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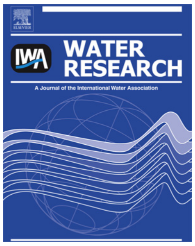

Successful application of nitritation/anammox to wastewater with elevated organic carbon to ammonia ratios

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ABSTRACT

The nitritation/anammox process has been mainly applied to high-strength nitrogenous wastewaters with very low biodegradable organic carbon content (<0.5 g COD·g N^{-1}). However, several wastewaters have biodegradable organic carbon to nitrogen (COD/N) ratios between 0.5 and 1.7 g COD·g N^{-1} and thus, contain elevated amounts of organic carbon but not enough for heterotrophic denitrification. In this study, the influence of elevated COD/N ratios was studied on a nitritation/anammox process with suspended sludge. In a step-wise manner, the influent COD/N ratio was increased to 1.4 g COD·g N^{-1} by supplementing digester supernatant with acetate. The increasing availability of COD led to an increase of the nitrogen removal efficiency from around 85% with pure digester supernatant to >95% with added acetate while the nitrogen elimination rate stayed constant (275 ± 40 mg N·L^{-1}·d^{-1}). Anammox activity and abundance of anammox bacteria (AMX) were strongly correlated, and with increasing influent COD/N ratio both decreased steadily. At the same time, heterotrophic denitrification with nitrite and the activity of ammonia oxidising bacteria (AOB) gradually increased. Simultaneously, the sludge retention time (SRT) decreased significantly with increasing COD loading to about 15 d and reached critical values for the slowly growing AMX. When the SRT was increased by reducing biomass loss with the effluent, AMX activity and abundance started to rise again, while the AOB activity remained unaltered. Fluorescent *in-situ* hybridisation (FISH) showed that the initial AMX community shifted within only 40 d from a mixed AMX community to "*Candidatus* Brocadia fulgida" as the dominant AMX type with an influent COD/N ratio of 0.8 g COD·g N^{-1} and higher. "*Ca*. Brocadia fulgida" is known to oxidise acetate, and its ability to outcompete other types of AMX indicates that AMX participated in acetate oxidation. In a later phase, glucose was added to the influent instead of acetate. The new substrate composition did not significantly influence the nitrogen removal nor the AMX activity, and "*Ca*. Brocadia fulgida" remained the dominant type of AMX. Overall, this study showed that AMX can coexist with heterotrophic bacteria at elevated influent COD/N ratios if a sufficiently high SRT is maintained.

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1. Introduction

The combination of nitritation and anammox is a cost saving alternative to conventional biological nitrogen removal via heterotrophic denitrification (Siegrist et al., 2008; Vlaeminck et al., 2012). Many studies have investigated various types of nitritation/anammox reactors with dedicated attention for the biomass growth mode as biofilm, flocs and/or granules (Vlaeminck et al., 2010), and the influence of process conditions such as the temperature (Vázquez-Padín et al., 2011), dissolved oxygen concentration (Strous et al., 1997), salinity (Liu et al., 2009) and inhibitory substances (Dapena-Mora et al., 2007).

The term "biodegradable organic carbon" refers to the difference between the dissolved COD in the influent and the dissolved COD in the effluent, i.e. the organic carbon, which is actually degraded in a reactor. So far, nitritation/anammox has mainly been applied for wastewaters with high ammonia concentrations and low concentrations of biodegradable organic substances such as digester supernatant, in which the ratio of biodegradable organic carbon to ammonia nitrogen (COD/N) is lower than 0.5 $\mathrm{g\,COD \cdot g\,N^{-1}}$ (Joss et al., 2009; van der Star et al., 2007; Wett, 2007). In contrast to this, complete heterotrophic denitrification via nitrite requires a COD/N ratio of more than 1.71 $\mathrm{g\,COD \cdot g\,N^{-1}}$. However, several wastewaters have COD/N ratios between 0.5 and 1.71 $\mathrm{g\,COD \cdot g\,N^{-1}}$ and thus, contain elevated amounts of organic carbon but not enough for heterotrophic denitrification. If no organic substrate should be dosed, anammox bacteria (AMX) are needed for complete biological nitrogen removal. The application of the nitritation/anammox process has, for example, already been proposed as a cost efficient method for nitrogen removal from urine (Udert et al., 2008; Wilsenach and van Loosdrecht, 2006). Stored urine has a theoretical COD/N ratio of approximately 1 $\mathrm{g\,COD \cdot g\,N^{-1}}$ (Udert et al., 2006) which can increase up to 1.5 $\mathrm{g\,COD \cdot g\,N^{-1}}$ (Bürgmann et al., 2011) due to ammonia volatilization during storage (i.e. a decrease of the ammonium concentration while the COD concentrations stays constant). AMX are also needed for a complete nitrogen removal in animal manure, especially piggery wastewater where the COD/N ratios can vary between 4 and 6.5 $\mathrm{g\,COD \cdot g\,N^{-1}}$, but this is sometimes also too low to achieve complete denitrification (Bernet and Béline, 2009). COD/N ratios of approximately 1 $\mathrm{g\,COD \cdot g\,N^{-1}}$ are also expected in the recently discussed integration of anammox into mainstream wastewater treatment (De Clippeleir et al., 2013; Winkler et al., 2012a).

The possible effects of COD on the nitritation/anammox process depend on the type of COD. Firstly, biodegradable organic carbon fosters the growth of heterotrophic bacteria (HET) which compete with ammonia oxidising bacteria (AOB) for oxygen and with AMX for nitrite. Additionally, more heterotrophic growth will lead to a higher sludge production, which in turn often leads to a higher sludge loss, and thus to a decrease in the sludge retention time (SRT). Sludge loss is the total amount of sludge which leaves the system, i.e. the sludge in the effluent, the sludge in the samples and in case the SRT is regulated, also the sludge which is removed to maintain a certain SRT. A low SRT is particularly critical for the slowly growing AMX. Several studies reported decreasing AMX and AOB activities with high COD concentrations in the influent. Chamchoi et al. (2008) for instance, reported that AMX were outcompeted by HET when the COD/N ratio in the influent of a complete anoxic system was higher than 2.0 $\mathrm{g\,COD \cdot g\,N^{-1}}$ (SRT not mentioned). Zhu and Chen (2001) observed a 70% reduction of AOB activity when the influent COD/N ratio of a nitrification reactor was between 1.8 and 3.5 $\mathrm{g\,COD \cdot g\,N^{-1}}$ (SRT not mentioned). On the other hand, Desloover et al. (2011) successfully operated a partial nitritation reactor with an influent COD/N ratio of 3.70 $\mathrm{g\,COD \cdot g\,N^{-1}}$ (SRT 1.7 ± 0.5 d) and a subsequent anammox reactor with an influent COD/N ratio of 1.60 $\mathrm{g\,COD \cdot g\,N^{-1}}$ (SRT 46 d). None of these studies discussed whether competition for nitrite, oxygen or space (low SRT) caused the observed problems and which were the crucial factors for a successful operation with elevated COD/N influent ratios. Often, important information to judge this, as e.g. the SRT and the type of COD, is missing.

Secondly, specific organic compounds can also be directly toxic for AOB and AMX. Complete inhibition of AOB was for instance observed in the presence of 1000 $\mathrm{mg \cdot L^{-1}}$ propionate, 1000 $\mathrm{mg \cdot L^{-1}}$ butyrate, 2000 $\mathrm{mg \cdot L^{-1}}$ acetate (Gomez et al., 2000), 3.7 $\mathrm{mg \cdot L^{-1}}$ phenol or 1.3 $\mathrm{mg \cdot L^{-1}}$ cresol (Dyreborg and Arvin, 1995). For AMX, Güven et al. (2005) observed a complete loss of activity in the presence of 23 $\mathrm{mg \cdot L^{-1}}$ methanol.

Thirdly, due to the ability of some anammox species to oxidise fatty acids while reducing nitrate (Güven et al., 2005; Kartal et al., 2007), fatty acids could foster AMX activity and increase their competitiveness. Apparently, the fatty acids are not integrated into biomass but are instead oxidised to carbon dioxide, while the carbon for growth is fixed from CO_2 via acetyl-CoA. The oxidation of fatty acids might only occur for the conservation of energy, which could in turn lead to a higher biomass yield for AMX (Kartal et al., 2008), but this hypothesis has not yet been proven.

So far, one-stage nitritation/anammox systems have mainly been operated with influent COD/N ratios below 0.5 $\mathrm{g\,COD \cdot g\,N^{-1}}$. In one of two studies which were performed with elevated COD/N influent ratios in biofilm systems, Chen et al. (2009) observed a significant reduction of the nitrogen removal efficiency (NRE) from 79% to below 52% when the influent COD/N ratio was increased from 0.5 to 0.75 $\mathrm{g\,COD \cdot g\,N^{-1}}$ (synthetic influent, type of COD and SRT not mentioned). In contrast to this, Jia et al. (2012) achieved almost 95% total NRE with an influent COD/N ratio of 1.28 $\mathrm{g\,COD \cdot g\,N^{-1}}$ (synthetic solution with glucose, SRT not mentioned). In case of suspended sludge systems, Lackner and Horn (2013) reached 80–85% total nitrogen removal with an influent COD/N ratio of 1.00 ± 0.27 $\mathrm{g\,COD \cdot g\,N^{-1}}$ (wastewater with high COD concentration, but type of COD and SRT are not mentioned) in a sequencing batch reactor. In our own previous studies with a suspended sludge system and five-times diluted urine (1.4–1.5 $\mathrm{g\,COD \cdot g\,N^{-1}}$) we observed process instabilities (Udert et al., 2008) and a population shift to a new stable state characterised with a low nitrogen elimination rate and limited AMX activity (Bürgmann et al., 2011). In most of these studies the type of COD is not known and especially in case of urine, it is not possible to separate the influence of the elevated COD/N influent ratio from the influence of other wastewater compounds on the process.

Higher SRTs and having several zones with different redox conditions (oxic and anoxic zones) are advantages of biofilm