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Removal of heavy metals and hydrocarbons by microalgae from wastewater in the steel industry

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ABSTRACT

Steel-industry effluents contain hazardous compounds such as heavy metals and hydrocarbons, which can damage the environment. Traditional remediation technologies generate secondary impacts and, therefore, developing more sustainable methods is imperative. In this work, the tolerance capacity of *Tetradesmus obliquus, Chlorella sorokiniana, Chlorella vulgaris, Arthrospira platensis* and *Arthrospira maxima* (spirulina) to steel hot-rolling wastewater was tested through their exposure to increasing concentrations of residue. Based on the results obtained, *A. maxima* was cultured in presence of wastewater and scaled to semi-industrial production systems. Influence of microalgae growth on wastewater composition was evaluated by the end of the experiment by measuring concentrations of contaminants of concern (iron and hydrocarbons). Results showed a reduction of hydrocarbons and iron of 75 and 97.9%, respectively. The findings obtained in this study stablish that spirulina cultures might be used as a novel and environmentally sustainable bioremediation tool for steel-industry wastewater.

1. Introduction

The increase in the world's population in the last century has led to an overexploitation and increase in the degradation of natural water sources, being industrial activities responsible for 23% of total water consumption [1,2]. The steel industry is one of the most water-demanding sectors, 28.6 m^3 is the average volume of water necessary to transform one ton of crude steel into the final product [3]. Those activities discharge wastewater with high pollutants, such as heavy metals, that need to be removed before discharging to natural water sources [4,5]. If untreated, these wastewater release toxic concentrations of heavy metals to the environment, which consequences have been difficult to forecast due to their long biological half-life [6]. Principally, toxicity of heavy metals lies on their bioaccumulation potential, increasing their concentrations along the food chain and showing major effects on upper trophic levels [7]. On this basis, since humans are on top of most of the food chains, we are the final accumulators of heavy metals. It has been detected that exposure to toxic concentrations of these compounds play a significant role in weakening defense mechanisms and increasing the risk of cancer development [8,9]. Furthermore, heavy metals cause a decrease on biodegradability of organic pollutants, making them to last longer on the environment and intensifying the effects of other toxic wastes [10].

Wastewater with high content of heavy metals are commonly treated by traditional chemical processes based on oxidation/reduction reactions and chemical precipitation (sodium and calcium carbonate are the most used chemical reagents). Most of metallic ions are separated as insoluble hydroxides, sulphides and carbonates, with a removal rate that can reach up to 97% in laboratory-scale experiments [11]. The efficiency of this technology depends on the concentration of heavy metals and can be ineffective when used at low concentrations. Moreover, this technique generates large quantities of harmful sludges that are difficult to dewater and manage, which makes it a non-environmentally friendly method [12]. Traditional physical methods are also used to treat industry wastewater, being adsorption the most common due to its simplicity of operation [13]. The vast variety of different adsorbent compounds makes it a very flexible method with a wide range of results. In particular, removal efficiency might vary from 70% to 98%, depending on the adsorbent used and the conditions [14]. Despite of the high efficiency of adsorption, many external factors affect the

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effectiveness of this technology, especially the presence of other pollutants, such as oils and greases [15]. In summary, traditional remediation procedures require large quantities of energy and reagents that produce toxic waste products, which are necessary to develop novel proceedings that are more sustainable and environmentally friendly [16,17].

Considering the urgency to find innovative and green wastewater treatments, the use of living organisms for effluent cleaning provides an alternative to traditional methods (bioremediation). The main advantages are the cost-efficiency rate, high public approval, and great results [18,19]. Several investigations have been carried out to prove how plants can remediate toxic wastes. Additionally, plants provide optimum habitats for a wide range of organisms, such as bacteria and fungi, that metabolise contaminants, enhancing the bioremediation process [20,21]. This symbiotic relation can lead to removal of heavy metals on wastewater with an efficiency up to 70% of metallic ions uptake and high long-term bioaccumulation potential [22]. Nevertheless, several studies have shown that slow biomass production and long planting/harvesting cycles derive into excessive remediation times, taking months and even years to see significative results [23,24]. The latter inconveniences may be avoided by using fast-growing photosynthetic organisms with an elevated surface-to-volume ratio, such as microalgae [25].Due to the recovery of high value compounds, such as pigments and antioxidants, through biorefinery of the microalgal biomass, microalgae bioremediation has shown to be a cost-effective tool [26]. Sarma et al. [27] demonstrated through the review of several studies that coupling microalgal bioremediation with biorefinery purposes can be a source of economic incomes during depuration. Furthermore, this novel technique could help industry avoiding weaknesses of traditional depuration systems, along with synthesis value-added compounds [28].The goal of this work is to evaluate the use of cyanobacteria and microalgae cultures, at semi-industrial scale, as a novel bioremediation system for steel industry wastewater. To do so, 5 species of microalgae and cyanobacteria were selected to bioremediate hot-rolling process wastewater. *Chlorella*, *Tetradesmus* and *Arthrospira* genres were selected according to their capacity to tolerate harmful wastewater and remove pollutants of concern [29,30]. To the best of our knowledge, this is the first study focused on industrial scale microalgae culturing for hot-rolling process wastewater bioremediation.

2. Materials and methods

This study was carried out in the facilities of Neoalgae Micro Seaweed Products (Gijón, Spain). Wastewater bioremediation through microalgae culturing depends on 2 factors: tolerance and uptake capacity. On this basis, experimental design was divided in tolerance assay and bioremediation.

2.1. Microalgae strains

The selection of the microalgae strains used during experimentation was done following the strains available in the Neoalgae Micro Seaweed Products catalogue (Gijón, Spain). Moreover, the choice was guided through the revision of the accessible bibliography, specially according to the investigations done by Rath [31] and Kalra et al. [32]. Two cyanobacteria, *A. maxima* (originally provided by the Spanish National Research Council, CSIC, Spain) and *A. platensis* (originally purchased to the Spanish Bank of Algae, BEA, strain n° 0005B), and three species of eukaryotic green microalgae, *C. vulgaris* (originally purchased to the Culture Collection of Algae and Protozoa, CCAP, United Kingdom, strain n° 211/109), *C. sorokiniana,* and *T. obliquus* (formerly known as *Acutodesmus obliquus*), were selected for this study. The latter two microalgae strains were isolated from a municipal solid waste landfill by Suarez-Montes et al. [33].

2.2. Culture media

For eukaryotic species, F/2 Medium was used as a growth medium for freshwater green microalgae and was provided as powder by Varicon Aqua (United Kingdom). The nutritional composition of this commercial medium is based on the standard F/2 medium described by Guillard in 1975 (as shown in Table 1) and was added 1 mL of each stock solution per litter of culture.

As reported by Suarez-Montes et al. [33], F/2 medium did not show optimum results for cyanobacteria, in terms of growth. Nevertheless, Morais et al. [35] observed that Zarrouk's medium provides optimum growth results for *Arthrospira* genre and so was chosen. This decision was made in order to achieve the best culture conditions for each specie before exposing it to wastewater medium. Chemical composition of Zarrouk's medium is described in Table 2 and addition was done by reason of 1 mL of each stock solution per liter of culture. However, environmental factors such as initial pH, temperature and photoperiod remained constant for the entire experimental set-up in order to reduce the variability among them.

All reagents were bought to Labbox Labware (Barcelona, Spain), with exception of: $NaHCO_3$, $NaNO_3$, K_2SO_4, $MgSO_4$, that were purchased to Vadequímica (Barcelona, Spain). NaCl was provided by Agrupasal (Asturias, Spain).

2.3. Wastewater composition

Wastewater was collected from the effluent of a steel processing facility in Spain. Samples were collected in 20 L inert plastic bottles and its composition is shown in Table 3. These samples were stored in a dark and cool room, without any filtering process, to preserve its characteristics.

Heavy metals analysis followed the ISO 15587-1:2002 requirements. Analysis was done by Inductively Coupled Plasma Mass Spectrometry (ICP-MS), following the methods described by Wilschefski and Baxter

Table 1
Chemical composition of F/2 medium (modified from [34]).

Solution A: Nitrate and phosphate solution (1 L)	
Nutrient	Amount (g)
$NaNO_3$ (98%)	84.15
$Na_2HPO_4 + H_2O$	6.0
$FeCl_3 + 6\ H_2O$	2.90
$Na_2EDTA + 2\ H_2O$	10.0

Solution B: Silicate stock solution (1 L)	
Nutrient	Amount (g)
$Na_2SiO_3 + 9\ H_2O$	33.0

Solution C: Trace metal stock solution (1 L)	
Nutrient	Amount (g)
$CuSO_4 + 5\ H_2O$	1.96
$ZnSO_4 + 7\ H_2O$	4.40
$Na_2MoO_4 + 2\ H_2O$	1.26
$MnCl_2 + 4\ H_2O$	36.0
$CoCl_2 + 6\ H_2O$	2.0

Solution D: Vitamin stock solution (1 L)	
Nutrients	Amount (mg)
Vitamin B1	400
Vitamin B12	0.002
Biotin	0.1

Table 2
Chemical composition of Zarrouk's medium.

Nutrient (purity)	Amount (g/L)
$NaHCO_3$ (>98%)	18.0
$NaNO_3$ (98%)	2.5
K_2HPO_4 (98%)	0.5
K_2SO_4 (99%)	1.00
NaCl (99.8%)	1.00
$CaCl_2 + 2\ H_2O$	0.04
Na_2EDTA (99%)	0.08
$MgSO_4 + 7\ H_2O$	0.2
$FeSO_4 + 7\ H_2O$	0.01
Micronutrient solution*	1 mL/L
*Micronutrient solution (g/L)	
H_3BO_3 (99%)	2.86
$MnCl_2 + 4\ H_2O$	1.81
$ZnSO_4 + 7\ H_2O$	0.22
$CuSO_4 + 5\ H_2O$	0.079
$(NH_4)_6Mo_7O_{24}$	1.00

Table 3
Wastewater chemical composition.

Heavy Metals	Amount (mg/L)
Arsenic (As)	<0.01
Cadmium (Cd)	<0.0002
Copper (Cu)	0.005
Chromium (Cr)	<0.004
Iron (Fe)	4.8
Mercury (Hg)	<0.1 μg/L
Nickel (Ni)	0.02
Lead (Pb)	<0.005
Zinc (Zn)	0.007

Hydrocarbons	Amount (mg/L)
C10–C40 (total)	0.44
C10–C16	<0.01
C16–C20	0.007
C20–C24	0.018
C24–C28	0.069
C28–C40	0.11

[36]. Total hydrocarbons in wastewater sample were measured by Gas Chromatography-Mass Spectrometry, as described by Brown et al. [37].

2.4. Lab conditions

Lab conditions were optimized for algal growth following the recommendations of the Food and Agriculture Organization of the United Nations [38]. Environmental settings were kept constant at a temperature of 25 °C and photoperiod of 16:8 h, with a continuous photon flux of 80–100 $\mu mol \cdot s^{-1} \cdot m^{-2}$. In addition, culture aeration was uninterrupted and provided by air pumps with a flow rate of 100 L/h. A preliminary acclimation set-up was carried out by growing the selected microalgae species in conical borosilicate flasks for 10 days, in order to achieve a full growth curve in all strains. All experimental groups were done by triplicates, including a standard group which did not include wastewater in its composition.

2.5. Tolerance/compounds uptake experiments

The initial testing started with a tolerance trial of five species of microalgae and cyanobacteria to different percentages of wastewater: 0, 10, 25, 50, and 75%. The initial inoculum added to the culture medium/media was 20% (regarding to final volume) and media were added following the quantities described in Section 2.2. These trials were done in triplicate with a final volume of 200 mL. Cultures were scaled up from 250 mL to 2 L and used as inoculum for pre-pilot volumes.

The following steps were focused on cyanobacteria belonging to the *Arthrospira* genus. Additionally, culture medium was changed for a modification of Zarrouk's standard medium through the modification of $FeSO_4$ content. This assay aimed to verify the impact of iron deprivation and excess on *Arthrospira* growth. *A. maxima* and *A. platensis* were grown under laboratory conditions and exposed to 5 concentrations of iron, according to the iron content in wastewater (Table 3). Thus, the experimental set up contained 5 concentrations of iron in culture medium: absence, control group with standard Zarrouk medium, 50%, 75% and 100% of total free iron. Reaching up to 4.8 mg/L and emulating iron content in wastewater. Cultures evolution was measured as described in Section 2.6.

Cultures were scaled up in 10 L bottles to semi-industrial closed systems called photobioreactors (PBR), which are mainly used for minimizing contamination in the culture media. PBRs consisted in vertical glass columns of 3 m high and 30 cm of diameter with bottom aeration, with a final volume of 100 L. pH during experiment oscillated between 9.5 (initial measure) and 10 (final measure) and temperature was not controlled since this section of the study was carried out inside a greenhouse.

2.6. Growth parameters

The cultures' growth was measured by optical density (OD) at 750 nm every 48 h using a spectrophotometer (BioChrome Libra S11), by taking 1 mL of each sample per replicate. Sampling was done under sterile conditions, and the sample was agitated with a vortex for 5 s before the optical density was measured. Background fluorescence was determined using a blank sample obtained by mixing the culture medium and wastewater following the experimental percentages. The presence of contaminant microorganisms and integrity of cellular membranes were checked every 2 days under an optical microscope (Bioblue BB.1153-PLi), and sampling was done by micro pipetting (200 μL) in sterile conditions.

2.7. Culture harvesting and biomass analysis

PBR cultures were harvested, after reaching the maximum cellular density during experiment, through centrifugation at 10,000 rpm in an industrial centrifuge. Target-effluent samples were taken and analysed as described in Section 2.3 to verify how the presence of algal cells altered the chemical composition of the initial wastewater. To verify biomass composition variation in the presence of wastewater, biomass samples were taken for analysis by ICP-MS.

2.8. Heavy metal and hydrocarbon uptake analysis

Toxic metallic ions and hydrocarbons were analysed, and the intake ratio was calculated in terms of the experimental uptake capacity (q) through a modification of the equation used by Tsekova et al. [39] regarding bioremediation with *Aspergillus niger* species:

$$q = \frac{V(Ci - Cf)}{m} \quad (1)$$

where q is the uptake capacity of the biomass (mg of metal/g of wet biomass), V is the final volume of the experiment (100L), Ci and Cf are initial and final concentrations of a given pollutant, respectively, and m is the total wet biomass harvested from the culturing system.

In addition, the removal efficiency (RE) of the hydrocarbons and heavy metals was evaluated from the initial composition of the wastewater and the final concentration of each compound in the wastewater, through Eq. (2) [39]:

$$RE = \left(\frac{Ci - Cf}{Ci}\right) \times 100 \quad (2)$$

2.9. Statistical analyses

Statistical analysis was performed using PAST software (version 4.05). Averages of triplicates were subjected to a Welch *t*-test and compared through a Tukey's range test at $p < 0.05$ to identify significant differences among the different groups within the experiment. All data were given as the average with standard deviation.

3. Results and discussion

3.1. Tolerance

All proposed species survived in the presence of percentages of wastewater lower than 75% during initial tolerance experiments. Furthermore, 75% trials showed a gradual decrease in terms of cellular density, reaching levels close to zero during the tolerance trials (Fig. 1). This mortality is visible in Fig. 1, which shows the evolution of chlorophyl content of the experimental cultures during that stage of experimentation.

Additionally, tolerance trials with 100% wastewater showed a gradual decrease of cellular density of the eukaryotic strains during the first 24 h of experimentation. This sudden death was clearly visible through microscopy analysis (Fig. 2). Results showed how *T. obliquus* cells persisted during the first 12 h exposed to 100% of wastewater. Nevertheless, 24 h microscopy analysis showed no viable cells, only being visible cellular structures with no detectable organelles. Likewise, *Chlorella* strains showed similar results, showing no viable cells after 24 h of exposal to 100% of residue.

Despite the unsatisfactory tolerance results of the chlorophyte strains, the cyanobacteria chosen for this study (genre *Arthrospira*) showed better results in terms of survival. According to Fig. 1, both *Arthrospira* species grew in presence of 75% of wastewater. Nevertheless, *A. platensis* growth seemed to be hindered by the presence of the residue. Chlorophyll content in *A. platensis* cultures increased unobtrusively along with culture time but absorbance measurements showed a maximum chlorophyll concentration 30% more than initial levels. This supports what stated by Önem et al. [40], who studied the toxic potential of free heavy metal ions on *A. platensis* growth. Nevertheless, the results obtained showed higher grow yield on *A. maxima*, which can be explained by a better tolerance ability to the determined wastewater. Moreover, Wang et al. [74] observed how minimum concentrations of a particular C15 hydrocarbon reduced the growth yield of some microalgal strains. On this basis, hydrocarbons present in wastewater could limit the survival of the species used during our study. Considering these results, Chlorophyta strains were discarded, and the following steps were focused on *A. maxima* and *A. platensis.* This decision was made due to the fact that dilution would result in a large volume of water to be handled and demand expansion of storage, and processing facilities.

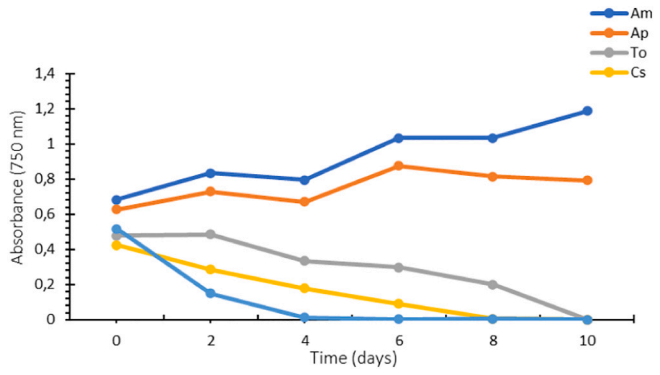

Fig. 1. Chlorophyl concentration evolution in 75% of wastewater during 2 L assay.

Our results support what was stated by Cui et al. [41]. Cyanobacteria, like *Arthrospira* genre, possess elevated binding affinity and binding sites for metallic ions, which gives them high potential as industrial wastewater bioremediators. Nevertheless, our results show how *A. maxima* grew with better results when compared to *A. platensis.* Previous studies have shown how excessive concentrations of heavy metals can produce toxic effects on cyanobacteria survival [40]. Considering the elevated concentration of iron in wastewater, it was decided to expose cyanobacterial strains to various iron stresses with no addition of wastewater. This test aimed to verify the growth performance of *A. platensis* and *A. maxima* cultured with different concentrations of iron. The objective of this assay was to discriminate the influence of free iron and oil hydrocarbons on cyanobacterial growth. Also, *Arthrospira* cultures were grown in iron deficiency conditions, under the assumption that cyanobacteria cells could not grow with optimum results in absence of this metallic ion. Our results agree with the studies of Das et al. [42] and Murwanashyaka et al. [43], the presence of metallic ions in mining wastewater represent potential micronutrients. These might serve as nutritional carbon sources for heterotrophic and autotrophic microalgae.

Spectrophotometric measures were taken every 48 h for two weeks (Fig. 3). Within the first 2 days, the optical density at 750 nm showed a slight decrease in cellular density in both *Arthrospira* species. This decrease was extended along the lag phase, where there was no increase in living cells. However, after the 4th day of culture, *A. maxima* reached the logarithmic/exponential phase, showing a progressive increase of cell population. By the 7th day, *A. maxima* cultures were able to double the initial optical density. This growth was sustained for 14 days, reaching a final optical density of 1.097. However, the *A. platensis* cultures did not enter the logarithmic phase until day 7, showing modest growth that led to a death phase. The results obtained during this phase of the study support the ones obtained during the tolerance trials (Fig. 1), which showed that presence of wastewater hindered *A. platensis* growth, when compared to *A. maxima*. Nevertheless, absorbance and microscopy measurements did not show cellular mortality. Previous studies have shown that presence of specific toxic pollutants hinders the biomass generation in spirulina (*Arthrospira*) cultures [44]. On this basis, a new experimental design was needed to discriminate the influence of iron stress on algal growth from the influence of hydrocarbons. Also, *A. maxima* and *A. platensis* were cultured through iron starvation, under the assumption that these species were able to chelate it and absorb it from the hot-rolling process wastewater (Fig. 3).

During experimentation, control groups of both species grew normally, verifying that culture conditions were optimum. For both species, absence of iron in culture medium triggered an initial descent in terms of cellular density, which was constant along the entire study, showing the importance of this ion as a micronutrient. Growth reduction due to iron deficiency is a well-known consequence, previous studies have already proved that iron presence promotes cyanobacteria growth in natural ecosystems [45,46]. Contrary to what was observed during tolerance trials (Fig. 1), *A. platensis* did not show a reduction in growth yield with presence of 4.8 mg/L of free iron. Moreover, both species showed great results in terms of absorbance measurements with 50, 75 and 100% of free iron. The results obtained agree with the ones of Molnàr et al. [47], who stated that *Arthrospira* genre is not negatively affected by increased free iron concentrations in culture medium. On this basis, Lu et al. [25] showed how slight supplementation of iron in *Arthrospira* cultures lead to a better performance in terms of metabolic bioremediation and growth. This result contradicts what was observed by Cepoi et al. [48], who observed how increasing quantities of metal ions reduce biomass production of these species. Nevertheless, our study clearly demonstrates how concentrations up to 4.8 mg/L of free iron did not hinder biomass generation in *Arthrospira* genre.

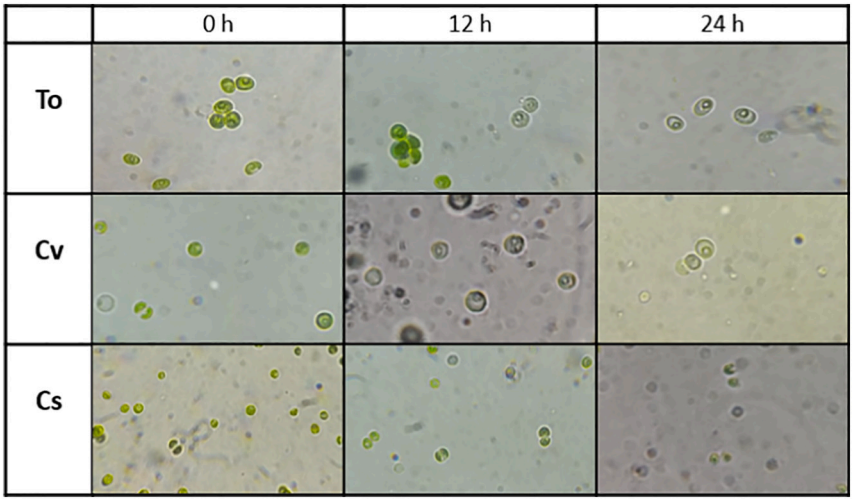

Fig. 2. Gradual cellular mortality of *T. obliquus*, *C. vulgaris* and *C. sorokiniana* during the first 24h of tolerance trials with 100% of wastewater.

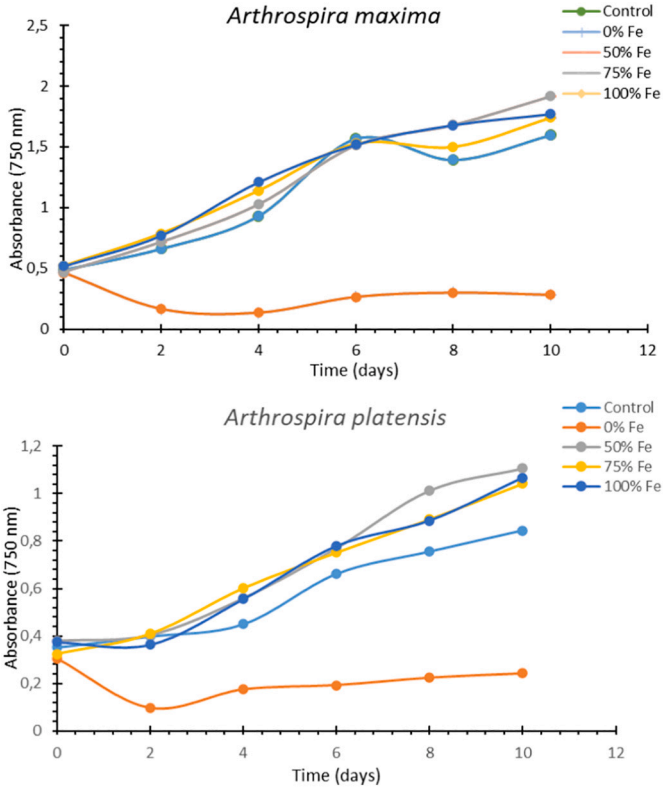

Fig. 3. Absorbance measurements during iron stress assay.

Absorbance and microscopy measurements did not show cellular mortality during iron stress experimentation. Furthermore, both *Arthrospira* species cultures were scaled up to 100 L PBRs for industrial systems scale trials. During this phase of investigation, experimental groups of *A. platensis* did not achieve high growth rates compared to control group. On the contrary, *A. maxima* grew with optimum results, achieving a maximum growth yield close to control group. The results obtained during this phase of the study support the ones obtained during the tolerance trials (Fig. 1), which showed that presence of wastewater hindered *A. platensis* growth, when compared to *A. maxima* (Fig. 4).

Our results showed how high amounts of free iron in wastewater did not reduce the survival of *A. platensis*. On the other hand, the survival limitation in wastewater could be explained by the presence of

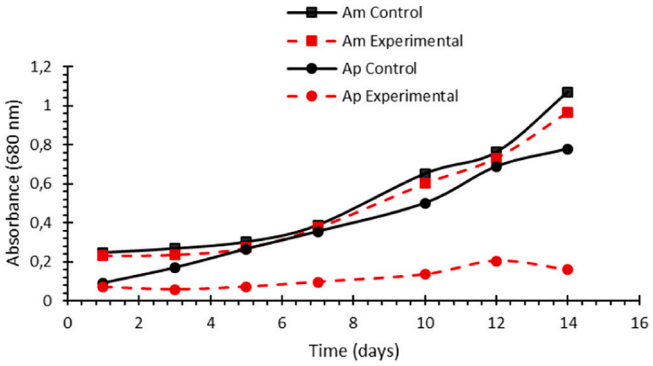

Fig. 4. *A. maxima* (Am) and *A. platensis* (Ap) optical density.

hydrocarbons. Our results relate to the ones obtained by López-Pacheco et al. [49]. In study, they observed how a particular C15 hydrocarbon can reduce *A. maxima* survival at similar concentrations to the ones in the wastewater used for our study. Furthermore, according to Gonzalez et al. [50], iron excess in culture media can lead to the formation of reactive oxygen species (ROS), derived from oxidative stress. Nevertheless, under the same conditions, *A. maxima* grew with no major issues, showing a higher potential to avoid wastewater toxicity when compared with *A. platensis*. However, the resistance of these organisms to determined wastewater does not establish that those organisms maximise the up taking of certain pollutants of interest. Furthermore, it is crucial to discriminate between tolerance to residues and their bioremediation.

Previous studies have shown that presence of toxic pollutants hinder the biomass generation during spirulina (*Arthrospira*) cultures [44]. On this basis, we assume that presence of elevated concentrations of hydrocarbons impede *A. platensis* cells from reproducing. Nevertheless, further studies are needed to discriminate the influence, on *A. platensis* growth, of hydrocarbons separately. Considering these results, *A. platensis* cultures were discarded due to their inability to grow in previous steps, and *A. maxima* cultures were harvested for further analysis.

3.2. Bioremediation

Iron ions in solution and long-chain hydrocarbons were the main components influenced by the biological treatment of wastewater (Table 4). These compounds showed a significant reduction in terms of

Table 4
Comparison of compounds between initial and final composition of wastewater.

Compounds	Initial composition	Final composition
Metals	Concentration (mg/L)	Concentration (mg/L)
Iron (Fe)	4.8	0.1
Hydrocarbons	Concentration (mg/L)	Concentration (mg/L)
C10–C40 (total)	0.44	0.11
C16–C20	0.007	0.089
C20–C24	0.018	<0.005
C24–C28	0.069	<0.005
C28–C40	0.11	<0.005

concentration.

3.2.1. Heavy metal removal

The obtained wet biomass was 1.5 ± 0.132 g/L, and the uptake capacity (q) and removal efficiency (RE) of iron by *A. maxima* were calculated through Eqs. (1) and (2). The uptake capacity reached up to 3.33 mg/g, meaning that each gram of wet biomass absorbed 3.33 mg of Fe^{2+} from wastewater in 2 weeks. Moreover, the results obtained by removal efficiency showed that *A. maxima* was able to remove 97.9% of the total iron content, which are similar to the iron removal ratio obtained by Serrà et al. [28]. Moreover, our results support the findings of Gong et al. [51], whose experience demonstrated the potential of *A. maxima* as a potent adsorbent for lead bioremediation. Their removal rate with dead biomass reached up to 84%. Moreover, Sadovsky et al. [52] also used dead *Arthrospira* (Spirulina) biomass for cerium ions remediation, obtaining great results in terms of removal rates. Despite of these good results, living organisms achieve better outcomes, optimizing bioremediation through microalgal adsorption and absorption activities. Considering the results obtained, the survival of *A. maxima* in large scale cultures is a critical point for long term wastewater bioremediation and so should be further studied.

Previous works demonstrated that iron must be added to solution with a chelating agent that makes ions more easily absorbable by microalgae cells [53]. On the contrary, other studies demonstrated that some species of microalgae and cyanobacteria can synthetize different molecules that serve as chelating compounds [54,55]. Moreover, presence of heavy metals inside the cytosol results in the binding of glutathione with metallic ions. This union forms a complex that is latter sequestered and accumulated into organelles [56]. On this basis, our results suggest that the variable proteomic expression under heavy metal stress can happen also in *A. maxima* during heavy metal remediation, but further investigations are needed.

This decrease in iron content was reflected in the biomass; while the iron content of the wastewater was reduced, the iron content of the biomass was further enhanced (increased by 285%). These results showed a statistically significant difference between control and outcomes from experimental groups ($p = 0.049$) (Fig. 5). Control samples of wastewater were taken from untreated residue after the same days of experiment. In case of the iron content of the biomass, control measurements were taken from *A. maxima* cultured under same conditions and culture systems as experimental cultures, but without presence of wastewater. This decision was taken in order to elaborate a reliable comparison among the data obtained.

Despite the significant increasement of total amount of iron in the experimental biomass (p = 0.049), previous studies achieved better results in terms of iron biomass enrichment. Akbarnezhad et al. [57] reached higher concentrations of iron in *A. platensis* biomass, up to 4465 ± 39.68 mg/kg. The differences on iron up taking are directly related to the concentration of free iron in culture media. For this reason, higher concentrations lead to a major up taking rate and more total iron inside the cytosol. Nevertheless, excessive metallic ions concentration (above the toxic threshold) derives into a decreased performance in terms of biomass generation and bioaccumulation [47]. For this reason, specific studies focused on overall survival of different microalgae strains are crucial before considering industrial application of these type of bioremediation systems. *A. maxima* present great potential in steel industry wastewater depuration due to their capability to adsorb heavy metal ions onto their surface, even when there is no metabolic activity [59,60]. Zada et al. [61] obtained similar results in terms of iron removal rates but using eukaryotic microalgae and simulated concentrations of Fe^{2+} in culture media. Our study completes their investigation by using a prokaryotic organism with elevated value for human nutrition, especially because of its high protein content and essential amino acids [62]. Iron enriched biomass resulting from this study represent an opportunity for iron supplementation, converting a residue into a potential product [57]. As far as we know, this is the first attempt of harnessing steel-industry wastewater for *Arthrospira* biomass generation. Nevertheless, biomass composition and security trials should be done before accepting the resulting biomass from a depuration system as food or source of supplementation. On this basis, *A. maxima* provides new opportunities as a novel organism in biological filters for wastewater inclusion into circular economy systems.

3.2.2. Hydrocarbon removal

Oil and hydrocarbon bioremediation by microalgae is a field that has been less studied than heavy metal removal. Nevertheless, Tremblay et al. [63] suggested that aerobic biodegradation of hydrocarbons is more efficient than the anaerobic pathway. On this basis, the presence of microalgae would have a synergic effect during oil bioremediation, demonstrating the importance of including photosynthetic organisms in this kind of techniques. Some marine microalgae can bioremediate contaminating compounds from oil spills, thus proving the existence of naturally occurring mechanisms for hydrocarbon assimilation in some photosynthetic microorganisms [64]. In the same way, it has been

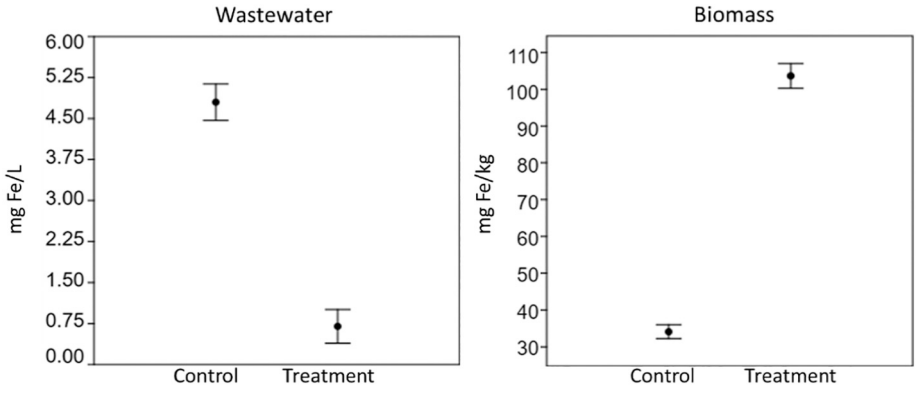

Fig. 5. Iron content in wastewater (mg/L) and wet biomass (mg/kg) of *A. maxima* (p = 0.049).

validated that some species of microalgae from the *Tetradesmus* and *Chlorella* genera can grow in oily industrial wastewater with great results in terms of cellular density [65,76].

Wang et al. [74] observed how a particular type of C15 hydrocarbon can produce a reduction on cellular growth in microalgae cultures at similar concentrations to those used for the present study. Nevertheless, during this investigation, *A. maxima* grew with optimum results and hydrocarbons showed a substantial decrease in terms of total concentration (Table 4). The uptake carried out by *A. maxima* showed that each gram of wet biomass absorbed up to 0.33 mg/L of hydrocarbons. In addition, hydrocarbon removal efficiency over 14 days appeared to be 75% over the total composition of wastewater. This result supports the ones presented in the work of Beigbeder et al. [66], regarding to phenolic compounds bioremediation. Such a reduction in total hydrocarbons was correlated with a significant reduction in long-chain hydrocarbons (20-carbons chain or longer). A significant decrease was observed in terms of how hydrocarbons ware affected by microalgae treatment (Fig. 6, $p = 0.04853$).

López-Pacheco et al. [49] observed the influence of a consortium of *A. maxima* and *C. vulgaris* on C15 hydrocarbons concentration. Their study yielded higher results in terms of removal rates when compared to the results obtained in this study. Nevertheless, our study focused on a mixed wastewater with elevated proportions of long-chain hydrocarbons, which were mostly removed. These results are visible on Fig. 6 c) and d). Our work showed how experimental groups were able to grow with good results in presence of residual hydrocarbons and induce a descent on them, supporting the conclusions of Kuttiyathil et al. [76] and stablishing the possibility of using organic molecules from industrial leftovers for mixotrophic (or heterotrophic) microalgae culturing. Considering the review study of Shrestha et al. [12], our results could be extrapolated to ionic flotation engineering systems as a novel biodegradable surfactant. By combination of *A. maxima* cultures with these systems, limitation of large-scale application is reduced, and removal rates of ionic flotation would be improved. Nevertheless, further works focused on the develop of a hybrid technique should be carried out.

Despite the previous results, the 16 to 20 carbon-chain hydrocarbons displayed a significant increase (maximum of 1257%) (Fig. 7). Specifically, they increased 0.105 mg/L per gram of wet biomass, assuming the absolute value provided in previous estimations.

Previous works observed hydrocarbons fragmentation mechanisms in different organisms. Naghdi et al. [67] reported that some fungal strains had the necessary mechanisms to break down large molecules, such as antibiotics, into small metabolites that could be further metabolised by microalgae. Also, López-Pacheco et al. [49] noticed that the reduction of hydrocarbons in culture media is correlated to a biotransformation process into smaller molecules. Based on the data obtained, *A. maxima* removed C28–C40 chain hydrocarbons, and C16–C20 chain hydrocarbons were multiplied by 12 after 2 weeks of biological treatment. The cellular insights of oils modification for bioremediation processes were elucidated by Girvan and Munro [68], who highlighted the importance of cytochrome P450 and its coupled biochemical reactions. These modifications in chemical structure changes of hydrocarbons molecules are close to the ones seen in non-photosynthetic microorganisms such as bacteria. This is directly related to the phylogeny proximity between bacteria and microalgae, which comes to be more evident in cyanobacteria as *A. maxima* [69,70]. Bearing in mind that

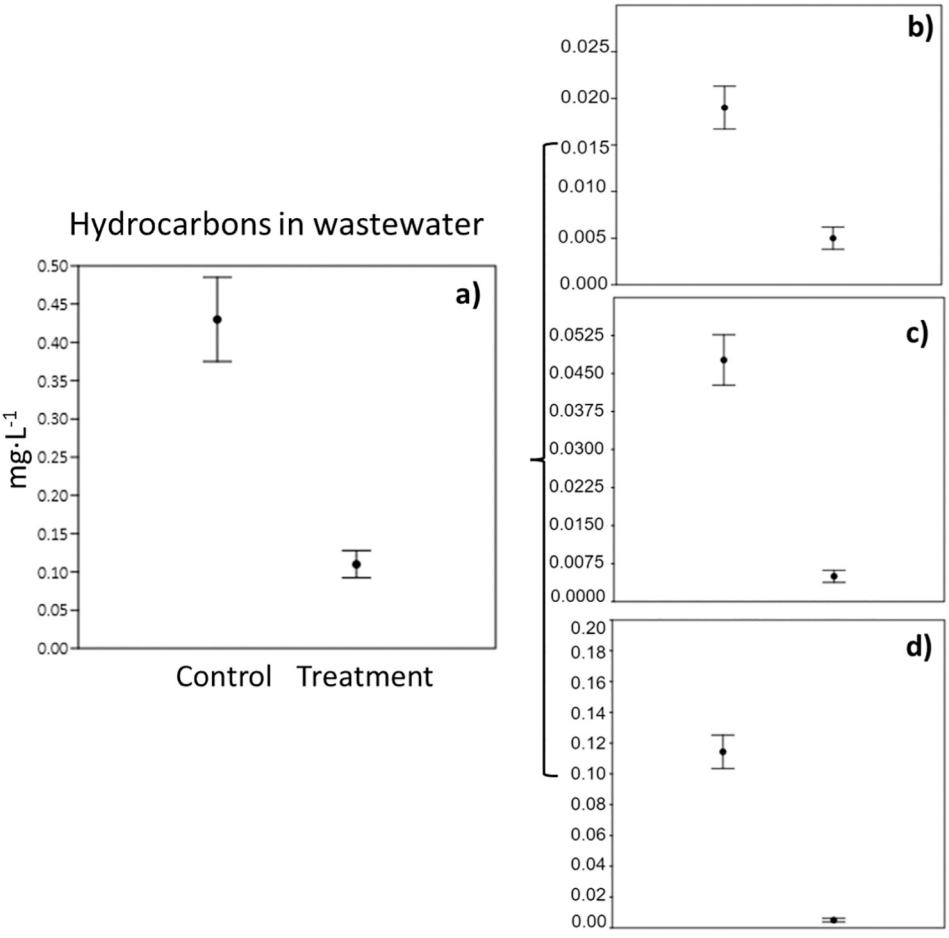

Fig. 6. Hydrocarbon reduction after *A. maxima* culture. a) Total number/concentration of hydrocarbons in both control and treatment wastewater; b) 20 to 24 carbon-chain hydrocarbons decrease; c) 24 to 28 carbon-chain hydrocarbons decrease; d) 28 to 40 carbon-chain hydrocarbons ($p = 0.04853$).

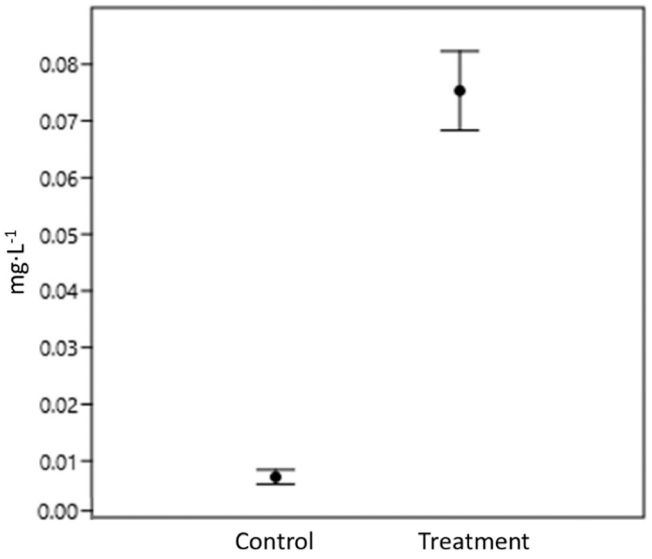

Fig. 7. 16 to 20 carbon-chain hydrocarbons increased in wastewater after *A. maxima* culture ($p = 0.04356$).

A. maxima belongs to Cyanobacteria phylum, modern omics approaches can provide higher efficiency in bioremediation reactions, as suggested by Correddu et al. [71]. To the best of our knowledge no work has been published on the role of microalgal extracellular mechanisms in the breakdown of long-chain hydrocarbons. On this basis, we encourage further works to focus on the role of microalgae bioremediation for this type of oily residues.

In conclusion, our results provide compelling evidence that *A. maxima* has great potential to become a novel method that could be used as an alternative to traditional wastewater treatments. Our research only focuses on biological aspects. However, techno-economic studies comparing the traditional and bioremediation systems combined to the feasibility of using microalgae systems should be conducted.

4. Conclusions

Steel hot-rolling wastewater remediation trough traditional systems is difficult, mainly due to the high concentrations of iron and hydrocarbons present in these types of residues. Nevertheless, this study has proven the successful acclimation to steel hot-rolling wastewater by the cyanobacteria *A. maxima,* along with the bioremediation of contaminants of concern.

Growth of this strain without iron addition/supplementation in nutritive media showed how *A. maxima* could utilize pollutants present in wastewater (mainly iron) as micronutrients. This result proves the possibilities of microalgae culturing as a novel way for compounds recovery from residues, approaching future industry to a circular economy system. Iron removal by the end of the study showed satisfactory results with a reduction of 97.5% of initial iron concentration. Even though this result is susceptible of being optimized, iron removal ratio obtained during this study is comparable to the ones resulted from techniques currently used by industry. Along with iron removal, experimental *A. maxima* biomass obtained got increased its iron content by more than 285%. This result indicates that iron up taking during bioremediation derives in biomass enrichment, in terms of iron content.

Similarly, the total hydrocarbons were reduced by 75% after 2 weeks of culture, particularly long-chain hydrocarbons. Contrary to traditional depuration systems, pollutants removal ratio was not hindered by the presence of hydrocarbons in wastewater, avoiding one of the main limitations in traditional steel industry wastewater remediation. In addition, the C16–C20 hydrocarbons increasement showed a correlation with long-chain hydrocarbons descent, which could be explained by the existence of extracellular mechanisms involved in hydrocarbon assimilation. Despite the fact that our study showed satisfactory results, further studies are needed to determine whether these findings could be applied at large scale systems without a reduction in performance.

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Acquisition of data: M. Blanco-Vieites, F. Delgado, M.Álvarez-Gil.

Analysis and/or interpretation of data: M. Blanco-Vieites, D. Suárez-Montes.

Category 2

Drafting the manuscript: M. Blanco-Vieites,

Revising the manuscript critically for important intellectual content: M. Blanco-Vieites, D. Suárez-Montes, A. Hernández Battez, E. Rodríguez.

Category 3

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M. Blanco-Vieites, D. Suárez-Montes, F. Delgado, M.Álvarez-Gil, A. Hernández Battez, E. Rodríguez.

Declaration of competing interest

No conflict of interest is existing.

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