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<td>Author(s)</td>
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The challenges of mainstream deammonification process for municipal used water treatment

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Abstract: The deammonification process combining partial nitritation and anaerobic ammonium oxidation has been considered as a viable option for energy-efficient used water treatment. So far, many full-scale sidestream deammonification plants handling high-ammonia used water have been in successful operation since Anammox bacteria were first discovered in 1990s. However, large scale application of this process for treating municipal used water with low ammonia concentration has rarely been reported. Compared to the sidestream deammonification process, the mainstream deammonification process for municipal used water treatment faces three main challenges, i.e. (i) high COD/N ratio leading to denitrifiers outcompeting Anammox bacteria; (ii) numerous difficulties in selective retention of AOB over NOB and (iii) sufficient accumulation of Anammox bacteria. Therefore, this paper attempts to provide a detailed analysis of these challenges and possible solutions towards sustainable mainstream deammonification process.

Keywords: Anaerobic ammonium oxidation; deammonification; ammonia-oxidizing bacteria; nitrite-oxidizing bacteria; soluble COD.
29 **Introduction**  

The biological nitrogen removal (BNR) process has evolved from the conventional nitrification-denitrification into anaerobic ammonium oxidation (Anammox) coupled with short-cut nitritation, known as deammonification. In general, the deammonification process can be classified as sidestream for high-ammonia used water (e.g. anaerobic digester liquor) and mainstream for low-ammonia used water (e.g. municipal used water). Large scale application of the sidestream deammonification process has been widely reported (Lackner et al. 2014), while the mainstream deammonification is being explored at its infancy stage (Regmi et al. 2014; Wett et al. 2013).

In general, municipal used water has high COD and low ammonia concentrations at the ratio of 10 to 14 for the raw used water and 7 to 10 for the settled used water (Henze et al. 2008). Evidences suggest that denitrifiers can compete with Anammox bacteria if the soluble COD (sCOD) to nitrogen ratio is larger than 0.5 in the anaerobic stage (Jenni et al. 2014). This means that the COD/N ratio must be reduced significantly for the deammonification process. For such purpose, a process configuration with two stages (e.g. A-B type process) has been adopted for mainstream deammonification of municipal used water. Organic matter should be ideally captured as much as possible at the stage A through various mechanisms (e.g. biosorption, storage etc.), and the stage A effluent with relatively low COD/N ratio is further treated at stage B by mainstream deammonification (Fig. 1). So far, chemically enhanced primary treatment (CEPT) and high rate activated sludge (HRAS) for carbon capture have been explored as the stage A in the mainstream deammonification process for the municipal used water treatment. Moreover, captured organic carbon is directly channeled to anaerobic digester (AD) for high-efficiency production of methane biogas (Kartal et al. 2010).

Selective retention of the main functional species is the major challenge in the stage B of mainstream deammonification process. A key step here is to selectively retain ammonia oxidizing bacteria (AOB) against nitrite oxidizing bacteria (NOB) to sustain nitritation which is prerequisite for Anammox. Anammox bacteria have been known as very slow-growing microbes, thus the retention of Anammox bacteria is another challenge faced in the mainstream deammonification process. Meanwhile, heterotrophic denitrifiers can easily out-compete Anammox bacteria for nitrite in the presence of organic...
matters (Chamchoi et al. 2008; Jenni et al. 2014; Xu et al. 2012). Several approaches have been explored
to promote retention of the slow-growing Anammox bacteria. For example, Anammox bacteria could be
selectively enriched in aggregated biomass (e.g. biofilm and granules), or they could be maintained
through selectively recycling Anammox biomass from the sidestream deammonification unit by cyclone
or screens (Wett et al. 2013).

This review aims to discuss the technical challenges, including (i) high COD/N ratio leading to
denitrifiers outcompeting Anammox bacteria; (ii) stable nitritation which needs selective retention of
AOB over NOB and (iii) sufficient accumulation of Anammox bacteria. Possible solutions towards
sustainable and stable mainstream deammonification for municipal used water treatment will also be
presented.

High COD/N ratio: a hurdle of the mainstream deammonification

Municipal used water typically has high COD/N ratio. This poses a big challenge for mainstream
deammonification as high COD promotes growth of denitrifying bacteria which compete with Anammox
bacteria for nitrite. In fact, according to the Gibbs free energy of denitrification and Anammox reactions,
denitrification is thermodynamically more favorable than Anammox in the presence of soluble
biodegradable COD (Kumar and Lin 2010). Furthermore, denitrifiers have growth rates and yields higher
than Anammox bacteria. Hence, Anammox bacteria cannot compete with denitrifiers in the situation
where soluble COD is sufficiently available.

As can be observed in Table 1, there is an inverse relationship between the nitrogen removal
through Anammox and substrate COD/N ratios. In order to sustain growth of Anammox bacteria against
denitrifiers, the ratio of biodegradable COD/N should be controlled at the level as low as possible, e.g. it
has been suggested that the soluble COD/N ratio should be below 0.5 for deammonification to occur
(Daigger 2014).

The concept of A-B stage process as illustrated in Fig. 1 has been developed to achieve
significant COD reduction at stage A. The ideal stage A unit must be able to maximize removal of total
COD, sCOD in particular, without significant impact on ammonia concentration. Organic matters present
in typical municipal used water can be classified into settleable particles (>100 µm), supra-colloidal particles (1µm-100 µm), colloidal (1 nm-1 µm) and soluble pollutants (< 1 nm) (Levine et al., 1991). According to these physical properties of dissolved and particulate organic matters in municipal used water, various options, including physical, chemical, biological or their combinations have been developed for high-efficiency removal and concentration of organic carbon at stage A.

Primary settling is a physical separation process by gravity, where most fine settleable solids can be removed. Generally, 50%-70% of suspended solids and 30%-40% of total COD can be removed through primary settling of raw sewage (van Haandel and van der Lubbe 2012). This implies that the COD/N ratio would be reduced to 7-10 after such a physical pretreatment, which is still too high for a sustainable Anammox growth (Table 1). To further reduce the COD/N ratio of municipal used water, CEPT has been practiced, in which chemical coagulants, typically iron or aluminium salts and/or organic polyelectrolytes are used for completely removing particulate COD. However, chemical coagulants are ineffective for removal of sCOD. In fact, it was demonstrated that particles larger than 1.2 µm could be removed efficiently by CEPT, but not particles smaller than 0.2 µm (Xu et al. 2006). In general, CEPT process can lower the total COD/N ratio to 3 - 6, which is still too high for Anammox bacteria to compete with denitrifiers during anaerobic step.

HRAS process has been suggested as a key unit of the stage A to reach an even lower soluble COD/N ratio. In the HRAS system, sCOD can be removed by high-efficiency intracellular storage or biosynthesis, and further separated together with the settled COD by biological flocculation. Normally 55-65% of the total COD, including 50-60% sCOD can be removed by a HRAS process operated at a short HRT of 0.5 hour and SRT of 0.5 day, respectively (Wett et al. 2007). The effluent of the stage A with low soluble COD/N ratio is treated in stage B for deammonification, while part of stage A sludge with captured COD is then directed to anaerobic digester for biogas production (Fig. 1).

Obviously, the HRAS as stage A has advantages of enhancing energy recovery and reducing aeration demand. However, the challenge associated with HRAS process is the unbalanced sludge flow between sludge directed to AD for enhanced biogas production and sludge returned to stage A for COD capture. This means that when more sludge is transferred for biogas production, less sludge will be available for COD degradation and COD capture. In addition, the sludge from stage A generally has a very poor settleability due to the shorter HRT and SRT applied (Bisogni and Lawrence 1971; Chan et al. 2011). As most of COD has been captured at stage A, excess sludge produced at stage B through
Deammonification would be largely reduced, due to slow growth rates of AOB and Anammox bacteria and low ammonia loading. This implies that part of excess sludge generated at stage B can be recycled to stage A for carbon capture. Therefore, it appears to be difficult to maintain a stable sludge concentration at stage A if the sludge is withdrawn for AD.

Instead of carbon capture, various anaerobic units e.g. upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) have also be employed as core methods to remove biodegradable organic matters in used waste with simultaneous biogas generation, hence can be considered as an alternative of stage A in the A-B stage-based deammonification process. However, dissolved methane in the anaerobic processes effluent may pose a new challenge to anaerobic pretreatment of low-strength municipal used water. The dissolved methane, if not properly controlled, may eventually be emitted causing greenhouse gas emission (Liu et al. 2013).

Selective retention of AOB against NOB: a challenge of mainstream deammonification

The short-cut nitrification is the core towards sustainable deammonification, indicating the necessity of selective retention and accumulation of AOB against NOB. This prerequisite is the biggest challenge in the mainstream deammonification process treating municipal used water due to the complementary metabolism of AOB and NOB populations.

Free ammonia (FA) and free nitrous acid (FNA) have commonly been used as control parameters of nitritation in sidestream deammonification with high ammonia (Park and Bae 2009). However, such FA-based control strategy is no longer effective mainstream deammonification of municipal used water with low ammonia concentrations of 15 to 50 mg/L (Negulescu 2011). pH control is another option but not an economically viable approach for nitritation due to high chemical costs. In general, high temperature can be considered as an effective control strategy for nitritation in warm climate as AOB grow relatively faster than NOB at temperatures higher than 25°C (Hellinga et al. 1998). As AOB grow faster than NOB, SRT can be controlled in a range shorter than NOB retention time but longer than AOB retention time. As such, NOB can be continuously washed out of the system. Although extensive efforts have been dedicated to various attempts including intermittent aeration (Ge et al. 2014), real-time control of aeration (Claros et al. 2012), inorganic carbon (NaHCO₃) (Tokutomi et al. 2010) and bioaugmentation with AOB (Bartrolí et al. 2011) to maintain sustainable mainstream nitritation, it is of yet-resolved challenge. So far, most commonly practiced engineering approaches towards nitritation are based on the
alternating dissolved oxygen (DO) control and short SRT (Wett et al. 2013).

Low DO below 0.5 mg/L is beneficial to selectively repress growth of NOB, due to their lower oxygen affinity compared to AOB (Laanbroek and Gerards 1993). This strategy has been adopted for the treatment of both high- and low-ammonium used water (Blackburne et al. 2008; Fernandes et al. 2013; Ruiz et al. 2003). However, recent studies showed that higher DO of around 1.5 mg/L was still beneficial to mainstream nitritation (Cao et al. 2013; Ge et al. 2014; Regmi et al. 2014). Using a tropical full-scale used water treatment plant in Singapore as the case study, Cao et al. (2013) showed that 75% of nitrite accumulation was achieved in the oxic units operated at the DO concentrations of 1.4-1.8 mg/L. So far, DO control has been one of the most effective ways towards nitritation as it has concomitant advantage of reducing aeration-associated operation cost.

Intermittent aeration in temporal or spatial fashion has been shown to be effective for selecting AOB against NOB. For example, 90-95% of nitrite accumulation was achieved through intermittent aeration in a SBR treating domestic used water (Guo et al. 2009; Zeng et al. 2009). This is probably due to the lag-time for NOB to respond the transition from anoxic to aerobic conditions, and inhibition of NOB by intermediate products (e.g. hydroxylamine) (Xu et al. 2012). Another approach is to use step-feed reactor configuration. Stable partial nitritation/denitritation had been reported in continuous plug-flow reactors with alternate anoxic and oxic (A/O) zones (Cao et al. 2013; Ge et al. 2014). However, it should be noted that such control strategy still needs further study in a sustainable mainstream deammonification process for municipal used water treatment.

Alternatively, top-up or bioaugmentation of AOB from sidestream to mainstream deammonification process can also be employed. This approach helps to faster start-up, maintain or recover the nitritation process. For instance, in Strass wastewater treatment plant, Austria, supplementation of AOB biomass from the sidestream deammonification system by a hydrocyclone helped to maintain a sustainable nitritation activity in the mainstream deammonification system (Wett et al. 2013). Indeed it is challenging to maintain a sustainable nitritation for low ammonia used water in full-scale application, which first requires inhibition of NOB versus AOB according to their different growth conditions, such as, DO, temperature and pH, and then followed by selective washout of NOB from the system according to SRT.

Challenges associated with effective retention of Anammox bacteria
In addition to the challenges discussed above, retention of Anammox bacteria is another challenge. Anammox bacteria grow extremely slow with a doubling time of about 11 days in a lab-scale experiment (Strous et al. 1998), and 25 days at temperature below 20°C (Hendrickx et al. 2012). Thus, a long SRT is essential for retention of Anammox biomass in the mainstream deammonification process, especially at temperatures below 20°C (Hendrickx et al. 2012; Lotti et al. 2014). Granular sludge and biofilm have been suggested for effective retention of Anammox biomass (Fernández et al. 2008). So far, Anammox granules and biofilm have been reported in various kinds of bioreactors including UASB, SBR, moving bed biofilm reactor (MBBR), dynamic membrane bioreactor (DMBR), and rotating biological contactor (RBC) (Christensson et al. 2011; Joss et al. 2009; Liu et al. 2008; Meng et al. 2014; Tang et al. 2011).

In general, the ammonia concentrations are very low in the municipal used water, so the development and maintenance of Anammox granules is very challenging. Hydrocyclone has been applied for separation of Anammox aggregates from waste sludge in the sidestream deammonification, which is then returned to the mainstream deammonification process (Wett et al. 2013). Through cyclone separation and return of Anammox aggregates, the SRT of Anammox bacteria can be significantly prolonged in the system. In fact, it was shown that larger aggregates from the underflow stream of a hydrocyclone exhibited much higher Anammox activity than the smaller ones (Nielsen et al. 2005; Vlaeminck et al. 2010). Anammox aggregates are generally larger but less compressible than the AOB and NOB which grow in smaller and more compressible flocs. According to such findings, selective separation and retention by screening or sieving have been developed for Anammox aggregates (De Clippeleir et al. 2013). Other methods for the retention of Anammox bacteria may include use of carriers or membrane systems.

Remarks

By reducing aeration demand and allowing carbon to be redirected to AD for biogas generation, deammonification application in the mainstream has the potential for a used water treatment plant to be energy neutral or even become net energy producers. Organic carbon and nitrogen are decoupled and treated individually in the different systems. To date, the HRAS process shows great promise for the removal of organic matters, including both soluble COD and particle COD. However, the stability and the effluent quality of HRAS process may need to be enhanced by CEPT method to achieve the required degree of COD/N in the deammonification stage so as to avoid inhibiting Anammox bacteria. Sustainable
mainstream deammonification remains a goal in the treatment of municipal used water, and current research in developing both mainstream nitritation control strategies and mainstream Anammox retention methods hold promise for attaining this goal in the near future.

**Acknowledgements**

We would like to thank the Public Utility Board (PUB) of Singapore for financial support of this research.

**References:**


granules under high DO and ambient temperature and subsequent Anammox for low C/N wastewater treatment. Chem Eng J 213(0):338-345


Figure and Table captions

Figure 1 Process configurations of the two-stage mainstream deammonification. PST: Primary Sedimentation Tank; FST: Final Sedimentation Tank.

Table 1 Effect of COD and COD/N on the Anammox bacteria
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<table>
<thead>
<tr>
<th>COD type</th>
<th>COD/N</th>
<th>Process</th>
<th>Effect on Anammox bacteria</th>
<th>References</th>
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<tr>
<td>Propionate</td>
<td>0.75</td>
<td>One-step granule</td>
<td>COD/N &gt;1.25, inhibit Anammox</td>
<td>(Chamchoi et al. 2008)</td>
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<td></td>
<td>-1.25</td>
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<tr>
<td>Fat milk</td>
<td>0.9-2</td>
<td>UASB granule</td>
<td>COD &gt; 300 mg/L, inhibit Anammox</td>
<td>(Chamchoi et al. 2008)</td>
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<td>Starch or peptone</td>
<td>1- 4</td>
<td>One-step granule</td>
<td>COD/N = 4, Anammox sharply dropped.</td>
<td>(Du et al. 2014)</td>
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<tr>
<td>Glucose</td>
<td>0.1-1</td>
<td>UASB granule</td>
<td>COD/N = 1 (COD=800) or COD/NO₃ &gt;1.46 (COD=700), Anammox disappeared</td>
<td>(Tang et al. 2013)</td>
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<tr>
<td>Sucrose</td>
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<td>One-step</td>
<td>TN removal by Anammox 68%, by Denitrification 8%</td>
<td>(Wang et al. 2010)</td>
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<td>Landfill leachate</td>
<td>0.87</td>
<td>One-step granule</td>
<td>Anammox decreased with COD/N increasing to 1.4</td>
<td>(Jenni et al. 2014)</td>
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<tr>
<td>Acetate or glucose</td>
<td>0.27</td>
<td>One-step granule</td>
<td>COD/N 0.5 to 0.75, TN removal rate decreased from 79% to 52%</td>
<td>(Chen et al. 2009)</td>
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<tr>
<td>Acetate</td>
<td>0.5</td>
<td>One-step biofilm</td>
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<td>0.75</td>
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