International Journal of Engineering Research-Online
A Peer Reviewed International Journal
Articles available online http://www.ijoer.in; editorijoer@gmail.com

Vol.9., Issue.4, 2021
July-August

RESEARCH ARTICLE

ISSN: 2321-7758

BIOREMEDIATION OF SLUDGE CONTAMINATED WITH USED LUBRICATING OILS

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DOI: 10.33329/ijoer.9.4.29

ABSTRACT
When sludge polluted with waste lubricating oil remnants is not adequately treated, it has a significant negative environmental impact. To reduce the concentration of these contaminants, bioremediation was recommended. The tests were conducted at the wastewater treatment plant's (WWTP) facilities, where native microbial consortia were evaluated before being added to biopiles made up of dehydrated sludge from domestic wastewater primary treatment (used as a source of organic matter), sludge from car washes, and sewage sludge from the city of Tajoura's industrial zone, Libya. More than seven microbial isolated, identified, and conserved microbial strains with total petroleum hydrocarbon (TPH) degrading potential. Each assembly was infected with a bacterial consortium at a concentration of $2.99x10^9$ CFU / ml of bacteria and fungal microorganisms such as Aspergillus spp., Fusarium spp., Trichoderma spp., and others in a series of pilot experiments. Temperature, pH, humidity, and oxygenation levels were all monitored at a concentration of 1x106 spores per millilitre. To confirm the behaviour of the medicines in question, two studies were done. The continuous variable TPH in ppm was evaluated in entire randomised blocks using the approach of linear mixed models, which indicated substantial differences between the control and test biopiles. TPH removal percentages of up to 94 percent in 120 days and 84 percent in 40 days were achieved, indicating a beneficial effect on the decontamination of industrial sewage sludge and sewage sludge using the consortiums of microorganisms under study.
Keywords: bioremediation, bio piles, used oils, total petroleum hydrocarbons, native microorganisms.

Introduction

The inadequate final disposal of contaminated sludge with deposits of used lubricating oils - composed of total petroleum hydrocarbons (TPH), polychlorinated biphenyls (PCBs), polycyclic aromatics (PAH), metals and other polluting compounds - cause a deterioration in the environment and the human health due to their carcinogenic, toxic and poisonous effects, they are considered substances of difficult biodegradation and are classified as hazardous waste by the regulations established in the Basel Convention (Arroyo et al., 2008). Today, worldwide there are various biological techniques in order to provide alternatives for decontamination of impacted areas in soil, air and water. Bioremediation is a decontamination process that uses a series of biochemical reactions by a population or consortium of microorganisms inoculated in the contaminated area, to convert the hydrocarbon structure into less toxic components (Benavides et al., 2006). This study was developed

from the macro-project of "Bioremediation of sludge contaminated with used oils", carried out by students of Bacteriology and Clinical Laboratory of the University of Santander (UDES), Bucaramanga. From samples of sludge contaminated with waste oil residues, the isolation, identification and maintenance of native strains of bacteria and fungi with hydrocarbon residues degrading capacity (TPH) were carried out; To carry out the decontamination of said waste, two tests were carried out, inoculating the biopiles made up of sludge from car washes and sewage sludge from an industrial zone mixed with dehydrated sludge obtained from the primary treatment of domestic wastewater. Among the techniques used in the bioremediation process, bioaugmentation and biostimulation are used. In this case, bioaugmentation was carried out with the addition of aqueous solutions in concentrations of $2.99x10^9$ CFU / ml of the selected native microorganisms, and biostimulation with the addition of Nitrogen, Phosphorus and Potassium (commercial presentation NPK 15-15-15); In addition, the monitoring of abiotic parameters such as temperature, pH, humidity, oxygenation (turning) was carried out.

Materials and method

Isolation, identification and maintenance of microorganisms

The samples were collected from different random points, taking 500 g at a depth no greater than 15 cm of oily sludge from car washes, sewage system sludge from industrial areas of the Tajoura, Libya, and stabilized water treatment sludge domestic waste, which was deposited in sealed plastic bags, kept in refrigeration until processing, and transported to the laboratories Wastewater Treatment Plant, Libya.

For the isolation, the samples are subjected to a pre-enrichment adding 100 g of each one of these in 250 ml of the modified basic broth medium (CBS) –Sodium Chloride (0.15g), Ammonium sulphate (0.3g), Potassium bi phosphate 0.37 g, 0.125 g, 0.125 g, Magnesium sulphate 0.075 g, Potassium nitrate (0.3 g) at room temperature, under constant stirring at a speed of 140 rpm, for a period of twelve days . From the sixth day, until the twelfth day, an inoculum was taken and sown by exhaustion in plates of MacConkey Agar (AMck), Nutritive Agar (AN), Cetrimide Agar, Blood Agar (AS) and Soil Infusion Agar 25% (It was obtained from 500 g of contaminated sludge in 100 ml of distilled water, it was stirred and left to rest for 24 hours, subsequently it was filtered and the filtrate was worked at 25%, and agar-agar qsp) was added; then it was incubated at 32ºC for 24-48 hours in Blood Agar (AS) at 32ºC for 24-48 hours in an atmosphere of 3-6% CO_2, and in Saboraud Agar at room temperature for 5 days for isolation of fungi.

The identification of the isolated fungi was carried out based on the morphological characteristics of the colonies (color, appearance, consistency, observation of the front and back of the colony, and the presence or absence of pigments and exudates) and the microscopic observation of characteristic structures of the genera used in this study (presence or absence of septa, hyphae pigmentation or not, and observation of asexual reproduction structures). The identification of bacteria was carried out from the biochemical series (triple sugar iron agar, citrate, SIM, urea, malonate, motility, phenylalanine, methyl red, Voges Proskauer, lysine iron agar) and a semi-automated system (BBL CRYSTAL-NF and gram positives as appropriate) and additional tests (cytochrome oxidase, catalase, Indole, OF oxidation-fermentation: glucose, sucrose, lactose, mannitol, maltose). After the identification, chimes were made in a modified medium (with 10% used lubricating oil) and sown in simple medium with glycerol for their conservation (refrigeration 4 ° C and freezing -20 ºC).

Preparation of the inoculum

After the identification of the microorganisms, degradability and compatibility tests were carried out. For the degradability test (qualitative), a modified liquid culture medium was used at different concentrations of burned oils (10, 15, 25 and 50%) to verify the removal of hydrocarbons, visually checking every four hours the presence or decrease of the oil layer on the surface of the medium due to the consumption of

fats by microorganisms with the formation of micelles (personal communication Nieto L. ICP, 2005).

The competitiveness or compatibility tests were carried out on modified agar, confronting all the microorganisms in massive sowing up to the middle of the box with a strain A and incubated at 37 ºC for 24 hours; After this time, in the half not seeded, the sowing was carried out by means of a perpendicular streak with another strain B, which was incubated again for 24 hours at 37 ºC; According to these results, compatible microorganisms were determined (Garzón et al., 2001).

The medium used to apply the bacterial pool to each biopile was prepared from 2 liters of water, 5% molasses, 0.1% mineral salts, 0.5% yeast extract, with a bacterial concentration of $2.99x10^9$ cfu / ml compared with the MacFarland scale, confirmed by the pour-plate counting technique, taking a volume of 1 ml of a dilution (from 101 to 108) in a sterile Petri dish, to which molten culture medium was added. (Plate Count), previously cooled to a temperature of 40ºC, mixed and incubated at 35-37ºC for 24 hours; the inoculation of the fungi (Aspergillus spp, Trichoderma spp, Fusarium spp) was carried out on days 15-45-60-75, the optimal concentration was 1x 106 spores / ml, which was obtained by inoculating the fungal microorganisms in liquid medium (Malta broth), incubated at room temperature for five days.

Construction of biopiles

In the first trial, five biopiles were used for the field tests. In the second trial eight biopiles were worked, each biopile constructed with a 15° inclination is observed, an area of 1 m2 with a weight 50 kg and polisombra for protection against solar rays; additionally, a tube was placed in the center with holes to facilitate the collection of leachates. Table 1 shows the first group made up of five biopiles, the treatment of these was planned for 40 days, each of them made up of a mixture of dehydrated sludge from domestic wastewater treatment and sludge from car washes. The inoculations of the bacterial pools were carried out on days 1-10-20 and 30, with the following microorganisms

The second group was made up of 8 biopiles made up of dehydrated sludge (from primary treatment of domestic wastewater) used as input of organic matter and sludge contaminated with used lubricating oils from the sewage system of the industrial zone.

Table 1. Group of 8 biopiles, bioaugmentation was applied on days 1-30-60-90. Total time 120 days

Biopile	Microorganism - code	Sludge ratio	TPH ppm
One	*Acinetobacter spp (I)*	1: 4 one part sewage sludge industrial zone-4 parts dehydrated sludge WWTP	20.58
	Micrococcus spp (M)		
	E. coli (C)		
	Bacillus spp (CU)		
	Rhizopus spp (D)		
Two	*Acinetobacter spp (I)*	1: 4 one part industrial sewage sludge-4 parts dehydrated sludge WWTP	20.61
	Citrobacter spp (CT)		
	Nocardia spp (CK)		
	Rhizopus spp (D)		
Three	*Pseudomonas aureuginosa (S1) Pseudomonas putida (S2)*	1: 4 one part sewage sludge industrial zone-4 parts dehydrated sludge WWTP	20.59
	Aspergillus spp (F)		
	Trichoderma spp (H))		

Four	*Acinetobacter spp (I)*	1: 4 one part sewage sludge industrial zone-4 parts dehydrated sludge WWTP	20.57
	Bacillus spp (CU)		
	Micrococcus spp (M)		
	Pseudomonas putida (S2)		
	Trichoderma spp (H)		
Five	*Pseudomonas aureuginosa (S1)*	1: 1 parts sewage sludge industrial zone and WWTP dewatered sludge	30.02
	E. coli (C)		
	Nocardia spp (CK)		
	Trichoderma spp) (H)		
Six	*Pseudomonas putida (S2)*	1: 1 parts sewage sludge industrial zone and WWTP dewatered sludge	30.02
	Acinetobacter spp (I)		
	Micrococcus spp (M)		
	Trichoderma spp (H)		
Seven	*Pseudomonas aureuginosa (S1)*	4: 1 parts industrial zone sludge and one part dewatered WWTP sludge	39.67
	Bacillus spp (CU)		
	Micrococcus spp (M)		
	Rhizopus spp) (D)		
Eight	*Acinetobacter spp (I)*	4: 1 parts industrial zone sludge and one part dewatered WWTP sludge	39.67
	Pseudomonas putida (S2)		
	Nocardia spp (CK)		
	Trichoderma spp (H)		

The two groups were monitored parameters such as temperature and humidity daily by the fist test and in the laboratory by gravimetric technique every eight days, to maintain a close level between 60-70%, if necessary tap water was added, pH every 10 days with the use of the potentiometer, count of viable microorganisms before and 72 hours after the addition of the bacterial inocula, addition of NPK nutrients (15-15-15) 100 g dissolved in water on days 8-16-24 after mounting and aeration (by manual turning) during the application of the microorganisms.

Physico-chemical analysis

TPH determinations were made by soxhlet extraction for 72 hours, concentration and determination by gravimetry (5520 D), determination of fats and oils by soxhlet extraction (ISO / TR 11046) and lead by atomic absorption, before, during and after treatment, in certified laboratories (Industrial Consultation Laboratory of the Industrial University of Santander, Chemical Laboratory of the University of Santander, Quality Control Laboratory of the Metropolitan Aqueduct of Bucaramanga and Laboratory of the PTAR- Río Frío).

Statistical analysis

In the two trials, the continuous variable TPH in ppm was analyzed using the method of linear mixed models in complete random blocks (Littell et al., 2006) where the block effect is given by time and is considered a random effect, and the The experimental factor to be evaluated is the type of treatment and it is considered a fixed effect. In the first trial, selected contrasts were made between biopile 1 and biopiles 2, 3, 4 and 5 (Littell et al., 2006).

RESULTS

22 important microorganisms were isolated in bioremediation processes in phase 1 of the project, including gram positives, gram negatives and fungi (see Annex), which were later used for the

formation of consortia and their application in the different biopiles to carry out the decontamination process of the TPH present in the contaminated sludge. The variation of pH and temperature throughout the process are observed in Table 2.

Table 2. Average pH and temperature readings in each of the biopiles for 30 days in the first test with 5 biopiles

Day	Biopile-1		Biopile-2		Biopile-3		Biopile-4		Biopile- 5	
	pH value	Temperature ºC	pH value	Temperature ºC	pH value	Temperature ºC	pH value	Temperature ºC	pH value	Temperature ºC
December, 29, 2020	7.9	22	6.2	23	7.3	23	6.4	23	7.2	23
January 10, 2021	8	24	6.9	23	7.3	23	6.5	23	7.3	23
January 11 2021	8.1	26	7.3	25	7.5	26	6.7	26	7.6	23
January 17 2021	8.2	26	7.1	25	7.3	26	6.7	26	7.5	24
January 26 2021	8.2	26	6.9	23	7.4	25	6.4	25	7.6	26

In the first trial, the effect of 5 different treatments for the degradation of polluting residues in sludge from car washes was evaluated in the response determined by the concentration of TPH in ppm, in an experimental period of 40 days with observations taken at days 0, 15, 30 and 40. Table 3 shows the initial and final concentrations of TPH and the removal percentages of TPH in each biopile.

Table 3. TPH removal percentage by the Soxhlet extraction method in 72 hours, to determine the effect of the microorganisms inoculated in each of the biopiles.

Biopila No	Inoculums	Initial TPH concentration (ppm)	Final TPH concentration (ppm))	% Removal	Removal Time (days)
1(negative control)	Without inoculum	21,274.50	19784.3	6.9	16
			19784.8	7	29
			19784.7	7	40
2	Bacteria: A. I. M Algae G	21,274.50	15956.88 12946.04	25	15
			11524.01	40	29
				46	48
3	Bacteria: E. I. CK Algae: H	21,274.50	14167.62 11898.015	34	15
			9726.12	45	29
				55	42
4	Bacteria: S1. S2 .I	21,274.50	12191.19	43	17

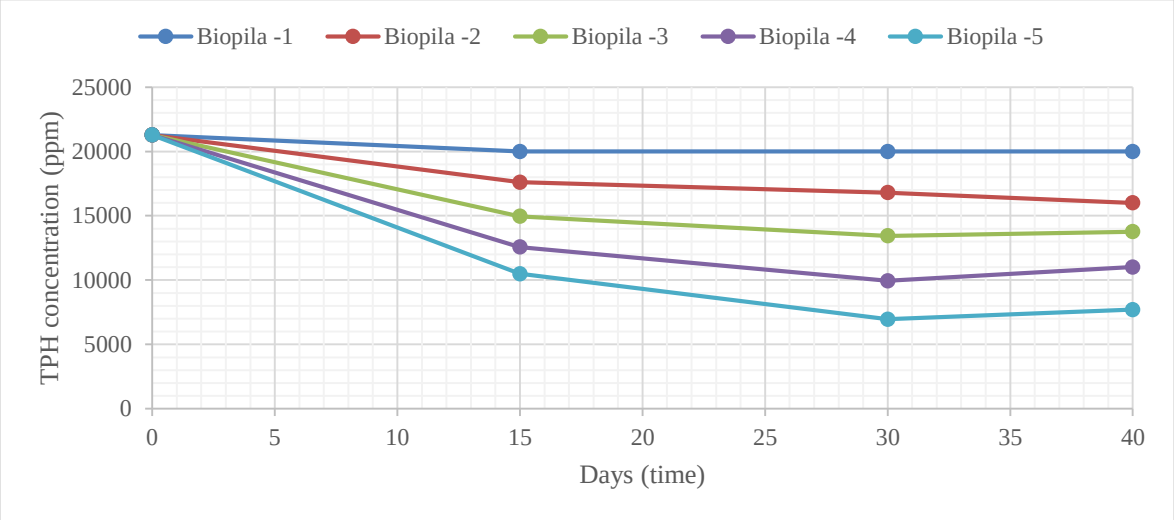
	Mushroom: H		5988.39	72	16
			2746.28	88	41
5	Bacteria: S1. S2. . Algae: H	21,274.50	14105.9	37	16
			7724.5	63.7	32
			5524.11	74.03	41

The pretreatment lead concentration in the contaminated sludge was 90 mg / l. The results obtained from the determination of Pb at 40 days post-treatment by the atomic absorption method are reported in Table 4.

Table 4. Post-treatment lead (Pb) results. Atomic absorption method

Biopila	**Pb(mg/l)**
One	69.98
Two	89.91
Three	79.98
five	59.97

Graph 1 shows the variation of TPH concentrations in ppm over time in the 5 biopiles. All biopiles started from the same TPH concentration, the control biopile presented a minimal decrease in TPH while biopiles 2, 3, 4 and 5 presented an apparently significant decrease in the 40 days of the experiment.



Graph 1. Variation of TPH concentrations in forty days.

To determine the existence of statistically significant differences between the treatments, a mixed linear model was fitted to the data (Table 5). The significance test of the model presented in Table 6 reveals significant differences between the treatments. Table 7 shows the selected contrasts comparing the control biopile with biopiles 2, 3, 4 and 5. The contrasts show evidence of significant differences at the 0.05 level of significance between the control biopile and biopile 2, and at the level of significance 0.01 between the control biopile and biopiles 3, 4 and 5.

Table 5. Estimates and standard errors of the parameters of the mixed linear model used for the analysis of complete random blocks of the trial in which the effect of 5 different treatments for the degradation of sludge from car washes was evaluated, in the determined response by the TPH concentration in ppm, in an experimental period of 40 days with observations taken at days 0, 15, 30 and 40.

Fixed effects			
Parameter	Estimated	Standard Error	p-value
Intercept	12156	2836.93	0.0234
Biopile 1	8000.57	2071.1	0.0023
Biopile 2	3269.11	2071.1	0.1404
Biopile 3	2110.56	2071.1	0.3283
Biopile 4	-1606.4	2071.1	0.453
Random effects			
Time 0	6305.34	2665.17	
Time 15	680.51	2665.17	
Time 30	-2649.9	2665.17	
Time 40	-4335.9	2665.17	

Table 6. Significance of the fixed effects of the treatment of the mixed linear model used for the analysis of complete randomized blocks of the trial in which the effect of 5 different treatments for the degradation of sludge from car washes was evaluated, in the determined response by the TPH concentration in ppm, in an experimental period of 40 days with observations taken at days 0, 15, 30 and 40.

Effect	GL num	GL Den	F value	p-value
Treatment	4	12	6.3	0.0057

Table 7. Contrasts between the negative control biopile and biopiles 2, 3, 4 and 5 at the end of the experimental period, for the tests in which the effect of 5 different treatments for the degradation of sludge from car washes is evaluated in the response determined by the concentration of TPH in ppm, in an experimental period of 40 days with observations taken at days 0, 15, 30 and 40.

Contrast	GL num	GL Den	F value	p-value
Biopila 1 vs Biopila 2	1	12	5.25	<0.05 to <0.0006
Biopila 1 vs Biopila 3			8.18	
Biopila 1 vs Biopila 4			21.61	
Biopila 1 vs Biopila 5			14.84	

Analysis of the significance of the fixed effects of the model reveals that at least one of the parameters of the linear mixed model is significant.

The contrast table reveals significant differences between the control biopile and biopiles 3, 4 and 5 at significance levels α = 0.05, and between the control biopile and biopiles 4 and 5 at the significance level α = 0.01 , demonstrating that the treatment carried out and the application of mixed culture of microorganisms was efficient in the decontamination of the sludge with TPH residues.

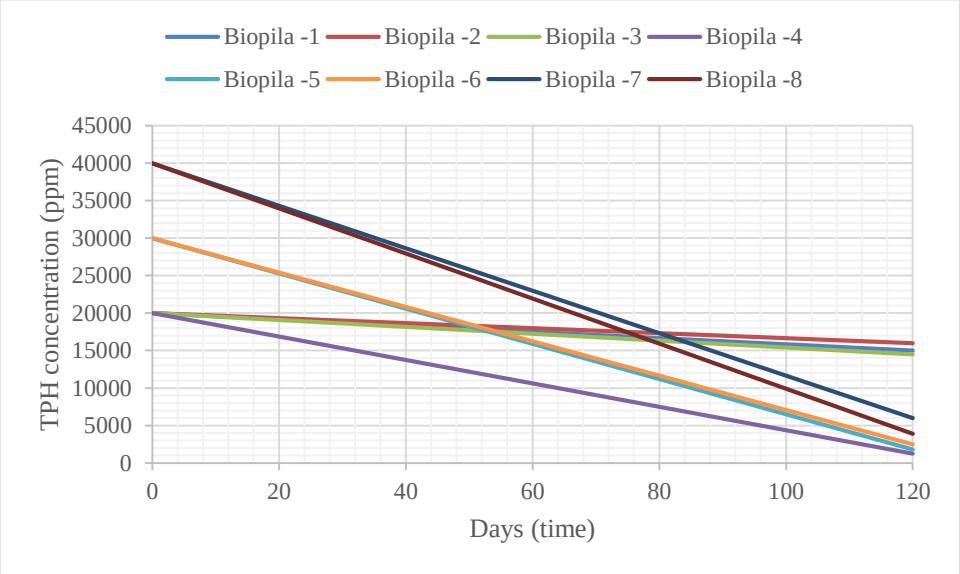
In the second trial, the effect of 8 different treatments for the degradation of sludge contaminated with TPH from the sewerage system of the industrial zone of Bucaramanga was evaluated through the concentration of TPH in ppm (parts per million) in an experimental period of 120 days with observations taken at days 0 and 120. Table 8 shows the treatments under investigation and the TPH removal percentages from each treatment at the end of an experimental period of 120 days.

Table 8. TPH removal percentage by the Soxhlet extraction method for 72 hours, concentration and gravimetric determination to determine the removal efficiency of the microorganisms inoculated in each of the biopiles in a period of 120 days.

No. Biopiles	Inoculums	Ratio: Contaminated sludge / dewatered sludge	Initial TPH concentration (ppm)	Final TPH concentration (ppm)	% Removal
1	Bacteria & Algae	01:04	21.6	11.6	48.5
2	Bacteria:I, CT, CK Mushrooms: H	01:04	21.6	891	95.5
3	Bacteria: S1, S2 Fungi: F, H	01:04	21.6	982	95.5
4	Bacteria: I, CU, M, S2 Mushrooms: H	01:04	21.6	1.08	94.6
5	Bacteria: S1, C, CK Mushrooms: H	01:01	31.15	1.94	94.7
6	Bacteria: S2, I, M Mushrooms: D	01:01	31.16	2.87	90.4
7	Bacteria: S1, CU, M Mushrooms: D	04:01	38.67	7.63	80.1

8	Bacteria: I, S1, CK Hongos: H	04:01	38.67	5.11	87.1

Graph 2 shows the change in TPH concentration in ppm over a period of 120 days. All the biopiles under study showed a decrease in the TPH concentration in the experimental period, with a higher concentration being observed in biopiles 7 and 8.



Graph 2. TPH concentration in a period of 120 days.

A mixed linear model, estimates and standard errors were fitted to the data from the second trial. The model revealed that there are no significant differences between the estimates of the effects and this finding, and it was confirmed in the general test of the model presented in Table 8. The conclusion for this experiment is that there is no effect of the different biopiles on TPH concentration in ppm, considering as random effects the two time measurements at 0 and 120 days of the experimental period.

Table 9. Estimates and standard errors of the parameters of the mixed linear model used for the analysis of complete random blocks of the experiment in which the effect of 8 different treatments for the degradation of sludge from the city's sewage system was evaluated, in the response determined by the concentration of TPH in ppm, in an experimental period of 120 days with observations taken at days 0 and 120.

Effect	Estimated	Standard error	Pr> | t |
Intercept	22385	12518	0.3246
Biopile 1	-6785	5,718.26	0.2741
Biopile 2	-11640	5,718.26	0.0812
Biopile 3	-11595	5,718.26	0.0822
Biopile 4	-11545	5,718.26	0.0833
Biopile 5	-6340	5,718.26	0.3042
Biopile 6	-5875	5,718.26	0.3384

Biopile 7	1260	5,718.26	0.8319
Random effects			
	Estimated	Standard error	
	11761	11931	
Time 1	-11761	11931	

Table 10. Significance of the fixed effects of the treatment of the mixed linear model used for the analysis of complete random blocks of the experiment in which the effect of 8 different treatments was evaluated for the degradation of sludge from the city's sewage system, in the response determined by the concentration of TPH in ppm, in an experimental period of 120 days with observations taken at days 0 and 120.

Effect	GL num	GL Den	F value	p-value
Treatment	7.1	7.1	1.59	0.281

The analysis shows that there is no effect of the different biopiles on the concentration of TPH in ppm, considering as random effects the two measurements of time at 0 and 120 days of the experimental period.

The initial lead concentration in the contaminated sludge was 122.2 mg / l, and in the post-treatment sludge 88.95 mg / l. The determination of the chemical oxygen demand and the lead concentration of the leachates produced during the treatment of each biopile is necessary to carry out an adequate elimination. Table 11 shows the COD data, referring to less than 2000 mg / Lt. The low COD levels indicate that the contaminants present in the mixtures were optimally degraded by the inoculated microorganisms. In biopiles 2, 6 and 7, where results of more than 1000 mg / Lt are observed, although it is not a considerable concentration, it indicates that there is the presence of contaminants that were possibly carried away by leaching. Table 11 shows the resulting lead concentrations, in some cases they are high, so a subsequent treatment of these leachates should be planned.

Table 11. Determination of post-treatment fats and oils by the gravimetric method by Soxhlet extraction; lead by the atomic absorption technique of post-treatment leachates and COD concentrations by spectrophotometry of the post-treatment leachates from the second test with 8 biopiles

Biopila	Fats and oils mg / l	Lead mg / l	COD mg / lt
1	300	150.8	523
2	676.6	110.3	1074
3	373.3	189.5	382
4	1896	116.7	234
5	2563	253.8	514
6	11.913	97.2	1077
7	15.075	282.3	1222
8	2650	171.07	533

DISCUSSION

Studies carried out for the North American Petroleum Institute (API) [2] on the treatment of

soils contaminated with oil residues show that 70-90% is removed at different times with concentrations between 10,000 to 50,000 ppm, the concentrations of this study found between 19,000 and 39,000 ppm achieving similar removals between (74 to 95%) in the two trials in 40 and 120 days.

The experimental phase of the bioremediation work on soils contaminated with used lubricating oils (hazardous waste) and the evaluation of microbial consortia with the ability to degrade hydrocarbon residues and their application, show the high similarity that they present in time and removal percentages in the two groups of biopiles, taking into account the inoculated microorganisms and the proportions of sludge, where it is compared with the results obtained by other authors [5-6], demonstrating the great importance of microbial inoculum for transform and use the pollutant as an energy source in the bioremediation process.

Important percentages (74-95%) of decontamination were achieved in short times of 40 and 120 days compared with the times of 9-12 months of the work carried out by Tettamanti et al. (2003); Similarly, higher removals were obtained than that reported in the hydrocarbon bioremediation work of the sediments of the Havana Bay of Núñez et al. (2005) of 48% with the isolated mixed culture of said bay.

Experiences of bioremediation of soils by biological techniques in the province of Santa Cruz (2002), and works carried out by Total Petroleum Hydrocarbon Criteria Working Group Series[9] in contaminated soils with concentrations between 20,000-55,000 ppm as fats and oils were degraded between 2 and 3 years (Brisio, 2005); Other studies by Huesemann and Moore (1993) show that 93% of saturated hydrocarbons and 74% of aromatics were degraded in sandy soil in Michigan (USA) with an initial concentration of 30,000 ppm TPH, the study it also indicates that the polar fraction was resistant to degradation during the 5 months that the study lasted.

Isolated and identified strains of contaminated sludge with hydrocarbon degrading capacity have been reported in the literature; within them we have gram negative (*Pseudomonas aeruginosa, Citrobacter freundii, Proteus mirabilis, Proteus vulgaris, Proteus penneri, Acinetobacter iwoffi, among others) and gram positive (Oerskovia species, Bacillus brevis, Nocardia spp., Actinomyces spp. and Bacillcoc megaterium, Micrococcus megaterium. spp.) and strains of fungi (Aspergillus spp., Trichoderma spp., Fusarium spp. and Rhizopus spp).* The degradability, competitiveness and compatibility tests carried out on these bacteria and fungi allowed us to evaluate that Pseudomonas aureuginosa and Psedomonas putida are microorganisms with high TPH degrading capacity, without diminishing the importance of other microorganisms [1,5].

The use of mixed cultures or bacterial pool favors a greater degradation with the use of biostimulation (Cardenas, 2006), being the time used by this author and the removal percentages of TPH similar to those achieved in this work.

The application of sludge from domestic wastewater treatment used as sponge and energy input at a concentration of 25%, and an initial concentration of TPH of 21,274.5 ppm, is a good option since TPH removals of 45 , 8, 54.28, 74.03 and 87.09% in 40 days, the differences are given according to the pool of microorganisms applied; These contrast with the results reported by Cárdenas et al. (2004) in concentrations of 30% of sludge obtaining lower removal percentages such as 41.98 and 47.46% in three months and an initial concentration of 4,000 ppm of TPH.

Conclusions.

With this work, considerable percentages of TPH removal were achieved between 74.03 and 87.09% in 40 days and 87, 94 and 95% in 120 days with the application of the microbial consortia obtained and the methodology applied in the process of bioremediation in field tests. It was possible to show that these residues deposited on the soils are not degraded without carrying out an adequate treatment. The use of native microorganisms represents an alternative to reduce pollution generated by hazardous waste and recovery of impacted areas.

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