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Plant residues — A low cost, effective bioremediation treatment for petrogenic hydrocarbon-contaminated soil

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HIGHLIGHTS

- Evaluation of the impact of 4 types of plant residues on the bioremediation of aliphatic hydrocarbons in contaminated soil.
- Development of a cost effective and green technology for hydrocarbon remediation in terrestrial environments.
- Greater understanding of the microbial mechanisms in this bioremediation system.

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ABSTRACT

Petrogenic hydrocarbons represent the most commonly reported environmental contaminant in industrialised countries. In terms of remediating petrogenic contaminated hydrocarbons, finding sustainable non-invasive technologies represents an important goal. In this study, the effect of 4 types of plant residues on the bioremediation of aliphatic hydrocarbons was investigated in a 90 day greenhouse experiment. The results showed that contaminated soil amended with different plant residues led to statistically significant increases in the utilisation rate of Total Petroleum Hydrocarbon (TPH) relative to control values. The maximum TPH reduction (up to 83% or 6800 mg kg^{-1}) occurred in soil mixed with pea straw, compared to a TPH reduction of 57% (4633 mg kg^{-1}) in control soil. A positive correlation (0.75) between TPH reduction rate and the population of hydrocarbon-utilising microorganisms was observed; a weaker correlation (0.68) was seen between TPH degradation and bacterial population, confirming that adding plant materials significantly enhanced both hydrocarbonoclastic and general microbial soil activities. Microbial community analysis using Denaturing Gradient Gel Electrophoresis (DGGE) showed that amending the contaminated soil with plant residues (e.g., pea straw) caused changes in the soil microbial structure, as observed using the Shannon diversity index; the diversity index increased in amended treatments, suggesting that microorganisms present on the dead biomass may become important members of the microbial community. In terms of specific hydrocarbonoclastic activity, the number of *alkB* gene copies in the soil microbial community increased about 300-fold when plant residues were added to contaminated soil. This study has shown that plant residues stimulate TPH degradation in contaminated soil through stimulation and perhaps addition to the pool of hydrocarbon-utilising microorganisms, resulting in a changed microbial structure and increased *alkB* gene copy numbers. These results suggest that pea straw in particular represents a low cost, effective treatment to enhance the remediation of aliphatic hydrocarbons in contaminated soils.

1. Introduction

Petrogenic hydrocarbons are an important energy source in our daily life. In addition to being a major source of energy, petrogenic hydrocarbons are also the raw material for many products such as plastics, paints, and cosmetics (Cohen, 2002). According to statistics from British Petroleum (2011), global petroleum consumption reached 87.4 million barrels per day by 2010. As a result of this massive use, industrial activities and unexpected spillage during transportation and storage the release of substantial amounts of hydrocarbon derivatives into the natural environment is unavoidable (Militon et al., 2010; Sarkar et al., 2005; Serrano et al., 2008). For example, it is estimated that 1.3 to 8.8 million tons of petrogenic hydrocarbons go into the marine environment annually (Berthe-Corti and Nachtkamp, 2010). Due to the negative effects of petrogenic hydrocarbons such as its toxicity, mutagenicity and carcinogenicity to many living organisms (Peng et al., 2009; Sheppard et al., 2011; Tang et al., 2010), developing safe, efficient and cost-effective methods for cleaning up contaminated soils is a global aim. Remediation of petrogenic hydrocarbon-contaminated soils can be carried out using a range of physico-chemical and bioremediation methods (Tang et al., 2010). However, physico-chemical methods

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(e.g., landfilling and incineration) are labour intensive and costly when compared to bioremediation methods (Kirkpatrick et al., 2006).

One biological method is phytoremediation, using plants and their rhizosphere microorganisms to biodegrade contaminants in water, sediments, soils, and air (Germida et al., 2002; Pilon-Smits, 2005). Although a number of studies have shown that a broad range of hydrocarbon contaminants can be subjected to phytoremediation (Banks et al., 2003; Chen et al., 2003; Gaskin et al., 2008; Kim et al., 2006; Phillips et al., 2006), the toxicity associated with these pollutants to the plants often limits the application of this technology. For this reason, finding new methods based on the application of non-living plant biomass may be desirable. Treatment of contaminated soil, mediated through the addition of inexpensive plant residues (such as hay and straw) may be an alternative method for the bioremediation of hydrocarbons in contaminated soil. Plant residues such as pea straw, wheat straw and hay have been previously used in bioremediation studies (Adetutu et al., 2012; Barathi and Vasudevan, 2003; Callaham et al., 2002; Hultgren et al., 2009; Zhang et al., 2008) but the results obtained have generally been inconclusive in terms of the benefit of the addition of the lignocellulosic waste to the rates of remediation. For example, adding wheat straw to creosote-contaminated soil did not increase the degradation of phenanthrene and pyrene compared to the control soil without amendment (Hultgren et al., 2009). This was explained by the fact that the addition of wheat straw stimulated microorganisms which were not involved in the degradation of PAH in the aged creosote soil. In another report, Adetutu et al. (2012) investigated the effect of different carbon sources (Tween 80, sawdust, compost and pea straw) on ^{14}C-hexadecane mineralisation. They found that in the presence of sodium azide (an inhibitor of bacterial activity) ^{14}C-hexadecane mineralisation was insignificant and fungal species which were growing on the amended carbon sources were unable to mineralise hexadecane. In contrast, when fungal growth was inhibited by the addition of nystatin, only the addition of Tween 80 significantly increased ^{14}C-hexadecane mineralisation compared to either control soil or soil from microcosms amended with sawdust, compost and pea straw. It was concluded that the decreased bacterial ^{14}C-mineralisation in the microcosms amended with either sawdust, compost or pea straw may have been a result of the fact that microorganisms involved in the degradation of hexadecane preferred to use these additional carbon sources rather than hexadecane as energy source or used both at the same time.

In contrast, a number of reports have shown positive effects of the addition of plant residues on the bioremediation of hydrocarbons (Barathi and Vasudevan, 2003; Rojas-Avelizapa et al., 2007; Zhang et al., 2008). Adding 20% wheat bran or sugarcane bagasse led to an increase in the degradation rate of aliphatic and aromatic hydrocarbons compared to the control in a bioremediation of fresh crude oil-contaminated soil. In this instance the degradation of aliphatic hydrocarbons increased from 37% in the control to 94% in contaminated soil amended with wheat bran, with the authors suggesting that the incorporation of the straw results in increased aeration in addition to nutrient addition (Barathi and Vasudevan, 2003). In fact the incorporation of lignocellulosic waste into oil contaminated soil may result in increased oil degradation through a number of interactions (Fig. 1). Plant residues contain biopolymers such as lignin, cellulose and hemicellulose (Trigo and Ball, 1994); the lignin fraction can act as an absorber of organic pollutants (e.g., PAH) in soil (Wang et al., 2007) and its presence may result in inhibition of the movement of organic pollutants into groundwater (Zhang et al., 2008). The polysaccharides released from decayed cellulose and hemicellulose can stimulate the biodegradation of petrogenic hydrocarbons through increased growth and activity of the soil microflora (Zhang et al., 2008). Physically, the plant residues can also improve soil properties, in terms of aeration, moisture, nutrition and structural properties, all of which may lead to the acceleration of hydrocarbon bioremediation. Finally, plant residues also have their own associated microbial community which may contribute to the degradation of hydrocarbons (Fig. 1). However what is apparent from the literature to date is that the effect of the type of plant residue on remediation has not been studied. This may at least partly account for the discrepancies reported in the literature regarding the beneficial effects of their addition on rates of bioremediation.

In general, the microflora (especially bacteria and fungi) play a vital role in any bioremediation of contaminated soil (Sheppard et al., 2011). Monitoring the soil microbial community is therefore an important part of a bioremediation project. The assessment of the activity and diversity of the microbial community is based on culture-dependent and culture-independent methods. Recently, culture-independent methods such as PCR-DGGE-sequencing, cloning sequencing, RT-qPCR and metagenomic analysis have been widely employed at the research level for assessment of the microbial community and for monitoring the presence of hydrocarbon degradation genes in the soil microbial community (Adetutu et al., 2012; Makadia et al., 2011; Phillips et al., 2008; Zhang et al., 2012). However these technologies have not yet been applied to studies on the impact of plant residues on the microbial community during bioremediation of a hydrocarbon-contaminated soil. The aim of this study was to investigate the potential of plant residues for enhancing the degradation of petrogenic contaminants in soil. This study will, for the first time clarify the potential of this low cost sustainable technology. In addition, molecular techniques have been used together with traditional microbiological techniques to further investigate the changes in microbial diversity in this contaminated soil.

2. Materials and methods

2.1. Experimental design

Uncontaminated soil sampled from Flinders University (South Australia) was used throughout. Collected soil (0–25 cm depth) was passed through a 4 mm sieve and used within 7 days. Particle-size analysis was carried out according to the methods described by Indorante et al. (1990); other parameters such as soil moisture, pH and organic matter content were determined using standard methods (Rayment and Higginson, 1992). The characteristics of the soil are listed in Table 1.

The experiment was conducted in a greenhouse at Flinders University with natural light conditions at 27–30 °C (during the warm season). To prepare aliphatic hydrocarbons, a mixture of diesel fuel and engine oil (60% diesel/40% engine oil) was used. This mixture was added to sieved soil to achieve a 1% (10,000 mg kg^{-1} soil) contaminant level. The soil was mixed thoroughly using a mechanical mixer. Four types of chopped plant residues were thoroughly mixed with 1 kg contaminated soil (2.5% w/w) and placed in plastic pots. The plant residues used were alfalfa hay mixed (Hm), pea straw mixed (Pm), wheat straw mixed (Wm) and various residues (Mm); containing 20% hay, 37.5% pea straw, 37.5% wheat straw and 5% gypsum. Another set of treatments involved placing the same amount of residues on the surface of the soil: alfalfa hay covered (Ht), pea straw covered (Pt), wheat straw covered (Wt) and various residues covered (Mt). This represented a layer approximately 3 cm thick on the surface of the soil. Controls including clean soil (i.e. uncontaminated) and control soil (contaminated soil without plant residues) were also included. Each treatment was replicated three times. All pots were watered based on 60–70% water holding capacity throughout the experiment. After 90 days, 100 g of each treatment soil was sampled and the following analyses were carried out.

2.2. Total petroleum hydrocarbon, microbial enumerations and fluorescein diacetate (FDA) hydrolytic activity of the soil

Total petroleum hydrocarbon (TPH) concentration in soil was measured according to the method described by the International Standard Organisation (ISO/DIS GC-method) as described by Makadia et al. (2011). Gas Chromatography was applied to determine the TPH concentrations of the soil samples. Gas Chromatography was carried