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Wastewater treatment using microbial enzymatic mixture

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ABSTRACT

The effectiveness of the use of microbial enzymes as a wastewater treatment method depends on factors such as temperature, pressure, and oxygenation level, among others. However, very few scientific studies have provided real data regarding the evaluation of the applicability of this type of treatment. This research was therefore carried out to evaluate the effect of temperature and aerobic treatment time on the level of treatment of wastewater pollutants, with a mixture composed of enzymes and aerobic microorganisms under environmental conditions in the city of Huancayo, Peru. For this purpose, synthetic wastewater samples were prepared, considering a value of $COD_{initial}$= 800 mg L^{-1}. One group of experiments was conducted at a 20°C controlled temperature and another group without control. The following average kinetic constants were obtained as results (K_o= 1.662 d^{-1}, Kc=2.53 mg COD mg^{-1} VSS) when the T°= 20°C. For the experiments where the T° was not controlled, the average constants were: K_o= 1.217 d^{-1}, K_c=1.24 mg COD mg^{-1} SSV. We observed from the measurements that the pollutant removal was adjusted to the kinetic model proposed by Orozco, and the biomass production was adjusted to the stoichiometric equation of biomass production with endogenous respiration. Therefore, we concluded that the effect of temperature control and treatment time on the level of purification caused by the microbial enzymatic product is significant at a 95% confidence level.

Keywords: aerobic purification, biomass, digesting bacteria, kinetics, microorganisms.

Purificação de águas residuais a partir de uma mistura enzimática microbiana

RESUMO

A eficácia da utilização de enzimas microbianas como método de tratamento de águas residuais depende de factores tais como temperatura, pressão, nível de oxigenação, entre outros. No entanto, muito poucos estudos científicos forneceram dados reais sobre a avaliação da aplicabilidade deste tipo de tratamento. Neste sentido, esta investigação foi realizada para avaliar o efeito da temperatura e do tempo de tratamento aeróbico no nível de purificação dos poluentes das águas residuais, com uma mistura de enzimas e microrganismos aeróbicos, em condições ambientais da cidade de Huancayo, Peru. Para este efeito, foram preparadas amostras de águas residuais de forma sintética, considerando um valor de $DQO_{initial}$= 800 mg L^{-1}. Um grupo de experimentos foi conduzido a uma temperatura controlada de 20°C e outro grupo sem controle. Foram obtidas como resultados as seguintes constantes cinéticas médias (K_o= 1.662

d^{-1}, K_c=2.53 mg DQO mg^{-1} SSV), quando o T°= 20 °C. Enquanto que, para as experiências em que o T°, ases constantes médias eram: K_o= 1,217 d^{-1}, K_c=1,24 mg DQO mg^{-1} SSV. A partir das medições, observou-sé que a remoção de poluentes foi ajustada ao modelo cinético proposto por Orozco, e a produção de biomassa foi ajustada à equação estequiométrica da produção de biomassa com respiração endógena. Assim, concluiu-se que o efeito do controlo da temperatura e do tempo de tratamento no nível de purificação causado pelo produto enzimático microbiano é significativo a um nível de confiança de 95%.

Palavras-chave: bactérias de digestão, biomassa, cinética, microrganismos, purificação aeróbica.

1. INTRODUCTION

Environmental impacts are due to the multiple activities carried out by human beings. In this sense, many of the by-products generated are not adequately disposed of, as is the case of wastewater. Depending on the industry in which they are generated (pharmaceuticals, personal care, pesticides, among others), the load of pollutants that carry with them various suspended and dissolved substances increase. This damages the natural bodies of water that come into contact with them, representing a major obstacle in water treatment plants due to their high stability and resistance to physicochemical and biological degradation (Zdarta *et al.*, 2022).

The types of pollutants in wastewater include pathogenic microorganisms, pharmaceuticals, and pesticides. Needless to say, the risks associated with them are detrimental to human health, flora and fauna, as well as other environmental components. According to (Wiegner *et al.*, 2021), in recent years more than 120 million cases of gastroenteritis have been reported worldwide. On the other hand, the presence of other potentially toxic contaminants (cadmium, chromium, copper, nickel, etc.) and those released into the soil during crop production are serious threats to environmental sustainability (Singh *et al.*, 2022).

The removal of these pollutants is a complicated process, even if conventional treatments such as activated sludge are applied, since their prevalence time and resistance to natural biodegradation processes are considerable (Zdarta *et al.*, 2022). Likewise, treatments such as bioremediation and phytoremediation are very efficient and environmentally friendly, while physical-chemical methods are techno-economic alternatives (Singh *et al.*, 2022). Concerning alternative technologies, the use of bio adsorbents has increased in drinking water and wastewater treatment, as well as nanomaterials that have shown excellent performance due to their chemical properties (Hakimi *et al.*, 2022), since the way they are prepared provides better absorption capacities, reduces costs and the magnetic collection system is more optimal (Xu *et al.*, 2021).

Therefore, the use of highly efficient biocatalysts such as enzymes is an important alternative for the removal of these resistant pollutants (Feng *et al.*, 2021), as they help to reduce their toxicity. When we refer to enzymes, we are talking about complete organism's alternative to plants or other microorganisms that take longer to develop and show greater sensitivity to adverse conditions in wastewater or are at risk of degradation during treatment (Azizan *et al.*, 2021). However, this purifying action of aerobic microorganisms can be affected in many cases by factors such as temperature, pressure, pH, oxygenation level, agitation, geometric shape and characteristics of the enclosures or containers where the biological action takes place, as well as the presence of suspended and dissolved solids in the water, such as non-biodegradable long-chain substances, oils and fats, among others (Marin *et al.*, 2021).

So, with the aim of improving the understanding of the pollutant removal process and biomass production within the secondary aerobic decontamination treatment, engineers and researchers in these topics proposed several mathematical relationships defined by formulas between the parameters that characterize a polluted water mixture and bacteria (Meek, 2022).

These mathematical relationships also allow predicting the performance of microorganisms in the purification process, which is used in the design of water treatment plants. The equations that allow explanation and serve as a basis for those used in substrate removal and biomass production were developed by Michaelis, Monod, Contoins, among the most notable authors. Meanwhile, for substrate removal and biomass production by microorganisms, there are models such as those proposed by Eckenfelder, McKinney, Lawerence & Mc Carty and Orozco. Each one is for particular purification conditions, such as substrate abundance, starvation and substrate limitation (Orozco, 2014).

Thus, in this research we proposed to determine and evaluate the performance of a bacterial enzymatic product in biodepuration with and without temperature control under the environmental conditions of Huancayo. Taking into account that, in aerobic type treatments, microorganisms are used to develop the work of metabolization of the different pollutants and produce a greater number of microorganisms.

2. MATERIALS AND METHODS

2.1. Wastewater preparation

This research was carried out in the suburban region of the city of Huancayo, Peru (12.03° S; 75.20° W). To obtain the wastewater samples, it was necessary to add the amounts indicated in Table 1, recording a COD= 800 mg L^{-1}. The volume of water prepared for each experiment was 10 L.

Table 1. Substances and quantities in grams that make up the synthetic wastewater.

Substance	Mass added/volume of liquid (g L^{-1})
Peptone	0.378
Sucrose	0.091
Starch	0.212
Ammonium sulfate	0.0455
Dibasic sodium phosphate	0.009

In addition, for each experiment it was necessary to condition the temperature of the drinking water to 89°C for a period of 5 min, then wait for this sample to cool down and reach T°= 20°C in order to eliminate the dissolved chlorine. Then, 10 L of the previously conditioned water was poured into a 20 L plastic container, while the substances described above were added. In this way, the liquid medium or synthetic wastewater was obtained for the pollutant purification tests.

2.2. Purification tests

With the previously prepared synthetic wastewater sample, 10 L were poured into the stirred-tank bioreactor for each experiment. Then the temperature and dissolved oxygen level sensors were activated, as well as the air flow solenoid valve and the bioreactor stirrer at a speed of 10 rpm (in the case of experiments without temperature control, the sensor that records this variable was deactivated). After that, time was allowed for oxygenation of the liquid medium and heating (for temperature-controlled experiments) up to the levels of 3 ppm of dissolved oxygen and 20°C, respectively. Then the previously adapted microbial enzyme mixture was inoculated and the time was counted thereafter. It should be noted that each experiment was carried out for 5 days; therefore, after inoculation of the microorganisms, samples of the

completely mixed liquor were taken every 24 hours. The sample volume collected at each sampling was 50 mL. For this purpose, the entire mixture was previously stirred in the bioreactor at a speed of 30 rpm for a period of 30 seconds and the completely mixed liquor samples taken daily were filtered to determine the volatile suspended solids (VSS). Meanwhile, the liquid obtained (filtrate) was subjected to soluble substrate analysis by the spectrophotometric method.

2.3. Data processing techniques

The VSS (biomass) and COD (soluble substrate S) data obtained daily were used to calculate the level and percentage of purification. For this purpose, the decrease in the concentration of soluble substrate (COD) with respect to the initial concentration was determined. The percentage of purification was calculated with the percentage of the fraction between the decrease of soluble substrate and the measured soluble substrate at the beginning of each experiment and based on the kinetic model proposed by (Orozco, 2014), where the substrate (S) concentration in the reactor, present in time, is related in terms of $S\,V^{-1}\left(\frac{S}{X}\right)$, as follows (Equation 1):

$$-\frac{ds}{xdt} = \frac{K_0\left(\frac{S}{X}\right)}{K_c+\left(\frac{S}{X}\right)} \quad (1)$$

Both constants, kinetic constant of maximum unit removal rate (K_0) and Contoins constant (K_c) are of interest to predict the evolution of the substrate concentration and can be estimated with experimental data from the purification tests in a batch reactor. For this purpose, they must be adapted to Equation 2, linearized using the Lineweaver-Burk method, as shown in the following Equation 2:

$$\frac{Xdt}{S_0-S} = \frac{K_c}{K_0}.\left[\frac{1}{\left(\frac{S}{X}\right)}\right] + \frac{1}{K_0} \quad (2)$$

For this aim, the biomass concentration in the reactor (X) and substrate concentration in the reactor (S), data for the 5 days were plotted on a Cartesian diagram, where the values of $\bar{X}\,S^{-1}$ were located on the *x* axis, and $-\bar{X}\,\Delta t\,\Delta S^{-1}$ on the axis *y*. Then, a linear regression was performed with the points indicated in the diagram, obtaining the kinetic constants of substrate removal K_o y K_c. Finally, hypothesis testing was conducted through the analysis of variance to verify the effects of the two manipulated factors on the purification percentage. This analysis was performed at a 95 % confidence level.

3. RESULTS AND DISCUSSIONS

The data shown below are those obtained from the practical tests and those estimated from Equations 1 and 2, such as kinetic coefficients and purification percentages. Tables 2 and 3 contain the data from the experiments considering the control and non-control of the temperature variable.

In the case of dissolved oxygen concentration, this was maintained in a range of 2 to 3 ppm, and the evolution of each parameter can be seen graphically in Figures 1 and 2:

Table 2. Data from temperature-controlled experiments.

Time	Substrate soluble	Biomass SSVLM	Substrate soluble	Biomass SSVLM	Substrate soluble	Biomass SSVLM
	REPLICA I	REPLICA I	REPLICA II	REPLICA II	REPLICA III	REPLICA III
Days	S COD (mg L^{-1})	X (mg L^{-1})	S COD (mg L^{-1})	X (mg L^{-1})	S COD (mg L^{-1})	X (mg L^{-1})
0	800	100	800	100	800	100
1	458	341	423	360	467	312
2	160	403	190	400	184	411
3	90	462	120	462	106	459
4	50	489	65	489	68	478
5	22	490	32	491	35	481

Table 3. Data from experiments without temperature control.

Time	Substrate soluble	Biomass SSVLM	Substrate soluble	Biomass SSVLM	Substrate soluble	Biomass SSVLM
	REPLICA I	REPLICA I	REPLICA II	REPLICA II	REPLICA III	REPLICA III
Days	S COD (mg L^{-1})	X (mg L^{-1})	S COD (mg L^{-1})	X (mg L^{-1})	S COD (mg L^{-1})	X (mg L^{-1})
0	800	100	800	100	800	100
1	590	198	575	210	546	206
2	356	278	331	270	380	290
3	186	356	156	368	150	398
4	105	412	94	401	80	411
5	40	413	34	402	25	411

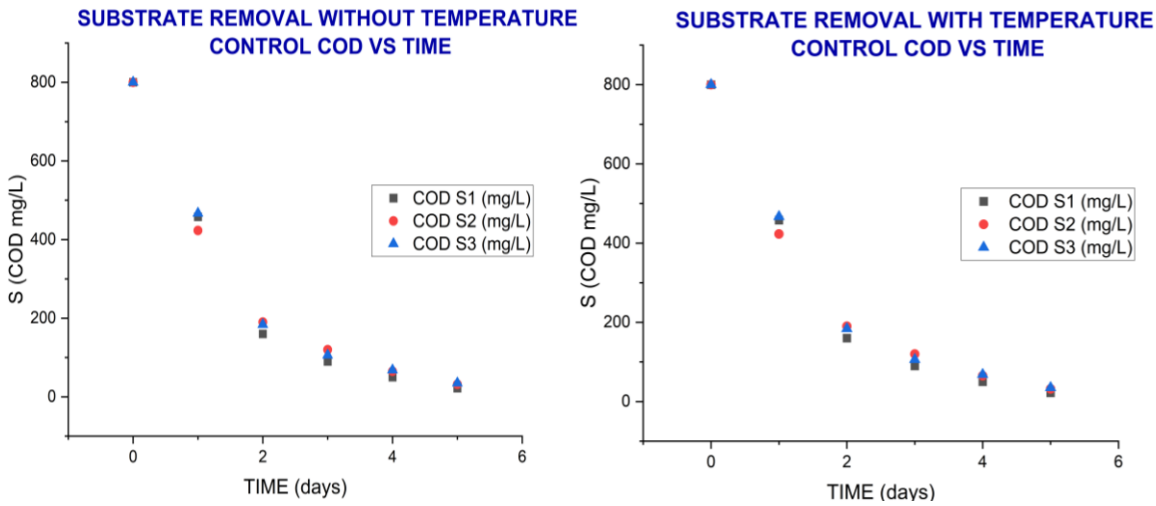

Figure 1. Substrate removal data with and without temperature control.

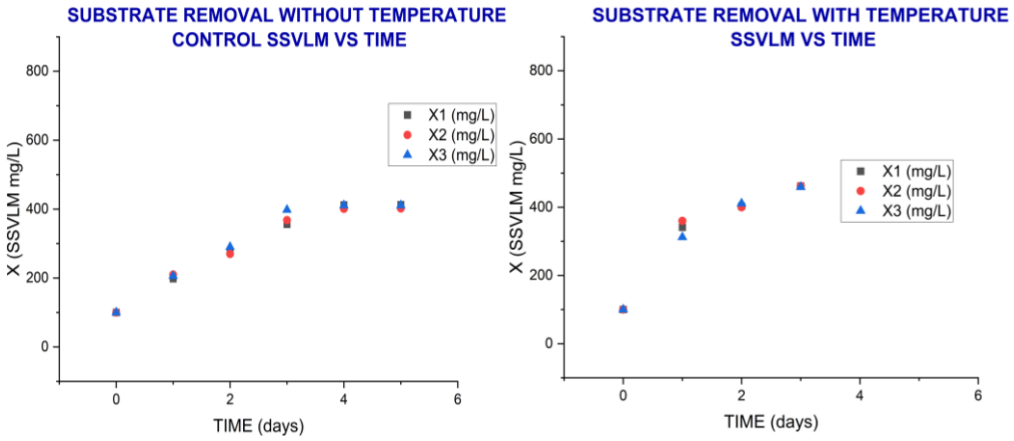

Figure 2. Biomass production data with and without temperature control.

3.1. Kinetic coefficients for substrate removal and biomass production

Plotting the results of ($-Xdt\ dS^{-1}$) vs ($S\ X^{-1})^{-1}$ to determine substrate removal kinetic constants K_o and K_c; as well as the results of ($dX\ X^{-1}dt$) vs ($-dt\ X^{-1}dt$) to find the endogenous coefficient (K_e) and the biomass production coefficient (Y), the following Figures 3 and 4 are obtained:

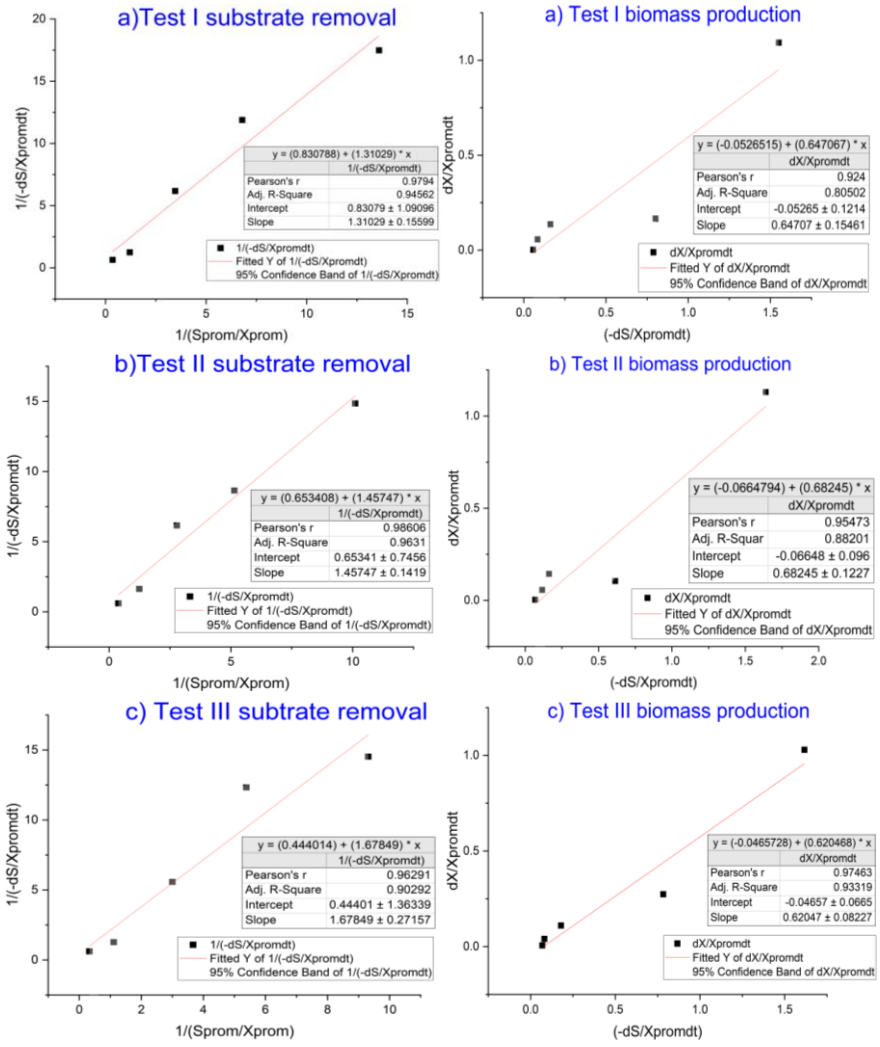

Figure 3. Kinetic constants of temperature-controlled tests. a) Test 1, b) Test 2 and c) Test 3.

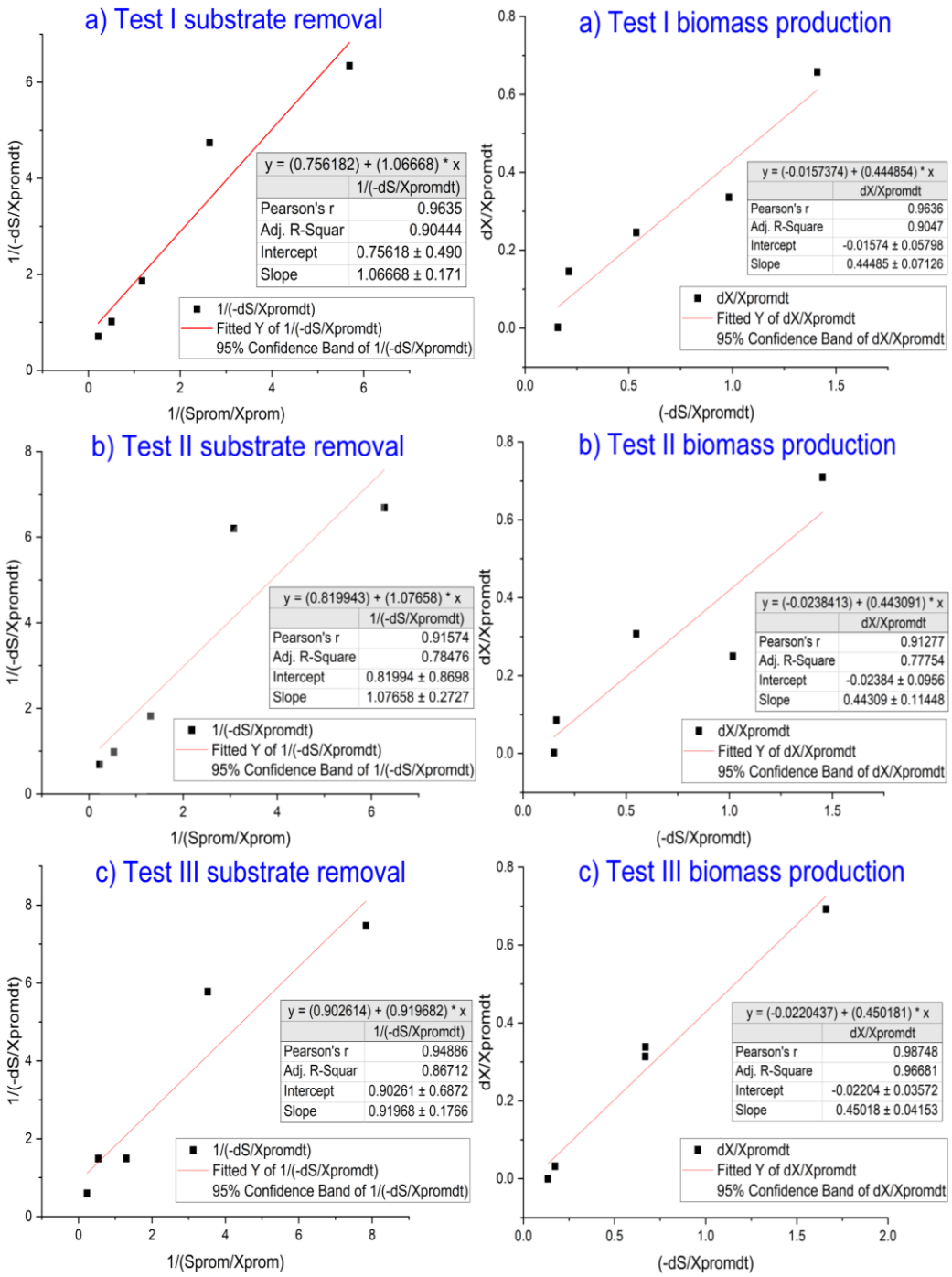

Figure 4. Kinetic constants of tests without temperature control. a) Test 1, b) Test 2 and c) Test 3.

According to the average results of the kinetic constants, it is observed that when the purification process is carried out maintaining T°=20°C, the K_o unit removal rate is higher than when the purification process is carried out without temperature control, by 26.8% with respect to the highest obtained. This behavior was to be expected, since the average ambient temperature during the day in the city of Huancayo is 19°C and at night it is around 10°C, which means that the speed of the purification process decreases at night and reaches its peak during the day. In this sense, aerobic biodepuration, at a temperature of 20°C, must be conducted at higher rates, since the enzymatic activities of these biocatalysts can be influenced by the thermal

variations in this area. In this regard, Belorkar and Jogaiah (2022) mentioned that the performance of many enzymes increases for every 10°C temperature increase.

Likewise, in the work developed by Hidalgo *et al.* (2023), from thermal pretreatment temperatures of 100, 130, 180 and 210°C, with a residence time of 24 h and two types of biomass material, they observed the reduction of methanogenic activity by increasing the pretreatment temperature, which is somewhat similar to what was reported in this research.

With respect to the average endogenous coefficient K_e, it is found that when the purification process is carried out at a constant temperature of 20°C, the value of 0.055 d^{-1} is 2.62 times the K_e average obtained for the case of tests without temperature control. This represents 1.46 times that obtained when the temperature was not controlled, where it was 0.446 mg SSV mg^{-1} COD. Similar results were obtained by Ramdani *et al.* (2010), who following batch test modeling, indicated endogenous waste decomposition rates of 0.005 d^{-1} and 0.012 d^{-1} for the anaerobic unit and the aerated and non-aerated alternating conditions, respectively. Also, Friedrich and Takács (2013) conducted twelve batch aerobic digestion experiments for activated sludge from seven water resource recovery facilities (WRRFs), reporting that endogenous decomposition rates vary significantly between values of 0.059 d^{-1} and 0.500 d^{-1} .

This means that when the process is carried out at 20°C, the consumption of biomass by endogenous respiration is higher at low temperatures. Meanwhile, in relation to the biomass production coefficient, when the temperature was maintained at 20°C, it was 0.65 mg SSV mg^{-1} COD.

3.2. Level and percentage of purification achieved in the tests

The purification level was calculated by obtaining the difference between the substrate concentration at the beginning of the experiment and the existing concentration. Whereas, the percentage represents the percentage of the fraction between the purification level and the initial substrate concentration in each experiment. The results are shown in Table 4:

Table 4. Level and percentage of purification.

	Time Days	**S_o-S_{end} (mg COD)**	**% purification**	**S_o-S_{end} (mg COD)**	**% purification**	**S_o-S_{end} (mg COD)**	**% purification**
		I	I	II	II	III	III
With temperature control	1	342	42.75	377	47.125	333	41.625
	2	640	80	610	76.25	616	77
	3	710	88.75	680	85	694	86.75
	4	750	93.75	735	91.875	732	91.5
	5	778	97.25	768	96	765	95.625
No temperature control	1	210	26.25	225	28.125	254	31.75
	2	444	55.5	469	58.625	420	52.5
	3	614	76.75	644	80.5	650	81.25
	4	695	86.875	706	88.25	720	90
	5	760	95	766	95.75	775	96.875

Figure 5 shows a plot of the results of the percentage of purification versus the time in

which it was tested:

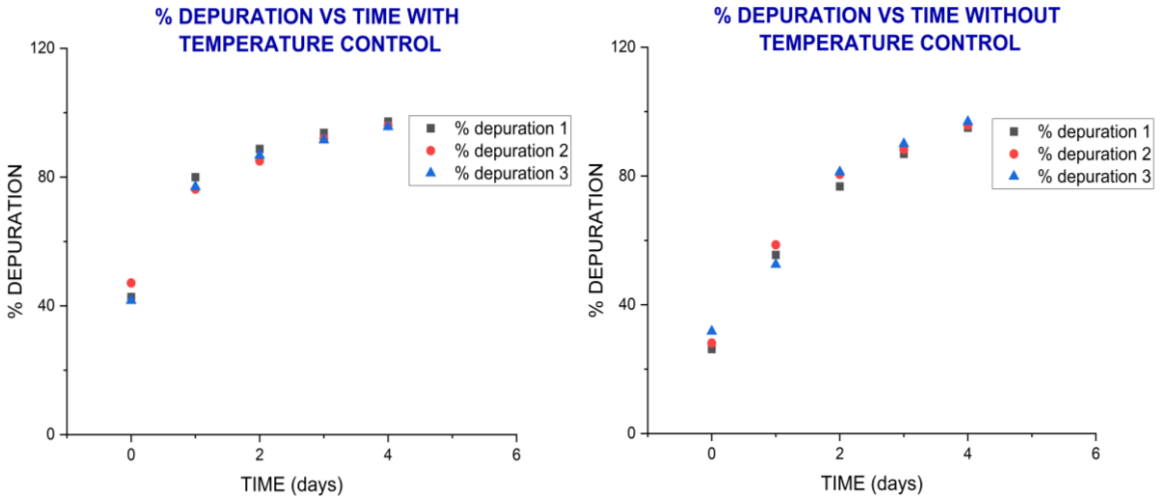

Figure 5. Percentage of purification vs time.

In the aerobic wastewater treatment with microorganisms, one of the factors that affect this process is the temperature, and in this particular case the mixture of enzymes and biodigester bacteria, despite the climatic variations of the city of Huancayo, had good performance in the process of substrate removal and biomass production. This is also confirmed by Bohutskyi *et al.* (2018), as they note that wastewater treatment efficiency, biomass productivity, sedimentability, composition, digestibility and methane potential vary in response to environmental conditions.

It is important to note that enzymes, as indicated by the manufacturer of this product, are very important to carry out this process because they allow the decomposition of substances such as oils, fats and proteins of large molecular size so that the bacteria can metabolize them without any difficulty. The critical role that temperature plays in enzyme function, in the context of evolution (as well as in biotechnology), means that this field is very active (Arcus *et al.* (2020). This is also confirmed by Wapshott-Stheli and Grunden (2021), who point out that thermal stability allows enzymes to remain active longer during industrial processes and that proteins from thermophilic and thermotolerant organisms such as *Bacillus methanolicus* are generally resistant to high temperatures ranging from 35°C to 65°C.

In addition, according to the experiences of researchers related to the kinetics of biomass removal and production, the models that best fit in several real cases are those of Lawrence & McCarty and Orozco in the removal of substrate. Accordingly, Montoya *et al.* (2020) point out that the effect of temperature is significantly more important than the effect of substrate in terms of molar mass reduction. Similarly, Esparza *et al.* (2022) mention that the yield of biomass increases as the operating temperature decreases in batch reactors fed with synthetic substrate. Jagaba *et al.* (2022) suggest that the modified Stother kinetic model is more appropriate for the description of the experimental data in terms of microbial growth parameters.

The results obtained were used to perform the analysis of variance, and it was statistically accepted, at a 95% confidence level, since the time and temperature control in the synthetic wastewater purification processes with the mixture of enzymes and aerobic biodigester bacteria is significant. To this it must be added that at a temperature of 20°C the effect on the speed of purification is greater, as well as the reaction time.

4. CONCLUSIONS

In the field of wastewater treatment, the application of enzymes has developed enormously,

since they serve as an auxiliary means of support to break down large molecules that are difficult to biodegrade by microorganisms in a biological reactor. Therefore, their mechanism of action in the purification of pollutants is based on the cooperation of these agents with biosorbent microorganisms. In this sense, according to the results obtained in this research, we concluded that if we maintain a temperature of 20°C the speed and level of purification is greater than when the process is carried out without temperature control. This is because the behavior of the concentration of water pollutants during the process of pollutant removal with the mixture of enzymes and aerobic microorganisms decreases and adjusts to the kinetic model proposed by Orozco, whose average kinetic constants at a temperature of 20°C were K_o= 1.662 d^{-1}, K_c=2.53 mg COD mg^{-1} SSV. While when the temperature was not controlled, the average constants were: K_o= 1.217 d^{-1} and K_c=1.24 mg COD mg^{-1} SSV, allowing us to establish that the effect of temperature control and treatment time on the level of purification of wastewater pollutants with the mixture of enzymes and microorganisms are significant at a 95% confidence level.

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