Ecotoxicology and Environmental Safety 159 (2018) 317–323

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Responses of atrazine degradation and native bacterial community in soil to *Arthrobacter* sp. strain HB-5

Jianpeng Gao[1], Peipei Song[1], Guanying Wang, Jinhua Wang, Lusheng Zhu, Jun Wang*

College of Resources and Environment, Key Laboratory of Agricultural Environment in Universities of Shandong, Shandong Agricultural University, Tai'an 271018, PR China

ARTICLE INFO

Keywords:
Atrazine
Degrading strain HB-5
Soil microbes
Biolog

ABSTRACT

The bioremediation of soil contaminated with organic pesticides is a safe and effective approach to remove pollutants from the soil. However, whether the invasion of foreign aid organisms affects the local organisms has received increasing attention in recent years. Therefore, the purpose of this study was to examine the degradation ability of atrazine by the strain HB-5 and evaluate its effects on natural bacterial communities in a miniature pot experiment. Results showed that HB-5 accelerated the degradation of atrazine and the degradation half-life of atrazine was 3.3 times less than the natural soil. Additionally, HB-5 increased the quantities of indigenous bacteria, the microbial biomass carbon and the Shannon, Simpson and McIntosh diversity indices of soil microbes in its early stage of use, But these parameters in soil treated with HB-5 decreased to values as low as those found in the control at the later stage of incubation. These suggested that the bacteria vanished as atrazine was completely removed. These results demonstrated that Arthrobacter sp. strain HB-5 had great potential and would be an effective and environmental friendly technique to remove atrazine from the contaminated soil.

1. Introduction

Atrazine, a common triazine herbicide, is a selective inhalation-type herbicide that can be used on crops both before and after the seedling stages (Smith et al., 2005). Atrazine has become one of the world's most popular herbicides because of its high efficiency, low toxicity, low cost and wide range of applications (Yan et al., 2015). However, atrazine is resistant to degradation in water and is relatively stable in soil, which may cause phytotoxicity to subsequent crops. The residual atrazine and related metabolites threaten both the stability of the ecosystem and human health (Chamberlain, 2011). Due to the extensive use and toxicity of atrazine, it is particularly important to find safe and efficient solutions to repair atrazine contamination.

Many approaches have been taken to remove atrazine from the environment, including chemical treatment, incineration, adsorption, and bioremediation (Ghosh and Philip, 2006). Compared with other treatment technologies, bioremediation is more advantageous because it can completely remove organic contaminants with low operation costs. Thus, bioremediation is considered as one of the preferred methods for atrazine removal from the environment (Wan et al., 2006). The *Nocardia* sp. strain was first isolated as an atrazine-degrading bacterial strain in 1985 (Giardi et al., 2006). Since then, intensive works have been conducted with respect to atrazine biodegradation (Mandelbaum et al., 1995; Katz et al., 2000; Hu et al., 2004; Dai et al., 2007; Li et al., 2008a, 2008b). There have been reports of the isolation and identification of many microorganisms capable of degrading atrazine, including *Pseudomonas* (Fernandes et al., 2014), *Rhodococcus* (Fazlurrahman et al., 2009), *Acinetobacter* (Singh et al., 2004), *Arthrobacter* (El Sebaï et al., 2011), *Bacillus* (Wang et al., 2014), *Agrobacterium* (Struthers et al., 1998), etc. However, the biodegradation efficiencies of these bacteria are not efficient enough in most cases. It is necessary to isolate new bacterium which has much higher atrazine removal efficiency than the common bacteria.

To optimize the atrazine removal of degrading bacteria, the specific bacteria strain has to be active when inoculated into soil. Indigenous microorganisms may impact exogenous microbial species in the soil (Boon et al., 2003; Xu et al., 2008; Wu et al., 2011). But reports of the effects of biodegradable atrazine on indigenous microbial communities have rarely been reported.

The bacterial strain *Arthrobacter* sp. HB-5, which can degrade over 95% of atrazine at concentrations of 200 mg L^{-1} within 48 h, was separated from the production wastewater and sludge from the pesticide plant by our laboratory. The objectives of this study were to (1) elucidate the efficient of degradation of atrazine using the HB-5 strain; (2)

* Corresponding author.
E-mail address: jwang@sdau.edu.cn (J. Wang).
[1] Jianpeng Gao and Peipei Song are co-first authors.

https://doi.org/10.1016/j.ecoenv.2018.05.017
Received 27 November 2017; Received in revised form 20 April 2018; Accepted 8 May 2018
Available online 21 May 2018
0147-6513/

Table 1
Basic physico-chemical properties of the tested soil.

Soil type	Black soil
pH	7.61 ± 0.11
TOC ($g\,kg^{-1}$)	17.63 ± 1.60
Available N ($mg\,kg^{-1}$)	132.31 ± 13.65
Available P ($mg\,kg^{-1}$)	18.42 ± 1.26
Available K ($mg\,kg^{-1}$)	127.50 ± 10.57
Clay (< 0.002 mm %)	10.45 ± 2.33
Sand (> 0.05 mm %)	31.96 ± 4.56
Silt (0.05–0.002 mm %)	57.72 ± 2.74
Water holding capacity (WHC)	18.56 ± 1.98

understand the microbial quantity, community functional diversity and microbial biomass responses of the native microbial community to the introduction of HB-5; and (3) clarify the degradation characteristics and ecological safety of the atrazine-degrading bacterium HB-5 to provide guidelines for the bioremediation of atrazine with HB-5.

2. Materials and methods

2.1. Materials

Soils untreated with atrazine were collected from the experimental fields of Shandong Agricultural University, Tai'an city, Shandong Province, China. The surface soil (0–20 cm) was collected, air-dried and sieved through < 0.425 mm mesh, after which it was stored at 4 °C for the subsequent use. The physical and chemical properties of the soil samples were listed in Table 1.

The HB-5 strain was isolated from the wastewater and sludge from a pesticide company. HB-5 can use atrazine as its sole carbon source. The HB-5 strain belongs to genus *Arthrobacter*, as identified by 16S rDNA technique (Liu, 2003).

Atrazine (98%) was purchased from DIMA TECHNOLOGY INC., USA. Other chemicals were of analytical grade or higher.

2.2. Experimental design

Ninety grams of soil were weighed in a 125-ml brown bottle. The treatments included natural soil (C), natural soil + atrazine (A), natural soil + degrading bacteria HB-5 (H), and natural soil + atrazine + degrading bacteria HB-5 (AH). The concentration of atrazine was $10\,mg\,kg^{-1}$ soil, and the concentration of degrading bacteria HB-5 was $4\,g\,kg^{-1}$ soil. The soil was watered to field water holding capacity. Each treatment was incubated in darkness at a controlled temperature of 25 °C. Soil samples were collected and analyzed at 0, 7, 14, 21, 28, 42, 56 and 70 days after incubation (DAI).

2.3. Analytical determination

2.3.1. Determination of atrazine in soil

The extraction and determination of atrazine in soil followed previous methods with modification (Wang et al., 2007). Briefly, 25 g of soil was shaken with 70 ml of acetone for 1 h and then vacuum filtered. This procedure was repeated twice with 30 ml of acetone. The filtrate was combined and transferred to a separatory funnel and extracted with 50 ml of 3% sodium chloride and 150 ml of $CHCl_3$. The mixture was well shaken and then allowed to stand for 30 min, and $CHCl_3$ was collected in a 250-ml flask. This procedure was repeated twice, and all the $CHCl_3$ was combined in the 250-ml flask. Ten milliliters of ligarine was then added to the flask, and the mixture was dried and brought to 10 ml with acetone.

Atrazine was determined using gas chromatography (GC, Agilent 7890B; Mcro-ECD detector; the capillary column HP-5, size: 30 m * 0.32 mm * 0.25 μm). The operation parameters included (1) injection temperature at 250 °C, (2) column temperature programed with initial temperature of 140 °C for 3 min, followed an increase of $50\,°C{\cdot}min^{-1}$ up to 260 °C, where it was held for 2 min, and (3) splitless injection at a volume of 1 μL. The GC has high accuracy and sensitivity to atrazine measurements, with recoveries between 90.35 (± 8.59%) and 93.58 (± 7.54%) at atrazine concentrations of 0.1, 1.0 and $10.0\,mg\,kg^{-1}$.

The degradation of atrazine was described by the first-order kinetics equation.

$$-dc/dt = K{\cdot}c;\ C = C_0 {\bullet} e^{-kt};$$

where C_0 is the initial concentration of atrazine, $mg{\cdot}kg^{-1}$; C is the concentration of atrazine at different incubation times, $mg{\cdot}kg^{-1}$; t is the sampling time; and k is the degradation constant. The degradation half-time was computed as the degradation rate within a known period of time.

2.3.2. Microbial measurement

The bacteria were cultured at 30 °C and separated and counted after 2 d. The fungi were cultured in Martensa medium at 28 °C and counted after 5 d. Each soil sample was tested three times, and the average number of microorganisms was calculated as the number of microorganisms. Relative colony count was computed as the difference processed in the soil microbial quantity minus the contrast in the soil microbial quantity (Sun et al., 2014).

2.3.3. The determination of soil microbial biomass carbon

Soil microbial biomass carbon was analyzed using the fumigation-extraction method (Li et al., 2008a, 2008b). Briefly, fresh soil was extracted with $0.5\,mol{\cdot}L^{-1}$ K_2SO_4 solution after fumigation for 24 h. The microbial biomass carbon was oxidized with excessive potassium dichromate and sulfuric acid, and unreacted potassium dichromate was titrated with ferrous sulfate. The microbial biomass carbon content was calculated based on the amount of potassium dichromate consumed.

2.3.4. The determination of soil microbial diversity

The Biolog microplate method is a quick and easy method for analyzing the functional diversity of microbial communities. Soil microbial diversity was measured using Biolog microplates (ECO) (Zhao et al., 2014). Ten grams of dried soil was added to 90 ml of sterilized 0.85% NaCl solution and sealed with parafilm. The suspension was shaken in darkness at 200 rpm for 1 h at room temperature and left to stand for 10 min. One milliliter of supernatant was added to 9 ml of sterilized 0.85% NaCl solution and vortexed. The solution was diluted further to yield a 10^{-3} dilution;. 150 μL 10^{-3} soil dilutions were inoculated onto Biolog microplates and placed in a thermostat incubator (25 ± 1 °C). Each treatment was repeated three times. The absorbance values of the samples were measured at 590 nm with a microplate reader at 4 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h and 168 h.

The Shannon, McIntosh and Simpson diversity indices were obtained with the following equations:

Shannon index, H': $H' = -\sum P_i \ln(P_i)$;

where P_i is the proportion of absorbance at the *i*th unit to that of the whole plate;

McIntosh index, U: $U = \sqrt{\sum n_i^2}$;

where n_i is the relative proportion of absorbance at the *i*th unit to that of the whole plate; and

Simpson index, $1/D$: $D = \sum \frac{n_i(n_i-1)}{N(N-1)}$;

where n_i is the ratio of absorbance of the *i*th plate and N is the total absorbance values of the whole plate.