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The effect of inorganic carbon on microbial interactions in a biofilm nitritation–anammox process

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ABSTRACT

The overarching goal of this study was to determine the role of inorganic carbon (IC) in influencing the microbial ecology, performance and nitrogen turnover by individual microbial communities of a biofilm based combined nitritation–anammox process. IC limitation was transiently imposed by reducing the IC input from 350% to 40% of the stoichiometric requirement for 40 days. The principal impact observed during IC limitation was the overgrowth of nitrite oxidizing bacteria (NOB) at the expense of anaerobic ammonia oxidizing bacteria (AMX). On the other hand, the concentrations of ammonia oxidizing bacteria (AOB) were relatively stable during the imposition of and recovery from IC limitation. The resulting dominance of NOB, in terms of their concentration and contribution to nitrite consumption over AMX, resulted, in turn, in a decrease in overall nitrogen removal from 78 ± 2.0% before IC limitation to 46 ± 2.9% during IC limitation. Upon recovery back to non-limiting IC input, it took an inordinately long time (about 57*HRT) for the N-removal to recover back to pre-limitation conditions. Even after recovery, NOB were still persistent in the biofilm and could not be washed out to pre-limitation concentrations. The emission of nitrous oxide (N_2O) and nitric oxide (NO), likely from AOB, transiently increased in concert with transient increases in ammonia and hydroxylamine concentrations during the period of IC limitation. Therefore, an unintended consequence of IC limitation in nitritation–anammox systems can be an increase in their greenhouse gas footprint, in addition to compromised process performance. Most emphasis to date on nitritation and anammox studies has been on the *nitrogen* cycle. The results of this study demonstrate that the differing strategies used by AOB, NOB and AMX to compete for their preferred assimilative *carbon* source can also significantly influence the microbial ecology, performance and carbon footprint of such processes.

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1. Introduction

Anaerobic ammonia oxidation (anammox) combined with partial nitritation (nitritation–anammox) is a cost-effective and energy efficient model for biological nitrogen removal (BNR) (Jetten et al., 2001). In the nitritation–anammox process, about half of the influent ammonia (NH_3) is converted to nitrite (NO_2^-) by ammonia oxidizing bacteria (AOB), followed by the anaerobic oxidation of the residual NH_3 to dinitrogen gas (N_2) by anaerobic ammonia oxidizing bacteria (AMX), with the production of a small amount of nitrate (NO_3^-) (Hao et al., 2002). Nitritation–anammox processes have been successfully applied at full-scale primarily for anaerobic digestion reject water post filtration (filtrate) or post centrifugation (centrate) at temperatures close to 30 °C, with nitrogen removal rates in the range 0.50–7.1 kg N/m^3/d (Joss et al., 2009; Kampschreur et al., 2009; van der Star et al., 2007).

The stability of engineered nitritation–anammox processes relies upon achieving an appropriate balance between the activities of the principal microbial protagonists therein, including AMX and AOB, while concomitantly minimizing the activity of nitrite oxidizing bacteria (NOB). Of these, AMX and AOB compete for NH_3 as an electron donor; AMX and NOB compete for NO_2^- as electron acceptor and donor, respectively; and AOB and NOB compete for oxygen (O_2) as preferred electron acceptor. Further, AMX, AOB and NOB all compete for inorganic carbon (IC) as the preferred assimilative carbon source. IC also serves to neutralize the acidity produced during AOB mediated NH_3 oxidation. While the impact of different N-species on AMX, AOB and NOB activity and overall process performance of anammox and nitritation–anammox processes have been widely considered, the significance of inorganic carbon supply in driving the microbial interactions among AMX, AOB and NOB has received limited attention thus far (Chen et al., 2012).

From a practical perspective, studies with high nitrogen loaded biological nitrogen removal (BNR) processes have shown that the IC levels in wastewater streams with high NH_4^+–N/COD ratio (1:1 to 4:1) can negatively impact AOB rates more than NOB rates (Guisasola et al., 2007). The negative impact of IC limitation on AOB was indeed reversed upon a pulse addition of bicarbonate (Guisasola et al., 2007). IC can also be limiting during the excessive use of flocculants such as ferric chloride during primary clarification (Tchobanoglous et al., 2003), and can further result from excessive CO_2 stripping during secondary treatment (Wett and Rauch, 2003). AOB, NOB and AMX are expected to respond differently to IC limitation. Both AOB and NOB to up-regulate CO_2 fixation pathways under IC limitation and NOB can further pursue a heterotrophic mode of growth during limiting concentrations of CO_2 (Bock, 1976; Luecker et al., 2010; Smith and Hoare, 1968; Wei et al., 2004). In contrast to AOB and NOB, AMX are not known to possess such adaptive mechanisms (Strous et al., 2006). Therefore, it could be hypothesized that AMX are likely the most vulnerable members of nitritation–anammox processes subject to IC limitation.

In addition, the impact of nitritation–anammox processes during the imposition of and recovery from IC limitation on the potential for emissions of nitric oxide (NO) and nitrous oxide (N_2O) needs to be characterized. Both gases have been implicated as products of imbalanced metabolism, especially in AOB (Yu et al., 2010). Although N_2O emissions from nitritation and anammox processes have been investigated (Kampschreur et al., 2009; Weissenbacher et al., 2010), the impact of IC limitation on gaseous-N emissions from these processes has not been studied.

Therefore, the overarching goal of this study was to determine the impact of IC limitation on the microbial ecology, performance and nitrogen turnover by individual microbial communities of a biofilm based combined nitritation–anammox process. The specific objectives were to: 1) track the changes in the microbial composition of the biofilm in response to the imposition of and recovery from IC limitation, 2) develop a mass balance based approach to quantify the specific contributions of AOB, NOB and AMX to overall substrate consumption profiles in the nitritation–anammox process, 3) investigate NO and N_2O emissions from the nitritation–anammox process during steady-state operation with non-limiting IC supply, followed by the imposition of and recovery from IC limitation.

2. Materials and methods

2.1. Reactor operation

A 6L lab-scale nitritation–anammox bioreactor was operated in biofilm mode with a hydraulic retention time (HRT) of 1.5 days for approximately 240 days at an influent N-loading of 0.32 ± 0.020 kg NH_4^+–N/m^3/d. About 2000 AnoxKaldnes K1 biocarriers were used for biofilm attachment at a volumetric fill capacity of 33%. The reactor temperature was maintained at 35 °C using a hot-plate and aeration was provided using laboratory air controlled at 0.15 LPM using an analog gas flow meter. Prior to the more intensive monitoring conducted during this study, the reactor was operated for 140 days (Figure S1, *Supplementary information*). After preliminary operation, this study consisted of pre-limitation monitoring (Phase I), followed by 40 days of IC limitation (Phase II), and 86 days of recovery (Phase III). During Phases I and III, the IC input was 0.45 kg HCO_3^-–C/m^3/d, corresponding to about 350% of the theoretical carbon demand for maintaining the biomass concentration and nitrogen removal in the nitritation–anammox process (as shown in *Supplementary information*). During Phase II, the IC input was reduced to 0.050 kg HCO_3^-–C/m^3/d or about 40% of the stoichiometric requirement. The influent media also consisted of 18 mM $(NH_4)_2SO_4$, 0.20 mM KH_2PO_4, 1.3 mM $MgSO_4 \cdot 7H_2O$, 1.2 mM $CaCl_2.2H_2O$, 1 mL each of trace elements solution as described previously (Park et al., 2010). To ensure a near optimal common physiological pH for the AOB. and AMX in the reactor (Park et al., 2010), the pH was maintained at 7.5 ± 0.03 with 1 M $NaHCO_3$ during Phase I and III, and with 1 M NaOH during Phase II.

Reactor performance was monitored by measuring the concentrations of ammonia (potentiometry, Thermofisher, Pittsburgh, PA), nitrite (diazotization-colorimetry), nitrate (potentiometry, Fisher, Waltham, MA) all according to Standard Methods (Eaton et al., 2005) and hydroxylamine (colorimetry) (Frear and Burrell, 1955) three times a week. Gaseous