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Toxicological interactions of ibuprofen and triclosan on biological activity of activated sludge

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HIGHLIGHTS

- Combined toxicity data of ibuprofen and triclosan to activated sludge were reported.
- Oxygen uptake was more strongly affected that enzymatic activity related to energy consumption.
- Ibuprofen was not toxic while triclosan exhibited high toxicity to activated sludge.
- Toxicity of mixtures showed similar profiles for both endpoints used.
- Antagonism was observed at low effect levels and synergisms at higher affected fractions.

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ABSTRACT

The growing use of pharmaceutical and personal care products increases their concentrations in the wastewater entering treatment plants and their levels into biological reactors. The most extended biological wastewater treatment is the activated sludge process. The toxicity of ibuprofen and triclosan, individually and combined, was studied by tracking the biological activity of the activated sludge measuring oxygen uptake rate and the inhibition of the esterase activity. Short-term exposure produced significant inhibition in oxygen uptake, with lower damage to enzymatic activity. Median effect values for oxygen uptake inhibition were 64 ± 13 mg L^{-1} and 0.32 ± 0.07 mg L^{-1} for ibuprofen and triclosan respectively using 125 mg L^{-1} activated sludge. For the inhibition of enzymatic activity values were 633 ± 63 mg L^{-1} for ibuprofen and 1.94 ± 0.32 mg L^{-1} for triclosan. Results indicated that oxygen uptake, related to primary activity of microorganisms, was more strongly affected than the enzymatic activity associated to energy consumption. Toxicity interactions were determined using the Combination Index-isobologram method. Results showed antagonism at lower values of affected population, after which the mixtures tended to additivity and synergism. For the case of enzymatic activity, the antagonism was less marked and the additivity range was higher.

1. Introduction

Emerging contaminants present significant research interest due to their systematic detection in wastewater [1,2] .The contamination originated from wastewater discharges impacts surface water quality due to the incomplete removal of many polar contaminants by conventional wastewater treatments [3]. Their toxicity to aquatic organisms has been frequently reported but there is still considerable lack of information on the effects of mixtures of these compounds on environments [4]. The occurrence of emerging pollutants in complex mixtures is another knowledge gap often recognized as a problem affecting ecosystems and drinking water supplies [5,6]. Among these compounds pharmaceutical and personal care products (PPCP) constitute a large and diverse set of chemicals, which include drugs and daily personal care products, widely used in large amounts throughout the world [7,8]. Additionally, many emerging compounds from these groups display pseudo-persistent exposure characteristics because their environmental dissipation rates are lower than discharge rates from effluent loadings, but higher than hydrologic retention times [9].

Among PPCP, ibuprofen (IBU) and triclosan (TCS) are particularly relevant compounds. The non-steroidal anti-inflammatory drug IBU is one of the most used active pharmaceutical ingredients

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worldwide [10,11]. TCS is a broad-spectrum antimicrobial agent widely used as antiseptic, disinfectant and preservative in many consumer products including cosmetics, household cleaning products and materials such as medical devices, textiles and plastic ware [12]. The occurrence of IBU and TCS is well-documented in influents and effluents of wastewater treatment plants (WWTP). The analgesic ibuprofen was detected at near 2 $\mu g L^{-1}$ in hospital wastewaters [13]. Kumar et al. [14] indicated a remarkably high concentration of TCS in the influent of a WWTP of 86.2 $\mu g L^{-1}$ in Savannah, Pal et al. [15] reported a maximum value of 7.1 $\mu g L^{-1}$ for IBU in WWTP effluents in Europe, with lower values in America and Asia. Martínez-Bueno et al. [16] reported maximum values for IBU and TCS in effluents of WWTP of 2.4 $\mu g L^{-1}$ and 3.7 $\mu g L^{-1}$ respectively. Rosal et al. (2010) [17] found maximum values of 4.1 $\mu g L^{-1}$ for IBU and 2.4 $\mu g L^{-1}$ for TCS in the influent of a WWTP. Both compounds have also been detected in freshwater compartments reaching the microgram per liter level [12,18]. The range at which IBU and TCS were detected in wastewater is typically in the tens of micrograms per liter and order of magnitude lower respectively, although both compounds have also been detected in freshwater compartments reaching the microgram per liter level.

Toxicant concentrations usually found in aquatic environments are at levels of μg/L or lower but considering the industrial environment of a wastewater treatment plant, the scenario is different. In the biological process of wastewater treatment, many dissolved contaminants are transferred to the solid phase, biomass or sludge, which is also an active microbial population responsible of pollutant removal. It was recognized that organic micro-pollutant concentrate in this phase up to 3 or 4 orders of magnitude with respect to aqueous media [19]. The risk for environmental compartments can be properly estimated by environmental risk assessment (ERA), which evaluates the probability of a compound to cause undesired environmental effects. In this sense, Ortiz de Garcia et al. [4] predicted the risk of 26 PPCPs in wastewater treatment plants and in the aquatic environment using the US EPA ecological structure–activity relationship (ECOSAR). Based on ecotoxicity data that included respirometry, the authors classified IBU as harmful and TCS as toxic to aquatic organisms.

Several studies highlighted the potential of IBU and TCS to promote adverse effects on aquatic organisms [20–24]. Despite the abundance of toxicological studies of PPCP using environmental organisms, surprisingly little attention has been paid to the microorganisms forming the activated sludge (AS) community [25]. Pasquini et al. [26] found that TCS at concentration as low as 0.1 $\mu g L^{-1}$ induced the overexpression of extracellular polymeric substances on AS, which is considered as stress response. The toxicity of pollutants to AS can be assessed by a number of methods apart from the simple monitoring of biomass growth or the counting of colony forming units. Oxygen uptake using respirometry, nitrification inhibition or adenosine triphosphate luminescence methods have been used for that purpose [27,28]. The inhibition of the activity of several enzymes such as dehydrogenases, phosphatases, glucosidases and esterases are also routinely used to measure specific alterations due to toxicants [29]. According to references [4,30–32], respirometry is the most realistic ecotoxicity test to evaluate the risk in a wastewater treatment plant and to detect the malfunction of biological reactors because they are direct indicators of the performance of the active biomass. The results are dependent on the particular characteristics of biomass, in turn affected by operational parameters, but they are essential to clarify the role of toxicants on sludge bioactivity. Additionally, esterase activity refers to a specific cellular function that requires intact membrane integrity [33] This biological reaction depends on the hydrolysis of FDA to fluorescein, by non-specific esterases present in different microbes [34], being a simple method to evaluate mixed populations such as those of activated sludge. The esterase activity is usually considered a measurement of cell viability, due to their direct relation with membrane integrity.

The co-occurrence of wastewater pollutants causes concern due to the potential interactive effects, such as synergistic or antagonistic toxicity, that may occur in the complex mixtures discharged with WWTP influents [5,35]. The interaction of several wastewater pollutants beyond the additivity described by the concentration addition (CA) method has been often neglected at low dose levels [36,37]. CA assumption has a clear practical value but there are clear evidences of non-additive behavior in many cases [38]. It has been recognized that non-additive behavior is concentration- and effect-level dependent, so experimental designs addressing this point are required [39]. For it, we used the combination index (CI)-isobologram equation method established by Chou and Talalay [40], a method that quantitatively assesses mixture effects over a wide range of effect levels. Using this method, our previous works showed that non-additive interactions occur in different mixtures with a general tendency to antagonism at low effect levels [41–44].

The aim of this study was to investigate the toxic effects of IBU and TCS on the AS from a WWTP by tracking oxygen uptake and the enzymatic esterase activity. TCS and IBU were selected in view of their widespread occurrence and the well-known negative effects exerted on aquatic organisms. The focus has been paid to the toxicological interactions between IBU and TCS using the combination index (CI)-isobologram method. To the best of our knowledge, it is the first work studying the combined action of organic micropollutants on AS bioactivity.

2. Materials and methods

2.1. Chemicals

Triclosan (TCS, $C_{12}H_7O_2Cl_3$, CAS No. 3380-34-5) and ibuprofen sodium salt (IBU, $C_{13}H_{17}NaO_2$, CAS No. 31121-93-4) both with 98% of purity were purchased from Sigma-Aldrich. The rest of chemical were also analytical grade and acquired from Sigma-Aldrich. Ultrapure water was generated from a Direct-Q^{TM} 5 Ultrapure Water Systems from Millipore (Bedford, MA, USA) with a specific resistance of 18.2 MΩ cm. Stock and working solutions of TCS, IBU and their mixtures were prepared in phosphate buffer and stored at 4 °C until use.

2.2. Activated sludge acclimatization and maintenance conditions

The bubble column reactor consisted of a transparent Plexiglass tube (12 cm inner diameter, 115 cm height and 4.5 L working volume) with a settler for sludge recirculation. The inoculum for the reactor start-up consisted of 500 mL collected from a municipal WWTP (Seville, Spain). Prior to exposure assays, the reactor operated continuously for 120 days under pseudo-steady state in order to achieve a homogeneous and stable composition of biomass. The activated reactor operated with Synthetic Sewage Feed, based on OCDE 209 standard medium: peptone, 160 $mg L^{-1}$; meat extract, 110 $mg L^{-1}$; urea, 30 $mg L^{-1}$; NaCl, 7 $mg L^{-1}$; $CaCl_2 \cdot H_2O$, 4 $mg L^{-1}$; MgSO4·7H2O, 2 $mg L^{-1}$ and K_2HPO_4, 28 $mg L^{-1}$. The synthetic influent was fed to the reactor continuously by a fixed speed peristaltic pump (hydraulic retention time 24 h). The air was supply thought a fine bubble diffuser and the dissolved oxygen (DO) concentration in the reactor was maintained at 3 $mg L^{-1}$ (measured using OD sensor, OXI 45+ CRISON). The reactor performance was monitored using the concentration of total suspended solids (TSS), chemical oxygen demand (COD) and ammonium nitrogen (NH_4-N). All the analyses were determined according to Standard Methods. Temperature was maintained at about 20 °C and pH was maintained at 8.0 ±0.1, close to the pK_a of TCS reported as 8.1 [45]. Acclimatiza-