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# Nitrate reduction by organotrophic Anammox bacteria in a nitritation/anammox granular sludge and a moving bed biofilm reactor

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ABSTRACT

The effects of volatile fatty acids (VFAs) on nitrogen removal and microbial community structure in nitritation/anammox process were compared within a granular sludge reactor and a moving bed biofilm reactor. Nitrate productions in both systems were lower by 40–68% in comparison with expected nitrate production. Expected sludge production on VFAs was estimated to be 67–77% higher if heterotrophs were the main acetate degraders suggesting that Anammox bacteria used its organotrophic capability and successfully competed with general heterotrophs for organic carbon, which led to a reduced sludge production. FISH measurements showed a population consisting of mainly Anammox and AOB in both reactors and oxygen uptake rate (OUR) tests also confirmed that flocculent biomass consisted of a minor proportion of heterotrophs with a large proportion of AOBs. The dominant Anammox bacterium was Candidatus "*Brocadia fulgida*" with a minor fraction of Candidatus "*Anammoxoglobus propionicus*", both known to be capable of oxidizing VFAs.



## 1. Introduction

Partial nitritation/anammox process is commonly used to remove nitrogen from ammonium rich wastewater. During this process ammonium is firstly oxidized to nitrite by ammonium oxidizers (AOB) (1) after which the remaining ammonium and nitrite are converted into dinitrogen gas by Anammox bacteria (2).

$$NH_4^+ + 1.5O_2 + 2HCO_3^- \rightarrow NH_4^+ + NO_2^- + 2CO_2 + 3H_2O \quad (1)$$

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (2)$$

Anaerobic ammonium oxidizing bacteria (Anammox) are capable of oxidizing ammonium with nitrite as electron acceptor. During this reaction a nitrate yield on ammonium of 0.26 $gNO_3$-N/$gNH_4$-N is expected due to the use of nitrite as electron donor for carbon dioxide reduction to biomass components. This leads to incomplete nitrogen removal in Anammox based nitrogen removal processes (Strous et al., 1999). Until recently it was considered that Anammox bacteria were obligate autotrophs not capable of converting organic carbon substrates. However, lately it was found that Anammox bacteria have the capacity to oxidize volatile fatty acids with nitrate as electron acceptor, while forming ammonium with nitrite as intermediate (Guven et al., 2005; Kartal et al., 2008). Anammox bacteria do not incorporate the fatty acids into biomass, but completely oxidize it into $CO_2$ thereby maintaining a low biomass yield (Kartal et al., 2007a). This organotrophic potential of Anammox bacteria has advantages for wastewater treatment. The conversion of fatty acids by Anammox bacteria prevents a high sludge production and allows the removal of nitrate yielding in lower total nitrogen effluent concentration (Winkler et al., 2012). Heterotrophic denitrifying bacteria oxidize fatty acids with either nitrate or nitrite as electron acceptor with a biomass yield on organic compounds is ca. 0.5 gVSS/gCOD. The produced biomass is an unwanted and costly by-product in wastewater treatment. Investigations showed that the most critical point in the competition between organotrophic Anammox and heterotrophs for acetate is the C/N ratio in the influent. If this ratio increases to values larger than 1 gCOD/gN the slow growing Anammox bacteria seem to lose the competition against general heterotrophs (Chamchoi et al., 2008; Guven et al., 2005; Pathak et al., 2007).

Simultaneous partial nitrification, Anammox and denitrification (SNAD) processes treating wastewater with an approximate ratio of VFA/N of 0.5 gCOD/$gNH_4$-N were developed in different reactor configurations, including UASB (Lan et al., 2011), non-woven rotating biologic contactor (NRBC) (Chen et al., 2009) and a sequencing batch reactor (Xu et al., 2010). All these studies were conducted at temperatures of 30–36 °C under constantly aerated conditions and partly

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