Effect of nitrogen amendment on respiration and respiratory quotient (RQ) in three hydrocarbon contaminated soils of different type

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ABSTRACT

Three soil types (sandy gravel, silty clay and sandy loam) from sites historically contaminated with total petroleum hydrocarbon (TPH) were amended with NH_4NO_3 at concentrations ranging from 16 to 2133 mg/kg $soil_{dry\ weight}$. Microbial activity was measured as O_2 consumption and CO_2 production in order to assess nitrogen limitation. Although activity was stimulated in all three soils under NH_4NO_3 amendment (after 72 h), the level of nitrogen required was soil specific. For the sandy gravel and silty clay soils, O_2 consumption and CO_2 production both showed enhanced microbial activity when amended with 16 mg/kg $soil_{dry\ weight}$ NH_4NO_3, whereas, these two parameters gave differing results for the sandy loam soil. Specifically, CO_2 production and O_2 consumption were stimulated with 66 mg/kg and 133 mg/kg $soil_{dry\ weight}$ of NH_4NO_3 respectively. In addition, respiratory quotient kinetic analysis suggested different decomposition processes occurring in this soil under different NH_4NO_3 amendment concentrations.

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1. Introduction

Ex situ bioremediation of petroleum based hydrocarbon contaminated soils is a well established remediation approach; particularly, as microorganisms capable of degrading such contaminants are often found to be present at sites (Hollender et al., 2003). However, various factors may limit the rate of degradation including a lack of essential nutrients such as nitrogen. Therefore, the addition of inorganic or organic nitrogen rich nutrients (biostimulation) is seen as an effective approach to enhance the bioremediation process (Hollender et al., 2003; Semple et al., 2006; Walworth et al., 2007) with positive effects of nitrogen amendment on microbial activity and/or petroleum hydrocarbon degradation being widely demonstrated (Liebeg and Cutright, 1999; Jørgensen et al., 2000; Margesin et al., 2000; Brook et al., 2001; Margesin and Schinner, 2001; Riffaldi et al., 2006; Margesin et al., 2007). Alternatively, amendment with nitrogen (particularly inorganic fertilisers) can have no effect, or when applied at high concentrations even deleterious effects (Bento et al., 2005; Walworth et al., 2007). In terms of deleterious effects, inorganic nitrogen fertilisers composed on nitrate and ammonium salts increase the salt concentration of soil

pore water, lowering the soil osmotic potential and inhibiting microbial activity (Walworth et al., 2007). In addition, Sarkar et al. (2005) found a drop in microbial population in fertilizer-amended soils which was suggested to be due to toxic effects resulting from fertilizer-induced acidity.

In order to assess nitrogen requirement in soils for the purpose of enhancing bioremediation, approaches that can determine the effect of nitrogen amendment on microbial activity are potentially useful. Soil respirometry is a sensitive approach to determine microbial activity in soils and, as such, is a valuable technique for bioremediation treatability testing (Hollender et al., 2003; Plaza et al., 2005). However, discrepancy exists on the most appropriate parameter to measure. For example, measuring CO_2 production provides data on mineralization and has the advantage over O_2 measurements of greater sensitivity due to its low background concentration in the atmosphere (Dilly, 2001). By contrast, O_2 is less susceptible to sources and sinks, and is preferred for in situ measurements (Miles and Doucette, 2001). In addition, oxygen consumption can also be measured in soil slurries (Aspray et al., 2007), widening the range of automated respirometers which can be used for soil bioremediation treatability testing.

Measured together, O_2 consumption and CO_2 production may provide additional information for bioremediation treatability testing. To date few studies have investigated this. Hollender et al. (2003), however, have found that measuring both O_2 consumption and CO_2 production provides information on nitrifying process and incomplete oxidation of carbon sources. In addition, Dilly (2001)

state that besides the physiology of the microbial community, the respiratory quotient (RQ), defined as the ratio of mol CO_2 evolution per mol O_2 uptake may provide information on the composition of available substrates.

Therefore, the aim of our work was to monitor microbial activity in three different historically contaminated soils following amendment with inorganic nitrogen in order to determine whether soils were nutrient (nitrogen) limited, and if so, the concentration of NH_4NO_3 required to enhance activity. Microbial activity was monitored both as O_2 consumption and CO_2 production using the Columbus automated respirometer in order to compare results obtained for both parameters. In addition, RQ and RQ kinetics were determined in order to gain insights into decomposition processes occurring in the soils.

2. Methods

2.1. Soils

Contaminated soils were collected from three sites under redevelopment following excavation and initial processing with a 60 mm grid screen. Soil 99615 was taken from a former Shipyard located west of Glasgow, contaminated with diesel and mineral range organics. Located in Paisley (West of Scotland), soil 47001C was taken from a site with mixed historical industrial activity including but not limited to use as a haulage and packing yard. Finally, soil 99214 was taken from a former plant storage and haulage yard in Fife, Central Scotland.

2.2. Physico-chemical methods

The pH of soil samples was measured with a glass electrode using 5 g soil in 25 ml of 0.01 M $CaCl_2$.

2.3. Respirometry and microcosm set-up

An Oxymax® ER10 respirometer (Columbus Instruments, Ohio, USA) connected to a PC was used to measure and record respiration in soil microcosms. Field moist soils (50 $g_{dry\ weight}$) were sieved (2 mm) and placed in 250 ml Duran bottles. The samples were amended with 2 ml of deionised water containing NH_4NO_3 at concentrations 16, 33, 66, 133, 266, 533, 1066, 2533 mg/kg soil in addition to a control sample (amended with 2 ml of deionised water only). Previous experiments had determined that 2 ml of water was either stimulatory to respiration (preferential moisture content) or, at least, did not significantly inhibit CO_2 and O_2 measurement.

The respirometer O_2 and CO_2 gas analysers were calibrated prior to each experiment. Sampling parameters for individual chambers were as per the manufacturer's default settings except the sample time which was set at 360 s. Cumulative respiration (O_2 consumed and CO_2 produced (µl)) were determined over 72 h during incubated at 30 °C ± 2 °C. Experiments were conducted in triplicate on three separate occasions.

2.4. Total petroleum hydrocarbon (TPH) extraction and analysis

TPH extraction was carried out using a modification of a method previously reported (Schwab et al., 1999). Basically, 2 g of field moist soil was placed in a 50 ml Teflon® Oak-Ridge centrifuge tube (Nalgene®) together with 2.5 g anhydrous sodium sulphate. A 10 ml aliquot of dichloromethane (Chromasolv® Plus; Sigma–Aldrich, UK) was added to the sample and shaken for 20 min on a reciprocating shaker held at maximum speed. The tubes were centrifuged at 3000 rpm for 10 min (Super Minor; MSE (UK) Ltd.).

TPH extracts were analysed on a gas chromatograph flame ionisation detector (GC-FID) (Thermo Focus; Thermo, UK). An aliquot, 1 µl of each sample was introduced into the injection port held at a temperature of 290 °C and operated in the splitless mode (splitless time of 2 min). Initial oven temperature was 40 °C for 5 min after which the oven was heated up to 300 °C at 15 °C/min and held for 20 min. The detector temperature was maintained at 300 °C. The standard used for quantification of TPH was composed of straight chain alkanes with carbon numbers ranging from C10 to C40 (Restek, Bucks, UK). A 30 m Zebron HT inferno column was used for separation which had an internal diameter of 0.25 mm and film thickness of 0.25 µm (Phenomenex, Macclesfield, UK), operated under a constant flow of 1 ml/min of helium.

2.5. Enumeration of culturable bacteria

Numbers of culturable heterotrophic bacteria were determined as colony forming units per gram of soil (cfu g^{-1}). Essentially, 1 g of soil (dry weight) was mixed with 9 ml of 0.85% NaCl and mixed by vortex for 60 s. The suspensions were diluted in decimal steps and plated on nutrient agar (NA; Oxoid Ltd., Basingstoke, UK) in triplicate and incubated for 48–72 h at 25 °C.

2.6. Statistical analysis

Cumulative O_2 consumption and CO_2 production in treatments were compared to controls using analysis of variance (ANOVA) and turkey tests with SPSS 14.0. Treatment significance was determined at probabilities of 0.01.

3. Results

3.1. Characterisation of soil samples

Physico-chemical data and bacterial counts of the soil samples are summarised in Table 1. TPH contaminant concentrations varied widely between the three soils, the highest being 47001C and lowest 99615. In soils 99214 and 47001C, TPH contamination was composed predominantly of diesel and mineral range (C22–C36) organics (Table 2), and so comparable with 99615.

3.2. Basal respiration

Cumulative oxygen consumption (µl) and carbon dioxide production (µl) were assayed in the three soils over a period of 72 h. In terms of basal respiration, soil 47001C had the highest activity for both CO_2 (73,309 ± 3985 µl) and O_2 (58,737 ± 5019 µl). Second highest was soil 99214 with carbon dioxide and oxygen consumption, 17,484 ± 1515 µl and 5865 ± 1636 µl, respectively. Finally, soil 99615 had the lowest basal respiration (6030 ± 581 µl and 4506 ± 1396 µl), respectively. The lower activity in soils 99214 and 99615 resulted in greater standard deviation for both CO_2 (8.7% and 9.6%, respectively) and O_2 (27.9% and 31%, respectively),

Table 1
Physico-chemical characterisation and bacterial counts of soil samples

Sample ID	Soil type	pH ($CaCl_2$)	Moisture content (%)	Total number of bacteria (cfu/$g_{dry\ weight}$)	TPH concentration (mg/$kg_{dry\ weight}$)
99214	Silty clay	8.0	23.1	ND	~2000
99615	Sandy gravel	6.7	17.7	2.9×10^3	242
47001C	Sandy loam	7.4	33.2	1.3×10^4	9040

ND – not determined.