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ACCLIMATATION OF MICROBIAL BIOMASS TO EFFLUENTS FROM A SWINE SLAUGHTERHOUSE IN BATCH REACTORS

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ABSTRACT

Objective: To evaluate the acclimatization of microbial biomass in sequential reactors to optimize the treatment of wastewater from pig slaughterhouses.

Theoretical framework: The meat industry generates highly contaminated wastewater. Biological treatments, such as aerobic and anaerobic systems, are more efficient and sustainable than physicochemical ones.

Materials and methods: Industrial effluents were collected in a pig slaughterhouse and then characterized. The microbial biomass was collected from the same slaughterhouse and was subjected to an acclimatization process in a sequential batch reactor.

Results and discussion: The results showed that the wastewater from the pig slaughterhouse had high levels of BOD, COD and suspended solids, exceeding the limits established by local regulations. During the acclimatization process, the microbial biomass demonstrated a gradual improvement in its COD removal capacity, reaching efficiencies greater than 70%. The positive correlation observed between volumetric organic load and organic matter removal efficiency indicates that acclimated biomass has a greater capacity to treat effluents with high levels of organic load.

Research implications: Biomass acclimatization is essential to improve the efficiency of biological treatment in slaughterhouse effluents, reducing its environmental impact and the need for costly treatments.

Value/originality: This study proposes a more sustainable and efficient solution for the treatment of slaughterhouse wastewater, highlighting the importance of biomass acclimatization.

Keywords: Wastewater, Microbial Biomass, Biological Treatment, Pig Slaughterhouses, COD Removal.

ACLIMATAÇÃO DE BIOMASSA MICROBIANA A EFLUENTES DE UM ABATEDOURO DE SUÍNOS EM REATORES DE BATELADA

RESUMO

Objetivo: Avaliar a aclimatação da biomassa microbiana em reatores sequenciais para otimizar o tratamento de águas residuárias de matadouros de suínos.

Enquadramento teórico: A indústria da carne gera águas residuais altamente contaminadas. Os tratamentos biológicos, como os sistemas aeróbios e anaeróbios, são mais eficientes e sustentáveis do que os físico-químicos.

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Materiais e métodos: Os efluentes industriais foram coletados em um matadouro de suínos e posteriormente caracterizados. A biomassa microbiana foi coletada no mesmo frigorífico e submetida a um processo de aclimatação em reator batelada sequencial.

Resultados e discussão: Os resultados mostraram que as águas residuais do matadouro de suínos apresentavam níveis elevados de DBO, DQO e sólidos em suspensão, ultrapassando os limites estabelecidos pela regulamentação local. Durante o processo de aclimatação, a biomassa microbiana demonstrou uma melhoria gradual na sua capacidade de remoção de DQO, atingindo eficiências superiores a 70%. A correlação positiva observada entre a carga orgânica volumétrica e a eficiência de remoção de matéria orgânica indica que a biomassa aclimatada possui maior capacidade de tratar efluentes com elevados níveis de carga orgânica.

Implicações para a investigação: A aclimatação da biomassa é essencial para melhorar a eficiência do tratamento biológico em efluentes de matadouros, reduzindo o seu impacto ambiental e a necessidade de tratamentos dispendiosos.

Valor/originalidade: Este estudo propõe uma solução mais sustentável e eficiente para o tratamento de águas residuais de matadouros, destacando a importância da aclimatação da biomassa.

Palavras-chave: Águas Residuais, Biomassa Microbiana, Tratamento Biológico, Matadouros de Suínos, Remoção de DQO.

ACLIMATACIÓN DE BIOMASA MICROBIANA A EFLUENTES PROVENIENTES DE UN CAMAL DE CERDOS EN REACTORES POR CARGA

RESUMEN

Objetivo: Evaluar la aclimatación de biomasa microbiana en reactores secuenciales para optimizar el tratamiento de aguas residuales de mataderos de cerdos.

Marco teórico: La industria de la carne genera aguas residuales altamente contaminadas. Los tratamientos biológicos, como los sistemas aeróbicos y anaeróbicos, son más eficientes y sostenibles que los fisicoquímicos.

Materiales y métodos: Los efluentes industriales fueron recolectados en un matadero de cerdos para luego ser caracterizados. La biomasa microbiana se recolectó del mismo matadero y fue sometida a un proceso de aclimatación en un reactor secuencial por carga.

Resultados y discusión: Los resultados mostraron que el agua residual del matadero de cerdos tenía altos niveles de DBO_5, DQO y sólidos suspendidos, superando los límites establecidos por la normativa local. Durante el proceso de aclimatación, la biomasa microbiana demostró una mejora gradual en su capacidad de remoción de DQO, alcanzando eficiencias superiores al 70%. La correlación positiva observada entre la carga orgánica volumétrica y la eficiencia de remoción de materia orgánica indica que la biomasa aclimatada tiene una mayor capacidad para tratar efluentes con altos niveles de carga orgánica.

Implicaciones de la investigación: La aclimatación de biomasa es esencial para mejorar la eficiencia del tratamiento biológico en efluentes de mataderos, reduciendo su impacto ambiental y la necesidad de tratamientos costosos.

Valor/originalidad: Este estudio propone una solución más sostenible y eficiente para el tratamiento de aguas residuales de mataderos, resaltando la importancia de la aclimatación de la biomasa.

Palabras claves: Aguas Residuales, Biomasa Microbiana, Tratamiento Biológico, Mataderos de Cerdos, Remoción de DQO.

1 INTRODUCTION

The meat industry is one of the largest producers of organic waste in the food industry. According to Peters *et al.* (2010), the definition of meat is a product of all animals, such as calves, cows, pigs, sheep and other mantles that are not included in the poultry complex. These meats are also known as red meat. According to FAO statistics, the need for meat supply is felt more than ever due to population growth. Consequently, meat consumption has increased, leading to increased production of wastewater in slaughterhouses (Baker, 2016).

Slaughterhouses consume a large amount of processed water, due to the following stages that include cleaning, washing, cutting and dispatching (Asselin *et al.* 2008). Wastewater from the slaughtering process can present a high biological risk to humans and other animals due to the presence of pathogens, toxic chemicals, and pharmaceuticals used for plant cleaning (Bustillo and Mhervar, 2016). The discharge of this wastewater can negatively affect the quality of natural water. One such effect is the reduction of dissolved oxygen in surface waters, which is a consequence of the high load of organic material in water resources (Baker, 2016).

Slaughterhouse wastewater is highly polluted waste liquids containing high concentration of organic matter, suspended solids, nitrogen, phosphorus, oils and fats (Saghir and Hajjar, 2022). COD concentrations in animal slaughter effluents can range from 500 to 15900 mg/L, while nitrogen is usually found between 50 and 841 mg/L (Carrasquero and Diaz, 2024; Derakhshan *et al.* 2023). In addition, it is estimated that the final discharges of slaughtered animals represent an average of 15 to 20% of blood, which is equivalent to 1 to 2 kg BOD per 1000 kg live weight of each animal and can reach 5.8 kg BOD per ton live weight, if there is total discharge of blood (Carrasquero *et al.* 2019)

Conventional physicochemical treatment methods such as coagulation-flocculation and sedimentation are often insufficient to remove persistent and hazardous organic pollutants from these waste waters (Moles *et al.* 2020; Jewell *et al.*, 2016). Thus, biological treatment is widely used to treat this type of effluent because of its efficiency and relatively low cost (Abdelfattah *et al.* 2023). Biological processes are more sustainable than physicochemical processes, mainly because they are more cost-effective and generate stable, non-toxic products. The most used biological systems include aerobic suspended growth processes, such as the activated sludge process and the sequential batch reactor. Membrane processes, such as the membrane bioreactor, and anaerobic processes, such as the up flow anaerobic sludge blanket reactor and the anaerobic filter, are also applied (Aziz *et al.*, 2019).

The acclimatization of microbial biomass is a crucial step prior to the operation of biological reactors for wastewater treatment, especially in processes involving highly contaminated effluents such as those generated in the meat industry. The need for this process lies in the gradual adaptation of microorganisms to the specific conditions of the wastewater to be treated, which includes factors such as the concentration of organic pollutants, the presence of toxic compounds and nutrient loading (Yanqoritha *et al.* 2018; Angenent *et al.* 2002).

During acclimation, the microbial biomass develops an optimized capacity to break down the organic compounds present and withstand the reactor conditions, increasing the efficiency of the purification process. This is critical to ensure the success of the biological treatment, as a well-acclimated biomass can cope with variations in effluent composition, withstand loading shocks and maintain a constant degradation activity. In addition, acclimation ensures that microorganisms grow and reproduce in a controlled manner, which improves the operational stability of the system and the quality of the treated effluent, minimizing the presence of persistent residual pollutants (Miao *et al.*, 2019).

The objective of this research was to evaluate the process of microbial biomass acclimation in batch reactors to optimize the treatment of wastewater from swine slaughtering.

2 MATERIALS AND METHODS

2.1 INDUSTRIAL EFFLUENT COLLECTION

The effluents from the slaughtering process were collected in a swine (*Sus domesticus)* slaughterhouse located southwest of the Maracaibo municipality, Zulia state, Venezuela, respectively. Effluent collection was performed according to the patterns established in the Standard Method (APHA *et al.*, 2022), using the 1060 methodology on sample collection and preservation.

Collection was carried out manually through simple random sampling in the tanks located at the effluent discharge outlet. Sampling was carried out monthly by collecting the wastewater from the companies in clean, dark-colored, plastic containers with a capacity of 20-25 L. The samples were then transferred to the laboratory for analysis. The samples were then taken to the laboratory, characterized and stored under refrigeration at 4°C to guarantee their initial characteristics.

2.2 CHARACTERIZATION OF INDUSTRIAL EFFLUENT

The composition of industrial effluents is of vital importance for the effective practice of their subsequent treatment. Samples of each effluent were characterized according to the Standard Method (APHA *et al.*, 2022) as shown in Table 1. The determinations were performed in duplicate, except for COD, which was performed in triplicate due to the variability of the analysis results.

Descriptive statistics were used to express the results of the characterization, expressing the mean values and standard deviation of each physicochemical parameter, and making histograms, for a visual representation of the distribution of the data, providing a clear view of the frequency with which different concentrations of contaminants are present. In addition, a Pearson correlation analysis of the measured physicochemical characteristics was performed.

2.3 ORIGIN OF THE MICROBIAL BIOMASS

The biomass was collected manually in the wastewater collection tank located inside the slaughter, unloading and raw material transport area of the swine slaughterhouse, in clean, dark-colored 1 L containers. The collected sludge and the slaughterhouse wastewater were placed in a batch reactor for the growth, activation and adaptation of the biomass in a 50:50 ratio, applying continuous aeration with 24-hour cycles for two weeks.

Table 1

Parameters measured during the characterization of effluents.

Parameter	Method Number*	Method Type
BOD	5210	Potentiometric
COD	5220	Volumetric method (closed reflux) for chloride concentrations ≤ 2000 mg/L
TKN	4500-Norg B	Volumetric
$N\text{-}NH_4^+$	4500-NH3 D	Volumetric
NO_2^-	4500-NO2—B	Colorimetric
NO_3^-	4500-NO3—B	Colorimetric
Total phosphorous (TP)	4500-P C	Colorimetric
$P\text{-}PO_4^{-3}$	4500-P C	Colorimetric
Cl^-	4500 Cl- B	Argentometric
pH	4500 H+ B	Potentiometric
Total alkalinity	2320 B	Volumetric
Real color	2120-C	Colorimetric
TSS (Total suspended solids)	2540 D	Gravimetric
SS (Settleable solids)	2540-F	Volumetric

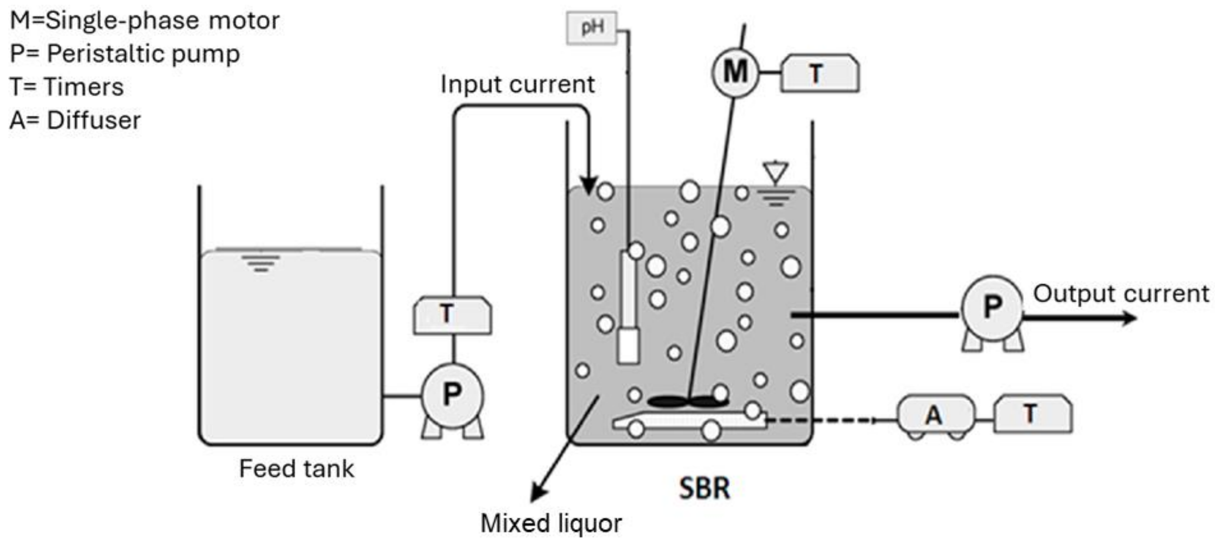
*APHA *et al.* (2022)

2.4 DESCRIPTION OF THE BATCH REACTOR

A laboratory scale sequential loading cylindrical reactor, 14.5 cm in diameter and 26 cm high, with a total device volume of 4 L operating in parallel, was used for the treatment of the pig slaughter effluent. The effective working volume was 2 L, with 30% adapted biomass and the rest wastewater. The reactor functioned in an automated manner for filling and discharging the water to be treated by means of a pumping system with one-way peristaltic pumps (Cole-Parmer, models 77202-60 and 77201-60), with time regulating devices (Excelline, model GTC-E-120AS9) and independent mechanical agitation and oxygen supply systems. All clarified effluent was removed from the reactor during the discharge phase (Figure 1).

Figure 1

Illustrated description of sequential batch reactor (SBR).



The influent inlet and effluent discharge from the reactor were carried out through flexible pipes of 6 mm diameter (Masterflex 06409-147), using one-way peristaltic pumps, with a constant flow rate of 93 mL/min for filling and discharge. The mixed liquor was also extracted. The mechanical agitation system consisted of a 15 W, 1300 rpm single-phase motor (General Electric, model WR60X165), assembled to a stainless-steel shaft with a four-wing blade that was immersed in the wastewater and sludge (mixed liquor). The engine rpm was maintained at 300 rpm using a potentiometer.

Air was supplied through a compressor (SeaStar, model HX-308-20) connected to a 5 mm transparent flexible tubing, linked in series with a diffuser device arranged at the bottom of the reactor, 45 cm long and 4 mm in diameter. This device supplied air in ascending form to the mixture contained in the reactor, guaranteeing a minimum concentration of dissolved oxygen of 2 mg/L during the aerobic phase.

2.5 ACCLIMATIZATION PROCESS

The biomass generated was used as inoculum in a reactor per load, with a useful volume of 2 L, in a 30:70 ratio (0.6 L of biomass and 1.4 L of industrial wastewater). Once inoculated, the reactor was subjected to a stabilization process with continuous aeration with an HRT of 24 hours, a CRT of 15 days and a sedimentation time of 30 minutes, maintaining the previously established criteria until the acclimatization process was completed. Biomass adaptation and stabilization of the reactor was carried out until the COD removal values obtained at the outlet were higher than 50% and good sludge sedimentation characteristics were observed (Carrasquero *et al.* 2024).

During adaptation, micronutrients were added to the wastewater to provide suitable conditions for the biomass to work, as suggested by Di Iaconi *et al.* (2002). The amount of nutrients added to the system was 1.5 mL per liter of mixed liquor (Table 2).

Table 2

Types and amount of nutrients added to the blended liquor in SBR.

Nutrient	Concentration (mg/L)
$NiSO_4.6H_2O$	0,5
$ZnSO_4.7H_2O$	0,5
$MnCl_2.4H_2O$	0,5
$FeCl_3.6H_2O$	0,73
$CoCl_2.6H_2O$	0,05
$CuSO_4.5H_2O$	0,005

For all cases, the pH and COD of the wastewater were measured at the inlet and outlet of the reactors by load. The organic matter removal efficiency during acclimatization was determined using Equation 1.

$$COD\ Removal\ (mg/L) = \left(\frac{CODi - CODo}{CODi}\right) \times 100 \qquad (1)$$

where:

COD_i is COD concentration at reactor inlet (mg/L); and
COD_o is COD concentration at outlet (mg/L).

Additionally, volumetric organic loads were determined using Equation 2, proposed by Mekonnen and Leta (2011). A Pearson correlation analysis was also performed between VOL and COD removed during effluent characterization.

$$VOL\ (\frac{g}{m^3} * d) = \frac{COD \times Vf}{Vr \times OCT} \qquad (2)$$

where:

COD: COD of raw wastewater (g/m^3);
Vf: volume of wastewater fed in each cycle (L);
Vr: useful volume of the reactor (L);
OCT: duration of the cycle in the SBR (d).

Similarly, a Pearson correlation analysis was performed between VOL and COD removed for the days of the acclimatization process.

3 RESULTADOS Y DISCUSIÓN

The effluent from the swine slaughterhouse presents high levels of contamination in several key parameters, such as BOD, COD, total phosphorus and settleable solids, which exceed regulatory limits (Table 3). Regarding organic matter concentration, the mean COD value was 7745 mg/L, higher than those reported by Saghir and Hajjar (2022), and Derakhshan *et al.* (2023), who found concentrations of 5350 and 4221 mg/L, respectively. Slaughterhouses use large volumes of water to clean floors, tools and animals, flushing a considerable amount of organic waste into the wastewater treatment system. This wash water includes traces of blood, fat, meat, excrement, and other wastes that contribute to the organic load and elevated BOD and COD levels.

Blood is rich in protein and has a high carbon and nitrogen content, which contributes significantly to BOD and COD levels. It is estimated that blood can contribute up to 60% of the total organic load in slaughterhouse wastewater (Derakhshan *et al.*, 2023). Similarly, fragments of meat, fat, and offal generated during slaughtering and processing are sources of organic matter that raise BOD and COD levels (Moles *et al.*, 2020).

Table 3

Composition of swine slaughterhouse wastewater.

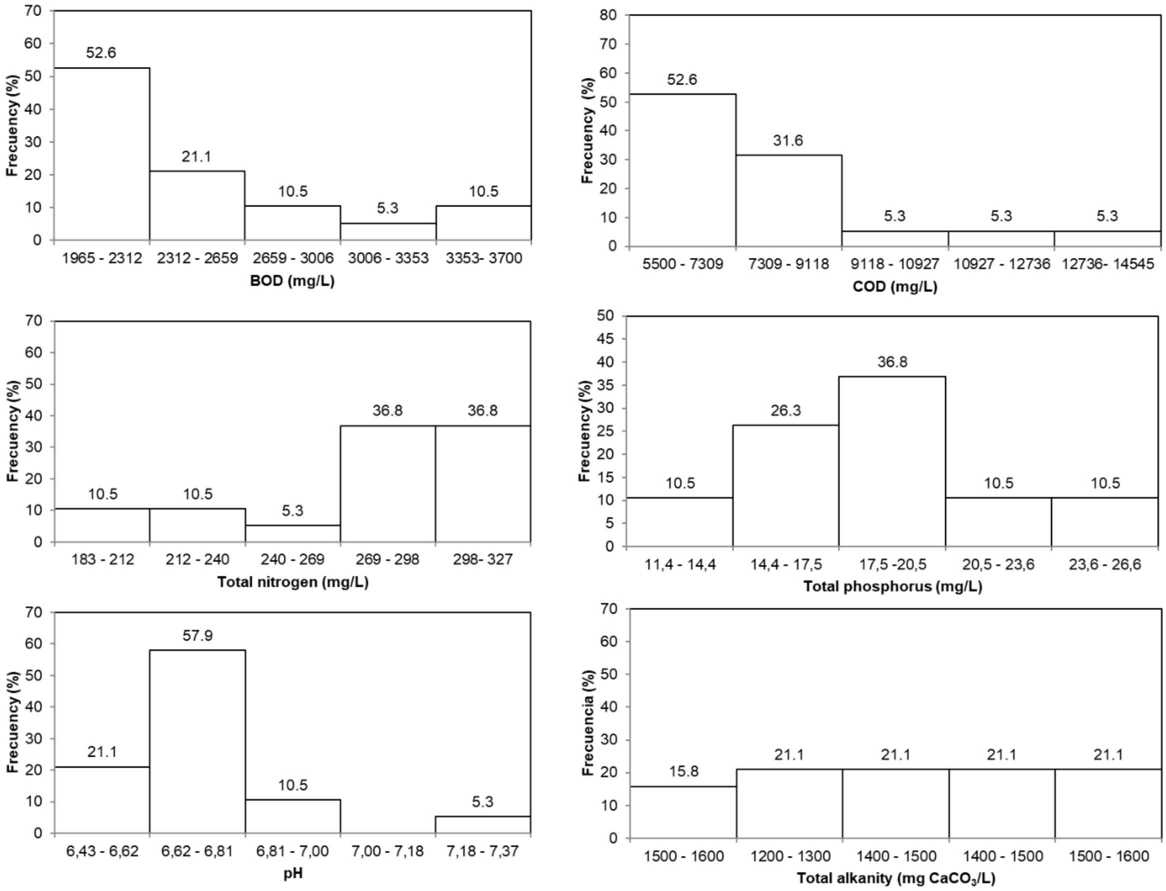
Parameter	Unity of expression	Value (mean ± SD)	Maximum Established Limites[1]
BOD	mg/L	2410± 513	60
COD	mg/L	7745 ± 2224	350
TKN	mg/L	2785± 39	-
$N\text{-}NH_4^+$	mg/L	173 ± 26	-
NO_X^-	mg/L	ND	10
Total phosphorous	mg/L	18,60 ± 3,16	9
$P\text{-}PO_4^{-3}$	mg/L	10,85 ± 2,96	10
Cl^-	mg/L	550± 28	1000
pH	-	6,77 ± 0,24	6 -9
Total Alkanity	mg $CaCO_3$/L	1376 ± 155	-
Real color	UC Pt-Co	350±70	500
Total suspendid solids	mg/L	3965 ±752	-
Sedimentable solids	mL/L	15,2 ± 0,2	1

n:18. n: number of repetitions ND: Not detectable. Detection limits: 1 mg/L. [1]Based on Decree 883 (1995).

The BOD concentration ranged from 1965 to 3700 mg/L (Figure 2). 52.6% of the analyzed samples were found in the lowest range (1965 - 2312 mg/L), the frequency decreased progressively in the higher ranges, down to 10.5% in the last two ranges (3006 - 3353 mg/L and 3353 - 3700 mg/L). Animal excreta, which may also be present in wastewater, are a significant source of readily biodegradable organic matter. These wastes increase the oxygen demand in the system, which raises the BOD. As for the biodegradability quotient (BOD/COD), this value was 0.31, suggesting that the effluent from this slaughterhouse has a significant fraction of organic matter that is difficult to biodegrade. This indicates the need to combine biological treatment with other technologies, such as advanced oxidation, to improve the overall treatment efficiency (Sing and Rashid, 2017; Selormey *et al.* 2021).

Figure 2

Frequency histograms of the parameters measured during characterization.



Total nitrogen concentrations ranged from 183 to 327 mg/L. Some 36.8% of the samples were in the 269 - 298 mg/L and 298 - 327 mg/L ranges. During animal processing, nitrogenous organic matter, such as proteins and amino acids, is broken down by microorganisms in the treatment system. This process releases ammonia as a by-product of the degradation. The variation in ammonia nitrogen levels will depend on the amount of organic matter and proteins present in the effluent (Mishra *et al.*, 2022). There was a very strong positive correlation (r = 0.939; p=0.000) between ammonia nitrogen and Kjeldahl total nitrogen (KTN), which was expected, as both are important components of total nitrogen (Table 4).

Similarly, there was a strong positive correlation (r=0.719: p=0.001) between total phosphorus and BOD. This suggests that as the amount of phosphorus in the effluent increases, so does the biodegradable organic load.

Tabla 4

Correlación de Pearson de los parámetros medidos en la caracterización.

	BOD	COD	KTN	$N\text{-}NH_4^+$	TP	$P\text{-}PO_4^{-3}$	pH	TA	Color	SS
COD	-.132									
p	.602									
KTN	.007	-.143								
p	.977	.570								
$N\text{-}NH_4^+$	.063	-.301	,939**							
p	.802	.224	.000							
TP	,719**	-.349	.215	.342						
p	.001	.156	.391	.164						
$P\text{-}PO_4^{-3}$	-.017	.009	-.003	.012	.241					
p	.947	.973	.990	.961	.336					
pH	-.145	-.209	.402	.397	.098	.073				
p	.566	.405	.098	.103	.698	.773				
TA	.291	.292	-.353	-.367	.110	-.121	-.118			
p	.242	.240	.151	.134	.665	.633	.641			
Color	-.368	.107	-.037	-.012	-,594**	-,621**	-.086	-.202		
p	.133	.673	.883	.963	.009	.006	.735	.422		
SS	.001	-,809**	-.030	.074	.127	-.285	.048	-.097	.159	
p	.996	.000	.905	.770	.615	.251	.849	.702	.527	
TSS	.244	.156	-.330	-.216	.080	-,521*	-.035	.164	.386	-.160
p	.329	.537	.181	.389	.753	.027	.891	.516	.114	.525

**. La correlación es significativa al nivel 0,01 (bilateral). *. La correlación es significante al nivel 0,05 (bilateral). TA: Total alkanity. SS: Sedimentable Solids. SST: Total Suspended Solids. p: p-value. n:18.

With respect to the values of physical parameters, the average concentration of total suspended solids and settleable solids were 3965 mg/L and 15.2 mL/L, respectively. Total suspended solids occur due to the dragging of large amounts of hair, coagulated blood and viscera remains that are discharged by the action of washing in the channels that receive the wastewater (Carrasquero and Díaz, 2024).

The average concentration of chlorides in the effluent was 550 mg/L, which can be attributed to the production of large amounts of urine by the animals during the stalling and stunning stages. This process contaminates the water with salts, such as chlorides, and nitrogenous compounds.

The pH values in swine slaughterhouse effluent are generally in the neutral to slightly acidic range. A slightly acidic pH can influence the effectiveness of biological wastewater treatment. If the pH falls below 6, the microbial activity responsible for organic matter degradation could be adversely affected, hindering the nitrification process and other biological treatments. Therefore, it is important to control the pH during effluent treatment to maintain an optimal environment for microorganisms.

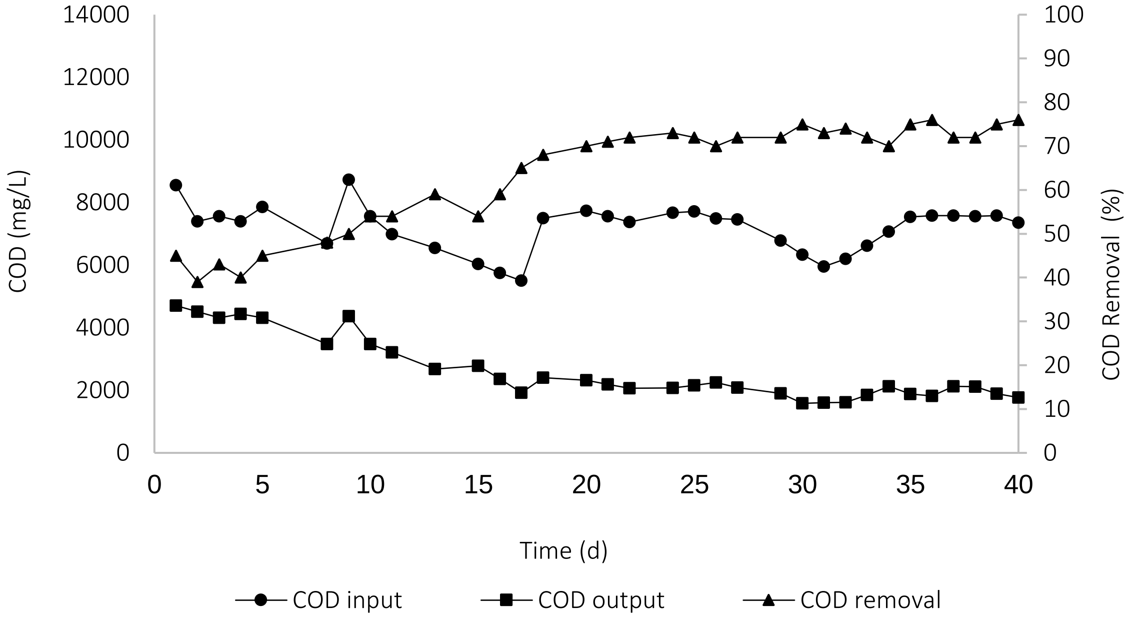
According to the results obtained in the effluent characterization, the wastewater generated by the swine slaughterhouse does not meet the necessary specifications for discharge into natural water bodies. Therefore, it is necessary to implement adequate treatment to reduce the levels of organic matter, nutrients, and solids to the limits allowed by current regulations (Decree 883, 1995).

The first stage of the biomass acclimation process (0-5 days) was carried out with an average VOL of 3.88 kg COD/(m^3.d). It was observed that as the experimental time in the reactor per load increased, the percentage of COD removal progressively increased, observing an average efficiency of 42.4% during the first five days of reactor operation (Figure 3). During the first days of the acclimatization process (until approximately day 10), the system presented fluctuations, which is common as the microbial biomass adjusts to the conditions of the medium.

Between on days 6 and 15, a COD removal of 48.8% was obtained, for the period between days 16 and 20, the percentage of COD removal in the reactor operating with a VOL of 4.63 kg COD/(m^3.d) was 64.5%, a value that increased to 71.2% from day 28, when the reactor operated at a VOL of 4.95 kg COD/(m^3.d). Values at the reactor outlet remained stable at around 2205 mg/L between days 30 and 35. At the end of acclimatization (day 40), the value at the reactor outlet was 2111 mg/L. This gradual increase in DOC removal indicates that the microbial biomass is acclimatizing effectively. As time passes, the microbial biomass becomes more efficient in degrading the organic matter present.

Figure 3

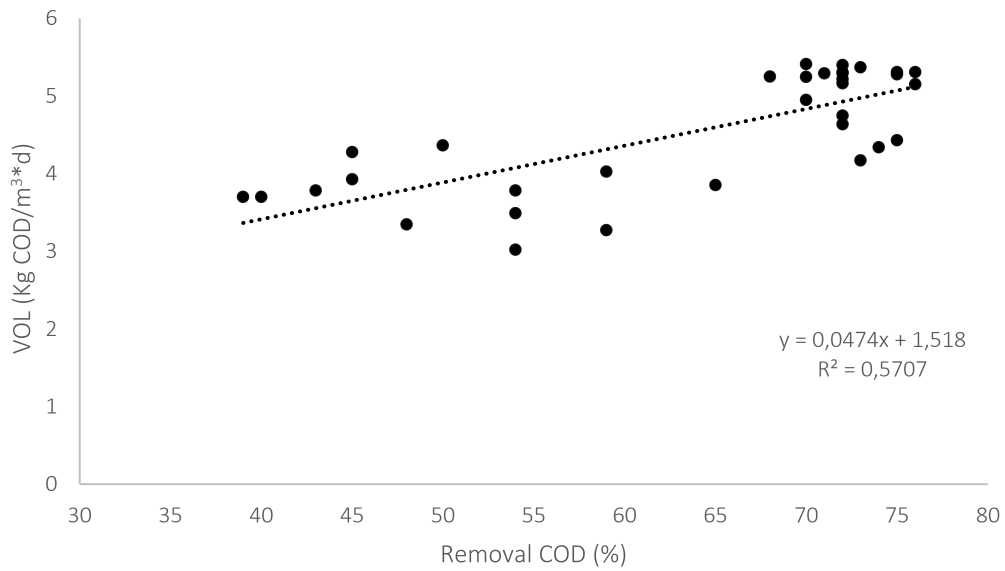
Acclimatization process.



A moderately positive correlation was found between volumetric COD load and percent COD removal, as the COD removal efficiency increased, so did the volumetric load treated. The coefficient of determination of 0.5707 indicates that approximately 57% of the variability in VOL can be explained by percent COD removal. Although the correlation is positive, the r^2 value suggests that the relationship is not extremely strong, which means that there are other factors besides DOC removal that could also be influencing the volumetric load. These factors could be the type of organic matter present in the effluent, the operating conditions of the system, and the ability of the system to adapt to variations in organic loading. To optimize performance, it would be beneficial to adjust these parameters and consider possible improvements in effluent pretreatment.

Figure 4

Correlation between volumetric organic load and organic matter removed.



4 CONCLUSIONS

Pig slaughterhouse effluents present high levels of organic contamination, as observed in the high concentrations of BOD, COD, total phosphorus, and suspended solids. These levels exceed regulatory limits, which makes it necessary to implement adequate treatments to prevent contamination of natural water bodies.

The acclimatization of microbial biomass in batch reactors is a fundamental process to optimize the biological treatment of these highly polluted effluents. During this process, the biomass improves its organic matter degradation capacity, resulting in a progressive increase in COD removal over time, reaching an efficiency of more than 70%.

There is a positive correlation between volumetric organic load and chemical oxygen demand removal, indicating that as the efficiency of the treatment system increases, so does its capacity to handle higher volumes of organic load. However, other factors, such as variability in effluent composition and system operating conditions, also influence this relationship.

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