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Research article

A new biostimulation approach based on the concept of remaining P for soil bioremediation

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ABSTRACT

C:N:P ratio is generally adopted to estimate the amount of nitrogen and phosphorus to be added to soils to accelerate biodegradation of organic contaminants. However, differences in P fixation among soils lead to varying amounts of available P when a specific dose of the element is applied to different soils. Thus, the application of fertilizers to achieve a previously established C:P ratio leads to biodegradation rates that can be lower than the theoretical maximum. In this study, we developed an equation to estimate the dose of P required to maximize organic contaminant biodegradation in soils as a function of remaining P (P-rem), using diesel as a model contaminant. The soils were contaminated with diesel and received six doses of P. CO_2 emission was used to estimate biodegradation of hydrocarbons. Biodegradation increased with P doses. The P level that provided the highest hydrocarbon biodegradation rate showed linear and negative correlation with P-rem. The result shows that the requirement for P decreases as the P-rem of the soil increases (or the P-fixing capacity decreases). The dose of P recommended to maximize hydrocarbon biodegradation rate in soil can be estimated by the formula P (mg/dm^3) = 436.5−5.39 × P-rem (mg/L).

1. Introduction

Biodegradation of organic contaminants in soils is stimulated by mineral nutrients (e.g. nitrogen and phosphorus), electron acceptors (generally oxygen) and the control of environmental variables such as temperature, pH, and moisture (Baptista et al., 2005).

Biostimulation is considered the most efficient method for the remediation of soils (Simpanen et al., 2016). The C:N:P ratio of 100:10:1 is generally adopted to estimate the amount of nitrogen and phosphate to be added in order to improve biodegradation (Cheng and Mulla, 1999; Roldán-Martín et al., 2007; Dandie et al., 2010). However, other ratios have been shown to be adequate for the biodegradation of organic pollutants (e.g. hydrocarbons) in soils (Ruberto et al., 2003; Leys, 2005; Zhen-Yu et al., 2008; Ramírez et al., 2009).

Variations in the optimum nutrient ratios possibly reflect differences in the behavior of nutrients in different soils. In the specific case of phosphorus, the element undergoes complex interactions with the constituents of soils (Novais and Smyth, 1999). For example, in tropical soils, P is strongly adsorbed, resulting in the fixation of the element on clay particles and its low availability (Raij, 1991; Novais and Smyth, 1999). The low availability of P, due to its strong sorption to soil constituents, can be considered one of the most important limitations to the biodegradation of organic matter in tropical soils (Cleveland et al., 2002; Orlander and Vitousek, 2005). Under these conditions, high P doses may be required to obtain an adequate concentration of the element in the soil solution to sustain microbial growth and, consequently, the biodegradation of organic contaminants.

The P sorption capacity of soils can be estimated through the remaining P (P-rem), a characteristic that depends on chemical, physical, and mineralogical properties of soils, besides organic matter content (Alvarez-Venegas et al., 2000; Santos et al., 2008). The P-rem measures the concentration of P which remains in solution after a given period of contact with the soil. This determination, proposed by Bache and Williams (1971), has been a practical solution to obtain an index of the P buffer capacity of the soil

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(Novais and Smyth, 1999). In Brazil, the determination of P-rem is made using a 10 mmol/L $CaCl_2$ solution containing 30–60 mg/L of P, which is kept in contact with the soil in a soil:solution ratio of 1:10. The concentration of 60 mg/L of P has been the most widely used. The amount of P which remains in solution depends on the combined action of the P concentration in the solution, contact time (Alvarez-Venegas et al., 2000), and the P adsorption capacity of the soil (Novais and Smyth, 1999).

The P-rem has been used to estimate the optimum doses of P to be added to sustain plant growth and production in Brazilian soils (e.g. Alvarez-Venegas et al., 1999). However, no report of the application of the P-rem concept to optimize the biodegradation of organic contaminants has been found.

The aim of this study was to develop an equation to estimate the P dose to be applied to sustain maximum biodegradation rates of organic contaminants in soils as a function of P-rem, using diesel as a model contaminant. Our hypothesis is that the dose of P required for maximum biodegradation rate is directly proportional to the P-fixing capacity of the soils.

2. Material and methods

2.1. Soil samples

Four soils were used in this study, selected based on their P fixation potential (assessed by P-rem) (Table 1). The soil with the highest P binding capacity was a Hapludox (P-rem = 11.6 mg/L); on the other side, the lowest P fixation capacity was that of a Quartzipsamment (P-rem = 52.8 mg/L).

2.2. Preparation of inoculum

An inoculum enriched in hydrocarbonoclastic microbial populations was prepared by adding diesel (50 mL/kg at 4-day intervals) to municipal solid waste compost. The diesel used in the experiment was the commercial Diesel S-500 (500 mg/Kg of S), produced and commercialized by Petrobras (Petróleo Brasileiro S.A.). Additives added to the product are not disclosed by the producer. The material was turned over daily, keeping the humidity at 60% of the maximum water holding capacity by replenishing the water lost by evaporation, estimated by frequent (every two days) weighing of the microcosms. After 20 days, the inoculum was stored at 6 °C (Leal, 2009). This inoculum was used to introduce a microbial community enriched in hydrocarbonoclastic populations in to the soils used in the bioremediation experiments. This inoculation strategy was adopted to overcome the expected low density or absence of hydrocarbon-degrading microbial populations in the soils, since we used air-dried B horizons stored for several months before use in the study.

2.3. Preparation of soil samples

After sieving through a 2.5 mm mesh sieve, the soil samples (500 g) received the inoculum (5 g/kg) and were placed into plastic bags. Then, a mixture of $CaCO_3$ and $MgCO_3$ (5:1 w/w) was added to adjust the pH to 7.0. Nitrogen (100 mg/dm^3) and potassium (120 mg/dm^3) were added as $(NH_4)_2SO_4$ and KH_2PO_4, respectively. In the treatments in which the soils did not receive P (zero P dose), K was added as KCl. After supplementation, the soils were thoroughly mixed until complete homogenization. Portions of 30 g were transferred to respirometric glass flasks (100 mL) and the humidity was adjusted to 60% of the WRC.

Six doses of P were added to each soil in the form of $NaH_2PO_4 \cdot H_2O$, discounting the amounts of P added as KH_2PO_4 (K source) (Table 1). Negative controls (non-contaminated soils) were prepared for each dose of P. These controls were used to estimate microbial activity attributed to the degradation of organic matter of the soil. P doses for each soil were relative (in %), namely, 0, 20, 40, 60, 80 and 100% the upper limit proposed by Alvarez-Venegas et al. (2000) (Table 1).

2.4. Incubation

After the application of nutrients, soil samples were incubated in the respirometric flasks at room temperature for 15 days, to allow the nutrients (in particular P) to react with the soil components and to stabilize the sorption processes. Soil moisture was maintained at 60% of the WRC. Control samples of 20 cm^3, with the application of the same P levels, were placed in plastic bags and incubated under the same conditions. After contamination with diesel (item 2.4), these samples were used to determine the P available (extracted by Melich-1) at the onset of the bioremediation experiment.

2.5. Soil contamination

After the incubation period, the soil samples were contaminated with diesel (20 mL/kg dry weight). The soil was then homogenized with a glass rod to ensure the distribution of the contaminant.

2.6. Respirometric analysis

The respirometric flasks were connected to a 55 channels respirometer equipped with an infrared gas analyzer (Sable Systems, NE, USA) with intermittent air flow (500 mL/min for 3 min in 6-min intervals), and kept immersed in a water-bath at 25 °C. The degradation of contaminants was accompanied by the emission of CO_2. The amount of CO_2 emitted by each treatment was deducted from CO_2 emitted by their respective uncontaminated controls (derived from soil organic matter biodegradation). The optimal dose of P for each soil was considered the one that resulted in the

Table 1
Chemical characteristics, water holding capacity and doses of P applied to the soils used in this study.

Soil[a]	N[b] (g/dm^3)	P (mg/dm^3)	K (mg/dm^3)	OM (g/dm^3)	WHC (mL/kg)	Doses of P (mg/dm^3)
Soil 1 (11.6)[c]	1.0	3.7	33	19.2	283	0; 95; 190; 290; 385; 480
Soil 2 (26.4)	3.2	7.7	113	60.1	296	0; 80; 165; 245; 330; 410
Soil 3 (29.4)	0.4	4.2	49	12.8	109	0; 80; 165; 245; 330; 410
Soil 4 (52.8)	1.0	22.6	121	19.2	79	0; 60; 120; 180; 240; 300

[a] Soil 1: Hapludox; soil 2: Dystrudepts; soil 3: Eutrudepts; soil 4: Quartzipsamment.
[b] N: nitrogen; P: phosphorus; K: potassium; OM: organic matter; WHC: water holding capacity, determined by the gravimetric method described by Blažka and Fischer (2014).
[c] Remaining P (mg/L) was evaluated by mixing the soil samples with 10 mM $CaCl_2$ containing 60 mg/L P (1:50 v/v). The mixture was stirred for 1 h and then the remaining P concentration was analyzed in the supernatant (Alvarez-Venegas et al., 2000).