Artigo

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REMOVAL OF HIGH-MOLECULAR WEIGHT POLYCYCLIC AROMATIC HYDROCARBONS

Ulrich Vasconcelos e Francisca P. de França*
Departamento de Engenharia Bioquímica, Escola de Química, Universidade Federal do Rio de Janeiro, CT, Bl. E, Ilha do Fundão, 21949-900 Rio de Janeiro – RJ, Brasil
Fernando J. S. Oliveira
Petróleo Brasileiro S. A., Av. Almirante Barroso, 81, 23º andar, 20031-004 Rio de Janeiro – RJ, Brasil

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Alternatives for the removal of high-molecular weight polycyclic aromatic hydrocarbons (HWM-PAH) from soil were tested by adding fertilizer or glycerol, as well as the combination of both. Experiments were carried out for 60 days in reactors containing a HWM-PAH-contaminated soil (8030 μg kg^{-1}), accompanied by pH monitoring, humidity control and quantification of total heterotrophic bacteria and total fungus. Fertilizer addition removed 41.6% of HWM-PAH. Fertilizer and glycerol in combination removed 46.2%. When glycerol was added individually, degradation reached 50.4%. Glycerol also promoted the increase of degradation rate during the first 30 days suggesting the HMW-PAH removal occurred through cometabolic pathways.

Keywords: bioremediation; polycyclic aromatic hydrocarbons; glycerol.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) comprise a class of molecules presented throughout the environment. PAH are formed by two or more condensed aromatic rings or cyclopentene arranged linearly, angularly or in groups. Such compounds originated from three main sources: vegetal, geochemical and anthropogenic.[1] Major concerns regarding PAH are related to their mutagenic and carcinogenic potential.[2-4]

According to their number of rings, PAH can be classified as low-molecular weight (LMW-PAH), i.e., with two and three rings or as high-molecular weight (HMW-PAH) with four or more rings.[5] The soil is the main reservoir of these compounds. As the PAH molecules are hydrophobic, they bind strongly to soil particles, therefore making the bioaccess difficult, due to a decrease decrease in bioavailability, especially in relation to the HMW-PAH.[6]

Microbial metabolism is the key factor for PAH removal from soils and comprises two distinct phases: first, mediated by the pollutant bioavailability and second, controlled by the relation of hydrocarbons sorption/desorption.[7] Degradation is influenced both by PAH concentration and mass transfer of contaminants from soil to microorganisms. Under suitable conditions, the microbiota utilize some strategies in an attempt to overcome the PAH persistence, such as: adhesion factor biosynthesis,[8] biofilm formation,[9] cometabolism[10] and increase of solubility by bioemulsifier/biosurfactant synthesis.[11]

PAH are not microbial preferred source of carbon due to its chemical structure, however, most microorganisms degrade the LMW-PAH while the HMW-PAH are degraded by cometabolism.[12] The United States Environmental Protection Agency (USEPA) lists 16 PAH of concern, ten of which have four to seven rings (HMW-PAH). Since PAH may represent a human and environmental health risk, the search for alternatives in order to reduce their elevated concentrations is an important endeavour.

Thermal desorption is considered an effective treatment among the commercially available techniques for PAH removal from soil, however, high temperatures used for this treatment are responsible for chemical reactions that modify the structure of PAH, such as intramolecular rearrangement and molecular weight growth by acetylene addition which contribute to the presence of residual PAH, sometimes in concentrations above the recommended safe levels.[13]

Biostimulation is an environmental-friendly alternative for the removal of PAH from soil. The technique is based on the stimulation of microorganims with degradation capabilities in order to promote the conversion of the contaminants into energy, biomass, metabolites and products.[14] The removal of pollutants is enhanced when certain conditions are met, such as neutral pH, water content around 15-20%, the presence of oxygen, and the addition of cosubstrates and essential nutrients in non inhibitory concentrations. Control and monitoring of these parameters comprise the technique of biostimulation leading to the establishment of proper environmental conditions in order to accelerate the biodegradation.[15] This work aimed to remove residual HMW-PAH in thermally treated soil by using the biostimulation techniques of fertilizer and glycerol supplementation.

EXPERIMENTAL

Soil

The soil was obtained from an industrial Thermal Desorption Unit. Samples were collected in appropriate containers and transported to the laboratory under refrigeration. Soil samples were analysed (Table 1) by applying the following assays: grain size distribution, pH in distilled water, water content and water-holding-capacity.[16-18] Total organic carbon, total nitrogen and total phosphorus were determined through the application of USEPA 9060, 351.2 and 365.3 methods, respectively.[19-21]

Soil extracts were obtained according to the USEPA 3540C method using dichloromethane and Soxhlet apparatus.[22] Samples clean-up were performed according to USEPA 3630C method by using silica gel column.[23] USEPA priority 16 PAH content was achieved

*e-mail: fpfranca@eq.ufrj.br

Table 1. Characterization of the soil samples

Analysis	Result
Texture (%)	
Clay (< 0.02 mm)	8 ± 6
Silt (0.002-0.02 mm)	33 ± 1
Fine sand (0.02-0.2mm)	22 ± 2
Medium sand (0.2-0.5 mm)	19 ± 1
Coarse sand (0.5-1.0 mm)	13 ± 4
Gravel (> 1.0 mm)	5 ± 5
Total Organic Carbon (mg kg^{-1})	23,000
Total N (mg kg^{-1})	1,397
Total P (g kg^{-1}g/kg)	777
TPH (mg kg^{-1})	699
16 EPA priority PAH (µg kg^{-1})	8430
10 HMW-PAH (µg kg^{-1})	8030
pH (in distilled water)	7.8 ± 0.1
Water-Holding-Capacity (%)	20.4 ± 0.1
Water content (%)	10.1 ± 0.1

using the USEPA 8270C method by gas chromatography (HP 5880A) coupled with mass spectrometry (EM 5987).[24] Determination of 10 HMW-PAH content corresponded to the sum of fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k] fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i] perylene and indeno[1,2,3-c,d]perylene. TPH content was determined according to the USEPA 8015B method using flame inductively detector gas chromatography (HP5880A), Table 2.[25]

Table 2. Parameters observed during PAH-HMW biodegradation

Systems	Deg	R-Deg[a]	R-Deg[b]	pH	Humidity (%)
1	41.6±0.2	33.0±1.0	91.0±2.0	7.5±0.3	16.9±2.0
2	46.2±0.1	121.0±1.0	17.0±1.0	7.6±0.1	16.0±1.8
3	50.4±0.1	90.0±1.0	60.0±1.0	7.5±0.1	17.0±1.2
4	24.±0.1	22.0±1.0	5.1±1.0	7.7±0.1	9.0±2.0

Deg – Degradation (%). R-Deg – Rate of degradation (µg/kg.day^{-1}). [a] – 0 to 30 days of bioprocess. [b] – 30 to 60 days of bioprocess

Bioremediation tests

Polyethylene reactors of 0.22 length x 0.22 width x 0.09 m height were used, filled with 2 kg of soil treated by thermal desorption, containing 8030 µg kg^{-1} of residual 10 HMW-PAH. The soil humidity was adjusted with sterile water to 70-80% of water-holding-capacity and the pH was monitored once every seven days. In order to guarantee oxygen supply, the soil was aerated manually every 72 h using a glass stick. Soil sampling for experiment analysis was carried out by collecting 10 g of the soil from three equidistant locations. This process aimed to mix and to homogenise the soil samples to achieve a unique final sample.

The HMW-PAH biodegradation experiments were carried out at a temperature of 28 ± 1°C for 60 days. The established conditions for each reactor are summarized in Table 3. Three systems were constructed, where native microbiota to the soil were stimulated by: 1- addition of NPK (10:10:10) fertilizer (Vitaplan, Capitão Leônidas Marques, Brasil); 2- combination of fertilizer and glycerol (Vetec, Rio de Janeiro, Brasil); and 3- glycerol addition.[26] A fourth system was constructed with no treatment by biostimulation and served as a control reactor. For the determination of abiotic loss, silver nitrate solution 10% (m/v) was used as a biocide.

Table 3. Summary of the established conditions in the reactors

Conditions	Systems			
	1	2	3	4
Fertilizer (g kg^{-1})	0.10	0.10	no	no
Glycerol (mg kg^{-1})	no	0.63	0.63	no
Humidity control	yes	yes	yes	no
pH monitoring	yes	yes	yes	yes

The microbial degradation was calculated as a percentage of the sum of the 10 HMW-PAH concentrations at intervals of 0, 30 and 60 days of bioprocess. The rate of HMW-PAH biodegradation from intervals, 0-30 days and 30-60 days were determined using the following formula:

$$C_i\text{-}C_f / \Delta t$$

where: C_i was the sum of the 10 HMW-PAH soil concentration in µg kg^{-1} at initial time; C_f was the sum of the 10 HMW-PAH soil concentration in µg kg^{-1} at final time; and Δt was the time in days. The concentration of THB and TF was determined by using the technique of counting the Colony Forming Units (CFU) at 0, 7, 15, 30 and 60 days. Agar Nutrient (Merk, Darmstadt, Germany) added with 50 mg L^{-1} nystatin (Sigma, St. Louis, USA) was used for THB determination and 4% Agar Sabouraud (Merk, Darmstardt, Germany) added with 50 mg L^{-1} ampicillin (Sigma, St. Louis, USA) was used for TF quantification. Plates were incubated at 28 ± 1 ºC for 48 and 72 h to THB and TF, respectively.

RESULTS AND DISCUSSION

The characteristics of the soil samples summarized in Table 1 demonstrates that the initial concentration of the USEPA 16 priority PAH was 8430 µg kg^{-1}, of which approximately 95% of the mass corresponded to the sum of the 10 HMW-PAH. The significant difference between the HMW-PAH and LMW-PAH was caused by thermal treatment applied to the soil, which promoted the removal of practically 100% of these compounds. The presence of residual LMW-PAH after thermal desorption was possibly associated with chemical reactions of the HMW-PAH during the thermal process.

Granulometric distribution of the soil samples mostly of silt and fine sand, however, the particles of sand were of different diameters. The soil contained a significant concentration of organic carbon, nitrogen and phosphorus, which did not promoted an inhibition of microbial growth. The C:N:P ratio of 100:6:3 is considered appropriate for hydrocarbon removal.[27] The addition of fertilizer to the soil has not altered this property. The pH was in an acceptable range to run the PAH microbial degradation, but required the adjustment of water content.

Figure 1 shows the HMW-PAH concentrations determined in the soil samples after 60 days of bioprocess. The decrease of HMW-PAH concentration observed during the studied intervals proved that a microbial process had occurred. Considering an abiotic loss estimated at 10%, the best result was obtained through biostimulation with glycerol.

Addition of fertilizer and glycerol to the soil, individually or associated, promoted the removal of 41.6-50.4% of HMW-PAH,

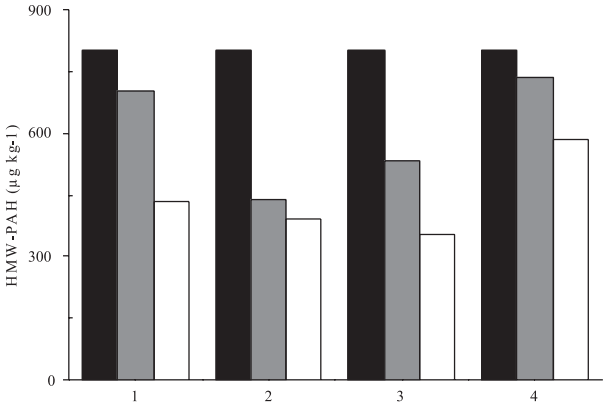

Figure 1. *HMW-PAH concentration during biostimulation tests. Addition of fertilizer (1); addition of fertilizer and glycerol (2); addition of glycerol (3); and control (4) at 0 days (■), 30 days (■) and 60 days (□)*

as shown in Table 2. According to the HMW-PAH biodegradation rate, determined during the period of 0-30 days, it was observed that the presence of glycerol, either combined with fertilizer or added individually was of significant importance for the removal of HMW-PAH, whereas fertilizer addition alone caused a slower removal. The increase of HMW-PAH biodegradation rate, related to the presence of glycerol was possibly associated with the fact that the compound served as a substrate for the synthesis of bioemulsifier and biosurfactant agents, as well as its action as a cosubstrate, considering that the HMW-PAH degradation occurs through mechanisms involving cometabolic pathways.

On the other hand, the minor rate of HMW-PAH degradation during the period of 0-30 days with the presence of fertilizer was possibly related to the initial consumption of microbiota from other carbon sources, such as lighter fractions of total petroleum hydrocarbons (TPH) as previously reported.[28] Most of the HMW-PAH uptake occurred later, between 30 and 60 days, corresponding to an increase of 64% in the biodegradation rate. It is important to highlight that HMW-PAH are not the preferred substrates of microorganisms and in most cases, these molecules are sorbed in the soil matrix, placing greater demands on microbial strategies of biodegradation.

For the period between 30 and 60 days, the rate of HMW-PAH biodegradation decreased significantly when compared to the first 30 days. A decrease of 86% occurred in the system with combined glycerol and fertilizer, and 33% in the system where only glycerol was applied. This drastic variation of HMW-PAH biodegradation rate was probably associated with the depletion of the glycerol and essential nutrients such as nitrogen and phosphorus.[29]

Decrease of pH compared with the initially determined value was observed in all reactors studied. This pH decrease may have been the result of microbial metabolism, but the soil buffer property may also have contributed to the pH decrease.

The results for THB and TF concentration are shown in Figure 2. It is of note that the curves possess similarities. Initially, the population was established around 10^7 and 10^5 CFU g^{-1} of soil for THB and TF, respectively, demonstrating that the soil nitrogen and phosphorus were concentrated at levels that guaranteed suitable nutrient supplies for the microbiota. The major increment of growth occurred on the seventh day, achieving almost one order of magnitude increase over initial values. After 60 days of experiments, the TBH and TF populations reached the observed values found in the control reactor, possibly due to the depletion of nutrients and the presence of toxic metabolites.

In soils PAH biodegradation is slower in comparison to other hydrocarbons. The HMW-PAH form a more complex group of molecules than the LMW-PAH and their biodegradation occurs through

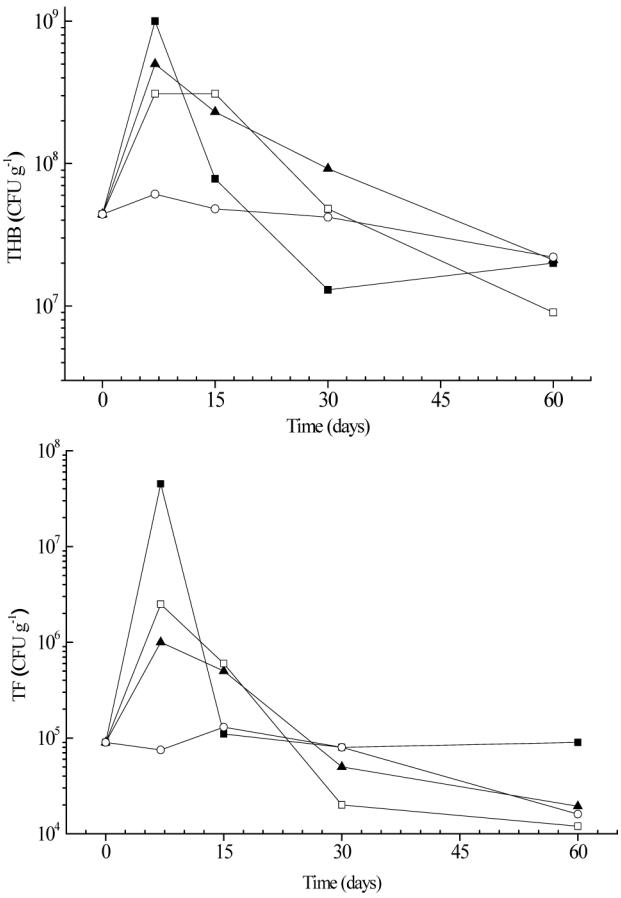

Figure 2. *Total heterotrophic bacteria (THB) and total fungi (TF) concentration during HMW-PAH biodegradation tests (■ – Addition fertilizer; □ - Addition of fertilizer and glycerol; ▲- Addition of glycerol; ○ – Control)*

cometabolic pathways, in most cases using LMW-PAH as cosubstrates. It is important to emphasize that the removal of persistent molecules in soil by cometabolism depends on the bioavailability of theses compounds.[30]

On the other hand, the non-assimilation of complex molecules by the microorganisms may determine the end point of several metabolic processes in hydrocarbon-contaminated soils.[31] In order to avoid negative impacts caused by the presence of toxic compounds, the microbial population uses a metabolic arsenal to guarantee its integrity and stability, through a mechanism known as tolerance. Tolerance acts as a mechanism of compensation against the caustic effects of the environment, where the microbial communities are established, increasing the chances of the removal of compounds with difficult degradation.[32]

Biostimulation with the application of fertilizer for the removal of hydrocarbons in soil are established and important methods for bioremediation and several examples are found in the literature.[33,34] On the other hand, the association of cosubstrates with to the fertilizer is establishing itself as a more efficient strategy for the removal of persistent hydrocarbons such as PAH. Glycerol appears as a potential cosubstrate candidate and good results have already been obtained when utilizing the compound in raw oil biodegradation.[35] Glycerol possesses important advantages compared to other cosubstrates: osmoregulatory properties being a preferred source of carbon in biosurfactant synthesis and availability on the market as a byproduct of the biodiesel industry, which decreases its cost and widens its scope of applications.[36-38]

Among the studied alternatives of native soil microbiota biostimulation for the removal of HMW-PAH, glycerol supplementation at

0.63 mg kg^{-1} of soil promoted the most positive effect on contaminants biodegradation.

CONCLUSION

The results describe alternatives for biodegradation of HWM-PAH-contaminated soil. The use of glycerol as a cosubstrate was the most suitable method of biostimulation of native microbiota and also increased the PAH biodegradation rate in 30 days of bioprocess.

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REFERENCES

1. Muckian, L.; Grant, R.; Doyle, E.; Clipson, N.; *Chemosphere* **2007**, *17*, 1535.
2. Mater, L.; Sperb, R. M.; Madureira, L. A. S.; Rosin, A. P.; Correa, A. X. R.; Radetski, C. M.; *J. Hazard. Mater*. **2006**, *136*, 967.
3. Enell, A.; Reichenberg, F.; Ewald, G.; Warfvinge, P.; *Chemosphere* **2005**, *61*, 1529.
4. Watanabe, K.; *Curr. Opin. Biotechnol*. **2001**, *12,* 237.
5. Daugulis, A. J.; McCracken, C. M.; *Biotechnol. Lett*. **2003**, *25*, 1441.
6. Gong, Z.; Alef, K.; Wilke, B. M.; Li, P.; *J. Hazard. Mater*. **2007**, *143*, 372.
7. Kaplan, C. W.; Kitts, C. L.; *Appl. Environ. Microbiol*. **2004**, *70*, 1777.
8. Johnsen, A. R.; Wick, L. Y.; Harms, H.; *Environ. Pollut*. **2005**, *133*, 71.
9. Burmølle, M.; Hansen, L. H.; Sørensen, B. J.; *Microb. Ecol*. **2007**, *54*, 352.
10. Carmichael, L. N.; Pfaender, F. K.; *Biodegradation* **1997**, *8*, 1.
11. Das, K.; Mukherjee, A. K.; *Bioresour. Technol*. **2006**, *98*, 1339.
12. Zhang, X. X.; Cheng, S. P.; Zhu, C. J.; Sun, S. L.; *Pedosphere* **2006**, *16*, 555.
13. Popp, N.; Schlömann, M.; Mau, M.; *Microbiology* **2006**, *152*, 3291.
14. Chaîneau, C. H.; Rougeux, G.; Yéprémian, C.; Oudot, J.; *Soil. Biol. Biochem*. **2005**, *37*, 1490.
15. Margesin, R.; Hämmerle, M.; Tscherko, D.; *Microbial. Ecol*. **2007**, *53*, 259.
16. ABNT; *NBR 7181. Solo – Análise granulométrica.*
17. EMBRAPA; *Manual de métodos de análise de solo,* SNLCS: Brasil, 1979.
18. Watwood, M. E.; White, C. S.; Dahn, C. N.; *Appl. Environ. Microbiol.* **1991**, *57*, 717.
19. USEPA; *Method SW-846 9060. Total Organic Carbon*.
20. USEPA; *Method 351.2. Determination of total Kjeldahl nitrogen by semi-automatic colorimetric*.
21. USEPA; *Method 365.3. Phosphorus, all forms (colorimetric, ascorbic acid, two reagent)*.
22. USEPA; *Method 3540C. Soxhlet extraction*.
23. USEPA; *Method 3630C. Silica gel cleanup*.
24. USEPA; *Method 8270C. Semivolatile organic compounds by gas chromatography/mass spectrometry*.
25. USEPA; *Method 8015B. Nonhalogenated organics by gas chromatography/flame ionization detector*.
26. Moorthi, P. S.; Deecaraman, M.; Kalaichelvan, P. T.; *Adv. Biotechnol.* **2008**, *8*, 34.
27. Franco, I.; Contin, M.; Bragato, G.; De Nobili, M.; *Geoderma* **2004**, *121*, 17.
28. Hesselsoe, M.; Bjerring, M. L.; Henriksen, K.; Loll, P. Nielsen, J. L.; *Biodegradation* **2008**, *19*, 621.
29. Ballaminut, N.; Matheus, D. R.; *Braz. J. Microbiol.* **2007**, *38*, 248.
30. Joner, E. J.; Hirmann, D.; Szolar, O. H. J.; Todorovic, D.; Leyval, C.; Loibner, A. P.; *Environ. Pollut*. **2004**, *128*, 429.
31. Chaillan, F.; Le Flèche, A.; Bury, E.; Phantavong, Y-H.; Grimont, P.; Saliot, A.; Oudot, J.; *Res. Microbiol.* **2004**, *155*, 587.
32. Baek, K. H.; Yoon, B. D.; Kim, B. H.; Cho, D. H.; Lee, I. S.; Oh, H. M.; Kim, H. S.; *J. Microbiol. Biotechnolol*. **2007**, *17*, 67.
33. Machín-Ramirez, C.; Okoh, A. I.; Morales, D.; Mayolo-Deloisa, K.; Quintero, R.; Trejo-Hernández, M. M. R.; *Chemosphere* **2008**, *70*, 737.
34. Oliveira, F. J. S.; de França, F. P.; *Appl. Biochem. Biotechnol.* **2005**, *121-124*, 593.
35. Zhang, G. L.; Wu, Y. T.; Qian, X. P.; Meng, Q.; *J. Zhejiang Univ. Sci*. **2005**, *6B*, 725.
36. Nevoigt, E.; Stahl, U.; *FEMS Microbiol. Rev*. **1997**, *21*, 231.
37. Batista, S. B.; Mounteer, A. H.; Amorim, F. R.; Tótola, M. R.; *Bioresour. Technol*. **2006**, *97*, 868.
38. Ooi, T.; Yong, K. C.; Hazimah, A. H.; Dzulkefly, K.; Wan Yunus, W. M. Z.; *J. Oleo Sci.* **2004**, *53*, 29.