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Ammonia-oxidizing microbial communities in reactors with efficient nitrification at low-dissolved oxygen

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ABSTRACT

Ammonia-oxidizing microbial communities involved in ammonia oxidation under low dissolved oxygen (DO) conditions (<0.3 mg/L) were investigated using chemostat reactors. One lab-scale reactor (NS_LowDO) was seeded with sludge from a full-scale wastewater treatment plant (WWTP) not adapted to low-DO nitrification, while a second reactor (JP_LowDO) was seeded with sludge from a full-scale WWTP already achieving low-DO nitrifiaction. The experimental evidence from quantitative PCR, rDNA tag pyrosequencing, and fluorescence in situ hybridization (FISH) suggested that ammonia-oxidizing bacteria (AOB) in the *Nitrosomonas* genus were responsible for low-DO nitrification in the NS_LowDO reactor, whereas in the JP_LowDO reactor nitrification was not associated with any known ammonia-oxidizing prokaryote. Neither reactor had a significant population of ammonia-oxidizing archaea (AOA) or anaerobic ammonium oxidation (anammox) organisms. Organisms isolated from JP_LowDO were capable of autotrophic and heterotrophic ammonia utilization, albeit without stoichiometric accumulation of nitrite or nitrate. Based on the experimental evidence we propose that *Pseudomonas*, *Xanthomonadaceae*, *Rhodococcus*, and *Sphingomonas* are involved in nitrification under low-DO conditions.

1. Introduction

Achieving energy independence has become a critical component of sustainability goals in the wastewater treatment industry. Simultaneously, the industry has to respond to pressures from increasingly stringent effluent regulations. In current practice, conventional biological nutrient removal (BNR) systems rely on high dissolved oxygen (DO) concentrations in portions of the treatment plant to accomplish oxidation of organic matter, nitrification, and phosphorus removal. Since aeration in BNR plants can account for nearly half of the energy used in BNR systems (Tchobanoglous et al., 2003), decreasing oxygen supply is one way to reduce energy consumption provided that reactors operated with lower DO can meet discharge regulations. Oxidation ditch-type reactors are one example of existing processes where nitrification occurs at low-DO and phosphorus removal can be

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established by cycling between low-DO and aerobic conditions (Zilles et al., 2002b).

It has been well documented that stable nitrifying reactors can be operated at DO concentrations below 0.5 mg/L (Bellucci et al., 2011; Liu and Wang, 2013; Park and Noguera, 2004), but it remains unclear which organisms are responsible for nitrification in such low-DO environments. Initial studies (Park and Noguera, 2004) focused only on ammonia oxidizing bacteria (AOB). However, the first study of ammonia-oxidizing archaea (AOA) in activated sludge (Park et al., 2006a) demonstrated their presence in full-scale oxidation-ditch type reactors with long solids retention times (SRTs) and having large portions of the activated sludge basin operated with low DO to achieve simultaneous nitrification and denitrification (SND). The same study did not find detectable amounts of AOA in treatment plants operated with conventional high-DO nitrification stages, opening up the possibility that AOA may play a role in low-DO nitrification. In other environments, studies comparing the relative abundance of AOB and AOA suggest that AOA may be more abundant than AOB when the concentrations of oxygen and ammonia are low (Erguder et al., 2009; Francis et al., 2005; Labrenz et al., 2010), in agreement with the initial observations in activated sludge (Park et al., 2006a). Nevertheless, recent studies regarding low-DO nitrification (Bellucci et al., 2011; Liu and Wang, 2013) have shown undetectable levels of AOA in low-DO lab-scale reactors. Thus, the comparative analyses of AOB and AOA in activated sludge have not produced consistent results of the effect of oxygen on the populations of AOB and AOA.

Other microorganisms that could be functionally important for nitrification in low-DO reactors, but have not been studied in detail, are anaerobic ammonia-oxidizing (anammox) bacteria and heterotrophic nitrifiers. Treatment processes that take advantage of the anaerobic metabolism of anammox organisms have been primarily developed for sidestreams with high ammonia concentrations (Strous et al., 1997), although there is interest in developing mainstream processes in which anammox contribute to nitrification in low-DO reactors (De Clippeleir et al., 2011). The influence of heterotrophic nitrification in activated sludge remains poorly understood. Most research regarding heterotrophic nitrification has focused on understanding whether specific heterotrophs have the ability to oxidize ammonia (Kim et al., 2005; Papen et al., 1989; Zhang et al., 2011), and very little research has focused on the influence heterotrophic nitrifiers may have in full-scale engineered systems.

In this study we aimed at investigating the microorganisms responsible for ammonia oxidation in low-DO reactors that received ammonia as the sole energy source. Specifically, we used quantitative PCR (qPCR) targeting the ammonia monooxygenase gene (*amoA*) to evaluate the relative abundance of AOA and AOB in low-DO lab-scale reactors seeded with sludge from different full-scale plants. In addition, we used tag pyrosequencing targeting the small subunit rRNA gene to gain a more comprehensive understanding of the communities in the reactors, and culturing to search for novel organisms potentially playing a role in low-DO nitrification.

2. Material and methods

2.1. Chemostat operation

Two chemostat reactors were operated in this study. Each reactor consisted of a 2 L glass vessel loosely sealed with parafilm. The influent flow rate in each reactor was 8.33 mL/h, producing 10-day solids and hydraulic retention times. DO was monitored and recorded every 5 min using a portable meter and an optical probe (WTW Multi 3410 Multiparameter Meter). The DO was maintained in the reactors below 0.3 mg/L by constantly flushing the headspace with a mixture of air and compressed nitrogen gas, and facilitating oxygen diffusion into the liquid by gentle mixing with a magnetic stirrer. Adjustments to the flow rate of each gas into the headspace were performed daily to ensure the desired DO was achieved. The reactors were fed a synthetic medium containing 30 mg NH_4^+-N/L, as described elsewhere (Park and Noguera, 2004). Under these conditions, the concentration of total suspended solids was always less than 10 mg/L (Park and Noguera, 2004). The reactors were operated at room temperature (between 21 and 23 °C).

2.2. Seed sludge

One of the ammonia-fed reactors (NS_LowDO) was seeded with sludge from the Nine Springs wastewater treatment plant (WWTP) (Madison WI), which uses a modified University of Cape Town process designed to achieve biological phosphorus removal (Zilles et al., 2002a). The plant is not operated to achieve significant denitrification, and therefore, an internal nitrate recycle flow is not used. The Nine Springs WWTP has approximately a 10-day SRT and 11 h hydraulic retention time. In the aerobic stage, DO reaches concentrations greater than 2 mg/L (Park et al., 2006b).

The other ammonia-fed reactor (JP_LowDO) was seeded with sludge from the Jefferson Peaks WWTP in Oak Ridge, NJ. Jefferson Peaks uses an MBR operated to achieve SND, in which greater than 90% of the treatment system has DO less than 0.2 mg/L (Littleton et al., 2013).

2.3. Sample collection and analytical tests

Biomass samples from the reactors were saved weekly by filtering between 50 and 100 mL of the cultures through 0.22-μm membrane filters (Millipore Laboratories, Billerica, MA) and then storing at −80 °C until DNA extraction. Culture supernatants were collected weekly, filtered through 0.45-μm membrane filters (Millipore Laboratories, Billerica, MA) and stored at −80 °C until analysis.

DNA was extracted using UltraClean® Soil DNA Isolation Kits (MoBIO Laboratories, Carlsbad, CA) and subsequently purified via ethanol precipitation. Extracted DNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and stored at −80 °C. Multiple extraction methods were tested since some degree of bias in community structure can be associated with the DNA extraction and purification method used (Bergmann et al.,