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Kinetics of microbial growth and degradation of organic substrates in subsoil as affected by an inhibitor, benzotriazole: Model based analyses of experimental results

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Abstract

Deep transport of degradable compounds through soils may occur if the metabolic activity in the soil profile is low; either by natural causes (low temperature during ice melt) or by toxic pollutants. De-icing chemicals (for roads and airports) represents a severe challenge to the soil's purifying capacity; rapid infiltration of contaminated water occurs in near-frozen soil, the contamination includes toxic compounds. Degradation experiments were conducted with toluene, and three compounds frequently used for de-icing; acetate, formate and glycol. The substrates were added to a subsoil (0.93 μmol substrate-carbon (C) g^{-1} soil, with ample amounts of nitrogen (N) and phosphorous (P)); and their mineralization was monitored in the presence of a toxic compound, benzotriazol (BTA) at various concentrations. BTA is commonly used as an additive in commercial de-icing fluids. A second and third dose of substrate was added after complete degradation of the previous one. The mineralization curves of the three consecutive doses were used to estimate kinetic parameters by fitting to a Monod-model. The model parameters estimated for each substance were the initial biomass C of the organisms growing on each substrate, C_{b0}, their maximum substrate uptake rates, V_{max}, their apparent substrate affinity, K_S, and growth yield, Y. The C_{b0} values for pristine soil were 4.9, 20.5 and 10 nmol C g^{-1} soil for formate, glycol and acetate, respectively, and 1–2 orders of magnitude lower for toluene. The K_S values were 1.1, 0.6, 2.5 and 0.13 mM for formate, glycol, acetate, and toluene, respectively. The high K_S values probably reflect diffusion limitations. The estimated yields (Y) in the absence of BTA were 0.032, 0.53 and 0.42 g biomass-C g^{-1} substrate-C for growth on formate, glycol and acetate, respectively. BTA invariably reduced the growth yield for organisms growing on the different substrates, and the yield reduction increased with increasing BTA concentrations (more than 50% reduction at 400 mg BTA l^{-1}). The degradation of the four substrates showed major differences in BTA-sensitivity, and there were strikingly weak signs (if any) of increasing BTA tolerance during growth in the presence of BTA (analyses of second and third dose experiments). The modelling of the consecutive substrate doses corroborated previous investigations of BTA effects on mineralization and community PLFA [Jia et al., 2006. Organic compounds that reach subsoil may threaten groundwater quality; effect of benzotriazole on degradation kinetics and microbial community composition. Soil Biology & Biochemistry 38, 2543–2556]. The results and the estimated Monod parameters are useful for predictive modelling of transport and degradation of pollutants as well as natural substances in sub-soils.

Keywords: Degradation; Growth; Kinetics; Model; Subsoil; Benzotriazole

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1. Introduction

Microbial degradation of soluble organic material in the vadose zone is crucial to avoid contamination of ground water aquifers, where degradable organic material may cause severe deterioration of the water quality by creating anaerobic conditions, accumulation of fermentation products and high concentrations of iron and manganese (Brown et al., 2000; Lovley and Anderson, 2000; Hansen et al., 2001; Wersin et al., 2001; Snyder et al., 2004). Organic pollutants in themselves deteriorate water quality by their bad taste/odour or toxicity (Keizer et al., 2001; Sarmah et al., 2004). The fate of organic pollutants has received much attention, and several models have been developed to analyse and predict the transport and degradation of organic compounds in the unsaturated (vadose) zone (Hunter et al., 1998; Lomander et al., 1998; French et al., 2001; Alfnes et al., 2004).

The degradation rate of organic contaminants depends on their quality as substrate for microbial growth. Repeated exposure to a contaminant which supports microbial growth will stimulate the degradation potential of the subsoil for such substances (Allen-king et al., 1994; Park et al., 2001). This implies that growth should be included explicitly in a degradation model. The need for including growth in degradation models is accentuated in all situations where the pristine soil contains few organisms which can degrade the substance in question. Surface soils contain large numbers of organisms which degrade natural substance, and for this reason, models for such systems can operate with first-order decay functions (Lundquist et al., 1999). In contrast, subsoils contain very few organisms in general, and even fewer organisms which can degrade most organic contaminants (Taylor et al., 2002). As a result, most degradation models for subsoils include growth (Alfnes et al., 2004; Mortensen and Jacobsen, 2004), even for the modelling of the degradation of naturally occurring substances (Roden and Urrutia, 1999).

Deicing fluids are potential subsoil contaminants in cold climates (Breedveld et al., 2003; Ramakrishna and Viraraghavan, 2005; Schaefer, 2006, Jia et al., 2006). Chemicals which have been used as deicers include inorganic salts and various low molecular weight organic substances. For instance, 1,2-propane diol (glycol), potassium acetate, sodium formate, and urea are widely used as runway- and aircraft de-icer/anti-icers at airports (French et al., 2001; Switzenbaum et al., 2001; Breedveld et al., 2003). As such, these compounds have a low toxicity and are readily degradable by microorganisms. Nevertheless, these compounds may penetrate deeply into the subsoil and possibly reach the aquifer, since they infiltrate rapidly during the cold season. One of the problems with commercial deicers is their additives, which may be more recalcitrant and more toxic than the main compounds (Corsi et al., 2006). One of these additives is benzotriazoles (BTA) (Cancilla et al., 1997; Breedveld et al., 2003). BTA and its derivates have been widely applied both as corrosion inhibitors in fluids and as UV light absorbers to protect polymers from photochemical deterioration (Reddy et al., 2000; Gugumus, 2002). They are applied to stabilize plastics, fibres, automotive coatings, and photographic paper (Crawford, 1999; Kuila et al., 1999), and the world production of BTA and its derivates is steadily increasing (Crawford, 1999). As a consequence, considerable amounts of benzotriazoles have leaked into the natural environment, both as direct leakage/spill from point sources and as more diffuse releases. BTA and its derivates have been found in snow, estuaries, rivers, and in groundwater (Fisher et al., 1995; Cancilla et al., 1998; Cornell et al., 2000; Reddy et al., 2000; Breedveld et al., 2003; Corsi et al., 2006). The observed toxicity of deicing chemicals has been attributed to BTA rather than the deicing agent itself (Cancilla et al., 1997; Cornell et al., 2000; Gruden et al., 2001). In addition BTA has been found to be recalcitrant to biodegradation (Rollinson and Callely, 1986; Hem et al., 2000; Tham and Kennedy, 2005; Jia et al., 2006). However, BTA is known to be light sensitive and photolytically degradable (Andreozzi et al., 1998; Hem et al., 2003). The infiltration of BTA with deicers into the subsoil may have a dual environmental effect; they represent a direct contaminant of the groundwater, and their toxicity may interfere with the degradation of other organic compounds (be it natural or man made).

On this background, we designed experiments to evaluate the effect of BTA on the degradation of a set of relevant organic pollutants found at airports. An incubation experiment with vadose soil (from 1 to 1.3 m depth) was designed, where respiration rates, microbial biomass and community composition (PLFA), were monitored as affected by substrates and different concentrations of BTA (Jia et al., 2006). In the present paper, the observed mineralization kinetics were analysed by a model of substrate uptake, growth, assimilation and respiration (mineralization). The objectives were to analyse the BTA inhibition patterns and to provide quantitative parameters for degradation rates and the inhibition of the mineralization rates of the selected compounds. Such parameters are needed in more complex transport/degradation models to predict the long-term effect on aquifers (French et al., 1999, 2001).

2. Materials and methods

2.1. Soil sampling and measurement of mineralization of first substrate dose

The soil was collected at 1–1.3 m depth from the research site Moreppen (Søvik et al., 2002) situated near Oslo International Airport, Gardermoen. A detailed description of the soil sampling and incubation strategies has been presented earlier (Jia et al., 2006). In short, four substrates; sodium formate, propylene glycol, potassium acetate (100 mg substrate-C l^{-1} soil water), and toluene (46 mg C l^{-1}) were used for the incubation experiment.