agronomy

Article

Exploring Different Pretreatment Methodologies for Allowing Microalgae Growth in Undiluted Piggery Wastewater

Alice Ferreira [1,*], Daniel Figueiredo [2], Rodrigo Cardeiras [1], Rui Nabais [1], Francisca Ferreira [1], Belina Ribeiro [1], Cláudia M. d. S. Cordovil [3], F. Gabriel Acién [4] and Luisa Gouveia [1,2]

[1] LNEG, National Laboratory of Energy and Geology I.P., Bioenergy Unit, Estrada do Paço do Lumiar 22, 1649-038 Lisbon, Portugal; rodrigo.cardeiras@gmail.com (R.C.); ruinabais94@gmail.com (R.N.); francisca.opf@gmail.com (F.F.); belina.ribeiro@lneg.pt (B.R.); luisa.gouveia@lneg.pt (L.G.)
[2] GreenCoLab—Associação Oceano Verde, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; danielfigueiredo@greencolab.com
[3] Forest Research Centre, School of Agriculture, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal; cms@isa.ulisboa.pt
[4] Chemical Engineering Department, University of Almeria, Ctra. Sacramento, s/n, 04120 Almería, Spain; facien@ual.es
* Correspondence: alice.ferreira@lneg.pt

Abstract: The overapplication of manure on agricultural soils leads to nitrogen and phosphorus discharge into the aquatic environment, resulting in serious eutrophication problems and decreased water quality. Piggery wastewater (PWW) can be treated by microalgae to recycle nutrients, but the toxic levels of ammonia and organic matter hinder their growth. Fresh water is usually used to dilute PWW, but it is a scarce resource. The implementation of a pretreatment step before microalgae-based treatment could make PWW suitable for microalgae growth. Electrocoagulation, ammonia stripping, photo-Fenton, and constructed wetlands were evaluated as pretreatment methods to reduce ammonia, chemical oxygen demand (COD), color, and total suspended solids. Moreover, the pretreated PWWs were tested to grow the microalga *Tetradesmus obliquus*. Photo-Fenton showed the best results among the other pretreatments, achieving removal efficiencies above 90%, except for ammonia. This resulted in *T. obliquus* being capable of growing on undiluted PWW, even at higher ammonia levels, achieving similar biomass productivity to synthetic medium (66.4 $\pm$ 17.8 mg$\cdot$L$^{-1}\cdot$day^{-1} and 60.1 $\pm$ 10.4 mg$\cdot$L$^{-1}\cdot$day^{-1}, respectively) almost doubling with pH control (116.5 mg$\cdot$L$^{-1}\cdot$day^{-1}). Thus, this pretreatment seems to be the most promising one to incorporate into microalgae-based treatment systems and must be further explored.

Keywords: swine wastewater; *Tetradesmus obliquus*; ammonia stripping; electrocoagulation; photo-Fenton; constructed wetland

Citation: Ferreira, A.; Figueiredo, D.; Cardeiras, R.; Nabais, R.; Ferreira, F.; Ribeiro, B.; Cordovil, C.M.d.S.; Acién, F.G.; Gouveia, L. Exploring Different Pretreatment Methodologies for Allowing Microalgae Growth in Undiluted Piggery Wastewater. *Agronomy* **2022**, *12*, 580. https://doi.org/10.3390/agronomy12030580

Academic Editor: Sung-Cheol Koh

Received: 19 January 2022
Accepted: 24 February 2022
Published: 26 February 2022

1. Introduction

Pork is an important source of protein around the world. According to recent estimates, the European Union is currently one of the largest pig producers, with an average of 148 million pig heads produced in the last 10 years [1]. This industry is estimated to generate 215–430.106 m^3/year (4–8 L/day/pig) of piggery wastewater (PWW) [2], which includes pig excreta combined with the water used to clean the hog housing sheds. Thus, it contains a high organic load, ammonium nitrogen, phosphorus, and recalcitrant organic compounds that hinder the performance of conventional treatment processes. PWW is commonly treated in stabilization ponds, where it is left stored for long periods before land application [3]. However, these generate high emissions of CO_2, methane (from anaerobic digestion), ammonia (by volatilization), and hydrogen sulfide (from decomposition), among others [4].

While PWW is a problem for piggeries to handle, for microalgae growth, it represents a valuable low-cost and ready to use source of nutrients. The assimilation of the nutrients from PWW in autotrophic microalgal biomass may provide alternative wastewater treatment for pig farming. The produced algal biomass can generate income as a source of animal feed or plant fertilizers and biostimulants for agriculture. Nevertheless, microalga cultivation in PWW still represents a challenge due to the high ammonia levels, high pH, dark color, and significant suspended solid content, resulting in high turbidity [4,5]. The increase in pH, due to microalgae photosynthesis and carbon assimilation, shifts the chemical equilibrium from NH_4^+ to NH_3, which is more toxic to microalgae cells [5,6]. In addition, the dark color and high turbidity reduce light penetration, lowering the amount of light available for autotrophic growth. The dilution of the PWW can reduce toxic nutrient concentrations to noninhibiting levels while promoting light availability to microalgae cells [7]. However, the use of fresh water, a scarce and precious resource, should be avoided, as it entails economic and ecological issues. This motivates the search for new strategies, such as the integration of a pretreatment process to minimize the toxicity of PWW and assure optimal microalgae growth.

Electrocoagulation (EC) is a popular method in wastewater treatment that has been widely studied for different WW sources owing to its versatility, simple setup, low footprint, and ecofriendly nature [8]. EC acts by destabilizing the charges of the pollutants through electrode-released cations (e.g., Fe^{2+} or Al^{3+}), which form hydroxides or polyhydroxides with a great affinity for dissolved substances or counterions to cause coagulation/adsorption phenomena [9]. Pollutants are aggregated into clusters and separated from the electrolytic mixture by sedimentation, due to the heavier weight of flocs, or by flotation with the help of microbubbles produced by water electrolysis. The key factors for EC are electrode type, electrode distance, current density, pH, the conductivity of the electrolyte, and time. Iron (Fe) and aluminum (Al) are the most widely used electrode materials because they are readily available and inexpensive, and they have high removal capacity [10]. However, the EC technology is not yet fully optimized to be considered a reliable water treatment technology, since most research is developed using small-scale batch reactors. One of the major challenges is developing a more systematic approach for EC reactor design, operation, and modeling to predict the EC performance and, thus, facilitate the scaling up to meet industrial capacity. Furthermore, research is required to evaluate the performance of EC units operating in continuous flow mode with more effective reactor design [11].

Ammonia stripping (AS) is a well-described method to reduce ammonium nitrogen levels of several wastewaters, due to its easy operation, high efficiency, and process stability [12,13]. However, the research on AS applied to PWW for ammonia removal is limited and not always consistent [13,14]. AS is based on the principle of mass transfer in which air is injected into the PWW to strip the free ammonia (NH_3-N) that is highly volatile. In this process, the wastewater is aerated after adjusting the pH over 8.5 using alkali [13]. Airflow rate, pH, and temperature are the most influencing parameters on the removal rates of ammonia [12,13,15]. Higher temperatures and pH have been reported to enhance the stripping efficiency, as they promote the formation of NH_3 [16]. Typical values found in the literature are pH 10–11, with a temperature up to 80 °C, and airflow rate ranging from 0.5 to 10 vvm ($L \cdot L^{-1} \cdot min^{-1}$) [14]. The AS process has several drawbacks, such as fouling problems, which affects stripping performance and entails high operation and maintenance costs, in addition to sludge production and high alkalinity of the effluent, and release of ammonia gas, which can cause additional environmental costs. This latter effect can be minimized by capturing the volatilized ammonia in an adjacent adsorbing unit containing an acid solution (e.g., sulfuric acid to produce ammonium sulfate, which can be used as fertilizer in agriculture) [12,13].

Advanced oxidation processes such as photo-Fenton (PF) have been drawing the attention of researchers for their advantages such as simpler process conditions (e.g., ambient temperature and pressure) and shorter reaction time, while being more economical and feasible than conventional methods [17]. In PF, hydrogen peroxide oxidizes Fe, which acts as a catalyst, yielding hydroxyl radicals. The oxidized Fe^{3+} is quickly reduced to Fe^{2+} by the action of UV–vis radiation, being regenerated cyclically. Hydroxyl radicals are responsible for the oxidation of organic matter due to their high oxidation potential ($E^0_{ox} = 2.8\ V$) [18]. They react rapidly in a nonselective manner, which enables the total degradation of organic contaminants, thus generating a smaller volume of solid waste [19]. Moreover, the hydroxyl radicals can oxidize ammonia to nitrate [20], which is less toxic for microalgae growth [6]. The H_2O_2/Fe ratio is highly important for the efficiency of the method to assure a complete reaction and the full consumption of H_2O_2. Furthermore, a prior acidification step is required since the reaction is more effective at acidic pH, which will later require increasing again the pH to neutral values. These pH variations all imply added operation costs. The introduction of UV light increases the reaction rate but requires extra costs, which could account for about 35%. For implementation of these methods, the toxicity of pretreated water must be always assessed since, depending on wastewater origin, the generated compounds might be more toxic than parental compounds. From the economic point of view, reported operation and maintenance values varied from 0.44 to 2.18 EUR·m^{-3} depending on the lamp used (not including personnel cost) [21]. Nonetheless, these illumination costs can be eliminated when coupling this process to open ponds under solar irradiation [22].

Constructed wetlands (CWs) are a natural, simple, and environmentally friendly technology with low construction and maintenance costs [23], especially suited for small farms in rural communities to allow higher nutrient use efficiency. CWs have several advantages such as the use of solar or wind energies, or gravity. This technique allows the direct use of the PWW, without dilution and the efficient removal of biochemical and chemical oxygen demand and ammoniacal nitrogen. The latter can be converted to nitrates by nitrification due to the passive aeration in these wetlands. The reduced nitrogen levels can be used for microalgae growth, which is a major advantage [24,25]. However, CWs require large areas, and they are vulnerable to climatic changes and temperature; furthermore, the substrates can be easily saturated and plugged, and their performance is highly affected by plant species [26].

The present work aimed to identify the most promising pretreatment methods to treat and enable undiluted PWW for microalgae growth at maximal biomass productivity. For this, the effect of the various operating parameters playing a key role on the pretreatment methodologies (pH, temperature, time, and reagent concentration) was investigated on the simultaneous degradation of color, COD, TSS, and ammonia nitrogen from PWW. Lastly, the growth of *Tetradesmus obliquus* was evaluated in the pretreated effluents resulting from optimized conditions.

2. Materials and Methods

2.1. Effluent Feedstock

The PWW 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) during the month of June and was stored at 4 °C until handling. The PWW corresponded to the liquid fraction of the pig slurry after solid–liquid separation. Pig slurry comprised pig excreta and water used to clean the housing facilities that accommodate animals at all stages of development, including sows and piglets. Batches of PWW served as substrate for microalga growth after different pretreatment methodologies as described below, at laboratory scale.

2.2. Electrocoagulation (EC)

The EC system included an external direct current power source (HY3005D, Mastech: Taipei, Taiwan) connected to a pair of flat electrodes plates (100 mm × 45 mm) immersed in 500 mL of PWW contained in 600 mL glass flasks. The electrodes were fixed at 1 cm to each other with a submerged surface area of 20 cm^2 (Figure 1). All EC tests were performed in batch mode, at 150 rpm (IKA Labortechnik ES5, Janke & Kunkel: Staufen, Germany), and room temperature. The initial pH was adjusted to 7 using 1 M HCl. The electrodes tested were aluminum, iron, and zinc due to their availability in the laboratory. The current density tested was 4, 12, and 20 mA·cm^{-2}. After 30 min of EC, the effluents were left to settle for 30 min. The supernatant was collected, and the pH was adjusted to 7.0 with 1 M HCl. The pretreated effluent was stored at 4 °C for further analysis and microalga cultivation experiments, as further described in Section 2.6.

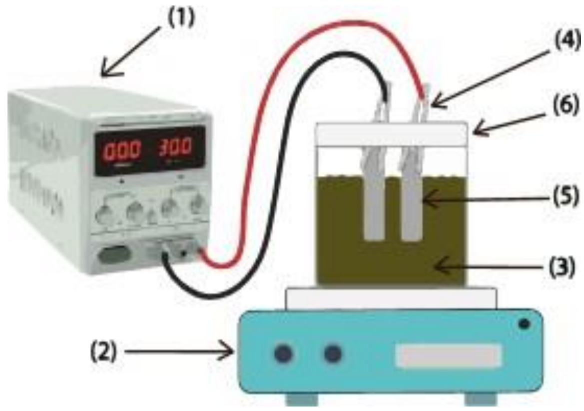

Figure 1. Schematic representation of the experimental set-up for electrocoagulation: 1—direct current power source, 2—magnetic stirrer, 3—piggery wastewater, 4—electrical wires and alligator clips, 5—electrode plates, and 6—stabilizing cap.

2.3. Ammonia Stripping (AS)

AS was performed in cylindrical 1 L bubble column reactors with a working volume of 0.8 L. The effects of pH (9, 10, and 11) and temperature (25 and 44 °C) were evaluated on the pollutant removal efficiency, especially ammonia. The temperature was increased by placing reactors in a thermally isolated box with an infrared lamp (Philips 250 W R40) connected to a temperature controller (Figure 2). The experimental runs were started after pH adjustments using 6 M NaOH. The reactors were bubbled with air at a fixed airflow of 7.5 vvm for 6 h. In the end, distilled water was added to compensate for the water loss during the process, and the pretreated PWW was left to settle. The supernatant pH was adjusted to 7.0 using 1 M HCl and stored for further analysis and microalga cultivation experiments.

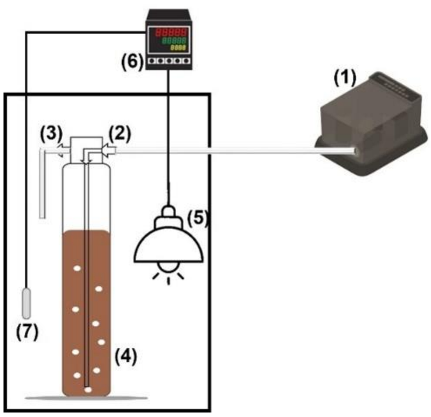

Figure 2. Schematic representation of the experimental setup for the ammonia stripping experiments: 1—air pump, 2—air entrance, 3—air exit, 4—bubble column reactor containing the wastewater, 5—infrared lamp, 6—set point controller, and 7—temperature probe.

2.4. Photo-Fenton Process (PF)

The photo-Fenton experiments were carried out in an installation setup designed and built in the Department of Renewable Energy at LNEG. The set consisted of a wooden box with an interior formed by two sections of a mirror, one containing the light source, which included four Eversun L40/79K lamps (Osram), with unit power of 40 W (8 W UVA (315–400 nm) and 0.04 W UVB (280–315 nm)), 59 cm long and 3.8 cm in diameter, arranged in a circle, and the other where the magnetic stirrer and reactor containing the PWW were located (Figure 3).

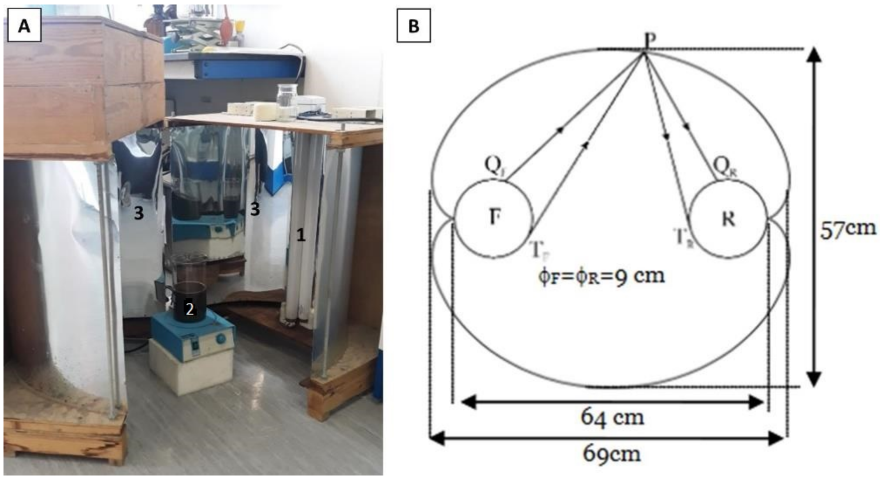

Figure 3. Experimental setup for the photo-Fenton experiments: (**A**) open montage (1—UV lamps; 2—reaction vessel containing the piggery effluent; 3—mirror section); (**B**) scheme of the optical device (F—energy source; R—reaction vessel; P—generic point in the mirror section; TF, QF—light source points; TR, QR—light receiving points).

The reactions were carried out, in batch mode, in a 1 L reactor with a working volume of 500 mL. The tested experimental conditions were chosen according to the available literature, as well as preliminary trials. Various concentrations of Fe^{2+} and H_2O_2 were tested: 0.2, 0.5, and 1.0 g $Fe^{2+}\cdot L^{-1}$ and 1, 4.9, and 10.5 g $H_2O_2\cdot L^{-1}$. Ferrous sulfate ($FeSO_4\cdot 7H_2O$) and hydrogen peroxide (35 wt.%) were used. The initial pH was first adjusted to 3 using cc. H_2SO_4, and then ferrous sulfate was added to the reactor. An acidic pH (2–4) is crucial to minimize the formation of secondary products less reactive, guaranteeing a high oxidation potential, as well as to avoid the precipitation of iron ions. The reactor was then placed under UV radiation for 120 min at room temperature and mixed at 400 rpm. To avoid the intensive formation of foam, H_2O_2 was added in dosages at different times: 1 $g\cdot L^{-1}$, 4.9 $g\cdot L^{-1}$, and 10.5 $g\cdot L^{-1}$, at intervals of 30 min. At the end of the process, the residual hydrogen peroxide was determined by iodometric titration with sodium thiosulfate (0.1 N). The pH was adjusted to 7.0 using 6 M NaOH, and the effluent was left to settle for 2 h. The supernatant was then filtered through a paper filter (11 µm, Whatman) to remove suspended iron precipitates. The pretreated effluent was stored for further analysis and microalga cultivation.

2.5. Constructed Wetland (CW)

The PWW was also submitted to a pretreatment using a lab-scale CW. This work was conducted by another team within the project ALGAVALOR consortium, and a full description of the experimental design and the results are detailed in Dias et al. (2020) [27]. They studied the removal of metals and nutrients on CWs microcosms to simulate a vertical subsurface flow CW. Experiments were performed at a laboratory scale using two types of porous substrate (lava rock and expanded clay), and two macrophyte species (*Typha latifolia* and *Phragmites australis*). The pretreated effluent resulting from the CW using *P. australis* and expanded clay was compared its potential for microalga cultivation and compared to other pretreatment methods of this work.

2.6. Microalga Cultivation

The microalga tested was *Tetradesmus obliquus* (ACOI 204/07, ACOI Culture Collection, Coimbra University, Portugal) that was previously shown to grow more efficiently on diluted (1/20) piggery effluent [28]. *T. obliquus* was inoculated in the different undiluted pretreated effluents at an optical density ($OD_{540\ nm}$) of 0.4 in 50 mL Erlenmeyer flasks with a working volume of 20 mL. Optimized parameters of EC, AS, PF, and CW for microalgae growth were used to compare each method, while Bristol synthetic medium and untreated PWW were used as controls. The pretreated effluents were further compared to diluted (1:2) PWW. The dilution was prepared with tap water. The cultures were maintained at 150 rpm and room temperature (23–25 °C) under constant fluorescent light (41 $\mu E \cdot m^{-2} \cdot s^{-1}$). The cultivation period was 15 days. All experiments were conducted in duplicate.

2.7. Culture Scale-Up

The microalga cultures that grew in the pretreated effluents (PWW-PF and PWW-CW) were scaled-up to 1 L bubble-column PBRs, with a working volume of 800 mL. *T. obliquus* was inoculated at 0.1 g $AFDW \cdot L^{-1}$, and synthetic medium (Bristol) was used as the control. Microalga cultures were maintained at room temperature (23–25 °C), under continuous fluorescent light (50 $\mu E \cdot m^{-2} \cdot s^{-1}$) and with an airflow of 0.6 vvm. The cultivation period was 16 days. Growth was monitored by daily measurements of $OD_{540\ nm}$, pH, and quantum yield (Qy) measured with the AquaPen-C AP 110-C (PSI, Drásov, Czech Republic). Qy corresponds to the photosynthetic efficiency of photosystem II in a dark-adapted state (Fv/Fm). Effects of pH control and weekly harvesting were then studied in PWW-PF cultures. pH was increased to 7 every time it fell below 5, according to daily measurements, using 1 M NaOH, while weekly harvesting was performed by centrifuging a certain volume of culture (4000× *g* for 5 min at 4 °C). The volume was determined on the basis of OD measurements at the harvesting time (cultivation cycles of 7 days) to assure a similar initial OD (around 0.2) in all the growing cycles, and the supernatant was returned to the reactor prior to the next growing cycle. In the end, average volumetric productivity (P_X) and removal efficiencies (RE) were calculated. All experiments were conducted in duplicate.

2.8. Wastewater Characterization

To calculate the RE of the tested pretreatment methodologies, the initial PWW and the pretreated effluents were characterized in terms of ammonia nitrogen, phosphorus, and COD, according to standard methods [29]. The characterization was always performed prior to and immediately after the pretreatment methodologies and microalga cultivation. Ammonium nitrogen was quantified using an ion-selective electrode Crison code: 96 63, using a Crison-Multimeter MM41. A commercial kit was used for the measurement of phosphorus (Phosver 3-Powder Pillows, Cat. 2125-99, HACH) using a HACH DR/2010 spectrophotometer, at 890 nm. COD determination was carried out according to the open reflux method—Method 5220-B [29]. Total suspended solids (TSS) were determined according to the standard methods—Method 2540-D [29]. Color was estimated by measuring the effluent absorbance at 540 nm, which was chosen after an initial absorbance scan over a wavelength range of 200–800 nm.

3. Results and Discussion

3.1. Electrocoagulation (EC)

According to Table 1, COD removal efficiencies of 35–41% were found for Zn electrodes, while TSS removal was 20.1% for 4 $mA \cdot cm^{-2}$. Al electrodes showed COD removals of 8–18%, while Fe electrodes obtained values of 6–22%. Contrarily, negative TSS RE values in Al and Fe electrodes were associated with higher TSS after EC pretreatment, further increasing with higher current density. This could be a result of electrode corrosion, as increased current leads to the release of higher Al^{3+} and Fe^{2+} contents. The COD removal can be attributed to the formation of hydroxides or polyhydroxides which coagulate and adsorb the pollutants [30]. Higher COD removals (50%) were found in the literature using Fe electrodes to treat PWW but at higher current densities (30 mA·cm^{-2}) [31]. The same study [31] reported low ammonia removals (3–10%) using Fe and Al electrodes, which was also obtained in the present work. This could be explained because $NH_4{}^+$ exists in the form of small molecules, which is not easy to flocculate [31]. Some authors reported the need for longer electrolysis time (up to 5 h) to increase turbidity and ammonia removal [32], while others reported efficient ammonia removal, but large differences were found among the published results [33]. EC is a very complex system with the dominant oxidation mechanism depending on the chemical composition, pH, anode material, chloride concentration, or operation conditions [34]. Synergic interactions between chemical and physical phenomena could also worsen EC operation, which is especially an issue in more complex wastewater [35].

Table 1. Removal efficiencies (%) of chemical oxygen demand (COD) and total suspended solids (TSS) obtained after electrocoagulation with different electrode materials and current densities.

Conditions		Removal Efficiency (%)	
Electrode Material	**Current Density (mA·cm^{-2})**	**COD**	**TSS**
Al	4	18.4 ± 2.6	−31.6 ± 2.2
	12	7.9 ± 2.6	−56.9 ± 0.0
	20	13.2 ± 2.6	−56.2 ± 7.9
Fe	4	6.0 ± 3.2	−5.4 ± 0.0
	12	9.3 ± 6.4	−19.2 ± 4.3
	20	22.2 ± 6.5	−33.0 ± 2.6
Zn	4	34.7± 3.1	20.1 ± 2.2
	12	37.8 ± 0.0	7.1 ± 2.2
	20	40.9 ± 3.1	9.3 ± 0.0

3.2. Ammonia Stripping (AS)

The pretreatment by AS was tested to reduce the toxic levels of ammonia in PWW. The effects of pH and temperature on the removal of ammonia are shown in Figure 4. As expected, the RE increased with pH, since alkaline environments shift the chemical equilibrium from $NH_4{}^+$ to gaseous NH_3, which is more volatile [13]. Thus, when the pH was raised from 9 to 11, ammonia RE increased to 30%. Moreover, temperature also had a positive effect on ammonia RE due to increased NH_3 solubility. However, this was less pronounced at pH 11, as raising the temperature from 25 to 44 °C only increased RE by 4% (67.8 ± 1.4% and 71.7 ± 2.6%, respectively). This may not be significant enough to justify the cost increment and technical difficulties associated with heating [12]. Thus, the pretreated effluent obtained by AS at pH 11 and 25 °C (room temperature) was selected to pursue microalga cultivation. However, the ammonium levels in this pretreated effluent were still higher than the general limit of toxicity for microalgae (around 150 mg·L^{-1}) [36]. Nonetheless, a dilution of at least 1:2 could be enough to overcome this limitation, which is far better than the previous dilution factors used by Ferreira et al. (2021) [28] and the literature in general (e.g., García et al., 2017 [2]; Godos et al., 2010 [37]; Molinuevo-Salces et al., 2016 [38]).

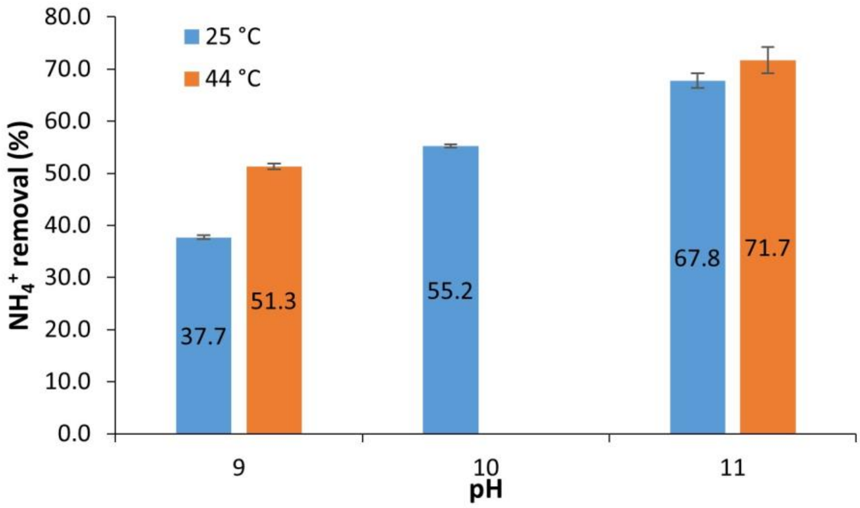

Figure 4. Ammonia removal efficiencies obtained after ammonia stripping with different pH and temperature. Values are presented as the mean ± mean deviation (*n* = 2).

3.3. Photo-Fenton (PF)

The main objective of photo-Fenton as a pretreatment was to decrease PWW color and organic load so that light could more easily penetrate the microalga culture and allow autotrophic growth, which was achieved. According to Table 3, for all tested conditions, the RE of color and COD was higher than 78% and 80%, respectively, showing the potential of PF to reduce the organic matter, which consequently decreased the effluent's color, generating clear effluents with a yellowish color. Moreover, the RE of TSS was higher than 85%, achieving near-complete removal (>98.5%) for the condition with the highest concentration of iron (1 g $Fe^{2+}\cdot L^{-1}$). These high RE values are due to the combination of oxidation with iron precipitation when pH is increased back to 7.0. The formation of denser flocs allows them to be better retained in the filter paper upon filtration, which generates a much clearer effluent. The RE of ammonia, on the other hand, was negligible in all tested conditions (below 10%), which could be due to low H_2O_2. Indeed, according to some authors, only at very high H_2O_2 concentration is it possible to degrade ammonia and generate N_2 and nitrate [39,40]. Moreover, organic nitrogen can be decomposed to form products such as molecular nitrogen, nitrate, nitrite, or ammonia, which could result in an increase in the latter in the PF pretreated wastewater [41].

According to residual H_2O_2 determinations, only at 1 g $Fe^{2+}\cdot L^{-1}$ was it possible to obtain a high decomposition of H_2O_2. Iron is an important factor for the PF reaction as it acts as a catalyst to decompose H_2O_2 and generate $OH^{\bullet}$ radicals. Furthermore, near-complete removal of H_2O_2 is indispensable because values higher than 10 $mg\cdot L^{-1}$ are toxic to microalgae [42]. In fact, the reaction is highly dependent on the Fe^{2+}/H_2O_2 ratio, which is why this is one of the most important parameters to consider when optimizing the process. Thus, looking at the residual H_2O_2 concentration (Table 2), Fe^{2+} plays a key role in H_2O_2 consumption, and only at the highest Fe^{2+} concentration was there complete consumption of H_2O_2 regardless of concentration. For lower Fe^{2+} concentrations, H_2O_2 was only half consumed at the dosage of 10.5 $g\cdot L^{-1}$.

Table 2. Removal efficiencies (%) after photo-Fenton (pH 3 and 120 min) in the tested conditions (Fe^{2+} and H_2O_2 concentrations) for color (given by absorbance measured at 540 nm), ammonia (NH_4^+), chemical oxygen demand (COD), and total suspended solids (TSS). The residual H_2O_2 present at the end of each run is also shown.

Conditions		Removal Efficiency (%)				Residual H_2O_2 ($g{\cdot}L^{-1}$)
Fe^{2+} ($g{\cdot}L^{-1}$)	H_2O_2 ($g{\cdot}L^{-1}$)	Color	COD	TSS	NH_4^+	
0.2	1.0	81.5 ± 5.0	82.3 ± 0.8	91.7 ± 0.5	7.99 ± 1.76	0.26
	4.9	83.3 ± 5.3	27.4 ± 0.0	85.0 ± 1.3	9.89 ± 2.57	3.21
	10.5	78.3 ± 1.2	22.3 ± 0.0	90.1 ± 0.8	3.76 ± 2.57	3.54
0.5	1.0	91.3 ± 1.6	84.8 ± 0.0	93.1 ± 0.1	2.03 ± 0.58	0.14
	4.9	92.2 ± 4.4	89.4 ± 0.4	92.7 ± 0.3	−1.29 ± 0.06	1.43
	10.5	88.9 ± 8.3	84.4 ± 1.3	93.4 ± 0.4	−4.13 ± 1.70	4.17
1.0	1.0	94.5 ± 1.1	88.7 ± 0.2	99.9 ± 0.1	−7.07 ± 0.52	0.26
	4.9	96.9 ± 0.7	91.6 ± 0.5	100 ± 0	−1.97 ± 0.29	0.30
	10.5	97.2 ± 0.5	92.6 ± 0.5	98.5 ± 0.1	0.69 ± 0.30	0.16

3.4. Microalga Growth in the Pretreated Effluents

3.4.1. Pretreatment Screening

The growth of the microalga *T. obliquus* was evaluated on each optimized pretreatment methodology (electrocoagulation, ammonia stripping, photo-Fenton, and constructed wetlands). Table 3 shows the composition of the pretreated effluents used for this experiment:

(i) Electrocoagulation using zinc electrodes and current density of 20 $mA{\cdot}cm^{-2}$.
(ii) Ammonia stripping at room temperature (25 °C) and initial pH of 11.
(iii) Photo-Fenton using 1.0 g $Fe^{2+}{\cdot}L^{-1}$ and 10.5 g $H_2O_2{\cdot}L^{-1}$.
(iv) Constructed wetlands using expanded clay as substrate and *P. australis*.

Table 3. Composition of the piggery wastewater (PWW) after the different pretreatment methodologies (EC—electrocoagulation; AS—ammonia stripping; PF—photo-Fenton; CW—constructed wetland) compared to raw PWW and diluted 1:20, in terms of chemical oxygen demand (COD), ammonia nitrogen (NH_4^+), phosphate (PO_4^{3-}), and color given by the absorbance at 540 nm. The pH was adjusted to 7 in all media before microalga inoculation.

PWW	COD ($mg\ O_2{\cdot}L^{-1}$)	NH_4^+ ($mg{\cdot}L^{-1}$)	PO_4^{3-} ($mg{\cdot}L^{-1}$)	TSS ($mg{\cdot}L^{-1}$)	Color
Raw	3759 ± 71	1500 ± 7	97.5	2575 ± 45	6.640
1:20	184.4 ± 7.1	64.6 ± 1.05	6.40	90.0 ± 0.00	0.300
AS	2766 ± 71	731.2 ± 1.1	81.0	1285 ± 125	2.830
EC	1489 ± 71	1329 ± 12	37.5	207.5 ± 27.5	1.070
PF	276.6 ± 14.2	1209 ± 11	0.560	37.5 ± 2.5	0.019
CW	319.1 ±7.1	122.6 ± 2.5	77.0	52.5 ± 2.5	0.157

Table 3 shows the initial composition of the various wastewaters used for cultivating *T. obliquus*. Except for ammonia, PF was the pretreatment that most decreased the contaminant load from the effluent, being very efficient in removing COD (92.6%) and color (99.7%). This method was also highly effective in removing phosphate (99.4%), mainly through precipitation with iron. However, this might not be so beneficial for microalgae growth since phosphorus is an essential macronutrient. CW allowed the highest reduction in ammonia (82%), but it must be considered that a dilution of 1:2.5 was applied to this effluent during the pretreatment process, decreasing the initial ammonia concentration to around 675 mg/L [27]. AS also allowed reductions in ammonia, TSS, and COD by 51.2%, 50.1%, and 26.4%. Lastly, EC was the least efficient method for all the evaluated parameters.

As can be seen in Figure 5, *T. obliquus* did not grow in undiluted PWW or AS and EC pretreated effluents. This observation was expected since the RE values on both processes were relatively low. In PWW pretreated with CW, there was an initial lag phase of 7 days, but growth started at a good rate, surpassing that in Bristol medium and 1:20 diluted PWW. The effluent pretreated with PF allowed the highest growth rate of all the media, presenting a steady growth throughout the cultivation period. By the 15th day, the microalga growing in CW came very close to the OD values of PF.

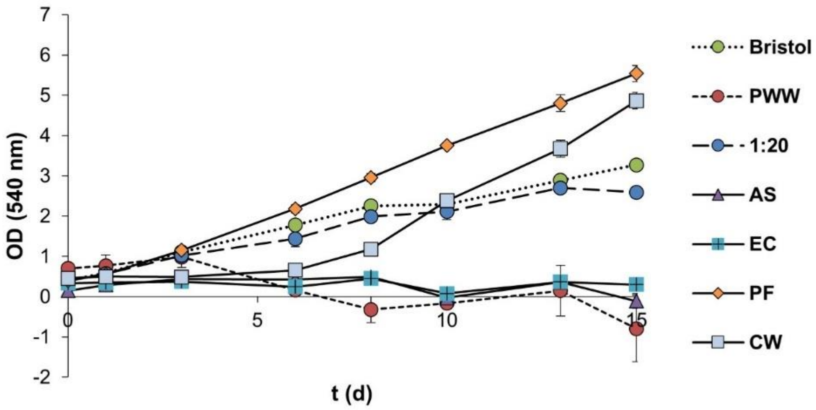

Figure 5. Growth curves for *Tetradesmus obliquus* cultivated in the pretreated piggery wastewaters: AS—ammonia stripping, EC—electrocoagulation, PF—photo-Fenton, and CW—constructed wetlands. Bristol medium, untreated PWW, and diluted (1:20) effluents were used for comparison. Values are presented as the mean ± mean deviation (n = 2).

3.4.2. Culture Scale-Up

Since CW and PF pretreatments allowed the growth of *T. obliquus*, further growth experiments were performed in 1 L bubble columns (Figure 6). *T. obliquus* achieved a final concentration of 1.46 ± 0.06 g·L^{-1} in PF, followed by 1.39 ± 0.32 g·L^{-1} in CW, compared to 0.92 ± 0.00 g·L^{-1} in Bristol medium (Figure 6a). Regarding the pH (Figure 6b), it increased with the microalga growing in CW, similarly to the Bristol medium. In PF effluent, on the other hand, the pH decreased to 6. This can be explained by the proton translocation out of the microalgal cell during ammonium uptake to maintain cell neutrality [43]. This could justify why *T. obliquus* can grow at high ammonia levels since, in this pH range, it is mostly in the form of NH_4^+. Although this was not the optimum pH range, the microalga achieved higher concentrations in PF effluent compared to the synthetic medium. A sudden drop in Qy suggested that the pretreated effluents first impacted the *T. obliquus* metabolism, but the cultures recovered by day 7, maintaining similar Qy levels to the culture in synthetic medium from then on (Figure 6c). Lastly, regarding wastewater treatment, *T. obliquus* could remove 37.3 ± 1.7% of NH_4^+ and 48.6 ± 1.7% of COD in PF effluent, while it reached complete NH_4^+ removal (99.8 ± 0.0%) and 19.5% ± 0.0% removal of COD in CW effluent (Figure 6d). However, it should be considered that both effluents differed significantly with respect to the starting levels of ammonia (CW had only about 10% of the ammonia present in PF). Furthermore, one should also consider the pH increase derived from the microalga growth in CW effluent, which promoted ammonia volatilization, contributing to its removal. In contrast, for PF, the removal could be completely attributed to the microalga assimilation.

Thus, as the pH drop did not significantly affect *T. obliquus* growth in the PF effluent, a more controlled pH could minimize contamination risks, especially from cyanobacteria that proliferate in alkaline pH values [44,45]. This could favor the production of monocultures, which is very difficult to achieve when cultivating in wastewater. In addition, PF can promote the removal of pathogens (due to the application of H_2O_2 and UV radiation), which could potentially decrease the downstream process costs involving disinfection of the biomass.

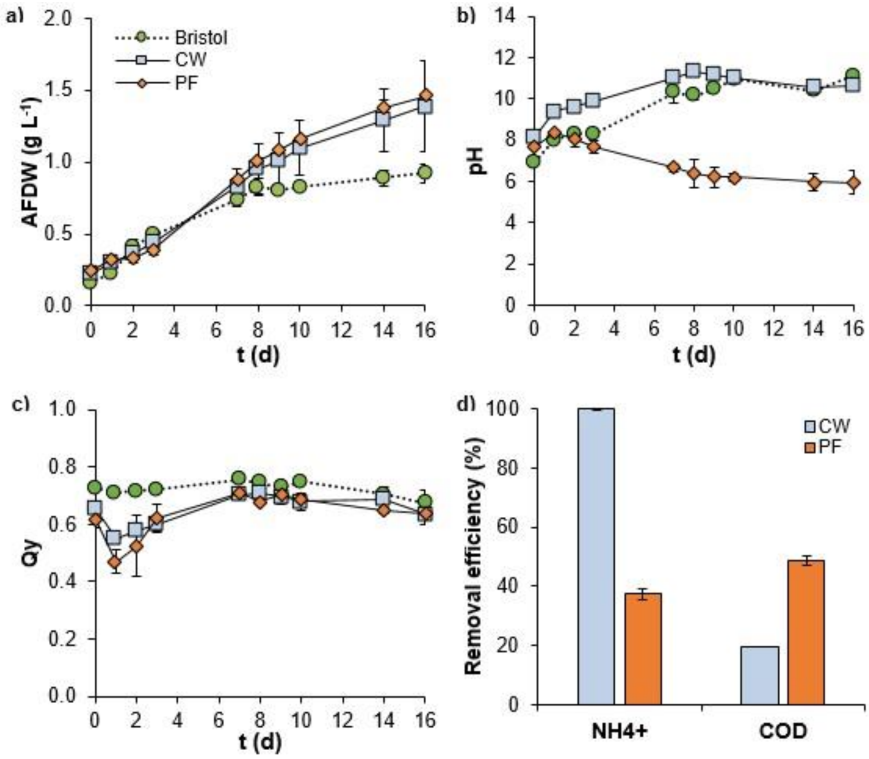

Figure 6. (**a**) Growth curve in ash-free dry weight (AFDW), (**b**) pH, (**c**) quantum yield (Qy), and (**d**) Removal efficiency of ammonia (NH_4^+) and chemical oxygen demand (COD) of *Tetradesmus obliquus* cultivation in piggery wastewater pretreated by photo-Fenton (PF) and constructed wetland (CW). Bristol medium was used as the control. Values are presented as the mean ± mean deviation (*n* = 2).

The microalga *T. obliquus* was shown to efficiently grow in the PF-treated effluent. Yet, the ammonia removal efficiency was rather low after 16 days, and the ammonia levels were still excessive according to Portuguese legislation [46]. Hence, to overcome this, some optimization strategies were applied to enhance microalga growth (Figure 7) and nutrient removal in the PF effluent (Figure 8), including pH control and weekly harvesting to overcome possible cell shadowing at higher concentrations.

Controlling the pH between 5 and 7 (PF-pH; Figure 7c) seemed to have a positive effect on the microalga growth (Figure 7a) and quantum yield (Figure 7e). After 9 days, the Qy started to decrease in all conditions, but the pH control (PF-pH) allowed the culture to maintain a similar Qy to the Bristol culture (Figure 7e). While the *T. obliquus* grown in PF (66.4 ± 17.8 $mg \cdot L^{-1} \cdot day^{-1}$) achieved similar productivity in Bristol medium (60.1 ± 10.4 $mg \cdot L^{-1} \cdot day^{-1}$), with pH control (PF-pH), it almost doubled (116.5 $mg \cdot L^{-1} \cdot day^{-1}$) (Figure 8a). The improvement verified in the microalga growth can be explained by the fact that the maintenance of a more controlled pH prevented chloroplast acidification, which can inhibit photosynthesis and microalgal growth [47].

On the other hand, weekly harvesting allowed two cycles of growth of 7 days each (first cycle: 7 days; second cycle: 7–14 days). No growth was verified at the third cycle (14–21 days) (Figure 7b). Without pH control, the average productivity was 122.1 ± 3.1 $mg \cdot L^{-1} \cdot day^{-1}$ at the first cycle and 85.1 ± 7.6 $mg \cdot L^{-1} \cdot day^{-1}$ at the second cycle (Figure 8a). With pH control, the productivities were similar, 123.2 ± 8.3 and 89.0 ± 6.0 $mg \cdot L^{-1} \cdot day^{-1}$ at the first and second cycles, respectively (Figure 8a). The marginal effect of the combination of pH control with weekly harvesting in the growth of *T. obliquus* might be related to similar pH values among both conditions (PF-H and PF-pH + H), which meant that it was only necessary to adjust the pH was at the beginning of each cycle (Figure 7d). Likewise, a high Qy was maintained in the first two cycles and did not differ appreciably among both conditions. By the third cycle, however, it decreased drastically, where no growth was observed (Figure 7f). Moreover, the cultures started to change color to yellow, associated with the production of carotenoids, possibly due to nutrient limitation (phosphorus).

As already mentioned, pH control was highly effective in improving average productivity (Figure 8a). Furthermore, weekly harvesting (PF-H and PF-pH + H) allowed the microalga to grow at least two times in the same effluent, achieving productivities that, when added to the previous cycle, allowed a bigger production of biomass. This suggests that PF effluent can be recycled at least once for microalga cultivation. Regarding removal efficiencies, pH control alone did not affect pollutant removal (Figure 8b). The weekly harvesting, however, seemed to have a more significant effect (from 29 ± 7% to 43 ± 3% for ammonia and from 22 ± 1% to 39 ± 6% for COD). The removal efficiencies were further improved when both strategies are applied (50 ± 3% for ammonia, 46 ± 6 % for COD) (Figure 8b). In addition, light intensities higher than 50 $\mu E \cdot m^{-2} \cdot s^{-1}$ could further promote the microalga growth as shown by some studies where improved microalga productivity and biomass composition were observed upon increasing light intensity to 150 and 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ [48], as well as under solar radiation [49].

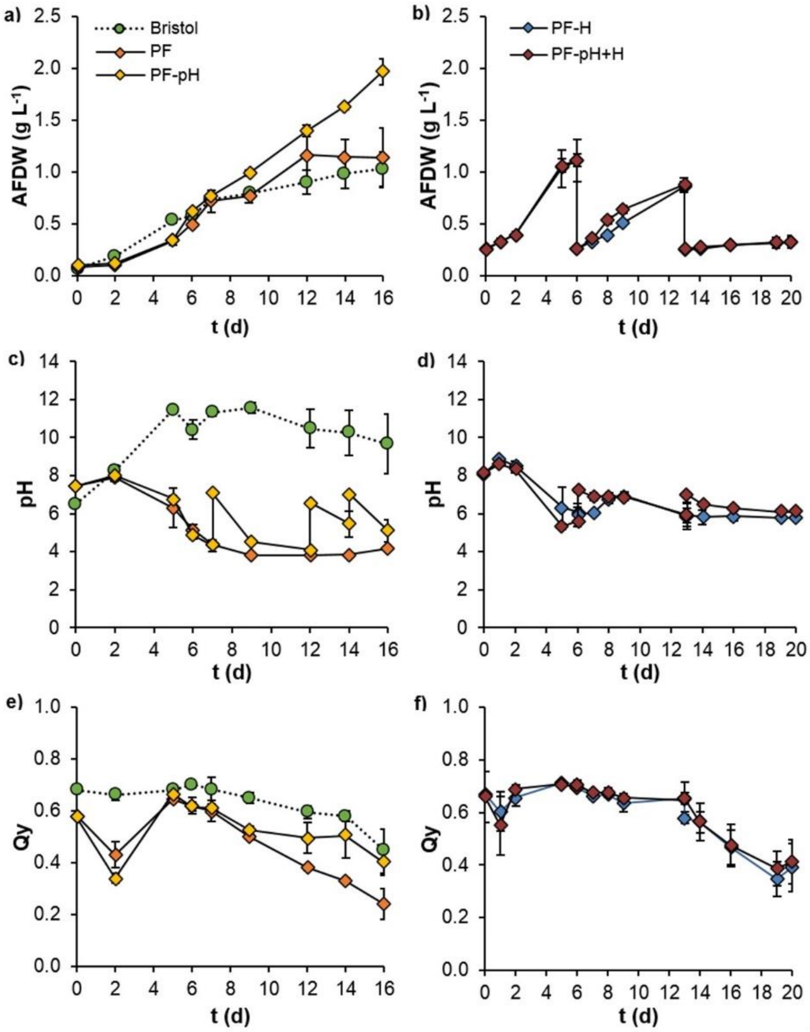

Figure 7. (**a**,**b**) Growth curves in ash-free dry weight (AFDW), (**c**,**d**) pH, and (**e**,**f**) quantum yield (Qy) of *Tetradesmus obliquus* in piggery wastewater pretreated by Photo-Fenton with combined optimization strategies (PF: no pH control or weekly harvesting; PF-pH: only pH control; PF-H: only weekly harvesting; PF-pH + H: pH control and weekly harvesting). Bristol medium was used as the control. Values are presented as mean ± mean deviation (n = 2).

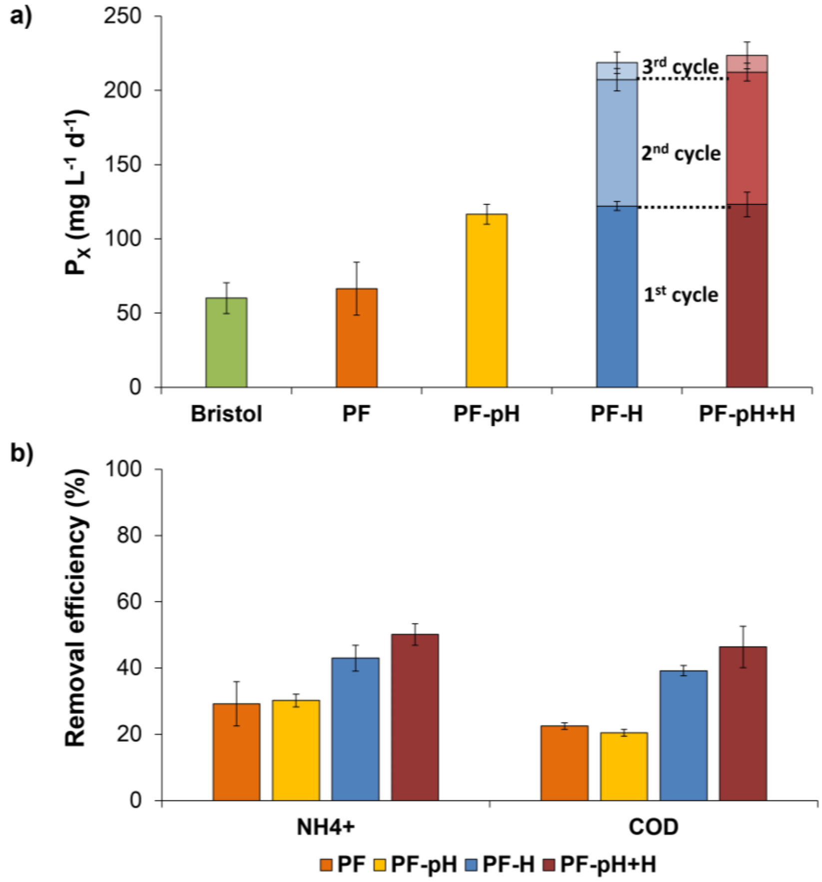

Figure 8. (**a**) Average biomass productivity and (**b**) removal efficiencies of ammonia (NH_4^+) and chemical oxygen demand (COD) after growth of *Tetradesmus obliquus* grown in piggery wastewater pre-treated by Photo-Fenton with combined optimization strategies (PF: no pH control or weekly harvesting; PF-pH: only pH control; PF-H: only weekly harvesting; PF-pH+H: pH control and weekly harvesting). Bristol medium was used as the control. Values are presented as the mean ± mean deviation ($n = 2$).

4. Conclusions

Microalgae are promising tools for recovering nutrients from wastewaters, providing clean water and valuable biomass that can be applied in agriculture to reduce water and fertilizer requirements, respectively. However, the high concentration of pollutants in wastewater from specific sources, such as pig farms, can inhibit their growth. Hence, the optimization of these systems by introducing an adjacent chemical process to improve the main treatment with microalgae could be very promising and a sustainable solution to a better management of piggery wastewater with potential nutrient recovery for agricultural use. Photo-Fenton is a rapid process that can partially or completely oxidize organic substances, minimizing their toxicity for the subsequent treatment with microalgae. This work revealed that PF was the best pretreatment for piggery wastewater, as it not only enhanced *Tetradesmus obliquus* productivity but could also promote biomass safety and quality for sustainable agriculture. The possibility of generating value from the wastes generated in the pig farms that can be injected back into the agricultural production system is a favorable strategy and an important part of the concept of the circular economy.

Author Contributions: Conceptualization, A.F. and L.G.; methodology, A.F., D.F., R.C., R.N., F.F. and B.R.; validation, A.F., D.F. and L.G.; formal analysis, A.F. and L.G.; investigation, A.F., D.F., R.C., R.N., F.F. and B.R.; data curation, A.F., D.F., R.C. and R.N.; writing—original draft preparation, A.F.; writing—review and editing, A.F., D.F., B.R., C.M.d.S.C., F.G.A. and L.G.; supervision, C.M.d.S.C.,

F.G.A. and L.G.; funding acquisition, L.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the project ALGAVALOR—Lisboa-01-0247-FEDER-035234, supported by the Operational Program for Competitiveness and Internationalization (COMPETE2020), by the Lisbon Portugal Regional Operational Program (Lisboa 2020), and by the Algarve Regional Operational Program (Algarve 2020) under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF); by the Biomass and Bioenergy Research Infrastructure (BBRI)—LISBOA-01-0145-FEDER-022059, supported by the Operational Program for Competitiveness and Internationalization (PORTUGAL2020), by the Lisbon Portugal Regional Operational Program (Lisboa 2020), and by the North Portugal Regional Operational Program (Norte 2020) under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF); and by the Red CYTED P319RT0025—RENUWAL—Red Iberoamericana para el Tratamiento de Efluentes con Microalgas. Alice Ferreira is pleased to acknowledge her PhD grant no. SFRH/BD/144122/2019 awarded by Fundação para a Ciência e Tecnologia.

Acknowledgments: The authors would like to thank Valorgado's owners for allowing the access to their facilities to collect the effluent. The authors would also like to thank Graça Gomes and Natércia Sousa (LNEG) for laboratorial assistance and maintenance of the microalgae cultures.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Eurostat Number of Pigs 2009–2019. Available online: https://ec.europa.eu/eurostat/databrowser/view/tag00018/default/table?lang=en (accessed on 2 September 2020).
2. García, D.; Posadas, E.; Grajeda, C.; Blanco, S.; Martínez-Páramo, S.; Acién, G.; García-Encina, P.; Bolado, S.; Muñoz, R. Comparative evaluation of piggery wastewater treatment in algal-bacterial photobioreactors under indoor and outdoor conditions. *Bioresour. Technol.* **2017**, *245*, 483–490. [CrossRef] [PubMed]
3. McGahan, E.J.; Phillips, F.A.; Wiedemann, S.G.; Naylor, T.A.; Warren, B.; Murphy, C.M.; Griffith, D.W.T.; Desservettaz, M.; McGahan, E.J.; Phillips, F.A.; et al. Methane, nitrous oxide and ammonia emissions from an Australian piggery with short and long hydraulic retention-time effluent storage. *Anim. Prod. Sci.* **2016**, *56*, 1376–1389. [CrossRef]
4. Nagarajan, D.; Kusmayadi, A.; Yen, H.W.; Dong, C.D.; Lee, D.J.; Chang, J.S. Current advances in biological swine wastewater treatment using microalgae-based processes. *Bioresour. Technol.* **2019**, *289*, 121718. [CrossRef] [PubMed]
5. Ayre, J.M.; Moheimani, N.R.; Borowitzka, M.A. Growth of microalgae on undiluted anaerobic digestate of piggery effluent with high ammonium concentrations. *Algal Res.* **2017**, *24*, 218–226. [CrossRef]
6. Salbitani, G.; Carfagna, S. Ammonium Utilization in Microalgae: A Sustainable Method for Wastewater Treatment. *Sustainability* **2021**, *13*, 956. [CrossRef]
7. Azov, Y.; Goldman, J.C. Free Ammonia Inhibition of Algal Photosynthesis in Intensive Cultures. *Appl. Environ. Microbiol.* **1982**, *43*, 735–739. [CrossRef] [PubMed]
8. Tahreen, A.; Jami, M.S.; Ali, F. Role of electrocoagulation in wastewater treatment: A developmental review. *J. Water Process Eng.* **2020**, *37*, 101440. [CrossRef]
9. Cerqueira, A.; Russo, C.; Marques, M.R.C. Electroflocculation for textile wastewater treatment. *Brazilian J. Chem. Eng.* **2009**, *26*, 659–668. [CrossRef]
10. Sahu, O.; Mazumdar, B.; Chaudhari, P.K. Treatment of wastewater by electrocoagulation: A review. *Environ. Sci. Pollut. Res.* **2014**, *21*, 2397–2413. [CrossRef] [PubMed]
11. Moussa, D.T.; El-Naas, M.H.; Nasser, M.; Al-Marri, M.J. A comprehensive review of electrocoagulation for water treatment: Potentials and challenges. *J. Environ. Manag.* **2017**, *186*, 24–41. [CrossRef]
12. Kinidi, L.; Tan, I.A.W.; Abdul Wahab, N.B.; Tamrin, K.F.B.; Hipolito, C.N.; Salleh, S.F. Recent Development in Ammonia Stripping Process for Industrial Wastewater Treatment. *Int. J. Chem. Eng.* **2018**, *2018*, 3181087. [CrossRef]
13. Folino, A.; Calabrò, P.S.; Zema, D.A. Effects of Ammonia Stripping and Other Physico-Chemical Pretreatments on Anaerobic Digestion of Swine Wastewater. *Energies* **2020**, *13*, 3413. [CrossRef]
14. Folino, A.; Zema, D.A.; Calabrò, P.S. Environmental and Economic Sustainability of Swine Wastewater Treatments Using Ammonia Stripping and Anaerobic Digestion: A Short Review. *Sustainability* **2020**, *12*, 4971. [CrossRef]
15. Kim, E.J.; Kim, H.; Lee, E. Influence of Ammonia Stripping Parameters on the Efficiency and Mass Transfer Rate of Ammonia Removal. *Appl. Sci.* **2021**, *11*, 441. [CrossRef]
16. Zhang, L.; Lee, Y.W.; Jahng, D. Ammonia stripping for enhanced biomethanization of piggery wastewater. *J. Hazard. Mater.* **2012**, *199–200*, 36–42. [CrossRef]
17. Jain, B.; Singh, A.K.; Kim, H.; Lichtfouse, E.; Jain, B.; Singh, A.K.; Kim, H.; Lichtfouse, E.; Treatment, V.S. Treatment of organic pollutants by homogeneous and heterogeneous Fenton reaction processes. *Environ. Chem. Lett.* **2019**, *16*, 947–967. [CrossRef]

18. Sánchez Pérez, J.A.; Román Sánchez, I.M.; Carra, I.; Cabrera Reina, A.; Casas López, J.L.; Malato, S. Economic evaluation of a combined photo-Fenton/MBR process using pesticides as model pollutant. Factors affecting costs. *J. Hazard. Mater.* **2013**, *244–245*, 195–203. [CrossRef]
19. Babuponnusami, A.; Muthukumar, K. A review on Fenton and improvements to the Fenton process for wastewater treatment. *J. Environ. Chem. Eng.* **2014**, *2*, 557–572. [CrossRef]
20. Pani, N.; Tejani, V.; Anantha-Singh, T.S.; Kandya, A. Simultaneous removal of COD and Ammoniacal Nitrogen from dye intermediate manufacturing Industrial Wastewater using Fenton oxidation method. *Appl. Water Sci.* **2020**, *10*, 1–7. [CrossRef]
21. Rueda-Márquez, J.J.; Levchuck, I.; Manzano, M.; Sillanpää, M. Toxicity Reduction of Industrial and Municipal Wastewater by Advanced Oxidation Processes (Photo-Fenton, UVC/H2O2, Electro-Fenton and Galvanic Fenton): A review. *Catalysts* **2020**, *10*, 612. [CrossRef]
22. Soriano-Molina, P.; Plaza-Bolaños, P.; Lorenzo, A.; Agüera, A.; García Saánchez, J.L.; Malato, S.; Sánchez Pérez, J.A. 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.* **2019**, *366*, 141–149. [CrossRef]
23. Abbasi, H.N.; Xie, J.; Hussain, S.I.; Lu, X. Nutrient removal in hybrid constructed wetlands: Spatial-seasonal variation and the effect of vegetation. *Water Sci. Technol.* **2019**, *79*, 1985–1994. [CrossRef] [PubMed]
24. Srivastava, P.; Yadav, A.K.; Garaniya, V.; Abbassi, R. Constructed wetland coupled microbial fuel cell technology: Development and potential applications. In *Biomass, Biofuels, Biochemicals: Microbial Electrochemical Technology: Sustainable Platform for Fuels, Chemicals and Remediation*; Elsevier: Amsterdam, The Netherlands, 2018; pp. 1021–1036. ISBN 9780444640529.
25. Harrington, C.; Scholz, M. Assessment of pre-digested piggery wastewater treatment operations with surface flow integrated constructed wetland systems. *Bioresour. Technol.* **2010**, *101*, 6950–6960. [CrossRef] [PubMed]
26. Nuamah, L.A.; Li, Y.; Pu, Y.; Nwankwegu, A.S.; Haikuo, Z.; Norgbey, E.; Banahene, P.; Bofah-Buoh, R. Constructed wetlands, status, progress, and challenges. The need for critical operational reassessment for a cleaner productive ecosystem. *J. Clean. Prod.* **2020**, *269*, 122340. [CrossRef]
27. Dias, S.; Mucha, A.P.; Duarte Crespo, R.; Rodrigues, P.; Almeida, C.M.R. Livestock Wastewater Treatment in Constructed Wetlands for Agriculture Reuse. *Int. J. Environ. Res. Public Health* **2020**, *17*, 8592. [CrossRef] [PubMed]
28. Ferreira, A.; Melkonyan, L.; Carapinha, S.; Ribeiro, B.; Figueiredo, D.; Avetisova, G.; Gouveia, L. Biostimulant and biopesticide potential of microalgae growing in piggery wastewater. *Environ. Adv.* **2021**, *4*, 100062. [CrossRef]
29. *APHA Standard Methods for the Examination of Water and Wastewater*, 20th ed.; American Public Health Association: Washington, DC, USA, 1998; Volume 51, ISBN 0002-9572.
30. Ghernaout, D.; Ghernaout, B.; Kellil, A. Natural organic matter removal and enhanced coagulation as a link between coagulation and electrocoagulation. *New Pub Balaban* **2012**, *2*, 203–222. [CrossRef]
31. Chen, R.F.; Wu, L.; Zhong, H.T.; Liu, C.X.; Qiao, W.; Wei, C.H. Evaluation of electrocoagulation process for high-strength swine wastewater pretreatment. *Sep. Purif. Technol.* **2021**, *272*, 118900. [CrossRef]
32. Lei, X.; Maekawa, T. Electrochemical treatment of anaerobic digestion effluent using a Ti/Pt–IrO_2 electrode. *Bioresour. Technol.* **2007**, *98*, 3521–3525. [CrossRef]
33. Lourinho, G.; Brito, P.S.D. Electrolytic Treatment of Swine Wastewater: Recent Progress and Challenges. *Waste Biomass Valorization* **2020**, *12*, 553–576. [CrossRef]
34. Yavuz, Y.; Ögütveren, Ü.B. Treatment of industrial estate wastewater by the application of electrocoagulation process using iron electrodes. *J. Environ. Manag.* **2018**, *207*, 151–158. [CrossRef]
35. Holt, P.; Barton, G.; Mitchell, C. Electrocoagulation as a wastewater treatment. *Third Annu. Aust. Environ. Eng. Res. Event* **1999**, *1000*, 41–46.
36. Collos, Y.; Harrison, P.J. Acclimation and toxicity of high ammonium concentrations to unicellular algae. *Mar. Pollut. Bull.* **2014**, *80*, 8–23. [CrossRef] [PubMed]
37. Godos, I.d.; Vargas, V.A.; Blanco, S.; González, M.C.G.; Soto, R.; García-Encina, P.A.; Becares, E.; Muñoz, R. A comparative evaluation of microalgae for the degradation of piggery wastewater under photosynthetic oxygenation. *Bioresour. Technol.* **2010**, *101*, 5150–5158. [CrossRef] [PubMed]
38. Molinuevo-Salces, B.; Mahdy, A.; Ballesteros, M.; González-Fernández, C. From piggery wastewater nutrients to biogas: Microalgae biomass revalorization through anaerobic digestion. *Renew. Energy* **2016**, *96*, 1103–1110. [CrossRef]
39. Goi, A.; Trapido, M. Hydrogen peroxide photolysis, Fenton reagent and photo-Fenton for the degradation of nitrophenols: A comparative study. *Chemosphere* **2002**, *46*, 913–922. [CrossRef]
40. Zoh, K.D.; Stenstrom, M.K. Fenton oxidation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). *Water Res.* **2002**, *36*, 1331–1341. [CrossRef]
41. Velásquez, M.; Santander, I.P.; Contreras, D.R.; Yáñez, J.; Zaror, C.; Salazar, R.A.; Pérez-Moya, M.; Mansilla, H.D. Oxidative degradation of sulfathiazole by Fenton and photo-Fenton reactions. *J. Environ. Sci. Health-Part A Toxic/Hazardous Subst. Environ. Eng.* **2014**, *49*, 661–670. [CrossRef]
42. Pulgarin, A.; Giannakis, S.; Pulgarin, C.; Ludwig, C.; Refardt, D. A novel proposition for a citrate-modified photo-Fenton process against bacterial contamination of microalgae cultures. *Appl. Catal. B Environ.* **2020**, *265*, 118615. [CrossRef]
43. Eustance, E.; Gardner, R.D.; Moll, K.M.; Menicucci, J.; Gerlach, R.; Peyton, B.M. Growth, nitrogen utilization and biodiesel potential for two chlorophytes grown on ammonium, nitrate or urea. *J. Appl. Phycol.* **2013**, *25*, 1663–1677. [CrossRef]

44. Mutoti, M.; Gumbo, J.; Jideani, A.I.O. Occurrence of cyanobacteria in water used for food production: A review. *Phys Chem Earth* **2022**, *125*, 103101. [CrossRef]
45. Ji, X.; Verspagen, J.M.H.; Stom, M.; Huisman, J. Competition between cyanobacteria and green algae at low versus elevated CO_2: Who will win, and why? *J. Exp. Bot.* **2017**, *68*, 3815–3828. [CrossRef] [PubMed]
46. Decree-Law No 236/98 Decree-Law 236/98 of the Portuguese Ministry of the Environment of 1 August establishing water quality standards. *Diário da República I Série-A* **1998**, 3676–3722.
47. Solovchenko, A.; Khozin-Goldberg, I. High-CO_2 tolerance in microalgae: Possible mechanisms and implications for biotechnology and bioremediation. *Biotechnol. Lett.* **2013**, *35*, 1745–1752. [CrossRef]
48. Nzayisenga, J.C.; Farge, X.; Groll, S.L.; Sellstedt, A. Effects of light intensity on growth and lipid production in microalgae grown in wastewater. *Biotechnol. Biofuels* **2020**, *13*, 1–8. [CrossRef]
49. Metsoviti, M.N.; Papapolymerou, G.; Karapanagiotidis, I.T.; Katsoulas, N. Effect of Light Intensity and Quality on Growth Rate and Composition of *Chlorella vulgaris*. *Plants* **2020**, *9*, 31. [CrossRef]