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Biodegradation of methyl parathion in the presence of goethite: The effect of *Pseudomonas* sp. Z1 adhesion

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ABSTRACT

In this research, the influence of goethite on biodegradation kinetic of methyl parathion was investigated in the presence of *Pseudomonas* sp. Z1. Semipermeable membrane experiments were performed to demonstrate the role of adhesion of degrading bacteria to surface of goethite in biodegradation of methyl parathion. Sorption of methyl parathion and bacteria onto goethite particles were also measured to assess the distribution of methyl parathion and bacteria between water and goethite surface. The first-order degradation rate constant of methyl parathion in different concentrations of goethite was in the order of 0.1 g L^{-1} > 0.01 g L^{-1} > 0 g L^{-1} > 1 g L^{-1} > 20 g L^{-1}, suggesting the presence of low concentrations of goethite accelerated the biodegradation of methyl parathion and high concentrations of goethite inhibited this biodegradation process. According to the result of semipermeable membrane experiment, when no bacterial attachment occurred in the system, the promotive effect of 0.1 g L^{-1} goethite for microbial degradation was disappeared and the inhibition effect of 20 g L^{-1} goethite increased. The results clearly demonstrated that the adhesion of bacteria to goethite was beneficial to the biodegradation of methyl parathion. The information obtained is of fundamental significance for the understanding of microbial degradation of organic pollution in soil.

1. Introduction

The fate of organic compounds in soil is of great environmental concern due to their adverse effects on organisms, including human beings. The microbial degradation is an important pathway for organic compounds elimination in soil. The availability of organic compounds to bacteria plays a key role in biodegradation. The existence of mineral particles can greatly alter the availability of organic compounds to microorganisms because organic pollutants that enter the soil environment become associated with soil solid particles (Katayama et al., 2010). Generally, soil particles sorbed organic compounds were unavailable for biodegradation without prior desorption (White and Alexander, 1996). For example, Weissenfels et al. (1992) reported that adsorbed PAH on the soil was completely protected from degrading bacteria. However, some evidences suggested that sorbed contaminants can be degraded by microorganisms, or at least that desorption into bulk solution is not a prerequisite for biodegradation (Calvillo and Alexander, 1996). Guerin and Boyd (1992) found that initial mineralization rates of naphthalene in soil slurries consistently exceeded the rates expected if only dissolved substrate were available and concluded that sorbed naphthalene was directly available to bacteria. Furthermore, it was observed that the existence of solid particles would lead to a higher biodegradation rate because sorbed contaminants could be degraded by attached bacteria. Poeton et al. (1999) showed that phenanthrene and fluoranthene were degraded at a higher rate in tests with sediment present than in tests without sediment present. They suggested that sorbed phenanthrene and fluoranthene were accessed by bacteria by attachment to the sediment particles. Park et al. (2003) postulated that enhanced mineralization rate of atrazine in the Houghton muck soil was due to the attachment of cells to soil particles. In soil environment, bacteria are often associated with mineral particles, and these interactions have profound impacts on microbial degradation of organic compounds (Chenu et al., 2002; Alekseeva et al., 2011). However, the role of attachment of bacteria to surface of soil mineral particles in biodegradation of organic pollution is not fully understood and direct experimental evidence is needed to demonstrate the influence of bacterial adhesion on biodegradation. Methyl parathion is a widely used organophosphate pesticide. Studies on the microbial degradation of methyl parathion have been reported (Pakala et al., 2007; Pino and Penuela, 2011). A *Pseudomonas* sp. was isolated that can co-metabolically degrade

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methyl parathion (Chaudhry et al., 1988). Rani and Lalithakumari (1994) isolated *Pseudomonas putida* that could hydrolyze methyl parathion and the degradation pathway had been described.

Iron oxides widely exist in the solid phase of soil. Bacteria are tent to attach on the surface of Fe oxide minerals, because of their opposite surface charge in most environments (Collins and Stotzky, 1992; Cornell and Schwertmann, 1996). The activity of bacteria which associated with iron oxide particles can differ dramatically from planktonic bacteria. For example, siderophore-producing bacteria grew rapidly on 8.6 nm hematite because hematite particles <10 nm could be adsorbed on the surface of bacteria to offer one possible pathway for Fe acquisition to siderophore-producing bacteria (Dehner et al., 2011). Given that the interactions between microorganism and Fe oxide particles occur naturally, it is important to understand how Fe oxide particles affect the microbial degradation of organic pollutions. However, literature on this subject is scarce, and the interactions between microbial adhesion on iron oxides and biodegradation remains unclear.

In present work, the effect of goethite on the biodegradation of methyl parathion, a typical organophosphorus pesticide, was investigated. The mechanisms regarding the effects of interfacial interactions among the goethite, pesticide and degrading bacteria on biodegradation were assessed by the biodegradation kinetics, methyl parathion adsorption and bacterial adhesion on goethite surface. Furthermore, a semipermeable membrane experiment was carried out to assess the role of degrading bacteria adhesion in biodegradation.

2. Material and methods

2.1. Methyl parathion

Methyl parathion (purity >99%) was purchased from the Pesticide Research Institute in Shanghai, China.

2.2. Mineral

Goethite was synthesized according to the method outlined by Atkinson et al. (1967). Briefly, 200 mL of 2.5 M NaOH was slowly titrated into a solution containing 50 g $Fe(NO_3)_3 \cdot 9H_2O$ in 825 mL deionized water. After aging for 24 h at 60 °C, the suspension was centrifuged at 8000 rpm for 10 min. The precipitates were washed with deionized water and 95% alcohol until free of Na^+ ions, and finally dried at 110 °C for 24 h. The prepared mineral was characterized by X-ray diffraction and ground to pass a 100 -mesh sieve for subsequent use.

2.3. Bacteria and growth conditions

The bacterium (*Pseudomonas* sp. Z1) capable of using methyl parathion as the sole carbon source and energy was isolated from the soil near Shanongda Pesticides Company in Hubei, China.

P. putida was grown in 100 mL of sterile mineral salts media (MSM, 3.0 g L^{-1} K_2HPO_4, 1.5 g L^{-1} KH_2PO_4, 0.01 g L^{-1} NaCl, 0.1 g L^{-1} $MgSO_4$, 0.001 g L^{-1} $FeSO_4 \cdot 7H_2O$ and 1 g L^{-1} $(NH_4)_2SO_4$, pH 7.0–7.2) containing yeast extract (200 mg L^{-1}) and methyl parathion (100 mg L^{-1}) and cultivated at 28 °C with shaking in a rotary shaker at 180 rpm for 10 h. Cells were harvested and washed twice with sterile Phosphate Buffered Saline (PBS, pH 7.0) and suspended in sterile MSM for subsequent use. This washing was not significantly decreased the variability of the bacteria.

2.4. Adsorption of methyl parathion and P. putida on goethite

A batch of known amount of goethite and 4 mL MSM were added to flasks and dispersed by ultrasonication. Then methyl parathion stock solutions were freshly prepared with MSM and were added to obtain the final suspension with methyl parathion concentrations from 0 to 50 mg L^{-1}. The mixture was shaken at 28 °C for 3 h in the dark and centrifuged at 20,000 × *g* for 10 min, and the supernatant was filtered through a 0.45 μm membrane syringe filter. The concentration of methyl parathion in the supernatant was determined by high-performance liquid chromatograph (HPLC) with a diode array detector at 275 nm wavelength and analyses were performed on a 4.6 mm × 250 mm reverse phase C18 column using methanol-water (70:30) as a mobile phase. The flow rate was 1.2 mL min^{-1} and the injection volume was 20 μL. The amount of methyl parathion adsorption on the goethite was calculated by the amount of methyl parathion added and the remaining in the supernatant. Control experiments (with bacterial cells or without minerals) at each concentration were also conducted in order to deduct the amount of methyl parathion sorption on bacterial cells and the flasks or degradation during equilibrium process. Calibration curve for methyl parathion was obtained preparing seven level concentrations (0.5, 1, 2, 5, 10, 20, 50 mg L^{-1}).

Batch experiments were also conducted to measure *P. putida* adsorption onto goethite. A known amount of bacteria was suspended in 30 mL MSM and placed in contact with a known weight of goethite. The mixture was shaken at 28 °C for 120 min and 5 mL of sucrose solution (60% by weight) was injected. The obtained mixture was centrifuged at 4000 rpm for 15 min. The suspension of unattached bacteria in the supernatant was pipetted and determined by the method developed by Jiang et al. (2007).

2.5. Biodegradation kinetics of methyl parathion

The batch degradation assays were carried out in a series of flasks contained 4 mL MSM and different concentrations of goethite (0, 0.01, 0.1, 1 and 20 g L^{-1}). The bacteria were introduced into the system to reach a concentration of 10^6 cells mL^{-1}. A known amount of methyl parathion stock solution prepared in MSM was then added to the flasks until 8 mL to obtain an initial methyl parathion concentration of 50 mg L^{-1}. The flasks were incubated in the dark at 28 °C, 180 rpm for 10 h. Samples were taken out every hour and centrifuged. The supernatant were examined by HPLC. The pellets were extracted by methanol which can recover near 100% methyl parathion from goethite surface for 3 times of extraction and all supernatant was pooled for analysis. The flask, goethite and methyl parathion solution were presterilized by autoclaving or filtrating before use. The control experiments were carried out as described above but without bacterial inoculants.

2.6. Semipermeable membrane experiment

To study the influence of bacterial adhesion on biodegradation, a semipermeable membrane experiment was carried out using dialysis bag (MWCO, 8000–14,000) which methyl parathion and MSM can freely pass across the dialysis bag while bacteria and goethite cannot. Three treatments were designed to analyze the biodegradation rates when bacteria were not attached onto the surface of goethite (Table 1). The dialysis bags were placed in the flasks

Table 1
Semipermeable membrane experiment design and first-order rate constants of methyl parathion.

Treatment	Inside	Outside	k (h^{-1})	R^2
1	Bacteria	MSM, MP	0.035	0.98
2	Bacteria	MSM, MP, goethite 0.1 g L^{-1}	0.028	0.95
3	Bacteria	MSM, MP, goethite 20 g L^{-1}	0.018	0.99

MP: methyl parathion; k: first-order rate constant; R: correlation coefficient.