Bioremediation of diesel fuel-contaminated soils in the Atacama Desert, Chile

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Chapter 3

BIOREMEDIATION OF DIESEL FUEL-CONTAMINATED SOILS IN THE ATACAMA DESERT, CHILE

Godoy-Faúndez, A.[a,e], Antizar-Ladislao, B.[b], Franke-Whittle, I.[c], , Turrión-Gómez, J.L.[d], Perez-Roepke, L.[e]

[a] Pontificia Universidad Católica de Chile, Dept. Ingeniería Química y Bioprocesos, Av. Vicuña Mackenna 4860, Santiago, Chile.
[b] Universidad de Cantabria, Dept. Ciencias y Técnicas del Agua y del Medio Ambiente, 39005 Santander, Spain.
[c] Universität Innsbruck, Institut für Mikrobiologie, Technikerstrasse 25, A-6020 Innsbruck, Austria.
[d] Universidad de Salamanca, CIALE, Dept. Microbiología y Genética, Av. Campo Charro, lab-301, 37007 Salamanca, Spain.
[e].Universidad Andrés Bello, Av. República 220, Santiago, Chile.

ABSTRACT

Since the 1980s, composting has been used as an alternative for bioremediation in open sites, with the microbial communities involved being a focal point of study. The use of this innovative bioremediation approach has been necessary due to recent changes in waste management regulations around the world, which focus towards more integrated, practical, sustainable and economic schemes than landfilling.

Currently, not much literature on the feasibility of bioremediation technologies to soils contaminated with mixtures of organic compounds exists. Even less information on the application of composting as a bioremediation technology is available. This chapter will include a case study on the successful application of in-vessel composting of a fuel-contaminated (diesel and gasoline) desert-mining soil/sawdust in the Atacama Desert (Chile). The physical and chemical factors that influence the distribution, abundance, richness diversity and evenness of microbial communities during composting of a fuel-contaminated soil during bioremediation will be discussed.

This chapter will also provide a review of the research conducted to date using molecular tools for the biomonitoring of changes in microbial communities as an indicator of remediation and restoration of oil-contaminated soils. In particular, the investigation of microbial community structure changes based on molecular tools including terminal restriction fragment length polymorphism (T-RFLP), BIOLOG EcoPlate™ and the COMPOCHIP microarray will be presented. The advantages and disadvantages of certain molecular tools for environmental management will also be discussed. Future needs for bio-monitoring of microbial community structure changes during bioremediation of oil-contaminated soils will be identified, and a discussion of the technological management, regulatory and sustainable aspects of using composting as a bioremediation technology will be included.

I. INTRODUCTION

For both commercial and human health reasons, it is important to identify and clean up sites that have become heavily contaminated by accidental oil spills around the world. Thus, a variety of different physical and chemical treatments have been applied in the treatment of contaminated soils (Lundstedt *et al*. 2006, Negri *et al*. 2004). Over the last twenty years, biological treatments, such as bioremediation, an inexpensive and environmentally-friendly approach, have shown promising results in the field (Antizar-Ladislao *et al*. 2004, Semple *et al*. 2001). The bioremediation of organic compounds results in the mineralization or transformation of harmful waste materials to more stable and harmless forms through the actions of microbial communities that use such compounds as carbon and energy sources. The success of the process is dependent on the biodegradability of the contaminant and the existing environmental conditions (physical-chemical factors). Furthermore, physical and chemical factors influence the distribution, abundance, richness, diversity and evenness of microorganisms.

Both *in-situ* and *ex-situ* bioremediation techniques exist. Among ex-situ techniques, closed systems (in-vessel systems or bioreactors) have been reported to be highly successful, where the control and optimization of environmental conditions increase the speed of the biological degradation process and the effectiveness of removal (Antizar-Ladislao *et al*. 2006a). Thus, the development and optimization of new treatment technologies using closed systems applied to contaminated soils are nowadays the focus of numerous studies.

However, there are still a limited number of published studies on the applicability of bioremediation techniques using an in-vessel composting system of contaminated soils with mixtures of organic compounds, and the majority of these studies have been conducted on soils contaminated with mono-compounds at laboratory scale and rarely applied to a real-case scenario. Additionally, most of previous studies have used bioremediation systems as a black box, whereby the microbial communities have not been investigated in detail. Thus, future efforts should include studies concerning changes in the diversity of microbial communities, based on advanced molecular tools allowing a greater understanding of bioremediation systems and potentially allowing for a high-quality predictor for the course of the bioremediation process (Franke-Whittle *et al*., 2005).

In this chapter, we will present a case study of the application of in-vessel composting, as a bioremediation treatment technology of a fuel-contaminated desert-mining soil/sawdust in the Atacama Desert (Chile). This chapter will present the fate of contaminants and changes in the microbial communities during in-vessel composting and will also discuss the relationship of the microbial community changes with physical and chemical parameters.

In summary, this chapter will determine optimal operation conditions during in-vessel composting of contaminated desert soils and contaminated sawdust, and will include discussion of technological, regulatory and sustainable aspects of treatment technologies to decontaminated soils in Chile.

II. BIOREMEDIATION OF SOILS CONTAMINATED WITH FUEL OILS

A. Desert soils contaminated with fuel oils

Deserts are arid regions, and thus desert soils are normally exposed to evaporation rates potentially twice as great as the precipitation. The world's deserts are divided into four categories: subtropical, cool coastal deserts, cold winter deserts and polar regions. Desert soils contaminated with fuel oils, were collected in the Atacama desert, Chile. The Atacama Desert is a cool coastal desert, characterized by cool temperatures because of offshore ocean currents, and is also the world driest desert after Antarctica.

Diesel oil or fuel is a complex mixture consisting basically of paraffinic, olefinic and aromatic hydrocarbons and, in smaller amounts, molecules containing sulphur, nitrogen, metals, oxygen, etc. Diesel oils are composed of molecules with 8-40 carbon atoms and are usually heavier, more viscous and less volatile than gasoline.

Desert soils may become contaminated by fuel due to various reasons, mainly because of accidental spills. Petroleum contamination is recognized as a significant threat to desert soils, however documented

research on the environmental consequences of terrestrial spills in deserts is still scarce. In fact, up to date, there are only a few reports on the characterization and treatment of fuel contaminated desert soils. One particular case is located is that of desert soil being contaminated with oil in Kuwait (Arabian Desert) following Iraqi invasion in the early 1990s. This case has been the subject of a study and application of treatment technologies (Balba 1998, Al-Daher 2001). Another study was conducted in the Antartic polar desert, where the effects of a small temperature increase on the removal of crude oil and diesel fuel contamination under various sub-Antarctic conditions was investigated (Delille *et al*. 2004).

Due to the limited number of studies available on desert soils, their characterization once pollution has occurred, and posterior feasibility (treatment) studies, the aim of this chapter is to present the results obtained in a study of characterization and treatment of fuel-contaminated desert-soils and sawdust in the Atacama Desert, Chile. Additionally, necessary information will be provided for those readers who are not familiar with this particular topic.

B. Bioremediation approaches commonly used

Soils contaminated with mixtures of organic compounds may be treated using a wide range of available techniques (Table 1), although they are often treated by solid-phase technologies (CLU-IN 2007). In general, different approaches have been applied for the bioremediation of contaminated soils, and can be categorised as in-situ or ex-situ, depending directly on the location of the treatment in reference to the location of the contamination. In-situ treatments do not require additional costs of excavations and confinement; nevertheless they present important disadvantages, for example a high variability and thus difficulty to achieve a steady state and adequate decontamination end point as well as a difficult control process and operational verification. Ex-situ treatments mainly involve the containment of the contaminated soil or transport of it to a hazardous waste landfill (HWL) for further treatment or some type of containment system. This sentence is too long and complex. Ex-situ treatments normally require shorter times for maximum decontamination, facilitated by the optimization of operational parameters, normally at lab scale and pilot plant scale. Additionally, ex-situ treatments offer the advantage of adding external agents which may enhance the biodegradation of the target contaminants, i.e., oxygen if the treatment is more efficient under aerobic conditions, nutrients and inocula to stimulate resident microbial communities, surfactants to facilitate contaminant bioavailability, etc. Two major decision criteria to select in situ or ex situ treatment are cost, including initial investment and operational cost, and time necessary to clean-up the contaminated site. These will be dependent on the type of contaminant or mixtures of contaminants, extension of contamination, type of soil, environmental risk, human exposure and others. In general, in-situ treatments are cheaper but present uncertainty regarding the final results, whereas ex-situ treatments are more expensive but offer a better control of operational parameters and process (USEPA 2002).

A widely used in-situ bioremediation approach is bio-venting, where aerobic biodegradation is enhanced by adding air or oxygen, water, nutrients, surfactants and/or microorganisms in order to achieve a final maximum removal of the target contaminant or mixture of contaminants (Dejonghe *et al*. 2001, USEPA 2002). Phytoremediation, which is defined as the process by which plants are used to remove, degrade, contain or render harmless environmental contaminants (Cunningham *et al.* 1996) is an attractive alternative to disruptive and expensive engineering remediation methods (Macek *et al.* 2000, Kechavarzi *et al.* 2007).

Among most widely used ex-situ bioremediation approaches to treat fuel-contaminated soils are biopiles, landfarming and composting. The use of biopiles has been used particularly in the petroleum industry (Guerin 2000), where contaminated soils are aerated and mixed with pretreated soils, nutrients and/or microorganisms as inocula to enhance contaminant biodegradation. Landfarming is another widely used ex-situ treatment that involves soil excavation and relocation in a remote site in thin layers. With the use of agriculture machinery the contaminated soil is ploughed facilitating a better contact between the contaminated soil, nutrients and resident microbial communities as well as a periodic aeration (Guerin 1999). The contaminated soil can be also irrigated to facilitate appropriate moisture content. A successful ex-situ bioremediation treatment currently under investigation for a systematic and efficient application to contaminated soils is in-vessel composting. In this treatment, contaminated soils

is excavated and mixed with organic amendments (e.g. green waste, sludge) which provides an appropriate carbon to nitrogen to phosphorous balance and may also stimulate residential microbial communities (Antizar-Ladislao *et al*. 2004).

Table 1. Treatment technologies applied to fuel contaminated soils

Technology	***Development status*** [1]	***Use rating***	***Cleanup time***	***Technology function***
	In situ biological treatment			
Bioventing	Full	Wide	NA	Destruct
Enhanced biodegradation	Full	Wide	NA	Destruct
Phytoremediation	Pilot	Limited	More than 3 years	Destruct
	In situ physical / chemical treatment			
Chemical oxidation	Full	Limited	NA	Destruct
Electrokinetic separation	Full	Limited	NA	Destruct
Fracturing	Full	Limited	NA	Extract
Soil flushing	Pilot	Limited	NA	Extract
Soil Vapor Extraction	Full	Wide	NA	Extract
Solidification / Stabilization	Full	Limited	NA	Extract/ Destruct
	In situ thermal treatment			
Thermally enhanced SVE	Full	Limited	> 3 years	Extract
	Ex situ biological treatment (assuming excavation)			
Biopiles	Full	Wide	0.5-1 year	Destruct
Composting	Full	Wide	0.5-1 year	Destruct
Landfarming	Full	Wide	> 1 year	Destruct
Slurry phase biotreatment	Full	Limited	0.5-1 year	Destruct
	Ex situ physical / chemical treatment (assuming excavation)			
Chemical extraction	Full	Limited	0.5-1 year	Extract/ Destruct
Chemical reduction / oxidation	Full	Limited	0.5-1 year	Destruct
Dehalogenation	Full	Limited	NA	Destruct
Separation	Full	Limited	> 1 year	Extract
Soil Washing	Full	Limited	0.5-1 year	Extract
Solidification / stabilization	Full	Limited	> 1 year	Immob.
	Ex situ thermal treatment (assuming excavation)			
Hot gas decontamination	Full	Limited	< 0.5 year	Destruct
Incineration	Full	Limited	< 0.5 year	Destruct
Pyrolysis	Full	Limited	< 0.5 year	Destruct
Thermal desorption	Full	Wide	< 0.5 year	Extract
	Other treatment			
Excavation and off-site disposal	NA	Wide	< 0.5 year	Extract/ Immob.

[1] Full scale: technology has been used in real site remediation.
NA = Not Available.

However, little literature exits on the bioremediation of mixtures of commercial products typically found in real consecutive accidental spills. The rate of biodegradation of such contaminated wastes

would depend on biological and physicochemical factors, such us low numbers of microbes, insufficient oxygen or nutrient availability, temperature and water availability (Antizar-Ladislao *et al*. 2005, 2006a). The rate of biodegradation would also depend on several physicochemical properties related to the contaminant (i.e. hydrophobicity, volatility, polarity), solid matrix (i.e. organic matter, porosity) (Mader *et. al* 1997), and its bioavailability (Antizar-Ladislao *et al*. 2006b, Semple *et al*. 2003). Additionally, biodegradation of fuel oils following real consecutive accidental spills is a complex process that depends on the nature and amount of contaminants (aliphatic and aromatic hydrocarbon mixtures) present (Mrayyan and Battikhi 2005).

a. Composting as a sustainable bioremediation approach

Composting is an aerobic process whereby organic materials are biologically decomposed. Heat is produced during the process, and conventional composting processes typically comprise four major stages. The four stages (mesophilic, thermophilic, cooling and maturation) vary in temperature and in the composition of the microbial communities (Bolta *et al*. 2003, Liang *et al*. 2003, VanderGheynst and Lei 2003).

Composting systems are generally divided into three categories: windrow, static pile, and in-vessel. In the windrow approach, the solid waste mixtures (soil and amendment) are composted in long rows and aerated by convective air movement and diffusion. The mixtures are periodically mechanically turned to expose the organic matter to ambient oxygen. This approach to composting leads to a characteristic fluctuating temperature regime as core temperatures will fall upon turning due to the introduction of cooler air masses and the release of entrapped heated air, and will rise when maximum microbial activity resumes. Over time, peak temperatures fall as the level of substrates decline. Windrowing is a simple mechanical system with no effective control of either oxygen or temperature levels within the organic mass undergoing decomposition. The technique, though popular commercially due to its ease of implementation and relative low cost of installation, has been criticized microbiologically since considerable periods of time elapse when either oxygen, temperature or both will become limiting for microbial diversity and decomposition.

In the static pile approach, piles of solid waste mixture, often with bulking agents (wood chips, straw) as a matrix improver, are aerated by using a forced-aeration system, installed under the piles to maintain a minimum oxygen level throughout the compost mass. Aeration may be on a timed basis (Epstein 1997) leading to high core temperatures and a severely restricted microbial diversity or via temperature feedback control (Antizar-Ladislao *et al*. 2004). This latter approach is based on a greater understanding of the microbial ecology involved whereby the control of air flow is dictated by reference to an upper temperature limit above which fans blow continuously until heat loss through latent heat of vaporization of water reduces the core temperature to an acceptable operational level. In both the Epstein (1997) and the Finstein *et al.* (1986) static systems, there are substantial variations in temperature throughout the composting mass and the cooler edges are routinely covered with a thick layer of finished compost acting as a thermal blanket to ensure that these edges reach regulatory temperature levels. Although the Finstein *et al.* (1986) system allows for the control of maximum core temperature, and in theory supplies an excess of oxygen for decomposition, both static approaches suffer from a gradation of temperature (and oxygen) throughout the organic waste mass. In static systems, active composting will occur over a three to four week period depending on the nature of the substrate being processed. This active phase is often followed by a two to three month period of maturation.

In-vessel composting takes place in a partially or completely enclosed container in which environmental conditions can be controlled. Enclosed vessels more closely approximate a laboratory incubator where the organic mass and its associated microflora should be exposed to a more even temperature profile. Control of temperature in in-vessel systems is usually achieved through recycling of exhaust gases with intermittent mixing of fresh air to maintain an agreed temperature (Antizar-Ladislao *et al*. 2004, Fraser and Lau 2000). In theory, such systems should allow for excellent control of temperature within the vessel and considerably less variation of temperature through the composting mass. However, in-vessel systems have serious limitations in general composting due to limited throughput and high installation costs (Das and. Keener 1994). Because of this, they are often used as a

form of pre-treatment "bioreactor" for up to 5 days prior to conventional composting,(Epstein 1997, Haug, 1993, Sasek *et al*. 2003).

The application of composting as a bioremediation technology to treat hazardous wastes has been shown to be effective in biodegrading polycyclic aromatic hydrocarbons (PAHs) (Antizar-Ladislao *et al*. 2004, 2006a, Canet *et al*. 2001), chlorophenols (Laine and Jorgensen 1997), polychlorinated biphenyls (Michel *et al*. 2001), explosives (Gunderson *et al*. 1997) and petroleum hydrocarbons (Al-Daher *et al*. 2001, Balba *et al*. 1998) at laboratory and/or field-scales. At present, few studies can be found on the applicability of composting to contaminated desert mining soils (subject of case study in Section III) with low organic matter content, high mineral content and high salinity (Mader *et al*. 1997, Rhykerd *et al*. 1995). Literature on the bioremediation of desert soils in Kuwait (Al-Daher *et al*. 2001, Balba *et al*. 1998), refers to open systems where *in-situ* landfarming, bioventing and composting treatment technologies were implemented (Al-Awadhi *et al*. 1996, Al-Daher *et al*. 1998) to treat more than 20 Mm^3 of oil accumulated in the desert soil following the Iraqi invasion of Kuwait in the early 1990s. Research with Kuwait desert soils indicates that biodegradation of total petroleum hydrocarbons above 60% can be reached following eight months of compost soil piles treatment.

b. Role of fungal and bacterial microorganisms during bioremediation

Fungi, although representing almost 75% of the microbial soil biomass and accounting for up to 100 meters of hyphae per gram of dried soil (Kästner and Mahlo 1996), have been less studied than bacteria in bioremediation processes. A review by Martín-Moreno *et al*. (2004) documents well the literature on the use of fungi in bioremediation and in the recovery of soils. Basidiomycetes, which cause white rot decay of wood have been employed in bioremediation for the degradation of a variety of environmental persistent pollutants such as PAH, and produce unspecific extracellular enzymes for the oxidation of aromatic rings (Kirk *et al*. 1992, Paszczynski and Crawford 1995, Bogan *et al*. 1996a, Bogan *et al*. 1996b).

White rot fungi in different combinations have been used in the biodegradation of PAHs in microcosms containing wheat straw and non-sterile coal-tar contaminated soil (Canet *et al*. 2001). Results suggested that the degradation of PAH was an indirect consequence of native microflora metabolism rather than an effect of the addition of the fungal species. Within this same perspective, litter-decomposing fungi have been studied for their ability to grow on different lignocellulosic substrates as well as for their ability to secrete lignolytic enzymes and degrade PAHs (Steffen *et al*. 2006). This work showed that the agaric basidiomycetes *Stropharia rugosoannulata* and *Stropharia coronilla* were the most efficient PAH degraders among the different species used. Other studies have been conducted using other fungi, such as the deuteromycete fungus *Cladosporium sphaerospermum*. This fungus, isolated from soil of an aged gas manufacturing plant, was able to degrade 23% PAHs, including high molecular weight PAHs in non sterile soils after four weeks, correlating well fungal establishment with laccase secretion and PAH degradation (Potin *et al*. 2004). The evaluation of the arbuscular mycorrhizal fungi (AMF) occurrence has been also tested in soil samples from a landfarming area used for fifteen years for petrochemical waste treatment (de Paula *et al*. 2006). Results showed that a significant growth of different plant species occurred when AMF were present. These AMF could be contributing to phytoremediation of soils containing not only PAHs but also heavy metals that are a human health risk (Gohre and Paszkowski 2006).

Similar approaches have been used to study PAH degradation, such as the inoculation of soils with basidiomycetous and ascomycetous fungi to induce the degradation of PAHs either derived from commercial or lignite tar (Grams *et al*. 1999). It was observed that 22 to 38% PAH could no longer be recovered from pasteurized soils inoculated with the mentioned fungi, three to 10 days after spiking. Additionally, *Irpex lacteus* and *Pleurotus ostreatus* used for the bioremediation of PAH-contaminated soils (Bhatt *et al*. 2002) was able to remove up to 67% of fluorine, 56% of phenanthrene and 49% of anthracene. The use of three species of white rot fungi (Novotny *et al*. 1999), including *Pleorotus ostratus* showed the highest PAH degradation activity reducing the levels of anthracene, pyrene and phenanthrene by 81-87%, 84-93% and 41-64% within two months, respectively. The presence of

cadmium or mercury did not alter the ability of *Pleorotus ostratus* to degrade PAHs (Baltrian *et al*. 2000).

The removal of aromatic herbicides by fungi isolated from pesticide contaminated soils is reported in a study by Bordjiba *et al*, (2001). The most frequently found species in the contaminated soils were *Aspergillus fumigatus, A. niger, A. terreus, Absidia corymbifera* and *Rhizopus microsporus* var *microsporus*, species not sensitive to pesticides.

Trichoderma, an anamorphic Hypocreaceaea (class Ascomycetes) is commonly found in the environment, and especially in soils (Wessels 1997). It is a well known biocontrol microorganism of several plant diseases (Benítez *et al*. 2004, Harman *et al*. 2004, Montealegre *et al*. 2005, Pérez *et al*. 2002), and has also proved to be useful in soil bioremediation (Esposito and da Silva 1998), in colonization of corn rhizosphere in pesticide containing soils (Rigot and Matsumura 2002) and in the degradation of phenols (Karetnikova and Zhirkova 2005). *Trichoderma* would thus appear to be a candidate for the accomplishment of multiple roles in polluted soils: colonization of plant roots, bioremediation of soils and biocontrol of plant diseases.

Unlike bacteria, fungi do not depend on continuous water-filled pathways for their translocation and spread in unsaturated-water soils. This is because of the expression and exposure of hydrophobins (Wessels 1997), small hydrophobic proteins that allow fungi to breach water - air interfaces and to grow in the pores formed between soil aggregates, that may also be penetrated by filamentous fungi through the use of special-shaped hyphae that disperse the soil (Wösten *et al*. 1999). Negligible bacterial movement can be detected in soils where vectors such as flowing water (Wong and Griffin 1976), earthworms (Singer *et al*. 2001) or plant roots (Kuiper *et al*. 2001) are absent.

The coexistence of fungi and bacteria in soil, and their metabolic cooperation in different systems has been discussed (Kohlmeier *et al*. 2005). The extensive colonization of fungal hyphae by bacteria suggests that fungi play an important role in bacterial movement and growth, by the formation of growth-promoting exudates (Arora and Gupta 1993). On this basis, "bioavailability" of bacteria to pollutants can be achieved using fungi to mobilize bacteria, rather than to provide bioavailability by mixing bacteria and contaminants (Semple *et al*. 2004). The combination of the fungus *Penicillium janthinellum,* bacterium *Stenotrophomonas maltophilia* VUN 10,010 and bacterial consortium VUN 10,009, isolated from separate creosote- and manufactured-gas-plant-contaminated soils, allowed a significant degradation of pyrene, chrysene, benz[*a*]anthracene, benzo[*a*]pyrene and dibenz[*a,h*]anthracene as well as bacterial growth. This process scarcely occurred in the absence of the fungus (Boonchan *et al*. 2000). The co-culture of any of these four bacteria (*Pseudomonas aeruginosa*, *Ralstonia picketii, Pseudomonas* sp. and *Psedomonas cepacea)* with any of the four fungi (*Penicillium* sp*., Trichoderma viride, Alternaria tenuis* and *Aspergillus terrus*) grown on sugarcane bagasse, showed a synergism in phenanthrene removal that depended on the bacteria-fungus co-culture, as compared to the pollutant removal by fungi that ranged from 35% to 50%, and the removal by bacteria that only reached 20% (Chávez-Gómez *et al*. 2003).

More recently, the use of *Pythium ultimum*, a fast growing oomycete that exhibits hydrophilic mycelial surfaces and of *Rhexocercosporidium*, an ascomycete that exhibits hydrophobic mycelial surfaces, with a naphthalene and phenanthrene-degrading *Pseudomonas putida*, showed that an efficient translocation of the bacteria occurred in the presence of *Pythium ultimum*. The study also showed that effective phenanthrene degradation occurred only in the presence of the fungal mycelia (Wick *et al*. 2007). The development and spread of fungal hyphae into the soil would be useful to facilitate a bacterial degradation approach of pollutants that are present not only at the soil surfaces but also deep inside the soils. A hyphal network could provide a physical space that could be used by bacteria to penetrate into the soil, taking advantage of the mechanisms used by fungi to grow and colonize the soils.

From this point of view, biocontrol fungi such as those belonging to the *Trichoderma* genera, resistant to different type of pollutants, could be used both to colonize soils and mobilize biocontrol bacteria (Montealegre *et al*. 2005) preventing the development of phytopathogens, and providing a network for mobilization of pollutant degrading bacteria (Leben 1984).

More studies about the roles of fungi concurring during bioremediation processes are necessary, in order to better understand the synergistic relationships between bacterial and fungal communities.

C. Bioremediation monitoring

a. Microbiological tools

An important first step towards understanding the roles of various microorganisms including bacteria, actinomycetes and fungi in a particular environmental sample is a determination of the composition and relative abundances of different microorganisms present in the environment. Until the 1980's, the determination of microbial community structures and the identification of microorganisms in environmental samples was conducted using culture-based studies, and different microorganisms were identified based on their phenotype. However, modern molecular techniques estimate that less than 1% of microbial species can grow under standard laboratory conditions (Amann *et al*. 1995, Kaeberlein *et al.* 2002, Ward *et al*. 1992). Other approaches to analyze the composition of microbial communities exist and are capable of discriminating between different bacterial strains. One such approach is the immunological direct-detection method enzyme-linked immunosorbent assay (ELISA) (Watanabe *et al.* 1996). Although this immunological method has a high resolution, it is not useful for a wide identification of microorganisms. Chemical biomarkers such as phospholipid fatty acids (PLFA) can also be used in the characterization of microbial communities present in contaminated waste mixtures under bioremediation conditions (Antizar-Ladislao and Russell 2007).

Molecular techniques based on the comparative analysis of 16S ribosomal DNA sequences are being widely used in microbial ecology studies, and have allowed the discovery of many novel and non-culturable bacteria. Furthermore, molecular techniques may help to discriminate between most of the microorganisms present in an environmental sample by analyzing the microbial genotype. The 16S rRNA gene encodes a RNA molecule of the ribsosome and is the most commonly used DNA sequence for the detection and phylogenetic analysis of microorganisms (Santos *et al.* 2004). The gene is approximately 1500 bp in length, is highly conserved, being found in all *Bacteria* and *Archaea*, and contains both variable and conserved regions. The gene is not laterally transferable and is the best molecular chronometer known (Woese *et al*. 1987). Large and rapidly growing databases of 16S rRNA gene sequences also exist.

Amplification of the 16S rRNA gene, followed by cloning, screening of the clone library and sequence analysis is becoming a commonly used approach used for microbial community analysis. Due to the power of the polymerase chain reaction (PCR) to amplify very small amounts of DNA, bacteria present at very low levels in an environmental sample are now detectable. The exponential nature of the PCR amplification process is very sensitive to any disturbance of amplification efficiency, and this can easily result in major PCR bias (Felske *et al*. 1998). PCR bias can occur such that certain templates within a mixed community type will inherently be favored in amplification. The use of PCR to amplify target DNA however greatly increases the detection sensitivity of microorganisms in an environmental sample in comparison to non-PCR based methods. Also, the physical, chemical and biological steps involved in the molecular analysis of an environmental sample are another contributing bias in microbial community analysis (von Wintzingerode *et al*., 1997). Despite these potential pitfalls in molecular ecology, the application of molecular tools has resulted in great advances into the knowledge of microbial diversity. The clone library approach has many advantages over other molecular methods, as it provides precise identification and quantification of the phylotypes present in samples. The cloning approach has been taken by many researchers to investigate the microbial diversity of numerous environmental samples including lab scale anaerobic digesters (Chouari *et al*. 2005), Antarctic desert soils (Smith *et al*. 2006) and deep-sea carbonate crusts (Heijs *et al*.2006).

Amplified 16S rDNA can also be subjected to DGGE, a method that can be used to assess the diversity of a microbial community through the separation of PCR-amplified 16S rDNA over a denaturing gradient. DGGE was originally invented to detect point mutations in medical studies (Fisher *et al*. 1979), but has been frequently used in recent years to investigate microbial communities in diverse environmental samples such as composts, and bioremediated environments (Green *et al*. 2004, Haruta *et al*. 2004). During DGGE, PCR products migrate through a polyacrylamide gel, encountering increasingly higher concentrations of chemical denaturant. Upon reaching a threshold denaturant concentration, the lower melting domains of the double-stranded PCR product begin to denature at

which time migration slows dramatically. Different patterns of bands will be produced as a result of differing sequences of DNA (from different bacteria) denaturing at different denaturant concentrations. Each band theoretically represents an individual bacterium present in the community. Bands can be excised from gels, and the DNA extracted, PCR amplified, and sequenced. Thus, the identity of members of the microbial community of a particular sample can be determined.

Temperature gradient gel electrophoresis (TGGE) is a method similar to DGGE, but instead uses a temperature gradient for separation of different DNA molecules. A high concentration of chemical denaturant is included in the gel, with the concentration being constant over the entire gel, such that increasing temperature is required for full denaturation. TGGE has been used to assess the diversity of microbial communities in different environments, including zinc-polluted soils and mealy-bugs (Brim *et al*. 1999, Franke-Whittle *et al*. 2005).

Terminal restriction fragment length polymorphism (T-RFLP) is another well known method for comparison of rDNAs. The method involves the amplification of DNA using universal 16S rDNA primers and the subsequent digestion of the product with restriction enzymes. Due to the differing sequences of different bacteria, the occurrence and location of the restriction sites will not be the same, and as a result, various patterns will be produced upon digestion. Sequence databases exist and can be searched for taxon-specific restriction sites (Moyer *et al*., 1996). Tiquia (2005) used T-RFLP's to study microbial community dynamics in manure composts and found that the bacterial and fungal community profiles changed over the composting process. Bacterial communities showed a higher diversity compared to fungal communities. The results of their study demonstrated that distinctive community patterns from manure composts could be rapidly generated using T-RFLP analysis and used to monitor the dynamics of microbial communities qualitatively and quantitatively.

Microscopic detection of specific microbial cells achieved by fluorescence *in situ* hybridisation (FISH) is a technique which can be used to detect and localize the presence or absence of specific microorganisms within a sample. It uses fluorescent probes which bind only to DNA in cells with which the probes show a high degree of sequence similarity. FISH, targeting the 16S rRNA gene, allows the detection and enumeration of specific phylogenetic groupings of microorganisms, without prior isolation. Jjemba *et al. (*2006) investigated soil bacteria that degrade PAHs in soils, using probes specific for the *Alpha-*, *Beta-* and *Gammaproteobacteria.* They reported that using the group specific probes, bacteria of the respective sub-classes could be detected and were distinguishable.

In summary, the application of molecular methods has resulted in great advances into the knowledge of microbial diversity, since molecular methods can be applied to various environments. However, in the course of experimentation, it should always be considered that the results obtained using these methods may include biases caused by the extraction efficiency of nucleic acids, PCR error, or other unknown factors. In particular, the application of such analytical methods to the biodegradation process is sometimes limited by the properties of compost, such as huge amounts of organic compounds and the presence of humic acids (Stach *et al.* 2001). These factors can make it difficult to purify nucleic acids. Other limitations include the difficulty of conducting quantitative studies with some environmental samples, the lack of discrimination between living and non-living microorganisms, the impossibility to discern between strains, the limited amount of known sequences, and the fact that most molecular methods are labour-intensive. Molecular methods often require the use of traditional microbiology which allows the isolation of microorganisms from specific environmental conditions. Then, their DNA, RNA, proteins and biochemical reactions can be investigated, and thus it is possible to isolate microorganisms involved in the bioremediation process during composting. Additionally, RNA extraction from soils and compost can be complicated and it is very difficult to discriminate between the majority of the microbial species present in an environmental sample. Other limitations include those arising from uncultivable microorganisms, which restrict the understanding of relationships of microbial communities with their surrounding environment (Kaeberlein *et al.* 2002). In summary, a polyphasic approach, including traditional cultivation and molecular methods, is desirable in order to understand the technical biases and limitations of each method, and the results obtained should be interpreted with extreme caution.

To completely understand the various processes that may occur during bioremediation of contaminated wastes using composting approaches, a more general and functional approach is required which would allow the investigation of microbial genes present in an environmental sample. Thus, new

techniques have emerged, which search for genes responsible for contaminant biodegradation during bioremediation. These are:

- *Metagenomics*. The direct sequencing of environmental samples has provided valuable insight into the lifestyles and metabolic capabilities of uncultured organisms occupying various environmental niches. Recent efforts include sequencing of individual large-insert bacterial artificial chromosome (BAC) clones as well as small-insert libraries made directly from environmental DNA (Tringe *et al.* 2005).
- *Proteomics.* Mass spectrometry-based proteomic methods are applied to evaluate gene expression, identify key activities and examine partitioning of metabolic functions (Ram *et al*. 2005).
- *Tagged mutagenesis*. A single mutant strain is marked by a tag signal and identified by parallel selection. Thus, genes expressed by microorganisms can be identified during exposure to conditions in their natural environments (Groh *et al.* 2005).
- *Environmental gene expression analysis*. Hybridization based microarrays with soil cDNA allow for the quantitative identification of biodegradation genes, and thus a characterisation of activated genes (Rhee et al. 2004). Additionally, probes varying in taxonomic specificity can be used, allowing for rapid classification, and perhaps quantification of the organisms present within a sample (Bae *et al.* 2006). Other methodologies applied to the quantification of functional genes in soils are MPN-PCR, competitive-PCR and real time PCR (Sharma *et al.* 2007).
- *Substrate-induced gene expression screening* (SIGEX). Relevant substrates generally induce catabolic-genes, and, in many cases, their regulatory elements (Uchiyama *et al.* 2005).

The use of individual microbes for complex environment tasks such as bioremediation of contaminated soils represents a great challenge for environmental biotechnology. Foremost, there is substantial discord between the laboratory conditions where the organism is manipulated and the *in situ* environment that is targeted by the microbial community. The application of molecular techniques such as those described in this section have allowed for a better understanding of the dynamics and composition of different microbial communities in the environment. Nevertheless, discovery of new gene pools involved in the biodegradation of pollutants and the development of new techniques such as enzymatic bioremediation is still a challenge which impedes a complete understanding of the microbiological processes involved during bioremediation of contaminated soils (Alcalde *et al.* 2006). Thus, newly advanced, robust and easy-to-use biomonitoring tools need to be developed.

b. Cutting-edge biomonitoring tools

The recent introduction of nucleic acid microarrays for the identification of bacteria in microbial ecology studies provide a powerful tool for the parallel detection of 16S rRNA genes, and have proved useful in microbial studies of phylogenetically diverse microbial groups (Gushin *et al*. 1997, Loy *et al*. 2002, Small *et al*. 2001). Microarray technology was first described in the late 1990s, and since then the technology has become more and more commonly used in various fields of research. DNA microarrays are based on the relatively old technology of DNA hybridization between two complementary strands of nucleic acids. The core innovation of the microarray technique is the ability to attach nucleic acids to a solid matrix in a precise location to create a densely packed array (Maskos and Southern 1992, Southern *et al*. 1999). DNA microarrays exploit the preferential binding of complementary, single-stranded nucleic-acid sequences (Brazma *et al.* 2000). Arrays are composed of many discretely located probes spotted onto a solid surface, each probe being specific for a given strain, subspecies, species, genus, or higher taxon (Loy and Bodrossy 2005). Each spot represents a unique probe sequence. Spots are usually 100-200 µm in size and are located within 200-500 µm of each other (Call *et al.* 2003).

DNA array technology offers the possibility to analyze an entire array of microorganisms concerning their presence or absence in a particular sludge sample in a single experiment. The extracted DNA from samples to be hybridized is labelled with fluorescent dyes such as Cy3 and Cy5 (Brazma *et al,* 2000). Hybridization with the labelled samples is followed by washing steps of differing stringency and scanning using a high-resolution scanner (Call *et al.* 2003). The relative expression levels of the

genes and abundance of a target in the sample and in control populations can be estimated from the fluorescence intensities and colours of each spot (Brazma *et al.* 2000).

Microarrays do however, also have their drawbacks. Various methodological deficiencies, such as the laborious protocols for the preparation of target molecules and insufficient detection limits constrict a broad application of microarrays in the analysis of microbial communities (Zhou, 2003, Peplies *et al.* 2004). Biases resulting from DNA extraction procedures as well as from PCR also exist (von Wintzingerode *et al.* 1997, Vianna *et al.* 2005). Weaker fluorescent signals can represent either a low concentration of a particular gene within the DNA extract or alternatively, a gene having sufficient but less than perfect similarity within the printed oligonucleotide (Dubois *et al.* 2004). Target secondary structure may also account for lower hybridization signals (Wang *et al.* 2002). Nonetheless, this technology has been applied and successfully used to study microbial communities in several environmental habitats such as a hypersaline cyanobacterial mat from a lake in Sinai, Egypt (Loy *et al.* 2002), ready-to-eat vegetable salads (Rudi *et al.* 2002), landfill methanotroph communities (Stralis-Pavese *et al.* 2003), soils (Small *et al.* 2001), and faecal samples (Wang *et al.* 2002). Diagnostic microarrays can also be used to investigate the effect of soil microbial diversity on soil fertility and the effect of agricultural practices on soil microbial communities (Bodrossy, 2003).

A microarray spotted with 369 different probes specific to microorganisms that have been previously reported in the degradation process of organic waste during composting, for plant, animal and human pathogens, and for plant disease suppressive bacteria was developed by Franke-Whittle *et al.* (2007). The COMPOCHIP 16S rRNA microarray was developed with the aim of being able to provide a direct characterization of the bacteria present in a particular compost sample. Composts contain a large and diverse community of microorganisms that are involved in the digestion of organic wastes (Franke-Whittle *et al.*, 2005). The COMPOCHIP array was applied to three different compost types (green compost, manure mix compost, and anaerobic digestate compost) of different maturity (2, 8 and 16 weeks), in order to determine if there were any differences in the microorganisms in the 3 compost types and maturity stages. Fig. 1 shows the COMPOCHIP microarray hybridized with DNA extracted from a 2 week green compost.

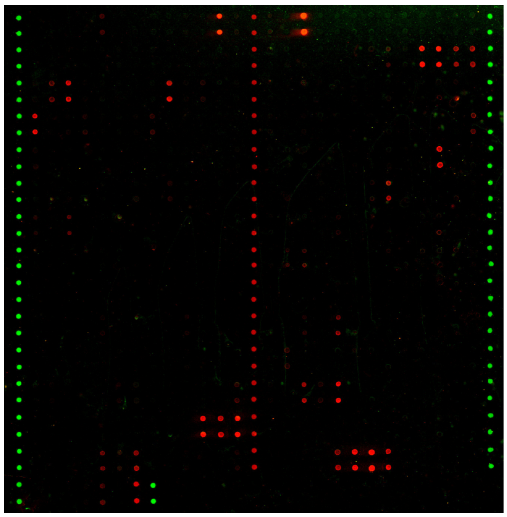

Figure 1. COMPOCHIP microarray image following hybridization of Cy5-labeled DNA extracted from 2 week green compost. Signals obtained for the print controls (Cy3-labeled), hybridization controls (central column of spots) and the different probes are evident.

Multivariate analysis showed that the bacterial composition of the 3 composts were different at the beginning of the composting process and became more similar upon maturation (Fig. 2). Certain probes were found to be more influential in discriminating between different composts. Interestingly, the 2 week manure mix compost was found to have the highest number of positive signals of all compost

samples, and higher signals for many probes than found in any other sample. The diversity and intensity of signals then decreased significantly over the following weeks, such that not many signals were detected in the 16 week manure mix compost sample. For both the anaerobic digestate and green composts, many of the probes gave hybridization signals above the threshold in the 2 and 8 week samples. In fact, higher signal to noise ratio (SNR) values were obtained in the 8 week sample for many of the probes than in the 2 week samples. The 16 week samples were found to closely resemble the 16 week manure mix compost sample, not yielding many signals with SNRs above the threshold. This study showed the potential usefulness of the COMPOCHIP microarray in determining microbial communities in composts. This microarray could also be applied to other soil and compost samples in order to gain an impression of the microbes present in a single test. A microarray containing oligonucleotides targeting key microorganisms in various bioremediation processes could be designed and would allow a direct monitoring of the microbial communities present in a bioremediation process.

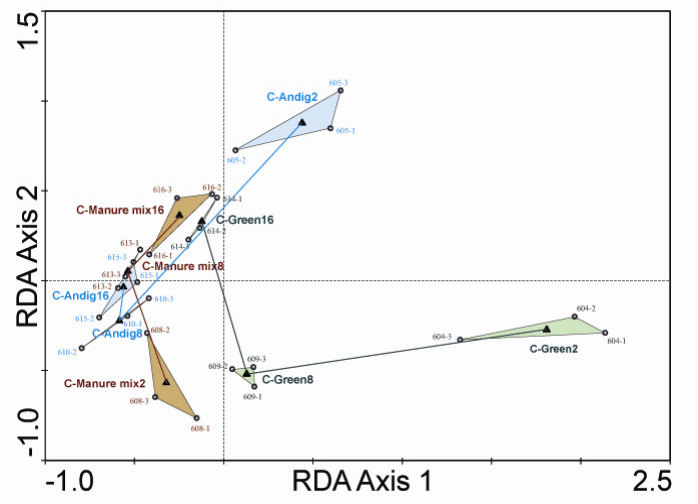

Figure 2. 2D-ordination-plot of three compost communities evolving over time analyzed by canonical analysis (redundancy analysis-RDA). The means for each sample are shown together with the three replicates.

Sole-carbon-source tests can also be used to monitor changes in microbial communities. Such tests are non-molecular, and reportedly reflect the composition of a microbial community. The method first described by Garland and Mills (1991), involves a commercially available microtiter plate (BIOLOG), which can be used to simultaneously test the utilization of 95 substrates as sole carbon sources. Utilization of a carbon source is indicated by colour development of a redox indicator dye, and changes in the overall patterns of carbon source utilization rates can be assessed by multivariate statistics (Baath *et al*., 1998). The BIOLOG test was originally developed for the classification of bacterial isolates based on the ability of the isolates to oxidize the different carbon sources. However, this method has also been adapted and used to characterize the metabolic capacities of a microbial community. BIOLOG has been commonly used to detect differences between microbial communities in soil and the rhizosphere (Garland 1996, Pfender *et al*. 1996, Zak *et al*. 1994). Due to the necessity of faster analysis of microbial communities, the initial BIOLOG plate GN was modified and redesigned at the request of a group of microbial ecologists. The result was a new BIOLOG microplate, the EcoPlate^{TM}, which contains 31 of the most useful carbon sources found in soils and used by microorganisms. The carbon sources are present in triplicate, and the 31 different C-sources (amines, aminoacids, carbohydrates, carboxylic acids and polymers) allow community level physiological profiles (CLPPs) to be established based on the capacity of microorganisms to utilize these different C substrates. In addition, it is feasible to establish the average metabolic response of a sample by calculating the average well colour development. Despite

the simplicity and low cost of using CLPPs for the characterization of different samples, relatively few reports of the application of this methodology in compost studies exist.

III. Bioremediation of contaminated desert soils with diesel fuel by in-vessel composting in the Atacama Desert, Chile

Chile is a regional economic leader in South America. Its economy is ore-dependent and it is considered to be a mining country due to continuous investments in the mining industry. Chile is currently the worlds leading extractor of copper, and copper sales represent approximately 40% of its commercial balance and around 9% of its Gross Internal Product. Relations between the mining industry, environment and society have not been smooth due to continuous environmental impacts and confrontations between the industry and human communities. Topics of debate range from the type of exploitation (e.g. open pit mine) to cultural disturbances. Nevertheless, the Chilean population has accepted the mining industry as a very important development motor.

In Chile there are two principal mining companies, CODELCO (Copper Corporation) a Governmental Company, and Minera Escondida Ltd (MEL), a private investment of BHP-Billiton, the world's largest diversified resources company. MEL owns Chiles most important copper mine. The mine is located in the Atacama region (north of Chile; 170 km SE of Antofagasta; 3,100 m above sea level), and is responsible for the production of 8% of the world's copper. The mine has an annual income tax of approximately US$ 140 million and investments total more than US$ 4 billion. The construction of the mine in the Atacama region started in 1988, and the first batch of ore was processed in 1990. MEL's production of copper at the Atacama region represents 20% of total copper production in Chile, and includes a work-force close to 2,300 people, 1,900 permanent jobs through outsourcing, and 8,000 additional permanent jobs in diverse productive activities associated to the mining industry, especially in the North of Chile. The role of Minera Escondida Ltd. is vital to the Chilean economy, since its production accounts for 2.5% of the country's Gross Internal Product (Minera Escondida, 2007).

Despite the economic benefits that the mining industry offers to Chile, environmental concerns related to the production of hazardous wastes exist. It is well known that the mining industry has a negative environmental impact, due to continuous excavations, dust production, noise emission by heavy machinery, and disturbance of flora and fauna. A non-evident impact is that resulting from the daily operation of heavy machinery, where repetitive spills of fuel oils have occurred during the repair and maintenance of machinery. Soils and sawdust have frequently been used as cheap and readily available adsorbent materials to help in the clean up of the spilled fuel oils, which consist of complex mixtures of aliphatic and aromatic hydrocarbons. This common practice in the mining industry has caused a real case environmental problem of current concern, whereby large amounts of hazardous wastes, comprised mainly of desert mining soils and sawdust contaminated with fuel oils, have accumulated over time and need to be cleaned-up.

Our present aim is to minimize the environmental impact caused by mining activities in Chile, and in particular in the Atacama region, which may be extended to other regions around the world. Little literature exits on the degradation and fate of mixtures of commercial products typical in real consecutive accidental spills in desert mining soils, under bioremediation conditions. In-vessel composting bioremediation technology is presented as a potential treatment option, that can be optimized to produce results complying with current environmental laws, and that minimizes human and animal exposure to contaminated sites.

A. Physicochemical characteristics of desert mining soils in the Atacama Desert, Chile

Chile has principally a Mediterranean climate, however, in the North, the Atacama desert exists, one of the worlds most important deserts, comprising the worlds biggest mining cluster and industry of copper and other ores. The Atacama Desert is an extreme, arid and temperate desert that extends from 20°S to 30°S along the Pacific coast of South America. Soils in the Atacama desert are taxonomically characterized as either aridisol, inceptisol or entisol, and have a thin development of A horizon above

thin B horizons. Atacama desert's soils often also have carbonate accumulation in a K horizon and are closer in composition to mother rock due to a continuous eolic erosion and a low pluvial activity typical of dry climates (USDA 1999). The low pluvial activity increases only in summers with the "Bolivian Winter" (Miller 1976). Additionally, soils are hyper-saline and this is one of the main reasons why these soils are dedicated principally to mining activities. The organic matter content is lower than 0.1% and concentrations of copper and sulphur are higher than the typically reported agronomic values (Thompson 1978). Minor and major essential elements are present at concentrations lower than that typical of agronomic soils.

B. Historical contamination

The maintenance of machinery and trucks at Minera Escondida Ltd. has resulted in continual accidental spills of mixtures of commercial fuel oils (diesel and gasoline). Typically, these fuel oils have been 'cleaned up' by absorption with sawdust and native desert mining soils in order to contain the contaminants and minimize the detrimental environmental and human impact of these contaminants. The contaminated mixtures of soil and sawdust are contained and disposed of in HWL. The HWL is normally exposed to environmental conditions typical of the desert climate, with daily oscillations of temperature between 0°C during the night and above 40°C during the day, and the hazardous wastes are not exposed to direct-solar radiation. This common practice in the mining industry has caused a real case environmental problem of current concern, where large amounts of these hazardous wastes have accumulated over time and need to be disposed of.

C. Bioremediation strategy

Due to the limited number of case studies available on the use of bioremediation technologies to treat contaminated desert soils and the need to decontaminate large amounts of hazardous wastes in the Atacama desert (Chile), this research sought to determine the feasibility of bioremediation by aerated in-vessel composting of mixtures of desert mining soil and sawdust contaminated with fuel oils as a bioremediation strategy to clean-up fuel contaminated wastes. The specific objectives of the project were to determine the effectiveness of different ratios of contaminated soil to contaminated sawdust (S:SD) contained and disposed of for two years in a HWL. Another objective was to find correlations between the percentage of contaminant removal and various physical and chemical parameters which may indicate the best conditions for maximum contaminant removal. A third objective was to investigate the microbial community changes during in-vessel composting treatment.

Our experimental design set-up consisted of the implementation of 30 laboratory-scale cylindrical aerated composting reactors operated continuously for 56 days with a moisture content close to 50% and maintained at mesophilic temperatures. Contaminated desert-soil and sawdust were treated at different ratios of contaminated soil to contaminated sawdust, S:SD 1:0, 3:1, 1:1, 1:3 and 0:1 (2,000 g total composting mixture, wet weight). Prior to filling in the composting reactors, contaminated wastes were separately passed through a 5 mm sieve followed by a 2 mm sieve. Atmospheric air warmed to 60 ^{0}C was introduced (pump of 1.1 KW and a maximum flow of 550 m^3 h^{-1}) to each reactor and circulated through an internal perforated piping to ensure sufficient oxygen concentration in the reactors (Fig. 3). Temperature was monitored with K type thermocouples, connected to a system OPTO 22 and stored in an ASCII file in a PC Pentium III. Recorded temperatures throughout the length of the treatment showed that the composting reactors reached mesophilic temperatures of 30 ± 2^{0}C at the surface of the substrate in contact with the head space and 40 ± 2^{0}C at the bottom of the substrate. Additionally, gas streams from the inlet and exhaust were continuously monitored for carbon dioxide production as evidence of aerobic biodegradation. Each reactor was homogenized on a daily basis to avoid stratification and oxygen content limitation. Sterilized distilled water (SDW) was added when needed to maintain 50% moisture content in all reactors during the treatment.

Sampling was conducted in triplicate after 0, 14, 28, 42 and 56 days. The samples were collected using a core tube (70 cm long, 5 cm i.d.) in order to obtain stratified samples at different radial distances

in each reactor. Each sample was separately manually homogenized with a stainless steel spatula in a sterile bag and stored in an amber glass flask for further analysis.

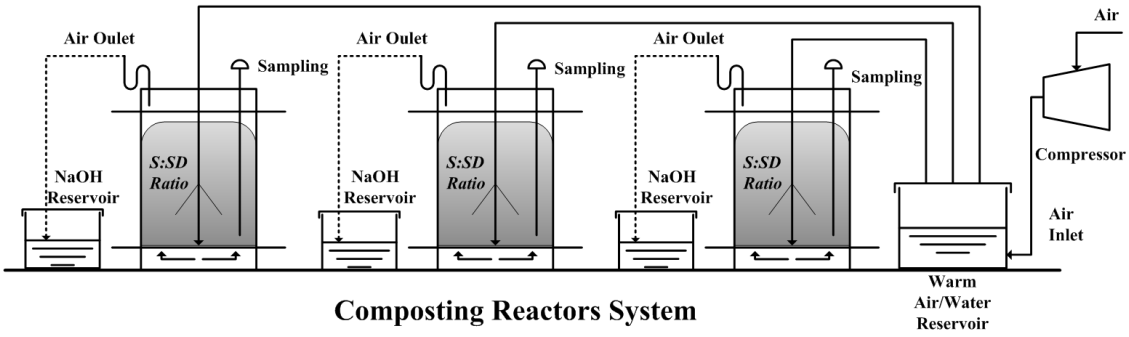

Figure 3. Schematic laboratory set up of in-vessel composting reactors.

Our criterion was that the autochthonous microbial communities were acclimatized to cold and warm temperatures and to the presence of pollutants. The corresponding abiotic controls comprised the same S:SD ratios, but reactors were radiated with Gamma rays of a Cesium 137 source to 25 kGy doses three times using an Experimental Nuclear Reactor (Chilean Commission of Nuclear Energy, CCHEN).

Chemical and microbiological analyses were performed using standard procedures (Sadzawka *et al*. 2000, USEPA 1986). pH, E.C., moisture content, organic matter content, nitrogen content, oligoelements and heavy metals, organic contaminants, and cultivable heterotrophic bacteria were monitored. Additionally, changes in the microbial communities, number of bacterial cells and percentage of live cells were determined using fluorescence microscopy by directs counts and a fluorescence microplate reader by LIVE/DEAD BacLight Bacterial Viability kit (Molecular Probes). Terminal-restriction fragment length polymorphism (T-RFLP) and BIOLOG Ecoplate^{TM} tests were conducted, and diversity indexes calculated using the Shannon-Wiener index (H´). The average well colour development (AWCD) was determined for the BIOLOG Ecoplates^{TM}.

Finally statistical analyses were conducted. A two-factor ANOVA test (factor 1, treatment or S:SD ratio; factor 2, time) applied for each degradation curve to elucidate the effect of S:SD ratio treatment and time of the removal of fuel oil. A t-test was conducted to investigate possible differences between initial and final values of diesel fuel content in each treatment and final diesel fuel content following different treatments. Principal Components Analysis (PCA) and Linear correlations (r) within-groups of physical and chemical parameters and removal percentage in different reactors were also investigated to determinate correlations between treatments.

D. Changes in physicochemical conditions

Since pH is one of the key factors for microbial metabolism, its value in the composting of substrates was monitored every 14 days (Fig. 4). Before treatment, all reactors were found to have pH values of 7.0 -7.5 and no statistical differences were found between reactors. Once the treatment started, pH changes were observed in the reactors with significant lower pH at lower S:SD ratios. Statistical significant differences were found between initial and final times ($p<0.05$ by t-test) in the majority of the reactors. Only the reactors S:SD - 1:1 and S:SD - 3:1 presented no statistical differences among them. No pH changes were observed in the abiotic controls (data not shown).

Lower pH values in treatments of lower S:SD ratios could be explained by the formation of canalizations in the presence of sawdust. This may have facilitated the aeration of the contaminated mixture promoting aerobic biodegradation, and thus CO_2 production and anaerobic zones where fermentation processes may have occurred, acidifying the mixtures. On the other hand, in the reactor with contaminated desert soil only (S:SD 1:0), the pH tendency was to rise over time.

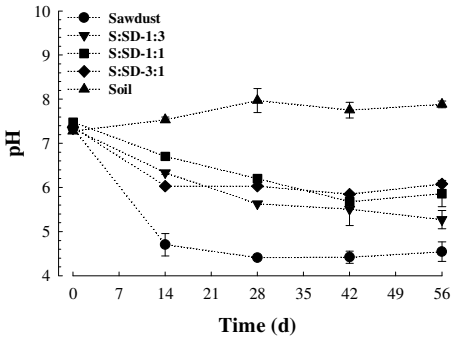

Figure 4. Temporal changes of pH.

Soils in the northern Chile Atacama desert are hyper-saline (electrical conductivity; EC > 10.0 mmhos cm^{-1}) and this is one of the main reasons for why these soils are dedicated principally to mining activities. Increasing levels of salinity in the reactors treating mixtures of contaminated desert soil and contaminated sawdust were observed over time (data not shown). This indicated a higher content of free ions in the composting mixtures over the duration of the experiment. In the reactors containing sawdust, the EC remained at a constant levels (≈12 mmhos cm^{-1}), however, in the reactor with S:SD - 1:0, the EC progressively decreased during the treatment to values closer to 7 mmhos cm^{-1}. Thus, no significant effect of salinity was observed in the composting treatments ($p>0.05$, correlation index and PCA) in this study.

Major and minor elements were monitored since they are essential for the catabolic activity of microorganisms. Thus, fuel oils degradation and biomass production depend directly of an accurate balance of these nutrients. Levels of Mn and available N, P, K were higher (range between 1,000 and 10,000 ppm) in the contaminated soil, sawdust and mixtures of contaminated soil and contaminated sawdust at various S:SD ratios as compared to normal values of B and Zn (30 - 180 ppm, respectively). Nevertheless, available N, P and K concentration values were low in all reactors as compared to reported agronomic values (data not shown). Initial N content was different in the reactors depending on the S:SD ratio, and the highest consumptions of total N was observed in the reactors with a S:SD 1:0, 1:3 and 3:1. The tendency of total N was to reach a steady state and similar concentration values ($p>0.05$) in the final points in all reactors (Fig. 5a). Total N did not change in the abiotic controls during treatment (data not shown). In the present study, it was assumed that indigenous soil microbial populations were adapted to the fuel oils present in the soil and to the environmental conditions. Values of available nutrients in this study were lower than those values typically reported in composting processes, which was not surprising because desert soils are mainly composed of mineral surfaces with low organic matter contents The levels of S and Cu were higher than those normally encountered in typical desert soils, and it was assumed that autochthonous microbial communities were adapted to the presence of high levels of S and Cu.

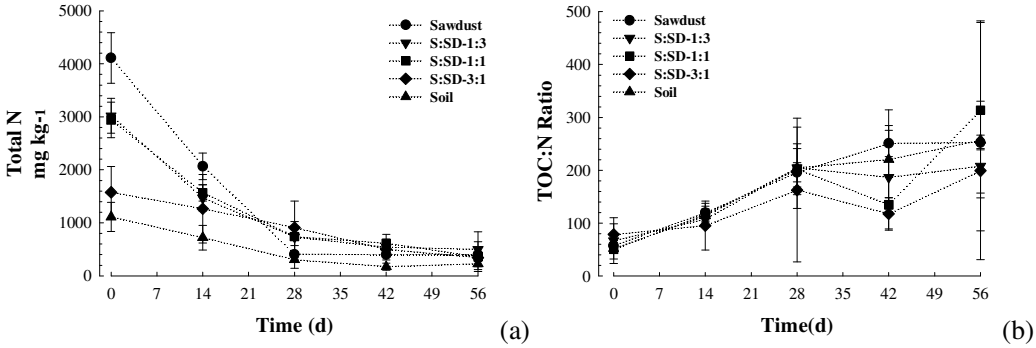

Figure 5. Temporal changes of: (a) N and (b) ratio TOC:N.

With respect to organic matter content, the composting reactors with mixtures of contaminated desert soil and contaminated sawdust contained a higher organic matter content compared to the composting reactors with only contaminated desert soil. This is most likely due to the capacity of sawdust to adsorb organic contaminants. Thus, composting reactors with a S:SD ratio of 3:1, 1:1 and 1:3 had organic matter contents of 24%, 30% and 49% respectively; the control reactor (S:SD 1:0) presented an organic matter content of 14%, a high value in comparison with typical non-contaminated desert soils (Atacama and Kuwait region, organic matter content < 1%). TOC/N ratios were within the range 50:1 and 100:1, higher ratios than those normally encountered in a composting process (Al-Daher *et al*. 1998). Temporal increases in TOC/N ratios similar in all reactors indicated that the temporal concentration of N decreased over time as compared to the temporal concentration of organic matter content (Fig. 5b). TOC/N ratios decreased from day 28 to day 42 in the reactors with a S:SD ratio of 3:1, 1:1 and then increased again. This temporal decrease needs further investigation. Temporal trends of total N and TOC/N ratios (Fig. 5a, b) indicate that N may have been a limiting nutrient in the composting reactors.

E. Changes in diesel fuel concentration

In order to follow the biodegradation of fuel oils present in the contaminated soil and sawdust mixtures, total hydrocarbon concentration was monitored. Different initial concentrations of fuel oil were observed in the reactors with higher initial hydrocarbon concentrations present in sawdust (Fig. 6a). The initial concentration of fuel oils in the mixtures corroborated the higher adsorbing capacity of sawdust. Following 56 days of treatment, different biodegradation curves were obtained in all reactors, with most of the biodegradation occurring during the first 42 days of treatment (Fig. 6a). Biodegradation curves indicated a similar trend among composting reactors with S:SD ratios of 1:0 and 3:1 with smooth decreases reaching contaminant removal between 30% and 40% after 42 days of continuous treatment (Fig. 6b). Reactors with S:SD ratios 1:1 and 1:3 with higher levels of sawdust presented higher removal rates. The reactor with a S:SD ratio 1:0 presented the lowest removal rate (60%). The concentration of fuel oils remained unchanged in the abiotic controls (data not shown). These removal percentages are similar and concordant with those reported in a previous commercial diesel oil contamination study (Marchal *et al*. 2003).

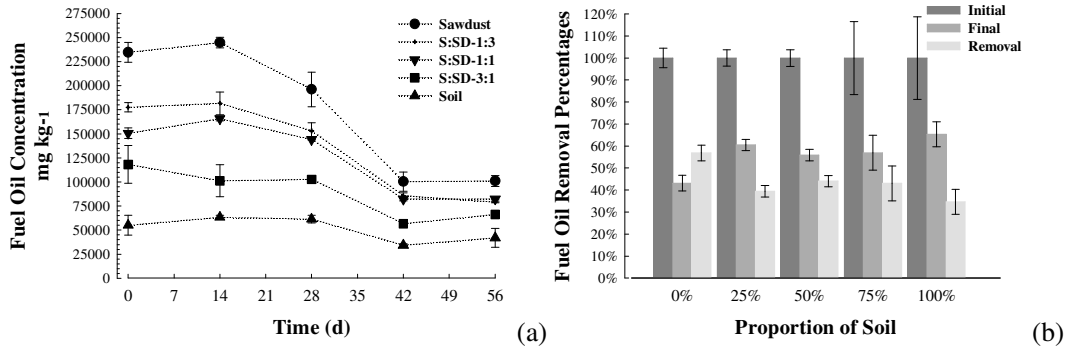

Figure 6. (a) Temporal changes in fuel oil concentration in experimental reactors, and (b) Fuel oil removal rates in the experimental reactors.

F. Changes in microbial communities

There have been few reported studies whereby molecular tools have been used to identify microorganisms or diversity of microbial communities in desert soils (Ros *et. al* 2006). Studies conducted in the Atacama Desert have until very recently focused on the investigation of the diversity of

heterotrophic bacteria based on 16S rRNA gene sequences, in order to improve our current understanding of the diversity of bacteria living in extreme environments. Such environments are close to their limit of dryness to sustain microbial life, and thus they have been compared with soils from the surface of Mars (Mars-like soils) (Navarro-González *et al*. 2003), a focus of diverse studies by NASA (Clements, 2002).

These microorganisms are typically the first organisms to react to chemical and physical changes in the environment. For example, it has been observed that an increase in the moisture gradient along the Atacama region results in a greater bacterial diversity of heterotrophically culturable bacteria (Clement 2002) and it is then more likely to find principally autotrophic (litotrophic) heterotrophic microorganisms. Drees *et al.* (2006) investigated the bacterial community structure of the Atacama Desert and reported a predominance of *Gemmatimonadetes* and *Planctomycetes*, which is unusual when compared to typical soil populations. The unique phylogenetic distribution of the organisms identified in the study of Drees *et al.* (2006) and in studies of other arid soils would suggest that the hyperarid environment does select for specific groups of bacteria. Nevertheless, more research is still needed to obtain a better knowledge of the microbial community composition in the Atacama Desert, and especially of the presence of heterotrophic bacteria in mineral soils.

The study presented in this chapter has used traditional and cutting-edge biomonitoring tools to investigate microbial community structure changes during bioremediation of fuel-contaminated soils in the Atacama desert, where the organic degradation take place in a similar fashion to a natural Martian environment (Stocker and Bullock, 1997). Direct counts and the percentage of viable bacteria indicated that count numbers were high at the beginning of the treatment and that no significant changes were observed during the course of the treatment (over 10^6 cells gr^{-1}). Additionally, viable bacteria comprised over 60% of total number of cells (Fig. 7a, b). These preliminary results indicated that most probably resident microbial communities were adapted to the presence of organic contaminants following consecutive accidental spills and storage of the contaminated wastes in the LHM, prior to the composting treatment.

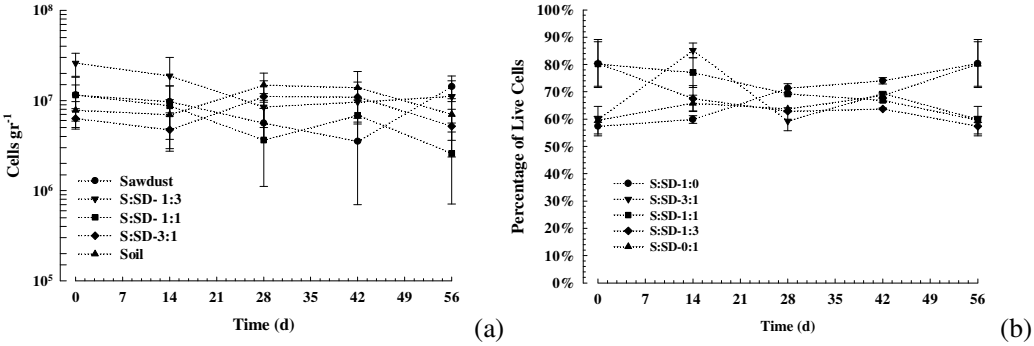

Figure 7. Temporal changes of: (a) Cell per gram of soil and (b) percentage of live cells in the experimental reactors.

When cutting-edge biomonitoring tools were applied to the Atacama desert soil samples, contradictory results were obtained, indicating that the changes in number of phylotypes were not in agreement with the changes in the metabolic activity (Fig. 8a, b). In the reactors where only soil was treated, an increase in diversity with a maximum reached after 42 days of treatment was seen, according to BIOLOG EcoPlate™. According to T-RFLP, maximum diversity was reached after 28 days of treatment and then decreased to a minimum after 42 days of treatment. The reactors where only sawdust was treated presented a trend in diversity similar to that of only soil according to BIOLOG EcoPlate™, but constant to a value close to 3 according to T-RFLP. Additionally, reactors where only sawdust was treated had, according to T-RFLP, higher diversity than all other samples.

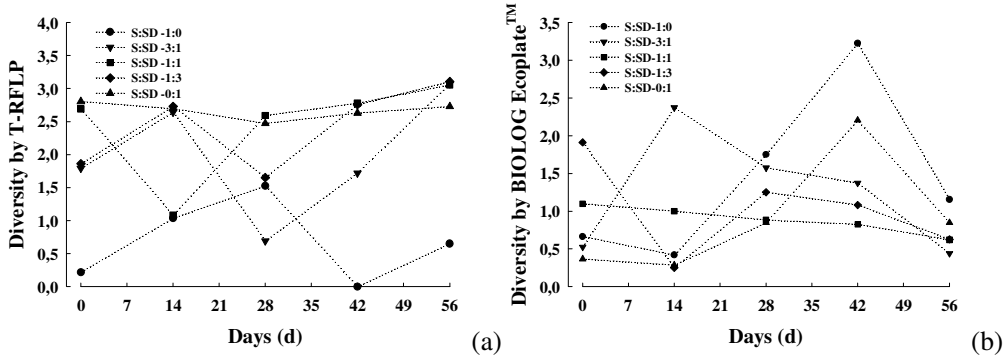

Figure 8. Temporal changes of diversity by (a) T-RFLP and (b) BIOLOG EcoPlate™ in the experimental reactors.

The AWCD indicated that the microbial activity in composting reactors increased with treatment duration (Fig. 9). According to these results, the reactors with a S:SD ratio 3:1 presented the highest metabolic activity during the length of the treatment and the reactors with a S:SD ratio 0:1 presented the lowest metabolic activity. AWCD results were in agreement with the biodegradation curves (Fig. 6a), and thus higher microbial activities resulted in higher fuel removal rates. Finally, higher microbial activities are observed towards the end of the treatment process and at higher sawdust contents in the composting mixture.

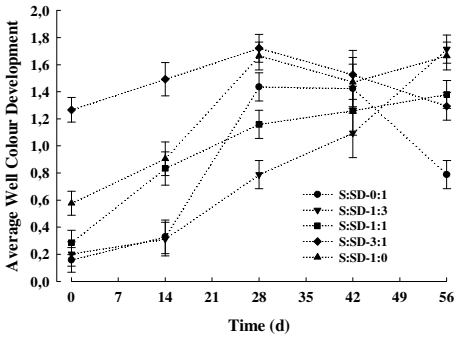

Figure 9. Temporal changes of AWCD in the experimental reactors.

G. Optimal conditions for maximum removal of fuel diesel

Statistical analysis using the results of different treatments indicated that 2 factors explained 83.6% of the total variance. Factor Time explained 73.3% of the total variance, and factor Treatment (S:SD ratio) explained 10.3% of the total variance. These results were confirmed with PCA analysis considering all physicochemical factors, where the reactors are dispersed in two axes that explain over 70% of variance (Fig. 11). Comparison of the results of the abiotic and experimental reactors, indicated that all treatments presented significant differences, indicating that biodegradation was the main mechanism of contaminant removal from fuel oil contaminated soils and sawdusts. The mesophilic temperatures used in this experiment were lower than those required for volatilization (Loehr *et al.* 2001), which further supports the results obtained.

A linear correlation analysis including the various physicochemical parameters monitored in this study during treatment indicated that the removal of fuel oils was significantly influenced by pH, TOC/N ratio and total N. The reactor with a S:SD ratio 1:0 presented a significant correlation of fuel oils removal with TOC:N ratio which may indicate that N may have been a limiting factor for fuel oils

biodegradation during treatment. The reactor with a S:SD 1:3 also presented a significant correlation of fuel oils removal with the total N present, which may have had some nutrient implications during treatment. The other reactors presented no significant correlations with the physicochemical parameters monitored. These correlations may indicate that the main mechanism of fuel oils removal during treatment is aerobic biodegradation with subsequent production of CO_2 resulting in lower pHs, as observed in this study

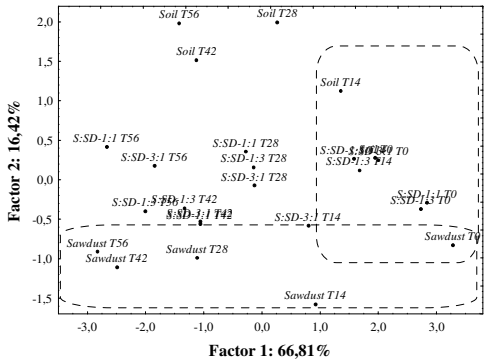

Figure 11. Effect of Factor 1 (time) and Factor 2 (treatment, S:SD) on fuel oils removal in all treatments investigated.

The major difference between treatments was observed for treatments with only soil (S:SD 1:0) and mixtures of contaminated soil and contaminated sawdust. No statistical difference was observed between treatments with a S:SD 1:1 and 1:3 and both were statistically different from treatments with a S:SD 0:1 and 3:1. Furthermore, the reactors with S:SD ratios 0:1 and 1:3 presented a significant difference between the initial and final fuel oil concentration ($p<0.005$). These results may suggest a lower biodegradation capability of residential microorganisms to biodegrade the fuel oils present in the contaminated soil and thus the importance of mixing both soil and sawdust, for a higher removal of fuel oils in soil. Additionally, diesel fuel initially present in the contaminated soil could be more recalcitrant than the fuel oil initially present in the contaminated sand. Treatment or S:SD ratio thus is the factor that more strongly affects the removal of fuel oil under in-vessel composting conditions of aged fuel oil contaminated mixtures of soil and sawdust. Soil to organic amendment ratios has proved to be necessary for in-vessel composting optimization (Antizar-Ladislao *et al*. 2006a). For an elevated operation yield (i.e. higher amount of contaminated mixture treated), a low S:SD ratio is advantageous, since a high S:SD ratio may show inhibitory effects on the residential microbial populations.

H. Regulatory and sustainable aspects of in-vessel composting as a bioremediation technology in Chile.

Environmental concern due to the presence of hazardous wastes is relatively new in Chile. Current Chilean legislation contemplates the application of bioremediation technologies for environmental decontamination (Ministry of Health 2004). Nevertheless, the existing legislation which is the only one that contemplates the integral management, manipulation and disposal of hazardous wastes, includes bioremediation technologies, such as composting as a valid alternative practice. At present there is not a coherent or clear guide to implement the existing normative. In this present year, 2007, a Ministry of Environment has been created in Chile with the mission of designing protocols to implement environmental policies. These protocols will include measures to treat contaminated wastes including contaminated soils, facilitating an appropriate application of the environmental policies among administrations, industry and citizens. Nowadays in Chile there is also a lack of technical expertise within the field of the application of bioremediation technologies. An undergoing constant discussion among administration, industry and citizens in Chile should lead to solutions to resolve environmental problems of current concern.

The feasibility study presented in this chapter provides evidence that the application of in-vessel composting as a bioremediation technology to treat hazardous wastes is a real treatment alternative to decontaminate wastes. The application of this bioremediation technology is encouraged by economic pressures since the cost of disposal of hazardous wastes in a HWL are high (more than US$100 per cubic meter of wastes) and by regulatory pressures over the mining industry, as they are enforced to present plans of closing and abandonment of the mine.

VI. Final remarks

Whilst present research on the application of bioremediation technologies to fuel-contaminated desert soils is still scarce, the investigations presented in this chapter have proved that composting is a good sustainable environmental technology that may be used to remove organic contaminants from fuel-contaminated desert soils, sawdust and mixtures. This bioremediation technology has proved to be beneficial for the amelioration of contaminated soils by reducing the concentration of organic contaminants, which promotes soil sustainability and soil re-use in contrast to other treatment technologies, i.e., landfill or incineration approaches. Additionally, a number of cutting-edge biomonitoring tools can be used to assess the extent of physical and chemical environmental changes during the application of bioremediation technologies.

The coexistence of fungi and bacteria in soil, and their metabolic cooperation in different systems should be considered in future bioremediation technologies, mainly based on the fact that extensive colonization of fungal hyphae by bacteria may play an important role in bacterial movement and growth in soil substrates. Fungi thus increase the "bioavailability" of bacteria to pollutants. In addition, biocontrol fungi such as those belonging to the *Trichoderma* genera (resistant to different type of pollutants) could be used for multiple purposes: colonization of soils to provide a network for the mobilization of pollutant degrading bacteria used in bioremediation processes, and/or of biocontrol bacteria to prevent development of phytopathogens. Nevertheless, more studies are necessary to establish the role of concurring fungi during a bioremediation process, considering that bacterial communities and fungal microbiota can co-operate and participate synergistically.

The bioremediation of desert mining soils in the Atacama Desert was possible using aerated in-vessel composting under controlled conditions, where the heterotrophic microorganisms were capable of degrading organic compounds. This is in concordance with other authors who studied desert soils and Martian conditions. The removal curves obtained had a tendency towards stabilization over time but with different end points, showing the differential capacity of absorption of the different composts due to presence of sawdust. These differences in the final concentration can be explained by differences in the degree of absorption, microbial communities present, interactions between them, structural properties of the pollutants and controlled conditions of treatment. The differential end points may however also be due to N limitation, where the right balance of nutrients must be reached.

Two principal factors, time and the presence of sawdust, explain the performance of bioremediation. The sawdust could be allowing major levels of removal (absorption) and the time may perhaps show that adaptation of the microbial communities for bioremediation does not happen straight away. Biomonitoring tools used in this study proved to be useful and complementary. Nevertheless, it is suggested that the application of the COMPOCHIP microarray could result in a better resolution of microbial communities present in the samples. The presence of adapted microbial communities and accessibility to aged contaminants was essential for experimental treatment of the Atacama desert soils. In conclusion, this study has further raised the importance of support with experimental data to the Chilean Legislation and support of the application of cutting edge tools in the bioremediation of desert soils.

References

Al-Awadhi N., Al-Daher R., El-Nawawy A., Balba M.T. (1996) Bioremediation of oil-contaminated soil in Kuwait .1. Landfarming to remediate oil-contaminated soil, *J. Soil Contamination* 5: 243-260.

Alcalde M., Ferrer M., Plou F.J., Ballesteros A. (2006) Environmental biocatalysis: from remediation with enzymes to novel green processes, *Trends Biotechnol.* 24:281-7.

Al-Daher R., Al-Awadhi N., El-Nawawy A. (1998) Bioremediation of damaged desert environment using the windrow soil pile system in Kuwait, *Environ. Int.* 24: 175-180.

Al-Daher R., Al-Awadhi N., Yateem A., Balba M.T., El-Nawawy A. (2001) Compost soil piles for treatment of oil-contaminated soil, *Soil Sed. Contamination* 10: 197-209.

Amann R.I., Ludwig W., Schleifer K.H. (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59: 143-69.

Antizar-Ladislao B., Lopez-Real J.M., Beck A.J. (2004) Bioremediation of polycyclic aromatic hydrocarbon (PAH)-contaminated waste using composting approaches, *Crit. Rev. Env. Sci. Tecnol.* 34: 249-289

Antizar-Ladislao B., Lopez-Real J., Beck A.J. (2005) Laboratory studies of the remediation of polycyclic aromatic hydrocarbon contaminated soil by in-vessel composting, *Waste Manag.* 25: 281-289.

Antizar-Ladislao B., Lopez-Real J., Beck A.J. (2006a) Degradation of polycyclic aromatic hydrocarbons (PAHs) in an aged coal-tar contaminated soil under in-vessel composting conditions, *Environ. Pollut.* 141: 459-468.

Antizar-Ladislao B., Lopez-Real J., Beck A.J. (2006b) Investigation of organic matter dynamics during in-vessel composting of an aged coal-tar contaminated soil using fluorescence excitation-emission spectroscopy, *Chemosphere* 64: 839-847.

Antizar-Ladislao B., Russell N.J. (2007) In-vessel composting as a sustainable bioremediation technology of contaminated soils and waste. In: *Environmental Research Trends*. Editor: Marvin A. Cato., Nova Science Publishers, Inc. New York.

Arora D. K., Gupta S. (1993) Effect of different environmental conditions on bacterial chemotaxis toward fungal spores, *Can. J. Microbiol*. 39: 922-931.

Baath E., Diaz-Ravina M., Frostegard A., Campbell C. (1998) Effect of metal-rich sludge amendments on the soil microbial community, *Appl. Environ. Microbiol.* 64: 238-245.

Bae J.W., Park Y.H. (2006) Homogeneous versus heterogeneous probes for microbial ecological microarrays, *Trends Biotechnol*. 24:318-323.

Bhatt M., Cajthaml T., Sasek V. (2002) Mycoremediation of PAH-contaminated soils, *Folia Microbiol (Praha)* 47: 255-258.

Balba M.T., Al-Daher R., Al-Awadhi N., Chino H., Tsuji H. (1998) Bioremediation of oil-contaminated desert soil: The Kuwaiti experience, *Environ. Int.* 24: 163-173.

Baltrian P., in Der Wiesche C., Gabriel J., Nerud F., Zadrazil F. (2000) Influence of cadmium and mercury on activities of ligninolytic enzymes and degradation of polycyclic aromatic hydrocarbons by *Pleurotus ostreatus* in soil, *Appl. Environ. Microbiol.* 66: 2471-2478.

Benítez T., Rincón A. M., Limón M. C., Codón A. C. (2004) Biocontrol mechanisms of *Trichoderma* strains, *Intl. Microbiol.* 7: 249-260.

Bodrossy L. (2003) Diagnostic oligonucleotide microarrays for microbiology. In: *A beginners guide to microarrays*. Pg 43-84. Editor: Blalock E. Kluwer Academic Publishers, Massachusetts USA.

Bogan B.W., Schoenike B., Lamar R.T., Cullen D. (1996a) Manganese peroxidase mRNA and enzyme activity levels during bioremediation of polycyclic aromatic hydrocarbon contaminated soil with *Phanerochaete chrysosporium*, *Appl. Environ. Microbiol.* 62: 2381-2386.

Bogan B.W., Schoenike B., Lamar R.T., Cullen D. (1996b) Expression of *lip* genes during growth in soil and oxidation of anthracene by *Phanerochaete chrysosporium*, *Appl. Environ. Microbiol.* 62: 3697-3703.

Bolta S.V., Mihelic R., Lobnik F., Lestan D. (2003) Microbial community structure during composting with and without mass inocula, *Compost Sci. Util.* 11: 6-15.

Boonchan S., Britz M.L., Stanley G.A. (2000) Degradation and mineralization of high-molecular-weight

polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures, *Appl. Environ. Microbiol.* 66: 1007-1019.

Bordjiba O., Steiman R., Kadri M., Semadi A., Guiraud P. (2001) Removal of herbicides from liquid media by fungi isolated from contaminated soil, *J. Environ. Qual.* 30: 418-426.

Brazma A., Robinson A., Cameron G., Ashburner M. (2000) One-stop shop for microarray data. *Nature* 403: 699-700

Brim H., Heuer H., Krogerrecklenfort E., Mergeay M., Smalla K. (1999). Characterisation of the bacterial community of a zinc-polluted soil. *Can. J. Microbiol.* 45: 326-338.

Call D.R., Borucki M.K., Loge F.J. (2003) Detection of bacterial pathogens in environmental samples using DNA microarrays. *J. Microbiol. Meth.* 53: 235-243.

Canet R., Birnstingl J.G., Malcolm D.G., Lopez-Real J.M., Beck A.J. (2001) Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by native microflora and combinations of white-rot fungi in a coal-tar contaminated soil, *Bioresour. Technol*. 76: 113-117.

Chávez-Gómez B., Quintero R., Esparza-García F., Mesta-Howard A. M., Zavala Díaz de la Cerna F. J., Hernández-Rodríguez C. H., Guillén T., Poggi-Varaldo H. M., Barrera-Cortés J., Rodríguez-Vásquez R. (2003) Removal of phenanthrene from soil by co-cultures of bacteria and fungi pregrown on sugarcane bagasse pith, *Bioresour. Technol.* 89: 177-183.

Chouari R., Le Paslier D., Daegelen P., Ginestet P., Weissenbach J., Sghir A. (2005) Novel predominant archaeal and bacterial groups revealed by molecular analysis of an anaerobic sludge digester. *Environ. Microbiol.* 7: 1104-1115.

Clements M. (2002) Studies of the Culturable Bacterial Diversity Along the North-south Moisture Gradient of the Atacama Desert. NASA. AMES. Second Astrobiology Science Conference NASA AMES Research Center - April 7 - 11, 2002. http://www.astrobiology.com/.

CLU-IN (2007) Technology Innovation Program, United States Environmental Protection Agency. http://www.clu-in.org/techfocus/

Cunningham S.D., Andersson T.A., Schwab A.P., Hsu F.C. (1996) Phytoremediation of soils contaminated with organic pollutants, *Adv. Agronomy* 56: 55-113.

Das K., Keener H.M. (1997) Numerical model for the dynamic simulation of a large scale composting system*, Transactions ASAE* 40: 1179-1189.

de Paula A.M., Soares A.R.F.S., Siquiera J.O. (2006) Biomassa, atividade microbiana e fungos micorrízicos em solo de "landfarming" de residuos petroquímicos, *R. Bras. Eng. Agric. Ambiental* 10: 448-455.

Dejonghe W., Boon N., Seghers D., Top E., Verstraete W. (2001). Bioaugmentation of soils by increasing microbial richness: missing links, *Environ. Microbiol.* 3: 649-657.

Delille D., Coulon F., Pelletier E. (2004) Effects of temperature warming during a bioremediation study of natural and nutrient-amended hydrocarbon-contaminated sub-Antarctic soils, *Cold Regions Sci. Technol.* 40: 61-70.

Drees K., Neilson J.V., Betancour J.L., Quade J., Henderson D.A., Pryor B.M., Maier R.M. (2006) Bacterial community structure in the hyper-arid core of the Atacama Desert, Chile, *Appl. Environ. Microbiol*. 72:7902-7018.

Dubois J.W., Hill S., England L.S., Edge T., Masson L., Trevors J.T., Brousseau R. (2004) The development of a DNA microarray-based assay for the characterization of commercially formulated microbial products, *J. Microbiol. Meth.* 58: 251-262.

Epstein E. (1997) *The Science Of Composting*, Technomic Publishing Company, Lancaster.

Esposito E., da Silva M. (1998) Systematics and environmental application of genus *Trichoderma*, *Crit. Rev. Microbiol*. 24: 89-98.

Felske A., Akkermans A.D.L., De Vos W.M. (1998). Quantitation of 16S rRNAs in complex bacterial communities by multiple competitive reverse transcription-PCR in temperature gradient gel electrophoresis. *Appl. Environ. Microbiol.*, 64: 4581-4587.

Finstein M.S., Miller F., Strom P.F. (1986) Waste treatment composting as a controlled system. In: H. Rehm, G. Reed (Eds.), *Biotechnology*, Verlag Chemie, Weinheim, Federal Republic of Germany, pp. 363-398.

Fisher S.G., Lerman L.S. (1979) Length-independent separation of DNA restriction fragments in two-dimensional gel electrophoresis, *Cell* 16: 191-200.

Franke-Whittle, I.H., Klammer S.H., Insam H. (2005) Design and application of an oligonucleotide microarray for the investigation of compost microbial communities, *J. Microbiol. Meth.* 62:37-56.

Franke-Whittle I.H., Knapp B.A., Fuchs J., Kaufmann R., Insam H. (2007) Changes in the bacterial community of different composts over time, investigated using the COMPOCHIP DNA microarray, (Submitted for publication)

Fraser B.S., Lau A.K. (2000) The effects of process control strategies on composting rate and odor emission, *Compost Sci. Util.* 8: 274-292.

Garland J.L., Mills A.L. (1991) Classification and characterisation of heterotrophic microbial communities on the basis of patterns of community level-sole-carbon-source-utilization, *Appl. Environ. Microbiol.* 57: 2351-2359.

Garland, J.L. (1996) Patterns of potential C source utilization by rhizosphere communities, *Soil Biol. Biochem.* 28: 223-230.

Godoy-Faúndez A., Antízar-Ladislao B., Reyes-Bozo L., Camaño A., Saéz-Navarrete C. (2007). Bioremediation of contaminated mixtures of desert mining soil and sawdust with fuel oils by aerated in-vessel composting in the Atacama Region (Chile), (Submitted for publication)

Gohre V., Paszkowski U. (2006) Contribution of the arbuscural mycorrhizal to heavy metal phytoremediation, *Planta* 223: 115-1122.

Grams G., Voigt K-D., Kirsche B. (1999) Degradation of polycyclic-aromatic hydrocarbons with three to seven aromatic rings by higher fungi in sterile and unsterile soils, *Biodegradation* 10: 51-62.

Green, S.J., Michel Jr. F.C., Hadar Y., Minz D. (2004) Similarity of bacterial communities in sawdust- and straw-amended cow manure composts, *FEMS Microbiol. Lett.* 233: 115-123.

Groh J.L., Luo Q., Ballard J.D., Krumholz L.R. (2005) A method adapting microarray technology for signature-tagged mutagenesis of Desulfovibrio desulfuricans G20 and Shewanella oneidensis MR-1 in anaerobic sediment survival experiments, *Appl. Environ. Microbiol*. 71:7064-7074.

Guerin T.F. (1999) Bioremediation of phenols and polycyclic aromatic hydrocarbons in creosote contaminated soil using ex-situ landtreatment, *J. Hazard. Materials*, 1999, 65: 305-315.

Guerin T.F. (2000) The differential removal of aged polycyclic aromatic hydrocarbons from soil during bioremediation, *Environ. Sci. Pollut. Res.*, 2000, 7: 19-26.

Gunderson C.A., Kostuk J.M., Gibbs M.H., Napolitano G.E., Wicker L.F., Richmond J.E., Stewart A.J. (1997) Multispecies toxicity assessment of compost produced in bioremediation of an explosives-contaminated sediment, *Environ. Toxicol. Chem.* 16: 2529-2537.

Guschin, D.Y., Mobarry, B.K., Proudnikov, D., Stahl, D.A., Rittmann, B.E., Mirzabekov, A.D. (1997) Oligonucleotide microchips as genosensors for determinative and environmental studies in microbiology. *Appl. Environ. Microbiol.* 63: 2397-2402.

Harman G.E., Howell C.R., Viterbo A., Chet I., Lorito M. (2004) *Trichoderma* species – opportunistic, avirulent plant symbionts, *Nature Reviews* 2: 43-56.

Haruta S., Kondo M., Nakamura K., Chanchitpricha C., Aiba H., Ishii M., Igarashi Y. (2004) Succession of a microbial community during stable operation of a semi-continuous garbage-decomposing system, *J. Biosci. Bioeng.* 98:20-27.

Haug R.T. (1993) *The Practical Handbook of Compost Engineering*, Lewis Publishers, CRC Press, Boca Raton, Florida.

Heijs S.K., Aloisi G., Bouloubassi I., Pancost R.D., Pierre C., Sinninghe Damsté J.S., Gottschal J.C., Van Elsas J.D., Forney L.J. (2006) Microbial community structure in three Deep-Sea carbonate crusts. *Microbial Ecol.* 52: 451-462.

Jjemba P.K., Kinkle B.K., Shann J.R. (2006) In-situ enumeration and probing of pyrene-degrading soil bacteria, *FEMS Microbiol. Ecol*. 55: 287-298.

Kaeberlein T., Lewis K., Epstein S.S. (2002) Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment, *Science* 296:1127-1129.

Karetnikova E.A., Zhircova A.D. (2005) Degradation of phenols formed during lignin pyrolysis by microfungi of genera *Trichoderma* and *Penicillium*, *Izv. Akad. Nauk. Ser. Biol.* 5: 539-544.

Kästner M., Mahro B. (1996) Microbial degradation of polycyclic aromatic hydrocarbons in soils affected by the organic matrix of compost, *Appl. Microbiol. Biotechnol.* 44: 668 – 675.

Kechavarzi C., Pettersson K., Leeds-Harrison P., Ritchie L., Ledin S. (2007) Root establishment of perennial ryegrass (*L-perenne*) in diesel contaminated subsurface soil layers, *Environ. Pollut.* 145: 68-74.

Kirk T.K., Lamar R.T., Glaser J.A. (1992) The potential of white-rot fungi in bioremediation. In: S. Mongkolsuk, P.S. Lovett, J.E. Trempy (Eds.) *Biotechnology and environmental science-molecular approaches*. New York, Plenum Press. Pp. 131 – 138.

Kohlmeier S., Smits T.H.M., Ford R.M., Keel C., Harms H., Wick L.Y. (2005) Taking the fungal highway: mobilization of pollutant-degrading bacteria by fungi, *Environ. Sci. Technol.* 39: 4640-4646.

Kuiper I., Bloomberg G.V., Lungtenberg B.J.J. (2001) Selection of a plant-bacterium pair as a novel tool for rhizostimulation of polycyclic aromatic hydrocarbon-degrading, *Mol. Plant-Microbe Interact.* 14: 1197-1205.

Laine M.M., Jorgensen K.S. (1997) Effective and safe composting of chlorophenol-contaminated soil in pilot scale, *Environ. Sci. Technol.* 31: 371-378.

Leben C. (1984) Spread of plant pathogenic bacteria with fungal hyphae, *Phytopathology* 74: 983-986.

Liang C., Das K.C., McClendon R.W. (2003) The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend, *Bioresour. Technol*. 86: 131-137.

Loehr R.C., Smith A., Corsi R.L. (2001), VOC and SVOC emissions from slurry and solid phase bioremediation processes, *Pract. Periodical of Haz., Toxic, and Radioactive Waste Mgmt*. 5: 211-224.

Loy A., Lehner A., Lee N., Adamczyk J., Meier H., Ernst J., Schleifer K.H., Wagner. M. (2002) Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment, *Appl. Environ. Microbiol.* 68: 5064-5081.

Loy A., Bodrossy L. (2005) Highly parallel microbial diagnostics using oligonucleotide microarrays, *Clinica Chimica Acta* 363: 106-119.

Lundstedt S., Persson Y., Oberg L. (2006) Transformation of PAHs during ethanol-Fenton treatment of an aged gasworks' soil, *Chemosphere* 65: 1288-1294.

Macek T., Mackova M., Kas J. (2000) Exploitation of plants for the removal of organics in environmental remediation, *Biotechnol. Adv.* 18: 23-34.

Mader B.T., UweGoss K., Eisenreich S.J. (1997) Sorption of nonionic, hydrophobic organic chemicals to mineral surfaces, *Environ. Sci. Technol.* 31: 1079-1086.

Marchal R., Penet S., Solano-Serena F., Vandecasteele J.P. (2003) Gasoline and diesel oil biodegradation, *Oil Gas Sci. Technol*. 58: 441-448.

Martín-Moreno C., González-Becerra A., Blanco-Santos M. J. (2004) Tratamiento biológico de suelos contaminados: contaminación con hidrocarburos. Aplicaciones de hongos en tratamientos de biorrecuperación, *Rev. Iberoam. Micol.* 21: 103-120.

Maskos U., Southern E.M. (1992) Parallel analysis of oligodeoxyribonucleotide (oligonucleotide) interactions. I. Analysis of factors influencing oligonucleotide duplex formation, *Nucleic Acid Res.* 20: 1675-1678.

Michel F.C., Quensen J., Reddu C.A. (2001) Bioremediation of a PCB-contaminated soil via composting, *Compost Sci Util.* 9: 274-284.

Mihial D., Viraraghavan T., Jin Y. (2006) Bioremediation of Petroleum-Contaminated Soil Using Composting, *Practice Periodical Hazard. Toxic Radioactive Waste Manag.* 10:108-115

Miller A. (1976) *Climates of Central and South America*, W. Schwerdtfeger, Ed. (Elsevier, Amsterdam), pp.113–145.

Minera Escondida (2007) http://www.escondida.cl/Escondida/ingles/index.html.

Ministry of Health (2004) Decreto Supremo No. 148: Reglamento Sanitario Sobre Manejo de Residuos Peligrosos (Sanitari normative on handling hazardous wastes), Santiago, República de Chile.

Montealegre J., Herrera R., Velásquez J. C., Silva P., Besoaín X., Pérez L. M. (2005) Biocontrol of root and crown rot in tomatoes under greenhouse conditions using Trichoderma harzianum and

Paenebacillus lentimorbus. Additional effect of solarizaton, *Electronic Journal of Biotechnology* 8: Issue of December 15, 2005. http://www.ejbiotechnology.info/content/vol8/issue3/full/7/

Moyer C.L., Tiedje J.M., Dobbs F.C., Karl D.M. (1996) A computer-simulated restriction analysis of bacterial small-subunit rRNA genes: efficacy of selected tetrametric restriction enzymes for studies of microbial diversity in nature. *Appl. Environ. Microbiol.* 62: 2502-2507.

Mrayyan B., Battikhi M.N. (2005) Biodegradation of total organic carbons (TOC) in Jordanian petroleum sludge, *J. Hazard. Mater.* 120: 127-134.

Navarro-González R., Rainey F., Molina P., Bagaley D., Hollen B., de la Rosa J., Small A., Quinn R., Grunthaner F., Cáceres L., Gomez-Silva B., McKays C. (2003). Mars-Like Soils in the Atacama Desert, Chile, and the Dry Limit of Microbial Life, *Science* 302: 1018-1021.

Negri M., Manfredini A., Saponaro S., Sorlini C., Bonomo L., Valle A., Zanardini E. (2004) Solid phase treatment of an aged soil contaminated by polycyclic aromatic hydrocarbons, *J. Environ. Sci. Health A – Toxic/Hazard. Substances Env. Eng.* 39: 1-17.

Novotny C., Erbanova P., Sasek V., Kubatova A., Cajthaml T., Lang E., Krahl J., Zadrazil F. (1999) Extracellular oxidative enzyme production and PAH removal in soil by exploratory mycelium of white rot fungi, *Biodegradation* 10: 159-168.

Paszczynski A., Crawford R. L. (1995) Potential for bioremediation of xenobiotic compounds by white-rot fungus *Phanerochaete chrysosporium, Biotech. Prog.* 11: 368 – 379.

Peplies, J., Chun Kwan Lau S., Pernthaler J., Amann R., Glockner F. O. (2004) Application and validation of DNA microarrays for the 16S rRNA-based analysis of marine bacterioplankton. *Environ. Microbiol.* 6: 638-645.

Pérez L.M., Besoaín X., Reyes M., Pardo G., Montealegre J. (2002) The expression of extracellular fungal cell wall hydrolytic enzymes in different *Trichoderma harzianum* isolates correlates with their ability to control *Pyrenochaeta lycopersici*, *Biological Res.* 35: 401-410.

Pfender, W.F., Fieland V.P., Ganio L.M., Seidler R.J. (1996) Microbial community structure and activity in wheat straw after inoculation with biological control organisms, *Appl. Soil Ecol.* 3: 69-78.

Potin O., Veignie E., Rafin C. (2004) Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by *Cladosporium sphaerospermum* isolated from an aged contaminated soil, *FEMS Microbiol. Ecol.* 51: 71-78.

Ram R.J., Verberkmoes N.C., Thelen M.P., Tyson G.W., Baker B.J., Blake R.C. 2nd, Shah M., Hettich R.L., Banfield J.F. (2005) Community proteomics of a natural microbial biofilm, *Science* 308:1915-1920.

Rhee S.K., Liu X., Wu L., Chong S.C., Wan X., Zhou J. (2004) Detection of genes involved in biodegradation and biotransformation in microbial communities by using 50-mer oligonucleotide microarrays, *Appl. Environ. Microbiol.* 70:4303-4317.

Rhykerd R.L., Weaver R.W., McInnes K.J. (1995) Influence of salinity on bioremediation of oil in soil, *Environ. Pollut.* 90: 127-130.

Rigot J., Matsumura F. (2002) Assessment of the rhizosphere competency and pentachlorophenol-metabolizing activity of pesticide-degrading strain of Trichoderma harzianum introduced into the root zone of corn seedlings, *J. Environ. Sci. Health B.* 37: 201-210.

Ros M., Goberna M., Moreno J.L., Hernandez T., García C., Insam H., Pascual J.A. (2006) Molecular and physiological bacterial diversity of a semi-arid soil contaminated with different levels of formulated atrazine, *Appl. Soil Ecol.* 34: 93-102.

Rudi K., Flateland S.L., Hanssen J.F., Bengtsson G., Nissen H. (2002) Development and evaluation of a 16S Ribosomal DNA array-based approach for describing complex microbial communities in ready-to-eat vegetable salads packed in a modified atmosphere, *Appl. Environ. Microbiol.* 68, 1146-1156.

Sadzawka A., Grez R., Mora M., Saavedra N., Carrasco M., Rojas C. (2000) *Métodos De Análisis Recomendados Para Los Suelos Chilenos*, Chilean Normative for soils. http://www.sag.cl.Chile.

Santos S.R., Ochman H. (2004) Identification and phylogenetic sorting of bacterial lineages with universally conserved genes and proteins, *Environ. Microbiol.* 6:754-759.

Sasek V., Bhatt M., Cajthaml T., Malachova K., Lednicka D. (2003) Compost-mediated removal of polycyclic aromatic hydrocarbons from contaminated soil, *Arc. Environ. Contam. Toxicol.* 44: 336-342.

Seymour R.M., Donahue D., Bourdon M., Evans J.R., Wentworth D. (2001) Intermittent aeration for in-vessel composting of crab processing waste, *Compost Sci Util.* 9: 98-106.

Semple K.T., Reid B.J., Fermor T.R. (2001) Impact of composting strategies on the treatment of soils contaminated with organic pollutants, *Environ. Pollut.* 112: 269-283

Semple K.T., Morriss A.W.J., Paton G.I. (2003) Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis, *Eur. J. Soil Sci.* 564: 1-10

Semple K., Doick K., Jones K., Burauel P., Craven A, Harms H. (2004) Defining bioavailability and bioaccessibility of contaminated soil and sediments, *Environ. Sci. Technol*. 15: 229A-231A.

Sharma S., Radl V., Hai B., Kloos K., Fuka M.M., Engel M., Schauss K., Schloter M. (2007) Quantification of functional genes from procaryotes in soil by PCR, J. Microbiol. Methods 68:445-452.

Singer A.C., Jury W., Luepromchai E., Yahng C. S., Crowley D. E. (2001) Contribution of earthworms to PCB bioremediation, *Soil Biol. Biochem*. 33: 765-776.

Small J., Call D.R., Brockman F.J., Straub T.M., Chandler D.P. (2001) Direct detection of 16S rRNA in soil extracts by using oligonucleotide microarrays. *Appl. Environ. Microbiol.* 67: 4708-4716.

Smith J.J., Tow L.A., Stafford W., Cary C., Cowan D.A. (2006) Baterial diversity in there different Antarctic cold desert mineral soils. *Microbial Ecol.* 51: 413-421.

Southern E.M., Mir K., Shchepinov M. (1999) Molecular interactions on microarrays, *Nature Genetics Supplement* 21:5-9.

Stach J.E.M., Bathe S., Clapp J.P., Burns R.G. (2001) PCR-SSCP comparison of 16S rDNA sequence diversity in soil DNA obtained using different isolation and purification methods, *FEMS Microbiol. Ecol.* 36: 139-151.

Steffen K.T., Schubert S., Tuomela M., Hatakka A., Hofrichter M. (2007) Enhancement of bioconversion of high-molecular mass polycyclic aromatic hydrocarbons in contaminated non-sterile soil by litter-decomposing fungi, *Biodegradation* 18: 359-369.

Stoker C.R., M. Bullock A. (1997) Organic degradation under simulated Martian conditions, *J. Geophys. Res.*, *102*: 10,881-10,888.

Stralis-Pavese N., Sessitsch A., Weilharter A., Reichenauer T., Riesing J., Csontos J., Murrell J. C., Bodrossy L. (2004) Optimization of diagnostic microarray for application in analysing landfill methanotroph communities under different plant covers, *Environ. Microbiol.* 6: 347-363.

Thompson L., Troeh F. (1978) *Soils and soil fertility*, 4th Edition, McGraw-Hill, New York.

Tiquia S.M. (2005) Microbial community dynamics in manure composts based on 16S and 18S RDNA T-RFLP profiles, *Environ. Technol.* 26: 1101-1113.

Tringe S.G., von Mering C., Kobayashi A., Salamov A.A., Chen K., Chang H.W., Podar M., Short J.M., Mathur E.J., Detter J.C., Bork P., Hugenholtz P., Rubin E.M. (2005) Comparative metagenomics of microbial communities, *Science* 308: 554-557.

Uchiyama T., Abe T., Ikemura T., Watanabe K. (2005) Substrate-induced gene-expression screening of environmental metagenome libraries for isolation of catabolic genes, *Nat. Biotechnol*. 23:88-93.

USDA (1999) Soil taxonomy, a basic system of soil classification for making and interpreting soil surveys, U.S. Government Printing Office.

USEPA (1986) U.S. Environmental Protection Agency, *Test Method for Evaluating Solid Waste*, SW-846, 3th Edition, USEPA, Washington, DC.

USEPA (2002) U.S. Environmental Protection Agency, *Application, Performance, and costs of biotreatment technologies for contaminated soils* EPA/600/R-03/037, USEPA, Washington, DC.

USEPA (2003) U.S. Environmental Protection Agency, *Guide for Conducting Treatability Studies Under CERCLA: Biodegradation Remedy Selection* EPA/540/R-93/519b. USEPA, Washington, DC.

VanderGheynst J.S., Lei F. (2003) Microbial community structure dynamics during aerated and mixed composting, *Transactions American Society Agricultural Engineers* 46: 577-584.

Vianna M.E., Horz H.P., Gomes B.P.F.A., Conrads G. (2005) Microarrays complement culture methods for identification of bacteria in endodontic infections, *Oral Microbiol. Immunol.* 20: 253-258

von Wintzingerode F., Gobel U.B., Stackebrandt E. (1997) Determination of microbial diversity in environmental samples: Pitfalls of PCR-based rRNA analysis. *FEMS Microbiol. Rev.* 21: 213-229.

Wang, R.-F., Beggs M.L., Robertson L.H., Cerniglia C. E. (2002) Design and evaluation of oligonucleotide-microarray method for the detection of human intestinal bacteria in fecal samples, *FEMS Microbiol. Lett.* 213: 175-182.

Wessels J.G.H. (1997) Hydrophobins, proteins that change the nature of the fungal surface, *Adv. Microbiol. Physiol.* 38: 1-45.

Watanabe K., Hino S. (1996) Identification of a functionally important population in phenol-digesting activated sludge with antisera raised against isolated bacterial strains, *Appl. Environ. Microbiol*. 62:3901-3904

Wick L.Y., Remer R., Würz B., Reichenbach J., Braun S., Schäfer F., Harms H. (2007) Effect of fungal hyphae on the access of bacteria to phenanthrene in soil, *Environ. Sci. Technol*. 41: 500-505.

Woese C.R. (1987) Bacterial evolution, *Microbiol. Rev.* 51: 221-271.

Wong P.T.G., Griffin D.M. (1976) Bacterial movement at high matric potentials-II. In fungal colonies, *Soil Biol. Biochem*. 8: 219-223.

Wösten H.A.B., van Wetter M.A., Lugones I.G., van der Mei H.C., Busscher H.J., Wessels J.G.H. (1999) How a fungus escapes the water to grow into the air, *Current Biol.* 9: 85-88.

Zak J.C., Willig M.R., Moorhead D.L., Wildman H.G. (1994) Functional diversity of microbial communities: a quantitative approach, *Soil Biol. Biochem.* 26: 1101-1108.

Zhou J. (2003) Microarrays for bacterial detection and microbial community analysis, *Curr. Opin. Microbiol.* 6: 288-294.