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Co-metabolic oxidation of pharmaceutical compounds by a nitrifying bacterial enrichment

Anwar Dawas-Massalha [a], Shirra Gur-Reznik [a], Sofia Lerman [a], Isam Sabbah [b,c], Carlos G. Dosoretz [a,*]

[a] Civil and Environmental Engineering, Technion – Israel Institute of Technology, Haifa, Israel
[b] The Galilee Society Research & Development Center, Shefa-Amr, Israel
[c] Braude College for Engineering, Karmiel, Israel

HIGHLIGHTS

- Transformation of pharmaceuticals at trace concentration could be driven by nitrifying activity.
- The transformation pattern observed is consistent with ammonia monooxygenase activity.
- An inverse correlation between the rate of transformation and concentration was found.
- A linear correlation between calculated cell-diffusive flux and transformation rates was found.
- AMO transformation appears as good indicator of biodegradability potential of trace organics.

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ABSTRACT

The biotransformation of five selected pharmaceuticals ibuprofen (IBP), ketoprofen (KTP), carbamazepine (CBZ), dexamethasone (DXM) and iopromide (IOP) by a stable nitrifying enrichment culture was investigated at concentrations ranging between 25 μg/L and 2 mg/L. Complete biotransformation was observed only for IBP and KTP, although, an inverse correlation between transformation rate and concentration was found. The transformation pattern observed is consistent with ammonia monooxygenase (AMO) activity. The metabolic succession of the compounds according to the biotransformation rates was: IBP > KTP > DXM > CBZ > IOP. A linear correlation between the calculated diffusive flux of the model compounds across a bilayer membrane and their biotransformation rates was found. Our results support the concept that augmentation with nitrifying activity can enhance the removal of trace organic pollutants during effluent treatment. Furthermore, ammonia-oxidizing activity appears as a good indicator for estimation of potential of biodegradability of pharmaceuticals, especially at low concentrations.

1. Introduction

The presence and fate of pharmaceutically active compounds (PhACs) in the water environment have been recognized as one of the emerging issues in environmental sciences due to their potential to cause undesirable ecological and human health effects (Ternes, 2005). Their high-level of persistence and low adsorption properties have led PhACs being found ubiquitously in water environments and water distribution systems. For example, carbamazepine (CBZ) has been detected in groundwater (Drewes et al., 2000) while diclofenac was found in tap water (Heberer et al., 2002). Because these chemicals are often present in wastewater at concentrations ranging from 0.1 to 10^5 ng/L (Ternes, 2007; Oulton et al., 2010) they cannot support growth of microorganisms, capable of mineralizing them, during biological wastewater treatment.

The removal of PhACs from water and wastewater is essential to prevent environmental contamination and their possible adverse effects. Although the direct or indirect effect of pharmaceuticals in the aquatic system is not yet well established, lab studies exemplify their potential adverse effect. Spread of antibiotic accelerates their evolutionary resistance in naturally occurring bacteria, as reported for ciprofloxacin (Marti et al., 2014); some drugs have the potential to accumulate in soils and perhaps adsorb into crops during irrigation with recycled water as reported for carbamazepine (CBZ) (Shenker et al., 2011). Although chronic effect are not yet clear, estrogenicity due to outspreading of natural and synthetic estrogens has been one of the best evaluated effects,

* Corresponding author. Address: Faculty of Civil and Environmental Engineering, Technion – Israel Institute of Technology, Haifa 32000, Israel. Tel.: +972 4 8294962; fax: +972 4 8228898.
E-mail address: carlosd@tx.technion.ac.il (C.G. Dosoretz).

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whereas acute toxicity was demonstrated for non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen (IBP) and ketoprofen (KTP) and β-blockers such as propranolol and metoprolol (la Farre et al., 2008).

Ecotoxicological data is still needed to evaluate what PhACs removal threshold is sufficient. There are instances where even a 90% removal rate may be cause for concern. Laboratory studies have shown similar concentration levels can induce adverse ecotoxicological effects on aquatic organisms. For example, triclosan and ciprofloxacin concentrations as low as 0.012–1.5 mg/L were found to induce a strong concentration-dependent decline in genus diversity of algal communities sampled upstream and downstream of a wastewater treatment plant (WWTP) in Kansas (Wilson et al., 2003). A number of studies have shown that the biodegradation of PhACs in wastewater are likely to be due to co-metabolic activity (Batt et al., 2006; Tran et al., 2009; Khunjar et al., 2011). This means that PhACs can be degraded in the obligate presence of a growth supporting substrate (primary substrate) (Tran et al., 2009). Studies published on the co-metabolism of PhACs are currently limited. Several studies on the removal of xenobiotics have shown that the degradation can be enhanced by augmentation through the addition of organic carbon sources or other nutrient substances, such as nitrogen and phosphate, as well as mineral constituents (Boiesen et al., 1993; Fava et al., 1995). It was also suggested that primary substrates not only serve to sustain biomass production, but also act as an electron donor for the co-metabolism of the non-growth substrate (Tran et al., 2009). Recent studies reported that the ammonia oxidizing bacteria (AOB) in a nitrifying activated sludge system were responsible for the elimination of these chemicals, driven by ammonia monooxygenase (AMO) (Batt et al., 2006; Yi and Harper, 2007). The co-metabolism of PhACs by nitrifiers may be important for their degradation because AMO has a relatively wide substrate specificity (Tran et al., 2009; Khunjar et al., 2011).

The main objective of this study was to establish the basis for the biotransformation of selected pharmaceuticals at environmental (μg/L range) and above-environmental (mg/L range) concentrations by ammonia oxidizing bacteria.

2. Methods

2.1. Chemicals and media

All chemical were of analytical quality. The pharmaceuticals model compounds used in this study were ibuprofen (IBP), ketoprofen (KTP), carbamazepine (CBZ) and dexamethasone (DXM), all purchased from Sigma–Aldrich, and iopromide (IOP) which was purchased from Ehrenstorfer GmbH.

2.2. Nitrifying bacterial enrichment

The nitrifying bacterial enrichment was obtained from a fluidized bed-continuous nitrifying reactor operated for many years in the laboratory of M. Green at the Technion (Tarre and Green, 2004; Green et al., 2005). The bacteria were originated from activated sludge. The culture was maintained in a bench scale-continuous reactor (2 L) filled with crushed basalt rock (approx. 2 mm in size) as the carrier material for the immobilized biomass. The activity of the biomass was periodically checked for AMO activity. The feed medium consisted of a solution of NH_4Cl (70 mg/L as N), $NaHCO_3$ (885 mg/L) and KH_2PO_4 (5 mg/L) in tap water, bubbled with air. No organic carbon sources were added to the medium. The feed flow rate was maintained at approx. 14 L/day. Prior to each biotransformation experiment, the required amount of colonized basalt carriers was removed from the reactor, bacteria were detached by vortexing to obtain a suspended biomass, followed by settling of the basalt pieces and separation of the suspended bacteria. The separated biomass was further concentrated by sedimentation in a graduated cylinder for two hours, after which the medium was discarded, the biomass tested for AMO activity and used for the experiments.

2.3. Biotransformation of PhACs with nitrifying bacteria

The biotransformation experiments were conducted in batch mode using 250 mL sterilized Erlenmeyer flasks filled with 100 mL medium. The medium consisted of tap water supplemented with 5 mg/L KH_2PO_4 and 50 mg/L $NaHCO_3$ to keep the pH at approximately 7.5–8.0 during the incubation period and, unless otherwise stated, 5 mg/L NH_4Cl (as N). Initial ammonia concentration was optimized in preliminary experiments as indicated below. The medium was spiked with the target PhACs as indicated, which consisted on the only source of organic carbon added to the medium. The stock solutions of IBP, KTP, CBZ, and DXM were made in methanol. In this case, a volume from the stock was taken and transferred to an empty flask, after which the methanol was evaporated and the medium was added. The IOP stock solution was made in ultrapure water. In all experiments, two types of controls were performed to validate the results: (i) flasks without bacteria to evaluate intrinsic degradation of the model compounds i.e., chemical stability; (ii) flasks supplemented with 2 g/L of the bacteriostatic biocide sodium azide (NaN_3) + 10 mg/L of the AMO inhibitor allylthiourea (ATU), in order to distinguish between biosorption and biodegradation. All experiments were performed at least in triplicates.

The flasks were incubated in an orbital shaker gently agitated at 80 rpm at a temperature of 25 °C. Samples for determination of ammonia and PhACs concentration were filtered through a 0.22 μm syringe filter.

2.4. Biomass viability

Biomass viability was determined using the Alamar Blue Cell Viability assay (Promega, 2013). The active reagent, resazurin, is a water-soluble dye which is converted by viable cells to resofurin, a fluorescent end product. Nonviable cells do not reduce the indicator dye, and thus do not generate a fluorescent signal. The procedure employed followed the manufacturer's instructions.

2.5. Determination of PhACs biotransformation

Biotransformation of model PhACs was tracked by liquid chromatography–multiple stage/mass spectrometry (LC–MS/MS). Analyses were essentially performed according to the method of Vanderford et al. (2003) with some custom modifications as described by Gur-Reznik et al. (2011). Injections were performed on an Agilent 1200 HPLC (Hewllet Packard) system coupled with an ion spray interface to an API 3200 triple quadrupole mass spectrometer (Applied Biosystems). A LiChroCart Purospher Star RP-18 (Merck) endcapped column (4.6 mm × 15 cm, 5 μm pore size) was used with a binary gradient of 0.1% (v/v) formic acid in water (A) and HPLC grade methanol (B). The gradient was as follows: 5% B held for 4 min, stepped to 100% by 9 min and held for 5 min, then linearly decreased to 5% B during 3 min. A 2-min equilibration step at 5% B was used at the end of each run to bring the total run time per sample to 19 min. Electrospray ionization was used in positive ion mode for KTP, CBZ, DXM and IOP, and in negative ion mode for IBP. The compounds were detected in multiple-reaction monitoring (MRM) mode. Precursor and product ions, as well as dwell times and voltages were used for the identification of the model compounds. An injection volume of 25 μL was used for all model