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BENZENE BIODEGRADABILITY TESTS ON A RESIDUAL GRANITIC SOIL

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ABSTRACT

Benzene is a component of most fuels and an important industrial solvent. It is widely spread in soils, sediments and groundwater near diesel/gasoline service stations, refineries and in other industrial areas. For benzene, as other BTEX's, the most used in-situ remediation technology is Soil Vapour Extraction followed by bioremediation. In the present work, a bacterial consortium was developed for the systematic study of the benzene bioremediation of soils contaminated. The effectiveness of biodegradation was studied in a residual granitic soil. The developed microorganism consortium was collected from a protection area of crude storage tank**s** in a refinery. Our results showed that the developed consortium was efficient in degrading benzene (about 18 days for soil remediation contaminated with 140 mg of benzene per kg of residual granitic soil in lab-scale studies).

1. INTRODUCTION

One of the major sources of petroleum hydrocarbons contamination in soils and groundwater is the leaking from underground storage tanks (USTs) [1, 2]. When this occurs, the products may spread through the subsoil in all phases: adsorbed in the mineral grains, volatized in the gaseous phase or dissolved in the water phase.

There are several technologies that have been applied to remediate soils contaminated with petroleum hydrocarbon in the vadose zone. Soil Vapour Extraction (SVE) is one of these technologies [3], normally used for the remediation of unsaturated zones contaminated with high concentrations of volatile organic compounds (VOCs). However, this technology is not the most effective when low concentrations of VOCs or non-volatile organic compounds (NVOCs) are present [4].

Bioremediation is an effective, economical and environmentally friendly technology for petroleum hydrocarbons, its success depending on the ability of the inoculated microbial degraders to remain active in the natural environment. This is generally achieved via bioaugmentation, biostimulation or using both these measures [5, 6].

Bioventing is a mixed technology that combines SVE with bioremediation; the air flow is used to remove VOCs through volatization and to enhance the aerobic biodegradation of semivolatile organic compounds (SVOCs), NVOCs and residual VOCs left in the soil by SVE [2, 4].

The aim of this work is to study the effectiveness of benzene biodegradation in a residual granitic soil (Srγ). The consortium of microorganisms was obtained from a soil contaminated with crude.

2. MATERIALS AND METHODS

2.1. Chromatographic analysis

Pro-analysis benzene were obtained from Panreac Quimica SAU, with purity≥ 99,5%.

The quantification of benzene was made by isothermal (200 ºC) gas chromatography [7]. Determinations were performed in a GC-Shimadzu-2010 chromatograph equipped with a FID and a TRB-5 Teknokroma column (30 m × 0.25 mm ID; 0.25 µm). The carrier gas was N_2; the sample injection was applied in splitless mode. The operating temperature for both the injector and the detector was 250 ºC. 100 µL of air phase was injected onto the gas chromatograph equipment.

2.2. Soils preparation and characterization

The soil used to develop the bacteria consortium (BSoil) was collected from the protection area of crude storage tanks in a refinery. This soil had a large grain size distribution with relevant clay fraction and a great amount of organic

matter. The total petroleum hydrocarbons (TPH) was determined using the colorimetric method (Remediaid test kits from Chemetrics), with a result value of 14,7 g of TPH per kilogram of BSoil.

The samples of Srγ were collected in Porto region from a recent slope excavation, at 2-3 m depth. This soil is common in the subsurface of the northern Portuguese territory. The geotechnical characterization tests indicated 7% of clays, 28% of silts, 60% of sands and 5% of gravel. Particle density, bulk density and porosity were 2.68, 1.06 and 60%, respectively. Physical and chemical characterization revealed a pH 4.56, soil conductivity at 25 ºC of 66 µS/cm, very low organic matter (≤ 0.81%) and loss on ignition of 11.80%. Major elements were determined by X -ray energy dispersive fluorescence with the following results: SiO_2 (50.72%), Al_2O_3 (23.64%), Fe_2O_3 (7.02%), MnO (0.08%), CaO (0.10%), MgO (2.29%), Na_2O (< 0.20%), K_2O (2.33%), TiO_2 (1.50%), P_2O_5 (0.21%).

The Srγ was previously dried in an oven for 72 hours at 50 ºC for bioremediation tests.

2.3. Benzene biodegradation tests

The studies of benzene biodegradation were performed in three different stages: initially in mineral liquid medium (MMA) [7] in sterilized Erlenmeyer flasks, afterwards in a Srγ in sterilized Erlenmeyer flasks and finally in a Srγ in columns.

In order to developed the selected consortium and adapt the microorganisms to the contaminant, successive transfers (T1, T2 and T3) were carried out in liquid phase (Figure 1). The cultures were developed aerobically in MMA [7], in sterilized Erlenmeyer flasks (500 mL) closed with Teflon valves (MininertTM, VICI®, Valco instruments) and incubated at 28°C, with shaking (150 rpm).

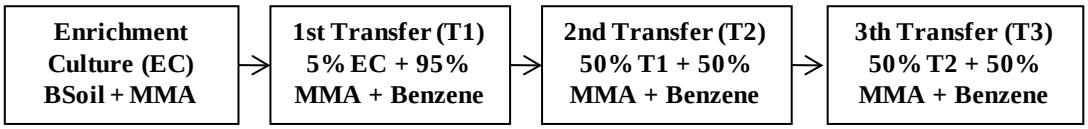


Figure 1 – Successive transfers in liquid phase tests

Various contamination levels of benzene were tested to select the most adequate working concentration range (14, 18, 21 and 28 mg/100 mL inoculums). Benzene concentration was monitored and the incubation period ended when the benzene concentration in the gas phase reached 0.5 mg per litre of air. At the end of the experiment pour-plate technique was used for Colony Forming Units (CFU) counting [7].

The studies in soils were first performed using sterilized Erlenmeyer flasks (500 mL) closed by Mininert valves. Each flask was prepared with 160 g of dry Srγ sample and 40 mL inoculum of the second transfer of the enrichment culture, resulting in 200 g of wet soil with a moisture content of 20%. Different benzene supplements were used: 88, 105 and 140 mg of benzene per kg of wet Srγ. These soil tests were referenced by Sr_88, Sr_105 and Sr_140, respectively. Non inoculated media was used for blank tests (Blank88, Blank105 and Blank140) to account for the abiotic degradation of benzene. The tests were done at constant temperature: 26 ºC. The incubation period was limited by a residual concentration of 0.5 mg benzene per litre of air.

Studies in columns were made using a lab-scale reactor. It was a cylindrical stainless steel column, 50 cm height and 10 cm inner diameter with two lateral sampling points. The contamination grade selected was 140 mg of benzene per kg of wet Srγ.

The column was loaded with 2000 g of wet Srγ (1600 g of dried soil and 400 mL of inocula) contaminated with 280 mg of benzene. These soil tests were referenced by Column_1 and Column_2. A non-inoculated media was used for blank tests to account the abiotic degradation of benzene: CBlank (1600 g of dried Srγ, 400 mL of mineral medium and 280 mg of benzene) and CBlankEst (1600 g of dried sterilized Srγ, 400 mL of mineral medium and 280 mg of benzene). The tests were carried out at room temperature (21-23 ºC), that was monitorized inside and outside the column. The incubation time was dictated by the value defined as the residual concentration of benzene in the gas phase (0.5 mg of benzene per liter of air). At the end of the experiment the biomass (CFU) was quantified [7].

3. RESULTS

3.1. Tests in liquid medium

According to the results obtained in the different transfers (Figure 2) all contamination levels were suitable for the bacterial consortium used. In the first transfer the remediation period was longer but all tests showed high remediation efficiency; thus, the consortium was considered appropriate for all the concentrations tested. The microorganisms after first transference showed higher degradation. This is in accordance with the expected as it is known that, whenever there is a stress, most of the microorganisms exhibit an explicitly different mechanisms, which provide them higher tolerance and resistance.

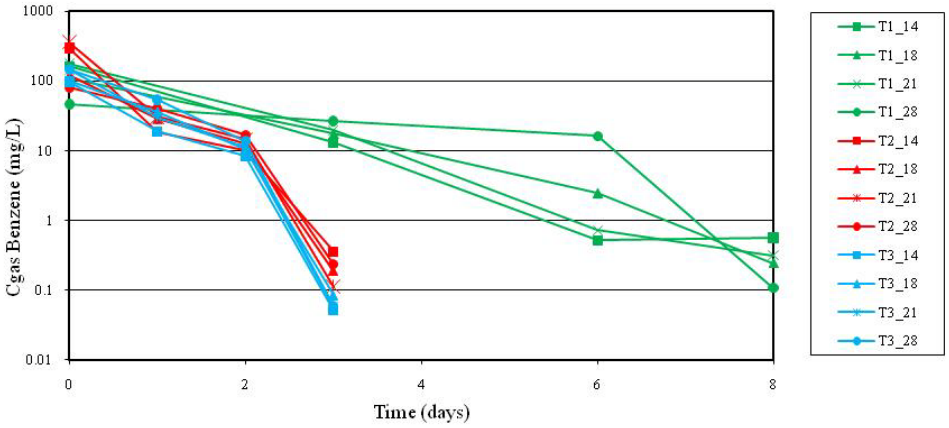
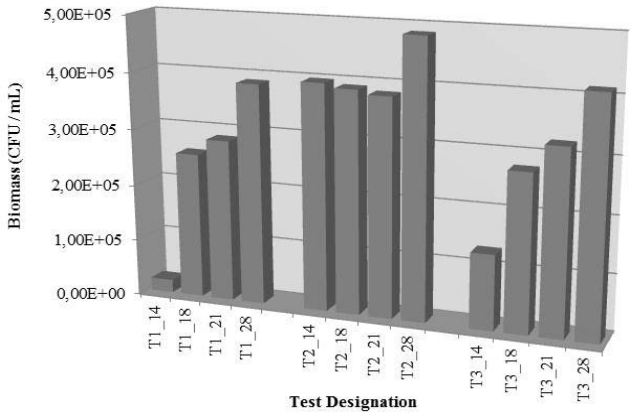


Figure 2 – Benzene degradation in different transfers. First transfers (T1), second transfers (T2) and third transfers (T3) with contamination levels of 14, 18, 21 and 28 mg of benzene /100 mL inoculum, respectively.

The CFU evaluations (Figure 3) demonstrated that the highest bacterial growth was obtained in the second transfer and that, in the same transfer, it increases with the amount of substrate (benzene) added to the culture. Based on these results the inoculum obtained in the second transfer with 28 mg of benzene per 100 mL of inoculum (T2_28) was considered as the most appropriate for further studies on Sry.

Figure 3 – Tests in liquid medium - biomass quantification at the end of the experiment



3.2. Tests in a residual granitic soil samples

The studies of the soil bioremediation in flasks (Figure 4) showed that the endogenous microorganisms of the Sry were not able to degrade benzene. The remediation time to reach the stipulated low concentration for the different benzene supplements used was identical (about 9 days).

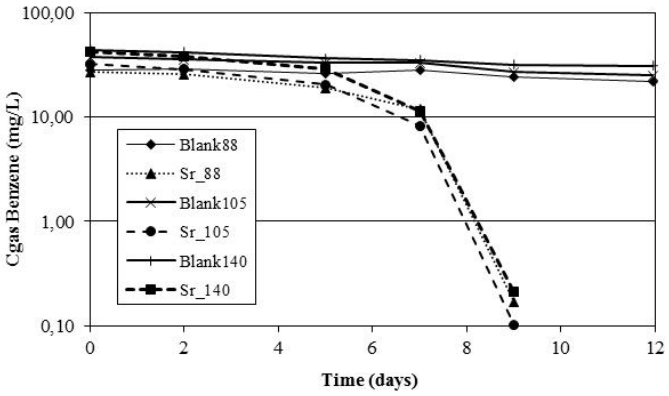


Figure 4 - Benzene degradation tests in flasks contaminated with 88, 105 and 140 mg of benzene per kg of residual granitic soil (dashed lines; Sr_XX) and non-inoculated tests (BlankXX; continuous line).

The results obtained in the columns tests are plotted in Figures 5 and 6. These results indicated identical benzene degradation comparatively with those obtained in flasks but with higher biomass and longer remediation time. One parameter that contributed for these periods was the different temperatures in the experiments. Both “Blanks” (sterile

and not sterile) displayed similar results evidencing that endogenous microorganisms of the granitic soil are not able to degrade benzene.

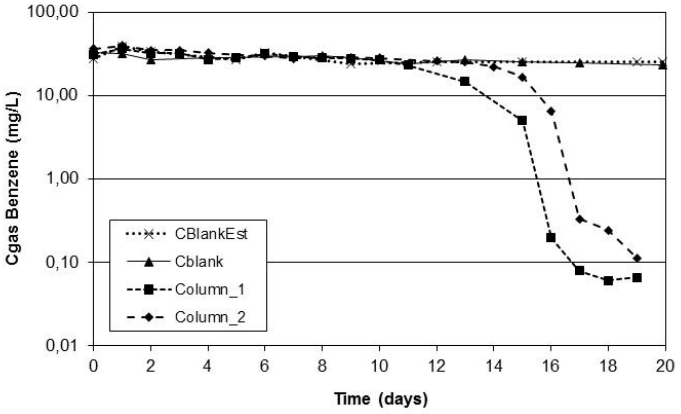


Figure 5 - Benzene degradation tests in columns contaminated with 140 mg of benzene per kg of residual granitic soil (Column_1 and Column_2) and non-inoculated test (CBlank and CBlankEst) with the same benzene concentrations.

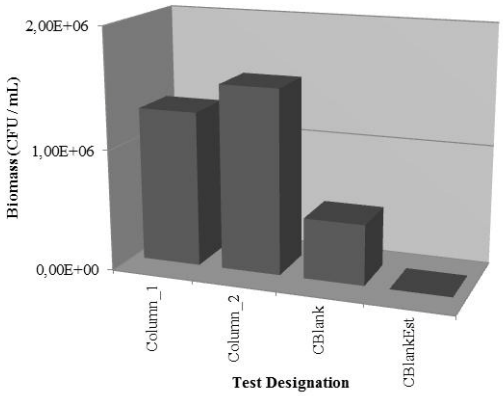


Figure 6 – Column tests - biomass quantification at the end of the experiment.

4. CONCLUSIONS

The bacterial consortium developed proved to be suitable for the degradation of benzene in the considered concentration range. The number of microorganisms that grew in liquid medium was lower than for Srγ.

The final benzene concentration in the gas phase in all tests performed was below the residual concentration of 0.5 mg benzene per litre of air. The results obtained in the soil tests, for both scales considered, and in liquid medium showed that the remediation period was longer for the soil, but the final remediation efficiency was the same for all of them. Therefore, the consortium developed proved to be able to biodegrade benzene in both media at the tested scale. This method may also be helpful for benzene remediation in natural soils (Srγ) using "green" technology.

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