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Effect of Inoculum Concentration on the Degradation of Diesel 2 by a Microbial Consortium

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Abstract: The objective was to determine the effect of inoculum concentration on the degradation of Diesel 2 by a microbial consortium called BIOT.PD001. For this, five systems were designed (in triplicate), which Contained Davis Minimum Medium, 5% Diesel 2 as a carbon source, and a suspension of the microbial consortium BIOT.PD001 (9×10^8 cells/mL) in concentrations of 2, 4, 6, 8, and 10% of the final volume. The monitoring of the degradation of Diesel 2 was carried out indirectly through the bacterial counts by the plate count method, the Biochemical Oxygen Demand (BOD_5) by the Winkler Method modified according to Alsterberg, and the concentration of total fats by Gerber's method. The retention time was 15 days. It was observed that the percentage of efficiency of the process increases as the concentration of inoculum increases, obtaining the highest percentage of efficiency (94.77%) when using 10% of inoculum (v/v), while when using inoculum concentrations of 2 and 4% (v/v), the efficiency percentages are the lowest, (68.4 and 66.6%, respectively). On the other hand, the variance analysis indicated that there is a significant difference between the averages of these values. The regression analysis indicated that the inoculum concentration significantly affects the efficiency of Diesel 2 degradation and that this is 86% explained by a linear regression model. There is a linear relationship between the inoculum concentration of the BIOT.PD001 microbial consortium and the BOD_5 tend to decrease as a function of time. It is concluded that the inoculum concentration significantly affects the efficiency of the degradation of Diesel 2 by the BIOT.PD001 consortium.

Keywords: consortium; Diesel 2; bioremediation; inoculum concentration; biodegradation; BOD_5

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1. Introduction

Oil is one of the most important non-renewable resources, not only because it is considered the energy source par excellence but also because it is the raw material for other by-products [1]. Among petroleum derivatives, Diesel 2 is the most consumed fuel in Peru and is used mainly in transportation, as well as in industry and power generation. Its consumption increased between 2000 and 2013 due to the growth of thermoelectric generation based on this fuel and the increase in the automotive diesel fleet [2]. Diesel is composed of aliphatic compounds (64%) (mainly cycloalkanes and n-alkanes), aromatic compounds (35%), and olefinic compounds (1%) [3].

One of the negative impacts of oil exploitation is caused by oil spills, and Peru is not exempt from this problem, where the Amazon region has been the most affected in the last four decades [4]. Although oil extraction in the Peruvian Amazon began in 1932, between 2003 and 2010, this extraction expanded territorially from 7.1 to 41.2% [5]. The first records of spills that we have are between the years of 1960 and 1970 when an

American company (currently part of Chevron) began the search for oil in the Peruvian and Ecuadorian Amazonian territory, and during the extraction, they released high volumes of toxic waste to streams and soils [6]. In 2000, a spill of approximately 5000 barrels of oil was recorded in the lower Marañón, near the native community of San José de Saramuro, and later in 2010, a spill occurred in the same area at the Pluspetrol base where 374 barrels were dumped [7]. On the other hand, in the coastal zone to the north of Peru, there are economically important oil zones for the country. However, the General Directorate of Captaincies and Coast Guards indicates that from 2008 to 2019, there have been six spills from ships and platforms of oil companies from the ports and adjacent areas [8]. Recently, the Peruvian coast was affected by the oil spill from the La Pampilla refinery belonging to the Repsol company, where 11,900 barrels were spilled, with an extension from the beaches of Ventanilla to Chancay in the north of Lima, and the damage caused to the ecosystem is still unknown [8–10].

Due to the environmental contamination problems that these oil spill accidents represent and the poor management of urban hydrocarbons, it is necessary to apply decontamination methods that are economical and friendly to the environment. There are various methodologies aimed at reducing their impact on the environment. However, the traditional physical or chemical methods used to treat contaminated environments can contribute to contamination due to their toxicity and recalcitrance to the biodegradation of hydrocarbons. Given this, a better alternative arises, the bioremediation process, in which microorganisms capable of naturally degrading toxic compounds are used as an environmentally friendly alternative to removing contaminants [11,12].

Among the bacteria with high hydrocarbon degradative capacity, the following are reported: *Brevibacterium*, *Spirillum*, *Xanthomonas*, *Alcaligenes*, *Arthrobacter*, *Nocardia*, *Flavobacterium*, *Vibrio*, *Achromobacter*, *Acinetobacter*, *Micrococcus*, *Pseudomonas* sp., *Ps. aeruginosa*, *Ps. mendocina*, *Ps. aureofasciens*, *Serratia rubidae*, *Bacillus* sp., *Brevibacterium*, *Corynebacterium*, *Flavobacterium*, *Sphyngomonas* sp., among others, while, among the yeasts, *Candida*, *Rhodotorula* and *Sporobolomyces* stand out, etc., which reduce the concentration of hydrocarbons and at the same time are safe for health and the environment. Some of these microorganisms produce emulsifiers and biosurfactants that reduce the surface tension between the oil and the aqueous medium, facilitating microbial access to the insoluble carbon source for its degradation. On the other hand, some algae and protozoa have been reported as biodegrading agents of petroleum hydrocarbons [12–15].

In order for microorganisms to carry out the biodegradation of hydrocarbons in the soil, there are a series of environmental parameters, such as humidity, aeration, pH, temperature, nutrients, electron acceptors, etc., which must be within optimal ranges for this purpose. The same goes for the different nutrients, as well as the population density, to be able to carry out the process with greater efficiency [14]. The study of microbial communities that take part in the in-situ biodegradation of hydrocarbons and the dynamics of their populations in biodegrading consortia is growing remarkably in the area of microbial ecology and constitutes a challenge for microbiologists. The reason for this is that most of the species that make up microbial communities are nonculturable. Many of these microorganisms have peroxidase and oxygenase activities, which allow the more or less specific oxidation of some oil fractions. This oxidation changes the properties of the compounds, making them susceptible to secondary attack and facilitating their conversion to carbon dioxide and water. On some occasions, it is not necessary to reach mineralization. Rather oxidation is sufficient to significantly reduce its toxicity or increase its solubility in water, increasing its bioavailability [15–19].

Degradation of petroleum hydrocarbons can be made more effective by using microbial consortia, as these are better adapted to contaminated environments and perform better than individual strains [20,21]. This was demonstrated when working with the Effective Microorganisms consortium to remediate sewage sludge from an oil refinery [22] and with the bacterial consortium made up *of Escherichia coli*, *Klebsiella* sp., *Lactobacillus* sp., *Enterobacter* sp., *Proteus* sp., and *Serratia* sp. used diesel, kerosene, premium motorcycle oil,

and motor oil as carbon sources. In this way, they demonstrated their potential to be used in hydrocarbon biodegradation processes [23]. Likewise, the association of *Rhodococcus* and *Mycolicibacterium* (formerly *Mycobacterium*) makes them the most versatile and efficient degraders of hydrocarbons. Many *Rhodococcus* strains biodegrade a wide variety of alkanes, including n-hexadecan, n-heptadecan, kerosene, and pristane [24]. On the other hand, in the city of Trujillo (Peru), studies have been carried out on the isolation and identification of oil-degrading microorganisms [25], and the effect of the inoculum and substrate on the degradation of oil in drinking water has been studied. Artificial sea with the mixed bacterial culture [26]. In previous works, the efficiency of Diesel oil degradation was investigated by the Efficient Microorganisms consortium in aerated and stirred tank bioreactors and also in bioreactors with biofilms, finding in both cases more than 50% efficiency [27].

The BIOT.PD001 microbial consortium used in this research is made up of bacteria of the genus *Bacillus*, *Micrococcus*, *Pseudomonas*, and *Rhodopseudomonas*, isolated from active sludge from the Covicorti-Trujillo Water Treatment Plant. In previous research, it has been shown that these bacteria have the metabolic potential for the degradation of hydrocarbons. Thus, *Pseudomonas* has a well-known trajectory as a hydrocarbon degrader; *Rhodopseudomonas* is a phototropic bacterium capable of growing on halocarboxylic acids in the presence of CO_2, and of degrading and recycling different aromatic compounds, surviving through reductive dehalogenation mechanisms and assimilation of the resulting acid. Whereas *Micrococcus* and *Bacillus* have the ability to degrade polycyclic aromatic hydrocarbons, such as naphthalene, anthracene, phenanthrene, and dibenzothiophene (DBT) [28,29]. The processes by which microorganisms degrade petroleum hydrocarbons occur in four steps. First, the microorganisms secrete surfactants that emulsify the hydrocarbon, then this emulsified adsorbs to the surface of the microorganism and then enters the cell through active or passive transport or endocytosis. Finally, within the cell, the hydrocarbon is degraded by enzymes [30].

As for the concentration of inoculum used, there is much diversity in its use, so some researchers use it at 1% [31], while others prefer to use concentrations ranging from 5 to 10% of the total volume of the fermenter, obtaining having each of them satisfactory results in terms of degradation of oil and derivatives [32,33]. It is very important to work with the proper concentration of inoculum since if the concentration is too low, the process is delayed, and if it is too high, the bacteria can inhibit their metabolism due to the quorum sensing phenomenon [34]. For this reason, the purpose of this study is to evaluate the effect of the inoculum concentration on the degradation of Diesel 2 by the microbial consortium BIOT.PD001, under laboratory conditions, in order to define the optimal concentration of inoculum that can be used in other investigations of biodegradation of diesel by these microorganisms, postulating the hypothesis that the effect of the concentration of inoculum on the degradation of Diesel 2 will be to increase the efficiency of the bioprocess in a directly proportional way.

2. Materials and Methods

2.1. Study Material

BIOT.PD001 microbial consortium, isolated from activated wastewater sludge at the Covicorti Treatment Plant in Trujillo (Peru)., which is composed of bacteria of the genera *Bacillus*, *Micrococcus*, *Pseudomonas*, and *Rhodopseudomonas*.

2.2. Methods

2.2.1. Inoculum Preparation

Each bacterium of the microbial consortium was inoculated separately in Trypticase Soy Broth and incubated at room temperature 22 ± 1 °C for 24 h; After that time, each culture was centrifuged at 3500 rpm for 10 min, and the cells were washed twice with Sodium Chloride Solution 0.85% (p/v). Then they were resuspended in NaCl 0.85% solution, and microbial suspensions were adjusted in nephelometer tubes to 3 McFarland (9×10^8 cell/mL)

with sterile saline water. Then, these suspensions were mixed in equal proportion to obtain a suspension with a final concentration of viable cells of 9×10^8 cells/mL.

2.2.2. Preparation of Evaluation Systems

Five Stirred Tank Bioreactors (STRs) were built Following the geometric dimensions shown in Figure 1. Bioreactors were sterilized using 5% sodium hypochlorite and were exposed to ultraviolet light for 2 h. After each STRs was fed with Davies Minimum Medium (Glucose 10 g, Ammonium chloride 1.0 g, sodium chloride 1.0 g, $MgSO_4.7H_2O$ 0.2 g, KH_2PO_4 3.0 g, Na_2HPO_4 3.0 g and distilled water 1000 mL), in this medium, glucose was replaced by 5% Diesel 2 (v/v) as a carbon source [35]. On the other hand, the inoculum was added to each bioreactor in concentrations of 2, 4, 6, 8, and 10%, respectively. Each bioreactor operated with a stirring of 120 rpm and 0.5 vvm of aeration, with a final working volume of 1.5 L. Each experiment was performed in triplicate.

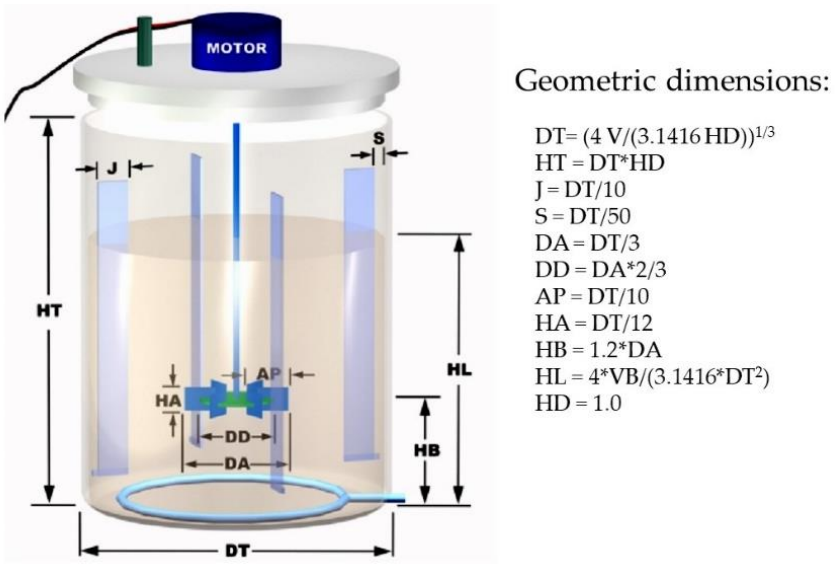

Figure 1. Stirred tank Bioreactor design.

2.2.3. Indirect Determination of Degradation of Diesel 2

At time zero and every 24 h, 50 mL samples were taken from each of the bioreactors where the following was determined: Biochemical oxygen [36] demand: Winkler method modified by Alsterberg. Microbial biomass production: Count of microorganisms by the seeding method by incorporation in Agar Soya Trypticase plates [37]. The concentration of Diesel 2 was evaluated at the beginning and at the end of the process, using the Gerber Method for the determination of total fats [38,39]. The hydraulic retention time was 14 days, and three replicates of each experiment were carried out.

2.2.4. Determination of Bioprocess Efficiency

The efficiency was evaluated according to the residence time of the hydrocarbon in the bioreactor and its consumption during the treatment [40].

$$\% \text{ efficiency} = MOc/MOi \times 100 \quad (1)$$

$$MOc = MOi - MOf \quad (2)$$

where:

MOc = Diesel 2 consumed (% oil v/v)
MOi = Initial concentration of Diesel 2 (% oil v/v)
MOf = Final concentration of Diesel 2 (% of oil v/v)
* Diesel 2 concentrations were determined by the Gerber method for total fats

2.3. Analysis of Data

For data analysis, the Microsoft Excel program was used, as well as the statistical package Minitab.16; IBM SPSS Statistics.22 and Statistica.10, which were used to calculate the percentages of efficiency, generation of dispersion and regression graphs, and regression analysis (the linear regression model was used, $R^2 = 0.86$ based on the estimation curvilinear for having the highest R^2 values) and ANOVA, followed by Duncan's post hoc test to compare efficiency means using the variables that influence the diesel degradation process 2 by the BIOT.PD001 microbial consortium.

3. Results

Table 1 shows the percentages of efficiency and the growth rates obtained for each concentration of inoculum of the bacterial consortium applied in the degradation of Diesel 2, where the highest percentage of degradation efficiency was for the group with 10% inoculum (94.77%), followed by the group with 8% inoculum (86.67%); the lowest percentage was obtained in the group with 4% inoculum (66.67% efficiency). In turn, in terms of growth rate (day^{-1}), calculated by means of least squares, it was in the groups with 6 and 8% inoculum where the highest value was observed (3.03 and 3.84, respectively).

Table 1. Average values of the percentage of efficiency in the degradation of Diesel 2 and growth rate when using different concentrations of inoculum of the BIOT.PD001 consortium in the degradation of diesel oil 2 in an aerated and stirred bioreactor.

Inoculum Concentration (%)	% Efficiency		Growth Rate (Day^{-1})
	Average	Standard Deviation (SD)	
2	68.46	1.94	2.61
4	66.67	1.53	1.80
6	74.10	3.59	3.03
8	86.67	1.86	3.84
10	94.77	1.78	2.58

Table 2 shows the result of the variance analysis, where the sum of squares column shows the variability both between groups and within groups, evidencing that the variation or deviation from the mean between groups was greater than that calculated within the groups of each level of the independent variable (inoculum concentration); In turn, the probability associated with the F statistic turned out to be significant (*p*-value of the test = 0.00 < 0.05), confirming that in at least 2 of the 5 groups studied, the experimental means are different, with a level of 95% confidence.

Table 2. Analysis of variance for comparison of average percentages of efficiency in the degradation of Diesel 2, obtained using five concentrations of inoculum of the microbial consortium BIOT.PD001 with three experimental replications.

	Sum of Squares	Gl	Root Mean Square	F	Sig.
Inter-groups	1772.05	4	443.01	86.41	0.00
Within groups	51.27	10	5.13		
Total	1823.31	14			

F table = 3.48 ($\alpha = 0.05$, 4, 10, 1 tail).

After having found a significant difference through the ANOVA test, Table 3 shows the results of Duncan's Post hoc test, which generated four subsets with a statistically significant difference. Subset 1 (composed of level 2 and 4% inoculum, with equal means of 66.66 and 68.46% efficiency, respectively); subset 2 (composed of the 6% level of inoculum, with an average efficiency of 74.1); subset 3 (composed of the 8% level of inoculum, with

an average of 86.6 efficiencies) and subset 4 (composed of the 10% level of inoculum, with an average of 94.76 efficiencies).

Table 3. Duncan's test for comparison of average percentages of efficiency in the degradation of Diesel 2 was obtained using five concentrations of inoculum of the microbial consortium BIOT.PD001 with three experimental repetitions.

Concentration	N	Subset for Alpha = 0.05			
		1	2	3	4
4.00	3	66.67			
2.00	3	68.47			
6.00	3		74.10		
8.00	3			86.67	
10.00	3				94.77
Sig.		0.35	1.00	1.00	1.00

The means for the groups in the homogeneous subsets are shown. a Uses the sample size of the harmonic mean = 3.00.

Table 4 shows the regression analysis used to explain the influence of the inoculum concentration of the BIOT.PD00 microbial consortium on the degradation efficiency of diesel oil 2, finding that our calculated F statistic (84.91) is greater than the critical value F table (4.67), concluding that the regression model as a whole is statistically significant; in turn, a better fit was found with the linear model, where a coefficient of determination $R^2 = 0.86$ was obtained.

Table 4. Analysis of variance in the linear regression model to evaluate the effect of the inoculum concentration of the microbial consortium BIOT.PD00 on the degradation efficiency of Diesel 2.

	Sum of Squares	Gl	Root Mean Square	F	Sig.
Regression	1581.23	1	1581.23	84.91	0.00
Residue	242.09	13	18.62		
Total	1823.31	14			

F table = 4.67 ($\alpha = 0.05$, 1, 13, 1 tail).

Figure 2 shows the growth curves generated by each concentration of inoculum used from day zero (inoculation) to day 14 (end of treatment).

Figure 3 shows the BOD_5 parameter as an indirect indicator of the degradation of Diesel 2, where treatment with 2% inoculum decreases demand (<50 mg O_2/L) after day 11, for then remain constant, while for the treatments with 8 and 10% of inoculum the tendency is to decrease (<50 mg O_2/L) after the 14th day of the experiment.

Figure 4 shows the percentage reduction in BOD_5, where the treatment that used a 2% bacterial inoculum achieves a maximum reduction in the BOD_5 parameter in the system on day 11, to then remain constant until day 14; the same happened with the inoculum of 4 and 6%. However, the treatments with the highest percentage of inoculum (8 and 10%) on day 14 still maintain a tendency to continue reducing said parameter, presenting a better performance in the Diesel 2 biodegradation process by batches.

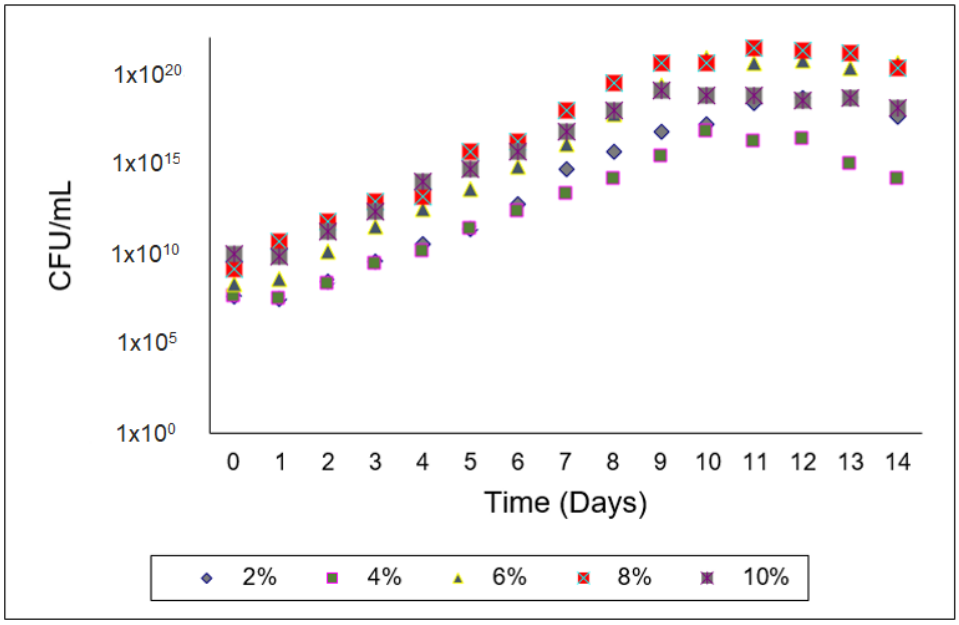

Figure 2. Variation profile of microbial biomass when using different concentrations of inoculum from the BIO.PD001 microbial consortium, in the degradation of Diesel 2, in an aerated and stirred bioreactor.

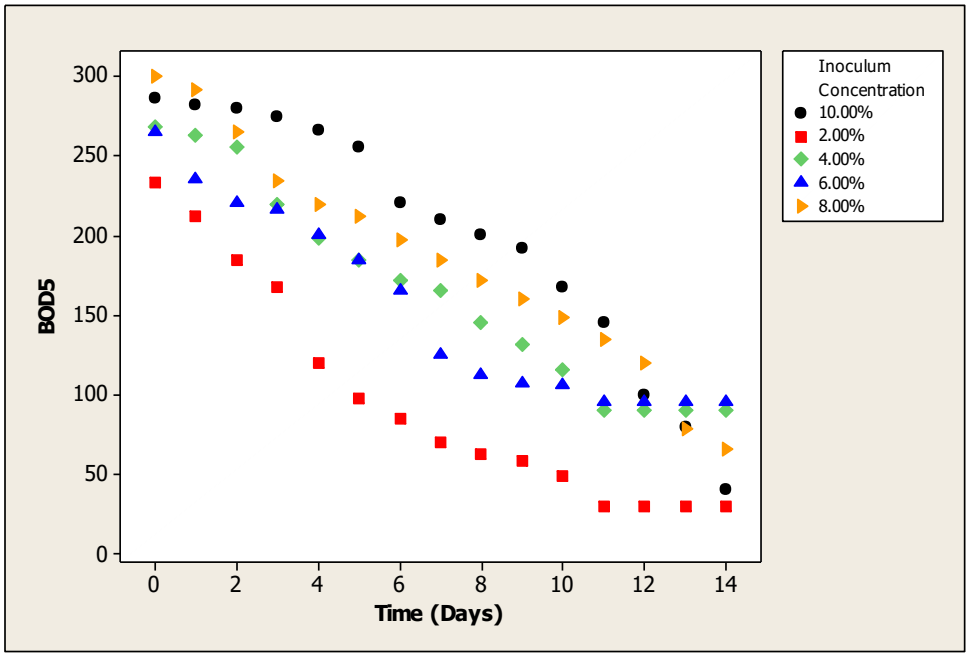

Figure 3. Profile of the variation of the Biochemical Oxygen Demand (BOD_5) when using different concentrations of inoculum of the BIO.PD001 microbial consortium, in the degradation of Diesel 2, in an aerated and stirred bioreactor.

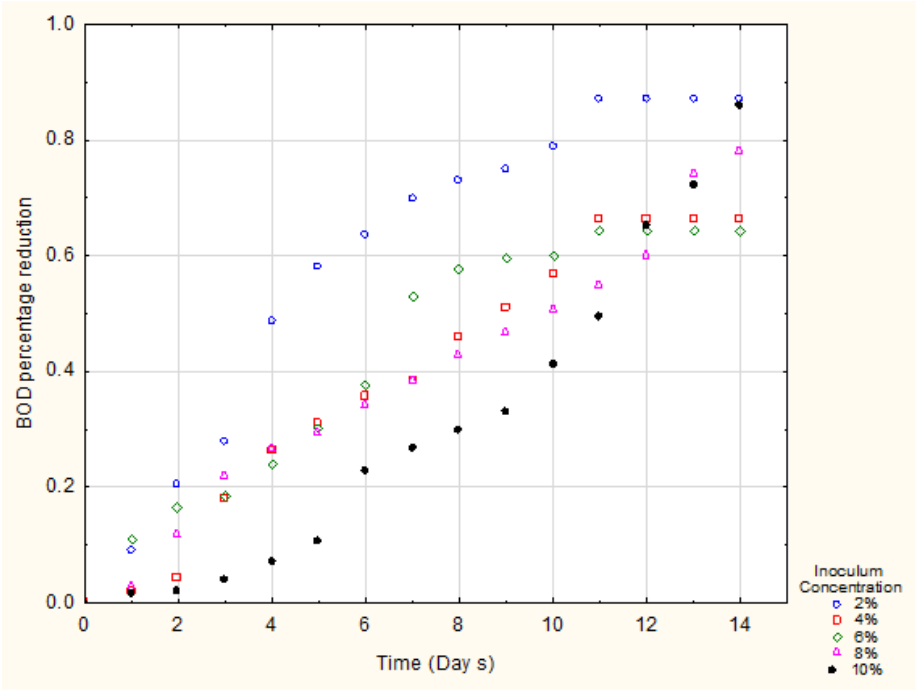

Figure 4. Multiple regression diagram of the BOD_5 reduction percentage over time when using five concentrations of inoculum (% v/v).

4. Discussion

The result shows an increase in the efficiency of the biodegradation of Diesel 2 when the inoculum concentration increases (Table 1), obtaining the highest efficiency percentage (94.77%) when 10% inoculum (v/v) is used, while when using inoculum concentrations of 2 and 4% (v/v), the efficiency percentages are those lowest (68.4, and 66.6% respectively). However, according to Badis (2016), a microorganism is considered to degrade oil and diesel if its degradation rate is greater than or equal to 5% [41]. In addition, after the analysis of variance, it is indicated that there is a significant difference between the efficiency averages of the five inoculums used (sig. $0.00 < 0.05$) (Table 2). However, the Duncan Test for multiple comparisons shows the formation of four subsets with a significant difference between the means of the efficiency percentages, which are: subset 1 (inoculum concentration of 2 and 4%), subset 2 (6%), subset 3 (8%) and subset 4 (10%) (Table 3). Therefore, when evaluating the degradation efficiency of Diesel 2 using the Gerber method, it is suggested that a high cell density be used for a better degradation process. Consequently, one of the most important factors for the process would be to ensure that the rate of biological transformation is fast enough to meet cleanup goals.

However, when evaluating the process through a linear regression analysis, it is found that 86% (R^2) of the variation in efficiency (D.V.) obtained after 14 days is explained by the variation in the concentration of inoculum (V.I.), in addition, the Pearson correlation coefficient indicates that there is a high degree of correlation between the independent and dependent variable, in turn, the result of the ANOVA analysis turned out to be significant, which indicates that both variables would be linearly related (Table 4).

In the results (Figure 2), the behavior of the variation of microbial biomass over time is observed for each of the five experimental groups, where an exponential increase can be seen from day 1 to day 10, to then pass to a stationary phase (flattening of the curve) and a fall until day 14, which lasted the observations in the laboratory, in all cases the behavior of the biomass was similar (increase in biomass as a function of time) and according to Tao et al. (2016), the biodegradation of hydrocarbons will depend on the survival of microorganisms after their inoculation, so it is important to monitor bacterial growth [42]. In turn, when calculating the growth rate for each experimental group using least squares (Table 1),

it can be seen that the highest growth rates are obtained when working with inoculum concentrations of 6% (3.03 day^{-1}) and 8% (3.84 day^{-1}), while the speed decreased for the inoculum concentration of 10% (2.58 day^{-1}), despite the fact that with this concentration the highest percentage of Diesel 2 degradation efficiency was obtained. Something similar occurred in the study by Birch et al. (2017) [43], where the velocity constants that were above 1 d^{-1} in water samples from four different places belonging to urban streams and a rural lake contaminated with five chemical substances derived from petroleum belonged to those samples that they had a lower initial indigenous bacterial density. This could be explained because when working with microbial communities or consortia, some factors arise that govern the dynamics of the various microbial populations, such as food and space limitations, competition between them, and unfavorable physical conditions. When the supply of soluble organic substrate is depleted, the bacterial population is less successful in replication, and the number of individuals tends to decrease [43].

On the other hand, the phenomenon of quorum sensing must also be taken into account, which is the ability of bacteria to communicate with each other, and that. They can be censused or counted when the cell density is very high. It produces the regulation of gene expression in response to fluctuations in cell population density, producing and releasing chemical signals called autoinducers that increase in concentration as a function of cell density [44]. In these systems, a decoy molecule known as an autoinducer is regularly used, which is produced individually by each of the cells of the colony, then the autoinducer is transported to the outside of the cell, where it accumulates progressively with increasing the number of bacteria in the colony, when a high concentration of the autoinducer is reached, is detected by special receptor molecules, which in turn activate the expression of genes that respond to the cell density of the colony [45].

In turn, the consumption of diesel oil has also been evaluated in this study indirectly through the Biochemical Oxygen Demand (BOD_5), which is based on the amount of oxygen needed to convert the oxidizable material into stable final products, being one of the most important parameters for the control of water pollution, which is used as a measure of organic pollution, as a basis for estimating the oxygen necessary for biological processes and as an indicator of process performance [46]. Thus, Figure 3 shows that the BOD_5 parameter progressively decreases over time for the five different experimental groups, where the curves with the steepest slopes (−17.129 and −15.671) belong to the groups with 8 and 10% inoculum, respectively, which shows that these two concentrations of inoculum degrade organic matter at a faster rate, with decreases ranging from 300 to 66 mg O_2/L (8% inoculum) and from 286 to 40 mgO_2/L (10% inoculum). Said variation would indicate that the diesel oil is being consumed by these bacteria since, by requiring a smaller amount of oxygen for the oxidation of the organic matter present, it would correspond to the presence of a smaller amount of degradable organic matter [47].

These results are similar to those reported by other researchers using BOD_5 as an indirect indicator of hydrocarbon degradation. Thus, Patiño et al. (2021) observed a decrease in BOD_5 over 15 days (inoculation on day zero, end of treatment on day 15), which occurred proportionally to residual oil, where this variation was from 40.41 to 6.2 mg of O_2/L (Treatment 1), from 87.24 to 5.6 mg of O_2/L (Treatment 2), from 72.92 to 5.8 mg of O_2/L (Treatment 3) [48]. In turn, in the bioreactor tests carried out for this study, a constant concentration of 5% Diesel 2 was used as a carbon source, which did not limit the growth of the strains of the consortium used. However, Mansur et al. (2020) observed that diesel oil survival when using the AQ5-05 strain decreased as the hydrocarbon concentration increased, from 2% (v/v) to 3.5% (v/v), maintaining its ability to degrade working up to 3% (v/v) of diesel at 10 °C and for seven days [49]. While Lee et al. (2018) obtained a diesel degradation of up to 56.45%) with the AQ5-05PBD strain, which was produced at a pH of 7.25 to 7.68 and at 15 to 20 °C, after evaluating the COD reduction efficiency observed a 99.9% removal and a slight reduction to 96% between days 57 and 66, respectively, in the presence of 320 mg/L diesel as the sole carbon source [50].

However, the authors refer that the biodegradation of petroleum hydrocarbons is complex and generally requires different microbial species or consortiums with particular enzymatic capacities that accelerate the rate of petroleum degradation [51,52]. Thus, Morales et al. (2022) used a bacterial inoculum from two strains of the pure culture, performing the degradation test in flasks with 30 mL of mineral medium supplemented with 300 µL of diesel for eight days, achieving 97.9 and 96.2% % degradation of diesel [53]. While Liu et al. (2022) evaluated seven consortia of oil-degrading bacteria through a diesel degradation test, inoculated in sterilized seawater with diesel oil to simulate the process of oil contamination and bioremediation, the diesel degradation rate obtained from the individual way of seven bacteria was 8.9, 18.7, 77.1, 11.6, 18.5, 44.1 and 13.4%, respectively, this showed that it is very difficult to a single strain uses all the components of the oil, unlike a bacterial consortium that usually has an efficiency yield greater than 86.2% at 16 days [54]. That is why Fathepure (2014) mentions that the tendency of diesel compounds to biodegrade follows a decreasing order (N-alkanes > branched alkanes > monoaromatic > cycloalkanes > polyaromatic), considering N-alkanes as very unstable. In the environment, less toxic and easily used by bacteria as a carbon source [55,56].

Furthermore, it should be noted that the concentration of contaminants has a high correlation with the bacterial species employed since different bacterial species will have different responses to contaminant exposure. Therefore, bacteria isolated from a polluted environment tend to be more efficient in the degradation of contaminants [57]. As in the case of Morales et al. (2017), who reported that six bacteria isolated from oil-contaminated soil could degrade the hydrocarbon in wastewater, including Serratia marcescens (C7S3A), which showed the highest percentage of emulsification (74%) and consequently a higher diesel degradation (96%) and at the consortium level they degraded 97% of the total diesel [53]. In conclusion, the main advantage of the biodegradation technique is that it is environmentally friendly, leaving almost no harmless substances in the environment after treatment [58]. Bacteria that are involved in the degradation process will naturally break down after application; therefore, they will not pollute the environment anymore.

5. Conclusions

The inoculum concentration significantly affects the efficiency of Diesel 2 degradation by the BIOT.PD001 consortium, since a positive linear relationship was observed between the inoculum concentration of the BIOT.PD001 consortium and the Diesel 2 degradation efficiency, reaching the highest percentage of efficiency (94.77) with the highest concentration of inoculum (10%). The degradation of Diesel 2 is reflected in the growth rate of the bacteria, which was higher when working with 8% inoculum, which suggests that growth may be regulated by the quorum sensing phenomenon. However, the percentage of efficiency (86.67%) achieved is also considered adequate. Likewise, it was observed that there is a linear relationship between the inoculum concentration of the BIOT.PD001 consortium and the Biochemical Oxygen Demand, which indicates that the organic matter is consumed as the concentration of bacteria increases.

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