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Removal of selected endocrine disrupters in activated sludge systems: Effect of sludge retention time on their sorption and biodegradation

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ABSTRACT

The fate of three endocrine-disrupting compounds (EDCs), namely 4-*n*-nonylphenol (4-*n*-NP), triclosan (TCS) and bisphenol A (BPA), was studied in activated sludge process. The effect of sludge retention time (SRT) on their sorption and biodegradation was also investigated. Three continuous-flow activated sludge systems were used, operating at SRT of 3, 10 and 20 days, respectively. Batch experiments with biomass originated from the aforementioned systems showed that 4-*n*-NP and TCS are significantly sorbed on the suspended solids, while BPA to a lesser extent. The sorption affinity of target compounds did not seem to be affected by SRT, with the exception of 4-*n*-NP for which higher values of sorption coefficients were calculated at SRT of 3 days. The operation of continuous-flow systems in the presence of 10–40 $\mu g\ L^{-1}$ of EDCs indicated that more than 90%, 94% and 97% of BPA, TCS and 4-*n*-NP, respectively, can be removed during the activated sludge process in systems operating at SRT of 3–20 days. Determination of EDCs in the dissolved and particulate phase and calculation of their mass flux revealed that biodegradation was the principal removal mechanism of EDCs. For SRT values used in this study, this parameter does not seem to affect significantly percentage biodegradation of BPA. On the other hand, biodegradation of 4-*n*-NP and TCS was enhanced at SRT of 20 days. Calculation of biotransformation rates showed that the highest values for all EDCs were calculated at SRT of 3 days.

1. Introduction

Several synthetic organic compounds that have been classified as endocrine disrupters (EDCs) are commonly detected in municipal and industrial wastewater. Among these, nonylphenol (NP), bisphenol A (BPA) and triclosan (TCS) present significant research interest due to their extensive use and their physicochemical and toxicological properties.

NP is a biotransformation product of nonylphenol ethoxylates (NPnEOs); an important group of non-ionic surfactants that are widely used in many commercial and household functions, including detergents, cosmetic products and textiles (Birkett and Lester, 2003). Due to its formation in sewer system (Ahel et al., 1994), NP is commonly detected in influent wastewater of sewage treatment plants (STPs) at concentrations ranging from few $\mu g\ L^{-1}$ to more than 50 $\mu g\ L^{-1}$ (Planas et al., 2002). NP has been reported to cause a number of estrogenic responses on aquatic organisms (Birkett and Lester, 2003) and it has been listed as a priority substance in the Water Framework Directive (EU, 2001). This compound is a mixture of different branched and linear chain isomers (*ortho*-, *meta*- or *para*-), with the most common ring isomers being the *para* isomers (4-NPs). In several recent studies investigating fate and toxicity of NP, 4-*n*-NP has been used as the representative compound of this category (Ying et al., 2003; Stasinakis et al., 2008a).

BPA is widely used for the production of flame retardants, polycarbonate and epoxy resins. These products are used in food and drink packaging, as additives in thermal paper and in dental fillings. As a result, BPA is often detected in municipal and industrial wastewater (Stasinakis et al., 2008b). It has been shown that BPA possesses weakly estrogenic activity, as well as antiandrogenic activity (Birkett and Lester, 2003). TCS is a broad spectrum antimicrobial and preservative agent that is widely used in personal-care products. Typical concentrations of TCS in influent wastewater of STPs range between 0.5 and 30 $\mu g\ L^{-1}$ (Stasinakis et al., 2008b).

So far, the occurrence and removal of the aforementioned EDCs in STPs has been well documented through several studies around the world (Nakada et al., 2006; Heidler and Halden, 2007; Stasinakis et al., 2008b). However, there are fewer data for their fate in activated sludge process and the role of sorption and biodegradation on their removal (Tanghe et al., 1998; Stasinakis et al., 2007;

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Kim et al., 2007; Zhao et al., 2008). On the other hand, among different operating conditions describing activated sludge process, sludge retention time (SRT) is commonly considered important for the removal of xenobiotics during wastewater treatment (Huang et al., 2008), affecting their sorption and/or biodegradation (Kim et al., 2005; Hashimoto and Murakami, 2009). Regarding the target compounds, so far there is a lack of data for the effect of SRT on TCS fate, while there are limited data for the role of SRT on NP and BPA removal (Johnson et al., 2005; Clara et al., 2005).

The objective of this study was to investigate 4-*n*-NP, BPA and TCS fate in activated sludge process and to evaluate the role of SRT on their sorption and biodegradation. Three series of sorption batch experiments were performed using biomass originating from activated sludge systems, operating at SRT of 3, 10 and 20 days, respectively. These SRT values are similar to those used in highly-loaded, conventional and low-loaded activated sludge systems, respectively. Data from these experiments were fit to the Freundlich isotherm models and values of distribution (K_d) and sorption coefficients (K_F and n) were determined for each SRT and target compound. Continuous-flow activated sludge systems were also operated in the presence of 10–40 $\mu g\ L^{-1}$ of the target compounds and SRT of 3, 10 and 20 days. This concentration range is similar to that commonly determined in influent wastewater of STPs. Concentrations of the target compounds were determined in the dissolved and particulate phase using gas chromatography–mass spectrometry (GC–MS) and mass balances were performed to estimate the role of sorption and biodegradation on EDCs removal. Based on these results, biotransformation rates were calculated for the target compounds at different SRT values.

2. Methods

2.1. Batch experiments

Batch experiments were performed to investigate EDCs sorption by activated sludge. Activated sludge samples were initially taken from a nitrifying municipal STP (Mytilene, Greece) and inactivated by pasteurization at 103 °C for 3 h in order to prevent biodegradation (Andersen et al., 2005). Three batch reactors (300 mL stoppered conical flasks) were filled with 200 mL of inactivated sludge and they were spiked with 20 μL of 4-*n*-NP, BPA and TCS, respectively, to obtain a final concentration of 100 $\mu g\ L^{-1}$. The batch reactors were wrapped in aluminum foil to prevent possible photodegradation of EDCs and put on a shaker at 125 rpm. Homogenized samples (5 mL) were taken at increasing time intervals (0–8 h) for the determination of dissolved and particulate EDCs and calculation of equilibrium time. Based on these results, a mass balance was performed for target compounds in order to check sludge inactivation. To quantify any potential abiotic losses of EDCs, similar experiments were performed using potable water and samples were taken at the beginning and at the end of the experiment.

To determine adsorption isotherms, three series of batch experiments were performed using inactivated biomass originating from lab-scale activated sludge systems. These systems were operated at SRT of 3, 10 and 20 days, devoid of the target compounds, as it is described in detail below. For each SRT and each target compound, seven batch reactors were used and filled with 100 ml of inactivated sludge. Flasks were exposed to a wide range of concentrations on the order of 10, 40, 80, 120, 200, 350 and 500 $\mu g\ L^{-1}$, respectively. Batch reactors were put on a shaker, while samples were taken at equilibrium and EDCs concentration was detected in the dissolved phase.

All the experiments were performed at 22.0 ± 1.0 °C, pH was equal to 7.5 ± 0.2, while concentration of mixed liquor suspended solids (MLSS) was ranged between 1000 and 1200 $mg\ L^{-1}$.

2.2. Continuous-flow experiments

Three lab-scale continuous-flow activated sludge systems were operated at SRT of 3 (System A), 10 (System B) and 20 days (System C), respectively. The aerobic reactors of all systems were cylindrical, continuously fed, plastic tanks with a liquid volume of 6 L. Each aerobic reactor was connected to a 4 L cylindrical clarifier that had a recycle discharge in the bottom and a side tube for effluent discharge. Solids recycle flow (7.2 $L\ days^{-1}$) and wastewater flow (7.2 $L\ days^{-1}$) were driven by peristaltic pumps, while aeration and efficient mixing in aerobic reactors were provided using porous ceramic diffusers.

At the start of the experiment, activated sludge from a nitrifying municipal STP (Mytilene, Greece) was used to seed the reactors. All systems were operated on synthetic wastewater containing CH_3COOH, NH_4Cl, K_2HPO_4 and KH_2PO_4 (Stasinakis et al., 2003) for biomass acclimatization to the feed (Phase A). The duration of Phase A was 15, 30 and 60 days for System A, System B and System C, respectively. The main characteristics of the influent wastewater were COD 358 ± 46 $mg\ L^{-1}$ and NH_4–N 38.4 ± 11.0 $mg\ L^{-1}$. The initial total (dissolved + particulate) concentration of EDCs in inoculated biomass was 1.4 $\mu g\ L^{-1}$, 1.2 $\mu g\ L^{-1}$ and 1.9 $\mu g\ L^{-1}$ for 4-*n*-NP, TCS and BPA, respectively. Up to the end of Phase A, target compounds concentration was reduced to values lower than methods' limits of detection (LODs). After biomass acclimatization, a mixture of EDCs was added to the synthetic wastewater to provide concentration of 10–40 $\mu g\ L^{-1}$ of each compound for a period equal to 4 SRT for System A and 3 SRT for Systems B and C (Phase B). Samples were regularly collected from influents, effluents and aeration tank of each activated sludge system and concentrations of EDCs were detected in the dissolved and particulate phase.

During the experiments, all systems were maintained at 24.2 ± 1.8 °C in the dark, dissolved oxygen (DO) in aerobic reactors was kept above 4.0 $mg\ L^{-1}$ and pH was ranged to 7.7 ± 0.2. Hydraulic residence time (HRT) in aerobic reactors was 10 h, while SRT in each system was maintained constant by daily wasting of mixed liquor directly from the aerobic reactors.

2.3. Chemicals and analytical methods

Stock solutions of the target compounds were prepared in methanol HPLC grade (Merck, Germany) by weighting appropriate amounts of 4-*n*-NP (99.5%, Dr. Ehrenstorfer-Schafers, Germany), BPA (>97%, Buchs, Switzerland) and TCS (>97%, Fluka, Germany) and stored at −18 °C. Working solutions were prepared by diluting proper volumes of stock solutions in Milli-Q reagent grade water.

To control the operation of continuous-flow activated sludge systems, analyses of influent and effluent COD (dissolved, COD_{dis} and total, COD_{tot}), NH_4–N, suspended solids in the effluents (SS_{out}), MLSS, mixed liquor volatile suspended solids (MLVSS) and sludge volume index (SVI) were regularly performed, according to Standard Methods (APHA, 1998). Moreover, DO, temperature and pH values were measured daily using portable instruments.

For the investigation of EDCs fate in activated sludge process, samples originated from batch and continuous-flow experiments were filtered through pre-ashed glass-fiber filters (GF/F, Whatman, UK) and filtrates were stored in the dark at 4 °C until solid-phase extraction (SPE) (normally 24 h after filtration). For the determination of EDCs in the particulate phase, filters were oven dried in 60 °C until constant weight and stored at −18 °C.

The analytical methods for the determination of EDCs in dissolved and particulate phase were developed and optimized by the authors (Gatidou et al., 2007). The developed procedure included either SPE (liquid samples) or sonication (solid samples), while for the qualitative and quantitative analysis a Hewlett Packard Gas Chromatograph 5890 Series II connected to a Hewlett