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Biodegradation of effluent contaminated with diesel fuel and gasoline

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Abstract

We studied the effects of fuel concentration (diesel and gasoline), nitrogen concentration and culture type on the biodegradation of synthetic effluent similar to what was found at inland fuel distribution terminals. An experimental design with two levels and three variables (2^3) was used. The mixed cultures used in this study were obtained from lake with a history of petroleum contamination and were named culture C_1 (collected from surface sediment) and C_2 (collected from a depth of approximately 30 cm). Of the parameters studied, the ones that had the greatest influence on the removal of total petroleum hydrocarbons (TPH) were a nitrogen concentration of 550 mg/L and a fuel concentration of 4% (v/v) in the presence of culture C_1. The biodegradability study showed a TPH removal of $90 \pm 2\%$ over a process period of 49 days. Analysis using gas chromatography identified 16 hydrocarbons. The aromatic compounds did not degrade as readily as the other hydrocarbons that were identified.

Keywords: Biodegradation; Mixed cultures; Diesel oil; Gasoline; Hydrocarbons

1. Introduction

Leaks and accidental spills are a regular occurrence during the exploration, production, refining, transport and storage of petroleum and petroleum products. In Brazil, the distribution infrastructure for petroleum, petroleum derivatives and ethyl alcohol includes 22 inland terminals (one of which is in the city of Uberlândia), 30 waterway terminals, 6 collector centers for alcohol, 13 refineries and several pipelines [2]. At the inland fuel distribution terminals, countless tank trucks are filled daily with gasoline and diesel fuel for nation-wide distribution. During the filling process some fuel may spill on the fill pad, also called a patio. In some cases, the water from washing the patios and the trucks flows into ditches where it is then allowed to run into small lakes or ponds located at the rear of the terminals.

The aromatic compounds (including the lighter weight compounds known as BTEX, benzene, toluene, ethylbenzene and xylene) make up from 10 to 59% of the gasoline (mass/mass), while the aliphatic hydrocarbons make up from 41 to 62%. Aromatics are usually more toxic than aliphatics with the same number of carbon atoms and they are typically found at higher concentrations in water because their solubility is three to five times greater [7]. Diesel oil or fuel is a complex mixture consisting basically of paraffinic, olefinic and aromatic hydrocarbons and, in smaller amounts, molecules containing sulfur, nitrogen, metals, oxygen, etc. Diesel oils are composed of molecules with 8–40 carbon atoms and are usually heavier more viscous and less volatile than gasoline.

Reducing hydrocarbon concentrations either in regularly discharged effluent, or in media contaminated by accidental fluid releases, is a significant challenge. Mechanical (e.g. skimming) and chemical (e.g. surfactants and dispersants) methods have limited effectiveness and can be expensive. Several researchers have studied the use of microorganism to decompose petroleum products and have shown this to be a promising technological alternative [3,10,15,19,21,27,30; etc.]. Microbiological activity is affected by a number of environmental factors including energy sources, donors and acceptors of electrons, nutrients, pH, temperature and inhibition by the substratum or metabolites. These parameters influence how quickly microorganisms adapt to the available substratum [25].

The goal of this study was to adapt two mixed cultures extracted from lake sediment that was contaminated with diesel oil and gasoline and to select the best culture and

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the best operational conditions among the ones studied. An experimental design with two levels and three variables was used. The subsequent objective was to evaluate the ability of the selected culture to biodegrade synthetic effluent made up of diesel oil and gasoline as a function of the operation time, using a 4 L capacity batch stirred tank reactor.

2. Materials and methods

2.1. Source of the microorganisms

Two mixed cultures were used (C_1 and C_2) were obtained from sedimented from a lake that received wash water containing diesel oil and gasoline from an inland fuel distribution terminal. Sediment was sampled according to the Associação Brasileira de Normas Técnicas (ABNT) standardized producers [23]. The lake was divided into eight sections of approximately the same surface area with samples being collected from each one of these sections. Samples were collected both from the sediment surface (C_1) which is in immediate contact with laker water and from a depth of 30 cm (C_2) (Fig. 1). Each sample from either the sediment surface or 30 cm was composited with the other sediment samples from the same depth from each of the eight sections. After collection and mixing the samples were submitted for microbiological quantification analysis. Sulfate reducing bacteria (SRB) were found in both C_1 and C_2 cultures. The presence of such microorganisms indicates that conditions are anaerobic, since these bacteria are only present where oxygen is absent or at very low concentrations, between 0.1 and 1.0 mg/L of oxygen.

2.2. Culture extraction adaptation and maintenance

The addition of the mixed cultures to the liquid medium was carried out in 500 mL Erlenmeyers flasks containing 10 g of sediment and 100 mL of mineral medium (MM) with a composition (g/L) of: $0.5KH_2PO_4$; $1.4Na_2HPO_4$; $0.6NH_4NO_3$; $0.1MgSO_4{\cdot}7H_2O$; $0.02CaCl_2{\cdot}2H_2O$; $0.03MnSO_4{\cdot}H_2O$. The pH of the MM was 7.0. Gasoline and diesel oil were obtained from inland terminal and added to this medium in the proportion of 1:1 (v/v), as the only carbon source. The system was sealed with a rubber stopper to avoid the loss of volatile hydrocarbons and incubated for 48 h at room temperature ($30 \pm 2\,^{\circ}C$), with continuous agitation in a shaker at 150 rpm.

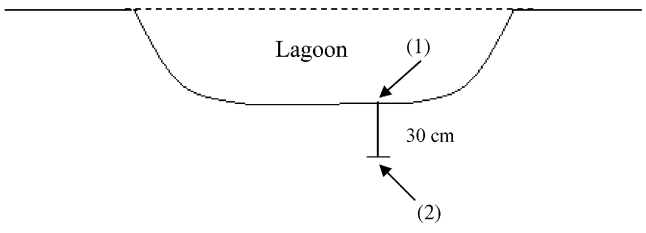

Fig. 1. Scheme of the contaminated lagoon. Surface of the soil at the bottom of a lake contaminated by petroleum derivatives where was collected the sample C_1. Soil collected from a depth of 30 cm in the same where was collected the sample C_2.

Once the cultures were established in liquid medium, they were adapted to the presence of fuel by increasing the gasoline and diesel concentrations from 0.5 to 6.0% (v/v) in successive cultivations. The inoculum volume used in each successive step was 10 mL and the MM volume was 100 mL. At the end of this adaptation period, the bacteria were capable of tolerating and consuming fuel at 6% of the medium volume, which corresponds to the largest degree of contamination identified in the lake. Manual aeration of the reactors was performed for three 3 min at every 48 h of operation.

The mixed cultures were maintained at $5 \pm 1\,^{\circ}C$ in the mineral medium with a 6% fuel concentration, after being grown at $30 \pm 1\,^{\circ}C$ for 24 h.

2.3. Experimental design

This phase of the study examined the influence of fuel and nitrogen concentration and of culture type on the biodegradation of the synthetic effluent. Results were used in the subsequent larger-scale experiment.

An experimental design with two levels and three variables (2^3) was used. The following parameters were fixed during the experiment: room temperature ($30 \pm 2\,^{\circ}C$), agitation (150 rpm), volume (100 mL), operation time (28 days) and composition of the mineral medium (same as described above except for the concentration of ammonium nitrate). *Statistic 5.0* software was used to evaluate the data. For the purpose of statistical and correlation analyses the independent variables were X_1 is the fuel concentration, X_2 the nitrogen concentration and X_3 the culture type (Table 1). The response or dependent variables were total petroleum hydrocarbons (TPH) removal and quantification of aerobic facultative bacteria at 0, 7, 14, 21 and 28 days.

As shown in Table 1, we carried out 8 experiments in 3 sets for a total 24 trials. Each experiment tested the combinations of the parameters established for the inferior (−1) and superior (+1) levels, according to the experimental design adopted.

The experiments were conducted under the same operational conditions used in the adaptation stage. In this stage, the reactors were manually opened and agitated for 3 min each every 48 h under a laminar flow hood.

Table 1
Extended matrix of the experimental design 2^3

Experiment	Fuels, X_1 (%, v/v)	Nitrogen, X_2 (g/L)	Mixed cultures, X_3
1	Abiotic losses		
2	4 (−1)	0.21 (−1)	C_1 (−1)
3	6 (+1)	0.21(−1)	C_1 (−1)
4	4 (−1)	0.55 (+1)	C_1 (−1)
5	6 (+1)	0.55 (+1)	C_1 (−1)
6	4 (−1)	0.21 (−1)	C_2 (+1)
7	6 (+1)	0.21(−1)	C_2 (+1)
8	4 (−1)	0.55 (+1)	C_2 (+1)
9	6 (+1)	0.55 (+1)	C_2 (+1)

Observation—X_1: fuels concentration (g/L); X_2: nitrogen concentration (g/L); X_3: mixed cultures; (−1): value of the low-level variables X_1, X_2 and X_3; (+1): value of the high-level variables X_1, X_2 and X_3.

After aeration, the stopper was quickly replaced. Because of the potentially high biochemical oxygen demand (BOD) of the mixture and the brief aeration period it is possible that the system may have become anaerobic for part of the incubation cycle. The nitrogen concentration of the MM, in the form of ammonium nitrate was adjusted based on the 6% (v/v) fuel ratio to obtain an approximate C:N ratio of 100:5 [4]. Richard and Vogel [18], studying the capacity of diesel fuel biodegradation by consortium of cultures in liquid medium, used a nitrogen concentration of 350 mg/L. We adopted higher (550 mg/L) and lower (210 mg/L) concentrations with the objective of trying to optimize the concentration of nitrogen used in the medium. These nitrogen levels bracket the concentration used by Richard e Vogel [18] as well as by Rocha et al. [8], who used a concentration of 340 mg/L in liquid medium for diesel fuel biodegradation.

Control treatments for these studies measured losses due to abiotic processes. Control reactors in sets of three for each experiment were operated under the same conditions (fuel concentration, nitrogen concentration and culture type) of biodegradation reactors, but sodium azide was added to them in the beginning of the process to inhibit biodegradation of the medium by the microorganisms.

2.4. Measurement of total petroleum hydrocarbons (TPH)

To measure TPH hydrochloric acid was added to 10 ml of effluent to achieve a pH of 2 or less. TPH was then extracted from the acidic mixture using the solvent S-316 (dimer/trimer chlorotifluor ethylene). Quantification of the extracted material was completed using an infrared spectrophotometer (Horiba, USA model OCMA-350). This spectrophotometric is adapted to measure aliphatic and aromatic hydrocarbons, independently from the number of carbon atoms in the compound. During the analysis, absorbances associated with CH, CH_2 and CH_3 are measured at a wavelength close to 3.4 μm specifically CH (3.8 μm), CH_2 (3.42 μm) and CH_3 (3.50 μm). Associated software then converts absorbances into total hydrocarbon content [29].

2.5. Quantification of the bacteria

The aerobic facultative bacteria were quantified on Bacto-agar medium (Difco Laboratories, 0001). The pour-plate method was used and cultures were incubated at $30 \pm 1\,^{\circ}C$ for 48 h. Results were expressed as colony forming units (CFU/mL).

2.6. Isolation and identification of the bacteria present in mixed culture C_1

Isolation was carried out according to the method described by Cunha and Leite [5] and modified for the conditions of this study. With the culture adapted to medium containing 6% (v/v) fuel the pour-plate method was used. The inoculum was added to filter paper in dishes containing mineral medium M_2 (consisting [g/L] of: 5.0NaCl, 1.0K_2HPO_4, 1.0$NH_4H_2PO_4$, 1.0$(NH_4)_2SO_4$, 0.2$MgSO_4 \cdot 7.0H_2O$ and 3.0KNO_3), plus the fuel mixture as the only carbon source. Dishes were incubated for 7 days at $30 \pm 2\,^{\circ}C$. Microorganisms capable of growing in the presence of the fuel mixture were isolated and identified according to the Bergey Manual [11].

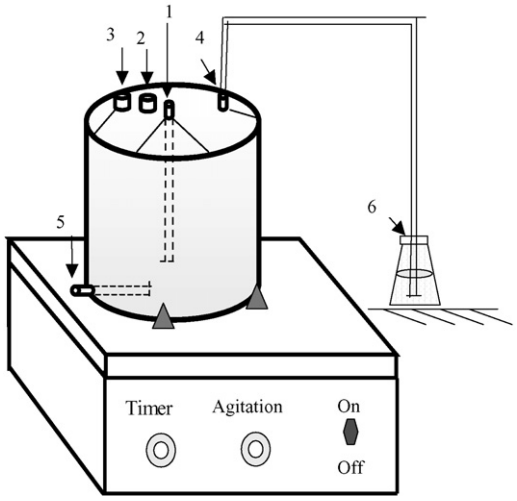

Fig. 2. Scheme operation unit. (1) Collector of samples in inox perforated tube; (2) entrance for measurer of temperature; (3) entrance for measurer pH; (4) exit of gas; (5) entrance of air in inox perforated tube; (6) Erlenmyer collector of escape gases with solvent S-316.

2.7. Study of the kinetics of the diesel oil and gasoline biodegradability—larger scale

In this stage, the kinetics of the biodegradability of the contaminants were studied on a larger scale, using the best parameters obtained in the earlier experiment. For this experiment, 4 L reactors were used containing a net volume of 1824 mL, consisting of 1600 mL mineral medium, 160 mL to the inoculum of the C_1 mixed culture and 64 mL of the hydrocarbon mix: 32 mL of diesel oil and 32 mL of gasoline (2%, v/v). The reactors, made of acrylic plastic and sealed with rubber stoppers, had four connections adapted to the top part and a connection in the base, as shown in Fig. 2. Air was supplied to the reactor by a pipeline with distribution valves and the flow was measured using a rotameter connected to the airline. Intermittent aeration was completed only every 48 h with a flow of 3 L/min for 3 min. The system was kept at room temperature ($30 \pm 2\,^{\circ}C$) and agitated continuously at 150 rpm.

TPH analyses were performed every 2 days of operation and microbiological analysis every 14 days. The pH was monitored daily. Identification of individual hydrocarbon compounds was conducted on the initial sample (time = 0 day) and final sample (time = 49 days) using a gas chromatograph.

2.8. Chromatographical analyses

Gas chromatography (GC) analyses were done at the end of the experiments to evaluate degradation potential and to identify some of the hydrocarbons of the fuel mixture (diesel oil and gasoline). Before each analysis, samples of the mixture were extracted with *n*-hexane P.A., by adding 10 mL of solvent and 50 mL of the sample into a 100 mL separation funnel. After

Table 2
Columns and condition of the chromatography

Conditions	Columns	
	Varian CP-porabond Q	J and W Scientific DB-1
Dimension ($L \times D$)	50 m × 0.32 mm	60 m × 0.25 mm
Carrier gas	He	He
Detector temperature	TCD[a] and FID[b] em série a 250 °C	FID: 340 °C
Injector temperature	250 °C	275 °C

L: length of the column, D: diameter of the column.
[a] TCD: thermal conductivity detector.
[b] FID: fire ionization detector.

phase separation, a sample of the organic phase was removed with a volumetric pipette and put in a sealed flask for subsequent analysis.

GC analyses were conducted using a SHIMADZU model GC17A GC equipped with a Varian CP-porabond (50 m × 0.32 mm) capillary column and two detectors in series: a thermal conductivity detector (TCD) followed by a flame ionization detector (FID). Capillary columns and the GC conditions used in the calibrations for the analyses of light compounds (column Varian CP-poranbond Q) and heavier compounds with more than five carbon (column J and W Scientific DB-1) are shown in Table 2.

Chromatogram peaks were identified and quantified using the retention time and response factors of these compounds, correlating chromatographic areas to molar concentrations, obtained after injections of standards contained in the Supelco Piano kit. The Piano kit is composed of standards for paraffinic, isoparaffinic, aromatic, naphthalenic and olefinic compounds.

3. Results

3.1. Adaptation of the cultures

Fuel concentrations in the culture medium were progressively increased as long as TPH removal remained greater than 70%, on average. The time needed to reach this level of removal was 15 days for each increment in the fuel concentration. Microorganisms studied in the two cultures (C_1 and C_2) degraded the contaminants, with TPH removal efficiencies greater than 70% in the several fuel concentrations (Table 3). This degree of removal efficiency suggest that the mixed cultures from the lake had already been exposed to contaminants and their enzymatic systems were pre-adapted to metabolizing various hydrocarbon molecules. Okerentugba and Ezeronye [24], state that microbiological communities exposed to hydrocarbons will adapt to the exposure through selective enrichment and genetic changes, resulting in an increase in the ratio of hydrocarbon-degrading versus non-degrading bacteria. Lakha et al. [27] indicates that several environmental factors can trigger or suppress gene expression associated with the enzymatic activities that degrade hydrocarbons. The pre-exposure of microorganisms makes them better suited to degrade hydrocarbons through higher growth and reproduction and more efficient metabolism thus maximizing the rate of TPH removal from the culture media. To degrade hydrocarbons, therefore, it is advantageous to use native microorganisms cultured from areas with historical contamination. This approach is likely to reduce or eliminate of the initial lag phase, which can be long and optimize overall process time.

Increasing the concentration of pollutant did not interfere significantly with the TPH removal efficiency, for either of the two mixed cultures. This sustained removal efficiency is likely related to the gradual increase of more effective degrading strains as fuel concentration rose and sensitive lineages died.

The advantages of using mixed cultures instead of pure cultures in biodegradation processes have been amply demonstrated [9,21]. These advantages could be attributed to synergistic interactions among the members of the association, which can be complex and favor petroleum degrading mechanisms. This is possible when one species removes toxic metabolites of another species that began the biodegradation process, or when two species work in succession with the first partially degrading compounds and the second finishing the job. Richard and Vogel [18], in their study of the kinetics of diesel oil biodegradation, reported the following maximum values for TPH (%) removal: 64.1 for an isolated *Acromobacteria anthropi* culture and 90 for a consortium (at the end of 50 days of process, using a mineral medium and an initial diesel oil concentration of 1%, v/v). The lowest level of TPH removal was identified when isolated microorganisms such as *Pseudomonas fluorescense* (named P2 and P25) were used, resulting in TPH removal of less than 20% after 50 days.

3.2. Experimental planning

Results of the quantification of aerobic facultative bacteria and TPH removal for the reactors with cultures C_1 and C_2 are presented in Table 4. The parameters with a significance level greater than 10% in the Student t-test were discarded.

Table 3
TPH removal efficiency by C_1 and C_2 cultures in different fuels concentration at 15 days of process

Cultures	TPH removal (%) with 0.5%, v/v of contaminants	TPH removal (%) with 1%, v/v of contaminants	TPH removal (%) with 2%, v/v of contaminants	TPH removal (%) with 4%, v/v of contaminants	TPH removal (%) with 6%, v/v of contaminants
C_1	82 ± 3	83 ± 4	84 ± 2	81 ± 3	83 ± 2
C_2	72 ± 2	75 ± 3	74 ± 3	76 ± 4	78 ± 2
Initial TPH (mg/L)	2500 ± 200	4000 ± 220	6800 ± 300	8000 ± 410	11000 ± 580

Table 4
Results of the quantification of aerobic bacteria in 28 days of the experiment

X_1	X_2	X_3	CFU/mL[a]	Removal of TPH (%)[b]
−1	−1	−1	$(1.8 \pm 0.6) \times 10^9$	80.0 ± 2.0
+1	−1	−1	$(5.0 \pm 0.1) \times 10^6$	69.0 ± 3.0
−1	+1	−1	$(1.1 \pm 0.1) \times 10^{10}$	90.0 ± 3.0
+1	+1	−1	$(5.0 \pm 0.1) \times 10^5$	70.0 ± 4.0
−1	−1	+1	$(9.5 \pm 0.9) \times 10^9$	85.0 ± 3.0
+1	−1	+1	$(7.8 \pm 0.8) \times 10^9$	83.0 ± 2.0
−1	+1	+1	$(5.9 \pm 0.7) \times 10^{10}$	97.0 ± 2.0
+1	+1	+1	$(5.8 \pm 0.8) \times 10^{10}$	95.0 ± 2.0

[a] $R^2 = 0.979$.
[b] $R^2 = 0.946$.

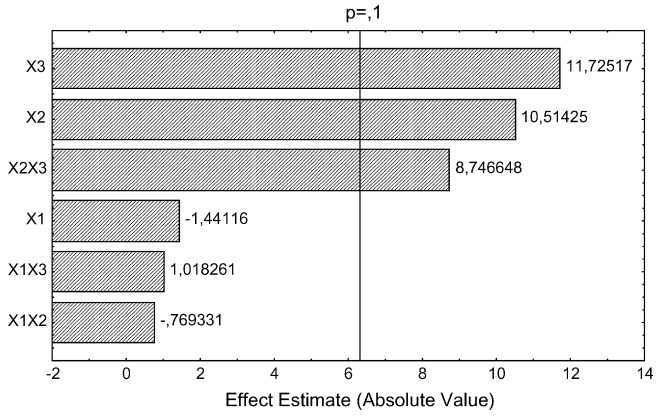

Fig. 3. Pareto diagram of the variables studied in the growth of aerobic bacteria in 28 days of the experiment.

The Pareto charts illustrating the responses of microorganisms and TPH removal over 28 days are shown in Figs. 3 and 4. Using experimentally derived we obtained Eqs. (1) and (2), which represent models defining the significant independent variables that affect (1) the concentration of aerobic facultative bacteria and (2) TPH (%) removal, respectively. The greatest TPH removal in the control reactors was 2.2% indicating that abiotic processes play only a very minor role in petroleum degradation. The TPH removal charts were calculated taking into account the abiotic losses.

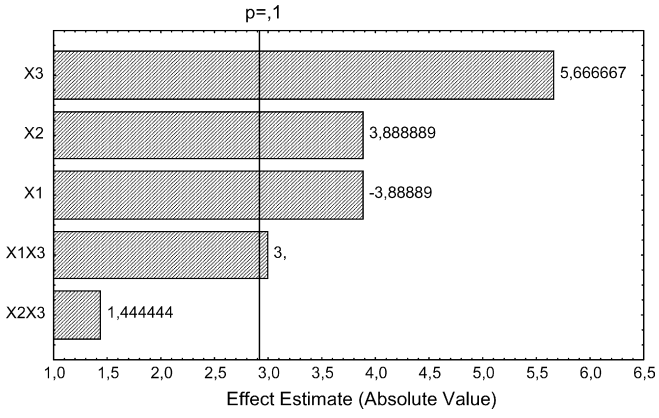

Fig. 4. Pareto diagram of the studied variables in the removal efficiency of TPH in the 28 days of the experiment.

The Pareto chart in Fig. 3 illustrates which factors significantly affected the growth of aerobic facultative bacteria, according to the degree of confidence already mentioned. The bars to the right of the dotted line express significant parameters and the numbers presented in these columns correspond to the Student *t*-figures. Culture type (X_3), nitrogen concentration (X_2) and the interaction between them (X_2X_3) were statistically significant, with culture type having the greatest influence. Both factors were positively correlated with bacterial growth meaning an increase in growth at 28 days was caused by the increase in nitrogen concentration ($X_2 = +1$) and the culture type C_1 ($X_3 = +1$).

Nitrogen and phosphorus availability within the medium limits most of the microbial processes because these elements are necessary for the synthesis of phosphorus-containing lipids and nucleic acids, as well as the completion of reactions that involve energy exchange [6]. In general, systems with petroleum hydrocarbons contamination have a high C/H ratio, which hinders microbial processes. For that reason, in most cases, it is necessary to correct this ratio to improve the hydrocarbon decomposition rate and to minimize environmental impacts.

It was necessary to correct the general equation by replacing variable X_3 with +1, seeking to maximize the response, in order to obtain the adjusted Eq. (1). This procedure was adopted because variable X_3 is qualitative and the adjusted equation is a function of the quantitative variables only

$$B = 3.7 \times 10^{10} + 2.8 \times 10^{10} X_2 \quad (1)$$

Fuel concentration (X_1), nitrogen concentration (X_2), culture type (X_3) and the fuel–culture interaction (X_1X_3) were all shown to significantly affect TPH removal (Fig. 4). Culture type had the greatest absolute (positive or negative) influence and the mixed culture C_1 produced the greatest degradation of hydrocarbons. Fuel concentration was negatively correlated to TPH removal that is, TPH removal decreased when a higher fuel concentration was used. However, the increase in fuel concentration did not result in a net inhibition of TPH removal, which is shown by the positive effect of the X_1X_3 interaction. That the degradation processes can be inhibited by the presence of the pollutants (substratum) at toxic levels is well established and reported in the literature [25,16,17,12]. Del'arco and de França [16] clearly show that the biodegradation of petroleum is inhibited when the concentration of crude oil in the reactors is tripled.

Because the adjusted equation should be a function of quantitative not qualitative variables. Eq. (2) was adjusted by replacing variable X_3 with +1 in the general equation, seeking to maximize the TPH removal. Thus Eq. (2), which is a TPH removal response, becomes a function of the variables fuel concentration (X_1) and nitrogen concentration (X_2), given by

$$R(\%) = 90.0 - 7.8X_1 + 4.4X_2 \quad (2)$$

After 28 days of operation 97.9% of the variability in cell growth data was explained by the independent variables include in Eq. (1) and 94.6% of the variability in TPH removal was explained by the independent variables included in Eq. (2). Given these

results, it is possible to conclude that C_1 is better than C_2 for degrading diesel oil and gasoline in liquid medium. The best maximum fuel concentration in the medium is 4% (v/v) and the preferable nitrogen concentration is 550 mg/L.

In agreement with Cunha and Leite [5], for a treatment *in situ*, it is important to take into account the detailed evaluation of the local microbiological population, the availability of nutrients and the necessary time for a remediation technology.

3.3. Identification of culture C_1

Since culture C_1 presented the greatest potential to decompose diesel oil and gasoline, the microorganisms present in the culture were identified. Six microorganisms were isolated, and from these four were identified: *Pseudomonas* sp., *Serratia* sp., *Klebsiella* and *Bacillus* sp. These four genera that occur in C_1 are facultative, suggesting that short-duration aeration (3 min) at 48-h intervals favored the growth of microorganisms with facultative metabolism. In addition *Pseudomonas*, *Serratia* and *Bacillus* are biosurfactant producers and, according to Pruthi and Cameotra [32], the superior performance of cultures (containing these genera) at degrading hydrocarbons is likely related to the production of biosurfactants (amphipathic molecules that reduce the surface tension of the medium), thus enhancing the bio-availability of the compounds to the bacteria.

Pseudomonas has frequently been found at sites polluted by petroleum and petroleum derivatives [9,13,18,28], suggesting this genera effectively metabolizes hydrocarbon molecules and may dominate bacterial populations in media where petroleum pollution has occurred. *Serratia* species have also been identified as providing excellent degradation of petroleum and derivatives [1,20,31]. *Bacillus* has also frequently been reported as an effective agent for the degradation of hydrocarbons [20,21,24,9]. Ghazali et al. [9], isolated *Bacillus* sp. and *Pseudomonas* sp. from hydrocarbon-polluted soils and studied these genera relative to their potential to biodegrade crude oil, benzene, ethylbenzene, *o*-xylene, *n*-tetradecano, octanol and decanol. Okerentugba and Ezeronye [24] isolated *Bacillus*, *Klebsiella*, *Pseudomonas* from rivers polluted by hydrocarbons and refinery effluent to study the potential use of these microorganisms to degrade petroleum.

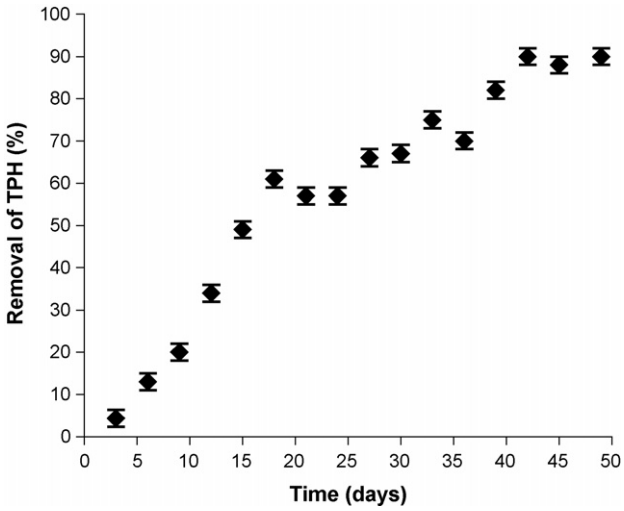

Fig. 5. Removal of TPH in the medium containing 4% (v/v) contaminant by the C_1 culture as a function of operation time.

3.4. Study of biodegradability of diesel oil and gasoline under the best operational conditions

The study of the biodegradability of a 4% diesel oil/gasoline mixture at was carried out in an enlarged scale, using the C_1 culture at a nitrogen concentration of 550 mg/L. The study duration was selected so as to maintain a constant level of TPH removal (Fig. 5).

At the end of 49 days TPH efficiency was $90 \pm 2\%$ (Fig. 5). Degradation behavior suggests a preferential consumption of the more easily degradable compounds during the first 20 days. After 20 days, there was an increase in the rate at which TPH concentration was reduced suggesting a change in either specific enzymatic systems or a change in the efficiency of the systems being used.

After treatment for 49 days olefins (decene and 2-methyl-1,3-butadiene), naphthenics (isopropylcyclopentane), paraffins (C11 and C13) and isoparaffins (2-methylpentene) were completely decomposed (Fig. 6). Cycloalkenes (naphthenics) are usually resistant to microbial attack. However, some authors [26,22] have demonstrated that substituted and non-substituted

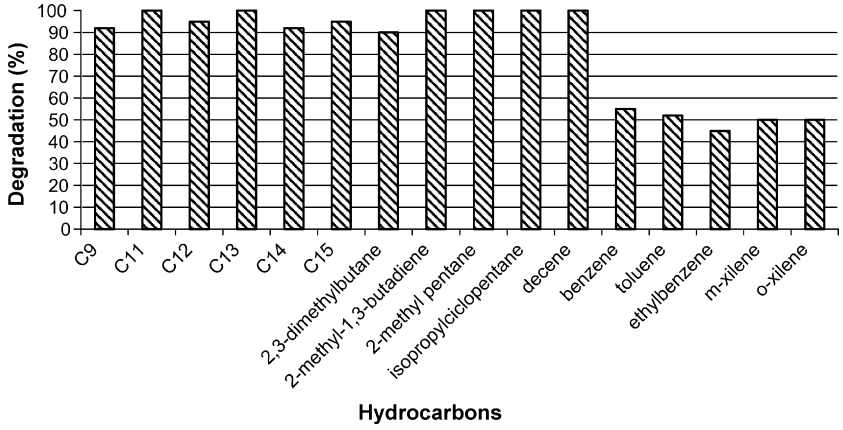

Fig. 6. Biodegradation of identified hydrocarbons in the synthetic waste after 49 days of the experiment.

cycloalkenes can undergo oxidative and co-oxidative degradation. Some non-substituted cycloalkenes are co-metabolized, forming cetones and alcohols. These compounds are oxygenated and the biodegradation takes place by the cleavage of the rings. On the other hand, the degradation of the substituted cycloalkenes appears to happen through oxidation. In these cases, the initial attack is at the substitution position, forming intermediate compounds (cyclical compound of carboxylic and aromatic acid) that undergo ring cleavage [26,22]. The compounds n-alkane C9, C12, C14, and the isoparaffin 2,3-dimethylbutane were degraded by about 90–95%.

The aromatic compounds identified (benzene, toluene, ethylbenzene, *m*-xylene, *o*-xylene) showed low biodegradability in face of the microbial attack when compared to the paraffinic and isoparaffinic, with removal rates varying from 45 to 55%. This low biodegradability is in agreement with the degree of susceptibility suggested by Leahye and Colwell [14]. These researchers verified that *n*-alkanes compounds were the most susceptible to microbial attack, followed by branched alkanes, aromatics of low molecular weight and cycloalkenes, which were the least susceptible to the attack.

Cunha and Leite [5] studied the biodegradation of gasoline by isolated and mixed cultures. Mixed culture studied presented the following degradation indices (percentage): 51.4% for toluene, 46.3% for the ethylbenzene, 49.4% for *n*-C9, 53.4% for *n*-C11, 50.4% for *n*-C12 and 50.0% for *n*-C13. Richard and Vogel [18], in the study of diesel oil biodegradation by a consortium, of microorganisms, obtained total degradation of the *n*-alkanes identified (*n*-C10 to *n*-C23). The greatest amount of residue identified consisted of branched alkanes such as pristane and phytane after 50 process days.

The fact that the paraffinic and isoparaffinic compounds in this study had lower biodegradability than the naphthalenic and olefinic molecules can be related to the fact that a larger number of former compounds were identified.

4. Conclusions

Results of this study indicate that cultures coming from the same polluted site but from different depths can exhibit different degradation characteristics when added to effluents containing hydrocarbon contaminants. The C_1 culture, demonstrated greater potential to treat fuel-terminal effluent by removing the greatest amount of TPH and having the highest microorganism growth (4%, v/v fuel concentration, nitrogen at 550 mg/L and a process time of 28 days). Since the C_1 culture was derived from surface sediments from the lake, it is likely that those epibenthic organisms were exposed to higher petroleum concentrations and thus better adapted to metabolize hydrocarbons. Aromatic compounds appeared to degrade less than other hydrocarbons.

After biodegradation under the best operational conditions, in a 4 L capacity batch stirred tank reactor, the chromatographical analyses showed that the aromatic compounds presented a lower biodegradability when compared to the other hydrocarbons identified.

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