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Feasibility of nitrogen and phosphorus removal from treated wastewater using microalgae and potential microalgae use as biofertilizer

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ABSTRACT

Wastewater treatment plants reduce the impact of eutrophication; however, alternatives or complements are needed because their efficiency is limited to 50 % by outdated guidelines and lack of technologies. This research determines the economic feasibility of microalgae treatments to bioremediate and valorize nitrogen (N) and phosphorus (P) from various wastewater sources in Antioquia, Colombia as a microalgae biofertilizer. *Chlorella Sorokiniana* (Chl), *Spirulina Platensis* (Spi) and *Scenedesmus* sp. (Scn) were cultured in 250 mL Erlenmeyer flasks with blends of inoculum, synthetic and real wastewater, 12:12 white light photoperiod (110 μM m^{-2} s^{-1}), 120 rpm and different concentrations of N:P. Synthetic wastewater and inoculum blends indicate that ammonia was consumed (up to 98.9 %) and oxidized to nitrite, which accumulates (up to 7.58 mg N/L), forming toxic aquatic environments, fluctuating pH and inhibiting nitrate uptake by microalgae (<14 % removal) and growth. The blends show high N and P consumption in most cases (73–99 % for both), nitrite removal (>98 %), high nitrate consumption and higher biomass yields of 882, 1197 and 1040 mg/L for Chl, Scn and Spi respectively. Blends in 20 L photobioreactors with 2:8 inoculum-wastewater ratios determine that the main operating expenses are energy for the 4 L/min air pump 50 %, 20 % for collection and 15 % for labor. The fastest growing microalgae was *Scenedesmus*, with annual profits of $4 M USD and OPEX 1.0 USD/Kg when processing 5184m^3 of effluent from a municipal wastewater plant, with a sales price of $22 USD/Kg microalgae. A limitation in its large-scale application is the space needed for greater effluent collection.

1. Introduction

Wastewater treatment plants (WWTP) have been developed to reduce the impacts on natural water and avoid issues related to sanitation and threats to human health. The proper use and disposal of water are critical to ensuring the subsistence of both biodiversity and human beings and become more difficult nowadays because of the increase in world population and the scenery of climate change [1], This added to the quality of the existing infrastructure and the obsolete guidelines for water treatment [2], which limits treatment efficiencies to 50 % of wastewater worldwide [3].

This issue has driven the development of physicochemical treatments such as coagulation and flocculation, filtration, and more modern technologies such as Advanced Oxidation Processes (AOP) [4], membrane technologies [5], and Membrane Bioreactors (MBR) stand out. Bioremediation, a form of biological treatment, has become a key solution as it employs biological organisms to eliminate or neutralize environmental contaminants through metabolic processes, Additionally, it is more cost-effective than other technologies that use chemical and physical treatments [6], since bioremediation has a removal efficiency for nitrogen and phosphorus between 80 and 100 %, other treatment systems like septic tanks, conventional or extended sludge systems, Upflow Anaerobic Sludge Blanket (UASB) has efficiencies below 30 % [7,8], while the use of ozonation, ultraviolet radiation or photocatalysis are efficient (close to 80 %) but faces challenges in energy consumption and membrane replacements [9].

This is particularly acute in developing countries; according to Pérez Mesa et al. [10], access to qualified water is being threatened by the pollutants discharged by domestic and industrial activities that do not have adequate treatment systems. In the case of Colombia, there are technical impossibilities to reduce the eutrophication risks generated by secondary effluent wastewater systems. According to these studies, only

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10 % of the nutrients (nitrogen and phosphorus) generated by industry and the population are removed, additionally, only 20 % of municipalities in Colombia have public water and sewage service coverage above 90 %, which means only 221 municipalities in the country. Meanwhile, 6.8 % of towns reported coverage is below 15 % [11].

Using microalgae for wastewater treatment presents a significant advantage to improving installed wastewater treatment systems, since 1980 microalgae have been studied by their demonstrated capacity to capture greenhouse gases like carbon dioxide (CO_2) and nutrients like nitrogen (N) and phosphorus (P) in the form of valuable biomass, in addition to reducing pollutants and pathogens and saving energy in wastewater processes [12] Nowadays, strains like *Chlorella Sorokiniana* [13], *Scenedesmus* sp. [14] and *Spirulina* [15] have been included in WWTP to enhance the reduction of chemical and biochemical oxygen demand (COD & BOD), nitrogen and phosphorus species, and heavy metals in effluents.

Microalgae have extensive applications in the food industry due to their high nutritional value, they are also used for energy purposes in the production of biofuels such as bioethanol, biomethane, biohydrogen, and especially biodiesel, due to their high lipid content and high biomass productivity; however, the costs of nutrients added through commercial fertilizers represent strong limitations. Microalgae biomass could also be used as biofertilizer, as it improves soil by increasing ion concentrations in crops and the organic matter content in the soil due to the production of polysaccharides [16].

Another application is bioremediation or phytoremediation of agricultural effluents containing pesticides and heavy metals [17]. They can oxidize or degrade pesticides in effluents, forming different non-toxic spices [18], while heavy metals were absorbed through ion exchange [19], with potential removal efficiencies between 70 % and 95 % and can have high tolerances of up to 100 mg/L for some heavy metals [17].

According to the existing knowledge of the ability of microalgae to capture nitrogen and phosphorus, the development gap in WWTPs in Colombia and their strong influence on eutrophication processes and threats to biodiversity, this research aims to improve nutrient removal performances in different wastewater effluents by using them as nutrient sources for the biomass production of *Chlorella sorokiniana*, Scenedesmus sp. and Spirulina platensis employing municipal wastewater effluents, poultry and dairy industries in Antioquia, Colombia. This research highlights that improving or implementing wastewater treatment systems for nutrient removal using algae-based technologies could valorize current wastewater residues as potential biofertilizers, representing economic benefits for industries and reducing the pressure on the ecosystem.

2. Materials and methods

Microalgae *Chlorella Sorokiniana* (Chl) [20], *Scenedesmus* sp. (Scn) [21] and *Spirulina platensis* (Spi) [22] were selected from previous research [10] and were available at the Center of Innovation of Cements ARGOS (CAPI) at EAFIT. The microalgae inoculum was prepared from frozen strains at −15 °C to solid BBM (Basal Bold medium). After 8 days, each alga was transferred to 50 mL flask with liquid BBM and allowed to grow for 15 days, repeating the process to 500 mL and then 2 L flask.

The inoculum was transferred to synthetic wastewater for 15 days by diluting 200 mL of MI to 1 L. Once they were adapted, their growth and nutrient uptake were evaluated according to procedures described in Section 2.1 for synthetic wastewater. The procedures described in Section 2.2 introduce different dilutions of municipal, poultry, and dairy wastewater effluents blended with synthetic wastewater where each alga exhibits maximum biomass production. At the end in numeral 2.3 essays for each strain and wastewater were carried out in 20 L photobioreactors (PBR) to establish the mass balance of the process and estimate the operational profits.

2.1. Synthetic wastewater

The microalgae biomass production and nutrient uptake were evaluated at different concentrations of reactive phosphorus (OP), nitrite (NO_2^-), nitrate (NO_3^-), and ammonia (NH_3^+). The medium employed is a modification of Bolds basal modified medium [23] and is described in Table 1. Glucose is added to simulate COD, nitrogen, and phosphorus concentrations and their species were modified to simulate average concentrations found in domestic wastewater effluents in Antioquia, Colombia as stated by Pérez Mesa, et al. Municipal wastewater has a composition and parameters that most closely resemble the conditions established in this preparation.

A synthetic wastewater medium was prepared to achieve nitrogen and phosphorus concentrations according to the experimental design described in Table 2, these values were selected according to average concentrations found in municipal wastewater effluents in Antioquia from 2019 to 2022, established in previous works by Perez Mesa et al. [24]. The experiment was carried out in a 250 mL flask with 3 replicates, where 10 mL of adapted inoculum was blended with 40 mL of synthetic wastewater, which were covered with gauze to prevent the entry of dust and facilitate adequate respiration of the microalgae. This experiment

Table 1
Synthetic wastewater preparation for microalgae inoculation.

Molecular formula	Reactive	Concentration
		mg/L
Organic matter solution (OMS): Transfer 500 mL to 1 L of medium		
$C_5H_9NO_4$	Glutamic acid	286
$C_6H_{12}O_6$	Glucose	286
$C_{12}H_{25}NaO_4S$	Sodium dodecile sulphate	10.6
$CaCl_2\cdot 2H_2O$	Calcium chloride	50
$MgSO_4\cdot 7H_2O$	Magnesium sulphate	150
Phosphorus stock solution (PSS): (Transfer volume to achieve desired P concentration)		
KH2PO4	Monopotassium phosphate	308
K2HPO4	Dipotassium phosphate	165
Total phosphorus (mg P/L)		100
Nitrogen stock solution (NSS): Transfer volume to achieve desired N concentration		
KNO_3	Potassium nitrate	1443.64
$NaNO_2$	Sodium nitrite	2.06
$C_2H_5NO_2$	Glycine	600.27
$SO_4(NH_4)_2$	Ammonium sulphate	943.405
Total nitrogen (mg N/L)		500

Molecular formula	Reactive	Concentration
		g/L
Micronutrients stock solution 1 (MSS1): Transfer 1 mL to 1 L of medium		
H_3BO_3	Boric acid	2.86
$MnCl_2\cdot 4H_2O$	Chloride manganese	1.81
$ZnSO_4\cdot 7H_2O$	Zinc sulphate heptahydrate	0.222
$(NH4)_6Mo_7O_{24}\cdot 4H_2O$	Ammonium molybdate	0.285
$CuSO_4\cdot 5H_2O$	Copper sulphate	0.079
$CoCl_2\cdot 6H_2O$	Cobalt chloride	0.0404
Micronutrients stock solution 2 (MSS2): Transfer 1 mL to 1 L of medium		
$2H_2O\cdot EDTA\cdot Na_2$	EDTA Disodium	10
KOH	Potassium hydroxide	6.2
Micronutrients stock solution 3 (MSS3): Transfer 1 mL to 1 L of medium		
$FeSO_4\cdot 7H_2O$	Iron sulphate	4.98
H_2SO_4	Sulphur acid (98 %)	0.1 ml/L

Table 2
Screening experimental design to synthetic wastewater.

Experiments[a]	Nitrogen (mg/L)	Phosphorus (mg/L)	Microalgae
0	60	5	None
1	25	2	Scn
2	25	2	Chl
3	25	2	Spi
4	25	7	Scn
5	25	7	Chl
6	25	7	Spi
7	120	2	Scn
8	120	2	Chl
9	120	2	Spi
10	120	7	Scn
11	120	7	Chl
12	120	7	Spi
13	60	5	Scn
14	60	5	Chl
15	60	5	Spi
16	150	10	Scn
17	150	10	Chl
18	150	10	Spi
19	20	1.5	Scn
20	20	1.5	Chl
21	20	1.5	Spi

[a] Duplicate experiments were carried out.

was carried out with an ACTUM HD 4000-L orbital shaker at 120 rpm for 12 days, light/dark photoperiod 12:12 using white light at 110 μM m^{-2} s^{-1} without CO2 bubbling.

To track the possible effect of other organisms and behavior of parameters without algae in the experiment, a blank without algae inoculum is performed in the center of the experimental design (60 mg N/L:5 mg P/L). One of the replicates for each alga was analyzed on day 1 to establish the initial concentrations through methodologies described in Table 3.

2.2. *Real wastewater evaluation*

From the results obtained in Section 2.1, the best result for microalgae and synthetic wastewater was selected (Scn: 25 mg N/L:2 mg P/L; Chl 150 mg N/L:10 mg P/L; Spi 60 mg N/L:5 mg P/L) was employed to introduce real municipal (ARD_m) and poultry ($ARnD_p$)wastewater. Initially, algae were adapted with 10 % of wastewater for 8 days, then 30 % and 50 %, followed by a screening experiment at different dilutions with 3 replicates as stated in Table 4. Operational conditions, agitation and light were replicated and adopted as stated in 2.1 for 12 days. At day 1 analysis was also performed to establish initial conditions according to those described in Table 3.

Municipal, dairy and poultry wastewater employed for the experiments was characterized to identify their chemical composition, which results are shown in Table 5:

Table 3
Methodologies implemented to estimate concentrations in synthetic wastewater.

Analysis performed	Units	Reference
Biomass (As total suspended solids SST)	mg SST/L	S.M. (2540 D)
Chemical oxygen demand (COD)	mg O_2/L	S.M. (5220 D)
Ammonia	mg N-NH_3/L	S.M (4500 NH3 B, C)
Phosphorus	mg P/L	S.M. (4500 - P B, E)
Nitrites	mg N-NO_2^-/L	SM. (4500 B)
Nitrates	mg N-NO_3^-/L	Test 1-65 NANOCOLOR 918-65, MACHEREY NAGEL
pH	U pH	S.M. (4500-H+ B)

Table 4
Screening essay for microalgae in ARD_m and $ARnD_p$ wastewater.

Experiments	Wastewater employed	Proportions of essays (AR/M/I)*	Microalgae employed
0	Medium blank	0 %/100 %/0 %	None
1	ARD_m	100 %/0 %/0 %	None
2	$ARnD_p$		
3	ARD_m	30 %/60 %/10 %	Chl, Scn, Spi[a]
4	$ARnD_p$		
5	ARD_m	50 %/40 %/10 %	
6	$ARnD_p$		
7	ARD_m	80 %/10 %/10 %	
8	$ARnD_p$		
9	ARD_m	90 %/0 %/10 %	
10	$ARnD_p$		

* AR: Real wastewater (% vol.); M: Synthetic wastewater (% vol. of medium for algae); I: Inoculum (% vol.)

[a] Spirulina inoculum was 20 %.

2.3. *Determination of operational costs (OPEX)*

For the subsequent evaluation in 20-liter photobioreactors (PBRs), the optimal dilution was selected by identifying the one with the lowest biomass production cost per kilogram, calculated based on the raw material costs.

2.3.1. *Microalgae growth and nutrient uptake evaluation*

To determine operational utilities, the microalgae cultures obtained and adapted in previous essays were transferred to 20 L vertical photobioreactor (PBR), where dairy wastewater was included ($ARnD_d$), by diluting 2 L of inoculums and 2 L of wastewater and were conditioned for 8 days for these experiments. Each inoculum obtained for Chl, Scn, and Spi was filled with ARD_m, $ARnD_p$ and $ARnD_d$ wastewater respectively to 20 L final volume with a water column height of 1 m (H) and a density of 1000 kg/m^3 (ρ_w). Wastewater was collected and transported to installations with dry ice and employed the same day to avoid significant changes in composition. Microalgae was cultivated in Medellín, with an average temperature (12 °C night/25 °C Day) and solar radiation (4850 Wh/m^2/day) and bubbling with atmospheric compressed air (Q_{air} = 4 L/min; Efficiency (E) =85 %) to ensure agitation. Samples were taken at the beginning of the experiment and every 3 days until reached death phase to track nutrient removal efficiencies and biomass production; height was measured to estimate evaporation loss and correct concentration results.

Removal efficiency calculation for compound *i.*

$$\gamma_i = \left(\frac{C_{1i} - C_{2i}}{C_{1i}}\right) \tag{1}$$

Required pump pressure for aeration in 40-liter PBR.

$$P_W = \rho_w g H \tag{2}$$

Required potency to pump air in photobioreactors.

$$U_t = \frac{(P_W{*}Q_{air}{*}FBR)}{E} \tag{3}$$

2.3.2. *Operational expenses determination*

Data collected in 20 L PBR was used to estimate the operational expenses (OPEX) required to their function, based on the specific requirements outlined in Table 6 for each type of microalgae, flow and initial pH for wastewater employed. The main considerations to estimate the operational costs of the process was raw material, the energy costs consider the use of aerators, and their required potency was estimated using Eq. (3) and confirmed with ASPEN PLUS, for a flow of 4 L/

Table 5
Global wastewater characterization.

Parameter	Method	Municipal	Poultry	Dairy
Nutrients				
Total Kjeldahl nitrogen (mg/L)	SM 4500 Norg B, SM 4500 NH3 B,C	59.2	30.5	17.7
Nitrites (mgN-NO_2^-/L)	SM. (4500 B)	<0.005	0.008	0.19
Nitrates (mg N-NO_3^-/L)	Test 1-65 NANOCOLOR 918-65, MACHEREY NAGEL	<1	<1	<1
Ammoniacal nitrogen (mg/L)	S.M (4500 NH3 B,C)	50.3	<5.0	2.37
Phosphates (mg P-PO_4/L)	S.M. (4500 - P E)	5.6	3.6	1.1
Phosphorus (mg P/L)	S.M. (4500 - P B, E)	7.6	4.6	1.9
Other chemical parameters				
pH	S.M. (4500-H+ B)	7.4	6.5	7.1
COD (mg O_2/L)	S.M. (5220 D)	172	428	1132
BOD5 (mg O_2/L)	S.M. (5210 B) ASTM D888-18	39.7	289.9	580.2
Total hardness (mg $CaCO_3$/L)	S.M. (2340C)	48.4	56.4	63
Calcium hardness (mg $CaCO_3$/L)	S.M. (3500 Ca B)	40.6	30.1	37.8
Conductivity (μS/cm)	S.M. (2510 B)	410	910	2050
Total acidity (mg $CaCO_3$/L)	S.M. (2310 B)	21.6	56.1	80
Total alkalinity (mg $CaCO_3$/L)	S.M. (2320 B)	136.3	177.9	<20.00
Phenols (mg/L)	S.M. (5530 B, D)	<0.05	<0.05	<0.05
Detergents (mg SAAM/L)	S.M. (5540C)	0.5	0.7	0.43
Fluorides (mg F-/L)	Test 1-42 NANOCOLOR 918142, MACHEREY NAGEL	1.1	0.4	0.7
Total solids (mg TS/L)	S.M. (2540 B)	233	702	1404
Metals				
Total beryllium (mg Be/L)		<0.010	<0.01	<0.010
Boron (mg B/L)		<0.100	<0.1	<0.050
Cadmium (mg Cd/L)		<0.003	<0.003	<0.003
Total calcium (mg Ca/L)		16.3	12.1	15.18
Total cobalt (mg Co/L)		<0.05	<0.05	<0.05
Copper (mg Cu/L)		<0.05	<0.05	<0.05
Tin (mg Sn/L)		<0.050	<0.05	<0.05
Strontium (mg Sr/L)		<0.050	0.053	<0.050
Lithium (mg Li/L)		<0.01	<0.01	0.001
Magnesium (mg Mg/L)		2.1	6.4	6.1
Manganese (mg/L)	EPA 200.7	0.1	0.1	0.4
Molybdenum (mg/L)		<0.05	<0.05	<0.05
Nickel (mg/L)		<0.02	<0.02	<0.02
Silver (mg Ag/L)		<0.05	<0.05	<0.05
Lead (mg Pb/L)		<0.01	<0.01	<0.01
Potassium (mg K/L)		9.5	32.3	64.8
Selenium (mg Se/L)		<0.01	<0.01	0.016
Total Silicon (mg Si/L)		7.6	8.7	0.74
Sodium (mg Na/L)		16.4	73.2	72.4
Total titanium (mg Ti/L)		<0.05	<0.05	<0.05
Vanadium (mg V/L)		<0.01	<0.01	<0.01
Zinc (mg Zn/L)		0.08	<0.05	<0.05
Chromium (mg Cr/L)	EPA 3015A	<0.05	<0.05	<0.010
Antimony (mg Sb/L)	EPA 3015A-SM 3120B	<0.010	<0.010	<0.010
Barium (mg Ba/L)	EPA 3015A-SM 3120B	0.058	<0.01	0.012
Total Mercury (mg/L)	SM 3112 B	<0.001	<0.001	<0.001
Arsenic (mg As/L)	SM (3030 K)	<0.010	<0.010	<0.010
Aluminum (mg Al/L)	SM 3030 B, SM. 3500 - Al B	4.7	<0.1	1.727
Total Iron (mg Fe/L)	SM 3030 G, SM 3500 Fe B	1.9	6.1	1.3

Table 6
Main tecno-economic considerations for microalgae cultures requirements.

Alga		Chl[a]	Spi[a]	Scn[a]
Consideration	Units			
Process specifications				
Reactor volume	m^3	5184		
Required pH	UpH	8	10	8
Regulatory solution (RS)	g KOH/year	225	23,261	225
Medium (Md)	% (Vol/vol.)	2 %	2 %	2 %
Inoculum	% (Vol/vol.)	20	20	20
Residual wastewater	% (Vol/vol.)	78	78	78
Economic assumptions				
Raw materials (RM)	USD/kg	Md + RS		
Energy consumption (Ut)	Kw/H	99.7		
Labour costs (Lc)[b]	USD/kg	0.30		
Quality control (Qc)	USD/kg	Lc * 15 %		
Maintenance (Mn)[c]	USD/kg	(RM + Ut + Qc) * 5 %		
Harvesting (Hst)[b]	USD/kg	(RM + Ut + Lc + Qc + Mn) * 15 %		

[a] Chlorella medium: 150 mg N: 10 mg P/L; Spirulina medium: 60 mg N: 5 mg P/L; Scenedesmus medium: 25 mg N: 2 mg P/L

[b] Heuristics adapted to Colombian technician salary in of $7143 USD/Year, from B. Columbia [25].

[c] For 333d/year according to heuristics for chemical design plants stated by Peters & Timmerhaus [26].

min for 24 h/day for 40 L PBR, considering a hypothetic reactor volume of 5184m^3; the energy prices was provided for a local supplier at $0.1014 USD/KwH.

2.3.3. Utilities estimation as biofertilizer

To determine a price for dry microalgae biomass, a theoretical value was determined, comparing the actual market values of biofertilizers with similar compositions, based on the sum of nitrogen (C_{Ni}) and phosphorus (C_{Pi}) concentrations (% wt./wt.) of the biomass, according to Eq. (4). Once the nutrients value in the market was established, the average was employed to determine a potential value for microalgae biomass according to the nutrient composition determined in the experiments according to Eq. (5). Average results were shown in Table 7.

Average nutrients price in commercial biofertilizers

$$P_{N+P} = \frac{\sum_{i=1}^{n}\left(\frac{MP_i}{C_{N_i}+C_{Pi}}\right)}{n} \tag{4}$$

Estimated biomass price according to N+P composition

$$B_p = P_{N+P} * \left(C_{N_i}+C_{P_i}\right) \tag{5}$$

Table 7

Nitrogen and phosphorus compositional price for market biofertilizer price and obtained microalgae.

Commercial fertilizer	N (C_N) (% wt./wt.)	P (C_P) (% wt./wt.)	Market price (MP) (USD/kg)	Nutrients price (P_{N+P}) (USD/kg)
Biofertilizer A	3.2 %	0.0 %	7.65	239.08
Biofertilizer B	2.0 %	1.1 %	9.26	299.65
Biofertilizer C	0.8 %	0.8 %	3.64	233.62
Biofertilizer D	6.0 %	1.4 %	17.14	232.42
Average	3 %	1 %	9.42	**251.19**

This study	N (C_N) (%wt./wt.)	P (C_P) (%wt./wt.)	Biomass price (B_p) (USD/kg)
Chlorella[a]	5.8 %	0.9 %	16.83
Scenedesmus[a]	8.2 %	0.8 %	22.61
Spirulina[a]	5.6 %	0.7 %	15.93

[a] This value corresponds to the average concentrations obtained for biomass obtained in municipal, poultry and dairy wastewater at minimum OPEX/kg. The price employed for operational profits over time was calculated for biomass obtained every day for each alga.

3. Results and analysis

3.1. Growth evaluation in synthetic wastewater

According to Table 8 Chlorella exhibited the highest biomass production, particularly under nutrient-rich conditions, with a notable increase of 519.7 %. It also demonstrated a strong capability for COD removal, achieving a 93 % reduction, which was superior to the blank at 82.9 %. Phosphorus removal was more efficient at low concentrations, peaking at 82.6 % in one experiment, while ammonium was nearly completely consumed (>99 %) in several conditions. However, nitrite levels tended to rise in the absence of ammonia-oxidizing bacteria (AOB), likely contributing to the accumulation of nitrites, whereas nitrate showed minimal changes across experiments, which is coherent with literature when ammonia and nitrate are present [27].

Scenedesmus also showed an increase in biomass, though less pronounced than Chlorella, with the greatest growth in the 25 N: 2P concentration. The COD reduction was substantial, with the highest removal rate of 91.5 % observed in the 120 N: 7P concentration. Phosphorus and ammonia removal were most efficient at low concentrations, with 94.2 % and > 75 % removal, respectively. However, a rise in nitrites was linked to the N ratio, particularly in the 120 N: 2P concentration, where ammonia consumption was higher, suggesting a relationship between these nutrient levels and nitrite accumulation.

Spirulina achieved the highest biomass increase in the 25 N: 2P concentration and demonstrated significant phosphorus removal, reaching 98.2 % in the 120 N: 7P concentration. Ammonia removal was nearly complete at low concentrations, while nitrite levels generally increased in most concentrations except for 20 N: 1.5P, where 98 % removal occurred. Nitrate removal was notable (>98 %) in specific conditions, with pH fluctuations varying across experiments, reaching a high of 16.4 % in 150 N: 10P.

The results indicate that Chlorella stands out for its high biomass production and nutrient removal efficiency, particularly under nutrient-rich conditions, achieving significant COD reduction (93 %) and complete ammonium consumption in several conditions. However, its limited ability to reduce nitrates and the accumulation of nitrites, likely due to the absence of ammonia-oxidizing bacteria (AOB), highlights a potential limitation in nutrient dynamics. Scenedesmus also demonstrated notable performance in COD, phosphorus, and ammonia removal, though it showed a more pronounced nitrite increase related to the N ratio. Spirulina, while excelling in phosphorus and nitrate removal in certain conditions, exhibited similar trends in nitrite accumulation and pH fluctuations, suggesting that nutrient ratios and microbial interactions play a key role in the efficiency of these microalgae species in wastewater treatment.

Table 8

Changes in nutrient composition and biomass in synthetic wastewater.

Experiment	Biomass		COD		Reactive phosphorus		Ammonia		Nitrite		Nitrate		pH	
	(mg SST/L)		(mg O_2/L)		(mg P/L)		(mg N-NH_4^+/L)		(mg N-NO_2^-/L)		(mg N-NO_3^-/L)		(U pH)	
	Day 1	Day 12	Day 1	Day 12	Day 1	Day 12	Day 1	Day 12	Day 1	Day 12	Day 1	Day 12	Day 1	Day 12
Chlorella sorokiniana														
60N:5P (blank)	0	39	378	65	5.0	2.6	24	26.87	0.06	0.11	24.00	24.37	6.72	8.04
20N:1.5P	66	279	344	66	2.5	0.9	11	<1.00	0.18	2.58	7.86	6.84	7.06	7.40
25N:2P	86	270	344	34	3.4	1.2	13	<1.00	0.18	2.73	9.46	13.07	7.19	6.74
25N:7P	82	302	344	27	9.3	4.8	17	<1.00	0.19	1.02	9.46	10.55	7.23	7.21
60N:5P	64	305	419	34	5.7	3.5	21	10.94	0.24	2.78	20.66	20.40	6.97	6.58
120N:2P	98	386	412	34	3.6	0.6	42	17.14	0.21	1.80	36.07	40.26	7.40	7.36
120N:7P	90	372	412	29	8.7	4.6	43	30.79	0.21	1.66	35.81	41.05	6.83	7.23
150N:10P	72	446	412	35	11.6	6.4	54	39.46	0.22	0.38	50.01	52.62	7.17	7.10
Scenedesmus sp.														
20N:1.5P	56	152	344	31	2.5	<0.15	9	1.74	0.46	0.22	7.86	7.18	7.21	8.63
25N:2P	60	224	344	30	2.6	<0.15	11	<1.00	0.09	0.22	9.46	9.37	7.20	8.76
25N:7P	100	175	344	36	6.8	4.4	13	2.69	0.08	0.28	9.46	9.46	7.30	8.27
60N:5P	90	110	412	33	5.1	3.0	23	18.01	0.09	0.23	20.66	20.66	7.00	8.31
120N:2P	54	192	412	44	2.7	0.5	40	29.29	0.11	7.58	38.17	37.97	7.40	6.59
120N:7P	54	110	412	35	7.0	4.7	43	37.96	0.11	0.29	34.23	33.79	6.92	8.12
150N:10P	88	186	412	39	9.5	5.6	54	46.29	0.13	0.40	52.90	50.14	7.25	8.26
Spirulina platensis														
20N:1.5P	63	739	344	40	1.8	<0.15	3	<1.00	0.03	0.01	9.73	0.19	9.83	9.00
25N:2P	66	903	344	40	2.3	<0.15	6	<1.00	0.04	0.07	11.00	0.14	9.57	8.70
25N:7P	102	239	344	39	5.0	1.6	5	<1.00	0.04	1.73	10.00	9.16	9.92	9.04
60N:5P	59	402	412	29	4.2	1.5	16	1.31	0.08	1.73	20.89	18.04	10.41	9.21
120N:2P	59	519	412	63	4.0	<0.15	33	10.17	0.04	1.94	38.55	37.37	10.42	9.02
120N:7P	69	550	412	56	6.6	<0.15	31	23.11	1.17	2.65	42.62	36.46	10.24	9.63
150N:10P	67	373	412	48	7.6	3.7	45	33.12	1.52	2.34	52.49	51.44	10.69	8.94

3.2. Real wastewater evaluation

Wastewater experiments shown in Table 9 revealed that all microalgae strains (Chlorella, Spirulina, and Scenedesmus) demonstrated increased biomass production in various types of wastewaters, outperforming the results observed in synthetic wastewater. This suggests increased adaptability and productivity when exposed to real wastewater, thus microbiological analyses were performed; these analyses suggest a higher adaptability and productivity of the microalgae, probably due to favorable interactions with bacteria and fungi. Microbiological results showed significant values for bacteria such as *Escherichia coli*, with <1 CFU/100 mL in Spirulina and Scenedesmus, and 2 CFU/100 mL in Chlorella. Total coliforms were 985 CFU/100 mL in Spirulina, while Scenedesmus and Chlorella showed much higher values, with 6,130,000 CFU/100 mL and 31,000 CFU/100 mL, respectively. In addition, 14,136 CFU/100 mL of thermotolerant coliforms were found in Scenedesmus and 1,989,000 CFU/100 mL in Chlorella, supporting the theory that these microbial interactions may favor algal growth. Due to time and resource constraints, these microbiological analyses were performed only at the end of each experiment, using municipal wastewater as the medium.

Chlorella had the highest biomass production in ARD_m and $ARnD_p$ wastewater with 882.50 and 705.79 mg SST/L, respectively. While COD removal was consistent with synthetic wastewater, reaching a minimum of 68.7 %, phosphorus removal was significantly higher, with Chlorella and Scenedesmus achieving >92.7 % and > 91.9 % in ARD_m and $ARnD_p$, respectively. Ammonia removal was also improved, with Scenedesmus showing up to 96.7 % efficiency in ARD_m. Nitrite concentrations were notably reduced, often nearing the method quantification limit (LOQ), with removal rates close to 99 %, while nitrate removal was similarly effective, particularly when concentrations were below 12.32 mg/L, with some experiments yielding over 84 % removal.

The results suggest that real wastewater enhances the biomass production and nutrient removal efficiency of microalgae compared to synthetic wastewater. Chlorella showed the greatest adaptability, with high biomass and nutrient uptake, particularly in ARD_m and $ARnD_p$. Nitrite and nitrate removal were significantly improved in real wastewater conditions, with nearly complete removal in several experiments. These findings highlight the potential for integrating microalgae into wastewater treatment processes, especially when combined with natural microbial communities, as this could enhance both productivity and nutrient removal efficiency.

3.3. Determining operational profits

According to data obtained, Chlorella demonstrates nitrogen removal peaking at 84,12 % by day 13 while phosphorus reached 95.21 % at day 10, but both nitrogen and phosphorus efficiencies decline by day 22 associated to cells death while SST peak was reached at day 15 with 795 mg/L. Spirulina shows the highest nitrogen removal (99.55 % by day 27) and steadily improving phosphorus efficiency, with SST rising rapidly to 4022.49 mg/L by day 31, indicating robust growth. Scenedesmus achieves consistent phosphorus removal (100 % from day 6 onward) and stable nitrogen reduction, though its SST fluctuates after day 13. Overall, Spirulina leads in nitrogen removal, Scenedesmus in phosphorus removal, while SST trends suggest varying biomass growth and aggregation patterns across the microalgae.

In the second set of experiments, which involves phosphorus-deficient $ARnD_p$, Chlorella, Spirulina, and Scenedesmus are evaluated for their nitrogen (γ_N) and phosphorus (γ_P) removal efficiency, alongside suspended solids (SST). Chlorella exhibits moderate nitrogen removal, peaking at 67.48 % by day 6, though it struggles with phosphorus

Table 9

Changes in nutrient composition and biomass in synthetic - real wastewater blends.

Experiment[a]	Biomass (mg SST/L)		COD (mg O_2/L)		Reactive phosphorus (mg P/L)		Ammonia (mg N-NH_3+/L)		Nitrite (mg N-NO_2-/L)		Nitrate (mg N-NO_3-/L)		pH (UpH)	
	Day 1	Day 12	Day 1	Day 12	Day 1	Day 12	Day 1	Day 12	Day 1	Day 12	Day 1	Day 12	Day 1	Day 12
ARD_m														
BK 0/100/0	6	23	309	33	4.4	1.8	26.7	26.2	0.02	2.09	21.4	17.8	7.1	8.7
BK 100/0/0	96	106	183	22	6.6	3.4	45.9	43.8	0.02	2.03	<1	<1	7.3	8.2
Chl 30/60/10	93	644	245	12	6.7	<0.15	47.8	11.6	1.6	0.74	32.6	42.8	7.8	5
Chl 50/40/10	112	830	211	48	4.9	<0.15	44.3	8.2	2.12	1.44	20.4	8.2	7.8	10.8
Chl 80/10/10	137	883	168	30	4.2	<0.15	34.4	4.4	1.39	0.02	5.3	<1	7.9	10.4
Chl 90/0/10	131	655	208	23	4.6	<0.15	44.7	2.9	3.76	1.06	<1	<1	7.9	10.9
Scn 30/60/10	77	1115	318	33	2.3	0.2	19	1.9	0.63	0.01	6.3	<1	8.6	10.6
Scn 50/40/10	125	1148	205	46	2.8	<0.15	27.5	3.8	1.03	0.01	4.1	<1	8.4	10.8
Scn 80/10/10	138	1198	171	54	3.8	1.2	39.1	4.4	1.94	0.01	1.1	<1	8.1	10.9
Scn 90/0/10	139	1158	196	48	4.4	0.3	44	1.5	1.68	0.01	<1	<1	7.9	10.9
Spi 30/50/20	77	1040	318	57	3.5	<0.15	26.2	<1	3.28	0.03	8.3	<1	10.1	10.1
Spi 50/30/20	89	865	242	21	5.4	0.4	28.5	2.2	3.18	0.01	4.9	<1	10.1	10.1
Spi 70/10/20	123	845	193	<15	4.1	<0.15	32.3	2.9	3.15	0.02	2.2	<1	10.2	10
Spi 80/0/20	139	860	193	<15	4.4	<0.15	42.4	2.9	1.6	0.01	<1	<1	10.2	10.1
$ARnD_p$														
BK 0/100/0	0	20	330	16	5.8	4.1	21.4	20	0.06	2.15	21.3	21.5	7.1	8.7
BK 100/0/0	122	66	153	<15	2.8	1.9	11.2	17.3	0.01	3.88	<1	<1	7.3	8.2
Chl 30/60/10	122	544	232	24	6.9	2	51.9	24.2	0.03	0	24.4	13	7.8	5
Chl 50/40/10	140	550	162	33	5.4	1.1	30.1	12.8	0.03	<0.005	16.9	2.6	7.8	10.8
Chl 80/10/10	177	706	150	23	3.3	<0.15	18.5	1.7	0.01	<0.005	4	0.9	7.9	10.4
Chl 90/0/10	169	636	110	28	2.6	<0.15	8.3	1.3	0.01	<0.005	<1	<1	7.9	10.9
Scn 30/60/10	166	716	113	<15	2.3	<0.15	11.2	2.2	0.02	0.01	5.7	<1	8.6	10.6
Scn 50/40/10	176	708	110	<15	2.4	<0.15	12.6	<1	0.01	<0.005	3.8	<1	8.4	10.8
Scn 80/10/10	205	825	153	22	2.5	<0.15	11.2	2.2	0.01	<0.005	<1	<1	8.1	10.9
Scn 90/0/10	196	972	138	33	2.6	<0.15	14.1	<1	0.01	<0.005	<1	<1	7.9	10.9
Spi 30/50/20	144	608	312	35	1.9	<0.15	14.1	1.2	20.15	8.47	12.3	<1	10.1	10.1
Spi 50/30/20	154	755	284	66	2	<0.15	9.7	<1	13.12	0.02	8.6	<1	10.1	10.1
Spi 70/10/20	183	833	306	69	2.2	<0.15	6.8	<1	16.86	0.01	2.7	<1	10.2	10
Spi 80/0/20	138	622	239	57	2.3	<0.15	6.8	<1	4.03	0.01	1.4	<1	10.2	10.1

[a] Chl 30/60/10 means that chlorella's experiment was performed with 30 % (vol/vol.) wastewater effluent, 60 % (vol/vol.) synthetic wastewater and 10 % (vol/vol.) microalgae inoculum.

removal (0 % throughout), likely due to the phosphorus deficiency and quantification methods limitations. SST fluctuates significantly, peaking at 711.77 mg/L by day 8 before dropping. Spirulina initially performs poorly, with negative nitrogen removal (up to −169.15 % by day 4) and inconsistent phosphorus removal, though it gradually improves, reaching 98.25 % nitrogen removal by day 22, albeit with limited phosphorus uptake (11.08 %), suggesting difficulties to adapt in those conditions. SST follows a complex trend, peaking at 663.55 mg/L by day 22. Scenedesmus, despite the phosphorus limitation, consistently removes nitrogen efficiently, reaching 99.71 % by day 22, with phosphorus levels fixed at 78.57 %, except for a notable drop on day 17. SST remains more stable, peaking at 552.64 mg/L.

For dairy wastewater was observed a similar behavior with municipal wastewater, with a significant increase in biomass production in the first days, especially for chlorella and Scenedesmus reaching 1530 and 1245 mg SST/L respectively the first 10 to 15 days, while spirulina shows difficulties to adapt. However, due to the high presence of bacteria in this wastewater type, the transition to death phase was accelerated, observing algae death in few days. This must be considered as an important risk if this technology is considered to this type of industry due to the loss of biomass in short times.

In the Graphic 1 is shown the experimental results for the nitrogen and phosphorus concentrations (%wt./wt.) in dried biomass over time. From these results can be identified a common behavior in the microalgae strains, were nitrogen and phosphorus increases in the first days with concentrations varying according to each strain, with nitrogen going from 2 to 7 % for Chl., 5 to 10 % for Scn and 7.5 to 10 % for Spi, while phosphorus goes from 0.85 to 2.8 % for chlorella, 1 to 2 % for spirulina, while Scenedesmus shown a tendency to keep concentrations between 0.6 and 1.5 %, all with fewer variations according to wastewater, and then reduces consistently until algae death. To ensure continuous nutrient removal, it would be necessary to implement a continuous flow system that allows for the constant replacement of wastewater (providing nutrients) and biomass harvesting. This approach would maintain an optimal residence time to produce nutrient-rich biomass, thereby achieving the best possible yield. These results have a deep impact to adequately determine biofertilizer price, taking into account the optimal harvesting times to ensure a high nutrient content in microalgae.

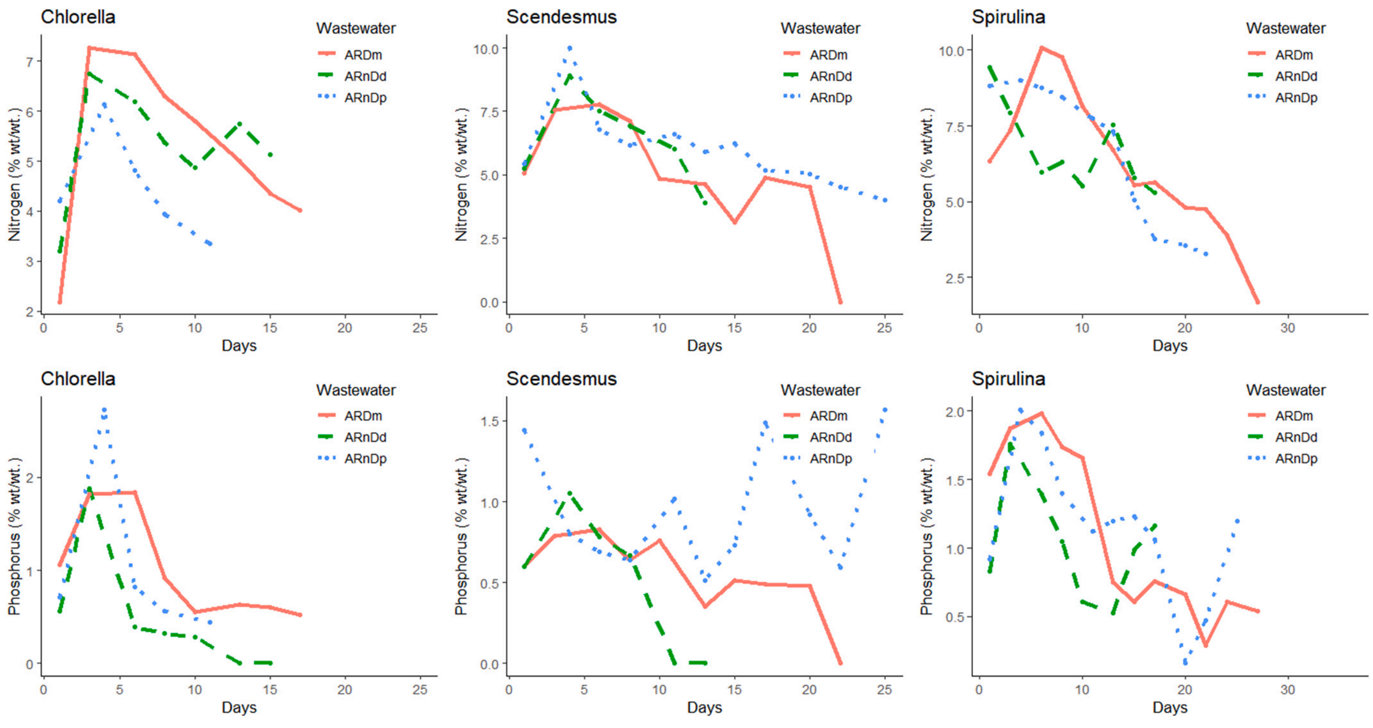
The Graphic 2 describes the changes in biomass production over time in the photobioreactors for each wastewater employed and summarizes the feasible operational profits per year that could be obtained according to the costs estimated shown in Table 10, the price stablished for the biofertilizer in Table 7 and the nutrients composition reported in Graphic 1. From results shown can be observed that biomass production and nutrients concentration in biomass vary according to the strain and the wastewater employed, were Scenedesmus has shown the best operational profits close to $4MUSD/Year, with a retention time close to 3 days.

Analyzing the distribution of operational expenses (OPEX) obtained in Table 10 for the algae strains across different wastewater (ARD$_m$, ARnD$_p$, and ARnD$_d$), several key trends emerge. The distribution of costs remains with utilities and labor costs as the major important factors, varying according to strain and wastewater. Poultry wastewater has the lowest OPEX compared to other wastewater, however, the utilities observed suggests that only Scenedesmus could represent real benefits in this wastewater due to his capacity to has a stable growth in low phosphorus conditions or must be required the addition of nutrients for Chlorella and Spirulina, increasing raw materials costs.

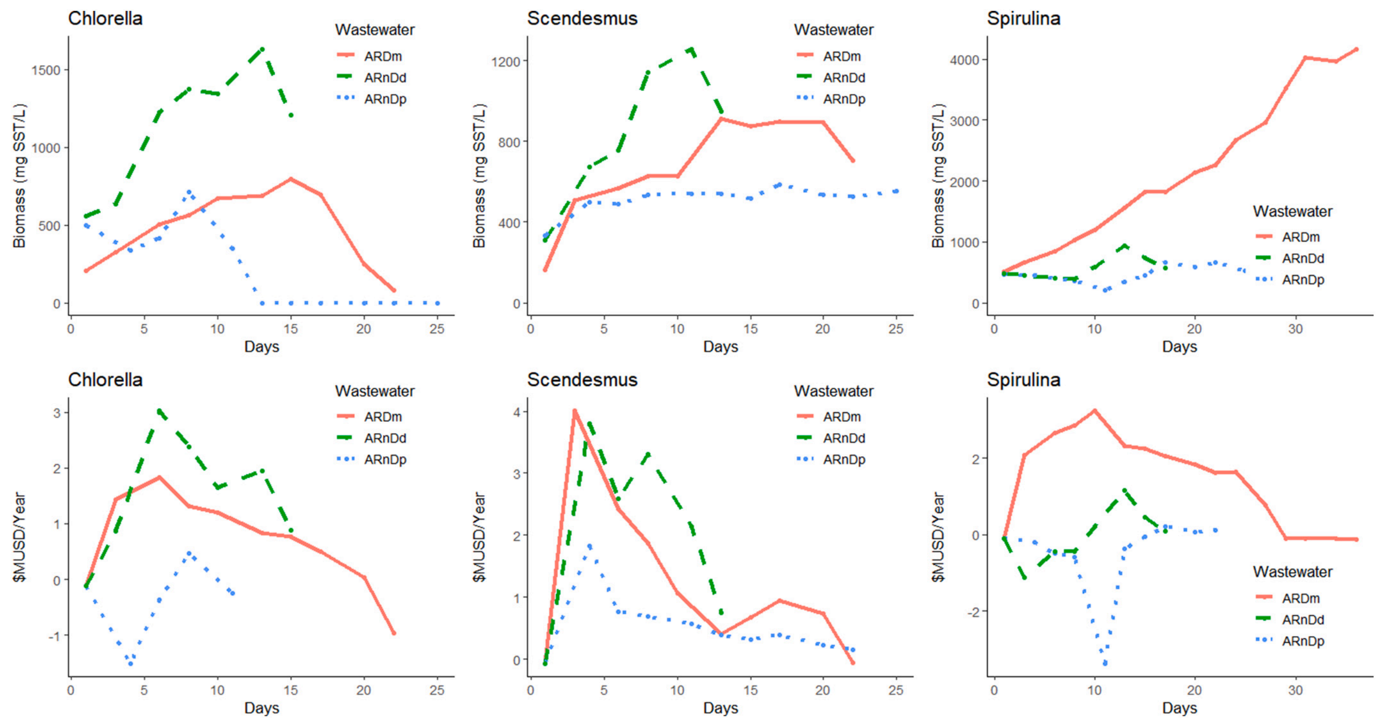
Municipal wastewater is shown to be the most complete in terms of nutrients for microalgae growth, allowing to evaluate all strains, where Scenedesmus show the highest operational expenses and the greatest utilities, followed by Spirulina and chlorella. For ARnD$_d$ there was a consistent increase in biomass production and utilities for Scenedesmus and chlorella than Spirulina and other wastewater, however, there was a deep decrease in biomass after the growth phase, which could imply high risks of losses if collection time is not controlled properly, these results emphasize the importance of strategic decision-making when selecting which algae to cultivate for optimal cost efficiency and profitability.

4. Discussion

The effectiveness of microalgae such as *Chlorella Sorokiniana*,



Graphic 1. Nitrogen and phosphorus concentration in dried biomass over time for algae and wastewater.



Graphic 2. Biomass production and operational profits for microalgae strains using different wastewater as nutrient source.

Table 10
Operational expenses and selling price obtained for microalgae cultures in wastewater effluents evaluated for minimum OPEX.

Algae	Chl	Scn	Spi
ARD_m			
Raw material (%)	5.11	6.93	4.30
Utilities (%)	57.95	41.16	52.37
Direct labor (%)	17.18	30.20	22.73
Maintenance (%)	4.14	4.14	4.14
Quality lab (%)	2.58	4.53	3.41
Harvesting (%)	13.04	13.04	13.04
OPEX (USD/year)	$139.448	$196.321	$154.289
Biomass (ton/year)	80	198	152
OPEX (USD/kg)	1.74	1.32	1.09
$ARnD_p$			
Raw material (%)	6.98	7.34	3.53
Utilities (%)	63.35	58.18	73.07
Direct labor (%)	10.86	15.03	5.41
Maintenance (%)	4.14	4.14	4.14
Quality lab (%)	1.63	2.26	0.81
Harvesting (%)	13.04	13.04	13.04
OPEX (USD/year)	$127,561	$138,886	$110,592
Biomass (ton/year)	46	70	20
OPEX (USD/kg)	2.75	1.98	5.52
$ARnD_d$			
Raw material (%)	8.30	3.97	7.70
Utilities (%)	16.82	18.74	36.31
Direct labor (%)	50.18	52.26	33.75
Maintenance (%)	4.14	4.14	4.14
Quality lab (%)	7.53	7.84	5.06
Harvesting (%)	13.04	13.04	13.04
OPEX (USD/year)	$191,544	$178,155	$128,543
Biomass (ton/year)	192	179	59
OPEX (USD/kg)	1.00	0.99	2.15

Scenedesmus sp. and *Spirulina platensis* in bioremediation processes are pivotal for sustainable water treatment technologies. This study assessed their capability to remove nitrogen and phosphorus species from water under varying nutrient ratios, shedding light on their biomass production and their impact on water quality parameters. In the case of SPI, low carbon levels seriously hamper the denitrification process, combined with the fact that SPI exhibits a low nutrient absorption capacity, which could lead to nitrite accumulation [28].

4.1. Discussion for synthetic wastewater

From data collected in synthetic wastewater can be observed that nitrogen species assimilation depends of their concentration, where ammonium is prioritized by microalgae, particularly under conditions of elevated concentrations, however, there is and existent limitation for nitrate uptake in ammonium presence (Removal yield <16.20 % in most of cases), which is consistent with data reported by M. Carletti et al. [27]. High ammonium concentrations (>8 ± 11 mg NH_3-N/L) suppress the activity of nitrite-oxidizing bacteria (NOB) in favor of ammonia-oxidizing bacteria (AOB), leading to an accumulation of nitrites in the system, which consequently inhibits biomass growth as can be observed in results with nitrite accumulation and reduction in biomass production, just like was stated by V. Pozzobon et al. [29].

Previous studies suggest that phosphorus concentrations exceeding 7 mg P/L do not significantly impact biomass productivity. However, although *Spirulina* has shown high efficiency in phosphorus removal, this behavior is also linked to abiotic processes such as precipitation associated to phosphorus oxidation as was observed in blanks and identified by S. Wang et al. [30]. Additionally, phosphorus uptake is closely tied to the daily absorption rate, as shown in Table 5 at the 150 N:10P experiment, where only 50 % of the initial concentration was absorbed after 12 days [31].

It's important to mention that the results from the synthetic wastewater experiments highlight critical environmental implications of nutrient assimilation by microalgae. Ammonium (NH_4^+) is preferentially assimilated at high concentrations, but this leads to the suppression of nitrite-oxidizing bacteria (NOB) and subsequent nitrite accumulation, which inhibits biomass growth and poses a risk of nitrite toxicity to aquatic ecosystems [32]. Additionally, nitrate uptake is limited in the presence of ammonium which could exacerbate eutrophication if left unchecked. Phosphorus removal, particularly by Spi, is efficient but influenced by abiotic processes such as precipitation, potentially leading to phosphorus re-release into the environment in rainy periods [33]. These findings underscore the need for balanced nutrient management in wastewater treatment systems to prevent nutrient overload, which leads to uncontrolled microalgae growth and accelerates environmental degradation, threatening biodiversity.

4.2. Discussion for real wastewater

The results obtained clearly demonstrate the influence of wastewater composition on the nitrogen removal efficiency by strains, because nutrient removal depends on the availability of the other, as microalgae cannot remove N without the presence of P in wastewater, or the other way around, because both nutrients are essential for their growth, but also the removal of N and P by microalgae depends on the concentrations of these nutrients in the microalgae biomass, since the nutrient uptake capacity depends on this ratio [34], where the presence of AOB avoids nitrite accumulation [35]. *Chlorella*, *Spirulina* and *Scenedesmus* exhibited a remarkable ability to utilize the nitrogen present in municipal wastewater, outperforming other species. This suggests that the microbial community and the specific matrix of nitrogenous compounds in this wastewater create a favorable environment for nitrogen uptake by these microalgae [36]. However, results from the poultry industry shows a decrease in nitrogen removal efficiency, which could be attributed to inhibitory substances as chlorine compound used for cleaning procedures, implying the absence of bacteria that reduces the bioavailability of nitrogen in the form of ammonia [37], as it has the lowest initial concentration compared to other wastewaters [38]. Dairy wastewater exhibited an accelerated growth and death of algae, indicating a fragile balance between the nutrient's availability and the microalgae-bacteria consortia, establishing limiting factors that should be evaluated to adapt this technology [39], This could be addressed through a pretreatment process aimed at reducing the initial microbial load in dairy effluents. Since these effluents are characterized by high microbial growth, it is essential to minimize it to prevent detrimental competition with algal growth [40].

Phosphorus removal efficiency varied considerably depending on the type of wastewater and microalgae necessities [41]. Municipal wastewater proved to be the most suitable medium for this process, suggesting that the microbial community present in it facilitates phosphorus uptake [36]. On the other hand, Spirulina showed a clear dependence on phosphorus for its growth and survival, highlighting the importance of this nutrient for the development and productivity of this microalgae strain [42], these results must be carefully considered to properly use spirulina, which could be more effective in wastewater with high phosphorus concentrations, or there will be needed the addition of it. A key factor for Spirulina growth is pH, as it directly affects biomass yield. A pH range of 8.5 to 10 is recommended, as high pH levels tend to increase free radicals, causing oxidative stress in the cells. However, it has been shown that at these pH levels, antioxidant activity (radical scavenging capacity) improves, enhancing growth and optimal nutrient absorption for Spirulina [43].

The findings of this study provide an interesting perspective on the relationship between nutrient removal efficiency and the accumulation of these nutrients in microalgal biomass. The positive correlation found between removal rates and the amount of nitrogen and phosphorus stored in the biomass suggests that the ability of microalgae to incorporate these nutrients into their cells is a determining factor in the effectiveness of bioremediation processes [44] and biofertilizers production due to changes in composition over time.

The results highlight the significant environmental impact of using microalgae for wastewater treatment, being particularly studied in municipal, poultry, and dairy wastewater. Chlorella, Scenedesmus and Spirulina demonstrated high nitrogen and phosphorus removal efficiencies in municipal wastewater, indicating a favorable environment for nutrient uptake, which could help to mitigate eutrophication risks associated to secondary wastewater effluents. However, there is a significant effect according to the wastewater employed, as was observed phosphorus deficient poultry wastewater or high organic loaded dairy wastewater. These findings underscore the importance of tailoring treatment strategies based on wastewater composition to maximize environmental benefits.

4.3. Discussion for financial approach

It is essential to select the appropriate strain based on the type of wastewater, as this factor directly impacts operational costs, Likewise, the biomass produced is a key factor, since the OPEX is calculated based on the kilogram of biomass generated. This value is affected, since, according to the principles of large-scale economy, the higher the production volume, the lower the unit costs [45]. OPEX tends to perform more favorably during residence times between 6 and 12 days, when operational efficiency is maximized, partly due to the higher protein content in the microalgae. In the initial days, profitability was limited by low biomass production, while towards the end, efficiency declines due to microalgae inactivity caused by nutrient depletion, implying energy consumption without value generation.

A critical driver of operating expenses (OPEX) is energy consumption, which varies in response to government policies and the country's energy situation. Across all scenarios, utility costs make up the largest share of operating expenses, which is coherent with this process due to the constant aeration requirement to ensure algae suspension, it's possible that other type of reactors as raceway could represent lower energy consumption improving this index. Labor costs was also representative in operational costs, mainly associated to fouling of algae and cleaning procedures, highlighting the need to optimize energy use to reduce total operating expenses and improve process efficiency, which could be related to reactor geometry, and could be achieved also by implementing other photobioreactor as raceway that could improve capital investments studies.

Biofertilizers for microalgae can't be competitive directly with conventional fertilizer products, for this reason, increases in their price must be justifiable with different benefits that provide the uses of biofertilizer from microalgae, like bioremediation of soil. Also, it is important to note that new regulations regarding nitrogen (N) and phosphorus (P) concentrations in discharged wastewater may significantly impact enterprises in the future, which will drive to the enhancement for nutrient removal in wastewater treatment processes.

5. Conclusions

From this work can be concluded that the selected microalga strains have the potential to generate economic benefits for wastewater from domestic, dairy and poultry industries, not only by reducing nutrients concentrations or reducing environmental taxes for nonaccomplishment of regulatory framework but creating the possibility to generate value from biomass obtained valorized as biofertilizers, which has an estimated OPEX around 1 USD/Kg and a possible selling price around 20 USD/Kg, which represent an important utility that could support capital investments. It's important to recognize that the economic viability for microalgal cultivation processes is conditioned to parameters such as strain employed, wastewater characteristics, hydraulic residence time, algal growth kinetics and nutrient uptake rate, which determines labor costs and energy consumption, being the most critical factors to control and ensure process profitability.

The inadequate management of nutrient disposal in water bodies leads to nitrite accumulation in ecosystems and is accelerated due to algae activities, being critical in absence of nitrite-oxidizing bacteria, causing negative effects in aquatic biota, threatening biodiversity. Chlorella, Scenedesmus and spirulina has shown that microalgae can remove and use nitrogen and phosphorus from wastewater to biomass production, which could significatively reduce nitrogen and phosphorus presence in water bodies and reduce eutrophication risks.

The experiments using real wastewater, including municipal, poultry, and dairy effluents, reveal that it is a suitable nutrient source for microalgae biomass production. The advancement in these technologies for tertiary wastewater treatment drastically reduces nutrients present in wastewater with efficiencies closer or higher to 90 %, not only decreasing eutrophication risks associated to wastewater effluents, but also represents and reduction of operational costs to produce microalgae biomass and their transformation to bioproducts such as biofuels, biofertilizers, bio pigments among others. However, wastewater must be characterized to ensure the nutritional requirements for optimal algae growth or avoid inhibitory or toxic substances such as heavy metals.

Future research should optimize their cultivation conditions, determine the optimum initial concentration of the inoculum and the minimum required nutrient levels, and integrate them into practical bioremediation systems to maximize their efficacy and scalability in real-world applications. The implementation of microalgae as a tertiary treatment could effectively address the issues faced by current treatment plants, which fail to remove excess contaminants and nutrients adequately. This improvement is evidenced by the removal percentages achieved, indicating a greater capacity to treat these pollutants. Additionally, future regulations on nitrogen and phosphorus levels in wastewater discharges could significantly affect costs, making efficient nutrient removal even more important.

CRediT authorship contribution statement

Alejandro Pérez Mesa: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Paula Andrea Céspedes Grattz:** Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Juan José Vidal Vargas:** Writing – original draft, Validation, Investigation, Formal analysis. **Luis Alberto Ríos:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **David Ocampo Echeverri:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Alejandra María Miranda Parra:** Writing – review & editing, Supervision, Resources, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Alejandro Perez Mesa reports financial support was provided by Colombia Ministry of Science Technology and Innovation. Alejandro Perez Mesa reports financial support was provided by Alejandro Angel Escobar Foundation. Alejandro Perez Mesa reports financial support was provided by University of Antioquia. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

[1] C. He, Z. Liu, J. Wu, X. Pan, Z. Fang, J. Li, B.A. Bryan, Future global urban water scarcity and potential solutions, Nat. Commun. (2021), https://doi.org/10.1038/s41467-021-25026-3.
[2] S. Naidoo, A.O. Olaniran, Treated Wastewater Effluent as a Source of Microbial Pollution of Surface Water Resources, International Journal of Environmental Research and Public Health 2014, Vol. 11, Pages 249–270 11 (2013) 249–270. doi: https://doi.org/10.3390/IJERPH110100249.
[3] M.P. Shah, Wastewater treatment: molecular tools, techniques, and applications, wastewater treatment: molecular tools, Techniques, and Applications (2022) 1–192, https://doi.org/10.1201/9781003165057/WASTEWATER-TREATMENT-MAULIN-SHAH/ACCESSIBILITY-INFORMATION.
[4] J.L. Wang, L.J. Xu, Advanced oxidation processes for wastewater treatment: formation of hydroxyl radical and application, Crit. Rev. Environ. Sci. Technol. 42 (2012) 251–325, https://doi.org/10.1080/10643389.2010.507698.
[5] E.O. Ezugbe, S. Rathilal, Membrane Technologies in Wastewater Treatment: a review, Membranes (Basel) 10 (2020) 89, https://doi.org/10.3390/membranes10050089.
[6] S.F. Ahmed, M. Mofijur, T.A. Parisa, N. Islam, F. Kusumo, A. Inayat, V.G. Le, I. A. Badruddin, T.M.Y. Khan, H.C. Ong, Progress and challenges of contaminate removal from wastewater using microalgae biomass, Chemosphere 286 (2022) 131656, https://doi.org/10.1016/J.CHEMOSPHERE.2021.131656.
[7] M. Von Sperling, C. de Lemos Chernicharo, Biological wastewater treatment in warm climate regions volume II, water intelligence, Online 4 (2005) 9781780402703, https://doi.org/10.2166/9781780402703.
[8] F.A. Nasr, B. Mikhaeil, Treatment of domestic wastewater using conventional and baffled septic tanks, Environ. Technol. 34 (2013) 2337–2343, https://doi.org/10.1080/09593330.2013.767285.
[9] E. David Ugwuanyi, Z. Queen, S. Nwokediegwu, M.A. Dada, M.T. Majemite, A. Obaigbena, Review of emerging technologies for nutrient removal in wastewater treatment, Https://Wjarr.Com/Sites/Default/Files/WJARR-2024-0520.Pdf 21 (2024) 1737–1749, https://doi.org/10.30574/WJARR.2024.21.2.0520.
[10] S.L. Wear, V. Acuña, R. McDonald, C. Font, Sewage pollution, declining ecosystem health, and cross-sector collaboration, Biol. Conserv. 255 (2021) 109010. https://www.sciencedirect.com/science/article/pii/S0006320721000628.
[11] IDEAM, Estudio Nacional del Agua 2022, Bogotá, 2023.
[12] B. Molinuevo-Salces, B. Riaño, D. Hernández, M.C. García-González, Microalgae and wastewater treatment: advantages and disadvantages, Microalgae Biotechnology for Development of Biofuel and Wastewater Treatment (2019) 505–533, https://doi.org/10.1007/978-981-13-2264-8_20/TABLES/4.
[13] J.B. García-Martínez, J.E. Contreras-Ropero, N.A. Urbina-Suarez, G.L. López-Barrera, A.F. Barajas-Solano, V. Kafarov, C. Barajas-Ferreira, D.M. Ibarra-Mojica, A. Zuorro, A simulation analysis of a microalgal-production Plant for the Transformation of inland-fisheries wastewater in sustainable feed, Water (Switzerland) 14 (2022), https://doi.org/10.3390/w14020250.
[14] N.D. Devi, X. Sun, L. Ding, V.V. Goud, B. Hu, Mixotrophic growth regime of novel strain Scenedesmus sp, DDVG I in municipal wastewater for concomitant bioremediation and valorization of biomass, J Clean Prod 365 (2022) 132834, https://doi.org/10.1016/J.JCLEPRO.2022.132834.
[15] F. Almomani, R.R. Bohsale, Bio-sorption of toxic metals from industrial wastewater by algae strains Spirulina platensis and Chlorella vulgaris: application of isotherm, kinetic models and process optimization, Sci. Total Environ. 755 (2021), https://doi.org/10.1016/j.scitotenv.2020.142654.
[16] M.L. Ortiz Moreno, K.X. Sandoval Parra, L.V. Solarte Murillo, Chlorella, ¿un potencial biofertilizante? Orinoquia 23 (2019) https://doi.org/10.22579/20112629.582.
[17] M.A. Abdel-Razek, A.M. Abozeid, M.M. Eltholth, F.A. Abouelenien, S.A. El-Midany, N.Y. Moustafa, R.A. Mohamed, Bioremediation of a pesticide and selected heavy metals in wastewater from various sources using a consortium of microalgae and cyanobacteria, Slov. Vet. Res. 56 (2019) 61–73, https://doi.org/10.26873/SVR-744-2019.
[18] T.P. Cáceres, M. Megharaj, R. Naidu, Biodegradation of the pesticide Fenamiphos by ten different species of green algae and Cyanobacteria, Springer Nature (2008), https://doi.org/10.1007/s00284-008-9293-7.
[19] N. Mirghaffari, E. Moeini, O. Farhadian, Biosorption of cd and Pb ions from aqueous solutions by biomass of the green microalga, Scenedesmus quadricauda, J Appl Phycol 27 (2015) 311–320, https://doi.org/10.1007/S10811-014-0345-Z.
[20] UTEX 1230 *Chlorella sorokiniana* | UTEX Culture Collection of Algae, (n.d.). https://utex.org/products/utex-1230?variant=30991207858266 (accessed September 29, 2024).
[21] UTEX EE101 Scenedesmus sp. | UTEX Culture Collection of Algae, (n.d.). https://utex.org/products/utex-ee-0101?variant=30991248359514 (accessed September 29, 2024).
[22] UTEX LB 2340 Spirulina platensis | UTEX Culture Collection of Algae, (n.d.). https://utex.org/products/utex-lb-2340?variant=30992067067994 (accessed September 29, 2024).
[23] J. Stein, Handbook of Phycological methods, in: Culture Methods and Growth Measurements., in: CANADIAN PHYCOLOGICAL CULTURE CENTRE, Cambridge University, 1975, p. 448.

[24] P. Mesa, S. Molina, O. Echeverri, A. Alvarado-Lassman, C. Velasco-Santos, J. Manuel Méndez-Contreras, A. Pérez Mesa, J. Cesar Saldarriaga Molina, L. Alberto Ríos, E. Ocampo Echeverri, D. Ocampo Echeverri, Diagnosis of Nutrient Discharges and Management Alternatives in Developing Countries and the Use of Microalgae as a Potential Solution: A Case Study from Different Provinces in Antioquia, Colombia, Water 2024, Vol. 16, Page 2215 16 (2024) 2215. doi:https://doi.org/10.3390/W16162215.

[25] B. Columbia, A.O. Alabi, M. Tampier, Microalgae Technologies & Processes for Biofuels / Bioenergy Production in 1 (2009) 5.

[26] Max. Peters, Timmerhaus Klaus, Diseño de Plantas y Su Evaluación Económica Para Ingenieros Químicos -2da Ed., 1991.

[27] M. Carletti, E. Barbera, F. Filippini, E. Sforza, Effect of ammonium/nitrate ratio on microalgae continuous cultures: species-specificity of nutrient uptake and modelling perspectives, Journal of Water Process Engineering 58 (2024) 104762, https://doi.org/10.1016/J.JWPE.2023.104762.

[28] H. Wan, K. Wang, X. Luo, C. Zhang, K. Deng, S. Lin, J. Xie, Q. Luo, X. Lei, L. Ding, Algal-mediated nitrogen removal and sustainability of algal-derived dissolved organic matter supporting denitrification, Bioresour. Technol. 407 (2024) 131083, https://doi.org/10.1016/J.BIORTECH.2024.131083.

[29] V. Pozzobon, C. Na, A. Moreaud, E. Michiels, W. Levasseur, Nitrate and nitrite as mixed source of nitrogen for Chlorella vulgaris: growth, nitrogen uptake and pigment contents, Bioresour. Technol. 330 (2021) 124995, https://doi.org/10.1016/j.biortech.2021.124995.

[30] S. Wang, X. Jin, Q. Bu, L. Jiao, F. Wu, Effects of dissolved oxygen supply level on phosphorus release from lake sediments, Colloids Surf. A Physicochem. Eng. Asp. 316 (2008) 245–252, https://doi.org/10.1016/J.COLSURFA.2007.09.007.

[31] G. Markou, I. Chatzipavlidis, D. Georgakakis, Effects of phosphorus concentration and light intensity on the biomass composition of Arthrospira (Spirulina) platensis, World J. Microbiol. Biotechnol. 28 (2012) 2661–2670, https://doi.org/10.1007/S11274-012-1076-4/METRICS.

[32] F.B. Jensen, Nitrite disrupts multiple physiological functions in aquatic animals, Comp. Biochem. Physiol. A Mol. Integr. Physiol. 135 (2003) 9–24, https://doi.org/10.1016/S1095-6433(02)00323-9.

[33] M.C. Ramos, I. Lizaga, L. Gaspar, A. Navas, The impacts of exceptional rainfall on phosphorus mobilisation in a mountain agroforestry catchment (NE, Spain), Catena (Amst) 216 (2022) 106407, https://doi.org/10.1016/J.CATENA.2022.106407.

[34] A. Beuckels, E. Smolders, K. Muylaert, Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment, Water Res. 77 (2015) 98–106, https://doi.org/10.1016/J.WATRES.2015.03.018.

[35] Y. Zhou, Y. Zhu, J. Zhu, C. Li, G. Chen, A comprehensive review on wastewater nitrogen removal and its recovery processes, Int. J. Environ. Res. Public Health 20 (2023), https://doi.org/10.3390/IJERPH20043429.

[36] C.C. Tang, Y.R. Hu, Z.W. He, Z.H. Li, Y. Tian, X.C. Wang, Promoting symbiotic relationship between microalgae and bacteria in wastewater treatment processes: technic comparison, microbial analysis, and future perspectives, Chem. Eng. J. 498 (2024) 155703, https://doi.org/10.1016/J.CEJ.2024.155703.

[37] K. Fukami, T. Nishijima, Y. Ishida, Stimulative and Inhibitory Effects of bacteria on the Growth of Microalgae, 1997.

[38] X. Li, W. Li, J. Zhai, H. Wei, Q. Wang, Effect of ammonium nitrogen on microalgal growth, biochemical composition and photosynthetic performance in mixotrophic cultivation, Bioresour. Technol. 273 (2019) 368–376, https://doi.org/10.1016/J.BIORTECH.2018.11.042.

[39] P. Singh, S.S. Mohanty, K. Mohanty, Comprehensive assessment of microalgal-based treatment processes for dairy wastewater, Front. Bioeng. Biotechnol. 12 (2024) 1425933, https://doi.org/10.3389/FBIOE.2024.1425933/BIBTEX.

[40] G. Gramegna, A. Scortica, V. Scafati, F. Ferella, L. Gurrieri, M. Giovannoni, R. Bassi, F. Sparla, B. Mattei, M. Benedetti, Exploring the potential of microalgae in the recycling of dairy wastes, Bioresour Technol Rep 12 (2020) 100604, https://doi.org/10.1016/J.BITEB.2020.100604.

[41] E.M. Salgado, A.F. Esteves, A.L. Gonçalves, J.C.M. Pires, Microalgal cultures for the remediation of wastewaters with different nitrogen to phosphorus ratios: process modelling using artificial neural networks, Environ. Res. 231 (2023) 116076, https://doi.org/10.1016/J.ENVRES.2023.116076.

[42] G. Abozied, A.Z. Sabah, D.A. Marrez, M.A. Ali, Impact of Phosphorus Sources on Biomass and Pigments Content of Spirulina platensis 28 (2024) 785–798, https://doi.org/10.21608/EJABF.2024.350992.

[43] M.M.S. Ismaiel, Y.M. El-Ayouty, M. Piercey-Normore, Role of pH on antioxidants production by *Spirulina* (*Arthrospira*) *platensis*, Braz. J. Microbiol. 47 (2016) 298–304, https://doi.org/10.1016/J.BJM.2016.01.003.

[44] M.A. Yaakob, R.M.S.R. Mohamed, A. Al-Gheethi, G.A. Ravishankar, R.R. Ambati, Influence of nitrogen and phosphorus on microalgal growth, Biomass, Lipid, and Fatty Acid Production: An Overview, Cells 10 (2021) 1–19, https://doi.org/10.3390/CELLS10020393.

[45] J.A. Clark, Economies of scale and scope at depository financial institutions, A Review of the Literature 73 (2014) 16–33. https://www.researchgate.net/publication/5043462.