17th International Conference on Environmental Science and Technology

Athens, Greece, 1 to 4 September 2021

Study on the fate of the chemical uncoupler 3,3′,4′,5-tetrachlorosalicylanilide (TCSA) in activated sludge process and investigation of its effects to different aquatic organisms

Gatidou G.[1,*], Hatzopoulos P.[1], Chhetri R.K.[2], Kokkoli A.[2], Giannakopoulos[2], Arvaniti O.S.[1], Andersen H.R.[2] and Stasinakis A.S.[1]

[1]Water and Air Quality Laboratory, Department of Environment, University of the Aegean, Mytilene 81100, Greece
[2]Department of Environmental Engineering, Technical University of Denmark, Miljøvej 115, Kgs. Lyngby, 2800, Denmark

*corresponding author: G. Gatidou
e-mail: ggatid@env.aegean.gr

Abstract
This study examines the biodegradation of 3,3′,4′,5-tetrachlorosalicylanilide (TSCA) by activated sludge and investigates its effects to different aquatic organisms. According to the experimental results, the fate of the target compound in activated sludge systems is governed by the mechanisms of sorption and biodegradation, while almost 90% of TCSA is expected to be removed in an aerobic activated sludge system operating with hydraulic residence time of 10 h. Ecotoxicity experiments shown that TSCA toxicity decreased from Daphnia > Vibrio > Lemna, while an ecological threat is possible in rivers where treated wastewater is diluted up to 100-fold.

Keywords: TSCA; biodegradation; activated sludge; effects; EC50; bioassays

1. Introduction

TCSA (3,3′,4′,5-tetrachlorosalicylanilide) has been widely used as metabolic uncoupler in activated sludge systems aiming to the reduction of the produced sludge (Ferrer-Polonio et al., 2017). Additionally, it is used as preservative and as bacteriostat in several products (O'Neil, 2006). Beside the wide use of this compound, so far, no data exists for its fate during activated sludge process as well as for its ecotoxicity in the aquatic environment. Previous articles have solely focused to its effects on COD removal and nitrification in activated sludge systems (Ferrer-Polonio et al., 2019).

The main objective of this study was to examine the effects of TCSA to different aquatic organisms (*Daphna magna*, *Vibrio fischeri*, *Lemna minor*) and to investigate the ability of activated sludge to biodegrade this compound. A mass balance model was used to predict its concentration in treated wastewater while a preliminary risk assessment was applied using Risk Quotient methodology to examine the possible ecological threat for the aquatic environment.

2. Materials and Methods

2.1. Experimental protocols

The effects of TSCA to *Daphna magna* were studied applying the immobilization test described by ISO (2012). OECD Guideline 221 was applied for the investigation of TSCA toxicity to *Lemna minor* (OECD, 2006), while the toxicity test with the photobacterium *Vibrio fischeri* conducted according to ISO 11348-3 (2007). Biodegradation and sorption of target compound was studied in aerobic batch experiments according to the protocol described by Mazioti et al. (2015).

2.2. Analysis

Analysis of TSCA in the dissolved and particulate phase was based on previously developed method (Samaras et al., 2011) and included solid phase extraction (SPE) and ultrasound sonication, respectively. The chromatographic analysis and quantification in HPLC-DAD were based on a previously developed method with slight modifications (Li et al., 2016).

2.3. Calculations

EC50 of TSCA to different bioassays were calculated according to the relevant protocols. First-order kinetics were applied for calculating the biodegradation rate constant (k) of TSCA. A mass balance was used for predicting its concentration in treated wastewater of an aerobic activated sludge system. Risk Quotient methodology was applied for assessing the ecological threat in rivers where different dilution of treatment wastewater occurs.

3. Results

Among different tested bioassays, the highest toxicity of TSCA was noticed for *Daphna magna* (48-h LC50: 0.054 mg L^{-1}), followed by *Vibrio fischeri* (15-min

EC50: 0.392 mg L^{-1}) and *Lemna minor* (7-d EC50: 5.74 mg L^{-1}). Concerning its fate in activated sludge systems, TSCA can be biodegraded under aerobic conditions. A half-life equal to 7.3 h was calculated, while its sorption distribution coefficient was equal to 3.34 L·g^{-1}. Use of mass balances showed that 90% of this compound is expected to be removed in an aerobic activated sludge system, mainly via biodegradation. A preliminary risk assessment of TSCA using the Risk Quotient methodology showed possible ecological threat in rivers where wastewater is diluted up to 100-fold.

Conclusions

TSCA is expected to be partially removed via biodegradation and sorption in a conventional biological wastewater treatment system. The Ecotoxicity experiments showed that the EC_{50} values of TSCA range between 0.054 mg L^{-1} *(Daphna magna*) and 5.74 mg L^{-1} (*Lemna minor*). These values indicate that an ecological threat is possible due to the occurrence of this compound in small rivers and streams.

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