Rapid start-up of one-stage deammonification MBBR without addition of external inoculum

Linda Kanders, Daniel Ling and Emma Nehrenheim

ABSTRACT

In recent years, the anammox process has emerged as a useful method for robust and efficient nitrogen removal in wastewater treatment plants (WWTPs). This paper evaluates a one-stage deammonification (nitritation and anammox) start-up using carrier material without using anammox inoculum. A continuous laboratory-scale process was followed by full-scale operation with reject water from the digesters at Bekkelaget WWTP in Oslo, Norway. A third laboratory reactor was run in operational mode to verify the suitability of reject water from thermophilic digestion for the deammonification process. The two start-ups presented were run with indigenous bacterial populations, intermittent aeration and dilution, to favour growth of the anammox bacterial branches. Evaluation was done by chemical and fluorescence in situ hybridization analyses. The results demonstrate that anammox culture can be set up in a one-stage process only using indigenous anammox bacteria and that a full-scale start-up process can be completed in less than 120 days.

Key words | anammox, deammonification, full scale, start-up, thermophilic digestion

INTRODUCTION

Interest in anammox-based processes has grown over the last decade due to its energy efficient ability to transform ammonia to nitrogen gas using a ‘short cut’ in the nitrogen cycle. The increasing nitrogen load per capita (Tumlin & Mattsson 2013), increasing population and thus increased demand for sewage treatment, in combination with stricter effluent standards, are increasing the demand for nitrogen treatment in wastewater streams. In addition, there is a general focus on reducing the cost and increasing the energy efficiency of water treatment.

Anammox in combination with nitritation (deammonification) has proven to be a reliable technology for nitrogen removal from highly concentrated, warm (25–35°C) nitrogen wastewater streams with relatively low carbon content (sCOD/N < 1) (Lackner et al. 2014). However, due to its potential for energy savings in the nitrogen removal cycle, extensive research has also been carried out on nitrite shunt processes in combination with anammox in the main stream of wastewater treatment plants (WWTPs) (Wett et al. 2013; Xu et al. 2015). As well as saving energy by requiring 60% less aeration (Wett 2007), the process also consumes a smaller amount of chemicals, in terms of external carbon or alkalinity buffer. Altogether, this leads to a substantially lower carbon footprint when implementing the process in a side stream of a WWTP.

The majority of full-scale projects reported so far have used external inoculums of anammox bacteria (Li et al. 2004; Wett 2007; Jardin & Hennerkes 2012; Christensson et al. 2013; Lackner et al. 2015) because of their slow growth rate. However (Kanders et al. 2014) recently published preliminary results that showed that it is not the addition of inoculum in a biofilm system that shortens the start-up time. On the contrary, it appeared that the bacteria originated from the reject water itself. This theory is also supported by other research groups (Zekker et al. 2013), although reactors based on these results have had rather long (>300 days) start-up times. Ni et al. (2014) and Ibrahim et al. (2016) among others refer to ‘long start-up times’ without inoculum when discussing earlier research, and estimate anammox doubling times of 11–30 d (Jetten et al. 2009; Van de Graaf et al. 1996).

The vast majority of anammox projects have focused on the treatment of reject water from mesophilic digestion of wastewater sludge. Until now few deammonification/anammox plants have been combined with thermophilic digesters. This is thought to be because some studies at
laboratory/pilot scale have reported difficulties in combining anammox start up and higher temperatures (Dosta et al. 2008). Higher temperature treatment (i.e. higher than mesophilic) can cause increased inhibition of ammonium oxidizing bacteria (AOB) as well as the anammox bacteria (Figdore et al. 2011) due to increased inert organics. Furthermore, there is a potential of inhibiting the seeding anammox bacteria due to inactivation in a thermophilic digester (Dosta et al. 2008).

In summary, several research groups have reported full-scale start-up that required external inoculum or long start-up times (>150 days). However, there are few reports on the possibility of using indigenous bacterial communities from the wastewater for the full-scale anammox process (Zekker et al. 2015; Kanders et al. 2014). Therefore, this study focuses on reducing the start-up time of an anammox process based on thermophilic digestion reject water without external inoculum. Replicate laboratory-scale experiments were set up without external inoculum as a proof of concept. The start-up was subsequently repeated at full scale in a sidestream treatment at a WWTP with thermophilic digesters.

The aim of the study was to investigate whether an inoculum free, one-stage moving bed biofilm reactor (MBBR) deammonification process, could be set up reproducibly, first at laboratory scale, and then at full scale, and operated on reject water from the thermophilic digesters at Bekkelaget WWTP in Oslo.

MATERIAL AND METHODS

Character of the reactors can be seen in Table 1.

Laboratory reactor set-up

The laboratory set-up consisted of two parallel 3.0 L glass reactors with top mounted mixing and a water bath for heating (30 °C). The reactors were fed with reject water from Bekkelaget WWTP, see below. Reactor ‘Laboratory Operation’ (LO) was filled with 1.5 L carriers as used in earlier work (Jiangsu Yulong, type YL-I, 385 m² m⁻³ stated protected surface, China) (Kanders et al. 2014) with a deammonification film of 5.0 g TS/L_carriers. Reactor ‘Laboratory Start-up’ (LS) was filled with 1.5 L carrier material (HXF12KLL, 700 m² m⁻³ stated protected surface, Germany) from the full-scale partial nitritation reactor (as described below) with a starting biofilm mass of 8.5 g TS/L_carriers. The reactors were intermittently aerated with an aeration pump and a bottom diffuser stone. The intermittent aeration was operated with a time relay and changed manually in order to keep reactor operation and oxygen concentration stable, the latter around a set point of 3.0 mg O₂L⁻¹. Reactors were covered with insulation material to maintain a stable temperature and for protection from light, and were operated in a continuous feeding mode. Where applicable, tap water was used to dilute the reject water. The reactors were placed at Lund University, Lund, Sweden.

**Full-scale reactor set-up**

The Bekkelaget WWTP is located in a rock cavern in Oslo. The plant began operation in 2001 and treats wastewater from 300,000 person equivalents (p.e.) of which 30% is industrial load. The main line consists of screens, sand and fat trap, pre-sedimentation, four parallel activated sludge lines with nitrogen removal followed by post-sedimentation, and finally sand filters. The main line can treat 2 m³ s⁻¹, a further 2 m³ s⁻¹ can be treated chemically if needed. The plant has two thermophilic digesters, volume 4,000 m³, each treating primary and biological sludge together with external fats, oil and grease. Digested sludge is dewatered and spread on agricultural land, and the reject water is recirculated back to the influent. The reject water carries 15–30% of the internal nitrogen load. When a carbon source is added, alkalinity is limiting for nitrogen removal and thus lime is added to the biological step during the colder half of the year.

A separate reject water treatment was operated for partial nitritation with a carrier material (of type HXF12KLL) in two 505 m³ parallel reactors from April 2013–August 2014. After successful laboratory results, the ability to use pre-heated dilution water was added in 2014. The dilution water, consisting of the main effluent water, could be maintained at 30 °C without using external heat by recovering heat from the reject water and excess heat from the main blowers. As in the laboratory reactors, the aeration was run intermittently with an oxygen set point of 5.0 mg O₂L⁻¹ during aeration. Only one reactor, denoted ‘Full-scale Start-up’ (FS), was used for deammonification start-up in 2014. The other reactor continued to run as a nitritation reactor.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Laboratory-scale experiments, 2013</th>
<th>Full scale, 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Operational (LO)</td>
<td>Start-up (LS)</td>
</tr>
<tr>
<td>Volume (m³)</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>Days of operation</td>
<td>128</td>
<td>128</td>
</tr>
</tbody>
</table>

*Continued operation, the first 183 days reported in this paper.
Reject water composition

The reject water characteristics for all three experiments are presented in Table 2.

Reject water was transported every third week from Bekkelaget WWTP to Lund University and stored at 4 °C. Reject water batches were equilibrated to room temperature when used in experiments and replaced every 3–5 days. No chemicals were added to the reactor or influent during operation.

Analytical procedures

Where appropriate, analysis results are presented as averages with standard deviation. Samples were analysed for NH₄-N, NO₂⁻N and NO₃⁻N, and soluble chemical oxygen demand (sCOD) with Dr Lange cuvettes (LCK 303; LCK 342; LCK 339; LCK 314; Hach Lange, Germany) and read in a spectrophotometer at either Lund University (Dr Lange Lasa 100) or Bekkelaget WWTP (Hach Lange DR 3900). Samples were filtered with GF/A filters at Lund and with GF/C filters at Bekkelaget (glass microfiber filter, Whatman).

In laboratory-scale experiments, the biofilm total solids (TS) from the carriers were measured at day 3, 25, 45 and 73 by drying and weighing 20 carriers. The carriers were then soaked in 37 vol% HCl for at least 4 hours and then rinsed, dried and weighed again. Full-scale reactor samples were analysed at day 8, 31, 73, 107 and 134 with the same procedure but using 50 carriers each time.

Temperature, dissolved oxygen and pH in the full-scale reactors were measured with SC1000, LDO and pH meters (Hach Lange, Germany). The same parameters were measured 3–5 times a week using HQD 40 and Intellical LDO101 and Intellical pHC 101 (Hach Lange, Germany) in the laboratory-scale reactors.

Alkalinity and total suspended solids (TSS) analyses were conducted according to standard methods 2320 and 2540D (APHA 2005).

Fluorescence in situ hybridization

Growth of the anammox bacteria on the carrier material during the first weeks, when no nitrogen reduction was expected, was monitored using a commercially available fluorescence in situ hybridization (FISH) test kit with standardized methodology (Snaidr et al. 1997) and prepared solutions (VIT®ANAMMOX, vermicon AG, Germany). The test kit detects the following anammox branches: Brocadia, Kuenenia, Scalindua, Candidatus Anammoxoglobus propionicus and Candidatus Jettenia asiatica, which covers the range of bacteria reported to be responsible for anammox (Kartal et al. 2007; Oshiki et al. 2005). Sample preparation and in situ hybridization was all according to the manual of the VIT®ANAMMOX test kit (vermicon AG, Germany) with one exception: the biofilm removed from carriers was pre-treated with sonication for 30–60 s at cycle 0.5 and 20% amplitude, instead of ultraturraxx as given in the manual. In the test, cells were excited and examined under a Nikon Optishot 2 fluorescence microscope. Viable cells appear green through a Nikon B2A filter while anammox bacteria appear red through a Nikon G2A filter. The percentage of viable area covered with anammox bacteria was analysed in at least ten randomly selected captured fields and an average percentage of anammox area was calculated. This analysis was performed at day 60, 73 and 109 for the full-scale reactors and at day 25, 45 and 73 for the laboratory-scale reactors. For validation during the full-scale start-up, a sample of biofilm was sent to vermicon for additional FISH analyses at day 36. The detection limit of this method ranges at 1,000 cells/ml.

Calculations

The amount of free ammonia (FA) was calculated according to Anthonisen et al. (1976). The nitrogen loading rate (NLR) and nitrogen reduction rate (NRR) were calculated from the incoming ammonium concentration, inflow and the total (protected) area of the carriers in the reactor.

NLR was calculated according to the following:

\[
NLR = \frac{NH_4^-N_{in} \times Flow_{in}}{Area_{carrier \ material} \ m^2d}
\]
The percentage ammonium reduction, measured as NH$_4$-N, was estimated as follows:

$$R_{NH4} = \frac{100 \times (NH4-N_{in} - NH4-N_{reactor})}{NH4-N_{in}} [\%]$$ (2)

The percentage inorganic nitrogen reduction was estimated according to the following:

$$R_{inorg} = \frac{100 \times (NH4-N_{in} - NH4-N_{reactor} - NO2-N_{reactor} - NO3-N_{reactor})}{NH4-N_{in}} [\%]$$ (3)

NRR was calculated as follows:

$$NRR_{NH4} = NLR \times R_{NH4} \left( \frac{gN}{m^2d} \right)$$ (4)

or

$$NRR_{inorg} = NLR \times R_{inorg} \left( \frac{gN}{m^2d} \right)$$ (5)

**Definition of start-up phase**

The start-up phase was divided into two phases. The culture was designated as being in the first phase until the first signs of anammox were detected in the FISH analyses and the first gap was measured in the nitrogen balance (Kanders et al. 2014). After this point, the process was designated as being in the second phase. Influent was diluted with tap water to maintain favourable concentrations of FA (<5 mg/L) and NO$_2$-N (40–60 mg/L) during start up to maintain AOB activity and promote growth of anammox bacteria. The start-up phase ended when nitrogen reduction reached >80% with undiluted influent. After this point, the process was designated as being in the continuous operation phase.

**RESULTS AND DISCUSSION**

**Laboratory reactor performance**

The performance of the laboratory-scale reactors LO and LS is shown in Figure 1(a) and 1(b) and Table 3 for 120 and 127 days of operation respectively. Reactor LO was run in a continuous operation mode. This reactor used active biofilm with anammox on carrier material from a previous experiment run on mesophilic reject water (Kanders et al. 2014) and was initially fed with diluted reject water to adapt the culture gradually. Stable conditions and sufficient nitrogen reduction were reached within 11 days, and thus the dilution was discontinued at this point. The load to the reactor was adjusted to maintain a high removal rate (Figure 1(c)). The average load was 3.3 ± 1.6 g Nm$^{-2}d^{-1}$ and the reduction of inorganic nitrogen was 83.0 ± 6.9%, calculated as described in Equations (1) and (5). The temperature was kept stable at 29.9 ± 0.1 °C and the pH varied around 7.35 ± 0.41. The influent was diluted 6-fold on day 43 due to a high concentration of FA in the reactor (11 mg/L), to reduce the FA to below 3.0 mg/L.

FISH analyses showed a high proportion of anammox bacteria in the biofilm (Table 3). No signs of inhibition from the thermophilic reject water were seen either from the chemical analyses, which showed high reduction or a substantial decrease in anammox biofilm.

In reactor LS, the first phase was found to last for 70 days and the second phase lasted from days 71–127, based on the start-up strategy presented in the previous section. The influent concentration of ammonium to reactor LS was controlled by addition of tap water. The load was kept stable at 1.0 ± 0.1 g Nm$^{-2}d^{-1}$ until day 70, when the first reduction in inorganic nitrogen was detected and FISH analysis showed the first signs of anammox bacteria. Nitrite reduction was the first indication of inorganic nitrogen reduction. Figure 1(c) shows a sudden removal rate increase from approximately 5% at day 50 to the same level as the LO reactor by day 70. The dilution was subsequently gradually phased out until the reactor was fed with undiluted reject water at day 127. The reduction of inorganic nitrogen between days 70–127 was 71 ± 15%. Temperature varied around 29.8 ± 0.5 °C and pH was in the range of 7.24 ± 0.56 over the entire period.

FISH analyses indicated the first signs of anammox at day 45 and a rapid increase around day 73 (Table 3).

**Full-scale operation performance**

The full-scale reactor transitioned from first phase to second phase around day 80, and the second phase lasted until day 120 (Figure 2). From day 121, the reactor operated with high nitrogen conversion rates and data were reported until day 178. Thus the dilution of the reject water was terminated at day 121 when the start-up phase of the full-scale plant was considered complete.

Figure 2(a) shows the nitrogen fractions of the diluted influent and in the reactor for the first 178 days of operation. Due to the limited capacity of the dilution water pump, a
slight increase of the influent concentrations can be seen up to day 80. As a result, the load set point of the system had to be reduced in order to avoid an excessive increase in the concentrations in the reactor. Consequently, the plant was run with low pH (<7.0) for 7 days due to excessive aeration.
During the first phase, the inorganic reduction in the FS reactor varied from 5–20% from the start (due to denitrification) but increased significantly around day 70, due to anammox activity. After 120 days the reduction of both ammonium and inorganic nitrogen exceeded 80%.

The levels of anammox bacteria and TS on the carriers are shown in Table 3. An additional sample was sent to an external laboratory (vermicon AG, Germany) for AOB, nitrite oxidizing bacteria (NOB) and anammox analysis (as defined in the FISH method) on day 36 to monitor potential growth of NOBs or unexpected growth of anammox. The sample showed a high proportion (30%) of active (intense colour) AOBs and low levels of NOBs (<1%) indicating an effective aeration strategy. The standard probe set-up for anammox did not detect any ‘anammox’ at this stage, indicating that they were not present or below the detection limit of 1,000 cells per ml. However, unusually high levels of Planctomycetes were detected by the specific gene probe for this group, with a suspicious anammox-like cluster formation (18%). Thus, it is possible that although the analysis with the anammox probe was negative, a so far unidentified anammox population was present in the sample, or at least one that does not belong to the proposed anammox branches: *Brocadia, Kuenenia, Scalindua, Candidatus Anammoxoglobus propionicus* and *Candidatus Jettenia asiatica*. Subsequent FISH analyses performed with the standard probe set-up for anammox first detected anammox at day 73, and there was a rapid increase of anammox in the next sample on day 109.

As intended, the results from the FS reactor reflected the previous results from the LS reactor. The measured pH, temperature, oxygen concentration during aeration, aeration time, hydraulic retention time (HRT) and load in the reactors during the first and second phases are shown in Table 4. The load in the FS reactor did not reach as high as the corresponding loads in the LS reactor due to unstable conditions, such as the temporary low temperatures and variations due to the limited dilution capacity.

### The length of the start-up period

Results from both the laboratory reactor LS and the full-scale reactor FS indicate that there is a critical abundance of anammox bacteria in the influent reject water sufficient to achieve non-seeded deammonification. Both laboratory reactors LS and LO demonstrated equal performance with >80% ammonia reduction from day 72 onwards. The full-scale reactor showed a similar performance from day 120, i.e. start-up lasted 48 days longer. The main differences between laboratory-scale and full-scale reactors were that the load during the first part of start-up (first phase) was 30% lower in the FS reactor and the average temperature in the FS reactor was 0.8 °C lower and showed larger fluctuations (Table 4). However, the main differences occurred between day 85–113, with instability and temperature fluctuations during the early days of the second phase. Only two analyses were conducted between day 85–107 due to holidays and there were no on-line measurements to rely on yet. Because of the unreliability of the process information, no operational changes were applied during this period. Subsequently, from day 103–113 the main blower which supplied heat to the dilution water circuit stopped working, resulting in low temperatures in the reactor (22–25 °C). The planned load increase was therefore put on hold until the temperature increased again. Higher temperatures promote growth, therefore this fall in temperature would have had a significant effect on the start-up of the FS reactor. Overall it was more challenging to maintain optimal and stable

<table>
<thead>
<tr>
<th>Reactor Day</th>
<th>Laboratory-scale Operational (LO)</th>
<th>Laboratory-scale Start-up (LS)</th>
<th>Full-scale Start-up (FS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anammox sp. (%)</td>
<td>Carrier TS (g/L)</td>
<td>Anammox sp. (%)</td>
</tr>
<tr>
<td>25</td>
<td>91 ± 9</td>
<td>4.6</td>
<td>nd</td>
</tr>
<tr>
<td>45</td>
<td>91 ± 14</td>
<td>8.1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>73</td>
<td>79 ± 23</td>
<td>10.4</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

³This sample was taken at day 36 and sent to an external laboratory (vermicon, Germany) for analysis. nd – no anammox detected.
Figure 2  (a) The ammonium concentration in the diluted influent and the nitrogen compounds in the full-scale reactor during start-up. The influent concentration to the reactor is shown as a line above with each measurement point marked. The inorganic nitrogen (NH$_4$-N, NO$_2$-N and NO$_3$-N) in the reactor is shown as a sum below with the same measurement interval as the influent. The gap between the line and the merged columns is the inorganic nitrogen reduction (also shown in % in Figure 2(b)). (b) Reduction of inorganic ammonium and ammonium. The line represents a 7 day rolling average of the reduction. (c) The volumetric ammonium load and reduction during start up.
conditions in the full-scale system. If the same beneficial boundary conditions could have been maintained in the full-scale set-up the start-up time is likely to have been shorter.

The results achieved in this study, without seeding, are similar to those reported by Lackner et al. (2015), in which the reactor was seeded with sludge from neighbouring plants (40 m$^3$ inoculum of 550 m$^3$ total volume) and achieved 80% inorganic nitrogen reduction after 150 days of operation.

It cannot be ruled out that anammox strains were already growing during the nitritation phase of the reactor, even though no nitrogen reduction was detected. However, given the high NO$_2$ (>400 mgL$^{-1}$) and continuous aeration (3.0 mg L$^{-1}$), it is highly unlikely that there were large quantities of anammox, and the FISH analyses suggest that there were fewer than the detection limit of 1,000 cells mL$^{-1}$. However it is outside the scope of this study to make a direct link between cell numbers and nitrogen reduction performance.

**Anammox growth rate**

Since most previous publications have shown very low growth rates on anammox, laboratory- and especially full-scale plants tends to use inoculum so as not to risk very long start-up times (>6 months). This study, together with previous research (Kanders et al. 2014), show that <100 days would be enough with presented conditions to establish anammox biofilm, which indirectly also indicates higher growth rates. This is shown with chemical analysis as well as supporting FISH-analyses in this study. Higher growth rates are also supported by Lotti et al. (2015) who recently showed that anammox doubling times as short as 3 days can be achieved in a membrane one-stage anammox reactor. The anammox growth rate, and hence the start-up time, seems to depend on the growth conditions rather than the initial anammox bacteria inoculum, provided the influent carries some indigenous anammox bacteria. The stability and robustness of the performance during start up also seems to be dependent on the technique selected for maintaining the age of the sludge. Membranes or MBBRs, rather than sludge or granules, may make it easier to maintain a critical amount of bacteria within the system, benefitting inoculum growth.

**Inoculation sources**

The initial anammox bacteria must either come from external seeding or from the influent. If external seeding is used the amount of anammox seeding bacteria is critical: the higher the sludge concentration and volume, the shorter the start-up time. To minimise the volume of transports of external seeding, Ali et al. (2015) suggested an immobilised micro-seed and defined the minimal amount of anammox bacteria (0.33 gVSS L$^{-1}$) that was needed for a short start-up ('about a month') in a high-loaded upflow anaerobic sludge blanket (UASB) (10 kg N m$^{-3}$d$^{-1}$) at laboratory scale. However the same study concluded that due to diffusion limitations the anammox inside the granules were not contributing to nitrogen removal. This implies that the indigenous anammox have an important role, and although low in abundance, they represent a continuous supply which is well distributed in the overall bacterial community.

The anammox family is diverse and complex, and our knowledge of these bacteria is still developing (Oshiki et al. 2015). The success of inoculation depends on the adaptivity of the anammox culture to the new environment so that the culture is able to flourish with the new influent.

### Table 4: Operational data from the laboratory-scale and full-scale reactors during first and second phase

<table>
<thead>
<tr>
<th>Reactor</th>
<th>LS</th>
<th>FS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase</strong></td>
<td><strong>First</strong></td>
<td><strong>Second</strong></td>
</tr>
<tr>
<td>Time (d)</td>
<td>1–70</td>
<td>71–127</td>
</tr>
<tr>
<td>pH (−)</td>
<td>7.39 ± 0.21</td>
<td>7.01 ± 0.78</td>
</tr>
<tr>
<td>T (°C)</td>
<td>29.7 ± 0.6</td>
<td>29.8 ± 0.4</td>
</tr>
<tr>
<td>O$_2$ (mgL$^{-1}$)</td>
<td>2.9 ± 0.70</td>
<td>2.5 ± 1.3</td>
</tr>
<tr>
<td>Aeration (min h$^{-1}$)</td>
<td>15 ± 2.6</td>
<td>34 ± 4.5</td>
</tr>
<tr>
<td>HRT (h)</td>
<td>17 ± 3</td>
<td>27 ± 12</td>
</tr>
<tr>
<td>NLR (g NH$_4$-N m$^{-2}$d$^{-1}$)</td>
<td>1.0 ± 0.1</td>
<td>1.8 ± 0.7</td>
</tr>
</tbody>
</table>

*During aeration.*
Some anammox strains may be more suited than others to biofilm systems, and other strains may be more suited to sludge systems. It is also possible that some anammox strains grow better on mesophilic reject water and others may prefer thermophilic or industrial streams with different water compositions. This is indicated in Jenni et al. (2014), where the mixed anammox community shifts towards a homogenous ‘Candidatus Brocadia fulgida’ culture as the inlet water composition changes. As well, in Park et al. (2010), two different reactor types were commissioned with inoculum different from each other resulting in similar anammox cultures after long-term operation (200 d), since influent originated from the same source. A third example of this is the characterization of eight anammox reactors where all plants had one dominant anammox culture each, although some of them originated from the same seeding source (Hu et al. 2010). Until these effects are fully characterised, it seems prudent to cultivate the indigenous anammox that is already in/adapted to the influent. The influent also has the potential to restart the culture if needed at some point.

**Denitrification**

The results of the laboratory trial suggest that denitrification plays a minor role in nitrogen removal. This is apparent in Figure 1(a), where the sum of nitrite and ammonia in the reactor corresponds to the influent ammonia in the first phase. In Figure 1(c), the inorganic nitrogen reduction was approximately 5% in the first phase before anammox activity starts. In the second phase, when anammox begins to produce nitrate, the fraction of nitrate to reduced ammonium was 2.4 (± 2.3)%. This was lower than the theoretical value of 11%, indicating that some nitrate is consumed by denitrifying heterotrophs.

In the full-scale reactor, the denitrification was somewhat higher in the first phase (Figure 2(b)), approximately 5–15%. The difference is most likely due to the easily degradable organics in the reject water being consumed during transportation to the laboratory and storage, whereas in the full-scale reactor the reject water was fresh and contained more degradable organics suitable for heterotrophic growth. Indications are that some of the particle COD degrades to soluble COD, and some of these are oxidized fully during aeration. This study did not include a full mass balance of the TSS, particle COD and soluble COD, which would have clarified the fate of these compounds. The COD content in the influent is contributing to a higher total nitrogen removal rate, not only by reducing the NO₃ produced by the anammox reaction but also by contributing to alkalinity, which does not appear to limit the system in the full-scale reactor.

**CONCLUSION**

The scope of this paper was to fully investigate the potential of a one-stage non-inoculated start-up of the anammox process. The results show that inoculation is not necessary to start up a full-scale deammonification process under the presented conditions. The start-up was demonstrated using reject water from thermophilic anaerobic digestion. This effect was shown to be reproducible in the laboratory and at full scale. The start-up took 120 days at full scale and 72 days at laboratory scale.

**ACKNOWLEDGEMENTS**

The authors would like to thank the Division of Biotechnology at Lund University and especially Olle Holst for making it possible to set up the laboratory reactors. Furthermore, we would like to thank the three Masters students Therese Areskough, Johanna Norup and Tove Rydén Sonesson at LTH, Lund for their thorough laboratory work and Bevas personnel, especially Jeanette Lund, Tommy Angeltvedt and Morten Rostad Haugen, for finding anammox as interesting as the authors did.

This research is partly financed by a Piia scholarship from Vinnova (Swedish Governmental Agency for Innovation Systems), with co-funding from Purac AB and ABB AB, which made the writing of this article possible.

**REFERENCES**


