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Evaluation of a microbial enzyme complex on the rate of aerobic biodegradation of domestic wastewater

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ABSTRACT

The present study evaluated the impact of the concentration of a microbial enzyme complex on the aerobic purification rate of domestic wastewater. A diverse and active microbial enzyme complex allows the efficient degradation of organic pollutants present in wastewater. In addition, environmental conditions, such as nutrient availability and optimum temperature, favor enzymatic activity and the performance of the purification process. For this purpose, aerobic depuration tests were carried out in a bioreactor considering three concentration levels of the microbial enzyme complex (1.0, 1.5, and 2.0 g/L), a controlled temperature of 17°C, and a dissolved oxygen concentration of 3.0 ppm. The adaptation process of the microorganisms lasted two days, with a daily decrease in the Chemical Oxygen Demand (COD) of 62.8, 71.1, and 77.9 ppm. Therefore, the removal rate increased with the concentration of the microbial enzyme complex, and the treatment time had a significant effect on the speed of purification.

1. Introduction

Unlimited population growth, urbanization, and intensive industrial activities have led to high rates of freshwater consumption and the production of wastewater laden with hazardous contaminants such as solvents, detergents, organic matter, pathogens, and others [1,2]. These contaminants pose a serious threat to anthropogenic activities, economic growth, water security, and global ecosystem health [3-5]. Therefore, finding effective and sustainable solutions to deal with

these contaminants through treatment and reuse processes is a mandatory measure for the competent authorities in the management of water resources [6]. In this regard, aerobic biodegradation processes are one of the most used treatment methods since they use microorganisms capable of decomposing, metabolizing, and using water contaminants as a source of energy and carbon for their growth [7-9]. In natural environments, microorganisms and their enzymes play a key role in the decomposition of organic matter, a process fundamental to nutrient cycling

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and the health of the aquatic ecosystem. In wastewater treatment systems, biological processes that take advantage of the enzymatic activity of microorganisms are used to remove organic contaminants through different strategies, such as biological oxidation, anaerobic digestion, and aerobic biodegradation, to maximize efficiency in the removal of undesirable organic compounds [10]. In addition, recent studies showing the bacterial capacity to eliminate organic compounds resistant to biological decomposition are highlighted. For example, [11] investigated the biological removal of nitrogen and phosphorus from domestic wastewater by inserting an anaerobic/anoxic holding tank in the sludge return line of a modified MLE-OSA system, highlighting the ability to remove specific wastewater pollutants. Also, [12] studied the biodegradation of crude oil by *Bacillus subtilis* isolated from contaminated soils in warm climate areas, demonstrating its ability to degrade complex organic compounds, such as crude oil, under specific environmental conditions. Another study by [13] investigated the ability of *Bacillus cereus* to biodegrade crude oil in hot areas. This study also highlighted the effectiveness of certain bacterial strains in the removal of persistent organic pollutants, [14] studied the biodegradation of methyl tert-butyl ether (MTBE) by the indigenous *Bacillus cereus* strain RJ1 isolated from soil, highlighting the importance of microbial diversity and adaptation of certain bacteria to degrade specific chemical compounds in the environment. The use of microbial enzymes has recently been shown to be one of the most effective alternative approaches to biodegradation since they accelerate chemical reactions and facilitate the conversion of contaminants into less harmful or even innocuous products [15]. For example, for organic and inorganic contaminants, synthesized enzymes, such as hydrolases, oxidoreductases, oxygenases, and peroxidases, are used to degrade actinomycetes, fungi, and blue-green algae [16]. In this sense, the Monod equation is a fundamental tool in the mathematical modeling of aerobic biodegradation processes. In the context of biodegradation of organic compounds in municipal

wastewater, it is used to understand how the concentration of these compounds influences the growth rate of the microorganisms responsible for their degradation and, therefore, the efficiency of biodegradation [17]. Therefore, it is important to control factors that may influence the bioconversion of this process such as temperature, suspended solids, concentration and types of dissolved substances, oils, fats, dissolved oxygen concentration, types of microorganisms, agitation technique, and oxygenation of water, among others [18-20]. The biodegradation of pollutants by a mixture of microorganisms and enzymes is a fundamental process to improve environmental quality and ensure water purification in receiving bodies. Based on this premise and with the objective of optimizing this biological process to counteract the negative effects of harmful substances, the main purpose of this study was to evaluate the rate and speed of aerobic biodegradation. The focus was on a specific mixture of microorganisms and enzymes, which was referred to as the microbial enzyme complex. This analysis was carried out with the intention of improving wastewater treatment processes.

2. Materials and methods

2.1. Experimental procedure

The activities carried out for the enzymatic biodegradation tests included conditioning and testing equipment and instruments, as well as the preparation of wastewater samples. For this purpose, the experimental equipment included a storage container (Figure 1).

2.2. Preparation of wastewater samples

The wastewater samples were artificially prepared and therefore referred to as synthetic wastewater. These samples were obtained for each test as follows: 25 L of chlorine-free drinking water was poured into the container of the experimental equipment, and then the mixer was activated to achieve a homogeneous mixture while simultaneously adding sucrose (2.35 g), starch (5.5 g), peptone (10.45 g), ammonium sulfate (1.8 g), and ammonium phosphate (0.25 g).

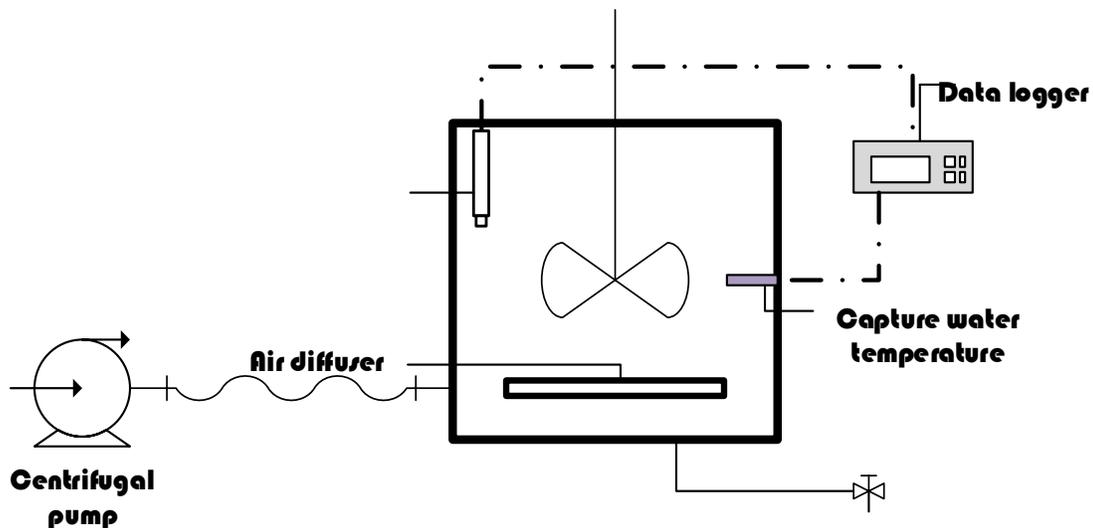


Fig. 1. Experimental equipment.

These substances were selected based on their ability to simulate the chemical and nutritional composition of domestic wastewater. For example, sucrose and starch are carbon sources that simulate the presence of carbohydrates and soluble organic matter found in wastewater, such as sugars and starches from food and organic waste. Peptone is a source of carbon and nitrogen that represents the complex organic matter present, such as proteins and peptides, from food remains and organic matter; ammonium sulfate and phosphate are sources of inorganic nitrogen and phosphorus, respectively. These nutrients are essential for microbial growth and are present in the form of inorganic compounds and minerals [21-23]. Likewise, it was necessary to ensure that the substances were completely dissolved to guarantee that the resulting mixture was homogeneous before using it as synthetic wastewater in the experimental process.

2.3. Biological purification experiments

After checking and testing the equipment and with the synthetic wastewater sample ready in the container, the experimental process started. For this purpose, the mixer was activated while 3.0 g of Na_2SO_3 and 1.0 g of CoCl were simultaneously added to minimize the dissolved oxygen concentration following what was mentioned by [24]. Afterward, the reference level was regulated at 3 ppm (set point) and the mixer rotation speed 54 RPM. Next, with the temperature and dissolved oxygen control elements activated, the microbial

enzyme complex (1.0, 1.5 and 2.0 g/L) was added (Figure 2). As the dissolved oxygen level decreased, the solenoid valve was activated to allow air into the diffuser.



Fig. 2. Enzyme complex label.

2.4. Rate of enzymatic biodegradation

The rate of enzymatic biodegradation can be described by kinetic equations that relate the rate of disappearance of the contaminant to the system conditions. One of the most commonly used kinetic models to describe biodegradation is the first-order exponential decay model, which is expressed by the following equation:

$$\frac{dC}{dt} = -k * C \quad (1)$$

where dC/dt is the rate of change of contaminant concentration with respect to time, C is the contaminant concentration at time t , and k is the biodegradation rate constant. This equation indicates that the rate of change of contaminant concentration is proportional to the current contaminant concentration in the system. The contaminant concentration exponentially decreases as time progresses until a minimum or non-detectability level is reached. The relationship between these two factors implies that the rate of biodegradation reaction can be influenced by both the nature of the contaminant and the availability of dissolved oxygen in the water. Pollutants that are more readily biodegradable may have a faster reaction rate under high dissolved oxygen conditions, while more persistent pollutants may require higher levels of dissolved oxygen or specific conditions to achieve an optimal biodegradation rate. Also, it is possible that, in some cases, more complex kinetic models are required to describe the biodegradation of certain contaminants adequately. Therefore, in order to associate this variable with the chemical oxygen demand, the following equation was used:

$$R = \frac{\Delta COD}{\Delta t} \quad (2)$$

where R is the rate of biodegradation, ΔCOD is the decrease in chemical oxygen demand after one day of treatment, and Δt is the treatment time interval.

2.5. Statistical analysis

The evaluation of the variation of the daily rate of biodegradation due to the effect of the factors indicated above was carried out using a two-factor analysis of variance [25]. The significance level was 0.05. The values of F_o , referred to as the percentage likelihood ratio F observed with the results, were used to compare with the values of F obtained from probability distribution tables. This comparison was used to measure the dispersion of the data with respect to the total mean. This test was used to evaluate if the means are statistically the same or if the means of the observed variable are the same when a factor is modified, then that factor does not affect it; in some cases, this is called null hypothesis testing [26]. This is rejected if F_o is greater than the F of probability distribution, at significance level α , degrees of freedom of the

factor being evaluated, and at degrees of freedom of the error ($F_{\alpha, G.L. \text{ factor}, G.L. \text{ mistake}}$).

3. Results and discussions

3.1. Presentation of collected chemical oxygen demand (COD) measurements

The COD data obtained were according to the applied methodological design and the concentrations of the microbial enzyme complex in which the experiments were carried out. Figure 3 shows the typical behavior of COD reduction with R1, R2, and R3 representing the repetition of the experiment, which could be understood as the adaptation period (two days) that the microbial enzyme complex undergoes. In addition, it adjusted to a straight line until approximately the fifth day, indicating that the microorganisms had access to a sufficient amount of substrate for their metabolism during the mentioned period [27]. However, an opposite trend was observed after the fifth day, when the COD slowed down, resembling cases of aerobic biodegradation where there was a limitation of substrates [28]. This suggested that as available substrates were depleted, microorganisms experienced a decrease in metabolic activity. It was possible that the decrease in COD after day five was related to the scarcity of substrates and the reduction in the availability of nutrients needed for microbial growth and metabolism, as suggested by [29-30], who pointed out that this availability influenced the rate and efficiency of aerobic purification. Furthermore, they suggested that treatment time and substrate availability were critical factors to consider when designing and optimizing wastewater treatment processes using microbial complexes and communities. The following was obtained when comparing the COD averages shown, as well as the correlation (Figure 4): The COD data listed are in triplicate for each combination of levels, according to the methodological design (Table 1). The table shows that as the concentration of the microbial enzyme complex increased, the daily reduction in COD was greater. This was observed in the slopes of the equations obtained by linear regression. On average, COD decreased daily by 62.8 ppm, 71.1 ppm, and 77.9 ppm at microbial enzyme complex concentrations of 1.0 g/L, 1.5 g/L, and 2.0 g/L,

respectively. These findings support the idea put forward by [31] They mentioned that a higher concentration of the microbial enzyme complex led to greater enzyme activity and, therefore, a better capacity to degrade the contaminants present in domestic wastewater since microorganisms produce a variety of enzymes that break down organic compounds and reduce COD. This, in

combination with the concentration of the microbial enzyme complex, had an important role in the efficiency of the aerobic purification process, as confirmed by [32]. Therefore, these results suggested that by adjusting the concentration of the microbial enzyme complex, it was possible to optimize the rate of contaminant reduction.

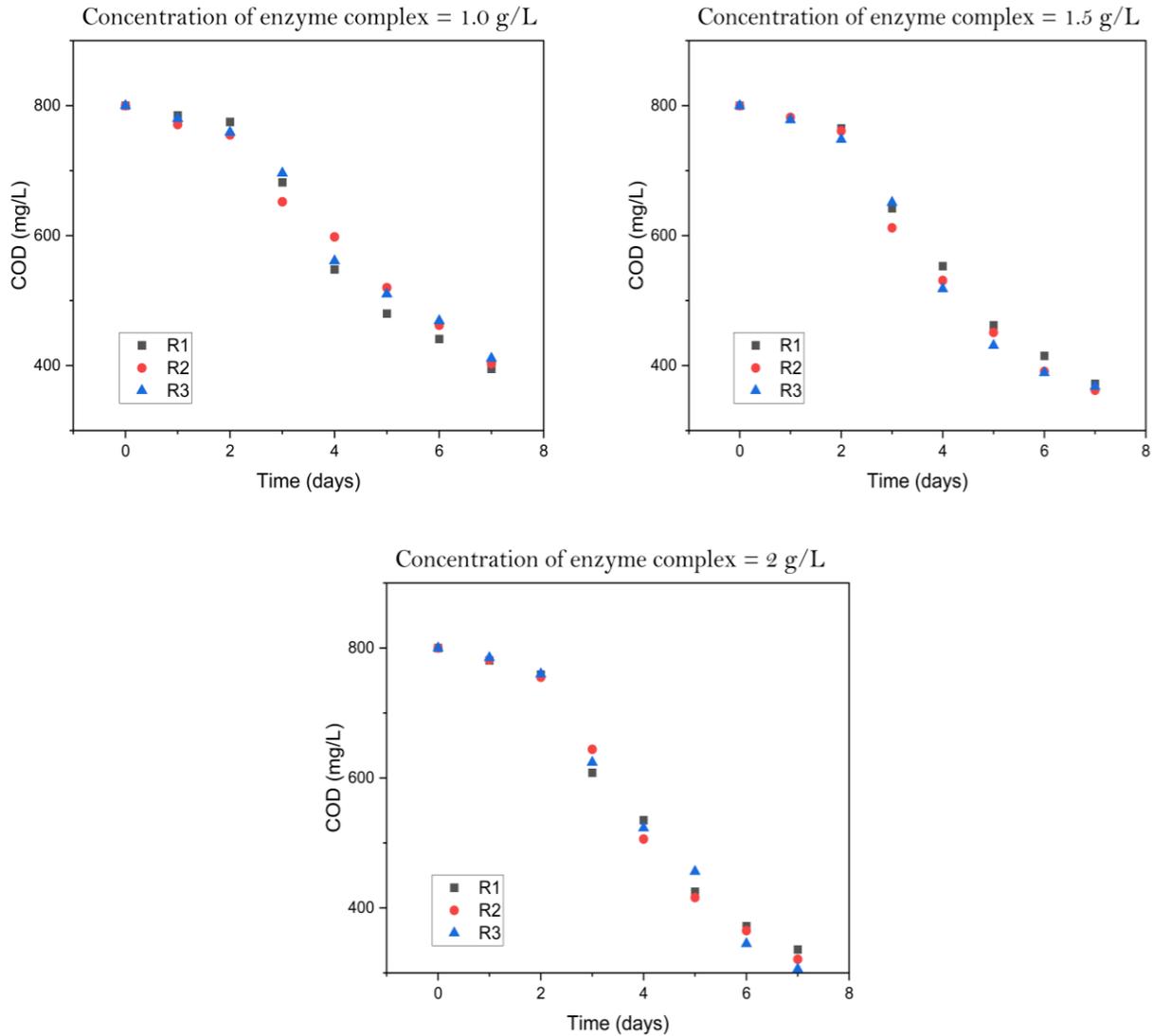


Fig. 3. Evolution of COD, according to data collected at different concentrations of enzyme complex: a) C1=1.0 g/L, b) C2=1.5 g/L, and c) C3=2.0 g/L.

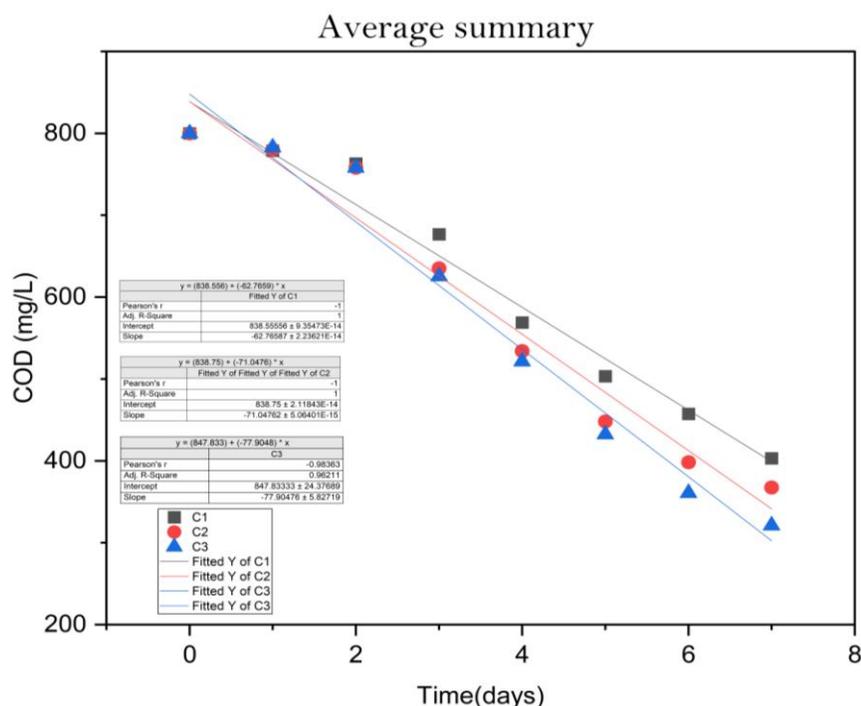


Fig. 4. Average COD of the three microbial enzyme complex concentrations.

Table 1. Data collected at various concentrations of microbial enzyme complex.

Concentration	Time (days)	COD (mg/L)		
		I	II	III
1.0 g/L	0	800	800	800
	1	785	771	780
	2	775	755	759
	3	682	652	696
	4	548	598	561
	5	480	520	510
	6	441	462	469
	7	395	403	411
1.5 g/L	0	800	800	800
	1	780	782	778
	2	765	761	748
	3	642	612	651
	4	553	531	518
	5	462	451	431
	6	415	391	389
	7	372	362	368
2.0 g/L	0	800	800	800
	1	781	782	785
	2	759	755	760
	3	608	644	624
	4	535	506	523
	5	425	416	456
	6	372	365	345
	7	336	321	306

3.2. Rate of enzymatic biodegradation

The results obtained on the rate of enzymatic biodegradation are shown in Figure 5. Each figure

presents the three experimental replicates (I, II, and III). Figure 6 shows the average results in a single graph.

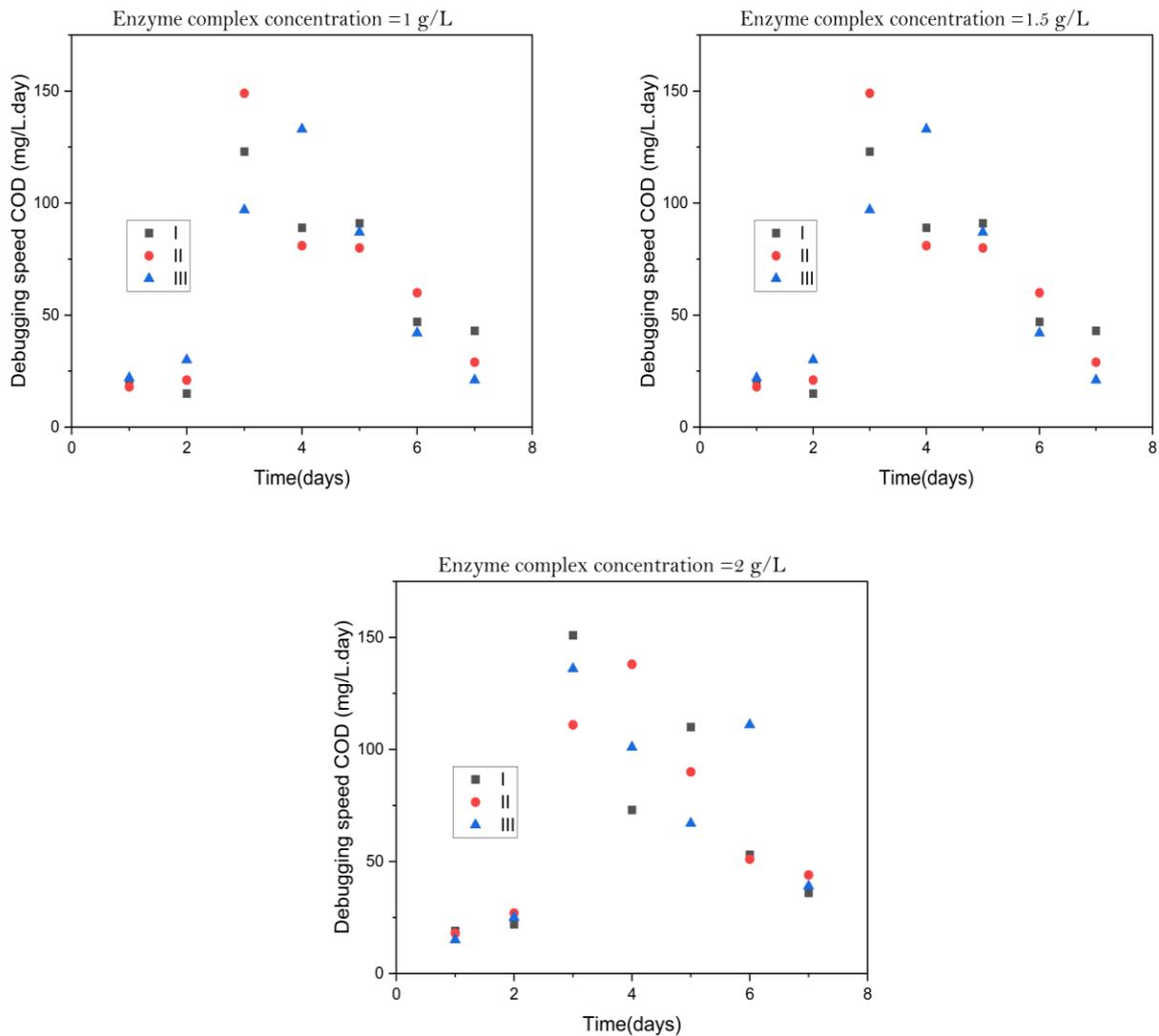


Fig. 5. Results of speed of clearance under different concentrations of enzyme complex: a) C1=1.0 g/L b) C2=1.5g/L c) C3=2.0 g/L.

As can be seen, the rate of purification tended to increase from the second day, reaching its maximum on the third day. This occurred because the microorganisms had access to a greater amount of dissolved biodegradable matter and increased in number thanks to their growth and replication. Subsequently, this trend decreased progressively until the seventh day. These patterns coincided with the adaptation process, as evidenced above in the COD data. The final Figure 6 shows that the concentration of 2.0 g/L of

microorganisms displayed a faster rate of purification; however, the one obtained at a concentration of 1.5 g/L did not differ significantly. In other words, the rate of purification was higher when the concentrations of the microbial enzyme complex were high. Similar results were obtained by [33-34] when they worked with other types of contaminants present in textile wastewater samples. They observed a similar rate of purification in the days after the second and third days of adaptation of the microorganisms. It is

important to mention several factors that can favor the rate of purification. One example is the presence of a diverse and abundant microbial enzyme complex since they allow a greater degradation of the contaminants present in the wastewater; also, an adequate level of dissolved oxygen is essential to maintain optimal metabolic activity and facilitate the oxidation of the contaminants [35]. In addition, the availability of essential nutrients can also influence the rate of purification [34], as shown in Figure 3. On the other hand, factors such as temperature, pH, and hydraulic residence time were not evaluated in this research, but they could interact with each other, and their optimization will depend on the type of contaminant present in the wastewater and the specific characteristics of the treatment system [28]. It is important to consider and control these factors in future research in order to attain greater achievement in efficient and effective purification. On the other hand, a mathematical relationship between time (X_1), microbial complex concentration (X_2), and rate of purification (Y), by means of a multiple linear regression analysis of the results from the third day, allowed for establishing a significant relationship between these variables (Table 2).

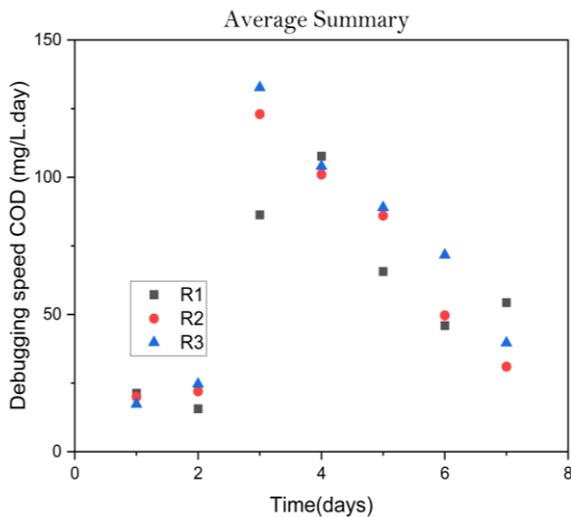


Fig. 6. Average speed of purification of the three concentrations of microbial enzyme complexes.

After evaluating the results of the rate of purification for each case and their respective replicates. Finally, the F_0 values obtained are shown in Table 3. The comparison showed that the factor

that affected the rate of purification was time, while the concentration of the microbial enzyme complex, as well as the interaction of both factors, had a non-significant effect.

Table 2. Multiple linear regression.

Variable	Coefficients
Intercept	152.633333
X_1	-19.311111
X_2	15.4

Table 3. Summary of the analysis of variation in speed results.

Factor	Sum of	Degrees	Medium	F_0
Time	35313.761	6	5885.626	4.0240
Concentrati	1448.9841	2	724.4920	0.4953
Interaction	3322.5714	12	276.8809	0.1893
Error	61430.333	42	1462.626	-
Total	101515.65	62	-	-

The F-statistics for each factor are shown in Table 4.

Table 4. Statistics F.

Factor	F (0.05,6,42)	F_0
Time	F (0.05,6,42)	2.34
Concentration	F (0.05,2,42)	3.23
Interaction	F (0.05,12,42)	2.00

4. Conclusions

The research examined the effect of the concentration of a microbial enzyme complex on the efficiency of aerobic purification of domestic wastewater. A significant decrease in chemical oxygen demand (COD) was observed from the second day of treatment, with average values of 62.8, 71.1, and 77.9 ppm for microbial enzyme complex concentrations of 1.0 g/L, 1.5 g/L, and 2.0 g/L, respectively. In addition, a mathematical relationship was established between time (X_1), microbial enzyme complex concentration (X_2), and COD (Y). The results indicated that the relationship could be expressed as $Y = 152.6 - 19.31 X_1 + 15.4 X_2$, considering a correlation coefficient of 0.93. Regarding the evaluation of the significance of the effects of the concentration of the microbial enzyme complex and time on the rate of aerobic purification, this study concluded that time was the factor that had a significant effect on the rate of purification. This study provides strong evidence that the concentration of the microbial enzyme complex positively influences the rate of aerobic purification of domestic wastewater. These findings have important implications for designing

and optimizing wastewater treatment processes, which can contribute to a more efficient and sustainable management of water resources.

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