Research Article

Received: 23 November 2009 Revised: 11 February 2010 Accepted: 31 March 2010 Published online in Wiley Interscience: 10 June 2010

(www.interscience.wiley.com) DOI 10.1002/jctb.2425

# Petroleum-contaminated soil remediation in a new solid phase bioreactor

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## Abstract

**BACKGROUND: Oil spills are a frequent source of environmental contamination. As a consequence, remediation of soils, waters and sediments is a great challenge in this area of research. This study aims at using a new type of soil bioreactor (13 L bench-scale and 800 L pilot-scale) to treat tropical soil contaminated with petroleum. Additionally, it includes the evaluation of the effectiveness of two auxiliary techniques: bulking agent addition (sawdust) and biostimulation using two different nitrogen sources (sodium nitrate and urea).**

**RESULTS: The best result in bench- and pilot-scale bioreactors were reached when using urea as a nitrogen supplement and bulking agent addition. Removal of 20 to 35% of total petroleum hydrocarbon (TPH) was achieved within 42 days. The molecular fingerprinting performed with 16S-PCR analysis associated with denaturing gradient gel electrophoresis (DGGE) was used to evaluate changes in the pattern of the bacterial community for all experimental conditions tested. The results revealed that the use of urea caused a smaller change in the dominant bacterial community structure than the treatments using nitrate, showing that this analysis can be a useful complementary tool to evaluate the impact of treatment strategies applied to hydrocarbon-contaminated soil.**

**CONCLUSIONS: These new solid phase bioreactors showed satisfactory results in the tropical soil bioremediation process, proving that the homogenization system interferes with crude oil biodegradation efficiency. This new technology can be used as an isolated treatment as well as in association with other classically employed bioremediation technologies.**


**Keywords:** bioremediation; petroleum; soil; bioreactor; DGGE

## INTRODUCTION

The Brazilian petroleum industry, mainly refineries and oil production areas, faces the possibility of leaks of crude oil reaching water bodies and soil. Thus, the need to develop and apply efficient technologies for treating soils contaminated with petroleum hydrocarbons, which present a large organic contamination, becomes increasingly more urgent, especially regarding reducing the time for remediation and processing costs. The use of bioremediation techniques is one of the most promising strategies to be adopted for the aforementioned treatment. According to Providenti *et al.*[1], bioremediation, in contrast to physical and chemical processes, is considered a safe, efficient and inexpensive method for removing dangerous pollutants. In the most common bioremediation applications, soil naturally occurring microorganisms are stimulated by enhancing the oxygen supply, nutrients concentration and moisture content to degrade the organic contaminants, such as petroleum hydrocarbons.

Among the main technologies used in bioremediation, the following have also been mentioned: bioventing, biosparging, bioslurping, phytoremediation, landfarming, monitored natural attenuation, biopiles and bioreactors.[2–15] These can be associated with specific techniques that aim at increasing the microbial activity such as biostimulation, bioaugmentation, the addition of biosurfactants and the incorporation of bulking materials.[16]

The use of bioreactors has emerged as an interesting alternative. Among the main advantages there is the possibility of continuous monitoring of system performance, the control of optimal processing conditions, wich is essential for maintaining microbial activity, and reduction of remediation time.[15,17–18] Among other factors that strengthen this trend is the fact that

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**Table 1.** Physicochemical properties of the contaminated soil used in this study

| Parameters | Value |
|---|---|
| Texture | Sandy loam |
| (%) sand (weight) | 73 |
| (%) silt (weight) | 14 |
| (%) clay (weight) | 11 |
| Organic matter (g $kg^{-1}$) | 58.0 |
| Total N (g $kg^{-1}$) | 2.3 |
| Available P (mg $kg^{-1}$) | 13.0 |
| pH | 6.4 |
| Bulk density (g $cm^{-3}$) | 1.2 |
| Water holding capacity (%) | 28 |

microbial mobility in the soil is restricted. This fact reduces their access to nutrients, affecting the biodegradation of those organic contaminants.[1] On the other hand, in a bioreactor, such a drawback can be completely overcome through the use of a suitable homogenization/mixing system.

Thus, the utilization of bioreactors becomes an even more promising alternative, particularly for tropical soil contamination, such as those found in Brazil, due to the limited applicability of other classical bioremediation techniques (for instance soil washing and biopiles) for that type of soil. These methods generally provide poor mixing systems to deal with low permeability soil, hindering the incorporation of oxygen and nutrients for the biodegradation process to occur. When using a bioreactor treatment, this problem is overcome through efficient homogenisation and aeration systems. Thus, the present work aims at developing a solid phase horizontal bioreactor of innovative design for the treatment of different kinds of soils contaminated with petroleum hydrocarbons, particularly tropical soils, in bench- and pilot-scale prototypes.

## EXPERIMENTAL

### Contaminated soil

The soil used in the experiments was previously doped to simulate an accidental contamination. The crude oil (paraffin oil °API 24.1 – average organic composition: 43.68% saturated, 15.18% aromatics, 21.8% asphaltenic compounds) was added to the natural soil in order to reach an oil content of 5.4% (w/w). The soil and crude oil samples were taken from the on-shore petroleum exploration field located in the northeast region of Brazil. After 72 h of contamination, the soil was used in biodegradation experiments and its physicochemical characterization is shown in Table 1. Despite being classified as a sandy loam soil, high content of organic matter, naturally present, confers mechanical characteristics similar to those of a typical clay soil.

### Biodegradation experiments in different treatment systems

Soil contamination was accomplished according to the previously described method, and the moisture content was adjusted to 50% of the water holding capacity (WHC) (14% moisture content).

*Experiments in the bench-scale bioreactor*

Experiments were carried out in a bench-scale prototype (Fig. 1) with dimensions of 240 × 420 × 323 mm, containing 8 kg of contaminated soil and agitated (4 rpm) for 42 days.[19] The agitation system was composed of a central axle and five perpendicular axles equipped with blades configured as shown in Fig. 2.

Correction of the nitrogen content in the soil was done using sodium nitrate or commercial grade urea so as to maintain a C : N : P ratio of 100 : 10:0.39. This ratio was defined as optimal in previous studies[19] and, therefore, there was no need to add an extra source of phosphorus to the system. Sawdust was added as a bulking material at a concentration of 10% (w/w). The specific conditions for each test are shown in Table 2.

*Experiments in the pilot-scale bioreactor*

The experiments were conducted in a U-shaped bioreactor with dimensions of 800 × 1200 × 1000 mm, made out of mild steel, with a total volume of 876 L (Fig. 3(a)). The blades configuration is the same as the bench-scale prototype bioreactor (Fig. 2).

The bioreactor was connected to a computerized control system that uses ELIPSE SCADA® software to supervise and automate the system components (agitation, aeration, introduction of additives), and to control the process parameters (temperature, moisture content, and $CO_2$ concentration) (Fig. 3(c)).

Two tests were accomplished reproducing, in pilot-scale, the best two selected experimental conditions from the bench-scale. The specific conditions for each test are described in Table 2.

### Analytical methods

The biodegradation experiments were monitored by quantifying TPH (total petroleum hydrocarbons) in the soil and oil degrading microorganisms. To quantify the degrading microorganisms the most probable number (MPN) technique were used, in agreement with the method described by Venosa and Wrenn[20] and Petrović *et al*.[21] Quantification of TPH in the soil was made by gas chromatography according to the modified EPA 8015B method, and soil extraction was done according to the EPA 3550B method (United States Environmental Protect Agency[22]). The hydrocarbon degradation percentages were calculated taking into consideration to the initial time (T0).

### Complementary assays – evaluation of the microbial diversity

The objective of such experiments was to evaluate the structure of the dominant bacterial community in the soil before and after contamination with crude oil and also at the end of the four tests carried out in the bench-scale prototype and the two tests conducted in the pilot-scale bioreactor.

*Extraction of DNA from the treatment systems*

Extraction of DNA from the soil was accomplished using the FastDNA® SPIN Kit for soil (BIO101, California, USA). The protocol was used according to the manufacturer's instructions and the extracted DNA was kept at −20 °C before being analysed.

*16S-PCR*

The primers U968f-GC1 ('clamp' + 5′ AAC GCG AAG AAC CTT AC 3′) and L1401r (5′ GCG TGT GTA CAA GAC CC 3′) were used in this stage.[23] The mixtures had a final sample volume of 50 µL with the following concentrations of each reagent: 1× *Taq* polymerase enzyme buffer (Promega), 2.5 mmol $L^{-1}$ $MgCl_2$ (Promega), 200 µmol $L^{-1}$ dNTPs (Promega), 10 $\rho$mols of each primer (Oligos), 5 µg BSA (Sigma), 1% formamide (Fluka), 3.75



  

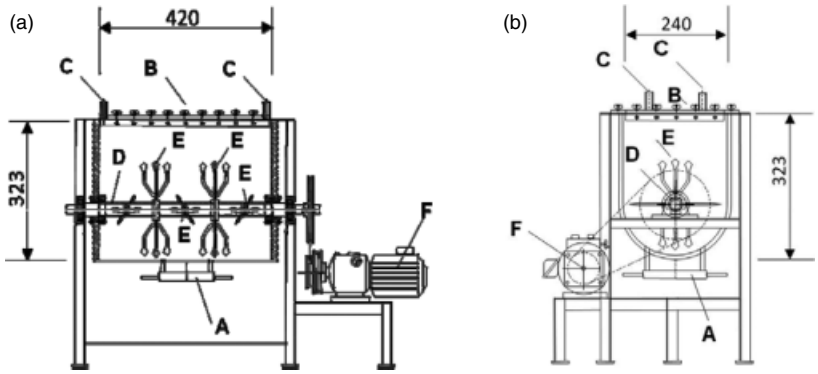


**Figure 1.** Bench-scale prototype of the solid phase bioreactor: (a) front view; (b) side view. A - discharge of material, B - cover, C - duct for air and reagents entry and $CO_2$ exit, D - central axle, E - blades, F - engine. All dimensions are in millimeters.

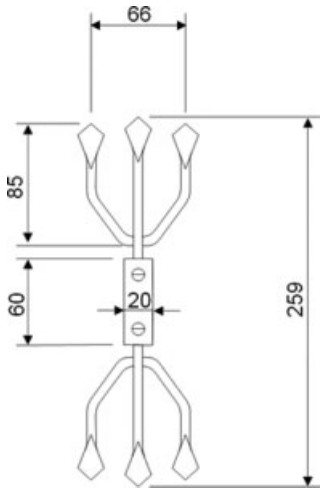


**Figure 2.** Configuration design of blades installed in the bench- and pilot-scale bioreactors.

U of Taq polymerase (Promega) and sterile Milli-Q water. For each reaction, 1 μL of DNA was added.

The PCR program used was initiated with a DNA denaturation cycle at 94 °C for 4 min, followed by 30 cycles at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min. The final extension cycle was 10 min at 72 °C. The products were verified by 1.2% agarose gel electrophoresis (w/v).

*DGGE (denaturing gradient gel electrophoresis)*

The experiments were conducted using the DCode™ Universal Mutation Detection System (BIO-Rad Richmond, VA, USA). The DGGE gels were prepared with a 6% (w/v) polyacrylamide solution in tris-acetate buffer (pH 8.3). A 40–70% gradient of denatured chemicals was used for the 16S-DGGE gel, which was composed of formamide and urea. Electrophoresis was conducted using the same buffer (TAE, pH 8.3) at 60 °C and 75V for 18 h. The gels were later stained with SYBR GREEN (Molecular Probes, Oregon, USA), observed under UV light and photographed with the image analysis system STORM (Pharmacia, Amersham). Using the digitized gel images, an analysis was done to generate a band profile, using Image Quant software (v. 5.2). The bands were considered for matrix construction when the peak height, referring to an intensity, did not exceed 1% of the sum of all the identified heights, in accordance with the protocol described by Iwamoto *et al.*[24] From the matrix showing the presence and absence of the bands identified, a cluster analysis was performed. Calculations of similarity were based on Pearson's coefficient.[25] The UPGA clustering method was used to calculate the clustering of the dendrograms generated for each gel using *Statistical Software for Windows* v. 7.0, (Statsoft, USA).

## RESULTS AND DISCUSSION

Among the main advantages associated with the use of bioreactors for treating contaminated tropical soils, the following can be mentioned: the possibility of reducing processing time; overall control and maintenance of the operational conditions (pH, temperature, aeration and moisture content); the possibility of effectively monitoring the process; reduced area required for assembling the system; control of atmospheric emissions and the water generated from the process; the possibility for directly introducing additives to the reactor (water, microorganisms, surfactants, nutrients, pH corrections, co-substrates); maintenance of an adequate degree of mixing (continuous or discontinuous agitation); the possibility of soil treatment with a large content of fine particles; and finally, the reduction of direct contact between the reactor content (pollutant) and the environment during the treatment process, which represents an advantage from an environmental and security point of view.

The selection of bioreactor configuration, as well as the associated bioremediation techniques (bio stimulation, bio augmentation, incorporation of bulking agent, dosage of biosurfactants) must be conducted while taking into consideration the characteristics of the soil to be treated, the nature of the contaminant, the microorganisms involved, the importance of aeration and suitable agitation, among others. For the treatment of contaminated soil without prior removal of the finest soil fractions, the use of solid phase horizontal bioreactors is recommended, as those used in the development of this study. Solid phase bioreactors are particularly appropriate for treating material with low water content (around 50% (w/w) of water holding capacity). In these types of bioreactors, the moisture content is maintained only at levels sufficient to maintain the microbial activity.[4] Despite the economic advantages associated with the reduction of water incorporation, relatively few studies have explored solid phase bioreactors as a function of the harmful effects that water limitation can have on microbial metabolism.[15] Among the configurations of horizontal solid phase reactors, two sub-configurations are shown that differ in the way they mix: rotating drums and fixed drums. In the first,



  

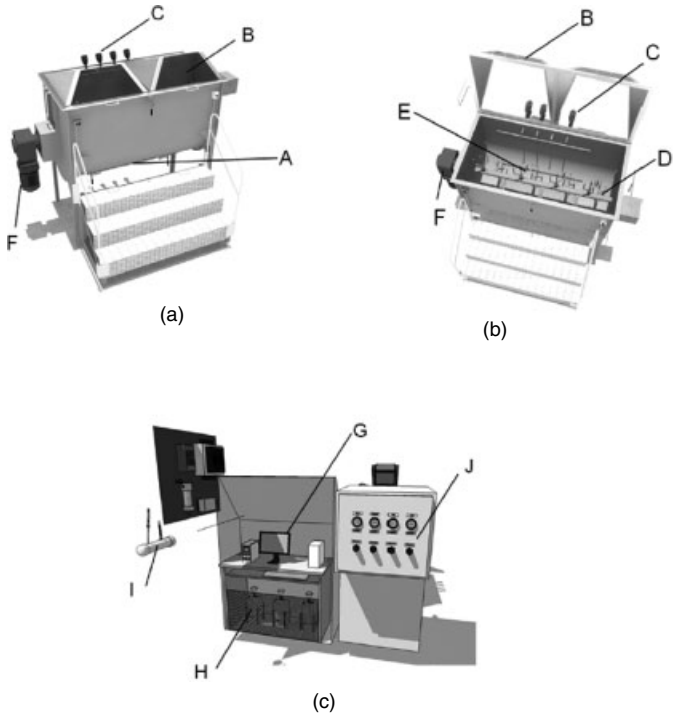


**Figure 3.** Pilot-scale solid phase bioreactor: (a) body of the bioreactor; (b) upper view of the agitation system; (c) control system. A - discharge of material, B - cover, C - duct for air and reagents entry and $CO_2$ exit, D - central axle, E - blades, F - engine, G - computerized control system, H - tanks for water and reagents, I - monitoring probes, J - pneumatic and electric control.

**Table 2.** Tests of bioremediation in the bench-scale and pilot-scale bioreactor

| Parameters | Bench 1 | Bench 2 | Bench 3 | Bench 4 | Pilot 1 | Pilot 2 |
|---|---|---|---|---|---|---|
| Occupancy rate (% useable volume) | 40 | 40 | 40 | 40 | 50 | 50 |
| Soil mass (kg) | 8 | 8 | 8 | 8 | 400 | 400 |
| Moisture content (%WHC) | 50 | 50 | 50 | 50 | 50 | 50 |
| pH Correction (pH 7) | No | No | No | No | No | No |
| Nutritional correction (C : N : P)[a] | 100 : 10 : 0.39[b] | 100 : 10 : 0.39[b] | 100 : 10 : 0.39[c] | 100 : 10 : 0.39[c] | 100 : 10 : 0.39[b] | 100 : 10 : 0.39[b] |
| Addition of bulking agent | No | Yes | No | Yes | No | Yes |
| Assay time (days) | 42 | 42 | 42 | 42 | 42 | 42 |
| Agitation | | | | | | |
| rpm | 4–5 | 4–5 | 4–5 | 4–5 | 4–5 | 4–5 |
| Cycles | 2 | 2 | 2 | 2 | 2 | 2 |
| Time | 15 min per day | 15 min per day | 15 min per day | 15 min per day | 15 min per day | 15 min per day |
| Aeration | | | | | | |
| (L $min^{-1}$) | 20 | 20 | 20 | 20 | 20 | 20 |
| Cycles | 1 | 1 | 1 | 1 | 1 | 1 |
| Time | 1 h per day | 1 h per day | 1 h per day | 1 h per day | 1 h per day | 1 h per day |

[a] Original phosphorous content of the soil.
[b] Nitrogen content corrected with sodium nitrate.
[c] Nitrogen content corrected with urea.

homogenisation of the system is promoted by rotation of the drum as a whole, around its axle, while in the second, mixing occurs due to the movement of a central axle of varied geometry, like the one used in this study.[18]

### Bench-scale bioreactor tests

The bioremediation tests in the bench-scale prototype reproduced the processing conditions previously tested in microcosms (use of biostimulation technique associated with the addition of a bulking material) and, additionally, evaluated the effectiveness of nitrogen source substitution (sodium nitrate instead of urea).

The results obtained by monitoring the TPH concentrations (mg TPH $g^{-1}$ soil) are shown in Table 3, as well as the respective TPH removal percentages reached for these parameters. No significant difference was detected as a result of bulking material addition (TEST Bench 2) compared with the result obtained through biostimulation (TEST Bench 1), when the sodium nitrate was used as nitrogen source. In that case, the addition of sawdust



  

**Table 3.** TPH results for the bioremediation tests in bench and pilot scale bioreactors

| Assay Identification | TPH (mg TPH $g^{-1}$ soil) Initial | Final | TPH removal (%) | Daily removal rate (mg TPH $g^{-1}$ soil $day^{-1}$) |
|---|---|---|---|---|
| Bench 1 | 22.24 ± 0.07 | 19.46 ± 0.02 | 12.48 ± 0.04 | 0.07 ± 0.04 |
| Bench 2 | 19.46 ± 0.04 | 16.93 ± 0.04 | 12.98 ± 0.04 | 0.06 ± 0.04 |
| Bench 3 | 21.67 ± 0.05 | 18.66 ± 0.10 | 13.86 ± 0.08 | 0.07 ± 0.08 |
| Bench 4 | 32.38 ± 0.05 | 26.04 ± 0.02 | 19.58 ± 0.04 | 0.15 ± 0.04 |
| Pilot 1 | 45.06 ± 0.03 | 37.97 ± 0.01 | 15.73 ± 0.02 | 0.14 ± 0.02 |
| Pilot 2 | 36.91 ± 0.10 | 23.94 ± 0.08 | 35.14 ± 0.09 | 0.26 ± 0.09 |

Bench 1 - biostimulation with sodium nitrate; Bench 2 - biostimulation with sodium nitrate and the addition of sawdust; Bench 3 - biostimulation with urea; Bench 4 - biostimulation with urea and addition of sawdust. Pilot 1 - biostimulation with urea; Pilot 2 - biostimulation with urea and the addition of sawdust.

**Table 4.** Results from counting the degrading microorganisms in the bench-scale and pilot- scale bioreactor

| Time (weeks) | Degrading microorganisms (log MPN $g^{-1}$ soil) Bench 1 | Bench 2 | Bench 3 | Bench 4 | Pilot 1 | Pilot 2 |
|---|---|---|---|---|---|---|
| 0 | 2.30 | 4.95 | 4.78 | 2.30 | <2.00 | 1.00 |
| 1 | 4.78 | 5.00 | 3.78 | 3.30 | <2.00 | 2.60 |
| 2 | 4.78 | 3.78 | 4.78 | 5.30 | 2.00 | 2.48 |
| 3 | 4.78 | 5.78 | 3.48 | 4.48 | 2.00 | 2.70 |
| 4 | 3.85 | 3.78 | 3.90 | 4.00 | 1.00 | 3.70 |
| 5 | 1.95 | 2.48 | 4.78 | 2.48 | 2.30 | 4.90 |
| 6 | 1.70 | 3.00 | 4.30 | 4.00 | 2.48 | 4.78 |
| 7 | 2.48 | 3.00 | 3.90 | 4.70 | 2.70 | 2.70 |

Bench 1 - biostimulation with sodium nitrate; Bench 2 - biostimulation with sodium nitrate and the addition of sawdust; Bench 3 - biostimulation with urea; Bench 4 - biostimulation with urea and the addition of sawdust.

increased the TPH removal efficiency by only 4% and the daily TPH removals were nearly equal (0.07–0.06 mg TPH $g^{-1}$ soil $day^{-1}$). The main benefit observed when sawdust was added was a better soil homogenisation inside the bioreactor.

When the nitrogen source was replaced by urea (TEST Bench 3) the TPH removal was enhanced from 12.48% to 13.86% using only the biostimulation technique, representing an increase of about 11% in the TPH removal efficiency, although the daily TPH removal rate was nearly the same (0.07 mg TPH $g^{-1}$ soil. $day^{-1}$). However, biostimulation using urea, along with the addition of sawdust (TEST Bench 4) enhanced TPH removal to 19.58%, which represented a significant increase on the daily TPH removal rate from 0.06 to 0.15 mg TPH $g^{-1}$ soil $day^{-1}$, thus proving the effectiveness of adding bulk material to accelerate the bioremediation process in the bioreactor prototype.

The results obtained from monitoring the crude oil degrading microorganism concentration are shown in Table 4. Generally, there was a variation in the degrading microorganism population density throughout the whole 7 weeks of test for the four experimental conditions tested. Only the bench-scale prototype 4, which incorporated urea and sawdust as bulking material, showed a significant increase in the population of degrading microorganisms at the end of the test (7 weeks) in relation to the initial population.

At the end of the tests conducted in the bench-scale bioreactor prototype it was demonstrated that the use of urea as a complementary nitrogen source and the addition of sawdust were essential to improve the oil biodegradation process. These conditions were then adopted for complementary tests in the pilot bioreactor.

### Pilot bioreactor tests

The results of TPH analyses for the two tests conducted in the pilot bioreactor are shown in Table 3.

The TPH removal percentages were around 16% for the first test (PILOT 1), while for the second test (PILOT 2), the TPH removal was increased to 35%, representing a 2.3 times higher efficiency after adding bulking material. The addition of sawdust almost doubled the rate obtained with the addition of urea only (from 0.14 to 0.26 mg TPH $g^{-1}$ soil $day^{-1}$), with an increase of 84.7% in the daily removal rate, indicating a positive effect on the acceleration of the soil bioremediation process. A similar result was obtained by Seabra and co-workers while treating crude oil bearing soil in biopiles, in which the authors showed that most TPH degradation occurred in the first 8 weeks (56 days) of treatment (0.28 to 0.36 mg TPH $g^{-1}$ soil $day^{-1}$),[13] but only reaching 28.6% increase in the daily removal rate. Comparing the results of TPH removal in this soil during the monitored natural attenuation process (data not shown), it was found that the increase of degradation in the pilot scale bioreactor was 5 times higher, confirming the reduction in treatment time afforded by the use of the proposed system.[26]

Additionally, it was verified that when scaling-up the bioreactor the degradation rate was doubled after adding sawdust, probably due to an increase of soil porosity. In the bench-scale tests, the maximum value obtained was 0.15 mg TPH $g^{-1}$ soil $day^{-1}$ (BENCH 4), whereas in the pilot bioreactor, using the same experimental conditions, the degradation rate reached 0.26 mg TPH $g^{-1}$ soil $day^{-1}$.

The results from counting the degrading microorganisms throughout the two tests are shown in Table 4.

The slow and gradual adaptation process of the microbial population to the crude oil can be seen by the results obtained during weekly monitoring of the bioreactor content for the two conditions tested. However, it was observed that in the second test, in which sawdust was added to biostimulation, the growth of the degrading microorganisms population was more pronounced in weeks 5 and 6, reinforcing the beneficial effect of adding bulking material, and, thus, causing the highest reduction of contaminant concentration, as previously discussed.



  

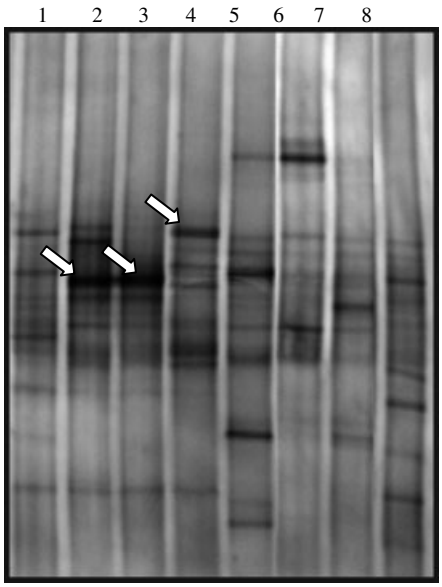


**Figure 4.** 16S-DGGE gel for the different evaluated treatments. 1 - initial contaminated soil referring to T0; 2 - final sample PILOT 1; 3 - final sample PILOT 2; 4 - final sample BENCH 4; 5 - final sample BENCH 3; 6 - final sample BENCH 2; 7 - final sample BENCH 1; 8 - uncontaminated soil.

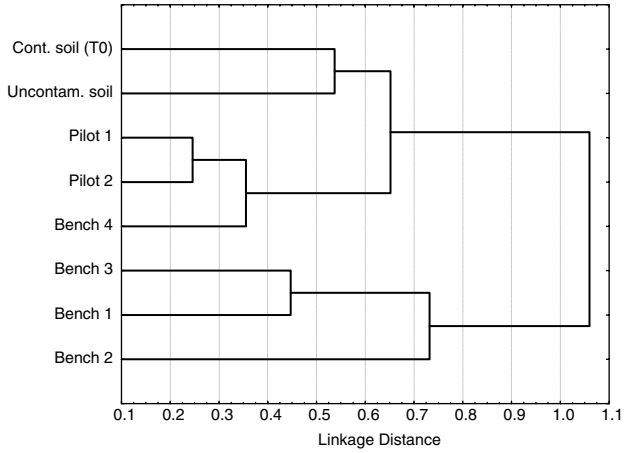


**Figure 5.** Dendrogram obtained for the 16S-DGGE gel with the evaluated treatments. Cont. soil.(T0) - initial contaminated soil; Uncont. soil. - uncontaminated soil; Bench 1 – bio-stimulation with sodium nitrate; Bench 2 – bio-stimulation with sodium nitrate and the addition of sawdust; Bench 3 – bio-stimulation with urea; Bench 4 – bio-stimulation with urea and the addition of sawdust; Pilot 1 – bio-stimulation with urea; Pilot 2 – bio-stimulation with urea and the addition of sawdust.

It was shown that the results obtained in these first tests in the pilot bioreactor are indicative of the technical viability of using the proposed system for the treatment of tropical soils contaminated with petroleum hydrocarbons.

*Evaluation of microbial diversity*

The objective of the experiments conducted at this stage was to evaluate the structure of the dominant bacterial community in the soil before and after contamination with crude oil (initial contaminated soil) and also at the end of the four different tests conducted in the bench-scale bioreactor prototype, and in the two tests conducted in the pilot bioreactor. This evaluation offers complementary information on the applicability of the adopted biological treatment.

The independent culture techniques, such as 16S-PCR DGGE, allow one to evaluate 90–99% of the bacterial population that cannot be detected by conventional culture methods. Thus, it is possible to verify more significant alterations in the structure of the dominant bacterial community after the treatments, helping to define the best strategy to be used.[27]

The band profile of the DGGE gel obtained with the PCR products for each sample evaluated is shown in Fig. 4. It was possible to verify a variation in the structure of the bacterial community for all samples tested, which can be better understood from the dendrogram shown in Fig. 5.

According to the dendrogram obtained for the matrix constructed from the 16S-DGGE gel (Fig. 5), it is possible to observe the formation of three distinct groups. The first group is formed by uncontaminated soil and the initially contaminated soil. The initially contaminated soil is probably similar to the uncontaminated soil because the simulated contamination was recent, and did not have time to affect significantly the soil original microbial population. The final samples from the tests conducted in the pilot bioreactor (PILOT 1 and PILOT 2), as well as the final sample of the fourth test in the bench prototype bioreactor (BENCH 4), form the second group, showing a significant change in microbial community in relation to the uncontaminated soil. It is noteworthy that the final samples mentioned represent conditions in which urea was used as the nitrogen source, indicating that its use caused a smaller alteration in the microbial community than the treatments using nitrate (final samples BENCH 1 and BENCH 2). Although the final sample of the third test in the prototype bioreactor (BENCH 3) incorporated urea as nitrogen source, it was more distant in relation to the second group, which contains samples from the pilot-scale reactor, showing a smaller change in the microbial community profile as the bioreactor scales up. It was verified from the analysis of the dendrogram that the second group was composed of conditions with the largest percentage of TPH removal (35.14% PILOT 2; 15.73% PILOT 1 and 19.58% BENCH 4). In the third group, the grouped conditions had a TPH removal percentage less than 14% (12.48% BENCH 1; 12.98% BENCH 2; 13.86% BENCH 3).

Several studies have been undertaken to evaluate the bacterial community of petroleum hydrocarbons contaminated soil using different fingerprinting techniques.[28,29] The DGGE technique, in particular, has been applied to confirm variations in the structure and diversity of the dominant microbial community in soil and water, before and after contamination, with different pollutants, and after the proposed treatments.[30–33]

Cunha and co-workers[34] used molecular biology techniques to evaluate the impact of contamination of gasoline blended ethanol in tropical soil (Rio de Janeiro/Brazil) and the treatments implemented (biostimulation or bioaugmentation) on the microbial community. Molecular fingerprinting conducted using the 16S-PCR technique, associated with the DGGE technique, showed that in a 240 h period, changes observed in the structure of the community for these treatments were less than that provided by natural attenuation alone. According to the authors, these results strengthen the current thought that the use of bioremediation has excellent potential for treatments of sites contaminated with hydrocarbons.

It was possible to verify the increased intensity of some bands initially present in the uncontaminated soil for different samples evaluated in the present study, as well as in the initial contaminated soil, (arrows in Fig. 4), which could represent the enrichment of degrading bacterial populations. That increased intensity was also



  

verified by Evans *et al*.[23] who related the specialisation of the dominant bacterial community to the presence of the organic contaminants.

The results obtained indicate that analysis of the structure of dominant bacterial community can, therefore, be used as a complementary tool to evaluate the impact of the biological treatment applied to soil contaminated with petroleum hydrocarbons.

## CONCLUSIONS

Results for TPH biodegradation efficiency in the studied soil, in bench- and pilot-scale bioreactors, indicate that the proposed treatment system is efficient for treating contaminated tropical soils, reflecting, directly, on the treatment costs, as well as minimising the health and environmental risks associated with prolonged exposure to those residues. It should be noted that the low moisture content required for soil treatment in the bioreactor has the main consequence of not generating waste that would need further treatment.

Increasing the scale of the bioreactor resulted in a two-fold increase in biodegradation efficiency. In the bench-scale reactor, the maximum value obtained was 19.58%, whereas in the pilot bioreactor, in the same experimental conditions, the efficiency reached 35.14%, implying that the automation and control systems installed in the pilot bioreactor influenced, positively, on the soil remediation process as a whole, making its operation easier and safer.

The best result in the pilot-scale bioreactor was obtained by adding both urea and sawdust, giving an increase in TPH removal from 15.73% to 35.14%, emphasizing the effectiveness of adding a bulking material to accelerate the bioremediation process in the prototype bioreactor.

The associated use of molecular tools, especially the DGGE technique, with conventional methods for evaluating the effectiveness of the biological treatment of petroleum bearing soil, showed a smaller alteration in the dominant bacterial community using urea instead of nitrate, confirming the choice of urea as the best nitrogen source to be used.

According to the results obtained, the solid phase bioreactor can be used as an isolated treatment technology as well as in association with classically employed bioremediation technologies.

## ACKNOWLEDGEMENTS

This work was sponsored by PETROBRAS (Petroleo Brasileiro S.A), with technical and institutional support from the Centre for Mineral Technology (CETEM/MCT), the Laboratory of Molecular Microbial Ecology of IMPPG/UFRJ and the CENPES/PETROBRAS, particularly to the technician Frederico Landa for performing the chromatographic analyses.

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