Eurasian J Soil Sci 2015, 4 (3) 153 - 160

Eurasian Journal of Soil Science

Journal homepage : http://fesss.org/eurasian_journal_of_soil_science.asp

Assisted bioremediation tests on three natural soils contaminated with benzene

Maria Manuela Carvalho [a,b,*], Maria Cristina Vila [b,c], Cristina Delerue-Matos [a], Maria Teresa Oliva-Teles [a], António Fiúza [b,c]

[a] REQUIMTE, Institute of Engineering of Porto, Polytechnic Institute of Porto, Porto, Portugal
[b] CIGAR, Faculty of Engineering, University of Porto, Porto, Portugal
[c] CERENA, Technical Institute of Lisbon, Lisbo, Portugal

Abstract

Bioremediation is an attractive and useful method of remediation of soils contaminated with petroleum hydrocarbons because it is simple to maintain, applicable in large areas, is economic and enables an effective destruction of the contaminant. Usually, the autochthone microorganisms have no ability to degrade these compounds, and otherwise, the contaminated sites have inappropriate environmental conditions for microorganism's development. These problems can be overcome by assisted bioremediation (bioaugmentation and/or biostimulation). In this study the assisted bioremediation capacity on the rehabilitation of three natural sub-soils (granite, limestone and schist) contaminated with benzene was evaluated. Two different types of assisted bioremediation were used: without and with ventilation (bioventing). The bioaugmentation was held by inoculating the soil with a consortium of microorganisms collected from the protection area of crude oil storage tanks in a refinery. In unventilated trials, biostimulation was accomplished by the addition of a nutrient mineral media, while in bioventing oxygen was also added. The tests were carried out at controlled temperature of 25 ºC in stainless steel columns where the moist soil contaminated with benzene (200 mg per kg of soil) occupied about 40% of the column's volume. The processes were daily monitored in discontinued mode. Benzene concentration in the gas phase was quantified by gas chromatography (GC-FID), oxygen and carbon dioxide concentrations were monitored by respirometry. The results revealed that the three contaminated soils were remediated using both technologies, nevertheless, the bioventing showed faster rates. With this work it was proved that respirometric analysis is an appropriate instrument for monitoring the biological activity.

Article Info

Received : 15.07.2014
Accepted : 18.01.2015

Keywords: bioremediation, bioventing, respirometry, soils, benzene

Introduction

Population growth and the resultant development of large high-density urban populations, together with parallel global industrialization, have placed major pressures on our environment, potentially threatening environmental sustainability. This has resulted in the buildup of chemical and biological contaminants throughout the biosphere but most notably in soils and sediments (Ward et al., 2004). The large-scale manufacturing, processing and handling of chemicals have led to serious surface and subsurface soil contamination with a wide variety of hazardous and toxic compounds. The resultant accumulations of the

* Corresponding author.
REQUIMTE, Institute of Engineering of Porto, Polytechnic Institute of Porto, Rua Dr. António Bernardino de Almeida, 431, 4200-072 Porto, Portugal
Tel.: +351228340500 E-mail address: mmc@isep.ipp.pt
e-ISSN: 2147-4249 DOI: http://dx.doi.org/10.18393/ejss.2015.3.153-160

various chemicals in the environment, particularly in the soils, are of significant concern because of their toxicity, including their carcinogenicity, and also because of their potential to bio-accumulate in living systems (Singh et al., 2004). Petroleum products are some of the most widely used chemicals; accidents and leakages are unavoidable and their constituents are often found in contaminated soils. According to the European Environment Agency (EEA) the potentially polluting activities throughout Europe are estimated at nearly 3 million and the soil contaminated sites are almost 250 000. Aromatic hydrocarbons were detected in 6% of such sites. If current trends continue, the number of sites needing remediation will increase by 50% by 2025 (EEA, 2010).

Benzene is a natural constituent of crude oil and is one of the most basic petrochemicals; it is present in most fuels; it is an important industrial solvent and is used as a chemical intermediate in the production of other chemicals (ATSDR, 2007; Morgan et al., 2009). Benzene is released to the soil environment through industrial discharges, waste disposal, fuel leaks or spillages (ATSDR, 2007). Upon release into the soil benzene will tend to disperse through the unsaturated zone eventually reaching the saturated zone (Morgan et al., 2009), causing degradation of the characteristics of soil and groundwater. Benzene is a multisite carcinogen to humans and no safe level of exposure can be recommended (WHO, 2000). The World Health Organization (WHO) found that the most significant adverse effects from prolonged exposure to benzene are hematotoxicity, genotoxicity and carcinogenicity (WHO, 2000). In European countries the official data about benzene background concentrations in soil is limited. However, based on the known releases of benzene into the environment documented by UK Environment Agency, the European Chemicals Bureau (ECB) estimates the value of 0.02 mg kg^{-1} for the benzene background soil concentration across Europe (Morgan et al., 2009a).

There are several technologies that have been applied to remediate soils contaminated with petroleum hydrocarbon in the vadose zone. Soil vapor extraction (SVE) is a technology normally used for the remediation of unsaturated zones contaminated with high concentrations of volatile organic compounds (VOCs) (Albergaria et al., 2008). However, when low concentrations of VOCs or non-volatile organic compounds (NVOCs) are present, this technology is not the most effective (Malina et al., 2002). Bioremediation is generally considered an environmental friendly and cost-effective technology for the removal of hazardous contaminants, where by-products are non-toxic (Singh et al., 2004). Bioremediation processes are among the best approaches to restoring contaminated soils; its success depends on the ability of microbial degraders to remain active in the contaminated environment and on the bioavailability of the contaminants to microorganisms. To improve the bioremediation process, besides a competent microbe able to degrade the contaminant carbon source, other parameters must be taken into account, e.g. water, oxygen, and usable nitrogen and phosphorous sources. The efficiency of the remediation process under natural conditions could be committed by the lack of any of the mentioned parameters. Strategies involving the addition of planted crops, nutrients, bioaugmentation and/or biostimulation have been reported (Fernandes et al., 2009; Lin et al., 2010; Tyagi et al., 2011) as allowing higher degradation rates. Due to limitations associated with bioaugmentation and biostimulation when applied individually, these techniques are emerging as complementary (Tyagi et al., 2011).

In this work three natural sub-soils were used to perform the bioremediation studies. Biostimulation (without and with ventilation - bioventing) and bioaugmentation with a microbial consortium was made for bioremediation of benzene contaminated soils. As the soil properties strongly affect the selection and the success of the remediation technology, its study was included in this work. Although Santhaveerana Goud et al. (2010) suggest that in soils with more than 10% of fines lower biodegradation rates were exhibited, in this study it was found that bioremediation rates are mainly affected by the mineralogical composition of the fine fraction.

Material and Methods

Materials and Analytical Methods

Benzene was of pro-analysis grade, obtained from Panreac Quimica SAU, with purity ≥ 99,5%. In this work three different sub-soils were studied: Limestone (CL), residual granitic (SR) and schist (XT), that are natural and no contaminated soils used for remediation tests. Soils samples were collected in the north of Portugal from fresh slope excavations, near the surface in the vadose zone. The samples were stored in appropriate containers at room temperature and protected from light. Before bioremediation tests, the samples were previously dried in an oven for 72 hours at 50 ^{0}C.

Another soil contaminated with hydrocarbons (BSoil) was used to extract the microbial consortium. It was collected from the protection area of crude storage tanks in a refinery. The sample was stored in a container at room temperature and protected from light.

Standard methodologies were used for the soil characterization. In CL, SR, and XT samples a large number of properties were evaluated: particle size distribution (LNEC E196/1966), plasticity (NP 143/1969), particle density (NP83/1965), bulk density (ASTM D4531-86), water content (NP84/1965), water-holding capacity (USSLS, 1954), permeability (ASTM D 2435/2004), pH and conductivity at 25^{0}C (Carter et al., 2006; Jones, 2001) and nitrogen and phosphorus content (Clesceri et al., 1998). The mineral composition was determinate using X-ray diffraction in an accredited external laboratory.

In the four soil samples (CL, SR, XT, and BSoil) total organic carbon (TOC) was determined by TOC-VCSN (Shimadzu) equipped with a Solid Sample Module SSM-5000A (Shimadzu) and total petroleum hydrocarbons (TPH) were quantified by colorimetric method using Remediaid test kits from Chemetrics. During experiments, the benzene in gas phase was monitored by isothermal (200 ºC) gas chromatography. The gas chromatography was performed in a GC-Shimadzu-2010 chromatograph equipped with a flame ionization detector (FID) and a TRB-5 Teknokroma column (30 m × 0.25 mm ID; 0.25 μm). The carrier gas was N_2; 100 μL of gas sample was injected in splitless mode. The operating temperature for both injector and detector was 250 ºC. Carbon dioxide and oxygen concentrations in the gas phase were determined using a respirometer (Servomex - 5200 Multipurpose) equipped with high accuracy paramagnetic transducers to measure oxygen and infrared detector with a single wavelength to quantify carbon dioxide.

Inoculum Preparation

In order to develop the selected microbial consortium obtained from BSoil, successive microbial cultures (transfers T1 and T2) were carried out in liquid phase. The cultures were developed aerobically in mineral media (MMA) containing 28 mg of xylene per 100 mL (Carvalho et al., 2010). Sterilized Erlenmeyer flasks closed with Teflon valves (MininertTM, VICI®, Valco instruments) were used. These cultures were incubated at 28°C, with shaking (150 rpm). The incubation period ended when the contaminant concentration in the gas phase reached 0.5 mg per liter of air. When the experiments ended the biomass was quantified. The number of Colony Forming Units (CFU) was counted using spread-plate technique where 0.5 mL of inoculum was sequentially diluted in sterile saline solution (0.85% NaCl; w/v), spread into LB medium agar and incubated at 28 ºC for 3 days.

The obtained microorganism's cultures were used in bioremediation tests for soil bioaugmentation.

Biodegradation Tests

The biodegradation tests were performed at controlled temperature of 25ºC in stainless steel columns (50 cm high, 10 cm inner diameter); the reactor was partially (≈40%) loaded with 2000 g of wet soil; the contamination was subsequently induced with 200 mg of benzene per kilogram of wet soil. Microbial transformations in soils are moisture dependent, the optimum soil moisture content for most aerobic processes ranges from 40 to 60 % of water-holding capacity (Margesin et al., 2005). In this study it was used about 50% of water-holding capacity; the soils moisture content were 11.0, 25.0 and 14.5 % in CL, SR and XT, respectively. In inoculated trials, enrichment cultures (inoculum) were used to humidify the previously dry soils and proceeding to its bioaugmentation and biostimulation; in the blank tests, sterilized water was used. In the bioventing tests, oxygen was supplied through the daily introduction of air into the system, by passing a flow rate of 20 mL min^{-1} for 15 minutes. The tests designation and specification are provided in Table 1.

Table 1. Biodegradation tests designation and specification

Soil sample	Bioremediation (tests without ventilation)		Bioventing (tests with ventilation)	
	Non inoculated	Inoculated	Non inoculated	Inoculated
Limestone	BbCL_Bz	BCL_Bz	BVbCL_Bz	BVCL_Bz
Granite	BbSR_Bz	BSR_Bz	BVbSR_Bz	BVSR_Bz
Schist	BbXT_Bz	BXT_Bz	BVbXT_Bz	BVXT_Bz

The concentrations of benzene in the gas phase were daily monitored in all bioremediation tests; the remediation times were dictated by the value defined as the residual concentration (0.5 mg of benzene per L of air). In bioventing tests the concentrations of CO_2 and O_2 in the gas phase were also monitored daily. At the

end of the experiment, the biomass (CFU) was quantified by previous extraction in sterile saline solution (0.85% NaCl; w/v) and determined by the method of serial dilution on LB plates.

The bioremediation efficiency was calculated based on relationships between the concentrations of benzene in different phases, obtained from sorption in previous studies (Carvalho, 2014). The Freundlich adopted parameters are presented in Table 2.

Table 2. Freundlich model fitting parameters (Carvalho, 2014)

Freundlich model equation	Limestone (CL)	Granite (SR)	Schist (XT)
$C_{soil} = K_F C_{gas}^n$	K_F= 2,9751 n = 0,718 R^2 = 0,9623	K_F= 0,3154 n = 1,155 R^2 = 0,9163	K_F= 0,5982 n = 0,969 R^2 = 0,8852

Results and Discussion

Soil Characterization

The soil properties before contamination are provided in Table 3. From the Table 3 it should be highlighted that:

a) The limestone (CL) displayed clay minerals (kaolinite) in its mineral composition, its fraction of fines was lower than schist and highest than granite, nonetheless it had the largest fraction of clay (particles diameter < 0.002 mm) and the greater plasticity index;

b) The granite (SR) exhibited abundance of clay minerals (kaolinite and montmorillonite) in its mineral composition, it had the lowest fraction of fines (<0.074 mm), its clay fraction was lower than limestone and highest than schist; however it had the greater clay activity. This soil presented the highest water-holding capacity, the larger porosity but the lowest permeability;

c) The schist (XT) exhibited the highest permeability and the largest fraction of fines (< 0.074 mm), nonetheless the fines are not plastic and the clay fraction is the lowest (3%). Clay minerals were not detected in schist. Its porosity is similar to limestone and it is lower than granite's;

d) The studied soils presented very low organic matter content and similarity on the permeability coefficients and on the pH, and consequently its effects on the remediation is invaluable.

Biodegradation Tests

The results of bioremediation and bioventing tests are presented in Figures 1 and 2. In Tables 4 and 5 are respectively presented the final biomass quantification results and the achieved efficiency in the different assays.

From the results, it is possible to remark that:

a) The microorganisms can degrade benzene, in both bioremediation and bioventing;

b) The lowest remediation time occurred in the bioventing tests, being the major difference (14 days) observed in granite;

c) For the same type of technology (bioremediation or bioventing), the remediation rate is faster in schist and slower in granite, the difference is more pronounced in bioremediation tests;

d) Respirometric parameters (CO_2 and O_2) proved to be good indicators of biological activity. Through the time evolution of these variables it is possible to detect the different steps in microbial activities, it is clearly the coincidence between the start of microbial activity (sudden increase in CO_2 and simultaneously decrease of O_2) and the benzene concentration drop (Cgas);

e) The final biomass is higher in the inoculated tests (B and BV) than in the blanks (Bb and BVb). In the same type of test, the lowest final biomass was observed in granite;

f) The final efficiency (Ef_f) was very high (≥ 99.3 %) and similar for all inoculated tests;

After 7 days, the calculated efficiency (Ef_7) was highest for schist and lowest for granite. Based on the efficiency after 7 days (Ef_7), the results revealed low rates of remediation for both limestone and granite, being more pronounced in non-ventilated tests which highlight the importance of venting on the process.

Table 3. Soils properties

Mineral Composition (decreasing order of relative occurrence)	CL	Calcite, kaolinite, mica, quartz and hematite
	SR	Kaolinite, muscovite, montmorillonite, quartz, potassium feldspars and hematite
	XT	Chlorite, mica, quartz and sodium feldspars
Grain size distribution	CL	Clay: 15 %; Silt: 30 %; Sand: 35 %; Gravel: 20 % < 0.074 mm = 47 %
	SR	Clay: 7 %; Silt: 28 %; Sand: 60 %; Gravel: 5 % < 0.074 mm = 37 %
	XT	Clay: 3 %; Silt: 79 %; Sand: 18 %; Gravel: 0 % < 0.074 mm = 87 %
Plasticity	CL	Plasticity index: 9; Clay activity: 0.6
	SR	Plasticity index: 7; Clay activity: 1
	XT	Plasticity index: non plastic
Water-holding capacity (WHC) Used water content (W) Porosity (η) Permeability (k)	CL	WHC = 20.1 %; W = 11 % η = 44 %; k = 7.91E-6 ms^{-1}
	SR	WHC = 46.5 %; W = 25% η = 60 %; k = 7.57E-6 ms^{-1}
	XT	WHC = 29.3 %; W = 14.5 % η = 47 %; k = 9.88E-6 ms^{-1}
Total petroleum hydrocarbon (TPH) Total organic compound (C) Nitrogen content (N) Phosphorus content (P)	BSoil	TPH = 296 mg kg^{-1} C = 1.33 %; N = 0.05 %; P = 0.04 % C : N : P = 120 : 4.5 : 3.6
	CL	TPH = 0. 46 mg kg^{-1} C = 0.651 %; N = 0.017 %; P = 0.068 % C : N : P = 120 : 3.1 : 12.5
	SR	TPH = 0.35 mg kg^{-1} C = 0.396 %; N = 0.022 %; P = 0.172 % C : N : P = 120 : 6.7 : 52.1
	XT	TPH = 0.55 mg kg^{-1} C = 0.498 %: N = 0.013%; P = 0.055 % C : N : P = 120 : 3.1 : 13.3
pH Condutivity	CL	pH = 6.8; Conductivity = 295.0 µS cm^{-1}
	SR	pH = 5.8; Conductivity = 24.3 µS cm^{-1}
	XT	pH = 6.1; Conductivity = 67.1 µS cm^{-1}
Biomass	CL	1.8 E+04 CFU $g^{-1}$of soil
	SR	4.1 E+04 CFU g^{-1} of soil
	XT	3.6 E+04 CFU g^{-1} of soil

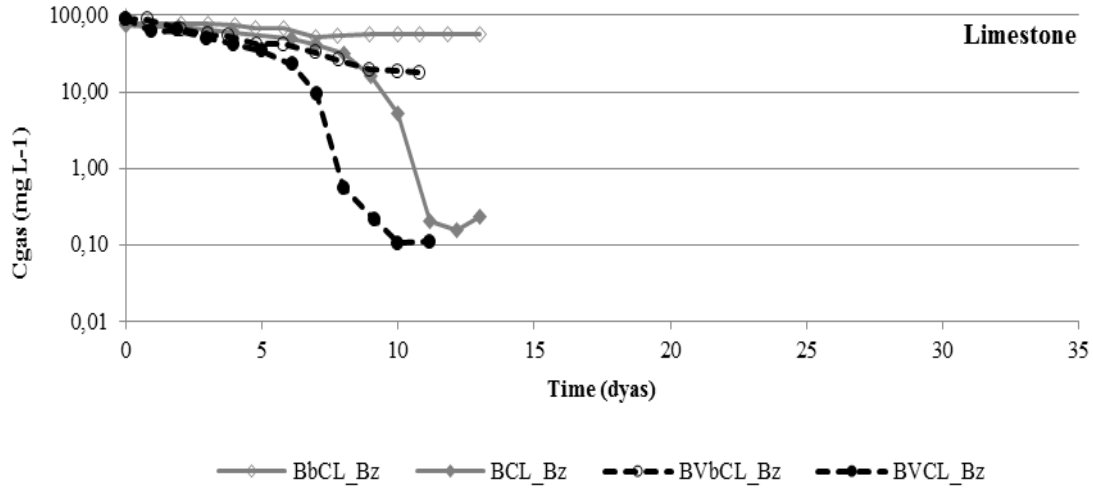
Table 4. Final biomass quantifications

Biomass (CFU g^{-1} of soil)					
BbCL_Bz	5.0E+4	BbSR_Bz	2.7E+4	BbXT_Bz	0
BVbCL_Bz	2.7E+4	BVbSR_Bz	2.6E+4	BVbXT_Bz	3.3E+4
BCL_Bz	4.6E+7	BSR_Bz	2.4E+6	BXT_Bz	9.1E+6
BVCL_Bz	7.7E+7	BVSR_Bz	1.2E+7	BVXT_Bz	8.3E+7

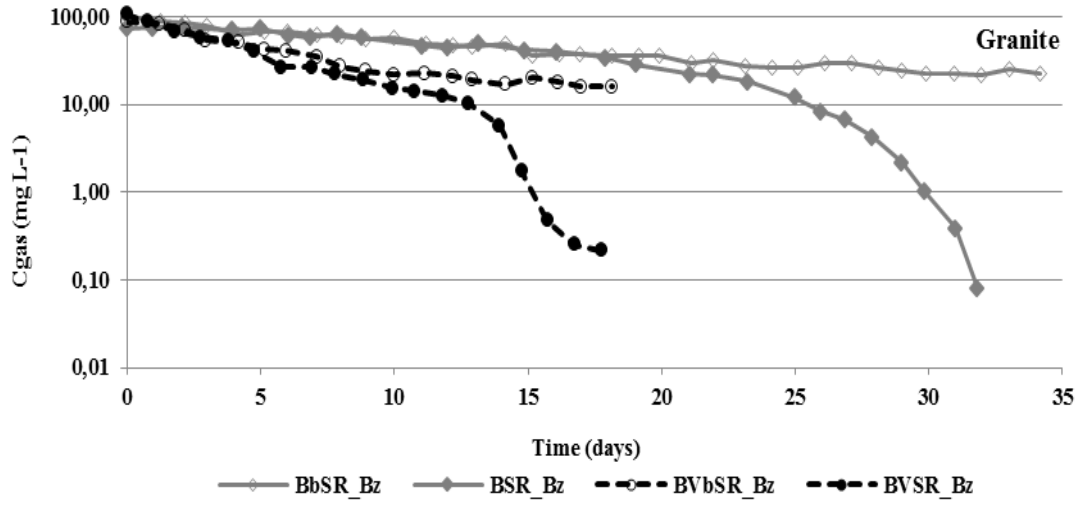
Table 5. Efficiency quantification

Test designation	Ef_7 (%)	Ef_f (%)	t (days)	Test designation	Ef_7 (%)	Ef_f (%)	t (days)	Test designation	Ef_7 (%)	Ef_f (%)	t (days)
BbCL_Bz	19.4	20.1	13	BbSR_Bz	13.9	27.6	32	BbXT_Bz	23.2	23.2	12
BVbCL_Bz	52.2	71.9	11	BVbSR_Bz	51.8	78.3	18	BVbXT_Bz	63.4	72.1	12
BCL_Bz	41.5	99.3	13	BSR_Bz	18.0	99.9	32	BXT_Bz	99.6	99.7	9
BVCL_Bz	84.0	99.6	11	BVSR_Bz	63.7	99.7	18	BVXT_Bz	99.7	99.7	7

Ef_7 – efficiency after 7 days, Ef_f – efficiency at the end of the tests, t –remediation time



(a)



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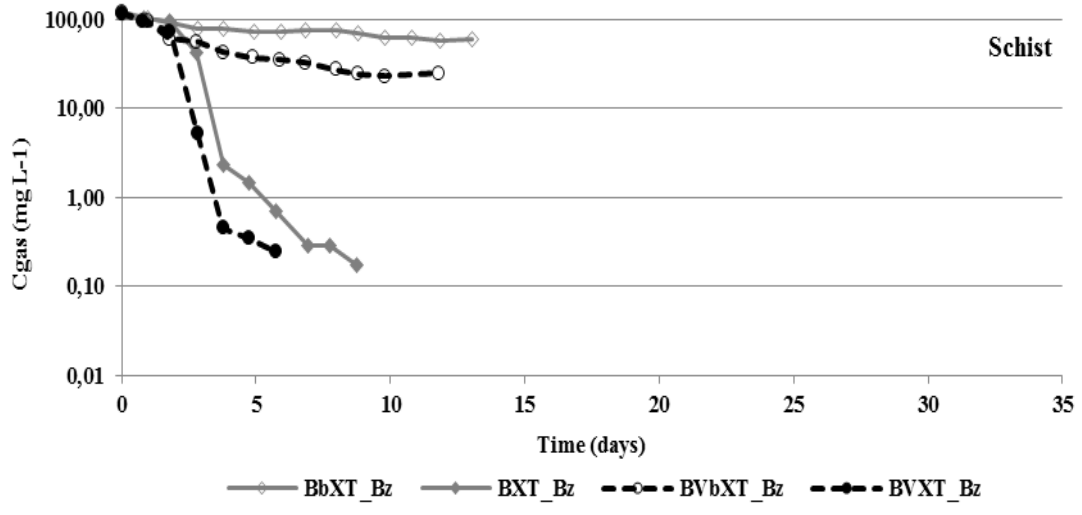
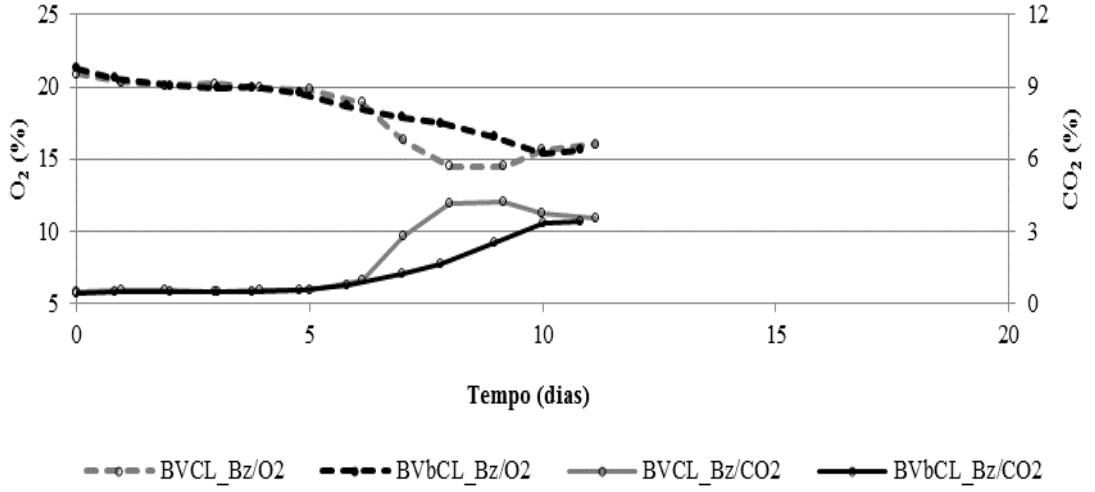
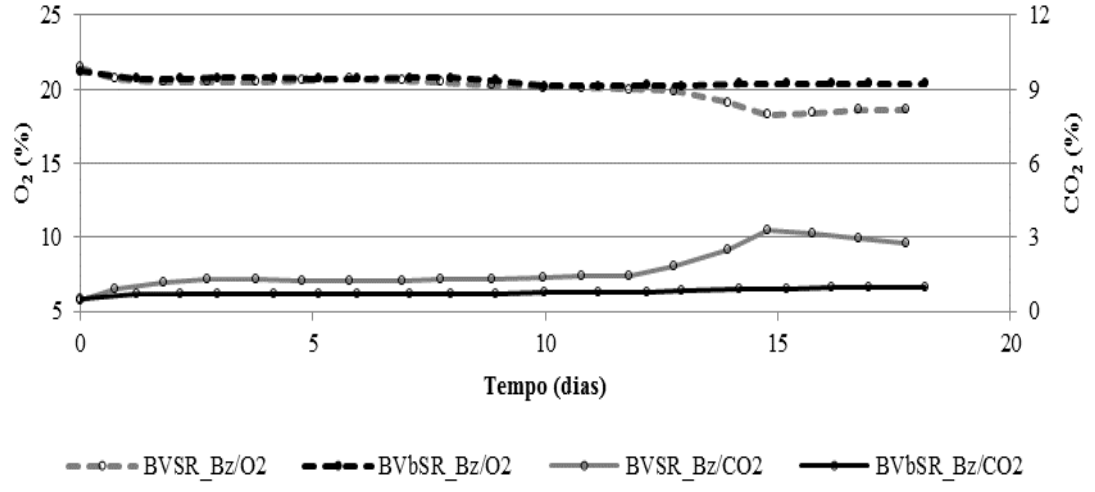


(c)

Figure 1. Time evolution of benzene concentration in gas phase (Cgas) from remediation tests performed in: (a) limestone; (b) granite and (c) schist. Legends of the graphics (tests designations) are in accordance with Table 1.



(a)



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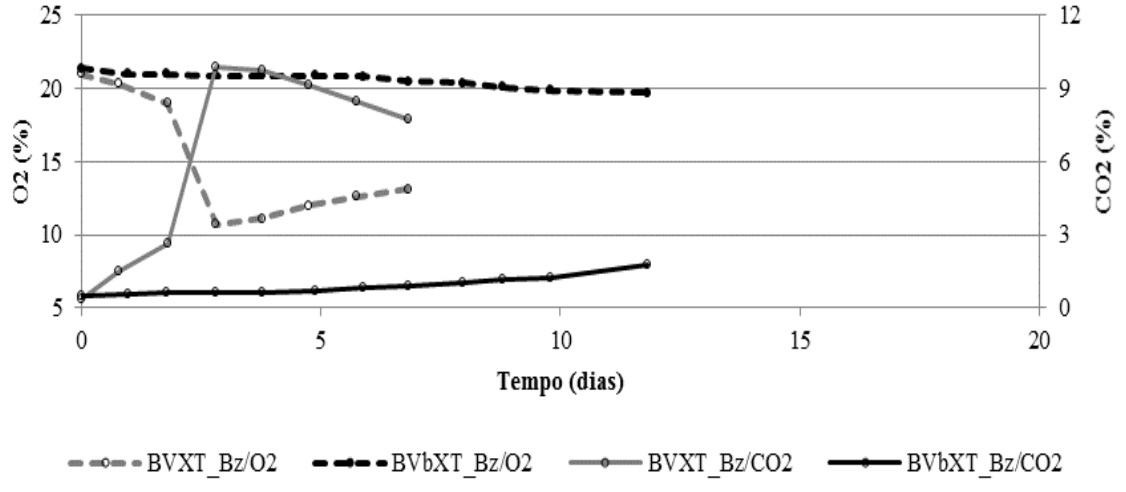

(c)

Figure 2. Time evolution of oxygen (O_2) and carbon dioxide (CO_2) in gas phase of remediation tests performed in: (a) limestone; (b) granite and (c) schist. Legends of the graphics (tests designations) are in accordance with Table 1.

Conclusion

The remediation efficiencies obtained for the two tested technologies (bioremediation without and with ventilation - bioventing) and for all the three sub-soils (limestone, granite and schist) were very high (≥ 99.3%).

Results allowed concluding that the effect of the diversity and abundance of clay minerals overcomes the effect of the fraction of fines. Limestone and granite have clay minerals in their mineralogical composition, being more abundant in granite, which also requires higher duration for achieving the target remediation efficiency in both technologies. On the other hand, schist has the highest fine fraction but without clay minerals, evidencing the lowest time required for remediation.

Bioventing tests allowed for higher remediation rates confirming the importance of oxygen supply on assisted bioremediation. This effect was more pronounced in the granite which presented the biggest difference for remediation times between bioremediation without ventilation and bioventing.

Acknowledgements

This work was supported by FEDER funds through the Operational Program for Competitiveness Factors - COMPETE and National Funds through FCT (the Portuguese Science and Technology Foundation) via the project PTDC/AAG-TEC/4403/2012 (ISIS).

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