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Short Communication

Effect of acclimation and nutrient supply on 5-tolyltriazole biodegradation with activated sludge communities

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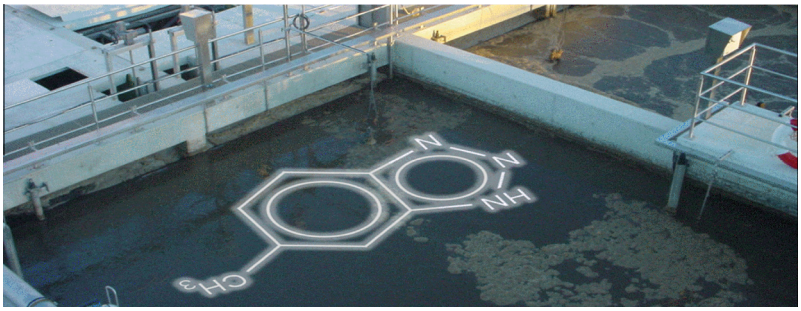
HIGHLIGHTS

- 5-Tolyltriazole biodegradation significantly improved by biomass acclimation.
- Biodegradation enhancement by non-specific sludge supernatant addition.
- Nitrogen addition also increased biodegradation rate.
- Degradation presumably starts by benzene ring cleavage serving as a carbon source.
- Heterocyclic N-containing ring is assumed not to be cleaved necessitating N-supply.

GRAPHICAL ABSTRACT



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ABSTRACT

The corrosion inhibitor 5-tolyltriazole (5-TTri) can have a detrimental impact on aquatic systems thus implying an acute need to reduce the effluent concentrations of 5-TTri. In this study, 5-TTri biodegradation was enhanced through acclimation and nutrient supply. Activated sludge communities (ASC) were setup in nine subsequent ASC generations. While generation two showed a lag phase of five days without biodegradation, generations four to nine utilized 5-TTri right after inoculation, with biodegradation rates from 3.3 to 5.2 mg L^{-1} d^{-1}. Additionally, centrifuged AS supernatant was used to simulate the nutrient conditions in wastewater. This sludge supernatant (SS) significantly enhanced biodegradation, resulting in removal rates ranging from 3.2 to 5.0 mg L^{-1} d^{-1} without acclimation while the control groups without SS observed lower rates of $\leqslant$2.2 mg L^{-1} d^{-1}.

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1. Introduction

The xenobiotic compound 5-tolyltriazole (5-TTri) is a commonly used corrosion inhibitor. 5-TTri, in combination with 4-TTri, is commercially available as tolyltriazole (TTri) and widely used in metal finishing and cooling systems (Verheyen et al., 2009), as anticorrosive in aircraft deicing and breaking fluids (Gruden et al., 2001) and in household dishwashing detergents (Vetter and Lorenz, 2013). Its high production volume and widespread usage, high polarity (logD 1.89), good water solubility and limited biodegradability, makes 5-TTri almost omnipresent in aquatic compartments (Liu et al., 2013). In river systems 5-TTri was detected up to 5.41 μg L^{-1} (Herzog et al., 2013).

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Chronic effects of 5-TTri occurred already at concentrations of 0.40 mg L^{-1} and showed adverse effects in the aquatic organism *Daphnia galeata* (Seeland et al., 2012). Therefore, 5-TTri is regarded as potentially hazardous for aquatic systems implying the need to optimize the removal efficiency of this compound. Especially wastewater treatment plants (WWTP) that are, besides other small diffuse entry paths from road runoffs, the major point source for 5-TTri into the aquatic environment (Asimakopoulos et al., 2013) are often incapable of completely removing 5-TTri during treatment (Stasinakis et al., 2013). Various laboratory biodegradation experiments with 5-TTri are described and showed that 5-TTri is biodegradable to a certain extent by microbial communities from activated sludge (Herzog et al., 2014b). However, these studies never aimed on acclimation or optimal nutrient availability to improve the activated sludge community (ASC). Information on how to enhance 5-TTri removal regarding biomass acclimation and nutrient composition is thus implicitly required and might help to improve the biodegradation of other benzotriazoles.

Therefore, this study evaluates efficient conditions to improve aerobic 5-TTri biodegradation by (A) acclimation of the AS communities to optimize 5-TTri biodegradation and (B) evaluating the effect of nutrient availability. A sludge supernatant, derived from activated sludge to simulate wastewater nutrient conditions, was applied as well as nitrogen containing compounds.

2. Methods

2.1. Chemicals

5-Tolyltriazole (5-TTri; CAS 136-85-6) was purchased from Sigma–Aldrich (Steinheim, Germany). All other media components were obtained from Merck KGaA (Darmstadt, Germany).

2.2. Experimental setup for biodegradation

Biodegradation was tested in different setups and three media being R2A-UV, MSM-SS and MSM (Table 1). All setups were supplied with 20 mg L^{-1} 5-TTri to ensure a fast acclimation and prepared in 100 mL glass bottles, filled with 20 mL media and covered with air-permeable aluminum caps. Shaking at 150 rpm ensured aerobic conditions. All experiments were operated in batch mode (no additional nutrients), and run for a maximum of 10 days in the dark to avoid photolysis. Temperature was kept constant at 20 °C (±2 °C); the pH was controlled to be around 7.5. Sterile setups (media with twice autoclaved AS) and abiotic setups (media without biomass) as controls were treated in the same manner as the biodegradation experiments.

2.3. Activated sludge inoculum

500 mL original activated sludge (AS) was obtained from stage 1 (high load stage) of a 2-stage municipal conventional activated sludge plant (CAS-M) (Herzog et al., 2014b), stored at 4 °C and was used within 24 h for reactor inoculation.

AS biodegradation potential was pre-evaluated in 150 mL R2A-UV media spiked with 20 mg L^{-1} 5-TTri and inoculated to a total biomass concentration of 3 g L^{-1} mixed liquor suspended solids. After biodegradation occurred, the experiment was stopped and 1.0 mL of the remaining biomass was used to inoculate subsequent acclimation and nitrogen supply experiments.

2.4. Acclimation procedure

Acclimation to 5-TTri was achieved in MSM over generations one to nine in the following manner: The first generation was inoculated with 1.0 mL pre-evaluated, acclimated AS and kept running until biodegradation occurred (after 15 days). The experiment was stopped and 1.0 mL of the reactor suspended biomass was used to inoculate a subsequent experiment termed generation two. After observing biodegradation this experiment was also stopped and used to prepare the third generation in the same manner as the second generation. These setups were repeated until generation nine, obtaining a highly selected biomass for 5-TTri biodegradation.

Biomass from all generations was harvested by centrifugation (20 min, 10,000*g*, 4 °C) and 1.0 mL of the re-suspended pellet (PBS) used to inoculate the sludge supernatant experiments.

2.5. Nutrient supply

2.5.1. Sludge supernatant

A supplement derived from AS was prepared by autoclaving (20 min, 121 °C) AS twice followed by centrifugation (10 min, 10,000*g*) and filtration of the supernatant at 0.45 μm to remove all solid particles. The obtained sludge supernatant (SS) was added to a total amount of 10% (v/v) to MSM media (Table 1) producing MSM-SS. These setups were inoculated with 1.0 mL reactor suspended solids obtained from generations 2 to 6 from the acclimation experiment.

2.5.2. Nitrogen

Three different nitrogen species, NH_4NO_3, $NaNO_3$ and NH_4Cl, were added to MSM media. Total N-concentrations of 25, 50, 100, 250 and 500 mg L^{-1} were used and supplied together with 20 mg L^{-1} 5-TTri.

2.6. Detection of biodegradation

After 30 min sedimentation, 200 μL supernatant was taken from the setups and used for UV-absorbance measurements (UV-AM) as described elsewhere (Herzog et al., 2014a) with the following changes applied: calibration was done with 1, 5, 10, 15 and 20 mg L^{-1} 5-TTri in high-purity water and the used media to evaluate measurement reliability and background absorbance.

Table 1
Media compositions, carbon–nitrogen ratio, nutrient applications and experimental setups.

Media	Components [g L^{-1}]	DOC:N ratio (DOC:N [mg L^{-1}])	Application	Experiment
R2A-UV (R2A media for UV-AM)	Casein peptone (1.0), glucose (0.5), potassium phosphate (0.3), soluble starch (0.3)	7:1 (880:120)	Optimal growth conditions, non-selective	Pre-evaluation
MSM (minimal salt media)	As MSM-CN, without sodium acetate and NH_4NO_3	–	Selection of bacteria, utilization of 5-TTri as sole C and N source	Acclimation and specific nitrogen supply
MSM-SS	MSM media supplied with 10% sludge supernatant	2.7:1 (162:62)	Simulation of wastewater conditions	Supply of sludge supernatant

Hoagland trace elements (0.1 mL L^{-1}) were added to all media.
pH was adjusted to 7.4 in all media.

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