-step biorefinery perspective. On this basis, our investigation achieves to transform pollutants from wastewater into 2 different types of bioproducts that could be further valorised. Moreover, the technology developed along this study facilitates the reduction in water pollution related to mining activities while recovering nutrients from generated wastewater. On this premise, added-value compounds extraction can play a key role in cost

minimisation strategies for biofuels production at an industrial scale. Nevertheless, techno-economic and life-cycle assessment should be analysed for pilot-scale optimisation. Furthermore, our work provides insights into the evolution of traditional sulphate treatment technologies into the circular economy paradigm. Altogether, the data provided could allow an ambitious biorefinery approach for microalgal-based wastewater bioremediation that needs to be further analysed by future works.

CRediT authorship contribution statement

M. Blanco-Vieites: Conception and design of study, Acquisition of data, Analysis and/or interpretation of data, Writing – original draft, Writing – review & editing. **V. Casado:** Conception and design of study, Analysis and/or interpretation of data, Writing – review & editing. **A. Hernández Battez:** Conception and design of study, Writing – review & editing. **E. Rodríguez:** Conception and design of study, Writing – review & editing.

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.

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References

Anerao, P., Kumar, H., Kaware, R., Prasad, K., Kumar, M., Singh, L., 2022. Algal-based biofuel production: Opportunities, challenges, and prospects. In: Chowdhary, P., Khanna, N., Pandit, S., Kumar, R. (Eds.), Bio-Clean Energy Technologies: Volume 1. Springer, Singapore, pp. 155–180. http://dx.doi.org/10.1007/978-981-16-8090-8_7.

Angela, P.P., Apurav, K.K., Kit, W.C., Shih-Hsin, H., Wei-Hsin, C., Jo-Shu, C., Rambabu, K., Fawzi, B., Pau, L.S., 2022. Continuous cultivation of microalgae in photobioreactors as a source of renewable energy: Current status and future challenges. Renew. Sustain. Energy Rev. 154, 111852. http://dx.doi.org/10.1016/j.rser.2021.111852.

Ayangbenro, A.S., Olanrewaju, O.S., Babalola, O.O., 2018. Sulfate-reducing bacteria as an effective tool for sustainable acid mine bioremediation. Front. Microbiol. 9, http://dx.doi.org/10.3389/fmicb.2018.01986.

Banerjee, S., Barman, S., Halder, G., 2017. Sorptive elucidation of rice husk ash derived synthetic zeolite towards deionization of coalmine waste water: A comparative study. Groundw. Sustain. Dev. 5, 137–151. http://dx.doi.org/10.1016/j.gsd.2017.06.004.

Belkin, S., Boussiba, S., 1991. Resistance of *Spirulina platensis* to ammonia at high pH values. Plant Cell Physiol. 32, 953–958. http://dx.doi.org/10.1093/oxfordjournals.pcp.a0.

Ben Ali, H.E., Neculita, C.M., Molson, J.W., Maqsoud, A., Zagury, G.J., 2020. Salinity and low temperature effects on the performance of column biochemical reactors for the treatment of acidic and neutral mine drainage. Chemosphere 243, 125303. http://dx.doi.org/10.1016/j.chemosphere.2019.125303.

Blanco-Vieites, M., Suárez-Montes, D., Hernández Battez, A., Rodríguez, E., 2022. Enhancement of Arthrospira sp. culturing for sulfate removal and mining wastewater bioremediation. Int. J. Phytoremediation http://dx.doi.org/10.1080/15226514.2022.2135680, (Published online: 20 2022).

Bressanelli, G., Perona, M., Saccani, N., 2019. Challenges in supply chain redesign for the Circular Economy: a literature review and a multiple case study. Int. J. Prod. Res. 57, 7395–7422. http://dx.doi.org/10.1080/00207543.2018.1542176.

Cercado, A.P., Ballesteros, F.C., Capareda, S.C., 2018. Biodiesel from three microalgae transesterification processes using different homogenous catalysts. Int. J. Technol. 9, 645–651. http://dx.doi.org/10.14716/ijtech.v9i4.1145.

Chai, W.S., Tan, W.G., Halimatul Munawaroh, H.S., Gupta, V.K., Ho, S.-H., Show, P.L., 2021. Multifaceted roles of microalgae in the application of wastewater biotreatment: A review. Environ. Pollut. 269, 116236. http://dx.doi.org/10.1016/j.envpol.2020.116236.

Chelgani, S.C., Parian, M., Parapari, P.S., Ghorbani, Y., Rosenkranz, J., 2019. A comparative study on the effects of dry and wet grinding on mineral flotation separation–a review. J. Mater. Res. Technol. 8, 5004–5011. http://dx.doi.org/10.1016/j.jmrt.2019.07.053.

Chittapun, S., Jonjaroen, V., Khumrangsee, K., Charoenrat, T., 2020. C-phycocyanin extraction from two freshwater cyanobacteria by freeze thaw and pulsed electric field techniques to improve extraction efficiency and purity. Algal Res. 46, 101789. http://dx.doi.org/10.1016/j.algal.2020.101789.

Colon, M., Iglesias, M., Hidalgo, M., Todolí, J.L., 2008. Sulfide and sulfate determination in water samples by means of hydrogen sulfide generation-inductively coupled plasma-atomic emission spectrometry. J. Anal. At. Spectrom. 23, 416–418. http://dx.doi.org/10.1039/B716302A.

Diniz, G.S., Silva, A.F., Araújo, O.Q.F., Chaloub, R.M., 2017. The potential of microalgal biomass production for biotechnological purposes using wastewater resources. J. Appl. Phycol. 29, 821–832. http://dx.doi.org/10.1007/s10811-016-0976-3.

Food and Agriculture Organization of the United Nations, 2021. 2.3 Algal production. In: Manual on the Production and Use of Live Food for Aquaculture. from https://www.fao.org/3/w3732e/w3732e06.htm Retrieved December 13, 2021, (n.d.) Rome, Italy.

Fernandes, T., Fernandes, I., Andrade, C.A.P., Cordeiro, N., 2020. Assessing the impact of sulfur concentrations on growth and biochemical composition of three marine microalgae. J. Appl. Phycol. 32, 967–975. http://dx.doi.org/10.1007/s10811-019-01946-y.

Figueira, F.da S., Moraes, C.C., Kalil, S.J., 2018. C-phytocyanin purification: Multiple processes for different applications. Braz. J. Chem. Eng. 35, 1117–1128. http://dx.doi.org/10.1590/0104-6632.20180353s20170160.

Garcia, J., Schultz, L.D., 2016. Determination of sulfate by conductometric titration: An undergraduate laboratory experiment. J. Chem. Educ. 93, 910–914. http://dx.doi.org/10.1021/acs.jchemed.5b00941.

Gastoldi, L., Ward, L.M., Nakagawa, M., Giordano, M., McGlynn, S.E., 2021. Changes in ATP sulfurylase activity in response to altered cyanobacteria growth conditions. Microb. Environ. (ISSN: 1342-6311) 36, 1347–4405. http://dx.doi.org/10.1264/jsme2.ME20145.

Giner Martínez-Sierra, J., Blas, O.Galilea.San., Marchante Gayón, J.M., García Alonso, J.I., 2015. Sulfur analysis by inductively coupled plasma-mass spectrometry: a reviewA. Spectrochim. Acta B 108, 35–52. http://dx.doi.org/10.1016/j.sab.2015.03.016.
Gorgich, M., Passos, M.L.C., Mata, T.M., Martins, A.A., Saraiva, M.L.M.F.S., Caetano, N.S., 2020. Enhancing extraction and purification of phycocyanin from *Arthrospira* sp. with lower energy consumption. Energy Rep. 6, 312–318. http://dx.doi.org/10.1016/j.egyr.2020.11.151.
Hadiyanto, H., Soetrisnanto, D., Marcelinus, Christwardana, 2014. Phytoremediation of palm oil mill effluent using *Pistia stratiotes* plant and algae *Spirulina* sp. for biomass production. Int. J. Eng. Trans. C: Aspects 27, 1809–1814. http://dx.doi.org/10.5829/idosi.ije.2014.27.12c.02.
Han, Y., Wu, C., Fu, X., Su, Z., Liu, M., 2021. Sulfate removal mechanism by internal circulation iron-carbon micro-electrolysis. Sep. Purif. Technol. 279, 119762. http://dx.doi.org/10.1016/j.seppur.2021.119762.
Hemalatha, M., Sravan, J.S., Min, B., Venkata Mohan, S., 2019. Microalgae-biorefinery with cascading resource recovery design associated to dairy wastewater treatment. Bioresour. Technol. 284, 424–429. http://dx.doi.org/10.1016/j.biortech.2019.03.10.
Hernández, E.V., Monje-Ramírez, I., Velásquez-Orta, S.B., Gracia-Fadrique, J., Ledesma, M.T.O., 2022. Surface activity of biomolecules released from microalgae harvested by ozone-flotation. Environ. Technol. Innov. 26, 102354. http://dx.doi.org/10.1016/j.eti.2022.102354.
Hoshino, R., Ogawa, M., Murakami, K., Wahyudiono Kanda, H., Goto, M., 2017. Extraction of lpids from wet *Arthrospira platensis* by liquefied dimethyl ether. Solvent Extr. Res. Dev. 24, 47–60. http://dx.doi.org/10.15261/serdj.24.47.
Hosseini, S.M., Usefi, M.M.B., Habibi, M., Pavizian, F., Van der Bruggen, B., Ahmadi, A., Nemati, M., 2019. Fabrication of mixed matrix anion exchange membrane decorated with polyaniline nanoparticles to chloride and sulfate ions removal from water. Ionics 25, 6135–6145. http://dx.doi.org/10.1007/s11581-019-03151-w.
Korhonen, J., Nuur, C., Feldmann, A., Birkie, S.E., 2018. Circular economy as an essentially contested concept. J. Clean. Prod. 175, 544–552. http://dx.doi.org/10.1016/j.jclepro.2017.12.111.
Kumar, M., Sun, Y., Rathour, R., Pandey, A., Thakur, I.S., Tsang, D.C.W., 2020. Algae as potential feedstock for the production of biofuels and value-added products: opportunities and challenges. Sci. Total Environ. 137116. http://dx.doi.org/10.1016/j.scitotenv.2020.137116.
Lage, S., Toffolo, A., Gentili, F.G., 2021. Microalgal growth, nitrogen uptake and storage, and dissolved oxygen production in a polyculture based-open pond fed with municipal wastewater in northern Sweden. Chemosphere 276, 130122. http://dx.doi.org/10.1016/j.chemosphere.2021.13.
Luengo, E., Condón-Abanto, S., Condón, S., Álvarez, I., Raso, J., 2014. Improving the extraction of carotenoids from tomato waste by application of ultrasound under pressure. Sep. Purif. Technol. 136, 130–136. http://dx.doi.org/10.1016/j.seppur.2014.09.008.
Marin-Batista, J.D., Villamil, J.A., Rodriguez, J.J., Mohedano, A.F., De la Rubia, M.A., 2019. Valorization of microalgal biomass by hydrothermal carbonization and anaerobic digestion. Bioresour. Technol. 274, 395–402. http://dx.doi.org/10.1016/j.biortech.2018.11.103.
Maurya, R., Zhu, X., Valverde-Pérez, B., Ravi Kiran, B., General, T., Sharma, S., Kumar Sharma, A., Thomsen, M., Venkata Mohan, S., Mohanty, K., Angelidaki, I., 2022. Advances in microalgal research for valorization of industrial wastewater. Bioresour. Technol. 343, 126128. http://dx.doi.org/10.1016/j.biortech.2021.126128.
Mera, R., Torres, E., Abalde, J., 2014. Sulphate, more than a nutrient, protects the microalga *Chlamydomonas moewusii* from cadmium toxicity. Aquat. Toxicol. 148, 92–103. http://dx.doi.org/10.1016/j.aquatox.2013.12.034.
Michael, A., Kyewalyanga, M.S., Lugomela, C.V., 2019. Biomass and nutritive value of Spirulina (*Arthrospira fusiformis*) cultivated in a cost-effective medium. Ann. Microbiol. 69, 1387–1395. http://dx.doi.org/10.1007/s13213-019-01520-4.
Mirhosseini, N., Davarnejad, R., Hallajisani, A., Cano-Europa, E., Tavakoli, O., Franco-Colín, M., Blas-Valdivia, V., 2021. Cultivations of *Arthrospira maxima* (Spirulina) using ammonium sulfate and sodium nitrate as an alternative nitrogen sources. Iran. J. Fish. 20, 475–489. http://dx.doi.org/10.22092/IJFS.2021.351071.0.
Mitra, M., Mishra, S., 2019. Multiproduct biorefinery from *Arthrospira* spp. towards zero waste: Current status and future trends. Bioresour. Technol. 291, 121928. http://dx.doi.org/10.1016/j.biortech.2019.121928.
Mohammadi, M., Mowla, D., Esmaeilzadeh, F., Ghasemi, Y., 2018. Cultivation of microalgae in a power plant wastewater for sulfate removal and biomass production: A batch study. J. Environ. Chem. Eng. 6, 2812–2820. http://dx.doi.org/10.1016/j.jece.2018.04.037.
Mohammadi, M., Mowla, D., Esmaeilzadeh, F., Ghasemi, Y., 2019. Enhancement of sulfate removal from the power plant wastewater using cultivation of indigenous microalgae: Stage-wise operation. J. Environ. Chem. Eng. 102870. http://dx.doi.org/10.1016/j.jece.2018.102870.
Mondal, M., Ghosh, A., Tiwari, O.N., Gayen, K., Das, P., Mandal, M.K., Halder, G., 2017. Influence of carbon sources and light intensity on biomass and lipid production of Chlorella sorokiniana BTA 9031 isolated from coalfield under various nutritional modes. Energy Convers. Manage. 145, 247–254. http://dx.doi.org/10.1016/j.enconman.2017.05.00.
Moraes, C.C., Sala, L., Cerveira, G.P., Kalil, S.J., 2011. C-phycocyanin extraction from *Spirulina platensis* wet biomass. Braz. J. Chem. Eng. 28, 45–49. http://dx.doi.org/10.1590/s0104-66322011000100006.
Mukherjee, S., Mondal, M., Banerjee, S., Halder, G., 2017. Elucidation of the sorptive uptake of fluoride by Ca 2+ -treated and untreated algal biomass of Nostoc sp. (BTA394): Isotherm, kinetics, thermodynamics and safe disposal. Process. Saf. Environ. 107, 334–345. http://dx.doi.org/10.1016/j.psep.2017.03.006.
Nagappan, S., Kumar, G., 2021. Investigation of four microalgae in nitrogen deficient synthetic wastewater for biorefinery based biofuel production. Environ. Technol. Innov. 23, 101572. http://dx.doi.org/10.1016/j.eti.2021.101572.
Nie, J., Sun, Y., Zhou, Y., Kumar, M., Usman, M., Li, J., Jihai, S., Wang, L., Tsang, D.C.W., 2020. Bioremediation of water containing pesticides by microalgae: Mechanisms, methods, and prospects for future research. Sci. Total Environ. 707, 136080. http://dx.doi.org/10.1016/j.scitotenv.2019.136080.
Northey, S.A., Mudd, G.M., Saarivuori, E., Wessman-Jääskeläinen, H., Haque, N., 2016. Water footprinting and mining: Where are the limitations and opportunities? J. Clean. Prod. 135, 1098–1116. http://dx.doi.org/10.1016/j.jclepro.2016.07.024.
Nur, M.M.A., Garcia, G.M., Boelen, P., Buma, A.G.J., 2019. Enhancement of C-phycocyanin productivity by *Arthrospira platensis* when growing on palm oil mill effluent in a two-stage semi-continuous cultivation mode. J. Appl. Phycol. 31, 2855–2867. http://dx.doi.org/10.1007/s10811-019-01806-9.
Oliveira, M.d., Monteiro, M., Robbs, P., Leite, S.G.F., 1999. Growth and chemical composition of *Spirulina maxima* and *Spirulina platensis* biomass at different temperatures. Aquac. Int. 7, 261–275. http://dx.doi.org/10.1023/A:1009233230706.
Ortiz-Villota, M.T., Romero-Morales, M.A., Meza-Rodríguez, L.D., 2018. La biorremediación con microalgas (*Spirulina máxima, Spirulina platensis* y *Chlorella vulgaris*) como alternativa para tratar la eutrofización de la laguna de Ubaque, Colombia. Rev. Investig. Desarro. Innov. Ing. 9, 163–176. http://dx.doi.org/10.19053/20278306.v9.n1.2018.8153.
Patnaik, R., Mallick, N., 2021. Microalgal biodiesel production: Realizing the sustainability index. Front. Bioeng. Biotechnol. 9, 620777. http://dx.doi.org/10.3389/fbioe.2021.620777.
Pearce, D.W., Turner, R.K., 1990. Economics of Natural Resources and the Environment. Johns Hopkins University Press, Baltimore MD.
Pez Jaeschke, D., Rocha Teixeira, I., Damasceno Ferreira Marczak, L., Domeneghini Mercali, G., 2021. Phycocyanin from Spirulina: A review of extraction methods and stability. Int. Food Res. J. 143, 110314. http://dx.doi.org/10.1016/j.foodres.2021.110314.
Pham, M.H., Pham, T.Y., Dinh, C.T., Bui, T.U., Tran, T.D., Nguyen, V.T., 2022. Microalgae and bioremediation of domestic wastewater. Curr. Opin. Green Sustain. 34, 100595. http://dx.doi.org/10.1016/j.cogsc.2022.100595.
Prasad, R., Gupta, S.K., Shabnam, N., Oliveira, C.Y.B., Nema, A.K., Ansari, F.A., Bux, F., 2021. Role of microalgae in global CO_2 sequestration: Physiological mechanism, recent development, challenges, and future prospective. Sustainability 13, 13061. http://dx.doi.org/10.3390/su132313061.

Purba, L.D.A., Othman, F.S., Yuzir, A., Mohamad, S.E., Iwamoto, K., Abdullah, N., Shimizu, K., Hermana, J., 2022. Enhanced cultivation and lipid production of isolated microalgae strains using municipal wastewater. Environ. Technol. Innov. 27, 102444. http://dx.doi.org/10.1016/j.eti.2022.102444.
Rawat, I., Bhola, V., Kumar, R.R., Bux, F., 2013. Improving the feasibility of producing biofuels from microalgae using wastewater. Environ. Technol. 34, 1765–1775. http://dx.doi.org/10.1080/09593330.2013.826287.
Rezania, S., Kamyab, H., Rupani, P.F., Park, J., Nawrot, N., Wojciechowska, E., Kumar, K.Y., Ghahroud, M.L., Mohammadi, A.A., Thirugnana, S.T., Chelliapan, S., Cabral-Pinto, M.M.S., 2021. Recent advances on the removal of phosphorus in aquatic plant-based systems. Environ. Technol. Innov. 101933. http://dx.doi.org/10.1016/j.eti.2021.101933.
Saeid, A., Chojnacka, K., 2016. Evaluation of growth yield of *Spirulina maxima* in photobioreactors. Chem. Biochem. Eng. Q. 30, 127–136. http://dx.doi.org/10.15255/CABEQ.2015.2269.
Salas-Montantes, C.J., González-Ortega, O., Ochoa-Alfaro, A.E., Camarena-Rangel, R., Paz-Maldonado, L.M.T., Rocha-Uribe, A., Soria-Guerra, R.E., 2018. Lipid accumulation during nitrogen and sulfur starvation in *Chlamydomonas reinhardtii* overexpressing a transcription factor. J. Appl. Phycol. 30, 1721–1733. http://dx.doi.org/10.1007/s10811-018-1393-6.
Selig, M.J., Malchione, N.M., Gamaleldin, S., Padilla-Zakour, O.I., Abbaspourrad, A., 2018. Protection of blue color in a spirulina derived phycocyanin extract from proteolytic and thermal degradation via complexation with beet-pectin. Food Hydrocoll. 74, 46–52. http://dx.doi.org/10.1016/j.foodhyd.2017.07.027.
Shin, H.Y., Shim, S.H., Ryu, Y.J., Yang, J.H., Lim, S.M., Lee, C.G., 2018. Lipid extraction from *Tetraselmis* sp. microalgae for biodiesel production using hexane-based solvent mixtures. Biotechnol. Bioprocess Eng. 23, 16–22. http://dx.doi.org/10.1007/s12257-017-0392-9.
Silva, S.C., Ferreira, I.C.F.R., Dias, M.M., Barreiro, M.F., 2020. Microalgae-derived pigments: A 10-year bibliometric review and industry and market trend analysis. Molecules 25, 3406. http://dx.doi.org/10.3390/molecules25153406.
Singh, D.V., Bhat, R.A., Upadhyay, A.K., Singh, R., Singh, D., 2021. Microalgae in aquatic environs: A sustainable approach for remediation of heavy metals and emerging contaminants. Environ. Technol. Innov. 21, 101340. http://dx.doi.org/10.1016/j.eti.2020.101340.
Souza da Silva de, S.P., Perrone, D., do Valle, A.F., 2022. Optimization of *Arthrospira maxima* cultivation for biomass and protein production and biomass technological treatment to color, flavor, and aroma masking for addition to food products. J. Appl. Phycol. 34, 65–80. http://dx.doi.org/10.1007/s10811-021-02601-1.
Sun, X.M., Ren, L.J., Zhao, Q.Y., Ji, X.J., Huang, H., 2018. Microalgae for the production of lipid and carotenoids: a review with focus on stress regulation and adaptation. Biotechnol. Biofuels 11, 272. http://dx.doi.org/10.1186/s13068-018-1275-9.
Sutherland, D.L., Ralph, P.J., 2019. Microalgal bioremediation of emerging contaminants - Opportunities and challenges. Water Res. 164, 114921. http://dx.doi.org/10.1016/j.watres.2019.114921.
Tan, H.T., Khong, N., Khaw, Y.S., Ahmad, S.A., Yusoff, F.M., 2020. Optimization of the freezing-thawing method for extracting phycobiliproteins from *Arthrospira sp*. molecules (Basel, Switzerland). Molecules 25, 3894. http://dx.doi.org/10.3390/molecules25173894.
Tavanandi, H.A., Mittal, R., Chandrasekhar, J., Raghavarao, K.S.M.S., 2018. Simple and efficient method for extraction of C-Phycocyanin from dry biomass of *Arthrospira platensis*. Algal Res. 31, 239–251. http://dx.doi.org/10.1016/j.algal.2018.02.008.
Thangiah, A., 2019. Spectrophotometric determination of sulphate and nitrate in drinking water at Asia-Pacific International University Campus, Muak Lek, Thailand. Rasayan J. Chem. 12, 1503–1508. http://dx.doi.org/10.31788/RJC.2019.1235201.
U.S. DOE, 2008. Algal Biofuels. Biomass Program. Department of Energy, Washington D.C.
Velenturf, A.P.M., Archer, S.A., Gomes, H.I., Christgen, B., Lag-Brotons, A.J., Purnell, P., 2019. Circular economy and the matter of integrated resources. Sci. Total Environ. 689, 963–969. http://dx.doi.org/10.1016/j.scitotenv.2019.06.449.
Wang, X., Liu, S.-F., Qin, Z.-H., Balamurugan, S., Li, H.-Y., Lin, C.S.K., 2020. Sustainable and stepwise waste-based utilisation strategy for the production of biomass and biofuels by engineered microalgae. Environ. Pollut. 114854. http://dx.doi.org/10.1016/j.envpol.2020.114854.
Wu, H., Shi, Y., Xia, Q., Zhu, W., 2014. Effectiveness of the policy of circular economy in China: A DEA-based analysis for the period of 11th five-year-plan. Resour. Conserv. Recycl. 83, 163–175. http://dx.doi.org/10.1016/j.resconrec.2013.10.0.
Xue, J., Balamurugan, S., Li, T., Cai, J.-X., Chen, T.-T., Wang, X., Li, H.-Y., 2021. Biotechnological approaches to enhance biofuel producing potential of microalgae. Fuel 302, 121169. http://dx.doi.org/10.1016/j.fuel.2021.121169.
Yong, W.K., Tan, Y.H., Sze-Wan, P., Lim, P.E., 2016. Response of microalgae in a changing climate and environment. Malaysian J. Sci. 35, 169–191. http://dx.doi.org/10.22452/mjs.vol35no2.7.
Yuvraj, 2022. Microalgal Bioremediation: A clean and sustainable approach for controlling environmental pollution. In: Arora, S., Kumar, A., Ogita, S., Yau, Y.Y. (Eds.), Innovations in Environmental Biotechnology. Springer, Singapore, pp. 305–318. http://dx.doi.org/10.1007/978-981-16-4445-0_13.
Zarrouk, C., 1966. Contribution à L'étude d'Une Cyanophycée Influence de Divers Facteurs Physiques et Chimiques sur la Croissance et la Photosynthese de *Spirulina Maxima* (Setch et Gardner) Geitler. University of Paris, Paris.
Zhang, M., Wang, H., 2014. Organic wastes as carbon sources to promote sulfate reducing bacterial activity for biological remediation of acid mine drainage. Miner. Eng. 69, 81–90. http://dx.doi.org/10.1016/j.mineng.2014.07.010.
Zhu, L., 2015. Biorefinery as a promising approach to promote microalgae industry: An innovative framework. Renew. Sustain. Energy Rev. 41, 1376–1384. http://dx.doi.org/10.1016/j.rser.2014.09.040.