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A Simulation Analysis of a Microalgal-Production Plant for the Transformation of Inland-Fisheries Wastewater in Sustainable Feed

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Abstract: The present research evaluates the simulation of a system for transforming inland-fisheries wastewater into sustainable fish feed using Designer® software. The data required were obtained from the experimental cultivation of *Chlorella* sp. in wastewater supplemented with N and P. According to the results, it is possible to produce up to 11,875 kg/year (31.3 kg/d) with a production cost of up to 18 (USD/kg) for dry biomass and 0.19 (USD/bottle) for concentrated biomass. Similarly, it was possible to establish the kinetics of growth of substrate-dependent biomass with a maximum production of 1.25 g/L after 15 days and 98% removal of available N coupled with 20% of P. It is essential to note the final production efficiency may vary depending on uncontrollable variables such as climate and quality of wastewater, among others.

Keywords: *Oreochromis* sp.; biomass; SuperPro; *Chlorella* sp.; inland fisheries

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1. Introduction

Aquaculture is now the world's fastest-expanding food-production sector, accounting for more than half of all fish consumed by humans [1], surpassing catch-fisheries production by 18.32 million tons, with a total value over USD 250 billion [2]. However, once considered a sustainable solution to fight malnutrition in low-income economies [3,4], its global growth has increased the demand for feed and water and generated new problems, such as high levels of untreated liquid and solid wastes [5,6].

Conventional methods for wastewater treatment, such as chemical flocculation, can generate other compounds in sewage sludge that are not effectively treated, eventually increasing their environmental impact. Therefore, it is essential to identify natural, biodegradable, nontoxic, affordable, and efficient alternatives for wastewater treatment [7]. The application of algae and cyanobacteria on wastewater treatment systems is one of the most innovative and sustainable alternatives for the sequestration of hazardous components [8] and reduction of environmental CO_2 [9], which are converted into biomass composed of industrially relevant metabolites, such as carbohydrates, proteins, lipids, carotenoids, and others [10]. Over the last ten years, different genera, including *Chlorella*, *Chlamydomonas*,

and *Spirulina*, have proven effective for the removal of organic matter [11,12], heavy metal ions [13], and phenolic compounds [14].

The global market for algal biomass and metabolites is expected to reach USD 1.143 billion by 2024, with a yearly growth rate of 7.39 percent [15]. Due to their natural colors, antioxidants, and other bioactive chemicals with valuable qualities, these microorganisms are effectively used in aquaculture hatcheries as animal-feed supplements [16]. However, their use can increase final-product prices due to species selection and expensive culture media. Using microalgae in a circular-bioeconomic approach for the aquaculture industry will deliver a twofold benefit: low-cost wastewater treatment and biomass for animal feed [17,18]. However, the operating conditions, biomass-production efficiency, and the effect of the concentration of N, P, and other nutrients on cell growth must be identified and analyzed.

In recent years, the application of specialized software, such as Aspen Plus, SuperPro, and MATLAB have made it possible to analyze the different processes of nutrient consumption and their transformation into total biomass and metabolites of interest [19]. Recent studies, such as the BIO_ALGAE model [20], address critical physical, chemical, and kinetic parameters governing the production of microalgae and bacteria in wastewater. This model has proven helpful in simulating bioremediation and microalgal production in aquacultural wastewater in a semicontinuous system with different environmental factors [21]. However, the different works in this field employ data from temperate production systems. To the best of the authors' knowledge, there are no available data from tropical areas. The present work focuses on the simulation analysis of a microalgal-production plant under different scenarios for transforming inland-fisheries wastewater into sustainable feed with a circular-economical approach.

2. Materials and Methods

2.1. Strain

Chlorella sp. (CHLO_UFPS010) from INNOValgae collection (Universidad Francisco de Paula Santander, Cúcuta, Colombia) was used in this study. *C. vulgaris* was grown in a 2 L glass flask with a working volume of 1.2 L containing Bold Basal medium [22]. The medium was mixed through the injection of filtered air with 0.5% (*v*/*v*) CO_2 at a flow rate of 0.78 L/min, 25 °C, and light–dark cycle of 12:12 h at 100 µmol/m^2 s for 30 days.

2.2. Experimental Design

Fisheries wastewater obtained from local *Oreochromis* sp. farmers (El Zulia, Norte de Santander, Colombia) was filtered twice and UV-sterilized [23]. After sterilization, the wastewater was supplemented with a known amount of biofertilizer until a concentration of NO_3 and PO_4 was reached (0.1 and 0.24 g/L, respectively). The alga was cultured (by triplicate) in a 9 L glass flask with a working volume of 7 L of UV-sterile supplemented wastewater. Each flask was mixed by injection of filtered air at a flow rate of 4.2 L/min and light–dark cycle of 12:12 h at 100 µmol/m^2 s for 40 days. Every five days, 50 mL of medium were axenically removed, and the biomass was concentrated using electroflotator equipment [24]. The recovered biomass was dried (50 °C, 12 h) and weighed. The cell-free medium was filtered and used for determination of NO_3 (HI 93728-01, HANNA) and PO_4 (HI 93713-01, HANNA). Kinetic constants for biomass production and NO_3 and PO_4 consumption were obtained from the results. The constants were described by linearizing the Monod equation:

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{Ks}{\mu_{max}} \times \frac{1}{s} \quad (1)$$

2.3. Process Description and Plant Simulation

The microalgal-production plant using fisheries wastewater was simulated using SuperPro Designer® software v8.0. (Intelligen, Inc., Scotch Plains, NJ, USA). In the upstream stage, *Chlorella* sp. was grown in Bold Basal Medium with the selected culture variables

shown in Table 1. Once the desired cell concentration was obtained, the cells (10% v/v) were transferred to photobioreactors (PBR) with higher working volumes (preinoculum). Once the PBR reached the optimal cell density, the cells were transferred (10% v/v) into two 5 m^3 raceways (20 d, (30 $\pm$ 2) °C).

Table 1. Biomass production variables.

Constants	Variable	Value	Units
X_o	Initial biomass	0.08	g/L
CO_2	CO_2 concentration	6	% v/v
N_o	Initial nitrate concentration	0.1	g/L
P_o	Initial phosphate concentration	0.2	g/L
I	Light intensity	100	µmol/m^2 s
Q	Air inlet	0.6	vvm

In the downstream process, the biomass produced was harvested by centrifugation and used to produce fish feed in two forms: pelletized biomass (dry) and live feed (liquid), depicted in Figure 1.

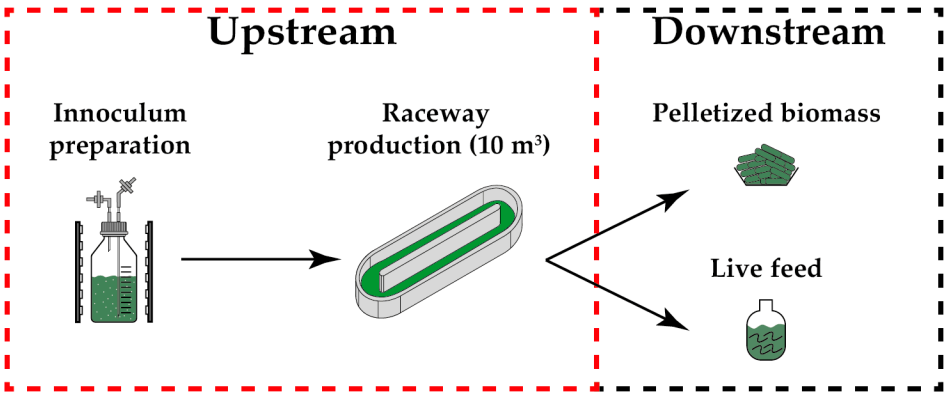


Figure 1. Process description of algal-based feed.

The production of *Chlorella* sp. biomass (Equation (2)) its consumption rate of NO_3 (Equation (3)), and its consumption rate of PO_4 (Equation (4)) were modeled by linearizing the Monod kinetics equation [25].

$$X_f = Xe^{\Delta t.\mu} \tag{2}$$

$$S_f = S_0 - \mu \times Y_{\frac{s}{x}} \times \Delta t \times X_0 \tag{3}$$

$$\mu = \frac{\mu_{max} \times s}{ks + s} \tag{4}$$

3. Results

3.1. Kinetics Constants for NO_3 and PO_4 Consumption

A computational model contains many factors that influence the development of the system to be evaluated. In this case, the simulation of a microalgal cultivation system using fisheries allows us to understand the behavior of this microorganism. According to experimental results, it was possible to obtain the NO^{3-} and PO^{4-} consumption constants that can be found in Figure 2, where the slope and intercept refer to ks/μ_{max} and $1/\mu_{max}$, respectively.

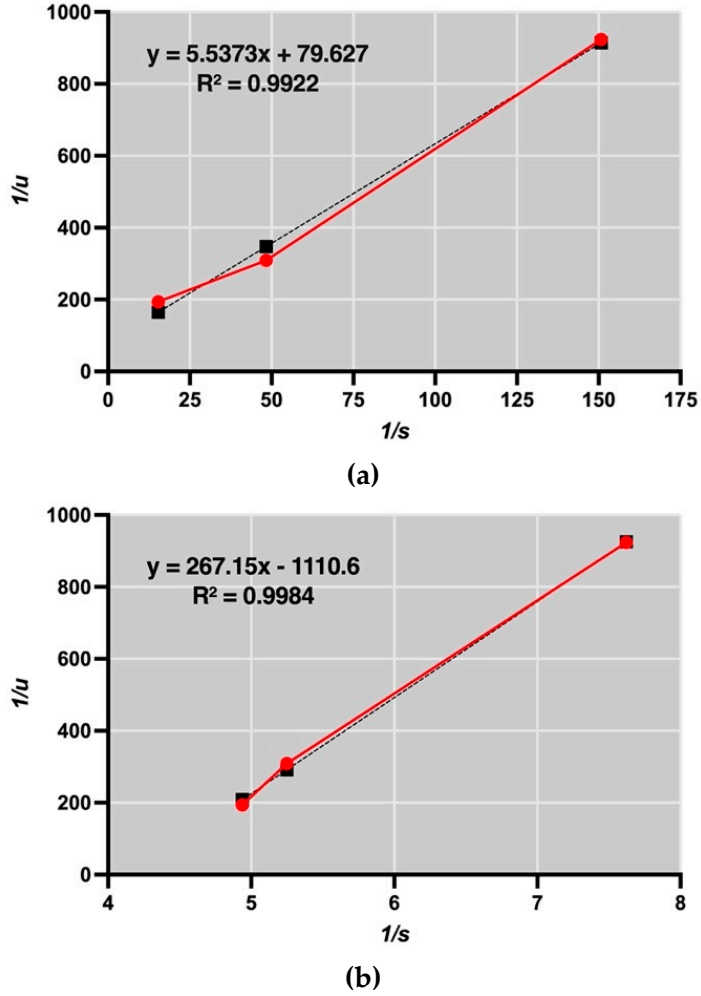


Figure 2. Consumption constant rates for NO_3 (**a**) and PO_4 (**b**).

Table 2 presents the results for the kinetic variables of the experimental culture of *Chlorella* sp. in fisheries wastewater. The effect of variables, such as light quality (light intensity and light–dark cycle) [26], pH [27], temperature [28], and the availability of carbon, nitrogen, and phosphorus [29,30], play a critical role in the productivity of biomass and specific metabolites, such as carbohydrates, proteins, and lipids [31,32]. According to Park et al. [33], the availability of these elements is fundamental in synthesizing diverse molecules that play an essential role in cellular metabolism.

Table 2. Kinetic variables from the experimental culture of *Chlorella* sp.

Constants	Variable	Value
μ	Specific growth rate	0.042
$Y_{N/X}$	Nitrate-consumption constant	0.23
$Y_{P/X}$	Phosphate-consumption constant	0.35

By correctly establishing the critical variables of the microalgal-growth process, it is possible to improve the precision between the experimental data and the data obtained through simulation, which establishes a reliable point for the optimization of the different processes in the cultivation of photosynthetic microorganisms [34,35]. Figure 3 shows the behavior of biomass production and NO_3 and PO_4 consumption according to the equations previously established. The results show the deviation between experimental and theoretical data is relatively low (0.29, 0.03, and 0.08 for biomass, NO_3, and PO_4,

respectively). However, it is worth mentioning that, for this case, inhibition by cell density, CO_2, light, and other variables that can have a positive or negative impact within the process were not considered.

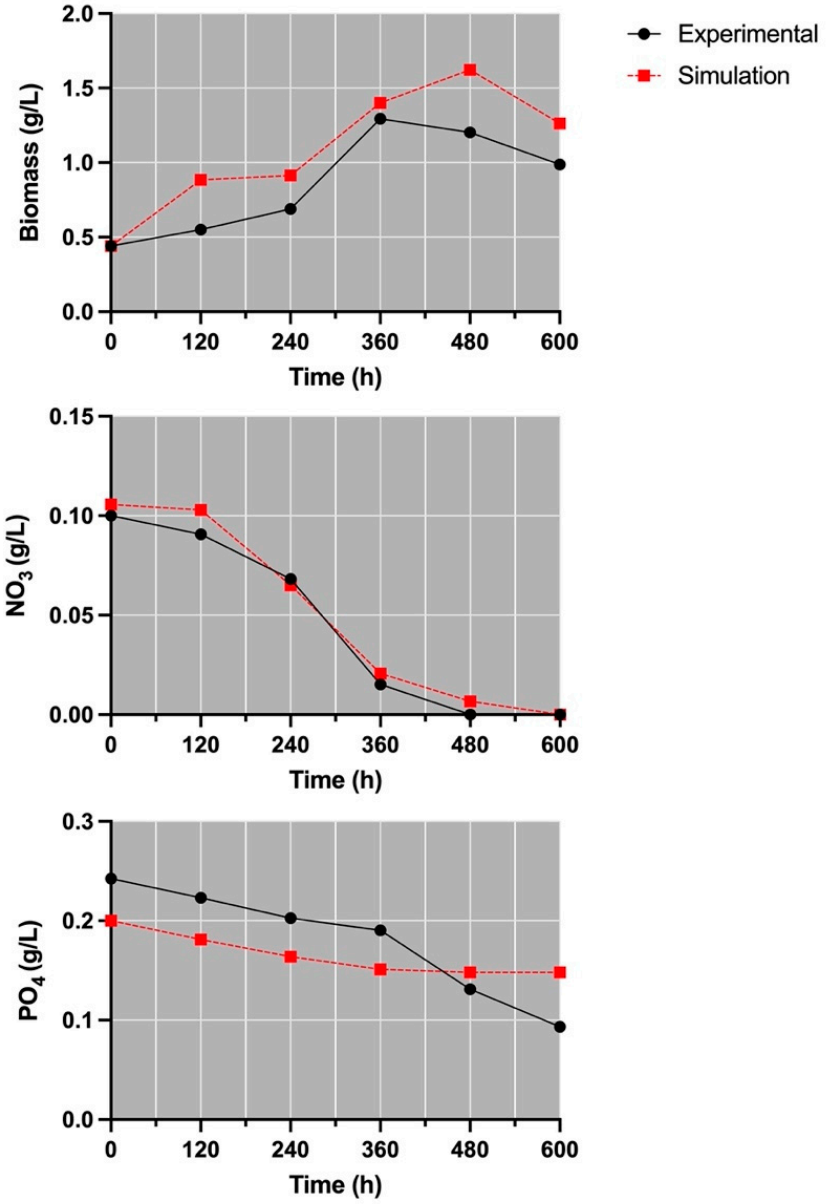


Figure 3. Graphical comparison between experimental and simulated data for biomass concentration and NO_3 and PO_4 uptakes by *Chlorella* sp.

3.2. Upstream

Figure 4 presents the upstream process of *Chlorella* sp.-biomass production using fish-farming wastewater supplemented with biofertilizer. This system consisted of preparing the culture medium, adaptation, scaling, and production of the microalgae in raceway reactors and has an annual production capacity of up to 180 m^3. The growth phase comprises seven reactors (four photobioreactors and three raceways) with residence times of 20 days and a final concentration of 0.8 g/L. This system was designed to operate in parallel to maintain a constant biomass production. Each reactor was inoculated using a concentration of 10% (v/v) of algae, except for the final product, which had an inoculum of 20% (v/v).

3.3. Downstream

Figure 5 shows the downstream process to produce pelletized biomass and live feed from *Chlorella* sp. Biomass harvesting is one of the critical points in microalgal production since this stage can consume up to 40% of the total production costs [36–39]. The live-feed process employs a solid-liquid separation system (centrifuge) that removes up to 40% of the total moisture. The concentrated biomass is then bottled (50 mL per unit) and distributed as feed for different types of fish requiring live phytoplankton diets. This process generates up to 64,697 bottles of feed every 20 days. For the dry-feed-production system, a centrifuge was used to remove up to 60% of the total humidity; the concentrated biomass passes through a fluidized bed dryer, allowing the relative humidity of the product to be reduced by up to 6%. Finally, the biomass is pelletized (1mg per pellet), reaching a final production of 5875 pellets per hour. To improve the impact on the water footprint of these processes, post-harvest water recirculation (highlighted in blue) was implemented for each system evaluated. This alternative allows a substantial reduction in production costs since not all nutrients are completely consumed [40–42].

3.4. Fixed Capital

The production costs (DFC) define the economic destiny of any production plant; they include the necessary expenses for the processing guidelines and functionality of each system involved [43]. Consequently, it defines the technical-economic feasibility of the process. Within the DFC, we can find the direct costs (TPDC), which refer to the acquisition, equipment, and functionality of the plant; the indirect costs (TPIC), which are the variables related to the construction; and, finally, the CFC, which are responsible for the safety and assurance of the project. Table 3 shows an increase in costs due to the inclusion of the medium recirculation. This increase is due to adding new equipment, which requires new spaces, materials, and trained personnel for its correct operation. In each process, other equipment is used, resulting in space, materials, and operational consumption demands.

Table 3. Fixed capital estimate for two scenarios of biomass production using *Chlorella* sp.

Fixed Capital Estimate	Pelletized Biomass		Live Feed	
	Normal	Optimized	Normal	Optimized
Total plant direct cost (TPDC) (physical cost)	118,639	128,955	102,577	116,059
Total plant indirect cost (TPIC)	75,373	81,928	71,523	73,735
Total plant cost (TPC = TPDC + TPIC)	194,012	210,883	174,100	189,794
Contractor's fee and contingency (CFC)	14,603	15,872	13,856	14,285
Direct fixed capital cost (DFC = TPC + CFC)	208,615	226,756	197,957	204,080

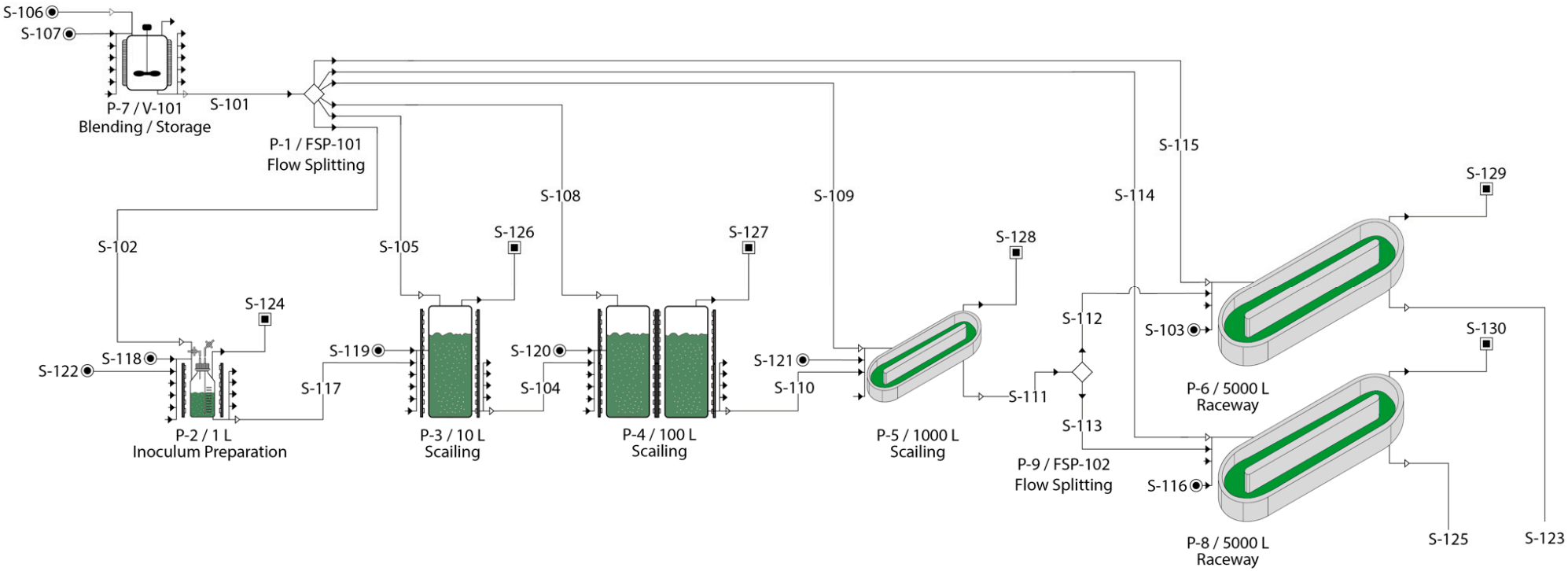


Figure 4. Flow diagram of the upstream-process production of *Chlorella* sp.

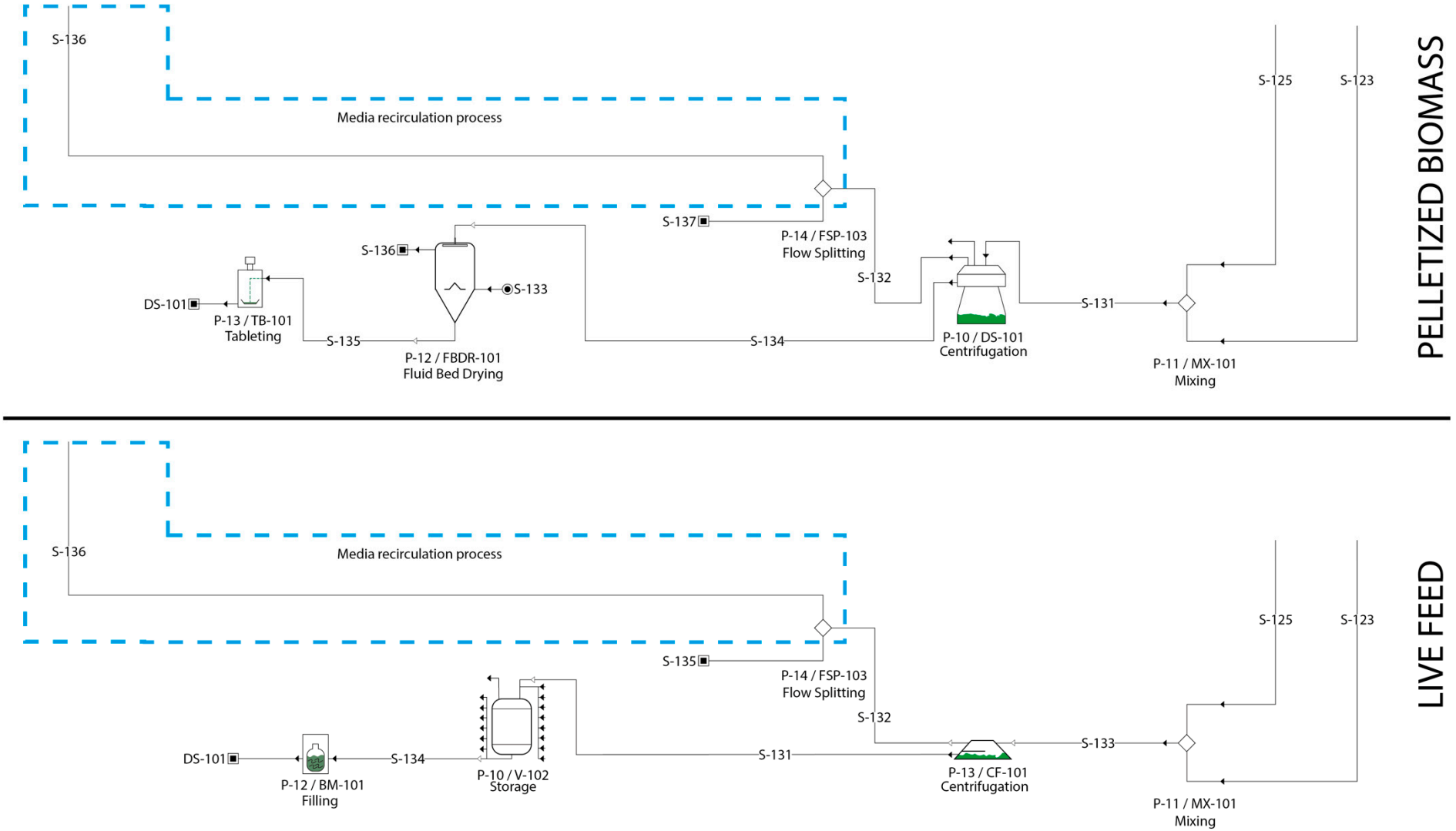


Figure 5. Flow diagram of the downstream process of pelletized biomass and live feed from *Chlorella* sp.

4. Discussion

The growth of the microalgae maintained a sigmoidal behavior typical of the implementation of kinetics with limited resources. The stationary phase took place between 0 and 120 h, at which time the cells had a process of adaptation to the available resources within the medium established in the experiment. Once this stage was completed, exponential growth began with approximately 360 h, reaching its maximum doubling rate of 1.2 g/L of biomass. Due to depletion of available nutrients and high cell density in the late exponential growth phase, the algal-growth rate reduced to a linear function, stabilizing at 0.8 g/L and reaching its stationary phase [32].

The application of simulations on industrial processes is an efficient solution for modeling and optimizing specific routes [44,45]. These techniques are based on predicting the behavior of the desired process through the calculation of the mass and energy balance of each section of the system [46]. By analyzing the different processes and their respective optimization, it is possible to develop new and better products that are economically competitive, as seen in Figure 6, where the optimization of the recirculation of the culture medium provides substantial improvement in its production of up to 20% for pelletized biomass and up to 80% for live feed. A significant result is a slight increase in TPDC for each process. This increase occurs because nutrients are still available in the culture medium, which decreases the production cost per cubic meter and improves the conversion rate of the nutrients present in the medium into usable biomass.

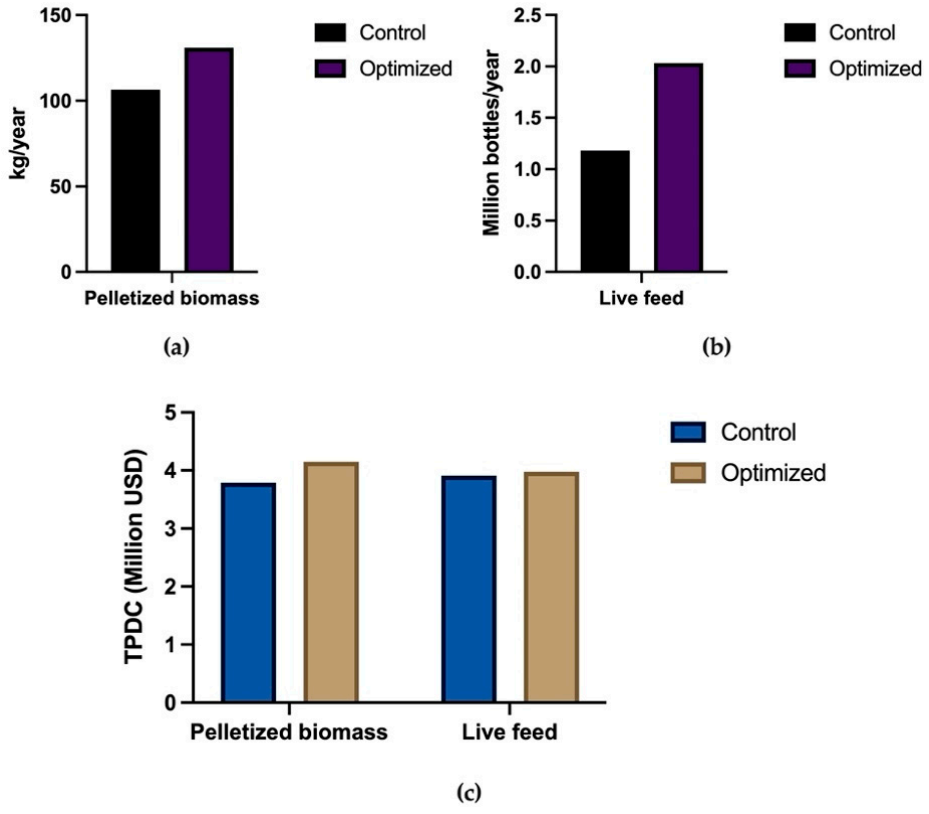
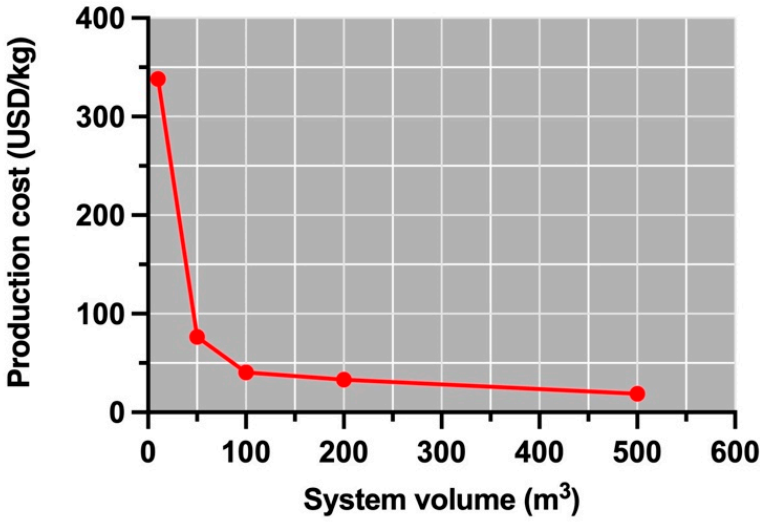


Figure 6. Biomass production for year-pelletized biomass (**a**) and live feed (**b**); total plant direct cost for two scenarios of biomass production (**c**).

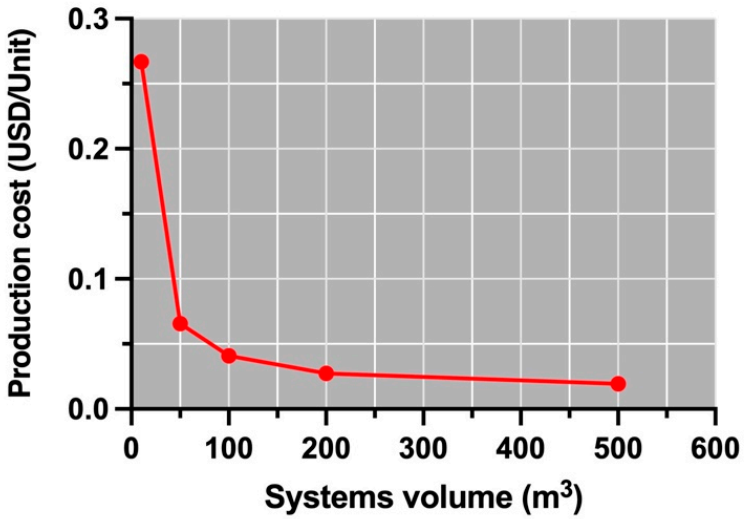
According to Ruiz et al. [47], the most critical variable that favors the profitability of products derived from microalgal biomass is the scale of the system, since increasing the capacity of the plant reduces the costs associated with production. Figure 7 summarizes the cost per kg/unit of biomass processed. Scaling defines the system's profitability for the two evaluated scenarios (pelletized biomass and live feed) using five different production

capacities (10, 50, 100, 200, and 500 m^3). The production cost was calculated according to Equation (5).

$$\text{Biomass cost} = \frac{\text{Production cost in USD}}{\text{Biomass production in kg}} \quad (5)$$



(**a**)



(**b**)

Figure 7. Production cost dependence on system volume for two scenarios of biomass production: (**a**) pelletized biomass and (**b**) live feed.

According to the results, in low scale systems (<50 m^3), the production costs are very high for pelletized-biomass- and live-feed-production systems (up to 338 USD/kg and 0.26 USD/unit, respectively) compared to those with operating volumes up to 500 m^3, of which biomass cost can be a fraction (18 USD/kg and 0.019 USD/unit, respectively). Finally, it is essential to highlight that other factors, such as pH, temperature, and light scattering in the reactor will affect the final productivity of the system [48–50].

5. Conclusions

The application of microalgae as a biotechnological tool for pollutant removal and water reuse in fish-farming systems is an essential strategy to increase the industrial sector's sustainability. According to the results of the SuperPro Designer software, by cultivating *Chlorella* sp. in fish-farming wastewater supplemented with N and P, it is possible to

produce up to 11,875 kg/yr (31.3 kg/d) with a production cost of up to 18 (USD/kg) for dry biomass and 0.19 (USD/bottle) for concentrated biomass. Similarly, it was possible to establish the kinetics of growth of substrate-dependent biomass with a maximum production of 1.25 g/L after 15 days and partial consumption of 98% of N and 20% of P. However, it is essential to note the final production efficiency may vary depending on uncontrollable variables, such as climate and quality of wastewater.

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