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Microbial activity balance in size fractionated suspended growth biomass from full-scale sidestream combined nitritation-anammox reactors

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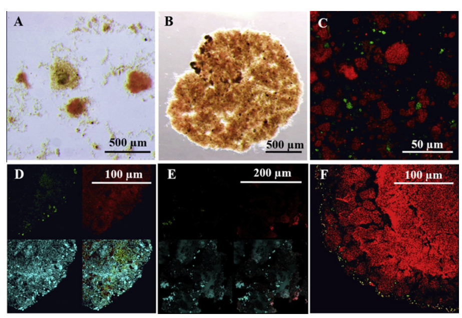
HIGHLIGHTS

- Abundance, distribution and activity of AOB and anammox in aggregates were studied.
- AOB were enriched in the floccular fraction, while anammox were enriched in granules.
- Higher ratios of anammox/AOB were found in plants with hydrocyclones.
- Substantial functional and population-level segregation in aggregates were found.

GRAPHICAL ABSTRACT



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ABSTRACT

The purpose of this study was to determine the abundance, distribution and activity of aerobic ammonia-oxidizing bacteria (AOB) and anammox in size fractionated aggregates from full-scale suspended growth combined nitritation-anammox sidestream reactors. Plants with or without a cyclone device were also studied to assess a purported enrichment of anammox granules. Specific aerobic ammonium oxidation rates ($p = 0.01$) and specific oxygen uptake rates ($p = 0.02$) were significantly greater in flocs than in granules. AOB abundance measured using quantitative FISH was significantly higher in flocs than in granules ($p = 0.01$). Conversely, anammox abundance was significantly greater in granules ($p = 0.03$). The average ratio of anammox/AOB in systems employing hydrocyclone separation devices was 2.4, significantly higher ($p = 0.02$) than the average ratio (0.5) in a system without a hydrocyclone. Our results demonstrate substantial functional and population-level segregation between floccular and granular fractions, and provide a key corroboration that cyclone separation devices can increase anammox levels in such systems.

1. Introduction

Single-stage combined nitritation-anammox processes select for both partial nitritation and anammox in the same reactor. Such processes are dependent upon the balanced activity and interactions of both aerobic ammonia-oxidizing bacteria (AOB) and anaerobic ammonia-oxidizing bacteria (anammox), which

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have been confirmed as an attractive option for nitrogen removal in high-strength wastewater due to low operational costs and energy consumption (Kartal et al., 2010). In the last few years, this process has evolved from lab reactors to full-scale wastewater treatment plants (Christensson et al., 2013; Joss et al., 2009). However, process instabilities and extended start-up periods have slowed widespread implementation of this promising technology (Joss et al., 2011). Increased knowledge of distribution and activity of microbial populations in combined nitritation-anammox biomass may assist in overcoming these barriers. The present study advances our understanding of the role of different microbial aggregates in combined nitritation-anammox processes.

Microbial aggregates in wastewater treatment systems are often in the form of loosely structured flocs, or dense granules. Granules are compact aggregates that do not flocculate under reduced hydrodynamic shear, have an approximately spherical shape, and settle significantly faster than flocs (Lemaire et al., 2008). In selected common unit processes at wastewater treatment plants (WWTPs), flocs and larger aggregates commonly coexist (Carvalho et al., 2006; Innerebner et al., 2007). Recent studies suggest that small and large aggregates may play different functional roles in the systems, particularly in granular nitritation-anammox processes (Gilbert et al., 2013; Hubaux et al., 2015; Vlaeminck et al., 2008; Volcke et al., 2010). Vlaeminck et al. (2010) demonstrated that the rate of activity and abundance of anammox increased with increasing aggregate size in biomass obtained from three combined nitritation-anammox reactors. Two of the reactors were composed primarily of large (>1 mm) dense granules and all of them contained a significantly higher fraction of granules than those assessed in this study. These results suggest that distribution of aggregate size and types may be important for successful nitrogen removal in full-scale suspended growth combined nitritation-anammox reactors. Thus far, however, our knowledge of microbial activity and population segregation in such suspended growth reactors is lacking.

Enhanced biomass retention in combined nitritation-anammox reactors is a considerable advantage, considering the slow growth rate of anammox. Hydrocyclone separation units are purported to be a good choice for effective retention of anammox-laden biomass, and such cyclones have recently seen deployment in the DEamMONification (DEMON) process, a suspended growth combined nitritation-anammox process (Klein et al., 2013; Lackner et al., 2014). Activity tests and modeling work indicate a doubling of anammox/AOB mass ratio in reactors with such cyclones (Wett et al., 2010b). However, to date no direct quantification of microbial populations in systems with and without hydrocyclones has verified this result.

In this study, several full-scale sidestream combined nitritation-anammox reactors with or without hydrocyclones for enhanced biomass separation were selected to assess the distribution and ratio of AOB and anammox in different microbial aggregates using quantitative fluorescence in situ hybridization (FISH) technology. Furthermore, the activity of AOB and anammox were also investigated in batch aerobic and anoxic assays. The specific objectives of this study were twofold: 1) to characterize population and activity segregation between aggregate fractions in biomass from full-scale suspended growth combined nitritation-anammox reactors; and 2) to quantitatively assess via molecular tools the increased retention of anammox biomass in systems employing hydrocyclone separators.

2. Materials and methods

2.1. Full-scale combined nitritation-anammox biomass

Representative mixed liquor samples were obtained from three full-scale combined nitritation-anammox reactors treating anaerobic digester supernatant: Werdhölzli WWTP (Zürich, Switzerland), Limmattal WWTP (Zürich, Switzerland) and ARA Thunersee WWTP (Thun, Switzerland). An overview of typical operating conditions for these plants is provided in Table 1. Both Limmattal and Thunersee reactors are operated with hydrocyclones intended to enrich granules. Hydrocyclones make use of centrifugal forces to select appropriate solids retention time (SRT) and retain denser microbial aggregates in the system (Wett et al., 2010a). The waste activated sludge is fed to a hydrocyclone from where the overflow containing floccular biomass is wasted and the underflow enriched in granules is recycled to the reactor (Nyhuis, 2013; Wett et al., 2013). Werdhölzli is operated as a sequencing batch reactor (SBR). Samples taken at different time points are referred to herein as Werd1 (Werdhölzli WWTP; April 25, 2012), Werd2 (Werdhölzli WWTP; October 5, 2012), Limma (Limmattal WWTP; May 8, 2012) and Thun (Thunersee WWTP, October 4, 2012). After sampling from the plant, the biomass was immediately separated into two fractions using a sieve with 0.5 mm pore size, and then washed with deionized water to remove residual dissolved reactor compounds. Samples were fixed immediately for quantitative FISH. Unfixed samples were stored temporarily at 4 °C prior to series of activity experiments (less than one week).

2.2. Aerobic and anoxic batch activity experiments

Aerobic and anoxic batch experiments were carried out to examine AOB and anammox activities. Both assays were adapted from the methodology employed by (Vlaeminck et al., 2007). Digester supernatant collected from the Werdhölzli WWTP was used for activity tests. The supernatant composition is as follows: pH 8.3 ± 0.2, NH_4^+-N 750 ± 25 mg/L, NO_2^--N < 0.5 mg/L, NO_3^--N 2 ± 1 mg/L, COD 450 ± 50 mg/L. For the ***aerobic activity tests***, biomass (a final VSS (volatile suspended solids) of around 2 g/L) was incubated in 250 mL Erlenmeyer flasks at 28 °C, with ammonium (using diluted digester supernatant, with a final NH_4^+-N concentration of 100 mg/L) as the substrate. A buffering solution (final concentrations 1 g $NaHCO_3$/L, 3.4 g KH_2PO_4/L and 4.4 g K_2HPO_4/L) was also added. Gastight anoxic serum vials (150 mL) were used in the ***anoxic batch experiments*** at 32 °C, with ammonium (diluted digester supernatant, with a final NH_4^+-N concentration of 100 mg/L) and nitrite (sodium nitrite, with a final NO_2^--N concentration of 100 mg/L) as substrates; a buffering solution was also added (final concentrations 1 g $NaHCO_3$/L and 0.04 g KH_2PO_4/L). The serum vials were sealed with rubber stoppers and flushed with N_2 gas for 30 min to obtain anoxic conditions. During incubation,

Table 1
Overview of the three full-scale suspended growth combined nitritation-anammox reactors investigated in this study. Operational conditions represent typical values for each reactor, per communications with system operators. All systems are in Switzerland.

Parameters	Werdholzli WWTP	Limmattal WWTP	Thunersee WWTP
Reactor type	SBR	SBR, with hydrocyclone[*]	SBR, with hydrocyclone[*]
Volume, m^3	1400	250	700
Influent NH_3^+-N, mg N/L	650 ± 50	1100	1100 ± 75
Nitrogen removal rate, g N/(L d)	0.9	–	0.3
Effluent NH_3^+-N, mg N/L	30 ± 10	70	155 ± 130
pH	7.8 ± 0.1	–	7.85–8.0
Temperature	30 ± 3	–	15–35
DO, mg/L	–	0.2–0.3	<0.4

[*] DEMON process.