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# Evaluation of *Scenedesmus dimorphus* under Different Photoperiods with Eutrophicated Lagoon Water

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**Abstract:** Given the need to improve bioenergy production processes, it is necessary to focus on low-cost culture media and environmental conditions of radiation and temperature. The *Scenedesmus dimorphus* species was cultured in eutrophicated lagoon water and Bayfolan 0.3% as culture media under four photoperiods with the objective of evaluating the biomass productivity, bioremediation capacity and influence of illumination on the composition and lipid content. It is concluded that the increase of light hours in the culture with eutrophicated lagoon water produces a decrease in the biomass productivity and COD removal percentage. The highest biomass productivity was obtained in photoperiod F1 (10.5:13.5) hours L:O, 0.053 ± 0.0015 g/L day and a removal of 95.6%. Bayfolan 0.3% with F2 (11.5:12.5) and F3 (12.5:11.5) did not show significant differences in the biomass productivity and COD removal. The increase in light hours in the photoperiod induced an increase of 1.01% and 2.84% of saturated fatty acids and 0.8% and 2.14% of monounsaturated fatty acids, as well as a decrease of 3.85% and 2.88% of polyunsaturated fatty acids in eutrophicated lagoon water and Bayfolan 0.3%, respectively.

**Keywords:** *Scenedesmus dimorphus*; photoperiod; mixotrophic; lagoon water



**Citation:** Pérez Bravo, S.G.; Castañeda Chávez, M.d.R.; Aguilera Vázquez, L.; Gallardo Rivas, N.V.; Morales Rodríguez, M.L.; Páramo García, U. Evaluation of *Scenedesmus dimorphus* under Different Photoperiods with Eutrophicated Lagoon Water. *Resources* **2023**, *12*, 140. https://doi.org/10.3390/resources12120140

Academic Editors: Daniele Duca, Giuseppe Toscano and Alessio Ilari

Received: 29 September 2023
Revised: 6 November 2023
Accepted: 21 November 2023
Published: 27 November 2023



## 1. Introduction

Microalgae are unicellular organisms containing carbohydrates, lipids and pigments. Their cultivation has been studied for the production of food, fuels and pharmaceuticals [1]. Renewable fuels, such as biodiesel and bioethanol, are promising and environmentally friendly alternatives [2]. They are considered third generation when produced from microalgal biomass [3]. For the exploitation of microalgal metabolites, it is necessary to decrease energy inputs, improve energy balances and reduce greenhouse gas emissions in the conversion processes to obtain more efficient production of biofuels [4], in addition to improving microalgal biomass production yields with low-cost culture media and decreased energy consumption during the process.

The factors that influence microalgal growth are nutrient availability and the C:N ratio in the culture medium, light penetration, carbon source, pH, salinity and temperature [5]. The light:dark cycles in microalgal culture influence the synthesis of organic compounds and nutrient metabolism [6].

Various artificial culture media and wastewater from different sources have been used for the cultivation of different species of microalgae, which are summarized in Table 1. Research has focused on biomass productivity under different metabolisms, finding higher productivities in mixotrophic than phototrophic cultures [7–9], in addition to the use of artificial culture media and urban and industrial wastewater, under a fixed photoperiod;

however, the use of eutrophicated natural water as a microalgal culture medium has not been investigated so far.

Little is known about the influence of the photoperiod on biomass productivity. Spirulina platensis shows the optimal biomass yield when using a photoperiod of 12.5:11.5 light:dark and drastically decreases its biomass when increasing the illumination to more than 12.5 [10]. Using artificial light in microalgae cultivation increases the cost of biomass production, so it is necessary to investigate microalgal growth under natural photoperiods.

**Table 1.** Biomass produced under different growing conditions.

| Microalgae | Culture Medium | Metabolism | Photoperiod L:D | Culture Conditions | Biomass Obtained | Reference |
|---|---|---|---|---|---|---|
| *Chlorella vulgaris* | Water, raw sewage | Mixotrophic | 12:12 | 37,000 lux, 25 °C, 16 days | 69.8 mg/Ld | [11] |
| | Water contaminated with 10 g/L of oil | Mixotrophic | 12:12 | 2000 lux, 25 °C | 0.41 g dry biomass | [12] |
| | Water contaminated with 20 g/L of oil | | | | 0.33 g dry biomass | |
| | Biological reactor wastewater | Mixotrophic | 12:12 | 180 μm/m$^2$/d, 24 ± 1 °C, Air 0.5 vvm | 20.3 mg/Ld | [13] |
| *Chlorella pyrenoidosa* | Dairy wastewater | Mixotrophic | 12:12 | 10 W/m$^2$, 25 °C | Not reported | [14] |
| *Scenedesmus acutus* | Raw wastewater | Mixotrophic | 12:12 | 37,000 lux, 25 °C, 16 days | 61.5 mg/Ld | [11] |
| *Scenedesmus* sp. | Domestic wastewater | Mixotrophic | 14:10 | 60 μm/m$^2$/d, 25 ± 2 °C, 7 days | 61.4 mg/Ld | [15] |
| | Leachate from sanitary landfill, 20% | Mixotrophic | 12:12 | 80 μm/m$^2$/d, air 4.5 L/min, 25 ± 2 °C | 3.9 mg/Ld | [9] |
| | Leachate from sanitary landfill, 60% | Mixotrophic | | | 319.9 mg/Ld | |
| | Leachate from sanitary landfill, 80% | Mixotrophic | | | 421.9 mg/Ld | |
| | Leachate from sanitary landfill, 100% | Mixotrophic | | | 163 mg/Ld | |
| | CHU | Phototrophic | | | 34.6 mg/Ld | |
| *Scenedesmus dimorphus* and *Scenedesmus minutum* | Municipal wastewater | Mixotrophic | 16:8 | 150 μm/m$^2$/d, 22 ± 2 °C | 16 mg/L | [16] |
| *Scenedesmus dimorphus* | Wastewater with lactic acid | Mixotrophic | 14:10 | 2500 lux, 25 °C, 10 days | 2.5 g/L | [17] |
| | Wastewater with lactic acid + 0.8 g/L de $NaNO_3$, 4 mg/L $K_2HPO_4$-3$H_2O$ | Mixotrophic | 14:10 | | 4.5 g/L | |
| | BG11 | Phototrophic | 12:12 | $CO_2$ atmospheric 11 L/min, 25 °C, 20 days | 96.5 mg/Ld | [7] |
| | BG11 + Apple pomace hydrolyzate 2% *w*/*v* | Mixotrophic | 12:12 | | 140.3 mg/Ld | |
| | BBM | Phototrophic | 16:8 | 120 μm/m$^2$/s 11 L/min air | 96.4 mg/Ld | [8] |
| | BBM + Hydrolyzed sugar cane bagasse 10 g/L | Mixotrophic | 16:8 | | 105.9 mg/Ld | |
| | BBM + Hydrolyzed sugar cane bagasse 5 g/L | Mixotrophic | 16:8 | | 119.2 mg/Ld | |

The chemical composition of the biomass is determinant for its industrial application; biomass rich in lipids is useful in the production of biodiesel, while that rich in carbohydrates is useful in the production of bioethanol. An effective tool for the determination of the biochemical composition of microalgae is Fourier transform spectroscopy (FT-IR), as it dissects functional chemical groups in different absorbance regions, lipids and proteins, and carbohydrates have characteristic absorbance in different frequency regions, which allows an elucidation of the biochemical composition of the biomass [18].

As described above, it is vital to investigate biomass production with low energy inputs and low-cost culture media. In this study, the influence of four different photoperiods on the biomass productivity of *Scenedesmus dimorphus* was evaluated; the light:dark cycles were selected because the geographical area where this research was conducted presents such photoperiods under natural conditions. The use of eutrophicated lagoon water and Bayfolan as the culture media has the dual purpose of evaluating the nutrient removal capacity and the biochemical composition of the microalgae.

Therefore, this work contributes to the scientific community by identifying whether the culture photoperiod influences the biomass productivity, nutrient removal capacity and biochemical composition of *Scenedesmus dimorphus* obtained by culturing it in eutrophicated lagoon water and Bayfolan.

## 2. Materials and Methods

### *2.1. Characteristics of the Culture Medium*

The eutrophicated water was taken from a lagoon called "El Conejo", located at coordinates latitude: 22.41881 and longitude: −97.87649, in the municipality of Altamira, Tamaulipas, Mexico. The water was subjected to autoclave heat treatment at 121 °C and 15 psi for 10 min. In addition, a 0.3% Bayfolan solution was used as a control medium. The species *Scenedesmus dimorphus* was provided by the Phycology Department of the Institute of Biology, UNAM.

### *2.2. Culture Conditions*

The microalgae were cultured in 1.5 L PET containers disinfected for 24 h with 0.14 mL/L of 5% sodium hypochlorite, followed by neutralization with 0.1 mL/L of 24.81% sodium thiosulfate (Fermont) and washing with sterile distilled water [19]. A culture volume of 1.25 L with 20% inoculum was used under the illumination of two 18 W Led lamps (lux) at room temperature. Table 2 describes the full factorial design of experiments: the first factor is the different culture mediums (lagoon water and Bayfolan); the second factor is the four different photoperiods.

**Table 2.** Experimental factorial design of eight treatments for two culture media and four photoperiods.

| Treatment | Culture Medium | Photoperiod (Light:Dark) |
|---|---|---|
| 1 | Eutrophicated lagoon water | F1 (10.5:13.5) |
| 2 | Eutrophicated lagoon water | F2 (11.5:12.5) |
| 3 | Eutrophicated lagoon water | F3 (12.5:11.5) |
| 4 | Eutrophicated lagoon water | F4 (13.5:10.5) |
| 5 | Bayfolan 0.3% | F1 (10.5:13.5) |
| 6 | Bayfolan 0.3% | F2 (11.5:12.5) |
| 7 | Bayfolan 0.3% | F3 (12.5:11.5) |
| 8 | Bayfolan 0.3% | F4 (13.5:10.5) |

This design allows for examination of the effect of each factor and their interaction on biomass production. The growth curve was performed by optical density at 685 nm in a UV/Vis spectrophotometer, model Cintra 303 (GBC, Regents Park, NSW, Australia). All the experiments were performed in triplicate.

### *2.3. Biomass Harvesting*

The biomass was left to sediment for 24 h and then centrifuged at 6000 rpm for 15 min in a centrifuge, model EBA 21 (Hettich, Saint-Laurent, QC, Canada), and the supernatant was eliminated. The samples were then lyophilized by freezing in 50 mL falcon tubes covered with aluminum foil with 5 holes. The process was carried out at −80 °C for 2 days, and then the samples were placed in a 2.5 L FreeZone lyophilizer benchtop freeze dryer (Labconco, Kansas, MO, USA).

### *2.4. Lipid Extraction*

Ultrasound-assisted extraction was performed at a 20 mL:g ratio, 20% amplitude, for 50 min, at room temperature in a UP200Ht ultrasonic processor (Hielscher, Teltow, Germany). The process was repeated three times, followed by filtration and evaporation of the solvent [12,20–22]. The solvent evaporation was performed in a Rotary evaporator, model R-134 (Buchi, Mumbai, India).

For the purification of the microalgae oil, 3 mL of $H_2SO_4$ 0.5M [6] was added to the sample to remove the chlorophyll, followed by centrifugation. The supernatant containing the chlorophyll was decanted, then the sample was washed twice with 5 mL of hexane and heated at 90 °C for 15 min; once cooled, it was centrifuged. The solvent was allowed to evaporate from the supernatant to obtain the lipid [11].

The solvents chloroform and methanol at 99.8% purity (Fermont, Playa del Carmen, México) were used.

### *2.5. Characterization*

The infrared spectra (FTIR) were obtained in a Fourier transform spectrometer, model spectrum 100 (PerkinElmer, Waltham, MA, USA). The measurements were performed in the mid-infrared in the range between 450 and 4000 $cm^{-1}$ for a total of 12 scans.

The lipid content was identified by gas chromatography using the AOAC 996.06 2001 methodology.

## 3. Results

The following tables present the results obtained under the four photoperiods studied: F1 (10.5:13.5 L:O); F2 (11.5:12.5 L:O); F3 (12.5:11.5 L:O); F4 (13.5:10.5 L:O). The data were analyzed using an analysis of variance (ANOVA) with $\alpha = 0.05$. In all cases in which the F test was significant, Tukey HSD analyses with $\alpha = 0.05$ were performed with the Excel statistical tool (Microsoft Office). Table 3 shows the biomass productivities obtained in g/Lday, while Table 4 shows the results of the analysis of variance (ANOVA) of these productivities.

**Table 3.** Biomass productivity in both culture media under the different photoperiods analyzed.

| **Factor B** | **Factor A—Biomass Productivity (g/L/Day)** | | | |
|---|---|---|---|---|
| **Growing Medium** | **F1 (10.5:13.5)** | **F2 (11.5:12.5)** | **F3 (12.5:11.5)** | **F4 (13.5:10.5)** |
| Eutrophicated lagoon water | 0.055 | 0.043 | 0.040 | 0.023 |
| | 0.054 | 0.050 | 0.033 | 0.025 |
| | 0.052 | 0.041 | 0.035 | 0.023 |
| Bayfolan at 0.3% | 0.038 | 0.036 | 0.033 | 0.023 |
| | 0.035 | 0.039 | 0.032 | 0.023 |
| | 0.036 | 0.036 | 0.033 | 0.026 |

**Table 4.** Results of ANOVA analysis of biomass productivities in both culture media under different photoperiods.

| Origin of Variances | Sum of Squares | Degrees of Freedom | Mean Squares | F | Probability | Critical Value for F |
|---|---|---|---|---|---|---|
| Sample | 0.000170 | 1 | 0.000170 | 27.48 | $8.04 \times 10^{-5}$ | 4.49 |
| Columns | 0.001514 | 3 | 0.000504 | 81.30 | $6.69 \times 10^{-10}$ | 3.23 |
| Interaction | 0.000385 | 3 | 0.000128 | 20.67 | $9.44 \times 10^{-6}$ | 3.23 |
| In-group | $9.93 \times 10^{-5}$ | 16 | $6.20 \times 10^{-6}$ | | | |
| Total | 0.002169 | 23 | | | | |

A two-factor ANOVA was performed with a significance level of 0.05. It was established that the $H_0$:biomass productivities were equal in all the photoperiods (Factor A), the $H_0$:biomass productivities were equal in both culture media (Factor B) and in $H_1$, there are no interactions between the two factors. Based on the results obtained, $H_0$ is rejected, that is to say, there is a statistically significant difference between the means of biomass productivity among the four photoperiods and the two culture media. $H_1$ is accepted, as there is interaction between both factors.

To identify the difference between the photoperiods, a one-factor ANOVA and Tukey's test were performed for each culture medium.

The results of the single factor ANOVA for the results of the biomass productivities in the eutrophicated lagoon water are shown in Table 5. Based on the results, the $H_0$ is rejected, i.e., there is a significant difference in the average biomass productivity among the four photoperiods evaluated. The results of Tukey's HSD test with a significance level of 0.05 are shown in Table 6 (HSD = 0.00816 and probability of $1.55 \times 10^{-5}$). Variation was observed between photoperiods F1 and F2, F1 and F3, and F1 and F4. In addition, in F2 and F3, F2 and F4, and F3 and F4, the greatest difference in the means was observed between photoperiods F1 and F4.

**Table 5.** Results of ANOVA analysis of biomass productivities obtained for eutrophicated lagoon water.

| Origin of Variances | Sum of Squares | Degrees of Freedom | Mean Squares | F | Probability | Critical Value for F |
|---|---|---|---|---|---|---|
| Between groups | 0.001471 | 3 | 0.00049033 | 50.29 | $1.55 \times 10^{-5}$ | 4.06 |
| Within groups | 0.000078 | 8 | 0.00000975 | | | |
| Total | | 11 | | | | |

**Table 6.** Tukey HSD test of biomass productivities for eutrophicated lagoon water in the analyzed photoperiods.

| | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| F1 | - | 0.0090 | 0.0177 | 0.0300 |
| F2 | - | - | 0.0087 | 0.0210 |
| F3 | - | - | - | 0.0123 |
| F4 | - | - | - | - |

A single factor ANOVA was performed for the results of the biomass productivities in Bayfolan 0.3%. As shown in Table 7, based on the results, the $H_0$ is rejected, that is, there is a significant difference in the average biomass productivity among the four photoperiods evaluated. The results of the Tukey HSD test with a significance level of 0.05 are shown in Table 8 (HSD = 0.00427 and probability of $1.22 \times 10^{-5}$). Variation was observed between photoperiods F1 and F4, F2 and F4, and F3 and F4.

**Table 7.** Results of ANOVA analysis of biomass productivities obtained in Bayfolan 0.3%.

| Origin of Variances | Sum of Squares | Degrees of Freedom | Mean Squares | F | Probability | Critical Value for F |
|---|---|---|---|---|---|---|
| Between groups | 0.000428 | 3 | 0.000142 | 53.54 | $1.22 \times 10^{-5}$ | 4.06 |
| Within groups | $2.13 \times 10^{-5}$ | 8 | $2.66 \times 10^{-6}$ | | | |
| Total | | 11 | | | | |

**Table 8.** Tukey HSD test of biomass productivities in Bayfolan 0.3%.

| | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| F1 | - | −0.0006 | −0.0030 | 0.0123 |
| F2 | - | - | −0.0023 | 0.0130 |
| F3 | - | - | - | 0.0153 |
| F4 | - | - | - | - |

As shown in Figure 1, the biomass productivity in crops grown with eutrophicated lagoon water decreased as light hours increased. On the other hand, in the crops grown with 0.3% Bayfolan, a slight increase in productivity was observed, reaching a maximum of 0.032 g/L ± 0.0005 in photoperiod three; however, as the light hours increased in photoperiod four, the productivity dropped drastically to 0.024 ± 0.0017 g/L/día.

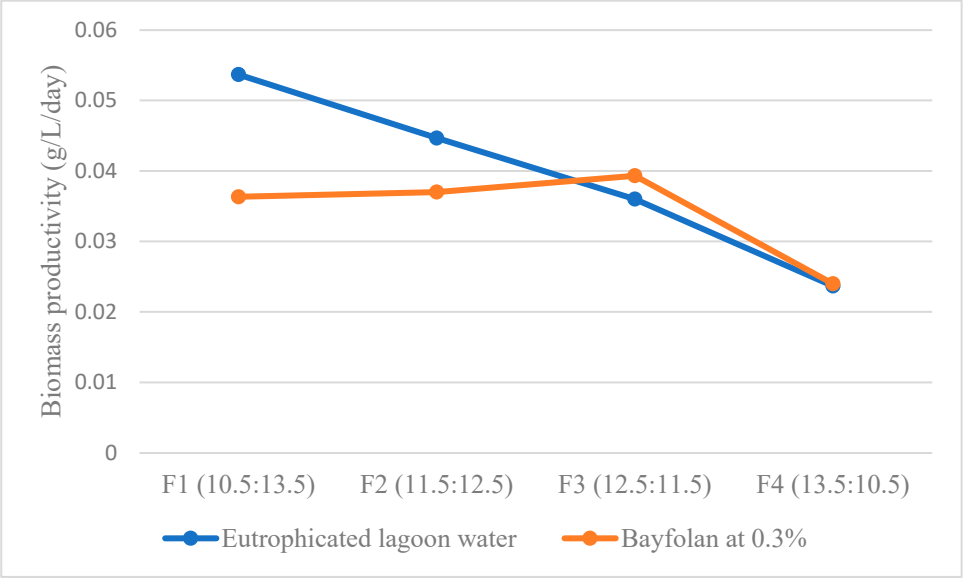



**Figure 1.** The behavior of biomass productivities in the analyzed photoperiods.

A calibration curve of absorbance versus the dry weight concentration of the *Scenedesmus dimorphus* biomass was performed. In Figure 2, the growth curves of *Scenedesmus dimorphus* in eutrophicated lagoon water are presented, and Figure 3 illustrates the growth curves in Bayfolan at 0.3%. Figure 2 shows a decrease in the biomass concentration in dry weight as the photoperiod light hours increased; the maximum concentration was 1.23 g/L in F1 and the minimum was 0.86 g/L in F4. The growth curves did not reach the stationary phase.

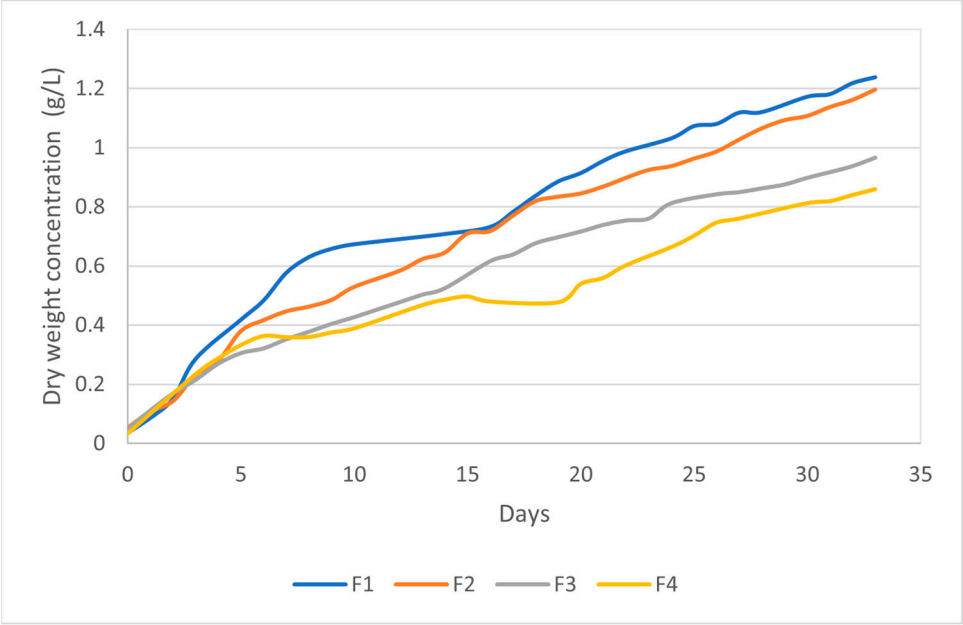



**Figure 2.** Growth curves of *Scenedesmus dimorphus* grown in eutrophicated lagoon water.

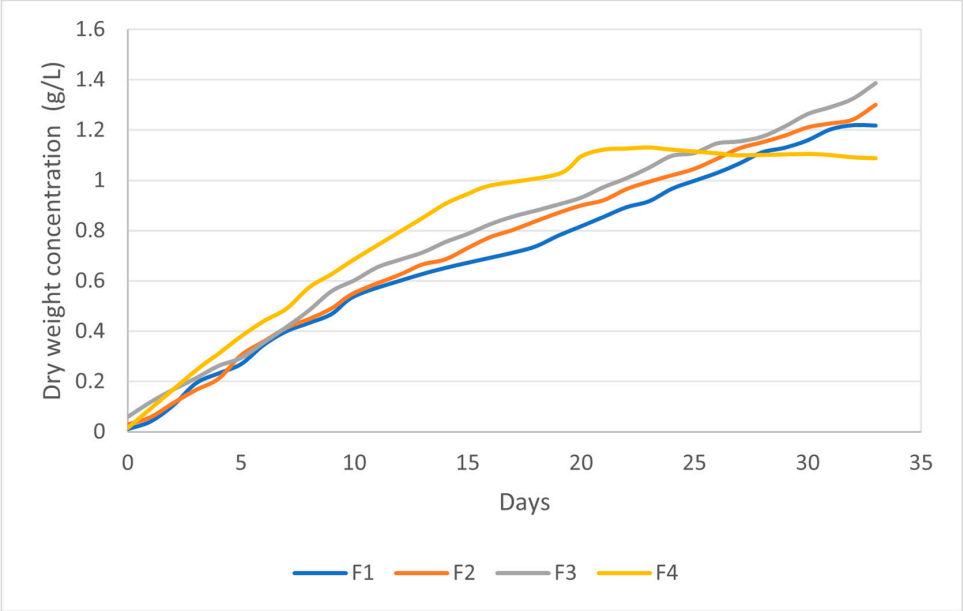



**Figure 3.** Growth curves of *Scenedesmus dimorphus* grown in 0.3% Bayfolan.

Figure 3 shows a maximum concentration of 1.38 g/L in F1 and a minimum concentration of 1.08 g/L in F4 at the end of the culture; however, in this culture medium, the stationary growth phase is not observed for the F1, F2 and F3 photoperiods. It is only observed in F4: on day 22, a concentration of 1.12 g/L is observed. This implies that the longer the light exposure time in the photoperiod, the faster the growth is, reaching its stationary growth phase followed by the death phase.

Table 9 shows the COD removal percentages in the photoperiods and culture media studied, while Table 10 shows the results of the analysis of variance (ANOVA) of these productivities. This was performed with a significance level of 0.05. It is established that the $H_0$:COD removal percentages are equal in all the photoperiods (Factor A), the $H_0$:COD removal percentages are equal in both culture media (Factor B) and in $H_1$, there are no interactions between the two factors. Based on the results obtained, $H_0$ is rejected, meaning

there is a statistically significant difference between the removal percentages among the four photoperiods and the two culture media. $H_1$ is accepted, as there is interaction between the two factors.

**Table 9.** Percentage of COD removal in both culture media under the different photoperiods analyzed.

| Factor B | Factor A—% Removal of Chemical Oxygen Demand | | | |
|---|---|---|---|---|
| | F1 (10.5:13.5) | F2 (11.5:12.5) | F3 (12.5:11.5) | F4 (13.5:10.5) |
| Eutrophicated lagoon water | 95.1 | 93.1 | 87.8 | 59.9 |
| | 97.1 | 91.9 | 82.5 | 61.0 |
| | 94.5 | 91.0 | 80.5 | 60.5 |
| Bayfolan at 0.3% | 59.5 | 89.0 | 87.2 | 43.6 |
| | 60.1 | 87.6 | 89.6 | 40.8 |
| | 61.2 | 89.1 | 89.7 | 45.1 |

**Table 10.** Results of the ANOVA analysis of the percentage of COD removal in both culture media under different photoperiods.

| Origin of Variances | Sum of Squares | Degrees of Freedom | Mean Squares | F | Probability | Critical Value for F |
|---|---|---|---|---|---|---|
| Sample | 868.806666 | 1 | 868.806666 | 247.25 | $3.75 \times 10^{-11}$ | 4.49 |
| Columns | 4986.05833 | 3 | 1662.01944 | 473.00 | $7.93 \times 10^{-16}$ | 3.23 |
| Interaction | 1380.29333 | 3 | 460.097777 | 130.94 | $1.80 \times 10^{-11}$ | 3.23 |
| In-group | 56.22 | 16 | 3.51375 | | | |
| Total | 7291.37833 | 23 | | | | |

The single factor ANOVA for the results of COD removal in eutrophicated lagoon water is shown in Table 11. Based on the results, $H_0$ is rejected because there is a significant difference in COD removal. The results of the Tukey HSD test with a significance level of 0.05 are shown in Table 12 (HSD = 5.46 and probability of $1.366 \times 10^{-7}$). Variation was observed between photoperiods F1 and F3 and F1 and F4, in addition to F2 and F3, F2 and F4, and F3 and F4. The greatest difference in means was observed between photoperiods F1 and F4.

**Table 11.** Results of ANOVA analysis of COD percentage removal obtained for eutrophicated lagoon water.

| Origin of Variances | Sum of Squares | Degrees of Freedom | Mean Squares | F | Probability | Critical Value for F |
|---|---|---|---|---|---|---|
| Between groups | 2240.995833 | 3 | 746.998611 | 170.77 | $1.36 \times 10^{-7}$ | 4.06 |
| Within groups | 34.99333333 | 8 | 4.37416666 | | | |
| Total | 2275.989167 | 11 | | | | |

**Table 12.** Tukey HSD test of percentage of COD removal for eutrophicated lagoon water in the analyzed photoperiods.

| | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| F1 | - | 3.5666 | 11.9666 | 35.1000 |
| F2 | - | - | 8.4000 | 31.5333 |
| F3 | - | - | - | 23.1333 |
| F4 | - | - | - | - |

The single factor ANOVA for the results of COD removal in Bayfolan 0.3% water is shown in Table 13. Based on the results, $H_0$ is rejected because there is a significant difference in COD removal among the four photoperiods. The results of the Tukey HSD test with a significance level of 0.05 are shown in Table 14 (HSD = 4.26 and probability of

$1.686 \times 10^{-9}$). Variation was observed between photoperiods F1 and F2, F1 and F3, F1 and F4, F2 and F4, and F3 and F4. The greatest difference in means was observed between photoperiods F3 and F4.

**Table 13.** Results of the ANOVA analysis of the percentage COD removal in Bayfolan 0.3%.

| Origin of Variances | Sum of Squares | Degrees of Freedom | Mean Squares | F | Probability | Critical Value for F |
|---|---|---|---|---|---|---|
| Between groups | 4125.35583 | 3 | 1375.11861 | 518.26 | $1.68 \times 10^{-9}$ | 4.06 |
| Within groups | 21.2266667 | 8 | 2.6533333 | | | |
| Total | | 11 | | | | |

**Table 14.** Tukey HSD test for % COD removal in Bayfolan 0.3%.

| | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| F1 | - | −28.3000 | −28.5600 | 14.4300 |
| F2 | - | - | −0.2600 | 42.7300 |
| F3 | - | - | - | 43.0000 |
| F4 | - | - | - | - |

Since the biomass showed significant differences in productivity under F1 and F4 photoperiods in both culture media, characterization with FT-IR spectrophotometry of the biomass and gas chromatography of the extracted lipids was performed. The samples were identified considering the culture medium and photoperiod. ALF1 and ALF4 refer to the biomass and lipid obtained from the culture in eutrophicated lagoon water under F1(10.5:13.5) and F4(13.5:10.5) photoperiods. BF1 and BF4 refer to the biomass and lipid obtained from the culture in 0.3% Bayfolan under F1(10.5:13.5) and F4(13.5:10.5) photoperiods.

Figure 4A shows the spectrum of the freeze-dried biomass that was cultured in eutrophicated lagoon water, while Figure 4B shows the spectrum of the one cultured in Bayfolan 0.3%. Table 15 shows the details of the signals obtained in the FTIR analysis of each of the samples grouped by wavelength range and their respective functional group.

**Table 15.** Signals obtained in FTIR spectrophotometer.

| ALF1 | ALF4 | BF1 | BF4 | Wavenumber Range $cm^{-1}$ | Functional Group | References |
|---|---|---|---|---|---|---|
| | | | **950–1200 Carbohydrate Band** | | | [18] |
| 1019 | 1021 | | | 980–1072 | C-O-C polysaccharides | [23,24] |
| | | 1036 | | | | |
| | | | 1053 | | | |
| | | 1036 | | 1030–1099 | P=O nucleic acids | [25] |
| | | | 1053 | | | |
| 1075 | 1074 | 1074 | | | | |
| 1075 | 1074 | 1074 | | 1070–1140 | C-O-C | [26] |
| 1151 | 1149 | | | 1134–1174 | C-O-C polysaccharides | [23] |
| | | | 1219 | 1210–1240 | P=O polysaccharides | [27] |
| | | 1240 | | | | |
| | | 1240 | | 1230–1244 | P=O polysaccharides | [25] |
| | | 1240 | 1231 | 1230–1310 | C-N secondary amide | [26] |
| | | 1262 | 1264 | | | |
| | | 1350 | | 1191–1356 | P=O polysaccharides, phosphodiester | [23] |

**Table 15.** *Cont.*

| ALF1 | ALF4 | BF1 | BF4 | Wavenumber Range $cm^{-1}$ | Functional Group | References |
|---|---|---|---|---|---|---|
| | 1379 | | | 1370–1398 | $CH_3$, $CH_2$, C-O proteins, and carboxyl groups | [25] |
| | | 1385 | 1381 | | | |
| | 1413 | 1401 | 1400 | 1390–1430 | C-N amide stretching | [26] |
| | | | 1405 | | | |
| | | | 1411 | | | |
| | 1413 | 1401 | 1400 | 1392–1460 | C-O carboxyl groups | [25] |
| | 1444 | | 1405 | | | |
| | | | 1411 | | | |
| | | | **1450–1720 Amino acid band** | | | [28] |
| | | | **1490–1710 Protein band** | | | [18] |
| | 1452 | 1454 | 1454 | 1450–1456 | $CH_2$, $CH_3$ Lipids and proteins | [23,25] |
| 1539 | 1536 | 1538 | 1533 | 1515–1570 | N-H secondary amide | [26] |
| 1546 | | | 1537 | | | |
| 1639 | 1630 | 1631 | 1634 | 1630–1680 | C=O secondary amide | [26] |
| | | | **2800–3000 Lipid band** | | | [18] |
| 2852 | 2851 | 2852 | 2851 | 2850–2960 | $CH_2$ symmetrical nucleic acids | [29] |
| 2874 | 2873 | 2871 | 2873 | 2960–2975 | $CH_3$ asymmetric Lipids | [25] |
| 2922 | 2919 | 2921 | 2919 | 2916–2936 | $CH_2$ asymmetric Lipids | [29] |
| | | 2955 | 2960 | 2952–2972 | $CH_3$ symmetrical Lipids | [29] |
| 3280 | 3281 | 3280 | 3281 | 3170–3370 | N-H secondary amide | [26] |
| 3285 | | | | | | |

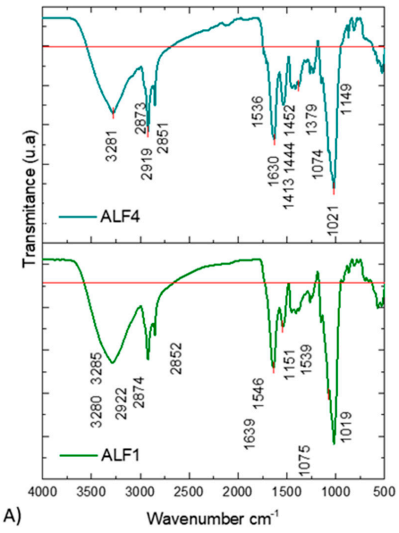
The identification and lipid content calculation were performed by gas chromatography using the AOAC 996.06 2001 methodology. The results are shown in Table 16.

**Table 16.** Fatty acid profile of *Scenedesmus dimorphus* biomass grown in eutrophicated lagoon water and Bayfolan 0.3%, under F1 (10.5:13.5) and F4 (13.5:10.5) photoperiods.

| | BF1 | BF4 | ALF1 | ALF4 |
|---|---|---|---|---|
| **Saturated Fatty Acids (%)** | **60.52** | **62.66** | **53.02** | **55.86** |
| Caprylic acid (C8:0) | 2.5 | 3.01 | 3.9 | 4.37 |
| Capric acid (C10:0) | 2.15 | 2.61 | 3.4 | 3.81 |
| Lauric acid (C12:0) | 2.40 | 2.73 | 3.57 | 4.08 |
| Tridecanoic acid (C13:0) | 1.33 | 1.5 | 1.86 | 2.03 |
| Myristic acid (C14:0) | 2.82 | 4.02 | 4.16 | 4.65 |
| Pentadecanoic acid (C15:0) | 1.26 | 1.65 | 1.81 | 2.01 |
| Palmitic acid (C16:0) | 18.17 | 18.71 | 11.59 | 13.09 |
| Margaric acid (C17:0) | 4.11 | 2.01 | 3.03 | 2.78 |
| Stearic acid (C18:0) | 9.66 | 8.35 | 11.24 | 8.79 |
| Arachidic acid (C20:0) | 2.73 | 3.44 | 4.37 | 5.13 |
| Heneicosanoic acid (C21:0) | 1.52 | 0 | 0 | 0 |
| Behenic acid (C22:0) | 7.87 | 9.71 | 0 | 5.12 |
| Tricosanoic acid (C23:0) | 1.21 | 1.46 | 4.09 | 0 |
| Lignoceric acid (C24:0) | 2.79 | 3.42 | 0 | 0 |

**Table 16.** *Cont.*

| | BF1 | BF4 | ALF1 | ALF4 |
|---|---|---|---|---|
| **Monounsaturated fatty acids** | **17.60** | **18.40** | **23.96** | **24.97** |
| Myristoleic acid (C14:1 cis 9) | 1.39 | 1.67 | 2.18 | 2.28 |
| Pentadecanoic acid (C15:1 cis 10) | 1.52 | 1.94 | 2.44 | 0 |
| Hexadecenoic acid (C16:1 cis 9) | 1.81 | 2.09 | 2.39 | 6.5 |
| Margaroleic acid (C17:1 cis 10) | 1.56 | 1.91 | 2.46 | 2.64 |
| Oleic acid (C18:1 cis 9) | 9.57 | 9.2 | 12.41 | 11.31 |
| Eicosenoic acid (C20:1 cis 11) | 1.75 | 1.59 | 2.08 | 2.24 |
| **Polyunsaturated fatty acids** | **21.87** | **18.99** | **23.02** | **19.17** |
| Linoleic acid (C18:2 cis 9, 12) | 12.05 | 9.81 | 11.55 | 10.36 |
| Gamma-linoleic acid (C18:3 cis 6,9, 12) | 1.59 | 1.97 | 2.13 | 2.45 |
| Alpha-linolenic acid (C18:3 cis 9, 12, 15) | 4.14 | 5.78 | 5.42 | 6.36 |
| Eicosadienoic acid (C20:2 cis 11, 14) | 1.23 | 0 | 0 | 0 |
| Eicosatrienoic acid (C20:3 cis 8, 11, 14) | 1.53 | 0 | 0 | 0 |
| Arachidonic acid (C20:4 cis 5, 8, 11, 14) | 1.33 | 1.43 | 1.89 | 0 |
| Docosahexaenoic acid (C22:6 cis 4, 7, 10, 13, 16, 19) | 0 | 0 | 2.03 | 0 |
| **Trans fatty acids** | **0** | **0** | **0** | **0** |



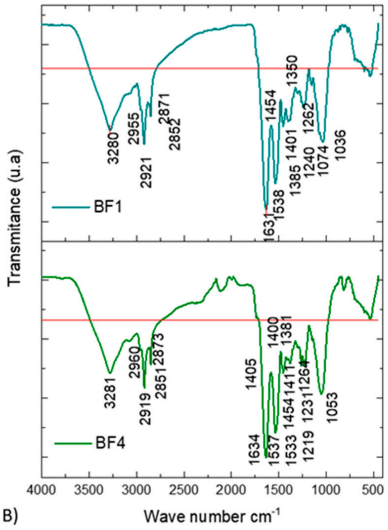




**Figure 4.** FTIR spectra of *Scenedesmus dimorphus* grown in eutrophicated lagoon water (**A**) and Bayfolan 0.3% V/V (**B**). F4 indicates photoperiod four (13.5:10.5) and F1 indicates photoperiod one (10.5:13.5).

## 4. Discussion

The genus *Scenedesmus* has the necessary characteristics to combine $CO_2$ fixation, lipid synthesis and water treatment [30]. The species *Scenedesmus dimorphus* was isolated from a wastewater effluent of the petrochemical industry of the city of Altamira, Tamaulipas, the study area of this research [31], which indicates its ability to develop under environmental conditions. Its growth potential has been investigated under different metabolisms in diverse culture media, which were summarized in Table 1, in which it can be observed that the investigations with *Scenedesmus dimorphus* have not varied based on the photoperiod, but by the addition of nutrients to change the phototrophic metabolism to mixotrophic, maintaining the same operating conditions. The biomass productivity of *Scenedesmus* dimorphus with residual water containing lactic acid is 2.5 g/L and is increased by adding 0.8 g/L $NaNO_3$ + 4 mg/L $K_2HPO_4$-$3H_2O$ up to 4.5 g/L biomass using the same conditions of 1500 lux and a 14:10 photoperiod [17]. When cultivated in the artificial culture medium BG11, it has a productivity of 96.5 mg/Ld and increases with the addition of apple pomace hydrolysate at 2% *w*/*v* up to 140.3 mg/Ld when cultivated with a 12:12 photoperiod and atmospheric $CO_2$ at a rate of 11 L/min [7]. Similarly, when cultivated in BBM, 96.4 mg/Ld are obtained. The production increases with the addition of 5 g/L of hydrolyzed sugarcane bagasse to 105.9 mg/Ld; however, the addition of 10 g/L of this nutrient decreases production to 105.9 mg/Ld because the turbidity of the substrate decreases the passage of illumination. These experiments were carried out under illumination of 120 µmol/$m^2$/s, a photoperiod of 16:8, and air supplementation [8].

Biomass productivity is a key factor for industrial applications of microalgae because they are photosynthetic organisms. Carbon and nitrogen elements are the most important in the metabolic pathway of microalgae [32]. The biomass productivities in eutrophicated lagoon water and 0.3% Bayfolan solution are different because eutrophicated lagoon water contains higher levels of nutrients and organic carbon; therefore, its metabolism is mixotrophic, and growth in 0.3% Bayfolan solution results in phototrophic metabolism.

The influence of the photoperiod on the growth of *Scenedesmus dimorphus* has not been evaluated previously; however, it was found that it has been cultivated in 12:12, 14:10 and 16:8 light:dark photoperiods. In this research study, it was found that the biomass productivity was different in the four photoperiods of study in lagoon water: the productivity was 0.053 g/L in photoperiod F1 (10.5:13.5), while 0.023 g/L was obtained in photoperiod F4 (13.5:10.5). In the Bayfolan 0.3% solution, no significant differences in productivity were observed among photoperiods F1, F2 and F3. Differences were only observed between photoperiods F1 and F4, where the productivities were 0.036 and 0.024 g/L, respectively.

The percentage of COD removal showed significant differences among the four photoperiods of study. The greatest difference was observed between F1 and F4, being 95.56 and 60.46%, that is, in photoperiod F1 (10.5:13.5), the greatest removal was obtained. On the other hand, in the Bayfolan 0.3% solution cultures, significant differences were also shown in the removal means. The greatest difference was observed between the F3 and F4 photoperiods: the removals were 88.83 and 43.16% respectively, that is, a greater removal was obtained in the F3 photoperiod (12.5:11.5). The results obtained in this research are related to those obtained in reported investigations, where the reported removal was 95.6% of COD by *Scenedesmus dimorphus* in wastewater with lactic acid [17]. Another study reported a removal of 95.9% of COD in domestic wastewater by *Scenedesmus* sp. [15], while *Chlorella vulgaris* was able to achieve 100% removal of COD in wastewater from a biological reactor [13].

The presence of the functional groups that form carbohydrates, amino acids, proteins and lipids was identified due to the fact that microalgae are organisms that are composed of these compounds [33]. Proteins are a sequence of amino acids, called the primary structure, where secondary amide bonds link the repeated units in a protein [26]. In the infrared spectrum, 9 amide bands can be observed, named A, B and I–VII. Among them, amide I is the most intense one, representing the C=O bond occurring in the 1600–1700 region

coupled to the N-H and C-N bending [28]. Amide I is observed in the 1585–1724 $cm^{-1}$ range, while amide II is in the 1490–1585 $cm^{-1}$ range [18].

The ALF1 sample does not present signals between 1191 and 1244 $cm^{-1}$ corresponding to the presence of nucleic acids. On the other hand, the confirmation of secondary amides is given by the presence of two C-N bonds and one N-H bond. Secondary amides are present in proteins, and despite having 4 signals in the N-H bonds, this does not present the confirmatory signals in C-N of a secondary amide; however, it presents the signals 1639, 3280 and 3285 $cm^{-1}$ of the C=O group and two N-H signals, confirming the presence of a primary amide [26].

Nitrogen is an essential component in the synthesis of proteins and nucleic acids in microalgae and is related to their growth [9] due to the fact that the culture in the lagoon water with F1 presented a higher concentration in mg/L in comparison with the one cultivated in F4. It is possible that the concentration of nitrogen in the culture was being depleted during the cultivation.

The biomass obtained from ALF4, BF1 and BF4 present signals corresponding to the presence of amide I [28] and amide II [18]; the presence of amides I and II is characteristic of protein chains [27]. Only sample BF4 presents three signals in the C-N range stretching amide and two signals in the N-H range of a secondary amide together with the C=O group of a secondary amide. Considering that secondary amides link the repeated units of the protein and two signals in C-N, one in N-H and the C=O should be present [26], it can be affirmed that only sample BF4 presents two protein-forming secondary amides. With respect to the 950–1200 $cm^{-1}$ band that characterizes the absorption of polysaccharides, it can be deduced that the microalgae cultivated in Bayfolan have a higher carbohydrate content. The use of Bayfolan favors the increase of pigments and proteins in the microalgae [13].

Regarding the representative bands of a lipid profile, the similarity of the signals among the samples was observed. The lipids have characteristic absorption bands, the absorption band of the C=O stretching of the ester and the vibration of the C-H stretching in the acyl chains around 2800–3000 $cm^{-1}$. This last one can characterize the lipid content [18]. The asymmetric and symmetric $CH_3$ and $CH_2$ groups in the 2800–3000 band are lipid hydrocarbons; these signals are representative of lipid accumulation in microalgae [27]. These results indicate that the samples have the same lipid profile.

The lipid profile showed the presence of saturated, monounsaturated and polyunsaturated fatty acids. The lipids obtained from the biomass cultured in Bayfolan 0.3% and in the eutrophicated lagoon water with photoperiod F4 (13.5:10.5) showed an increase in the percentage of saturated and monounsaturated fatty acids, in addition to a decrease in polyunsaturated fatty acids with respect to the lipid percentages obtained from the biomass cultured in photoperiod F1 (10.5:13.5). The increase in light hours in the photoperiod of cultivation influenced the fatty acid profile: caprylic, capric, capric, lauric, tridecanoic, arachidic, behenic and myristoleic fatty acids presented an increase, while stearic, oleic and linolenic acids presented a decrease. On the other hand, the palmitic acid content was constant in the % lipids obtained from the biomass grown in Bayfolan in both photoperiods. As for the lipid percentages obtained from the biomass grown in eutrophicated lagoon water under photoperiods F1 and F4, a higher palmitic acid content was observed in photoperiod F4 with respect to photoperiod F1. The fatty acid profile obtained from *Scenedesmus dimorphus* agrees with those obtained in related research. Among the lipids that have been obtained from this species are mainly C16 to C18 fatty acids, although C20 to C22 can also be obtained depending on the culture conditions [7,16,17].

The percentage of lipids found in the microalgae *Scenedesmus dimorphus* grown in Bayfolan 0.3% and eutrophicated lagoon water were 18.71 and 13.09% palmitic acid, 8.3 and 8.79% stearic acid, 9.2 and 11.31% oleic acid and 9.81 and 10. 36% of linoleic acid, respectively, which are lower than those reported by other studies, where in BG11 medium, wastewater with lactic acid and municipal wastewater had 24.23, 25.01 and 21.6% palmitic acid, 0, 53.24 and 23.3% oleic acid, and 44.01, 0 and 24.01% linolenic acid, respectively [16,17].

## 5. Conclusions

By increasing the number of hours of light, the cultures in the eutrophicated lagoon water produced a decrease in the biomass productivity and percentage of COD removal. The highest biomass productivity was obtained in photoperiod F1 (10.5:13.5) hours L:O, $0.053 \pm 0.0015$ g/Lday and a removal of 95.6%, while in the cultures grown in Bayfolan 0.3% under photoperiods F2 (11.5:12.5) and F3 (12.5:11.5) hours L:O, the biomass productivities and COD removal percentages were obtained without significant differences.

The same functional groups C=O, CH, $CH_3$ and $CH_2$ belonging to fatty acid formation were identified in the biomass of *Scenedesmus dimorphus* grown in both culture media under F1 and F4 photoperiods, by FTIR spectroscopy.

The main fatty acids identified by the gas chromatography technique were palmitic stearic, oleic and linolenic acids in both culture media. The increase in light hours in the photoperiod induced the increase of saturated and monounsaturated fatty acids, as well as the decrease of polyunsaturated fatty acids.

**Author Contributions:** Conceptualization, S.G.P.B. and M.d.R.C.C.; methodology, S.G.P.B. and L.A.V.; software, S.G.P.B. and M.L.M.R.; validation, L.A.V. and U.P.G.; formal analysis, N.V.G.R. and M.L.M.R.; investigation, S.G.P.B. and L.A.V.; resources, N.V.G.R. and U.P.G.; writing—original draft preparation, S.G.P.B.; writing—review and editing, M.d.R.C.C. and L.A.V.; visualization, M.L.M.R. and N.V.G.R.; supervision, M.d.R.C.C. and U.P.G. All authors have read and agreed to the published version of the manuscript.

**Funding:** We are grateful to CONACYT for the national grant 21542 awarded to the first author to study for a PhD in engineering sciences at the Tecnológico Nacional de México/Instituto Tecnológico de Ciudad Madero.

**Data Availability Statement:** Data are contained within the article.

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

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