Scenedesmus obliquus in poultry wastewater bioremediation

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ABSTRACT
Wastewater biological treatment with microalgae can be an effective technology, removing nutrients and other contaminants while reducing chemical oxygen demand. This can be particularly interesting for the meat producing industry which produces large volumes of wastewater from the slaughtering of animals and cleaning of their facilities. The main purpose of this research was the treatment of poultry wastewater using Scenedesmus obliquus in an economical and environmentally sustainable way. Two wastewaters were collected from a Portuguese poultry slaughterhouse (poultry raw – PR and poultry flocculated – PF) and the bioremediation was evaluated. The performance of microalga biomass growth and biochemical composition were assessed for two illumination sources (fluorescent vs LEDs). S. obliquus achieved positive results when grown in highly contaminated agro-industrial wastewater from the poultry industry, independently of the light source. The wastewater bioremediation revealed results higher than 97% for both ammonium and phosphate removal efficiency, for a cultivation time of 13 days. The saponifiable matter obtained from the biomass of the microalga cultures was, on average, 11% and 27% (m/malga) with PR and PF wastewater, respectively. In opposition, higher sugar content was obtained from microalga biomass grown in PR wastewater (average 34% m/malga) in comparison to PF wastewater (average 23% m/malga), independently of the illumination source. Therefore, biomass obtained with PR wastewater will be more appropriate as a raw material for bioethanol/biohydrogen production (higher sugar content) while biomass produced in PF wastewater will have a similar potential as feedstock for both biodiesel and bioethanol/biohydrogen production (similar lipid and sugar content).

1. Introduction
Global food production is estimated to be responsible for between 20% and 50% of anthropogenic environmental impacts [1] with the livestock sector accounting for more than the direct emissions from the transport sector [2].

While meat consumption is projected to increase by 70% by 2050, chicken meat has become one of the most extensively consumed food products in the world [3]. Portugal ranks ninth in chicken production among European Union countries and broiler chicken represents 75% of the total meat consumption in this country [4]. Nevertheless, chicken has been described as the most environmentally friendly meat in several Life Cycle Assessment (LCA) studies (e.g. [1]). González-García et al. [4] studied a Portuguese broiler chicken production unit, namely the environmental impacts from the processes involved in a slaughterhouse (e.g. transport, packaging and waste treatment), with direct water emissions (e.g. P, COD) playing a significant impact in eutrophication potential. In fact, the meat processing sector produces large volumes of wastewaters due to the slaughtering of animals and cleaning of the slaughterhouse facilities and meat processing plants. This sector uses 24% of the total freshwater consumed by the food and beverage industry and up to 29% of that consumed by the agricultural sector worldwide [5]. In poultry processing, water is used primarily for feather removal (scalding), bird washing (before and after
evisceration), chilling, cleaning and sanitizing of equipment and facilities, and for cooling of mechanical equipment such as compressors and pumps [6].

Wastewater treatment is one of the greatest global challenges for the sustainable development of our society, being directly associated with the water-food-waste nexus. The volume and hazardous nature of wastewater have been increasing due to human activities as population continues to grow, and industrialization and urbanization, cause pressure on water resources and increase the unregulated or illegal discharge of contaminated water.

The conventional wastewater treatment processes include both physicochemical (e.g. flocculation) and biological (anaerobic and aerobic) processes, usually requiring many chemicals and are very high energy-demanding [7,8]. For conventional biological wastewater treatments such as the activated water sludge process, the mechanical aeration process represents 45–75% of the total operation cost [8], while anaerobic digestion does not provide an efficient removal of nutrients. Moreover, the sludge generated by these processes may lead to secondary pollution due to the use of chemicals, and there is the direct emission of greenhouse gases (GHG). Animal effluents from slaughterhouses are usually pre-treated (e.g. screening, sedimentation, blood and fat separation), followed by physicochemical treatments, mainly by coagulation/flocculation, and/or secondary biological treatment [5].

The use of algae for the removal of contaminants, namely nutrients (C, N and P), coliform bacteria, heavy metals and the reduction of chemical and biological oxygen demand, removal and/or degradation of xenobiotic compounds and other contaminants [7] guarantees remarkable advantages, such as the reduction of energy, emissions, costs and hazardous sludge formation. The algae provide the O₂ used by heterotrophic and autotrophic microorganisms to oxidize and/or assimilate the organic carbon as well as nitrogen and phosphorus. Likewise, the algal biomass generated in the process can be used as a feedstock for biofuel production [9–11], as well as, bioplastics, biofertilizer/soil conditioner and feed, among others.

Another important advantage of an algal-bacterial system comes from the possibility to integrate wastewater (WW) treatment with biogas upgrading or flue gas treatment, which contributes to mitigate greenhouse gas (GHG) emissions and to enhance nutrient recovery from WW [12].

There are many reported cases where microalgae have successfully performed the biological treatment of different types of wastewater, such as urban [9,13], agroindustry (e.g. potato and coffee processing, fish feed and yeast production) [14], brewery industry [15,16], dairy [17,18] and swine manure [19]. In the case of poultry industry effluents, published studies (e.g. [20–23]) have been almost exclusively dedicated to microalgae phycocremediation of the liquid effluent resulting from the anaerobic digestion of poultry litter (mix of bedding material, manure and feathers resulting from intensive poultry production) rather than on the direct nutrient removal of wastewaters (from e.g. scalding, washing and cleaning operations).

The combination of waste management (WW treatment), mitigation of GHG and microalgae biomass production constitutes a cost-efficient and environmentally friendly alternative to their traditional counterparts. According to Brennan and Owende [24], the conjunction of wastewater treatment and biofuel production is probably one of the most economically and environmentally sustainable ways to produce bio-energy and bio-products. However, technical improvements and an optimization of the process must be conducted to ensure its economic viability and sustainability prior to the implementation of this technology in conventional wastewater treatment plants (WWTPs) [12].

Light-emitting diodes (LEDs) could be the lighting of the next generation as they are long-lasting, mercury-free and fast-responding diodes, emitting nearly monochromatic light at different wavelengths [25]. LED lights increase both the photosynthetic efficiency for growing microalgae and the power conversion efficiency by enhancing the total biomass production per input energy [26,27]. In addition, LEDs can provide a more sustainable control of light during microalgal growth and manipulation of the biochemical composition of biomass compared to broadband light (e.g. fluorescent lamps) [28,29].

This study aims to develop a sustainable and economical approach, as much as possible, by using microalgae to treat the poultry wastewater, as an alternative to the very expensive flocculation step currently performed by the poultry industry. The experiments were performed indoor using artificial lighting to minimize the high variability of outdoor conditions (e.g. temperature, light intensity and photoperiod). To reduce the energy costs associated with illumination, an LED light source was used for microalgae growth. Compared to fluorescent and other light sources, LED lighting has low energy consumption, long lifespan and low heat generation and, consequently, can influence the microalgae biomass characteristics [25,30]. The sugar and lipids content were assessed to evaluate the potential use of the produced biomass as feedstock for biofuels (biodiesel, bioethanol and biohydrogen).
2. Material and methods

2.1. Effluents

Two different wastewaters from a poultry slaughterhouse (AVIBOM AVICEOLA, SA in Torres Vedras, Portugal) were used in this study: PR (Poultry raw) and PF (Poultry flocculated). PR effluent results from the boiling of all the animal leftovers in water which are not sold to the final consumer (such as feathers, heads, paws, skin, guts, etc.). PF effluent corresponds to the PR after a primary flocculation treatment performed in the slaughterhouse, which represents a very high cost to the company.

2.2. Microalgae cultivation experiments

Different experiments using the microalga *Scenedesmus obliquus* (ACOI 204/07, Coimbra University Algotec, Portugal) were designed using PR and PF effluents from the poultry company and using different lighting sources (LEDs and fluorescent lamps). Taking into account the differences in the ammonium content present in both effluents, the PF effluent was diluted (PF (%)) in order to have a similar starting point. These experiments were compared with the ones carried out with the non-sterile Bristol medium (0.250 g/L NaNO₃, 0.175 g/L KH₂PO₄, 0.075 g/L K₂HPO₄, 0.075 g/L MgSO₄·7H₂O, 0.060 g/L Fe-EDTA, 0.033 g/L CaCl₂·2H₂O, 0.025 g/L NaCl and 1 mL/L trace elements solution CHU) [31]. Bubble column photobioreactors (PBRs) of 1-L (45 cm length and 5 cm diameter) were used to cultivate the microalgae under continuous artificial light and at room temperature (≈25°C). To compare the bioremediation efficiency and the biomass composition under different light sources, the reactors were illuminated with three white fluorescent (Philips TL-D 36W/54-765) lamps or three white LED (Master LED Tube T8 19W/865), which were positioned at about 18 cm from the reactors; these were 8 cm apart from each other (Figure 1). The intensity of the illumination was 100 µmol/(m² s) for both fluorescent and LED lamps, measured by the Phywe Lux-meter. The energetic consumption of both illumination sources was measured by an Ecowatt Chacon KGS 01-01.

The PBRs cultures had compressed aeration (atmospheric air, 0.035% CO₂), at 1vvm (L/(L·min)), controlled by an Aalborg Mass Flow meter GFM17, to promote agitation and supply carbon dioxide for microalgae growth [32].

In all cases, the experiments were performed in duplicate and an active inoculum of *S. obliquus* (Bristol medium, pH 7) was added to each PBR, to a certain volume (1/10) which guaranteed an initial optical density (OD₅₄₀ nm, Hitachi U-2000 spectrometer) around 0.15 for all PBRs.

All the experiments were conducted until N-NH₄⁺, P-PO₄³⁻ and COD polluting nutrients were depleted, according to the Portuguese legislation regarding water quality standards (DL n° 236/98 [33]): N-NH₄⁺ <10 mg/L, P-PO₄³⁻ <10 mg/L, COD < 150 mg O₂/L. Finally, the biomass was collected, dried and its biochemical composition was evaluated.

Concerning the growth of the cultures, the pH (WTW Inolab) was evaluated daily, while the cultures’ dry weight (DW) was determined at least three times a week (by samples filtration through GF/C filters (47 mm diameter, 1.2 µm porosity, VWR). As PR effluent was very turbid (and reddish) at the start of the trial (see Figure 1), the monitoring of the growth curves was done only by dry weight to avoid the turbidity and colour interference on the optical density measurement. The obtained filtrates were analysed in terms of ion depletion according to the methods described in the analysis section.

The growth kinetic parameters evaluated were the specific growth rate (µ) (Equation (1)) and the average biomass productivity (Pavg) (Equation (2)).

\[
\mu(g/L) = \frac{\ln X_t - \ln X_0}{t_x - t_0}, \quad (1)
\]

where \(X_0\) and \(X_t\) are the dry weight cell concentrations (g/L) at the beginning, \(t_0\) (d) and at the end of the exponential growth phase, \(t_x\) (d), respectively.

\[
P_{avg}(g/L d) = \frac{X_{max} - X_0}{t_1 - t_0}, \quad (2)
\]

where \(X_{max}\) is the maximum dry weight cell concentration (g/L) at the time \(t_1\) (time when the dry weight cell concentration was maximum) and \(X_0\) is the dry weight cell concentration at the beginning of cultivation time \(t_0\) (d).
The produced biomass was harvested by centrifugation (11,300 g at 4°C for 10 min; Heraeus Multifuge 3SR+). The concentrated biomass was oven dried (Memmert) at 70°C overnight until it reached a constant weight.

### 2.3. Analysis

#### 2.3.1. Wastewater characterization

The solid content of the WW was analysed according to Method 2540 described in Standard Methods for the Examination of Water and Wastewater [34]. Total solids (TS) are determined gravimetrically after drying of the WW sample at 105°C overnight. Volatile solids (VS) are calculated as the difference between TS and the ash residue of the sample. Total and volatile suspended solids (TSS and VSS) are determined as described for TS and VS but only on the filtered liquid fraction (<2.0 µm).

The ammonium, phosphate and nitrate ions concentrations were measured in the effluent as well as the COD (chemical oxygen demand), before and after the microalga bioremediation treatment. Ammonium ion concentration was analysed using a Crison multimeter MM411 with an Ion Selective Electrode NH4+. Commercial kits were used for the measurement of nitrate (Nitrate Cell Test 1.14542 – Spectroquant test kit, Merck) and phosphorus ions (Phosver 3-powder pillows, Cat. 2125-99, Hach) using a HACH DR/2010 spectrophotometer (\(\lambda_{\text{NO}_3^-} = 525\) nm and \(\lambda_{\text{PO}_4^{3-}} = 890\) nm). COD was analysed using the Open Reflux method – Method 5220-B [34].

The oil content on PR effluent was determined gravimetrically, after solvent (hexane) extraction.

#### 2.3.2. Microalgae biomass characterization

The produced microalgae biomass was analysed in terms of total sugars and lipids, the most relevant chemical components with potential application for biofuel production.

To extract and determine the total sugar content, a quantitative acid hydrolysis (500 mg of dried biomass with sulphuric acid) and the phenol-sulphuric method (with sulphuric acid) and the phenol-sulphuric method were combined, based on the descriptions by Hoebler et al. [35] and Dubois et al. [36], respectively.

The saponifiable matter, as well as the fatty acid composition of the microalgae biomass, was assessed through the BF\(_3\) method [37] directly on the dried biomass. The obtained lipid fractions were analysed by gas chromatography (GC) using a CP-3800 GC (Varian, U.S.A.) equipped with a 30-m SUPELCOWAX 10 capillary column (0.32 mm of internal diameter and 0.25 µm of film thickness); the injector (split 1:50) and the detector (flame ionization) temperatures were kept constant at 250°C; the oven temperature programme was started at 60°C for 2 min, increased by 10°C/min until it reached 200°C, then increased by 5°C/min until it reached 240°C and then kept constant at this temperature for 7 min; carrier gas (He) was kept at 0.4 atm. The relative percentage of each fatty acid methyl ester was determined from the peak areas (previously identified by fatty acid methyl ester standard injections). Saponifiable matter (FAME) content was calculated according to the European Standard 14103 [38]. The lipid fraction was also characterized in terms of its iodine value (IV) according to the EN 16300 [39].

### 2.4. Statistical analysis

All the trials and measurements were performed at least in duplicate and the results were evaluated using the SISVAR software [40] and the average comparison Scott-Knott (p < 0.05) test.

### 3. Results and discussion

#### 3.1. Wastewater characterization

The visual inspection of the poultry wastewaters showed that PR was heterogeneous and reddish while the PF was homogeneous and colourless. Table 1 shows the physicochemical characterization of these wastewaters. TS, total suspended solids (TSS), VS, VSS, organic load (COD) and inorganic constituents, namely phosphorus (P), in poultry raw wastewater (PR) presented significantly (p < 0.05) higher values than in the poultry flocculated wastewater (PF). However, the nitrogen (N) concentration, particularly in the form of ammonium ion (N-NH\(_4^+\)), in the PF wastewater was about double the amount found in PR wastewater. The oil content on PF was not evaluated as this effluent was previously flocculated at the poultry company. The oil content present on PR was low and therefore not justified its removal before the microalga cultivation experiments.

Table 1. The composition of raw (PR) and flocculated (PF) poultry wastewater. Values represent the average of at least two replicates.

| Wastewater     | Poultry raw (PR) | Poultry flocculated (PF) |
|----------------|------------------|--------------------------|
| Total suspended solids, TSS (g/L)* | 1.4\(^a\) | 0.1\(^b\) |
| Total solids, TS (g/L)* | 2.0\(^a\) | 0.7\(^b\) |
| Volatile solids, VS (g/L)* | 1.4\(^a\) | 0.1\(^b\) |
| Volatile suspended solids, VSS (g/L)* | 1.2\(^b\) | 0.03\(^b\) |
| Chemical oxygen demand, COD (mg O\(_2\)/L)* | 3 694.7\(^a\) | 97.5\(^b\) |
| N - NH\(_4^+\) (mg/L)* | 122.7\(^b\) | 259.3\(^a\) |
| P - PO\(_4^{3-}\) (mg/L)* | 27.9\(^b\) | 23.4\(^b\) |
| N - NO\(_3^-\) (mg/L)* | 0.2 | not detected |
| Oil content (mg/g) | 0.6 | not determined |

\(^a\)Means followed by different letter in the same line correspond to significant differences, Scott-Knott test, p < 0.05.
These results of wastewater characterization are in line with values from the literature concerning these kinds of effluents [41] and indicate that a bioremediation treatment (e.g. using microalgae) is required before PR and PF are discharged into water streams.

Considering that the ammonium recommended value for microalgae optimal growth is around 120 mg/L N-NH₃ [42], it was assumed that the PR wastewater (123 mg/L N-NH₃) could be used without any dilution. On the other hand, the poultry flocculated effluent (PF) should be diluted to ½ with fresh water.

### 3.2. S. obliquus growth/wastewater treatment

The microalga growth was performed under fluorescent and LED lighting. Analysing the microalga growth through the logarithmic curves presented in Figure 2(A, B), it is possible to verify that there are no substantial differences in *S. obliquus*’ growth when the two types of illumination were used. The cultures grown in PF (½) wastewater and Bristol medium presented typical growth curves, reaching the stationary phase by 10th–12th day. Due to the transparency of these two media (PF (½) and Bristol), the artificial light could penetrate the PBRs almost perfectly. On the other hand, the higher proportion of suspended solids in the PR wastewater might have interfered in the dry weight (DW) values, namely at the beginning of the assay (in both lighting types). The colour of the PR cultures during the experiments changed from reddish to green evidencing the growth of the microalgae and resulted in clear liquid. In this case, a final biomass concentration of 3.1 and 3.8 g/L was obtained for fluorescent and LED light, respectively. Thus, it should be noted that the highest biomass concentration was attained for microalgal growth in the raw poultry effluent (prior to the costly flocculation step), under LED lighting (lower energy scenario).

Table 2 presents growth kinetics parameters derived from the microalgae growth curves, under different conditions. The specific growth rates of the microalgae cultivated in PR wastewater were lower than the remaining, which should be related to the above mentioned highest initial DW values. However, in terms of average biomass productivities, no significant (p < 0.05) differences were found between cultures grown in PR and PF (½) wastewaters and Bristol medium. Therefore, it is possible to obtain the same amount of algal biomass by using poultry industry effluents, instead of using expensive chemical nutrients and potable water. This has evident advantages in terms of lowering the cost of microalgae production, besides additional environmental assets. Furthermore, in terms of light source, no significant differences (p < 0.05) were observed in the growth parameters, and the use of LEDs allowed to an energetic saving of 55%. These results showed that the LED light can be a suitable choice for indoor microalga cultivation.

In all cases, average biomass productivity values (0.08–0.13 g/L d) were higher than the ones obtained by Singh et al. [20] growing a *Scenedesmus* strain in the effluent of poultry litter anaerobic digestion (0.076 g/L d).

In all the experiments, after an initial alkalisation of the culture media, the pH remained in the range of 8–11 (Figure 2(C,D)) as it usually occurs in microalgae assays (without CO₂ injection for pH control).

The initial and final values of ammonium, phosphate and COD, as well as the bioremediation efficiencies, for both effluents and light sources are shown in Table 3. The removal efficiencies of ammonium and phosphate ions reached values of above 97%, allowing to legally dispose these effluents into surrounding streams in terms of these nutrients (Portuguese legal limit < 10 mg/L [33]). The light source does not seem to greatly affect the efficiency of the biological treatment, although a higher (p < 0.05) nitrogen bioremediation yield was attained in PR when using LED light (97.7%) in comparison to fluorescent light (97.1%). Very few have studied the impact of LED lighting on microalgal nutrient removal on wastewaters and effluents. Kim et al. [43] studied the effects of different LEDs wavelengths on microalga growth and nitrogen and phosphorus removal using *Scenedesmus* sp. for wastewater treatment. They have observed that N-removal rate could be increased using wavelength mixing with red and blue light and also that blue light was effective on phosphorus removal. Yan and Zheng [44] found similar results, with mixed LED light wavelength treatments yielding optimal *Chlorella* sp. growth and nutrient removal in biogas fluid.

*S. obliquus* also showed potential to reduce the high organic load of the PR effluent, with COD removal rates...
above 95% (final values around 160 mg O₂/L) (Table 3).
In PF (½) assays, COD values suffered only small variations, being the obtained values (around 50 mg O₂/L) below the limit imposed by the Portuguese legislation for wastewater disposal in aqueous streams (< 150 mg O₂/L).

In studies performed by Gupta et al. [45], S. obliquus also showed greater potential for removing organic carbon (76% COD removal) and nutrients (98% N-removal, 98% P-removal) from urban wastewater, with better results than the ones obtained using Chlorella sorokiniana (70% COD removal, 87% N-removal, 68% P-removal) [45]. A similar behaviour was described by Gouveia et al. [13] who presented maximum removals attained by S. obliquus of 95% for total nitrogen, 92% for phosphorus, and 63% for COD. Other researchers also referred to the potential of S. obliquus for urban [46,47] and brewery [16] wastewater treatment. Makarevičienė et al. [48] used Chlorella sp. and Scenedesmus sp. to treat the liquid fraction of digestate after biogas production and reported a removal efficiency of 91% for nitrogen (for both algae) and 94.7% and 95.6%, respectively, for phosphorus. When growing Chlorella sp., the BOD was reduced by up to 87.1% and with Scenedesmus sp. it was reduced by 92.1%. Wang et al. [49] stated a removal of nitrogen of approximately 80% and of

**Table 3.** Effect of lighting (fluorescent vs LEDs) on nutrient removal from raw and flocculated (diluted ½) poultry wastewaters by the microalga Scenedesmus obliquus. Values represent the average of at least two replicates.

| Lighting type | Effluent | Fluorescent | LED |
|---------------|----------|-------------|-----|
|               |          | PR (%)      | PF (½) | PR (%) | PF (½) | Portuguese legal limit |
| N - NH₄⁺ (mg/L) | Initial** | 122.7ᵃ | 129.6ᵇ | 122.7ᵃ | 129.6ᵇ | 10 |
|               | Final*   | 3.5ᵃ | 1.6ᵇ | 2.8ᵃ | 1.4ᵇ | |
| ηbioremediation (%)* | 97.1ᵃ | 98.8ᵇ | 97.7ᵇ | 98.9ᵇ | |
| P - PO₄³⁻ (mg/L) | Initial* | 27.9ᵃ | 11.7ᵇ | 27.9ᵃ | 11.7ᵇ | 10 |
|               | Final*   | 0.1ᵇ | 0.3ᵇ | 0.2ᵃ | 0.2ᵇ | |
| ηbioremediation (%)* | 99.3ᵃ | 98.3ᵇ | 97.5ᵃ | 97.4ᵇ | |
| COD (mg O₂/L) | Initial* | 3694.7ᵃ | 97.5ᵇ | 3694.7ᵃ | 97.5ᵇ | 150 |
|               | Final*   | 160 | 50 | 148 | 52 |
| ηbioremediation (%)* | 96 | 48 | 96 | 47 |

Note: Means followed by different letter in the same line correspond to significant differences, Scott-Knott test, *p < 0.05, **p > 0.05.

**Figure 2.** Effect of lighting source (fluorescent and LED) in microalga growth (A and B) and pH (C and D) for different Scenedesmus obliquus culture media: PR (poultry raw – ■) and PF (½) (poultry flocculated – □) wastewater, and Bristol (○). (Illumination intensity 100 µmol/(m² s), compressed aeration 1 vvm (L/(L min)).
The statistical analysis of fatty acid profiles of *Scenedesmus obliquus* biomass cultivated in raw and flocculated (diluted ½) poultry wastewaters has been observed when switch to a mixotrophic nutritional mode. Similar results have been observed when growing *Scenedesmus* and *Chlorella* strains in the effluent from poultry litter anaerobic digestion [20] and in urban wastewaters [9].

The use of different types of lighting (fluorescent vs. LED) did not significantly affect the chemical composition of the microalgal biomass (Table 4). The exception goes for when growing algae in PF (½) wastewater, *S. obliquus* could produce 21–25% (m/malg) of total sugars, values slightly lower than the ones for saponifiable matter (25–29% (m/malg)). In fact, the biomass from the PF (½) wastewater presented more than double the saponifiable matter i.e. lipids capable of being converted to esters (and lately biodiesel), compared to the samples obtained from PR wastewater. This is probably due to a stress situation caused by low nutrient concentration in PF (½) due to the dilution, which induces the synthesis of lipids and consequently saponifiable matter. The results indicated that with these industrial wastewaters, *S. obliquus* is capable of producing as much lipids as sugars and, consequently, could be used for both biodiesel and bioethanol/biohydrogen production, respectively. Furthermore, bioethanol can be directly produced from the lipid-extracted biomass, as described by Chng et al. [52].

3.3. *S. obliquus* biomass quality for biofuels

The final harvested *S. obliquus* biomass obtained from each experiment was characterized in terms of lipids and sugar content to evaluate their potential as a raw material for biofuel production. It is evident from Table 4 that the biomass produced in the PR effluent contains around three times higher sugars than saponifiable matter values, i.e. the values rose to more than 30% (m/malg) sugars compared to around 10% (m/malg) for saponifiable matter. A correlation between COD (in the wastewater) and sugar synthesis (in microalgae biomass) could be stated, as already described by Mandal and Mallick [50]. The higher concentration of COD means higher amount of organic-C which could promote the capacity of the microalgae to switch to a mixotrophic nutritional mode. Similar biomass composition has been observed when growing *Scenedesmus* and *Chlorella* strains in the effluent from poultry litter anaerobic digestion [20] and in urban wastewaters [9].

The strategy, in this case, should be focused on using this biomass as feedstock for bioethanol or biohydrogen fermentative processes. Previous studies with this *S. obliquus* strain have shown that yields around 0.19 g EtOH/g alga sugar and 57 mL H2/g VSalg can be attained [9,51].

Table 4. Effect of lighting (fluorescent vs LEDs) on biomass composition (total sugar, oil content and fatty acid profile) of *Scenedesmus obliquus* biomass cultivated in raw and flocculated (diluted ½) poultry wastewaters.

| Lighting type | Fluorescent | LED |
|---------------|-------------|-----|
|               | PR | PF (%) | PR | PF (%) |
| Total sugars (% m/malg)* | 36.2 | 21.6 | 31.9 | 24.8 |
| Saponifiable matter (% m/malg)* | 9.9 | 24.5 | 11.4 | 29.3 |
| Fatty acid composition (% m/mLipid fraction)* | |
| C14:0 | 0.5 | 0.5 | 0.5 | 0.4 |
| C16:0** | 19.0 | 21.8 | 19.2 | 22.6 |
| C16:1 | 2.8 | 1.8 | 3.6 | 1.6 |
| C18:0 | 1.3 | 2.2 | 1.3 | 2.3 |
| C18:1 | 19.8 | 39.7 | 24.6 | 41.2 |
| C18:2 | 18.4 | 11.8 | 14.5 | 11.0 |
| C18:3** | 10.4 | 10.0 | 10.7 | 9.5 |
| C20:0 | 0.4 | 0.1 | 0.5 | 0.2 |
| C22:0 | 0.1 | 0.1 | 0.1 | 0.2 |
| C22:1 | n.d. | n.d. | n.d. | 0.1 |
| C24:0 | n.d. | n.d. | n.d. | 0.1 |
| Non-identified | 27.3 | 11.9 | 25.1 | 11.0 |
| Saturated | 21.3 | 24.7 | 21.6 | 25.7 |
| Unsaturated | 51.4 | 63.3 | 53.3 | 63.4 |
| Iodine value (g I2/100 g)* | 78.6 | 82.5 | 77.5 | 81.0 |

Note: Means followed by different letter in the same line correspond to significant differences, Scott-Knott test. *p < 0.05; **p > 0.05; n.d. = not detected. The statistical analysis of fatty acid profile was only performed for the means of the main constituents.
which should be further explored. This agrees with studies by other authors reporting higher lipid yields for microalgae grown under green [54], blue [55] or red [56] LED lighting.

The biomass obtained from poultry raw wastewater (PR) was used as raw material for hydrogen production by dark fermentation with Enterobacter aerogenes [10]. In this process, a production yield of 378 mL H₂/gVS and a biogas purity (H₂/CO₂ volume ratio) of 6.7 were achieved which may result of the high sugar content present in the biomass. These results when compared to the ones obtained using biomass grown in urban, dairy, cattle or brewery wastewaters were higher [10].

The biomass (PR) was also evaluated in terms of its potential to produce bio-oil, bio-char and biogas by pyrolysis and the results were promising [57].

4. Conclusions

This research confirms the potential use of microalgae for efficient wastewater bioremediation for poultry effluents concomitantly with the production of valuable biomass, in an economical and environmentally sustainable way. The bioremediation efficiency through the microalga Scenedesmus obliquus was higher than 97% for both ammonium and phosphate removal. Ammonium depletion was reached after 9 and 15 days for PF (½) and PR wastewater, respectively, regardless of the light source. The biomass produced contained 9% (from poultry raw, fluorescent lamps) to 29% (m/malgae) (from poultry after flocculation, LEDs) of saponifiable matter, and 21% (from poultry after flocculation, fluorescent lamps) to 36% (m/malgae) (from poultry raw, fluorescent lamps) of sugars, which means that it could be used for biodiesel (lipids) and/or bioethanol/biohydrogen (sugar) production.

The positive results obtained with PR effluent using S. obliquus are particularly interesting for the poultry industry. The microalgae bioremoval process can reduce the need for flocculation, which has negative environmental and economic impacts. In addition, the use of LED lighting instead of fluorescent lighting was proved efficient and allowed saving 55% of electricity, with associated environmental and economic advantages.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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