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The Circular Economy Approach to Improving CNP Ratio in Inland Fishery Wastewater for Increasing Algal Biomass Production

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Abstract: In this work, the capacity of wastewater from an inland fishery system in Colombia (Norte de Santander) was tested as culture medium for *Chlorella* sp. and *Scenedesmus* sp. Due to insufficient N and P concentrations for successful algae growth, the effect of wastewater replenishment with NO_3, PO_4, and Na_2CO_3 or $NaHCO_3$ as a carbon source was analyzed using a three-factor nonfactorial response surface design. The results showed that the addition of $NaNO_3$ (0.125 g/L), K_2HPO_4 (0.075 g/L), KH_2PO_4 (0.75 g/L), and $NaHCO_3$ (0.5 and 2 g/L for *Chlorella* sp. and *Scenedesmus* sp. respectively) significantly increased the biomass of *Chlorella* sp. (0.87 g/L) and *Scenedesmus* sp. (0.83 g/L). Although these results show that the addition of other nutrients is not necessary (Na, Mg, SO_4, Ca, etc.), it is still essential to determine the quality of the biomass produced in terms of its application as a feed supplement for fish production.

Keywords: *Oreochromis* sp.; protein; lipids; carbohydrates; *Scenedesmus*; *Chlorella*; inland fisheries

Citation: García-Martínez, J.B.; Sanchez-Tobos, L.P.; Carvajal-Albarracín, N.A.; Barajas-Solano, A.F.; Barajas-Ferreira, C.; Kafarov, V.; Zuorro, A. The Circular Economy Approach to Improving CNP Ratio in Inland Fishery Wastewater for Increasing Algal Biomass Production. *Water* **2022**, *14*, 749. https://doi.org/10.3390/w14050749

Academic Editor: Feng-Min Li

Received: 29 December 2021
Accepted: 24 February 2022
Published: 26 February 2022

1. Introduction

Fish production is the source of animal protein with the highest growth rate; according to the Food and Agriculture Organization of the United Nations (FAO), between 2001 and 2018, aquaculture production grew by 5.3%, while fish consumption significantly increased to 19.4 kg per capita in 2017 [1]. This growth in both processing and consumption is attributed to their capacity to successfully provide safe food with a higher micronutrient yield with less environmental input [2].

Aquaculture production can be separated into two main categories, open (with constant water exchange) and closed systems (without water exchange) [3]. In the last few years, the inland open system has become an economical option for producing high-quality fish protein in Africa and Latin America, strengthening fish production on both continents [4,5]. However, its large water exchange volume has led to an elevated demand for freshwater and a high concentration of wastewater with significant levels of dissolved inorganic nitrogen and phosphorus that must be safely treated before its disposition [6]; however, a large volume of the wastewater is not treated correctly [7]. Such high levels of nutrients are caused by relatively high fish stocking densities, intensive feeding regimes, and leftovers of uneaten food. According to Crab et al. [8], up to 75% of food consumed is converted to nitrogen and phosphorus. The latter contributes to the sustained increase in organic residues and toxic compounds in aquatic systems [9]. Nutrients such as nitrogen

and phosphorus are the main ones responsible for eutrophication in water bodies nearby fishery production sites [10]; therefore, the removal of those nutrients is a critical step to prevent negative impacts on the local water environments [11].

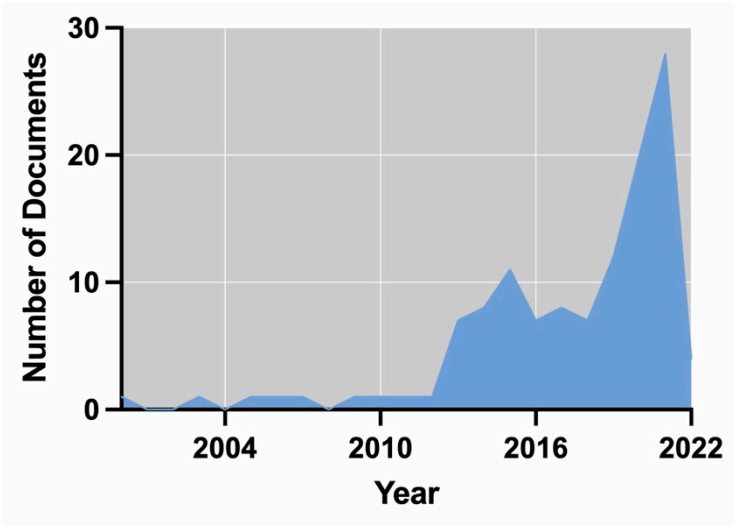
The biological denitrification of nitrate in aquaculture wastewater is an efficient process that depends on using organic matter inside the wastewater as a carbon source [12].

Unlike open systems, which require a constant water exchange, recirculation aquaculture systems (RASs) or "closed systems" are based on the treatment of post-consumption water for its subsequent re-entry into the culture ponds [13]. This system has the quality of reducing the water footprint in a sustained manner [14], while generating a solid residue (sludge) that can be used as a matrix for biogas production [15].

During the last 50 years, significant efforts have been made to remove different nutrients from this wastewater to avoid eutrophication of water bodies near the production systems and recirculate the treated water [8]. Currently, there is a great diversity of biological and chemical methods that have been used satisfactorily in the nutrient removal process, such as (1) biological processes for nitrogen removal including nitrification and denitrification [16] and (2) chemical processes including chemical precipitation for phosphorus removal [17]; the latter process, despite being helpful, is a less environmentally friendly technique since it leads to the formation of sludge that is highly polluting to the environment [18]. One alternative is the biological treatment of wastewater using microalgal cultures [19]. The use of microalgae and cyanobacteria is considered one of the most prominent technologies with the most significant contribution to the conservation of the environment [9]. This is due to their fast growth rate. They can grow in wastewater while reducing the concentration of harmful nutrients; moreover, their biomass can be used as feedstock for a wide range of industrial interest products [20].

The research and development on the application of aquaculture liquid waste as a source of nutrients to produce algal-based metabolites is a field that, in recent years, has attracted different researchers worldwide. Figure 1 shows the number of publications in the last 22 years (TITLE-ABS-KEY: aquaculture AND wastewater AND microalga), according to the Scopus database (Elsevier). It is possible to observe that, since 2012, the number of documents has exponentially increased up to a final number of 121 (including accepted manuscripts for 2022). China, Malaysia, the United States, and India dominate the scientific publication on the usage of aquacultural wastewater into usable metabolites and biomass.

Through the culture of microalgae into the wastewater, it is possible to remove the concentrations of nitrates, phosphates, and other nutrients present to obtain economically viable products for the national aquaculture sector, such as (1) protein and fat-rich feed, (2) biofertilizers, and (3) biofuels [18]. To date, the use of different strains of microalgae and cyanobacteria such as *Chlamydomonas* sp. [21], *Chlorella* sp. [3,8,22–33], *Dunaliella* sp. [23,33,34], *Isochrysis* sp. [33], *Nannochloropsis* sp. [10,23], *Navicula* sp. [23], *Oscillatoria* sp. [26], *Parachlorella* sp. [35], *Platymonas* sp. [31,36], *Scenedesmus* sp. [23,30], *Spirulina* sp. [37], *Synedra* sp. [38], *Tetraselmis* sp. [13,18,23,33,34,39], algal–bacterial biofilm [40], and mixed consortia of algal strains [41] for the removal of nutrients from fishery wastewater has been tested at a laboratory (<50 L) and demonstration scale (>200 L) in countries such as Belgium [5,42], China [16], Colombia [43,44], Denmark [41], Spain [15,45], South Africa [22,46], and Poland [19]. One interesting fact from those studies is that most authors worked with wastewater from RAS systems. There are few reports on the utilization of wastewater from inland fisheries with high water exchange rates. The present work evaluates wastewater from inland fisheries with high water exchange as a nutrient source to produce microalgae.



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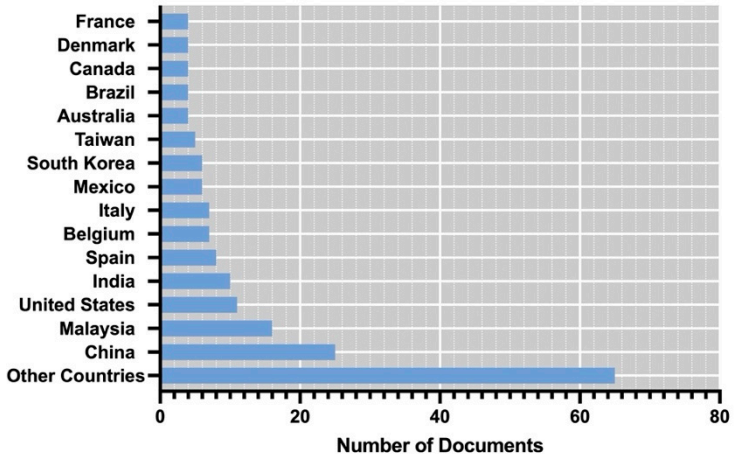


(**b**)

Figure 1. Evolution of the number of publications from 2000 to 2021 on the transformation of aquacultural wastewater using microalgal biotechnology (**a**) and their country of origin (**b**).

2. Materials and Methods

2.1. Fishery Wastewater

The untreated wastewater used in this study was obtained from a local company (El Manantial) in El Zulia (Norte de Santander, Colombia). The fish production (*Oreochromis* sp.) is carried out in aerated open ponds with constant water exchange throughout the year. The system uses water from a local river, filtered through a fine-mesh filter with a water replacement rate of 40% per day. The samples came from the fattening stage with a feed rate of approximately three rations for 7 g/unit·day (approximately 24% *w*/*w* of protein, and 2.5 *w*/*w* of lipids) until a culture density of 20–30 fish/m^3 was reached. The water was collected through 2019 (February to December) using a sterile amber borosilicate flask and transported at 20 °C. The wastewater was used immediately upon arrival and chemically analyzed (pH, turbidity, temperature, BOD_5, COD, total alkalinity, acidity, total hardness, calcium hardness, nitrates, and phosphates) according to standard methods for examining water and wastewater [47].

2.2. Strains

Chlorella sp. (CHLO_UFPS010) and *Scenedesmus* sp. (SCEN_UFPS015) from INNOValgae collection (UFPS, Colombia) were used as inoculate. The strains were pre-cultivated in a 2 L glass flask with a working volume of 1.2 L containing Bold Basal Medium [48]. The

media was mixed through the injection of filtered air with 0.5% (*v*/*v*) CO_2 at a flow rate of 0.78 L·min^{-1}, 25 °C, and a light/dark cycle of 12:12 h at 100 µmol·m^{-2}·s^{-1} for 30 days.

2.3. Experimental Design

The fishery wastewater was filtered twice according to Hawrot-Paw [19] and UV-sterilized using a device previously designed for this type of wastewater [43]. To identify the best solid inorganic source of carbon that enhanced the production of algal biomass and the removal of N and P, the wastewater was supplemented with different concentrations (0.8, 1.2, and 1.6 g/L) of either sodium carbonate (Na_2CO_3) or sodium bicarbonate ($NaHCO_3$) [44] before inoculation. Both strains were cultured (by triplicate) in a 2 L glass flask with a working volume of 1.2 L of UV-sterile wastewater. Each flask was mixed by injection of filtered air at a flow rate of 0.78 L·min^{-1} and light/dark cycle of 12:12 h at 100 µmol·m^{-2}·s^{-1} for 30 days. As controls, the strains were cultured in Bold Basal Medium (control BBM) and wastewater without carbon addition (control WW).

The biomass produced was harvested using an electroflotation device (10 aluminum electrodes, 20 min, 150 rpm, and 50 W) [49,50], washed trice with distilled water, freeze-dried, and stored (4 °C) until use. Lastly, the different components of biomass such as carbohydrates [51,52], proteins [53], lipids [54], carotenoids [55], and ash [56] were measured. The cell-free media were analyzed for their content of nitrates and phosphates.

2.4. Supplemented Wastewater

The effect of supplementing the wastewater with NO_3, PO_4, and the best carbon source obtained (either Na_2CO_3 or $NaHCO_3$) was analyzed using a nonfactorial response surface design with three factors, three levels, and two central points.

3. Results

3.1. Fishery Wastewater

Nutrients such as nitrogen and phosphorus are the main ones responsible for eutrophication in water bodies nearby fishery production sites [10]; therefore, the removal of those nutrients is a critical step to prevent negative impacts on the local water environments [11,57]. Wastewater was analyzed for the content of nitrates, phosphates, and other parameters required to identify the quality of the water (Table 1).

Table 1. Physicochemical characteristics of the wastewater.

Parameters	Units	Mean Value
pH	pH units	8 ± 0.1
Turbidity	NTU	20 ± 0.8
Temperature	°C	24 ± 0.5
Biochemical oxygen demand (BOD_5)	mg/L	25.3 ± 0.02
Chemical oxygen demand (COD)	mg/L	41 ± 0.05
Total alkalinity	mg/L $CaCO_3$	80.88 ± 4.2
Acidity	mg/L $CaCO_3$	6 ± 0.08
Total hardness	mg/L $CaCO_3$	90 ± 0.82
Calcium hardness	mg/L $CaCO_3$	62.5 ± 0.3
Nitrates	mg/L NO_3	80 ± 0.04
Phosphates	mg/L PO_4	70 ± 0.07

3.2. Effect of Carbon Source

According to the results, higher concentrations of sodium carbonate and sodium bicarbonate (>1 g/L) improved the final concentration of biomass in both strains in comparison with the strain's growth either in Bold Basal Medium (Control BBM) or in wastewater (control WW). Between both strains, *Chlorella* sp. grew slightly better in the wastewater (up to 0.51 g/L) than *Scenedesmus* sp. (0.41 g/L) supplemented with the different carbon

sources; however, sodium bicarbonate enhanced the production of biomass (Figure 2a) and protein (Figure 2b) compared to sodium carbonate.

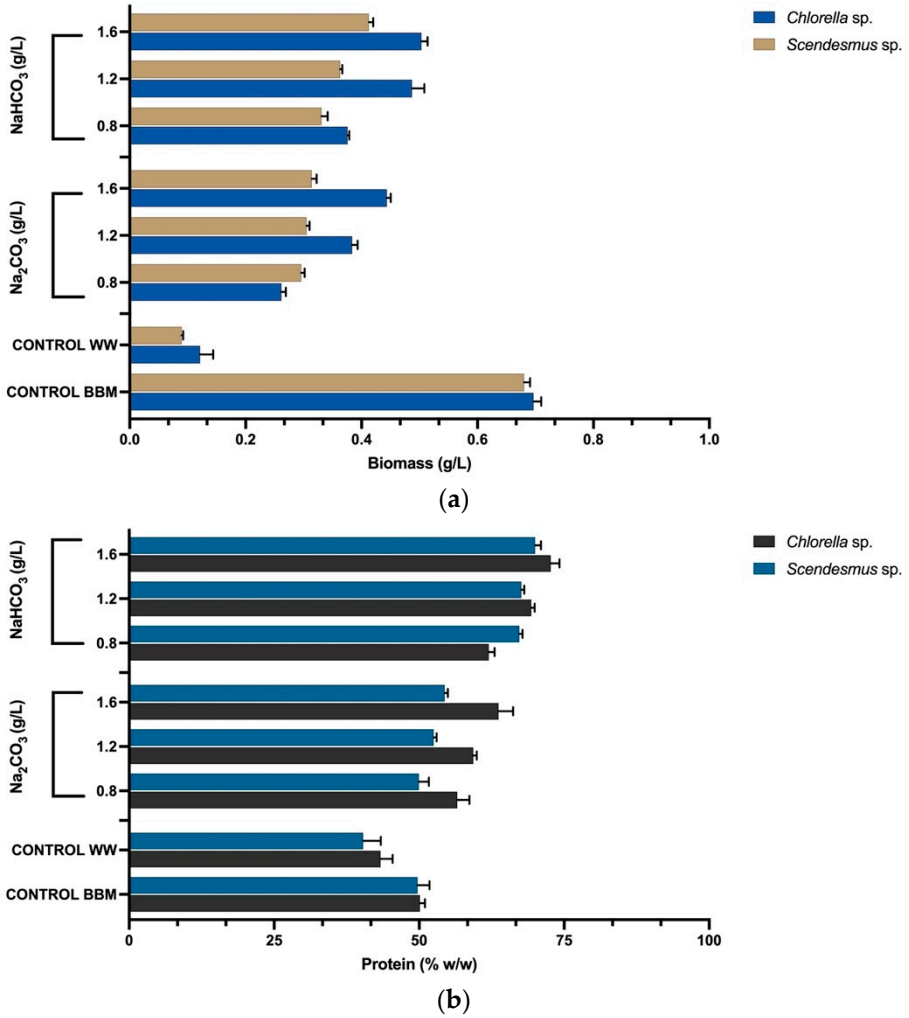
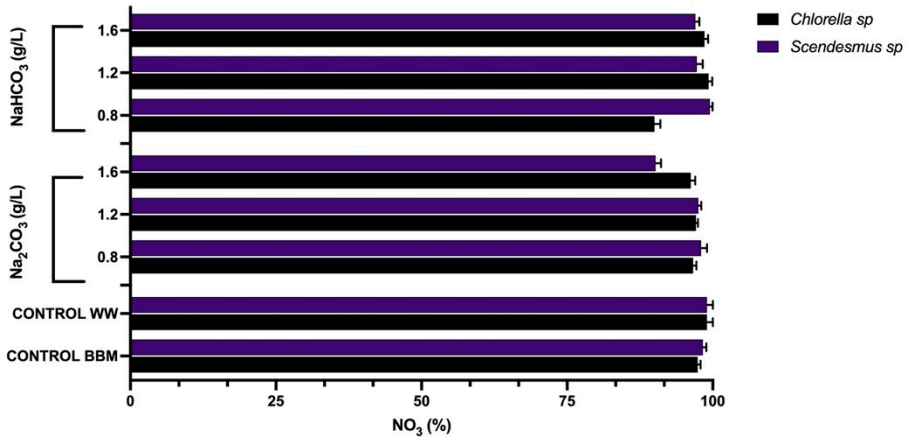


Figure 2. Biomass concentration (**a**) and protein content (**b**) using different sodium carbonate and sodium bicarbonate concentrations.

Unlike biomass, NO_3 and PO_4 removal from both strains remained relatively constant, even compared to the two controls (BBM and WW). According to the results for NO_3 uptake (Figure 3a), there was no significant difference between both carbon sources and controls. On the other hand, the removal of PO_4 (Figure 3b) seemed to be influenced by carbon source concentration. In the experiments with higher Na_2CO_3 and $NaHCO_3$, over 80% of PO_4 was consumed.



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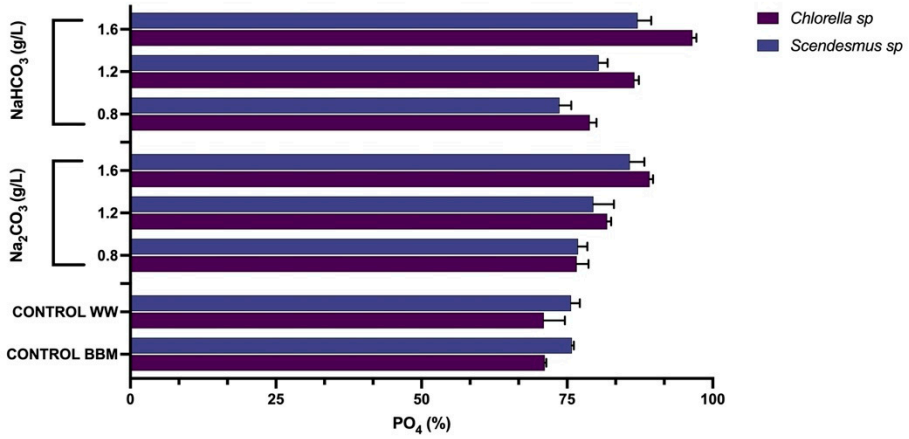

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Figure 3. Nitrate (NO_3) (**a**) and phosphate (PO_4) removal (**b**) using different concentrations of sodium carbonate and sodium bicarbonate.

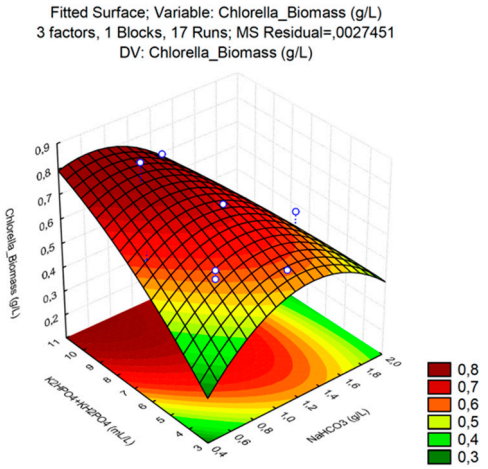
3.3. Supplemented Wastewater

The effect of supplementing the wastewater with N, P, and a carbon source was analyzed. To achieve this, the concentrations of $NaNO_3$, phosphate buffer (K_2HPO_4 and KH_2PO_4) present in the Bold Basal Medium [48], and sodium bicarbonate ($NaHCO_3$) as the carbon source were used. These components' effect was evaluated using a nonfactorial response surface design with three factors, three levels, and two central points. The resolved design can be found in Table 2.

The experimental data concerning the effects of the concentration of $NaNO_3$, $NaHCO_3$, and phosphate buffer (K_2HPO_4 and KH_2PO_4) on the production of biomass were fitted on two models: linear (L) and quadratic (Q) (Figure 4b,d). $NaHCO_3$ and phosphate buffer affected the final biomass concentration on both strains. Figure 4a,c presents the response surface plots for the effect of $NaHCO_3/K_2HPO_4$ + KH_2PO_4 in the concentration of biomass for *Chlorella* sp. and *Scenedesmus* sp. According to the results, relatively more significant concentrations of buffer phosphate (>10 mL/L) and low concentrations of $NaHCO_3$ (0.4—0.8 g/L) substantially increased the final concentration of biomass in *Chlorella* sp. On the other hand, relatively higher concentrations of buffer phosphate (>10 mL/L) and $NaHCO_3$ (>1.5 g/L) substantially increased the final concentration of biomass in *Scenedesmus* sp.

Table 2. Design of experiments for C/N/P ratio analysis.

Experiment	$NaNO_3$ (mL/L)	Phosphate Buffer		$NaHCO_3$ (g/L)
		K_2HPO_4 (mL/L)	KH_2PO_4 (mL/L)	
10 (C)	7	7	7	1.2
12	10.3	7	7	1.2
17 (C)	7	7	7	1.2
13	7	3,7	3,7	1.2
6	5	5	5	1.6
11	3.7	7	7	1.2
14	7	10.3	10.3	1.2
2	5	9	9	1.6
3	9	5	5	1.6
9	9	9	9	1.6
1	5	5	5	0.8
4	9	9	9	0.8
7	5	9	9	0.8
5 (C)	7	7	7	1.2
16	7	7	7	1.87
8	9	5	5	0.8
15	7	7	7	0.53



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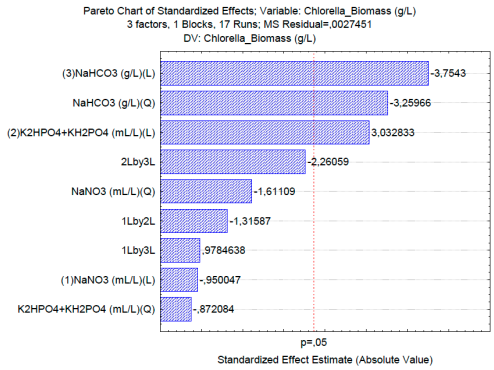

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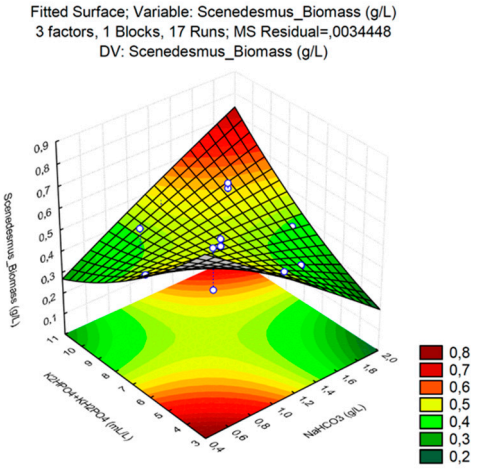

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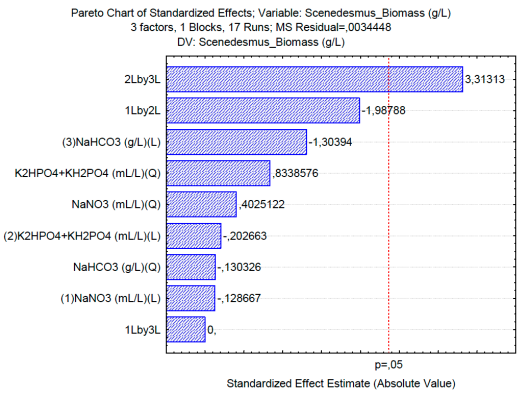


(**d**)

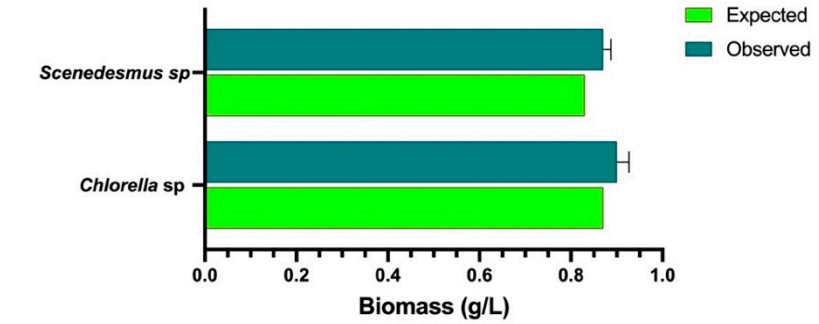
Figure 4. Surface response and Pareto charts for *Chlorella* sp. (**a**,**b**), and *Scenedesmus* sp. (**c**,**d**).

By analyzing the results from the interactions found between the variables (Figure 4a–d), the ratio $NaHCO_3$ to KH_2PO_4 + K_2HPO_4 was chosen. Table 3 represents the highest scenarios for biomass concentration; X is the concentration of $NaHCO_3$ (mL/L), and Y is the concentration of KH_2PO_4 + K_2HPO_4 (mL/L) while maintaining $NaNO_3$ at 5 mL/L (0.125 g/L of $NaNO_3$) for each strain. The production of biomass on *Chlorella* sp. and *Scenedesmus* sp. was further tested on a 50 L flat-plate PBR (0.78 $L \cdot min^{-1}$ of filtered air, 12:12 h light/dark cycle at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 30 days) using supplemented wastewater with the optimal conditions for carbon and phosphate source while maintaining $NaNO_3$ at 5 mL/L.

Table 3. Variables for optimal biomass concentration on both strains were studied.

Strain	Label	Variable	Value
Chlorella sp.	X	$NaHCO_3$ (g/L)	0.5
	Y	KH_2PO_4 + K_2HPO_4 (mL/L)	11
	$Z_{(expected)}$	Biomass (g/L)	0.87
Scenedesmus sp.	X	$NaHCO_3$ (g/L)	2
	Y	KH_2PO_4 + K_2HPO_4 (mL/L)	11
	$Z_{(expected)}$	Biomass (g/L)	0.83

Under the optimized conditions, the concentration of biomass produced was slightly higher than the expected result for *Chlorella* sp. and *Scenedesmus* sp. (0.9 and 0.87 g/L) (Figure 5a), with an interesting concentration of carbohydrates (22.5% and 23.5% *w*/*w*), proteins (45.8% and 42.8% *w*/*w*), lipids (13.6 and 12.2% *w*/*w*), total carotenoids (1.6% and 2.5% *w*/*w*), and ash (15.4% and 15.3% *w*/*w*) (Figure 5b). The removal of NO_3 and PO_4 (Figure 5c) showed that over 95% of the available NO_3 and up to 50% of PO_4 were consumed by both strains.



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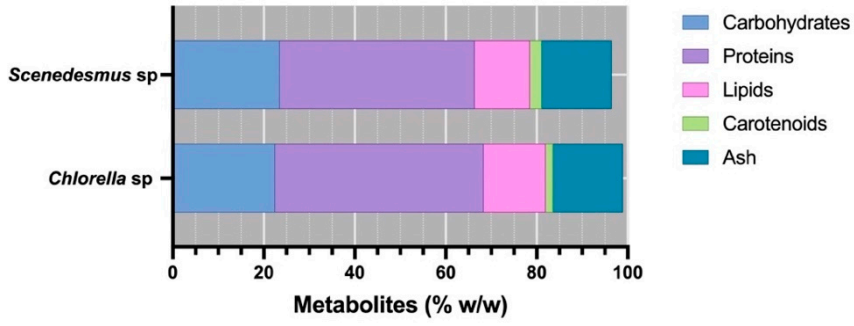
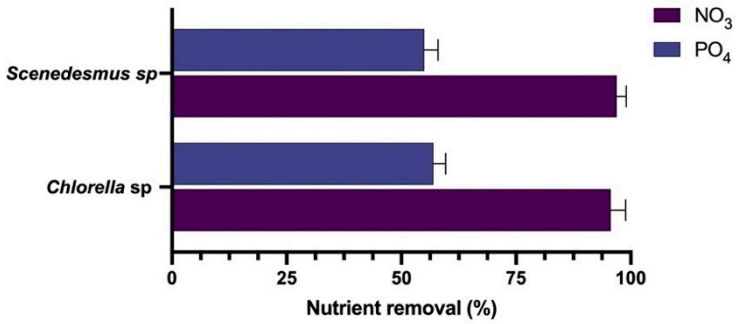


(**b**)

Figure 5. *Cont.*



(**c**)

Figure 5. Biomass production (**a**), its composition (**b**), and nutrient removal (**c**) under the optimized conditions for the supplemented wastewater for *Chlorella* sp. and *Scenedesmus* sp.

4. Discussion

4.1. Effect of Carbon Source

The analysis and selection of the carbon source are critical steps in the production of algal and cyanobacterial biomass; the correct concentration of the carbon source employed will enhance the synthesis and accumulation of the metabolites of interest [58]. Most of the studies found in the literature used CO_2 as the primary carbon source; however, sodium carbonate is the preferred carbon source on different culture media such as BG11. Other carbon sources such as sodium bicarbonate and sodium acetate have been studied as an alternative source for algal production. Lu et al. [59] found that sodium bicarbonate significantly reduced the content of N and P while producing up to 1.7 g/L of *Spirulina platensis* in raw swine wastewater. In our case, both strains grew better on the wastewater supplemented with up to 1.6 g/L of sodium bicarbonate; however, the final biomass concentration was relatively low (0.4–0.6 g/L) for both strains, which may have been due to lower levels of N and P available. In another study, Do et al. [60] tested the efficiency of a new strain of *Scenedesmus acuminatus* in high carbonate levels. The authors found that the strain was able to withstand up to 4.2 g/L of sodium bicarbonate with an exciting concentration of biomass (1.7 g/L) and high removal of N and P. Other works using sodium carbonate [61] and sodium bicarbonate [62] on *S. obliquus* found lower biomass concentration values (0.21 and 0.68 g/L respectively). Lastly, Barajas-Solano et al. [63] found that a relatively low concentration of sodium carbonate enhanced the biomass and hydrocarbon production on a Colombian strain of *Botryococcus braunni*.

4.2. Supplemented Wastewater

In the region of Norte de Santander (Colombia), most of the inland fisheries work under a constant exchange of water; this process allows the removal of high nutrient content from the production systems while maintaining oxygen levels, which helps the fish to grow and prevents the proliferation of blooms of toxic microorganisms. The latter explains the lower NO_3 and PO_4 found in the samples. Therefore, the wastewater must be supplemented with an external source of N and P to support the microalgal growth to produce fish feed, which may increase the cost of production. According to early results from our research group, an algal production plant of 500 m^3 using fishery wastewater supplemented with N and P can produce up to 11,875 kg/year (31.3 kg/day) with a production cost of up to 18 USD/kg for dry biomass (dry feed) and 0.19 USD/bottle for concentrated liquid biomass (live feed) [64].

In the present study, we sought to determine the concentration of N, P, and carbon that favored biomass production using a nonfactorial response surface design, an interesting tool for optimizing different processes, including algal production [65]. Our results show that the interaction between sodium bicarbonate and the phosphate source improved biomass production; to the best of our knowledge, this is the first report on the effect of P/C interaction on biomass concentration using wastewater from inland fisheries. Most of the

works published in the last few years focused on the viability of N, P, and the carbon source concentration (in the form of CO_2). Nitrogen and phosphate are destined to synthesize proteins and essential metabolites, while carbon is employed on nitrogen fixation and biomass production [63].

The application of microalgal cultures as a sustainable process for the removal of nutrients has been studied on wastewater from *Oreochromis niloticus* [3,20,22,24–26,38], *Lates calcarifer* [8], shrimp culture [29–31], *Mugil cephalus* [10,32], *Sparus aurata* [10,16], *Scophthalmus maximus* [39], and even synthetic wastewater [32,36]. According to the results, the percentage of NO_3 removed (>95%) is similar to that reported by most authors; however, the rate of PO_4 was lower than other authors since the wastewater was supplemented with external P.

The concentration of biomass produced is affected not only by the biological behavior of the strain but also by the source of the wastewater and the method in which the algae is grown. In general, the biomass concentration reported for different strains in a wide range of aquacultural wastewater ranges between 0.1 to 1 g/L. The highest recorded concentrations of algal biomass were reported by Han et al. [40], Guldhe et al. [22], and Tejido-Nuñez et al. [3] (2.52, 2.47, and 2.28 g/L, respectively); however, Han et al. used algae-bacteria consortia, while Gulde et al. grew the strain in heterotrophic mode, and Tejido-Nuñez et al. used non-sterile wastewater. Some other authors such as Malibari et al. [23] and Ge et al. [29] reported the lowest biomass concentration (<0.5 g/L); even when the authors evaluated a wide range of algal strains, the wastewater evaluated was obtained from shrimp culture (including *Penaeus* (*Litopenaeu*) *vannamei*). Therefore, this type of wastewater does not possess high nutrients for algal production. The different studies listed in Table 4 show similar levels of NO_3 and PO_4 removal, which implies that the different strains studied are capable of consuming those nutrients; however, unless the wastewater comes from recirculating aquaculture systems (RASs), such as the work of Dourou et al. [10], is supplemented with pulp wastewater [34], is enriched with biogas digestate [41], or is even enriched with a culture medium [37], this type of wastewater is not able to sustain large concentrations of algal biomass. Lastly, the protein content in those strains ranges between 24% and 60% (*w*/*w*), which makes them an exciting source for fish feed.

Table 4. Strains evaluated for removing nutrients (NO_3 and PO_4) and the concentration of biomass produced in different aquacultural wastewater.

Strain	Biomass Yield (g/L)	Protein Content (% *w*/*w*)	Source of Wastewater	NO_3 Removal (%)	PO_4 Removal (%)	Reference
Chlamydomonas sp.	0.66	n/a	*Oreochromis niloticus*	84.7	96	[21]
Chlorella sp.	0.058	n/a	*Shrimp culture*	>90	>90	[23]
	n/a	n/a	*Lates calcarifer*	–	99	[8]
Chlorella sp. NIVA CHL-137	0.39	n/a	*O. niloticus*	98.1	n/a	[24]
C. sorokiniana	2.47 [1]	24.57		84.51	73.35	[22]
C. sorokiniana 211/8K	0.476	n/a	*O. niloticus*	78	77	[25]
C. vulgaris	1.1	n/a		94.6	97.9	[21]
	0.58	n/a		95	81	[26]
	2.85	57	*catfish*	n/a	n/a	[27]
	n/a	n/a	*carps, trout, and sturgeon larvae*	92.23	89.25	[28]
	0.426	n/a	*Shrimp culture*	86.1	82.7	[29]
	n/a	n/a		99	n/a	[30]
C. vulgaris LH-1	1.1	n/a	*Marine aquaculture*	97.3	53.8	[31]
C. vulgaris 2714	1.12 [2]	n/a	*synthetic intensive aquaculture*	99	95	[32]
C. vulgaris CCAP 211/11B	1.31 [3]	49.96	*closed recirculating aquaculture system*	76.56	92.72	[34]
C. vulgaris CCAP 211/52	2.28	n/a	*O. niloticus*	99.8	99.7	[3]
Dunaliella sp.	0.061	n/a	*Shrimp culture*	>90	>90	[23]
D. tertiolecta	0.38	n/a		95.44	91.19	[33]
	0.329	n/a	*Mugil Cephalus*	96	99	[32]
Isochrysis galbana	0.16	n/a		66.02	91.93	[33]
Nannochloropsis sp.	0.073	n/a	*Shrimp culture*	>90	>90	[23]
N. gaditana	0.847	41	*Sparus aurata and Dicentrarchus labrax production*	92	87	[10]
Navicula sp. 1	0.083	n/a	*Shrimp culture*	>90	>90	[23]
Navicula sp. 2	0.077	n/a		>90	>90	

Table 4. *Cont.*

Strain	Biomass Yield (g/L)	Protein Content (% *w*/*w*)	Source of Wastewater	NO_3 Removal (%)	PO_4 Removal (%)	Reference
Oscillatoria okeni	0.38	n/a	*O. niloticus*	90	75	[26]
Parachlorella kessleri TY	0.275	n/a	*Aquaculture wastewater*	99	95.6	[35]
Platymonas helgolandica var. tsingtaoensis	1.85	n/a	*Synthetic Marine Aquaculture Wastewater*	55	80	[36]
Platymonas helgolandica	n/a	n/a	*Shrimp culture*	99	n/a	[31]
Scenedesmus obliquus	0.07	n/a		85	79	[32]
Sc. quadricauda NIVA-CHL 7	0.38	n/a	*O. niloticus*	98.7	n/a	[24]
Spirulina sp.	1.1 [4]	65.73		72.11	93.84	[37]
Synedra sp. FACHB-1712	0.37 [5]	n/a	*Freshwater aquaculture pond*	65.44	68.98	[38]
Tetradesmus obliquus SAG 276-1	1.98	n/a	*O. niloticus*	99.7	99.6	[3]
Tetraselmis sp.	0.081	n/a	*Shrimp culture*	>90	>90	[23]
T. chuii	1.38	39.8	*Sea bass*	99	n/a	[15]
T. suecica	1.97	50.2	*Sparus aurata*	99.8	98.7	[16]
	1.0	n/a	*Scophthalmus maximus*	95.7	99	[39]
	0.60	n/a	*M. cephalus*	94.40	96.06	[33]
	0.460	n/a		98	97	[34]
Algal–bacterial biofilm	2.52	n/a	*Fish and shrimp mixed culture*	95	99	[40]
Mixed consortia	1.99 [6]	n/a	*Aquaculture effluent*	99.5	99.2	[41]
Chlorella sp.	0.9	45.8	*Inland fish production (Oreochromis sp.)*	>95	52.3	This study
Scenedesmus sp.	0.87	42.8			51.4	

[1] Growth under heterotrophic conditions. [2] Co-cultivated with *Mucor indicus* 24905. [3] Mixture of pulp (60%) and aquaculture (40%) wastewater. [4] Wastewater supplemented with 25% (*v*/*v*) of Zarrouk medium. [5] N:P ratio adjusted to 6:1. [6] Enriched with biogas digestate.

5. Conclusions

In this study, we found that wastewater from inland fisheries dedicated to the production of *Oreochromis* sp. must be supplemented with an external source of N and P to work as a nutrient source for algal biomass production. Results from the optimization using nonfactorial response surface design show that the addition of $NaNO_3$ (0.125 g/L), K_2HPO_4 (0.075 g/L), KH_2PO_4 (0.75 g/L), and $NaHCO_3$ (0.5 and 2 g/L for *Chlorella* sp. and *Scenedesmus* sp. respectively) significantly increased the biomass of *Chlorella* sp. (0.87 g/L) and *Scenedesmus* sp. (0.83 g/L), where the $PO_4/NaHCO_3$ ratio increased the overall biomass production in both *Chlorella* sp. and *Scenedesmus* sp. Although these results show that the addition of other nutrients is not necessary, it is still critical to determine the quality of the biomass produced in terms of its application as a feed supplement for aquaculture production and the possible environmental impacts (positive or negative) generated by this type of process.

Author Contributions: Conceptualization, J.B.G.-M. and V.K.; methodology, A.F.B.-S. and C.B.-F.; software, A.F.B.-S.; validation, L.P.S.-T. and N.A.C.-A.; formal analysis, A.Z.; investigation, L.P.S.-T. and N.A.C.-A.; resources, A.F.B.-S. and C.B.-F.; data curation, A.Z.; writing—original draft preparation, J.B.G.-M.; writing—review and editing, A.F.B.-S. and A.Z.; visualization, C.B.-F.; supervision, V.K.; project administration, A.F.B.-S. and C.B.-F.; funding acquisition, A.F.B.-S. and C.B.-F. All authors have read and agreed to the published version of the manuscript.

Funding: This paper was partially supported by grants from Universidad Industrial de Santander (Colombia) and Newton–Caldas Fund Institutional Links, with the project "ALGALCOLOR: Bio-Platform for the Sustainable Production of Cyanobacterial-Based Colors and Fine Chemicals" ID 527624805.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We would like to express our sincere gratitude to Universidad Francisco de Paula Santander (Colombia), Universidad Industrial de Santander and Sapienza University of Rome for providing the equipment for this research and the Colombian Ministry of Science Technology and Innovation MINCIENCIAS for supporting national Ph.D. doctorates through the Francisco José de Caldas scholarship program.

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

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