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From piggery wastewater to wheat using microalgae towards zero waste

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ABSTRACT

Microalgae production is still expensive, driving the need to lower costs while strengthening the industry's environmental sustainability. Microalgae are recognized tools for efficient wastewater treatment, offering the recycling of nutrients and water for agriculture, and producing biomass rich in growth-promoting compounds to improve plant productivity and resistance to adverse conditions. The use of wastewater can reduce cultivation costs as it is a source of nutrients and water. Alternative low-cost methods can significantly decrease harvesting costs, which represents one of the most expensive steps of the whole process.

The goal of this work was to evaluate the potential of wastewater-grown microalga biomass for agriculture purposes. To reduce production costs, the microalga *Tetradesmus obliquus* was produced in pre-treated photo-Fenton (PF) piggery wastewater in combination with the use of different harvesting techniques - electro-coagulation, flocculation, and centrifugation, and different combinations. From the wastewater treatment process, two fractions (biomass and supernatant) were evaluated for germination and growth of wheat (*Triticum aestivum* L.) plants and compared to non-harvested microalga culture (MC), distilled water, and Hoagland (synthetic) solution. The concentrated resulting from PF was also tested as a biofertilizer.

The results confirm that both biomass and supernatants are useful for agricultural applications. The obtained biomass elicited a 20–105 % increase in germination index compared to the control, while supernatants were inhibiting. The opposite trend was observed at later stages of wheat growth, where the nutrient-enriched supernatants and the PF concentrate (PF-CC) increased the number of tillers (3–5) and leaves (30–42) after 83 days. Wheat plants treated with MC and PF-CC produced similar number of ears (3.4 ± 0.5 and 6.0 ± 4.1 ears per plant, respectively) than the synthetic control (5.7 ± 1.4) after 182 days. All fractions obtained from the process can be used in a zero-waste process.

1. Introduction

The growing world population has put immense pressure on agriculture and livestock production. To fulfil the rising nutritional demands, farmers have relied on the use of chemical fertilizers to enhance agricultural yields [1]. Poor management practices dependent on the excessive application of fertilizers have led to serious environmental issues like pollution of soil, air and water, soil salinity, increasing pest resistance, loss of soil fertility, threatening food security, biodiversity, and human health [1]. Animal products are an important protein source worldwide since they are complete in all essential amino acids compared to most plant-derived proteins [2,3]. However, their water footprint is substantially larger, and their production generates enormous volumes of highly pollutant wastewater (WW) that, when discharged without the proper treatment, can contaminate water bodies and endanger the surrounding ecosystems [4].

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Sustainable approaches in the agricultural and livestock sector are of maximal importance to improve global food security. Microalgae can offer solutions to both wastewater treatment and agricultural fertilizing issues. They can recover the nutrients from WW, making it a low-cost and readily available nutrient source [5]. However, agro-industrial effluents tend to be highly rich in organic matter, ammonia, and suspendend solids, which inhibits the growth of microalgae in complete medium. A pre-treatment step using photo-Fenton (PF) to treat piggery wastewater (PWW) before microalgae-based treatment that allowed the direct use of PWW for microalgae production, without any dilution with freshwater [6]. Photo-Fenton is an advanced oxidation method used for the degradation of organic pollutants in water. It involves the generation of highly reactive hydroxyl radicals through the interaction of hydrogen peroxide (H_2O_2) and iron (Fe^{2+}) ions in the presence of UV–vis radiation. During the process, iron is renerated cyclically between Fe^{3+} and Fe^{2+}. The hydroxyl radicals are extremely powerful oxidants ($E^0_{ox} = 2.8$ V) capable of breaking down a wide range of organic compounds into smaller, less harmful molecules. The efficiency of PF is highly dependent on the H_2O_2/Fe ratio to assure a complete reaction and the full consumption of H_2O_2. Furthermore, the reaction is more effective at acidic pH, between 2.8 and 3 [7,8]. The PF process offers several advantages, including its effectiveness in treating recalcitrant pollutants, short reaction time, and mild reaction conditions (room temperature and pressure) [9]. The introduction of UV light increases the reaction rate but requires extra energy costs. Nonetheless, these can be minimized using open ponds under solar irradiation [10].

Microalgae have biostimulant properties due to the action of growth-stimulating compounds (e.g., phytohormones, polysaccharides, amino acids, polyamines, and fatty acids), while other secondary metabolites with bioactive properties (e.g., antimicrobial, antiviral, and antioxidant compounds) can improve plant tolerance to stress or improve soil aggregation and stability [11,12].

Microalgae pose an opportunity for the agroindustry, but high production costs limit the biomass application to high-value products, while commodity markets, such as animal feed or biofertilizers remain economically unsustainable [13]. Harvesting is one of the most critical steps of the microalgae industry, accounting for up to 30 % of the total production costs [14]. Thus, more cost-efficient harvesting methods that are fast in industrial operations and do not compromise biomass quality are required [15].

A multi-step harvesting approach using flocculation and electrocoagulation has been shown as a promising tool for concentrating biomass and saving centrifugation costs [16]. Flocculation using chemical compounds and natural polymers, such as chitosan, could be a promising approach to concentrate microalgal biomass, with high biomass recovery efficiencies for strains with agricultural potential. Electrocoagulation (EC) with alternative electrodes (Fe, Mg, and Zn) could efficiently recover biomass with minimal energy and time requirements [17]. Both electrocoagulation and flocculation rely on the use of positively charged compounds that induce cell charge neutralization, promoting floc formation and biomass sedimentation [16]. Introducing chemical compounds, polymers, or metals to harvest microalgal biomass can result in contaminated biomass for high-value applications [18] but cheaper technologies such as flocculation or electrocoagulation have the potential to enrich biomass with beneficial elements for agricultural or animal feed markets. For example, chitosan has antimicrobial activity for pest control [19] and *Nannochloropsis* biomass after electrocoagulation was enriched with essential nutrients, whose bioavailability was beneficial in cherry tomato plants [20].

This work proposed the recovery of the nutrients from piggery wastewater (PWW) using microalga *Tetradesmus obliquus* and the complete use of the obtained fractions (biomass, supernatant and PF precipitate). The study aimed to reduce microalgal production costs by using a low-cost medium like WW and using two alternative harvesting methods to select the best combination for the germination and plant growth of wheat (*Triticum aestivum* L.), since this crop is used in swine feed formulations, supporting a circular bioeconomy.

Table 1
Piggery wastewater composition: pH, conductivity (k), total Kjeldahl nitrogen (TKN), ammonia (NH_4^+), phosphate (PO_4^{3-}), and chemical oxygen demand (COD).

pH	k (mS/cm)	TKN (mg N/L)	NH_4^+ (mg/L)	PO_4^{3-} (mg/L)	COD (mg O_2/L)
7.72	22.2 ± 0.3	1855 ± 0.3	1257 ± 14	198 ± 46	8305 ± 169

Table 2
Composition of piggery wastewater pre-treated by photo-Fenton: pH, conductivity (k), total Kjeldahl nitrogen (TKN), ammonia (NH_4^+), phosphate (PO_4^{3-}), and chemical oxygen demand (COD).

pH	k (mS/cm)	TKN (mg N/L)	NH_4^+ (mg/L)	PO_4^{3-} (mg/L)	COD (mg O_2/L)
7.72	19.1 ± 0.0	1302 ± 70	950 ± 28	0.15 ± 0.02	762 ± 41

2. Materials and methods

2.1. Effluent feedstock

The piggery wastewater was collected from a stabilization pond in the pig farm Herdade do Pessegueiro—Valorgado, at Glória do Ribatejo, Portugal (39°00′09.0″ N, 8°38′45.5″ W) on October 2021 and was stored at 4 °C until handling. PWW is the liquid fraction of the pig slurry, which is a mixture of pig excreta from animals at all stages of development, including sows and piglets, and the water used to clean the housing facilities, after a primary in situ solid-liquid separation. The composition of PWW is given in Table 1.

2.2. Photo-Fenton pre-treatment

The photo-Fenton experiments were carried out in batch mode in a 5 L reactor with a working volume of 4 L, placed in a setup designed and built in the Department of Renewable Energy at LNEG. The setup and optimized operation conditions were previously described [6]. The pH of PWW was initially adjusted to 3 using cc. H_2SO_4, and iron sulphate ($FeSO_4{\cdot}7H_2O$) was added (1.0 g Fe^{2+}/L). H_2O_2 (35 wt%) was added to the reactor at 10.5 g/L. PWW was exposed to the UV light for 120 min at room temperature and mixed at 400 rpm. At the end, the pH was adjusted to 7.0 using 6 M NaOH, and the pre-treated effluent was left to settle overnight. To remove suspended iron precipitates, the supernatant was filtered through a paper filter (11 μm, Whatman). The supernatant was then used for microalga cultivation and the concentrate (PF-CC) was stored at −18 °C for wheat trials.

2.3. Culture conditions

The microalga *Tetradesmus obliquus* (ACOI 204/07, ACOI Culture Collection, Coimbra University, Portugal) was cultivated in 5 L bubble-column photobioreactors (PBRs) using piggery wastewater pre-treated with photo-Fenton (PF-PWW) as the culture medium (Table 2). Cultures were kept growing during the experimental trial (6 months) and were harvested every 15 days for the plant experimental trials. Fresh culture medium was added after harvesting to maintain cultures at exponential growth. Cultures were maintained at room temperature (21 °C) with an air flux of 0.6 vvm under continuous fluorescent light (60 μE/m^2s). The cultures were inoculated with an initial concentration of 0.3 g/L (ash-free dry weight - AFDW).

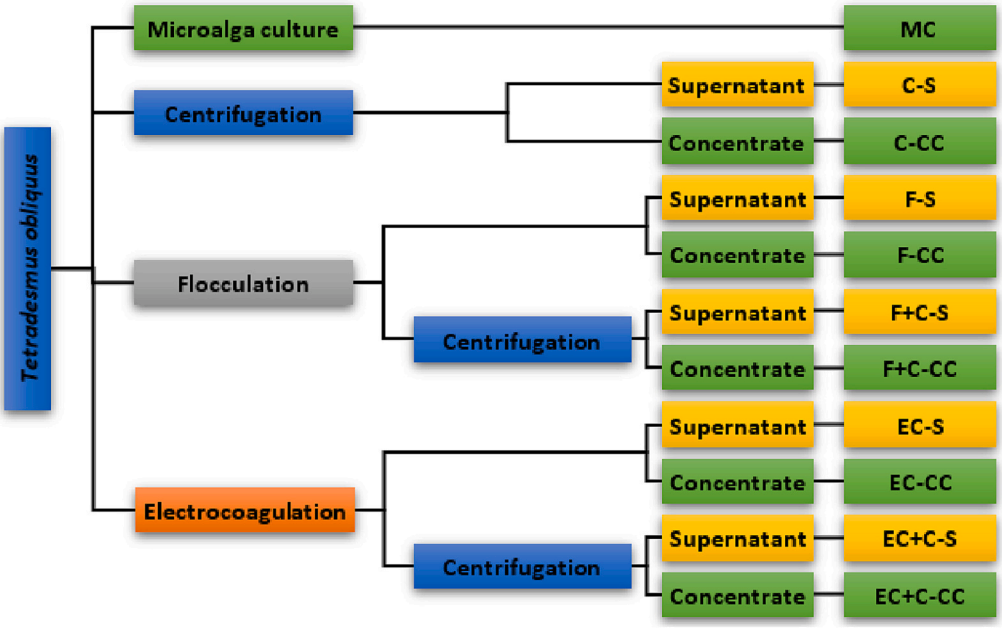

Fig. 1. Schematic diagram of the downstream process of the microalga *Tetradesmus obliquus*: direct microalga culture (MC), concentrated biomass (CC) and respective supernatant (S) harvested by centrifugation (C), flocculation (F), electrocoagulation (EC), flocculation + centrifugation (F + C), and electrocoagulation + centrifugation (EC + C).

2.4. Harvesting

T. obliquus was harvested using flocculation (F), electrocoagulation (EC), centrifugation (C), flocculation followed by centrifugation (F + C), and electrocoagulation followed by centrifugation (EC + C). The harvested biomass and respective supernatants were collected and stored at −18 °C and 4 °C, respectively, for further experiments and analysis.

2.4.1. Electrocoagulation (EC)

The EC setup was composed of an external direct current power source (HY3005D, Mastech, Taiwan) connected to a pair of flat electrode plates. The cathode was made of titanium coated with platinum while the anode was combined with zinc, magnesium, and iron plates. Anode and cathode electrodes were fixed parallel to each other at 1 cm apart and immersed in 500 mL of microalga culture using 600 mL glass flasks. EC trials were done in batch mode with cultures stirred at 150 rpm and using previously optimized conditions (initial pH of 8, current density of 55 mA/cm^2 for 8 min) [17]. After EC treatment the cultures were left to settle for 30 min and the biomass concentrate and supernatant were collected.

2.4.2. Flocculation (F)

Flocculation experiments were done in 2 L glass flasks using a Jar Test (AMF/60, Vittadini, Italy). The pH of microalga cultures was adjusted to 5.5 using 1 M HCl or 1 M NaOH [21]. The flocculant chitosan was added to the microalga culture at 25 mg/L (based on preliminary experiments) and stirred for 1 min at 150 rpm to allow flocculant dispersion. Stirring was then slowed down to 15 rpm for 5 min to allow the formation of microalgal flocs. Finally, the stirring was stopped, and the flocs were left to settle for 30 min.

2.4.3. Centrifugation (C)

Centrifugation was operated at 4000 ×*g* for 5 min at 4 °C (6-16KS, Sigma, Japan). The microalga culture was directly centrifuged, as well as the biomass concentrates from flocculation (F + C) and electrocoagulation (EC + C).

2.5. Biomass processing and biochemical analysis

Biomass samples of *T. obliquus* were freeze-dried (Heto Power Dry LL3000, Thermo Scientific, USA) for biochemical analyses of proteins, sugars, and lipids. Protein content was estimated through the Lowry method in samples previously treated with NaOH 0.1 M [22]. Lipid content was obtained gravimetrically after Soxhlet extraction with n-hexane for 6 h. Carbohydrates content was determined by subtracting the other fractions. Moisture and ash were determined gravimetrically by drying in an oven at 105 °C until constant mass and by incineration at 550 °C in a muffle furnace.

2.6. Agricultural trials

2.6.1. Preparation of irrigation solutions

The biomass harvested by individual or combined techniques was diluted with distilled water to prepare microalgae suspensions at a concentration of 0.2 g AFDW/L. The supernatants from each harvesting process were applied directly (no dilution). A total number of 11 treatments were tested (Fig. 1).

2.6.2. Germination

The germination tests were performed in Petri dishes using wheat (*Triticum aestivum* L.) seeds. Each Petri dish was lined with filter paper (Whatman No. 1) and 15 seeds were placed. A volume of 5 mL of each irrigation solution was added to the seeds. There were three replicates per treatment (45 seeds in total). Distilled water was used as the negative control. Seeds were incubated at 20 °C in the dark for 5 days in a growing chamber (FITOCLIMA S600 PL, Aralab, Portugal). The number of germinated seeds in each Petri dish was counted, and the root and shoot lengths were measured. The germination index (GI) was determined according to Zucconi et al. using Eq. (1) [23]:

$$GI\ (\%) = \frac{G \times L}{G_w \times L_w} \times 100 \quad (1)$$

where G and Gw correspond to the total number of germinated seeds and L and Lw to the root length for the tested conditions and the negative control (distilled water), respectively.

Table 3
Description of the commercial substrate (Terra de Montemor) and perlite (SIRO) used for the experiments.

Commercial substrate	Perlite
Organic Matter ……..…… 50 %	Pore volume ……..…….…… 95 %
pH ……..…… 5.3–6.4	Aeration capacity …… 65 %
Nitrogen …….. 1.1–1.2 %	Granulometry ……..…… 3–6 mm
Phosphorus …..……..…….... 0.7–1.1 %	Conductivity …….. 3–5 μS/cm
Potassium ..…….…..... 0.7–1.0 %	pH …… 6–7
Calcium ……..…..... 0.5–0.7 %	
Magnesium ……..…..... 0.1–0.2 %	
Moisture ……..…….....…. 50 %	

Table 4
Chemical composition of the mixture of soil and perlite (20:1) used for the experiments: pH, conductivity (k), organic matter (OM), potassium oxide (K_2O), phosphorus (P), carbon (C), ammonia (N-NH_4^+), and nitrate (N-NO_3), micronutrients (Cu, Fe, Mn, and Zn) and base saturation (Ca^{2+}, Mg^{2+}, Na^+, K^+).

pH	*k* (μS/cm)	OM (%C)	Macronutrients (mg/kg)			
			N-NH_4^+	N-NO_3	P	K_2O
5.63	834.5	12.23	77.55	<2.50	129.1	635.5

Microelements (mg/kg)			
Fe	Cu	Zn	Mn
131	2.09	5.58	219

Base saturation (%)				
GSB	Ca^{2+}	K^+	Mg^{2+}	Na^+
99.1	58.48	9.96	21.92	8.77

2.6.3. Wheat growth

The commercial substrate (Terra de Montemor) used in this trial was composed vegetation soil, bio humus, dry peat, and vitamins. Perlite (SIRO) was mixed with the substrate at a ratio of 1:20 (Table 3). The chemical composition of this mixture was determined (Table 4). The soil was slightly acidic, had no salinity effects, and was rich in macro and micronutrients.

2.6.4. Seedling preparation

After germination, 5 seedlings were randomly selected from each treatment, and transplanted to a mixture of perlite and soil (1:20). Seedlings were incubated in a growth chamber (FITOCLIMA S600 PL, Aralab, Portugal) for 15 days at 20 °C, under a light/dark photoperiod of 16 h/8 h. The tested irrigation solutions were the same as in the germination trials (Fig. 1). A positive control consisting of Hoagland solution at 1/4 strength (to avoid nutrient toxicity) was used. The plants were watered weekly with 5 mL of the respective solutions and whenever necessary with distilled water to maintain soil moisture.

2.6.5. Greenhouse trial

After 15 days, the seedlings were transplanted into pots (0.5 L) containing a mixture of perlite and soil (1:20). The growth trial was conducted in a greenhouse at Instituto Superior de Agronomia (38°42′28.697″ N, 9°11′6.187″ W) for 182 days. Throughout the trial, plants were transferred to successive bigger pots (1.6 and then 3 L) to avoid growth inhibition or root asphyxia. Plants were watered once a week with irrigation solutions (microalga suspensions and supernatants) and distilled water alternately. Distilled water and full Hoagland solution were used as negative and positive controls, respectively. The initial irrigation volume was 50 mL per pot (0.5 L), which was increased proportionally to the amount of soil used, as the size of the pots grew (1.6 L – 100 mL; 3 L – 200 mL) [24]. Plants were measured for root and shoot length, number of tillers, and leaves, 83 days after sowing (DAS). At this point, only plants showing significant growth were maintained (MC, PF-CC, and supernatant treatments), while plants treated with harvested microalga suspensions were ended. Although with reduced growth, the plants treated with distilled water (negative control) were maintained for comparison. At the end of the trial (182 DAS), the length of the shoots, fresh weight, and number of tillers and ears were measured for all plants. Soil samples were collected for mineral analysis before and at the end of the trial.

2.6.6. Analysis of soil, concentrate, irrigation solutions, and plants

Samples of soil, photo-Fenton concentrate (PF-CC) and plants (shoot and ears) were oven dried for 24 h at 65 °C, while the irrigation solutions were filtered prior to the analysis.

The pH and conductivity (*k*) were measured with a potentiometer. The organic matter content was determined through the total organic carbon method by dry combustion in the Ströhlein apparatus [25]. Micronutrients were quantified by atomic absorption spectrophotometry, after extraction with 0.5 M acetic acid solution [26]. For the quantification of the saturation bases, the Schollenberger method was applied, using ammonium acetate solution (NH_4OAc) 1 M buffered at pH 7 for the extraction, and NH_4^+ as the "index cation". Quantification was subsequently performed on an atomic absorption spectrophotometer (Skalar, Netherlands). The Egnér-Riehm method was used for the determination of extractable K and P. The quantification of phosphorus was performed through molecular absorption spectrophotometry in a segmented flow autoanalyser (Skalar, Netherlands), while potassium quantification was by flame photometry [27]. Mineral nitrogen was determined through molecular absorption spectrophotometry, in a segmented flow autoanalyser (Skalar, Netherlands) [28].

Soil permeability problems due to irrigation water were evaluated by calculating the Sodium Adsorption Ratio (SAR) according to Eq. (2) [29].

$$SAR = \frac{[Na^+]}{\sqrt{\frac{[Ca^{2+}]+[Mg^{2+}]}{2}}} \quad (2)$$

2.7. Statistical analysis

One-way ANOVA was used to evaluate the effects of irrigation solutions on the germination index and on the growth parameters of wheat plants. The *p*-values resulting from the sum of square analyses were used to describe the impact of the factors, while Tukey's post hoc test was used to detect differences among the tested conditions. For all tests, a significance level (α) of 0.05 was considered and outliers were initially disregarded using Minitab® 19.1.

3. Results

3.1. Biomass production

T. obliquus was able to grow in undiluted PWW pre-treated with photo-Fenton even at higher ammonia levels (950 ± 28), which was not possible in raw PWW (1257 ± 14). In this medium, the pH of the culture tended to decrease below the pk_a of NH_4^+/NH_3 (9.25), meaning that most is in the form of NH_4^+, which is less toxic to microalgae. This way, the microalga could achieve an average productivity of 66.4 ± 17.8 mg/L/d and removal efficiencies of 37.3 ± 1.7 % of NH_4^+, 100 ± 0.0 % of PO_4^{3-}

Table 5
Productivity (P_X) of *Tetradesmus obliquus* in pre-treated piggery wastewater and removal efficiencies of ammonium (NH_4^+), phosphate (PO_4^{3-}), and chemical oxygen demand (COD).

P_X (mg/L/d)	Removal (%)		
	NH_4^+	PO_4^{3-}	COD
66.4 ± 17.8	37.3 ± 1.7	100 ± 0.0	48.6 ± 1.7

and 48.6 ± 1.7 % of COD (Table 5).

The biomass harvested by centrifugation was rich in protein (>50 %, Fig. 2), as expected due to the high availability of N, especially in the form of ammonia [30]. A higher protein content could also result in higher content of amino acids known to have a biostimulant effect [31,32]. Moreover, the biostimulant effect could also come from carbohydrates, more specifically polysaccharides [33,34]. Flocculated biomass had a slightly higher composition of carbohydrates (28.4 % vs. 20.5 % when applying centrifugation), which could be accounting for the chitosan present in the biomass (Fig. 2). The low lipid contents of *T. obliquus* (<3 %, Fig. 2) can be explained by their active growth under non-limiting N concentration, which is mobilized for protein synthesis [30].

Electrocoagulation had a more significant effect on the biomass composition since the ash content was very high (about 60 %, Fig. 2). This was due to the metals released from the electrodes during EC [17]. Subtracting the ash, the protein, carbohydrate, and lipid contents were similar between F and EC biomass samples (58.3, 31.7, and 3.3 %, respectively, for F, 54.3, 32.4, and 3.5 %, respectively, for EC). From the agricultural point of view, the enriched biomass by metals could be beneficial for fulfilling the plant's nutrient requirements. However, the EC parameters should be further optimized to meet the proper metal contents within the biomass for target applications.

3.2. Composition of irrigation suspensions and photo-Fenton concentrate

The pH of all irrigation solutions was initially adjusted to 7, but the determined values were all slightly acidic (Table 6). MC, F-CC, EC-CC and EC + C-CC had conductivity values below 3000 μS/cm, presenting only mild to moderate restrictions. The supernatants had >10 times higher *k* values than the biomass suspensions, meaning they should have severe restrictions to their use. Since the *k* values for all irrigation suspensions were >5000 μS/cm, regardless of the SAR value, they presented no application restrictions. The SAR value was also used to assess the toxicity of Na. For all conditions, considering that surface irrigation was carried out, its use had severe application restrictions. Although there was a high amount of this ion in the irrigation suspensions, they also contained high levels of Ca and Mg, which provided an ion balance that minimizes infiltration problems and runoff risks.

The irrigation suspensions also contained relevant amounts of organic matter (OM), which can contribute to build soil structure and permeability (Table 6). There was a contribution to increasing infiltration and the impediment of surface runoff that can contaminate surrounding water bodies [29].

Macro and micronutrient analyses of irrigation solutions showed much higher concentrations in supernatants than in microalgae suspensions (Table 6). The most significant differences were in N contents since N-NH_4^+ concentrations in supernatants (57.6–571.1 mg/L) were much higher compared to harvested algal suspensions (2.1–10.1 mg/L).

Photo-Fenton concentrate (PF-CC) is acidic (4.96) and has a high *k* and OM (Table 7). It is rich in N in N-NH_4^+ form, P due to the precipitation with iron during the neutralization step of the photo-Fenton process, and very rich in K. As expected, it is enriched in Fe, which is added to the pre-treatment process.

An expected production of 8 t/ha was assumed (maximum expected production value according to Veloso et al. [29]) to assess the recommended NPK fertilization based on the initial soil composition (Table 8). For the assumed production value, P and K fertilization was not necessary since the initial soil was rich in both nutrients (Table 4). This meant that the irrigation solutions provided these nutrients in excess. In terms of N, it was necessary to supply 77.60 kg/ha to the soil. The quantity of N provided by each irrigation solution (MC, C-S, F-S, EC-S, F + C-S, and EC + C-S) and PF-CC during the whole trial was determined (Table 8).

3.3. Wheat plant trials

3.3.1. Germination index

The harvesting method significantly influenced the GI, with relevant increases compared to the control ($p < 0.05$). The microalga suspensions (directly or after each harvesting approach) showed a positive effect on the GI (Fig. 3). The highest GI were obtained on seeds watered with C

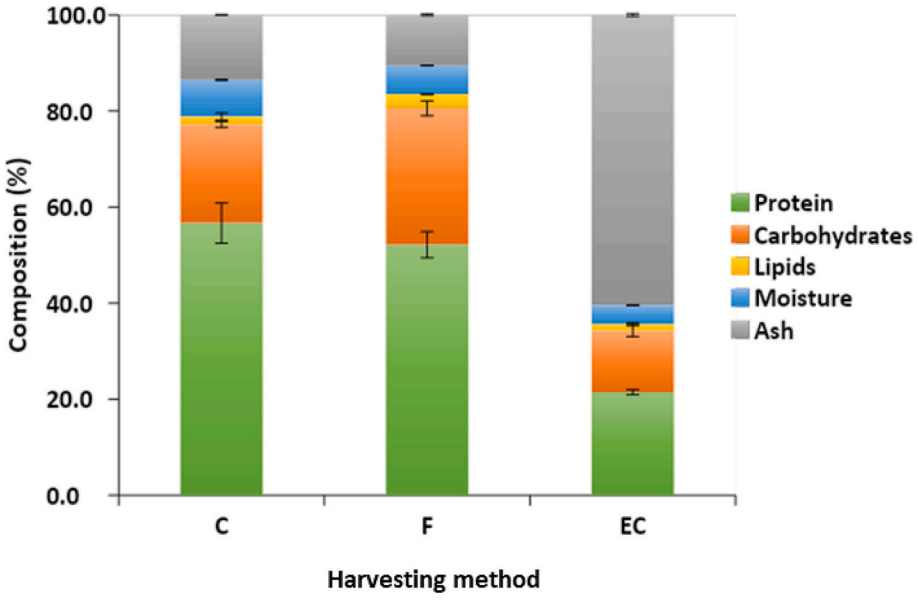

Fig. 2. Chemical composition of *Tetradesmus obliquus* biomass grown in piggery wastewater pre-treated by photo-Fenton after harvesting by centrifugation (C), flocculation (F), electrocoagulation (EC). Results are shown as mean ± standard deviation ($n = 2$).

Table 6
Composition of the irrigation solutions (biomass and supernatants): pH, conductivity (k), organic matter (OM), primary macronutrients ($N\text{-}NH_4^+$, $N\text{-}NO_3$, K), secondary macronutrients (Ca, Mg), micronutrients (Fe, Cu, Zn, and Mn), and beneficial elements (Na).

Parameter	Biomass (0.2 g/L)						Supernatant				
	MC	C	F	EC	F + C	EC + C	C	F	EC	F + C	EC + C
pH	6.15	5.95	6.27	5.41	6.42	4.58	5.61	6.66	4.85	6.15	5.73
k (μs/cm)	2835	4804	1028	1887	3314	1515	21,460	29,810	23,330	19,460	23,990
Primary macronutrients (mg/L)											
$N\text{-}NH_4$	57.62	10.05	4.13	2.1	7.72	3.72	551.66	557.64	474.44	571.06	466.78
$N\text{-}NO_3$	4.53	0.84	0.15	0.30	0.37	0.18	3.73	2.28	32.6	5.13	63.1
K	227.0	36.70	6.325	10.08	14.01	6.440	446.0	456.0	426.0	476.0	466.0
Secondary macronutrients (mg/L)											
Ca	14.0	5.22	2.22	5.80	5.88	2.52	>7	>7	>7	>7	>7
Mg	5.09	1.66	0.585	1.42	1.81	0.500	>7	>7	>7	>7	>7
Micronutrients (mg/L)											
Fe	0.0001	0.0251	0.0628	0.0463	0.0607	0.0564	0.2842	1.2156	0.0556	0.4496	0.071
Cu	0.0186	0.0001	0.0001	0.0001	0.0018	0.0003	0.2509	0.0470	0.2440	0.0156	0.2748
Zn	0.0782	2.4770	0.0444	0.0616	3.3157	0.9000	0.2118	3.3157	0.3898	2.8515	0.2943
Mn	0.2964	0.0881	0.0001	0.0009	0.4175	0.0001	2.5355	1.9228	2.5391	2.7761	2.4802
Beneficial elements (mg/L)											
Na	195.1	41.46	12.26	24.93	16.81	10.95	359.3	272.9	307.7	234.0	221.1

MC: Microalga culture; C: Centrifugation; F: Flocculation; EC: Electrocoagulation; F + C: Flocculation + Centrifugation; EC + C: Electrocoagulation + Centrifugation.

Table 7
Composition of concentrate resulting from the photo-Fenton process of piggery wastewater: pH, conductivity (k), organic matter (OM), primary macronutrients ($N\text{-}NH_4^+$, $N\text{-}NO_3$, K), secondary macronutrients (Ca, Mg), micronutrients (Fe, Cu, Zn, and Mn), and beneficial elements (Na).

pH	*k* (μS/cm)	OM (%C)	Macronutrients (mg/kg)			
			$N\text{-}NH_4^+$	$N\text{-}NO_3$	P	K_2O
4.96	45,980	21.65	3544	<2.50	188.0	25,475

Microelements (mg/kg)			
Fe	Cu	Zn	Mn
343	45.2	33.2	249

Base saturation (cmol(+)/kg)			
Ca^{2+}	K^+	Mg^{2+}	Na^+
5.03	19.7	3.02	19.6

and F biomass, 205 and 198 %, respectively. EC, F + C, and EC + C biomass samples had no significant effects on GI as compared to MC or the water control ($p > 0.05$). EC biomass accumulated electrode metals because of water electrolysis and coagulation, thus possibly affecting seed germination. However, no significant differences in GI for EC when compared to MC, C, and F ($p > 0.05$) suggested this was not the case here. Centrifuged biomass after flocculation (F + C) suggested no biostimulant effects on seed germination. The supernatants of each harvesting process showed no biostimulant effect, with the GI values being lower than the GI of the water control (Fig. 3).

3.3.2. *Wheat growth at tillering and stem elongation stage*

All plants watered with the supernatants showed a positive and similar effect on plant growth 83 DAS (Fig. 4). Plants watered with EC-S were able to develop a similar number of tillers than the positive control ($p > 0.05$) (Fig. 4a). A longer main tiller (approx. 40 cm) was observed when compared to the positive control (Hoagland solution, approx. 30 cm) ($p < 0.05$) (Fig. 4b). No significant differences were detected between the supernatants of the different harvesting techniques, with all treatments providing positive results for potential applications in agriculture. Plants watered with the harvested microalga suspensions, performed similarly to the negative control, with decreased plant growth ($p > 0.05$).

3.3.3. *Wheat growth at maturation stage*

3.3.3.1. Plant growth. No significant differences were detected in the fresh weight between all conditions and the positive control ($p > 0.05$), except for the negative control (Fig. 5a). The length of the shoots treated with the supernatants (C-S, F-S, EC-S, F + C-S and EC + C-S) were statistically different from the condition MC ($p < 0.05$). No differences in shoot length were observed between MC, PF-CC, and positive control ($p > 0.05$) (Fig. 5b).

No significant differences in number of ears were detected between the positive control and the PF-CC, MC, F-S, EC-S and EC + C-S conditions ($p > 0.05$). PF-CC was the condition that provided a more similar efficiency to that of the synthetic solution, since the ears are the main goal of wheat crop cultivation (Fig. 6).

3.3.3.2. Plant composition. The values obtained for the negative control (distilled water) were within the reference range for almost all nutrients. However, K was lower and Ca, Fe and Mn were higher (Table 9). The concentration of micronutrients, like Fe and Mn, easily pass from deficiency to toxicity. Thus, high values of Fe and Mn suggest toxicity for the

Table 8
Nitrogen supplied to soil (kg/ha) from the supernatants obtained after the different harvesting methods during the whole growth trial (182 days). Excess nitrogen is presented in the parenthesis.

Soil (kg/ha)	Recommended (kg/ha)[a]	Irrigation suspensions (kg/ha)						PF-CC
		MC	C-S	F-S	EC-S	F + C-S	EC + C-S	
182.4	260	155.9 (78.29)	1393 (1316)	1404 (1327)	1272 (1194)	1445 (1368)	1329 (1251)	83.92 (6.32)

[a] Based on an expected wheat production of 8 t/ha, according to Veloso et al. [29].

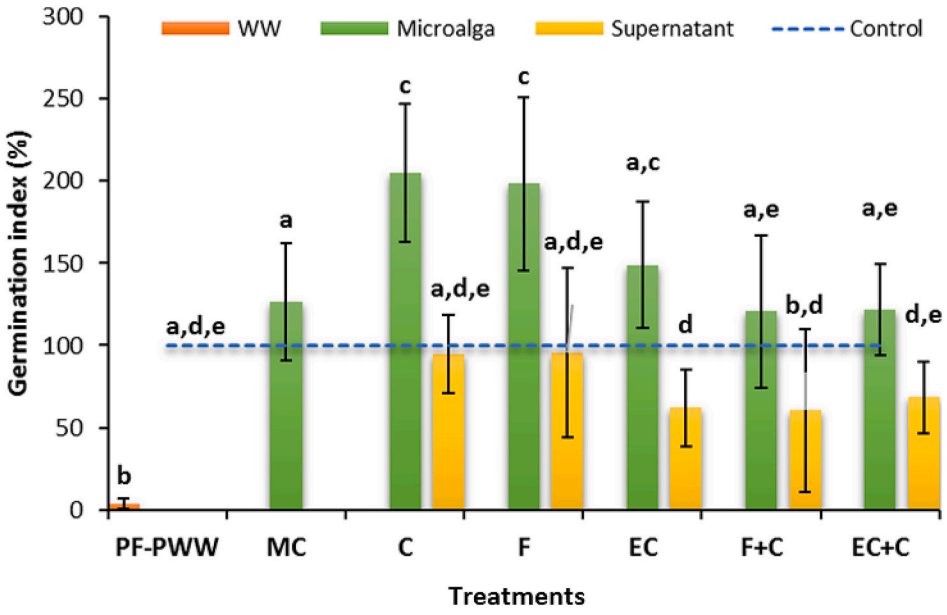

Fig. 3. Germination index (%) of wheat (*Triticum aestivum* L.) seeds treated with Tetradesmus obliquus from: direct culture (MC) (green) and supernatants (yellow) after harvesting by centrifugation (C), flocculation (F), electrocoagulation (EC), flocculation followed by centrifugation (F + C) and electrocoagulation followed by centrifugation (EC + C). Water as a control (blue) corresponds to the germination index of 100 %. PF-PWW is the photo-Fenton pretreated piggery wastewater prior to microalga-based treatment (orange). Different letters indicate significant differences among treatments (Tukey's test, $p < 0.05$), and results are shown as mean ± standard deviation ($n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

plant (Table 9). The positive control (Hoagland) has a higher N concentration and a lower P, Fe and Zn concentration. For the remaining concentrations, the values are in agreement with the reference ones [29]. The high concentration of Zn in plants (>70 mg/kg) watered with the supernatants from EC (EC-S and EC + C-S) is related to the accumulation of Zn in the supernatants due to electrode release during the harvesting process.

For PF-CC, all values were within the reference range [29], excluding N which was below (N: 17.5–30.0 g/kg). MC has the concentration of macronutrients (primary and secondary) and micronutrients within the expected range [29]. For all plants treated with supernatants, the values of N, P, Ca, and Mg were within range (N: 17.5–30.0 g/kg; P: 2–5 g/kg; Ca: 2–10 g/kg; Mg: 1.5–10 g/kg). The values of K, S, and Zn (K: 15.0–30.0 mg/kg; S: 1.5–6.5 g/kg; Zn: 20–70 mg/kg) were high. Mn levels were higher for plants treated with supernatants compared with the other conditions, but were within the expected range (16–200 mg/kg). B was high for all conditions (B: 1.5–4 mg/kg) [29], but still below toxicity levels [35]. For all conditions the values obtained (Table 9) are within the imposed maximum limits displayed in the Directive 2002/32/EC (Cd: 1 mg/kg of feed and Pb: 10 mg/kg of feed) in feed intended for animal feed [36].

It was not possible to characterize the ears, for all conditions, because there was not enough plant material to carry out the analyses (Table 10). For macronutrients, N and P, all conditions presented values within the reference interval, and were, in some conditions, slightly above [29]. For K, in plants treated with Hoagland, MC and PF-CC, the concentration of this primary macronutrient is low, and better results were found, in terms of the number of ears, for these same conditions. Similar results were found between MC, PF-CC and the positive control. For all conditions, micronutrients contents were present at concentrations similar to the reference values [29]. The Cd and Pb values were also below the maximum stipulated values (Table 10) [36].

3.3.4. Soil composition

A clear difference in the effect of microalgal suspensions and supernatants on the soil conductivity was observed after 83 days (Table 11). While the application of microalga suspensions reduced the initial k value (834.5 μS/cm) by less than half, the use of supernatants caused a significant increase to values higher than 5000 μs/cm (Table 11). These results indicate that the supernatants contained nutrients in more soluble ionic forms, which were more available for plant uptake. The use of supernatants enriched soil with potassium oxide (K_2O), and nitrogen (in both NH_4^+ and NO_3^- forms). There were no significant changes in metals among the different conditions, except for Zn, which was higher at EC and EC + C supernatant and biomass conditions. These results may be due to Zn being included as one of the anode plates used for electrocoagulation. Through the analysis of percentages of base exchanges, it was possible to confirm that the microalgal suspensions enriched the soil more in Ca^{2+} and Mg^{2+}, while the supernatants enriched the soil in K^+ and Na^+. To avoid excessive soil salinity, irrigation was alternated between the irrigation suspensions and distilled water.

All test conditions had slightly acidic pH, except for PF-CC which was neutral. Hence, all pH were adequate for plant growth. Soil salinity was measured by *k* and indicated whether there is the accumulation of salts in the soil. For the supernatants, high soil salinities were obtained, while for the controls, PF-CC and MC, soils had low salinities. The organic matter (OM) content was classified as being very high for all conditions. A high percentage of OM in the soil increases its water-holding capacity, making it available to plants. It also improves aeration and soil structure, favours nutrient availability and increases cation exchange capacity. The cation exchange capacity (CEC) is defined by the ability of the soil to absorb cations, with the most frequent values varying between 2 and 50 cmol(+)/kg [29]. All conditions were characterized by CEC values close to 100, which indicates that the soils had a high

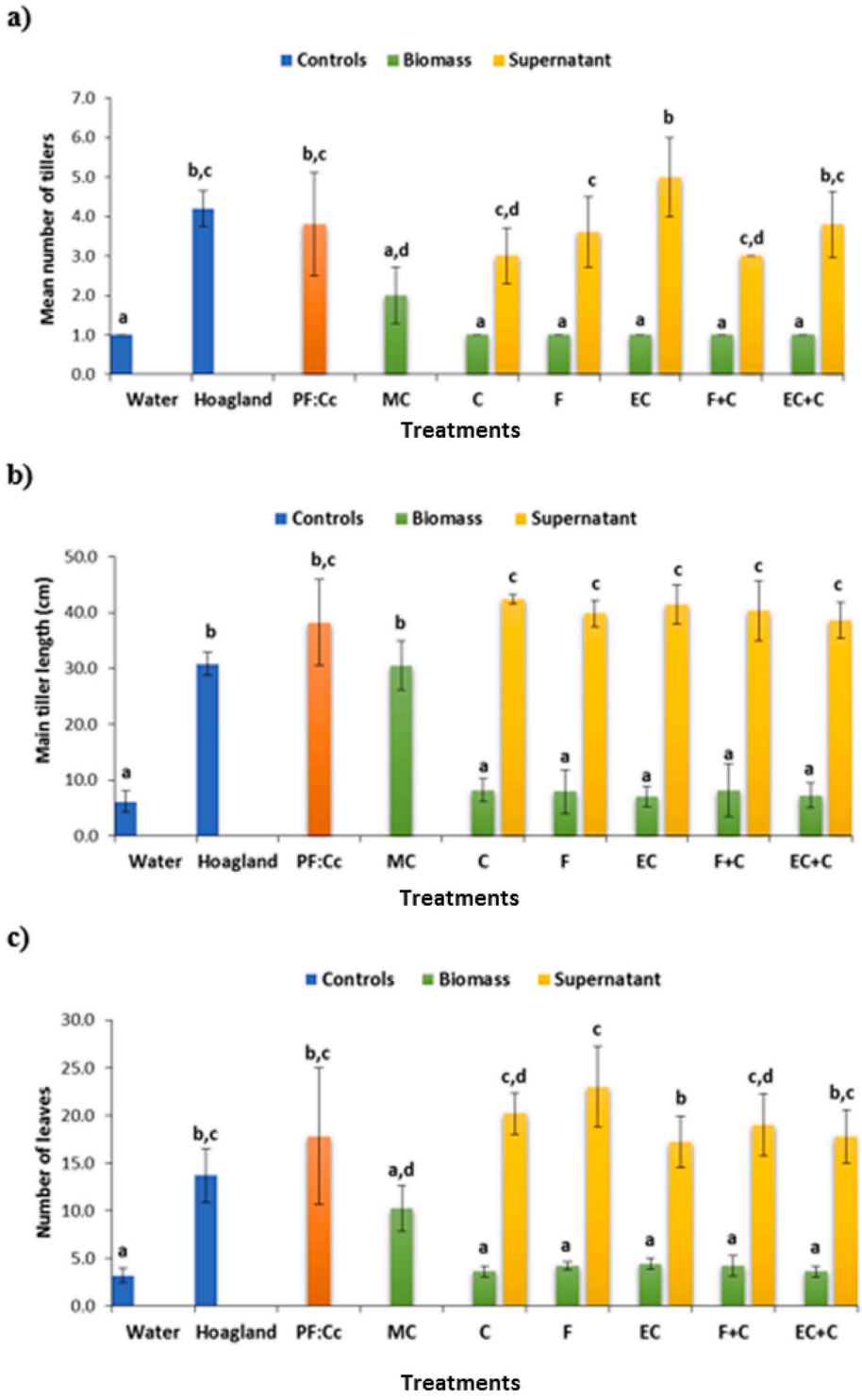

Fig. 4. Growth characteristic of *Triticum aestivum* L. 83 days after sowing: a) Mean number of tillers, b) Main tiller length, and c) Mean number of leaves treated with Tetradesmus obliquus (after 83 days): direct culture (MC), biomass (green) and supernatants (yellow) after harvesting by Centrifugation (C), Flocculation (F), Electrocoagulation (EC), Flocculation + Centrifugation (F + C), and Electrocoagulation + Centrifugation (EC + C). Distilled water and Hoagland solution were used as negative and positive controls, respectively (blue). PF-Cc is the concentrate from the photo-Fenton process (orange). Different letters indicate significant differences among treatments (Tukey's test, $p < 0.05$), and results are shown as mean ± standard deviation ($n = 5$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

capacity to retain nutrients and water.

An accumulation of N in soils was observed from 83 to 182 DAS, but was less pronounced in MC and PF-CC treated soils (Table 12). The levels of the other macronutrients were relatively stable throughout the experiment (Tables 11 and 12). There was a more pronounced enrichment of the soil in micronutrients. In PF-CC treated soil, the Fe content more than doubled, from 239 to 568 ppm between 83 and 182 DAS, which was justified by the high Fe content present in the concentrate that resulted from the photo-Fenton process (Table 7).

4. Discussion

4.1. Germination

The harvesting method significantly influenced the GI, with relevant increases compared to the control ($p < 0.05$). C and F biomass had significantly higher GI than the control ($p < 0.05$), 205 and 198 %, respectively. The positive GI results obtained with the C biomass (205 %) could be associated with the shear stress caused to cells, facilitating the release of bioactive compounds [37]. The same happened with low

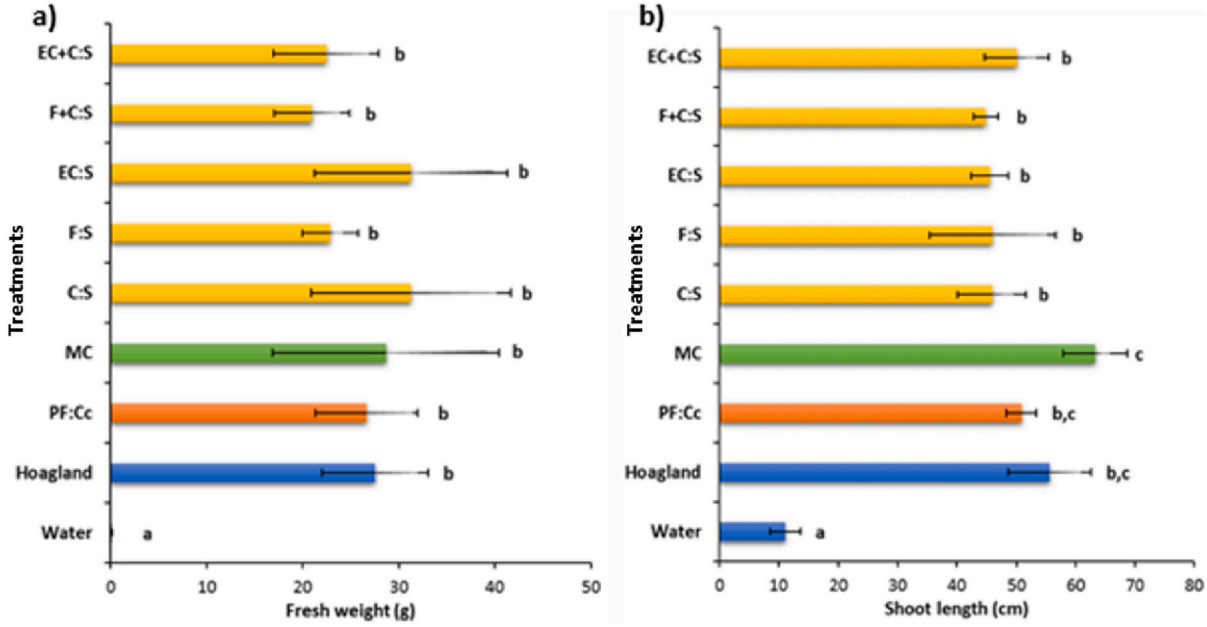

Fig. 5. Growth characteristic of *Triticum aestivum* L. evaluated at the end of the trial (182 days after sowing): a) Fresh weight and b) length of shoots treated with Tetradesmus obliquus: direct culture (MC), and supernatants (yellow) after harvesting by Centrifugation (C), Flocculation (F), Electrocoagulation (EC), Flocculation + Centrifugation (F + C), and Electrocoagulation + Centrifugation (EC + C). Distilled water and Hoagland solution were used as negative and positive controls, respectively (blue). PF-Cc is the concentrate from the photo-Fenton process (orange). Different letters indicate significant differences among treatments (Tukey's test, $p < 0.05$), and results are shown as mean ± standard deviation ($n = 5$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

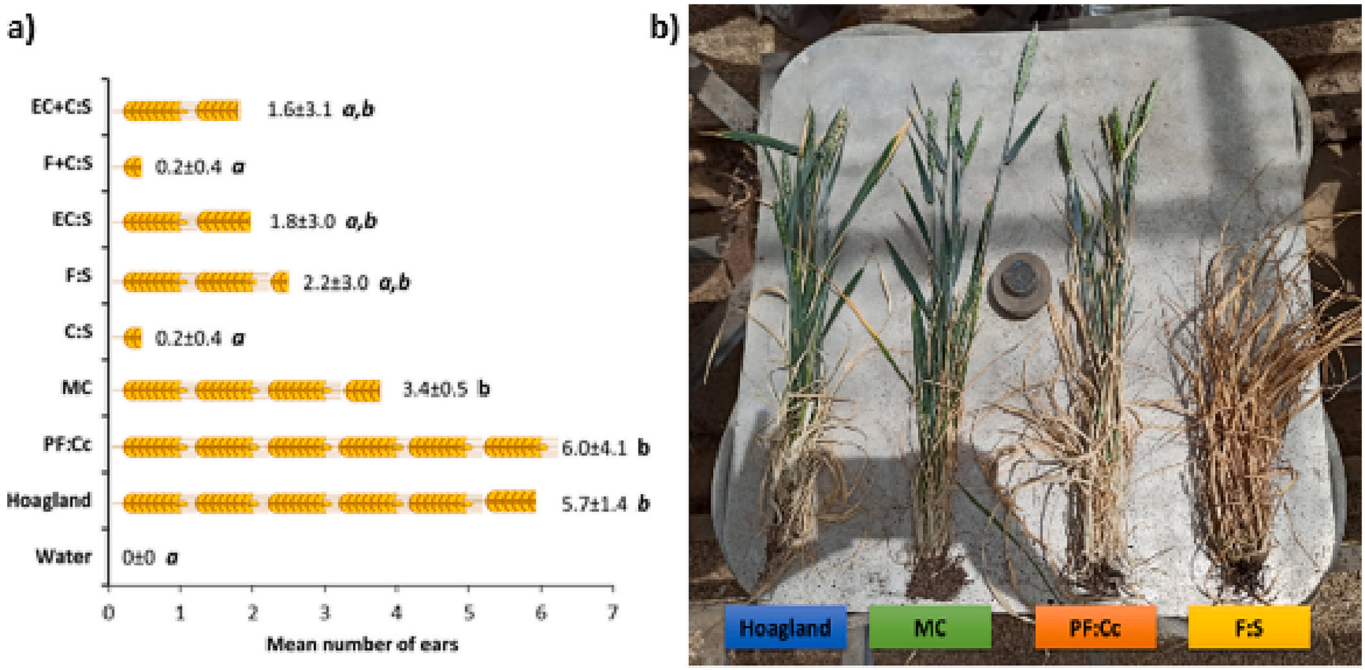

Fig. 6. Ear growth characteristics (182 days after sowing): a) Mean number of ears per wheat (*Triticum aestivum* L.) plant treated with *Tetradesmus obliquus*: direct culture (MC), and supernatants after harvesting by Centrifugation (C), Flocculation (F), Electrocoagulation (EC), Flocculation + Centrifugation (F + C), and Electrocoagulation + Centrifugation (EC + C). Distilled water and Hoagland solution were used as negative and positive controls, respectively. PF-Cc is the concentrate from the photo-Fenton process. Different letters indicate significant differences among treatments (Tukey's test, $p < 0.05$) and results are shown as mean ± standard deviation ($n = 5$). b) Photography showing the wheat plants treated with Hoagland solution (positive control), direct culture (MC), photo-Fenton concentrate (PF-CC), and supernatant from flocculation (F-S).

disruption treatments, where partial but not fully disrupted *T. obliquus* cells were more effective in boosting the GI of wheat [38]. Also an increase of 20 % in GI of wheat seeds treated with suspensions of *Chlorella sorokiniana* and spent medium after microalga cultivation, suggesting the microalga excretes bioactive molecules to the medium that can enhance germination [39]. The present GI values agree with those obtained in wheat seeds treated with *T. obliquus* grown in 5 % PWW (GI = 120 %) [40]. This can be related to microalgae synthesizing a diversity

Table 9
Chemical composition of wheat shoots treated with supernatants obtained after different harvesting methods: primary macronutrients (N-NH_4^+, N-NO_3, K), secondary macronutrients (Ca, Mg, S), micronutrients (Fe, Cu, Zn, Mn, and B), and beneficial elements (Na).

Parameter	H_2O	Hoagland	MC	C-S	F-S	EC-S	F + C-S	EC + C-S	PF-CC
Primary macronutrients (g/kg)									
N	–	15.7	21.9	33.6	29.3	30.0	32.8	30.8	9.89
P	3.66	5.41	4.51	4.24	3.97	3.87	3.92	4.03	2.16
K	8.97	23.9	26.3	48.2	48.7	42.1	45.9	44.3	25.4
Secondary macronutrients (g/kg)									
Ca	5.38	2.66	2.66	3.00	2.84	3.04	2.81	3.36	3.16
Mg	2.86	2.49	1.43	1.94	2.96	3.52	3.26	3.14	3.75
S	1.80	3.31	1.64	4.42	12.8	14.0	10.9	12.9	15.7
Micronutrients (mg/kg)									
Fe	339	138	161	197	205	216	253	202	198
Cu	19.9	15.2	16.4	18.8	18.7	17.4	18.8	17.3	14.6
Zn	63.0	71.9	34.7	62.8	88.2	108	79.2	117	30.1
Mn	145	61.9	62.8	180	202	192	186	185	66.3
B	8.32	6.68	12.2	27.2	26.3	22.6	23.3	23.6	7.26
Beneficial elements (g/kg)									
Na	3.59	0.278	1.00	15.2	16.6	13.3	17.2	18.1	0.845
Heavy metals (g/kg)									
Cr	9.54	6.50	5.95	5.71	5.86	5.56	5.99	5.02	6.36
Ni	2.92	1.46	1.73	1.65	2.00	1.43	2.55	2.15	1.35
Cd	0.161	0.299	0.292	0.217	0.259	0.287	0.263	0.234	0.0960
Pb	3.19	2.09	1.71	2.76	2.55	1.45	2.23	2.30	2.52

MC: Microalga culture; C: Centrifugation; F: Flocculation; EC: Electrocoagulation; F + C: Flocculation + Centrifugation; EC + C: Electrocoagulation + Centrifugation.

Table 10
Ear composition of wheat plants treated with supernatants obtained after different harvesting methods: primary macronutrients (N-NH_4^+, N-NO_3, K), secondary macronutrients (Ca, Mg, S), micronutrients (Fe, Cu, Zn, Mn, and B), and beneficial elements (Na).

Composition	Hoagland	MC	PF-CC
Primary macronutrients (g/kg)			
N	25.7	33.0	18.4
P	5.04	4.51	4.09
K	7.66	9.78	6.32
Secondary macronutrients (g/kg)			
Ca	0.593	0.649	0.53
Mg	1.86	1.35	1.11
S	2.13	2.03	1.52
Micronutrients (mg/kg)			
Fe	81.2	131	94.8
Cu	17.9	18.2	15.9
Zn	98.1	59.3	61.8
Mn	45.6	91.3	44.5
B	3.32	6.64	4.71
Beneficial elements (g/kg)			
Na	0.081	0.166	0.103
Heavy metals (g/kg)			
Cr	6.22	9.65	6.86
Ni	2.61	3.88	1.61
Cd	0.131	0.152	0.019
Pb	1.69	2.58	2.42

MC: Microalga culture; F: Flocculation; EC + C: Electrocoagulation + Centrifugation.

of biologically active molecules, such as phytohormones, which are known to influence seed germination [12,41].

The positive GI (198 %) results of F biomass could be related to the use of chitosan, which promote seed germination and seedling growth under low-temperature stress [42,43]. Chitosan is a non-toxic polymer derived from chitin, which is biodegradable, ecologically accepted, and food grade, therefore not compromising the applications of the microalgae biomass [44]. The cationic properties of chitosan facilitate microalgae harvesting and can also adsorb to plant surfaces, thus extending the contact time and increasing proximity between the plant and the microalgae biomass. This effect could also promote the efficacy of bioactive compounds in boosting seed germination.

When centrifugation is done after flocculation (F + C) or electrocoagulation (EC + C), the germination boositing effect of the biomass is reduced. In the first case, the centrifugal forces might break microalga-chitosan flocs, lowering the amount of chitosan present in the biomass. Free chitosan could also bind to plants and impede microalgae cells from binding to seedling tissues, decreasing the contact time and proximity of microalgae biomass. In EC + C, C might increase the toxic concentration of metals in the algal biomass which ultimately leads to a negative response of the GI [45].

In general, centrifugation is considered to be an expensive method of microalgae harvesting because it requires more energy, specialized equipment, and maintenance costs and can be more labor-intensive than chitosan flocculation. Chitosan flocculation can be a cost-effective method for large-scale microalgae harvesting, as it requires relatively simple equipment and it is easily scaled up. Although the cost of chitosan can vary depending on the supplier, it is generally considered to be a relatively low-cost chemical compared to other chemicals used in microalgae harvesting [46]. This way, flocculation seems to be the best method for harvesting biomass to be used for seed germination.

The direct use of the PF-PWW on wheat seeds indicated that the high concentration of nutrients has a phytotoxic effect as no seeds germinated (Fig. 3). The high concentration of NH_4^+ presents in the PWW, is usually attributed as the main toxicity factor in swine waste [47], with previous studies demonstrating a negative correlation between the NH_4^+ concentration and seed germination [48]. However, after microalgae-based treatment, the inhibitory effect is greatly reduced, with GI similar to the control ($p > 0.05$). The proximity of the supernatant's nutrient contents

Table 11
Intermediate composition of the soil treated with the different irrigation solutions (biomass and supernatants), photo-Fenton concentrate (PF-Cc) and controls (distilled water and Hoagland solution) 83 days after sowing: pH, conductivity (*k*), organic matter (OM), potassium oxide (K_2O), phosphorus (P), ammonia ($N\text{-}NH_4^+$), and nitrate ($N\text{-}NO_3$), micronutrients (Fe, Cu, Zn, and Mn) and base saturation (Ca^{2+}, K^+, Mg^{2+}, and Na^+).

Parameters	dH_2O	Hoagland	Biomass (0.2 g/L)					Supernatant				PF-Cc
			MC	C	F	EC	F + C	EC + C	C	F	EC	F + C	EC + C	
pH	6.4	6.1	6.0	6.4	6.4	6.5	6.7	6.5	6.1	5.7	5.9	5.9	5.9	6.7
k (μs/cm)	316	1005	1638	275	286	276	332	329	5394	6737	7650	5502	6832	1610
OM (%)	28.8	32.0	26.6	28.2	26.8	27.7	27.2	26.1	20.8	26.8	31.1	28.5	25.7	29.4
GSB (%)	98.3	98.8	98.8	99.1	98.5	98.8	98.8	98.5	99.0	99.6	99.5	98.9	98.9	98.7
Primary macronutrients (ppm)														
$N\text{-}NH_4$	<10	<10	<10	<10	<10	<10	<10	<10	1122	1142	1140	804	1212	108
$N\text{-}NO_3$	27.5	203.8	<10	<10	<10	<10	<10	<10	578	114	32.4	107	34.7	37.3
P	15.9	51.4	20.0	33.5	26.2	33.9	33.0	21.8	29.3	26.5	19.5	30.6	27.5	38.0
K_2O	830	1370	1600	670	670	890	740	870	4870	6330	5700	4700.0	5940	1630
Micronutrients (ppm)														
Fe	115	239	103	107	101	116	106	104	90.8	87.7	84.8	85.3	85.8	239
Cu	1.34	3.36	1.51	1.54	1.54	1.48	1.44	1.38	1.52	1.46	1.31	1.56	1.16	3.36
Zn	4.21	9.58	5.24	4.42	4.46	21.6	5.69	36.4	5.34	5.13	10.8	5.25	11.6	9.58
Mn	138	136	141	186	183	173	164	140	119	116	118	117	114	136
Base saturation (cmol(+)/kg)														
Ca^{2+}	65.3	60.4	55.3	65.4	65.5	65.5	64.4	61.6	44.2	48.9	46.2	44.8	44.5	59.9
K^+	5.60	8.38	8.62	4.98	4.51	4.32	6.40	6.97	16.2	13.6	15.3	13.9	15.0	8.49
Mg^{2+}	22.3	22.1	19.8	23.7	23.6	24.0	22.8	24.7	14.9	15.2	16.0	14.5	16.7	14.5
Na^+	5.03	7.96	15.0	5.00	4.93	4.93	5.22	5.18	23.8	21.9	22.1	25.8	22.8	15.8

MC: Microalga culture; C: Centrifugation; F: Flocculation; EC: Electrocoagulation; F + C: Flocculation + Centrifugation; EC + C: Electrocoagulation + Centrifugation.

to the toxicity limits could explain the decreased GI values [49]. Still, the nutrient contents in the supernatants may be more adequate for later stages of plant development (vegetative growth), since during germination, grain seeds degrade their own storage molecules to obtain energy [50].

4.2. Wheat growth

At an initial growth phase (growth chamber), the microalgal suspensions were sufficient for wheat growth. However, these same suspensions proved to be insufficient for more developed plants (Fig. 4), which is explained due to the low concentrations of macro and micronutrients (Table 6).

Improved growth occured in wheat plants supplemented with supernatants at later growth stages (Figs. 4 and 5) since they were richer in nutrients, especially N (Table 6). This element plays a key role in plant performance, it combines with carbon (C), hydrogen (H), oxygen (O), and sulphur (S) to create amino acids, which are the building blocks of proteins, and are used in the formation of the protoplasm, the site for cell division and thus for plant growth and development. N is also needed for all the enzymatic reactions in plants, and is a major constituent of the chlorophyll molecule, therefore being essential for photosynthesis [51].

The soil used for this experiment was rich in nutrients (Table 4), requiring only minimal N fertilization. Thus, the beneficial effect of the microalga or supernatants could be more pronounced when applied to poor soils, as it was evidenced with the application of *Chorella vulgaris* to marginal soils, replacing mineral fertilizers in wheat growth [52]. An accumulation of N in soils was observed from 83 (Table 11) to 182 DAS (Table 12), but was less pronounced in MC and PF-CC treated soils A high amount of N promotes plant growth, but when in excess, it is not completely absorbed by the plant, and in its nitric form is easily leached while contaminating the surrounding waters. The majority of N was found in the ammoniacal form ($N\text{-}NH_4$) which is a good indication, since this ion is more retained in the soil adsorption complex, there being less prone to soil leaching. Nitrate ($N\text{-}NO_3$) is quite soluble in water and is not retained in the soil. As it is quite mobile, it is dragged to the deeper layers of the soil, by percolation waters, but also by surface runoff waters [29]. Thus, it is convenient to have more nitrogen in the ammoniacal form than in the nitric form, which is verified in almost all soils analyzed, except the positive control soil (Hoagland's solution), where the nitrogen source is in the form of nitrate.

The high value of K (>30 g/kg) of plants was expected, since the supernatants also had a higher value (Table 5), and the plant assimilated this macronutrient (Table 9). K is necessary for carrying out protein synthesis and cell division [29]. Low content of this macronutrient can decrease the number of cell divisions and, consequently, plant growth, which can explain the reduced growth of plants with the negative control (Fig. 5). In terms of Fe, there is the biofortificartion of the ears of wheat plants treated with MC and PF-CC. An accumulation of Zn, Fe, Mn and Cu in wheat grains treated with cyanobacteria was also obtained previously [53].

Aside from the supernatants, the PF-CC also improved the wheat growth and ear production (Fig. 6). The valorization of this fraction is important since PF-CC is a by-product from photo-Fenton pre-treatment, and by evidencing its potential as an organic fertilizer, it can become a co-product on the promotion of sustainable agriculture by reducing the demand of chemical fertilizers. MC could also offer benefits since it is a less processed fraction of the microalga biomass production (no harvesting means lower costs), which improves the economic feasibility of the whole process. This result could be due to growth-promoting molecules produced by microalgae, which promote a better nutrient uptake by plants. An improved ear number of wheat plants treated with *Nostoc piscinale* in various field experiments carried out over three years [54]. A reduction of 75 % of chemical fertilization of wheat by applying microbial consortiums, including cyanobacteria species [55]. Improvement in plant weight of wheat treated with wastewater-grown microalga

Table 12
Final composition of the soil treated with the different supernatants, photo-Fenton concentrate (PF-Cc) and controls (distilled water and Hoagland solution) 182 days after sowing: pH, conductivity (k), organic matter (OM), potassium oxide (K_2O), phosphorus (P), ammonia ($N\text{-}NH_4^+$), and nitrate ($N\text{-}NO_3$), micronutrients (Fe, Cu, Zn, and Mn) and base saturation (Ca^{2+}, K^+, Mg^{2+}, and Na^+).

Parameters	dH_2O	Hoagland	MC	C-S	F-S	EC-S	F + C-S	EC + C-S	PF-Cc
pH	6.7	6.2	6.3	5.9	6.2	5.9	5.7	5.7	6.6
k (μs/cm)	406	552	1244	6512	7687	6366	4626	6345	667
OM (%)	22.7	22.7	22.0	22.7	18.0	23.1	24.8	21.8	27.7
GSB (%)	98.9	99.8	99.8	99.6	99.9	99.3	99.4	99.7	99.5
Primary macronutrients (ppm)									
$N\text{-}NH_4$	4.50	9.50	157	1662	1889	1347	871	1412	24.7
$N\text{-}NO_3$	9.20	136	13.1	99.9	112	123	133	137	18.6
P	68.5	101	35.9	48.8	51.5	39.8	29.9	37.7	64.0
K_2O	943	1129	1535	7305	5628	5351	4469	4817	959
Micronutrients (ppm)									
Fe	128	173	157	139	128	165	140	157	568
Cu	5.01	5.42	15.22	4.36	5.46	4.86	4.52	4.80	7.85
Zn	10.6	11.5	12.3	16.4	17.8	125	11.9	93.4	26.0
Mn	64.8	81.1	71.9	67.9	74.9	64.0	68.3	68.4	174.2
Base saturation (cmol(+)/kg)									
Ca^{2+}	23.8	27.4	25.2	31.3	35.3	28.1	30.6	31.3	26.7
K^+	2.78	2.92	3.55	14.1	15.1	13.7	9.90	15.6	2.34
Mg^{2+}	7.40	8.40	6.90	7.55	7.59	10.5	6.46	11.1	7.00
Na^+	2.99	2.43	6.90	29.3	32.4	29.2	22.6	30.0	4.60

MC: Microalga culture; C: Centrifugation; F: Flocculation; EC: Electrocoagulation; F + C: Flocculation + Centrifugation; EC + C: Electrocoagulation + Centrifugation.

suspensions (7.4–33 %) with 25 % N savings in chemical fertilization was also previously shown [56]. This could be due to growth-promoting molecules produced by microalgae, which promote a better nutrient uptake by plants. Thus, a promising strategy could be the combination of MC with PF-CC in order to provide both essential nutrients and bioactive compounds to improve the nutrient uptake and promote plant growth.

5. Conclusions

T. obliquus grown in PF-PWW improved seed germination and provided faster root and shoot growth. The various tested fractions (biomass, supernatant, and PF precipitate) were shown to serve different purposes during the different wheat growth stages. The use of centrifugation and flocculation as a harvesting method had a very positive impact on wheat seed germination, with the microalga biomass achieving a GI of 187 and 205 %, respectively. Supernatants from the microalga cultivation were also a valuable irrigation suspensions for agriculture to sustain plant growth and resulted in a higher number of leaves and tillers. PF precipitate was an important input of essential nutrients, achieving similar ear productivity compared to positive control. Ultimately, this strategy is not only crucial to develop more environmentally sustainable treatments for agro-industrial effluents but could contribute to minimize microalgal production costs by using a low-cost and readily available source of nutrients. The generation of treated water for irrigation and biomass will help alleviate the ever-growing pressure on water resources and reduce the dependence of non-renewable fertilizers for agriculture. Furthermore, microalga could be a vehicle for delivering the nutrients present in manure in a more controlled manner, avoiding nutrient runoff that contaminate aquatic environment.

CRediT authorship contribution statement

Conceptualization, A.F, D.F., K.Š. and L.G.; methodology, A.F., D.F., B.R., C.S. and L.G.; investigation, A.F., D.F., F.F., A.M., C.B., G.M., B.R., K.Š., C.S. and L.G.; writing—original draft preparation, L.G.; writing—review and editing, A.F., D.F., F.F., A.M., C.B., K.Š., C.S., G.A. and L.G.; visualization, A.F., D.F., F.F., A.M., and C.B.; supervision, C.S, G.A. and L.G.; project administration, L.G.; funding acquisition, C.S. and L.G. All authors have read and agreed to the published version of the manuscript.

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

The authors are unable or have chosen not to specify which data has been used.

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References

[1] N.K. Arora, Agricultural sustainability and food security, Environ. Sustain. 13 (1) (2018) 217–219, https://doi.org/10.1007/S42398-018-00032-2.

[2] M. Henchion, M. Hayes, A.M. Mullen, M. Fenelon, B. Tiwari, Future protein supply and demand: strategies and factors influencing a sustainable equilibrium, Foods 6 (2017) 53, https://doi.org/10.3390/FOODS6070053.

[3] M. van der Spiegel, M.Y. Noordam, H.J. van der Fels-Klerx, Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production, Compr. Rev. Food Sci. Food Saf. 12 (2013) 662–678, https://doi.org/10.1111/1541-4337.12032.

[4] R. Ibidhi, H. Ben Salem, R. Ibidhi, H. Ben Salem, Water footprint of livestock products and production systems: a review, Anim. Prod. Sci. 60 (2020) 1369–1380, https://doi.org/10.1071/AN17705.

[5] F.G. Acién, C. Gómez-Serrano, M.M. Morales-Amaral, J.M. Fernández-Sevilla, E. Molina-Grima, Wastewater treatment using microalgae: how realistic a contribution might it be to significant urban wastewater treatment? Appl. Microbiol. Biotechnol. 100 (2016) 9013–9022, https://doi.org/10.1007/s00253-016-7835-7.

[6] A. Ferreira, D. Figueiredo, R. Cardeiras, R. Nabais, F. Ferreira, B. Ribeiro, C.M.d. S. Cordovil, F.G. Acién, L. Gouveia, Exploring different pretreatment methodologies for allowing microalgae growth in undiluted piggery wastewater, Agron 12 (2022) 580, https://doi.org/10.3390/AGRONOMY12030580.

[7] J.A. Sánchez Pérez, I.M. Román Sánchez, I. Carra, A. Cabrera Reina, J.L. Casas López, S. Malato, Economic evaluation of a combined photo-Fenton/MBR process using pesticides as model pollutant. Factors affecting costs, J. Hazard. Mater. 244–245 (2013) 195–203, https://doi.org/10.1016/j.jhazmat.2012.11.015.

[8] A. Babuponnusami, K. Muthukumar, A review on Fenton and improvements to the Fenton process for wastewater treatment, J. Environ. Chem. Eng. 2 (2014) 557–572, https://doi.org/10.1016/j.jece.2013.10.011.

[9] B. Jain, A.K. Singh, H. Kim, E. Lichtfouse, B. Jain, A.K. Singh, H. Kim, E. Lichtfouse, V.S. Treatment, Treatment of organic pollutants by homogeneous and heterogeneous Fenton reaction processes, Environ. Chem. Lett. 16 (2019) 947–967.

[10] P. Soriano-Molina, P. Plaza-Bolaños, A. Lorenzo, A. Agüera, J.L. García Sánchez, S. Malato, J.A. Sánchez Pérez, Assessment of solar raceway pond reactors for removal of contaminants of emerging concern by photo-Fenton at circumneutral pH from very different municipal wastewater effluents, Chem. Eng. J. 366 (2019) 141–149, https://doi.org/10.1016/j.cej.2019.02.074.

[11] A. Ferreira, C.R.V. Bastos, C.M.d.S. Cordovil, F.G. Acién-Fernández, L. Gouveia, Algaeculture 4 agriculture: from past to future, Front. Agron. (2023), https://doi.org/10.3389/fagro.2023.1064041.

[12] D. Ronga, E. Biazzi, K. Parati, D. Carminati, E. Carminati, A. Tava, Microalgal biostimulants and biofertilisers in crop productions, Agronomy 9 (2019) 192, https://doi.org/10.3390/agronomy9040192.

[13] J.R. Benemann, I. Woertz, T. Lundquist, Autotrophic microalgae biomass production: from niche markets to commodities, Https://Home.Liebertpub.Com/Ind. 14 (2018) 3–10, https://doi.org/10.1089/IND.2018.29118.JRB.

[14] F. Fasaei, J.H. Bitter, P.M. Slegers, A.J.B. van Boxtel, Techno-economic evaluation of microalgae harvesting and dewatering systems, Algal Res. 31 (2018) 347–362, https://doi.org/10.1016/J.ALGAL.2017.11.038.

[15] A. Guldhe, R. Misra, P. Singh, I. Rawat, F. Bux, An innovative electrochemical process to alleviate the challenges for harvesting of small size microalgae by using non-sacrificial carbon electrodes, Algal Res. 19 (2016) 292–298, https://doi.org/10.1016/J.ALGAL.2015.08.014.

[16] C. Inostroza, N. El Bahraoui, R. Rivera-Tinoco, F.G. Acién, Uses of electro-coagulation-flocculation (ECF) for the pre-concentration of microalgae biomass, Process Biochem. 122 (2022) 1–7, https://doi.org/10.1016/J.PROCBIO.2022.09.012.

[17] D. Figueiredo, A. Ferreira, P. Quelhas, P.S.C. Schulze, L. Gouveia, Nannochloropsis oceanica harvested using electrocoagulation with alternative electrodes – an innovative approach on potential biomass applications, Bioresour. Technol. 344 (2022), 126222, https://doi.org/10.1016/J.BIORTECH.2021.126222.

[18] C.T. Matos, M. Santos, B.P. Nobre, L. Gouveia, Nannochloropsis sp. biomass recovery by Electro-Coagulation for biodiesel and pigment production, Bioresour. Technol. 134 (2013) 219–226, https://doi.org/10.1016/J.BIORTECH.2013.02.034.

[19] K. Xing, X. Zhu, X. Peng, S. Qin, Chitosan antimicrobial and eliciting properties for pest control in agriculture: a review, Agron. Sustain. Dev. 35 (2015) 569–588, https://doi.org/10.1007/S13593-014-0252-3/METRICS.

[20] D. Figueiredo, A. Ferreira, F. Gama, L. Gouveia, Nannochloropsis oceanica biomass enriched by electrocoagulation harvesting with promising agricultural applications, Bioresour. Technol. Reports 17 (2022), 100979, https://doi.org/10.1016/J.BITEB.2022.100979.

[21] D. Vandamme, I. Foubert, I. Fraeye, K. Muylaert, Influence of organic matter generated by *Chlorella vulgaris* on five different modes of flocculation, Bioresour. Technol. 124 (2012) 508–511, https://doi.org/10.1016/j.biortech.2012.08.121.

[22] O. Lowry, N. Rosebrough, A. Farr, R. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 165–275, https://doi.org/10.1016/S0021-9258(19)52451-6.

[23] F. Zucconi, M. Forte, A. Monaco, M. De Bertoldi, Biological evaluation of compost maturity, Biocycle. 22 (1981) 27–29.

[24] C.M.d.S. Cordovil, M.J. Goss, J. Coutinho, F. Cabral, Estimating short- and medium-term availability to cereals of nitrogen from organic residues, J. Plant Nutr. 35 (2012) 366–383, https://doi.org/10.1080/01904167.2012.639918.

[25] I. Póvoas, M.F. Barral, Métodos de Análise de Solos, in: Comun. Do IICT, Série Ciências Agrárias N°. 10, Lisboa, 1992.

[26] E. Lakanen, R. Ervio, A comparison of eight extractants for the determination of plant available micronutrients in soils, Acta Agral. Fenn. 123 (1971) 223–232. https://www.scirp.org/(S(czeh2tfqw2orz553k1w0r45))/reference/referencespapers.aspx?referenceid=2896004. (Accessed 29 September 2022).

[27] H. Egnér, H. Riehm, W.R. Domingo, Untersuchungen uber die chemische Bodenanalyse als Grundlage fur die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung, in: K. Lantbrukshögskolans Ann, 1960, pp. 199–215. https://scirp.org/reference/referencespapers.aspx?referenceid=1594975. (Accessed 29 September 2022).

[28] R.L. Mulvaney, Nitrogen—inorganic forms, in: D.L. Sparks, A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnston, M.E. Sumner (Eds.), Methods Soil Anal. Part 3 Chem. Methods, John Wiley & Sons, Ltd, 1996, pp. 1123–1184, https://doi.org/10.2136/SSSABOOKSER5.3.C38.

[29] A. Veloso, C. Sempiterno, F. Calouro, F. Rebelo, F. Pedra, I.V. Castro, M. da C. Gonçalves, M. da E. Marcelo, P. Pereira, P. Fareleira, P. Jordão, R. Mano, R. Fernandes, Manual de Fertilização das Culturas, 3rd ed., 2022.

[30] L.N. Nguyen, L. Aditya, H.P. Vu, A.H. Johir, L. Bennar, P. Ralph, N.B. Hoang, J. Zdarta, L.D. Nghiem, Nutrient removal by algae-based wastewater treatment, Curr. Pollut. Reports 8 (2022) 369–383, https://doi.org/10.1007/S40726-022-00230-X/FIGURES/3.

[31] Á.F. Mógor, V. Ördög, G.P.P. Lima, Z. Molnár, G. Mógor, Biostimulant properties of cyanobacterial hydrolysate related to polyamines, J. Appl. Phycol. 30 (2018) 453–460, https://doi.org/10.1007/s10811-017-1242-z.

[32] E. Navarro-López, A. Ruíz-Nieto, A. Ferreira, F. Gabriel Acién, L. Gouveia, Biostimulant potential of *Scenedesmus obliquus* grown in brewery wastewater, Molecules. 25 (2020) 1–16, https://doi.org/10.3390/molecules25030664.

[33] M. Joan Chanda, N. Merghoub, H.E.L. Arroussi, Microalgae polysaccharides: the new sustainable bioactive products for the development of plant bio-stimulants? World J. Microbiol. Biotechnol. 35 (2019) 1–10, https://doi.org/10.1007/s11274-019-2745-3.

[34] H. El Arroussi, N. El Mernissi, R. Benhima, I.M. El Kadmiri, N. Bendaou, A. Smouni, Microalgae polysaccharides a promising plant growth biostimulant, J. Algal Biomass Utln. 7 (2016) 55–63.

[35] S.A.G. Nejad, H. Etesami, The importance of boron in plant nutrition, Met. Plants (2020) 433–449, https://doi.org/10.1002/9781119487210.CH20.

[36] European Parliament and the Council of the European Union, DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 7 May 2002 on undesirable substances in animal feed, Off. J. Eur. Communities 140 (2002) 10–20.

[37] A.I. Barros, A.L. Gonçalves, M. Simões, J.C.M. Pires, Harvesting techniques applied to microalgae: a review, Renew. Sust. Energ. Rev. 41 (2015) 1489–1500, https://doi.org/10.1016/J.RSER.2014.09.037.

[38] A. Ferreira, D. Figueiredo, F. Ferreira, B. Ribeiro, A. Reis, T.L. da Silva, L. Gouveia, Impact of high-pressure homogenization on the cell integrity of *Tetradesmus obliquus* and seed germination, Molecules. 27 (2022) 2275, https://doi.org/10.3390/MOLECULES27072275.

[39] R. Kholssi, E.A.N. Marks, J. Miñón, O. Montero, A. Debdoubi, C. Rad, Biofertilizing effect of *Chlorella sorokiniana* suspensions on wheat growth, J. Plant Growth Regul. 38 (2019) 644–649, https://doi.org/10.1007/s00344-018-9879-7.

[40] A. Ferreira, L. Melkonyan, S. Carapinha, B. Ribeiro, D. Figueiredo, G. Avetisova, L. Gouveia, Biostimulant and biopesticide potential of microalgae growing in piggery wastewater, Environ. Adv. 4 (2021), 100062, https://doi.org/10.1016/j.envadv.2021.100062.

[41] A. Hussain, S. Hasnain, Phytostimulation and biofertilization in wheat by cyanobacteria, J. Ind. Microbiol. Biotechnol. 38 (2011) 85–92, https://doi.org/10.1007/s10295-010-0833-3.

[42] Y.J. Guan, J. Hu, X.J. Wang, C.X. Shao, Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress, J Zhejiang Univ Sci B 10 (2009) 427–433, https://doi.org/10.1631/jzus.B0820373.

[43] R. Li, J. He, H. Xie, W. Wang, S.K. Bose, Y. Sun, J. Hu, H. Yin, Effects of chitosan nanoparticles on seed germination and seedling growth of wheat (*Triticum aestivum* L.), Int. J. Biol. Macromol. 126 (2019) 91–100, https://doi.org/10.1016/j.ijbiomac.2018.12.118.

[44] Y. Xu, S. Purton, F. Baganz, Chitosan flocculation to aid the harvesting of the microalga *Chlorella sorokiniana*, Bioresour. Technol. 129 (2013) 296–301, https://doi.org/10.1016/j.biortech.2012.11.068.

[45] S. Visigalli, M.G. Barberis, A. Turolla, R. Canziani, M. Berden Zrimec, R. Reinhardt, E. Ficara, Electrocoagulation–flotation (ECF) for microalgae harvesting – a review, Sep. Purif. Technol. 271 (2021), https://doi.org/10.1016/j.seppur.2021.118684.

[46] Z. Yin, R. Chu, L. Zhu, S. Li, F. Mo, D. Hu, C. Liu, Application of chitosan-based flocculants to harvest microalgal biomass for biofuel production: a review, Renew. Sust. Energ. Rev. 145 (2021), 111159, https://doi.org/10.1016/j.rser.2021.111159.

[47] S.M. Tiquia, N.F.Y. Tam, Elimination of phytotoxicity during co-composting of spent pig-manure sawdust litter and pig sludge, Bioresour. Technol. 65 (1998) 43–49, https://doi.org/10.1016/S0960-8524(98)00024-8.

[48] J. Yuan, D. Chadwick, D. Zhang, G. Li, S. Chen, W. Luo, L. Du, S. He, S. Peng, Effects of aeration rate on maturity and gaseous emissions during sewage sludge

composting, Waste Manag. 56 (2016) 403–410, https://doi.org/10.1016/j.wasman.2016.07.017.
[49] M.D. Gerber, T. Lucia, L. Correa, J.E.P. Neto, É.K. Correa, Phytotoxicity of effluents from swine slaughterhouses using lettuce and cucumber seeds as bioindicators, Sci. Total Environ. 592 (2017) 86–90, https://doi.org/10.1016/j.scitotenv.2017.03.075.
[50] S. Liu, W. Wang, H. Lu, Q. Shu, Y. Zhang, Q. Chen, New perspectives on physiological, biochemical and bioactive components during germination of edible seeds: a review, Trends Food Sci. Technol. 123 (2022) 187–197, https://doi.org/10.1016/j.tifs.2022.02.029.
[51] J.A. Silva, R. Uchida, Essential nutrients for plant growth: nutrient functions and deificiency symptoms, Plant Nutr. Manag. Hawaii's Soils, Approaches Trop. Subtrop. Agric. (2000) 31–55.
[52] C. Schreiber, H. Schiedung, L. Harrison, C. Briese, B. Ackermann, J. Kant, S. D. Schrey, D. Hofmann, D. Singh, O. Ebenhöh, W. Amelung, U. Schurr, T. Mettler-Altmann, G. Huber, N.D. Jablonowski, L. Nedbal, Evaluating potential of green alga *Chlorella vulgaris* to accumulate phosphorus and to fertilize nutrient-poor soil substrates for crop plants, J. Appl. Phycol. 30 (2018) 2827–2836, https://doi.org/10.1007/s10811-018-1390-9.
[53] A. Rana, M. Joshi, R. Prasanna, Y.S. Shivay, L. Nain, Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria, Eur. J. Soil Biol. 50 (2012) 118–126, https://doi.org/10.1016/J.EJSOBI.2012.01.005.
[54] G. Takács, W.A. Stirk, I. Gergely, Z. Molnár, J. van Staden, V. Ördög, Biostimulating effects of the cyanobacterium *Nostoc piscinale* on winter wheat in field experiments, South African J. Bot. 126 (2019) 99–106, https://doi.org/10.1016/J.SAJB.2019.06.033.
[55] C. Ramírez-López, F.J. Esparza-García, R. Ferrera-Cerrato, A. Alarcón, R. O. Cañizares-Villanueva, Short-term effects of a photosynthetic microbial consortium and nitrogen fertilization on soil chemical properties, growth, and yield of wheat under greenhouse conditions, J. Appl. Phycol. 31 (2019) 3617–3624, https://doi.org/10.1007/s10811-019-01861-2.
[56] N. Renuka, R. Prasanna, A. Sood, A.S. Ahluwalia, R. Bansal, S. Babu, R. Singh, Y. S. Shivay, L. Nain, Exploring the efficacy of wastewater-grown microalgal biomass as a biofertilizer for wheat, Environ. Sci. Pollut. Res. 23 (2016) 6608–6620, https://doi.org/10.1007/s11356-015-5884-6.