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Effect of process parameters on the bioremediation of diesel contaminated soil by mixed microbial consortia

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ABSTRACT

This study focused on investigating the key parameters that influences carbon dioxide (CO_2) production during the bioremediation of diesel contaminated soil. The effects of diesel concentration, moisture content and biomass dose were investigated in batch experiments, for 20 days, to ascertain the CO_2 production and the amount of diesel mineralized. A regression model based on full factorial design of experiments was developed to predict the CO_2 production. Based on the *F* and *p* values from Analysis of Variance (ANOVA) results, the main effects of process parameters affected diesel bioremediation strongly than the 2-way and 3-way interaction effects. The highest total petroleum hydrocarbon (TPH) mineralized and the maximum CO_2 productions were ~3000 mg/kg-soil and ~10,000 mg/kg-soil, respectively, at a diesel concentration of 10,000 mg/kg-soil, 20% moisture content and a biomass dose of 275 mg/kg-soil.

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1. Introduction

Bioremediation of diesel contaminated soil by the use of microorganisms is an emerging environmentally friendly and cost effective technology. Diesel is a well known environmental pollutant that can easily contaminate soil and groundwater through their intrusion by various routes such as leakage from underground storage tanks, accidental spills, drilling sites, improper waste disposal practices, and leaching landfills (Roy et al., 2014). Hydrocarbon components released into the soil can be remediated by biodegradation processes (Chemlal et al., 2013). Biodegradation is defined as a biologically catalyzed oxidation or reduction reaction involving complex chemical compounds, based on either growth or co-metabolism. In the case of growth, organic pollutants are used as the sole source of carbon and energy which results in their complete degradation (mineralization). On the other hand, co-metabolism is defined as the metabolism of an organic compound in the presence of a growth substrate which is used as the primary carbon and energy source (Das and Chandran, 2011). Biodegradation of organic pollutants is governed by the activity of microorganisms such as bacteria, fungi, yeast and microalgae. These microorganisms co-exist with effective cooperation from the soil. However, the lack of microbial consortia strength (concentration) results in poor treatability of the soil contaminated with diesel (Maletić et al., 2011). Therefore, the inoculation of diesel contaminated soil with microbial consortia having high metabolic activity is a prerequisite to achieve effective bioremediation (Bento et al., 2005; Bundy et al., 2004; Rocha et al., 2000; Zanaroli et al., 2010).

Bioremediation of diesel contaminated soils can be achieved under *in-situ* or *ex-situ* conditions (Silva et al., 2015). *In-situ* bioremediation has proven to be cost-effective and often considered to be the safest method with limited disruption to field operations (Suja et al., 2014). Factors such as the availability of microorganisms with appropriate enzymatic and physiological capacity, favorable environmental and nutritional conditions for microbial growth and metabolism, the composition and concentration of the pollutants affects the efficiency of bioremediation processes. However, the bioavailability of active microbial consortia plays a major role to enhance diesel degradation rates. The lack of sufficient microbial population in diesel contaminated soil results

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in low biodegradation potential and slow biodegradation rates (Thompson et al., 2005).

An overview of literature on this topic indicated that biostimulation and/or bioaugmentation techniques enhance the efficiency of bioremediation of diesel contaminated soils. Biostimulation is the process in which phosphorus and nitrogen are introduced into the soil to initiate the growth of microorganisms in order to achieve faster biodegradation rates of diesel. On the other hand, bioaugmentation is the process in which potential microorganisms are inoculated in diesel contaminated soil to facilitate the biodegradation processes (Cerqueira et al., 2014; Silva-Castro et al., 2013; Sprocati et al., 2012). Besides, the addition of surfactant, hydrogen peroxide and other organic wastes such as wheat straw has shown to improve the bioremediation efficiency of diesel contaminated soils (Soleimani et al., 2013). In addition to biostimulation and bioaugmentation, chemical oxidation and electrokinetic oxidation techniques have also been suggested for the treatment of diesel contaminated soil (Falciglia et al., 2011).

Carbon dioxide (CO_2) production and their generation rate during bioremediation have been used as an index of microbial activity (Balba et al., 1998; Namkoong et al., 2002). It is a primary greenhouse gas emitted through the human activities and natural processes. An average global CO_2 flux from contaminated soil is about 68 ± 4 Pg-C/yr, whereas fossil fuel burning adds only about 5 Pg-C/yr. Thus, even a small change in the soil respiration flux may affect the annual loading of atmospheric CO_2 (Raich and Schlesinger, 1992). Soil organic matter contains approximately 1600 Pg carbon reservoir which is more than twice that of the atmospheric CO_2-C pool. Solomon et al. (2009) showed that the atmospheric CO_2 emission is likely to increase from current levels of 380 ppm (*v/v*) to peak values of 450–600 ppm (*v/v*) over the coming century and an irreversible sea level rise of at least 0.4–1.00 m is expected. Therefore, quantification of CO_2 gas that is emitted during bioremediation of diesel contaminated soil is of greatest concern from the sustainable bioremediation view point.

In this study, the main and interaction effects of process parameters on diesel mineralization (Dm, %) and subsequent CO_2 production (μmol) in bioaugumented soil using experiments designed by full factorial design was performed by considering the diesel concentration, moisture content and biomass dose as the main parameters.

2. Materials and methods

2.1. Soil sample

Soil samples were collected from the University of Ulsan (South Korea) garden at a depth of 8–10 cm and transported by a sterile plastic bag to the laboratory, and it was air dried and sieved using 1.18–425 μm sieve size in order to obtain homogenized soil samples prior to use.

2.2. Diesel

Diesel (density = 0.801 kg/L) sample was purchased from a local gasoline station and stored in the refrigerator (at 4 °C), under dark condition.

2.3. Microorganism

The mixed microbial consortium previously acclimatized to petroleum hydrocarbon was obtained from Ecophile, South Korea. It contained a consortia of *Pseudomonas* sp., *Yarroia* sp., *Acinetobacter* sp., *Corynebacterium* sp., *and Sphingomonas* sp. These cultures were concentrated through centrifugation (Eppendorf centrifuge 5804 R). The consortium had a total biomass concentration of 2.041 g/L.

2.4. Experimental design

A full factorial experimental design was formulated with three factors and two levels (2^3) in order to investigate the effect of diesel concentrations (X_1), moisture content (X_2) and biomass dose (X_3) on diesel mineralization and CO_2 production. Each factor was investigated at two levels (low and high) and it was assumed that the response is approximately linear over the range of the factor levels considered. The statistical model for a 2^k design would include k main effects, k two factor interactions, and (k/3) three factor interactions. The ranges of the independent variables and the levels investigated using factorial design are shown in Tables 1 and 2, respectively. Considering the biomass dose applied for the different experimental runs, the diesel to microorganism (F/M) ratio was varied from 10 to 300. All statistical calculations (F - Fischer's variance ratio, p - probability value, DF- degrees of freedom, Seq SS - sequential sum of squares and Adj MS - adjusted mean of squares) and analysis were done using the software MINITAB (Product version: Minitab® 17.2.1, PA, USA).

2.5. Batch biodegradation studies

Biodegradation experiments were conducted in batch incubations by varying the diesel concentration, moisture content, and biomass dose from low and high levels, in 160 ml glass serum bottles (Wheaton, New Jersy, USA) (Table 2). 25 g soil was spiked with the desired concentrations of diesel and mixed with concentrated microorganism consortium. Known amounts of surfactant (Tween 80, 0.2% *w/w*) and water was added to the soil. Tween 80 was added in order to increase the bioavailability of the hydrocarbons to the microorganisms. Nitrogen and phosphorus were also added to the soil at a ratio of Oil: N: P of 100: 1: 0.2. The soil was vortex mixed to obtain a homogenized mixture. The serum bottles were closed air tight using rubber septa and aluminum crimps and incubated at 25 °C. Batch vials were purged with CO_2 free air (1.2 L/min) by passing air through a KOH packed column followed by 4 M NaOH trap. A humidifier was used to prevent aspirated alkali solution and provide 100% humidified air to the vials at room temperature (Namkoong et al., 2002).

2.6. Control experiments

To determine the activity of natural indigenous microorganisms on the bioremediation process, three sets of experiments with initial diesel concentrations of 5000 mg/kg-soil, 10,000 mg/kg-soil and 15,000 mg/kg-soil, under field moist conditions were investigated. To understand the soil background, respiration experiments having sample moisture contents ranging from 10 to 30% were incubated together with the batch bioremediation experiments. In order to assess abiotic diesel losses through physical processes, experiments were also conducted using oven dried soil.

Table 1
Experimental parameters and levels investigated for the biodegradation of diesel.

Process parameters	Low level	High level	Centre point
Diesel concentration (mg/kg-soil), X_1	5000	15,000	10,000
Moisture content (%), X_2	10	30	20
Biomass dose (mg/kg-soil), X_3	50	500	275

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