Journal of Hazardous Materials 344 (2018) 200–209

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Research Paper

Biodegradation of triclosan in diatom *Navicula* sp.: Kinetics, transformation products, toxicity evaluation and the effects of pH and potassium permanganate

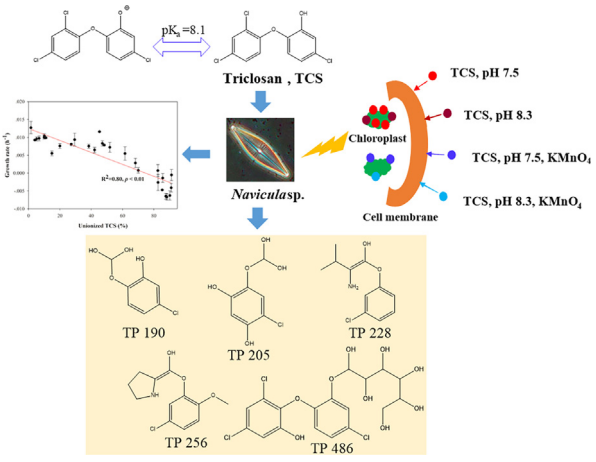
Tengda Ding[a,b], Kunde Lin[b], Mengting Yang[a], Lianjun Bao[c], Juying Li[a,*], Bo Yang[a], Jay Gan[d]

[a] *Shenzhen Key Laboratory of Environmental Chemistry and Ecological Remediation, College of Chemistry and Environmental Engineering, Shenzhen University, Shenzhen 518060, China*
[b] *State Key Laboratory of Marine Environmental Science, Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystem, College of the Environment and Ecology, Xiamen University, Xiamen 361005, China*
[c] *School of Environment, Guangzhou Key Laboratory of Environmental Exposure and Health, and Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, China*
[d] *Department of Environmental Sciences, University of California, Riverside, CA 92521, United States*

HIGHLIGHTS

- Triclosan posed high toxicity to *Navicula* sp.
- The pH had a significant influence on bioaccumulation and toxicity of triclosan.
- Higher unionized triclosan at pH 7.5 contributed to its higher toxicity.
- Presence of $KMnO_4$ reduced bioaccumulation and toxicity of triclosan in *Navicula* sp.
- Transformation pathways of triclosan are proposed based on 7 identified metabolites.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 June 2017
Received in revised form
11 September 2017
Accepted 20 September 2017
Available online 21 September 2017

Keywords:
Triclosan
Biodegradation

ABSTRACT

Triclosan (TCS) is one of the most widely used pharmaceutically active compounds and frequently detected in treated wastewater and the impacted aquatic environment. However, the fate and toxicity of TCS in aquatic organisms is poorly known, including in particular the potential for the formation of incomplete biological transformation products. In this study, TCS posed high toxic effects (e.g., growth inhibition and damage of photosynthesis) to typical freshwater diatom *Navicula* sp., with the 24 h and 72 h EC_{50} values of 173.3 and 145.6 $\mu g\,L^{-1}$, respectively. The bioaccumulation of TCS in diatom cells increased with the increasing exposure to TCS and showed to be time-dependent. The higher intracellular TCS lead to higher toxicity on *Navicula* sp. The intracellular TCS concentration and the growth inhibition of TCS in *Navicula* sp. at pH 7.5 was obviously higher than that at pH 8.3, which was likely due to the higher

* Corresponding author.
E-mail address: jyli@szu.edu.cn (J. Li).

http://dx.doi.org/10.1016/j.jhazmat.2017.09.033

Toxicity
pH and $KMnO_4$
Navicula sp.

abundance of unionized TCS in the culture. $KMnO_4$ reduced both bioaccumulation and toxicity of TCS in *Navicula* sp., especially at pH 8.3. A total of seven products were detected by liquid chromatography-tandem mass spectrometry (LC–MS/MS). These findings will provide a reference for the risk assessment of TCS in aquatic environment.

1. Introduction

The occurrence and fate of pharmaceutically active compounds (PhACs) in the aquatic environment was paid special attention in the last few decades due to their potential undesirable ecological and human health effects. Triclosan (5-chloro-2-(2, 4-dichlorophenoxy)-phenol, TCS) is widely used in medical and personal care products because of its high antimicrobial effectiveness. For example, up to 1000 tons of TCS was produced in Europe per year [1]. The widespread use of TCS over the last 40 years results in a massive discharge to wastewater treatment plants (WWTPs) and then in surface waters [2,3]. TCS was widely detected in wastewater (8.05 $\mu g\,L^{-1}$) [4], sludge (1965 $\mu g\,kg^{-1}$) [5], river (0.282 $\mu g\,L^{-1}$), groundwater (0.03 $\mu g\,L^{-1}$) [6] and sediments (41.7 $\mu g\,kg^{-1}$) [7]. TCS possesses a relatively high octanol-water partition coefficient ($\log K_{ow}$ of 4.8) [8,9], which can lead to its bioaccumulation in biota and biomagnification via food chain, ultimately threatening the safety of organisms. TCS was found to be highly toxic to *Daphnia magna*, fish (zebrafish, fathead minnows, bluegill sunfish) and green algae [10,11]. For example, Dann and Hontela [12] found that TCS showed toxic effects to green algae *Selenastrum capricornutum*, *Scenedesmus subspicatus*, and *Anabaena flosaquae* with EC_{50} values ranging from 1.4 to 4.7 $\mu g\,L^{-1}$. The bioaccumulation factors of TCS in *Cladophora* sp. was as high as 2100 [13]. At the base of the trophic food chain, algae such as diatoms represent a source of food for numerous organisms, and these microalgae are particularly relevant or seriously affected by exposure of xenobiotic pollutants in aquatic ecosystems [14]. *Navicula* sp. is one of the most common and occurring diatoms in freshwater and is often used to predict the toxicity and the bioavailability of xenobiotics in aquatic environments [15]. For example, Magnusson et al. [16] found that the growth of *Navicula* sp. was significantly inhibited by the herbicides (e.g., diuron, tebuthiuron, atrazine, simazine, and hexazinone) with the EC_{50} ranging from 2.6 to 157 $\mu g\,L^{-1}$. In addition, once released into the aquatic environment, transformations of TCS may occur, producing metabolites with different environmental behavior and ecotoxicological profile. For instance, Tohidi and Cai [17] reported that over 60% of TCS was biotransformed during the wastewater treatments, and three toxic/persistent metabolites (i.e., methyltriclosan, 2, 4-dichlorophenol, and 2, 8-dichlorodibenzoparadioxin) were found. Therefore, a simple exposure analysis of TCS on algae is not sufficient, the fate (e.g., biotransformation and its transformation products) of triclosan in the environment is required.

TCS is a chlorinated phenoxyphenol with a pK_a value of 8.1 [18]. The pH in surface waters (common range from 7 to 9) can thus influence on the speciation and the following toxicity and fate of TCS in aquatic organisms. Rowett et al. [19] indicated that TCS with neutral species are more toxic than the corresponding anionic TCS in crustacean *Gammarus pulex*. Lipnick [20] pointed out that the un-ionized species of TCS were more permeable than its ionized species to lipid membranes. Additionally, potassium permanganate ($KMnO_4$) is commonly used in wastewater treatments with high removal efficiency, comparative stability, relatively low cost, and ease of operation [21,22], resulting in its distribution in natural waters and potential toxic effects on non-target organisms. However, little information is available on the effects of pH and $KMnO_4$ on the toxicity and fate of TCS in algae.

The present study is to determine the toxicity and fate of TCS in a typical freshwater diatom *Navicula* sp. Transformation products are identified by liquid chromatography-mass spectrometric analysis, and the degradation pathways of TCS are proposed. Specific attention was also given to characterizing the influence of pH and $KMnO_4$ on toxicity and fate of TCS.

2. Materials and methods

2.1. Chemicals

Triclosan was purchased from Sigma-Aldrich (China). HPLC-grade methanol were obtained from Fisher Scientific (China). The hydrochloric acid (HCl), sodium hydroxide (NaOH) and potassium permanganate ($KMnO_4$) were purchased from the Sinopharm Chemical Reagent Co. Ltd. (China). A stock solution of TCS (100 $mg\,L^{-1}$) was prepared by mixing the TCS in methanol. A 10 $mg\,L^{-1}$ stock solution of $KMnO_4$ was prepared. The initial pH was adjusted by 0.1 M HCl or 0.1 M NaOH. All chemicals used in this study were of analytical or HPLC grade.

2.2. Toxicity assay

The diatom *Navicula* sp. cultures were inoculated into 500 mL sterile D1 medium. The constituents in D1 medium and cultivation procedure were described in our previous study [23]. Algal bioassays were conducted with the addition of 0, 10, 50, 100, 200, 400 and 800 $\mu g\,L^{-1}$ TCS for 72 h in triplicates. To explore the main environmental factors on toxicity of TCS, two pH values (7.5 and 8.3) were adjusted in the culture, and the toxicity of 400 $\mu g\,L^{-1}$ TCS with different $KMnO_4$ addition (0, 0.5, 1, 2, and 4 $mg\,L^{-1}$) was also investigated. The pH values commonly range from 7 to 9 in surface waters, two initial pH values (7.5 and 8.3) were demonstrated to be favorable conditions to culture freshwater algae *Navicula* sp. In addition, given that TCS has a pK_a value of 8.1, the pH of 7.5 and 8.3 were consequently selected for better elucidating the influence of the dissociated or pronated status of TCS on toxicity and fate of TCS in *Navicula* sp. Batch experiments were conducted in 100 mL Erlenmeyer flasks containing 50 mL D1 medium. The optical densities of the algal suspensions were determined to indicate the algal density at 680 nm in a UV-2550 spectrophotometer (Shimadzu, Japan). The cellular growth rates (d^{-1}) were calculated by fitting the cell numbers to an exponential function and the chlorophyll content of *Navicula* sp. was analyzed using hot methanol extraction as reported in our previous work [23].

2.3. TCS uptake in diatom cultures

The uptake of TCS by *Navicula* sp. was investigated in 100 mL Erlenmeyer flasks containing 50 mL sterilized D1 mediums amended with different concentrations of TCS (50, 200 and 800 $\mu g\,L^{-1}$). The pH values usually range from 7 to 9 in surface waters, therefore, the influence of two pH values (7.5 and 8.3) was investigated. Different addition of $KMnO_4$ (0.5 and 2 $mg\,L^{-1}$)