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Optimization and Performance Modeling of Constructed Wetlands for the Treatment of Slaughterhouse Effluents in Tropical Zones Using Response Surface Methodology

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Abstract

The meat industry generates wastewater with high organic matter loads, posing a significant environmental risk if not properly treated. The present study evaluated the performance of a horizontal subsurface flow constructed wetland (HSSF-CW) treating slaughterhouse effluents characterized by high-strength influent concentrations of 3570.51 ± 153.82 mg/L COD, 2114.33 ± 104.58 mg/L BOD_5, and 1173.77 ± 96.95 mg/L TOC. Furthermore, Response Surface Methodology (RSM) was employed to model and optimize the operational parameters. The independent variables considered were hydraulic retention time (HRT: 3, 5, and 10 days) and vegetation type (*Heliconia latispatha*, *Typha latifolia*, and polyculture). The results demonstrated a statistically significant improvement in treatment efficiency, achieving maximum removal efficiencies of 86.5% for COD, 89.4% for BOD_5, and 91.5% for TOC. The statistical models exhibited high accuracy ($R^2 \geq 0.996$, $p < 0.001$). Adjusted response surface equations identified the polyculture with a 5-day HRT as the most favorable operational scenario. These findings confirm that properly designed and operated constructed wetlands represent a viable and sustainable alternative for treating high-load agro-industrial effluents, contributing to the protection of receiving water bodies. Future research should focus on full-scale studies and the inclusion of critical parameters such as nutrients and pathogens for a more comprehensive system characterization.

Keywords: constructed wetlands; slaughterhouse wastewater; organic matter; response surface methodology; optimization; nature-based solutions

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1. Introduction

The meat processing industry, particularly slaughterhouses, generates effluents characterized by high organic loads, suspended solids, nutrients, and other potentially toxic compounds. Consequently, these discharges pose a significant environmental challenge, primarily driving eutrophication and the degradation of receiving water bodies [1]. In

this context, the effective treatment of slaughterhouse wastewater (SWW) is imperative to mitigate environmental impacts and minimize associated public health risks [2].

Constructed wetlands (CWs) have emerged as a sustainable and cost-effective alternative for wastewater treatment, as they mimic the self-purification mechanisms of natural wetlands [3,4]. Numerous studies have demonstrated the effectiveness of these systems in removing organic matter, nutrients, and pathogens from a wide range of effluents, including municipal, industrial, and agro-industrial wastewaters [5,6]. Specifically, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), and Total Organic Carbon (TOC) serve as critical parameters for assessing organic removal efficiency in these treatment systems [7,8].

Various studies have applied Response Surface Methodology (RSM) to optimize the operational parameters of CWs, evaluating key variables such as hydraulic retention time (HRT), organic loading rate (OLR), substrate type, and vegetation [9,10]. However, while these studies have contributed significantly to process optimization, the majority have focused on municipal or general industrial effluents. Consequently, there is a distinct gap in the literature regarding specific applications for SWW, which exhibits a pollutant profile that differs substantially from other wastewater types [11].

The reviewed literature indicates that, while models exist for optimizing COD removal in constructed wetlands [12], it remains necessary to broaden the analytical scope to include additional parameters such as BOD_5 and TOC. This is particularly critical in contexts where effluents are characterized by high loads of biodegradable organic matter and meat processing by-products. These distinct characteristics may necessitate tailored adjustments to both operational parameters and treatment system design [13].

Although CWs have been widely implemented for the treatment of agro-industrial effluents, significant challenges persist regarding their design optimization for high-strength wastewaters, specifically SWW [14,15]. The current literature indicates that, despite the proven efficacy of CWs in tropical regions, most existing design criteria rely on empirical rules or "black box" approaches [16]. These conventional methods often fail to adequately capture the complex, non-linear biochemical interactions occurring within the system, particularly when treating effluents characterized by high loads of biodegradable organic matter and meat processing by-products [17].

Moreover, although RSM has been effectively applied to optimize phytoremediation processes in recent years, existing studies have predominantly focused on municipal wastewater or monoculture vegetation systems [18,19]. Data regarding the mathematical modeling of polyculture systems treating high-strength industrial effluents remain sparse [20]. Specifically, the potential synergistic effects of combined plant species in handling high organic loads have not been adequately modeled using robust statistical tools [21]. Consequently, reliance on conventional "one-factor-at-a-time" (OFAT) experimental designs [22] limits the identification of optimal operational regions where the interaction between HRT and vegetation type maximizes treatment efficiency and system performance [23].

To address this knowledge gap, the novelty of the present study lies in transcending traditional experimental frameworks—such as completely randomized or simple factorial designs—by shifting towards statistical process optimization and the application of second-order models. This advanced methodological approach is applied to the treatment of SWW, an aggressive and under-studied effluent characterized by extreme organic loads and complex biochemical dynamics. Consequently, the primary objective of this research was the optimization and performance modeling of constructed wetlands for the treatment of slaughterhouse effluents in tropical zones using Response Surface Methodology. Specifically, a 3^2 experimental design was employed to evaluate the interactive effects of

HRT and vegetation type, ensuring high-precision prediction of removal efficiencies and effluent concentrations.

2. Materials and Methods

2.1. Study Site

The study was conducted at the facilities of the Tecnológico Nacional de México (TecNM), Misantla Campus, located in Veracruz, Mexico (Figure 1), between January 2022 and December 2023. The local climate is characterized as warm-humid, with a mean annual temperature of 22.7 °C and an average annual precipitation of 2036.4 mm. The study site is situated at an elevation of 300 m above sea level (m a.s.l.).

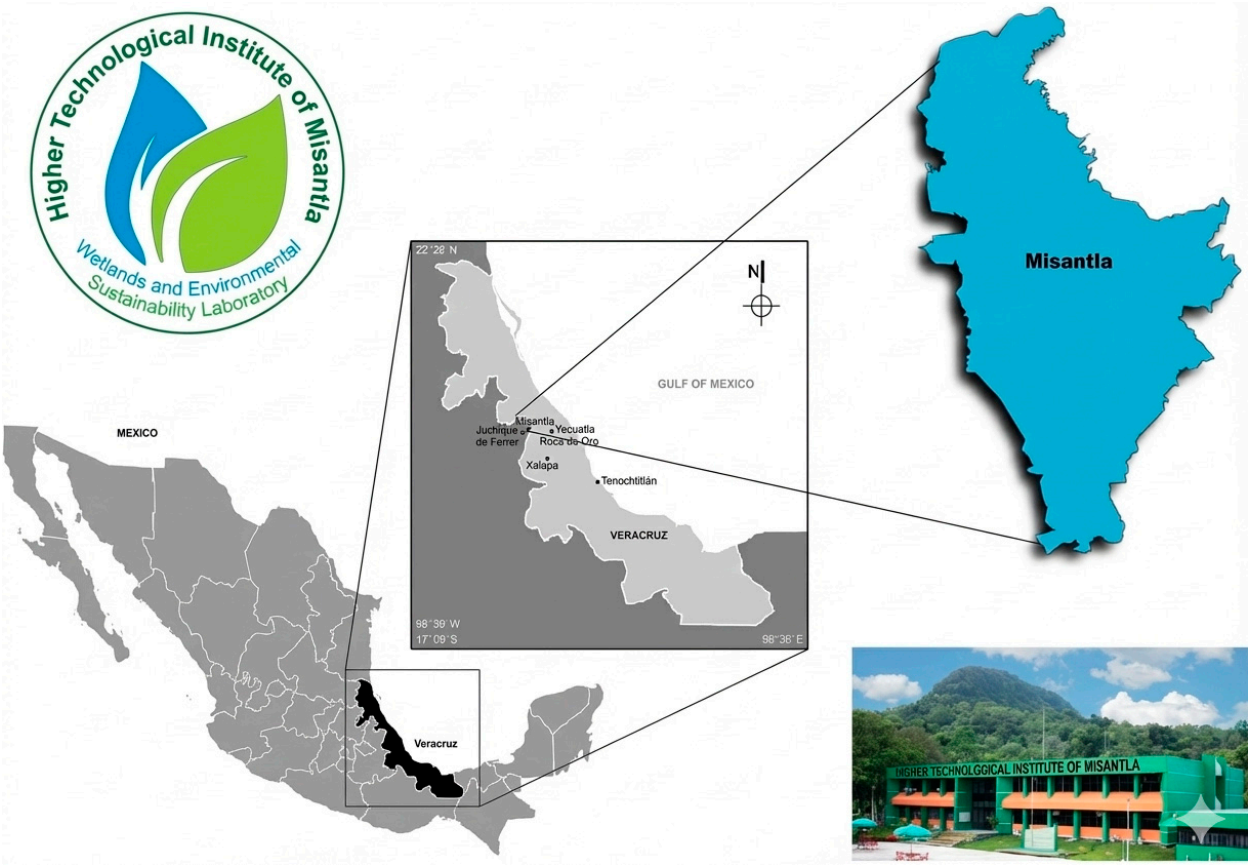


Figure 1. Description of the study site.

2.2. Configuration and Operation of the CW

The wastewater used in this study was collected from a local slaughterhouse located 1.2 km from the experimental site. The wastewater was stored in a 1200 L sedimentation tank, positioned 50 cm above the experimental units to facilitate gravity feeding. The experimental setup comprised eight mesocosm-scale Horizontal Subsurface Flow Constructed Wetland (HSSF-CW) units, constructed of reinforced concrete (Figure 2).

The HSSF-CW experimental units were constructed with dimensions of 150 cm × 40 cm × 100 cm (length × width × height). The outlet level was adjusted to maintain an effective water depth of 65 cm, resulting in a gross bed volume of 390 L. Based on a filter media porosity of 50%, the net working volume (pore volume) was calculated as 195 L per unit. The cells were arranged in a linear configuration, with the adjacent unit serving as an experimental replicate of the preceding one.

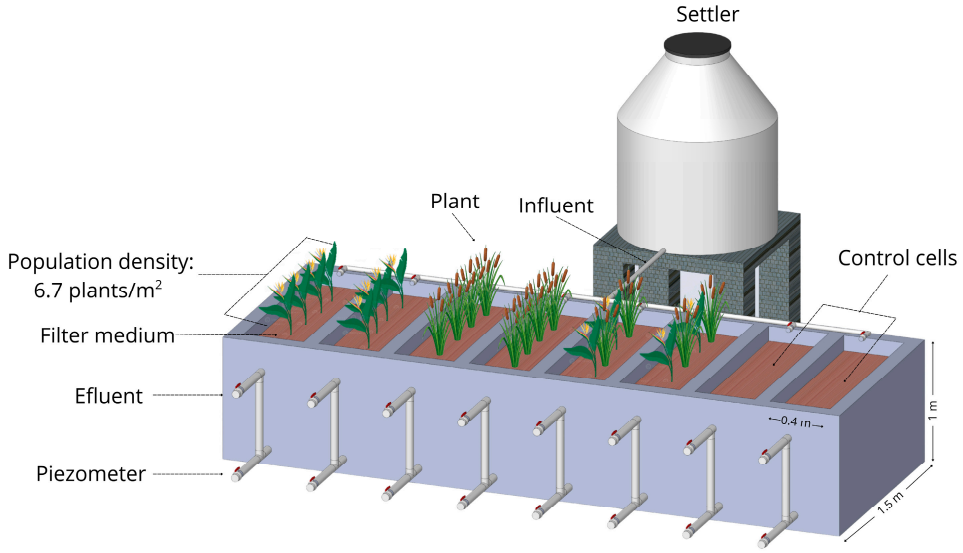


Figure 2. Experimental system design.

The experimental units were assigned to four treatments: an unplanted control, a polyculture (*Typha latifolia* + *Heliconia latispatha*), and monocultures of *Typha latifolia* and *Heliconia latispatha*, respectively. This experimental design allowed for the comparative assessment of treatment performance under replicated conditions.

The unplanted control unit, containing the same filter media, operated under identical conditions to the other cells, as all units were fed from a common influent source (the sedimentation tank).

This configuration enabled the assessment of the specific contribution of vegetation to COD, BOD_5 and TOC removal. Volcanic rock was selected as the support medium, characterized by a particle size ranging from 10–30 mm and an initial effective porosity of approximately 50%. The bed depth was maintained at 65 cm to ensure adequate subsurface flow conditions. Regarding surface morphology, the volcanic rock exhibits a rough texture and a specific surface area of 3–5 m^2/g, properties which significantly facilitate microbial attachment and biofilm development [24]. Moreover, its chemical composition predominantly comprises silicon dioxide (SiO_2, ~48–55%), aluminum oxide (Al_2O_3, ~15–18%), and iron oxide (Fe_2O_3, ~10–12%), providing active sites that support adsorption and ion exchange mechanisms within the system [25]. All units were evaluated under three HRT: 3, 5, and 10 days.

2.3. System Stabilization and Operation

All experimental runs were conducted under a sequential batch operation mode, utilizing the same HSSF-CW setup throughout the study. The establishment of the biological community relied on the native mixed microbial consortium naturally present in the slaughterhouse wastewater. No external commercial strains or specific algal inocula were introduced.

Prior to data collection, the HSSF-CW units underwent a three-month acclimatization and stabilization period to ensure vegetation establishment and biofilm maturation. During this phase, a stepwise adaptation protocol was implemented to mitigate potential shock loading and substrate inhibition. The SWW fraction was increased incrementally over consecutive months at volumetric ratios of 25%, 50%, and 75% (v/v), respectively, until reaching full-strength effluent (100%) for the experimental runs.

Post-acclimatization, the experimental trials were executed in accordance with the defined 3^2 factorial design matrix. The modulation of HRT levels (3, 5, and 10 days)

was controlled by adjusting the batch residence time while maintaining a constant net working volume [26]. To guarantee data reliability and minimize carry-over effects, a stabilization period was implemented after each HRT adjustment, ensuring the system reached pseudo-steady-state conditions prior to sampling [27].

The system was considered to have reached a pseudo-steady state after a minimum duration equivalent to three consecutive cycles (3 × HRT) had elapsed [28]. Operational stability was validated when the variability in effluent COD concentrations between consecutive sampling events remained below 10% [29]. Data obtained exclusively during this stable regime were incorporated into the statistical analysis.

2.4. SWW Characterization

Physicochemical analyses were performed to characterize the collected wastewater, specifically determining COD, BOD_5, and TOC. All analyses were conducted according to Standard Methods for the Examination of Water and Wastewater (SM 5220 for COD, SM 5210 for BOD_5, and SM 5310 for TOC). Additionally, water temperature (T), pH, and Electrical Conductivity (EC) were recorded using a Hanna HI9811-51 portable multiparameter meter (HANNA® Instruments, Woonsocket, RI, USA). Dissolved Oxygen (DO) was measured with a Hanna HI9146-04 m (HANNA® Instruments, Woonsocket, RI, USA). The characterization data is summarized in Table 1.

Table 1. Physicochemical characterization of the influent.

Parameter	Units	Mean ± SD *
COD	mg/L	3570.51 ± 153.82
BOD_5	mg/L	2114.33 ± 104.58
TOC	mg/L	1173.77 ± 96.95
TSS	mg/L	2047.45 ± 252.28
TN	mg/L	246.56 ± 8.47
TP	mg/L	42.31 ± 2.21
TC	Log NMP/100 mL	7.471 ± 1.201
FC	Log NMP/100 mL	5.462 ± 0.941
EC	µS/cm	1805 ± 610
DO	mg/L	0.7 ± 0.09
T	°C	27.2 ± 3.1
pH		8.01 ± 0.6

Note(s): * SD: standard error, TSS: Total suspended solids, TN: Total nitrogen, TP: total phosphorus, TC: total coliforms, FC: fecal coliforms.

To maintain hydraulic conductivity and mitigate clogging risks within the porous media, the slaughterhouse effluent was subjected to a multi-stage pretreatment protocol.

Initially, gross solids removal was performed using bar racks at the discharge source. Subsequently, the wastewater was passed through a fine mesh screen prior to entering the feed reservoir to retain smaller debris. This reservoir served a dual function as a storage tank and a primary sedimentation unit, facilitating the gravitational settling of suspended particulate matter. As a final polishing step, the supernatant was processed through an in-line filter located at the outlet valve before being directed into the wetland influent distribution system.

2.5. Experimental Design and RSM

A three-level full factorial design 3^2 was employed to evaluate and model the effects of two key independent variables, as outlined in Table 2:

Table 2. Variables and their levels in experimental design.

Variable	Units	Symbols	Coded Levels		
			−1	**0**	**1**
Vegetation	NA	X_1	*Heliconia latispatha*	*Typha latifolia*	Polyculture (*T. latifolia* + *H. latispatha*)
HTR	days	X_2	3	5	10

Note(s): X_1: Represents the Vegetation Type (categorical) and X_2: represents the HRT (numerical).

The selected response variables were effluent concentration (mg/L) and removal efficiency (%) for COD, BOD_5, and TOC. The experimental design comprised a total of 18 runs, with two replicates performed for each treatment combination to estimate the experimental error. These runs corresponded to different HRT combinations, as detailed in Table 3.

Table 3. A 3^2 factorial design matrix for the two test factors in uncoded units, along with observed (experimental) and predicted (model) responses for COD, BOD_5, and TOC removal in slaughterhouse effluents.

No.	Factors		COD (mg/L)		BOD_5 (mg/L)		TOC (mg/L)	
	X_1	X_2	Observed	Predicted	Observed	Predicted	Observed	Predicted
1	*H. latispatha*	3	2229.59	2193.88	1278	1256.85	650.79	639.06
2	*T. latifolia*	3	2607.47	2571.76	1501.76	1480.62	775.02	763.28
3	Policultivo	3	2295.05	2259.35	1316.76	1295.62	672.31	660.57
4	*H. latispatha*	5	1301.25	1265.55	728.27	707.13	345.61	333.87
5	*T. latifolia*	5	1366.71	1331	767.03	745.89	367.13	355.39
6	Policultivo	5	741.87	706.16	397.03	375.88	161.72	149.98
7	*H. latispatha*	10	2217.69	2181.98	1270.95	1249.8	646.88	635.14
8	*T. latifolia*	10	1970.73	1935.02	1124.71	1103.57	565.69	553.96
9	Policultivo	10	1033.47	997.76	569.7	548.56	257.58	245.84
10	*H. latispatha*	3	2158.18	2193.88	1235.71	1256.85	627.32	639.06
11	*T. latifolia*	3	2536.06	2571.76	1459.48	1480.62	751.54	763.28
12	Policultivo	3	2223.64	2259.35	1274.47	1295.62	648.83	660.57
13	*H. latispatha*	5	1229.84	1265.55	685.98	707.13	322.14	333.87
14	*T. latifolia*	5	1295.3	1331	724.75	745.89	343.65	355.39
15	Policultivo	5	670.46	706.16	354.74	375.88	138.24	149.98
16	*H. latispatha*	10	2146.28	2181.98	1228.66	1249.8	623.4	635.14
17	*T. latifolia*	10	1899.31	1935.02	1082.42	1103.57	542.22	553.96
18	Policultivo	10	962.05	997.76	527.41	548.56	234.1	245.84

Note(s): X_1: Represents the Vegetation Type (categorical) and X_2: represents the HRT (numerical).

The selection of HRT levels was driven by operational criteria specific to tropical environments, rather than a rigid arithmetic progression. The coded levels were defined to represent distinct treatment regimes within the experimental domain [30–32]:

- Low level (−1): 3 days, representing a high hydraulic loading rate (HLR) scenario to assess system resilience under hydrodynamic stress.
- Medium level (0): 5 days, serving as the baseline design parameter for organic matter removal, accounting for the accelerated biological kinetics typical of tropical temperatures.
- High level (+1): 10 days, corresponding to an extended residence time aimed at effluent polishing and enhanced pathogen inactivation.

Although this discretization results in unequally spaced intervals, the experimental design effectively explores the defined domain of interest [32]. This approach prioritizes engineering applicability over strict statistical symmetry, allowing for the characterization of non-linear responses and interaction effects within a practical operational range [33].

The mathematical relationship between the independent variables and the response variables was described using a second-order polynomial (quadratic) model, expressed as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j + \varepsilon$$

where

Y is the predicted answer.

X_i, X_j are the independent variables (factors: Vegetation, HRT).

β_0 is the constant coefficient (intersection).

β_i, β_{ii}, β_{ij} are the linear, quadratic, and interaction coefficients, respectively.

ε is the experimental error.

In contrast to HRT, which functions as a continuous variable, the Vegetation factor (X_2) represents a nominal categorical variable. For the purpose of mathematical modeling, this factor was encoded using discrete integer identifiers (−1, 0, +1). It is crucial to emphasize that this numerical encoding serves as a computational expedient and does not imply a biological continuum or an ordinal relationship [34]. Consequently, the inference drawn from the regression model is strictly constrained to these specific discrete levels, reflecting distinct plant configurations rather than a continuous function across the vegetation factor space.

For each established vegetation treatment, the system operated under HRT of 3, 5, and 10 days throughout the experimental period. Influent and effluent samples were collected at the conclusion of each treatment cycle for subsequent analysis, and the resulting data were averaged. Samples were refrigerated at 4 °C and analyzed within 24 h of collection. The contaminant removal efficiency was calculated using the following equation:

$$\xi(\%) = \frac{C_{in} - C_{ef}}{C_{in}} \times 100$$

where

C_{in}: Concentration in the influent (mg/L).

C_{ef}: Concentration in the effluent (mg/L).

ξ: removal efficiency (%).

2.6. Statistical Analysis and Model Validation

Experimental data were analyzed using STATGRAPHICS Centurion XVI.I and Python 3.13.8. Regarding the latter, Gemini (Google DeepMind) assisted in generating and debugging the scripts (utilizing libraries such as Matplotlib 3.10.8 and NumPy 2.4.1) for the graphical visualization of the RSM. A quadratic polynomial model including interaction terms was fitted to the data, and an Analysis of Variance (ANOVA) was performed to determine the statistical significance of the model terms ($p < 0.05$). Furthermore, model adequacy was evaluated through the coefficient of determination (R^2 and adjusted R^2), residual analysis to verify normality and homogeneity of variance, and the lack-of-fit test. Process optimization was performed using the desirability function to identify the operational conditions that maximized organic matter removal (COD, BOD_5, and TOC).

3. Results

3.1. Statistical Model Evaluation via 3^2 Design

A three-level factorial design (3^2) was selected to elucidate the relationship between the independent factors (X_1, X_2) and the response variables (Y). A second-order polynomial

(quadratic) model was developed to fit the experimental results, with coefficients determined via multiple regression analysis. The resulting model equations are presented below:

$$Y_1 = 1331.0 - 279.691x_1 - 318.372x_2 - 312.421x_1x_2 - 345.149x_1^2 + 922.388x_2^2$$

$$Y_2 = 0.6289 + 0.0767x_1 + 0.0883x_2 + 0.0875x_1x_2 + 0.0967x_1^2 - 0.2583x_2^2$$

$$Y_3 = 745.89 - 165.622x_1 - 188.527x_2 - 185.003x_1x_2 - 204.385x_1^2 + 546.202x_2^2$$

$$Y_4 = 0.6489 + 0.0767x_1 + 0.0883x_2 + 0.0875x_1x_2 + 0.0967x_1^2 - 0.258333x_2^2$$

$$Y_5 = 355.392 - 91.9467x_1 - 104.662x_2 - 102.704x_1x_2 - 113.465x_1^2 + 303.225x_2^2$$

$$Y_6 = 0.6989 + 0.0767x_1 + 0.0883x_2 + 0.0875x_1x_2 + 0.0967x_1^2 - 0.2583x_2^2$$

where

Y_1: COD concentration in the effluent (mg/L).
Y_2: COD removal efficiency (%).
Y_3: BOD_5 concentration in the effluent (mg/L).
Y_4: BOD_5 removal efficiency (%).
Y_5: TOC concentration in effluent (mg/L).
Y_6: TOC removal efficiency (%).

The developed model was employed to predict organic matter removal (COD, BOD_5, and TOC) in mesocosm-scale constructed wetlands treating slaughterhouse wastewater. A normal probability plot of the residuals was analyzed to verify the normality assumption. The residual diagnostics, illustrated in Figure 3, indicate that the residuals follow a normal distribution (aligning with the straight line), thereby satisfying the most critical assumption for statistical model validation. Consequently, the constructed quadratic model was deemed adequate.

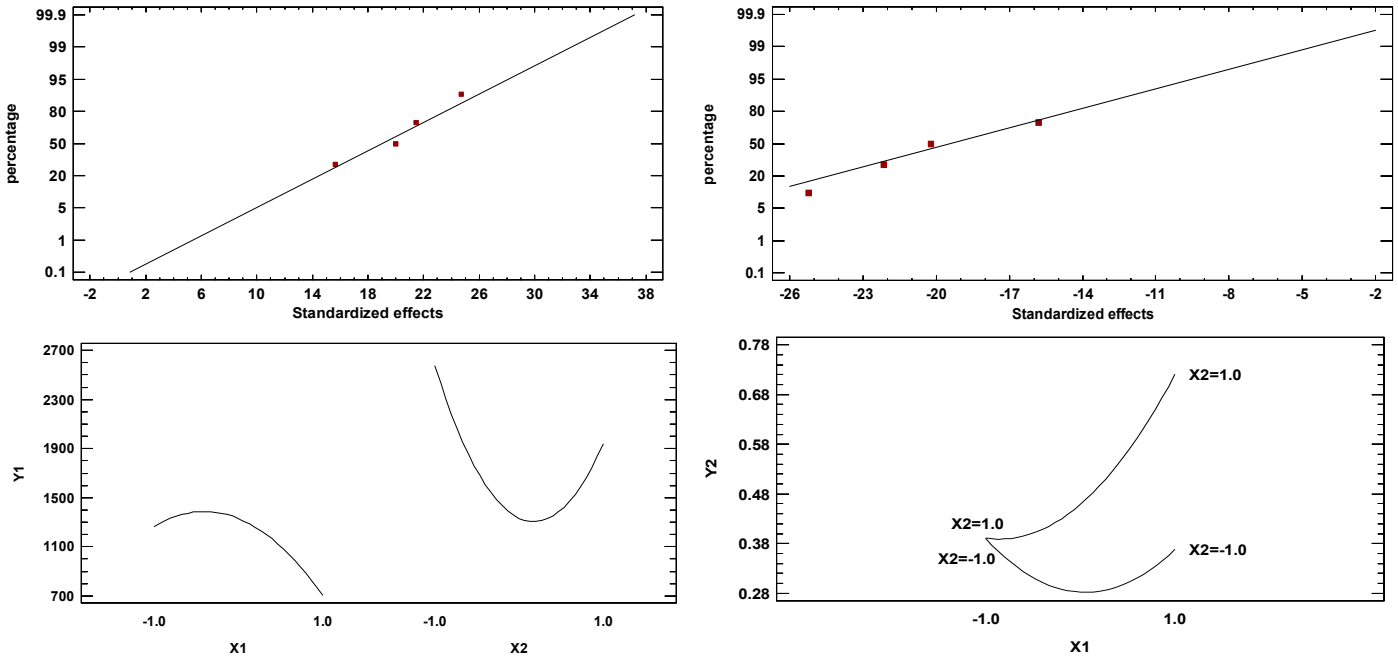


Figure 3. Normal probability plot of standardized residuals for the quadratic model, main effects, and interaction effects for COD removal efficiency (The lines connect the mean response values at each experimental level, illustrating the trends for the main effects and the interaction between factors).

The experimental versus predicted values for the response variables are presented in Table 2. Regarding the variability of the effects shown in Figure 3, the Standard Error of the

Estimate indicated that the residual standard deviation (serving as the global error margin for the model) ranged from ±14.38 to ±43.73 mg/L for effluent concentrations and was approximately ±1.24% for removal efficiencies. This low standard error confirms the high reliability of the main effects and interactions displayed.

Figure 4 presents the parity plots (observed vs. predicted values) for COD, BOD_5, and TOC removal efficiencies. As illustrated, the experimental data points exhibit a tight distribution along the unity line ($y = x$), demonstrating a high degree of agreement between the model predictions and the actual system performance. The minimal data dispersion and absence of significant outliers visually corroborate the high coefficients of determination ($R^2 > 0.99$) and low standard errors obtained from the ANOVA. Consequently, the second-order polynomial model is validated as robust and highly accurate for predicting organic matter removal within the studied experimental domain.

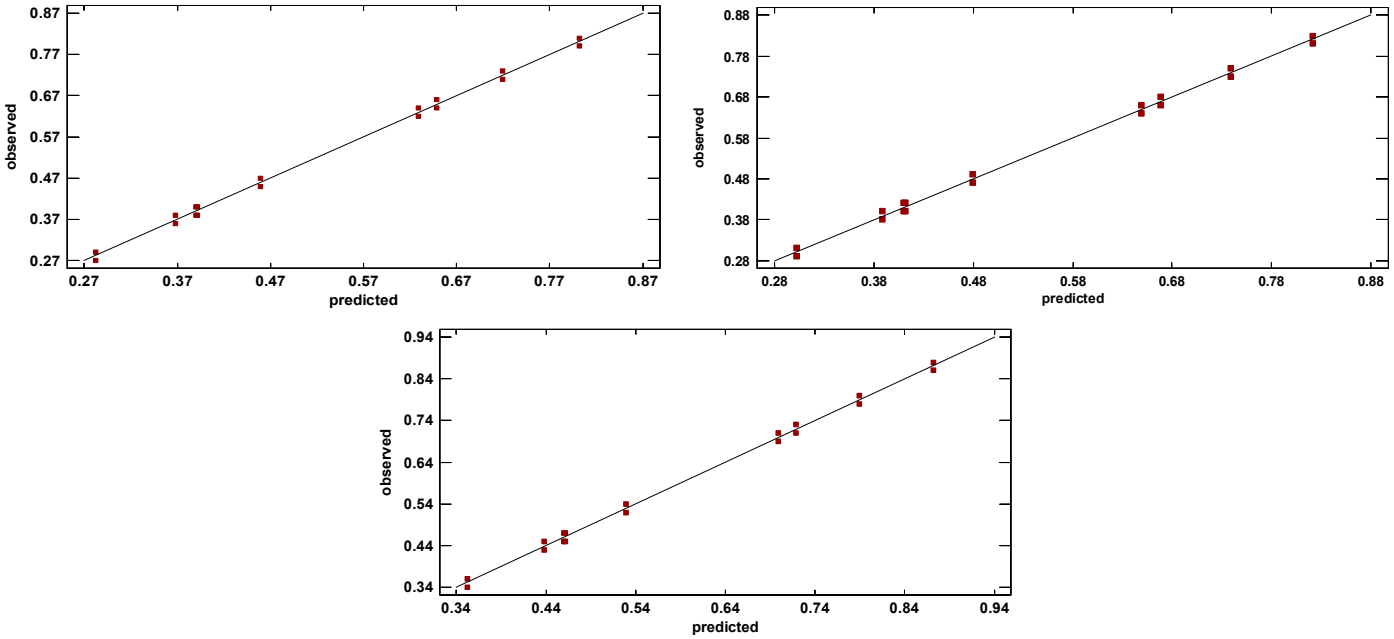


Figure 4. Observed versus predicted values for COD, BOD_5, and TOC removal efficiencies.

The experimental results demonstrate that removal efficiencies for COD, BOD_5, and TOC were not merely dependent on single factors but were driven by the interaction between the rhizospheric complexity and the hydraulic contact time. Observed removal efficiencies spanned a wide range, from 27% to 83%, underscoring that system performance is critically dependent on the equilibrium between hydraulic loading rates and biological degradation kinetics.

Overall, the polyculture system exhibited superior performance across all evaluated retention times, achieving maximum removal efficiencies of 81% for COD, 83% for BOD_5, and 88% for TOC. Notably, all these peak values were recorded at an HRT of 5 days. These results suggest that the combination of plant species induces a synergistic effect that enhances microbial activity and facilitates contaminant retention.

Conversely, *T. latifolia* exhibited the lowest removal efficiencies, particularly at an HRT of 3 days, recording minimum values of 27% for COD, 29% for BOD_5, and 34% for TOC. These findings suggest that this species requires a longer contact time to achieve performance levels comparable to the other evaluated configurations [35–37].

H. latispatha displayed intermediate yet consistent performance, with efficiencies showing significant improvement at an HRT of 5 days (66–73%). However, a slight decline was observed at an HRT of 10 days. This behavior can be attributed to the divergence between hydraulic retention and optimal biological kinetics [30]. From a biochemical

perspective, substrate limitation likely induced endogenous respiration and biomass decay, resulting in the secondary release of Soluble Microbial Products (SMPs) and nutrients into the bulk liquid [37]. Furthermore, the depletion of dissolved oxygen restricted oxidative pathways such as nitrification [38]. Simultaneously, physical limitations—specifically adsorption site saturation, evapotranspiration-driven concentration, and hydraulic short-circuiting caused by biofilm clogging—reduced the effective hydraulic efficiency, thereby negating the theoretical advantages of extended residence times [39–41].

Across all evaluated scenarios, an HRT of 5 days was identified as the optimal condition for maximizing the removal of both biodegradable and refractory organic matter. This finding is pivotal for the design of constructed wetlands, as it enables the optimization of land area requirements (footprint), costs, and overall efficiency, thereby avoiding prolonged retention times that do not necessarily yield significant improvements in system performance [41].

These results underscore the critical importance of considering both vegetation type and HRT in the design and operation of natural treatment systems. The appropriate selection of these factors can significantly enhance organic contaminant removal efficiency in HSSF-CW [42].

The ANOVA results for the proposed model are summarized in Table 4. These results demonstrate that the regression equations adequately describe the relationship between the response variables (COD, BOD_5, and TOC removal) and the significant input factors.

According to the ANOVA results (Table 4), the model F-values were 368.15 for COD, 368.14 for BOD_5, and 368.14 for TOC. These high F-values indicate that the majority of the variability in the responses is accounted for by the regression model, implying that the model terms exert a statistically significant influence on the response variables.

The associated *p*-value was used to assess whether the F-value was sufficiently high to indicate statistical significance. The *p*-values obtained for COD, BOD_5, and TOC removal were less than 0.05, confirming that the model is statistically significant at the 95% confidence level. This suggests that both vegetation type (X_1) and HRT (X_2), as well as their interactions, are critical factors for the effective removal of organic contaminants in constructed wetlands [26].

Furthermore, the low *p*-values associated with the quadratic (X_1^2, X_2^2) and interaction ($X_1 \cdot X_2$) terms further substantiate the importance of these factors, highlighting their significant influence on the treatment system's performance. This analysis corroborates the validity of the proposed model and confirms that the evaluated factors exert a significant influence on contaminant removal, thereby justifying its application for the design optimization of treatment systems [43].

To optimize COD, BOD_5, and TOC removal, establishing a model with a 'good fit' is essential to avoid misleading or inconsistent results. To assess the adequacy of the proposed model, the coefficient of determination (R^2), the F-test, and the Lack-of-Fit test were evaluated. The ANOVA results indicated that the response surface models were statistically significant ($p < 0.05$), exhibiting high adjusted R^2 values.

The COD model yielded an R^2 value of 0.997 and an adjusted R^2 of 0.995, indicating excellent goodness of fit. Similarly, the BOD_5 model exhibited an R^2 of 0.9965 and an adjusted R^2 of 0.9951, while the TOC model achieved an R^2 of 0.9964 and an adjusted R^2 of 0.9949. These results demonstrate that the proposed models effectively account for the variability in the response variables.

The model *p*-values were found to be less than 0.05, confirming their statistical significance at the 95% confidence level. This implies that the model variables—specifically vegetation type and HRT—are critical factors significantly influencing organic contaminant removal.

Table 4. ANOVA analysis of the quadratic model for COD, BOD_5, and TOC removal efficiencies.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F-Test	*p*-Value
COD					
X_1	938,724	1	938,724	368.15	0.000
X_2	1.22×10^6	1	1.22×10^6	477.02	0.000
X_1^2	476,512	1	476,512	186.88	0.000
X_1X_2	780,856	1	780,856	306.24	0.000
X_2^2	3.40×10^6	1	3.40×10^6	1334.67	0.000
Lack of Fit	9.7406×10^{-6}	3	3.24686×10^{-6}	0	0.9995
Pure Error	2.29×10^4	9	2549.85		
Correlated Total	6.84×10^6	17			
R^2 = 99.7%; $R_{aj}^2 = 99.5\%$					
BOD_5					
X_1	329,166	1	329,166	368.14	0.000
X_2	426,511	1	426,511	477.01	0.000
X_1^2	167,093	1	167,093	186.88	0.000
X_1X_2	273,807	1	273,807	306.23	0.000
X_2^2	1.19×10^6	1	1.19×10^6	1334.65	0.000
Lack of Fit	1.667×10^{-5}	3	5.55652×10^{-6}	0	0.9998
Pure Error	8047.15	9	894.128		
Correlated Total	2.40×10^6	17			
R^2 = 99.65%; $R_{aj}^2 = 99.51\%$					
TOC					
X_1	101,450	1	101,450	368.14	0.000
X_2	131,449	1	131,449	476.99	0.000
X_1^2	51,497.2	1	51,497.2	186.87	0.000
X_1X_2	84,384.5	1	84,384.5	306.21	0.000
X_2^2	367,782	1	367,782	1334.59	0.000
Lack of Fit	2.0834×10^{-5}	3	6.94468×10^{-6}	0	0.9997
Pure Error	2480.19	9	275.577		
Correlated Total	739,043	17			
R^2 = 99.64%; $R_{aj}^2 = 99.49\%$					

Note(s): X_1: Vegetation Type, X_2: HRT, F-test: Fisher's ratio, *p*-value: probability value, R^2: coefficient of determination, R_{aj}^2: adjusted coefficient of determination.

The signal-to-noise ratio, quantified by the 'Adeq Precision' parameter, is critical for assessing the model's ability to navigate the design space effectively. A ratio exceeding 4 is generally considered desirable. In this study, values of 7.44 for COD removal and 6.62 for both BOD_5 and TOC removal were recorded. These results indicate an adequate signal, thereby validating the applicability of the quadratic model for process optimization.

Consequently, the fitted quadratic model is suitable for navigating the design space and accurately predicting contaminant removal in constructed wetlands. Thus, this model serves as a robust tool for the optimization of the system's operational conditions. The experimental validation of this model, including confirmatory runs at the optimal conditions, is detailed in Section 3.2.

While the degradation of organic matter in CWs is fundamentally a biological process driven by the synergy between the filter media, microbial biomass, and vegetation, this study focuses on the macroscopic operational parameters that regulate these interactions [44].

HRT and Vegetation Type were selected as the independent experimental factors, constituting the primary governing variables of the system's performance [45]. HRT dictates the hydraulic residence time necessary for microbial kinetic reactions, while vegetation modulates the rhizospheric microenvironment (e.g., via radial oxygen loss and root exu-

dates) to enhance biological activity. The physicochemical properties of the support matrix were standardized across all experimental units to eliminate confounding effects, thereby isolating the specific contributions of macrophyte species and hydraulic loading rates to the treatment efficiency.

Table 3 presents the complete experimental matrix, contrasting the raw experimental data (Observed) against the model-predicted values derived from the 18 runs of the 3^2 factorial design. Additionally, this table summarizes the results of the Lack-of-Fit tests for COD, BOD5, and TOC removal. The F-values obtained indicated that the lack of fit was not statistically significant relative to the pure error. This implies that the variation in the data around the fitted model is consistent with random error rather than structural model deficiencies. This implies a significant correlation between the independent variables and the process response, indicating that the model is adequate for describing the relationship between the experimental factors and the observed responses.

For all evaluated parameters (COD, BOD_5, and TOC), the p-values > 0.9995 associated with the Lack-of-Fit test suggest that there is insufficient evidence to reject the null hypothesis regarding model adequacy. This indicates that the observed variations in the data are not significant relative to the pure error, confirming that the proposed model is suitable for describing the relationship between the input variables and the contaminant removal responses. Consequently, the Lack-of-Fit results underscore the model's quality and its capability to reliably predict system responses.

Therefore, based on the Lack-of-Fit test results and the obtained p-values, it is concluded that the quadratic model is adequate and fits the experimental data satisfactorily. The absence of significant lack of fit validates the reliability of the proposed model

3.2. Optimization of Operational Conditions

The objective of the optimization process was to identify the experimental conditions that maximize the removal efficiency of COD, BOD_5, and TOC from slaughterhouse effluents. The system performance was assessed through six response variables (Y_1–Y_6), expressed in terms of residual concentration (mg/L) and removal efficiency (%). To this end, the desirability function methodology was employed. The optimization criteria included minimizing the residual concentrations of COD, BOD_5, and TOC (Y_1, Y_3, and Y_5) while simultaneously maximizing their respective removal efficiencies (Y_2, Y_4, and Y_6). All response variables were assigned a weight and importance value of 3.0, reflecting the equal significance of each parameter regarding the quality of the treated effluent.

The optimization process yielded a maximum predicted desirability value of 0.9836, with a corresponding observed desirability of 0.9630 (Figure 5). Under these optimal conditions, the following response values were obtained: Y_1 = 741.87 mg/L, Y_2 = 0.79, Y_3 = 397.03 mg/L, Y_4 = 0.81, Y_5 = 161.72 mg/L, and Y_6 = 0.86. These results indicate highly efficient organic matter removal.

Comparing the experimental values with those predicted by the model under optimized conditions revealed a high degree of agreement, with deviations below 3%. This result validates the robustness of the fitted regression model and its accuracy in reproducing the system's behavior. The minimal contaminant concentrations (lowest values for Y_1, Y_3, and Y_5) and the maximal efficiencies achieved (values approaching 88% for Y_6) demonstrate the effectiveness of the evaluated system under these operating conditions.

In contrast, the least desirable conditions (e.g., Y_1 = 2607.47 mg/L, Y_2 = 0.27, Y_3 = 1501.76 mg/L, Y_4 = 0.29, Y_5 = 775.02 mg/L, and Y_6 = 0.34) yielded a desirability value of only 0.0204. This result underscores the system's marked sensitivity to operational conditions. This behavior highlights the need for precise control over the operational variables to maximize the performance of the constructed wetland.

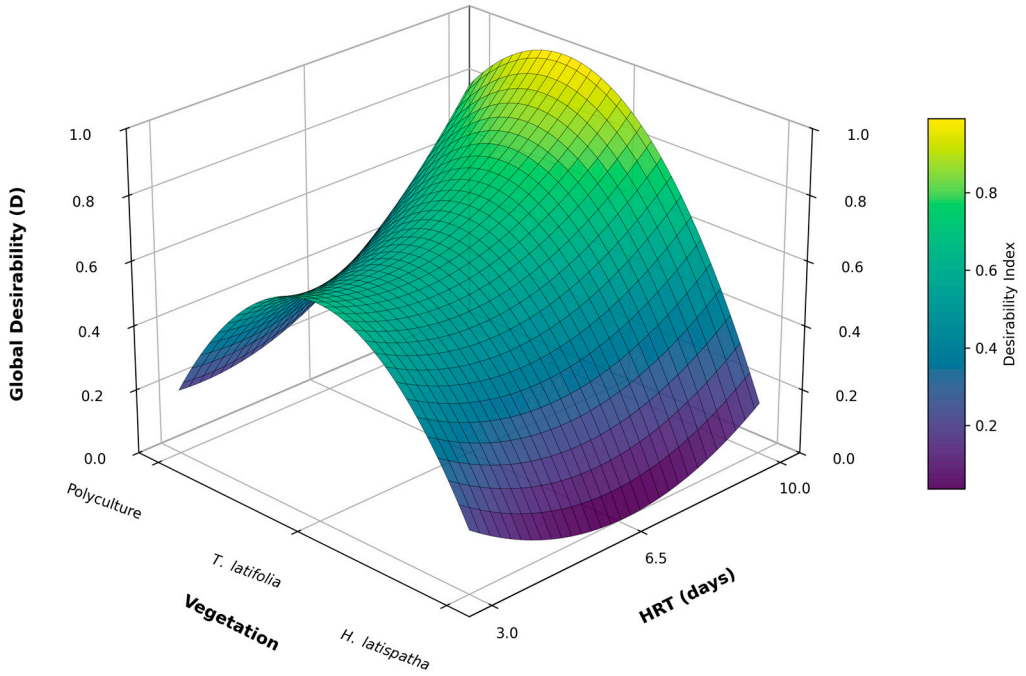


Figure 5. Desirability function for numerical optimization conditions.

These results demonstrate the potential of constructed wetlands as an efficient and sustainable technology for the treatment of slaughterhouse effluents, particularly when operated under optimized conditions. Furthermore, the minimal margin of error between the model predictions and the observed values confirms the adequacy of the employed response surface model for system prediction and optimization. Consequently, it can serve as a decision-support tool for the design and operation of similar treatment systems [46].

3.3. Relationship Between Responses and Variables Under Optimal Conditions

The objective of this study was to optimize the operational conditions of a horizontal subsurface flow constructed wetland to maximize the organic matter removal efficiency (COD, BOD_5, and TOC) while simultaneously minimizing its residual concentrations in the effluent (Figures 6 and 7). The derived response surface equations model the influence of HRT (X_1) and vegetation type (X_2) on the system's response variables: residual concentration and removal efficiency of COD, BOD_5, and TOC (Y_1–Y_6).

The effluent COD concentration (Y_1) exhibited a significant decrease with increasing HRT. This trend, associated with the quadratic coefficient, indicates that prolonged retention times favor the degradation of organic matter. Furthermore, vegetation type exerted a significant influence on system performance. The use of polycultures resulted in superior efficiencies, as evidenced by the positive interaction effect observed in Y_2. This finding suggests that optimal performance is achieved by combining a high HRT with plant diversity, thereby enhancing biofiltration and rhizodegradation mechanisms [47].

Regarding BOD_5, the effluent concentration (Y_3) decreased with increasing HRT, exhibiting a trend analogous to that observed for COD. The removal efficiency (Y_4) mirrored the behavior of Y_2, indicating a consistent pattern: maximal efficiencies were achieved under polyculture conditions and higher HRT. This finding reinforces the synergy between extended contact time and plant biodiversity in the rhizosphere, which facilitates the aerobic biodegradation of organic compounds [48].

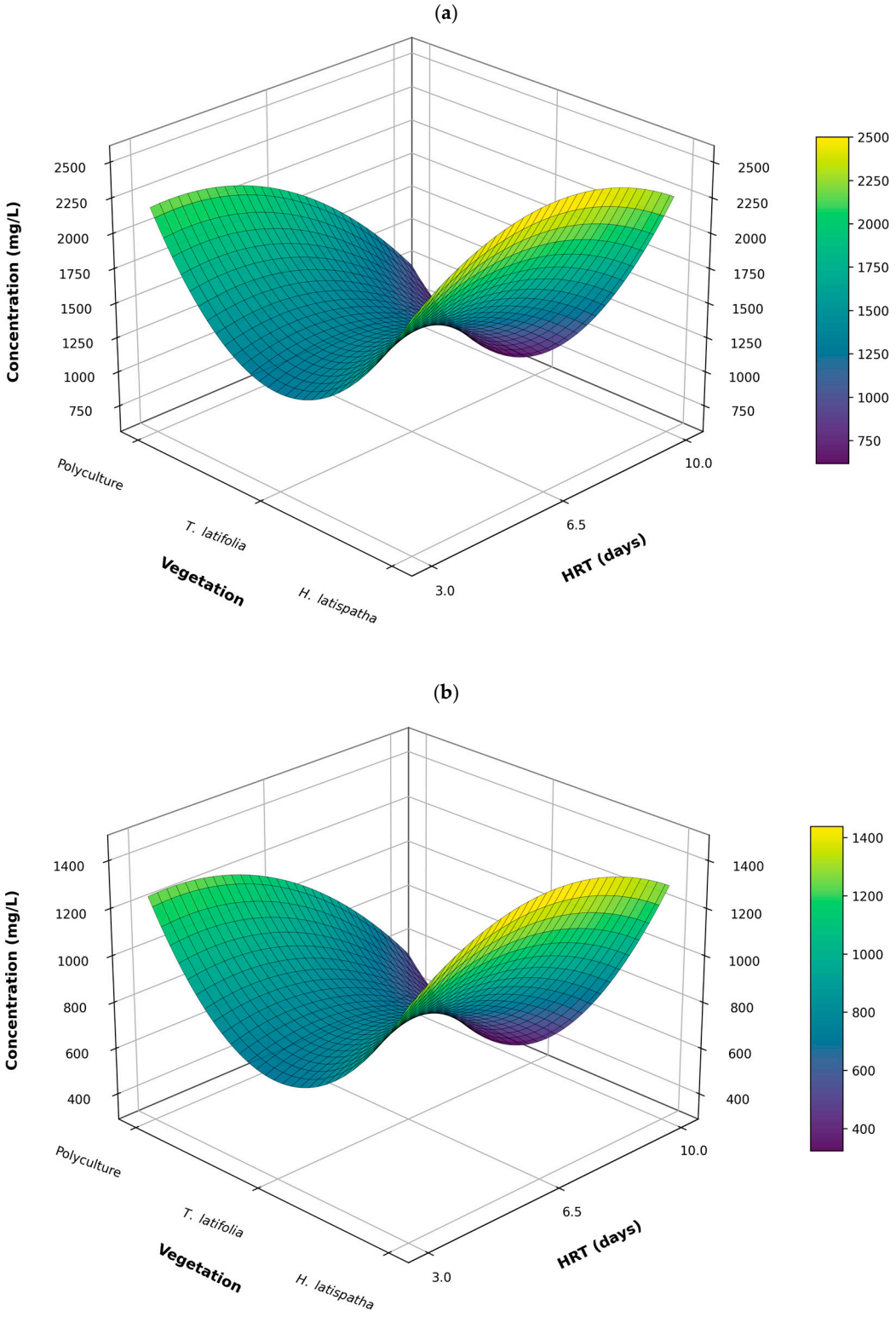


Figure 6. *Cont.*

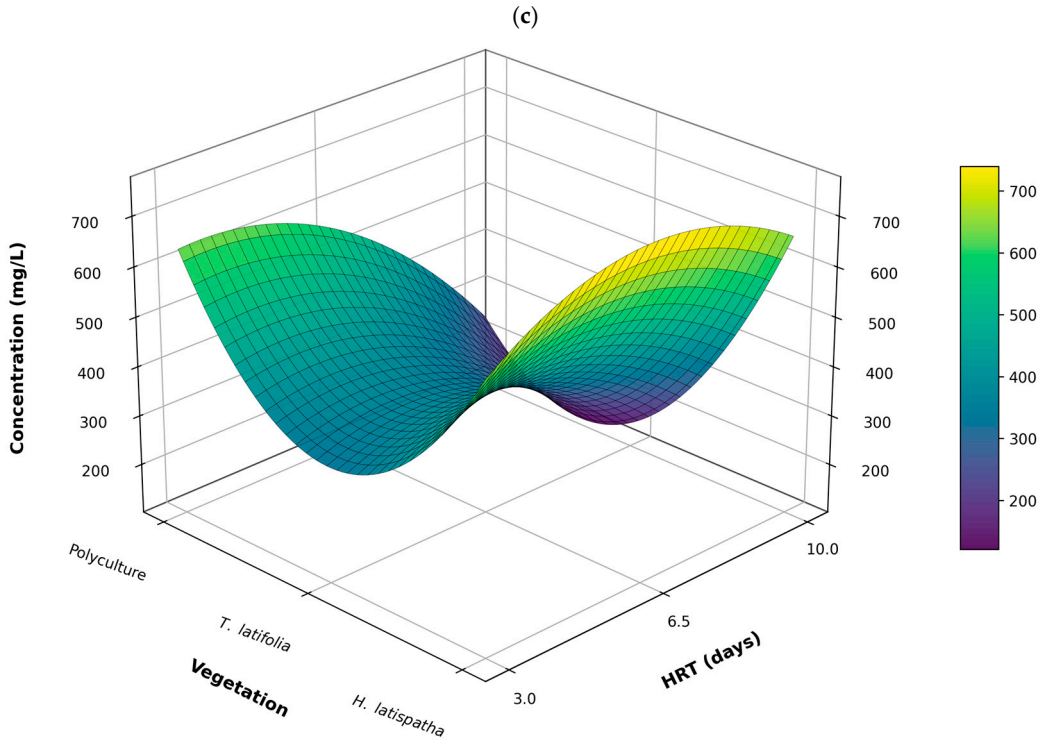


Figure 6. Response surface plots for the concentration (mg/L) of: (**a**) Y_1: COD, (**b**) Y_3: BOD_5, and (**c**) Y_5: TOC in slaughterhouse wastewater as a function of the independent variables X_1: HRT and X_2: vegetation.

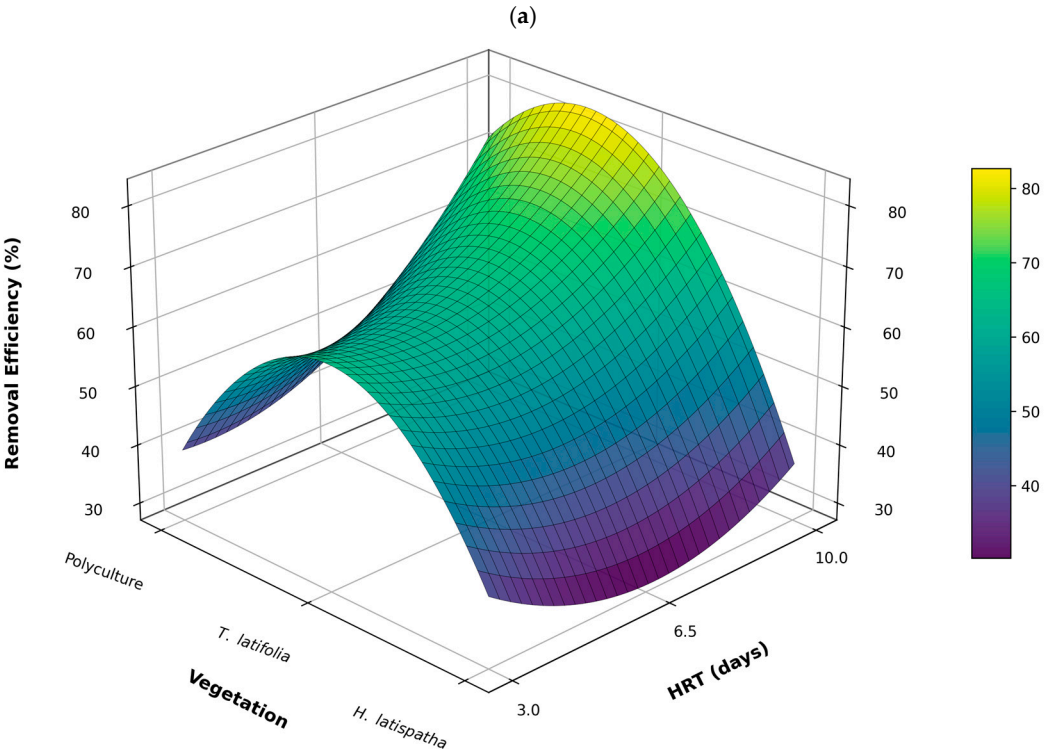


Figure 7. *Cont.*

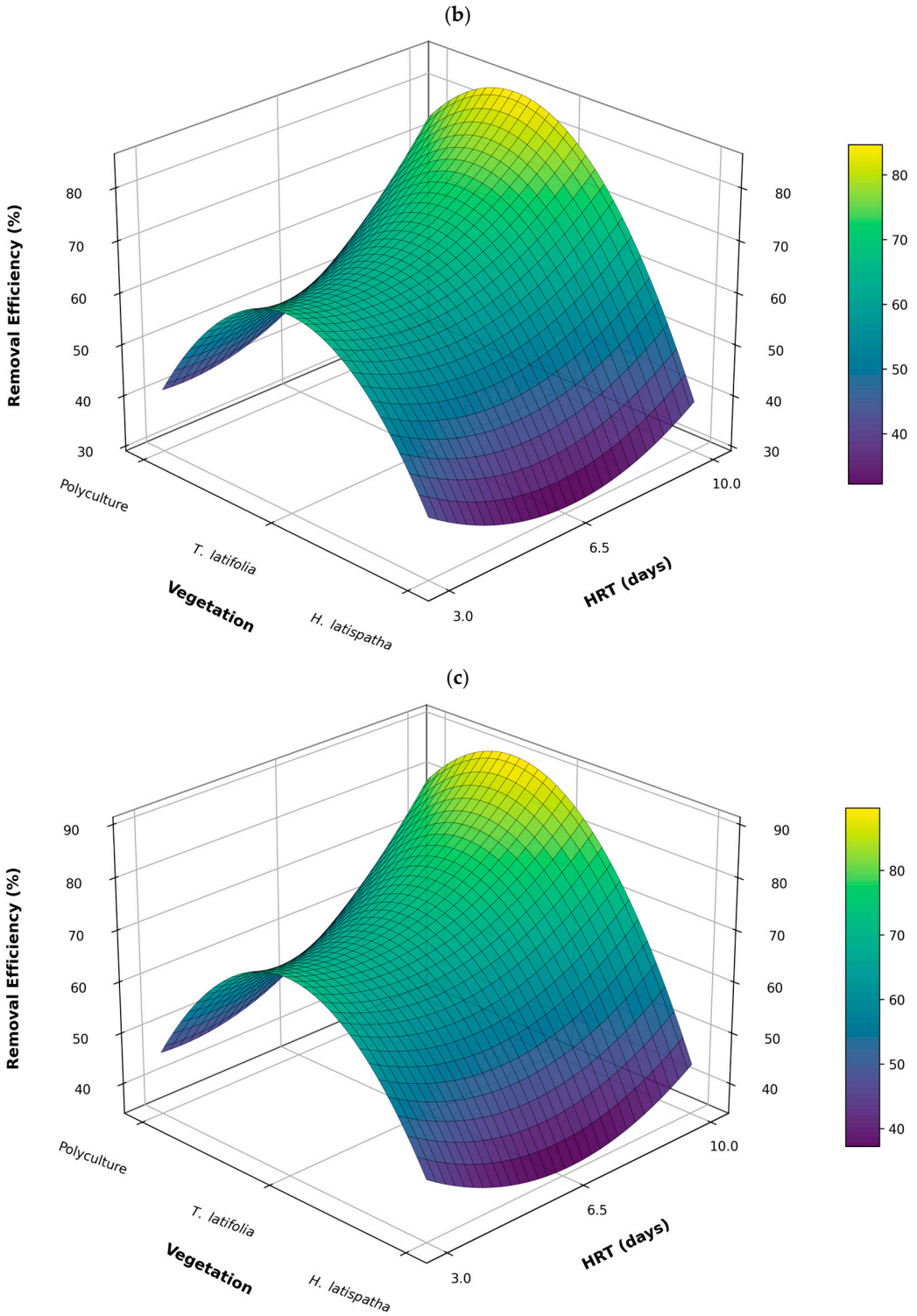


Figure 7. Response surface plots for the removal (%) of: (**a**) Y_2: COD, (**b**) Y_4: BOD_5, and (**c**) Y_6: TOC in slaughterhouse wastewater as a function of the variables: X_1: HRT and X_2: vegetation.

TOC exhibited a similar trend. Effluent concentration (Y_5) decreased with increasing HRT, while removal efficiency (Y_6) was maximized under polyculture conditions. The presence of both plant species may have enhanced oxygen transfer to the rhizosphere, providing a greater diversity of microhabitats for degrading microorganisms [49].

Collectively, the response surfaces indicated that HRT (X_1) is a critical variable for wetland performance. Longer retention times prolong the contact between wastewater, plants,

and substrate, thereby favoring biological degradation. Furthermore, vegetation type (X_2) exhibited a significant influence: the polyculture (*Typha latifolia* + *Heliconia latispatha*) yielded superior results compared to monocultures. This suggests that plant biodiversity may be a key factor in enhancing treatment efficiency within natural systems [50,51].

3.4. Comparison with Similar Studies

This study focused on optimizing the removal of organic matter (COD, BOD_5, TOC), as high organic load is the primary constraint in slaughterhouse wastewater. Consequently, parameters such as heavy metals, specific pathogens, and tertiary disinfection steps were outside the experimental scope. This study achieved removal efficiencies of up to 86.5% for COD, 89.4% for BOD_5, and 91.5% for TOC. While these values confirm the system's effectiveness, a critical comparison with literature reveals how factors such as system configuration, climatic conditions, and influent characteristics drive performance differences.

Influence of System Configuration. Several studies resort to hybrid systems to handle high organic loads. For instance, Galindo-Montero et al. [15] implemented a coupled system comprising an Upflow Anaerobic Sludge Blanket (UASB) reactor followed by an HSSF-CW, achieving 85% COD and 75% BOD_5 removal (HRT = 7.5 days). Notably, the present study achieved comparable, and in some cases superior, efficiencies (86.5% COD) using a single-stage HSSF-CW. This suggests that the specific polyculture arrangement (*T. latifolia* + *H. latispatha*) provided sufficient rhizosphere complexity to promote degradation rates typically requiring pre-treatment steps in other configurations.

Climatic and Environmental Factors. Climate plays a pivotal role in biological kinetics. Studies in varying climates, such as the poultry slaughterhouse treatment in Vietnam (78.7% COD removal) [52] and the municipal slaughterhouse study in Tanzania (75.9% COD removal) [53], report slightly lower organic removal rates. The superior performance observed in our study can be partially attributed to the tropical climatic conditions of the region (average temperatures > 25 °C). Higher temperatures significantly enhance metabolic rates and enzymatic activity of the microbial biomass, offering a kinetic advantage over systems operating in subtropical or temperate zones.

Technological Approach (Passive vs. Active). When comparing biological methods with advanced oxidation processes, the trade-offs become evident. A study in Iran combining a UASB with a Fenton process achieved a remarkable 98% COD removal [54]. While strictly more efficient, such active processes entail high operational costs (energy and reagents) and technical complexity. In contrast, the wetland system evaluated here, with ~88% efficiency, offers a balance between performance and sustainability, making it more suitable for rural or developing contexts where low maintenance is prioritized over polishing to potable standards.

Ultimately, while influent variability (e.g., blood content and C/N ratios) inherently affects absolute removal values across studies, the results confirm that optimizing HRT and vegetation diversity in a tropical single-stage wetland can yield effluents comparable to more complex hybrid systems.

4. Discussion

4.1. Statistical Validation and Model Robustness

The application of Response Surface Methodology (RSM) via a three-level factorial design proved to be a robust approach for modeling the complex biochemical dynamics of slaughterhouse wastewater treatment. The high coefficients of determination ($R^2 > 0.99$) and adjusted R^2 values obtained for COD, BOD_5, and TOC models indicate that the second-order polynomial equations explain over 99% of the variability in the process. This aligns

with ANOVA, where high F-values and p-values < 0.05 confirmed the statistical significance of both the linear and quadratic terms of vegetation type and HRT.

Crucially, the insignificant lack-of-fit ($p > 0.05$) and the normal distribution of residuals confirm that the models are free from structural bias and adequate for navigation within the design space. These findings corroborate the previous literature suggesting that quadratic models are superior to linear regressions for predicting non-linear biological processes in constructed wetlands, particularly when characterizing the removal of high-strength organic loads typical of agro-industrial effluents.

4.2. Mechanistic Influence of HRT and Vegetation Regimes

The results underscore the critical interdependence between HRT and vegetation diversity. The optimization process identified an HRT of 5 days as the operational optimum. This finding represents a kinetic equilibrium: distinct from an HRT of 3 days, where contact time is insufficient for adequate hydrolysis and diffusion of pollutants into the biofilm, and an HRT of 10 days, where the system likely approaches substrate limitation or experiences the release of secondary metabolites.

The superior performance of the polyculture system (*T. latifolia* + *H. latispatha*), achieving removal efficiencies between 81% and 88%, supports the hypothesis of synergistic rhizospheric interactions. While *T. latifolia* is known for its extensive root system that facilitates substrate filtration, its monoculture performance at low HRT was suboptimal (27–34% removal). The introduction of *H. latispatha* appears to enhance the diversity of the root-associated microbial community. This niche differentiation likely provides a broader range of redox conditions (aerobic micro-zones near roots and anoxic zones in the bulk media), facilitating the simultaneous degradation of readily BOD_5 and more recalcitrant compounds (COD, TOC). This observation is consistent with ecological theory suggesting that plant diversity enhances functional stability and treatment resilience in engineered ecosystems.

The removal efficiencies achieved in this study (up to 86.5% for COD and 89.4% for BOD5 under optimized conditions) are competitive with, and in some cases superior to, recent configurations reported in the literature. For instance, the system outperformed the hybrid constructed wetland evaluated by manufacturers in Vietnam (78.7% COD removal) and the municipal slaughterhouse wetland studied by Semba et al. in Tanzania (75.9% COD removal).

Notably, our HSSF-CW achieved results comparable to more complex hybrid systems, such as the UASB-CW configuration reported by Galindo-Montero et al. [15] (85% COD removal at 7.5 days HRT). While advanced oxidation processes (AOPs) combined with biological treatment in Iran achieved higher efficiencies (98% COD removal), the operational simplicity and low energy footprint of the proposed wetland system make it a far more sustainable solution for developing regions or rural agro-industry. Furthermore, our results align with Kumar et al. [55], who reported approximately 80% COD removal in food industry effluents, confirming the reproducibility of CW technology for this wastewater matrix.

The desirability function analysis, which yielded a global desirability of 0.9630, validates the feasibility of simultaneous optimization. By maximizing contaminant removal while minimizing effluent concentration, the model provides a practical tool for reducing the land area requirements (footprint) of wetland systems. Operating at an HRT of 5 days rather than the conventional 7–10 days allows for a significant reduction in reactor volume without compromising effluent quality.

From a broader sustainability perspective, the effectiveness of the polyculture system highlights the potential for using native, locally available macrophytes to construct re-

silient, low-cost treatment infrastructure. This approach not only ensures compliance with environmental discharge standards but also promotes biodiversity and ecosystem services.

4.3. Limitations and Future Research Directions

While this study successfully modeled steady-state performance, future research should investigate the long-term dynamics of the polyculture system, specifically focusing on biomass accumulation and potential clogging mechanisms over extended operational periods. Additionally, metagenomic analyses of the rhizospheric microbiome are recommended to elucidate the specific microbial pathways enhanced by the interaction between *T. latifolia* and *H. latispatha*. However, scaling up these findings to a pilot or full-scale continuous flow system would be essential to validate the hydraulic efficiency and economic viability under fluctuating industrial loading rates.

Furthermore, the application of HSSF-CWs for slaughterhouse wastewater treatment presents specific operational challenges due to high organic and nutrient loads. Prolonged loading can lead to rapid biofilm growth and solids accumulation within the porous media, increasing the risk of substrate saturation and clogging. As observed in similar high-strength effluent studies, this physical obstruction can reduce hydraulic conductivity and treatment efficiency over time. Therefore, the implementation of rigorous pretreatment (as applied in this study) and potential resting cycles are critical strategies to mitigate clogging and extend the lifespan of the system.

Finally, while the RSM effectively modeled the influence of controllable operational parameters, the inherent variability of biological systems in outdoor environments suggests the need for more robust optimization strategies. Future investigations should consider implementing Taguchi experimental designs, which distinguish between control factors (e.g., HRT, vegetation) and uncontrollable noise factors (e.g., dryness, rainfall, temperature gradients, influent fluctuations). By analyzing the Signal-to-Noise (S/N) ratio, subsequent studies could identify operational configurations that are not only efficient but also resilient to the environmental instability characteristic of tropical zones.

5. Conclusions

The results of this study demonstrate that CWs, under appropriate operational conditions, represent an effective and sustainable alternative for the treatment of wastewater generated in slaughterhouses. The application of RSM enabled the modeling and optimization of the removal of key organic pollution parameters, such as COD, BOD_5, and TOC.

Among the key findings, it was determined that HRT and vegetation type exerted a significant influence on treatment efficiency. Specifically, the use of polycultures (*Typha latifolia* and *Heliconia latispatha*) combined with an extended HRT significantly enhanced pollutant removal. The optimization process achieved maximum removal efficiencies of 86.5% for COD, 89.4% for BOD5, and 91.5% for TOC. These results significantly outperform the values reported in the technical literature for similar systems, validating the proposed configuration as a high-performance solution.

Response surface models allowed for the identification of optimal conditions to maximize COD, BOD_5, and TOC removal efficiencies. This provides a practical and reliable decision-making tool for the design and operation of CWs specifically tailored to the treatment of slaughterhouse effluents.

This study contributes to technical and scientific knowledge by validating the use of CWs as Nature-based Solutions (NbS), specifically applied to meat industry effluents, which have been scarcely addressed in the literature. Furthermore, it provides a holistic approach by integrating multiple water quality indicators and utilizing advanced statistical tools for their analysis and optimization.

Future research initiatives should prioritize the long-term hydrodynamic evaluation of these systems across varying scales (pilot and full-scale), specifically addressing the operational challenges imposed by high-strength slaughterhouse effluents. Given that prolonged organic loading can accelerate biofilm proliferation and solids accumulation—leading to pore occlusion and a critical reduction in hydraulic conductivity—strategies such as rigorous pretreatment protocols and the implementation of intermittent resting cycles must be investigated to mitigate bioclogging and extend substrate lifespan. Additionally, the experimental scope should be broadened to encompass the removal dynamics of nutrient species (nitrogen and phosphorus) and specific pathogens, while exploring alternative native macrophyte configurations.

Finally, while RSM provided a precise model of controllable parameters, the inherent instability of outdoor biological systems warrants the adoption of robust design strategies. Future studies should integrate Taguchi experimental methods to systematically decouple control factors from uncontrollable stochastic noise (e.g., precipitation variability, temperature gradients). By analyzing the Signal-to-Noise (S/N) ratio, subsequent research can identify operational configurations that ensure not only high treatment efficiency but also resilience against the environmental fluctuations characteristic of tropical agro-industrial zones.

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