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Original Article

Performance of anaerobic and aerobic biological reactors in psychrophilic wastewater treatment

Desempeño de reactores biológicos anaerobios y aerobios en tratamiento psicrofílico de aguas residuales

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Abstract

Introduction: this study evaluated the efficiency of organic matter removal in synthetic wastewater under psychrophilic conditions of 12°C, representative of the city of Pasto. Three types of biological reactors were used: upflow anaerobic sludge blanket reactor (UASB), attached-growth anaerobic reactor (AGAR), and aerobic trickling filter.
Objective: to determine the influence of reactor type and hydraulic retention time (HRT) on chemical oxygen demand (COD) removal in wastewater treatment under psychrophilic conditions.
Methodology: experiments were conducted with COD concentrations ranging from 96 to 1440 mg/L and different HRT levels. System performance was evaluated using statistical analysis based on a factorial experimental design and exploratory data analysis (EDA). These tools enabled the characterization of COD removal distribution across systems and the assessment of statistical significance in the observed differences.
Results: COD removal ranged from 75% to 87.5% across the systems. Both reactor type and HRT showed a significant influence on COD removal. In the UASB reactor, lower HRT favored higher COD removal, whereas in the AGAR and the trickling filter, no significant differences were observed between the HRT levels evaluated.
Conclusions: HRT was identified as a critical factor for organic matter removal efficiency under psychrophilic conditions, as it compensates for low-temperature effects depending on reactor type. Furthermore, statistical analysis indicated that pH was the determining factor for COD removal in the three systems studied. Optimization of HRT, together with other operational parameters, is essential to maximize the efficiency of biological treatment processes and ensure adequate effluent quality.

Keywords: Biological treatments, hydraulic retention time (HRT), organic matter removal, psychrophilic conditions, wastewater.

Resumen

Introducción: este estudio evaluó la eficiencia de remoción de materia orgánica en aguas residuales sintéticas bajo condiciones psicrofílicas de 12°C, propias de la ciudad de Pasto. Para ello, se utilizaron tres tipos de reactores biológicos: reactor anaerobio de flujo ascendente (RAFA), reactor anaerobio de crecimiento adherido (RACA) y filtro percolador aerobio.
Objetivo: determinar la influencia del tipo de reactor y del tiempo de retención hidráulica (TRH) en la remoción de la demanda química de oxígeno (DQO) en aguas residuales tratadas bajo condiciones psicrofílicas.
Metodología: los experimentos se desarrollaron con concentraciones de DQO en el rango de 96 a 1440 mg/L y con diferentes TRH. El desempeño de los sistemas se evaluó mediante un análisis estadístico basado en un diseño experimental factorial y en un análisis exploratorio de datos (AED). Estas herramientas permitieron caracterizar la distribución de la remoción de DQO en los distintos sistemas y establecer la significancia de las diferencias observadas.
Resultados: los sistemas alcanzaron remociones de DQO entre 75% y 87.5%. Se confirmó la influencia significativa tanto del tipo de reactor como del TRH. En el reactor RAFA, un TRH bajo favoreció una mayor remoción de DQO, mientras que en los reactores RACA y en el filtro percolador no se observaron diferencias significativas asociadas a este parámetro.
Conclusiones: el TRH se identificó como un factor crítico en la eficiencia de la remoción de materia orgánica bajo condiciones psicrofílicas, ya que permite compensar la influencia de la baja temperatura dependiendo del reactor utilizado. Asimismo, el análisis estadístico indicó que el pH constituyó el factor determinante en la remoción de DQO en los tres sistemas estudiados. La optimización del TRH y de otros parámetros operacionales resulta esencial para maximizar la eficiencia de los procesos biológicos y garantizar la calidad del efluente final.

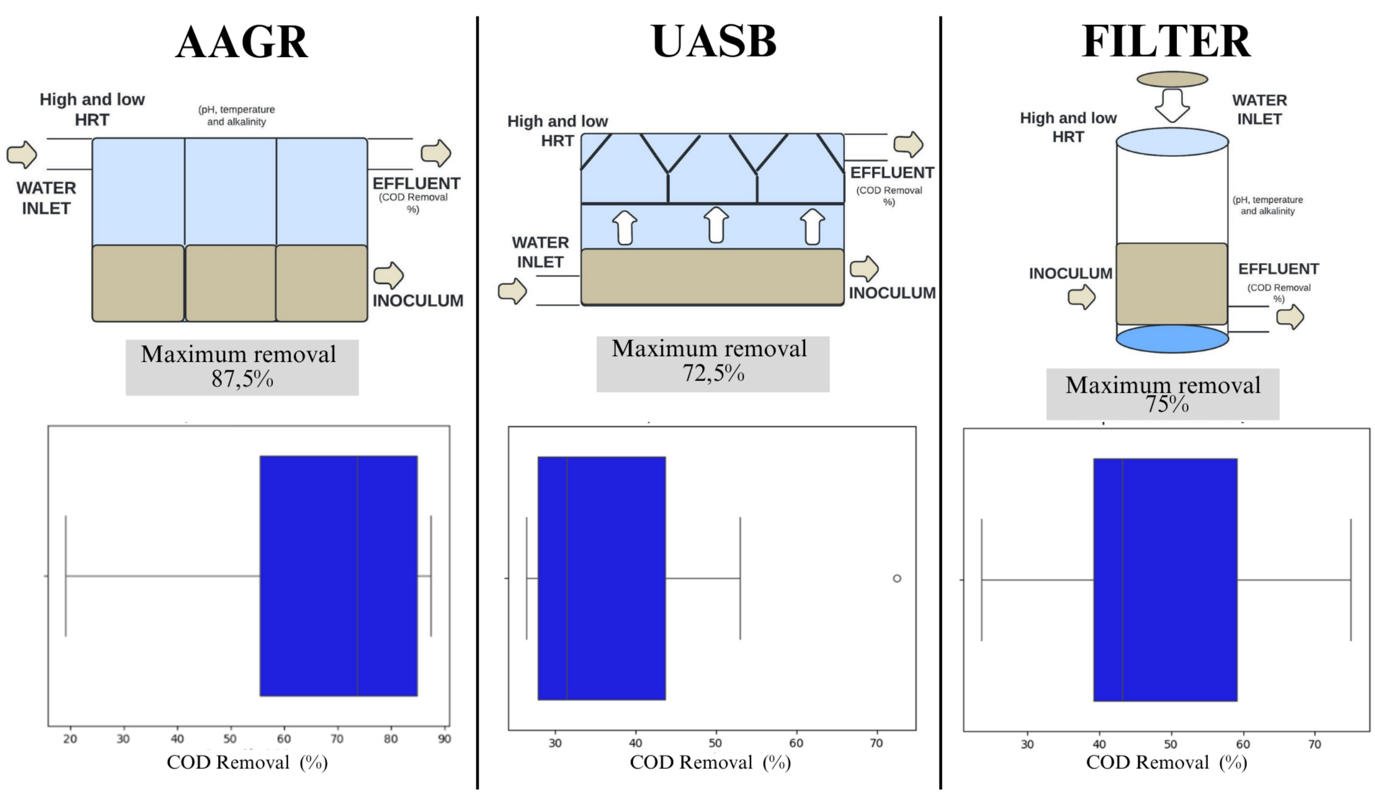
Palabras clave: Agua residual, condiciones psicrofílicas, remoción de materia orgánica, tiempo de retención hidráulica (TRH), tratamientos biológicos

Spanish version

Why was this study conducted?
The study aimed to evaluate the performance of anaerobic and aerobic biological reactors in removing organic matter from synthetic wastewater under psychrophilic (low-temperature) conditions, simulating those found in the city of Pasto. It sought to identify the most effective treatment system and optimal operational conditions for such environments.

What were the most relevant findings?
The most relevant results of the study indicate that pH is the most critical factor influencing COD removal efficiency in all three biological treatment systems. Its consistent impact across systems highlights the need for effective pH control to optimize performance. HRT had a significant effect only in the UASB reactor, pointing to the necessity of flow management in anaerobic processes. Although temperature and alkalinity showed trends, their effects were less consistent and reactor-dependent.

What do these findings contribute?
The use of multiple linear regression models proved effective for predicting optimal operating conditions. Additionally, inoculum acclimation was shown to be a key factor, especially in anaerobic systems where microbial adaptation is slower. These results contribute to the improvement and adaptation of wastewater treatment systems in cold climates, supporting the design of more sustainable and efficient solutions.



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Introduction

The sustainable management of wastewater is a critical global challenge due to its direct impact on environmental preservation and public health. In this context, biological treatments have emerged as an effective alternative for mitigating organic pollution in water bodies, offering solutions that not only comply with environmental regulations but also enable the reuse of treated water, promoting more sustainable and economically viable practices (1).

Water pollution is a significant global issue; it is estimated that approximately 48% of wastewater does not receive adequate treatment, resulting in the discharge of over one billion tons of untreated wastewater into aquatic ecosystems, introducing a variety of harmful contaminants (2). In Colombia, the situation is particularly alarming. A preliminary study conducted in 2020 analyzing the main biological wastewater treatment systems used in the country found that only about 30% of total wastewater is treated (3).

According to the 2015 report on basic consumption ranges by MINVIVIENDA, regarding domestic wastewater, the average household water consumption in Colombia was 10.60 m^3/month in cold climate zones and 15.44 m^3/month in warm climate zones. These discharges, along with those from industrial, agricultural, and other activities, contribute to high organic matter accumulation in rivers. A notable example is the Bogotá River, which receives approximately 165,525 tons of biochemical oxygen demand (BOD) and 375,743 tons of chemical oxygen demand (CD) annually, according to a national water study by IDEAM. The situation is even more critical in regions such as the Nariño department, where the lack of treatment infrastructure has led to the direct contamination of vital water sources, such as the Pasto River, with severe consequences for the environment and public health, as documented in the CORPONARIÑO comprehensive water resource management program.

Among the various technologies available for preventing and controlling water pollution, biological treatments stand out for their efficiency and sustainability. These processes use microorganisms to decompose organic matter and other contaminants, consuming less energy and generating fewer harmful by-products, thereby reducing environmental impact more effectively. Additionally, by lowering the concentration of pathogens and toxic substances, these treatments play a crucial role in preventing diseases associated with the consumption of contaminated water (4).

In this context, the use of synthetic wastewater in laboratory studies has gained importance, as it allows precise control over substrate composition and facilitates the comparison of optimization strategies in biological processes (5). Biological wastewater treatment systems, leveraging microbial activity, are highly efficient in removing suspended solids and degrading organic matter. Their adaptability to diverse environmental conditions, including low temperatures such as those in the city of Pasto, makes them a versatile technology for a wide range of applications (6).

A previous study conducted in Pasto demonstrated that while low temperatures can generate inhibitors that slow down the decomposition of organic matter, they also confer remarkable

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stability to the system. These findings, obtained in a geographical context identical to the present study, with distinct climatic characteristics, underscore the need to further investigate treatment systems adapted to cold environments (7). Advances in this field will not only optimize wastewater management in low-temperature regions but also drive the development of more efficient and sustainable technologies on a global scale.

Moreover, these systems provide a robust alternative for the biological oxidation of pollutants and the removal of nutrients such as nitrogen and phosphorus (8). Although significant progress has been made in wastewater treatment, addressing this particular topic would fill an important research gap, offering a more comprehensive and detailed understanding of the processes involved in cold environments, ultimately optimizing treatment strategies in regions with these climatic characteristics.

In Colombia, the most commonly used wastewater treatment methods include activated sludge, stabilization ponds, and constructed wetlands. These biological methods achieve a BOD_5, COD, and total suspended solids (TSS) removal efficiency of over 80%, with performance exceeding 90% when combined with anaerobic and aerobic systems, depending on wastewater and climatic conditions (3).

Given these considerations, the present study focuses on evaluating biological treatment systems under specific local conditions characterized by a psychrophilic climate. Both anaerobic systems—including an upflow anaerobic reactor (UASB) and an anaerobic attached-growth reactor (AAGR)—and an aerobic system, specifically a trickling filter, were studied. The research aimed to assess microbial adaptation using a synthetic substrate representative of domestic wastewater in Pasto to maintain controlled substrate composition, facilitating process comparisons. Key indicators such as organic load reduction, expressed as COD removal efficiency at different hydraulic retention times (HRT), were used to evaluate system performance. Through physicochemical and statistical analyses, the study sought to identify the most effective biological treatment for urban wastewater under local conditions.

Methodology

Description of the Treatment Systems

Two anaerobic reactors and one aerobic reactor were evaluated. The anaerobic reactors were the anaerobic attached-growth reactor (AAGR) and the upflow anaerobic sludge blanket (UASB) reactor. Both operate in the absence of oxygen (9), degrading organic matter into simpler chemical compounds through different phases, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis (10). The AAGR utilizes support media for the growth of a microbial biofilm, while the UASB reactor relies on a granular sludge bed suspended within the reactor. In both cases, organic matter degradation generates biogas as a by-product and facilitates solids sedimentation (11).

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On the other hand, an aerobic trickling filter was used, which operates in the presence of oxygen. This system consists of a porous bed where an aerobic microbial biofilm develops. Wastewater is distributed over the bed using a sprinkler, which also facilitates system aeration (9). Microorganisms within the biofilm degrade organic matter in the presence of oxygen, producing a treated effluent with a lower organic load. Figure 1 presents a basic schematic of the mentioned reactors.

The reactors used in this study were specifically designed and constructed for research purposes. The trickling filter employed polyethylene hose as the filtering medium due to its durability and biomass retention capacity, while the AAGR used polyurethane foam because of its porous structure, which facilitates microbial colonization. Both reactors were fabricated from transparent acrylic, allowing for easy visualization of internal processes. Methylene chloride adhesive was used to ensure a hermetic and durable bond, guaranteeing the structural integrity of the systems throughout the experiment. These design choices were crucial for obtaining reliable results.

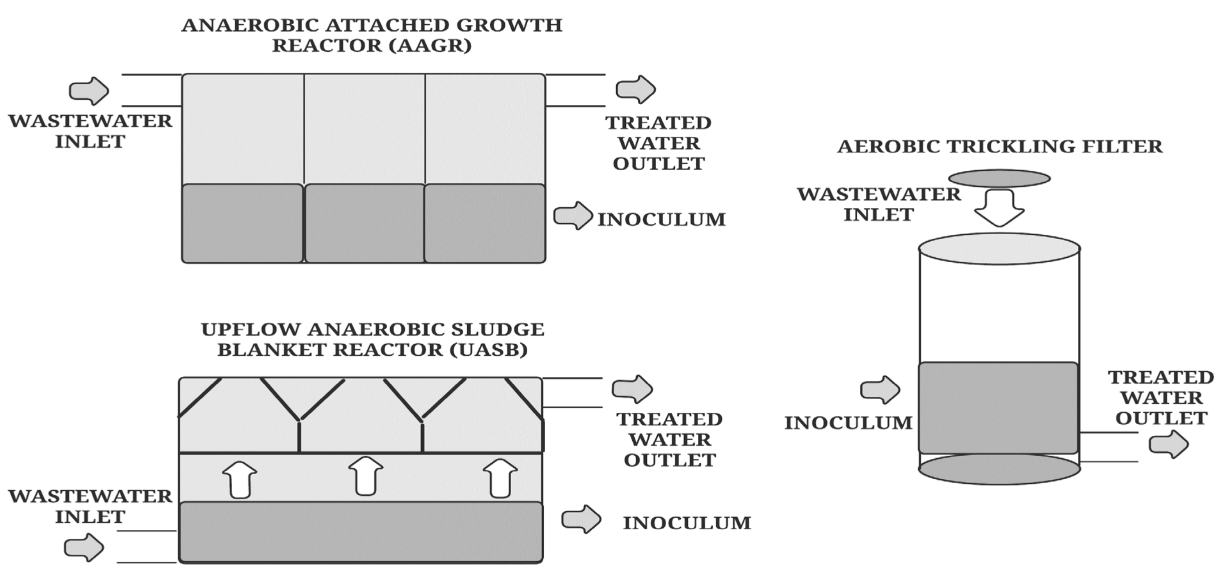

Figure 1. Schematic of Each Studied Reactor

Preparation of Synthetic Wastewater and Inoculum

Based on the characterization of different wastewater collectors in the city of Pasto, synthetic wastewater was prepared, considering that domestic wastewater has a high biodegradable component. Therefore, it was assumed that BOD_5 represents approximately 80% of COD, with an estimated value of 250 mg/L (12). The parameter ranges were determined based on the minimum and maximum values observed in the characterization of the Pasto River. Table 1 presents the quantities of components used for the preparation of synthetic wastewater.

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Table 1. Composition of Synthetic Wastewater

Component	Concentration, (mg/L)
Unflavored gelatin	0.17
Powdered milk	0.51
Glucose	0.94
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.01
Potassium dihydrogen phosphate (KH_2PO_4)	0.22
Ammonium sulfate ($(NH_4)_2SO_4$)	0.37
Sodium bicarbonate ($NaHCO_3$)	0.75

Source: (13)

The resulting solution was subjected to heating and continuous stirring for 15 minutes to ensure complete dissolution of the compounds, preventing the formation of clumps, particularly in components prone to agglomeration. Subsequently, the necessary dilution was performed, and the COD concentration of the final solution was determined.

Regarding the sludge acclimation process, the reactors were fed, and supernatant samples were analyzed to evaluate organic matter removal. Once the desired 80% removal efficiency was achieved, a portion of the sludge was extracted, and a mixture of 60% sludge and 40% water was prepared for further analyses.

The inoculation of the anaerobic reactors (UASB and AAGR) was carried out using sludge obtained from treatment plants handling sugar-processing wastewater and poultry-processing wastewater. For the aerobic reactor (trickling filter), the inoculum was obtained from an aerobic lagoon used for leachate treatment. The sludge was characterized by determining total solids (TS) concentrations using gravimetric methods described in the Standard Methods for the Examination of Water and Wastewater.

Data Collection Instruments

Field parameters such as pH and temperature were monitored in situ using a multiparameter device (Multi 3630 IDS), which was previously calibrated. The calibration process involved verifying the connections, rinsing the electrodes with distilled water, and immersing the sensors in the respective standard calibration solutions. Once the readings stabilized, the device was adjusted according to the manufacturer’s instructions. This procedure ensured the accuracy of the measurements for each established parameter.

The wastewater characterization included the measurement of total alkalinity, determined using the titrimetric method (Method 2320B), and COD, assessed using the open reflux dichromate method (Method 5220B), both described in the Standard Methods for the Examination of Water and Wastewater.

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Statistical Analysis to Determine Factor Influence and Optimization Process

The variables in this study were classified according to Stevens' scale (14), which differentiates between:

- Nominal variables (categories without a specific order)
- Ordinal variables (categories with a defined order)
- Interval variables (numerical values with meaningful differences but no absolute zero)
- Ratio variables (numerical values with an absolute zero).

This classification was essential for selecting the appropriate statistical tests and ensuring the validity of the analyses. A detailed classification of the variables is presented in Table 2.

Table 2. Classification of Variables According to Stevens' Scale

Variable Type	Variable	Numerical/ Non-numerical	Category	Stevens' Scale Classification
Response Variable	COD Removal (%)	Numerical	Continuous	Ratio
Factors	Reactor Type	Non-numerical	Categorical	Nominal
	HRT	Non-numerical	Categorical	Ordinal
Noise Factors	pH	Numerical	Continuous	Interval
	Temperature	Numerical	Continuous	Interval
	Alkalinity	Numerical	Continuous	Ratio
Design Factors	Flow Rate	Numerical	Continuous	Ratio
	Wastewater Composition	Numerical	Continuous	Ratio

This classification facilitated the selection of appropriate statistical tools, allowing for an accurate evaluation of the influence of each factor on COD removal efficiency and guiding the optimization of treatment processes.

Exploratory Data Analysis (EDA)

The data on COD removal (%), pH, temperature, and alkalinity obtained from the AAGR, UASB, and Trickling Filter reactors were subjected to descriptive analysis. Measures such as mean, standard deviation, minimum and maximum values, and percentiles were calculated for each combination of reactor type and hydraulic retention time (HRT) level.

To visualize and compare the distribution of these parameters—including COD removal between HIGH and LOW HRT levels for each reactor—boxplots were used. These graphical representations illustrate the median, quartiles (25%, 50%, and 75%), and outliers, which significantly deviate from the rest of the observations. This visual representation facilitates an understanding of variability and central tendency, providing a solid basis for selecting appropriate statistical tests and interpreting results.

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Influence of HRT Factor for Each Reactor

To determine whether HRT (HIGH and LOW levels) significantly influences COD removal, different statistical tests were conducted depending on the case. The first step involved assessing data normality using the Shapiro-Wilk test.

- If the data followed a normal distribution, Levene's test was used to check for homogeneity of variances.
- If variances were equal, a parametric ANOVA (Student's t-test) was performed to determine whether there were significant differences (14).
- If the data did not follow a normal distribution, a non-parametric ANOVA (Mann-Whitney U test) was applied to identify significant differences between groups.

The decision-making process for selecting the appropriate statistical tests when comparing mean (or median) COD removal between HIGH and LOW HRT levels is illustrated in the flowchart presented in Figure 2. (TRH Alto y Bajo).

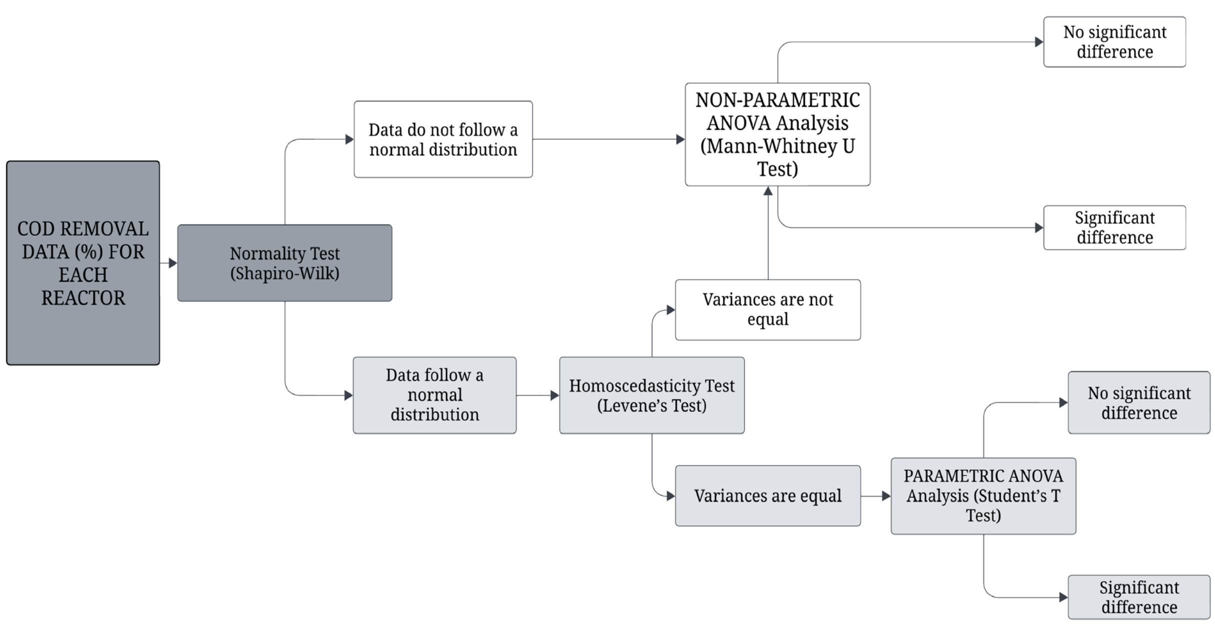

Figure 2. Statistical Analysis Strategy for Group Comparison

Influence of Noise Factors (pH, Temperature, and Alkalinity) for Each Reactor

To assess the influence of pH, temperature, and alkalinity on COD removal in each reactor, regression models were employed, considering both linear and non-linear relationships as appropriate. In cases where a non-linear relationship was identified, a second-degree polynomial regression model was implemented, incorporating linear terms, interaction terms, and quadratic terms to better capture the system's behavior. Conversely, when a linear relationship was assumed,

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a multiple linear regression model was applied, ensuring that all model assumptions were met, with a particular focus on residual normality. To facilitate the interpretation of results, Pareto charts were constructed, enabling the visualization of the magnitude and direction of the effects exerted by each variable and their interactions. These graphical analyses played a crucial role in identifying the most influential factors governing COD removal efficiency across the different reactor configurations.

Statistical Analysis for Optimization Processes in Each Reactor

A model-based optimization approach was implemented to establish the optimal operating conditions of the reactors, aiming to maximize COD removal efficiency. A multiple linear regression model was fitted to the experimental data, correlating COD removal (%) with pH, temperature, and alkalinity to determine the most influential factors in the process. Prior to model fitting, all independent variables were standardized to ensure an equitable contribution of each factor, thereby improving the robustness of the analysis.

An optimization algorithm developed in Python was employed to identify the pH, temperature, and alkalinity values that maximized COD removal, as predicted by the regression model. The optimization process was carefully constrained to the observed experimental data range for each variable, ensuring that the results remained realistic, applicable, and relevant to the specific conditions of the study.

Results and Discussion

Inoculum Collection and Acclimation

Table 3 presents essential data regarding the acclimation time (expressed in days) required for each reactor to achieve the target COD removal efficiency. This acclimation phase is a critical aspect of biological reactor performance, as it allows microorganisms to gradually adapt to the system's environmental conditions, ensuring their ability to develop the metabolic functions necessary for the efficient degradation of organic matter present in wastewater. The successful completion of this adaptation period is fundamental to achieving stable and effective treatment performance in the long term (15).

Table 3. Acclimation Period Required to Achieve Target COD Removal Efficiency

Reactor	**Days**	**COD Removal (%)**
Upflow Anaerobic Sludge Blanket (UASB)	28	70.0
Anaerobic Attached-Growth Reactor (AAGR)	12	90.8
Aerobic Trickling Filter	3	90.0

Based on the results, it can be inferred that the acclimation time of inoculum in biological reactors varies depending on their origin and the reactor conditions. The trickling filter inoculum, sourced from a similar aerobic environment, acclimated rapidly due to its prior adaptation to comparable

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conditions. In contrast, the inoculum from the anaerobic reactors, originating from different environments, required a longer adaptation period to adjust to the new operating conditions and the specific type of organic matter present in the system (16). Table 4 presents the sludge characterization results for each of the reactors studied.

Table 4. Sludge Characterization

Reactor	Total Solids (TS), mg/L
Upflow Anaerobic Sludge Blanket (UASB)	82,654
Anaerobic Attached-Growth Reactor (AAGR)	114,248
Aerobic Trickling Filter	13,356

The concentration of solids in the sludge suggests that higher total solids (TS) levels, as observed in the AAGR and UASB reactors, correlate with a longer acclimation period for microorganisms. This prolonged adaptation may be attributed to greater microbial diversity and the accumulation of inert solids within the system (17). In the case of the AAGR, the high total solids content is primarily due to the sludge collection point, as it was extracted from the bottom of a facultative lagoon, where organic matter decomposition led to a significant accumulation of settled solids, thereby increasing TS concentration.

Moreover, the presence or absence of oxygen also plays a crucial role in acclimation time. Anaerobic reactors require a specialized microbial community that can adapt to oxygen-free conditions, which prolongs the acclimation process. In contrast, aerobic reactors, where oxygen facilitates organic matter degradation, support a more diverse microbial community, enabling a faster adaptation, especially when the inoculum originates from a similar aerobic environment (17).

These findings align with previous studies on UASB acclimation (18), which report inoculum adaptation periods ranging from 19 to 25 days before reactor startup. In the present study, the acclimation times observed for the UASB and AAGR reactors fall within this range, reinforcing the notion that anaerobic microorganisms exhibit more complex and slower metabolic processes compared to aerobic communities. This metabolic complexity, along with the lower diversity and higher sensitivity of anaerobic microbial consortia to environmental changes, explains the longer adaptation periods required for anaerobic systems. Consequently, these factors justify the differences observed in acclimation times between the anaerobic and aerobic reactors evaluated (19).

Experimental Results: COD Removal Efficiency (%)

The COD removal efficiency reflects the performance of the reactors in wastewater treatment. Figure 3 illustrates the COD removal fluctuations observed in the AAGR reactor over a period of 150 days. Initially, the removal efficiency increases progressively, reaching approximately 80%, indicating a gradual adaptation of the microbial biomass. However, between days 40 and 60, a decline in efficiency is observed, which may be attributed to operational variations affecting system stability.

Following this phase, the reactor undergoes a new period of increased efficiency, yet around day 100, a drastic decline occurs, with COD removal dropping to nearly 20%. This significant decrease

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suggests possible disturbances in system performance, such as shifts in microbial activity, changes in influent characteristics, or transient imbalances in reactor conditions. Nonetheless, by day 140, the reactor recovers its performance, with efficiency stabilizing again above 80%, indicating resilience and adaptation of the microbial community to operational variations.

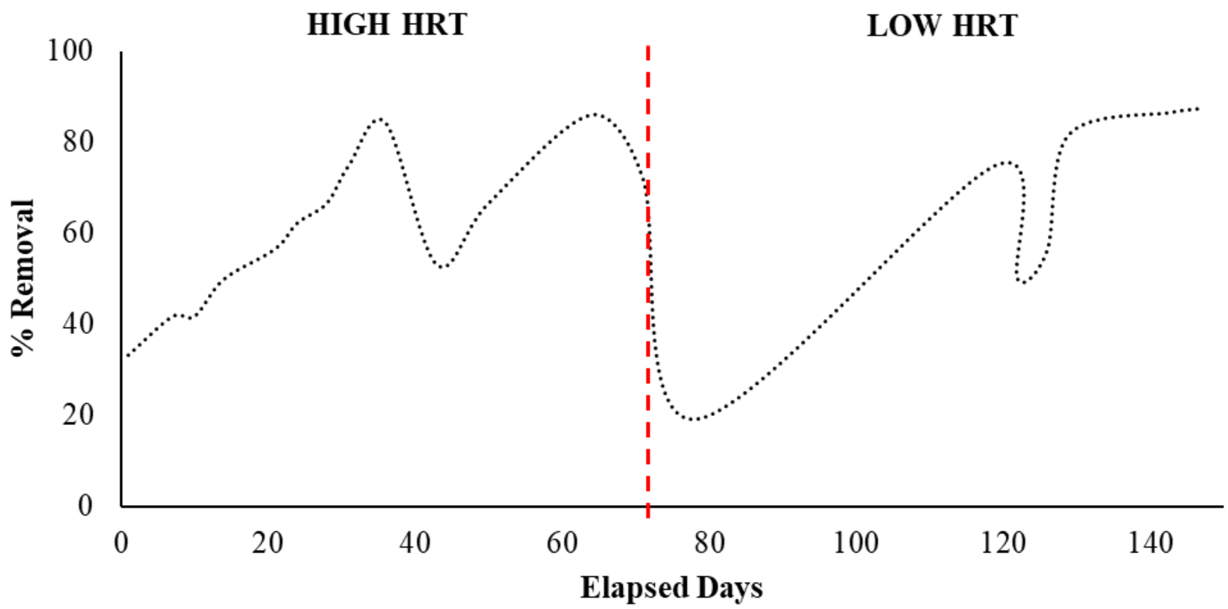

Figure 3. COD removal (%) for the RACA reactor

Among the anaerobic treatment systems evaluated, the UASB reactor also exhibited distinct COD removal dynamics, as shown in Figure 4. Initially, the removal efficiency remained stable, fluctuating between 20% and 30% during the first 160 days of operation. This initial period of low efficiency suggests a prolonged acclimation phase, likely due to the complex microbial adaptation process required for anaerobic degradation under the specific operational conditions.

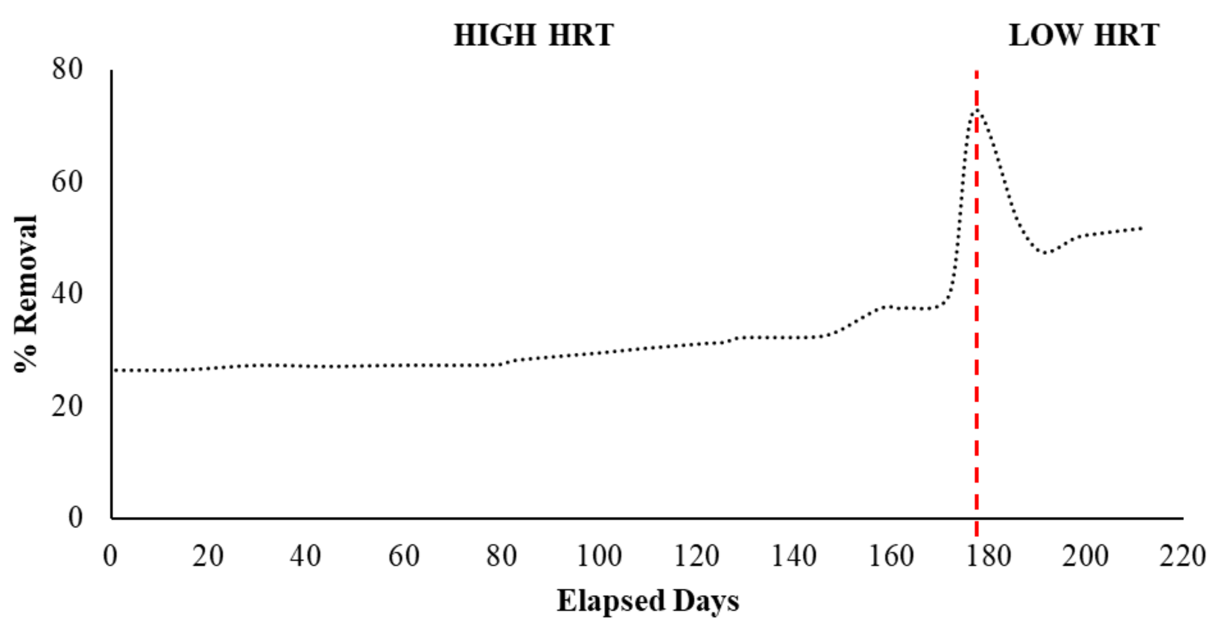

Figure 4. COD Removal Efficiency (%) for the UASB Reactor

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After day 160, a notable increase in COD removal efficiency was observed, reaching a peak of approximately 60%. However, this improvement was not sustained, as efficiency declined shortly after, eventually stabilizing between 40% and 50% toward the end of the experiment. These fluctuations indicate potential operational challenges or microbial community shifts, highlighting the need for further investigation into factors influencing reactor stability and long-term performance under psychrophilic conditions.

Conversely, in the aerobic system, represented by the trickling filter, the COD removal results are shown in Figure 5. The graph illustrates COD removal efficiency in an aerobic trickling filter over a period of 40 days. Several fluctuations in efficiency were observed throughout the operational period, with peak removal efficiencies close to 70% occurring on days 15 and 35.

However, between these peaks, the efficiency dropped significantly, reaching values around 40%. These variations suggest potential instabilities in reactor performance, which could be attributed to changes in organic load, microbial activity shifts, or operational conditions affecting system stability. Further analysis is required to determine the factors influencing performance fluctuations and to develop strategies for enhancing reactor consistency and efficiency over time.

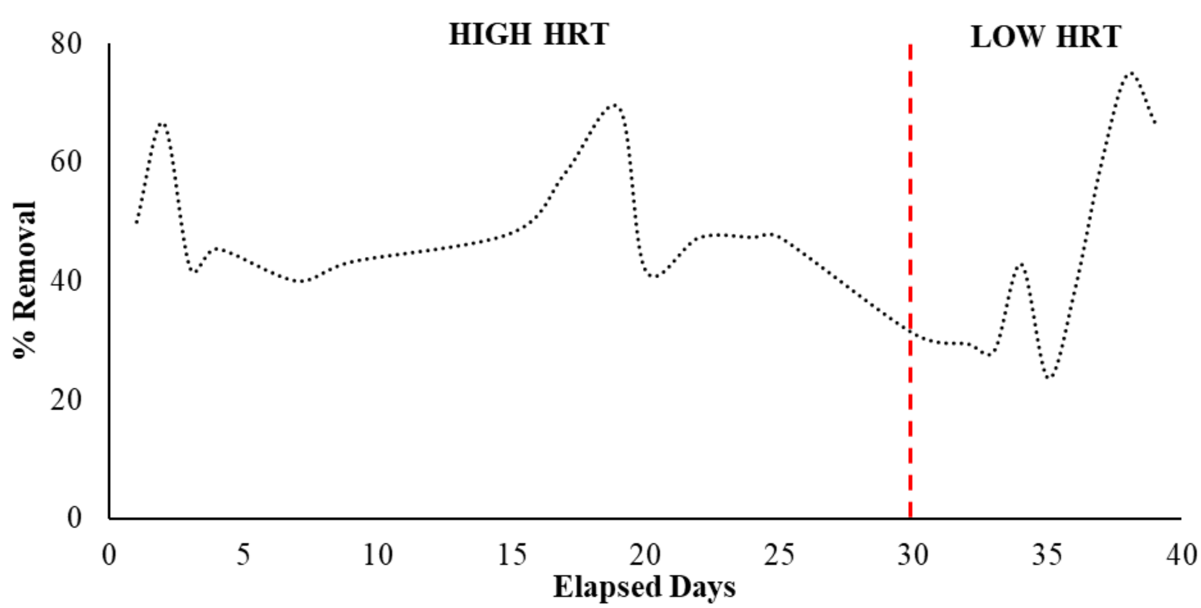

Figure 5. COD Removal Efficiency (%) for the Aerobic Trickling

Statistical Analysis to Determine the Influence of HRT

Exploratory Data Analysis (EDA)

A descriptive statistical analysis was conducted to characterize the distribution of COD removal efficiency data for each reactor under different HRT conditions. Measures of central tendency and dispersion were computed, providing an initial assessment of the efficiency of organic matter removal in the evaluated treatment systems. The detailed results of this analysis are presented in Table 5.

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Table 5. Exploratory Data Analysis

Reactor	HRT	Mean	Standard Deviation	Min	Q1 (25%)	Median (50%)	Q3 (75%)	Max
AAGR	High	72.50	13.56	53.13	66.67	72.21	84.38	86.12
	Low	76.20	25.02	19.21	53.13	75.00	84.25	87.50
UASB	High	33.08	10.33	26.44	27.35	30.70	32.82	72.50
	Low	50.55	2.08	47.37	50.00	50.76	51.71	52.92
TRICKLING FILTER	High	48.52	10.16	40.00	42.00	44.34	50.69	69.23
	Low	46.55	18.11	23.64	30.26	42.86	63.33	75.00

The preliminary results indicate that the AAGR reactor achieved the highest COD removal efficiency, with an average of 72.5%. To further evaluate the influence of HRT on COD removal, Figure 6 is presented, illustrating the effect of different retention times on the performance of the treatment systems.

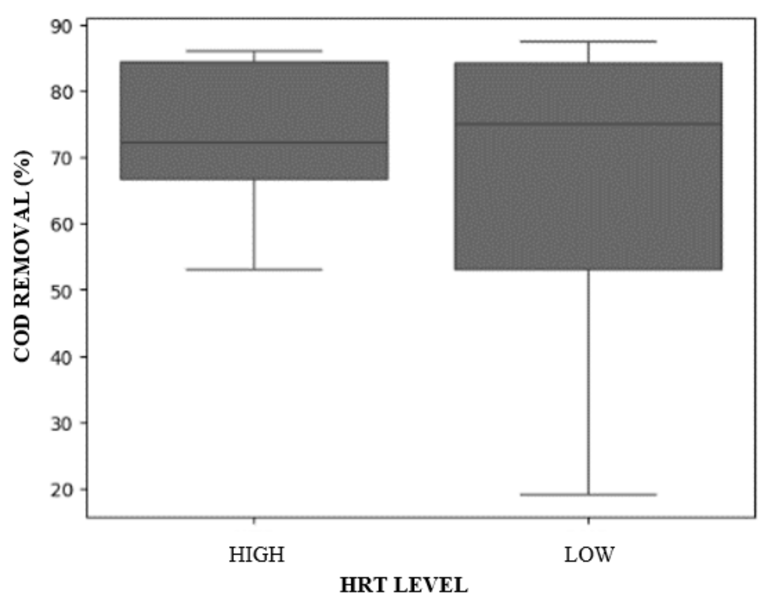

Figure 6. Boxplot for HRT Levels in the AAGR Reactor

The boxplot compares COD removal efficiency in the AAGR reactor under two HRT conditions: HIGH and LOW. Although the median COD removal is slightly higher under LOW HRT, the greater variability in the data suggests that the effect of HRT on reactor performance is not conclusive. Additionally, outliers are observed in both conditions, indicating the potential influence of external factors on system behavior.

In the case of the UASB reactor, the boxplot analysis (Figure 7) reveals a higher COD removal efficiency associated with LOW HRT, albeit with greater variability in results. Conversely, the HIGH HRT condition exhibits lower COD removal efficiency but shows greater consistency in the data, suggesting more stable operational performance.

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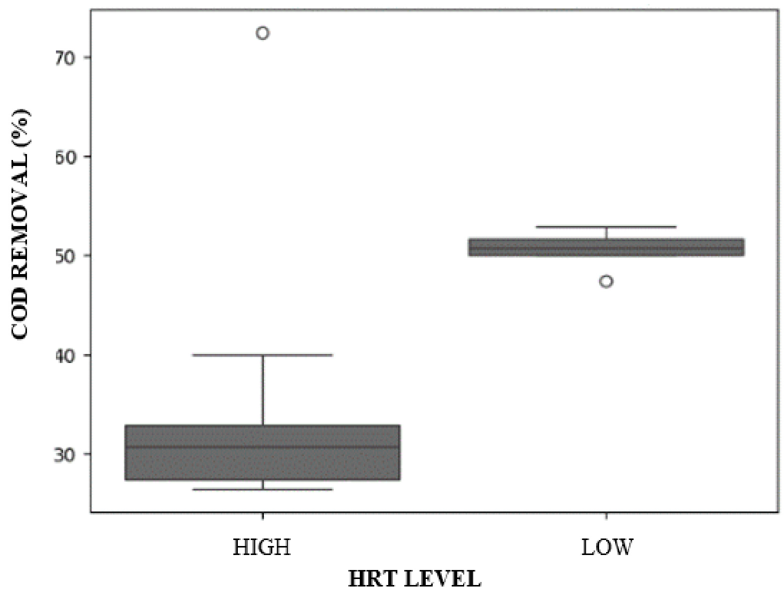

Figure 7. Boxplot for HRT Levels in the UASB Reactor

Finally, for the aerobic treatment system, represented by the trickling filter, the boxplot in Figure 8 indicates that HIGH HRT exhibits a slightly higher median COD removal efficiency, approximately 45%, with a more clustered data distribution, signifying greater consistency in performance. In contrast, LOW HRT presents a median COD removal of around 40% but with higher data dispersion, suggesting greater variability in treatment efficiency under this condition. Outliers are observed in both cases, indicating potential external events or operational fluctuations that may have influenced COD removal efficiency in specific instances.

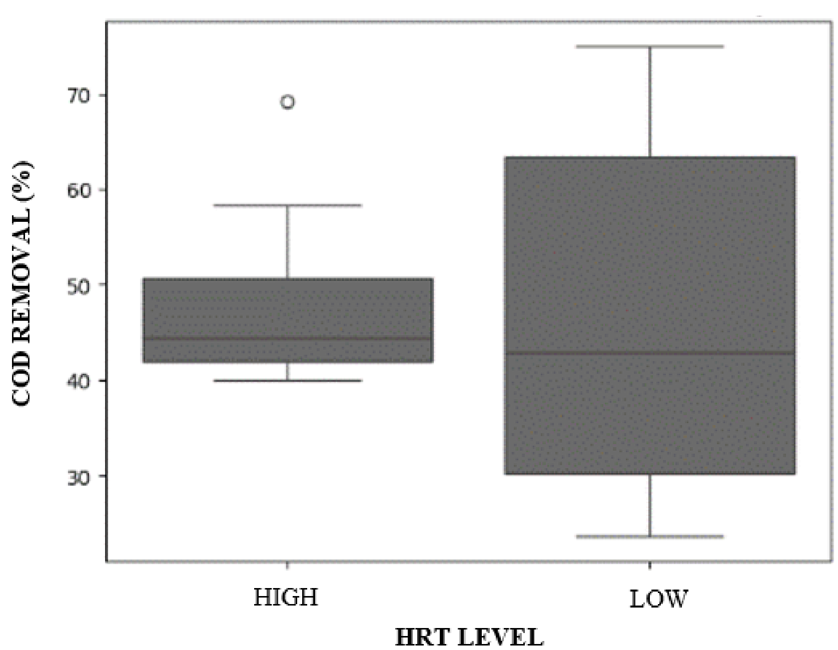

Figure 8. Boxplot for HRT Levels in the Trickling Filter

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Influence of HRT on Each Reactor: Data Mining

Following the exploratory data analysis, statistical tests were performed for each reactor to determine whether HRT (High or Low) significantly influenced COD removal efficiency. Initially, data normality was assessed using the Shapiro-Wilk test, while homogeneity of variances was evaluated with Levene's test. Based on these preliminary analyses, parametric (ANOVA, Student's t-test) or non-parametric (Mann-Whitney U test) methods were applied as appropriate to compare the means or medians of COD removal efficiency between the two HRT levels for each reactor. The results are summarized in Table 6.

Table 6. Statistical Tests to Determine the Influence of HRT on Each Reactor

FACTORS		**NORMALITY TEST**		**HOMOGENEITY TEST**		**ANOVA ANALYSIS**		
REACTOR TYPE	**HRT Level**	Shapiro-Wilk		Levene's Test		Parametric (Student's t-test) Non-Parametric (Mann-Whitney U)		
		P value	**Result**	**P valor**	**Result**	**Test**	**P value**	**Conclusion**
AAGR	High	0.616	Normal	0.35	Equal variances	Student's t-test	0.590	No significant difference
	Low	0.198						
UASB	High	0.000	Non-normal	Not applicable		Mann-Whitney U	0.0028	Significant difference
	Low	0.846	Normal					
TRICKLING FILTER	High	0.033	Non-normal	Not applicable		Mann-Whitney U	0.649	No significant difference
	Low	0.294	Normal					

The results indicate that HRT significantly influenced COD removal efficiency only in the UASB reactor, suggesting a complex interaction between physicochemical, operational, and biological factors specific to each treatment system. These findings align with a previous study (20), where a UASB reactor exhibited high sensitivity to flow variations, a phenomenon explained by the nature of anaerobic processes in this system. In UASB reactors, the granular biomass is more susceptible to washout under low HRT conditions, limiting the contact time required for effective organic matter degradation. Additionally, mass transfer limitations and slower reaction kinetics, which are intrinsic to anaerobic processes, further highlight the need for a longer HRT to optimize COD removal efficiency in this type of reactor (18).

In contrast, in the AAGR and Trickling Filter reactors, the lack of a significant HRT effect could be attributed to the well-established and adapted microbial biomass, efficient mass transfer mechanisms, and faster reaction kinetics (19). These factors contribute to greater stability, allowing microorganisms to maintain high organic matter degradation activity, even under lower HRT conditions. This stability is further enhanced by the presence of support media in the degradation

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process, which provides a stable microbial attachment surface, ensuring efficient treatment performance across different flow conditions.

Furthermore, the small difference between the evaluated HRT ranges (high and low) may have reduced the likelihood of detecting significant differences in these systems. Given that both HRT levels appear to support sufficient treatment efficiency, this could explain the absence of statistically significant effects in the AAGR and Trickling Filter reactors.

Statistical Analysis to Determine the Influence of Noise Factors (pH, Temperature, Alkalinity) on COD Removal

Exploratory Data Analysis (EDA)

The preliminary descriptive analysis of the AAGR reactor revealed considerable variability in operational parameters and COD removal efficiency. The pH remained within a slightly acidic to neutral range, with a mean of 7.53 and a standard deviation of 0.32, suggesting adequate control of this parameter. Temperature exhibited minimal fluctuation, ranging from 16°C to 18°C, with a mean of 17°C. Alkalinity displayed the highest variability, ranging from 88.97 to 195.38 mg/L, with a standard deviation of 31.56, indicating possible changes in influent composition or chemical dosing variations. In terms of COD removal, an average efficiency of 68.25% was observed, with values ranging from 19.2% to 87.5%. The distribution of these variables is visualized in the boxplots presented in Figure 9.

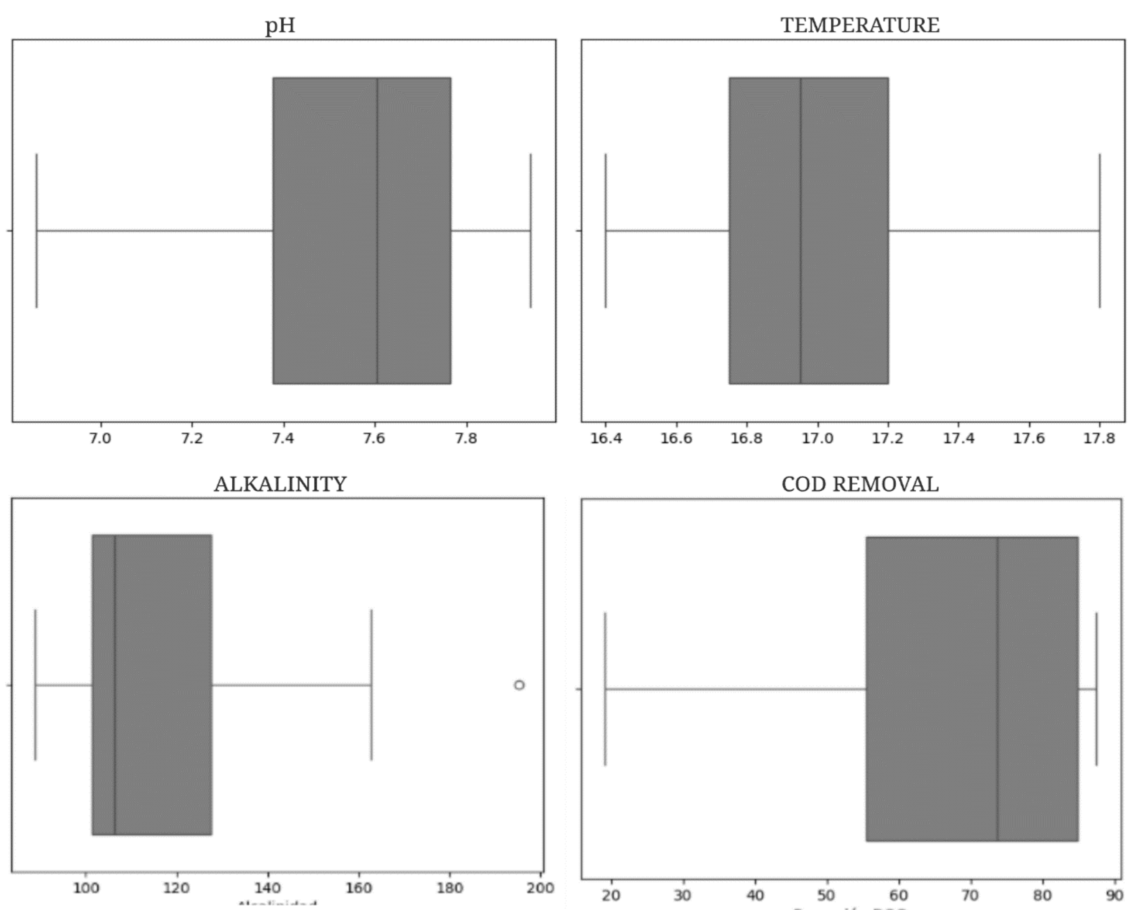

Figure 9. Boxplot of pH, Temperature, and Alkalinity for the AAGR Reactor

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The descriptive analysis of the UASB reactor showed moderate variability in operational parameters and COD removal efficiency. The pH remained primarily within a neutral range, with a mean of 7.37 and a standard deviation of 0.50. Temperature exhibited limited fluctuation, ranging from 15.5°C to 18.1°C, with a mean of 17.11°C. Alkalinity, however, showed greater variability, with values ranging from 17.36 to 195.3 mg/L and a standard deviation of 53.14. This variation can be explained by the organic matter degradation process, where the breakdown of organic compounds produces acids, such as carbonic acid, which react with alkaline compounds in the water, reducing alkalinity levels (21). The continuous production of acids and the subsequent consumption of alkalinity contribute to the observed fluctuations.

The average COD removal efficiency for the UASB reactor was 36.87%, with values ranging from 26.44% to 72.5%. A standard deviation of 11.94% indicates considerable data dispersion, suggesting that removal efficiency may be influenced by operational factors or influent characteristics. The distribution of these variables is presented in the boxplots shown in Figure 10.

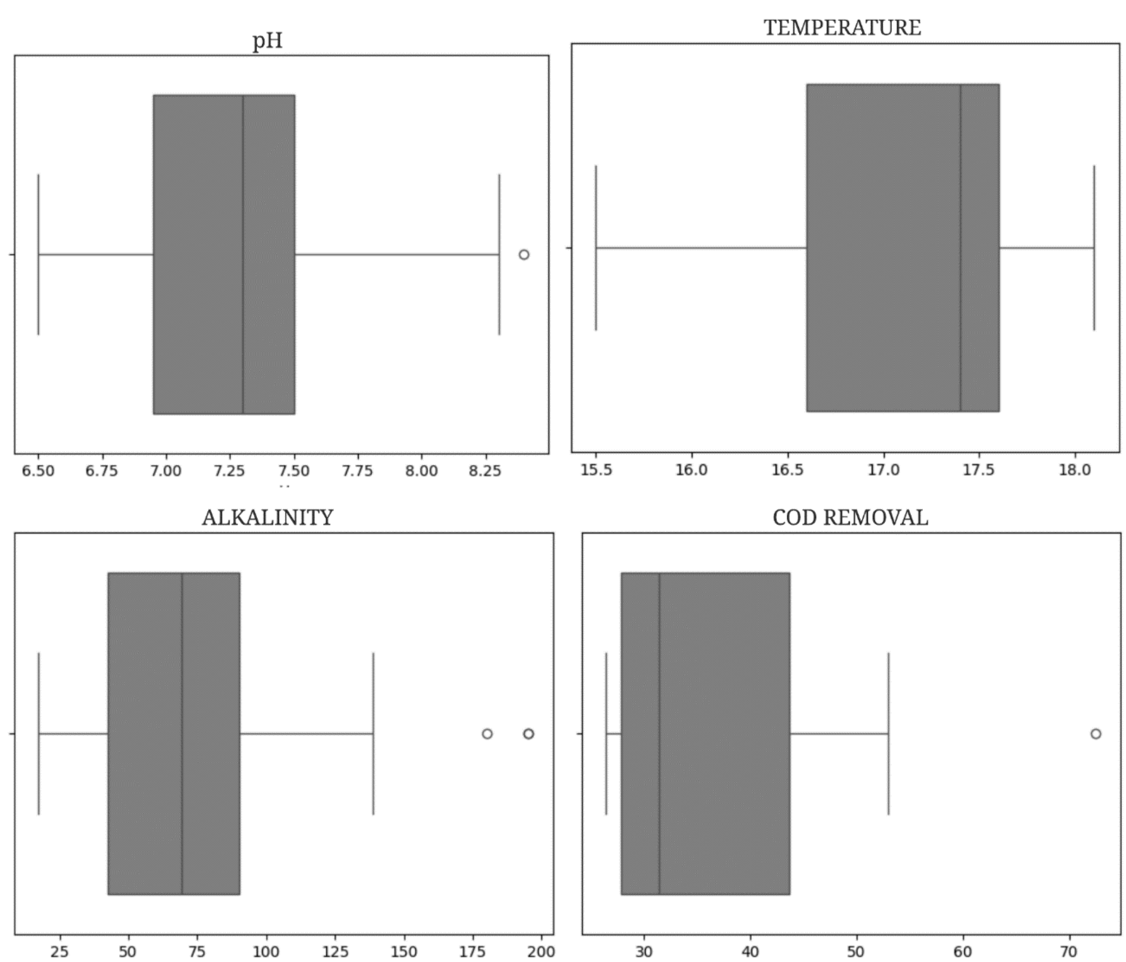

Figure 10. Boxplot of pH, Temperature, and Alkalinity for the UASB Reactor

The descriptive analysis of the aerobic trickling filter revealed that pH remained within a slightly alkaline range, with a mean of 7.76 and a standard deviation of 0.25, indicating an environment favorable for microbial activity. Temperature showed greater variability, ranging from 13.9°C to

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18.1°C, with a mean of 16.09°C and a standard deviation of 1.26, which may suggest environmental fluctuations or changes in operational conditions. Alkalinity also exhibited considerable variability, ranging from 4.34 to 26.04 mg/L, with a standard deviation of 6.36, which may be related to influent composition or neutralization processes within the system.

Regarding COD removal efficiency, an average of 47.38% was recorded, with values ranging from 23.64% to 75.00%. A standard deviation of 14.95% indicates significant data dispersion, suggesting that removal efficiency may be influenced by operational factors or influent characteristics. The distribution of these variables is illustrated in the boxplots shown in Figure 11.

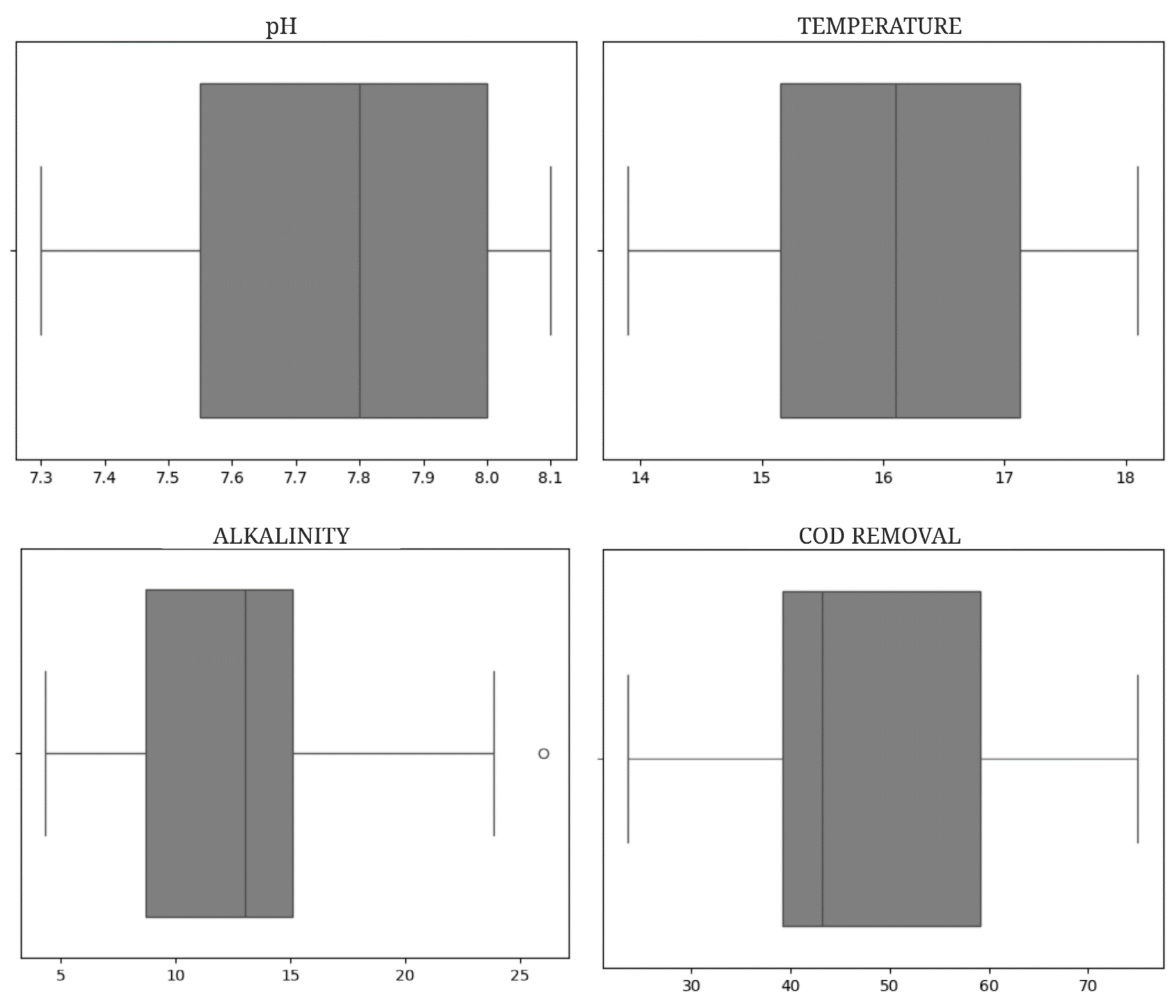

Figure 11. Boxplot of pH, Temperature, and Alkalinity for the Trickling Filter Reactor

Influence of Noise Factors (pH, Temperature, and Alkalinity) on COD Removal for Each Reactor

To assess the influence of independent variables on COD removal efficiency in each reactor, regression models were fitted, followed by the generation of Pareto charts. These charts allowed for a visual representation of the relative contribution of each factor to the observed variability in COD removal efficiency.

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For the anaerobic treatment systems, Pareto charts for the AAGR and UASB reactors are presented in Figures 12 and 13, respectively. The linear regression analysis revealed that pH is the dominant factor influencing COD removal efficiency, exhibiting an inverse relationship—meaning that higher pH values correspond to lower COD removal rates. While temperature and alkalinity were not statistically significant, they still showed notable trends. Alkalinity tended to have a direct relationship with COD removal efficiency, suggesting that higher alkalinity levels may contribute to a slight increase in organic matter degradation.

The Pareto chart highlights the predominant role of pH, demonstrating its relative magnitude compared to other variables, thereby confirming the importance of pH optimization in improving COD removal efficiency in AAGR and UASB reactors.

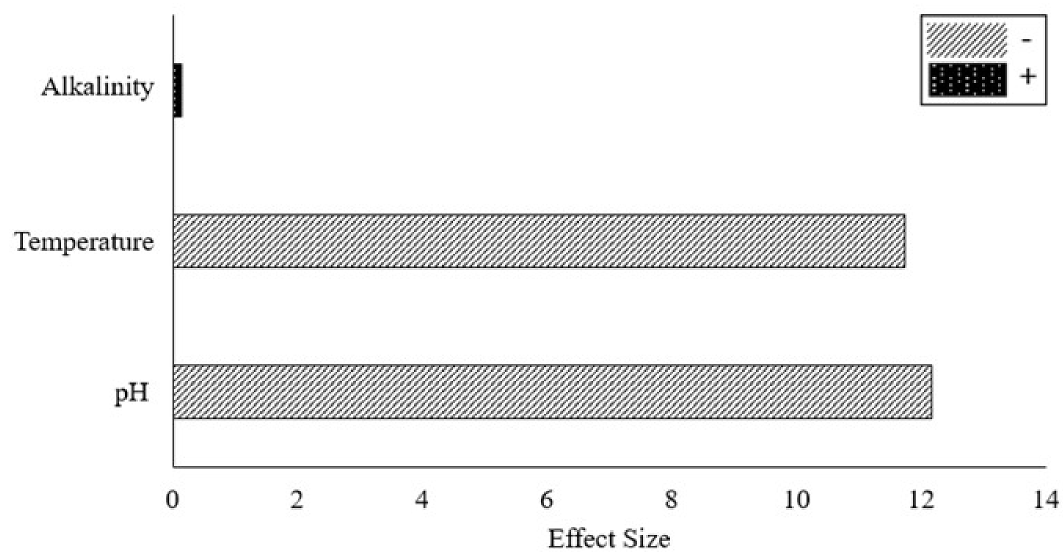

Figure 12. Influence of pH, Temperature, and Alkalinity on the AAGR Reactor

The inverse relationship between pH and COD removal efficiency in AAGR and UASB reactors can be attributed to both biological and chemical factors. An increase in pH may inhibit the microbial communities responsible for organic matter degradation, either directly or by favoring the proliferation of competing bacterial populations (22).

Additionally, elevated pH levels can alter system chemistry, affecting nutrient availability, the formation of toxic compounds, and the stability of microbial flocs, all of which can negatively impact COD removal efficiency (23).

The inverse relationship between temperature and COD removal efficiency could be explained by the fact that, although higher temperatures generally enhance biological reactions, they can also negatively impact microbial activity, particularly in psychrophilic conditions, such as the low environmental temperatures in Pasto (24).

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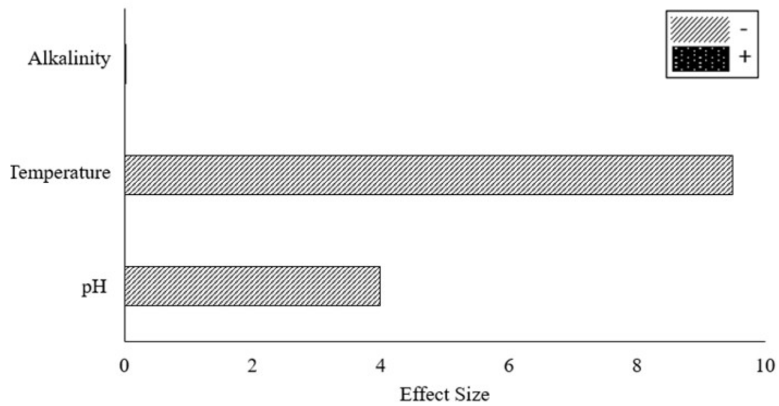

Figure 13. Influence of pH, Temperature, and Alkalinity on the UASB Reactor

Conversely, the direct relationship observed between alkalinity and COD removal efficiency suggests that a more alkaline environment may enhance organic matter degradation. Alkalinity acts as a pH buffer, helping to maintain stable conditions for microbial activity (24). Furthermore, some biological degradation processes require alkaline ions as enzymatic cofactors, which may explain the increased COD removal efficiency observed with higher alkalinity levels (25).

For the aerobic system, represented by the trickling filter, the Pareto chart is shown in Figure 14. The linear regression analysis for the trickling filter confirmed that pH is the key determinant in COD removal efficiency, showing a significant inverse relationship—higher pH levels correspond to lower removal efficiency. Although alkalinity exhibited a positive trend, its effect was not statistically significant, suggesting that its influence on COD removal efficiency may be limited or dependent on other factors. Temperature did not have a significant effect in this system.

The Pareto chart reinforces the strong impact of pH, as its effect size is significantly greater than that of the other variables. The analysis of all three biological reactors (AAGR, UASB, and Trickling Filter) confirms the crucial role of pH as a determinant factor in COD removal efficiency. In all cases, pH exerted a significant influence, highlighting the need for pH regulation strategies. Effective pH control can be achieved through various methods, including the addition of neutralizing agents (acids or bases), sludge recirculation systems, or pre-treatment stages that adjust the pH of the influent (26).

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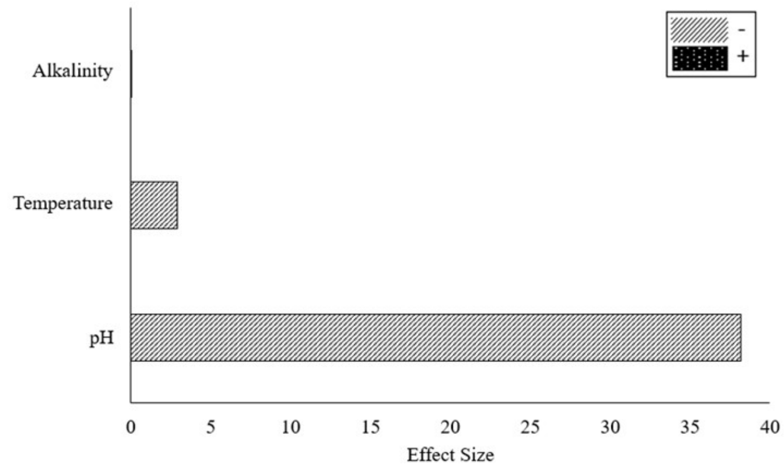

Figure 14. Influence of pH, Temperature, and Alkalinity on the Trickling Filter

Statistical Analysis for Optimization Processes in Each Reactor

Multiple linear regression models were used to optimize reactor operation, aiming to maximize COD removal efficiency by correlating it with pH, temperature, and alkalinity. The optimal operational values identified through these models are presented in Table 6.

Table 6. Optimal Operating Conditions for Each Reactor

REACTOR	**pH** (units)	**Temperature (°C)**	**Alkalinity (mg/L)**	**COD Removal (%)**
AAGR	6.86	16.40	195.30	96.52
UASB	6.50	15.50	195.30	79.51
TRICKLING FILTER	7.30	13.90	4.34	71.35

The AAGR reactor achieved the highest COD removal efficiency (96.52%) under slightly acidic conditions (pH 6.86), a temperature of 16.40°C, and an alkalinity of 195.30 mg/L. These results suggest that a slightly acidic environment and elevated alkalinity enhance microbial activity, promoting organic matter degradation. For the UASB reactor, COD removal reached 79.51% under similar alkalinity conditions (195.30 mg/L), but with a more acidic pH (6.50) and a slightly lower temperature (15.50°C). This difference in optimal conditions may be attributed to the distinct microbial community composition in UASB reactors, which are adapted to more acidic environments and lower temperatures (22).

A similar trend was reported in a study conducted in Mexico (27) where UASB reactors used for domestic and synthetic wastewater treatment achieved COD removal efficiencies ranging from 76% to 88%, demonstrating their high efficiency and potential when integrated into a treatment train.

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Finally, the aerobic trickling filter achieved a COD removal efficiency of 71.35%, but under notably different conditions. The optimal pH was slightly alkaline (7.30), the temperature was the lowest among the three systems (13.90°C), and alkalinity was significantly lower (4.34 mg/L). These findings indicate that the trickling filter is less sensitive to alkalinity variations and can operate efficiently within a wider pH range but at lower temperatures, aligning with the environmental conditions of Pasto.

Aerobic trickling filters are generally less efficient than anaerobic reactors in removing COD from wastewater due to several factors, including the nature of the organic matter, filter operating conditions, and biofilm formation, which can limit degradation efficiency. In contrast, anaerobic processes, owing to their diverse microbial communities and operating conditions, can degrade a wider range of organic compounds, leading to higher COD removal efficiencies (28). These findings align with the results of this study, where despite the faster acclimation process in the aerobic system, anaerobic reactors achieved higher COD removal efficiencies.

Conclusions

pH is the primary factor influencing COD removal efficiency in all three treatment systems (AAGR, UASB, and Trickling Filter). Statistical analysis and Pareto charts confirm the strong impact of pH, highlighting the need for pH regulation and control to optimize these treatment processes.

Temperature and alkalinity exhibited variable and less significant effects on COD removal, depending on the reactor type. While trends were observed, their statistical influence was inconsistent across all three systems.

Hydraulic retention time (HRT) significantly affected COD removal only in the UASB reactor. The greater sensitivity of this system to flow variations, due to the nature of anaerobic processes and the susceptibility of granular biomass to washout, underscores the importance of HRT optimization to achieve efficient organic matter degradation in UASB reactors.

Multiple linear regression models proved to be valuable tools for identifying optimal pH, temperature, and alkalinity conditions that maximize COD removal efficiency in each reactor. These models enable the prediction of treatment performance based on operational conditions, facilitating decision-making and process optimization.

Inoculum acclimation is a crucial step in the startup of biological reactors. The adaptation period of microbial communities to system conditions and the organic matter type varies depending on inoculum origin and reactor characteristics. Acclimation is generally longer in anaerobic systems due to the metabolic complexity of anaerobic microorganisms.

The findings of this study can be applied to optimize domestic wastewater treatment systems, particularly in regions with similar environmental conditions. A better understanding of organic matter degradation mechanisms under psychrophilic conditions allows for the design of more efficient and sustainable treatment processes, reducing operational costs and improving effluent quality through the integration of various treatment systems.

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Recommendations and Future Research

Based on this study, future research should explore the feasibility of combining treatment systems, such as UASB reactors followed by an aerobic system. Literature suggests that this integrated approach has high potential for COD removal efficiency and could enhance overall treatment performance.

Additionally, in comparative studies between different reactors, it is crucial to standardize operating conditions across all systems. Ensuring identical operational parameters guarantees reliable and meaningful comparisons, improving the accuracy of performance evaluations between different treatment technologies.

CrediT authorship contribution statement

Conceptualization - Ideas: Gloria Lucía Cárdenas Calvachi, Paola Andrea Ortega Guerrero. Data Curation: Gloria Lucía Cárdenas Calvachi, Paola Andrea Ortega Guerrero, Jennifer Jiménez Paz. Formal analysis: Jennifer Jiménez Paz, Carol Julieth Montezuma. Acquisition of financing: Gloria Lucía Cárdenas Calvachi, Paola Andrea Ortega Guerrero. Investigation: Gloria Lucía Cárdenas Calvachi, Paola Andrea Ortega Guerrero. Methodology: Gloria Lucía Cárdenas Calvachi, Paola Andrea Ortega Guerrero. Project Management: Gloria Lucía Cárdenas Calvachi, Paola Andrea Ortega Guerrero. Resources: Gloria Lucía Cárdenas Calvachi, Paola Andrea Ortega Guerrero. Software: Carol Julieth Montezuma. Supervision: Jennifer Jiménez Paz. Validation: Gloria Lucía Cárdenas Calvachi, Paola Andrea Ortega Guerrero, Jennifer Jiménez Paz. Writing - original draft - Preparation: Carol Julieth Montezuma, Jennifer Jiménez Paz. Writing - revision and editing - Preparation: Carol Julieth Montezuma, Jennifer Jiménez Paz.

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