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Impact of microbial physiology and microbial community structure on pharmaceutical fate driven by dissolved oxygen concentration in nitrifying bioreactors

Lauren B. Stadler [1], Nancy G. Love*

Department of Civil and Environmental Engineering, University of Michigan, 1351 Beal Avenue, EWRE, Ann Arbor, MI 48109, United States

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ABSTRACT

Operation at low dissolved oxygen (DO) concentrations (<1 mg/L) in wastewater treatment could save utilities significantly by reducing aeration energy costs. However, few studies have evaluated the impact of low DO on pharmaceutical biotransformations during treatment. DO concentration can impact pharmaceutical biotransformation rates during wastewater treatment both directly and indirectly: directly by acting as a limiting substrate that slows the activity of the microorganisms involved in biotransformation; and indirectly by shaping the microbial community and selecting for a community that performs pharmaceutical biotransformation faster (or slower). In this study, nitrifying bioreactors were operated at low (~0.3 mg/L) and high (>4 mg/L) DO concentrations to understand how DO growth conditions impacted microbial community structure. Short-term batch experiments using the biomass from the parent reactors were performed under low and high DO conditions to understand how DO concentration impacts microbial physiology. Although the low DO parent biomass had a lower specific activity with respect to ammonia oxidation than the high DO parent reactor biomass, it had faster biotransformation rates of ibuprofen, sulfamethoxazole, 17α-ethinylestradiol, acetaminophen, and atenolol in high DO batch conditions. This was likely because the low DO reactor had a 2x higher biomass concentration, was enriched for ammonia oxidizers (4x higher concentration), and harbored a more diverse microbial community (3x more unique taxa) as compared to the high DO parent reactor. Overall, the results show that there can be indirect benefits from low DO operation over high DO operation that support pharmaceutical biotransformation during wastewater treatment.

1. Introduction

Energy conservation is an increasingly desirable goal in wastewater treatment given rising concerns over fossil fuel energy resources and climate change. The most energy intensive process in activated sludge wastewater treatment is aeration, which makes up 45–75% of a conventional wastewater treatment plant's (WWTP's) total energy costs (Rosso et al., 2008). Therefore, one strategy for implementing more sustainable wastewater treatment involves a reduction in energy consumption by moving towards treatment that minimizes aeration (Flores-Alsina et al., 2011; Leu et al., 2009; Rosso et al., 2008). Conventional nitrifying activated sludge WWTPs typically operate with bulk liquid dissolved oxygen (DO) concentrations of greater than 2 mg/L to ensure complete nitrification and stable nitrifying populations (WEF, 2008), which results in a substantial energy demand for aeration. There is mounting evidence that stable carbon removal and nitrification can occur at low DO concentrations (<1 mg/L) (Jimenez et al., 2011; Schuyler et al., 2009), suggesting that substantial energy and cost savings are possible by reducing DO levels. Advances in sensor technology and aeration control in recent years have demonstrated that strategies such as ammonia-based aeration control can result in operation at DO concentrations between 0.5 and 1.0 mg/L (Rieger et al., 2014; Uprety et al., 2015). Low DO treatment also can also enable simultaneous nitrification and denitrification, resulting in supplemental carbon savings in addition to aeration energy savings

* Corresponding author. Department of Civil and Environmental Engineering, University of Michigan, 183 EWRE Building, 1351 Beal Avenue, EWRE, Ann Arbor, MI 48109, United States.
E-mail addresses: lauren.stadler@rice.edu (L.B. Stadler), nglove@umich.edu (N.G. Love).
[1] Present address: Department of Civil and Environmental Engineering, Rice University, 6100 Main Street, Houston, TX 77005, United States.

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(Daigger and Littleton, 2000; Jimenez et al., 2011).

Notable fundamental research on low DO wastewater treatment has focused on nitrification (Arnaldos et al., 2013; Bellucci et al., 2011; Liu and Wang, 2013; Park and Noguera, 2004); nitrifiers grow relatively slowly as compared to heterotrophs due to their chemolithoautotrophic metabolism and inferior oxygen scavenging ability. Selecting for nitrifiers that are adapted to low DO environments and taking advantage of improvements in mass transfer efficiencies during low DO operation can result in at least 20% less energy for aeration (Arnaldos and Pagilla, 2014). As we move toward low DO treatment processes, there is a need to better understand of how low DO environments affect the function and structure of nitrifying wastewater communities to ensure stable performance without compromising treatment outcomes.

Ammonia oxidizers, which perform the first step of nitrification using the enzyme ammonia monooxygenase (AMO), may also play a major role in pharmaceutical biotransformation during wastewater treatment. AMO can catalyze the oxidation of a relatively wide range of substrates (Arp et al., 2002). Several studies have shown that pharmaceutical loss, and specifically estrogen transformation, is enhanced in nitrifying activated sludge systems (Suarez et al., 2010; Tran et al., 2009; Vader et al., 2000), and have implicated the involvement of AMO. Further, pure culture work has shown the ability of AMO to biotransform compounds such as natural and synthetic estrogens, as well as bisphenol A (Khunjar et al., 2011; Shi et al., 2004; Sun et al., 2012). Many enzymes besides AMO also act as primary and/or secondary catalysts of oxidation reactions. WWTPs with long solids retention times (SRTs) that support the growth of nitrifiers also support the growth and activity of other slow growing heterotrophs that may be involved in pharmaceutical biotransformations (Vuono et al., 2016). Thus, while greater nitrifying activity may be indicative of environments capable of enhanced biotransformation, other microbial processes may be responsible for those biotransformations, many of which are poorly understood and not well-characterized.

Low DO treatment can impact the rate and extent of pharmaceutical biotransformation by 1) slowing the activity of the microorganisms involved in biotransformation because DO acts as a limiting substrate in respiration or catabolic reactions; and/or 2) selecting for a community that is more (or less) efficient at biotransformation. The objective of this work was to determine the impact of low DO treatment on pharmaceutical biotransformations by nitrifying communities, and specifically to discern between effects of DO concentration on microbial physiology (activity) and on microbial community structure (selection). This work focused on nitrifiers as they are thought to be particularly sensitive to DO limitation and because nitrifiers have been implicated in the biotransformation of many pharmaceuticals. Parent chemostat reactors were used to grow nitrifying enrichment cultures under low (~0.3 mg/L) and high (>4 mg/L) DO concentrations to understand how DO growth conditions impact microbial community structure. Short-term batch experiments using the biomass from the parent reactors were performed under low and high DO conditions to understand how DO concentration impacts microbial physiology. This experimental design allowed us to distinguish between long-term (microbial community structure) and short-term (physiologic) effects of DO on pharmaceutical biotransformations. Allylthiourea-inhibited batch experiments were also conducted to assess the link between nitrification and pharmaceutical biotransformations. This research will contribute to improvements in the design and operation of WWTPs using low DO-adapted metabolisms in wastewater treatment and a better understanding of the impact that energy efficient treatment strategies on pharmaceutical removal.

2. Materials and methods

2.1. Parent reactors and water quality analyses

Two parent chemostat reactors, a high DO and a low DO reactor, were operated for over a year. Each reactor consisted of a 19 L plastic container with a liquid volume of 12 L. The residence time in each reactor was 20 days, and this was achieved by intermittently feeding 25 mL of influent and wasting 25 mL every hour. The SRT was selected to support the growth of slow-growing microorganisms and ensure that nitrifiers, particularly in the low DO reactor, were not washed out. The influent to the reactors was a synthetic medium containing 195 mg-N/L as ammonia-N plus trace nutrients (influent details provided in Table A1, Appendix A). pH was controlled via base addition (30 g/L sodium bicarbonate) to maintain the pH at approximately 7.5. The high DO parent reactor was aerated constantly to maintain a DO concentration above 4 mg/L. The DO concentration in the low DO parent reactor was controlled using real-time feedback from an optical DO probe (Orion RDO® Pro, In Situ Inc.). The DO probe triggered the aerator to turn on when the DO dropped below the setpoint of 0.2 mg/L, which resulted in a DO concentration that fluctuated between 0.2 and 0.4 mg/L. The reactors were operated at room temperature (typically between 21 and 24 °C). Both reactors were seeded with sludge from the Ann Arbor, MI WWTP, which uses an anaerobic-aerobic process to achieve biological phosphorus removal and nitrification. The Ann Arbor WWTP has an SRT of approximately 9 days and operates at a DO concentration of 2–3 mg/L in the aerobic basins. Samples were collected from the parent reactors one or two times per week and analyzed for soluble nitrogen species concentrations (ammonia-N, nitrite-N, and nitrate-N) according to Standard Methods (methods 4500F-NH_3, 4500B-NO_2^-, and 4110; APHA et al., 2005). Samples were filtered through 0.45 μm nitrocellulose filters and stored at 4 °C until analysis (within 48 h of sampling for ammonia- and nitrite-N analyses, and within one week of sampling for nitrate analyses).

2.2. Pharmaceutical selection

The pharmaceuticals selected for investigation included: acetaminophen, acetyl-sulfamethoxazole, atenolol, caffeine, carbamazepine, 17α-ethinylestradiol (EE2), glyburide, ibuprofen, naproxen, sulfamethoxazole, trimethoprim, and venlafaxine (Table 1). These compounds were selected based on their prevalence in WWTP influents and effluents, structural characteristics, and reported low sorption to biomass such that biotransformation is likely the primary mechanism responsible for loss during treatment.

2.3. Pharmaceutical biotransformation batch experiments

Batch experiments were performed using the biomass taken from the low and high DO parent reactors (after over a year of operation) to determine the extent and rate of pharmaceutical biotransformation by each parent community. Batch experiments were performed for each parent biomass at two different DO concentrations: high DO (constantly aerated) and low DO (DO maintained between 0.2 and 0.4 mg/L). DO control in the low DO batch reactors during the batch experiments was performed by sparging with a mixture of nitrogen gas and air. Optical DO probes (WTW, Weilheim, Germany) were used to monitor the DO concentration throughout the batch experiments. Biomass from the parent reactors was concentrated and washed prior to initiating the batch experiments. For each batch experiment, 500 mL of the mixed liquor from a parent reactor was centrifuged (5 min at 5500 rpm,