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Surfactant Enhanced Washing of Soil Contaminated with Petroleum Hydrocarbons and Treatment of Produced Wastewaters Using a Biofilter

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Abstract

Wastewater generated by washing a real petroleum hydrocarbon-contaminated soil was treated using a submerged aerobic filter. The wastewater contained petroleum hydrocarbons, surfactants, and other compounds that leached during the soil washing process. The efficiency of the biological treatment using natural or synthetic surfactants on the hydrocarbon-contaminated soil washing process, and different operation temperatures (24, 28, and 32°C) were compared and the identification of the microbial consortium present in the biofilter was carried out. The best degradation efficiency (73% hydrocarbon removal) was obtained when the wastewater from the soil washing using locust bean gum was treated at the biofilter operated at 24°C. The microorganisms found in the microbial consortium in the biofilter were *B. subtillis, C. jeikeium, Pseudomonas sp., A. sobria, A. caviae, E. sakazakii.*

Keywords: Surfactants, submerged aerobic filter, wastewaters treatment, soil washing.

1 Introduction

Mexico is a country with an intense petroleum activity. Only during 2007, over 3 million crude oil barrels were produced daily [1]. However, besides its economic preponderance, the oil industry as a whole has had a large negative environmental impact. Soil contamination with petroleum hydrocarbons is very common [2]. Crude oil spills have produced severe contamination of soils with a wide variety of toxic and/or persistent oil derivatives. The usual treatment techniques for contaminated areas include the application of chemical, biological, or physical processes aimed to destroy the involved contaminants, modifying it to harmless by-products and/or reducing their concentration. A widely used methodology for remediation is *in-situ* soil washing consisting in dragging the contaminants present in soils by using a carrier solution [3]. Once the hydrocarbon-contaminated soils have been washed, the washing solution usually contains the added surfactants and the contaminants desorbed from the soil.

Several processes have been reported for the treatment of such wastewater. In particular, when surfactants are present, biological processes, ultrasonic irradiation, advanced oxidation, activated charcoal adsorption, and activated membrane reactors have been applied. Physical chemical processes are frequently used consisting in coagulation-flocculation-sedimentation coupled to activated sludge systems. Considerable removal of the main contaminants has been achieved by applying such process, nonetheless with high amount of sludge produced [4]. Advanced oxidation process applied to the same kind of wastewater have generated remarkable results for the removal of bulk contamination [5], [6] for a quite efficient treatment process without the generation of important amounts of sludge. The systems, however, may require expensive chemicals and the application of high amounts of energy.

Biological treatment is a cost-effective method suitable for wastewater restoration. However, to our knowledge, few works have used biodegradation as treatment for soil washing wastewater. Treatment of wastewater containing surfactants by biological processes (i.e. activated sludge), has some difficulties related with low degradation rates and foam production [7]. Use of aerated biofilters is a promising technique due to its capability to promote high biomass loads, easy operate and biodegradation rates higher than free cell systems [8]. The use of submerged aerobic filters has advantages as their high capacity for contaminant removal, efficient stable operation, acceptance and adaptation to organic and hydraulic load fluctuations, high biomass retention time, proper interaction between substrate and microorganism, and enough oxygen to maintain the system under aerobic conditions. The aim of this work is to show our results on integral remediation process for soils contaminated with petroleum hydrocarbons, including soil characterization, the surfactant enhanced soil washing assessments, production and characterization of wastewater and its treatment in an

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aerobic submerged filter and the microbiological characterization of the fixed-biomass system.

2 Methodologies

2.1 Soil characterization.

The soil used in this study was obtained from the Azcapotzalco ex-refinery, in Mexico City. The soil general characteristics were reported previously by [9], which are shown in Table 1. According to the size of soil particles, it was considered as sandy soil with gravel.

2.2 Soil washing assessments.

Portions of contaminated soil (6 g) were washed with 20 mL of a washing solution with different surfactants. Three natural (guar gum, locust bean gum, mezquite seed gum) and 12 synthetic surfactants (Surfacpol 203, Surfacpol G, Surfacpol A 1404, Emulgin 600, Tween 20, Brij 35, Tween 80, SDS, Polafix, SDBS, Texapon 40, Polafix CAPB) were tested for soil washing. Samples were placed into a 50 mL serological flasks and shaken at 150 rpm for 23 h in a mechanical shaker at room temperature. Then, movement ceased and the soil was allowed to settling by gravity and the solution was decanted. The washed soil was allowed to dry at room temperature for further analysis. The surfactants with the highest efficiency in TPHs removal in the 50-mL scale soil washing process were selected to generate the wastewater at bench scale to be used in the submerged aerobic biofilter.

Table 1. General characteristics and metal content in contaminated soil.

Parameter	Value	Parameter	Value
TPH (mg kg^{-1})	31,902	Cell counts (UFC g^{-1}_{soil})	$230x10^5$
pH	6.3	As	<Detection Limit
Humidity (%)	4.5	Cd (mg kg^{-1})	4.08± 0.27
Parameter < 2mm (%)	63	Cu (mg kg^{-1})	310.24± 5,19
Particles > 2mm (%)	37	Zn (mg kg^{-1})	165.92± 10.8
TOC (%)	0.27	Pb (mg kg^{-1})	32206.23± 1435.50
TOM (%)	0.46	Ni (mg kg^{-1})	8608± 798
Soluble phosphorus (mg L^{-1})	0.84	Na (mg kg^{-1})	1679.39± 267.30
Total nitrogen (%)	0.04	K (mg kg^{-1})	1376.02± 259.53
Cation exchange capacity (meq 100 $g.soil^{-1}$)	16.54	Ca (mg kg^{-1})	8029.65± 88.05

2.3 Determination of total petroleum hydrocarbons (TPH) in the washed soil.

TPHs were determined in the soil after washing with the different solutions tested according to the EPA 9071B method. Briefly, TPHs were extracted from the soil with hexane using a Soxhlet system. Thereafter, hexane was evaporated in a rotovaporator and TPHs were calculated based on weight differences.

2.4 Wastewater generation from the soil washing process.

Wastewater was obtained by washing 700 g of hydrocarbon-contaminated soil with 2.1 L of the washing solution with the previously selected surfactants. The sample was agitated with a Lightnin mixer using a A310 impeller in a cylindrical acrylic container during 2.5 h at 1300 rpm. Each sample was washed two times and was allowed to settling for 24 h. After this time the aqueous phase was separated and characterized for pH, conductivity, color, turbidity, biological oxygen demand (BOD_5), chemical oxygen demand (COD), total solids (TS), $CaCO_3$, methylene blue active substances (MBAS), grease and oils, and some metals (Al, Cr, Fe, Pb) following the Standard Methods [10].

2.5 Submerged aerobic biofilter.

The laboratory model employed consisted of packed glass columns, 14 cm height and 2.5 cm width, with a capacity of 25 mL filled with a support material with previous biofilm formation. The water to be recycled contained in Erlenmeyer flasks was placed in the lower part of the filter; a peristaltic pump (MasterFlex Cole Parmer Instrument Co, USA) with an approximate flow of 0.96 L min^{-1} was used for water recirculation.

Two variables were established for biofilter operation: temperature and surfactant concentration. Three temperatures were established, 24, 28, and 32°C; the surfactant at different concentrations (0.5 and 1% for TW80, and 0.1 and 0.2% for the locust bean gum). Temperature was monitored through a temperature controller (PolyScience), aeration was achieved by coupling stainless steel diffusers and an air pump (ELITE 799).

As support for biofilm formation, red volcanic rock (*Tezontle*) was used. The support was ground, washed, dried and then sieved (American mesh 10 and 20) to obtain regular sized particles. This material is very available, cheap, porous and resistant and has been used previously as a biofilter packing material [11]. The inoculum for biofilm formation was obtained from a municipal wastewaters treatment plant. The support (8.5 g) was inoculated with 50 mL of the wastewater from the treatment plant and 2 mL of TW80 as carbon source. The mixture was kept under agitation at 120 rpm at 28°C during 2 months with periodicals (every 2 weeks) addition of 2 mL of the inoculum and the surfactant (or the amount needed to keep the 50 mL volume).

2.6 Determination of the number and type of microorganisms in the biofilm.

The number of microorganisms present on the biofilm was determined by plate counting through obtaining 1 g of support. The support was poured it into sterile deionized water and vortexed, aiming at releasing the microorganisms adhered to the support. The culture medium was nutrient agar (Becton Dickinson), plates were incubated at 37 °C for 24 h. Those plates that showed 30 to 300 well-defined colonies and were not overlaying were taken as the basis to determine the total colony forming units per gram of support (CFU g^{-1}) [12].

Microorganisms isolation was performed also by the plate extension technique using brain-heart-infusion

(BHI, Becton Dickinson) as culture medium. Dilutions (up to 10^{-8}) were sown and plates were incubated at 37 °C for 24 h. From the obtained colonies, the most abundant strains in the microbial consortium were isolated by means of the cross-streak technique using BHI as culture medium; plates were incubated at 37 °C during 24 h. Every isolated strain was macroscopically examined to identify shape, margin, elevation, opacity, and pigmentation of colonies. Their Gram response was identified as well as the cellular morphology. Biochemical profiles were determined for the isolated colonies using the API 20E (BioMérieux, France) identification systems for the Gram negative microorganisms and the BBL Crystal for the Gram positive ones, to observe the fermentative oxidation of sugars, including the production of indole and oxidase [13]. The obtained information was compared with that reported in the Bergey´s Manual [14].

2.7 Soil washing wastewater treatment assessment in the submerged aerobic biofilter.

Wastewater from the soil washing (c.a. 100 mL) was recirculated in the submerged aerobic biofilter during 1 week for each treatment until the COD concentrations did not show a significant diminution. Degradation kinetics was measured through COD removal; electrical conductivity and pH. The microbial count on the biofilm was determined at the beginning and end of the process time as described previously. Different treatment conditions were tested for the soil washing wastewater according to the variables established in the biofilter operation.

3. Results and Discussion

3.1 Hydrocarbons removal from the contaminated soil and selection of surfactants and doses

From the 15 surfactants tested to washing the contaminated soil, the highest removal was obtained for the synthetic surfactants. From them, the highest TPHs removal was obtained with Brij 35 (56.78%), followed by TW80 (55.54%), whereas the lowest removal was obtained with Polafix CAPB (29.22 %). From the natural surfactants, locust bean gum yielded the highest TPHs removal (31.13%). The removal obtained with the other two natural surfactants tested was similar with 18.83% for the mezquite gum and 18.44% for the guar gum (Figure 1).

Since there was no significant difference in the removal obtained with Brij 35 and TW80, it was decided to use TW80 for the scale-up processes. Once the most efficient surfactants for TPHs removal had been chosen, different concentrations of them were used to choose the most adequate one to wash the contaminated soil to obtain the wastewaters to be cleansed by means of the biofilter. The best efficiency was obtained with TW80 at 0.5%, and at 0.1% when using the locust bean gum. Based on the results obtained in TPH removal using these concentrations, we chose the doses that yielded the best TPH removals from the contaminated soil. The chosen concentrations were 0.5% for TW80 and 0.1% for the locust bean gum.

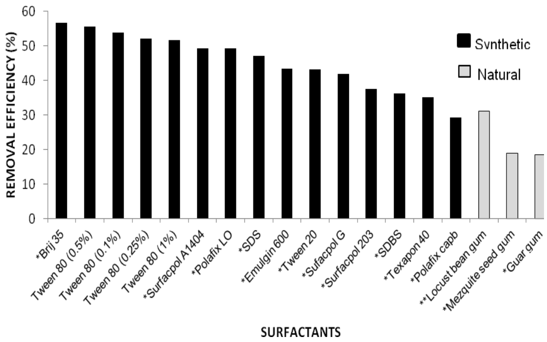

Figure 1. TPH removal obtained by washing the soil with different surfactants.

3.2 Characterization of the soil washing wastewater

Table 2 depicts the results for the characterization of the soil washing wastewater obtained for the best conditions tested. As shown, pH of the wastewater was almost neutral.

Conductivity values were quite similar for the TW80 and LBG effluents with values of 2,580 and 2,366 mg L^{-1}) The BOD/COD ratios were 0.197 (TW80) and 0.285 (locust bean gum), indicating that only 19.7% and 28.6 % of the matter could be degraded microbiologically, respectively. It has been reported that wastewater can be considered as easily biodegradable if the BOD/COD ratio is between 0.4 and 0.8 [15], [16], therefore, the sample to be treated could not be considered as easily biodegradable. The amount of metals i.e., Al, Cr, Fe, and Pb, was higher in the locust bean gum washing waters (65, 0.07, 39 and 43 mg L^{-1}, respectively) in comparison to those found in the TW80 wastewaters (23, 0.02, 11 and 20 mg L^{-1}, respectively), because this gum dragged a higher amount of the soil components due to its high capacity to bind to certain metals by the strong binding between metallic ions and the OH^- groups present in the locust bean gum's structure [17].

Table 2. Physicochemical parameters of the wastewater when using TW80 and locust bean gum LBG.

Parameter	Employed coagulant-flocculant	
	TW80	LBG
pH	6.51	7.08
Conductivity (μs)	2580.00	2366.00
Color (PtCo)	3625.00	5100.00
Turbidity (UNT)	525.00	862.50
BOD_5 (mg L^{-1})	289.64	360.89
COD (mg L^{-1})	1468.00	1264.00
TS	5.08	4.48
$CaCO_3$ (mg L^{-1})	22.50	27.00
MBAS (mg L^{-1})	0.015	0.11
Greases and oils (mg L^{-1})	6.00	25.20
Al (mg L^{-1})	23.62	65.12
Cr (mg L^{-1})	0.023	0.075
Fe (mg L^{-1})	11.25	39.23
Pb (mg L^{-1})	20.130	42.63
BOD_5/COD	0.197	0.285

The amount of grease and oil present in the LBG wastewaters (25.2 mg L^{-1}) resulted higher than that found in the TW80 wastewater sample (6 mg L^{-1}), while the MBAS concentration was obviously higher for the TW80-containing effluent than the amount registered in the LBG effluent. MBAS assessment indicates the amount of ionic surfactant present in residual waters. In is possible that LBG reacted with the galactomanana molecule as the ionic surfactants do. Total solids were very similar for both wastewaters (% for TW80 effluent and 4.48 for the LBG one).

In a recent work [18] it has been reported the characteristics of wastewaters generated when a synthetic surfactant solution (SDS) was employed to treat a soil contaminated with automotive oil. They found a COD and BOD values of 1,329 and 385 mg L^{-1}, respectively, giving a BOD/COD ratio of 0.29. On the other hand, the MABS content was of 122 mg L^{-1}, conductivity was of 1,107 S and hardness was 489 mg L^{-1} as $CaCO_3$·Pb, Fe, Cr and Al were present in concentrations of 0.4, 19, 0.07 and 24 mg L^{-1}.

3.3 Wastewater treatment in the submerged aerobic filter

For the wastewater obtained with TW80, the best biodegradation results were obtained when the biofilter was operated at 24°C, achieving a 62.5% TPHs removal and removal velocities of 6.20 mg L-1 h-1. Whereas the least favorable results were obtained by operating the biofilter at 28°C, achieving a removal velocity of 2.5 mg L-1 h-1 and a 28.3% TPHs removal efficiency. The microbial behavior in the system along the degradation kinetics showed some variations depending on the conditions under which the biofilter was operated; the best viable count was 108 CFU g^{-1}, whereas the lowest was of 105 CFU g^{-1} (Table 3).

Table 3. Results of the degradation kinetics of the contaminants in the wastewaters from the soil washing using TW80 or Locust bean gum.

Treatment	Degrad rate (mg L^{-1} h^{-1})	Rem. efficien-cy (%)	Count (CFU g $soil^{-1}$)	
			CVi	CVf
TW80 0.5%. 24°C	6.2	62.5	2.5×10^6	4.7×10^5
TW80 0.5%. 28°C	5.2	51.9	5.7×10^6	4.0×10^8
TW80 0.5%. 32 °C	4.7	41.3	4.0×10^8	2.5×10^6
TW80 1%. 28°C	22.0	63.1	4.7×10^5	3.6×10^6
LBG 0.1%, 28°C	2.2	39.9	2.7×10^6	2.2×10^6
LBG 0.1%, 32°C	3.9	38.1	2.0×10^6	3.5×10^6
LBG 0.1%, 24°C	6.6	73	2.2×10^6	2.0×10^6

CVi = initial viable count, CVf = final viable count,

A good correlation between temperature and degradation velocity, as well as with the degradation efficiency was observed. The higher the operation temperature of the biofilter, the lower was the velocity and the degradation efficiencies. When the surfactant's concentration increased, an increase in the degradation velocity was observed and the removal efficiency of the contaminant was very close to that obtained when operating the biofilter at 24 °C and concentration of the surfactant equal to 0.5%.

Table 4. Values of degradation kinetic constants for the contaminants present in the wastewaters generated by the soil washing.

Surfactant	Treatment	R^2	COD_0 (mg L^{-1})	k (h^{-1})
LBG	0.1% 24°C	0.9439	1581.5	0.009
	0.1% 28°C	0.4233	1232.7	0.003
	0.1% 32°C	0.9583	1442.4	0.003
TW80	0.5% 24°C	0.878	1581.4	0.005
	0.5% 28°C	0.5758	1423.9	0.003
	0.5% 32°C	0.6646	1587.8	0.002
	1% 28°C	0.6784	10573	0.002

Injection of air into the system did not showed any positive effect on the treatment, on the opposite, the degradation velocity and TPHs removal efficiency decreased as compared to the other treatments. The microbial behavior along the degradation kinetics showed some variations depending on the biofilter operation conditions, however, counts remained in the order of 10^6 CFU g^{-1} (Table 5). As observed for treated water in the case of the synthetic surfactant, decrease in color, turbidity, BOD, COD and metals was observed in the wastewater of the soil washing using locust bean gum after its treatment with the submerged aerobic biofilter (Table 5). From the degradation kinetics, based on the COD removal, equations with an inverse exponential tendency were determined:

$$y= C_O e^{-kt} \qquad (1)$$

where y is the COD concentration in the medium at time t, C_o is the initial concentration, and k is the reaction velocity constant (Table 4). These values allowed predicting the degradation values when using other operation conditions within the system. According to the k value (h^{-1}) obtained in equation (1), it can be said that introduction of air into the system did not improved reaction velocity, since the values of this constant did not showed any significant increase as compared to the treatments in which no air was introduced.

The BOD_5/COD relationship for the wastewaters generated with TW80 did not show any significant change, the value before the treatment was 19.7% whereas after the treatment this relation was of 11.5%, indicating that the amount of matter that could be degraded biologically had already been consumed almost completely. For the wastewater generated with the locust bean gum, the change was significant: the initial ratio was 28.6% and turns 85.16% after treatment, revealing an increase of biodegradation in the effluent.

Table 5. Comparison of the physicochemical parameters before and after treatment with the biofilter of the wastewater generated from the contaminated soil washing using TW80 or Locust bean gum.

Para-meter	TW80			LBG		
	Initial value	Final value	Change (%)	Initial value	Final value	Change (%)
pH (units)	6.51	7.36	-13.06	7.08	6.18	12.71
Condu-ctivity (Ms)	2580.00	788.00	69.46	2366.0	664.0	71.94
Color (PtCo)	3625.00	399.00	88.99	5100.0	456.0	91.06
Turbi-dity (UNT)	525.00	32.00	93.90	862.50	56.00	93.51
BOD (mg/L)	289.64	24.29	91.96	360.89	72.39	79.94
COD (mg/L)	1468.00	211.50	85.59	1264.0	85.00	93.28
TS (g/L)	5.08	718.70	-14053.21	4.48	558.9	-12381
$CaCO_3$ (mg/L)	22.50	238.32	-959.20	27.00	170.1	-530.00
MBAS (mg/L)	0.015	0.63	-4080.00	0.112	0.46	-309.82
Grease and oils (mg/L)	6.00	-	-	25.20	-	-
Al (mg/L)	23.62	0.047	99.80	65.12	2.45	96.24
Cr (mg/L)	0.023	<0.06	-	0.075	<0.06	-
Fe (mg/L)	11.25	2.95	73.73	39.23	3.35	91.46
Pb (mg/L)	20.13	0.72	96.41	42.63	0.87	97.96
BOD/COD	0.20	0.12	-	0.29	0.85	-

3.4 Microbial identification

Microbial identification of the most frequent colonies found in the viable counts from the wastewater used as inoculum and wastewater treated with the submerged aerobic biofilter for every tested surfactant was carried out. Identification was performed according to the colonial morphology, Gram staining, and the biochemical tests (API 20E and BBL Crystal). The microorganisms found are depicted in Table 6. Some of the strains reported in Table 6 have already been reported previously in hydrocarbon degradation processes.

Most hydrocarbon degrading bacteria belongs to the Gram-negative group [19], the lipopolysaccharides in their membranes help for the formation and stabilization of hydrocarbon emulsions in aqueous systems and contribute to increase the attacking surface on the contaminant, for its ulterior assimilation [20].

There is a lack of papers which treating contaminated hydrocarbon-soils with soil washing and the subsequent biological treatment, report the kind of microorganisms present in the biological system, Nevertheless, Iturbe *et al*. [21], reported the treatment of soils contaminated with petroleum hydrocarbons by biopiles. They found using miniaturized systems (API and crystal BBL) the following microorganisms in the mentioned soil: *Bacillus liqueniformis and cereus; Micrococcus sedentarius* and *luteus, Turicela otitis, Staphylococcus Schiefieri* and *Corynebacterium renale.*

In a recent work, the research group has been working with a 5 L column, very similar to the one employed here, and the evaluation of the microbiota has been carried out by molecular analysis of consortia. DGGE methodology has been employed in order to elucidate the microflora changes inside the biofilter, due to changes in the COD of the wastewaters, the surfactant concentration and the residence time [22].

4 Conclusions

The use of a submerged aerobic filter is feasible for the treatment of petroleum hydrocarbons-contaminated soils washing wastewater. The best results in contaminant removal from the soil using synthetic surfactants were obtained with TW80, whereas locust bean gum was the most efficient among the natural surfactants.

The best contaminants removal efficiencies in the wastewater generated by petroleum hydrocarbons-contaminated soils using natural (locust bean gum) and synthetic (TW80) surfactants was achieved when the biofilter was operated at 24°C. Removal of COD after treating the wastewater from the soil washing using TW80 was as high as 85.6% and 93.3% for locust bean gum. Treatment of the wastewater from the soil washing with locust bean gum induced a significant change in the BOD_5/COD ratio, changing from 28.6% to 85.16% after treatment.

The microbial population found in this study (*B. subtillis, C. jeikeium, Pseudomonas sp., A. sobria, A. caviae,* and *E. sakazakii.*) have been previously reported as hydrocarbon degrading, and some of them have been reported as surfactant producers.

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