Ecotoxicology and Environmental Safety 156 (2018) 48–55

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Biodegradation of four selected parabens with aerobic activated sludge and their transesterification product

Jing Lu, Haipu Li*, Yi Tu, Zhaoguang Yang*

Center for Environment and Water Resources, College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, PR China

ARTICLE INFO

Keywords:
Parabens
Activated sludge
Adsorption
Biodegradation
Transesterification

ABSTRACT

Parabens are preservatives widely used in foodstuffs, cosmetics and pharmaceuticals, which have led to elevated paraben concentrations in wastewater and receiving waters. Laboratory-scale batch experiments were conducted to investigate the adsorption and degradation of parabens in an aerobic activated sludge system. Results show that biodegradation plays a key role in removing parabens from the aerobic system of wastewater treatment plants, while adsorption on the sludge is not significant. The effects of parent paraben concentration, concentration of mixed liquor suspended solids (MLSS), initial pH and temperature on degradation were investigated using kinetic models. The data shows that the degradation of parabens could be described by the first-order kinetic model with the rate constant ranging from 0.10 to 0.88 h^{-1} at 25 °C and pH 7.0. Paraben degradation can be enhanced by increasing the MLSS concentration and temperature, or by decreasing the parent paraben concentration. Furthermore, the pH of the incubation system should be lower than 8.0. The half-lives of the parabens were estimated to range between 0.79 and 6.9 h, with methylparaben exhibiting the slowest degradation rate. During degradation in the present system, transesterification occurred, with methylparaben being the major transformation product in the incubation systems of ethylparaben, propylparaben and butylparaben. These results were confirmed by mass spectrometry and aliphatic alcohol additive experiments. This is the first discovery of paraben transesterification in an activated sludge system, and it is associated with trace methanol in the system.

1. Introduction

The most commonly used synthetic preservatives in food, cosmetics and pharmaceuticals are parabens, which are a family of compounds derived from *p*-hydroxybenzoic acid that possess different alkyl ester chains, such as methyl-(MeP), ethyl-(EtP), propyl-(PrP), butyl-(BuP) and benzyl-parabens (Díaz-Cruz and Barceló, 2015; Haman et al., 2015). Human exposure to parabens has been associated with adverse health outcomes, such as endocrine dyscrasia, immune dysfunction, and developmental and behavioral disorders (Kang et al., 2013; Karpuzoglu et al., 2013; Li et al., 2016). Moreover, conventional toxicity tests show that parabens are toxic to algae, invertebrates and fish (Yamamoto et al., 2011). The effluent from wastewater treatment plants (WWTPs) and untreated wastewater have been deemed as the main two sources of parabens in environment (Kasprzyk-Hordern et al., 2008; Kimura et al., 2014; Ramaswamy et al., 2011). Wang and Kannan (2016) found MeP, EtP, PrP and BuP could be detected at 0.1–1.2 ng/L in effluent from New York WWTPs, and even after an advanced process of ultrafiltration or ozone oxidation, MeP, EtP, PrP and BuP were still detected at 6.8, 0.3, 0.4 and 0.1 ng/L, respectively. Thus, to reduce its potential environmental impact, the transformation and removal of parabens in WWTPs have received much attention.

Generally, volatilization is not considered to be an important pathway for paraben removal in WWTPs due to their low volatility. Biodegradation and adsorption by activated sludge are two possible processes responsible for the fate of parabens (Haman et al., 2015; Liu and Wong, 2013). Li et al. (2015) investigated the occurrence and fate of parabens in an advanced WWTP and observed that they were mainly degraded in the anaerobic tank. Wu et al. (2017) found that biodegradation primarily occurred under aerobic conditions when they compared the biodegradation efficiency of MeP and PrP in activated sludge under aerobic and anaerobic conditions. In addition, Song et al. (2017) found that adsorption was the dominant removal mechanism of PrP in greywater during the first 30 h before biodegradation by aerobic attached-growth biomass. Furthermore, the group suggested that a trace amount of methanol may accelerate PrP biodegradation (Song et al., 2017). Gonzalez-Marino et al. (2011) reported that MeP and EtP was degraded faster than PrP and BuP when using activated sludge as

* Corresponding authors.
E-mail addresses: lihaipu@csu.edu.cn (H. Li), zgyang3@gmail.com (Z. Yang).

https://doi.org/10.1016/j.ecoenv.2018.02.078
Received 13 December 2017; Received in revised form 27 February 2018; Accepted 28 February 2018

an inoculum, while the opposite was observed when using raw wastewater. This disparity was associated with the different bacterial and enzymatic composition between the activated sludge and raw wastewater. Close and Neilsen (1976) found that MeP can repress propylparaben esterase, and that PrP can be used as a sole carbon source for *Pseudomonas cepacia*, indicating that the latter was more easily degraded. In these studies, microorganisms were isolated from different materials, such as non-sterile parabens solutions; an oil-in-water emulsion containing parabens as preservative additives (Amin et al., 2010; Close and Neilson, 1976); composite raw wastewater; activated sludge from WWTPs (Gonzalez-Marino et al., 2011); and a pilot scale biofilter for greywater treatment (Song et al., 2017). Therefore, we can reasonably speculate that the behavior of parabens significantly depends on the specific processing conditions, such as the inoculum, biodegradation processes, structure of parent compounds and operating parameters. In-depth investigations are required to elucidate the biodegradation and transformation mechanisms of parabens in WWTPs.

In the present study, laboratory-scale batch experiments were conducted to evaluate the role of adsorption and degradation in paraben removal using fresh aerobic activated sludge from a WWTP. The effects of mixed liquor suspended solid (MLSS) concentration, parent concentration, temperature and initial pH on the degradations of parabens within the mg/L range were investigated to provide quantitative information on paraben behavior in an activated sludge system. Finally, the transformation products and behaviors were investigated. Different paraben structures (MeP, EtP, PrP and BuP) were selected as the targeted compounds. Surprisingly, the phenomenon of paraben transesterification was found in the activated sludge from the WWTP; thus, the interaction between transesterification and alcohol was investigated. This work will provide important information for understanding the fate and transformation of parabens in aerobic activated sludge treatment systems.

2. Materials and methods

2.1. Chemicals

Analytical standards of MeP (99%), EtP (99%), PrP (99%) and BuP (99%) were purchased from Macklin (Shanghai, China). Methanol (HPLC grade) was obtained from Honeywell (Morristown, USA). Ethanol, sodium hydroxide (NaOH), hydrochloric acid (HCl) and sodium azide (NaN_3) were obtained from Hushi (Shanghai, China). The other mineral salts were of analytical reagent. Ultra-pure water was obtained from the Beijing Purkinje system (Beijing, China).

Stock solutions (1000 mg/L) of individual standards were prepared in methanol and then stored at − 18 °C in the dark. According to their aqueous solubility (Table S1), paraben stock solutions (100 mg/L), used to investigate transesterification products, were prepared in water and stored at 4 °C.

2.2. Biodegradation experiments

Fresh activated sludge was obtained from a municipal WWTP (Changsha, China). The MLSS of the fresh activated sludge was 11,000 ± 200 mg/L. Incubation solutions with 550–5500 mg/L of inoculum were prepared by centrifugation (2654 ×g, 3 min; Eppendorf Centrifuge 5424 R, Germany) using a specific volume of fresh activated sludge. The supernatant was discarded and then 100 mL mineral salts media (MSM) was added to the pellet to achieve the desired concentration. The MSM consisted of KCl (1.3 g/L), KH_2PO_4 (0.2 g/L), NaCl (1.2 g/L), NH_4Cl (0.5 g/L), $CaCl_2$ (0.08 g/L), $MnCl_2{\cdot}4H_2O$ (2.4 g/L) and $NaHCO_3$ (2.8 g/L) (Liu et al., 2011). The pH of the incubation solution was adjusted with 0.1 M HCl or NaOH solutions (Zeng et al., 2009).

Biodegradation of MeP, EtP, PrP and BuP was evaluated individually under designed conditions. A series of batch experiments were carried out in reactors made of sterilized 250-mL Erlenmeyer flasks containing 100 mL MSM and a desired concentration of activated sludge. The test compound was spiked into the incubation media by pipetting the desired volume of stock solution. The degradation kinetics of the parabens, as well as the effects of MLSS, initial pH and temperature on degradation was investigated. Unless otherwise noted, experiments were conducted under the following conditions: initial concentration of parabens 1.0 mg/L, temperature 25 °C, pH 7.0 and MLSS 2200 mg/L. The reactors were completely covered with aluminum foil to prevent photodegradation and plugged with sterile breathable membranes. The batch experiments were kept under aerobic conditions by shaking the reactors at 150 rpm to ensure a dissolved oxygen concentration greater than 3 mg/L (Tran et al., 2015). After static sedimentation for 2 min, 1.0 mL samples were collected from each reactor at time points 0.25, 0.5, 1, 2, 3, 4, 5, 8, 10, 18, and 24 h. Control reactors containing parabens without sludge were carried out to account for the adsorption of parabens onto the flask surface. The background concentration in the sludge ranged from 0.0048 to 0.010 mg/kg (the method is shown in SI-Text-1).

Biodegradation kinetics was based on the first-order equation, as follows:

$$ln(C_t) = ln(C_0 e^{-kt})$$

Where, C_0 is the initial concentration of the parabens; C_t is the concentration of the parabens at time t; and k is the first order rate constant. Using this equation, the half-life, $t_{1/2}$, can be calculated as $(ln\,2)/k$.

To evaluate the adsorption of the target parabens onto activated sludge, a series of batch experiments were performed with heat-inactivated activated sludge biomass under the same experimental conditions as the biodegradation tests with the maximum sludge concentration (5500 mg/L). The inactivation of the activated sludge was carried out at 121 °C for 20 min (Shen'an LDZX-50KB, Shanghai, China), followed by the addition of the metabolic inhibitor NaN_3 (1 g/L) to maintain sterility (Liu et al., 2011).

2.3. Analytical methods

Samples (1.0 mL) were centrifuged at 15,643 ×*g* for 15 min, after which 0.5 mL of supernatant was transferred to an amber vial for HPLC analysis. Chromatographic separation was performed on an Agilent 1260 HPLC system (Agilent Technologies, Palo Alto, USA) with an autoinjector and a UV detector at a wavelength of 254 nm. An AlltimaC18 column (250 × 4.6 mm, 5 μm, GRACE, USA) with a pre-column (AlltimaC18, 7.5 × 4.6 mm, 5 μm) was used to separate the analytes. Agilent Chemstation Software (OpenLab Control Panel 1260) was used to acquire data. An isocratic eluent was composed of methanol and water, with a methanol: H_2O (v/v) ratio of 70:30 for MeP and EtP; 75:25 for PrP; and 80:20 for BuP. The injection volume was 25 μL and the flow rate was 1.3 mL/min at ambient temperature. The limit of detection (LOD) of the HPLC method for MeP, EtP, PrP and BuP was 1.3, 0.56, 0.45 and 0.73 μg/L, respectively. Recovery (%) of the parabens in the experimental activated sludge was calculated from data obtained by analysis of three replicates of the selected parabens at 1.0 mg/L. The recoveries of the spiked MeP, EtP, PrP and BuP were 95.9%, 83.5%, 101.9% and 94.5%, respectively. Additionally, the samples were used to determine the transesterification products of parabens by performing high performance liquid chromatography electrospray triple-quadrupole mass spectrometry (HPLC-MS/MS) and gas chromatography mass spectrometry (GC-MS) analysis (analytical methods are shown in SI-Text-2, 3).

3. Results and discussion

3.1. Adsorption of parabens on sludge

After the activated sludge was heat-inactivated, the removal was