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Title: Performance of aerobic nitrite granules treating an anaerobic pre-treated wastewater originating from the potato industry.

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ABSTRACT

In this study nitrogen removal via nitrite > 80% was achieved after approximately 80 days in an Sequencing Batch Reactor (SBR) treating pre-treated industrial wastewater originating from the potato industry. Thereafter SBR performance was investigated during the formation of aerobic nitrite granules (ANG). The first granules appeared after 26 days leading to full granulation after 64 days. ANG showed excellent settling properties, as the Sludge Volume Index (SVI) went down to 16 mL/g and a SVI_{10}/SVI_{30} =1 was obtained. qPCR analysis showed that slow growing organisms, especially Polyphosphate Accumulating Organisms (PAO) were stimulated by an anaerobic feeding strategy. The average nitrogen removal was 95.3% over the entire operational period, and it mainly followed the "nitrite-route". Moreover, with ANG also phosphorus removal efficiencies up to 65.7% could be achieved. However, it has to be mentioned

that nitrous oxide was an important denitrification product, which implies some environmental concerns.

KEYWORDS: aerobic granular sludge; nitrite-pathway; nitrous oxide; industrial wastewater; control strategies, PAO

1. INTRODUCTION

Industrial wastewaters are characterized by a great variability in terms of (bio)chemical composition pH, temperature, intermittent and shock loadings which are all inseparably dependent of the variety in industrial activities (Rosenwinkel et al. 2014). During the last century, many activated sludge (AS) technologies such as conventional activated sludge (CAS) systems, multiple stages AS system, membrane bioreactor (MBR) systems, hybrid processes and discontinue-flow sequencing batch reactor (SBR) processes were implemented for industrial wastewaters (Rosenwinkel et al. 2014).

Typically, potato processing industries deal with high-strength wastewater as it contains high amounts of starch, proteins, amino acids and sugars. On one hand, this high organic load is favourable for primary anaerobic pre-treatment as this initial treatment step goes along with the production of biogas. However, because proteins and amino acids are a primary source of nitrogen (N) and potatoes contain 0.6 mg/g phosphorus (P) (Bosak et al., 2016), this wastewater contains a substantial amount of nutrients as well. Biological nutrient removal (BNR) processes require a sufficient fraction of easily biodegradable COD. Therefore, a significant bypass of raw potato processing wastewater is unavoidable to achieve an enhanced BNR performance.

A combination of simultaneous nitritation-denitritation and aerobic granular sludge (Bassin et al., 2012; Yilmaz et al., 2008) might be an interesting strategy to reduce the amount of bypass needed to purge anaerobic pre-treated wastewaters, or in general for wastewaters with a low COD/N ratio. The main advantages of a nitrogen removal via nitrite are: (1) 25% less oxygen demand during nitrification; (2) 40% less COD demand during denitrification, (3) 20% reduction in CO_2 emission and (4) 30-50% less sludge production (Peng and Zhu, 2006). In addition, aerobic granular sludge has the potential for simultaneous removal of nutrients in a single tank (N and P) (De Kreuk et al., 2005; Lochmatter et al., 2013). Simultaneous nitrification-denitrification can be achieved through the formation of anoxic zones, which in granules typically occur near the core as a result of the decreasing oxygen-gradient (Beun et al., 2001; Zeng et al., 2003). Furthermore, by periodically alternating anaerobic and aerobic conditions, phosphorus removal can be achieved as well (De Kreuk et al., 2005; Lochmatter et al., 2013). Under phosphate rich conditions, polyphosphate accumulating organisms (PAO) can store carbon sources such as volatile fatty acids (VFA) as polyhydroxyalkanoates (PHA) (Mino et al., 1998). These internal PHA storage pools can be used for denitrification in combination with P-removal through denitrifying polyphosphate accumulating organisms (DPAO) (Carvalho et al., 2007; de Kreuk and van Loosdrecht, 2004). However, (D)PAO compete for VFA with (denitrifying) glycogen accumulating organisms (D)GAO (Bassin et al., 2012; Oehmen et al., 2007). In contrast to (D)PAO, (D)GAO are unable to contribute to P-removal, which makes them less favorable for BNR processes.

In recent years, research regarding granulation and short-cut nitrogen removal processes mainly focused on partial-nitritation/anammox (Liang et al., 2015; Lotti et al., 2014; Wang et al., 2012). Only a few studies reported granulation and simultaneous nutrient removal on industrial wastewater (Arrojo et al., 2004; Cassidy and Belia, 2005; Liu et al., 2015; Wang et al., 2007). To the best of our knowledge, simultaneous BNR by the use of aerobic nitrite granules (ANG) has not been studied on industrial wastewater before.

Environmental concerns, related to BNR, such as the reduction of greenhouse gas emissions have become more and more critical. Nitrous oxide is an important greenhouse gas and contributes to the destruction of the stratospheric ozon layer (IPCC, 2007). Previous research (Kampschreur et al., 2009, Kishida et al., 2004, Wunderlin et al., 2012) showed that the main factors stimulating the formation of nitrous oxide are: (1) nitrite accumulation, (2) low dissolved oxygen (DO) concentrations and low (3) COD/N values. According to these findings, N_2O analysis should be taken into account for ANG.

This study, focuses on treating industrial wastewater originating from the potato industry, aims to: (1) obtain a degree of nitritation-denitritation for >80% in floccular sludge, (2) transform the floccular sludge into ANG, (3) analyse BNR performance of ANG, (4) monitor N_2O production and calculate emissions for ANG.

2. MATERIALS AND METHODS

2.1. Reactor Set-up And Operating Conditions

A lab-scale sequencing batch reactor (SBR) with H/D ratio of 1.74 was operated at room temperature (18-22°C). The reactor was inoculated with sludge originating from a

lab-scale reactor treating similar industrial wastewater and performing conventional BNR. The SBR process was controlled by a Siemens PLC, process settings and visualization were controlled by a specifically programmed LabVIEW (National Instruments, Austin, Texas, USA) supervision program. Table 1 summarizes the different experimental periods.

In experimental period, obtaining N-shortcut (ONS), during each cycle a total volume of 1L was fed to the reactor spread over 4 separate feeding steps. To obtain a profound nitrogen shortcut via nitrite, the typical SBR sequence during ONS started with of a pre-aerobic phase (15 min), to make sure the sludge was endogenous, and four consecutive anoxic feeding phases (30 min), each followed by an aeration step with variable duration (15-35 min). To end the SBR cycle, there was one additional anoxic (30 min) and aerobic (15-35 min) phase without feeding followed by a prolonged anoxic phase (90min), a settling phase (120 min) and effluent withdrawal (5 min).

During the ANG periods, starting from day 104, the SBR sequence consisted of a pre-aerobic phase (30 min), an extended anaerobic feeding phase (90 min), a maximum of 5 reaction (aerobic/anoxic) steps with variable duration (see 2.2), a settling phase (60 – 2.7 min) and effluent withdrawal (5 min). During reactor operation the volume exchange ratio (VER) was increased from 9% (working volume 11L) during experimental period ONS to 26% (working volume 13.5L) in experimental period "ANG – P II" (table 1).

2.2. Aeration control strategy

To obtain the nitrite pathway for BNR, an aeration control strategy, based on the oxygen uptake rate (OUR) was applied. It was already demonstrated by Blackburne et

al., 2008 and Lemaire et al., 2008 that a sharp drop in the OUR appears as soon as NH_4^+ oxidation is finished. This aeration control strategy was the main criterion to end the aeration period.

In order to promote aerobic granulation and retain nitrogen removal via nitrite, an additional control strategy based on pH-decrease was applied. This pH-slope method assured alternation between the first aerobic and anoxic period in order to control nitrite accumulation, to maintain nitrogen removal via nitrite, and to achieve a degree simultaneous nitritation-denitritation (SND) as high as possible. The subsequent aerobic phases (limited to 5) had a maximum time of 35 min and were also supervised by the OUR aeration control strategy.

The dissolved oxygen (DO) level in the aerobic step was controlled using an on-off regulation between 1.0 and 2.0 mg O_2 /L. The aeration rate was kept between 1.5 and 2.0 L air/min. A constant SRT of 20 days was maintained by periodically removing the excess sludge from the reactor.

2.3. Microbial activity measurements

A custom-build respirometer was controlled by a WAGO PLC (GmbH & Co. KG, Germany) and consisted of a DO probe (Hamilton, Switzerland) and air pump. It was supervised by a custom-build LabVIEW (National Instruments, Austin, Texas, USA) control program, which automatically calculated oxygen uptakes rates (OUR), wereafter the maximum specific oxygen uptake rates (SOUR) of AOB ($SOUR_{AOB}$) and NOB ($SOUR_{NOB}$) could be determined. Biomass was collected at the end of the SBR cycle and aerated until endogenous respiration $SOUR_{endo}$ was reached. Then, a pulse of nitrite ($NaNO_2$) was dosed to achieve an initial concentration of 10 mg NO_2^--N/L. After three

OUR measurements, a pulse of ammonium (NH_4Cl) was dosed to achieve an initial concentration of 10 mg NH_4^+-N/L. The DO was controlled between 4 mg O_2/L and 6 mg O_2/L. Based on Jubany et al., 2009, following calculations were used to evaluate the specific nitrogen removal rates (SR) and NOB/AOB activity ratio:

$SOUR_{NOB}$ (mg O_2/h. gVSS) = $SOUR_{NOB+endo}$ - $SOUR_{endo}$

$SR_{NOB} = SOUR_{NOB}/(1.14 \text{ mg } O_2/\text{mg N})$

$SOUR_{AOB}$ (mg O_2 /h. gVSS) = $SOUR_{AOB+NOB+endo}$ - $SOUR_{NOB+endo}$

$SR_{AOB} = SOUR_{AOB}/(3.43 \text{ mg } O_2/\text{mg N})$

NOB/AOB activity ratio (%) $=(SR_{NOB}/SR_{AOB}) \times 100$

This NOB/AOB activity ratio can be used in order to evaluate the nitritation-denitritation process, a low nitratation-rate (SR_{NOB}) will result in a low activity ratio, which is a good indicator for the performance of "nitrite-route" nitrification.

2.4.qPCR Procedure For Molecular Quantification

DNA extraction from sludge samples was conducted using the NaTCA method (McIlroy et al., 2009). Resulting DNA concentrations were analysed by a fluorometric quantification using an Qubit 3.0 Fluorometer (ThermoFisher Scientific, Waltham, Massachusetts). In order to determine the abundance of AOB, NOB, PAO and GAO specific target genes (table 2) were quantified through SYBR Green quantitative polymerase chain reaction (qPCR) assays. Real-time PCR was performed on a CFX96 Touch (BioRad, Hercules, California, USA). Standard curves were constructed by two

series of 10 fold dilutions of plasmids carrying the target genes, which varied between 1.0×10^3 and 1.0×10^8. Only standard curves with an R square > 0.99 were withhold. Furthermore, for each DNA sample, three replicates were run and all qPCR assays contained no-template control reactions. For every sample, the average target copy numbers per genome (table 2), MLVSS and specific DNA concentration, were used to calculate the amount of target cells per g biomass. This finally led to the NOB/AOB absolute quantification ratio.

2.5. The Industrial Wastewater

The wastewater was sampled bimonthly directly at the UASB reactor outlet of a potato processing enterprise and stored immediately at 4°C. Most of the easily degradable COD was consumed during the pre-treated anaerobic step. Therefore, in the three first operational periods (ONS, ANG I and ANG II), acetate was dosed to maintain an average COD/N ratio of 6.5. In ANG P III, part of the acetate was gradually substituted (10 – 50%) by raw wastewater (sampled at the USAB inlet, containing on average 6030 mg COD/L and 5080 mg SCOD/L), also to maintain an average COD/N ratio of 6.5. The average influent parameters throughout the different operational periods (ONS and ANG) were: 1546 ± 389 mg COD /L, 1334 ± 335 mg SCOD/L, 236.8 ± 26.9 mg NH_4-N/L, 53.8 ± 27.0 mg PO_4-P/L.

2.6. Analytical methods

All samples were filtered over a glass microfiber filter (particle retention 1.2 µm). Subsequently concentrations of phosphate (Hach Lange, Germany), ammonium (Hanna Instruments, Belgium), nitrite (Hach Lange, Germany), nitrate (Hanna Instruments,

Belgium) and COD (Hanna Instruments, Belgium) were analysed with standard cuvette tests (Hanna Instruments, Temse, Belgium). The evolution of the sludge morphology was examined using an MOTIC (Xiamen, China) microscope. Biomass concentration and sludge volume index (SVI) measurements were conducted according to the standard methods (APHA, 1998).

2.7. In-situ cycle measurements

During period ONS, in-situ cycle measurements during aerobic steps were carried out to determine the nitrite accumulation rate (NAR).

$$NAR\ (\%) = [NO_2^- \text{-}N]^{tn}_{t0} / ([NO_2^- \text{-}N]^{tn}_{t0} + [NO_3^- \text{-}N]^{tn}_{t0}) \times 100$$

With t_0 the start of the aeration phase and t_n the end of the aeration phase.

Subsequently, during the ANG periods, in-situ cycle measurements were performed during standard reactor operation. Grab samples were taken every 10 – 30 min in order to obtain profiles of nitrite, nitrate, ammonium and phosphate. N_2O profiles were monitored in the liquid phase using a Unisense (Aarhus, Denmark) micro sensor with data logging. A two-point calibration of the micro sensor was done before each measurement. A calculation example regarding N_2O emissions can be found in the supplementary data (A).

2.8. SND efficiency

Calculation of the simultaneous nitritation-denitritation (SND) was based on the total amount of NO_x-N formed, divided by the amount of NH_4-N oxidized. A distinction was made between SND_1, during the first aeration step, and the cumulative SND_c throughout the entire reaction cycle.

$$SND_1\ (\%) = ([NO^-_x\text{-}N]^{tn}_{t0} / [NH_4^+\text{-}N]^{t0}_{tn}) \times 100$$

with t_0 start of aeration 1 and t_n the end of aeration 1

$$SND_c\ (\%) = (([NO^-_x\text{-}N]^{t1n}_{t10} + [NO^-_x\text{-}N]^{t2n}_{t20} + [NO^-_x\text{-}N]^{t3n}_{t30}) / ([NH_4^+\text{-}N]^{t10}_{t1n} + [NH_4^+\text{-}N]^{t20}_{t2n} + [NH_4^+\text{-}N]^{t30}_{t3n})) \times 100;$$

With t_{10} start of aeration 1 and t_{1n} the end of aeration ; t_{20} start of aeration 2 and t_{2n} the end of aeration 2; ….

3. RESULTS AND DISCUSSION

3.1. Repression Of NOB And Achievement Of The Nitrite-pathway

The first experimental period, ONS lasted 103 days. During this period, the NAR was determined regularly. In the subsequent operational periods (ANG I – III) maintaining the nitrite-pathway was an important objective, but due to SND processes, it was unfeasible to determine the NAR during the ANG periods. Therefore, specific activity measurements, to determine SR_{AOB}, SR_{NOB} and to calculate NOB/AOB activity ratio, were carried out throughout the whole study. On day 0 (the inoculum), 99, 174, 205, 408, 427 and 455 measurements for molecular quantification by qPCR were conducted to verify the absolute populations of AOB.

Figure 1 shows the results of the NAR (during stage ONS), microbial activity measurements, molecular quantification, NOB/AOB activity/quantification ratio and nitrogen removal efficiency. It can be observed that, at the onset of ONS, nitrogen removal followed the conventional two-step nitrification. Through the use of the aeration control strategy, a continued increase in NAR and decline in the NOB/AOB activity ratio could be observed. As from day 79 the NAR reached 87.5%, NOB/AOB activity ratio went down below 30% and a quantification ratio of 0.093 was achieved. These results indicate that nitrification was shifted to a one step nitritation followed by a denitritation step to close the nitrogen removal loop. Aeration control strategies already proved their value for achieving nitritation-denitritation (Blackburne et al., 2008; Lemaire et al., 2008).

During the following operational periods (ANG PI – PIII), the nitrite-route for nitrogen removal could easily be maintained, the NOB/AOB activity ratio was below 25% almost constantly. Only at the start of ANG PIII, a slight increase of the NOB/AOB activity ratio to about 40% could be noticed. In addition, the NOB/AOB quantification ratio was below 0.05 for every sample taken during the ANG periods. Overall, a close relationship between NOB/AOB activity ratio and quantification ratio was observed.

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3.2. Factors Affecting The Transformation From Floccular Sludge Into ANG

An important aim of the present study was to transform the floccular sludge into ANG. Therefore, sludge characteristics as biomass concentration, SVI and morphology were examined during the different operational periods. In order to survey the microbial population, molecular quantification by qPCR was conducted.

The biomass concentration of the seed sludge was 6.31 gMLSS/L. During ONS, the VER and settling time were kept constant. As a result of these settings, biomass concentrations (ML(V)SS) sharply decreased (figure 2). The initial SVI values varied between 200 and 300 mL/g.

The formation and maintenance of the ANG granules can be divided in 3 consecutive periods (ANG - P I; ANG - P II; ANG - P III). During ANG - P I settling time was decreased from 25 min on day 104 to 5 min on day 208, while the VER was increased from 11% to 20%. The combination of these two factors resulted in a strong hydraulic selection pressure, which has been shown to lead to the selection of quickly settling particles (Liu and Tay, 2004). As figure 2 shows, the ML(V)SS fluctuated, while in

general a slightly rising trend is visible. The sludge morphology of the seed sludge showed irregular, loose and even filamentous structures (supplementary Fig. B.1(A)). At the start of ANG – P I (day 104), some preliminary aggregation was already visible. Granule development was especially clear from day 127 up to day 205 leading to well-shaped particles with dimensions up to 0,5 mm. Another parameter demonstrating well-formed granules is the SVI_{10}/SVI_{30} ratio (de Kreuk et al., 2007). Figure 2 shows the decreasing SVI values (at day 167, $SVI_{10} = SVI_{30} = 16$ mL/g). Due to some aeration issues a minor increase in SVI values was observed from day 174 until day 208 (average $SVI_{10} = 77$ mL/g; $SVI_{30} = 55$).

At the start of ANG - P II, the industrial wastewater was contaminated with anaerobic sludge originating from the potato enterprise UASB reactor. This contamination is clearly visible in figure 2, which shows that the MLVSS/MLSS ratio decreased and indicating an increase of inorganic matter in the reactor. With a slight delay, also the SVI values increased. From the microscopic point of view (supplementary Fig. B.1(A)), granule degradation and accumulation of loose and filamentous structures were observed. At day 226, the batch of wastewater was replaced. Nevertheless, it should be emphasized that a significant portion of the previously formed granules remained stable during this disturbance (supplementary Fig. B.1(A)).

ANG - P III was initiated with a reduction of the settling time from 6 min on day 307 to a final minimum of 3 min on day 362. As figure 2 shows, this modification affected the ML(V)SS values, which dropped significantly as results of biomass washout (effluent TSS not measured). Furthermore, sludge settling greatly improved, resulting in

decreasing SVI values (at day 384, SVI_{30} = 30), indicating highly granulated biomass. This was confirmed by microscopic analysis of sludge morphology (supplementary Fig.B.1(B)), demonstrating the presence of mature granules, between day 336 and 395, with sharp edges and dimensions varying between 0.5 and 1.0 mm. Because a slight drop in nitrogen removal (76% at day 405) was observed, the VER was decreased to 17% from day 405. This modification also affected sludge settleability (at day 419, SVI_{30} = 119), due to the growth of suspended biomass. However, again settleability (at day 440, SVI_{30} = 47) as well as nitrogen removal (98% at day 440) recovered fairly quickly.

Figure 3, shows the amount of target cells per g biomass for PAO and GAO. It can be observed that the inoculum already contained a fair amount of PAO , while the GAO population was much smaller. The specific reactor operation during ONS, completely turned over this balance (day 99). Subsequent, introduction of an anaerobic feeding phase in ANG PI – PIII definitely stimulated progressive growth of PAO, while the GAO population first showed a decline whereafter, starting from ANG PIII, the population recovered again. These observations are in line with the results of previous research from de Kreuk and van Loosdrecht, 2004 and Pronk et al., 2015, who stated that selection of slow growing organisms, such as PAO and GAO are one of the most important strategies to stimulate aerobic granulation. Both the rising PAO levels, and recovery of GAO, during ANG PIII, might be a consequence of the partial replacement of the acetate by raw wastewater, containing different VFA substrates (Oehmen et al., 2007).

During experimental period ANG PI – PIII, also phosphorus removal was examined. These results, together with the anaerobic PO_4-P release are summarized in table 3. A significant increase for both parameters can be observed between ANG PI and PII. Similar phosphorus removal efficiencies concerning the treatment of industrial wastewater with aerobic granular sludge were already reported by(Arrojo et al., 2004; Liu et al., 2015; Yilmaz et al., 2008).

3.3. The Effect Of ANG On The Sludge Loading Rate And SND

High specific conversion rates are one of the major benefits of aerobic granulation (Liu and Tay, 2004). Table 4 shows the average COD (gCOD/gVSS.day) and nitrogen sludge loading rates (mgNH_4-N/gVSS.day) for the different experimental periods. A progressive increase in both loading rates can be observed over the whole operational period, starting at 0.162 gCOD/gVSS.day during "ONS" up to 0.274 gCOD/gVSS.day in period ANG-P III. The enhanced nitrogen loading rate is even more remarkable, which was increased from 22.8 mgNH_4-N/gVSS.day to 54.2 mgNH_4-N/gVSS.day.

The possibility to employ aerobic granules for simultaneous nitrification-denitrification was already proved by Bassin et al., 2012; Kishida et al., 2006; Pronk et al., 2015. Moreover, SND was also observed in the present study, the results are summarized in table 3, which displays the average $\%SND_1$ and $\%SND_c$ for the different experimental periods. These results confirm the favourable effect of granulation, and COD storage, during the anaerobic phase on the SND (Gao et al., 2011; Guo et al., 2013; Zeng et al., 2003). Moreover, it is clearly visible that after the first loop (one alternation between

aerobic and anoxic steps), the stored organic carbon becomes more and more depleted, leading to a decrease in %SND_c in the following loops (SND_c).

3.4. Effect Of ANG On Nitrous Oxide Emissions

Nitrous oxide can be formed during autotrophic nitrification and heterotrophic denitrification. In both processes, N_2O acts as an inevitable intermediate. For ANG the two most important pathways leading to N_2O production (Kampschreur et al., 2009) are: (1) nitrifier denitrification and (2) heterotrophic denitrification. Nitrifier denitrification covers the reduction from NO_2^- by AOB under oxygen limiting conditions, low organic carbon content and increased nitrite concentrations (Wrage et al., 2001; Wunderlin et al., 2012). Incomplete heterotrophic denitrification leads to the formation of N_2O as main product instead of N_2. The key factors affecting this incomplete denitrification are high nitrite levels and the absence of sufficient carbon sources (Itokawa et al., 2001, Kishida et al., 2004). According to Quan et al., 2012, granules could be a trigger for enhanced N_2O emissions as well.

Due to the high solubility of N_2O in water, it was assumed that during non-aerated periods, the emissions were negligible. Therefore, emissions were only considered during aeration events. For experimental period ONS, N_2O profiles were assessed for one specific aeration step on day 59 and 97 (figure 4 A/B). The on/off aeration regulation affected N_2O formation, resulting in a specific saw - tooth profile. However, in general, a progressive increase in soluble N_2O could be observed for both experiments. In order to estimate N_2O emissions, calculations (supplementary data A) were performed, resulting in the cumulative N_2O - N emissions, which can be compared

to the total amount of NH_4^+ - N consumed. For both experiments, comparable results of approximately 4.0% emission as N_2O – N were obtained. As already stated before, the main trigger for these N_2O emissions is expected to be nitrifier denitrification caused by low DO and elevated NO_2^- concentrations.

During ANG – PIII, online N_2O measurements were conducted on day 308 and 449, next to a number of grab samples analysed offline, in order to obtain a general overview of the nitrogen mass balance (figure 5). Both types of measurements show a significant formation of N_2O during aerobic steps as well as a significant production of N_2O during anoxic periods. Due to subsequent aeration events, anoxic N_2O production caused strongly increased N_2O emissions (Kampschreur et al., 2008).

At day 308, the N_2O - N emission was 15.05 mg/L. According to figure 5A, nitrite was completely denitrified after the anoxic periods. However, high concentrations of dissolved nitrous oxide at the end of an anoxic period caused elevated N_2O emissions after the anoxic phases. In total 5.05 mg N_2O – N /L was emitted during the first aeration pulse after an anoxic phase, which represents 33.5% of the N_2O emitted. Similar results were obtained at day 449 as 5.20 mg N_2O – N /L (31.1%) of the total of 16.68 mg N_2O – N/L was emitted directly after the anoxic phase. However, the overall N_2O emission at day 449 was almost twofold compared with the emission on day 308. The availability of organic carbon compounds during aerobic periods might explain this difference, as the acetate was partially substituted by raw wastewater during ANG PIII. As most of the easily degradable carbon is stored intracellularly during anaerobic conditions, significant N_2O production is caused by simultaneous nitritation-denitritation. Similarly, Gao et al., 2016 found that the combination of (1) internal storage denitrification and (2) higher ammonium removal rates led to nitrite

accumulation. They suggested that under these conditions, incomplete denitrification occurs, which might at least partially explain why the emission at day 449 is almost twofold higher than the emission on day 308. These findings correspond with the results of previous studies (Lemaire et al., 2006; Zeng et al., 2003). Consequently, strategies to mitigate N_2O emissions for ANG should be investigated in more detail. Desloover et al., 2012 proposed several strategies to mitigate the N_2O emissions in biological nitrogen removal systems. Most strategies rely on minimizing N_2O precursors, as low DO, elevated NO_2^- concentrations, low COD/N ratio's. Yang et al., 2013 found that applying a step-feed on a partial nitrifying granular sludge reactor could reduce the N_2O emissions with 53.8%. The use of a step-feed might be a promising strategy for ANG processes as well. During period ONS, step-feed is used in order to obtain nitrogen shortcut, which coincides with the fact that N_2O emissions are significant lower than during the ANG periods. In addition, it can be interesting to investigated elevated DO set points and there might be a great potential in developing a control strategy, based on the dissolved N_2O accumulation in order to supervise the anoxic periods Recently, Dries, 2016 also emphasized that dynamic SBR cycles might be interesting, in order to develop novel N_2O mitigation strategies.

4. CONCLUSIONS

In conclusion, formation of ANG with pre-treated industrial wastewater, originating from the potato industry, is possible. Short-cut nitrification was achieved after approximately 80 days. After changing the operational strategy, granules became the dominant fraction from day 168. Additionally, the nitritation-denitritation pathway could be maintained at all times. BNR efficiencies for N and P reached 95.3% and

65.7%. Nevertheless, an important denitrification product of ANG seems to be nitrous oxide, which implies some environmental issues. Future investigation should examine how to mitigate N_2O formation and emission in more detail.

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Day	Period	VER (%)	Aim
1 - 103	obtaining N-shortcut (ONS)	9%	Repressing NOB; promoting AOB
104 - 208	ANG period I (ANG P I)	9% - 17%	Promoting granulation
209 - 306	ANG period II (ANG P II)	17% - 26%	Examination of granule stability
307 - 456	ANG period II (ANG P III)	26% - 17%	Recovering and maintaining ANG

Table 1: Overview different experimental periods.

Target	Cell copy number	Primers	Conditions	Reference
amoA ***Nitrosomonas***	2	amoA-1F / amoA-1R	98° - 3‘ [98° - 15“ / 56° - 15“ / 72° - 30“] x 40	Rotthauwe and Witzel, 1997
nxrB ***Nitrospira***	2	nxrBF169 / nxrBR638	98° - 3‘ [98° - 15“ / 56° - 15“ / 72° - 30“] x 40	Pester et al., 2014
16S rRNA ***Candidatus Accummulibacter phosphatis***	2	PAO651f / PAO846r	98° - 3‘ [98° - 15“ / 57° - 15“ / 72° - 15“] x 40	Fukushima et al., 2007
16S rRNA ***Candidatus Competibacter phosphatis***	5,66	GAOQ989f / GAM1278r	98° - 3‘ [98° - 15“ / 59° - 15“ / 72° - 30“] x 40	Crocetti R. et al., 2002

Table 2: Target, cell copy number, primers, qPCR conditions and references

	PO_4-P removal (%)	**Anaerobic PO_4-P release (mg PO_4-P/g.VSS)**
	Average ± SD	Average ± SD
ANG P I (day 104 – 208)	41.0 ±15.1% (n=15)	9.2 ± 9.5 (n=4)
ANG P II (day 209 – 306)	65.7 ± 18.1% (n=14)	21.1 ± 2.3 (n=3)
ANG P III (day 307 – 456)	61.5 ± 27.1% (n=21)	20.6 ± 6.4 (n=12)

Table 3: Average PO_4-P removal (%) and anaerobic PO_4-P release (mg PO_4-P/g.VSS) for the different experimental periods.

	COD loading rate (g COD/g.VSS.day)				N loading rate (mg NH4-N/g.VSS.day)			
	Min.	*Max.*	*Average + SD*	*%CV*	*Min.*	*Max.*	*Average + SD*	*%CV*
ONS (day 1 - 103)	0.0539	0.269	0.162 ± 0.0455	28	6.76	44.6	22.8 ± 8.46	37
ANG P I (day 104 - 208)	0.0918	0.291	0.195 ± 0.0456	23	16.3	45.5	29.6 ± 7.78	26
ANG P II (day 209 - 306)	0.109	0.303	0.203 ± 0.0463	23	20.0	56.4	40.9 ± 11.2	27
ANG P III (day 307 -)	0.130	0.444	0.274 ± 0.0677	25	34.2	81.1	54.2 ± 9.40	17

Table 4: Average COD and nitrogen loading rate during the different experimental periods.

	SND_1 (%)				SND_c (%)			
	Min.	*Max.*	*Average + SD*	*%CV*	*Min.*	*Max.*	*Average + SD*	*%CV*
ONS (day 1 - 103)	39.2	85.2	64.9 ± 14.5	22				
ANG P I (day 104-208)	45.0	87.0	72.4 ± 15.2	21	37.9	71.2	49.0 ± 14.3	29
ANG P II (day 209-306)	65.3	85.5	75.3 ± 10.1	13	44.0	66.1	55.1 ± 15.6	28
ANG P II (day 307 -)	48.9	88.6	74.0 ± 12.4	17	35.9	66.2	50.0 ± 9.3	19

Table 5: Average SND_1 and SND_c during the different experimental periods.

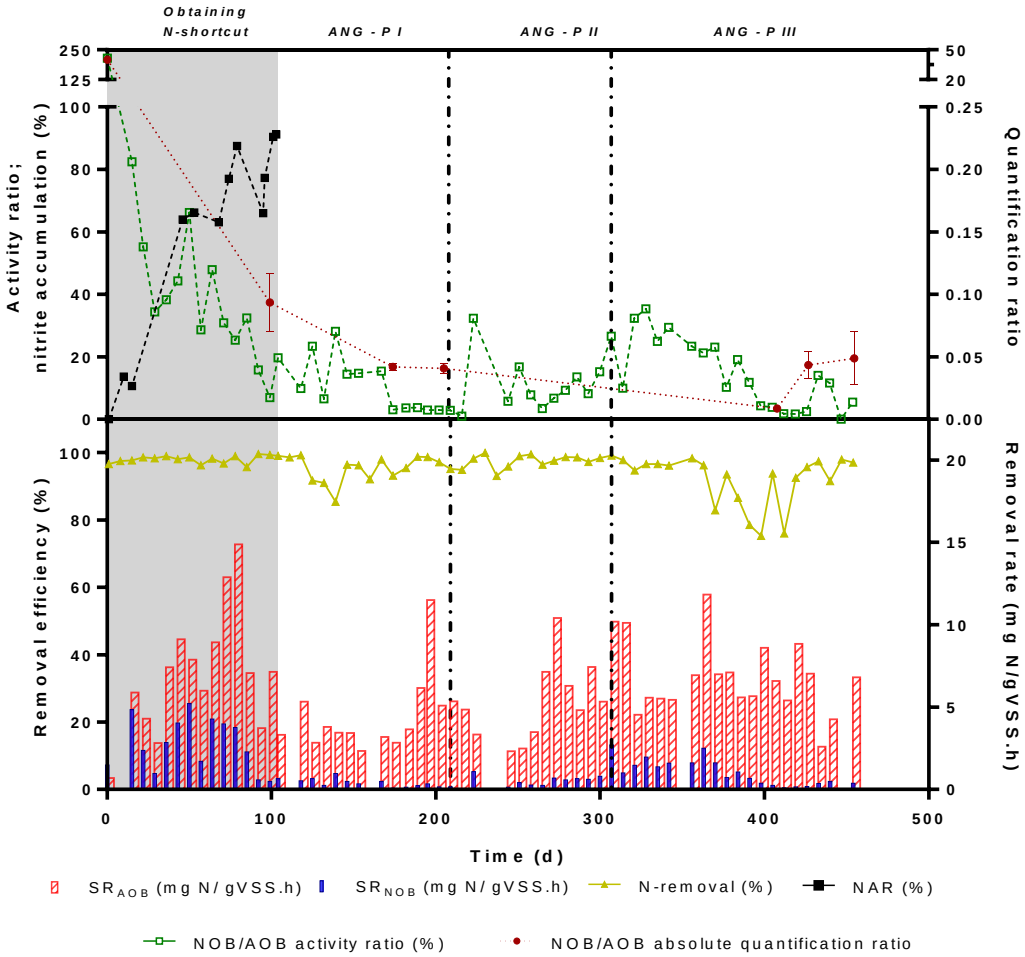

Figure 1. Nitrite accumulation rate (%), Specific nitrogen removal rates (SR) for AOB and NOB (mg N/gMLVSS.h), NOB/AOB activity ratio (%), NOB/AOB absolute quantification ratio and nitrogen removal (%) throughout the different experimental periods.

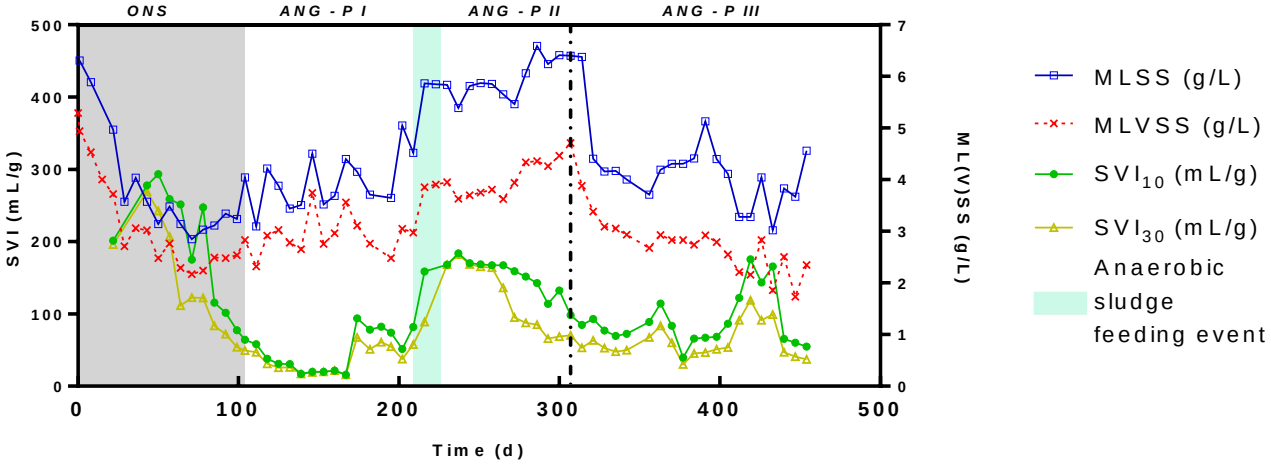

Figure 2. Evolution of sludge parameters ML(V)SS, SVI_{10} and SVI_{30} throughout the different operational periods (ONS – ANG P III)

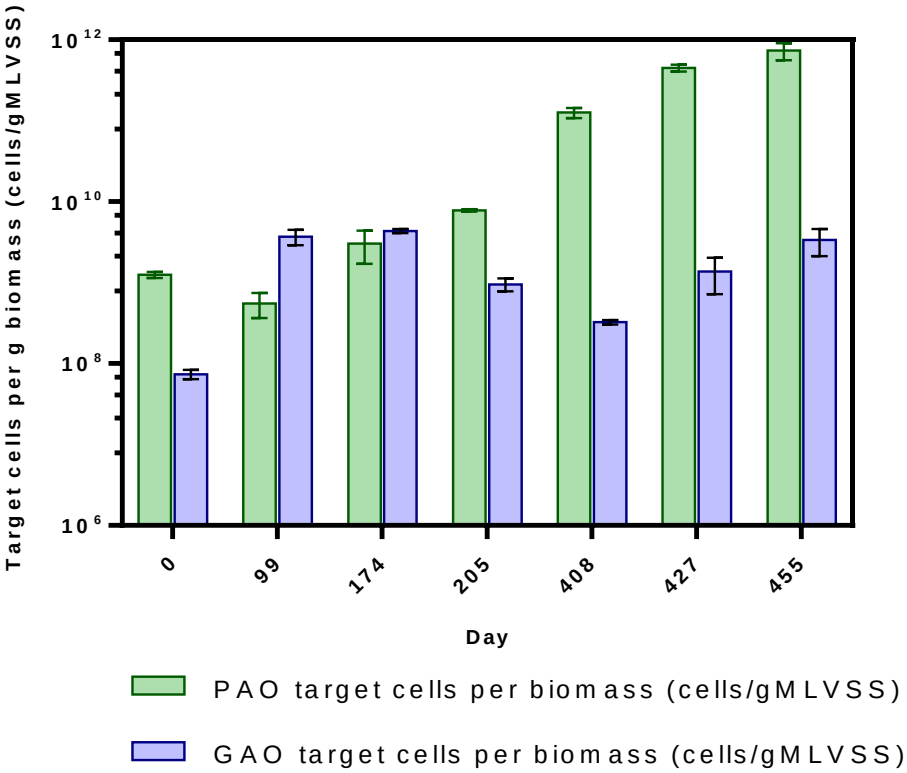

Figure 3. Evolution of specific PAO and GAO abundance (target cells per biomass) during the operational period.

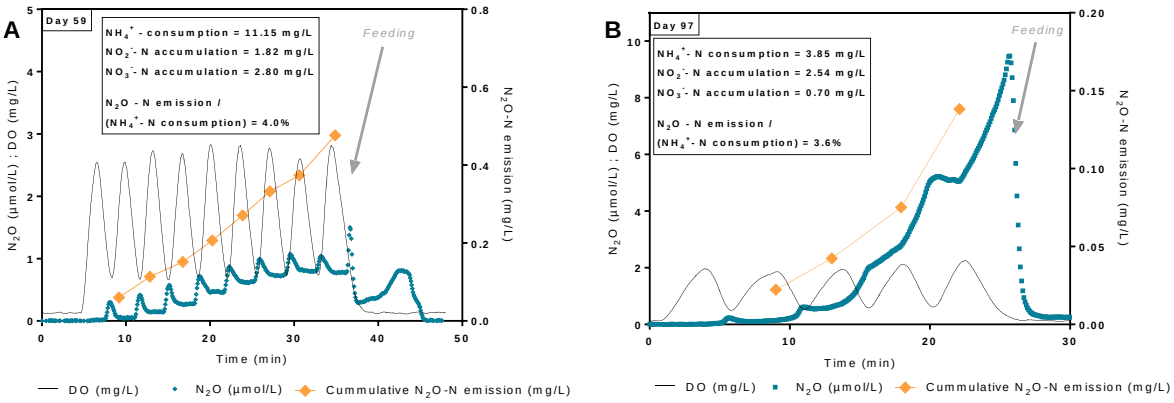

Figure 4. Nitrous oxide formation and emission on day 59 (A) and 97 (B).

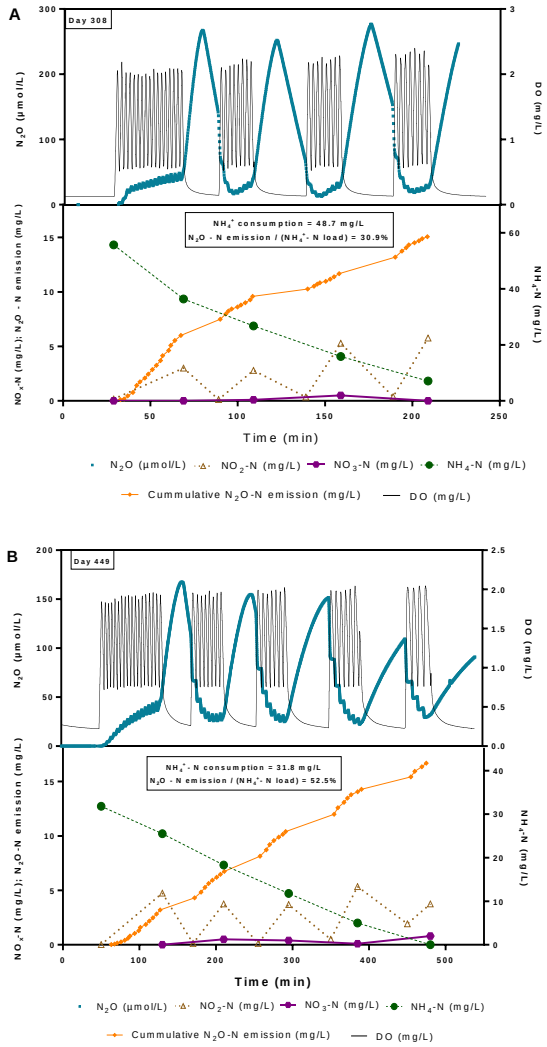

Figure 5. Online cycle measurements showing nitrous oxide formation and emission in combination with NO_2^-, NO_3^- and NH_4^+ offline grab samples on day 308 (A) and 445 (B).