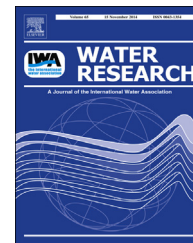




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Impact of coexistence of flocs and biofilm on performance of combined nitrification-anammox granular sludge reactors

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ABSTRACT

Nitrogen (N) removal from high-strength wastewater can be accomplished in single-stage combined nitrification-anammox reactors with suspended growth biomass composed of floccular sludge, granular sludge, or of any mix of these 2 different sludge fractions. To date, the influence of floccular biomass on granular sludge reactor performance and stability has not been investigated experimentally or numerically. To address this knowledge gap, two 1D multi-species models were developed in Aquasim to assess the importance of small levels of flocs in putatively granular sludge combined nitrification-anammox reactors for different bulk oxygen concentrations and organics loads. The models included the growth and decay of aerobic ammonium-oxidizing organism (AOO), nitrite-oxidizing organisms (NOO), heterotrophic organisms (OHO), and anammox organisms (AMO) in exclusively granular sludge reactors, and in granular sludge reactors with small levels (~5% of total biomass) of flocs. While maximum N removal efficiencies were similar for both model structures, floc addition led to a lower optimal dissolved oxygen concentration (DO) as well as a narrower maximum N removal peak, suggesting that small levels of floccular material may decrease process robustness to bulk oxygen changes. For some DO levels, this led to drastic efficiency drops. Furthermore, floc addition also led to substantial segregation in activity and microbial population distribution, with AOO, NOO and OHO concentrated in flocs and AMO concentrated in granules. Increased organic loading (COD:N = 4:3) improved maximum N removal efficiency in both model structures, but yielded substantially different predictions for optimal DO setpoint and process robustness to variations in DO. Taken together, our results indicate that even small levels of floccular biomass in biofilm reactors can have profound implications for reactor performance and optimization and for segregation of linked microbial processes, and suggest that the common practice of neglecting small levels of floccular material in biofilm models and in practice may lead to erroneous predictions.

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1. Introduction

Processes combining aerobic and anaerobic ammonium oxidation (so-called 'nitrification anammox') have been successfully implemented in sidestream reactors at full-scale wastewater treatment plants (Christensson et al., 2013; Joss et al., 2009; Wett, 2007) leading to lower energy requirements for nitrogen (N) removal from high-strength wastewater with low C/N ratios. However, unexplained process instabilities and slow start-ups continue to present challenges to implementation by practitioners (Joss et al., 2011). To overcome these barriers, a fundamental understanding of the underlying microbial mechanisms and spatial localization of microbial populations and activities is needed. The present study provides new insight into the role of different microbial aggregates in the autotrophic N removal process.

Autotrophic N removal can be carried out in various reactor configurations. Enhanced biomass retention in granular sludge reactors is a considerable advantage over other reactor configurations considering the slow growth rates of anammox. Even within granular sludge reactors, however, disparate types of aggregates can be present. Depending at least in part on granulation methodology, inoculation procedure, and mixing, the aggregates formed in granular sludge reactors can be flocs, small granules, large granules, or any mix of these (Arrojo et al., 2006; Schaubroeck et al., 2012; Vangsgaard et al., 2013; Vlaeminck et al., 2009). Flocs are defined as loose and permeable aggregates composed of microcolonies enmeshed in exocellular polymers (Scuras et al., 1998). Granules are compact, dense aggregates with an approximately spherical shape that settle significantly faster than flocs (Lemaire et al., 2008). It has been observed that flocs are present in putative granular sludge reactors and reciprocally granules in suspended sludge reactors, suggesting that these two types of biomass aggregates coexist more often than a priori supposed (Innerebner et al., 2007; Vlaeminck et al., 2009, 2010; Winkler et al., 2012b). Recent studies insinuate that small and large aggregates play different functional roles in the nitrification-anammox process (Vlaeminck et al., 2010; Volcke et al., 2010, 2012; Winkler et al., 2011), suggesting that the granule size distribution might be important for successful N removal. However, the influence of flocs on granular sludge reactor performance and stability has not been investigated yet experimentally nor numerically.

Successful autotrophic N removal is effectively a microbial balancing act that relies on the coordinated activities of the different types of organisms involved. For instance, sufficient oxygen should be available for aerobic ammonium oxidizing organisms (AOO), but excess oxygen should be avoided to prevent the growth of nitrite oxidizing organisms (NOO) and to avoid the direct reversible inhibition of anammox (AMO) by oxygen. In addition to these autotrophic organisms, heterotrophic organisms (OHO) seem to be involved in the process by growing either on decay products (Okabe et al., 2005) or on inlet exogenous organic carbon. Without any external organic source, it has been observed that OHO can account up to 23% of the total biomass in anammox biofilms (Ni et al., 2012) and up to 50% in autotrophic nitrifying biofilm (Kindaichi et al., 2004). However, interactions between AMO and OHO and the

effect of OHO activity on anammox processes are not clear yet (Kuenen, 2008; Lackner and Horn, 2013). On the one hand, organics may improve N removal efficiency in some instances via heterotrophic denitrification (Udert et al., 2008) or organotrophic anammox activity (Güven et al., 2005; Winkler et al., 2012b). On the other hand, organics can hamper the anammox process through competition between OHO and AOO for oxygen (Okabe et al., 1995), through inhibition (Jin et al., 2012) or through decreased SRT (Winkler et al., 2012a). The specific influence of different levels of exogenous organic carbon on N removal performance in granular combined nitrification-anammox reactors, with or without small fractions of floccular biomass, is as yet unclear.

This study aimed to assess the response in overall performance and in organization of microbial communities to different exogenous organic carbon loadings and bulk oxygen concentrations in two single-stage combined nitrification-anammox reactor configurations: a reactor containing exclusively granules, and the other containing ~95% (weight) of the biomass in form of granules and ~5% in form of flocs. To achieve this, we developed two multi-species 1D models that aimed to compare N removal efficiency in these two granular sludge reactors. In a first step, reactor efficiencies were computed for different bulk oxygen concentrations. In a second step, the impact of soluble and readily degradable organics in the inlet was investigated.

2. Materials and methods

2.1. Model structure

Two 1-D multispecies models were developed in parallel with the Aquasim software (Reichert, 1995) considering two different biomass aggregate structures. The first model included only granular biomass, while the second model structure included both granular and floccular biomass. Two primary properties differentiated these two aggregate fractions:

- 1) Biomass distribution was assumed to be heterogeneous in granules as it is standard in biofilm models, and homogeneous in flocs due to flocculation and deflocculation of floccular biomass; and
- 2) Granules were considered to be mass transfer (diffusion) limited, while mass transfer limitations were not explicitly modeled in flocs.

2.2. Granules modeling

Granules were considered to be symmetrical spherical biofilms in which the concentration of all the compounds was constant for any given radius. Granules had a constant porosity of 80% and grew from an initial radius of 50 μm up to 750 μm . The biofilm was assumed to be "rigid," meaning that there was no diffusive mass transport of solids in the biofilm. The only movement of solids was therefore caused by biomass growth in the biofilm. Granular biomass (particulate phase) was initially split into four equal fractions for each type

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