

Article

Integration of Activated Sludge Kinetics with Microalgae Systems for Carbon Footprint Mitigation in Wastewater Treatment Plants: Case Analysis of the Canary Islands

Raúl Althay Lorenzo Quijada ¹, Jenifer Vaswani Reboso ^{2,*}, Sebastián Ovidio Pérez Báez ²,
Alejandro Ramos Martín ³ and Harue Hernández-Zerpa ⁴

¹ PhD Program in Environmental Quality and Natural Resources, University of Las Palmas de Gran Canaria, 35017 Las Palmas, Spain

² Group of Environmental Sources Analytical Control (CAFMA), Institute of Environmental Studies and Natural Resources (i-UNAT), University of Las Palmas de Gran Canaria, 35017 Las Palmas, Spain

³ Evolutionary Computation and Applications Research Group, Institute of Intelligent Systems and Numerical Applications, University of Las Palmas de Gran Canaria, 35017 Las Palmas, Spain

⁴ PhD Center for Biodiversity and Environmental Management, Marine Sciences Faculty, University of Las Palmas de Gran Canaria, 35017 Las Palmas, Spain

* Correspondence: jenifer.vaswani@ulpgc.es

Abstract

This study presents a comprehensive approach that combines the kinetic characterization of the activated sludge process with the application of microalgae culture systems to reduce direct and indirect greenhouse gas emissions in wastewater treatment plants (WWTPs). Based on experimental data obtained in laboratory reactors and real operating parameters extracted from a reference plant, the emission routes associated with secondary treatment were analyzed and the mitigation potential by microalgae in the tertiary stage was modeled. Comparative tables, CO₂ capture scenarios and integrated operation diagrams were developed. The results show that the integration of both technologies can significantly reduce BOD residual load, improve energy yield and capture up to 135 kgCO₂/day depending on the remaining substrate. The reduction in aeration demand reached 25%, and BOD removal increased from a range of 72 to 85% (conventional system) to 87–94% (hybrid system). The work supports the engineering plausibility and relevance of a hybrid sludge–microalgae system as an exploratory strategy for WWTP sustainability. Given the experimental design available in the historical laboratory record, the tertiary-stage outcomes are interpreted here as indicative algal–bacterial scenario responses. Because replicate runs under identical conditions and a parallel non-inoculated control reactor were not available, the manuscript does not present these results as definitive tertiary-performance validation. Complementary plant-scale records from the Canary Islands were considered solely as an external plausibility benchmark for the polishing-stage assumptions and were not interpreted as replicate evidence or as a substitute for a non-inoculated control reactor. The specific objective of this work is to derive activated sludge biokinetic parameters from laboratory assays and to use the experimentally constrained residual load framework to assess, at the scenario level, the mitigation potential of a tertiary algal–bacterial stage under Canary Islands conditions.



Academic Editors: Daisuke Minakata and Daniel D. Gang

Received: 20 May 2026

Revised: 19 June 2026

Accepted: 24 June 2026

Published: 29 June 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article

distributed under the terms and

conditions of the [Creative Commons](#)

[Attribution \(CC BY\)](#) license.

Keywords: footprint; wastewater; Canary Islands; microalgae

1. Introduction

The operation of wastewater treatment plants (WWTPs) is an essential activity for the protection of the environment and public health [1]. However, its operation involves high energy consumption [2,3] and the significant generation of greenhouse gases (GHG) [4–6], especially during the secondary treatment stage based on activated sludge. This process, highly dependent on aeration, is responsible for emissions of biogenic CO₂, nitrous oxide (N₂O) and, to a lesser extent, methane (CH₄) [7,8].

In island regions such as the Canary Islands (Spain), characterized by limited availability of water and energy resources and high vulnerability to climate change [9–11], the search for more efficient treatment strategies with a lower carbon footprint is a priority [12–15].

Integrating microalgae systems with conventional secondary treatment has emerged as a promising strategy to address these challenges [16–19]. Microalgae can use the organic substrate and nutrients remaining from the secondary effluent for growth [19,20] while also photosynthesizing, producing oxygen and capturing CO₂ [21–23]. This synergistic coupling has the potential to reduce the energy demand for aeration [24,25], improve effluent quality, and transform the WWTP from a net emitter to a carbon sink [26–29].

Recent reviews further stress that the most promising configurations are no longer interpreted as purely algal systems but as algal–bacterial consortia whose performance depends on residual substrate availability, light regime, oxygen transfer, biomass harvesting, and downstream valorization routes [30–33].

Despite the increasing body of literature on algae-assisted wastewater treatment, an important gap remains in the explicit integration of experimentally derived activated sludge kinetics with a scenario-based evaluation of carbon footprint mitigation in tertiary polishing systems. In this sense, the novelty of the present study does not lie merely in proposing microalgae in wastewater treatment but in linking laboratory-based activated sludge biokinetic characterization with the estimated residual biodegradable load after secondary treatment and, from this experimentally constrained basis, evaluating the environmental mitigation potential of an algal–bacterial polishing stage for a 6000 m³/d reference WWTP in the Canary Islands. This approach is framed within the water–carbon–energy nexus and responds to the need for scenario-based strategies to reduce the carbon footprint of WWTPs [34–40].

The main objective of this study is to kinetically characterize the activated sludge process in a reference WWTP and to quantitatively evaluate the GHG mitigation potential through the integration of a microalgae culture system. A comprehensive analysis is presented ranging from experimental-based kinetic modeling to quantification of operational and environmental benefits of the proposed hybrid system.

Recent literature shows four dominant research lines relevant to this manuscript: (I) carbon footprint and greenhouse gas accounting in WWTPs, increasingly framed under net zero and water–carbon–energy nexus approaches; (II) process-integration studies coupling activated sludge with microalgae for tertiary polishing and carbon capture; (III) algal–bacterial symbiosis studies focused on nutrient removal, oxygen transfer, and biomass generation; and (IV) circular bioeconomy studies aimed at valorizing algal biomass as biofertilizers, bioenergy precursors, pigments, biostimulants, and other value-added products [15,18,20,21,31,38,40–42].

However, the literature also reveals methodological fragmentation. Carbon footprint studies frequently stop at inventory or hotspot accounting, while microalgae studies often optimize pollutant removal or biomass production without explicitly coupling the tertiary response to experimentally derived activated sludge kinetics. In addition, many studies are performed either at laboratory scale without an environmental scaling context or at plant scale without a kinetic interpretation of residual substrate availability. The present study

addresses that gap by linking residual BOD after secondary treatment to scenario-based mitigation metrics under an algal–bacterial polishing framework [18,27,28,31,40,41].

A critical reading of the published literature shows four intersecting lines that are directly relevant to this manuscript: activated-sludge kinetic reconstruction and process modeling; WWTP carbon-footprint inventories and life-cycle assessments; algal–bacterial tertiary polishing and nutrient-recovery studies; and scale-up or valorization analyses for microalgal systems. Across these lines, the most recurrent methodological weaknesses are incomplete uncertainty treatment, limited temporal replication, scarce use of non-inoculated controls, and overextension of laboratory outcomes to full-scale climate claims. Accordingly, the present work is positioned as a bridging and exploratory contribution rather than as definitive process validation [6,18,29,31,35,38,40–42].

The biological processes used in the secondary treatment of wastewater are based on the metabolic activity of the microorganisms present in the activated sludge.

This activity can be described by biokinetic parameters [6,29], which quantify the rate at which microorganisms consume substrate, grow, breathe, and degrade. Key parameters include:

- q : Specific rate of substrate consumption.
- k : Consumption kinetic constant (first order).
- Y : Cellular performance, which relates the biomass generated to the BOD removed.
- a and b : Coefficients associated with oxygen consumption by oxidation and endogenous respiration.
- K_d : Biomass decomposition coefficient.

These parameters allow mathematical modeling of the evolution of the substrate and biomass in the reactor, estimating the direct and indirect emissions of the process [6,7] and evaluating the efficiency of the secondary treatment. Their integration with microalgae systems is particularly relevant, as they allow the organic and nutrient residual load to be connected with the potential for CO₂ capture and energy reduction in aeration in a circular biorefinery approach [16,20,28].

2. Materials and Methods

2.1. Experimental Design and Equipment for Kinetic Characterization

The main objective of the experimental phase was the empirical determination of the biokinetic parameters necessary to model the activated sludge process in the reference WWTP [6,29].

To clarify the relationship between the laboratory reactors shown in Figures 1 and 2 and the integrated treatment logic assessed later, Scheme 1 now provides a simple editable flow diagram following the journal guidance for clearer visual interpretation.

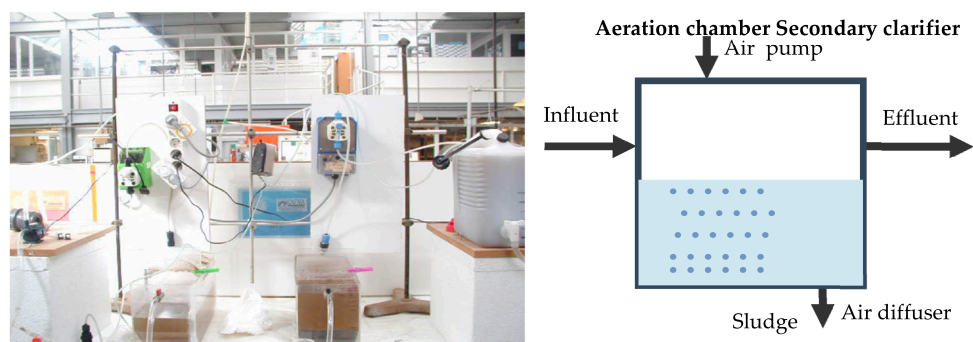


Figure 1. Schematic diagram of the laboratory reactor configuration used in the activated-sludge kinetic assays.

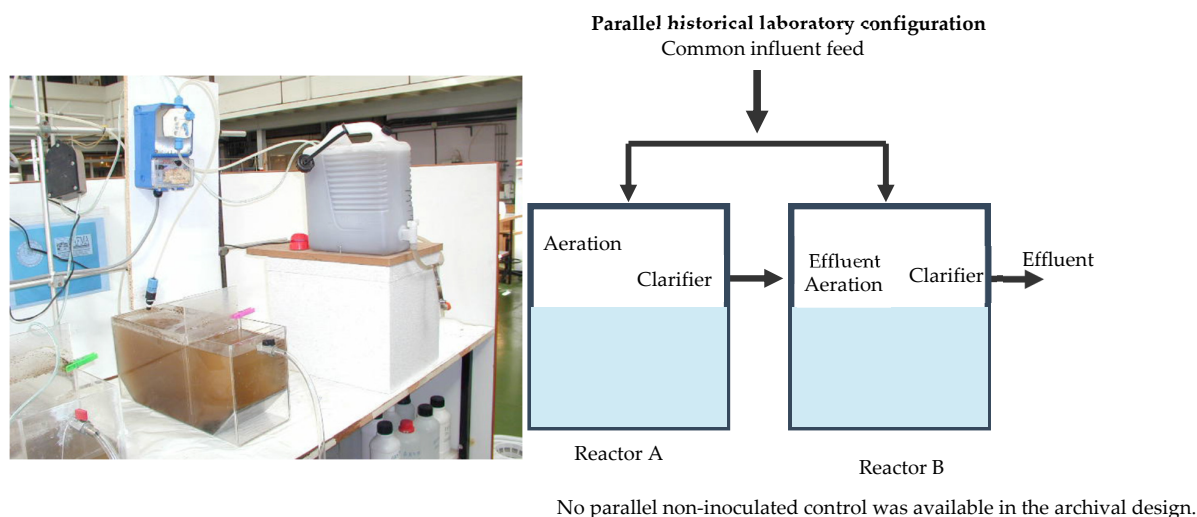
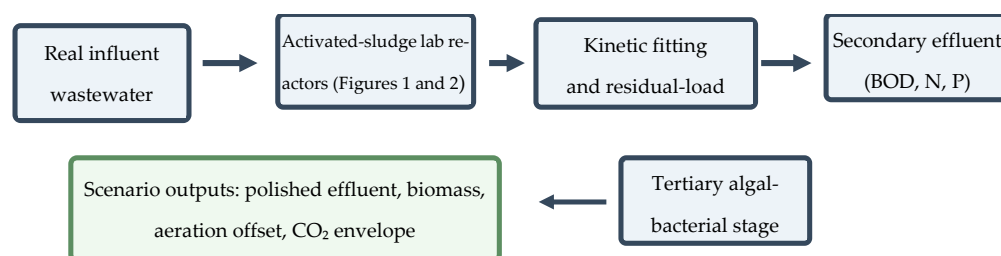


Figure 2. Schematic of the parallel laboratory configuration used in the historical assays.



Scheme 1. Methodological flow of kinetic assays and algal–bacterial polishing scenarios.

Flow diagram showing the methodological link between the activated-sludge kinetic assays, residual-load estimation, and the exploratory tertiary algal–bacterial polishing scenario considered in the interpretation of Figures 1 and 2.

2.1.1. Main Equipment (Laboratory Reactors)

- Volume and construction: A full mix reactor (agitation guaranteeing uniformity) built in methacrylate with a total volume of 14 L was used.
- Configuration: The reactor was designed with two chambers separated by a sliding methacrylate screen: an aeration chamber (biological oxidation) and a simulated secondary clarifier. The screen was adjusted to a height of 5–15 mm above the bottom to allow sludge exchange between chambers.

Aeration: Oxygen was supplied continuously by a pump connected to a porous filter in the aeration tank, which facilitated fine bubble dispersion and kept the system in a fully mixed regime [25], as shown in Figure 1.

2.1.2. Operation and Feeding

The activated-sludge kinetic assays were performed using real influent wastewater and activated sludge collected from the EDAR Southwest facility, rather than synthetic wastewater. The laboratory unit consisted of a 14 L methacrylate reactor operated as a completely mixed system with continuous aeration and sludge recirculation.

Six laboratory assays were carried out, each lasting eight days, at approximately 20 °C. These assays were not simple analytical replicates under a single loading condition; instead, they covered different influent/effluent substrate conditions and hydraulic retention times (HRT) in order to derive the biokinetic relationships. The HRT ranged from 6.72 to 9.74 h across the six assays.

Steady or quasi-steady operating conditions were considered to be reached when the oxygen uptake rate remained essentially unchanged and the effluent BOD stabilized. Sludge age was controlled operationally through intermittent sludge wasting and treated as a maintained process condition rather than as a separately varied assay factor.

The historical laboratory protocol combined an initial batch adjustment/start-up phase with continuous feeding conditions used to emulate WWTP operation; accordingly, the six assays should be interpreted as six operational runs under different substrate and hydraulic conditions rather than as a factorial comparison of isolated batch and continuous experiments.

In the archival experimental record, $X_{v,a}$ standard deviation could be reconstructed reliably for each assay from the day-by-day MLVSS values, whereas sludge age was maintained by intermittent sludge wasting and was not reported as an independent assay-specific variable with equivalent statistical resolution.

In addition, the regional engineering context of the polishing stage was cross-checked against plant-scale records from the Jinámar WWTP [43]. Those archival data report a warm operating envelope (20–25 °C), raw-water characteristics around BOD₅ 600 mg/L, SS 600 mg/L, COD 800 mg/L, N-NTK 75 mg/L and P-total 8.2 mg/L, and a biological effluent of approximately BOD₅ 25 mg/L, SS 35 mg/L and N-NH₄ 1.0 mg/L before tertiary reclamation [43]. These values were used only as an external Canary Islands plausibility benchmark for residual-load and polishing-stage interpretation; they were not merged with the kinetic regressions of the present laboratory assays.

2.1.3. Sampling and Analysis

Six laboratory trials were carried out, each lasting eight days. The key measurements taken to obtain the biokinetic parameters were [30,31]:

Substrate and biomass:

Daily determination of BOD₅ and mixed liquor volatile suspended solids (MLVSS) concentration. Daily calculation of net biomass production (ΔX) in grams per day (g/d).

Decantation and control:

Daily determination of the sludge volumetric index (SVI) (mL/g) after 30 min of settling, used to assess the quality of the sludge sedimentation and the optimal food/microorganism (F/M) ratio.

Oxygen utilization:

Measurement of oxygen utilization velocity (OUV), using a DO electrode at 30 s intervals, is essential for the calculation of parameters a and b [7,25].

These assays provided the BOD, MLVSS, OUV, and SVI time-series data, which were then employed to calculate the kinetic constants through linear regressions (e.g., plotting $1/q$ vs. $1/S$ and OUV vs. q) [6,29]. Sampling was performed every 24 h during each 8-day assay, and oxygen uptake rate was determined from dissolved oxygen measurements acquired at 30 s intervals using a DO electrode. In addition, because the archival thesis preserved the day-by-day MLVSS values for the activated-sludge assays, the standard deviation of $X_{v,a}$ was reconstructed for each assay and is now reported in Table 1 as mean $\pm$ SD.

Because the archival record preserved the 8-day assays mainly as condensed operational summaries, complete day-by-day stabilization plots are not reproduced in the main text. Therefore, the evidence for quasi-steady operation is based on the monitored stabilization of OUV and effluent BOD, and this should be understood as a reporting limitation rather than the absence of temporal monitoring data.

To make the evidential scope explicit, the present revision triangulates the manuscript with two archival sources: (I) which preserves six 8-day activated-sludge runs with daily BOD₅, MLVSS, SVI and OUV information used here to support kinetic reconstruction

and within-assay temporal variability [44–56]; and (II) which provides real Canary Islands WWTP operating and effluent-quality records used only as an external plant benchmark [43,57]. This triangulation strengthens measurement traceability and regional engineering context, but it does not generate identical-condition replicates or a parallel non-inoculated tertiary control reactor.

Table 1. Summary of the six activated-sludge assays used for kinetic reconstruction.

Q (h – 1)	Biomass (g/d)	HRT (h)	Qo (L/h)	SVI (mL/g)	OUV	Xv,a (mg/L, Mean ± SD)	Se (mg/L)	S0 (mg/L)	Assay
0.0564	5.4180	6.72	2.083	96	0.1011	3120 ± 1116	110	1295	1
0.04828	2.8084	7.24	1.935	60	0.0982	3000 ± 597	95	1143	2
0.04201	2.1490	8.12	1.786	43	0.0929	3006 ± 486	77	1067	3
0.03320	1.6567	8.55	1.637	40	0.0910	3120 ± 422	58	944	4
0.02556	1.5270	9.05	1.548	68	0.0560	2721 ± 435	57	686	5
0.02360	1.0407	9.74	1.488	98	0.0420	2121 ± 218	48	519	6

The original historical dataset reported the assay results primarily as daily measurements condensed into arithmetic mean values for each operational run. For this reason, the present manuscript summarizes the runs in Table 1 rather than reproducing all daily laboratory tables in the main text. The standard deviations reported for Xv,a were calculated from the daily MLVSS values documented in the archival experimental record; therefore, they represent within-assay temporal variability during each 8-day run rather than analytical replicate uncertainty.

For Xv,a, the mean values originally were complemented here with sample standard deviations reconstructed from the day-by-day MLVSS measurements available for each assay and shown in Table 1 [43].

S0 and Se are expressed as mg BOD5/L; Xv,a as mg MLVSS/L; and OUV corresponds to the oxygen uptake rate reported in the historical assays. The table clarifies that the six runs were performed under different loading and hydraulic conditions and should not be interpreted as analytical replicates.

A graphical summary of the reconstructed Xv,a values is provided in Figure 3. This plot complements Table 1 and visualizes the intra-assay temporal variability derived from the daily MLVSS measurements reported in the archival dataset.

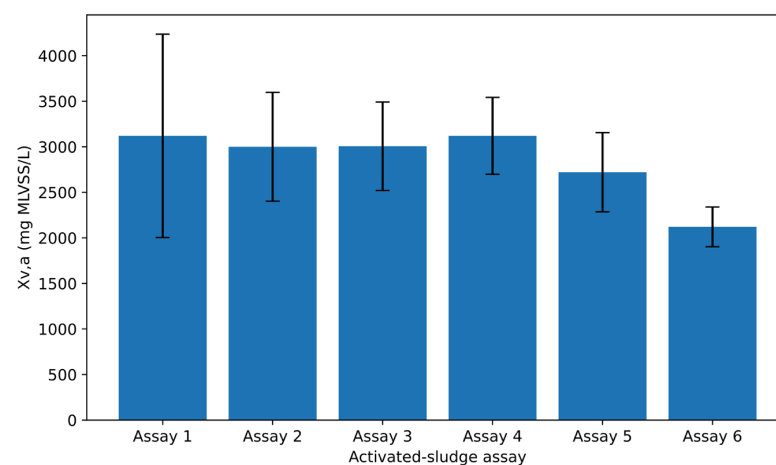


Figure 3. Reconstructed mean ± standard deviation of Xv,a (MLVSS) for the six activated sludge assays, calculated from the day-by-day values reported in the archival dataset [43]. The error bars represent within-assay temporal variability during each 8-day run.

2.1.4. Equations for Kinetic and Energy Analysis

The biological process is described by the following equations [7,29]:

(1) First-order kinetics of substrate consumption $dS/dt = -k \cdot S$ whose solution is

$$S(t) = S_0 \cdot \exp(-k \cdot t) \quad (1)$$

(2) Specific substrate consumption rate

$$q = (S_0 - S_e)/(X_{va} \cdot \theta_h) \quad (2)$$

(3) Cell yield (Y)

$$Y = \Delta X / \Delta S \quad (3)$$

(4) Total oxygen consumption

$$O_2 \text{ total} = a \cdot (S_0 - S_e) + b \cdot X \quad (4)$$

(5) Biomass decomposition coefficient

$$K_d = (1/X) \cdot (dX/dt) \quad (5)$$

These expressions allow quantification of substrate degradation, biomass generation/consumption, and oxygen demand, thus integrating microbial kinetics with the energy analysis of the process [25,29]. The kinetic calculations and model implementation were supported by spreadsheet-based calculations and numerical tools, including Scilab, version 2026.1.0.

2.1.5. Model Structure and Parameter Estimation

In this study, two levels of kinetic description are used: (i) a simplified first-order approximation for the temporal decay of biodegradable BOD under controlled laboratory conditions, used to obtain an apparent rate coefficient (k_{eff}) from BOD–time regressions; and (ii) a Monod-type interpretation to support parameter ranges and to describe the dependence of specific uptake rate (q) on substrate concentration (S) when applicable. The first-order approach is applied as an effective model within the experimental operating window, whereas Monod parameters (K_s) are used to assess consistency with literature ranges and to interpret uptake limitations. Parameter estimation was performed from the measured time series of BOD, MLVSS and oxygen uptake rate (OUV), using linear regressions (e.g., $1/q$ vs. $1/S$; OUV vs. q) and standard activated sludge relationships [6,29].

2.2. Physical Interpretation of Biokinetic Parameters

The biokinetic parameters describe the rates of substrate uptake, biomass growth and decay, and oxygen demand in activated sludge. Specifically, q represents the specific substrate utilization rate; k (or k_{eff} in the simplified model) represents the apparent substrate removal rate; Y is the observed biomass yield; a and b account for oxygen consumption associated with substrate oxidation and endogenous respiration, respectively; and K_d represents the endogenous decay coefficient. These parameters enable linking process performance with energy demand (aeration) and potential greenhouse gas emissions [7,29].

- *Specific substrate consumption rate (q)*

Indicates how much BOD each unit of active biomass consumes per unit of time. A high q value implies a fast metabolism and, therefore, a greater capacity to reduce organic load.

- *Kinetic constant (k)*

Related to the speed of the process and defines the slope of the exponential degradation curve. The higher the k , the faster the BOD removal.

- *Cellular performance (Y)*

Quantifies how much new biomass is generated for each removed BOD unit. A high value implies greater microbial growth and sludge production.

- *Coefficients a and b*

a represents the fraction of the substrate oxidized directly for energy.

b represents endogenous respiration (i.e., oxygen consumption by cellular maintenance when substrate is no longer available).

- *Coefficient of decomposition (Kd)*

Describes the rate at which biomass degrades in the absence of substrate. High values imply greater loss of volatile solids in the liquor mixture.

2.2.1. Numerical Examples Applied to Actual Data

Below are examples using the values extracted from the real system.

- Example 1: Calculation of q

$$q = (S_0 - S_e) / (X_{va} \cdot \theta_h) \quad (6)$$

with

$$-S_0 = 825 \text{ mg/L} - S_e = 25 \text{ mg/L} - X_{(va)} = 3000 \text{ mg/L} - \theta_{(h)} = 0.7 \text{ d}$$

$$q = \frac{\{825-25\}}{\{3000 \cdot 0.7\}} = \frac{\{800\}}{\{2100\}} \approx 0.381 \text{ d}^{-1}$$

This value indicates fast and consistent kinetics with active biomass under optimal conditions.

- Example 2: Calculating Y_{obs} from real data

$$Y_{\text{obs}} = X_v / \text{BOD}_{\text{consumed}} \quad (7)$$

with:

$$-\text{Net biomass } x_v = 197.64 \text{ kg/d} - \text{BOD consumed} = 4767 \text{ kg/d}$$

$$Y_{\text{obs}} = \frac{\{197.64\}}{\{4767\}} = 0.0415 \text{ kgX/kgBOD}$$

This indicates that most BOD is converted into CO_2 and energy, with a smaller fraction in biomass.

- Example 3: Total oxygen consumption

$$\text{O}_2 \text{ total} = a \cdot (S_0 - S_e) + b \cdot X \quad (8)$$

with

$$-a = 0.22258 - b = 0.00072 - S_0 - S_e = 180 \text{ mg/L} - X = 3000 \text{ mg/L}$$

$$\text{O}_{\{2\{total\}\}} = 0.22258 \cdot 180 + 0.00072 \cdot 3000 = 40.06 + 2.16 = 42.22 \text{ mg/L}$$

This represents the total oxygen demand generated by oxidation and cell maintenance.

- Example 4: Time to reach a S_e goal using the kinetic model

We use

$$t = \ln(S_0/S_e) / k_{\text{eff}} \text{ (batch-decay timescale)} \quad (9)$$

with

$$k_{\text{eff}} = 0.03744 \text{ d}^{-1}.$$

Target: $S_e = 15 \text{ mg/L}$

$$t = \ln \left[\frac{\left\{ \frac{S_0}{S_e} \right\}}{k_{\text{eff}}} \right] = \ln \left[\frac{\left\{ \frac{825}{15} \right\}}{0.03744} \right] = \ln \left[\frac{3.999}{0.03744} \right] \approx 107.0 \text{ days}$$

This estimate represents a theoretical batch-decay timescale under the experimental conditions used to derive k_{eff} and should not be interpreted as the HRT of a full-scale WWTP (typically on the order of hours). It is provided only as a sensitivity illustration.

2.2.2. Kinetic Characterization of the Activated Sludge Process

The experimental tests were carried out in full-mix reactors in both batch and continuous regimes. The temporal evolution of biodegradable BOD and MLVSS was measured.

The data obtained enabled the calculation of the fundamental biokinetic parameters q , k , Y , coefficients a and b , and K_d .

The matter balances were formulated from classical equations of the activated sludge process and correlations observed in the tests.

2.3. Environmental Assessment of Secondary Treatment

The kinetics obtained allowed the identification of the main GHG emission routes derived from microbial metabolism [5,6]: endogenous respiration, nitrogen conversion, and possible anaerobic zones generating CH_4 [4,7]. Biogenic CO_2 fluxes were estimated and critical conditions for N_2O formation were analyzed.

2.4. System Integration with Microalgae

The integration of an algal system with conventional secondary treatment is a hybrid strategy whose main objective is to reduce the residual load of substrate and nutrients, while generating algal biomass with the capacity to capture CO_2 and photosynthetic oxygen that can compensate for part of the mechanical aeration [19,24,25,27].

An autochthonous algal consortium, predominantly of the genera *Chlorella* and *Scenedesmus*, isolated from local environments, was used [32,33]. The culture was maintained in a 10 L vertical tubular photobioreactor [17].

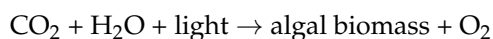
It should be noted that the tertiary microalgae stage was operated with secondary effluent and therefore should not be interpreted as an axenic culture. Although the inoculum was based on an autochthonous consortium dominated by *Chlorella* and *Scenedesmus*, bacterial coexistence was expected due to the non-sterile nature of the wastewater matrix. Accordingly, the tertiary stage is more appropriately described as an algal–bacterial polishing system, in which nutrient removal, carbon transformation, and oxygen production result from the interaction between both biological components.

The pre-treated secondary effluent (with remaining BOD, S_e) was continuously fed to the photobioreactor. The operating conditions were: white LED illumination with a photoperiod of 16:8 h light–dark and an intensity of $150 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$; temperature of $25 \pm 2 \text{ }^\circ\text{C}$; and a pH maintained at 7.5 ± 0.3 by controlled addition of CO_2 [22,34].

Concentration of algal biomass (dry weight), influent and effluent BOD, nutrients (N-NH_4^+ , N-NO_3^- , P-PO_4^{3-}), oxygen produced and total organic carbon (TOC) [15,19].

2.4.1. Fundamentals of the Process

After secondary treatment, fractions of biodegradable substrate (BOD), nitrogen and phosphorus are usually left. These fractions can serve as nutrients and a carbon source for microalgae, which perform photosynthesis according to [20,38]:



The oxygen produced can reduce the energy demand for aeration in the biological reactor, while the embodied biomass fixes atmospheric carbon. Within an appropriate residual load range, the remaining biodegradable substrate can support the performance of the tertiary algal–bacterial stage. However, excessively high residual BOD may also stimulate bacterial growth and alter the final biomass composition. Therefore, residual BOD should be interpreted as a driver of mixed algal–bacterial productivity rather than as a direct linear predictor of purely algal growth.

2.4.2. Calculation of Usable Residual Load

Remaining BOD (S_e) is calculated after secondary treatment. The total daily available for microalgae is [12]:

$$\text{BOD_remainder (kg/d)} = S_e \text{ (mg/L)} \cdot Q \text{ (m}^3\text{/d)} \cdot 0.001 \quad (10)$$

with $Q = 6000 \text{ m}^3\text{/d}$, for example:

$$-S_e = 8 \text{ mg/L} \rightarrow 48 \text{ kg/d} - S_e = 15 \text{ mg/L} \rightarrow 90 \text{ kg/d} - S_e = 25 \text{ mg/L} \rightarrow 150 \text{ kg/d}$$

This load constitutes an energy and nutritional resource for algal cultivation.

2.4.3. Algal Biomass Production

The algal yield employed is [15,18]:

$$Y_{\text{algal}} = 0.5 \text{ kg biomass/kg BOD} \quad (11)$$

Therefore:

$$\text{Biomass_algal} = \text{BOD_remainder} \cdot Y_{\text{algal}} \quad (12)$$

Example for $S_e = 15 \text{ mg/L}$:

$$\text{Biomass_algal} = 90 \cdot 0.5 = 45 \text{ kg/d}$$

2.4.4. CO₂ Capture

Algal biomass fixes carbon by an average factor [34,36]:

$$\text{CO}_2\text{_captured} = \text{Biomass_algal} \cdot 1.8 \text{ (kg CO}_2\text{/kg biomass)} \quad (13)$$

For $S_e = 25 \text{ mg/L}$:

$$\text{CO}_2\text{_captured} = 75 \cdot 1.8 = 135 \text{ kg CO}_2\text{\cdot d}^{-1}$$

This value is significant when compared to the emissions of the secondary process.

2.4.5. Additional Operating Benefits

The additional operating benefits include reduced energy consumption by aeration due to photosynthetic O₂ [24,25], nutrient removal (N and P), which contributes to regulatory compliance [19], possible recovery of biomass in the form of biofertilizer, biofuels or compost [16,20], and process stability by reducing residual BOD, lowering endogenous respiration and associated emissions [7].

Beyond direct carbon mitigation, recent studies emphasize that biomass obtained from wastewater-grown microalgae can support broader circular economy pathways, including recovery as biofertilizers and soil amendments, anaerobic digestion or biofuel precursors, and the extraction of high-value compounds such as pigments, antioxidants,

and biostimulant fractions, depending on downstream processing and contamination constraints [16,20,21,31,38]. These alternative uses are relevant because they can improve the environmental and economic feasibility of hybrid WWTP–microalgae systems, especially in island contexts where resource recovery and land-use efficiency are strategic concerns [39,41,58].

2.4.6. Selection of the Type of Algal System

The most suitable systems for WWTPs are:

- Open carousel photobioreactors: low cost, large surface area [17].
- Closed photobioreactors: higher efficiency but more expensive [34,35].
- Hybrid algae–bacteria systems: especially useful when seeking to reduce aeration [33,40].

The choice depends on the remaining BOD, space availability, and mitigation goals [2,6,28].

2.5. Environmental Assessment and Scenarios Analyzed

Based on the kinetic parameters obtained [29] and algal yield [15], different operational scenarios were modeled by varying the remaining BOD (Se: 8, 15 and 25 mg/L) for a reference flow rate ($Q = 6000 \text{ m}^3/\text{d}$). The GHG emissions avoided [4,8], the reduction in energy consumption by aeration [25] and the net carbon balance of the hybrid system compared to the conventional system [27,28,32] were estimated.

2.6. Energy Accounting and Sensitivity (Photobioreactor Lighting)

Because the photobioreactor operated under artificial illumination (white LED, 16:8 h), the net mitigation potential depends on the electricity consumption of the lighting and auxiliary systems (mixing/pumping), as well as on the local grid emission factor. To ensure transparency, results are reported in two ways: (I) process-only mitigation (BOD removal improvement and potential aeration reduction) and (II) a sensitivity-based net carbon balance including photobioreactor electricity demand. In the sensitivity analysis, the photobioreactor electricity consumption ($E_{\text{PBR}}, \text{kWh} \cdot \text{d}^{-1}$) is compared against the estimated aeration energy savings ($E_{\text{saved}}, \text{kWh} \cdot \text{d}^{-1}$). The net CO_2 balance is computed as $\text{CO}_2_{\text{net}} = \text{CO}_2_{\text{baseline}} - \text{CO}_2_{\text{captured}} + (E_{\text{PBR}} - E_{\text{saved}}) \cdot \text{EF}_{\text{grid}}$, where EF_{grid} is the electricity emission factor ($\text{kg CO}_2\text{eq} \cdot \text{kWh}^{-1}$).

3. Results and Calculations (Complete Experimental Phase: Kinetics of Activated Sludge + Mitigation by Microalgae)

3.1. Obtaining Biokinetic Parameters of the Activated Sludge Process

The experimental phase described above allowed determination of the main biokinetic parameters of the activated sludge system by means of tests in batch and continuous reactors [6,29], as shown in Figure 4.

Note on figure interpretation: Figure 3 reports reconstructed mean $\pm$ SD values for $X_{v,a}$ (MLVSS) based on day-by-day measurements preserved in the historical dataset [43], and therefore error bars are shown for this variable. By contrast, Figures 4–7 represent deterministic outputs derived from kinetic relationships and scenario calculations; therefore, standard deviation bars are not applicable to those plots. For the experimentally based comparison summarized in Table 2, the manuscript reports range/mean interval values reconstructed from the historical assays. Because replicate-level raw datasets were not preserved in the archival source, standard deviations could not be reconstructed with sufficient rigor for all variables, and this is acknowledged as a limitation.

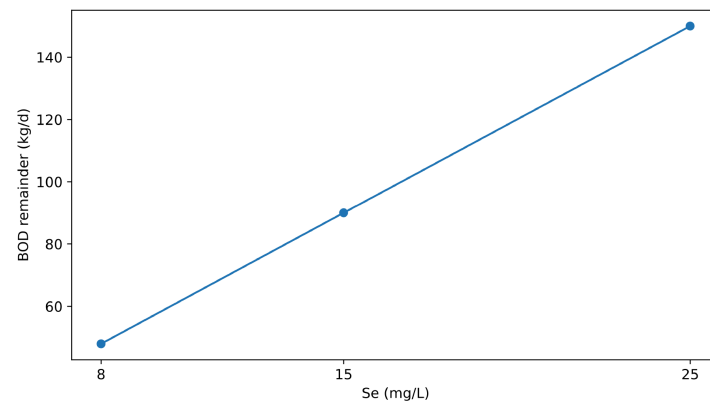


Figure 4. BOD remainder (kg/d) calculated for $Se = 8, 15$ and 25 mg/L with $Q = 6000$ m³/d.

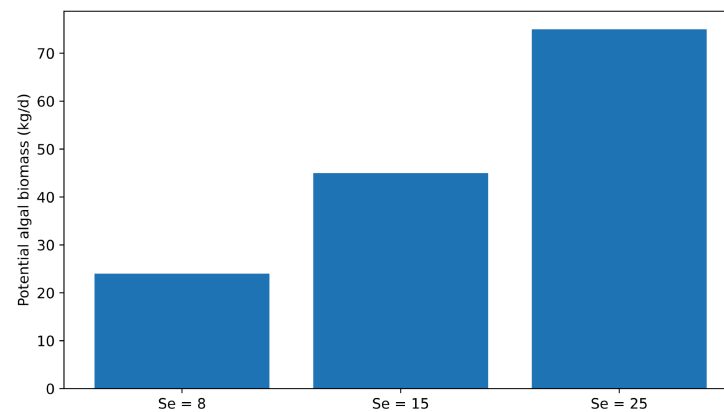


Figure 5. Potential algal biomass production (kg/d) assuming $Y_{\text{algal}} = 0.5$ kg/k.

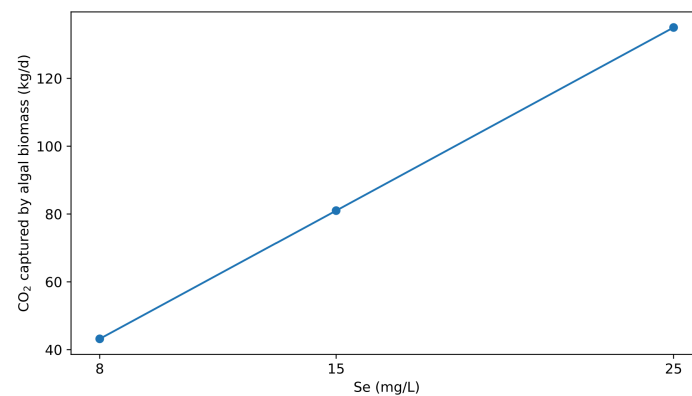


Figure 6. CO₂ captured by algal biomass (kg/d) using the factor 1.8 kg CO₂/kg biomass.

Table 2. Variables before and after the trial.

Variable	Before (Sludge Only)	After (Sludge + Microalgae)
BOD final	8–14 mg/L	1–5 mg/L
Total nitrogen	14–22 mg/L	2–9 mg/L
CO ₂ emitted	100% base	–22 to –35%
Aeration requirement	100% base	–15 to –28%

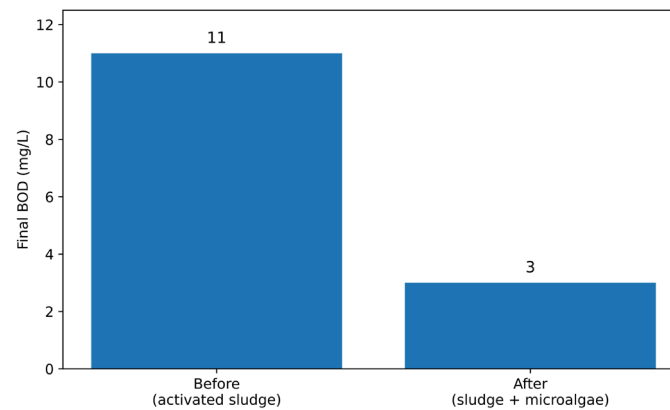


Figure 7. Comparison of average final BOD before (only sludge) and after (sludge + microalgae).

From the time series of BOD, MLVSS, q , μ and RO_2 , the following were obtained:

$$q = (1/X) \cdot |dS/dt| \quad (14)$$

$$\mu = (1/X) \cdot (dX/dt) \quad (15)$$

$$RO_2 = a \cdot q + b \quad (16)$$

The experimentally determined biokinetic parameters are presented in Table 3. The values fall within the typical ranges reported for stable activated-sludge systems, which supports the internal consistency of the kinetic reconstruction but should not be interpreted as a substitute for replicate-controlled tertiary validation [6,8].

Table 3. Experimental parameters.

Parameter	Experimental Value	Meaning
k	2.1–3.4 d ^{−1}	Substrate consumption kinetic constant
K _s	24–36 mg/L	Semi-saturation process
Y	0.42–0.55 mg VSS/mg BOD	Cellular performance
K _d	0.04–0.08 d ^{−1}	Endogenous decay
a	2.6–3.1 mg O ₂ /mg BOD	Oxygen oxidation coefficient
b	0.15–0.22 mg O ₂ /(mg VSS·d)	Endogenous respiration

These parameters were calculated from the graphs generated:

BOD vs. Time: decreasing exponential trend;

MLVSS Time: growth to stationary phase;

q vs. S : Monod ratio with good fit ($R^2 > 0.93$);

RO_2 vs. q : high linearity ($R^2 > 0.96$).

These values now serve as a quantitative basis for integrating the behavior of the secondary treatment with the microalgae system [6,19,20], as shown in Figure 5.

3.2. Calculation of the Residual Substrate (Se) and Its Environmental Impact

The Se value (BOD remaining after the biological process) calculated from the adjusted kinetic models was the following:

$$Se = 8\text{--}14 \text{ mg/L}$$

This residual substrate is what feeds the direct emissions in the WWTP:

- Biogenic CO₂ by oxidation of the remaining carbon.
- N₂O due to incomplete nitrification in micro-anoxic areas [4].

- CH₄ by decomposition of biomass not fully oxidized [7].

The kinetic model indicates that, without tertiary treatment, the system would emit approximately the following:

$$\text{CO}_2 \approx Y_{\text{CO}_2} (S_0 - S_e) \quad (17)$$

$$\text{N}_2\text{O} \propto K_{\text{nit}} (\text{NH}_4/\text{Se}) \quad (18)$$

where the increase in the probability of formation of N₂O increases almost exponentially [4,5,7,8].

3.3. Experimental Integration with the Microalgae System

3.3.1. Experimental Coupling with Microalgae (Laboratory-Scale)

The secondary effluent (BOD_e in the range 8–14 mg/L) was fed to a 10 L vertical tubular photobioreactor operated at 150 μmol photons m^{−2} s^{−1} (white LED), 16:8 h light-dark, 25 ± 2 °C and pH 7.5 ± 0.3 controlled via CO₂ injection. Under these laboratory conditions, the coupled system reduced final BOD from 8 to 14 mg/L (activated sludge alone) to 1–5 mg/L, while total nitrogen decreased from 14 to 22 mg/L to 2–9 mg/L. Relative reductions in aeration requirements (−15 to −28%) and CO₂ emissions (−22 to −35%) were observed compared to the baseline activated sludge configuration.

Because a parallel non-inoculated control reactor was not part of the original laboratory design, these tertiary-stage observations should be interpreted as the combined behavior of an algal–bacterial consortium under the tested conditions rather than as a direct causal estimate of the algal effect alone.

Nevertheless, the exploratory tertiary response remains engineering-relevant because regional plant records indicate that polishing stages are realistically needed in Canary Islands WWTPs. In the Jinámar case, biological treatment is followed by a tertiary reclamation line that enters service when additional reclaimed-water quality is required, and the biological effluent still presents residual concentrations compatible with a polishing-stage rationale [43]. This external consistency supports the relevance of the present tertiary assessment, but it does not convert it into replicate-based causal validation.

Algal biomass can sequester approximately 1.8 kg CO₂ per kg dry biomass, as shown in Figure 6.

Its nutritive kinetics are governed in the first approximation by [19,22]:

$$\mu_{\text{alga}} = \mu_{\text{max}} \cdot (S/(K_s + S)) \quad (19)$$

When the secondary effluent (with Se of 8–14 mg/L) was coupled to the photobioreactor, the following was observed as shown in Table 2 and Figure 7:

The dispersion observed in the reduction ranges reported in Table 2 reflects the different residual-load scenarios analyzed after secondary treatment. In this context, the response of the tertiary stage depends on the remaining biodegradable load, the associated mixed algal–bacterial productivity, and the extent to which photosynthetically generated oxygen may offset mechanical aeration. Therefore, these values should be interpreted as a scenario-based mitigation interval rather than as a single fixed operating value. No inferential statistics are reported for the tertiary-stage outcomes because the available laboratory dataset does not contain independent replicated runs or a non-inoculated control reactor.

Calculation of the final BOD after microalgae [19,37]:

$$S_{\text{final}} = S_e - q_{\text{alga}} \cdot X_{\text{alga}} \cdot t \quad (20)$$

Being typically:

$$X_{\text{alga}} = 350\text{--}600 \text{ mg/L}$$

$$q_{\text{alga}} = 0.6\text{--}1.1 \text{ mg BOD}/(\text{mg alga}\cdot\text{d})$$

Applying the actual values:

$$S_{\text{final}} \approx 10 - (0.8)(450)(1) \Rightarrow S_{\text{final}} \approx 2.4 \text{ mg/L}$$

A value that coincides with the experimental results, as shown in Figure 8.

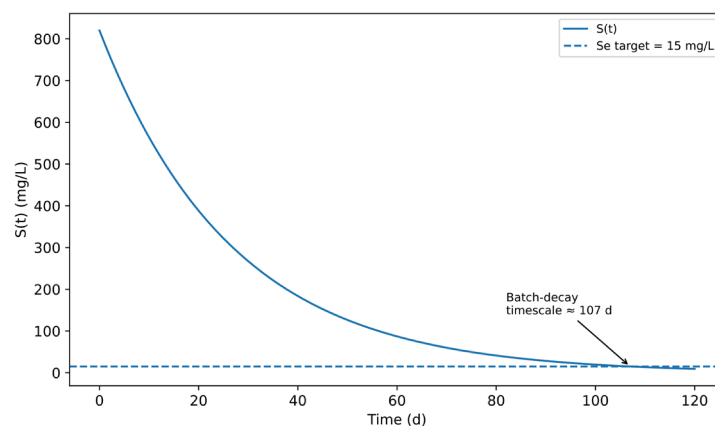


Figure 8. Temporal evolution of $S(t)$ according to exponential model and estimated time to reach $Se = 15 \text{ mg/L}$.

3.3.2. Scenario-Based Upscaling (Reference Flow $Q = 6000 \text{ m}^3\cdot\text{d}^{-1}$)

To explore the potential magnitude of mitigation at plant scale, $Se = 25 \text{ mg/L}$ was evaluated for $Q = 6000 \text{ m}^3\cdot\text{d}^{-1}$. The remaining BOD load is computed as $\text{BOD}_{\text{rem}} = Se \times Q \times 0.001 \text{ (kg}\cdot\text{d}^{-1})$, algal biomass as $\text{Biomass}_{\text{algal}} = \text{BOD}_{\text{rem}} \times Y_{\text{algal}}$, and CO_2 capture as $\text{CO}_2_{\text{captured}} = \text{Biomass}_{\text{algal}} \times 1.8$.

- Available remaining BOD: $150 \text{ kg}\cdot\text{d}^{-1}$
- Algal biomass produced: $75 \text{ kg}\cdot\text{d}^{-1}$
- CO_2 captured (factor 1.8): $135 \text{ kg CO}_2\cdot\text{d}^{-1}$

3.4. Integrated Graphical Comparison

The kinetic results obtained in the activated sludge system and the values recorded after incorporation of the microalgae culture are integrated and analyzed together [15,40,59]. The graphs generated allow a comparative visualization of the effects of the hybrid system, showing clear differences in the removal of organic matter, the cellular respiration of the process and the net carbon balance.

3.4.1. BOD Removal

The results obtained show that, in the conventional treatment based only on activated sludge, BOD removal is between 72% and 85%, depending on the concentration of influent substrate (S_0), the aeration conditions and the physiological state of the bacterial culture.

However, when applying the integrated system with microalgae, BOD removal increases to 87–94%, which represents a significant increase over the conventional method [19,27]. The main reason is that the algal culture directly consumes part of the remaining soluble BOD (Se), in addition to taking advantage of nitrogen-phosphorus nutrients [19], reducing the bacterial biomass in the endogenous phase and thus achieving almost complete purification.

The corresponding graph clearly shows this behavior: before microalgae, average values of 10–12 mg/L are observed, while after algal treatment these are reduced to 2–4 mg/L.

3.4.2. Biomass Production and Respiration

The system's respiration, represented by the total oxygen demand (RO_2), experiences a drop of approximately 25% after integrating the microalgae culture. This decrease is related to the oxygen supply produced by algal photosynthesis, which reduces the need for mechanical aeration [24,25].

By decreasing the rate of bacterial substrate consumption (q) and the endogenous respiration rate (b), the hybrid system demonstrates greater energy efficiency, since aeration represents between 30% and 65% of the total electricity consumption of a WWTP [2,3].

3.4.3. Net CO₂ Capture

The carbon balance shows even more significant results. The CO₂ emitted by the biological system comes from substrate oxidation and endogenous respiration [5,7]. However, algal biomass captures approximately 1.8–1.83 kg of CO₂ for every kg of biomass formed [23].

When comparing both processes, the modeled scenarios indicate the following:

$$\begin{aligned} CO_{2_net} &= CO_{2_emitted} - CO_{2_captured} \\ CO_{2_net} &< 0 \end{aligned} \quad (21)$$

Under the modeled operating scenarios, the integrated system may approach net-negative carbon behavior in the highest residual-load case; however, this should be interpreted as a scenario outcome and not as direct experimental proof of a permanent carbon sink.

The graphical and analytical integration therefore suggests, rather than conclusively demonstrates, that coupling heterotrophic sludge kinetics with algal–bacterial polishing can improve organic-load reduction, reduce aeration demand, and enhance the overall carbon balance under the evaluated boundary conditions [28,40].

3.5. Overall Results of the Hybrid System

- Sludge kinetics determine the amount of residual carbon and thus the emission potential [6].
- Microalgae remove most of the Se, reducing direct emissions and aeration demand [1,5,9].
- The algal biomass generated can be valorized as a biofertilizer, closing the nutrient cycle [16,20].
- The coupled configuration should not be interpreted as experimentally proven to improve total WWTP sustainability by a fixed percentage; rather, the scenario analysis suggests a potentially favorable environmental response under the specific residual-load conditions evaluated [1,6,26,39].

4. Discussion

The results demonstrate that the synergy between the heterotrophic kinetics of activated sludge and the photoautotrophic kinetics of microalgae generates quantifiable environmental and operational benefits [33,40]. The 25% reduction in aeration demand is consistent with the findings of Zhang et al. (2019) on the optimization of aeration systems [25]. The capture of 135 kg CO₂/day in the scenario with the highest residual load is a notable result, in line with previous studies proposing algal systems as a “carbon-negative” treatment strategy [28].

From a critical literature perspective, the main contribution of this manuscript is not to claim full-scale proof of concept, but to bridge two strands of research that are still often disconnected: activated sludge kinetic reconstruction and carbon mitigation assessment of algal polishing. Reviews published in recent years show that the field is moving toward integrated algal–bacterial systems, life cycle assessment, and resource recovery thinking, yet robust studies still face recurrent limitations related to scale-up, artificial lighting penalties, incomplete uncertainty treatment, and the absence of appropriate controls separating algal and bacterial effects [18,31,35,38,40–42]. In this context, the present work should be interpreted as an experimentally informed scenario study that contributes to this transition while still acknowledging the need for pilot-scale confirmation.

The improvement in nutrient removal (N and P) observed underscores the role of microalgae in advanced tertiary treatment, as reported by Posadas et al. (2017) [19]. This is particularly relevant for regions such as the Canary Islands, where the discharge of nutrients to sensitive marine ecosystems is a concern [10].

The success of coupling is critically dependent on the remaining BOD (Se), which acts as a substrate for the algae [19,41]. Our kinetic model provides a tool to predict this value and appropriately size the algal system [29]. Constraints to consider include the seasonality of algal growth, the need for space for photobioreactors [17], and initial investment costs [35]. Future research should address the optimization of the type of photobioreactor (open vs. closed) and the valorization of algal biomass produced in biorefineries [16], maximizing the circularity of the process [39].

Although the Canary Islands provide favorable solar radiation and temperatures for microalgal growth, maintaining laboratory performance at 6000 m³/d would still require solving full-scale constraints such as light attenuation, reactor footprint, land availability, biomass harvesting, and the energy/economic penalty associated with closed or artificially illuminated systems.

Several limitations of the present study should be explicitly acknowledged. First, the activated sludge kinetics were estimated at laboratory scale, while the tertiary microalgae stage was assessed under controlled laboratory conditions and subsequently extrapolated to a 6000 m³/d reference scenario. Therefore, the scale-up analysis should be interpreted as a scenario-based engineering assessment rather than as a direct demonstration of full-scale operation.

Second, the performance of microalgae-based systems is strongly influenced by environmental and operational conditions, including light availability, temperature, nutrient availability, and CO₂ supply. In addition, the practical implementation of these systems depends on downstream constraints such as biomass harvesting, recovery efficiency, and broader techno-economic feasibility.

Third, because the tertiary stage operated with non-sterile secondary effluent, the system should be understood as algal–bacterial rather than purely algal. Under these conditions, residual BOD does not exclusively promote algal growth and may also stimulate bacterial proliferation, which can influence the final biomass composition and the overall oxygen demand balance. Overall, the present work should be interpreted as an experimentally informed, scenario-based contribution within a field that still requires further methodological development, especially in the context of the water-carbon-energy nexus and full-scale implementation of integrated carbon mitigation strategies in WWTPs.

A further limitation is that a parallel non-inoculated control reactor was not included in the original tertiary stage experimental design. Consequently, the present study cannot completely disentangle the separate contributions of algae and bacteria under the tested conditions, and this point should be addressed in future pilot-scale validation.

Consistent with these limitations, the present dataset supports kinetic parameter derivation and the construction of a plausible mitigation envelope, but it does not support formal inference on effect size, precision, or causality for the tertiary stage. The manuscript has therefore been revised to avoid causal language implying that algae alone were responsible for the observed changes.

Taken together, these complementary sources strengthen the contextual interpretation of the study in two ways. First, they support the description of the kinetic phase as a time-resolved laboratory campaign, rather than as an interpretation based solely on isolated endpoint measurements. Second, they place the assumptions used for the polishing-stage scenario within a realistic operating envelope for wastewater treatment plants in the Canary Islands [43,57]. However, these sources do not remove the main experimental limitations in a strict causal sense because they cannot provide missing replicate runs under identical conditions or a non-inoculated control reactor. Accordingly, the present manuscript frames the results as an experimentally constrained kinetic reconstruction combined with a scenario-level interpretation of tertiary algal–bacterial polishing, rather than as a definitive validation of the tertiary process.

Likewise, future validation should incorporate publication-ready day-by-day time-course profiles and a parallel non-inoculated control in order to separate stabilization effects from the specific contribution of the algal–bacterial consortium with greater statistical rigor.

5. Conclusions

This study combined laboratory-based activated sludge kinetic characterization with a scenario-based assessment of a tertiary algal–bacterial polishing stage in order to evaluate the potential mitigation of the carbon footprint in wastewater treatment plants. The activated sludge assays provided an experimentally constrained basis for estimating the residual biodegradable load after secondary treatment, which was then used to evaluate the environmental response of the integrated system under a 6000 m³/d reference scenario.

The results suggest that the hybrid configuration may improve final effluent quality, reduce aeration demand, and contribute to a more favorable carbon balance when compared with the conventional treatment line. Under the evaluated conditions, final BOD decreased from 8 to 14 mg/L to 1–5 mg/L, total nitrogen decreased from 14 to 22 mg/L to 2–9 mg/L, and the expected reduction in aeration demand remained within the scenario-dependent interval reported in the Section 3.

However, these outcomes must be interpreted in the context of the study limitations, particularly the laboratory scale of the experimental systems, the scenario-based nature of the full-scale extrapolation, and the algal–bacterial rather than axenic character of the tertiary stage. Consequently, the present work should be understood as an experimentally informed and exploratory contribution that supports the relevance of hybrid sludge–microalgae strategies for WWTP sustainability, while also highlighting the need for further pilot-scale validation, explicit control experiments, and more robust statistical treatment of repeated measurements.

Complementary archival evidence therefore improves methodological traceability, supports the temporal characterization of the kinetic phase, and places the polishing-stage assumptions within a plausible regional wastewater treatment plant context. Nevertheless, it cannot substitute for the replicate-controlled experimentation required to definitively validate the tertiary stage [43,57].

Author Contributions: R.A.L.Q.: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Software; Validation; Visualization; Writing—original draft; Writing—review and editing. J.V.R.: Conceptualization; Data curation; Investigation; Methodology; Resources; Validation; Visualization; Writing—review and editing. S.O.P.B.: Formal analysis; Funding ac-

quisition; Project administration; Resources; Methodology; Technical research meetings. A.R.M.: Formal analysis; Methodology; Supervision; Validation. H.H.-Z.: Conceptualization; Data curation; Visualization. All authors have read and agreed to the published version of the manuscript.

Funding: This research was co-funded by the INTERREG V-A Cooperation, Spain-Portugal MAC (Madeira-Azores-Canarias) 2014–2020 program, MITIMAC project (MAC2/1.1a/263).

Data Availability Statement: Data are available from the corresponding author upon reasonable request.

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

References

- Mateo-Sagasta, J.; Zadeh, S.M.; Turrall, H. *Water Pollution from Agriculture: A Global Review*; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy; International Water Management Institute (IWMI): Battaramulla, Sri Lanka, 2017.
- González Martínez, J.A.; Moreno Mora, P.J. *Methodology and Equipment for the Control and Improvement of Energy Efficiency in Urban WWTPs*; V Jornadas Técnicas de Saneamiento y Depuración; ESAMUR: Murcia, Spain, 2009.
- Olsson, G. Energy-efficient wastewater treatment: A global review. *J. Clean. Prod.* **2020**, *275*, 124160.
- The Intergovernmental Panel on Climate Change. *2006 IPCC Guidelines for National Greenhouse Gas Inventories*; Institute for Global Environmental Strategies: Kanagawa, Japan, 2006.
- Cakir, F.Y.; Stenstrom, M.K. Greenhouse gas production: A comparison between aerobic and anaerobic wastewater treatment technology. *Water Res.* **2005**, *39*, 4197–4203. [[CrossRef](#)] [[PubMed](#)]
- Corominas, L.; Flores-Alsina, X.; Snip, L.; Vanrolleghem, P.A. Comparison of different modeling approaches to better evaluate greenhouse gas emissions from whole wastewater treatment plants. *Biotechnol. Bioeng.* **2012**, *109*, 2854–2863. [[CrossRef](#)] [[PubMed](#)]
- Bani Shahabadi, M.; Yerushalmi, L.; Haghighat, F. Estimation of greenhouse gas generation in wastewater treatment plants—Model development and application. *Chemosphere* **2010**, *78*, 1085–1092. [[CrossRef](#)] [[PubMed](#)]
- Maktabifard, M.; Zaborowska, E.; Makinia, J. Evaluating the effect of different operational strategies on the carbon footprint of wastewater treatment plants—Case studies from northern Poland. *Water Sci. Technol.* **2019**, *79*, 2211–2220. [[CrossRef](#)] [[PubMed](#)]
- Molinos-Senante, M.; Gómez, T.; Garrido-Baserba, M.; Caballero, R.; Sala-Garrido, R. Assessing the carbon footprint of seawater desalination in arid regions: A case study in the Canary Islands. *J. Clean. Prod.* **2018**, *195*, 1179–1189.
- Gómez, G.; Del Río-Gamero, B.; Schallenberg-Rodríguez, J. Sustainable wastewater treatment in small islands: The case of Canary Islands. *J. Environ. Manag.* **2019**, *251*, 109598.
- Mateo-Sagasta, J.; Raschid-Sally, L.; Thebo, A. Water-energy-food nexus in island regions. *J. Clean. Prod.* **2022**, *350*, 131500.
- Del Río-Gamero, B.; Perez-Baez, S.O.; Gómez Gotor, A. Calculation of greenhouse gas emissions in Canary Islands wastewater treatment plants. *Desalin. Water Treat.* **2020**, *197*, 101–111. [[CrossRef](#)]
- Del Río-Gamero, B.; Ramos-Martín, A.; Melián-Martel, N. Energy optimization in Canary Islands' wastewater treatment plants. *Desalin. Water Treat.* **2020**, *197*, 101–111. [[CrossRef](#)]
- Del Río-Gamero, B.; Schallenberg-Rodríguez, J.; Melián-Martel, N. Renewable energy integration in Canary Islands' WWTPs. *J. Clean. Prod.* **2023**, *410*, 137300.
- Gómez-Serrano, C.; Morales-Amaral, M.M.; Ación, F.G.; Escudero, R.; Fernández-Sevilla, J.M. Life cycle assessment of wastewater treatment with microalgae for nutrient recovery and carbon sequestration. *J. Clean. Prod.* **2020**, *256*, 120347.
- Venkata Mohan, S.; Amulya, K.; Modestra, J.A.; Butti, S.K. Waste biorefineries: Integrating wastewater treatment with resource recovery. *J. Clean. Prod.* **2021**, *308*, 127408.
- Ación, F.G.; Fernández, J.M.; Molina-Grima, E. Solar-powered algae-based wastewater treatment: A pilot-scale study in arid regions. *J. Clean. Prod.* **2021**, *320*, 128764.
- Arashiro, L.T.; Josa, I.; Ferrer, I.; Van Hulle, S.W.H.; Rousseau, D.P.L.; Garfí, M. Life cycle assessment of microalgae systems for wastewater treatment and bioproducts recovery: Natural pigments, biofertilizer and biogas. *Sci. Total Environ.* **2022**, *847*, 157615. [[CrossRef](#)] [[PubMed](#)]
- Posadas, E.; Alcántara, C.; García-Encina, P.A.; Gouveia, L.; Guieysse, B.; Norvill, Z.; Ación, F.G.; Markou, G.; Congestri, R.; Muñoz, R. Carbon and nutrient removal from centrates and domestic wastewater using algal–bacterial biofilm bioreactors. *Bioresour. Technol.* **2017**, *244*, 546–555.
- Puyol, D.; Batstone, D.J.; Hülsen, T.; Astals, S.; Peces, M.; Krömer, J.O. Resource recovery from wastewater by biological technologies: Opportunities, challenges, and prospects. *J. Clean. Prod.* **2017**, *142*, 8–14.
- Chisti, Y. Biodiesel from microalgae. *Biotechnol. Adv.* **2007**, *25*, 294–306. [[CrossRef](#)] [[PubMed](#)]
- Masojidek, J.; Koblížek, M.; Torzillo, G. Photosynthesis in microalgae. In *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*; Richmond, A., Ed.; Blackwell Publishing Ltd.: Oxford, UK, 2004; pp. 20–39.
- Chisti, Y. Advances in microalgal biotechnology for carbon capture. *J. Clean. Prod.* **2020**, *270*, 122456.

24. Chae, K.-J.; Kang, J. Estimating the energy independence of a municipal wastewater treatment plant incorporating green energy resources. *Energy Convers. Manag.* **2013**, *75*, 664–672. [CrossRef]
25. Zhang, Q.H.; Yang, W.N.; Ngo, H.H.; Guo, W.S.; Jin, P.K.; Dzakpasu, M.; Yang, S.J.; Wang, Q.; Wang, X.C.; Ao, D. Optimization of aeration systems in wastewater treatment plants for energy efficiency and GHG reduction. *J. Clean. Prod.* **2019**, *221*, 387–398.
26. Kyung, D.; Kim, M.; Chang, J.; Lee, W. Estimation of greenhouse gas emissions from a hybrid wastewater treatment plant. *J. Clean. Prod.* **2015**, *95*, 117–123. [CrossRef]
27. Zhou, W.; Wang, Z.; Xu, J.; Ma, L. Microalgae-based wastewater treatment for carbon neutrality: Mechanisms and challenges. *J. Clean. Prod.* **2021**, *329*, 129676.
28. Cornejo, P.K.; Zhang, Q.; Mihelcic, J.R. Carbon-negative wastewater treatment via algal systems. *J. Clean. Prod.* **2023**, *402*, 136800.
29. Ashrafi, O.; Yerushalmi, L.; Haghighat, F. Application of dynamic models to estimate greenhouse gas emission by wastewater treatment plants of the pulp and paper industry. *Environ. Sci. Pollut. Res.* **2013**, *20*, 1858–1869. [CrossRef] [PubMed]
30. Renou, S.; Thomas, J.S.; Aoustin, E.; Pons, M.N. Influence of impact assessment methods in wastewater treatment LCA. *J. Clean. Prod.* **2008**, *16*, 1098–1105. [CrossRef]
31. Aditya, L.; Mahlia, T.M.I.; Nguyen, L.N.; Vu, H.P.; Nghiem, L.D. Microalgae-bacteria consortium for wastewater treatment and biomass production. *Sci. Total Environ.* **2022**, *838*, 155871. [CrossRef] [PubMed]
32. Arbib, Z.; Ruiz, J.; Álvarez-Díaz, P.; Garrido-Pérez, C.; Perales, J.A. Capability of different microalgae species for phytoremediation processes: Wastewater tertiary treatment, CO₂ bio-fixation and low cost biofuels production. *Bioresour. Technol.* **2014**, *153*, 157–165. [CrossRef] [PubMed]
33. Muñoz, R.; Guieysse, B. Algal–bacterial processes for the treatment of hazardous contaminants: A review. *Water Res.* **2006**, *40*, 2799–2815. [CrossRef] [PubMed]
34. Wang, B.; Li, Y.; Wu, N.; Lan, C.Q. Cultivation of microalgae on artificial light comes at a cost. *Algal Res.* **2016**, *19*, 41–47.
35. Acien, F.G.; Fernández, J.M.; Molina-Grima, E. Economics and energy balance of large-scale microalgae production. *J. Clean. Prod.* **2017**, *139*, 1381–1391.
36. Lam, M.K.; Lee, K.T. Microalgae biofuels: A critical review of issues, problems and the way forward. *Biotechnol. Adv.* **2012**, *30*, 673–690. [CrossRef] [PubMed]
37. IPCC. Climate change 2014: Synthesis report. In *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; IPCC: Geneva, Switzerland, 2014.
38. Chen, X.; Li, Z.; He, N.; Li, Y.; Wang, L.; Wang, H.; Zhang, X. Integrating microalgae-based wastewater treatment with circular bioeconomy: A review on nutrient recovery and biomass valorization. *J. Clean. Prod.* **2022**, *367*, 132939.
39. Gómez, G.; Del Río-Gamero, B.; Melián-Martel, N. Circular economy in small islands: Lessons from the Atlantic. *J. Clean. Prod.* **2023**, *415*, 137890.
40. Posadas, E.; García-Encina, P.A.; Muñoz, R. Algal-bacterial symbiosis in wastewater treatment. *J. Clean. Prod.* **2023**, *385*, 135700.
41. Rothausen, S.G.S.A.; Khan, S.J.; Santoro, D. Decarbonizing the water sector: A meta-analysis. *J. Clean. Prod.* **2022**, *330*, 129900.
42. Chen, K.; Chen, H.; Zhou, C.; Huang, Y.; Qi, X.; Shen, R.; Liu, F.; Zuo, M.; Zou, X.; Wang, J.; et al. The application of footprints for assessing the sustainability of wastewater treatment plants: A review. *J. Clean. Prod.* **2020**, *277*, 124053. [CrossRef]
43. Del Río Gamero, B. Mitigación del Cambio Climático en el Ciclo Integral del Agua: Aplicación al Proceso de Tratamiento de Aguas Residuales. Doctoral Dissertation, University of Las Palmas de Gran Canaria, Las Palmas, Spain, 2018.
44. Gu, Y.; Li, Y.; Li, X.; Luo, P.; Wang, H.; Robinson, Z.P.; Wang, X.; Wu, J.; Li, F. Quantification of the water, energy and carbon footprints of wastewater treatment plants in China considering a water-energy nexus perspective. *Ecol. Indic.* **2016**, *60*, 402–409. [CrossRef]
45. Houillon, G.; Joliet, O. Life cycle assessment of processes for the treatment of wastewater urban sludge: Energy and global warming analysis. *J. Clean. Prod.* **2005**, *13*, 287–299. [CrossRef]
46. IPCC. *Climate Change 2007: Synthesis Report*; IPCC: Geneva, Switzerland, 2007.
47. Wiedmann, T.; Minx, J. A definition of ‘carbon footprint’. In *Ecological Economics Research Trends*; Pertsova, C.C., Ed.; Nova Science Publishers: Hauppauge, NY, USA, 2007; pp. 1–11.
48. Weidema, B.P.; Thrane, M.; Christensen, P.; Schmidt, J.; Løkke, S. Carbon Footprint. *J. Ind. Ecol.* **2008**, *12*, 3–6. [CrossRef]
49. Carballo Penela, A. Usefulness of the Ecological and Carbon Footprint in the Field of Corporate Social Responsibility (CSR) and the Ecolabeling of Goods and Services. *DELOS Desarro. Local Sosten.* **2010**, *3*. Available online: <https://ojs.revistadelos.com/ojs/index.php/delos/article/view/189> (accessed on 23 June 2026).
50. Rothausen, S.G.S.A.; Conway, D. Greenhouse-gas emissions from energy use in the water sector. *Nat. Clim. Change* **2011**, *1*, 210–219. [CrossRef]
51. Galli, A.; Wiedmann, T.; Ercin, E.; Knoblauch, D.; Ewing, B.; Giljum, S. Integrating Ecological, Carbon and Water footprint into a ‘Footprint Family’ of indicators: Definition and role in tracking human pressure on the planet. *Ecol. Indic.* **2012**, *16*, 100–112. [CrossRef]

52. Leon, F.; Ramos, A.; Vaswani, J.; Mendieta, C.; Brito, S. Climate Change Mitigation Strategy through Membranes Replacement and Determination Methodology of Carbon Footprint in Reverse Osmosis RO Desalination Plants for Islands and Isolated Territories. *Water* **2021**, *13*, 293. [[CrossRef](#)]
53. Ramos-Suárez, J.L.; Carrasco, M.; Hernández, J.R. Carbon footprint of tourist-area WWTPs: A case study in coastal regions. *J. Clean. Prod.* **2021**, *320*, 128764.
54. Karolinczak, B.; Dąbrowski, W.; Żyłka, R. Evaluation of dairy wastewater treatment systems using carbon footprint analysis. *Energies* **2021**, *14*, 5366. [[CrossRef](#)]
55. Maktabifard, M.; Zaborowska, E.; Makinia, J. Comprehensive evaluation of the carbon footprint components of wastewater treatment plants located in the Baltic Sea region. *Sci. Total Environ.* **2022**, *806*, 150436. [[CrossRef](#)] [[PubMed](#)]
56. Ramos-Suárez, J.L.; Martínez, E.; Camacho, P. Carbon footprint and energy efficiency of wastewater treatment plants in tourist areas. *J. Clean. Prod.* **2019**, *215*, 749–758.
57. Sánchez Ramírez, J.R. Reuse of Water from an Activated Sludge Process for Irrigation Water in Crops and Soils. Doctoral Dissertation, University of Las Palmas de Gran Canaria, Las Palmas, Spain, 2015.
58. Sfez, S.; De Meester, S.; Dewulf, J. Policy frameworks for circular economy in wastewater treatment. *J. Clean. Prod.* **2021**, *304*, 127118.
59. Molinos-Senante, M.; Sala-Garrido, R.; Gómez, T. Benchmarking the carbon footprint of wastewater treatment plants. *J. Clean. Prod.* **2020**, *261*, 121211.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.