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Changes in the microbial community during bioremediation of gasoline-contaminated soil

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ABSTRACT

We aimed to verify the changes in the microbial community during bioremediation of gasoline-contaminated soil. Microbial inoculants were produced from successive additions of gasoline to municipal solid waste compost (MSWC) previously fertilized with nitrogen-phosphorous. To obtain Inoculant A, fertilized MSWC was amended with gasoline every 3 days during 18 days. Inoculant B received the same application, but at every 6 days. Inoculant C included MSWC fertilized with N–P, but no gasoline. The inoculants were applied to gasoline-contaminated soil at 10, 30, or 50 g/kg. Mineralization of gasoline hydrocarbons in soil was evaluated by respirometric analysis. The viability of the inoculants was evaluated after 103 days of storage under refrigeration or room temperature. The relative proportions of microbial groups in the inoculants and soil were evaluated by FAME. The dose of 50 g/kg of inoculants A and B led to the largest CO_2 emission from soil. CO_2 emissions in treatments with inoculant C were inversely proportional to the dose of inoculant. Heterotrophic bacterial counts were greater in soil treated with inoculants A and B. The application of inoculants decreased the proportion of actinobacteria and increased of Gram-negative bacteria. Decline in the density of heterotrophic bacteria in inoculants occurred after storage. This reduction was bigger in inoculants stored at room temperature. The application of stored inoculants in gasoline-contaminated soil resulted in a CO_2 emission twice bigger than that observed in uninoculated soil. We concluded that MSWC is an effective material for the production of microbial inoculants for the bioremediation of gasoline-contaminated soil.

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Introduction

Accidental spills of gasoline and other petroleum products in the fuel distribution terminals, mainly during truck cleaning and distribution in pations, are a common cause of soil and water systems contamination in Brazil.[1] BTEX compounds (benzene, toluene, ethylbenzene, and xylenes) present in gasoline are highly toxic and harmful to human health; among these, benzene is a known carcinogenic substance.[2–4] The presence of ethanol in gasoline can increase the solubility of BTEX dissolved in the groundwater and thereby hindering its natural biodegradation by increasing the persistence of these compounds in the environment.[5,6] Ethanol can be biodegraded preferably than BTEX, which leads to consumption of oxygen that would be required for the degradation of hydrocarbons. Furthermore, ethanol may be toxic or inhibitory to degrading microorganisms of monoaromatic hydrocarbons.[5] Corseuil and Fernandes[7] indicated that contamination of water table caused by Brazilian commercial gasoline may be greater than that by conventional gasolines from other countries. This issue stems from the ability of ethanol to increase the solubility of petroleum hydrocarbons in water. The results of these experiments showed that, in the aqueous phase, fractions of 10–30% of ethanol can increase the concentration of benzene, toluene, and xylene (BTX). This effect of co-solvency responsible for the higher concentration of hydrophobic organic compounds in water is greater to xylenes, which are less soluble compounds among all BTX compounds and when ethanol is added, its solubility is enhanced. Thus, it is also likely that higher concentrations of ethanol in water aquifers facilitate greater solubility of polycyclic aromatic hydrocarbons (PAH), which are extremely hydrophobic and harmful to the human health.

Bioremediation has been applied for the recovery of environments contaminated with oil and oil products. Biotechnological processes applied in bioremediation are promising because they offer better cost benefit than physicochemical techniques, which are more expensive, difficult to implement, and require continuous monitoring to achieve desirable results.[8–10] Bioremediation techniques have great developmental potentials in Brazil, as the climatic conditions of this country are favorable for the biodegradation of contaminants.

Bioremediation includes mineralization or transformation of contaminants into less toxic forms by different groups of microorganisms.[11–13] This process, which tends to occur naturally, can be accelerated in two ways: (i) by biostimulation, which is based on stimulation of the catabolic activity of indigenous microorganisms by the addition of limiting nutrient minerals, supplying oxygen or other electron acceptors, and by maintaining suitable conditions of temperature, pH, and moisture and (ii) by bioaugmentation, which is based on the inoculation of a population or consortium of effective microorganisms for the degradation of contaminants.[9,14–16] The simplest form of bioremediation is natural attenuation, which includes only monitoring of the natural degradation of the contaminant in the contaminated environment without any intervention. The comparison between the three bioremediation strategies for soil contaminated with diesel demonstrated that the results were site-specific, varying with the soil type.[17] In this study, we demonstrated the efficiency of applying microbial inoculants enriched from municipal solid waste compost (MSWC) for the bioremediation of soil contaminated with gasoline. Respirometric tests were employed to evaluate the mineralization of gasoline in inoculants and in soil receiving gasoline contamination. Changes in the microbial community were assessed by analysis of the inoculants after storage for 103 days.

Material and methods

Soil contamination and characterization

The experimental soil was sifted through 5-mm mesh and identified to be extremely clayey by physical and chemical analyses (Table 1). After adjusting the moisture content of the soil to 60% of the WHC and the C:N:P ratio to 100:10:1 (assuming C added as gasoline), the soil was contaminated artificially with 20 mL/kg of gasoline containing approximately 22% ethanol.

Inoculants development

The inoculants were produced by enrichment of MSWC with gasoline (50 mL/kg). Inoculant A received gasoline application every 3 days for up to 18 days. The gasoline application interval for inoculant B was 6 days. The C:N:P ratio of the compost was adjusted to 100:10:2, considering the amount of carbon added in the form of gasoline in the first application. During inoculants enrichment, the moisture content of the soil was maintained at 40–60% of the WHC. The Inoculant C consisted of MSWC fertilized with N and P sources, but no gasoline. All treatment soils were stored under the same conditions.

Oil decomposition activity in the contaminated soil as a function of inoculant dose

Test treatments were constructed using gasoline contaminated soil (20 mL/kg) and inoculants A, B or C that were added

Table 1 – Physical–chemical characteristics of soil.

Characteristic	Unit	Value
Thick sand	%	12
Fine sand	%	11
Silt	%	4
Clay	%	73
pH (H_2O)	–	4.8
WHC	%	48.89
Org C	g/kg	32.0
N total	g/kg	0.6
P	mg/dm^3	0.5
K	mg/dm^3	39
Ca^{2+}	$cmol_c/dm^3$	0.33
Mg^{2+}	$cmol_c/dm^3$	0.01
Al^{3+}	$cmol_c/dm^3$	0.77

WHC, water holding capacity; Org C, organic carbon; $cmol_c$, cent mol of charge.

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