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Factors impacting biotransformation kinetics of trace organic compounds in lab-scale activated sludge systems performing nitrification and denitrification

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HIGHLIGHTS

- We examined TOrC biotransformation kinetics in nitrifying and denitrifying reators.
- TOrC biotransformation was linked to heterotrophic and autotrophic activity.
- TOrC biotransformation rates were not sensitive to the initial TOrC concentration.
- Readily biodegradable organic matter suppressed TOrC biotransformation rates.

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ABSTRACT

To predict TOrC fate in biological activated sludge systems, there is a need to accurately determine TOrC biodegradation kinetics in mixed microbial cultures. Short-term batch tests with salicylic acid, 17α-ethinylestradiol, nonylphenol, trimethoprim and carbamazepine were conducted with lab-scale activated sludge cultures in which the initial TOrC concentration (1 mg/L and 0.0005 mg/L) and readily biodegradable substrate concentrations were varied. The results indicate that pseudo-first order kinetic estimates of TOrC are not sensitive ($p > 0.05$) to the initial TOrC concentration as long as the initial TOrC concentration (S_0) to biomass (X_0) ratio (on COD basis) is below 2×10^{-3}. The presence of readily biodegradable organic matter suppresses TOrC biotransformation rates under nitrifying and denitrifying conditions, and this impact can be adequately described using a reversible non-competitive inhibition equation. These results demonstrate the importance of closely mimicking parent reactor conditions in batch testing because biotransformation parameters are impacted by *in-situ* carbon loading and redox conditions.

1. Introduction

Water resource reclamation facilities (WRRFs) act as barriers against the indiscriminate output of trace organic compounds (TOrC) to the environment [1]. Overall removal of TOrC at WRRFs is achieved via several mechanisms including volatilization, abiotic transformation, biotransformation and sorption to solids [2,3]. Over the past 10 years, our knowledge of TOrC fate through different unit processes in WRRFs has significantly advanced [4–8]. From this body of knowledge, it has been recognized that activated sludge treatment processes are robust options for reducing concentrations of synthetic organic compounds like TOrC [9–13].

TOrC biodegradation by activated sludge is dependent on multiple factors including, but not limited to, chemical compound configuration, bioavailability, the presence of alternative substrates (e.g. biodegradable dissolved organic carbon), nitrification capacity, redox conditions, microbial community composition and microbial growth state [14–25]. Given the complexity of TOrC biodegradation, it is important to define and utilize appropriate testing conditions to ensure that measured biotransformation kinetics are truly representative of the system being studied. Indeed, standardized protocols do exist for helping researchers perform these tests (e.g., Organization for Economic Co-operation and Development (OECD) Test number 314B–Biodegradation in activated sludge) [26,27]. Yet, there exists significant variability in reported

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TOrC biotransformation rates and efficiency throughout the literature [3,28]. While inherent biological variability is expected to contribute to the wide range of TOrC removal efficiency, we hypothesize that performing tests under conditions which differ from those of the *in situ* environment also impacts reported results.

The development of optimum TOrC substrate to biomass ratios (S_0/X_0 or *F:M* ratio) is a fundamental requirement for determining microbial biotransformation kinetics that appropriately describe biological systems [10,29]. Two approaches have been traditionally used for estimating biotransformation parameters for synthetic organic compounds. In the *intrinsic* test condition ($S_0/X_0 > 20$), a high concentration of substrate relative to the biomass concentration is used. This test is also referred to as a high *F:M* test and yields the maximum specific growth rate at which an organism can utilize a specific substrate. The biomass concentration typically increases during this test as growth is unbounded due to nutrient replete conditions [30]. In the *extant* test approach ($S_0/X_0 < 20$), a low concentration of substrate relative to the biomass concentration is used. This test is also referred to as a low *F:M* test and yields the specific growth rate that an organism is capable of achieving at a specific substrate concentration. During this test, the low substrate concentration inhibits growth, which typically leads to a constant biomass concentration [30]. In addition to defining initial TOrC to biomass ratios, it is important to understand how the presence of alternative, more readily biodegradable substrates can impact biotransformation rates: the presence or absence of these substrates can influence whether biotransformation is dictated by co-metabolism, fortuitious metabolism and/or mixed substrate utilization.

In this study, we utilize a standardized method that allows for the determination of TOrC biokinetics under conditions that most appropriately represent the system being studied. We employ five model TOrC (salicylic acid, 17α-ethinylestradiol, trimethoprim, carbamazepine, nonylphenol) for this study based on a combination of their occurrence, biodegradability, ease of analyses [8,15,31] as well as their projected ecotoxicological impacts [32] on receiving water bodies, and explored biotransformation of these TOrC under nitrifying and denitrifying conditions. We quantify the effect of the variable initial TOrC concentration to biomass ratios as well as the presence of readily biodegradable organic matter on TOrC biotransformation kinetics and provide recommendations for future testing.

2. Experiment

2.1. Chemicals and materials

Non-labeled TOrC (purity > 99%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) or Fisher/Thermo Scientific (Pittsburgh, PA). Deuterium labeled 17α-ethinylestradiol (EE2-d4), salicylic acid (d4-SA), nonylphenol (d4-NP), trimethoprim (d3-TMP) and carbamazepine (d10-CBZ) were purchased from C/D/N Isotopes, Inc. (Pointe-Claire, Quebec, Canada). Deuterated caffeine (d3-CAF) was used as an analytical internal standard to monitor instrument drift. Stock solutions of non-labeled TOrC were prepared in methanol or Nanopure™ water. Stock solutions containing 1000 mg COD/L or 10,000 mg COD/L of each labeled TOrC were also prepared in methanol. Deuterated TOrC stock solutions were used during solid-phase extraction (SPE). The chemical oxygen demand (COD) notation was utilized throughout this work to maintain consistency with existing activated sludge process models (ASM) that utilize the COD approach. Conversions between notations were performed using the following factors: 1 mg SA = 1.6 mg COD; 1 mg EE2 = 2.7 mg COD; 1 mg TMP = 1.5 mg COD; 1 mg CBZ = 2.2 mg COD; 1 mg NP = 3.0 mg COD.

2.2. Lab-scale activated sludge cultures

Mixed liquor from a WRRF employing a 5-stage biological nutrient removal configuration (SRT = 7–10 day, MLSS = 2500–3200 mg/L) was used to seed two separate chemostats; one of the chemostats was designated as a nitrifying activated sludge culture (NAS) exposed to aerobic conditions, while the second reactor was designated as a denitrifying activated sludge culture (DAS) and exposed to continuous anoxic conditions. These cultures were maintained in well-mixed chemostats operated at a solids retention time (SRT) of 10 days. Aeration for the NAS was provided with air pumps and fine bubble diffusers to maintain a dissolved oxygen concentration of 7–8 mg/L O_2. The pH for both reactors was maintained between 7.5 ± 0.1 using an automated controller (pH Controller, Omega Engineering, CT), pH electrode (Accumet epoxy, Orion Research, INC. Beverly, MA) and sodium bicarbonate (80 g/L) or sulfuric acid (1 N). Both reactors were mixed with magnetic stir bars. Further details regarding feed composition is provided in Table S1 and in an associated publication [33].

2.3. Lab-scale activated sludge cultures: long term experiments

Once the chemostats achieved steady state removal of nitrogen (defined as three consecutive days in which the effluent nitrogen concentration was not statistically different at the 95% confidence interval from the three previous time-points), non-labeled TOrCs (500 ng COD/L of each TOrC) were added to the feed of the respective chemostats. The reactors were then operated in this manner for 297 days (approximately 30 x SRT). Composite effluent samples from each chemostat were obtained over two days twice a month to monitor TOrC concentrations. These samples were centrifuged at $10{,}000 \times g$ for 30 min at 4 °C, after which the supernatant was filtered through 0.22 μm nylon filters and stored at −20 °C before being processed via SPE prior to analysis by liquid chromatography with tandem mass spectrometry (LC-MS/MS).

2.4. TOrC depletion assays

Biomass samples (1 L) from NAS and DAS reactors were harvested and supplemented with 5 mL of 50 g/L sodium bicarbonate buffer to achieve an initial pH of 7.5. An aliquot of the NAS biomass was added to amber glass vessels equipped with air pumps and fine bubble diffusers that were used to maintain aerobic conditions (DO between 7 and 8 mg/L (high DO condition) or at 1.5 mg/L (low DO condition)). Similarly, an aliquot of DAS biomass was added to amber glass vessels that were then purged with nitrogen gas for 30 min to ensure that the DO was below detection. After a 30-min period, the DAS vessels were sealed and a Tedlar™ bag filled with nitrogen gas was used to provide positive headspace pressure to ensure that anoxic conditions were maintained throughout the experiments.

2.4.1. Varied initial TOrC concentration to biomass ratio (S_0/X_0) experiments

NAS and DAS biomass aliquots prepared as described above were supplemented with each non-labeled TOrC. In these experiments, two TOrC concentrations (1 mg COD/L and 0.0005 mg COD/L) were tested in separate amber glass vessels. These two concentrations were selected to represent conditions in which excess TOrC is applied in batch tests (i.e., 1 mg COD/L) versus a scenario where low concentrations of TOrC are used in batch tests (i.e.,