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## PART II: Bioremediation

### Chapter 3

# BIOREMEDIATION OF TOCS PRESENT IN FUEL-CONTAMINATED DESERT MINING SOIL AND SAWDUST IN THE ATACAMA DESERT (CHILE)

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### ABSTRACT

Repetitive spills of fuels and lubricants during reparation and maintenance of machinery within Chilean mining industry constitute an unseen pollution of current environmental concern. These spills had subsequently been adsorbed by desert soils and sawdust used as cheap sorbent materials are considered hazardous wastes (Chilean legislation) and must be contained and disposed on a hazardous waste landfill. Alternative accepted treatments to landfilling consist in biological treatments such as bioremediation, a cost-effective opportunity for Chile. Nevertheless, it remains unknown if bioremediation of fuel-contaminated wastes is feasible under in desert mining soils. In this study we determined the feasibility of bioremediation by aerated in-vessel composting of an aged fuel-contaminated desert mining soil and sawdust. We investigated the removal of total organic compounds (TOCs) in a composting process at laboratory scale under controlled conditions of temperature, humidity and ventilation at five soil to sawdust ratios (S:SD, 1:0, 3:1, 1:1, 1:3, 0:1). Terminal-restriction fragment length polymorphism (TRFLP) tests were conducted in order to determinate the Richness and Diversity of Operational Taxonomic Units through treatment (O.T.U.). We relate removal curves to changes in the diversity of microbial communities determinate molecular tools. Different TOC removal curves were obtained and observed after 56 days of treatment. The highest (50%) and the lowest (35%) removal rate were found in reactor S: SD-0:1 and S: SD-1:0, respectively. Interaction between presence of sawdust and time factors, both as source of variation, was statistically significant on removal curves. No trends were found among changes in richness and diversity as well as in correlation between them and removal curves. However, higher levels of sawdust corresponded with an incremental number of O.T.U., diversity and higher removal's rate. Our results shown that removal TOCs are feasible desert soil at every ratio S: SD but with differential goal achievements and molecular profiles obtained are not a predictive tool related to abatement of pollution in this treatment.

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**Keywords:** Bioremediation, desert mining soils, TOC, Atacama Desert.

## 1. INTRODUCTION

Continuous fuel spills within the Chilean mining industry at Atacama Desert (Chile) and necessity of abatement pollution attempts have allowed using soils and sawdust as a low-cost locally available sorbent material, resulting in large amounts of fuel-contaminated materials disposed in landfills by hazardous wastes. Chilean legislation treats this fuel-contaminated material as hazardous waste and therefore, it should be contained or treated (Ministerio de Salud, Chile, 2004). These materials are accumulated on hazardous waste landfills over time and need to be cleaned-up. This research investigated the application of in-vessel composting of a fuel-contaminated desert-mining soil and sawdust in the Atacama Desert as a bioremediation treatment technology monitored by molecular tools.

## 2. MATERIALS AND METHODS

30 cylindrical poly-vinyl-chloride aerated composting reactors were set-up at laboratory-scale and operated continuously during 56 days at constant ventilation ( $16 \text{ L min}^{-1}$ ) by introduction of atmospheric air previously warmed at  $60^\circ\text{C}$  for maintain internal a mesophilic temperature range ( $30 \pm 2^\circ\text{C}$  at the top surface of the composting mixture in contact with the head space, and  $40 \pm 2^\circ\text{C}$  at the bottom). Moisture content was adjusted at 50%. Composting reactors were operated using 2000gr of five soil to sawdust ratios (S: SD, 1:0, 3:1, 1:1, 1:3, 0:1, on a dry weight basis) in triplicate previously mixed, homogenized and air-dried. Total organic carbon (TOC) concentrations were monitored according to Standard Methods (USEPA, 2005). Terminal-restriction fragment length polymorphism (TRFLP) was performed by isolation communitarian microbial DNA (DNA isolation kit, MOBIO), and used as the template for the polymerase chain reaction (PCR) using primer pairs 8F labeled by NED fluorochrome ( $5' \text{ AGA GTT TGA TCC TGG CTC AG } 3'$ ) and 1392R ( $5' \text{ ACG GGC GGT GTG TAC } 3'$ ) both designed for the 16S eubacterial rDNA sequences. PCR product was digested with the enzyme HhaI and restriction fragments. Restriction fragments were separated (capillary electrophoresis) and detected in Perkin Elmer ABI Prism 310 sequencer. For each microbial community, richness and diversity were also determined. Richness (S) was defined as the number of phylotypes (O.T.U.) and the Diversity expressed as Shannon-Wiener index ( $H'$ ) (Kowalchuk et al., 2004). Abiotic controls comprised the same ratios previously radiated with three doses of Gamma rays at 25 kGy. Two-factor ANOVA test (factor 1, treatment (sawdust percentage); factor 2, time) was applied for each removal curves to determinate their contribution as variation sources in removal fuel.

## 3. RESULTS

For study the feasibility of applying composting as a bioremediation treatment, bioreactors at lab scale were used (Godoy-Faúndez et al., 2007). Different removal curves were obtained. Highest TOC removals rates were observed in all reactors after 28 days of treatment (Fig.1). The highest removal rate was found in the reactor with 100% sawdust (56, 8%) and the lowest in

reactor with 100% soil (34, 6%). Reactors with ratios S: SD, 3:1, 1:1 and 1:3 had removal rates among 39 and 44% (data not shown). These results support the idea that microbial communities need turning on or lag phase before to activate their degradative metabolic activities. No changes were observed in abiotic controls displaying that mainly the removal TOCs could be due to biological degradation and not by mass transfer phenomenon.

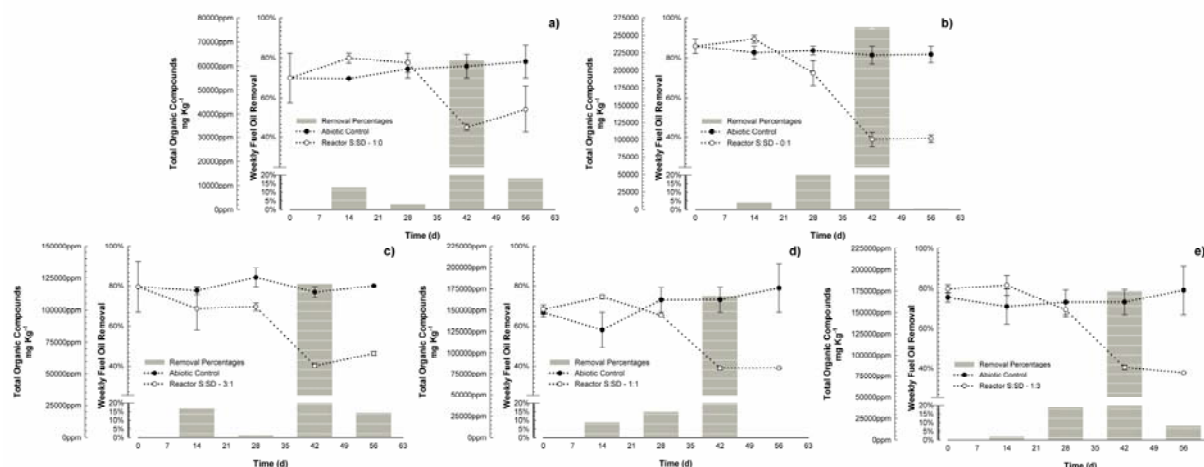


Figure 1. The figure show removal curves and weekly fuel oil removal percentages calculated as the remotion percentage among two consecutive measurements for every ratio Soil: Sawdust (S:SD) by week: a) Reactor with 100% soil; b) Reactor with 100% Sawdust; c) Reactor with Ratio S:SD-3:1; c) Reactor with Ratio S:SD-1:1; c) Reactor with Ratio S:SD-1:3.

For determining the influence of factors (presence of sawdust and time of treatment) on removal curves, Two-Way ANOVA was performed. In our experiment was not feasible separate both effects due to significant interaction between both parameters ( $p < 0.0001$ ) (Fig. 2). In abiotic controls, variations in removal curves could be due mainly to uncertainty associated on standard errors in detection's techniques. However, presence of sawdust and time of treatment are having a relevant effect on treatment.

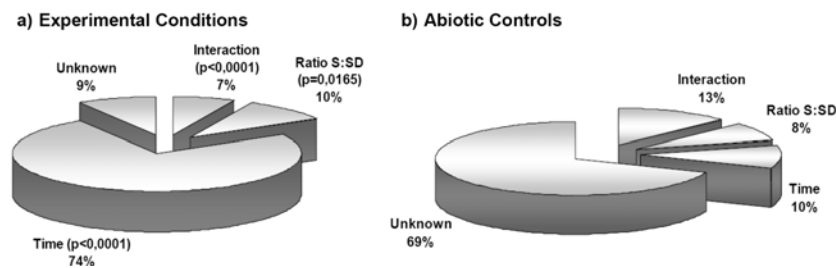


Figure 2. The figures show the effect of Time, Ratios S:SD and Interaction among factors as variation sources percentages on removal curves (changes and differences among removal curves, respectively) by Two-Way Repeated Measures ANOVA.

For determining if changes in molecular profiles can be used as bioindicator of process, community indexes such as Richness and Diversity were obtained by T-RFLP. Low number of O.T.U.s (fragments detected) were found in all reactor expressed as Richness index (Fig.3) perhaps due to a poor isolation of DNA or low abundance of microorganism in ratios S:SD. Nevertheless, higher levels of sawdust corresponded with an incremental number of O.T.U. coupled to higher diversities in microbial communities. Diversities changed throughout the treatment, but without correlations with the removal curves or removal rates per week (data not shown).

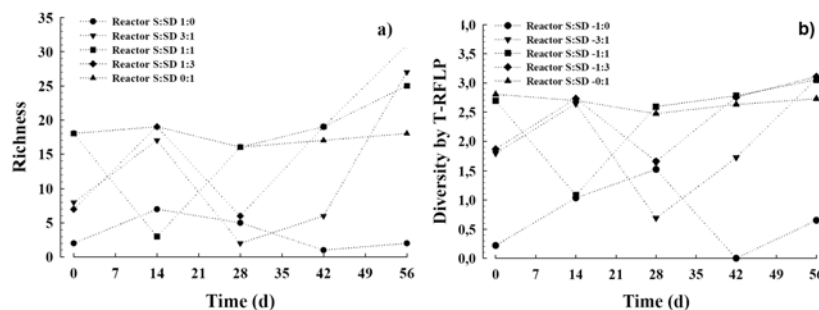


Figure 3. The figures show changes related to Richness and Diversity. a) Changes associated to Richness along of treatment. b) Changes associated to Diversity along of treatment

#### 4. DISCUSSION AND CONCLUSIONS

Our results indicate that bioremediation of desert mining soils and sawdust contaminated with fuel-oil is feasible to be applied. Different TOC removal curves were obtained after 56 days of treatment. All obtained curves have a lag phase with a huge decreasing in final times. These results are supported by large removal percentages after middle times. Aforementioned, is not correlated to number of fragments detected by molecular profiles found it. In our results, at higher levels of sawdust, Richness and Diversity was higher at compare with other ratios S: SD. Changes in profiling have not correlations with the removal curves. Higher removal rates were found in reactors with S: SD ratios 1:3 and 100% sawdust. These removal rates perhaps were feasible due to higher presence of sawdust as bulking agent (porous media) that could allow desorption processes as well as biodegradation supported by metabolism present in alochtonous microorganisms. No changes were found in abiotic controls suggesting that TOC removals in the treated reactors were mainly due to biodegradation mechanisms.

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