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Research article

Effect of feed starvation on side-stream anammox activity and key microbial populations

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ABSTRACT

The anaerobic ammonium oxidation (anammox) process is widely acknowledged to be susceptible to a wide range of environmental factors given the slow growth rate of the anammox bacteria. Surprisingly there is limited experimental data regarding the susceptibility of the anammox process to feed starvations which may be encountered in full-scale applications. Therefore, a study was established to investigate the impact of feed starvations on nitritation and anammox activity in a demonstration-scale sequencing batch reactor. Three starvation periods were trialled, lasting one fortnight (15 d), one month (33 d) and two months (62 d). Regardless of the duration of the starvation period, assessment of the ammonia removal performance demonstrated nitritation and anammox activity were reinstated within one day of recovery operation. Characterisation of the community structure using 16S rRNA and functional genes specific for nitrogen-related microbes showed there was no clear impact or shift in the microbial populations between starvation and recovery phases.

1. Introduction

Anaerobic digestion is a biological process which aims to convert organic substances into biogas and inorganic compounds. The majority of the inorganic content is in the form of ammonium (NH_4^+). After digestion the sludge is usually dewatered, for example by the means of centrifuges, to reduce the moisture content of the digested sludge. In some countries including Australia further drying of the dewatered sludge is required which can be achieved through processes such as air drying to produce biosolids for beneficial reuse. The ammonium rich dewatered centrate is commonly (or usually) recycled back to the main wastewater treatment plant, which increases the nitrogen load by 10–20% as the dewatered centrate can range between 100 and 1000 g N/m^3 (Lackner et al., 2008). Recently, the anammox process has attracted much attention as it offers many advantages compared to the conventional nitrification-denitrification process for treating high nitrogenous side-stream wastewater (Vlaeminck et al., 2007). These advantages include no external carbon source requirements, minimal surplus sludge production, and reduced greenhouse gas emissions (Lackner et al., 2008; Kartal et al., 2010; Park et al., 2010). As a result, the anammox process is widely accepted as an energy saving and cost-effective alternative to conventional processes and has been implemented at full scale as a side-stream nitrogen removal strategy (Lackner et al., 2014).

In addition to mechanical dewatering, sludge drying lagoons are also a commonly applied for dewatering after anaerobic digestion. The digested sludge is pumped into drying lagoons for solar drying and evaporation (Cheremisinoff, 2002). The ammonium rich supernatant formed from the dewatering of the solids is used as a water cap in order to minimise odour. In South Australia, the Bolivar wastewater treatment plant (WWTP) has the option to operate both sludge drying lagoons and mechanical dewatering as part of the sludge treatment process. The operation of the dewatering

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plant (i.e. centrifuges) is dependent upon the volatile solids (VS) loadings of the drying lagoons. These loadings are monitored to primarily prevent odour generation and to promote stabilisation. Due to the operational costs, mechanical dewatering is only operated if and when necessary, to ensure the drying lagoon loading restrictions are met. The dewatered centrate generated from the centrifuges is returned to the activated sludge process at the main plant, with a portion stored for the operation of a demonstration-scale reactor. Occasionally, the dewatered centrate is diverted to the drying lagoons to maintain the water cap. As a result, the supply of dewatered centrate for anammox treatment can be potentially impacted for long durations at a time (up to two months). Additionally, centrate supply can also be impacted when dewatering plants are offline due to breakdowns including centrifuges, pumps, polymer dosing units, and maintenance periods. While there is the capacity to store centrate for short periods of time (days) for the demonstration-scale reactor, there is currently no storage capacity to store an adequate supply of centrate to last long durations (weeks/months) especially if the nitritation-anammox process was to be implemented at full scale.

The prolonged shortage of feed supply may impact the anammox process. The impacts on the anammox species as well as the ammonia oxidizing archaea/bacteria (AOA & AOB) which are responsible for the precursor step of providing anammox with the substrate nitrite (NO_2^-) needs to be considered. For example, if anammox was to be implemented at WWTPs that have the option to dewater sludge using both mechanical and lagoon drying, or for when dewatering plants are offline.

It is widely documented that anammox bacteria are susceptible to environmental factors given their slow growth rate (Jin et al., 2012). However to date, published data about starvation impacts on nitritation and anammox process are limited and derived from trials conducted at the lab-scale fed with synthetic wastewater. A study by Vlaeminck et al. (2007) investigated the reactivation of oxygen-limited autotrophic nitrification/denitrification (OLAND) biomass from a lab-scale (44L) rotating biological contactor (RBC). While the study demonstrated successful preservation of the biomass (~35 g) with nitrate additives at 20 °C, the reactivation was only conducted at lab scale (100 mL working volume) using batch tests. Laboratory scale batch tests were also conducted by Carvajal-Arroyo et al. (2014) and Wu et al. (2015) to investigate starvation tolerance of anammox bacteria. Caravajal et al. (2014) investigated the impacts that 48 h substrate starvation periods had on anammox activity and demonstrated the re-establishment of feeding resulted in immediate recovery. In addition, Wu et al. (2015) found that in the absence of nitrite, anammox cultures were able to withstand a starvation period of four weeks at 36 °C. Whether or not these observations reflect the recovery and performance of anammox that are in full-scale systems, which are not exposed to synthetic media and potentially starved for a longer duration, remains unclear. Furthermore, quantitative data on the impacts that starvation has on the abundance of key functional microbial organisms in these systems has not been characterised.

Accordingly, the aim of this study was to determine if the anammox and nitrifying populations in a demonstration-scale pilot-plant could survive and regain activity after enduring starvation periods under conditions that are representative of full-scale operation. In this study, starvation was defined by the deprivation of new centrate feed and importantly absence of nitrite (NO_2^-) which is required for the anammox reaction. Three starvation time periods were trialled including Phase I (15 d), Phase II (33 d), and Phase III (62 d). Nitritation and anammox activity were assessed by investigating the ammonium removal contributions and efficiencies during aeration and anoxic phases respectively. In addition, quantitative polymerase chain reaction (qPCR) was used to assess the impact of starvation on key microbial populations by quantifying total bacterial and archaeal 16S rRNA, and six functional genes specific for nitrogen removal including amoA, nxrB, and nirS (Kim et al., 2013).

2. Materials and methods

2.1. Pilot plant set-up

Experiments were conducted in a single 4.7 m^3 sequencing batch reactor (SBR) based on Suez Environnements's Cleargreen™ technology (Fig. 1). The SBR operates the suspended growth process where the nitritation and anammox reactions occur within one SBR, separated by time. To achieve this, the process involves 4 sub cycles comprised of feeding (with dewatered centrate), 5–50 min aeration (for nitritation), and 30–60 min anoxic mixing (for anammox). The four sub cycles are followed by a settling phase (20 min) and decant phase (4–20 min).

The SBR is equipped with a mechanical stirrer and online instrumentation including NH_4^+, and NO_3^- (WTW Varion plus), pH and redox potential (Endress and Hauser, Orbisint), and temperature sensors (Endress and Hauser, iTEMP).

2.2. Operational conditions during reference and recovery periods

On average the influent ammonium concentration of the centrate to the bioreactor was maintained between 400 and 500 mg N/L NH_4^+-N during normal operating periods. The process control system of the pilot plant controlled the aeration phase of the SBR to a target dissolved oxygen (DO) concentration of <1 mg/L. The SBR was operated at 28 °C which was maintained by a reverse cycle water conditioner (Climaveneta) and heating/cooling water jacket that was built into the wall of the SBR.

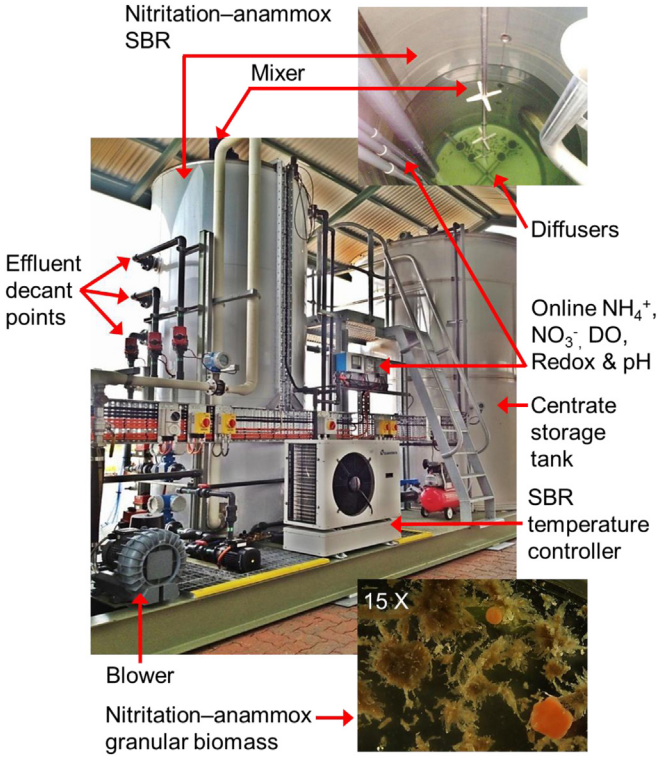

Fig. 1. Nitritation-anammox pilot plant showing the external and internal features of the SBR. Bottom insert showing 15× magnification of the biomass.