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Nejayote and Food Waste Leachate as a Medium for *Scenedesmus acutus* and *Haematococcus pluvialis* Production: A Mixture Experimental Design

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Abstract: The wastewaters of nejayote and food waste leachate are polluting effluents with a high load of organic matter that cause great problems when discharged to water receptors. In this work, we investigated the treatment of nejayote wastewaters and food waste leachate for the production of microalgae *Scenedesmus acutus* and *Haematococcus pluvialis*. For *Scenedesmus acutus*, treatment with 10% food waste leachate and 90% growth medium resulted in a concentration of 5.34 g/L in 20 days (μmax = 0.16/day). Meanwhile, 10% nejayote and 90% medium growth produced 4.45 g/L at 20 days (μmax = 0.13/d). A significant reduction of up to 82.6% ammonium, 84.1% orthophosphate, and 87.25% COD was also observed between the different treatments. For *Haematococcus pluvialis*, the treatment of 90% food waste leachate and 10% growth medium produced a concentration of 4.73 g/L at 6 days (μmax = 0.71/day), while the mixture of 25% najayote, 25% food waste leachate, and 50% growth medium produced a concentration of 5.5 g/L at 20 days (μmax = 0.25/dat). A reduction of up to 97.8% ammonia, 97.4% orthophosphate, and 73.19% COD was also recorded. These findings demonstrated the potential to cultivate microalgae and extract biomolecules for commercial purposes.

Keywords: nejayote; food waste leachate; microalgae

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1. Introduction

Due to population growth and industrialization, the demand for domestic and industrial water has increased in cities. There was, therefore, an increase in the amount of wastewater spilled into the sewer system. The reuse of wastewater has become important as it increases the availability of water and thus promotes the conservation of water resources. The objective of wastewater treatment is to reduce specific contaminant concentrations to safe levels for reuse or the discharge of sewage into the environment [1]. Contaminated water generated by various activities, such as industrial and municipal, is called wastewater. Wastewater treatment plants help to remove contaminants from water to comply with water quality standards and regulations [2].

The use of microalgae for wastewater treatment has gained global attention. This interest is due to the fact that, in addition to purifying nutrients from wastewater, it also generates biomass for various applications [3]. Microalgae are photosynthetic organisms, and among their different industrial applications are food, feed, cosmetics, pharmaceuticals, and wastewater treatment. In addition to the increased interest of researchers in recent years, wastewater treatment is considered beneficial for the environmental and economic areas [4]. These microorganisms have the ability to survive in different environmental conditions as well as grow in various wastewater sources, for example, municipal, industrial, domestic, agricultural, and saline wastewater mixtures. Microalgae absorb toxic nutrients and heavy metals in wastewater that help in their growth, resulting in wastewater treatment [5]. In the 1970s, microalgae crops began to be used as a tertiary treatment of wastewater. For the use of microalgae in this process and in the production of biofuels, it is desired that they possess certain characteristics, such as a higher growth rate and productivity, a high lipid content, greater tolerance to possible contaminants and toxic compounds present in treated wastewater, a high tolerance for NH_4^+, high O_2 generation rates, and a high reduction in CO_2. Although these characteristics are of vital importance, the growth of microalgae is the limiting factor for the efficient removal of nutrients and contaminants from an algae and bacterial culture [6]. Because microalgae grow in a liquid medium, they need several steps to obtain biomass. The main steps in the processing of microalgal biomass are cultivation, collection, and extraction. Generally, the growth of microalgae requires a sufficient supply of a source of carbon and light for photosynthesis to occur. In addition to the sources of organic carbon, salts, vitamins, nitrogen, and phosphorus for the growth of microalgae, a balance between CO_2, pH, temperature, and light intensity is important [7].

A thermo-alkaline process known as nixtamalization is utilized to remove the pericarpium from maize grains, either completely or partially. The by-products nixtamal and nejayote are generated as a result of this procedure. Nixtamal grains, which are silky in texture, are utilized as constituents in the production of nixtamalized maize products. In contrast, nejayote is the alkaline water that is produced when the cereals are boiled. In Latin America, where nixtamalized products are regarded as an absolute necessity, this by-product is produced in significant quantities due to the high demand for it. The conventional nixtamalization method requires as much as 7.5 L of water per kilogram of maize, which leads to the formation of nejayote [8]. This wastewater causes an environmental problem due to its alkalinity and biological demand for oxygen (DBO). Unfortunately, smaller processors discharge nejayote directly into the wastewater, while larger processors use considerable resources to treat nejayote aerobically and/or anaerobically, thereby decreasing the DBO and chemical oxygen demand (COD), but sometimes without achieving the desired degree of cleaning [9]. Liquidation can be defined as a contaminated liquid or wastewater that is created by the drainage of rainwater, is filtered through accumulated pollutants, and passes to the soil and nearby areas. The content of the dump can vary depending on the waste content, water content, type, and age of the landfill, as well as climatic conditions [10]. Recently, possible solutions have been sought for the use of leachate by extracting its organic content and nutrients. One of the interesting approaches that leachate has had is the production of biomass from microalgae as a source of renewable energy. In addition to the decrease in nutrients from the biomass generated by the growth of microalgae, the generated biomass is a by-product that can be marketed to produce biofuels, cosmetics, feed, and fertilizers, among others. Unfortunately, this is limited by its pathogenic properties, so it is necessary to disinfect the leachates [11].

Various studies evaluated the production of *Chlorella* sp. in a growing medium with different concentrations of lixiviates and found a reduction in the growth of microalgae at concentrations greater than 10%. Other studies highlighted the importance of the initial biomass concentration in a cultivation medium with a sanitary filling liquefied for greater efficiency in biomass productivity, nutrient elimination, COD, and phenol. One feature of these studies was that they were carried out in a laboratory in batches, controlling temperature and radiation [12].

Therefore, the aim of this study was to investigate the use of a mixture of nejayote and food waste leachate as a medium of cultivation for two types of microalgae and to assess their potential to reduce nutrients in both types of waste. For this purpose, the combination of both wastewaters, taking into account its high contaminant content, was used to demonstrate its potential as a medium of growth for microalgae. The goal was to find the best response variables using an experimental mixing design and see what happened to cell growth when these two types of wastewaters were mixed together in a lab setting.

2. Materials and Methods

2.1. Algal Strains and Cultivation Conditions

Scenedesmus acutus and *Haematococcus pluvialis* were obtained from the crop collection of the Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mexico. The strain was preserved in the baseline medium Bold (BBM), containing the following compounds in mg/L: $NaNO_3$ (250), $CaCl_2{\cdot}2H_2O$ (25), $MgSO_4{\cdot}7H_2O$ (75), K_2HPO_4 (75); KH_2PO_4 (175), and NaCl (25). The concentration of trace elements in (mg L^{-1}) was the following: EDTA (50), KOH (31), $FeSO_4{\cdot}2H_2O$ (4.98), H_3BO_3 (11.42), $ZnSO_4{\cdot}7H_2O$ (8.82), $MnCl{\cdot}4H_2O$ (1.44), MoO_3 (0.71), $CuSO_4{\cdot}5H_2O$ (1.57), and $Co(NO_3){\cdot}6H_2O$ (0.49). The initial pH of the medium was adjusted to 9 with the addition of base (0.1 mol/L NaOH) and acid (0.1 mol/L H_2SO_4) and sterilized at 121 °C for 20 min before inoculation. The microalgae were grown at a temperature of 25 ± 1 °C and under continuous fluorescent light of 40 µmol photons/$m^2{\cdot}s$, measured with a photometer (Sensor Quantum 6 Bar, Spectrum Technologies, USA), and with a light/dark cycle of 16/8 h. Filtered air was supplied from the bottom of the bioreactor using an air compressor at a flow rate of 1.5 L/min. Microalgal cells were used during the exponential growth phase to inoculate the experiments. The culture was manually shaken 2 times a day.

The experiments were carried out in batches using 1000-mL borosilicate bottles. At the beginning of each series of experiments, a calculated volume of pre-cultivated cell suspension was inoculated for a total working volume of 500 mL and an initial biomass concentration of 0.5 g/L. Table 1 shows an experimental design based on a mixture of extreme vertices with five distinct combinations of food waste leachate, bold basal medium (BBM), and nejayote. Over the course of the 20-day experiment, 20 mL samples were taken every 4 days on a regular basis to evaluate the pH in each treatment without making any adjustments, quantify nutrients, and measure biomass growth using spectrophotometry at 680 nm. A schematic of the experimental protocol with the treatment (T1–T5) requirements is shown in Figure 1.

Table 1. Extreme vertex experimental mixture design for the evaluation of food waste leachate and nejayote as *S. acutus* and *H. pluvialis* culture media.

OrderEst	Run Order	TipoPt	Nejayote (%)	Leachate (%)	BBM (%)
11	1	1	0	90	10
8	2	1	90	0	10
7	3	1	0	10	90
2	4	1	0	10	90
1	5	1	0	90	10
14	6	1	10	0	90
10	7	0	25	25	50
5	8	0	25	25	50
15	9	0	25	25	50
6	10	1	0	90	10
3	11	1	90	0	10
4	12	1	10	0	90
9	13	1	10	0	90
13	14	1	90	0	10
12	15	1	0	10	90

Note(s): OrderEst: standard order of the runs, TipoPt: identifies the center points and cube points.

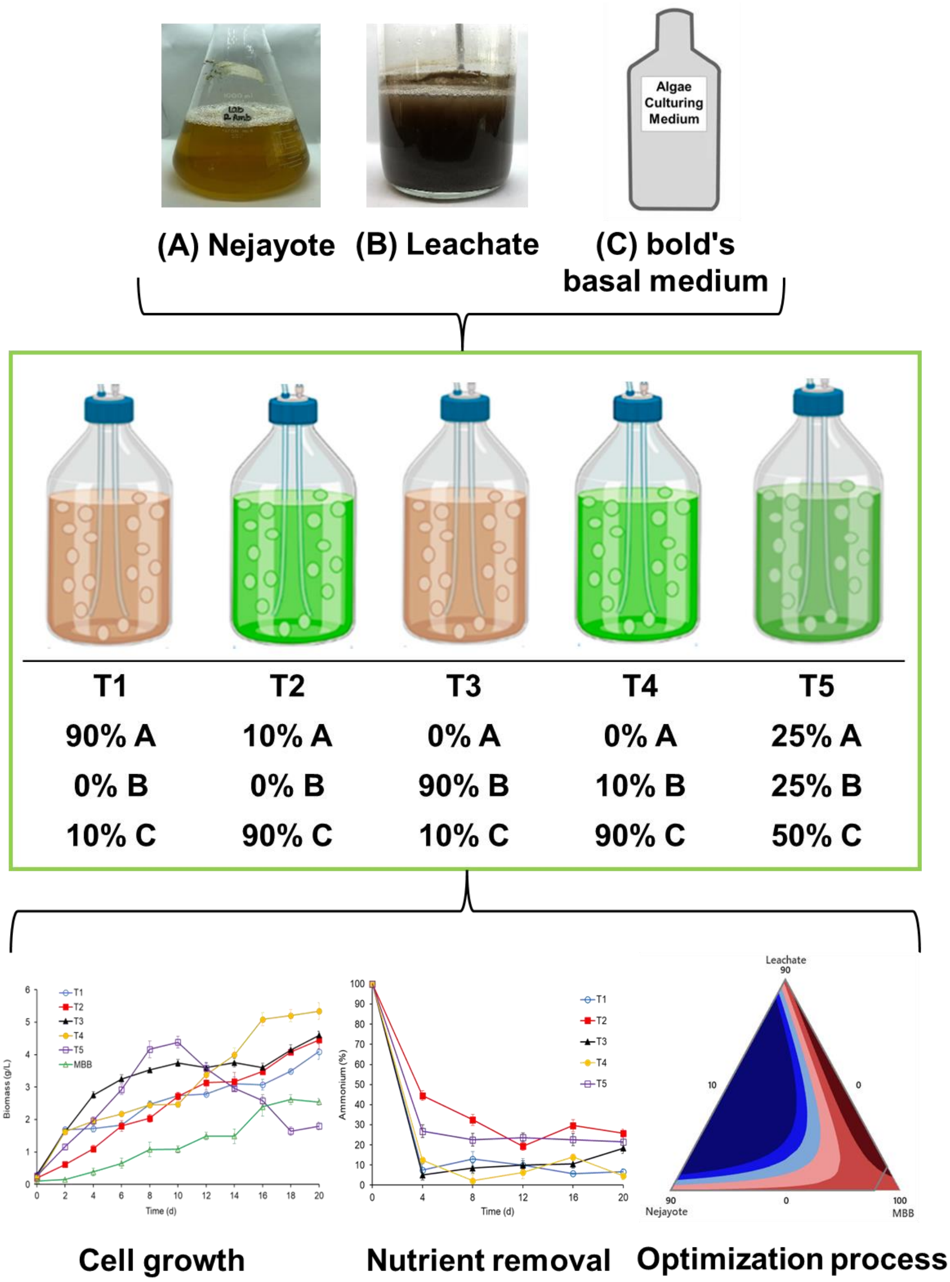

Figure 1. Experimental protocol in this study.

2.2. Waste Streams Source

The food waste leachate was collected from the storage tank after the primary treatment (solid separation) of a local "la estrella" market in Nuevo León, Mexico. The nejayote was obtained from the maize processing company "Maseca", which is situated in the same region. Both waste stream sources were filtered using 0.45 µm nylon microfilters to remove fine particles in suspension before characterization. Subsequently, the samples were stored at −20 °C, and the physicochemical properties of the waste were evaluated.

2.3. Analytical Procedures

For each set of experiments, samples were extracted every 2 days and analyzed for biomass concentration, PO_4^{3-}, NH_4^+, and soluble chemical oxygen demand (COD). Biomass concentration was monitored every 2 days through the correlation between optical density (OD) and the dry weight of the suspension solids (SS) of algae biomass for each species. The OD was measured at 680 nm using a spectrophotometer (Thermo GENESYS

10-Vis, USA), and the dry weight of the algae biomass was determined gravimetrically as SS according to the standardized method 2450-D APHA.

The pH was measured using a pH meter (Orion Star A215, Thermo Scientific, USA) using the 4500-H method. The phosphate concentration (PO_4^{3-}) was measured by the amino acid method (HI93717, Hanna Instruments, USA). Ammonium nitrogen (NH_4^+) was measured using the Nessler method (HI93733, Hanna instruments), and soluble chemical oxygen demand was analyzed using the closed-flow method, 5220-D APHA. The alkalinity, total solids, total volatile solids, and total suspended solids were measured according to the APHA 2320-A, 2450-B, 24-50-E, and 24-50-D methods, respectively. The liquid samples were filtered through a 0.45 µm filter to remove microalgae cells and other particles before analysis. All the chemicals used were of analytical quality. All samples were carried out quadruplicate and expressed as an average with standard deviation (SD).

2.4. Statistical Analysis

The experimental data were examined using Minitab 19. To optimize the responses relating to biomass production, ammonium nitrogen, orthophosphate, and COD, a Dunnett analysis and extreme vertex mixing design were employed. The objective was to determine the optimal levels of the concentration of food waste lixiviate and nejayote that would provide the best biomass growth and the greatest reduction in nutrients. In addition, mixing surface graphs were generated for each of the variables analyzed.

3. Results and Discussion

3.1. Sample Characteristics

The nutritional composition and pH values of the nejayote and food waste leachate are shown in Table 2. The pH of the sewage was alkaline. Both samples contained significant amounts of nitrogen; food waste leachate showed a relatively higher amount in the form of ammonium, orthophosphates, and COD of 290, 17.8, and 4788 mg/L, respectively. However, food waste leachate exhibited low sulfate concentrations, alkalinity, and hardness. The result of this work was consistent with the results of some previous studies [13–16]. The nejayote wastewater was characterized. The pH was found to be 8.6, the total solids were 10 g/L, the COD was 1798 mg/L, ammonia was 23.91 mg/L, and orthophosphates were 8.9 mg/L [17–20].

Table 2. Characteristics of the food leachate and nejayote sample.

Parameter	Units	Food Leachate	Nejayote
Total dissolved solids	(g/L)	1.97 ± 0.05	9.66 ± 0.08
Total solids	(g/L)	2.16 ± 0.09	10.04 ± 0.18
Total volatile solids	(g/L)	1.31 ± 0.02	7.02 ± 0.21
Total fixed solids	(g/L)	0.86 ± 0.01	3.03 ± 0.14
Volatile dissolved solids	(g/L)	1.73 ± 0.03	6.75± 0.08
Fixed dissolved solids	(g/L)	0.24 ± 0.01	2.90± 0.10
Ammonium	(mg NH_4-N/L)	290 ± 8.16	23.91 ± 2.66
Nitrate	(mg NO_3-N/L)	260 ± 23.15	146.25 ± 17.78
Nitrite	(mg NO_2-N/L)	1446 ± 37.48	1927.73 ± 59.44
COD	(mg O_2/L)	4788 ± 68.29	1798.33 ± 43.03
Orthophosphates	(mg PO_4^{3-}/L)	17.8 ± 0.32	8.9 ± 0.03
Electric conductivity	(S/m)	5.65 ± 0.08	3.03 ± 0.03
pH		9.1	8.6
Sulfates	(mg SO_4^{2-}/L)	40.05 ± 1.64	473.39 ± 14.77
Turbidity	NTU	2390 ± 86.74	944 ± 47.09
Alkalinity	(mg $CaCO_3$/L)	731 ± 17.03	2466.66 ± 400.04
Hardness	(mg $CaCO_3$/L)	640 ± 20.78	2050.00 ± 173.20
Chloride	(mg Cl^-/L)	0.251 ± 0.02	0.127 ± 0.01

3.2. Biomass Growth of S. acutus and H. pluvialis in Nejayote and Food Leachate

Before investigating the culture of these microalgae using nejayote and food waste leachate, a preliminary cultivation of both microalgae in BBM was conducted to investigate their behavior under optimal conditions. Twenty days were used for monitoring the development of both microalgae. The trend of the development curve (Figure 2a) was consistent with the findings of other studies that stabilization occurred around day 15 [21]; nonetheless, our study's findings show that, in the case of *S. acutus*, stabilization started after day 16. Furthermore, the growth kinetics of our sample matched those of the *S. acutus* growing in the central region of BG-11, as reported previously [22,23]. Furthermore, the obtained μmax growth rate = 0.12 was comparable to that of previous research that found μmax = 0.15 [24]. Nevertheless, on day 20, the concentration of *H. pluvialis* increased to nearly 3.3 g/L (Figure 2b). The *H. pluvialis* growth curve tendency observed in this study is in accordance with the results of other studies [25,26]. The ability of different nejayote and food waste leachate combinations (T1–5) to act as a growing medium for *S. acutus* and promote growth was investigated. Every treatment showed a distinct growth pattern (T1–5). The highest growth rate, statistically established to be T4 (10% food waste leachate + 90% BBM + 0% nejayote), was observed to reach 5.34 g/L on day 20. T3 (90% food waste leachate + 10% BBM + 0% nejayote) was shown to be the second-best promoter of biomass growth, with 4.59 g/L on day 20. As prospective growing means for *S. acutus*, T2 and T1 (90% nejayote) showed less favorable results (Figure 2a). Around day 16, the growth increases, and in T4, the growth trend exceeds the growth curve with the BBM. Nevertheless, the biomass obtained in the T1–4 treatments was higher than the biomass that the BBM would be able to produce. After 20 days, T5's growth decreased to 1.79 g/L from a peak of 4.38 g/L on day 10. Our result of 5.34 g/L generated in T4 is higher than that of a study that used landfill leachate for the growth of biomass from *Scenedesmus* sp. The best result was attained with an 80% landfill leachate, producing 4.21 g/L. Compared to our study [27], we found that treatment with 100% landfill leachate did not yield excellent results, and the higher amounts were below 1.63 g/L. *Scenedesmus* sp. growth curves (μmax = 0.6) [27] most closely approach the T5 and T3 growth curves (μmax = 0.39, Table 3). The nejayote and food waste leachate concentrations each showed different growth rates of *H. pluvialis* (T1–T5). In terms of statistics, T5 yielded the highest results, obtaining 6.02 g/L in 16 days. T2 had a positive concentration of 5.11 g/L on day 12, while T3 had a positive concentration of 5.98 g/l on day 10. When used as a medium of cultivation for *H. pluvialis*, treatments with 90% nejayote demonstrated an increase in the growth of cells, comparable to the outcomes shown with *S. acutus* (Figure 2b). The growth curve with the BBM was not as pronounced as the growth trend in T1–T5, however in T2–T5, the growth peak was reached on days 10–16 rather than day 20. The nejayote and food waste leachate concentrations each showed different growth rates of *H. pluvialis* (T1–T5). In terms of statistics, T5 yielded the highest results, obtaining 6.02 g/L in 16 days. T2 had a positive concentration of 5.11 g/L on day 12, while T3 had a positive concentration of 5.98 g/L on day 10. When used as a medium of cultivation for *H. pluvialis*, treatments with 90% nejayote demonstrated an increase in the growth of cells, comparable to the outcomes shown with *S. acutus* (Figure 2b). The growth curve with the BBM was not as pronounced as the growth trend in T1–T5, however in T2–T5, the growth peak was reached on days 10–16 rather than day 20. Previous research has demonstrated that *H. pluvialis* can reach up to 9–10 g/L [28,29]. As demonstrated in Figure 2b, T3 produced a higher value of μmax = 0.71/d on day 6 for *H. pluvialis* than did cell growth from other treatments. A study found that cultivated *H. pluvialis* produced a μmax of 0.68/d, which was less than the μmax found in this investigation (Table 3) [30]. Additionally, treatments containing 90% nejayote did not function optimally as a growing medium for *S. acutus* or *H. pluvialis*, indicating that these concentrations are not ideal for promoting the growth of microalgae. The mixture design for cell growth's contour graph (Figure 2c) indicates that using more food waste leachate than nejayote was the most effective way to use nejayote and leachate as a medium for cultivating *S. acutus*. Furthermore, the contour chart (Figure 2d) for *H.*

pluvialis demonstrates that any wastewater may be utilized as a medium for *H. pluvialis* culture, and that a higher biomass yield representing a higher waste consumption in our study can be obtained with a smaller quantity of BBM. BBM was used in the formulation of test media for every experiment for two reasons: first, to let light into the crop mattress; and second, to dilute the organic load to find the concentration at which it yields the optimum results for cell development with both wastewaters. The growth kinetics of both microalgae, as shown in Figure 2a,b, can be modified using a nonlinear regression model (Table 3). With a high concentration of food waste leachate in the early phases of the microalgae's growth, it is clear that for *S. acutus*, the model fits well in T3 and T5 from days 0 to 6. Moreover, it was shown that in T1–T5, the model could explain the growth behavior of the microalgae in H. pluvialis. In every treatment, the pH was maintained between 8 and 10 without being impacted by the development of any microalgae cells.

Table 3. Logit regression model of *S. acutus* and *H. pluvialis* cell growth with respect to treatment of nejayote and food waste leachate proportion over a predetermined period of time.

Treatment	Period (d)	Biomass Concentration (g/L)	Maximum Specific Growth Rate (1/d)	Maximum Cell Productivity (g/L/d)	R^2	Model Equation
			Scenedesmus acutus			
BBM	0–6	0.65 ± 0.16	0.29	0.09	0.92	
	0–20	2.54 ± 0.09	0.12	0.12	0.95	$Y = \frac{3.60+(-0.31-3.60)}{1+e^{((t-12.91)/5.94)}}$
T1	0–6	1.82 ± 0.05	0.31	0.25	0.67	
	0–20	4.09 ± 0.23	0.01	0.19	0.91	$Y = \frac{3.34+(-104.38+3.34)}{1+e^{((t+39.69)/9.66)}}$
T2	0–6	1.80 ± 0.17	0.37	0.26	0.98	
	0–20	4.45 ± 0.26	0.15	0.21	0.98	$Y = \frac{6.60+(-217.30-6.60)}{1+e^{((t+74.05)/20.79)}}$
T3	0–6	3.25 ± 0.12	0.39	0.48	0.96	
	0–20	4.59 ± 0.13	0.13	0.21	0.75	$Y = \frac{4.00+(-203.34-4.00)}{1+e^{((t+18.29)/4.42)}}$
T4	0–6	2.17 ± 0.04	0.38	0.32	0.82	
	0–20	5.34 ± 0.16	0.16	0.25	0.95	$Y = \frac{4.61+(-185.70-4.61)}{1+e^{((t+48.08)/11.60)}}$
T5	0–6	2.92 ± 0.14	0.39	0.44	0.99	
	0–20	1.79 ± 0.11	0.09	0.13	0.06	$Y = \frac{3.00+(0.22-3.00)}{1+e^{((t-3.05)/1.04)}}$
			Haematococcus pluvialis			
BBM	0–6	1.19 ± 0.18	0.19	0.13	0.88	
	0–20	3.30 ± 0.12	0.10	0.14	0.95	$Y = \frac{3.41+(-99.84-3.41)}{1+e^{((t+77.20)/20.80)}}$
T1	0–6	2.53 ± 0.18	0.35	0.31	0.89	
	0–20	4.23 ± 0.12	0.19	0.11	0.89	$Y = \frac{3.42+(-156.19-3.42)}{1+e^{((t+26.45)/6.28)}}$
T2	0–6	2.12 ± 0.08	0.30	0.33	0.99	
	0–20	4.44 ± 0.12	0.20	0.13	0.82	$Y = \frac{4.71+(0.38-4.71)}{1+e^{((t-6.81)/2.03)}}$
T3	0–6	4.73 ± 0.31	0.71	0.40	0.95	
	0–20	4.62 ± 0.23	0.20	0.11	0.49	$Y = \frac{5.44+(0.45-5.44)}{1+e^{((t-4.15)/0.94)}}$
T4	0–6	2.15 ± 0.11	0.30	0.33	0.92	
	0–20	3.04 ± 0.17	0.13	0.11	0.72	$Y = \frac{3.92+(-1.08-3.92)}{1+e^{((t-3.20)/4.27)}}$
T5	0–6	3.48 ± 0.05	0.51	0.36	0.95	
	0–20	5.50 ± 0.33	0.25	0.13	0.89	$Y = \frac{5.59+(-331.41-5.59)}{1+e^{((t-43.94)/9.89)}}$

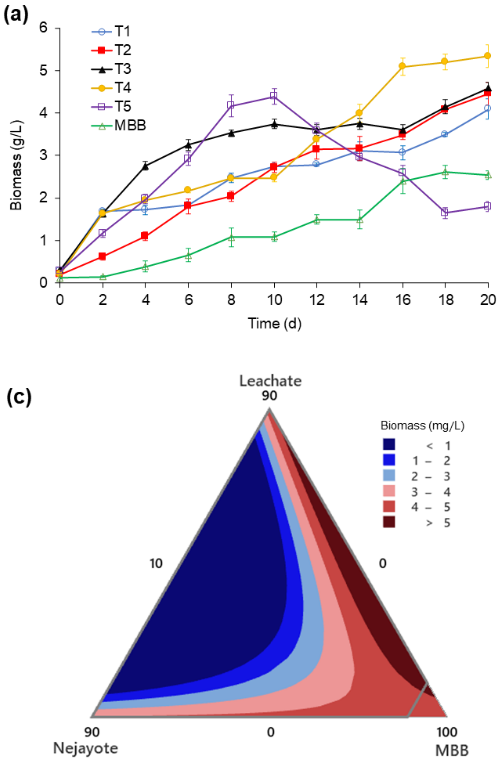

Figure 2. Growth curves in treatments (T1–5) and BBM of (**a**) *S. acutus* and (**b**) *H. pluvialis*. Mixture contour plots of (**c**) *S. acutus* and (**d**) *H. pluvialis*.

3.3. Efficiency in Nutrient Uptake

3.3.1. Ammonium (NH_4^+)

In order to understand how nitrogen behaved in each experiment and how microalgae might use this source to enhance growth, the NH_4^+ variable was examined. The NH_4^+ value decreased in both microalgal treatments when compared to the starting value (Figure 3). Because the microalgae require it as a source of nitrogen for growth and metabolism, this reduction occurred. Since the residues underwent sterilization, abiotic factors like nitrifying bacteria cannot eliminate them, indicating that the microalgae are responsible for the nutrient elimination mechanism [31]. The NH_4^+ level drops the most in the first 4 days with both microalgae. Other studies have found similar results with the strains *Scenedesmus* sp. [32] and *Chlorella vulgaris* [33]. For *S. acutus*, the decrease in this value is contrasted with the microalgae growth from T1 to T5 on day 8 (Figure 3a). The NH_4^+ reductions of T3, T4, and T5, which demonstrated a larger reduction at the end of treatments of 71.9, 82.6, and 71.3%, respectively, did not differ statistically (Figure 3c). The treatment was analyzed using the ANOVA-Dunnett statistical approach with day 0 serving as the control group. NH_4^+ was found to have significantly decreased in T2, T3, and T4 over the course of 4 days, with T2 decreasing to 51.9% of 12.6 mg/L, T3 decreasing to 76.8% of 188.6 mg/L, and T4 decreasing to 82.6% of 34.1 mg/L. T5 experienced a decrease to 83.3% of 90.4 mg/L over the course of 12 days, and T1 did not significantly differ, declining to 26.1% of 16.3 mg/L in 4 days. According to the contour chart in Figure 3e, using a higher proportion of nejayote than food waste leachate is a good way to use *S. acutus* for NH_4^+ reduction. This will result in values of less than 10 mg/L of NH_4^+ after 20 days. In particular, it can be shown that the procedure yields the lowest values in the nejayote and BBM areas based on the surface's form. For *H. pluvialis*, NH_4^+ was eliminated significantly in all treatments (Figure 3b), with T4 resulting in a larger reduction. In terms of statistics, the ANOVA-Dunnett analysis revealed that the levels of NH_4^+ in T1 had significantly decreased, falling to 92.5% from 16.53 mg/L in 4 days; in T2, the levels had decreased to 80.6% from 12.58 mg/L in 12 days; in T3, the levels had decreased to 94.9% from 187.42 mg/L in 4 days; in T4, the levels had decreased to 97.8% from 34.09 mg/I in 8 days; and in T5, the amounts had dropped to 78.5% from 89.90 mg/L in 16 days (Figure 3d). Other investigations using wastewater from potato juice showed similar findings, with *H. pluvialis* being able to eliminate at least 83.4% of NH_4^+ [34]. The contour chart (Figure 3f) indicates that a medium with a larger proportion of nejayote is the optimum choice for reducing the NH_4^+ of nejayote and food waste leachate with *H. pluvialis*.

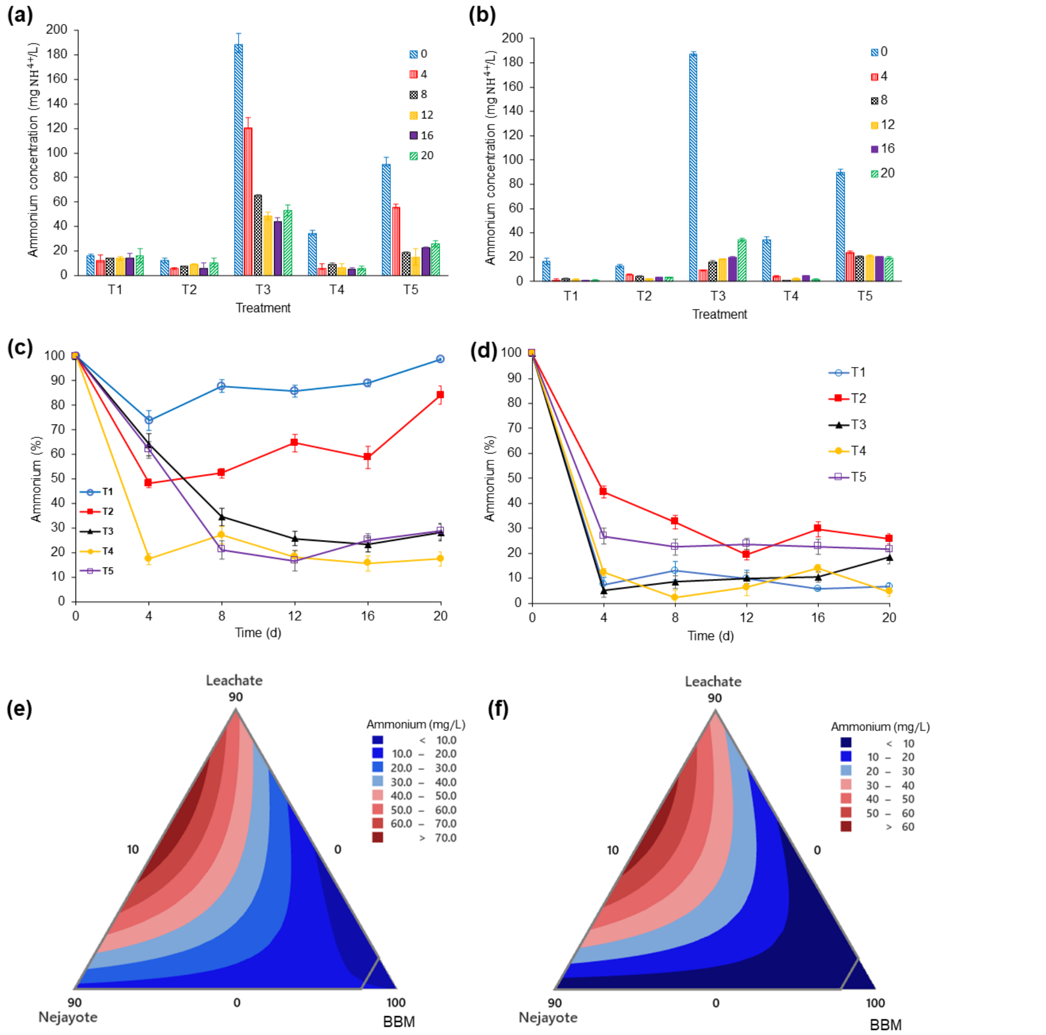

Figure 3. Ammonium removal profile in quantification for (**a**) *S. acutus* and (**b**) *H. pluvialis*. Ammonium removal profile in percentage (%) according to the initial value for (**c**) *S. acutus* and (**d**) *H. pluvialis*. Mixture contour plots in ammonium quantification for (**e**) *S. acutus* and (**f**) *H. pluvialis*.

3.3.2. Orthophosphate (PO_4^{3-})

PO_4^{3-} consumption was measured throughout the experiment. For *S. acutus*, all treatments demonstrated a reduction in PO_4^{3-} relative to the starting value (Figure 4a), suggesting that PO_4^{3-} started to decrease as microalgae began to grow. Furthermore, every treatment had a distinct reduction ratio for this value (Figure 4a). Consequently, the T2 and T4 treatments resulted in a further decrease. It is commonly recognized that when exposed to high concentrations of pollutants, such as phosphorus, different microalgae exhibit different behaviors [35]. Between the T1 and T5 treatments, there were statistically no changes in PO_4^{3-} reductions. As a control group, the ANOVA-Dunnett statistical analysis performed on day 0 revealed a substantial decrease in PO_4^{3-} in T1–T5 starting from the time the microalgae stopped growing. The T2 and T4 treatments showed the largest reductions, going from 10.5 mg/L to 84.1% and 83% to 19.6 mg/L, respectively (Figure 4c). Similar findings about the growth of *Scenedesmus* sp. microalgae, which may reduce phosphorus by 99%, were reported in other studies [36]. The optimum strategy to use *S. acutus* to minimize PO_4^{3-} is to use more nejayote than food waste leachate, as shown by the contour graph in Figure 4c. As a result, PO_4^{3-} values will be less than 2 mg/L. Specifically, it can be shown that the procedure yields the lowest values in the nejayote and BBM areas and a smaller percentage in the food waste leachate area, depending on the surface's form.

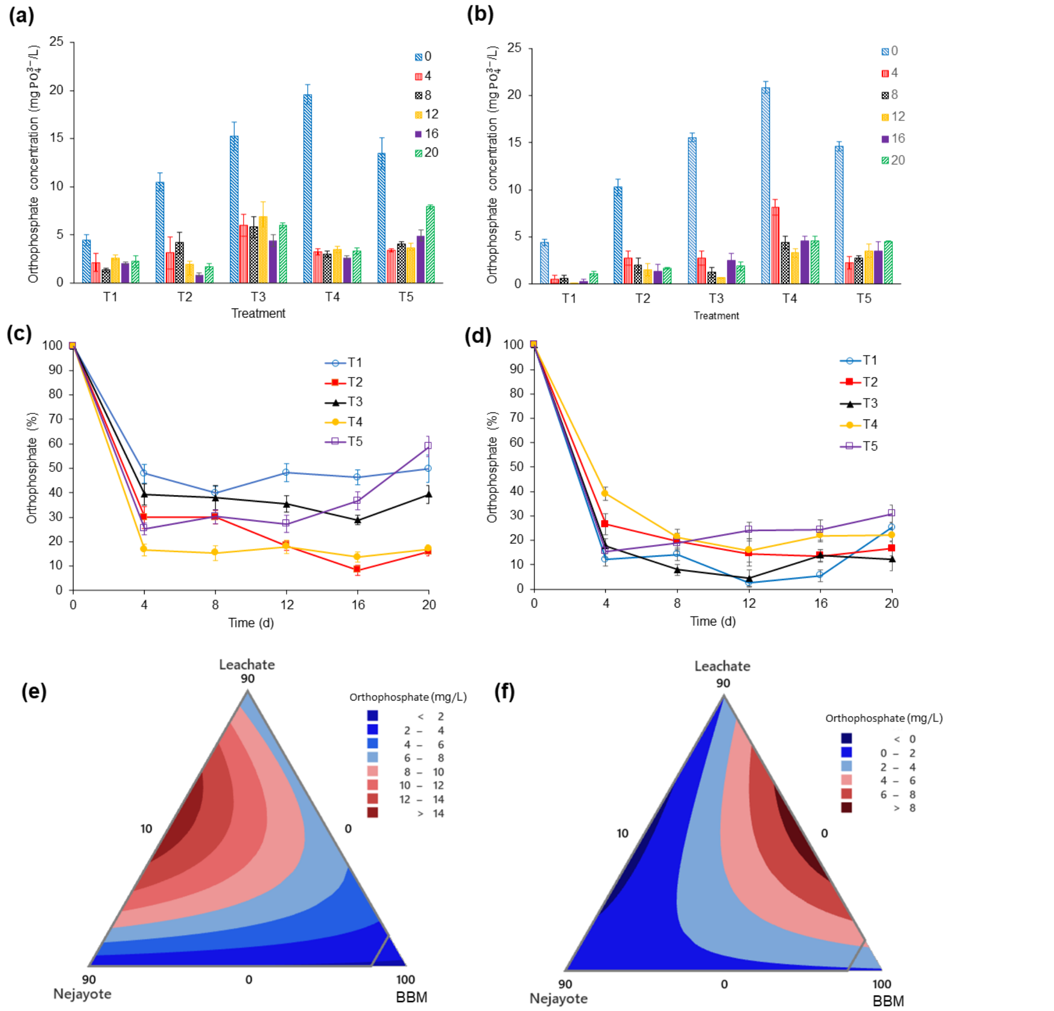

Figure 4. Orthophosphate removal profile in quantification for (**a**) *S. acutus* and (**b**) *H. pluvialis*. Orthophosphate removal profile in percentage (%) according to the initial value for (**c**) *S. acutus* and (**d**) *H. pluvialis*. Mixture contour plots in orthophosphate quantification for (**e**) *S. acutus* and (**f**) *H. pluvialis*.

Concerning *H. pluvialis*, the overall elimination of PO_4^{3-} was notable across all treatments; the decrease in this value corresponds to the growth of microalgae through the treatment (Figure 4b). Consequently, the T4 treatment was the area where the reduction was the largest. For the treatment taken as a control group on day 0, the ANOVA-Dunnett statistical analysis revealed a significant decrease in TP in T1, which decreased by up to 97.4% from 4.4 mg/L in 12 days, in T2, which decreased by up to 86.4% from 10.28 mg/L in 16 days, and in T3, which decreased by up to 95.12% from 15.57 mg/L in 12 days. T4 also experienced a significant reduction in TP, decreasing by up to 84.2% from 20.88 mg/L within 12 days, and T5 reduced by up to 84.6% from 14.63 mg/L in 4 days (Figure 4d). Similar results with home wastewater were observed in previous investigations, when *H. pluvialis* was able to decrease at least 98% of total phosphorus [37]. Based on the contour chart (Figure 4f), the most effective method of employing *H. pluvialis* to reduce the PO_4^{3-} of nejayote and food waste effluent is to increase the amount of nejayote used.

3.3.3. Soluble Chemical Oxygen Demand (COD)

Both microalgae's COD characterization during the experiment indicates a decline in all treatments (T1–5) (Figure 5a,b). The culture of both strains showed the largest reduction in COD during the growth and stationary stages; these findings are consistent with a previous investigation [38]. Furthermore, according to previous publications, the airing time during wastewater treatment significantly decreased COD; similar behavior was seen in T5, where cell growth was not primarily present in the middle by the end of the experiment [39]. For the treatment taken as a control group on day 0, the ANOVA-Dunnett statistical analysis indicated a significant reduction in COD in T1, which decreased to 69.69% from 1.668 mg/L in 20 days; in T2, to 68.29% from 280 mg/L in 20 days; in T3, to 86.53% from 4.362 mg/L in 20 days; in T4, to 87.25% from 1.295 mg/L in 8 days; and in T5, to 86.39% from 1.863 mg/L within 20 days (Figure 5c). These results are similar to those of [40] where the COD reduction of 82% achieved in this investigation was superior than the result obtained with *Scenedesmus* sp. [40]. Comparable amounts of food waste leachate, nejayote, and BBM were shown to result in decreased COD values, as seen by the surface mixture design graph shown in Figure 5e. Based on this finding, using similar amounts of nejayote and food waste leachate, which result in a COD concentration of less than 300 mg/L, would be the best option for the treatment composition when using *S. acutus* in the context of COD reduction.

The statistical analysis carried out in the case of *H. pluvialis* revealed a significant decrease in COD in all treatments. The T1, T2, and T4 treatments showed the least amount of decrease. With a decrease of up to 73.19% from 4357 mg/L on day 20, T3 was the treatment that achieved the greatest COD reduction (Figure 5b). *C. vulgaris* was able to reduce COD by at least 60% in other investigations involving water discharges from lixiviates [41]. This research yielded similar results. The lowering behavior of this value can be seen throughout the experiment in the interaction chart (Figure 5d) of the treatments with regard to the removal of COD, leading to a better COD reduction outcome in the treatment of T3. T5 was the other treatment where COD was significantly reduced. Using equal quantities of food waste leachate and nejayote is the optimal way to use *H. pluvialis* for reducing DQO in both products, according to the contour chart (Figure 5e). For lowering the COD value, the ratio of both wastewaters to the blue region is ideal. For the treatment taken as a control group on day 0, the ANOVA-Dunnett statistical analysis revealed a significant reduction in COD in T1, which decreased to 29.43% from 1636 mg/L in 20 days; in T2, to 71.73% from 287 mg/L in 8 days; and in T3, to 73.19% from 4357 mg/L at 20 days; in T4, to 56.16% from 1294 mg/L in 8 days; and in T5, until 56, 46% from 1874 mg/L at 8 days. Higher COD values in this microalgae affected the maximum cell growth in T3 (4357 mg/L) and T5 (1874 mg/L), suggesting that COD concentration has a significant role in cell growth [42]. These results are comparable to those seen with the *H. pluvialis* strain in palm oil mill effluent [43]. Additionally, the greatest drop in this variable is obtained in treatments with the highest initial amounts of COD.

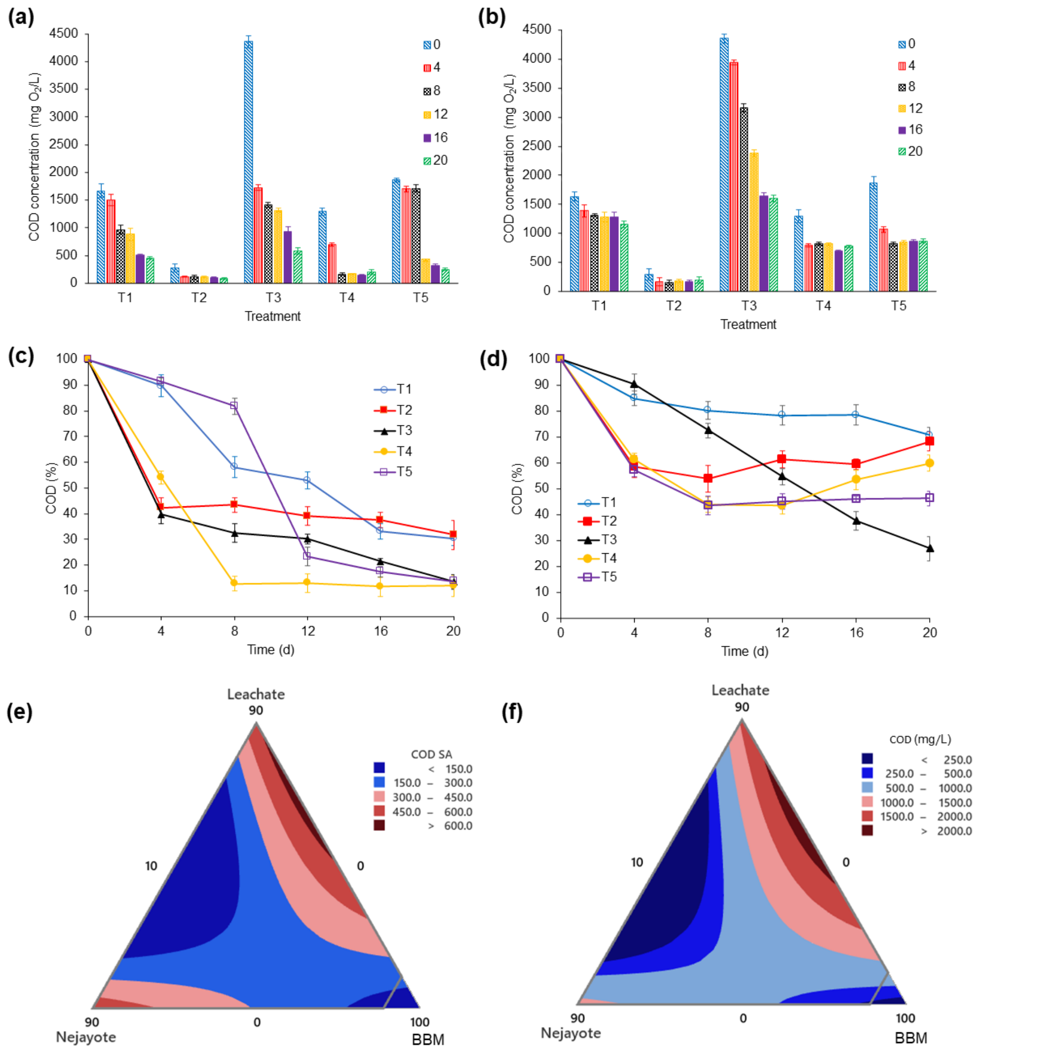

Figure 5. COD removal profile in quantification for (**a**) *S. acutus* and (**b**) *H. pluvialis*. COD removal profile in percentage (%) according to the initial value for (**c**) *S. acutus* and (**d**) *H. pluvialis*. Mixture contour plots in COD quantification for (**e**) *S. acutus* and (**f**) *H. pluvialis*.

3.4. Determination of the Optimal Conditions for the Growth of S. acutus and H. pluvialis and the Reduction in Nutrients

Mix ratio optimization is used to determine the combination of input variable configurations that collectively maximize a single response or a group of responses. It is beneficial to select the most favorable operating conditions that will maximize the process. The optimal solutions derived from the predicted responses of the microalgae *S. acutus* and *H. pluvialis* are shown in Table 4. Nejayote proved to be the most suitable wastewater for nutrient reduction and biomass growth in *S. acutus* and *H. pluvialis*, while food waste leachate proved to be the most effective for *S. acutus* biomass growth. A combination of nejayote and food waste leachate can be effective for the growth of *H. pluvialis* biomass. These findings also demonstrated that the strain of microalgae affects the results, so it is preferable to use a single type of wastewater for *S. acutus* and a combination of two types of water for *H. pluvialis* when grown under controlled conditions. Based on experimental data, the highest biomass growth values for *S. acutus* were 6.64 g/L and 4.45 g/L (for the biomass growth and nutrient uptake combination), whereas the higher values for *H. pluvialis* were 5.5 g/L and 4.44 g/L (for the biomass growth and nutrition uptake combination). Additional experiments were conducted using the optimum compositions determined to validate the model. The optimal results for biomass growth were determined to be 6.76 g/L and 5.68 g/L for *S. acutus* and *H. pluvialis*, respectively. For the combination of biomass growth and nutrient uptake, optimal results were determined to be 4.32 g/L and 4.76 g/L for *S. acutus* and *H. pluvialis*, respectively. The experimental results were determined to be consistent with the prediction. The small discrepancy in the results can be attributed to alterations in the composition of the substrates used for the validation of the experiments.

Table 4. Optimization by mixture design response optimizer.

Procedure	Mixture Composition (%)			Predicted Responses			
	Nejayote	Food Waste Leachate	BBM	Biomass Growth (g/L)	NH_4^+ (mg/L)	PO_4^{3-} (mg/L)	COD (mg/L)
				Scenedesmus acutus			
Biomass growth	0	46	54	6.64	—	—	—
Nutrient uptake	10	0	90	—	10.6	1.7	89
Biomass growth and nutrient uptake	10	0	90	4.45	10.6	1.7	89
				Haematococcus pluvialis			
Biomass growth	25	25	50	5.5	—	—	—
Nutrient uptake	10	0	90	—	3.24	1.7	196.66
Biomass growth and nutrient uptake	10	0	90	4.44	3.24	1.7	196.66

Using microalgae as a remediation system has several advantages over other technologies. Controlling temperature and light intensity allows for large-scale microalgae growth. This method is cost-effective and requires minimal maintenance compared to other technologies. Microalgae are photosynthetic organisms that utilize contaminants to generate food and release oxygen, thereby aiding in the aerobic degradation of these substances. Cultivating microalgae in polluted environments allows them to adapt to harsh conditions and develop a higher tolerance to toxic substances. As a result, they become more efficient at removing contaminants from water bodies. Microalgae have an amazing ability to transform contaminants using their enzymes. This transformation process not only helps to remove contaminants but also produces valuable compounds like biofuels, omega-3 fatty acids, pigments, amino acids, and sugars. Microalgae have the potential to outperform bacteria and fungi when it comes to breaking down organic pollutants. Furthermore, algae can help capture carbon that other organisms would otherwise release as carbon dioxide. Microalgae have the remarkable ability to capture carbon from the atmosphere at an impressive rate (1.83 kg of CO_2/kg of biomass). They also outperform land crops in terms of biomass productivity, with a 40–50% higher rate. Additionally, microalgae are highly effective in removing pollutants, achieving a removal rate of 80–100%.

4. Conclusions

Stress conditions were applied to the batch experiments for the valorization of nejayote and food waste leachate in order to determine the influence of the mixture components' proportions alone. The relative proportions of each component in the mixture are crucial in a co-substrate experiment. Nejayote and food waste leachate contain a significant amount of organic and inorganic substances that could damage the environment. Therefore, they need to be treated before being discharged into water bodies.

According to the data obtained in this study, the most effective option to promote cell growth in *S. acutus* is to use a greater amount of food waste leachate compared to nejayote. The optimal result was achieved by combining 10% food waste leachate and 90% BBM, resulting in a concentration of 5.34 g/L. Similarly, a combination of 10% nejayote and 90% BBM produced ceran results at 4.45 g/L. Also, the most effective combination for reducing COD was found to be 10% food waste leachate and 90% BBM, resulting in an 87% reduction from the initial concentration of 1295 mg/L. The reduction in PO_4^{3-} was achieved by combining 10% nejayote and 90% BBM, resulting in an 84.1% reduction from an initial concentration of 10.5 mg/L. For NH_4^+ reduction, the most effective mixture consisted of 10% food waste leachate and 90% BBM, resulting in 83% reductions of 34.1 mg/L.

In the case of *H. pluvialis*, the combination of nejayote and food waste leachate is the preferred option to promote cell growth. The optimal result is achieved by combining 25% nejayote, 25% food waste leachate, and 50% BBM, resulting in 5.5 g/L. These results indicate the potential of these residues to be used as a medium for growing these microalgae. However, the most effective combination to reduce NH_4^+ was a mixture of 10% food waste leachate and 90% BBM, resulting in a 97.8% reduction from 34.09 mg/L. To reduce PO_4^{3-}, the optimal mixture was 90% nejayote and 10% MBB, resulting in a 97.4% reduction from an initial concentration of 4.4 mg/L. Finally, to reduce COD, the best mixture was 90% food waste leachate and 90% BBM, resulting in a reduction of up to 73.19% from an initial concentration of 4357 mg/L. Our findings indicate that food waste leachate is more suitable for the growth of *S. acutus*, while nejayote can be used for *H. pluvialis*.

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