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Active heterotrophic biomass and sludge retention time (SRT) as determining factors for biodegradation kinetics of pharmaceuticals in activated sludge

Marius Majewsky [a,*], Tom Gallé [a], Viviane Yargeau [b], Klaus Fischer [c]

[a] Resource Center for Environmental Technologies (CRTE) – CRP Henri Tudor, 66, rue de Luxembourg, 4221 Esch-sur-Alzette, Luxembourg
[b] Department of Chemical Engineering, McGill University, 3610 University Street, Montreal, Quebec, Canada H3A 2B2
[c] Department of Analytical and Ecological Chemistry, University of Trier, Behringstr. 21, 54296 Trier, Germany

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ABSTRACT

The present study investigates the biodegradation of pharmaceutically active compounds (PhACs) by active biomass in activated sludge. Active heterotrophs (X_{bh}) which are known to govern COD removal are suggested as a determining factor for biological PhAC removal as well. Biodegradation kinetics of five polar PhACs were determined in activated sludge of two wastewater treatment plants which differed in size, layout and sludge retention time (SRT).

Results showed that active fractions of the total suspended solids (TSS) differed significantly between the two sludges, indicating that TSS does not reveal information about heterotrophic activity. Furthermore, PhAC removal was significantly faster in the presence of high numbers of heterotrophs and a low SRT. Pseudo first-order kinetics were modified to include X_{bh} and used to describe decreasing PhAC elimination with increasing SRT.

1. Introduction

The removal of pharmaceutically active compounds (PhAC) during wastewater treatment has become a major concern in water research during the last decade. Biodegradation during activated sludge treatment has been identified as a major elimination pathway in particular for hydrophilic non-persistent PhACs in a variety of studies. To assess PhAC breakdown in individual activated sludges, biodegradation rates are mostly determined in lab-scale tests where microbial biomass is a key parameter. Biomass is usually approximated by the amount of total (or volatile) suspended solids (TSS) which can be easily determined by routine measurements. Recent studies proposed pseudo first-order reaction kinetics to describe PhAC removal (Maurer et al., 2007; Wick et al., 2009). This reaction is governed by the amount of biomass and the biodegradation rate constant k_{biol}. However, a major drawback of utilizing TSS is that only a fraction of the suspended solids can be considered as viable biomass while an inert fraction is also present (Cronje et al., 2002). Only the viable fractions are responsible for biological removal processes and biodegradation rates should therefore be expressed in terms of active biomass. While this has been successfully achieved, e.g. for COD and NH_4^+ transformations by classifying activated sludge bacteria into heterotrophic and autotrophic fractions, the issue of identifying bacteria (groups) that are responsible for xenobiotic biodegradation processes still remains. In this context, slow growing specialized bacteria and diversified enzymes were suggested to enhance PhAC removal. These bacteria are believed to be retained in sludge in significant numbers in wastewater treatment plants (WWTPs) that operate above a critical sludge retention time (SRT) of 10 days (referred to 10 °C) (Clara et al., 2005). The concept of critical SRTs was developed for implementing the nitrification process in biological wastewater treatment systems and has been adopted for PhAC removal (Kreuzinger et al., 2004).

In contrast, Stasinakis et al. (2010) found the highest biotransformation rates of endocrine disruptors at a low SRT of 3 days and Gaulke et al. (2009) found no difference for 17α-ethinylestradiol at two different SRTs suggesting that heterotrophic bacteria capable of degrading PhAC are present both at low and high SRTs. Furthermore, biodegradation rates of aminopolycarboxylic acids, which have been suggested to be promoted by heterotrophic microbial activity, were significantly higher at low SRTs (Majewsky et al., 2010). The SRT is a design criterion for WWTPs and strongly related to microbial growth. Nonetheless, the relation between SRT, microbial community structure and xenobiotic degradation performance is not fully understood and controversial findings have been reported (Clara et al., 2005; Gaulke et al., 2009; Kraigher et al., 2008; Saikaly et al., 2005; Schaar et al., 2010; Stasinakis et al., 2010).

* Corresponding author. Tel.: +352 42 59 91 670; fax: +352 42 59 91 555.
E-mail addresses: marius.majewsky@tudor.lu (M. Majewsky), tom.galle@tudor.lu (T. Gallé), viviane.yargeau@mcgill.ca (V. Yargeau), fischerk@uni-trier.de (K. Fischer).

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Table 1
Physico-chemical properties and application of the selected compounds.

	CAS	Log K_{ow}[a]	Molecular weight[a] (g mol^{-1})	Water solubility[a] (mg L^{-1})	K_d secondary sludge (L kg TSS^{-1})	Application
Caffeine	58–08–2	−0.07	194.19	2.16×10^4	–	Psychostimulant
Carbamazepine	298–46–4	2.45	236.28	112	1,2[c]	Anti-epileptic drug
Diclofenac	15307–79–6	0.7[b]	296.16	2.37	16[c]	Non-steroidal anti-inflammatory drug
Paracetamol	103–90–2	0.46	151.17	1.4×10^4	<1[c]	Non-steroidal anti-inflammatory drug
Sulfamethoxazole	723–46–6	0.89	253.28	610	260[d]	Antibiotic

[a] SRC Database.
[b] Jones et al. (2002).
[c] Ternes et al. (2004).
[d] Göbel et al. (2005).

The presented study focuses on the active heterotrophic biomass which governs COD removal, suggesting a determining factor for biological PhAC removal as well. It aims at contributing to the refinement of biodegradation rate estimations and at explaining variability of the latter between WWTPs. The interrelationship of PhAC removal capacity with operational process parameters such as SRT and hydraulic retention time (HRT) was investigated. A spectrum of five different hydrophilic pharmaceuticals was chosen (Table 1) that contains a variety of molecular structures with heterocyclic and aromatic rings and different functional groups. The selected substances carbamazepine (CBZ), diclofenac (DCF), sulfamethoxazole (SMX) and paracetamol (PCT) as well as caffeine (CAF) have been detected widely in concentrations up to the μg L^{-1} level in wastewater influents (Heberer, 2002; Zhang et al., 2008). These compounds range from persistent to easily biodegradable chemicals. Partitioning of the investigated compounds onto biomass particles by adsorption is usually not significant in the overall elimination (Ternes et al., 2004) and was therefore not subject to this study. Only sulfamethoxazole has a tendency to adsorb on secondary sludge particles with a solid–water distribution coefficient (K_d) of 260 L kg TSS^{-1} (Göbel et al., 2005). This would account for a maximum 10% of the total elimination efficiency, dependending on the sludge production (Ternes et al., 2004).

PhAC biodegradation kinetics were determined in activated sludge from two Luxembourg WWTPs that differed in size, layout, HRT, SRT and organic loading rates, suggesting significant differences in the level of heterotrophic microbial activity. A simultaneous estimation of viable heterotrophic biomass fractions and degradation kinetics was achieved by combining batch experiments with respirometry. Recent research successfully demonstrated the applicability of such a set-up in order to account for both, micropollutant biodegradation and process kinetics of activated sludge (Olmez-Hanci et al., 2011). The present study raised the question, whether pharmaceutical attenuation can be attributed to heterotrophic activity and therefore linked to treatment process parameters.

2. Methods

2.1. Sampling and bioreactor

Activated sludge (2 × 20 L) was taken from the aerated tanks of the two Luxembourg WWTPs studied, Mamer and Boevange, in May 2009. Aliquots (2.4 L) were used for respirometer experiments. The respirometer used to study the removal of the PhACs consisted of a 3 L jacketed bioreactor (Ochs GmbH, Germany) maintained at a temperature of 20 ± 1 °C. A Metrohm GPD 751 Titrino controlled the pH at 7.5 ± 0.2 during the experiment by addition of hydrochloric acid or sodium hydroxide. The dissolved oxygen concentration, monitored using a LDO probes from Hach-Lange, was automatically maintained between 3 and 6 mg O_2 L^{-1} by an aeration pump controlled by a program written in LabView®. The oxygen uptake rate was calculated from the depleting dissolved oxygen concentration during non-aeration phases by moving window linear regressions (n = 10). The input of atmospheric oxygen via the liquid surface was taken into account in the model used for simulation ($K_La = 4.5 \times 10^{-3}$ min^{-1}).

2.2. Estimation of active heterotrophic biomass

Activated sludge aliquots (2.4 L) were decanted into the respirometer at the same day of sampling to estimate the active heterotrophic biomass content. The sludge was left for 8–12 h until it reached the endogenous phase before the experiment was started, to make sure that no residual substrate was present. Autotrophic microorganisms were inhibited during the respirometry experiment by addition of N-allylthiourea (concentration c = 10 mg L^{-1}). The amount of active heterotrophic biomass X_{bh} was estimated from modeling simulations of the oxygen uptake rate (OUR) responses to defined spikes of sodium acetate (c = 60 g L^{-1}, V_{added} = 2.5 ml) as presented in details elsewhere (Plattes et al., 2007; Vanrolleghem et al., 1999). Simulations were realized by use of the activated sludge model no. 1 (ASM1) within the wastewater treatment modeling software GPS-X from Hydromantis (Hamilton, Canada). Heterotrophic yields were calculated from theoretical ($COD_{theoretical}$ = 70.6 mg O_2 L^{-1}) and experimental COD of sodium acetate spikes. Default values were used for the decay rate (b_h = 0.62 d^{-1}, Henze et al., 2000). The growth of biomass during the experiment was negligible due to the small amounts of sodium acetate added. Subsequently, biodegradation tests described in the following section were performed with the same sludge.

2.3. Biodegradation experiments

The pharmaceuticals carbamazepine, diclofenac, sulfamethoxazole, paracetamol and caffeine (purchased from Dr. Ehrenstorfer GmbH, Germany) were added as a mixed stock solution (c = 1.2 mg L^{-1} in H_2O, V_{added} = 2 ml) to the bioreactor resulting in an initial concentration of 1 μg L^{-1} (V_{sludge} = 2.4 L). In order to make biodegradation rates directly comparable, the same synthetic substrate was used together with PhAC spikes in each experiment. The synthetic substrate consisted of a mixture of sodium acetate, ammonium chloride and sodium dihydrogen phosphate monohydrate with a ratio of C:N:P of 100:50:1, corresponding to typical carbon to nutrient ratios occurring in domestic wastewater. The substrate was added (V_{added} = 21.2 ml) at a concentration of $COD_{theoretical}$ = 736.8 mg O_2 L^{-1}, thereby avoiding nitrogen or phosphorus from becoming limiting factors. The amount added ensured that the synthetic primary substrate was permanently present in excess during the period of the biodegradation test (5–6 h) and controlled by real-time OUR measurements. Samples of 10 ml were