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Treatment of Wastewaters Generated by Surfactant-Enhanced Washing of Soils in an Aerobic Biofilter Inoculated with a Consortium of Hydrocarbon Degraders

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Authors' contributions

This work was carried out in collaboration between all authors. Author MZS performed the analysis and wrote the first draft of the manuscript. Authors EIGP and LGT managed the analyses of the entire study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The aims of this work were 1) To evaluate the performance of a submerged biofiltration system for the treatment of a surfactant-enriched wastewater that had been generated by a soil washing process. 2) To evaluate the effect of the flux and organic load over the performance of the system. 3) To determine the microbial evolution as an effect of the flux at different lengths of the biofilter by using a denaturing gradient gel electrophoresis (DGGE) analysis.
Study Design: A three factorial design was used to evaluate the effect of different fluxes and organic loads over the performance of a continuously operated submerged aerobic biofilter. The DGGE technique was employed to determine microbial changes in the biofilter.
Place and Duration of Study: The study was carried out at the Bioprocess Laboratory, Bioprocesses Department UPIBI-IPN, Mexico. The experimental stage lasted approximately eight months and the DGGE analysis four months more.
Methodology: Contaminated soil was physicochemical and microbiologically characterized. A total of 70 kg of contaminated soil was washed using a 1:3 ratio

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soil/surfactant solution (0.5% Sulfopon 30-SP30). The surfactant-enriched wastewater was then treated in a submerged biofilter. The biofiltration system consisted of a column with a length of 50 cm and diameter of 12 cm. The biofilter was packed with *tezontle* with an average diameter of 0.2-0.4 cm and 70% void space. The biofilter working volume was 4.5 L. The samples of the packing material for the DGGE analysis were obtained from the ports located along the biofilter: at the wastewater inlet, at the middle of the column and at the outlet. After DNA extraction with a Power Soil DNA Isolation Kit (MO BIO), PCR (polymerase chain reaction) analysis was conducted. The 16S rRNA gene was amplified using universal bacterial primers. The data obtained by DGGE analysis for the microbial population developed in the biofilter were further analyzed by the Jaccard similarity coefficient.

Results: The soil contained 14,704 mg/kg TPH. BTEX compounds were not found, and only two different PAHs were found in the soil samples: benzo-fluoranthene and benzopyrene, at concentrations of 0.1280 and 0.0682 mg/kg of soil, respectively. During the surfactant-aided soil washing, the highest removal percentage of the oil removed from the soil was 59% with 0.5% SP30. The wastewater generated after the soil washing process contained, in average 1,329 mg COD/L and 211 mg/L of grease and oil. Higher COD removals were obtained at a flux of 0.4 L/h for both of the COD initial concentrations. While the highest removal was 78.27%, determined at an initial COD concentration of 300 mg/L. When applying fluxes of 0.28 and 0.40 L/h at a higher initial COD concentration, the COD removals were increased; this was not the case for a flux of 0.63 L/h. For a given initial COD concentration, the removal efficiencies were higher for lower fluxes. Analysis of the similarity between the microbial populations for varying fluxes and levels along the length of the biofilter was determined by the Jaccard (JI) index. The results showed that the initial microbial populations (t_0) have low similarities with the developed microbial populations at the different conditions tested.

Conclusion: Both the flux and the initial COD concentration had an impact on COD removal and the microbial concentration in the column. The COD removal percentages were similar at fluxes of 0.28 and 0.63 L/h. The highest removal percentage of 78.27% was obtained at a flux of 0.4L/h; this finding was in agreement with the highest microbial count and the specialization of microbial populations (less diversity). In general, it was shown that the flux had an effect on changes in microbial population. Greater effects were observed on the microbial population due to the position along the reactor, e.g., the greatest differences were found at the different levels of the biofilter.

Keywords: Biofilter; DGGE; heavy oil fraction; surfactants.

1. INTRODUCTION

Hydrocarbon contamination affects the fertility and productivity of soil because it modifies the soil's physical, chemical and biological characteristics. Additionally, contamination can cause soil compaction and acidity [1]. Many technologies are used to restore hydrocarbon-contaminated soils [2]. One of these technologies used in soil remediation is surfactant-aided soil washing. As hydrocarbon molecules are attached to the soil particles, the surfactants help by increasing the mobility and solubility of hydrocarbons in aqueous media [3]. Thus, surfactants are able to improve the mass-transfer of hydrocarbons from soil into the aqueous phase [4,5].

Surfactant-aided soil washing has yielded good results, however, the efficiency of the process shows strong dependence on the soil composition, surfactant structure and pollutant properties [6]. This technology has been classified as an economical, easy-to-apply and effective process to remediate crude oil contaminated soils [7]. Hence, there is an increased interest in its implementation [8 and 9]. By using this technology, Iturbe et al. [10] obtained a TPH (total petroleum hydrocarbons) removal of approximately 92% when treating contaminated soils, with initial concentrations up to 17,238 mg/kg; the surfactant used to achieve this removal percentage was Canarcel TW80 (0.5% v/v). In another study, contaminated soils with hydrocarbons were washed using SDS (sodium dodecyl sulfate) as a washing solution, obtaining removals of over 90% [3].

Although surfactant-aided soil washing shows higher removals of TPH, a large amount of wastewater is produced during the process because oil is transferred to the liquid phase. Subsequently, the resulting wastewater must be treated to remove contaminants, thus enabling the proper recycling or disposal of the water [11].

Several methods for treating these wastewaters have been reported in the literature, based on biological and physicochemical principles. The treatment of wastewater containing surfactants has been widely reported by biological methods as activated sludge or with submerged biofilters [12,13,14], by ultrasonic irradiation [15], by advanced oxidation [11,16], by activated carbon [17], by membrane biological reactors [18] and by permeable reactive barriers [19].

One technology that is attracting greater attention is biofiltration, as this process could achieve higher removal of pollutants, which are subsequently completely metabolized. Biofiltration is considered an environmentally friendly technology. Contaminants are removed by biofilters due to the degradation abilities of the microbial communities constituting the biofilm, which are presumed to experience a strong selection pressure in favor of those members that can metabolize the pollutants or their metabolic products [20,21].

Mijaylova et al. [22] studied the performance of a biofilter packed with volcanic rock and inoculated with activated sludge, these authors reported an organic matter removal greater than 80%. Recently, Zamudio et al. [13] found COD removals up to 72% when treating contaminated wastewater produced in a soil washing process using natural surfactants. In this work, it was found that the degradation of the pollutants was due to the activity of different types of microorganisms, such as *Pseudomonas aeruginosa, Enterobacter sakazakii* and *Bacillus subtilis,* that were present in the biofilm. Those strains are considered to be hydrocarbon-degrading microorganisms.

Biofilm composition and activity are two important parameters for the successful operation and control of fixed film processes in water and wastewater treatment. Widely used parameters for biofilm characterization are biofilm thickness, total dry weight and total cell count. These parameters are, however, not sufficient to describe biofilm composition and activity [23]. Despite its central role in biofiltration, the ecology of the microbial community in biofilters remains unknown, primarily because of the difficulty of making detailed observations. Recently developed molecular techniques allow the determination of community composition from DNA extracted from the biofilter without the need to culture the organisms [24]. This knowledge could be used to improve control of the reactor behavior and to design enhanced processes.

Many techniques are available to identify the microorganisms that are involved in a biological process (such in biofiltration systems), but the most commonly used today are those based on molecular biology. These techniques can detect and identify microorganisms based on certain molecular markers that can be used to identify microorganisms that degrade or transform pollutants [25].

Previous studies of the community structure in biofilters, most of them in wastewater treatment biofilters, have relied on the following methods to elucidate community structure: (a) culture methods [26]; (b) cloning and sequencing of conserved housekeeping genes, e.g., 16S ribosomal RNA [27 and 28] or functional genes [29]; (c) chemical fingerprints [30]; (d) nucleotide probes [27]; and (e) polymerase chain reaction (PCR)-based fingerprint techniques (e.g., denaturing gradient gel electrophoresis, DGGE) [31]. Each of these methods provides information on the structure of the microbial community being studied [24].

In this work, a contaminated soil was physicochemical and microbiologically (by microbial counts) characterized. A microbial consortium of hydrocarbon degraders isolated from decontaminated soil was enriched and pre-adapted to the pollutant and then inoculated into a submerged biofilter. The biofiltration system was evaluated for treatment of surfactant-enhanced wastewater resulting from soil washing. Finally, the DGGE technique was applied to characterize the diversity of microorganisms present in the biofilter.

2. MATERIALS AND METHODS

2.1 Soil Characterization

The contaminated soil was obtained from an old auto repair facility located at Mexico City (Mexico), the soil was dried at room temperature (25ºC) for 3 days, and then the characterization was carried out. The determination of TPH, some compounds such as PAHs (polycyclic aromatic hydrocarbons) and BTEX (benzene, toluene, ethyl benzene and xylene), was performed based on Mexican standard methodologies that establish the conditions for soil analysis (NOM-021-SEMARNAT-2000, EPA 9071B and EPA1664A, as suggested in NOM-138-SEMARNAT-2003).

The microbial communities of the soil were characterized to identify heterotrophs and hydrocarbon degraders. Plate counts for heterotrophic bacteria were made on Petri dishes containing nutritive agar (Bioxon). For the detection of hydrocarbon-degrading bacteria, a mineral medium containing (mg/L): 8.5 KH_2PO_4, 21.75K_2HPO_4, 33.4 $Na_2HPO_4.7H_2O$, 1.7 NH_4Cl, 22.5$MgSO_4.7H_2O$, 27.5 $CaCl_2$, and 0.25 $FeCl_3.6H_2O$ and supplemented with different carbon sources (diesel, petroleum and waste automotive oil) was used. These substrates were applied using moistened filter paper. The bacteria were incubated at 30ºC over a period of 48 hours to 96 hours for heterotrophic and degrading bacteria. The mineral medium is an adaptation of that suggested by the Mexican standard for the determination of BOD (NMX-AA-028, as suggested in NOM-001-SEMARNAT-1996).

2.2 Wastewater Generation

Seventy kilograms of contaminated soil were washed using a 1:3 ratio of soil to surfactant solution (0.5% SP30: Sulfopon 30, an anionic surfactant). SP30 was chosen after a series of assessments as previously described [32].

2.3 Biofiltration System

2.3.1 Inoculum

Enrichment of the microbial consortia was performed in a 20 L reactor. A total of 50 g of contaminated soil was added to 10 L of mineral medium and waste automotive oil (10 g per week) as a carbon source to allow pre-adaptation of the microbial culture to the pollutants present in the soil. The culture was incubated at room temperature with constant aeration. Microbial counts were determined by the plate technique. Once the culture reached a hydrocarbon degrader count of $1x10^{10}$ CFU/ml (colony formation units per ml of media) in approximately 3 weeks, it was used to inoculate the biofilter.

2.3.2 Experimental set up

The biofilter consisted of a column that was 50 cm long and 12 cm in diameter (two columns were employed for the experiments, they were simultaneously run. The average of the data obtained in both columns is reported). There were 3 sample ports along the reactor to take samples of the packed material, one at the wastewater inlet (1), one at the middle (3) and one at the outlet (5). The biofilter was packed with *tezontle* (a very porous red volcanic rock) with an average diameter of 0.2-0.4 cm and 70% void space. The biofilter working volume was 4.5 L. To monitor the performance of the system, liquid samples of the wastewater stream were regularly collected at the inlet and outlet.

Bacterial immobilization was facilitated by up flow recirculation of the previously adapted microbial culture at a rate of 0.2 L/ h for three weeks. The initial degrading microorganism count was approximately $1x10^{8}$ CFU/g support.

2.4 Wastewater Treatment in the Biofilter

Different dilutions of the wastewater produced during the soil wash process at an initial concentration of 1,329 mg COD/L were prepared by mixing the wastewater stream with tap water to obtain 2 different initial COD (chemical oxygen demand) concentrations (300 and 480 mg COD/L). During the wastewater treatment tests in the biofilter, 3 different fluxes (0.28, 0.4 and 0.63 L/h), 2 different initial COD concentrations (300 and 480 mg COD/L) and a surfactant concentration of 0.5% SP30 were evaluated. These parameters were established to evaluate the efficiency of column treatment on the wastewater. The wastewater was fed in the up flow direction; therefore, the bottom of the biofilter was the inlet and the top the outlet, as previously mentioned. The biofilter operation time was 150 h per experiment.

Samples were taken every 5 h on average to determine the reduction of COD during the process.

2.5 Microbial Characterization of the Biofilter

A 2.0 g sample of the biofiltration system (packet material with biofilm) was taken when the system was operating at steady state conditions at the different evaluated conditions. The samples were mixed with 20 mL sterile saline solution and triturated to reduce the particle size by grinding in a mortar under sterile conditions. All the material was then vortexed to obtain a biomass solution. Biomass solution was centrifuged at 6000 rpm for 15 min, the supernatant was decanted and the concentrated biomass was used for DNA extraction

following the recommendations of the MOBIO Powersoil DNA isolation kit. Extracted DNA was quantified by using a Spectrophotometer at 280 nm and stored at -20ºC.

After DNA extraction PCR (polymerase chain reaction) was conducted. The 16S rRNA gene was amplified using universal bacterial primers as reported by Yu and Morrison [33], amplifying the three hyper variable regions (V3-V5). The PCR reaction proceeded as follows: initialization temperature at 95ºC for 9 minutes, denaturation at 94ºC for 1 minute, hybridization at 55ºC for 1 minute, polymerization at 72ºC for 1.5 minutes, extension at 72ºC for 10 minutes and a final temperature of 4ºC; there were 30 cycles from the denaturation temperature to the polymerization temperature.

DGGE tests were performed to establish differences among the microbial communities found in the soil and the microbial communities developed in the packing material at 3 different locations along the biofilter (inlet, middle and outlet) and at three different fluxes. Polymerase chain reaction amplicons (2–20 µl equivalent to 100–500 ng DNA for metagenomic samples) were separated by DGGE on 8% polyacrylamide gels with a linear gradient of 30–70% denaturant (100% denaturant was 40% [v/v] formamide plus 42% [w/v] urea) using the DCodeTM System (Bio-Rad, Hercules, CA, USA. Electrophoresis was carried out at 90 V, for 16 h at 60ºC in 1× TAE buffer. Gels were stained with Gel Red^{TM} Nucleic Acid Gel Stain, 10,000X in water (Biotium, Inc. CA, USA) and visualized in a Transilluminator KODAK Gel Logic 440 Digital Imaging System (KODAK, USA).

2.5.1 Data analysis

The data obtained by DGGE analysis for the microbial population developed in the biofilter were further analyzed by the Jaccard similarity index. The similarity coefficients are frequently used to study the coexistence of species or the similarity between samples obtained at different conditions and constitutes a first approach to estimate the diversity of bacterial species. A matrix of similarity coefficients, either between species or locations, is used to analyze changes in microbial populations over time or at different locations [25]. Jaccard's index is one of the most useful and widely used indices to determine similarity between binary samples. Jaccard's index may be expressed as previously reported [25]:

$$J = \frac{n_{AB}}{n_A + n_B - n_{AB}} \quad (1)$$

where n_{AB} is the number of bands in samples A and B, n_A is the number of bands present in sample A, and n_B is the number of bands present in sample B.

3. RESULTS AND DISCUSSION

3.1 Soil Characterization

During characterization, it was determined that the soil contained 14,704 mg/kg TPH. BTEX compounds were not found, and only two different PAHs were found in the soil samples: benzo-fluoranthene and benzopyrene, in concentrations of 0.1280 and 0.0682 mg/kg of soil, respectively.

When using different hydrocarbon carbon sources, the resulting microbial counts were $1x10^8$ CFU/g, $1.5x10^8$ CFU/g and $1x10^8$ CFU/g for diesel, petroleum and waste automotive oil, respectively. These microbial counts were higher than that determined with only the nutritive

agar ($4x10^7$ CFU/g). The highest microbial count was found when petroleum was the only energy and carbon source. These data suggest that the bacterial community is mainly composed of hydrocarbon-degrading bacteria. This finding could be partially explained by the exposure of the microbial populations to the waste automotive oil on site for a long period of time (20-30 years). As reported by Zacarias-Salinas et al. [32], the most common activities performed at this automotive repair facility include replacement of automotive fluids (e.g., motor oil, radiator coolant, transmission fluid, and brake fluid), replacement of non-repairable equipment, and repair of fixable equipment. The waste oil, duty lube oils and greases were disposed in an underground storage tank for a long period of time. Leaks and spills in the storage tank were the major cause of soil contamination, and it was found when the company suspended activities. The place was dismantled to become a residential zone.

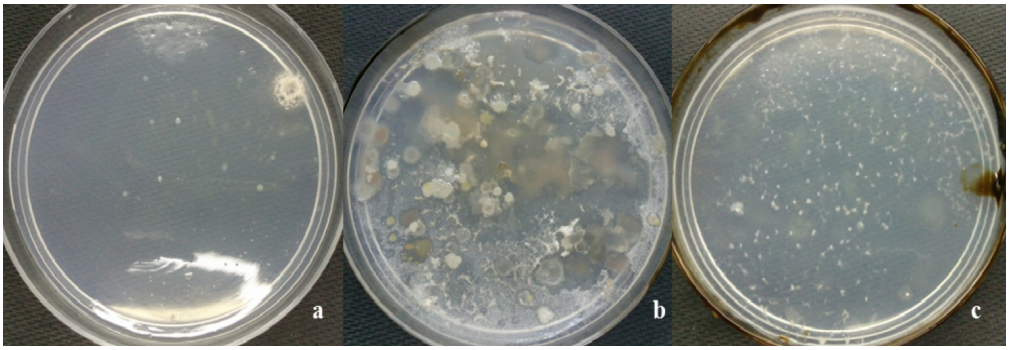

Fig. 1. Bacterial colonies of hydrocarbon degraders in different carbon sources: a) diesel, b) petroleum and c) waste automotive oil

The values obtained in the microbial counts were the same order of magnitude as those found for hydrocarbon degraders in the study reported by Iturbe et al. [10]. These authors obtained a microbial count of a PAH-contaminated soil with values of $1.8x10^8$ CFU/g for heterotrophic bacteria and $5.4x10^8$ CFU/g, $1x10^8$ CFU/g and $5.6x10^8$ CFU/g for diesel, petroleum and oil, respectively. In other work, Bogart and Hemmingsen [34] reported CFU counts of a wide variety of studied soils. Values of 2.8 to $2.9x10^7$ CFU/g of soil were found for urban and garden soils; these values were definitively lower than that found in soil contaminated with grease and oil ($4.5x10^7$ CFU/g). In
Fig. **1**, the macroscopic characteristics of the different hydrocarbon-degrading bacteria using different substrates are depicted. The characteristics of the colonies grown in diesel and waste automotive oil were similar. Colonies were observed with circular and irregular form; convex, pulvinate and flat elevation; entire and curled margins; and colors of white, translucent and beige.

3.2 Wastewater Generation

During the surfactant-aided soil washing, the highest removal percentage of the oil removed from the soil was 59% with SP30 at a concentration of 0.5%. The wastewater generated after the soil washing process contained 1,329 mg COD/L, 385 mg/L of BOD_5, conductivity of 1,107 μS, hardness as $CaCO_3$ of 489 mg/L, MBAS of 122 mg/Land 211 mg/L of grease and oil. Additionally, Pb, Fe, Cr and Al were detected in low concentrations, i.e., 0.40, 19.05, 0.07 and 24.21 mg/L, respectively.

3.3 Wastewater Treatment in the Biofilter (Biofilter Performance Evaluation)

The biofilter was continuously operated for 60 days. Throughout the operation time, three different fluxes, two COD initial concentrations and a fixed value of 0.5% of SP30 (washing solution) were used. Higher COD removals were obtained at a flux of 0.4L/h with both of the COD initial concentrations, while the highest removal was 78.27 %, determined at an initial COD concentration of 300 mgCOD/L. When applying fluxes of 0.28 and 0.40L/h at a higher initial COD concentration, the COD removals were increased; this was not the case for a flux of 0.63 L/h. For a given COD initial concentration, the removal efficiencies were higher for lower fluxes. The lowest removal percentages were obtained at a flux of 0.63L/h (Table 1).

Table 1. COD removal at the biofilter with three different fluxes and two initial COD concentrations (iCOD), and CFU count at three biofilter levels (obtained using the heterotrophs medium described in materials and methods section), 1: inlet, 3: middle and 5: outlet. Inlet organic loads as mgCOD/m^2.d are also depicted

Flux L/h	Inlet organic load mgCOD/m^2.d	Initial mgCOD/L	COD removal (%)	CFU/g support		
				1	3	5
0.28	0.64	300	55.28	$3.5x10^5$	$1.7x10^5$	$4.0x10^5$
0.28	1.02	480	47.00	$4.3x10^5$	$9.4x10^6$	$1.6x10^6$
0.40	0.91	300	78.27	$2.3x10^5$	$1.6x10^6$	$2.5x10^4$
0.40	1.46	480	59.65	$4.3x10^5$	$3.1x10^7$	$5.6x10^4$
0.63	1.44	300	40.52	$1.3x10^4$	$2.4x10^4$	$1.0x10^6$
0.63	2.30	480	43.26	$1.1x10^4$	$1.4x10^4$	$7.9x10^6$

As shown in Table 1, the inlet organic loads used to operate the biofilter ranged between 447.84 and 1612.80 mg COD/m^3.d. These values are lower than those reported for a submerged biofilter which treats municipal wastewaters [22]. In this study, the evaluation of different organic loads ranging from 0.8 to 6 g COD/m^2.d was reported. The authors found that the COD removals obtained during the experiments were correlated with the initial organic load and the type of packaging material used (low density polyethylene, polypropylene, polyurethane and *tezontle*).The units (mgCOD/m^2.d) reported in this work consider a specific area of the packaging material (700 m^2/m^3). On the other hand, the COD contained in the wastewater for the present work are less biodegradable than that used in the above mentioned paper (municipal wastewaters).

3.4 Microbial Characterization of the Biofilter

At the lowest flux (0.28 L/h), a homogeneous microbial distribution was observed along the length of the biofilter, with concentrations of approximately 10^5 CFU/g support (Table 1). In general, higher microbial counts (9.4 x 10^6, 1.6 x 10^6 and $3.1x10^7$) were obtained at the middle section of the reactor for two of the evaluated fluxes 0.28 and 0.4 L/h. Moreover, the higher biomass concentrations were determined at a flux of 0.4 L/h with both of the COD initial concentrations. While the highest value ($3.1X10^7$ CFU/g support) was found at the middle of the biofiltration system at a flux of 0.4 L/h and an initial COD of 480mgCOD/L. Higher microbial counts at a flux of 0.4 L/h were associated with the higher removal percentages obtained (78.27 and 59.65 % for initial COD concentrations of 300 and 480 mgCOD/L, respectively) as seen in Table 1.

The higher microbial counts determined at the middle section of the reactor at a flux of 0.4 L/h could be partially explained because the pollutant concentration was higher at the bottom of the reactor, corresponding with the inlet of the wastewater. In this section of the biofilter, the substrate was initially metabolized, and then metabolic intermediates could be released to be used for the microorganisms in the middle section of the biofilter. Moreover, at this level, the highest biomass concentration was determined ($3.1X10^7$ CFU/g support).

At the highest flux, the microbial counts were generally lower than those obtained at the 0.28 and 0.4L/h fluxes, especially at the inlet and middle parts of the reactor (levels 1 and 3). These data suggest that the biofilm may have been physically reduced by the increase in flux (shear rate).

Fig. 2. presents some of the observed DGGE profiles. The soil DNA sample was located in lane 1; in this sample, 16 main bands (OTUs) were detected. At the initial time (t_0), when the biofilm was developed but before continuous operation started, fewer bands were present than for the soil sample. A total of 5 bands disappeared, including band A.

Analysis of the similarity of the microbial population among fluxes and among the levels along the length of the biofilter was determined by the Jaccard (JI) index, as shown in Analysis of similarities showed that the microbial population at a flux of 0.4 L/h is different among the 3 levels of the biofilter, with low JI values. Some similarities were found between the band patterns for the microbial population at fluxes of 0.4 L/h and 0.28 L/h, particularly in samples 0.28-1 and 0.4-1, with JI of 0.7. The band profile at the inlet was similar to the one obtained at the inlet with a flux of 0.28 L/h, except for an increase in the intensity.

In lane 0.4-3, fewer bands were detected, which could be caused by an increase in a predominant microbial population. The microbial counts at this location were the highest, indicating an increase in specialized microorganisms. Additionally, this was correlated with higher biomass concentration, as discussed in the above section.

Similarly as found for the flux of 0.4 L/h, for a flux of 0.6 L/h there were low similarities between the microbial populations at the different levels of the biofilter. However, some similarities between the microbial populations were also found between samples 0.4-3 and 0.63-1, and samples 0.4-5 and 0.63-1, showing JI values of 0.87 and 0.7, respectively. In general, at the highest flux (0.63 L/h), there were fewer bands, indicating the possible elimination of some of the microbial population, most likely due to shear phenomena. This explanation was also supported by the microbial count.

Table 2. The results showed that the initial microbial populations (t_0) have low similarities with the developed microbial populations at the different conditions tested (low values of JI).These results were expected due to the adaptation of the microbial consortium to the operational conditions of the system. Fewer but more specialized microbial species could be expected after an adaptation period. However, bands B and C are present in almost all of the samples. A slight similarity was found between the initial consortium developed in the biofilter support (t_0) and the microbial population in sample 0.4-5 (JI=0.7).

When comparing the microbial populations that developed at the same flux but at different levels of the biofilter, data showed that the highest similarity was found between samples 0.28-3 and 0.28-5 (corresponding with the middle and outlet sections of the biofilter, at a flux

of 0.28 L/h), with corresponding JI of 0.9. As seen in Fig. 2, at a flux of 0.28 L/h, the bands pattern is similar in different levels of the biofilter; the bands E, F and G remained constant. These results are in agreement with the microbial counts, which showed a homogeneous distribution along the reactor at a flux of 0.28 L/h. However, the middle and top sections of the biofiltration system (labeled 0.28-3 and 0.28-5, respectively) showed higher similarity, this was supported by the Jaccard´s index. In these two levels band I appears, indicating slightly higher diversity at the middle and top sections of the biofilter.

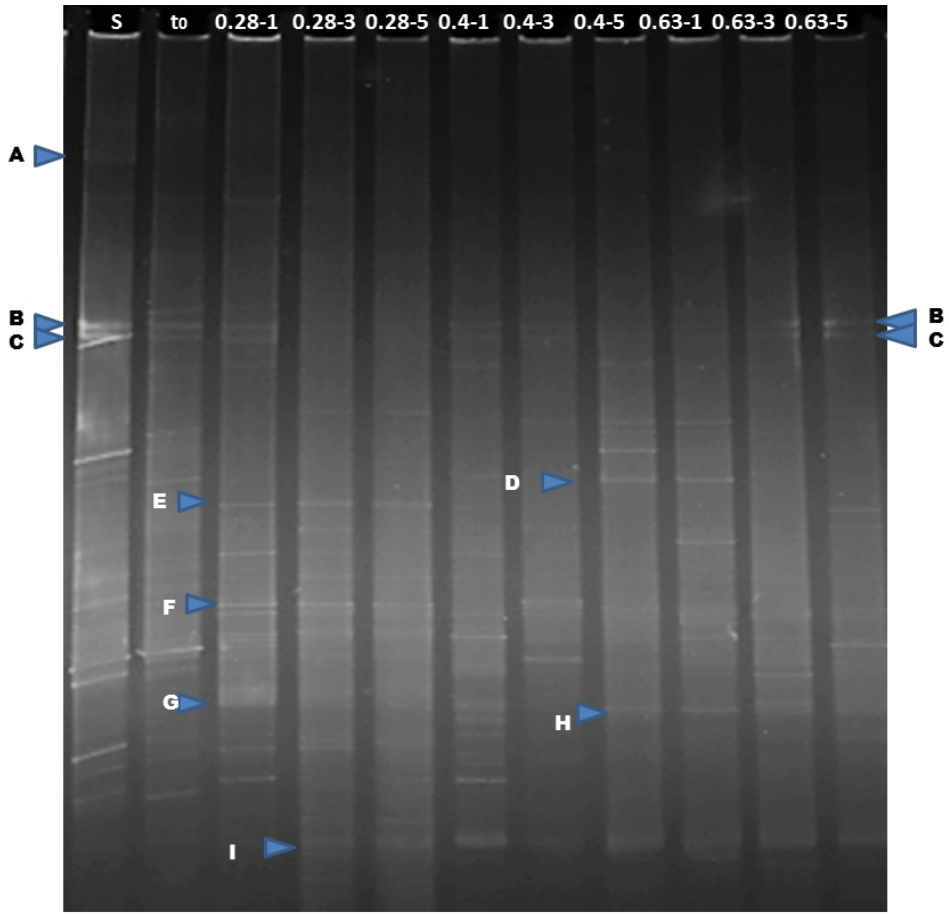

Fig. 2. DGGE patterns of amplified 16S rDNA fragments from the packing material samples in the biofilter operation. The first two lanes are labeled as S (soil) and t_0 (initial time of operation);in subsequent lanes, 0.28, 0.40 and 0.63 correspond to the different fluxes, and the suffixes 1, 3 and 5 correspond to different levels in the biofilter. All correspond to an initial COD concentration of 480 mg/L and 0.5% SP30. Letters are described in the text

Analysis of similarities showed that the microbial population at a flux of 0.4 L/h is different among the 3 levels of the biofilter, with low JI values. Some similarities were found between the band patterns for the microbial population at fluxes of 0.4 L/h and 0.28 L/h, particularly in samples 0.28-1 and 0.4-1, with JI of 0.7. The band profile at the inlet was similar to the one obtained at the inlet with a flux of 0.28 L/h, except for an increase in the intensity.

In lane 0.4-3, fewer bands were detected, which could be caused by an increase in a predominant microbial population. The microbial counts at this location were the highest, indicating an increase in specialized microorganisms. Additionally, this was correlated with higher biomass concentration, as discussed in the above section.

Similarly as found for the flux of 0.4 L/h, for a flux of 0.6 L/h there were low similarities between the microbial populations at the different levels of the biofilter. However, some similarities between the microbial populations were also found between samples 0.4-3 and 0.63-1, and samples 0.4-5 and 0.63-1, showing JI values of 0.87 and 0.7, respectively. In general, at the highest flux (0.63 L/h), there were fewer bands, indicating the possible elimination of some of the microbial population, most likely due to shear phenomena. This explanation was also supported by the microbial count.

Table 2. Jaccard index between samples at the three different fluxes and at three biofilter levels, 1: inlet, 3: middle and 5: outlet

	0.28-1	**0.28-3**	**0.28-5**	**0.40-1**	**0.40-3**	**0.40-5**	**0.60-1**	**0.60-3**	**0.60-5**
t_0	0.47	0.27	0.22	0.4	0.4	0.61	0.33	0.36	0.37
0.28-1	-	0.46	0.38	0.7	0.41	0.58	0.45	0.36	0.41
0.28-3	-	-	0.9	0.38	0.28	0.33	0.21	0.23	0.38
0.28-5	-	-	-	0.31	0.3	0.27	0.23	0.25	0.41
0.40-1	-	-	-	-	0.45	0.63	0.5	0.4	0.45
0.40-3	-	-	-	-	-	0.63	0.87	0.55	0.33
0.40-5	-	-	-	-	-	-	0.7	0.33	0.28
0.60-1	-	-	-	-	-	-	-	0.44	0.36
0.60-3	-	-	-	-	-	-	-	-	0.27

4. CONCLUSION

Soil characterization showed that even with a high concentration of THPs, the microbial activity of hydrocarbon degraders is still high. However, the diversity of microorganisms decreased due to the presence of many compounds found in waste automotive oil. It was possible to clean the contaminated soil using the appropriate surfactant and dose.

The resulting wastewater contained 1,329 mg/L COD and 211 mg/L grease and oils. The wastewater was treated efficiently on the column, which was previously inoculated with the bacteria present in the contaminated soil. Both the flux and the initial COD concentration had an impact on the COD removal and the microbial concentration in the column. The COD removal percentages were similar at fluxes of 0.28 and 0.63L/h (47 and 43%, respectively). The higher removal percentages of 59.65 % and 78.27 % were obtained at a flux of 0.4L/h for both of the initial COD tested. This was in agreement with the highest microbial count and a specialized microbial population (less diversity, but specialized microorganism).

DGGE technology proved highly suitable for the characterization of the microorganisms present inside the packed column. The characterization of the changes in the microbial community inside the biofilter leads to better understanding of the system. In general, it was shown that the flux had an effect on changes in microbial population. Greater effects were observed on the microbial population due to the position along the reactor.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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