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Bioventing and Enhanced Bioremediation of Natural Soils Contaminated by Biofuels

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Abstract

Soil is a natural resource, vital to human survival due the number of socio-economical and environmental functions that performs, that every day is subject to innumerous anthropogenic pressures.

With the growing production and use of biofuels, it is important to understand its fate when spilled in the soil and its influence in the bioremediation of other common contaminants.

Research that led to this dissertation intended to address the bioremediation of soils contaminated with blends of benzene and biofuels (butanol).

For this propose, biodegradation tests were made in liquid medium, and a microbial consortium was developed to use in bioventing and bioremediation tests that were performed in columns filled with different soil types.

Bioremediation and bioventing tests were monitored through the time evolution of the contaminant concentration in the gas phase.

The results obtained showed that soils can be remediated with the technologies used, with smaller remediation times when using bioventing. The presence of butanol (common biofuel) decreased the remediation time of benzene in the bioventing tests, but increased it in the bioremediation ones.

Key Words: Soil, Biofuels, Bioventing, Bioremediation, Benzene

Resumo

O solo é um recurso natural, vital para a sobrevivência humana devido ao grande número de funções socioeconómicas e ambientais que desempenha, que diariamente é sujeito a inúmeras pressões antropogénicas.

Com a crescente produção e uso de biocombustíveis, é importante perceber o seu destino quando existem derrames nos solos e a sua influencia na bio-remediação de outros contaminantes.

A investigação que conduziu a esta dissertação pretende abordar a temática de solos contaminados com misturas de benzeno e biocombustíveis (butanol).

Para o efeito, foram realizados testes de biodegradação em meio liquido, desenvolvido um consorcio microbiano para usar nos testes de bio-ventilação e bio-remediação, que foram realizados em colunas, com diferentes solos.

Todo o processo foi monitorizado através da evolução temporal da concentração de contaminante na fase gasosa.

Os resultados obtidos permitiram verificar que os solos utilizados podem ser remediados com as tecnologias selecionadas, sendo o tempo de remediação menor quando usada a bio-ventilação. O butanol diminuiu o tempo de remediação do benzeno, nos testes de bio-ventilação, mas aumentou o tempo de remediação nos testes de bio-remediação.

Palavras chave: Solo, Biocombustíveis, Bio-ventilação, Bio-remediação, Benzeno

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Abbreviations, Acronyms And Symbols

ASTM American Society for Testing and Materials

Bsoil Soil contaminated with crude

CFU Colony Forming Units

CL Limestone

CO_2 Carbon dioxide

C_p Contaminant concentration

DDI Distilled and demineralized water

EC Enrichment Cultures

EEA European Environment Agency

EU European Union

FID Flame Ionization Detector

GC Gas Chromatography

H Henry's law constant

ISO International Organization for Standardization

K_{CO} Organic carbon partition coefficient

K_{ow} Octanol-water coefficient partition

K_s Soil-water coefficient partition

LB	Lysogeny Broth
MMA	Mineral liquid medium
N	Nitrogen
NAPL	Non Aqueous Phase Liquid
O_2	Oxygen
P	Phosphorous
SR	Residual granitic soil
SVE	Soil Vapor Extraction
TOC	Total Organic Carbon
toe	tone of oil equivalent
TPH	Total Petroleum Hydrocarbons
V_c	Contaminant volume
VOC	Volatile Organic Compounds
V_r	Erlenmeyer flask volume
ρ	Wet bulk density
ρ_c	Contaminant density

Chapter 1 - Introduction

In this chapter is done an introduction to the theme, explaining its importance and the scientific areas involved in the problematic of soil remediation.

1.1. Aims and outline of the dissertation

The investigation that led to this dissertation intended to address the bioremediation of soils contaminated with blends of benzene and biofuels.

As it any other fuel, failures during production, transport of this biofuel can result in soil contamination. This is particularly problematic because it is not yet know which remediation technologies will be effective for the cleanup of those contaminated sites.

There is very little information about the bioremediation of soils contaminated with biofuels, and in particular about butanol, an emergent biofuel. Even less information exists about bioremediation of soils contaminated with blends of benzene (a carcinogenic compound, used worldwide) with butanol.

It is known that geological nature and physico-chemical properties of the soil play a significant role in the applicability of remediation technologies, although this aspect is seldom considered in the literature.

Therefore the main goals of this dissertation are:

- Isolate and develop a microbial consortium capable of degrade benzene and butanol blends;
- Evaluate the ability of the developed microbial consortium to degrade the benzene and butanol blends;
- Evaluate the efficiency of bioventing when applied to different types of soils contaminated with benzene and butanol blends;
- Evaluate the influence of butanol in the benzene biodegradation.

1.2. Dissertation organization

This dissertation is organized in four chapters, References and Appendixes.

In chapter 1 it is drawn an introduction to the theme, explaining its importance and the scientific areas involved in the problematic of soil remediation.

In chapter 2 is done a description of the materials, reagents and equipment used and are presented the methods used to realize the collection and preparation of the soils, the quantification of the contaminants, quantification of Oxygen (O_2) and Carbon Dioxide (CO_2), the microbial consortium development and the bioremediation and bioventing tests.

In chapter 3, are presented the results obtained in tests carried out, and a discussion of them.

In chapter 4 are presented the conclusions of the dissertation, from the results obtained.

In the Appendix are presented the experimental data and the calculations made to obtain the results of chapter 3.

1.3. Biofuels

According to Directive 2003/30/CE, a biofuel is a liquid or gaseous fuel that is used for transportation and is produced from biomass [1]. Biofuels are a renewable energy source and can be used as an alternative to the conventional fuels produced from non-renewable sources (fossil fuels). They are made from a variety of materials, such as plant matter, agriculture crops, forest by-products, and municipal wastes through biochemical or thermochemical processes [2].

With increases in energy consumption, it can be expected that the production and use of biofuels in Europe will increase. The production of biofuels in 2010 reached 105 billion litters (28 billion gallons US) worldwide, an increase of 17% from 2009 [3].

One of the major consumers of biofuels is the transportation sector, which is one of the biggest contributors of environment pollution. Figure 1 shows the biofuel consumption for the transportation sector in the Europe Union (EU) in 2010, in mega tones of oil equivalent (Mtoe).

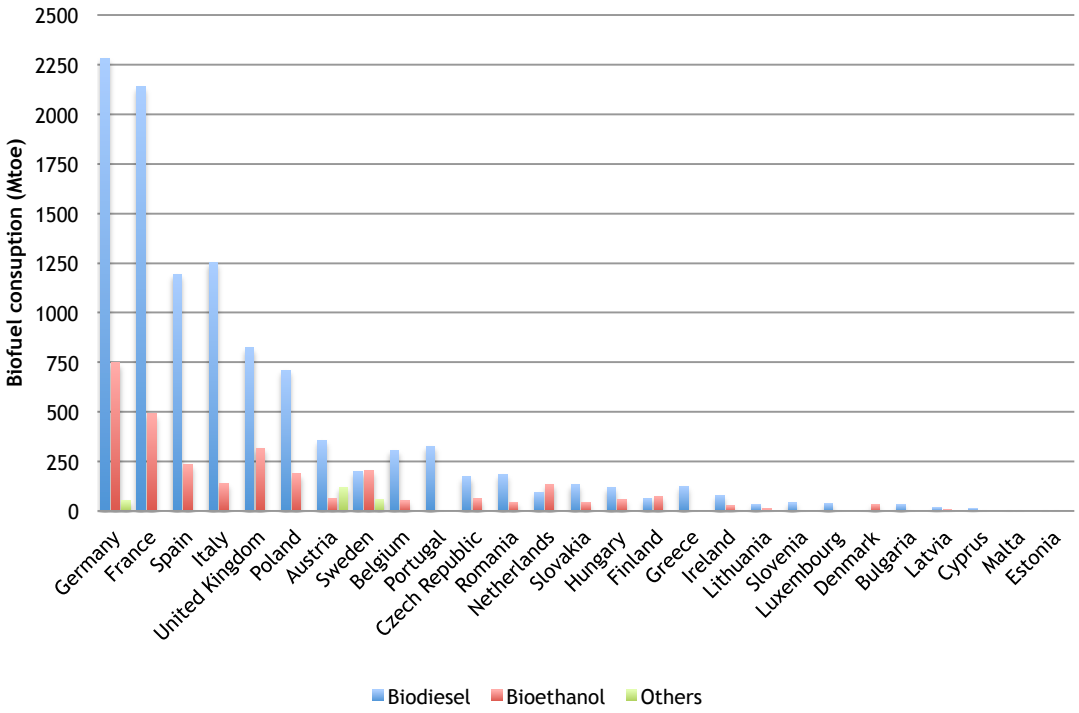


Figure 1 - Biofuel consumption for transport in Europe Union in 2010 (in Mtoe) [3]

One of the ways to reduce those emissions is to replace mineral-based fuels by bio-origin renewable fuels [4]. A wide variety of fuels can be produced from bio-products. Figure 2 gives an overview of the conversion routes and fuel produced.

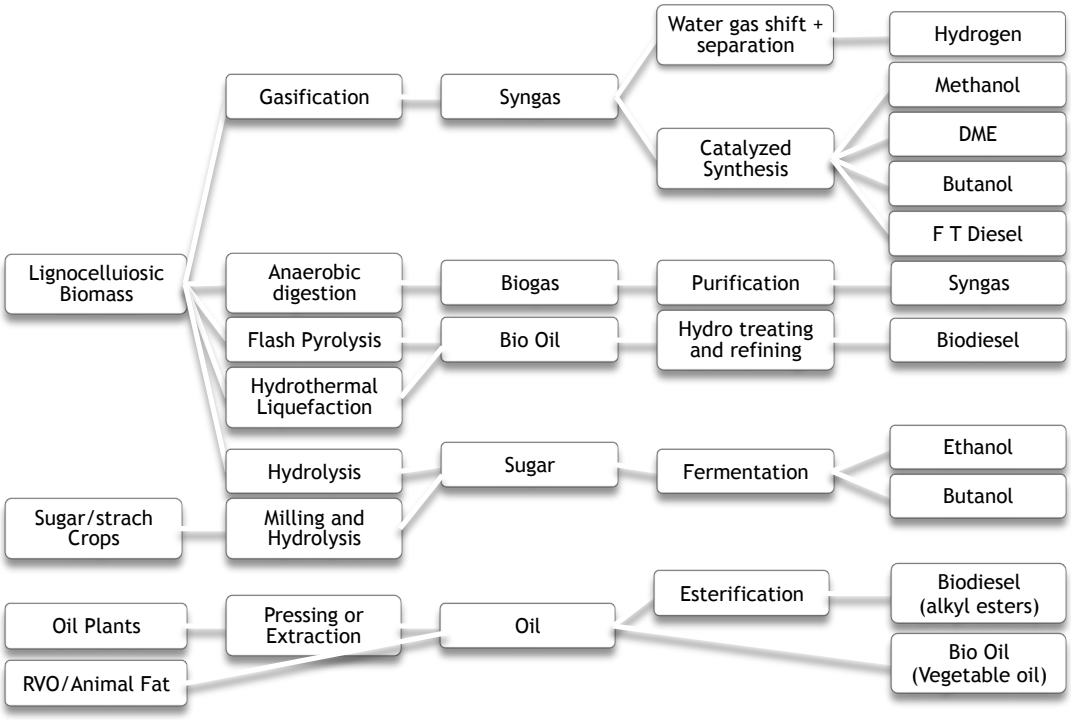


Figure 2 – Overview of conversion routes to biofuels, Adapted [4, 5]

The availability of renewable agriculture biomass for the production of biofuels is not evenly distributed around the globe. For example, most of the production of corn is in the United States [5]. With the increase demand for corn for the production of ethanol, the prices increased to a point where producing ethanol became cost prohibitive.

Recent research demonstrated that butanol could be produced at lower prices from several substrates [5]. Its use as a biofuel has only been reported since 2005, when a car was driven across the United States using butanol instead of gasoline [6].

Butanol provides a number of advantages when compared with other biofuels such as biodiesel or ethanol, such as: (1) it can be used in pure form or as a blend in gasoline, (2) no modification in the existing car engines are necessary when used as a blend or as a sole fuel, (3) it is simpler to handle when compared to ethanol due to its lower vapor pressure, (4) it is not hygroscopic and therefore allows blending with gasoline at the refineries, (5) it is less corrosive, (6) it has a higher energy content and (7) the dibutyl ether derivative has the potential for a diesel fuel [6].

1.4. Soil

According to International Organization for Standardization (ISO), soil can be defined as the upper layer of the Lithosphere and is transformed by weathering and physical/chemical and biological processes [7]. It is also the interface of the Earth with the atmosphere.

The soil formation process is extremely slow, and is therefore considered a non-renewable resource [8]. It is vital to human survival due the number of socio-economical and environmental functions that performs. For example, is the medium for plant growth, which is the main food source, both, for humans and animals, plays a very important role in the hydrological cycle, serves as filter for water, substrate for the transformation of various substances such as carbon and nitrogen [9].

The soil consists, as a complex and variable mixture of solid, gaseous and liquid materials. Just in Europe, more than 320 types of soils were identified, according to its functions, e.g. agriculture [8]. It is composed by three distinct phases: solid, gas and liquid.

The Figure 3 shows the relative proportions of the four principal soil components.

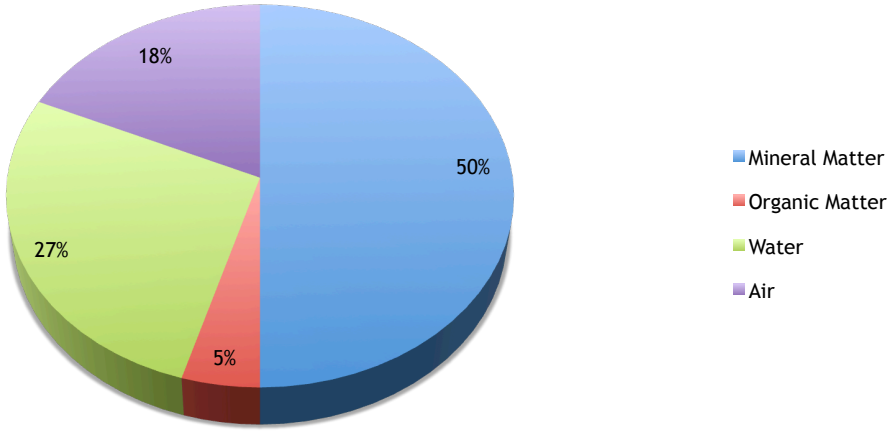


Figure 3 - Relative proportions of the four principal soil components, Adapted [10]

1.4.1. Solid phase

The solid phase of the soil is constituted by organic and inorganic matter. The inorganic fraction consists essentially in fine minerals subdivided according to the size, as illustrated in Table 1.

Table 1 – Classification of minerals by size, Adapted [11]

Classification	Description	Particle size
Clay	Microscopic particles of colloidal mineral nature, laminated layers or plates	>0.002 mm
Silt	Fine particles of minerals from the bedrock	0.002-0.075 mm
Sand	Intermediate particles of minerals from the bedrock	0.075-2 mm
Gravel	Coarse particles of minerals from the bedrock	2-75 mm

Soils can be classified by texture through the percentage of each mineral constituent. Using a Feret triangle it is possible to determine the texture of a given soil. Unfortunately, an international agreement has not yet been reached on the definition of the texture classes. Figure 4 shows the Feret triangle used by the Agriculture Department of the United States of America.

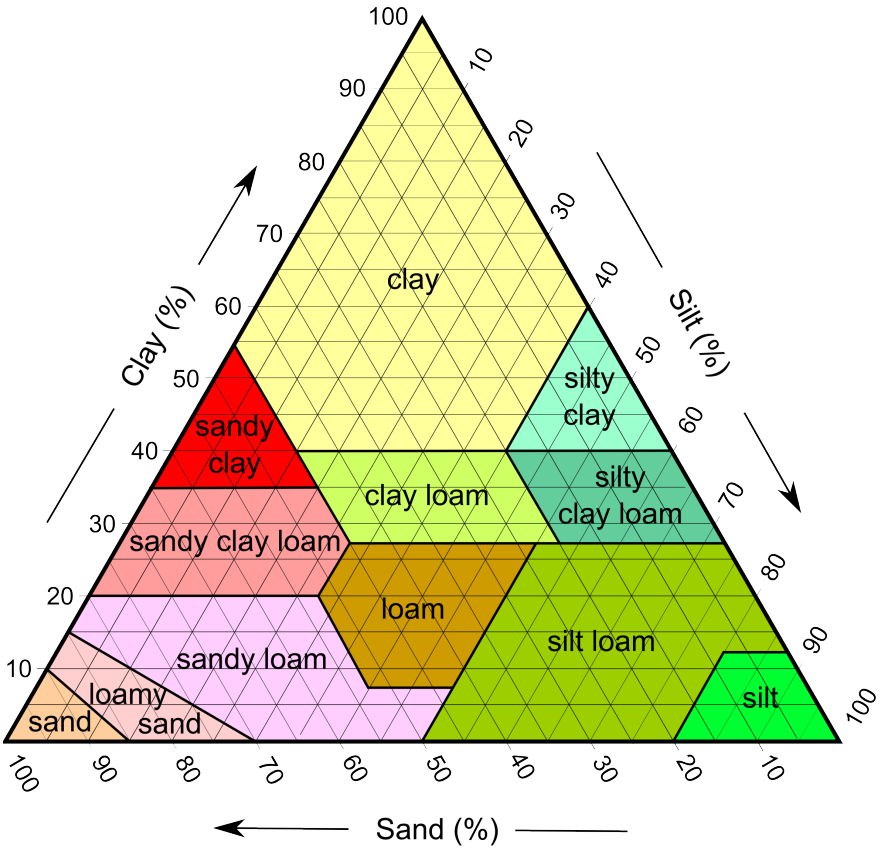


Figure 4 – Feret triangle used in the determination of soil texture classes, Adapted [12]

The texture of a soil is very important due its influence in the water and air permeability and therefor will determine the applicability of specific soil remediation technologies [11].

Focusing now in the soil's organic matter, it essentially comes from the animals and plants existing on the soil, since vegetation is the primary source of its formation [12]. The organic fraction of the soil constitutes a very complex system where animals and plants can be found in various states of decomposition, including excreted products from living organisms and microbiological organisms. It is essentially composed by a mixture of carbohydrates, proteins, lipids and resins and it is the main energy source for the living organisms in the soil [12]. The organic matter accumulates mainly in the upper layer of the soil.

1.4.2. Liquid phase

The liquid phase of the soil is extremely important because it is the main dispersion medium for nutrients and contaminants in soil. It is important to emphasize that there is a constant transfer of water between the atmosphere, the surface and the subsoil, as part of the hydrologic cycle [11].

When the water reaches the ground, part is infiltrated in the soil and reaches the infiltration zone or unsaturated zone. From here the water can continue its downward movement until it reaches the saturated zone, a zone constituted by only two phases, solid and liquid.

Figure 5 represents the different zones of the soil.

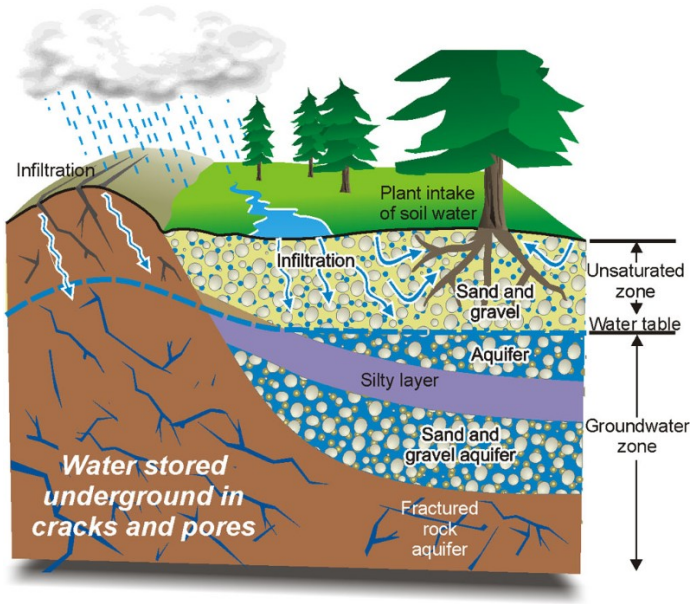


Figure 5 – Different zones of the soil [13]

The layer of the subsoil that allows the water movement is called an aquifer. Aquifers are normally formed by a layer of geologic material with high permeability that allow water flow.

There are two different types of aquifers depending on the permeability of the surrounding layers: unconfined and confined.

- Unconfined aquifers, are a permeable and partially saturated geological formation with a bottom boundary with a very low permeability [11].
- Confined aquifers are a permeable and completely saturated geological formation, with the bottom and top boundary with a vey low permeability [11].

The Figure 6 represents these two types of aquifers.

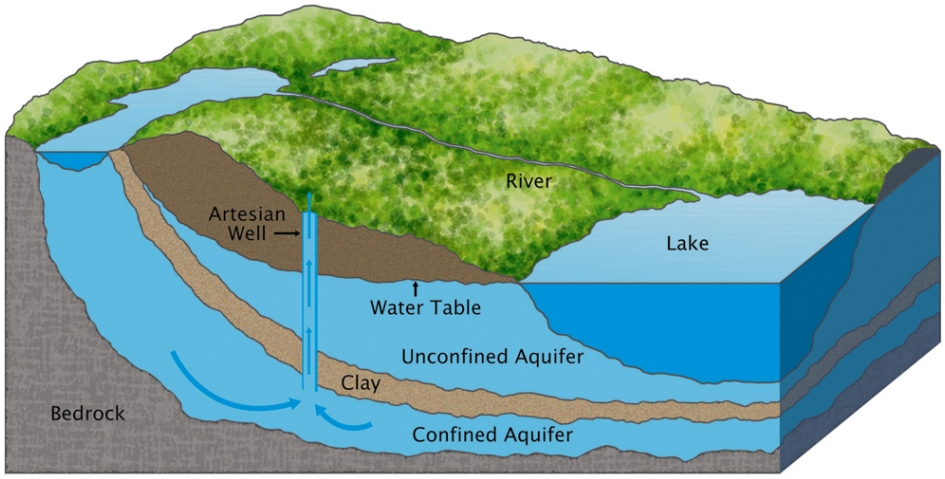


Figure 6 – Saturated and unsaturated aquifers [14]

The liquid phase of a soil is not only constituted by water, but also by a Non Aqueous Phase Liquid (NAPL) when contaminants are present [9].

1.4.3. Gas phase

The gas phase of the soil is mainly constituted by air that fills the soil pores. The presence of air in the soil is particularly important due to the presence of O_2, which is very important for biological processes. Therefore, air is responsible for the nature and intensity of the biochemical reactions that occur, but affecting some soil characteristics such as organic matter proportion, pH, quantity and state of nutrients. Since air is in the pore space, it is in

equilibrium with the water that is also present in the soil. If the quantity of water increases, the quantity of air will inevitably decrease [12].

Air renewal is of great importance since it replenishes O_2 and removes CO_2 byproducts of metabolic activity present in the soil. Without this, the ideal conditions for the fauna and flora would cease to exist [12].

Gas exchange between the soil and atmosphere preferably occurs by diffusion but other mass transfer mechanisms can occur. Gas diffusion will occurs when there is a gradient of partial pressure in the air constituents. In the majority of the soil, the diffusion is a continuous process because the consumption of the O_2 and CO_2 production by plants and microorganisms is also a continuous process [12]. The O_2 concentration in the soil decreases with the depth of the soil, until the 60 centimeters were the O_2 concentration is practically zero.

The temperature difference between the soil and the atmospheric air, as well as between the different soil layers, is other way of gas exchange, because it causes convective currents responsible for air movement.

1.5. Soil Contamination

Soil degradation is a serious problem in Europe. It is caused or magnified by human activities such as industries, inappropriate agriculture activities, tourism, and growth of urban areas. This has a direct impact on water and air quality, biodiversity, climatic changes, and can even jeopardize food safety and human health [8, 15]

Soil contamination occurs when a chemical enters to the soil affecting its natural characteristics. This alteration can be brought by a voluntary or involuntary act. The physicochemical properties of those substances and the soil itself influence how the contaminant is distributed [9].

Contamination can remain confined to the soil or can move to other environment compartments. It can even reach animals and humans. Figure 7 shows the pathways of contaminants to the different environment compartments.

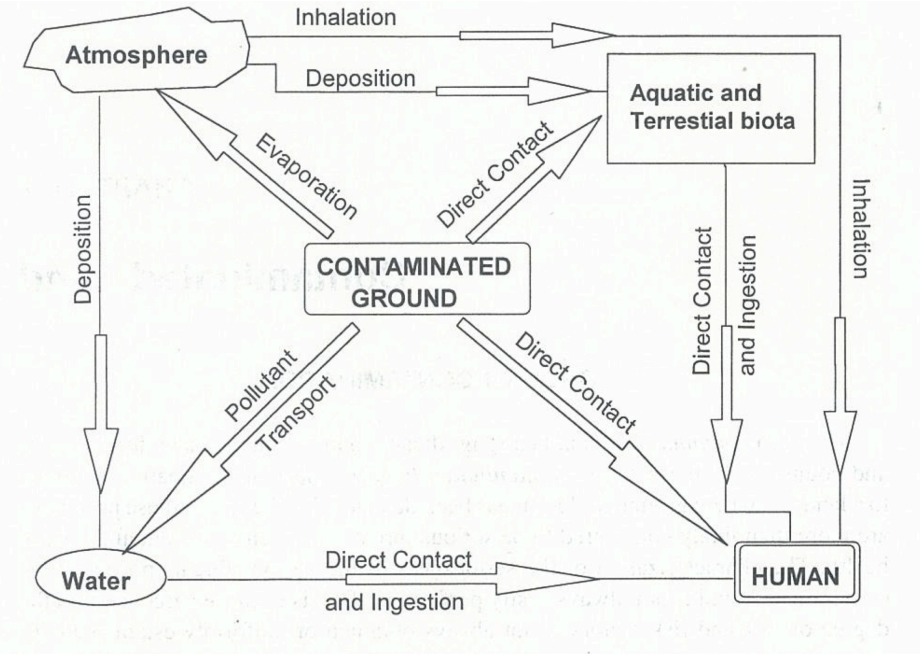
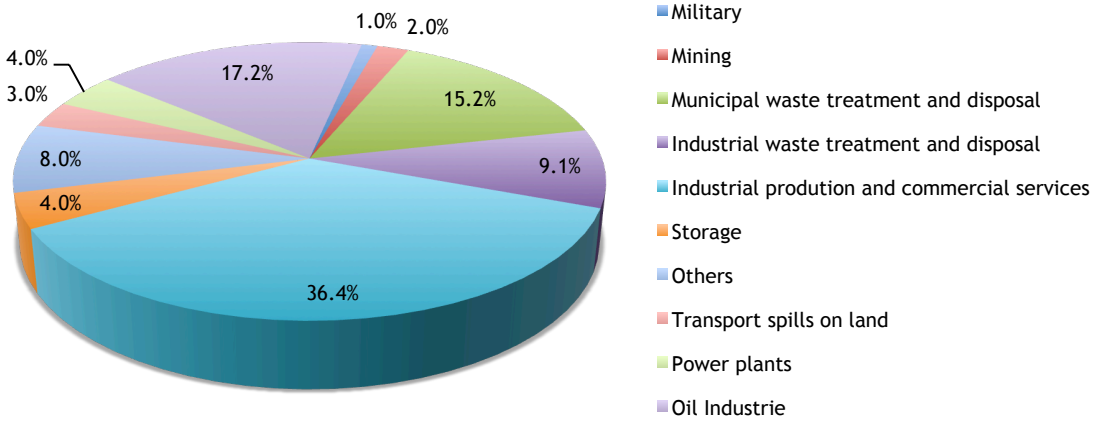


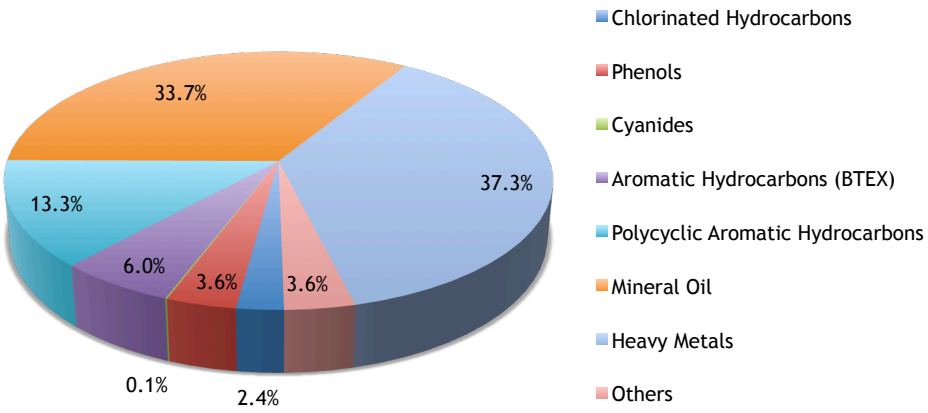
Figure 7 - Pathways from contaminated ground to biotic receptors [16]

According to the European Environment Agency (EEA), there are approximately 3 million sites where potentially polluting activities have taken place [17]. This data only takes into account countries that have information regarding soil contamination. Portugal is one of the countries that belong to the group that had not provided this information. With the improvements in data collection, the number of recorded polluted sites is expected to grow in the next years, if no changes are made in legislation.

Figure 8 shows the principal activities responsible for soil contamination, and the principal contaminants.



(a)



(b)

Figure 8 – a) Activities responsible for soil contamination, b) principal contaminants, Adapted [18]

As shown in Figure 8 the situation concerning contaminated soil is far from desirable. According to the "Thematic strategy for soil protection" [8], the Member States are obliged to take the appropriate measures to prevent soil contamination by dangerous substances, and to draw up a list of sites polluted by dangerous substances when concentration levels pose a significant risk to human health and the environment.

1.5.1. Contaminants distribution by the soil phases

When a contaminant hits the soil, it tends to infiltrate and percolate through it. Depending on the contaminant and soil characteristics, the contamination can be restricted to the upper layers of the soil or it can continue and infiltrate deeper in the soil. A large variety of phenomena can occur, such as transport, retardation, attenuation and increase in movement that can affect the contaminant's distribution in the different soil phases [15]. In most cases a contaminant is normally distributed in all the soil phases. Figure 9 shows the mass transfer processes between the different soil phases.

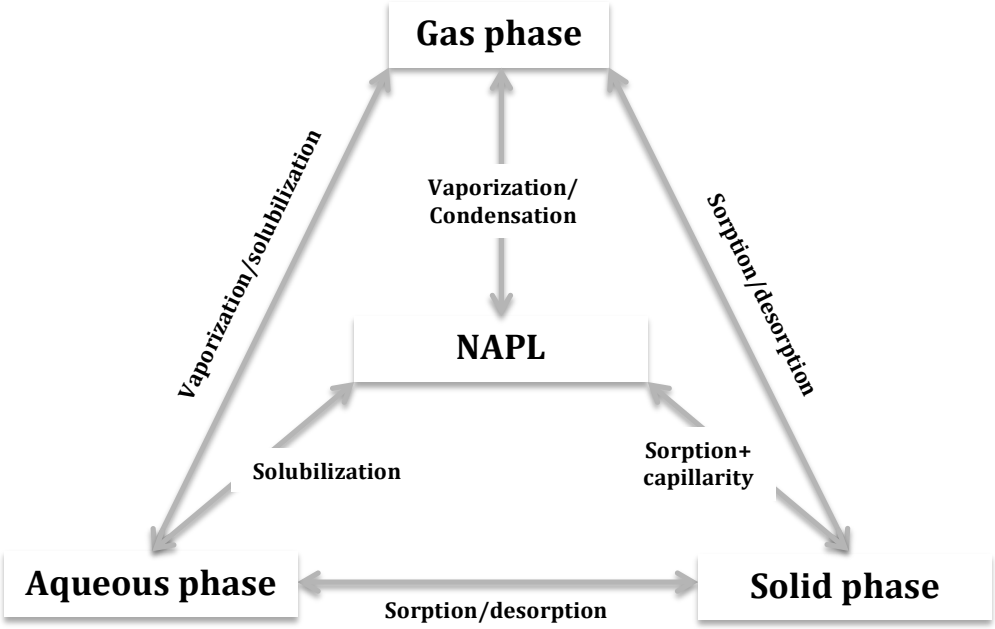


Figure 9 - Mass transfer processes between the different soil phases, adapted [9]

The contaminant's properties that most influence its distribution in soils are: (1) vapor pressure; (2) water solubility and (3) Henry's law constant (H). The soil properties that most influence the contaminant's distribution are: (1) organic matter content, (2) moisture content and (3) its texture. [9].

The contaminants distribution can be evaluated through partition coefficients. Partition coefficients are empirical constants that describe how a contaminant is distributed between two phases [15]. In soil contamination, the octanol-water partition coefficient (K_{ow}), the soil-water partition coefficient (K_s), and the organic carbon partition coefficient (K_{CO}) are considered the most important.

1.6. Contaminants

1.6.1. Benzene

Benzene is an aromatic hydrocarbon, liquid at room temperature with a sweet odor, highly volatile, colorless, and is slightly soluble in water [19]. Benzene is one of the most used chemicals worldwide as a gasoline additive, a solvent and as a raw material to produce

several other chemicals. It is mostly produced from petroleum. Table 2 shows a summary of the main properties of benzene.

Table 2 - Physical and chemical properties of benzene [15]

Chemical formula	C_6H_6	**Boiling point (°C)**	81.1
State of matter	Liquid	**Vapor Pressure at 25°C (mm Hg)**	95.2
Appearance	Colorless	**Water solubility at 25°C ($g\ L^{-1}$)**	1.80
Odor	Sweet	**Henry´s law constant at 25°C ($atm\ m^3\ mol^{-1}$)**	5.48×10^{-3}
Molecular weight ($g\ mol^{-1}$)	78.11	**Log K_{OW}**	2.13
Density at 15°C	0.8787	**K_{CO}**	85

The main sources of benzene in the soil are spills from storage tanks and deposit of residues, industrial spills, but there are also natural emissions such as gas emissions from volcanoes and forest fires [19].

Volatilization from moist soil surfaces is expected to be an important fate process based upon a Henry's Law constant of 5.48×10^{-3} atm m^3 mol^{-1}. Benzene may volatilize from dry soil surfaces based upon its vapor pressure [20]. Benzene can be biodegraded under aerobic and anaerobic conditions. Aerobic biodegradation can be an important process for the natural decay of benzene present in soil and groundwater [19].

Table 3 summarizes the existing information of the health effects of benzene in humans and animals.

Table 3 - Effects of benzene on human and animals health, Adapted [19]

Type of contact	Death	Systemic			Immunologic/Lymphoretic	Neurologic	Reproductive	Developmental	Genotoxic	Cancer
		Acute	Intermediate	Chronic						
Human										
Inhalation	●	●	●	●	●	●	●	●	●	●
Oral	●	●	●			●				●
Dermal	●	●								
Animal										
Inhalation	●	●	●	●	●	●	●	●	●	●
Oral	●	●	●	●	●	●	●	●	●	●
Dermal		●	●							●

1.6.2. Butanol

Butanol is an alcohol, liquid at room temperature, colorless, flammable and with a rancid sweet odor [21]. Butanol is mostly used as an organic solvent and as an intermediate to produce other organic chemicals. It can be produced from petrochemicals and occurs naturally as a product of fermentation of carbohydrates. Butanol can also be used as biofuel as a blend with gasoline or in pure form.

Table 4 shows a summary of the main properties of butanol.

Table 4 - Physical and chemical properties of butanol [21, 22]

Chemical formula	C_4H_9OH	**Boiling point (°C)**	118
State of matter	Liquid	**Vapor Pressure at 25°C (mm Hg)**	7.00
Appearance	Colorless	**Water solubility at 20°C (g L^{-1})**	90
Odor	Rancid Sweet	**Henry´s law constant at 25°C (atm m^3 mol^{-1})**	8.81×10^{-6}
Molecular weight (g mol^{-1})	74.12	**Log K_{OW}**	0.88
Density at 15°C (g mL^{-1})	0.81	**K_{CO}**	72.0

The main sources of butanol in the soil are spills from storage tanks, volatilization from solvents, industrial spills, and sewage treatment and from the manufacture of some food products and beverage. Butanol also occurs naturally in some pulses vegetables such as dried beans, split peas, and lentils [21].

Volatilization from moist soil surfaces is expected to be an important fate process based upon a Henry's Law constant of 8.81×10^{-6} atm m^3 mol^{-1}. Butanol may volatilize from dry soil surfaces based upon its vapor pressure [23]. It is a highly biodegradable compound [21].

1.7. Soil Remediation

Soil remediation can be a difficult, slow and expensive process [15]. Due to the risk to human health caused by soil contamination, a significant development in soil remediation technologies has been done in last decades. There are several technologies that can be used alone, together or sequentially. These include *ex situ* or *in situ* and some of them may be applied in both situations [9]. In *Ex situ* technologies, the soil is excavated and transported to other location to perform the treatment (prepared bed systems, or in-tank systems). This location has been designed to enhance treatment and/or prevent spread of contaminants to underground water [24]. *In situ* technologies consist of treating contaminated soil in place, without the need to transport the soil somewhere else to perform the treatment.

Soil remediation technologies can be classified as: (1) physical, (2) chemical, (3) biological and (4) thermal [15]. Figure 10 shows a summary of the principal soil remediation technologies.

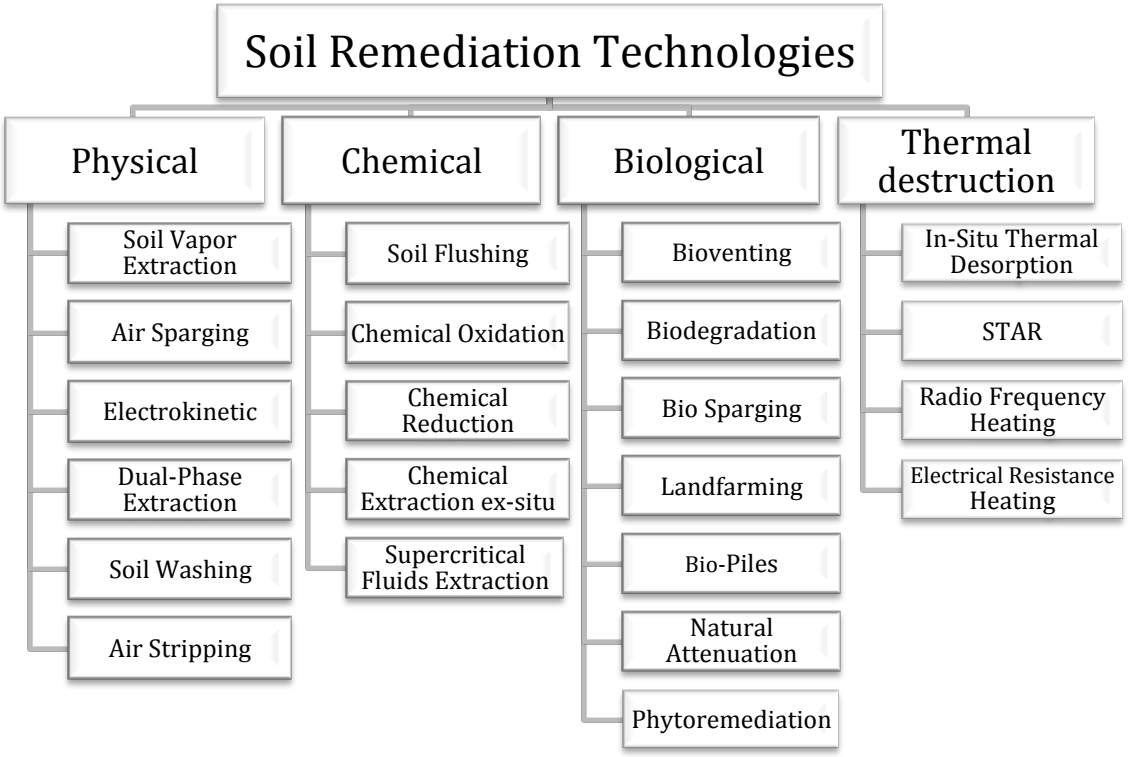


Figure 10 - Soil Remediation Technologies, Adapted [11, 15]

Physical and chemical technologies make use of known chemical properties or reactions coupled with material transformation of the contaminant to separate or contain the contamination. In physical processes, the contamination is transferred to another phase and in chemical processes, the chemical structure of the contaminant is altered, transforming it into a less toxic product, or more easily withdrawn from the solid phase [25].

For biological technologies (bioremediation) the contaminants are transformed by the microbial metabolism into innocuous substances, such as water, CO_2, fat acids and biomass. This process can occur in aerobic or anaerobic conditions. If the process depends only on the natural capacity of the soil to degrade the contaminants without altering the current conditions or adding nutrients it is called intrinsic bioremediation. If the activity of indigenous microbial populations is enhanced by adding additives such as O_2 (or other electron acceptors), nutrients, biodegradable carbonaceous substrates, bulking agents, and/or moisture the process is called enhanced bioremediation [26]. These type of technologies are normally slower than physical and chemical technologies but can be cheaper [25].

In the thermal destruction technologies, high temperatures are used to burn, decompose, destroy or promote the contaminants transfer to another phase. These type of technologies are very fast but very expensive due the high energy cost [25].

The choice of the best technology is not easy and in each case several criteria have to be considered. These include the contaminant's properties, soil characteristics, location of the contamination, minimum concentration desired, maximum time of remediation and cost [15].

1.8. Bioventing

Bioventing is an *in-situ* remediation technology that uses indigenous microorganisms to biodegrade contaminants present in the unsaturated zone [27]. This process is similar to the soil vapor extraction (SVE) but while SVE removes contaminants especially through volatilization, bioventing promotes the biodegradation of contaminant *in-situ* and normally without the necessary need for further treatment of the air extracted from the wells [27]. Both volatilization and biodegradation occur during SVE and bioventing but in SVE the main mechanism of remediation is volatilization, while in bioventing the main mechanism is biodegradation [11].

Bioventing can be applied to most volatile contaminants that are biodegradable under aerobic conditions. Figure 11 shows the contaminants that are most likely to be successfully remediated using bioventing.

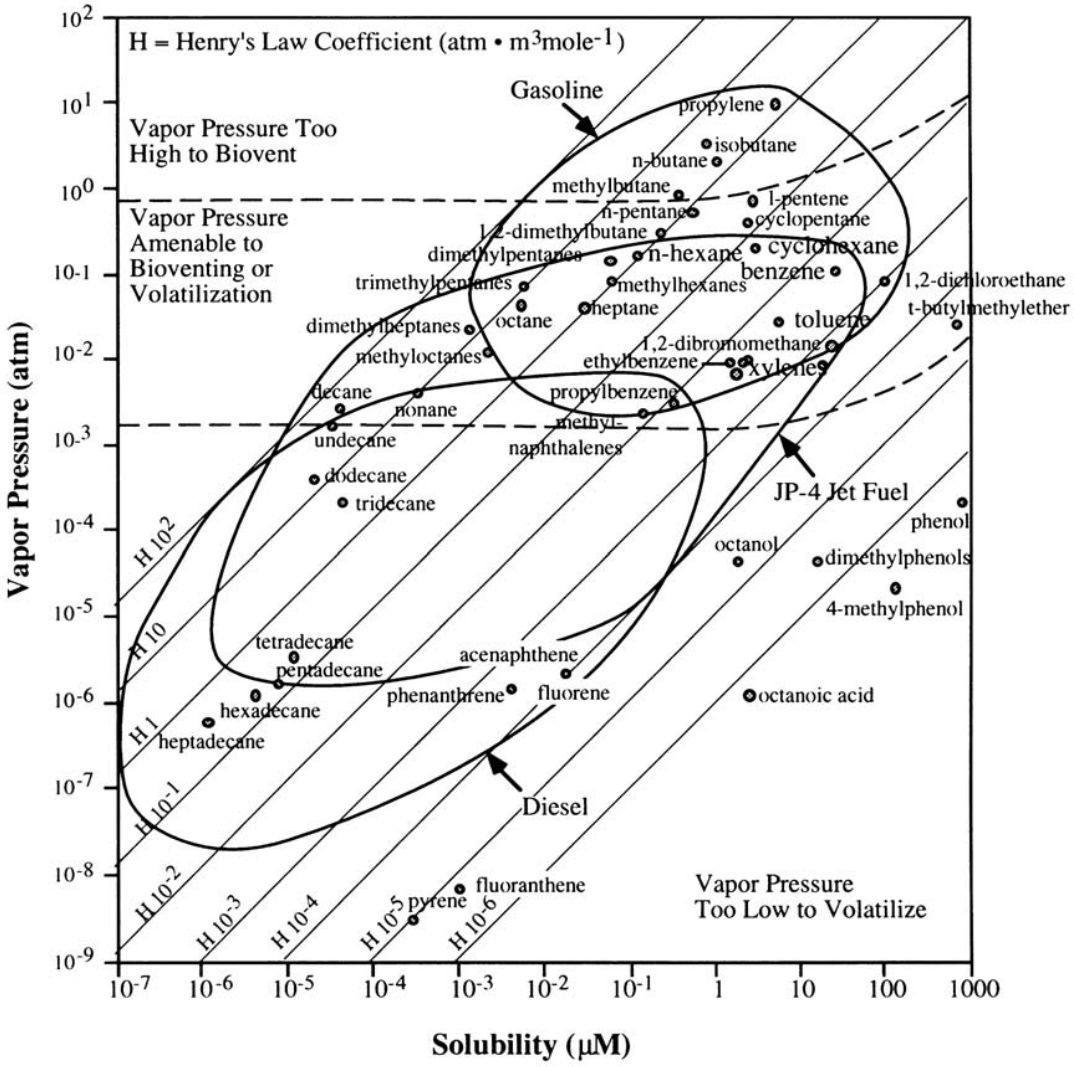


Figure 11 – Contaminants that can be treated using bioventing [28]

Microbial activity is enhanced by supplying air or O_2 using extraction or injection wells and sometimes by adding nutrients [27, 28]. Figure 12 illustrates a typical bioventing system.

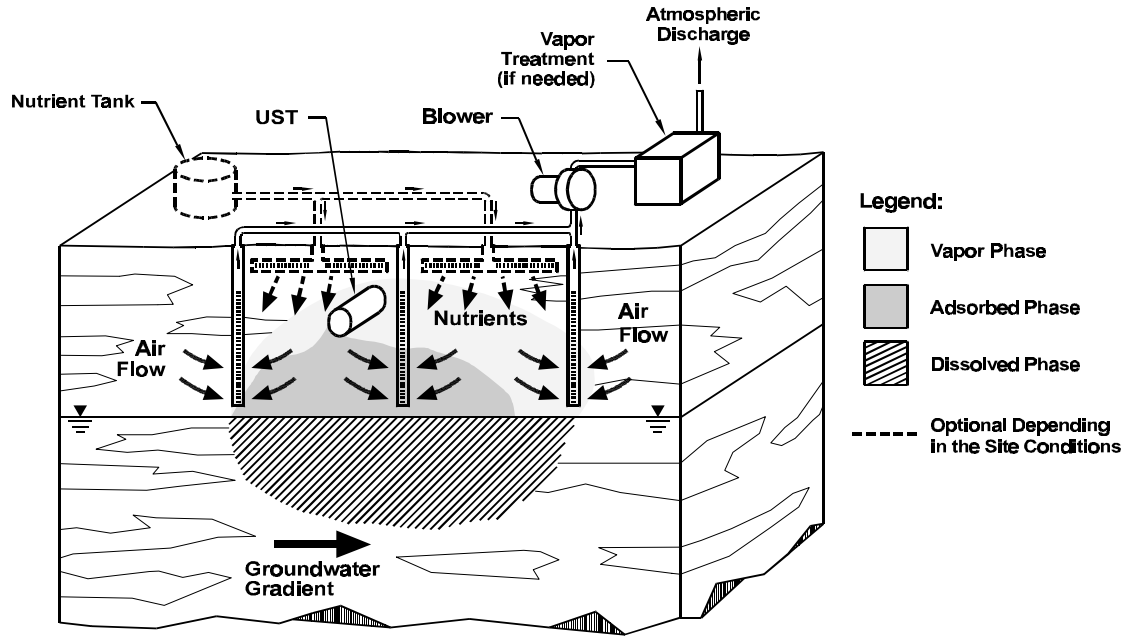


Figure 12 - Typical bioventing system using vapor extraction [27]

Many configurations can be adopted for a bioventing system. The principal components of the system are:

- Air extraction system
    - Pumps: are used to promote the air movement through the soil and can be vacuum pumps or blowers.
    - Air extraction wells: can be vertical or horizontal depending on site-specific needs and conditions. Horizontal wells are more efficient than vertical ones at shallower depths. The number of wells needed depends of the permeability of the soil and the contaminant concentration. A higher number of wells should be used in areas with higher contaminant concentration in order to decrease the remediation time [11].
    - Air injection wells: are similar to air extraction wells. They can be active injection wells, forcing compressed air into the soils using a blower, or passive injection wells, when used in combination with extraction wells, only to provide a pathway for the atmospheric air easily flow through the soil [27].

- Air treatment system: used when the concentration of volatile organic compounds (VOC) in the extracted air exceeds the established air quality limits. The most common treatments are: granular activated carbon, catalytic oxidation and thermal oxidation [27].

- Nutrients supply system: many methods can be used to deliver nutrients into the soils, if they are not present in enough concentrations. Some of these methods are injected through horizontal wells similar to those used for air extraction, and irrigation systems like sprinklers [27].

- Surface seals: can be included in the bioventing system to prevent surface water infiltration, to reduce fugitive emissions and to prevent short-circuiting of air flow [27].

- Groundwater pumps: can be used to drawdown the phreatic level, if the contamination is located at a site with a shallow groundwater table, or to expose contaminated soils in the capillary or saturated zone [27].

Bioventing, just any other technology, has advantages and disadvantages. Table 5 summarizes some of those advantages and disadvantages.

Table 5 - Advantages and disadvantages of bioventing, adapted [27]

Advantages	Disadvantages
Uses readily available equipment, easy to install	High contaminant concentration may be toxic to microorganisms
Creates minimal disturbance to site operations, can be used to address inaccessible areas	Not applicable in certain site condition (e.g., low soil permeability)
Requires short treatment times: usually 6 months to 2 years under optimal conditions	Cannot always achieve low cleanup standards
Is cost competitive	
Easily combinable with other technologies (e.g., air sparging, SVE)	
May not require costly offgas treatment	

1.8.1. Factors that influence the applicability of bioventing

The most important factors for the applicability of bioventing are the biodegradability of the contaminants and the intrinsic permeability of the soil [11, 29]. Treatability tests are

normally used to determine whether a contaminant will be biodegraded with conditions mimicking what is present at the site. In general, most of the organic compounds usually detected at these sites, (e.g. petroleum hydrocarbons and oxygenated solvents) are easily biodegraded under aerobic conditions [29]. The intrinsic permeability of the soil determines the rate at which O_2 can be supplied to the microorganisms responsible for biodegradation. These microorganisms, mostly bacteria, use O_2 to metabolize organic matter and produce CO_2 and water [27]. The values for intrinsic permeability can vary from 10^{-13} to 10^{-5} cm^2 for the most soil types. Table 6 shows the relation between the intrinsic permeability values and the effectiveness of bioventing.

Table 6 – Intrinsic permeability and Bioventing effectiveness [27]

Intrinsic Permeability (cm^2)	Bioventing Effectiveness
$k \geq 10^{-8}$	Effective
$10^{-8} \geq k \geq 10^{-10}$	May be effective, needs further evaluation
$k < 10^{-10}$	Not effective

There are other factors that influence the applicability of bioventing and can be found in Table 7.

Table 7 - Key parameters used to evaluate the applicability of bioventing, Adapted [27]

Site characteristics	Contaminant characteristics
Soil structure and stratification	Chemical structure
Microbial presence	Concentration and toxicity
Soil pH	Vapor pressure
Moisture content	Product composition and boiling point
Soil temperature	Henry's law constant
Nutrient concentration	
Depth to groundwater	

Chapter 2 - Materials and Methods

In this chapter is done a description of the materials, reagents and equipment used and are presented the methods used to make the collection and preparation of the soils, the quantification of the contaminants, quantification of oxygen (O_2) and carbon dioxide (CO_2), the microbial consortium development and the bioremediation and bioventing tests.

2.1. Reagents

The contaminants used in the laboratorial tests were benzene and butanol, both Pro-analysis, obtained form Panreac Quimica SAU, with purity ≥ 99.5%.

The mineral liquid medium (MMA) used in microbial cultures, was prepared with chemical reagents obtained from Merk, all Pro-analysis, with the following composition: $(NH_4)_2SO_4$ (3.8 mmol L^{-1}), KNO_3 (1.02 mmol L^{-1}) and $NaNO_3$ (8.2 mmol L^{-1}) as Nitrogen source, Na_2HPO_4 (6.0 mmol L^{-1}), KH_2PO_4 (4.0 mmol L^{-1}), $CaCl_2.H_2O$ (0.47 mmol L^{-1}), NaCl (0.14 mmol L^{-1}), $MgSO_4.7H_2O$ (0.41 mmol L^{-1}), $N(CH_2CO_2H)_3$ (0.52 mmol L^{-1}), $FeSO_4.7H_2O$ (2 mg L^{-1}), $ZnSO_4.7H_2O$ (0.1 mg L^{-1}), $MnSO_4.H_2O$ (0.03 mg L^{-1}), H_3BO_3 (0.3 mg L^{-1}), $CoSO_4.7H_2O$ (0.24 mg L^{-1}), $CuSO_4.5H_2O$ (0.01 mg L^{-1}), $NiSO_4.7H_2O$ (0.02 mg L^{-1}), $NaMoO_4.2H_2O$ (0.03 mg L^{-1}), $Ca(OH)_2$ (0.5 mg L^{-1}) and Ethylenediaminetetraacetic acid (5 mg L^{-1}) [15].

The saline solution (0.85 %) used in the cultures dilutions was prepared with NaCl, Pro-analysis, from Merk.

The solid medium Lysogeny Broth (LB) used in the Petri dishes, was prepared with Tryptone (10 g L^{-1}), Yeast extract (5 g L^{-1}) and agar-agar (20 g L^{-1}), all from Liofilchem, for bacterial uses, and 10 NaCl (g L^{-1}) Pro-analysis from Merk.

The saline solution, MMA and LB were sterilized in autoclave (121 °C and 1 atm for 15 minutes) before any use.

2.2. Equipment

In the course of the laboratorial work, several equipment were used. In Table 8 is a list of that equipment.

Table 8 - Equipment used in the laboratorial work

Equipment	Brand	Model
Magnetic Stirrers	Heidolph	Unimax 1010
Autoclave	Panasonic	MLS-3020U
Analytical Balance	Chyo	JL
Semi-analytical Balance	Kern	EW1500-2M
UV-cleaner box	Biosan	UVC/T-M-AR
Thermostat Cabinets	WTW	TS1006-I and TS606/2-i
Mass Flow Controller	AALBORG	GFC17
Gas Chromatography	Shimadzu	GC-2014
Incubator	BINDER	FD
Instrumental TPH Kits	Chemetrics	Remediaid test kits
pH Meter	WTW	Inolab
O_2 and CO_2 Analyzer	Servomex	5200 Multipurpose

Figure 13 shows some of the laboratorial equipment used in the curse of the laboratorial work.

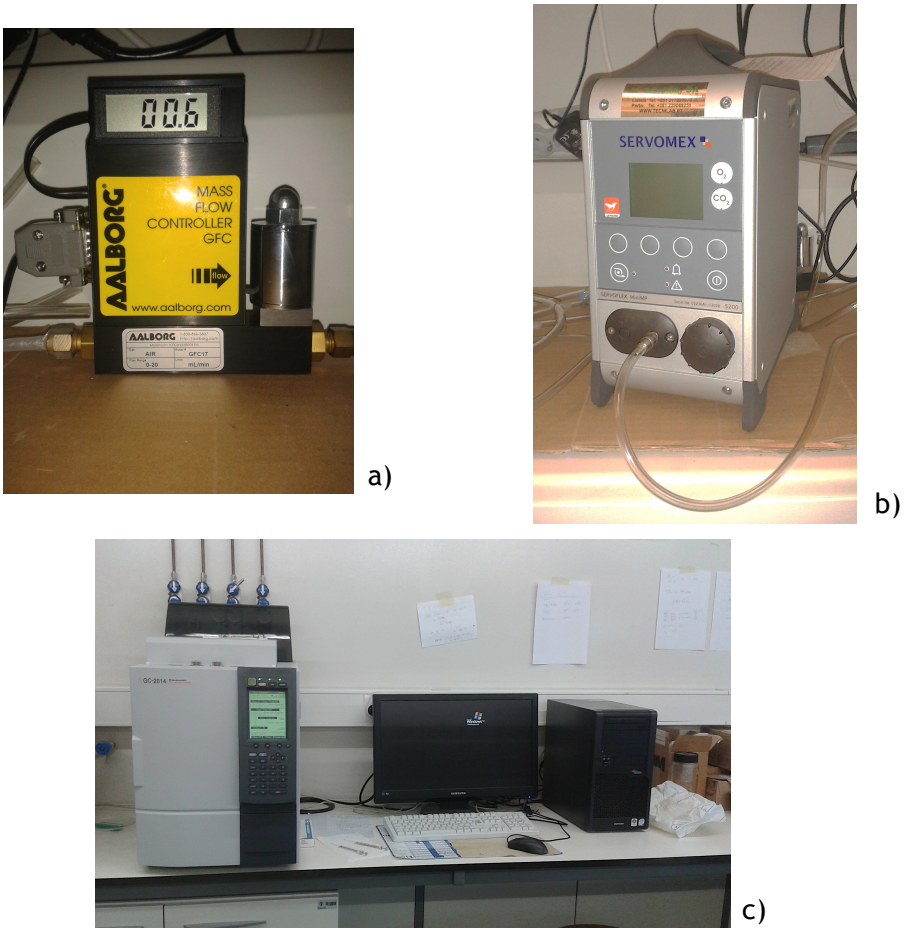


Figure 13 – Laboratorial equipment; a) Mass Flow Controller; b) O_2 and CO_2 Analyzer; c) Gas Chromatography

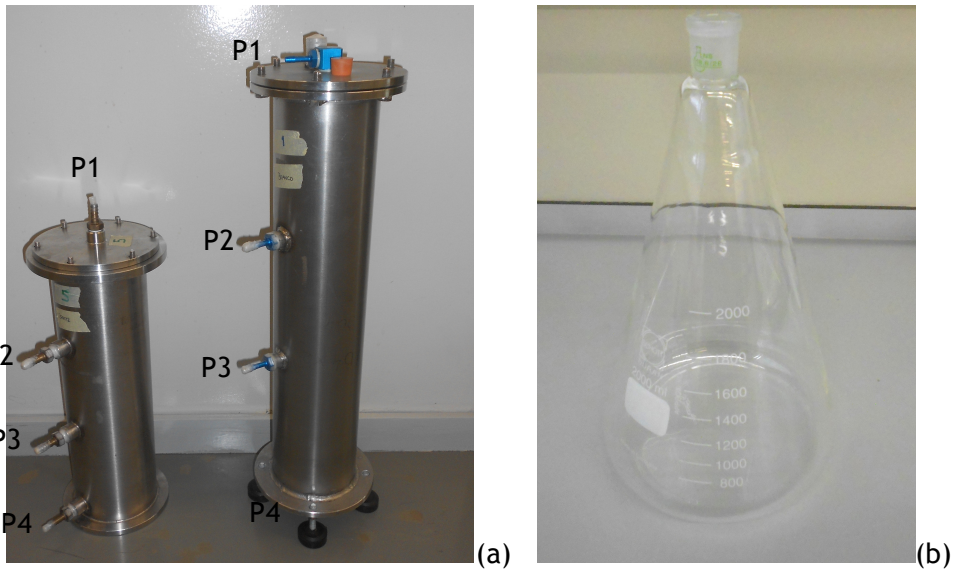
2.3. Reactors

The bioremediation tests in mineral liquid medium were performed in Erlenmeyer flasks autoclavable of 500 mL, 1000 mL and 2000 mL closed with Teflon valves (Mininert®).

In the preparation of the Enrichment Cultures (EC) Erlenmeyer flasks of 250 mL were used.

The bioremediation and bioventing tests in column were performed in two sets of cylindrical stainless steel bioreactors, with an internal diameter of 10 cm and an internal height of 50 and 35.5 cm. The column with 50 cm had four sampling ports, one in the top (P1), two equally divided by the lateral wall (P2 and P3) and one in the bottom (P4). The column with 35.5 cm had four sampling ports too; one in the top (P1), two equally divided by the lateral wall (P2 and P3) and one near the base (P4).

In the Figure 14 are presented the reactors used.



(a) (b)

Figure 14 – Reactors used in the experimental work; (a) stainless steel columns; (b) Erlenmeyer flasks

2.4. Collection and preparation of the soils

In this work three soils were used, two natural (residual granitic soil and a soil contaminated with crude) and one prepared soil (limestone). The prepared soil is a natural soil which particle size was controlled in laboratory to meet a prefixed particle size.

The soil contaminated with crude (Bsoil), was used exclusively in the preparation of the Enrichment cultures, without suffering any preparation. Was stored in appropriate containers, at room temperature and away from the light.

The residual granitic soil (SR) was dried and stored in an appropriate container, at room temperature and way from the light. The characterization of this soil was performed in previous work reported in [15].

The limestone soil (CL) was fragmented, dried and stored in an appropriate container, at room temperature and way from the light. Those soils were subsequently sieved to separate different size fractions from which the test pieces were produced with controlled particle size distribution. This particle size distribution was calculated to meet a similar particle size distribution as the SR. Table 9 resumes the particle size distribution of all soil types (SR and CL).

Table 9 - Particle size distribution of limestone

Particle size (mm)	Soil mass (g)	
	Column height = 50.0 cm	Column height = 35.5 cm
>2.380	324	230
2.38-0.850	540	383
0.850-0.425	324	230
0.425-0.212	234	166
0.212-0.105	162	115
0.105-0.074	54	38
<0.074	162	115
Total	1800	1278

The residual granitic soil and the limestone soil were submitted to two cycles of sterilization (121 °C and 1 atm for 15 minutes) and cooled at the UV-cleaner box, to ensure aseptic conditions, before the preparation of the bioventing and bioremediation columns.

2.5. Quantification of the contaminants in the gas phase

The qualification of the contaminants was performed by gas chromatography (GC), equipped with a 60/80 Carbopack B support (2.4 m × 3.18 mm × 2.1 mm) column and a Flame Ionization Detector (FID). The gases used were hydrogen and air for the detector and nitrogen as carrier gas, with a flow of 1 mL min^{-1}.

The chromatographic analysis was performed in isothermal mode, with the column at 150 °C, the detector at 200 °C, and the injector at 200 °C. The volume injected was 200 µL.

Several calibration curves were made during the laboratorial work in order to embrace the contaminants concentration range. The patterns were prepared using 500 mL Erlenmeyer flasks closed with Mininert® valves. The contaminants were injected with Hamilton 701N 10 µL syringes. The flasks were left to stand overnight in order to the contaminant volatilizes completely and the equilibrium be reached. With the concentration of contaminant and the area peak was possible draw the calibration curves. This methodology was applied to butanol and benzene.

The concentration on the patterns was determined using the Equation 1.

Equation 1: $$C_p = \frac{V_c \times \rho_c}{V_r}$$

Where C_p is the contaminant concentration in the pattern (mg L^{-1}), V_c is the contaminant volume (µL), ρ_c is the density of the contaminant (mg μL^{-1}) and V_r is the Erlenmeyer flask volume (L). The Erlenmeyer flask volume was calculated from the weighing of the distilled and demineralized water (DDI) mass necessary to completely fill the flask.

2.6. Oxygen and carbon dioxide quantification in the gas phase

The quantification of the O_2 and CO_2 in the gas phase was performed by respirometry. The respirometer was equipped with an infrared detector, for CO_2 determination, and a Paramagnetic detector for O_2 and an internal pump that allowed the gas transport. The respirometer was used in the bioventing tests in open circuit mode. The respirometry analyses was done daily, for 15 minutes in the column tests for granitic and limestone tests being the circuit closed for the rest of the time, simulating a pulsed venting regime.

2.7. Total Petroleum Hydrocarbons determination

The Total Petroleum Hydrocarbons (TPH) determinations were made using Instrumental TPH Kits. The method is based on reactions of alkylation of Friedel-Crafts and colorimetry. The soil sample (5 g) is put in contact with anhydrous sodium sulfate, that works as drying agent, and then is added dichloromethane that extracts the TPH's from the soil. The mixture is stirred and left to sediment, and then the supernatant is decanted to other test tube with

Florisil. The liquid phase is extracted and added aluminum chloride (reaction catalyst) that leads to the development of color, which intensity varies with the concentration of TPH's present on the soil. The absorbance is measured on a colorimeter, at the end of a certain time, and the TPH's concentration (mg of TPH's by Kg of soil) is calculated using a calibration curve provided with the equipment [30].

2.8. Biodegradation tests in liquid medium

In order to distinguish and isolate the types of microorganisms capable to degrade both benzene and butanol, two transfers (T1 and T2) were carried out in liquid phase, with EC, MMA and contaminants (benzene and butanol). These transfers were made from an Enrichment culture, prepared in 250 mL Erlenmeyer flasks where were added 10 g of Bsoil and 90 mL of MMA. This flasks were incubated overnight at 28 °C and stirred at 150 rpm. The first transfer was performed in six, 500 mL Erlenmeyer flasks, all sterilized and closed with Mininert® valves. The inoculum used in this transfer was the EC, in the proportion 5:95 (V:V) with MMA (5 mL of EC and 95 mL of MMA), and contaminated with benzene, butanol and mixture of both. The second transfer was performed in six, 1 liter Erlenmeyer flasks, all sterilized and closed with Mininert® valves. The inoculum used in this transfer was the T1, in the proportion 50:50 (V:V) with MMA (100 mL from T1 and 100 mL of MMA) , and contaminated again with benzene, butanol and a mixture of both. Both transfers were incubated at 28 °C and stirred at 150 rpm. Figure 15 shows how the transfers were performed. Figure 16 shows an example of an enrichment culture and some material used in the quantification of the CFU´s.

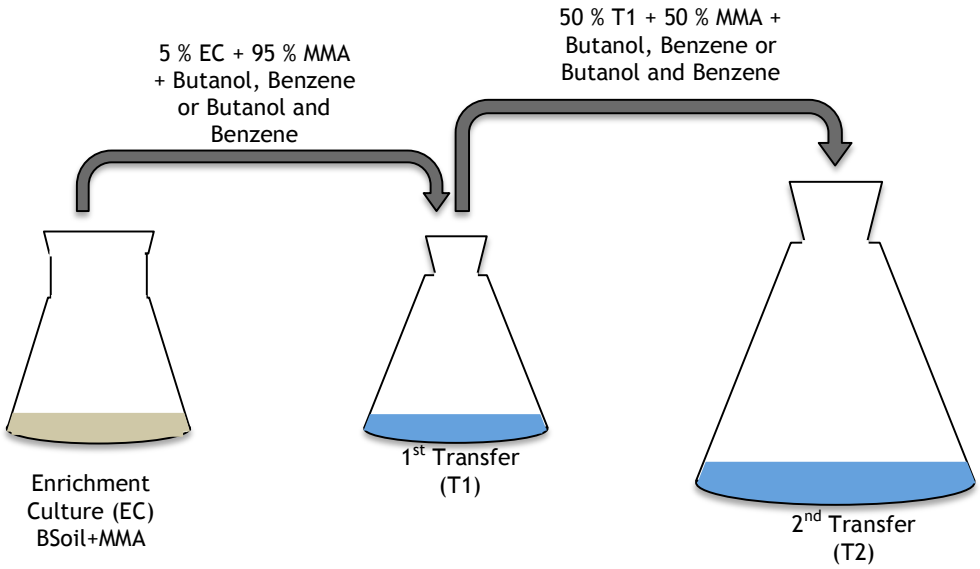


Figure 15 - Successive transfers in liquid phase


a)


b)

Figure 16 – a) Enrichment culture; b) material used for the quantification of microbial population

The concentration in the gas phase was monitored by GC. The sample (200 µL) was collected with an ILS 250 µL syringe. The incubation period ended when the concentrations of the contaminants reached 0,5 mg L^{-1}. At the end of each test, the microbial population was quantified, by counting the CFU, with successive dilutions in sterile saline solution (10^{-3} until 10^{-6}). Table 10 resumes the biodegradation tests performed in liquid medium.

Table 10 - Syntheses of the biodegradation test in liquid medium

Enrichment culture	Contaminant	Contaminant concentration (mg L^{-1})	1st transfer	2nd transfer
EC1	Benzene Butanol	210 210	T1Bz210Bt210A	T2Bz210Bt210A
	Benzene Butanol	210 210	T1Bz210Bt210B	T2Bz210Bt210B
	Benzene Butanol	210 0	T1Bz210Bt0A	T2Bz210Bt0A
	Benzene Butanol	210 0	T1Bz210Bt0B	T2Bz210Bt0B
	Benzene Butanol	0 210	T1Bz0Bt210A	T2Bz0Bt210A
	Benzene Butanol	0 210	T1Bz0Bt210B	T2Bz0Bt210B

The value of 210 mg L^{-1} was adapted the contaminant concentration used in previous work developed in this subject [15].

2.9. Inoculum preparation

In order to prepare the inoculum used in the bioventing and bioremediation tests, two transfers (T1 and T2) were carried out in liquid phase, with EC, MMA and contaminants (benzene and butanol) to isolate and develop a microbial consortium capable of degraded the selected contaminants. These transfers were made from an Enrichment culture, prepared in 250 mL Erlenmeyer flasks in which were added 10 g of Bsoil and 90 mL of MMA. This flasks were incubated overnight at 28 °C and stirred at 150 rpm.

The first transfer was performed in five, 1 liter Erlenmeyer flasks, all sterilized and closed with Mininert® valves. The inoculum used in this transfer was the EC, in the proportion 5:95 (V:V) with MMA (14 mL of EC and 266 of MMA), and contaminated with both benzene and butanol. The second transfer was performed in five, 2 liter Erlenmeyer flasks, all sterilized and closed with Mininert® valves. The inoculum used in this transfer was the T1, in the proportion 50:50 (V:V) with MMA (280 mL from T1 and 280 mL of MMA), and contaminated with both benzene and butanol. Both transfers were incubated at 28 °C and stirred at 150 rpm. Figure 17 shows how the transfers were performed.

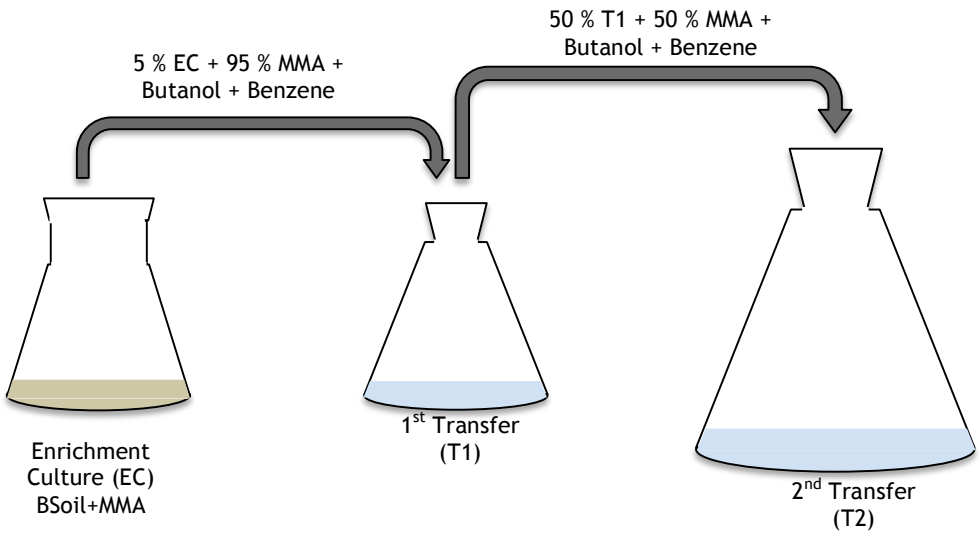


Figure 17 - Successive transfers in liquid phase

The concentration in the gas phase was monitored by GC. The sample (200 µL) was collected with an ILS 250 µL syringe. The incubation period ended when the concentrations of the contaminants reached 0,5 mg L^{-1}. At the end of each test, the microbial population was quantified, by counting the CFU, with successive dilutions in sterile saline solution (10^{-3} until 10^{-6}).

Throughout the work, two inocula were prepared, form two enrichment cultures, in order to have enough inoculum to perform the bioremediation and the bioventing tests in columns. Table 11 resumes the tests made to obtain the inoculum.

Table 11 - Syntheses of the test made to prepare the inoculum

Inoculum/ Enrichment culture	Contaminant	Contaminant concentration (mg L^{-1})	1st transfer	2nd transfer
Inoculum 1 EC2	Benzene Butanol	210 210	T1Bz210Bt210A	T2Bz210Bt210A
	Benzene Butanol	210 210	T1Bz210Bt210B	T2Bz210Bt210B
	Benzene Butanol	210 210	T1Bz210Bt210C	T2Bz210Bt210C
	Benzene Butanol	210 210	T1Bz210Bt210D	T2Bz210Bt210D
	Benzene Butanol	210 210	T1Bz210Bt210E	T2Bz210Bt210E
Inoculum 2 EC3	Benzene Butanol	210 210	T1Bz210Bt210A	T2Bz210Bt210A
	Benzene Butanol	210 210	T1Bz210Bt210B	T2Bz210Bt210B
	Benzene Butanol	210 210	T1Bz210Bt210C	T2Bz210Bt210C
	Benzene Butanol	210 210	T1Bz210Bt210D	T2Bz210Bt210D
	Benzene Butanol	210 210	T1Bz210Bt210E	T2Bz210Bt210E

The value of 210 mg L^{-1} was adapted the contaminant concentration used in previous work developed in this subject [15].

2.10. Bioventing and bioremediation tests

In the following it will be designated as bioventing the enhanced biodegradation tests performed with ventilation, and as bioremediation the enhanced biodegradation tests performed without ventilation.

The bioventing tests were all performed in stainless steel columns with 50 cm high (total volume of 3917 mL), containing 2000 mL of wet soil. The bioremediation tests were all performed in stainless steel columns with 35.5 cm high (total volume of 2788 mL), containing 1420 mL of wet soil. In all the tests the contamination as obtained by blending benzene and butanol. The soils used were granite with 25 % of water content and limestone with 11.1 % water content. The tests performed were inoculated and non-inoculated (sterilized blanks) incubated at 25 °C. In the non-inoculated tests the water content was obtained adding sterilized DDI to the dry soil. In the inoculated tests the water content was obtained adding the inoculum prepared in the second transfers.

The preparation of the columns followed the next steps:

1. Columns disinfection with ethyl alcohol (96 % V:V);
2. Placing the septa or vales in all the sampling ports of the column;
3. Introduction of the pre-sterilized soil and mixed with the inoculum or the sterilized DDI in the column;
4. Placing the cover of the column in place;
5. Placing the column in the horizontal;
6. Contamination of the column, using the sampling ports P2 and P3 (Figure 14), 50 % in each port;
7. Agitation of the column inverting it three times;
8. Placing the column in the vertical position inside the thermostatic cabinet at 25 °C.

The bioventing columns installation used in the laboratorial tests consisted of a respirometric circuit composed of the bioreactor (column), flowmeter controller, O_2 and CO_2 analyzer and a computer to receive and store the data (O_2 and CO_2 concentrations), 1 record/minute. The installation is illustrated in the Figure 18.

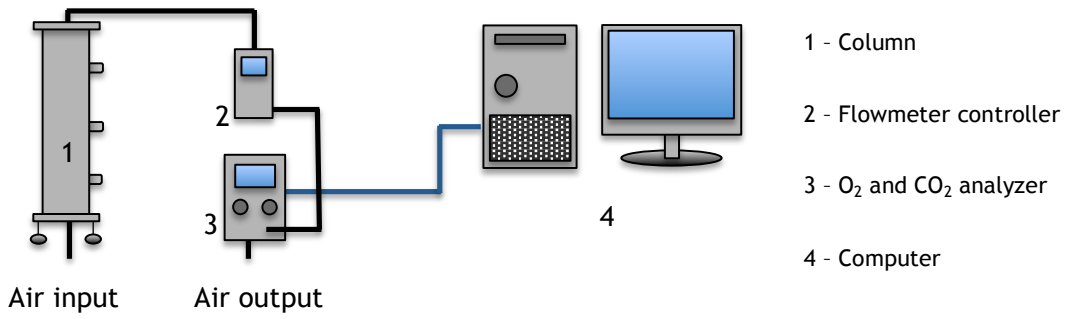


Figure 18 – Bioventing columns installation

The O_2 and CO_2 analyzer (3) is equipped with a pump that promotes the circulation of air through the entire installation. The flow of air used was 20 mL min^{-1} and was controlled by the Flowmeter controller (2). The air enters the bottom of the column (P4), passes through

the soil and exits through the top of the column (P1), airing the soil. The air dragged out of the column passes by the Mass Flow Controller and them by the O_2 and CO_2 analyzer, that monitors the concentrations of this two compounds. The air that exits the analyzer is expelled by the exhaust system of the laboratory. It was placed at the entrance pipe a cellulose acetate filter (0.20 µm pores) to prevent the entry of particles. The respirometry analyses was done daily, for 15 minutes. After the respirometry analyses the contaminants concentration was performed by GC. The sample (200 µL) was collected at the sampling port 2 (P2) with an ILS 250 µL syringe. The tests performed until the contaminants concentration reached 0.5 mg of contaminant by kilogram of soil for three consecutive days. At the end of each test, the microbial population was quantified, by counting the CFU, with successive dilutions in sterile saline solution (10^{-2} until 10^{-4}). In the designation of each test are referred the test is ventilated or not (BV for ventilated and B for non-ventilated), N for the non-inoculated tests, the typo of soil (SR for granitic soil and CL for limestone) and the contaminants concentration (Bz for benzene, Bt for butanol and the numbers 1 for 210 mg of contaminant by kilogram of soil, 05 for 105 mg of contaminant by kilogram of soil and 0 for 0 mg of contaminant by kilogram of soil).

Table 12 resumes the bioventing and biodegradation tests made with granitic and limestone soils.

Table 12 - Syntheses of the bioventing and bioremediation tests

Test designation	Type of test	Ventilation time (min)	Contaminant	Contaminant concentration (mg Kg^{-1})
BVNSRBz1Bt1	Non-inoculated	15	Benzene Butanol	210 210
BVSRBz1Bt0	Inoculated	15	Benzene Butanol	210 0
BVSRBz1Bt1	Inoculated	15	Benzene Butanol	210 210
BVSRBz1Bt05	Inoculated	15	Benzene Butanol	210 105
BNSRBz1Bt1	Non-inoculated	0	Benzene Butanol	210 210
BSRBz1Bt0	Inoculated	0	Benzene Butanol	210 0
BSRBz1Bt1	Inoculated	0	Benzene Butanol	210 210
BSRBz1Bt05	Inoculated	0	Benzene Butanol	210 105
BVNCLBz1Bt1	Non-inoculated	15	Benzene Butanol	210 210
BVCLBz1Bt0	Inoculated	15	Benzene	210

			Butanol	0
BVCLBz1Bt1	Inoculated	15	Benzene Butanol	210 210
BVCLBz1Bt05	Inoculated	15	Benzene Butanol	210 105
BNCLBz1Bt1	Non-inoculated	0	Benzene Butanol	210 210
BCLBz1Bt0	Inoculated	0	Benzene Butanol	210 0
BCLBz1Bt1	Inoculated	0	Benzene Butanol	210 210
BCLBz1Bt05	Inoculated	0	Benzene Butanol	210 105

Chapter 3 - Results and Discussion

In this chapter, results obtained are presented and a discussion of them. The experimental data used in this analysis can be find in the Appendix.

3.1. Soil characterization

In this work three soils were used: two naturals (residual granitic soil and a soil contaminated with crude) and one prepared soil (limestone).

3.1.1. Soil used as inoculum source (Bsoil)

The soil used in the inoculum preparation was collected from the retention basins of the storage tanks of a refinery. Figure 19 shows a sample of the soil (Bsoil).

Figure 19 - Bsoil sample

Table 13 shows the results obtained in the characterization tests, for total organic carbon (TOC), total petroleum hydrocarbons (TPH), nitrogen (N) and phosphorus (P). Bsoil is a sandy soil heavily contaminated with crude.

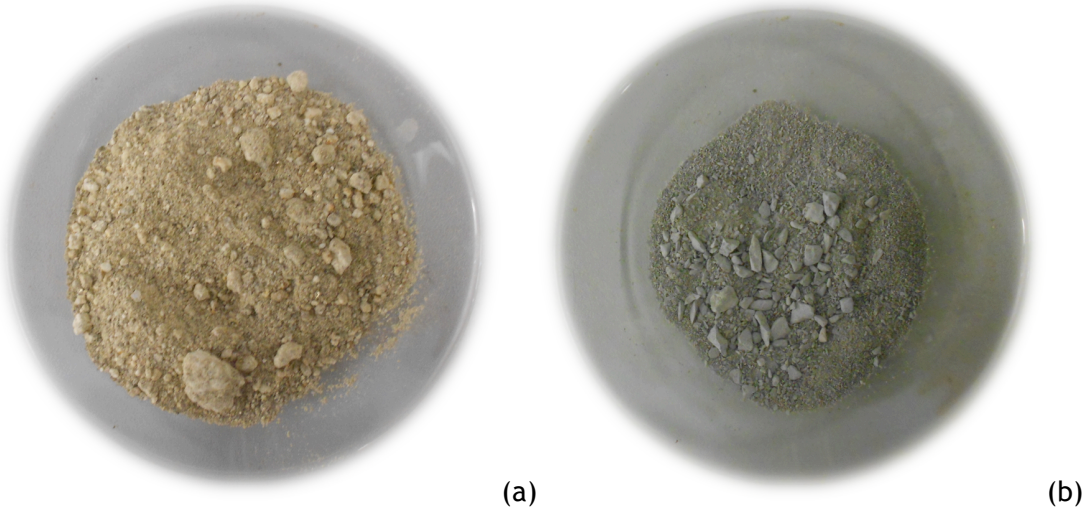
Table 13 - Bsoil properties [15]

Soil	TOC (%)	TPH (g Kg-1)	N (%)	P (%)
Bsoil	1.33	2.96	0.05	0.04

3.1.2. Granite and limestone

The selected soils represent lithologic units with relevance to Portugal. The granitic soil (SR) was collected in Porto zone and the limestone (CL) was collected in the vicinity of Coimbra.

Figure 20 shows a sample of SR and CL.



(a) (b)

Figure 20 – Samples of the soils used; (a) granite (SR); (b) limestone (CL)

Table 14 shows the results obtained in the characterization tests, the American Society for Testing and Materials (ASTM) classification, wet bulk density (ρ), total organic carbon (TOC), total petroleum hydrocarbons (TPH), nitrogen (N) and phosphorus (P).

Table 14 – Granite (SR) and limestone (CL) properties [15]

Soil	ASTM classification	ρ (Kg m^{-3})	TOC (%)	TPH (mg Kg-1)	N (%)	P (%)
SR	SM – Silty sands	1060	0.396	0.35	0.022	0.172
CL	SC -Clayey sands	1530	0.651	0.46	0.017	0.068

3.2. Biodegradation tests in liquid medium

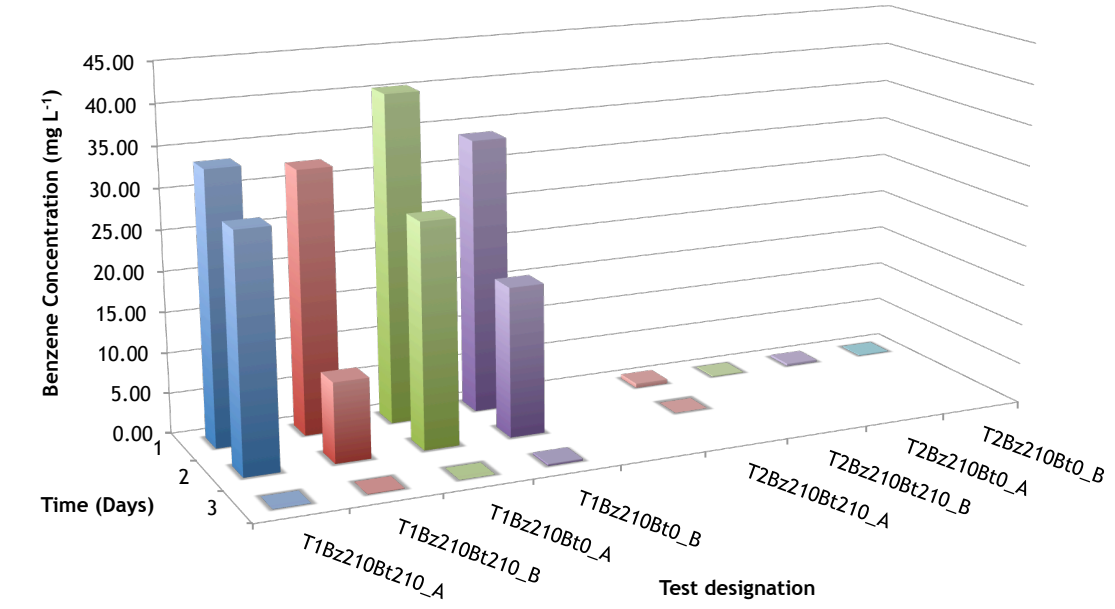
The biodegradation tests in liquid medium were performed in order to distinguish and isolate the types of microorganisms that specifically degrade benzene and butanol, and the time that it takes to biodegrade benzene, butanol and a blend of the two. In this subchapter the results obtained in those tests are presented, including the time evolution of the concentration of benzene and butanol in the gas phase, and the biomass at the end of the test. The experimental data used in this analyze can be found in Appendix 2.

The results obtained in these first tests showed that:

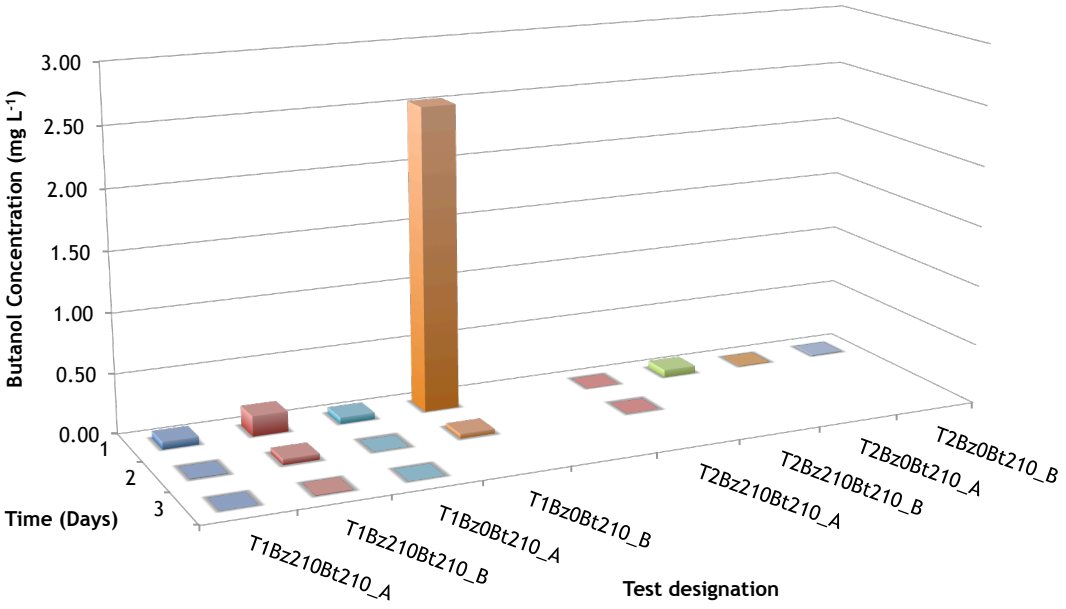
- The microbial consortium was capable of degrading benzene, butanol and a blend of the two, in the selected concentration (210 mg L^{-1});
- Butanol was always biodegraded faster than benzene;
- The concentration of 0.5 mg L^{-1} (final concentration of the test) was quickly reached in the second transfer for all tests;

Butanol always showed lower concentrations in the gas phase when compared with benzene concentrations in the beginning of all tests. This suggests that a large part is diluted in the aqueous phase, due to its high water solubility (90 g L^{-1}).

Figure 21 shows the time evolution of the concentration of benzene and butanol in the gas phase for the tests performed in liquid medium. Figure 22 shows the biomass concentration (in Colony Forming Units (CFUs per mL) at the end of the test. Figure 23 shows the visual aspect of the microorganisms that appeared in the test with butanol and, benzene separately.



(a)



(b)

Figure 21 – Biodegradation tests in liquid medium; (a) evolution of the benzene concentration in gas phase in the 1st and 2nd transfer, (b) evolution of the butanol concentration in gas phase in the 1st and 2nd transfer

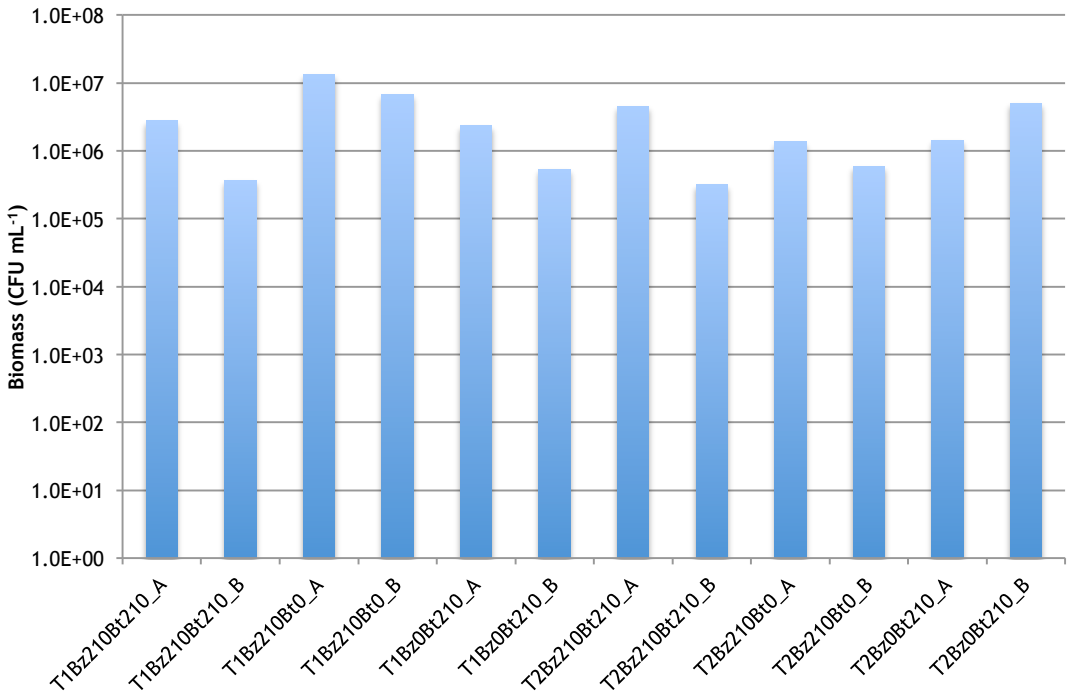
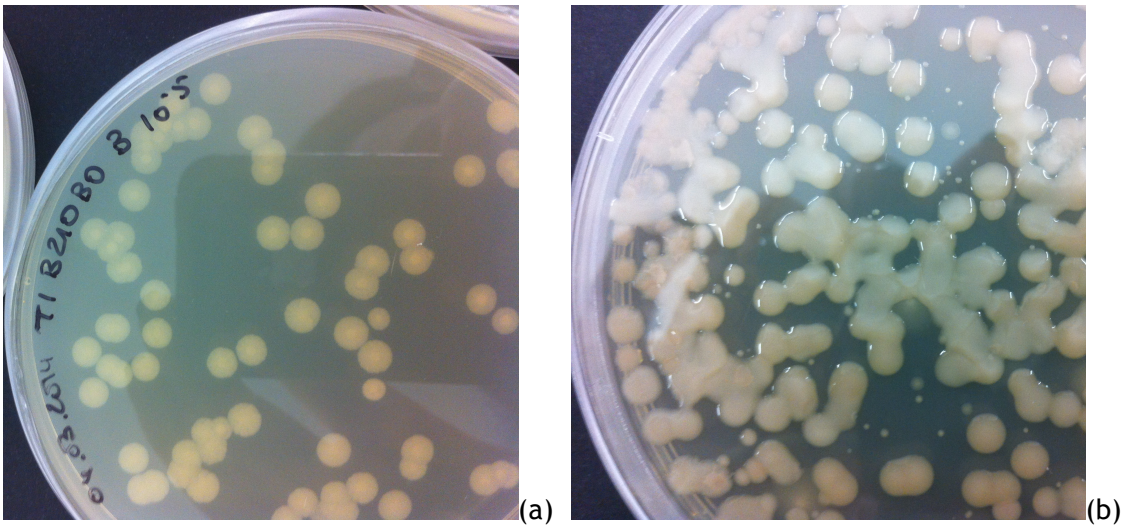


Figure 22 - Biomass quantification at the end of the tests



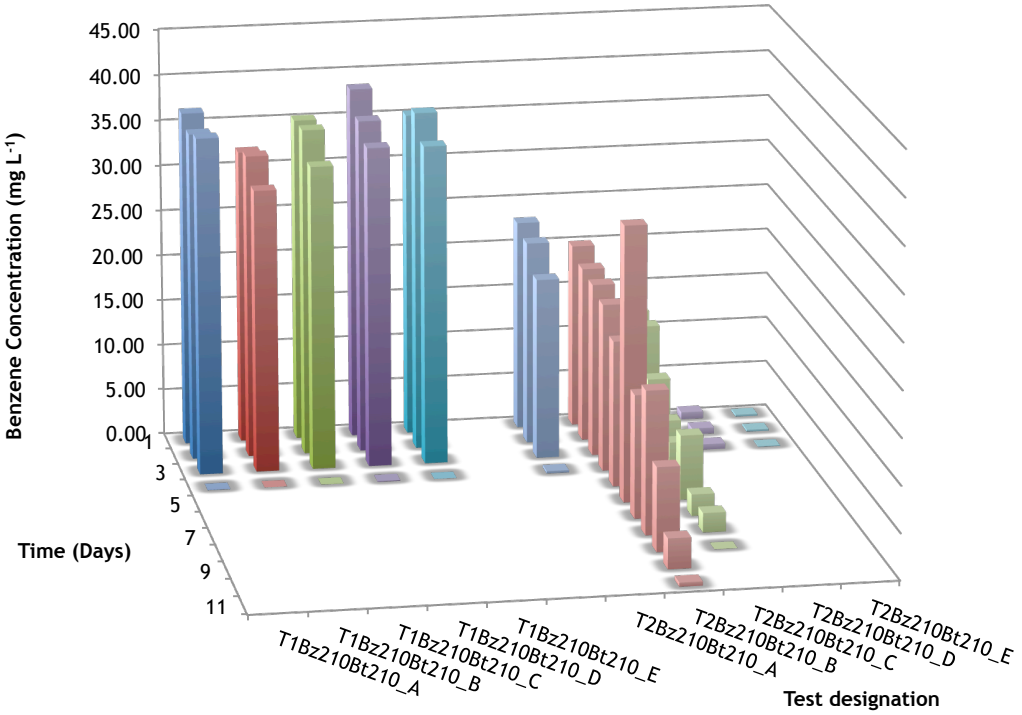
(a) (b)

Figure 23 – Microorganisms found in the biodegradation tests in liquid medium; (a) test with benzene as carbon source; (b) test with butanol as carbon source

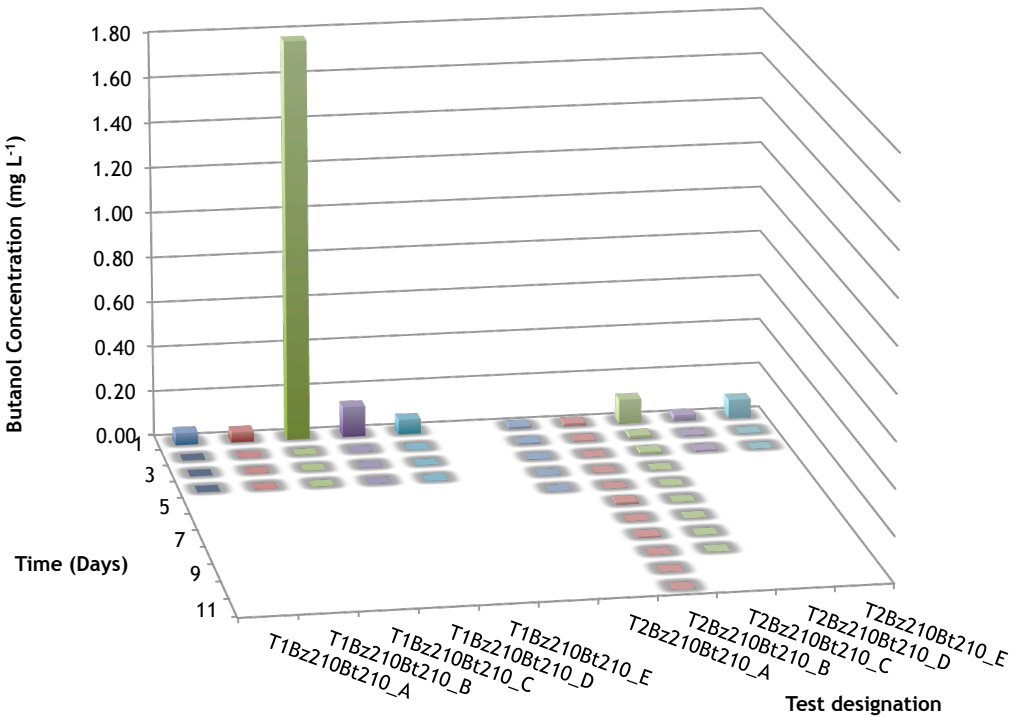
3.3. Inoculum preparation

The inoculum preparation was done in liquid medium, with the objective of isolating and developing the microbial consortium capable of degrade benzene and butanol, and to use in bioventing and bioremediation tests. In this subchapter the results obtained in the monitoring and the preparation of the inoculum are presented. In addition, the time evolution of the concentration of benzene and butanol in the gas phase and the biomass at the end of the test are also shown. The experimental data used in this analysis can be found in Appendix 3.

During this work, two inocula were prepared from two different enrichment cultures, and both were prepared using the method described in the subchapter 2.9 of this work. Figure 24 and Figure 25 shows the time evolution of the concentration of benzene and butanol in the gas phase for the two inocula.

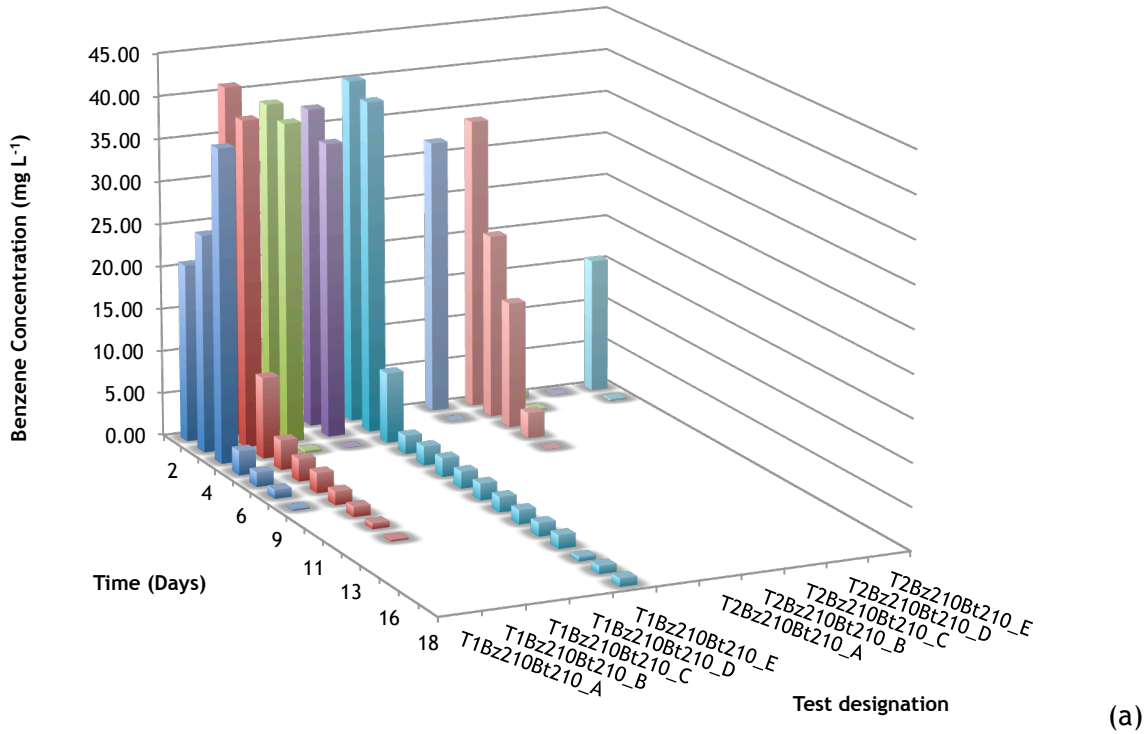


(a)

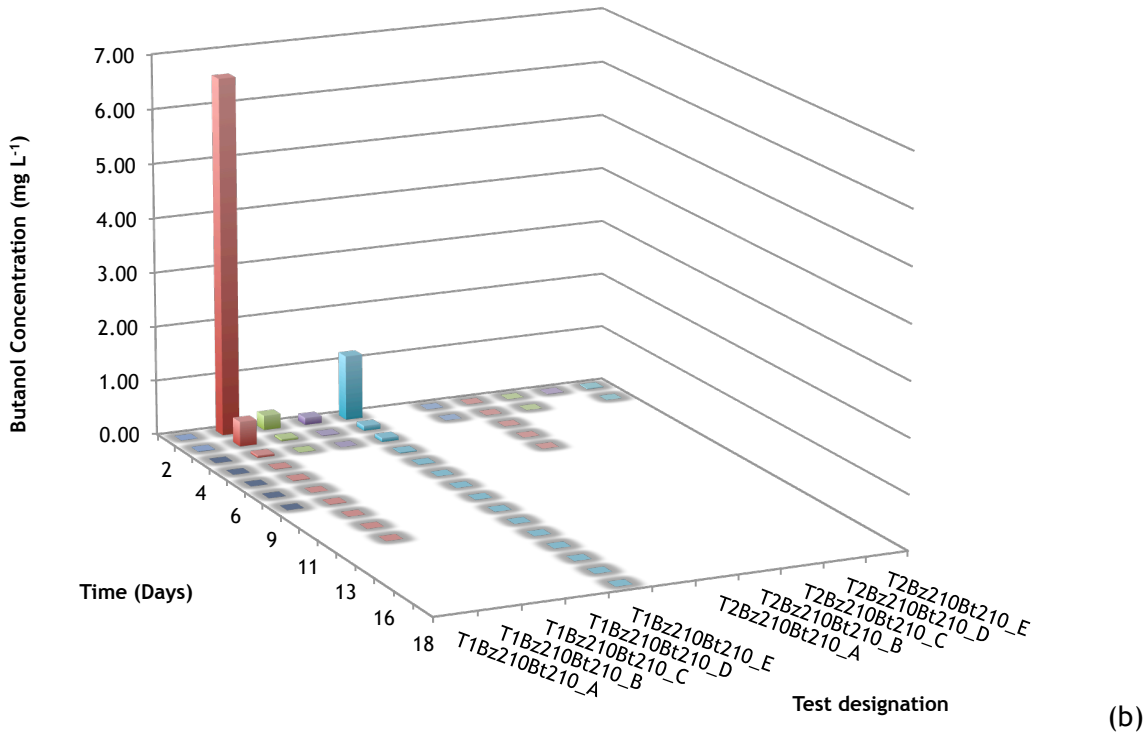


(b)

Figure 24 – Inoculum 1; (a) evolution of the benzene concentration in the gas phase in the 1st and 2nd transfers, (b) evolution of the butanol concentration in the gas phase in the 1st and 2nd transfers



(a)



(b)

Figure 25 - Inoculum 2; (a) evolution of the benzene concentration in the gas phase in the 1st and 2nd transfers, (b) evolution of the butanol concentration in the gas phase in the 1st and 2nd transfers

The results obtained in the inoculums preparation showed that:

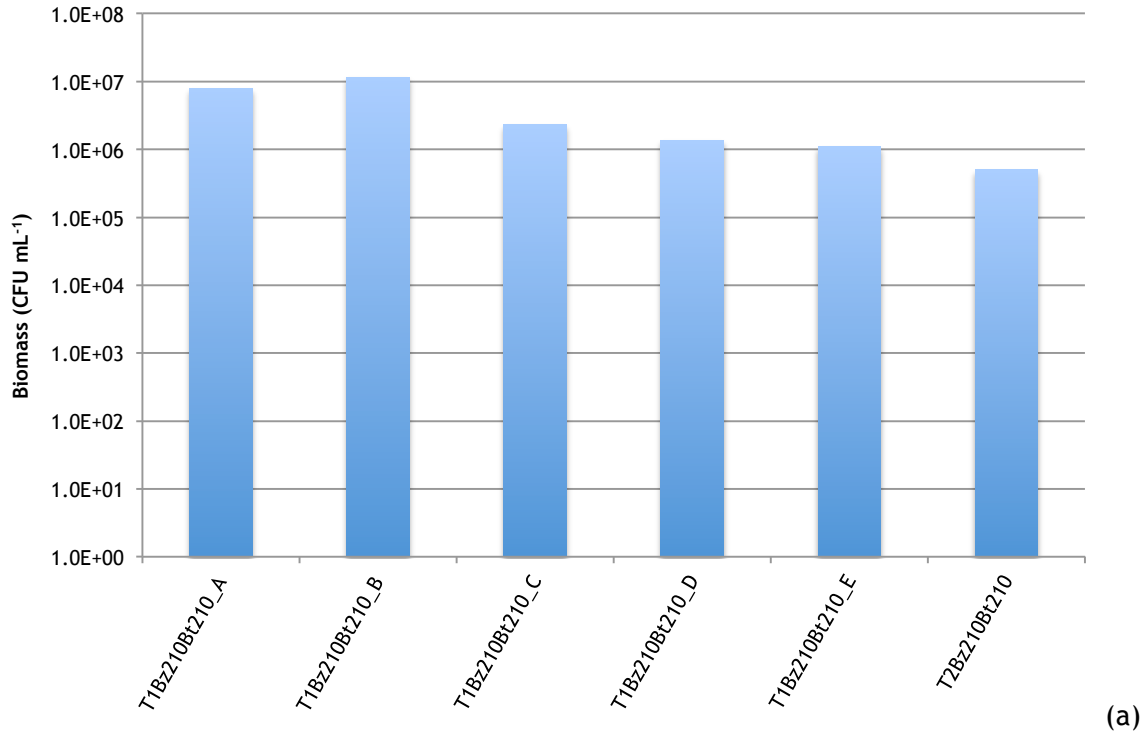
- Butanol was biodegraded faster than benzene in the two inocula;
- The concentration of 0.5 mg L^{-1} (final concentration of the test) was quickly reached in the first transfer in inoculum 1, while in inoculum 2, that concentration was reached faster in the second transfer;

Like in the biodegradation test performed in liquid medium, the butanol concentration was, from the beginning of the tests, very low when compared with the benzene concentration.

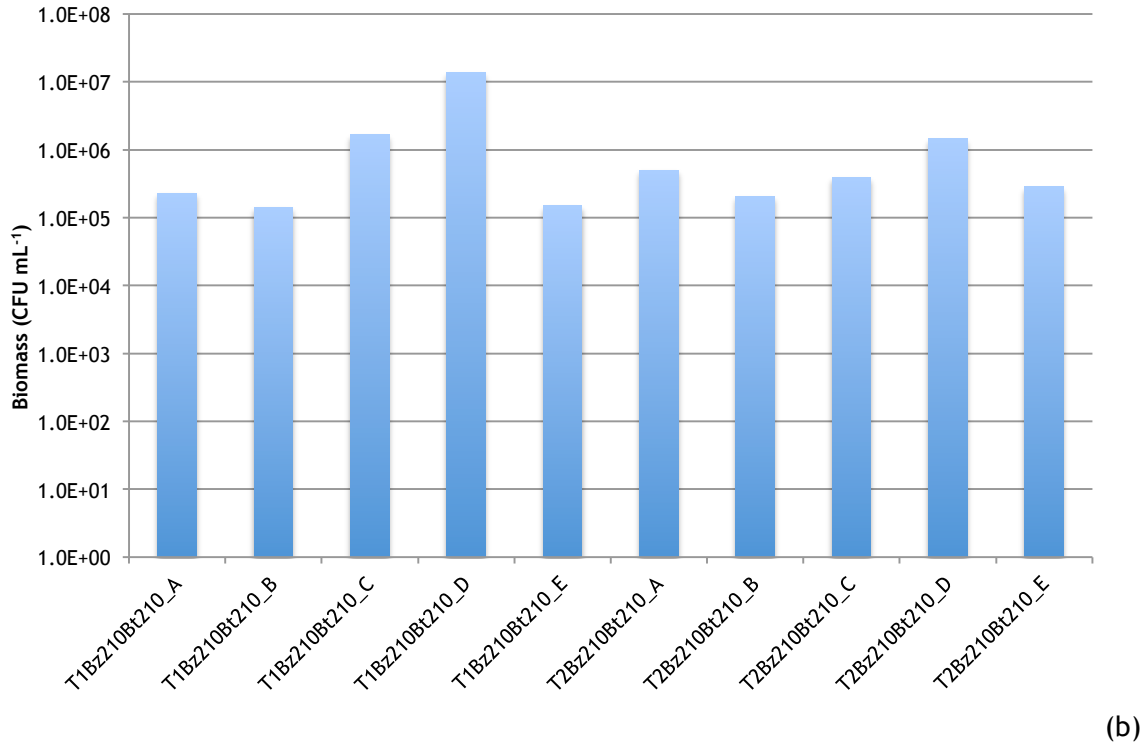
During the course of the work, the order that the injections in the GC were made were found to influence the results. If an injection of a sample with high contaminant concentration were made before an injection of a sample with a lower concentration, the result of the second would be higher than the actual value. Therefore, in order to try avoid this kind of experimental errors, samples that potentially had higher contaminant concentrations were injected first.

The TPH concentration was also measured in the liquid phase in order to determine the contaminant concentration (benzene plus butanol) remaining in the inoculum. The concentrations were very low for all the tests.

Figure 26 shows the biomass at the end of the test. For the second transfer for inoculum 1, the biomass was only quantified in the final flask with all the inoculum.



(a)



(b)

Figure 26 – Biomass quantification at the end of the tests; (a) Inoculum 1; (b) Inoculum 2

3.4. Bioventing and bioremediation tests

As mentioned in the section 2.10 of this work, bioventing is defined as enhanced biodegradation tests performed with ventilation, and bioremediation is defined as enhanced biodegradation tests performed without ventilation.

The results are organized by soil type (SR and CL). For each soil, inoculated bioventing tests (BV), non-inoculated bioventing tests (BVN), inoculated bioremediation tests (B) and non-inoculated bioremediation tests (BN) were performed. The results shown include the time evolution of the concentration of benzene and butanol in the gas phase, the biomass at the final of the test, the remediation time and the theoretical remediation efficiency at the end of the test. In the case of the bioventing test, it is also presented the time evolution of the O_2 and CO_2 concentration. The experimental data used in this analysis can be found in Appendix 4

3.4.1. Tests on Granitic soil

The results obtained in the bioventing and bioremediation tests performed with granitic soil (SR), relating the GC analyses (gas phase), are shown in Figure 27 to 29.

The results obtained in the bioventing and bioremediation tests (Figure 27 and Figure 28) shown that:

- Like in the biodegradation test in liquid medium and in the inoculum preparation, the concentration of butanol was always very low since the beginning of the tests;
- The benzene concentration in the non-inoculated bioventing test (BVNSRBz1Bt1) was slightly decreasing over time. In the beginning of test, its decrease was similar to the inoculated bioventing tests, maintaining a constant evolution during the remaining test time. This fact suggests that the losses should be exclusively related to advection. The O_2 and CO_2 concentrations suggest that there is no microbial activity (Figure 29 (b)). The biomass quantification showed some microbial growth indicating that probably the two steps of sterilization of the soil was not sufficient. This test was stopped when the bioventing test was stopped (19 days) so was not given the necessary time to accomplish complete remediation. The theoretical remediation efficiency for this test was 95.3 %, which demonstrates the great importance of vapor extraction for the remediation of benzene, since it is a very volatile compound;

- In the inoculated bioventing tests (BVSRBz1Bt0, BVSRBz1Bt1 and BVSRBz1Bt05), the difference between the tests is the butanol concentration (0 mg L^{-1}, 210 mg L^{-1} and 105 mg L^{-1} respectively). The test with 105 mg L^{-1} of butanol was the fastest to reach 0.5 mg L^{-1} of benzene for three consecutive days (13 days), followed by the test with 210 mg L^{-1} (15 days) and the last was the test with no butanol (19 days). This suggests that the presence of butanol contributes to the decrease of benzene remediation time. The theoretical remediation efficiency was higher than 99.8 % for the entire inoculated bioventing test, for both butanol and benzene.

- In the non-inoculated bioremediation test (BNSRBz1Bt1), the benzene concentration was slightly decreasing over time, although with a lower rate than the corresponding bioventing one. Biomass quantification (Figure 29 (b)) also does not show microbial activity. The theoretical remediation efficiency for this test was 93.0 %.

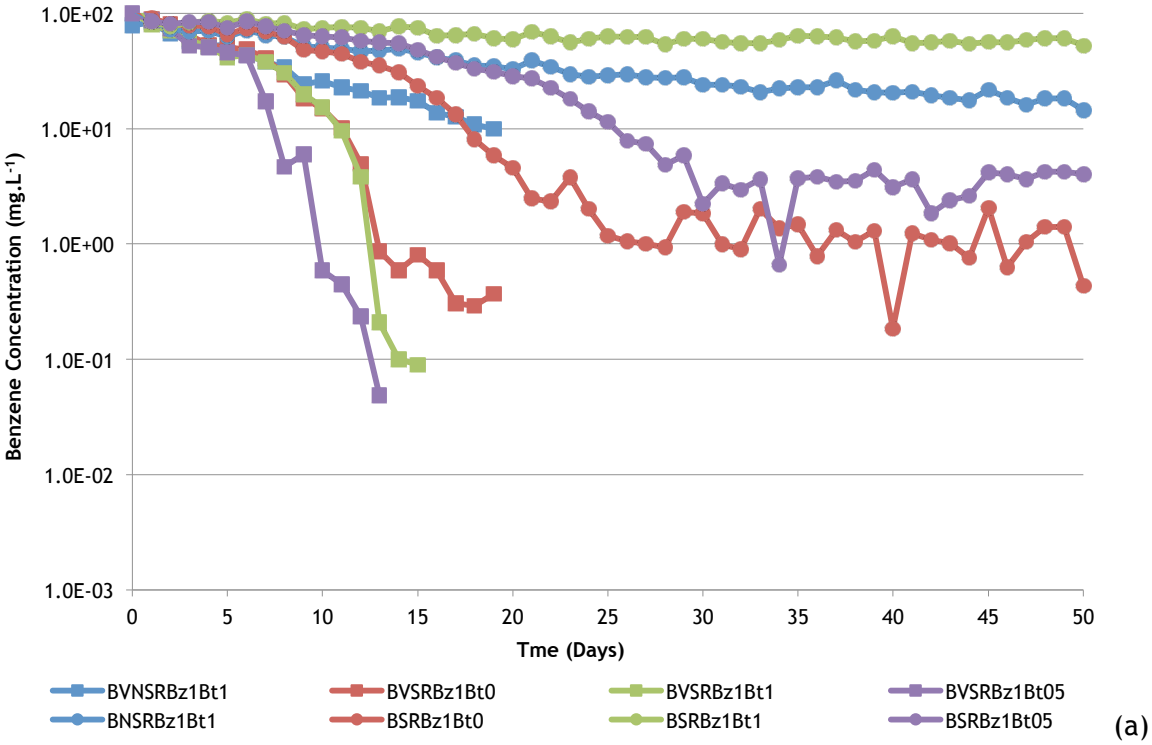
- The inoculated bioremediation tests (BSRBz1Bt0, BSRBz1Bt1 and BSRBz1Bt05) were stopped after 50 days, once benzene concentrations were similar for several days, even without reaching the concentration of 0.5 mg L^{-1} for three consecutive days. The test with the lowest concentration after the 50 days was the BSRBz1Bt0 with a theoretical remediation efficiency of 99.8 %, followed by the BSRBz1Bt05 with 98.1 %.

- In the BSRBz1Bt1 test, the microbial consortium seems to have not worked, with only a slight decrease in concentration. Since this test did not work, it was expected that its behavior was similar to the BNSRBz1Bt1, but the benzene concentrations were always higher, therefore, an error in the contamination of this column probably should have occurred.

- The butanol concentration, since day 24, for most of the tests, was no longer detected by the GC, so the values do not appear in the Figure 27 (b).

- Respirometry described adequately the biodegradation process. The O_2 and CO_2 concentrations variations registered correspond to the different phases of the biodegradation process.

- Biomass quantification (Figure 29 (b)) show similar results in the bioventing and bioremediation tests.



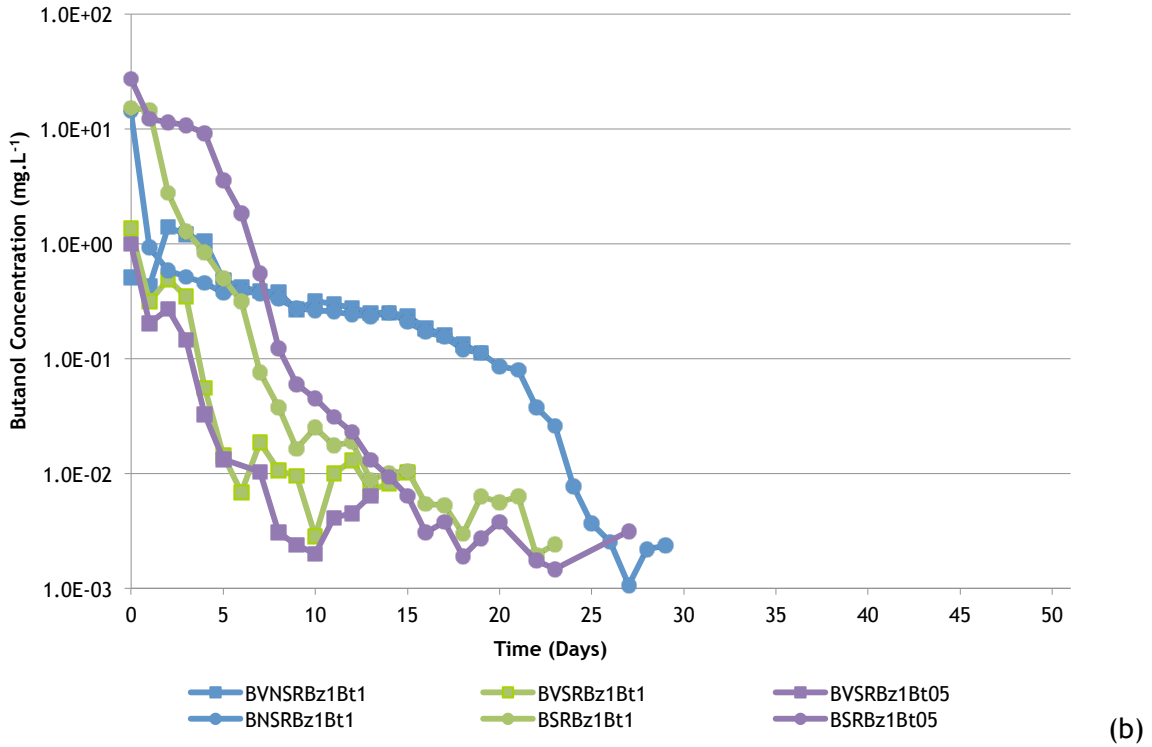
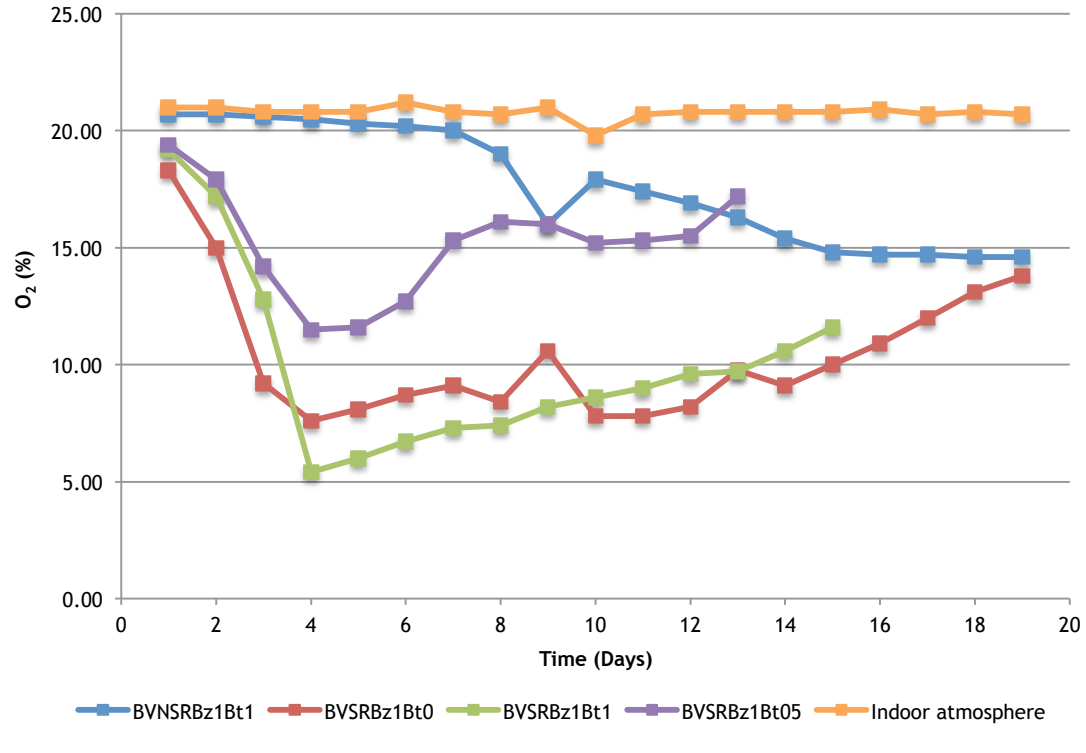
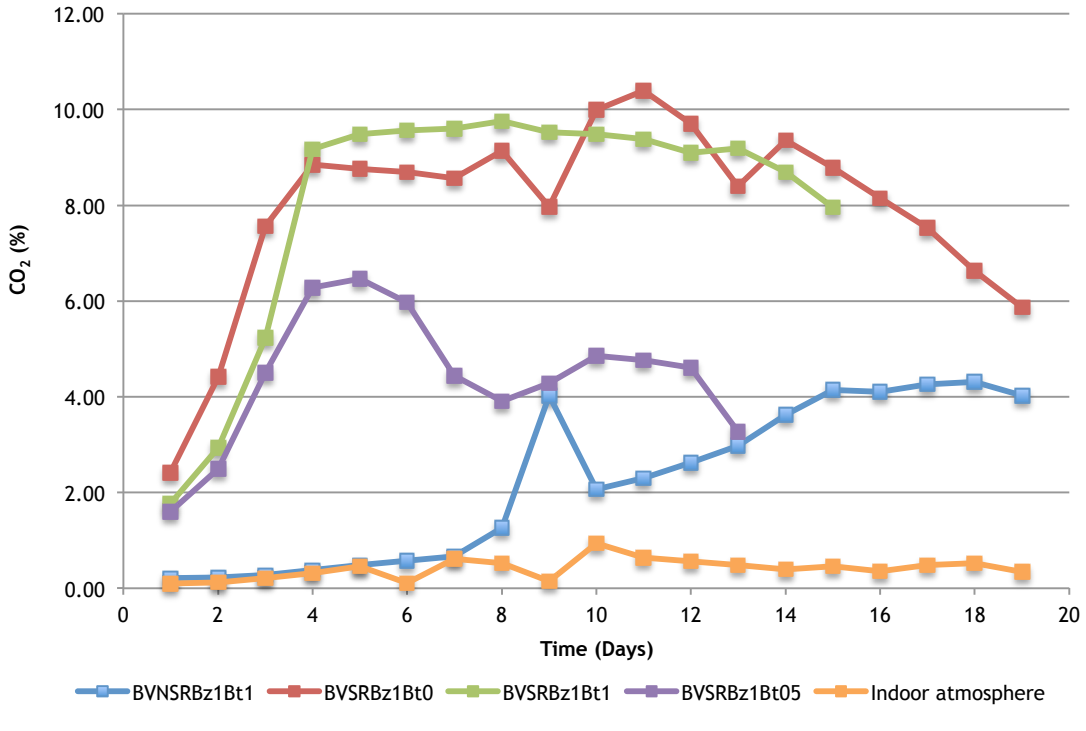


Figure 27 - Bioventing and bioremediation tests in granite (SR); (a) time evolution of the concentration of benzene in gas phase; (b) time evolution of the concentration of butanol in gas phase

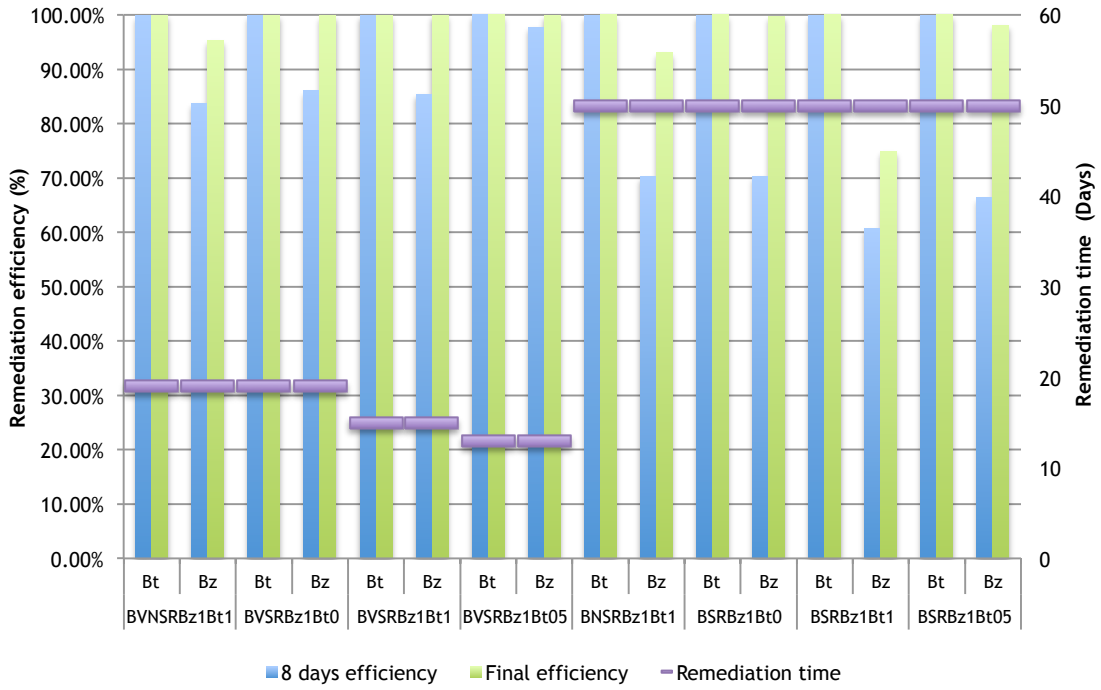


(a)

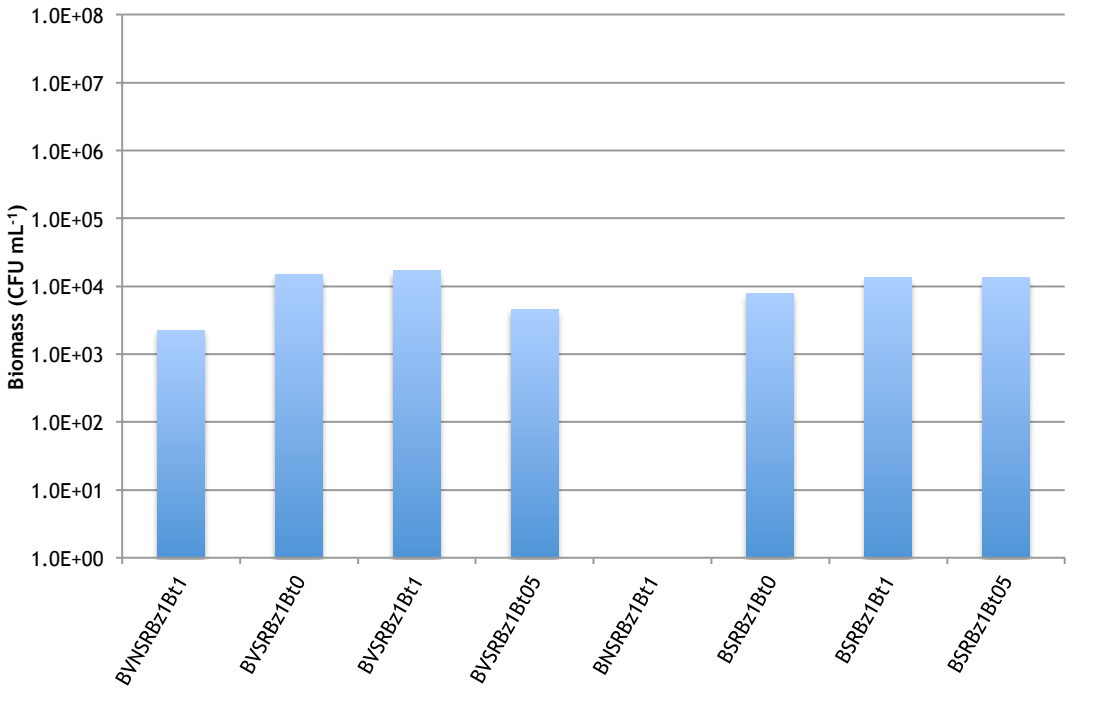


(b)

Figure 28 - Bioventing and bioremediation tests in granite (SR); (a) time evolution of O_2; (b) time evolution of CO_2



(a)



(b)

Figure 29 - Bioventing and bioremediation tests in granite (SR); (a) Remediation efficiencies and remediation time; (b) Biomass quantification at the end of the tests

At the end of the bioremediation tests, O_2 and CO_2 concentrations inside the columns were measured (Table 15).

Table 15 - O_2 and CO_2 concentration at the end of the bioremediation tests

Test designation	BNSRBz1Bt1		BSRBz1Bt0		BSRBz1Bt1		BSRBz1Bt05	
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
Concentration (%)	18.90	2.39	1.70	12.00[1]	2.80	12.00[1]	16.40	5.00

[1] Maximum CO_2 valor measured by the O_2 and CO_2 Analyzer, the real value is possibly higher

Regarding the values in Table 15, it can be seen that the O_2 concentrations are very low and the CO_2 concentrations are very high. In the BVSRBz1Bt1 test, the low O_2 concentration and the high CO_2 concentration might be an explanation why the microbial consortium did not work. The concentrations for the BNSRBz1Bt1 test suggest the absence of microbial activity.

Comparing these results with that presented in previous work developed on this subject [15], the following conclusions can be state:

- The remediation time in the inoculated bioventing test with benzene only (BVSRBz1Bt0) (20 days) was about the same when compared with bioventing tests reported in [15] (18 days);
- For the inoculated bioventing tests with butanol and benzene (BVSRBz1Bt1 and BVSRBz1Bt05) when compared with bioventing tests reported in [15], the remediation time was shorter, which again indicates that the presence of butanol reduces the remediation time of benzene;
- For all the inoculated bioremediation tests (BSRBz1Bt0, BSRBz1Bt1 and BSRBz1Bt05), the remediation time was longer (50 days), when compared with [15] (32 days);

The TPH concentration was also measured in the solid phase in order to determine the contaminant concentration (benzene plus butanol) remaining in the soil. Figure 30 shows the concentration of TPH in the solid phase for the bioventing and bioremediation tests.

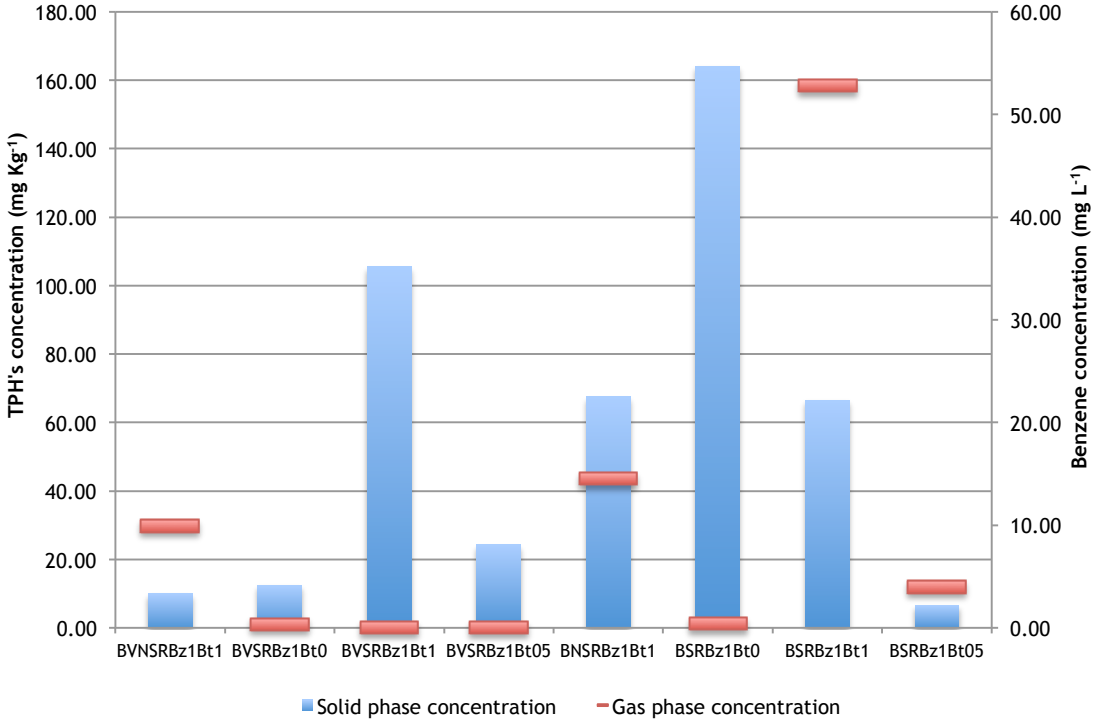


Figure 30 - Concentration of TPH in the solid phase and benzene concentration in gas phase for granite (SR)

The analysis of the Figure 30 shows that, although the benzene and butanol concentrations in gas phase were, in most of the tests, very low, the concentrations in the solid phase were, in some tests, very high. This can be a very important result because using only the contaminant concentrations in gas phase may not be enough to know if the remediation process is working properly.

The remediation efficiencies were recalculated by using the concentration of TPH in the solid phase and the benzene concentration in the gas phase at the end of each test, were. Figure 31 shows a comparison between the remediation efficiencies, with the gas phase only and the gas phase plus solid phase.

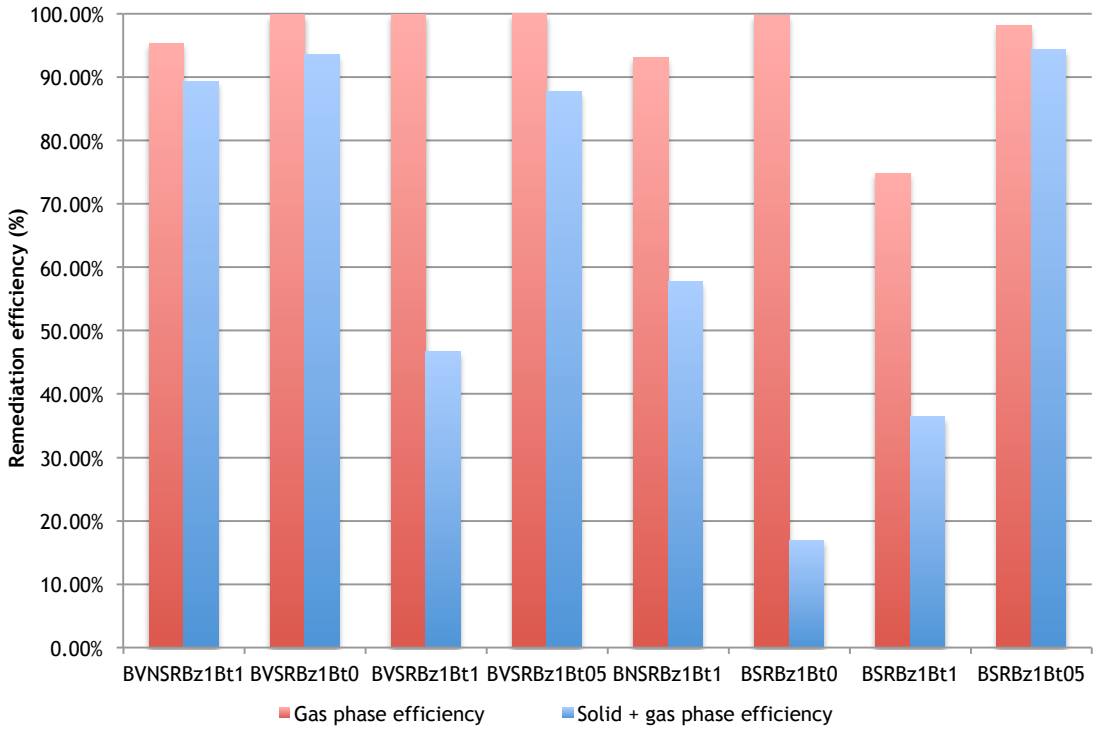


Figure 31 - Remediation efficiencies in gas phase only and in solid + gas phase

As shown in Figure 31, when is taking into account the contaminant remaining in the solid phase, the remediation efficiencies are substantially lower.

3.4.2. Tests on Limestone

The results obtained in the bioventing and bioremediation tests performed with limestone (CL), relating the GC analyses (gas phase), are shown in Figure 32 to 34.

The results obtained in the bioventing and bioremediation tests (Figure 32 and Figure 33) shown that:

- Like in the biodegradation test in liquid medium and in the inoculum preparation, the concentration of butanol was always very low from the beginning of the tests;
- In the non-inoculated bioventing test (BVNCLBz1Bt1), the benzene concentration was slightly decreasing over time, maintaining a constant evolution during the test. This fact suggests that the losses should be exclusively related to advection. The O_2 and CO_2 concentration and the biomass quantification also do not show microbial activity

(Figure 33). This test was stopped simultaneously with the other bioventing tests were stopped (21 days) so it was not given the time needed to accomplish complete remediation. The theoretical remediation efficiency for this test was 95.9 %, which demonstrates the great importance of vapor extraction for the remediation of benzene, since it is a very volatile compound;

- In the inoculated bioventing tests (BVCLBz1Bt0, BVCLBz1Bt1 and BVCLBz1Bt05) the difference between the tests is the butanol concentration (0 mg L^{-1}, 210 mg L^{-1} and 105 mg L^{-1} respectively). The test with 105 mg L^{-1} of butanol was the fastest to reach 0.5 mg L^{-1} of benzene for three consecutive days (8 days), followed by the test with 210 mg L^{-1} (10 days) and the last was the test with no butanol (18 days). This suggests that butanol decreases benzene remediation time. The theoretical remediation efficiency was higher than 99.7 % for the entire inoculated bioventing test, for both butanol and benzene.

- In the non-inoculated bioremediation test (BNCLBz1Bt1), the benzene concentration was slightly decreasing over time, although with a lower rate than the corresponding bioventing one. The biomass quantification (Figure 34) also does not show microbial activity. The theoretical remediation efficiency for this test was 88.1 %.

- The inoculated bioremediation tests (BCLBz1Bt0, BCLBz1Bt1 and BCLBz1Bt05) were stopped after 22 days when the benzene concentration in the BCLBz1Bt0 and the BCLBz1Bt05 tests reached 0.5 mg L^{-1} for three consecutive days. In both tests, the behavior is very similar which demonstrates that lower butanol concentrations do not interfere in benzene remediation, with a theoretical remediation efficiency above 99.0 %. In the BSRBz1Bt1 test, the microbial consortium seems to have not worked, with only a slight concentration decrease, showing similar behavior to the non-inoculated test (BNCLBz1Bt1).

- Sometimes butanol concentrations were not possible to be detected by the GC, therefore some values do not appear in the Figure 32 (b).

- Respirometry described adequately the biodegradation process. The O_2 and CO_2 concentrations variations registered correspond to the different phases of the biodegradation process.

- Biomass quantification (Figure 34 (b)) shows a higher biomass amount in the bioremediation tests than in the bioventing tests.

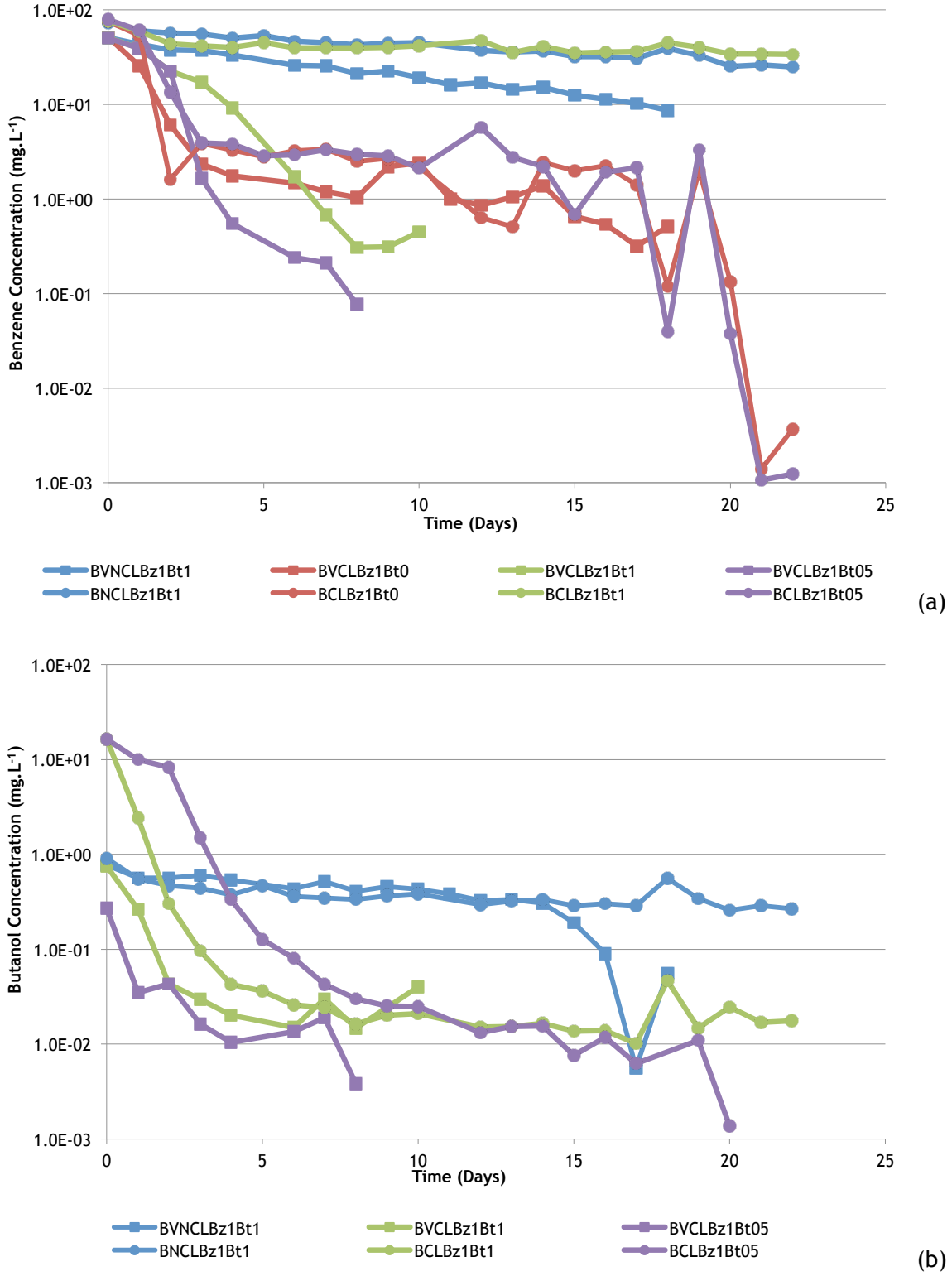
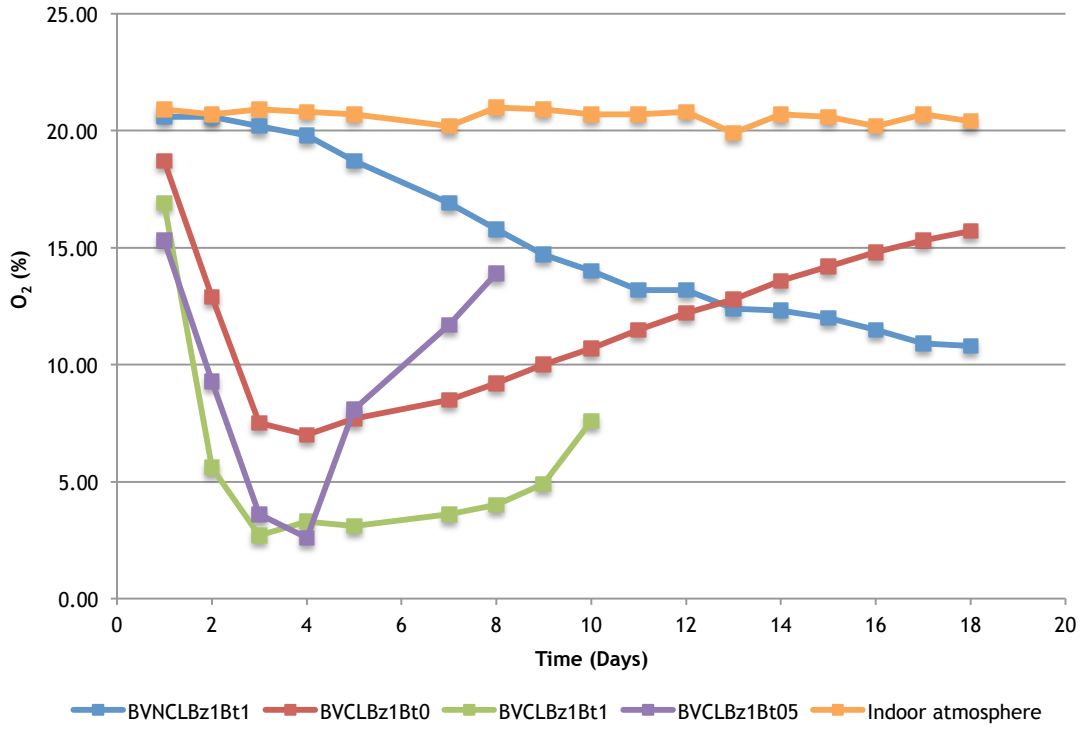
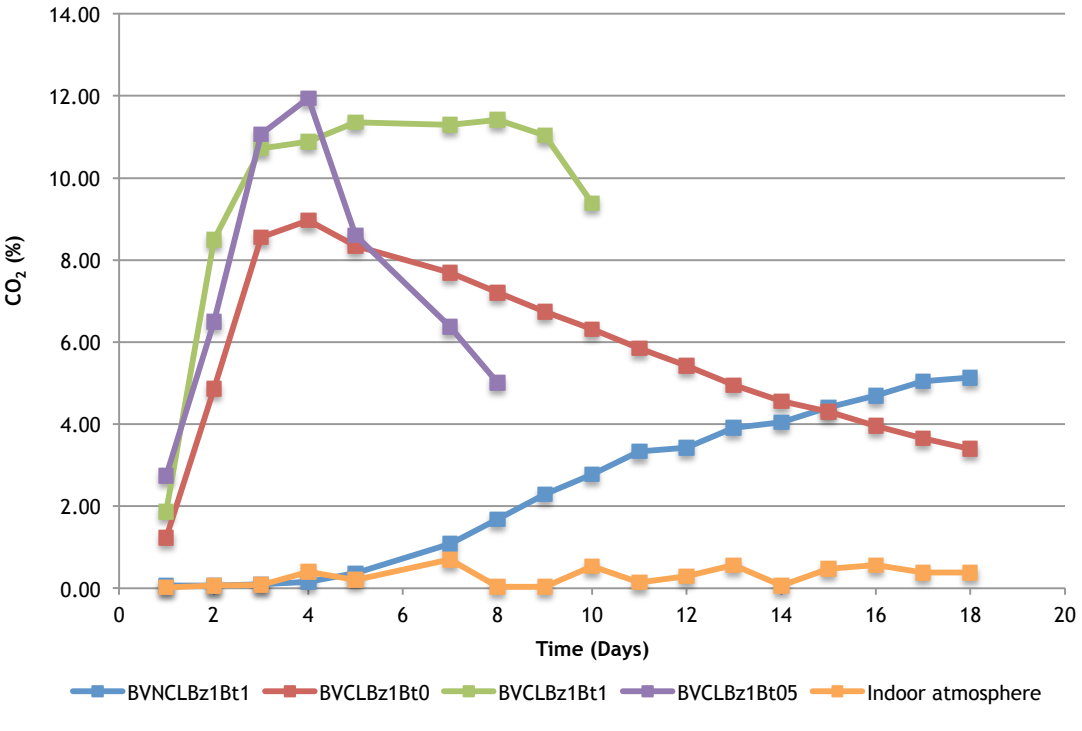


Figure 32 - Bioventing and bioremediation tests in limestone (CL); (a) time evolution of the concentration of benzene in gas phase; (b) time evolution of the concentration of butanol in gas phase

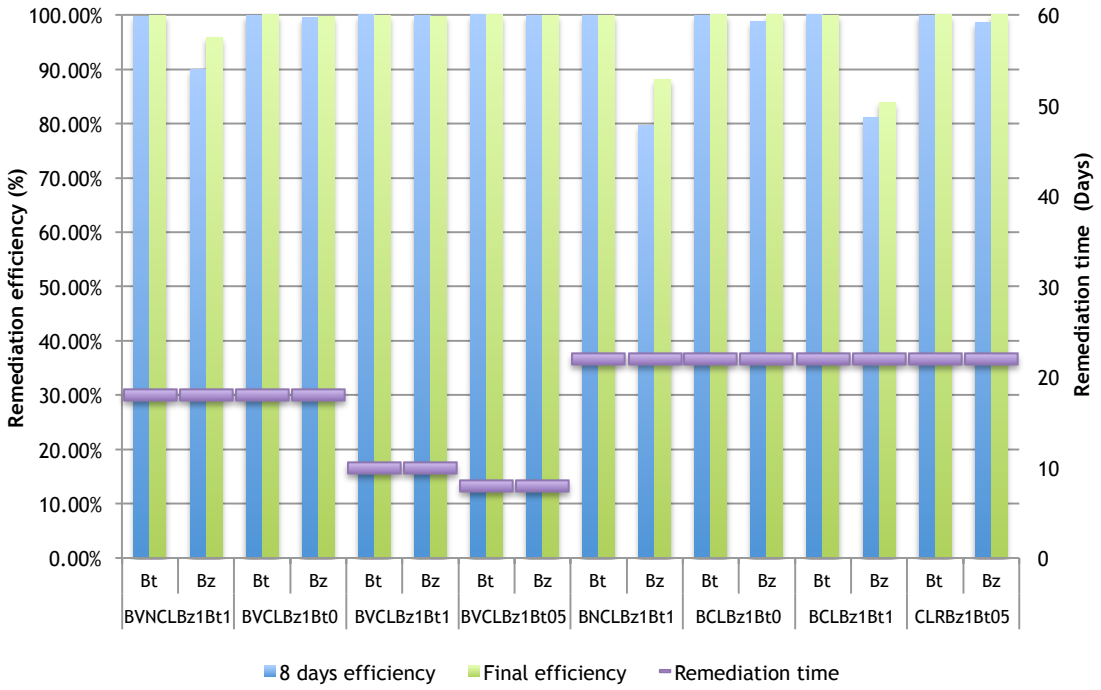


(a)

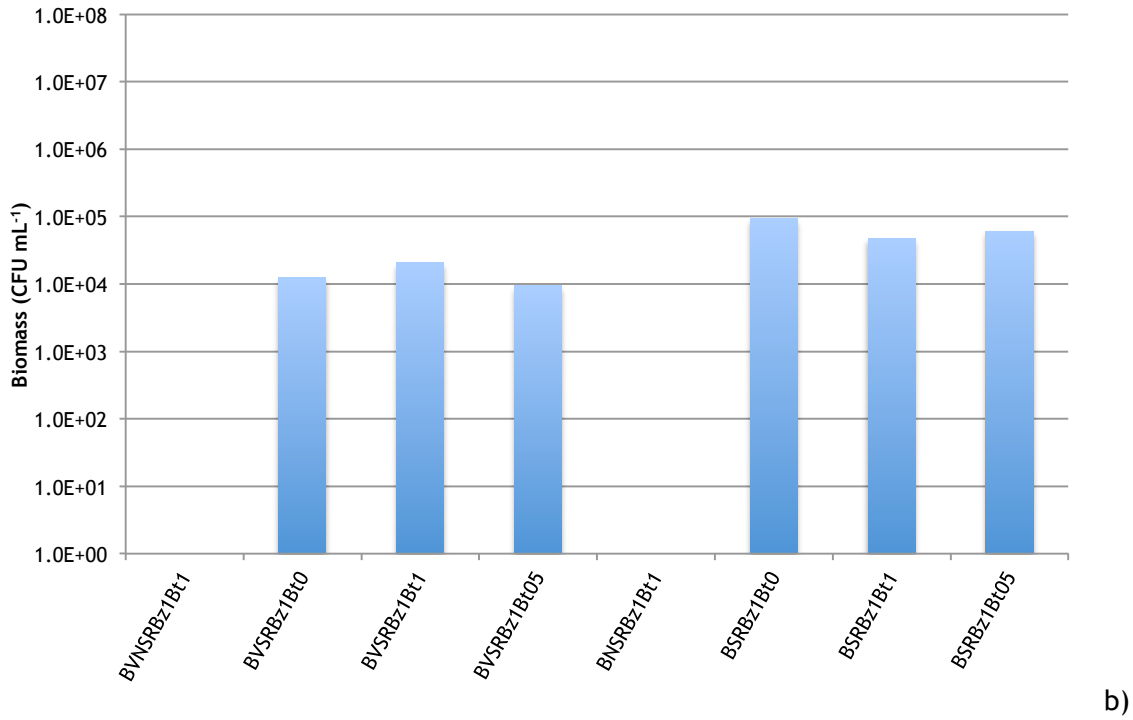


(b)

Figure 33 - Bioventing and bioremediation tests in limestone (CL); (a) time evolution of O_2; (b) time evolution of CO_2



(a)



b)

Figure 34 - Bioventing and bioremediation tests in limestone (CL); (a) Remediation efficiencies and remediation time; (b) Biomass quantification at the end of the tests

At the end of the bioremediation tests, O_2 and CO_2 concentrations inside the columns were measured (Table 16).

Table 16 - O_2 and CO_2 concentration at the end of the bioremediation tests

Test designation	BNCLBz1Bt1		BCLBz1Bt0		BCLBz1Bt1		BCLBz1Bt05	
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
Concentration (%)	21.00	0.07	17.80	0.84	4.90	8.70	6.00	7.49

Regarding the values in Table 16, it can be shown that O_2 concentrations in the tests with butanol (BCLBz1Bt1 and BCLBz1Bt05) present lower O_2 and higher CO_2 concentrations when compared with the test without butanol (BCLBz1Bt0). The concentrations for the BNCLBz1Bt1 test suggest the absence of microbial activity.

Comparing the results with that presented in previous work developed in this subject [15], the following conclusions can be state:

- The remediation time in the inoculated bioventing test with benzene only (BVCLBz1Bt0) (18 days) was longer when compared with bioventing tests reported in [15] (11 days);
- For inoculated bioventing tests with butanol and benzene (BVCLBz1Bt1 and BVCLBz1Bt05) when compared with bioventing tests reported in [15], the remediation time was shorter, which again indicates that the presence of butanol reduces the remediation time of benzene;
- For all the inoculated bioremediation tests (BCLBz1Bt0, BCLBz1Bt1 and BCLBz1Bt05), the remediation time was longer (22 days), when compared with [15] (13 days);

The TPH concentration was also measured in the solid phase, in order to know the contaminants concentration (benzene plus butanol) remaining in the soil. Figure 35 shows the concentration of TPH in the solid phase for the bioventing and bioremediation tests.

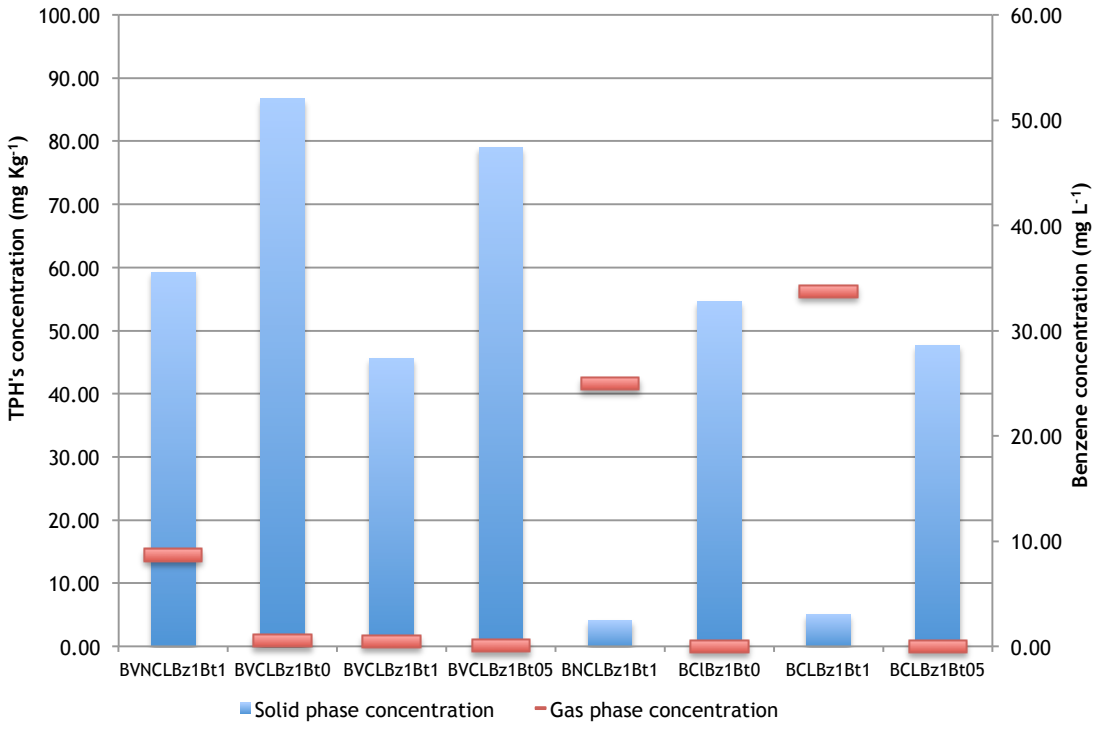


Figure 35 - TPH's concentration in solid phase and benzene concentration in gas phase for limestone (CL)

The analysis of the Figure 35 shows that, although the benzene and butanol concentrations in gas phase were, in most of the tests, very low, in the solid phase the concentrations were, in some tests, very high. This can be very important because only using the contaminant concentrations in gas phase is not enough to know if the remediation process is working properly.

The remediation efficiencies were recalculated with the concentration of TPH in solid phase and the benzene concentration in the gas phase at the end of each test. Figure 36 shows a comparison between the remediation efficiencies, in the gas phase only and in gas phase plus solid phase.

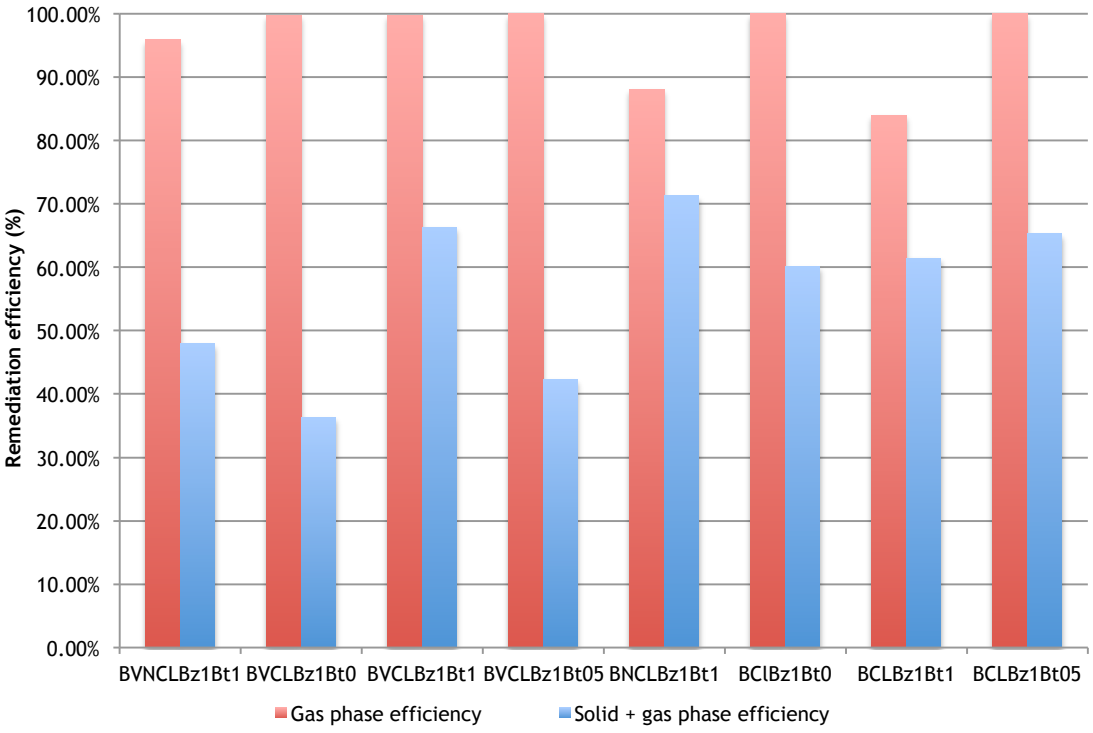


Figure 36 - Remediation efficiencies in gas phase and in solid + gas phase

As shown in Figure 36, the remediation efficiencies are substantially lower when taking into account the contaminant remaining in the solid phase.

3.4.3. Soils comparison

Figure 37 was made in order to compare the theoretical remediation efficiencies and the remediation times of the two soils used. As can be seen in the Figure 37 (a), the theoretical remediation efficiency was always higher for limestone (CL), with the exception of the non-inoculated bioremediation test (BNBz1Bt1). The remediation time was, for all the tests, lower for limestone (CL), since the bioremediation tests were 28 days faster.

As can be seen in the Figure 37 (b), biomass growth in the bioventing test was very similar for the two soils, while bioremediation tests were always higher for CL.

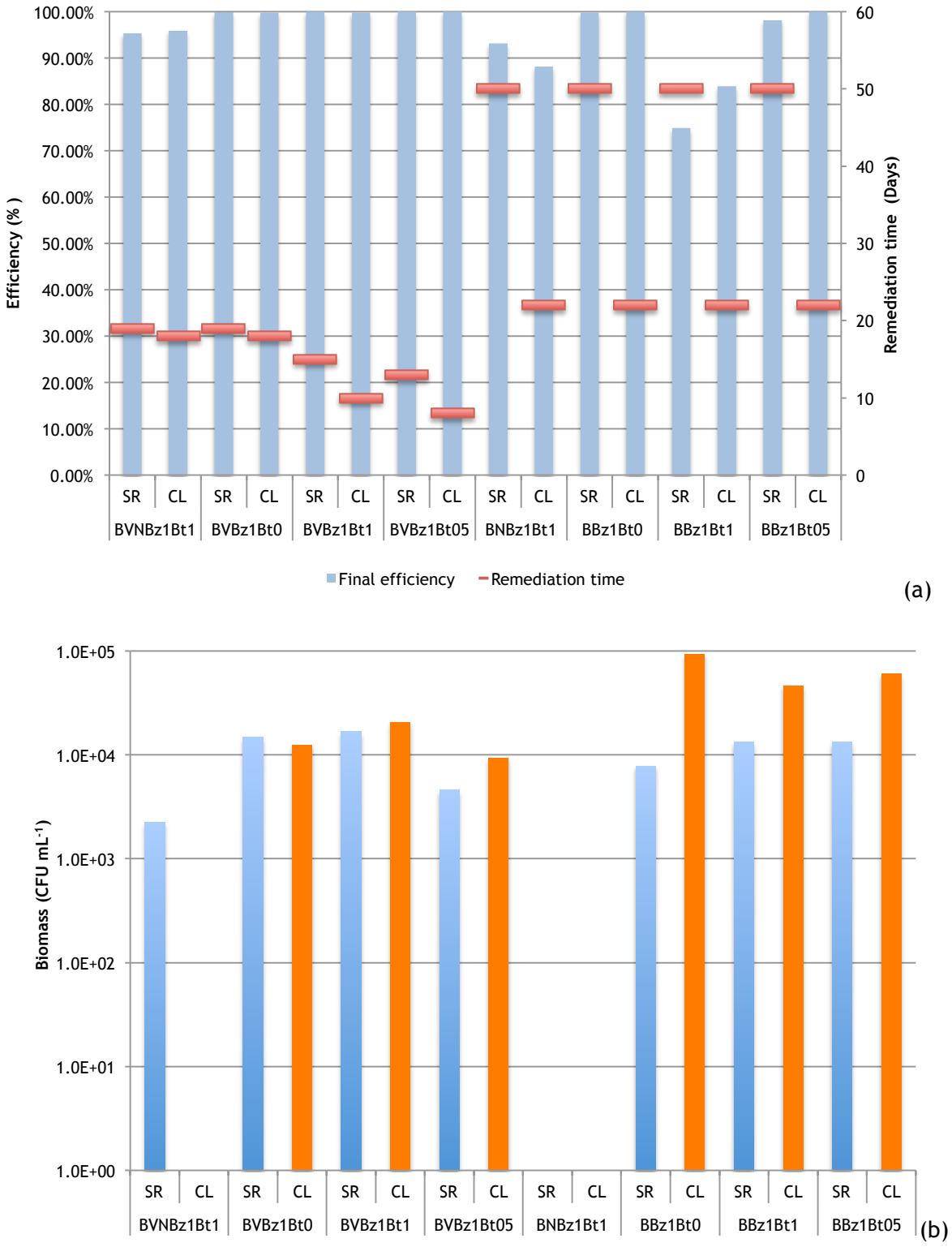


Figure 37 - Comparison between the two soils used; (a) theoretical remediation efficiency and remediation time; (b) Biomass quantification at the end of the tests

Chapter 4 - Final considerations

4.1. Conclusions

The selected contaminants, benzene (Bz) and butanol (Bt), showed different behaviors in the two different soils, granite and limestone (SR and CL).

The analytical methodology used to control contaminants concentration in gas phase proved to be appropriated and simple to use once developed.

The selected microbial consortium was capable of degrading the contaminants and a blend of the two at a maximum concentration of 210 mg L^{-1}.

The biodegradation tests in liquid medium allowed to distinguish the different types of microorganisms that degrade benzene and butanol.

Butanol always showed low concentrations in the gas phase since the beginning of all the tests when compared with benzene concentrations, and this suggests that a large part of it is diluted in the aqueous phase, due to its high water solubility (90 g L^{-1}).

The inoculum developed and used in the bioventing and bioremediation tests proved to be successful in obtaining very high remediation efficiencies in almost all the tests (only exception was the bioremediation test with the highest concentration of butanol).

The remediation time of the bioremediation test for SR was substantially faster than the bioventing tests (19 days to 50 days). For CL, the remediation time was faster in the bioventing tests but the difference was smaller (18 days to 22 days).

The bioremediation test in both soils did not work for the highest concentration of butanol. This can possibly be explained by the low O_2 and high CO_2 concentrations at the end of the test, which may indicate that the quantity of O_2 inside the column was not enough to degrade all of the contaminant that was present.

Respirometry described adequately the biodegradation process. The O_2 and CO_2 concentrations variations registered correspond to the different phases of the biodegradation process.

When compared the results with other works in the same field, the presence of butanol in the soil decreased the remediation time of benzene in the bioventing tests. In the bioremediation tests, the result was the opposite, butanol increased the remediation time of benzene.

The analysis of TPH in the soil at the end of the bioventing and bioremediation tests revealed that, in most of the tests, a high concentration of contaminant remained in the soil, which may indicate that control of the process only by the contaminant concentration in the gas phase may not be enough.

When the efficiencies were recalculated taking into account the contaminant remaining in the solid phase, they were much lower with values between 35 and 70%.

4.2. Recommendations for future work

To better understand some of the results obtained in this work, the following is recommended:

- Use different ventilation times, and different ventilation regimes in the bioventing tests;
- Perform sorption studies of the contaminants and soils used;
- Use different soils and different blend of contaminants;
- Perform mathematical modeling to describe the remediation processes.

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Appendix

Appendix 1 - Calibration curves

1. Butanol

Table 17 - Values of the peak area used to make the butanol calibration curve

Concentration (mg L^{-1})	Area
0.000	0.00E+00
0.254	1.26E+05
0.635	5.86E+05
2.541	2.67E+06
7.622	7.66E+06
12.704	1.21E+07

Slope=9.70E+05

Intercept=0

Coefficient of determination=0.9994

2. Benzene

Table 18 - Values of the peak area used to make the benzene calibration curve

Concentration (mg L^{-1})	Area
0.000	0.00E+00
0.275	4.77E+05
0.687	1.04E+06
1.375	2.40E+06
4.124	8.01E+06
6.874	1.26E+07
20.621	3.96E+07
27.494	4.80E+07
41.241	6.27E+07
54.988	1.09E+08
82.482	1.60E+08

Slope=1.88E+06

Intercept=0

Coefficient of determination=0.9949

Appendix 2 - Biodegradation test in liquid medium results

Table 19 - Results obtained for the contaminant concentration in gas phase, biodegradation test in liquid medium, 1st transfer (mg L^{-1})

Test	T1Bz210Bt210_A		T1Bz210Bt210_B		T1Bz210Bt0_A		T1Bz210Bt0_B		T1Bz0Bt210_A		T1Bz0Bt210_B	
Days	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz
1	0.06	33.48	0.17	32.37	0.00	40.38	0.00	33.83	0.05	1.02	2.53	0.14
2	0.00	28.90	0.05	9.81	0.00	27.53	0.00	18.43	0.00	0.34	0.04	0.06
3	0.00	0.02	0.00	0.05	0.00	0.01	0.00	0.24	0.00	0.01		

Table 20 - Results obtained for the contaminant concentration in gas phase, biodegradation test in liquid medium, 2nd transfer (mg L^{-1})

Test	T2Bz210Bt210_A		T2Bz210Bt210_B		T2Bz210Bt0_A		T2Bz210Bt0_B		T2Bz0Bt210_A		T2Bz0Bt210_B	
Days	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz
1	0.00	0.54	0.06	0.11	0.00	0.29	0.00	0.09	0.00	0.01	0.01	0.00
2	0.00	0.00										

Table 21 – Biomass quantification at the end of the tests

Test	T1Bz210Bt210_A	T1Bz210Bt210_B	T1Bz210Bt0_A	T1Bz210Bt0_B	T1Bz0Bt210_A	T1Bz0Bt210_B
Biomass (CFU mL^{-1})	2.75E+06	3.68E+05	1.32E+07	6.73E+06	2.34E+06	5.31E+05
Test	T2Bz210Bt210_A	T2Bz210Bt210_B	T2Bz210Bt0_A	T2Bz210Bt0_B	T2Bz0Bt210_A	T2Bz0Bt210_B
Biomass (CFU mL^{-1})	4.52E+06	3.16E+05	1.35E+06	5.85E+05	1.43E+06	5.05E+06

Appendix 3 - Inoculum preparation results

1. Inoculum 1

Table 22 - Results obtained for the contaminant concentration in gas phase, 1st inoculum preparation, 1st transfer (mg L^{-1})

Test	T1Bz210Bt210_A		T1Bz210Bt210_B		T1Bz210Bt210_C		T1Bz210Bt210_D		T1Bz210Bt210_E	
Days	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz
1	0.05	36.58	0.05	32.04	1.77	35.31	0.14	38.50	0.07	35.36
2	0.00	35.73	0.00	33.08	0.00	35.68	0.00	36.42	0.00	37.06
3	0.00	36.73	0.00	30.81	0.00	33.15	0.00	34.94	0.00	34.87
4	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00

Table 23 - Results obtained for the contaminant concentration in gas phase, 1st inoculum preparation, 2st transfer (mg L^{-1})

Test	T2Bz210Bt210_A		T2Bz210Bt210_B		T2Bz210Bt210_C		T2Bz210Bt210_D		T2Bz210Bt210_E	
Days	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz
1	0.01	22.92	0.01	20.04	0.11	12.42	0.03	0.78	0.08	0.21
2	0.00	22.18	0.00	19.06	0.01	12.37	0.00	0.66	0.00	0.21
3	0.00	19.70	0.00	18.87	0.01	8.12	0.01	0.60	0.00	0.08
4	0.00	0.26	0.00	18.27	0.00	5.18				
5			0.00	15.87	0.00	4.52				
6			0.01	30.00	0.00	7.00				
7			0.00	13.37	0.00	2.34				
8			0.01	15.54	0.00	2.09				
9			0.00	9.09	0.00	0.02				
10			0.00	3.29						
11			0.00	0.44						

Table 24 - Biomass quantification at the end of the tests

Test	T1Bz210Bt210_A	T1Bz210Bt210_B	T1Bz210Bt210_C	T1Bz210Bt210_D	T1Bz210Bt210_E
Biomass (CFU mL^{-1})	8.00E+06	1.16E+07	2.30E+06	1.36E+06	1.10E+06
Test	T2Bz210Bt210				
Biomass (CFU mL^{-1})	5.00E+05				

2. Inoculum 2

Table 25 - Results obtained for the contaminant concentration in gas phase, 2nd inoculum preparation, 1st transfer (mg L^{-1})

Test	T1Bz210Bt210_A		T1Bz210Bt210_B		T1Bz210Bt210_C		T1Bz210Bt210_D		T1Bz210Bt210_E	
Days	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz
2	0.00	20.73	6.57	41.05	0.26	38.53	0.13	37.45	1.18	40.26
3	0.01	25.45	0.45	38.31	0.04	37.41	0.01	34.53	0.09	38.90
4	0.00	36.73	0.04	9.45	0.00	0.29	0.00	0.03	0.07	8.21
5	0.00	2.82	0.01	3.45					0.02	2.13
6	0.00	1.66	0.01	2.61					0.00	2.20
7	0.00	1.01	0.00	2.34					0.00	2.16
9	0.00	0.17	0.00	1.56					0.00	1.97
10			0.00	1.17					0.00	1.94
11			0.00	0.67					0.00	1.75
12			0.00	0.21					0.00	1.64
13									0.00	1.53
14									0.00	1.55
16									0.00	0.49
17									0.00	0.91
18									0.00	0.93

Table 26 - Results obtained for the contaminant concentration in gas phase, 2nd inoculum preparation, 2nd transfer (mg L^{-1})

Test	T1Bz210Bt210_A		T1Bz210Bt210_B		T1Bz210Bt210_C		T1Bz210Bt210_D		T1Bz210Bt210_E	
Days	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz
1	0.00	31.90	0.00	33.93	0.00	1.06	0.00	0.01	0.02	15.61
2	0.00	0.00	0.00	21.45	0.00	0.18			0.00	0.21
3			0.00	14.70						
4			0.00	2.97						
5			0.00	0.01						

Table 27 - Biomass quantification at the end of the tests

Test	T1Bz210Bt210_A	T1Bz210Bt210_B	T1Bz210Bt210_C	T1Bz210Bt210_D	T1Bz210Bt210_E
Biomass (CFU mL^{-1})	2.28E+05	1.43E+05	1.66E+06	1.39E+07	1.50E+05
Test	T2Bz210Bt210_A	T2Bz210Bt210_B	T2Bz210Bt210_C	T2Bz210Bt210_D	T2Bz210Bt210_E
Biomass (CFU mL^{-1})	4.98E+05	2.03E+05	3.90E+05	1.45E+06	2.86E+05

Appendix 4 - Bioventing and biodegradation tests results

1. Granitic soil

Table 28 - Results obtained for the contaminant concentration in gas phase, bioventing tests, granitic soil (mg L^{-1})

Test	BVNSRBz1Bt1		BVSRBz1Bt0		BVSRBz1Bt1		BVSRBz1Bt05	
Days	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz
0	0.51	79.18	0.08	109.93	1.36	101.88	1.00	99.94
1	0.43	82.75	0.02	89.67	0.32	79.68	0.20	87.19
2	1.40	66.08	0.54	80.58	0.49	71.00	0.27	73.68
3	1.21	57.06	0.47	65.01	0.35	58.32	0.15	52.20
4	1.05	51.87	0.11	53.24	0.06	51.16	0.03	50.75
5	0.48	53.94	0.04	50.69	0.01	41.52	0.01	45.87
6	0.42	43.50	0.06	48.47	0.01	43.73	0.00	42.88
7	0.39	37.98	0.06	41.14	0.02	37.99	0.01	17.25
8	0.38	34.03	0.03	29.28	0.01	30.60	0.00	4.66
9	0.27	24.47	0.02	18.32	0.01	19.93	0.00	5.97
10	0.32	25.73	0.01	15.04	0.00	15.33	0.00	0.59
11	0.30	22.94	0.03	10.19	0.01	9.65	0.00	0.45
12	0.28	21.14	0.02	4.96	0.01	3.84	0.00	0.24
13	0.25	18.60	0.02	0.87	0.01	0.21	0.01	0.05
14	0.25	18.76	0.02	0.59	0.01	0.10		
15	0.24	17.36	0.03	0.80	0.01	0.09		
16	0.18	13.70	0.01	0.59				
17	0.16	12.59	0.01	0.31				
18	0.13	10.89	0.01	0.29				
19	0.11	9.91	0.01	0.37				

Table 29 - Results obtained for the contaminant concentration in gas phase, bioremediation tests, granitic soil (mg L^{-1})

Test	BNSRBz1Bt1		BSRBz1Bt0		BSRBz1Bt1		BSRBz1Bt05	
Days	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz
0	14.28	110.65	1.39	111.75	15.21	135.43	27.42	172.75
1	0.93	87.40	0.06	91.21	14.47	83.98	12.13	85.17
2	0.59	72.60	0.07	79.90	2.77	76.70	11.52	80.80
3	0.52	69.81	0.04	79.27	1.29	83.52	10.82	83.65
4	0.46	70.44	0.06	77.18	0.84	86.20	9.19	83.68
5	0.38	62.61	0.00	65.73	0.50	83.22	3.56	75.17
6	0.39	71.86	0.04	74.51	0.31	89.88	1.84	85.09
7	0.37	64.93	0.03	69.37	0.08	80.67	0.55	77.15
8	0.33	62.50	0.02	62.34	0.04	82.62	0.12	70.67
9	0.27	51.82	0.02	48.05	0.02	72.49	0.06	64.48
10	0.26	50.44	0.03	46.97	0.03	75.26	0.04	63.52
11	0.26	49.44	0.03	44.79	0.02	76.45	0.03	61.37
12	0.24	47.22	0.02	37.99	0.02	75.51	0.02	58.11
13	0.23	47.64	0.02	35.46	0.01	70.19	0.01	55.95

14	0.25	49.63	0.02	30.91	0.01	77.20	0.01	55.48
15	0.21	45.54	0.01	23.36	0.01	75.45	0.01	48.38
16	0.17	41.49	0.01	18.43	0.01	63.81	0.00	41.79
17	0.15	38.96	0.01	13.24	0.01	64.87	0.00	37.02
18	0.12	35.16	0.01	8.08	0.00	66.39	0.00	33.22
19	0.11	34.70	0.01	5.86	0.01	60.59	0.00	31.07
20	0.09	32.82	0.01	4.57	0.01	59.62	0.00	28.45
21	0.08	38.88	0.01	2.50	0.01	69.28	0.00	27.09
22	0.04	34.38	0.00	2.33	0.00	62.93	0.00	22.71
23	0.03	29.53	0.00	3.81	0.00	56.14	0.00	18.04
24	0.01	28.23	0.00	2.02	0.00	60.48	0.00	14.18
25	0.00	28.83	0.00	1.18	0.00	63.02	0.00	11.42
26	0.00	29.66	0.00	1.06	0.00	62.60	0.00	7.85
27	0.00	27.87	0.00	1.01	0.00	62.67	0.00	7.44
28	0.00	27.47	0.00	0.93	0.00	53.66	0.00	4.83
29	0.00	27.99	0.00	1.90	0.00	59.83	0.00	5.83
30	0.00	23.89	0.00	1.86	0.00	60.13	0.00	2.21
31	0.00	23.96	0.00	0.99	0.00	56.66	0.00	3.37
32	0.00	23.23	0.00	0.90	0.00	54.87	0.00	2.94
33	0.00	20.75	0.00	2.02	0.00	55.34	0.00	3.64
34	0.00	22.35	0.00	1.37	0.00	58.70	0.00	0.66
35	0.00	22.83	0.00	1.48	0.00	64.37	0.00	3.70
36	0.00	22.83	0.00	0.78	0.00	63.10	0.00	3.85
37	0.00	26.34	0.00	1.33	0.00	61.44	0.00	3.46
38	0.00	21.77	0.00	1.05	0.00	57.19	0.00	3.51
39	0.00	20.64	0.00	1.30	0.00	58.00	0.00	4.38
40	0.00	20.46	0.00	0.18	0.00	63.47	0.00	3.11
41	0.00	20.95	0.00	1.24	0.00	55.40	0.00	3.66
42	0.00	19.49	0.00	1.08	0.00	55.71	0.00	1.85
43	0.00	18.61	0.00	1.02	0.00	57.86	0.00	2.40
44	0.00	17.53	0.00	0.75	0.00	54.40	0.00	2.62
45	0.00	21.66	0.00	2.06	0.00	56.38	0.00	4.20
46	0.00	18.57	0.00	0.63	0.00	55.71	0.00	4.01
47	0.00	16.15	0.00	1.04	0.00	58.64	0.00	3.64
48	0.00	18.39	0.00	1.41	0.00	61.18	0.00	4.23
49	0.00	18.39	0.00	1.41	0.00	61.18	0.00	4.23
50	0.00	14.53	0.00	0.43	0.00	52.80	0.00	4.02

Table 30 - O_2 and CO_2 concentration in the bioventing tests (%)

Test	BVNSRBz1Bt1		BVSRBz1Bt0		BVSRBz1Bt1		BVSRBz1Bt05		Indoor atmosphere	
Days	O2	CO2	O2	CO2	O2	CO2	O2	CO2	O2	CO2
1	20.70	0.21	18.30	2.42	19.20	1.77	19.40	1.60	21.00	0.10
2	20.70	0.22	15.00	4.42	17.20	2.94	17.90	2.49	21.00	0.12
3	20.60	0.28	9.20	7.57	12.80	5.23	14.20	4.50	20.80	0.21
4	20.50	0.38	7.60	8.85	5.40	9.17	11.50	6.28	20.80	0.31
5	20.30	0.48	8.10	8.76	6.00	9.48	11.60	6.47	20.80	0.46
6	20.20	0.57	8.70	8.69	6.70	9.57	12.70	5.97	21.20	0.11
7	20.00	0.67	9.10	8.57	7.30	9.60	15.30	4.44	20.80	0.62
8	19.00	1.26	8.40	9.13	7.40	9.76	16.10	3.91	20.70	0.53
9	16.00	4.02	10.60	7.97	8.20	9.53	16.00	4.28	21.00	0.15

10	17.90	2.07	7.80	9.99	8.60	9.48	15.20	4.85	19.80	0.94
11	17.40	2.30	7.80	10.40	9.00	9.38	15.30	4.76	20.70	0.64
12	16.90	2.63	8.20	9.70	9.60	9.10	15.50	4.61	20.80	0.56
13	16.30	2.97	9.76	8.40	9.70	9.19	17.20	3.27	20.80	0.49
14	15.40	3.63	9.10	9.36	10.60	8.69			20.80	0.39
15	14.80	4.14	10.00	8.78	11.60	7.96			20.80	0.46
16	14.70	4.10	10.90	8.15					20.90	0.35
17	14.70	4.26	12.00	7.53					20.70	0.49
18	14.60	4.31	13.10	6.63					20.80	0.53
19	14.60	4.02	13.80	5.87					20.70	0.34

Table 31 - Biomass quantification at the end of the tests

Test	BVNSRBz1Bt1	BVSRBz1Bt0	BVSRBz1Bt1	BVSRBz1Bt05
Biomass (CFU mL^{-1})	2.25E+03	1.48E+04	1.68E+04	4.60E+03
Test	BNSRBz1Bt1	BSRBz1Bt0	BSRBz1Bt1	BSRBz1Bt05
Biomass (CFU mL^{-1})	0.00E+00	7.80E+03	1.33E+04	1.33E+04

Table 32 - Values used to calculate the remaining contaminant concentration in the soil

Test	BVNSRBz1Bt1	BVSRBz1Bt0	BVSRBz1Bt1	BVSRBz1Bt05	BNSRBz1Bt1	BSRBz1Bt0	BSRBz1Bt1	BSRBz1Bt05	
Solid phase									
Soil mass	1.80	1.80	1.80	1.80	1.28	1.28	1.28	1.28	Kg
Density	1060.00	1060.00	1060.00	1060.00	1060.00	1060.00	1060.00	1060.00	Kg m^{-3}
Volume	1.70E-03	1.70E-03	1.70E-03	1.70E-03	1.21E-03	1.21E-03	1.21E-03	1.21E-03	m^{-3}
Initial contaminant concentration	210.00	210.00	210.00	210.00	210.00	210.00	210.00	210.00	mg L^{-1}
Initial contaminant mass	356.60	356.60	356.60	356.60	253.19	253.19	253.19	253.19	mg
Final contaminant concentration	10.06	12.38	105.45	24.31	67.46	164.07	66.45	6.67	mg Kg^{-1}
Final contaminant mass	18.10	22.29	189.81	43.76	86.21	209.68	84.93	8.52	mg
Liquid phase									
Volume	0.20	0.20	0.20	0.20	0.14	0.14	0.14	0.14	L
Gas phase									
Volume	2.03	2.03	2.03	2.03	1.44	1.44	1.44	1.44	L
Contaminant concentration	9.91	0.37	0.09	0.05	14.53	0.43	52.80	4.02	mg L^{-1}
Contaminant mass	20.10	0.75	0.18	0.10	20.93	0.62	76.06	5.79	mg
Total									
Contaminant mass	38.21	23.03	189.99	43.86	107.14	210.31	160.98	14.31	mg
Solid + gas phase efficiency	89.29	93.54	46.72	87.70	57.68	16.94	36.42	94.35	%
Gas phase efficiency	95.28	99.82	99.96	99.98	93.08	99.79	74.86	98.09	%

2. Limestone

Table 33 - Results obtained for the contaminant concentration in gas phase, bioventing tests, limestone (mg L^{-1})

Test	BVNCLBz1Bt1		BVCLBz1Bt0		BVCLBz1Bt1		BVCLBz1Bt05	
Days	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz
0	0.77	50.58	0.04	51.22	0.75	50.58	0.27	50.17
1	0.56	43.27	0.03	25.67	0.26	39.12	0.03	39.24
2	0.56	37.46	0.07	6.08	0.04	22.64	0.04	22.64
3	0.60	36.98	0.04	2.35	0.03	17.08	0.02	1.64
4	0.54	33.27	0.03	1.76	0.02	9.17	0.01	0.54
6	0.44	25.98	0.03	1.47	0.01	1.72	0.01	0.24
7	0.51	25.39	0.03	1.19	0.03	0.68	0.02	0.21
8	0.41	21.11	0.02	1.04	0.01	0.31	0.00	0.08
9	0.46	22.59	0.06	2.19	0.00	0.31		
10	0.43	19.05	0.08	2.39	0.04	0.45		
11	0.38	16.06	0.03	1.00				
12	0.33	16.84	0.00	0.86				
13	0.33	14.35	0.03	1.04				
14	0.31	15.14	0.04	1.38				
15	0.19	12.57	0.03	0.65				
16	0.09	11.38	0.01	0.54				
17	0.01	10.30	0.00	0.32				
18	0.06	8.64	0.01	0.52				

Table 34 - Results obtained for the contaminant concentration in gas phase, bioremediation tests, limestone (mg L^{-1})

Test	BNCLBz1Bt1		BCLBz1Bt0		BCLBz1Bt1		BCLBz1Bt05	
Days	Bt	Bz	Bt	Bz	Bt	Bz	Bt	Bz
0	0.91	73.41	0.05	74.57	16.40	76.37	16.37	78.67
1	0.55	60.06	0.04	53.76	2.44	59.06	10.00	60.92
2	0.47	57.28	0.02	1.61	0.30	43.75	8.25	13.46
3	0.44	55.27	0.04	3.85	0.10	41.88	1.49	3.93
4	0.37	49.99	0.03	3.29	0.04	40.19	0.33	3.83
5	0.47	53.65	0.02	2.78	0.04	44.72	0.13	2.85
6	0.36	46.46	0.03	3.23	0.03	39.46	0.08	2.94
7	0.35	44.65	0.03	3.35	0.02	39.67	0.04	3.34
8	0.33	42.68	0.02	2.48	0.02	39.73	0.03	2.95
9	0.37	44.36	0.03	2.70	0.02	40.34	0.03	2.86
10	0.38	44.86	0.02	2.17	0.02	41.45	0.02	2.13
12	0.30	37.07	0.00	0.63	0.02	46.90	0.01	5.69
13	0.32	35.77	0.00	0.51	0.02	35.45	0.02	2.74
14	0.33	36.85	0.03	2.41	0.02	41.19	0.02	2.18
15	0.29	31.87	0.02	2.00	0.01	34.83	0.01	0.68
16	0.30	31.96	0.02	2.25	0.01	35.78	0.01	1.91
17	0.29	30.65	0.02	1.39	0.01	36.41	0.01	2.15
18	0.56	38.98	0.00	0.12	0.05	45.02	0.00	0.04
19	0.34	32.84	0.02	2.00	0.01	39.86	0.01	3.31

20	0.26	25.33	0.00	0.13	0.02	34.26	0.00	0.04
21	0.29	26.31	0.00	0.00	0.02	34.02	0.00	0.00
22	0.27	24.95	0.00	0.00	0.02	33.75	0.00	0.00

Table 35 - O_2 and CO_2 concentration in the bioventing tests (%)

Test	Zero		BVNSRBz1Bt1		BVSRBz1Bt0		BVSRBz1Bt1		BVSRBz1Bt05	
Days	O2	CO2	O2	CO2	O2	CO2	O2	CO2	O2	CO2
0										
1	20.60	0.06	18.70	1.23	16.90	1.87	15.30	2.74	20.90	0.02
2	20.60	0.07	12.90	4.86	5.60	8.50	9.30	6.49	20.70	0.07
3	20.20	0.10	7.50	8.56	2.70	10.72	3.60	11.07	20.90	0.08
4	19.80	0.15	7.00	8.96	3.30	10.88	2.60	11.95	20.80	0.40
5	18.70	0.37	7.70	8.34	3.10	11.35	8.10	8.60	20.70	0.20
7	16.90	1.08	8.50	7.69	3.60	11.30	11.70	6.37	20.20	0.70
8	15.80	1.68	9.20	7.21	4.00	11.42	13.90	5.02	21.00	0.04
9	14.70	2.29	10.00	6.74	4.90	11.03			20.90	0.03
10	14.00	2.78	10.70	6.31	7.60	9.39			20.70	0.54
11	13.20	3.33	11.50	5.84					20.70	0.14
12	13.20	3.43	12.20	5.43					20.80	0.29
13	12.40	3.91	12.80	4.95					19.90	0.57
14	12.30	4.05	13.60	4.56					20.70	0.06
15	12.00	4.41	14.20	4.30					20.60	0.48
16	11.50	4.69	14.80	3.95					20.20	0.57
17	10.90	5.04	15.30	3.66					20.70	0.39
18	10.80	5.14	15.70	3.39					20.40	0.38

Table 36 - Biomass quantification at the end of the tests

Test	BVNCLBz1Bt1	BVCLBz1Bt0	BVCLBz1Bt1	BVCLBz1Bt05
Biomass (CFU mL^{-1})	0.00E+00	1.25E+04	2.08E+04	9.35E+03
Test	BNCLBz1Bt1	BCLBz1Bt0	BCLBz1Bt1	BCLBz1Bt05
Biomass (CFU mL^{-1})	0.00E+00	9.35E+04	4.68E+04	6.11E+04

Table 37 - Values used to calculate the remaining contaminant concentration in the soil

Test	BVNCLBz1Bt1	BVCLBz1Bt0	BVCLBz1Bt1	BVCLBz1Bt05	BNCLBz1Bt1	BClBz1Bt0	BCLBz1Bt1	BCLBz1Bt05	
Solid phase									
Soil mass	1.80	1.80	1.80	1.80	1.28	1.28	1.28	1.28	Kg
Density	1530.00	1530.00	1530.00	1530.00	1530.00	1530.00	1530.00	1530.00	Kg m^{-3}
Volume	1.18E-03	1.18E-03	1.18E-03	1.18E-03	8.35E-04	8.35E-04	8.35E-04	8.35E-04	m^{-3}
Initial contaminant concentration	210.00	210.00	210.00	210.00	210.00	210.00	210.00	210.00	mg L^{-1}
Initial contaminant mass	247.06	247.06	247.06	247.06	175.41	175.41	175.41	175.41	mg
Final contaminant concentration	59.23	86.74	45.60	79.01	4.09	54.65	5.10	47.67	mg Kg^{-1}
Final contaminant mass	106.61	156.13	82.08	142.22	5.23	69.84	6.51	60.93	mg
Liquid phase									
Volume	0.20	0.20	0.20	0.20	0.14	0.14	0.14	0.14	L
Gas phase									
Volume	2.55	2.55	2.55	2.55	1.81	1.81	1.81	1.81	L
Contaminant concentration	8.64	0.52	0.45	0.08	24.95	0.00	33.75	0.00	mg L^{-1}
Contaminant mass	22.05	1.32	1.14	0.20	45.19	0.01	61.12	0.00	mg
Total									
Contaminant mass	128.66	157.44	83.23	142.42	50.42	69.84	67.63	60.93	mg
Solid + gas phase efficiency	47.92	36.27	66.31	42.35	71.26	60.18	61.44	65.26	%
Gas phase efficiency	95.88	99.75	99.79	99.96	88.12	100.00	83.93	100.00	%