Promotion of nitrifiers through side-stream bioaugmentation: a full-scale study
F. Stenström and J. la Cour Jansen

ABSTRACT

Bioaugmentation of nitrifiers from a side-stream treatment is an efficient method for boosting the mainstream process at a wastewater treatment plant (WWTP). Although this technology has been known for several years, the number of full-scale applications for it is limited. For a WWTP approaching its critical nitrogen load capacity, the benefits are doubled if the introduced side-stream treatment for digester supernatant is combined with bioaugmentation. Not only is the nitrogen load to the mainstream process decreased by 10–25%, but the mainstream process is also boosted with nitrifiers, increasing the nitrification capacity. In this full-scale study, the increment of the nitrification rate is examined in the mainstream process at different temperatures and at different flow rates of returned activated sludge to the side-stream treatment. Our results show that the nitrification rate in the mainstream process was increased by 41% during the coldest period of the study, implying that the examined WWTP could treat considerably higher nitrogen loads if bioaugmentation were permanently installed.

Key words | bioaugmentation, biological wastewater treatment, digester supernatant, inoculation, sequencing batch reactor

INTRODUCTION

The dewatering of digested sludge at wastewater treatment plants (WWTPs) results in a separate stream referred to as digester supernatant. Typical characteristics of this stream are a temperature of 25–35 °C, an ammonium concentration of 500–1,500 mg NH₄-N L⁻¹, and a flow rate that is equal to 0.5–1% of the influent flow rate to the WWTP. These properties make this stream favorable for separate treatment and imply high removal rates. Separate treatment of the digester supernatant has been applied since the 1930s (Rudolfs & Gehm 1939). Biological treatment via nitrification–denitrification was introduced as a separate treatment during the 1970s (Prakasam & Loehr 1972). In recent decades, however, several new processes have been developed, all intended to be more environmentally friendly and energy efficient. New chemical and biological routes are used for the transformation of ammonium nitrogen to nitrogen gas, meaning that less aeration and a decreased carbon source are needed (reviewed in Schmidt et al. 2005).

The main groups of bacteria involved in biological nitrogen removal (BNR) are nitrifiers and denitrifiers. Nitrifiers grow considerably more slowly than do denitrifiers, meaning that the volumes for nitrification represent most of the volume of the biological reactors. Furthermore, nitrifiers are more sensitive to cold temperatures than denitrifiers, implying that the difference in required basin volumes becomes even more obvious during cold seasons. Nitrifiers typically constitute <4% of the biomass in biological reactors at WWTPs for municipal wastewater with BNR (Ekama & Wentzel 2008). To ensure a sufficient quantity of nitrifiers, there must be a high solids retention time (SRT). The SRT, and thus the nitrification volume, is usually the limiting factor for nitrogen removal at WWTPs. Increasing the quantity of nitrifiers would enable the same quantity of nitrogen to be treated in smaller reactors, enable more nitrogen to be treated in reactors of the same volume, or enable reduced SRTs, which in turn would reduce the energy consumption for aeration. Nitrifiers can be enriched through inoculation, also known as bioaugmentation. Methods for nitrifier cultivation/inoculation to the mainstream process are often subdivided into two types: offsite and onsite. Commercial, offsite-produced nitrifiers can be purchased, although they are considered too expensive for continuous...
use (Parker & Wanner 2007). Onsite-produced nitrifiers are typically cultivated (i) in a side-stream treatment, (ii) in the lane for returned activated sludge (RAS), or (iii) in a parallel train. The InNitri® process (Kos 1998) and the BABE® process (Zilverentant 1999; Salem et al. 2003) are process configurations incorporating cultivation in side-stream treatments. Process configurations with the cultivation of nitrifiers in the RAS lane include the BAR® process (Novák et al. 2003) and the ScanDeNi® process (Rosén & Huijbregsen 2003). Augmentation from parallel trains has been studied by Plaza et al. (2001) and Neethling et al. (1998).

The benefits of the separate treatment of warm digester supernatant are obvious in regions with colder climate. The temperature correction factor for the specific growth rate of nitrifying bacteria ranges from 1.072 to 1.127 (Head & Oleszkiewicz 2004). This implies that, given a temperature difference of 20 °C between the digester supernatant and the mainstream process, the required nitrification volume must be four to 10 times larger if the digester supernatant is treated in the mainstream process instead of separately. If the digester supernatant is treated using conventional nitrification–denitrification in the side-stream and then conveyed to the mainstream process, nitrifiers from the side-stream can exert a bioaugmentation effect. Nevertheless, since there are several environmental differences between these two streams regarding factors such as temperature, alkalinity, ammonium concentration, and pH, different species of nitrifiers could be cultivated. The effects of bioaugmentation and an increased nitrification rate in the mainstream process can thereby be limited. To ensure side-stream growth of the bacteria that grow best in the mainstream process, a small share of RAS can be recirculated to the side-stream treatment in order to achieve the same type of nitrifying bacteria in the two different streams (Salem et al. 2003). The bioaugmentation effect will then be enlarged, resulting in higher nitrification rates in the mainstream process. This approach of deliberately promoting a monoculture of nitrifiers, as in the BABE® process, may have a drawback when compared to multicultural processes such as the InNitri® process, which is probably better able to withstand inhibiting compounds (Leu & Stenstrom 2010). The nitrification rate of a system is influenced by the kinetics of the biochemical reactions and the quantity of nitrifiers. When bioaugmentation is used, it primarily affects the quantity of nitrifiers, not the kinetics.

The SRT is crucial for bioaugmentation in both the side-stream treatment and the mainstream process. In the side-stream treatment, the amount of active nitrifiers is dependent on the SRT: with longer SRTs, fewer active nitrifiers are transferred to the mainstream process due to decay (Van Loosdrecht & Henze 1999). Consequently, the SRT in the side-stream treatment should be kept low to ensure a large proportion of active nitrifiers and, thereby, as large an inoculation effect as possible in the mainstream process. To take advantage of the additional nitrifiers in the mainstream process, the SRT should be kept low there as well. In a simulation study, Salem et al. (2003) stated that it is sufficient if the actual SRT in the mainstream process is equal to half of the critical SRT required to maintain a stable nitrification process when using bioaugmentation. Moreover, the temperature in the mainstream process also has a great impact on the magnitude of bioaugmentation: the colder the mainstream process, the greater the inoculation effect of the added nitrifiers (Salem et al. 2005).

Among the different processes available for treating digester supernatant, bioaugmentation from a separate reactor is the only process that obviously improves the mainstream process. Consequently, when a separate treatment of the digester supernatant is combined with a deliberate inoculation to the mainstream process, there is a double effect: reducing the total nitrogen (TN) load in the digester supernatant and boosting the mainstream process. In spite of this advantage, few studies involve full-scale bioaugmentation of nitrifiers to the mainstream process (Pei et al. 2005). Some modelling approaches have been carried out to reveal how different magnitudes of the RAS flow rates to the separate treatment (Salem et al. 2003) and different temperatures in the mainstream process (Salem et al. 2005; Berends et al. 2005) affect the boosting of nitrifiers to the mainstream process. Nevertheless, to the authors’ knowledge there has not been reported any results from full-scale studies that reveal these facts. This full-scale study investigates the inoculation effect from the treatment of digester supernatant in a sequencing batch reactor (SBR) to the mainstream process, under different mainstream process temperatures and different RAS flow rates from the mainstream process to the SBR.

**MATERIALS AND METHODS**

**The WWTP and ordinary operation conditions**

This study was performed at Slottshagen WWTP, which treats municipal wastewater from the city of Norrköping, Sweden. The plant is designed for 200,000 population equivalent (PE), defined as 70 g BOD₇ PE⁻¹ d⁻¹, a TN load of 2,240 kg d⁻¹, and a flow rate of 2,000 m³ h⁻¹, where BOD₇
stands for biochemical oxygen demand. The actual load corresponds to approximately 70% of the BOD$_7$ and TN capacity, and approximately 95% of the flow rate. The WWTP is an activated sludge plant comprising pre-precipitation, biological reactors with pre-denitrification and contact stabilization, and post-precipitation. The biological treatment in the mainstream process consists of two separated trains, in this study called the Augmented Train and the Reference Train. Two-thirds of the influent flow rate is directed to the Augmented Train and one-third is directed to the Reference Train. The RAS flow rate averages approximately 95% of the influent flow rate. The digested sludge is dewatered in centrifuges, and the digester supernatant is piped to a buffer tank holding a water volume of 200 m$^3$, from which it is pumped into an SBR. The flow of the digester supernatant typically constitutes 0.5% of the flow of influent to the WWTP, whereas the TN load to the SBR averages about 15% of the TN load to the plant. Treated water from the SBR is directed to the Augmented Train of the biological treatment. Because the mainstream process consists of two completely separated trains, an accurate comparison is possible between the Augmented Train, boosted with nitrifiers from the SBR, and the Reference Train, not boosted.

The biological treatment in the mainstream process is composed of a modified Ludzack-Ettinger process, in addition to aerated stabilization reactors on the RAS flow. The typical composition of the influent/effluent to/from the biological treatment is: TN, 30/6 mg L$^{-1}$; ammonium (NH$_4^+$-N), 23/2 mg L$^{-1}$; nitrate (NO$_3^-$-N), 1/2 mg L$^{-1}$; chemical oxygen demand, 220/35 mg L$^{-1}$; phosphate (PO$_4^{3-}$/P), 1/0.2 mg L$^{-1}$; and BOD$_7$, 115/2 mg L$^{-1}$, where the first number in each ratio refers to the influent and the second to the effluent. Ethanol is added as an external carbon source when needed, and is dosed only when the nitrate in the effluent from the biological treatment is higher than a pre-set value. The concentration of mixed liquor suspended solids (MLSS) is controlled based on the water temperature, indirectly controlling the SRT in the system. A separate pump is used for the withdrawal of waste activated sludge (WAS), pumping from the RAS stream in order to maintain the required MLSS concentration in the reactors. The flow rate from the frequency-controlled WAS pump is set manually.

The SBR operates during the cold season, from November to May. The covered reactor is operated with post-denitrification and has an effective volume of 1,000 m$^3$, constituting 3% of the volume of the biological treatment in the mainstream process, or 9% of the volume of the biological reactors in the Augmented Train. The SBR is run with a cycle length of 8 h; a normal cycle without the recirculation of RAS to the SBR is shown in Figure 1. For further information about the WWTP and the SBR, see Stenström et al. (2014).

The experimental plan

The study was performed from December 2013 to May 2014. The SBR was started for the season at the end of October 2013, using activated sludge from the mainstream as inoculum. A stable process was established after 2 weeks. A pipe was temporarily installed for pumping RAS from the Augmented Train to the SBR; the process scheme for the experimental set-up is shown in Figure 2.

The flow rate of RAS to the SBR was stepwise increased relative to the flow rate of the digester supernatant to/from the SBR: starting with 0%, it was stepped up to 10%, 35%, and finally 100%, corresponding to 0.08%, 0.28%, and 0.80% of the RAS flow rate in the Augmented Train, respectively. The RAS was pumped to the SBR during the denitrification phase. Nitrification tests were performed roughly every second week on water from the Augmented Train and the Reference Train. The samples were collected from the last aeration basin in each train. On most occasions when nitrification tests were performed, multiple water samples were collected and analyzed to enable mass balances and other calculations. The temperature in the mainstream process varied from 8 °C to 15 °C and in the SBR from 24 °C to 35 °C (Figure 3). The temperature in the SBR was not noticeably affected by the stepwise increased share of cold RAS. The heat generated by biological reactions (Jewell & Kabrick 1980),

![Figure 1](https://iwaponline.com/wst/article-pdf/74/7/1736/458716/wst074071736.pdf)

Figure 1 | The phases of a normal SBR cycle. During decantation, there is a short period (seconds) for the withdrawal of excess sludge.
enhanced by ethanol dosage, kept the temperature at approximately 30°C. The lowest temperature in the SBR occurred in mid March and coincided with a week-long blower failure. The cyclic pH fluctuations during these days were greatly diminished, confirming the reduced biological activity.

In order to inoculate as large an amount of nitrifiers as possible, the SRT in the SBR was decreased during the last 2 months of the study. This was accomplished by omitting the sedimentation phase and letting the mixers operate during the decantation phase, so that the SRT was equal to the hydraulic retention time (HRT). Although the aim was to decrease the SRT in the mainstream process during the last months of the study, this was not possible due to practical restrictions in the WAS treatment system.

Nitrification tests were performed in the laboratory. A 400 mL portion of each sample was continually aerated at 20°C to a dissolved oxygen concentration of 5.0–6.5 mg L⁻¹ during the test. Ammonium was added to an initial concentration of about 40 mg N L⁻¹, along with nutrients and alkalinity. Samples of 7 mL were withdrawn every 20 minutes and were immediately filtered. Samples were analyzed for NH₄⁺-N, NO₃⁻-N, and NO₂⁻-N. The ammonium utilization rate was determined from the production rate of nitrate plus nitrite, and as a control, also determined from the utilization rate of ammonium. Concentrations of MLSS and mixed liquor volatile suspended solids were determined to enable calculations of utilization rates related to suspended solids. The nitrification tests were performed in accordance with Kristensen.
et al. (1992). Chemical analyses were performed using commercial cuvette test kits (Hach Lange, Düsseldorf, Germany) and a Xion 500 spectrophotometer (Hach Lange); test kits LCK 303 and LCK 504 were used for NH₄⁺-N, LCK 341 and LCK 342 for NO₂⁻-N, and LCK 359 for NO₃⁻-N.

Background to calculations

The increase of nitrifiers in the Augmented Train, added from the SBR, can be determined by calculating the mass of nitrifiers produced per day in the Augmented Train, the total mass of active nitrifiers in the SBR, and the mass of active nitrifiers conveyed from the SBR to the Augmented Train.

Assuming steady-state conditions, the mass of nitrifiers produced per day in the Augmented Train is given by

\[
M \Delta X_A = Y_A F N_a, \tag{1}
\]

where

\[
M \Delta X_A = \text{mass per day of nitrifiers generated (kg VSS d}^{-1}; VSS = \text{volatile suspended solids}),
\]

\[
Y_A = \text{yield coefficient for nitrifiers (kg VSS kg NH}_4\text{N}_{\text{red}}^{-1}),
\]

and

\[
F N_a = \text{mass per day of ammonium reduced (kg NH}_4\text{N}_{\text{red}}^{-1} \text{ d}^{-1}) \ (\text{Ekama & Wentzel 2008}).
\]

The mass of active nitrifiers in the SBR reactor is given by

\[
M X_A = \frac{F N_a Y_A SRT}{(1 + b_{AT} SRT)}, \tag{2}
\]

where

\[
M X_A = \text{mass of active nitrifiers in the reactor (kg VSS)},
\]

\[
F N_a = \text{mass per day of ammonium reduced (kg NH}_4\text{N}_{\text{red}}^{-1} \text{ d}^{-1}),
\]

\[
SRT = \text{sludge retention time (d)}, \text{ and}
\]

\[
b_{AT} = \text{specific endogenous respiration rate for nitrifiers at T}^ \circ \text{C (d}^{-1}) \ (\text{Ekama & Wentzel 2008}).
\]

Since the active nitrifiers are evenly distributed in the VSS, the mass of nitrifiers conveyed per day from the SBR to the Augmented Train is equal to

\[
\frac{M X_A (Q_{eff} X_{eff})}{V_{SBR} X_{SBR}} \ (\text{kg VSS d}^{-1}), \tag{3}
\]

where

\[
Q_{eff} = \text{effluent flow rate from the SBR to the Augmented Train (m}^3 \text{ d}^{-1}),
\]

\[
X_{eff} = \text{concentration of VSS from the SBR to the Augmented Train (kg VSS m}^{-3}),
\]

\[
V_{SBR} = \text{total volume of the SBR (m}^3), \text{ and}
\]

\[
X_{SBR} = \text{concentration of VSS in the SBR (kg VSS m}^{-3}).
\]

RESULTS AND DISCUSSION

Nitrification tests

Figure 4 shows the results from the nitrification tests in the mainstream process. The first two nitrification tests, at weeks 49 and 51, were performed without any recirculation...
of RAS over the SBR – that is, no deliberate boosting of nitrifiers to the mainstream process. Higher nitrification rates were found in the Reference Train for weeks 49 and 51, a finding that is consistent with observations by plant operators that the Reference Train outperforms the Augmented Train. The reason for this outperformance remains unclear since the sludge age and the individual volumes of the anoxic and aerobic basins are similar in the different trains. The fluctuations of nitrification rates in the Reference Train follow the fluctuations of temperature quite well; however, this pattern is not as obvious for the Augmented Train.

After week 51, a recirculation of RAS over the SBR was introduced: starting with 10% of the digester supernatant flow rate, stepping up to 35% and finally to 100%. To equalize the variations in individual data from separate weeks, the results were grouped into categories based on the RAS flow rate to the SBR. Furthermore, since the nitrification rates of the two trains differed before the RAS recirculation was started, the results were normalized against the category, with 0% RAS recirculation as a base (Figure 5). As shown in Figure 5, the nitrification rates in the Augmented Train increased compared with those in the Reference Train. Divided into categories, the increments were: 17% with 10% RAS flow rate; 41% with 35% RAS flow rate; 25% with 35% RAS flow rate and short SRT; and 22% with 100% RAS flow rate and short SRT. The largest increments coincided with the lowest temperatures in the mainstream process, which is in accordance with the simulation results in Berends et al. (2005), indicating that a low temperature in the mainstream process has a greater impact on bioaugmentation than the share of the RAS flow rate to the SBR.

To reveal how the increased nitrification rates from the bioaugmentation correlated with the temperature in the mainstream process, weekly uncategorized and normalized data were used, with week 49 as a base. The normalized nitrification rates in the Augmented Train were compared with those in the Reference Train. Figure 6 shows that increased nitrification rates between the trains for 35% RAS to the SBR are gathered along a curve, with the increment increasing as the temperature decreases. Results for 10% and 100% RAS to the SBR are found in the graph at each side of the curve for 35%. These suggest that a higher RAS recirculation to the SBR implies a larger increment of the nitrification rate. The highest studied RAS recirculation of 100% is consistent with what is recommended in Van Loosdrecht (2008). In this comparison, it was found that the highest increment in the nitrification rate for the Augmented Train coincided with the lowest temperature and was 58% higher than in the non-bioaugmented Reference Train. This result is in the same range of increased nitrification rate as was found in a full-scale study by Salem et al. (2004).

Results of calculations

Given these two assumptions – (i) that the SBR contains the same type of nitrifiers as the Augmented Train, which is in agreement with findings in Salem et al. (2004), and (ii) that steady-state conditions prevail both in the SBR and in the
Augmented Train – the calculation of the increased quantity of nitrifiers in the Augmented Train, conveyed from the SBR, should be in the same range as the measured increment of the nitrification rate in the Augmented Train. The daily mass of nitrifiers produced in the Augmented Train was calculated according to Equation (1). The total mass of nitrifiers in the SBR and the daily conveyed mass of nitrifiers from the SBR to the Augmented Train were calculated according to Equations (2) and (3), respectively. The values of the parameters in these equations were selected according to commonly observed values (Metcalf & Eddy 2003): \( Y_A \) was set to 0.12 g VSS g NH4-N\(^{-1}\); \( b_{AT} \) was set to 0.08 g VSS g VSS\(^{-1}\) d\(^{-1}\); and the temperature correction coefficient (\( \theta \)) for \( b_{AT} \) was set to 1.04.

Calculations of the nitrifiers transferred from the SBR to the Augmented Train were performed for the weeks when water samples were collected, excluding weeks 49 and 51 since the bioaugmentation had not yet started then. Figure 7 shows that there is a relatively good consistency between measured data and calculated results. The largest difference occurred in week 5. This discrepancy could be explained by the fact that the flow rate of the digester supernatant had been higher for a long period prior to week 5, but had been decreased to a lower range just a few SBR cycles before the samples were taken.

**CONCLUSIONS**

The results from this full-scale study indicate that the temperature in the bioaugmented process is the most important parameter for the magnitude of bioaugmentation; the lower the temperature, the greater the bioaugmentation impact. When the results were grouped together in different categories, the largest increment in the nitrification rate was 41%, but when the results were studied for an individual week, the largest increment was 58%. Both of these results coincided with the lowest water temperature in the bioaugmented mainstream process.
Increasing the RAS flow rate from the mainstream process to the side-stream treatment has a positive impact on the magnitude of bioaugmentation, although this is limited compared to the impact of temperature on the bioaugmented process. When uncategorized and normalized weekly data were used, and the increase in nitrification rates for the Augmented Train were compared with those of the Reference Train in a temperature range of 12–15 °C, the highest nitrification rate was obtained for 100% RAS recirculation to the SBR, followed by decreasing results for 35% and 10% RAS recirculation.

Preliminary results from molecular methods show a higher abundance of nitrifiers in the Augmented Train than in the Reference Train. This is to be further investigated.

ACKNOWLEDGEMENTS

We wish to thank the Swedish Water and Wastewater Association (project no. 12-105) and Veolia Water Technologies AB for financially supporting this study. We would also like to thank the staff of Slottshagen WWTP for their consistent support and help.

REFERENCES


First received 5 February 2016; accepted in revised form 30 June 2016. Available online 25 July 2016