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Coupling of partial nitritation and anammox in two- and one-stage systems: Process operation, N_2O emission and microbial community

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ABSTRACT

Advances in research and technological development in the field of wastewater treatment encourage the implementation of engineered autotrophic nitrogen removal (ANR) systems based on the coupling of partial nitritation (PN) and anaerobic ammonium oxidation (anammox). Such processes can be conducted in two independent dedicated reactors (i.e., two-stage system) or, alternatively, in the same reactor under limited aeration (i.e., one-stage system). In this investigation, both configurations were successfully tested using the sequencing batch reactor (SBR) technology. Processed wastewater was supernatant from a sewage sludge anaerobic digester containing about 1 g NH_4^+-N/L and 0.3 g PO_4-P/L. Pre-conditioning of the supernatant through dilution and magnesium phosphates (e.g., struvite) precipitation favored the anammox process performance under both configurations. The N loading rate (NLR) applied in the PN reactor was $\leq$1.3 g N/(L·d) with nitrite production efficiencies of about 48%, whereas the N removal rate (NRR) in the anammox reactor was 0.43–0.56 g N/(L·d). On the other hand, in the one-stage reactor, the NRR was approximately 0.27 g N/(L·d). Estimated emissions of nitrous oxide (N_2O) in such bioreactors ranged from 0.4 to 4.6% of the N loaded. Comparatively, similar NRRs were achieved for both reactor configurations but the one-stage system used a smaller reaction volume, ran at a lower NLR, and emitted N_2O at a lower rate than the two-stage system. The microbial community in both systems was dominated by aerobic ammonium-oxidizing bacteria of the genus *Nitrosomonas* and the anammox species Ca. *Brocadia sinica*.

1. Introduction

Liquid effluents such as anaerobic digester supernatants, landfill leachates, and some industrial waste streams are rich in nitrogen (N), mostly as ammonium (NH_4^+), and contain low amount of readily biodegradable organic carbon (OC) (Van Hulle et al., 2010; Wett et al., 2009). This low OC:N ratio determines the kind of treatment potentially applicable to reduce their N content. The conventional bioprocess of nitrification-denitrification (NDN) targeting N removal by converting the ammonium into dinitrogen gas (N_2) is inefficient in such scenario owing to the high energy demand for aeration and the requirement of an external biodegradable OC source for completing denitrification (Malamis et al., 2014). Alternatively, different processes aiming to N recovery are applicable (Mehta et al., 2015), but their final interest usually increases with the N content in the wastewater. In this context, completely autotrophic N removal (ANR) based on anaerobic ammonium

Abbreviations: ALK, alkalinity; anammox, anaerobic ammonium oxidation; ANR, autotrophic N removal; AOB, ammonium-oxidizing bacteria; BLR, biomass loading rate; BOD_5, 5-d biochemical oxygen demand; COD, chemical oxygen demand; DO, dissolved oxygen; EC, electrical conductivity; F, flow rate; FA, free ammonia; FNA, free nitrous acid; HRT, hydraulic residence time; IC_{50}, half maximal inhibitory concentration; N, nitrogen; NDN, nitrification-denitrification; NLR, N loading rate; NOB, nitrite-oxidizing bacteria; NPE, nitrite production efficiency; NPR, nitrite production rate; NRE, N removal efficiency; NRR, N removal rate; OC, organic carbon; OTU, operational taxonomic unit; P, phosphorus; PN, partial nitritation; RAA, relative anammox activity; SBR, sequencing batch reactor; sNRR, specific NRR; TIC, total inorganic carbon; TKN, total Kjeldahl N; TS, total solids; VER, volume exchange ratio; VS, volatile solids; VVM, volume per volume per minute; WWTP, wastewater treatment plant.

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oxidation (anammox) is particularly interesting in terms of energy consumption if such nitrogenous content is lower than 2 g NH_4^+-N/L (Magrí et al., 2013).

Application of ANR implies the combination of both partial nitritation (PN) and anammox processes (Bagchi et al., 2012; Lackner et al., 2014; Van Hulle et al., 2010). For the PN process, target is to aerobically convert ammonium into nitrite (NO_2^-) at a molar rate of 57% (Eq. (1)) according to the typical anammox reaction stoichiometry (Eq. (2); adapted from Strous et al., 1998). Owing to the fact that complete nitrification is usually led by two different microbial groups; i.e., nitritation ($NH_4^+ \rightarrow NO_2^-$) by ammonium-oxidizing bacteria (AOB) and nitratation ($NO_2^- \rightarrow NO_3^-$) by nitrite-oxidizing bacteria (NOB), activity of the second group must be suppressed to avoid production of nitrate (NO_3^-). Several methods have been suggested to control such suppression in nitrifying bioreactors, among them (i) combination of mesophilic temperature (~35 °C) and short solids residence time (Magrí et al., 2007), (ii) high concentration of free ammonia (FA, NH_3) (Magrí et al., 2012b), (iii) high concentration of free nitrous acid (FNA, HNO_2) (Pedrouso et al., 2017), and (iv) low concentration of dissolved oxygen (DO) (Li et al., 2011). Regarding the anammox process, it consists in anoxically oxidize the ammonium into dinitrogen gas using nitrite as the final electron acceptor (Eq. (2)).

$$1\ NH_4^+ + 0.86\ O_2 \rightarrow 0.57\ NO_2^- + 0.43\ NH_4^+ + 0.58\ H_2O + 1.14\ H^+ \quad (1)$$

$$1\ NH_4^+ + 1.32\ NO_2^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 2.03\ H_2O \quad (2)$$

The integrated PN-anammox process can be conducted either in independent dedicated reactors (i.e., two-stage systems) (Dosta et al., 2015; Scaglione et al., 2015; van Dongen et al., 2001) according to Eqs. (1) and (2), or concomitantly in the same reactor (i.e., one-stage systems) (Furukawa et al., 2006; Jeanningros et al., 2010; Sliekers et al., 2002) under DO limiting conditions according to Eq. (3) (Eq. 1+ 2). In these three equations the biomass is not included. Jaroszynski and Oleszkiewicz (2011) reported, for the two-stage systems, higher risk of instability owing to run under high N concentrations but very high potential for process intensification and optimization, as optimal conditions can be provided independently. Otherwise, the same authors described the one-stage systems as more simple in configuration but with higher requirements in process control owing to the complex interaction between microbial populations; i.e., aerobic and anaerobic ammonium-oxidizers, nitrite-oxidizers and heterotrophs. Indeed, microorganisms coexisting in such systems will tend to self-organization in specific structures forming granules or biofilms where conditions are more favorable for their development. The sequencing batch reactor (SBR) technology has widely been used for the implementation of ANR systems considering suspended sludge, granular sludge and fixed biofilms (Daverey et al., 2012; Dosta et al., 2015; Kampschreur et al., 2009; Magrí et al., 2012b; Miao et al., 2016). However, and despite the potential interest, comparative studies involving the two aforementioned engineered configurations (i.e., two-stage vs. one-stage), also including potential environmental impacts like nitrous oxide (N_2O) emission, and microbial community issues, are not frequent in the scientific literature.

$$1\ NH_4^+ + 0.86\ O_2 \rightarrow 0.44\ N_2 + 0.11\ NO_3^- + 1.45\ H_2O + 1.08\ H^+ \quad (3)$$

Because of the slow growth rate of the anammox bacteria, with doubling times of 2.1–11 d at ~30 °C (Lotti et al., 2015; Strous et al., 1998), and their specialized metabolism (Strous et al., 2006), the anammox systems are particularly sensible to process perturbations and inhibitory events. A wide range of compounds have been identified as inhibitors of the anammox process including N substrates (nitrite, ammonium), oxygen, organic matter (usually referred as chemical oxygen demand, COD), salts, heavy metals, phosphate (PO_4), and sulfide (S^{2-}), among others (Jin et al., 2012). In order to ensure appropriate process performance in anammox systems, the inhibitory events must be prevented, and the concentration thresholds for the limiting compounds must be particularly assessed since they are often case-specific varying from one system to another. Concerning the biomass structure, both granules and biofilms act as protective environments, increasing the tolerance to inhibitory compounds in comparison to suspended cell cultures (Flemming et al., 2016; Liu and Tay, 2004).

Conventional biological N removal treatments may emit substantial amounts of greenhouse gases such as N_2O which is formed during both the nitrification and denitrification reactions (Alinsafi et al., 2008; Massara et al., 2017; Tallec et al., 2006). Emission of N_2O in ANR systems has also been reported, although such gas is not produced directly through the anammox metabolic pathway (Strous et al., 2006). Thus, N_2O emission in these systems is mostly related to the activity of both nitrifying and denitrifying microorganisms (Kampschreur et al., 2009; Okabe et al., 2011; Rodriguez-Caballero and Pijuan, 2013). In this regard, emissions have been reported as variable, dependents on reactor operational parameters such as (i) nitrite accumulation, (ii) DO limiting conditions, (iii) biodegradable OC availability, and (iv) pH in the liquid bulk, among others. Additionally, chemical side-reactions may also result in significant N_2O emissions; e.g., nitrite reduction induced by ferrous ion (Kampschreur et al., 2011). The one-stage systems are usually reported as lower N_2O emitters than the two-stage systems (Campos et al., 2016).

The aim of this study is the application of ANR (PN-anammox) for treating supernatant from an anaerobic digester processing municipal sewage sludge by considering two possible configurations; i.e., two-stage and one-stage systems. SBRs are used for the implementation of the process. A comparative assessment is conducted taking into account process performance, N_2O emission, and the potential limiting effect of phosphate. The conditioning of the supernatant based on phosphate precipitation as magnesium phosphate is considered. Additionally, the microbial community within the reactors is characterized by means of 16S rRNA gene high-throughput sequencing.

2. Material and methods

2.1. Inoculum sources

The nitrifying sludge used for conducting PN was enriched from activated sludge obtained from the municipal wastewater treatment plant (WWTP) in Liffré (Brittany, France). Such facility performs N removal by conventional NDN and applying intermittent aeration. The anammox sludge was obtained from an upflow reactor packed with a fabric material to promote the attached growth of the biomass, and continuously fed with synthetic mineral medium, running at the Irstea laboratory in Rennes. The dominant anammox bacterial species was identified as Candidatus *Brocadia sinica* (Connan et al., 2017). Immediately after collecting the sludge from the upflow reactor, the specific N removal rate (sNRR) at 35 °C averaged 1.0 ± 0.2 g N/(g VS·d) (VS, volatile solids).

2.2. Short-term phosphate inhibition test

The short-term effect of phosphate on the activity of the anammox sludge was assessed in a batch test using glass bottles (total volume: 250 mL). The anammox sludge was extracted from