International Biodeterioration & Biodegradation 119 (2017) 626–630

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Anaerobic degradation of atrazine

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ARTICLE INFO

Article history:
Received 2 September 2016
Received in revised form
27 September 2016
Accepted 3 October 2016
Available online 10 October 2016

Keywords:
Atrazine
Biodegradation
Sulfate reducing condition
Nitrate reducing condition
Mineralization
Soil slurry reactor

ABSTRACT

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is the most frequently used herbicide in U.S. agricultural crop production. Unfortunately, the heavy use of this herbicide has contaminated soil, sediments, and aquatic ecosystems. Anaerobic degradation of atrazine was studied under sulfate and nitrate-reducing conditions using enrichment cultures developed from an atrazine contaminated soil. The soil samples were enriched using mineral salt media with either nitrate or sulfate as electron acceptors in the presence of atrazine under strict anaerobic conditions. The enriched samples were experimented with atrazine as either the sole source of carbon or nitrogen and also under co-metabolic conditions with molasses as co-substrate. The results revealed that atrazine was removed under both electron acceptor conditions. However, the atrazine degradation efficiency was significantly higher under sulfate reducing conditions than the nitrate reducing conditions. Under sulfate reducing conditions, atrazine removal was faster when molasses was used as co-substrate. The metabolic analysis showed that atrazine was mineralized and the major metabolites observed include hydroxy atrazine, cyanuric acid, chloride, and ammonium. A soil slurry reactor with atrazine contaminated soil showed more than 99% removal of atrazine within 70 days of incubation.

1. Introduction

Many xenobiotic chemicals introduced into the environment for agricultural and industrial use are nitro-substituted aromatics. Nitro groups in the aromatic ring are often implicated as the cause of the persistence and toxicity of such compounds. Nitroaromatic compounds enter soil, water, and food by several routes such as use of pesticides, plastics, pharmaceuticals, landfill dumping of industrial wastes, and the military use of explosives (Kalderis et al., 2011). Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is the most frequently used herbicide in U.S. agricultural crop production (Park et al., 2003). About 2 billion kg of chemicals are used as pesticides each year in the United States and agricultural usage accounts for about 77% (Park et al., 2003). Atrazine is a highly effective triazine herbicide that has been used extensively for the control of broadleaf weed species since it was first introduced in 1958 (Scott et al., 2009). Atrazine has also been shown to be environmentally persistent, where the half-life of atrazine in soil has been found to be between 4 and 57 weeks, and atrazine has been detected in both surface and ground waters in several countries at concentrations up to 4.6 μM (Scott et al., 2009). Atrazine and its metabolites have been found in ground and surface waters at levels exceeding the EPA's maximum contaminant level of 3 ppb (Neumann et al., 2004).

Some pesticides accumulate in nature because they exceed rates of dissipation, which includes microbial and chemical degradation (Park et al., 2003). A limited bioavailability may lead to unexpected pesticide persistence in soils, and therefore may increase the likelihood of ground or surface water contamination (Park et al., 2003). The bioavailability of pesticides and organic contaminants has been found to be a potential limitation to the full bioremediation of contaminated soils. Generally, soil-sorbed organic contaminants and pesticides have been considered unavailable for biodegradation without prior desorption. However, some evidence suggests that sorbed contaminants can be degraded by microorganisms (Park et al., 2003).

Atrazine is a relatively mobile herbicide that produces a reversible inhibition of photosynthesis. It does not bioaccumulate significantly, but it is relatively persistent and subject to abiotic and biotic breakdown. Some metabolites are phytotoxic but usually will be less than the parent compound. Residue concentrations in streams and rivers in agricultural watersheds are occasional, but there are major peaks in spring and early summer following applications of atrazine. The effects of atrazine were examined on the sexual development of African clawed frogs (Hayes et al., 2002).

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http://dx.doi.org/10.1016/j.ibiod.2016.10.002
0964-8305/

Atrazine (>0.01 ppb) was shown to induce hermaphordism and demasculized the larynges of exposed males. It was hypothesized that atrazine induced aromatase which causes the conversion of testosterone to estrogen. This disruption of steriodogenesis best explains the demasculinization of the male larynx and the production of hermaphrodites. Atrazine and other environmental factors may be a factor in the decline of amphibians worldwide (Hayes et al., 2002).

It has also been suggested that atrazine may be carcinogenic (Scott et al., 2009). Due to their broad specificity, atrazine and related triazine herbicides can also be toxic to nontarget photosynthetic species. Phytoplankton are the most sensitive organisms, followed, in decreasing order of sensitivity, by macrophytes, benthic invertebrates, zooplankton, and fish (Solomon et al., 1996). Atrazine inhibits photophosphorylation but typically does not result in lethality or permanent cell damage in the short term (Solomon et al., 1996). It has been recommended that site-specific risk assessments be conducted to assess possible ecological effects in the context of the uses to which these ecosystems are put and the effectiveness and cost–benefit aspect of any risk mitigation measures that may be applied (Solomon et al., 1996).

Ecological observations suggest that sulfate-reducing and methanogenic bacteria might metabolize nitroaromatic compounds under anaerobic conditions if appropriate electron donors and electron acceptors are present in the environment (Boopathy, 2007). Under anaerobic conditions, the sulfate-reducing bacterium, *Desulfovibrio* sp. (B strain) transformed TNT to toluene (Boopathy and Kulpa, 1992; Boopathy et al., 1993) by reduction and deamination reactions. Gorontzy et al. (1993) reported that under anaerobic conditions, methanogenic bacteria reduced nitrophenols and nitrobenzoic acids. Preuss et al. (1993) demonstrated conversion of TNT to triaminotoluene by a *Desulfovibrio* sp.

The anaerobic bacterial metabolism of nitroaromatics has not been studied as extensively as of aerobic pathways, perhaps because of the difficulty in working with anaerobic cultures and perhaps the slow growth of anaerobes. Hallas and Alexander (1983) showed successful transformation of nitrobenzene, nitrobenzoic acid, nitrotoluene, and nitroaniline by sewage sludge under anaerobic conditions. Boopathy and Kulpa (1994) isolated a methanogen, *Methanococcus* sp. from a lake sediment, which transformed TNT to 2,4-diaminonitrotoluene. This organism also transformed nitrobenzene and nitrophenol. The intermediates observed were amino derivatives of the parent compounds. According to some reports, the reductive transformation of nitroaromatic compounds leads to detoxification of the substance (Boyd et al., 1983; Battersby and Wilson, 1989). The observation of sulfate reducers and methanogenic bacteria by many workers (Boopathy et al., 1993; Boopathy and Kulpa, 1994; Boopathy, 2007; Gorontzy et al., 1993; Preuss et al., 1993) suggests that these organisms could be exploited for bioremediation under anaerobic conditions by supplying proper electron donors and electron acceptors. Recent reports on the degradation of nitro aromatic compounds indicate the usefulness of mixed bacteria in soil and nutrient conditions for maximum removal of these compounds (Markis et al., 2010; Ziganshin et al., 2010; Montgomery et al., 2011; Rylott et al., 2011; Mutter et al., 2012; Solyanikova et al., 2012).

There are very limited reports available on anaerobic degradation of atrazine compared to aerobic condition. Therefore, this study was conducted to study anaerobic degradation of atrazine under sulfate and nitrate reducing conditions using atrazine-contaminated soil. The results indicated under sulfate-reducing condition atrazine was degraded significantly by sulfate reducing bacterial consortium.

2. Materials and methods

2.1. Soil

The contaminated soil was collected from the Rebeca Sugarcane Farm in Schriever, Louisiana, USA. The atrazine concentration in the soil ranged from 40 to 325 mg/kg of soil. The soil had a total organic carbon content of 4.1%. The pH of the soil was 6.7.

2.2. Chemicals

Atrazine and cyanuric acid were obtained from Sigma Chemicals, St. Louis, USA. All other chemicals were of reagent grade.

2.3. Enrichment cultures

The atrazine contaminated soil sample collected from the sugarcane farm was enriched under anaerobic conditions using either sulfate or nitrate as electron acceptors. The anaerobic technique described by Balch and Wolfe (1976) was used throughout the study. The medium used for the enrichment consisted of the following components (mM): KH_2PO_4 (2.94), K_2HPO_4 (1.15), NH_4Cl (9.35), NaCl (10.27), $MgCl_2$ (0.5), $CaCl_2$ (0.34), molasses (0.1%) and yeast extract (0.1 g/L). For sulfate and nitrate reducing conditions 20 mM of sodium sulfate and 20 mM of potassium nitrate served as electron acceptor respectively. The culture bottle received 70 ppm of atrazine. After preparation, 100 ml medium was dispensed into bottles and made anaerobic as described by Balch and Wolfe (1976) and autoclaved. One gram of atrazine contaminated soil was added to each culture bottle and incubated for 15 days at ambient temperature (20–22 °C). A 5-ml culture was transferred to fresh corresponding medium. After five transfers, experiment was conducted on the sixth enrichment culture.

2.4. Atrazine degradation study

Atrazine degradation study was conducted under various conditions, namely, atrazine as the sole carbon source, atrazine as the sole nitrogen source, and under co-metabolism. For atrazine as the sole carbon source, the same medium described above was used without molasses and yeast extract and for atrazine as the sole nitrogen source, ammonium chloride was not added to the culture medium. Molasses at 0.1% (V/V) served as co-substrate for co-metabolic condition. All experiments were conducted in triplicates with triplicate abiotic control. Bacterial growth and atrazine concentration were monitored periodically.

2.5. Soil slurry study

To determine whether the sulfate reducing enrichment culture that is developed in the previous study could metabolize atrazine in the contaminated soil, an experiment was conducted with atrazine contaminated soil collected from the sugarcane farm mentioned above. The atrazine concentration in the soil ranged from 40 to 325 mg/kg of soil. The medium described above was prepared in 2.5 L anaerobic bottles with a working volume of 2 L. Atrazine contaminated soil (15% W/V in soil slurry) was added to the bottles with the medium. The bottles were autoclaved and a 5% inoculum of sulfate reducing enrichment culture was added. In the control bottles, the cells were heat-inactivated (autoclaved). Triplicate soil slurry reactors were maintained. Mixing was achieved at 150 rpm using a bench top shaker (Fisher Scientific, St. Louis, MO). A sample of soil slurry was analyzed periodically for atrazine concentration.