

The increase of process stability in removing ammonia nitrogen from wastewater

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ABSTRACT

This work focuses on the removal of ammonia nitrogen pollution from wastewaters in a two-stage laboratory model based on a combination of the nitrification and anammox processes with the biomass immobilized in a polyvinyl alcohol (PVA) matrix. Owing to the immobilization approach inside the PVA pellets, the bacterial activity remained nearly unchanged on an abrupt change in the environmental conditions. The nitrification kinetics were significantly dependent on the dissolved oxygen concentration. The critical dissolved oxygen concentration at which the nitrification process using the immobilized bacterial culture stops is 0.6 mg/L. The volumetric rate of nitrogen removal by the anammox bacteria was 158 mg/(L·d). The technology presented is well-suited for removing high ammonia nitrogen concentrations (≥ 300 mg/L).

Key words | ammonia nitrogen, anammox, immobilization, nitrification, wastewater

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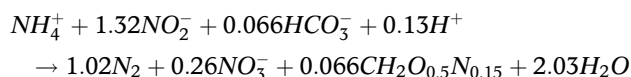
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INTRODUCTION

Ammonia nitrogen is routinely removed from wastewaters by biological nitrification and denitrification, which are processes requiring both appreciable investment costs (reactor) and appreciable running costs (aeration, organic substrate dosing). The anammox (anaerobic ammonium oxidation) process, developed in the 1990s (Mulder *et al.* 1995; Van de Graaf *et al.* 1995), is financially beneficial (Jetten *et al.* 2001). The anammox bacteria are able, autotrophically and in anoxic conditions, to stimulate the reaction between the ammonia and nitrite nitrogen, producing nitrogen gas. The anammox process can be conveniently combined with partial nitrification, where one half of the ammonia nitrogen entering the process is biologically oxidized in the presence of oxygen to nitrite, which reacts with the remaining ammonia nitrogen within the anammox process, producing nitrogen gas (which is released to air). This can be described by the equation (Riffat 2012):



where the formula $\text{CH}_2\text{O}_{0.5}\text{N}_{0.15}$ stands for the anammox bacteria biomass.

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This process combination is called CANON (completely autotrophic nitrogen removal over nitrite) (Schmidt *et al.* 2003). Anammox bacteria have been detected in a number of wastewater treatment plants (Schmidt *et al.* 2002). Attention has recently also been paid to overall community structure and anammox species using the genome-based metagenomics (Bhattacharjee 2017). Many papers focusing on the nitrification-anammox process in the treatment of wastewaters have been published (e.g. Isaka *et al.* 2013; Kosari *et al.* 2014; Lackner *et al.* 2014; Li & Sung 2015; Fuchs *et al.* 2017). A low anammox bacteria growth rate (Jetten *et al.* 2001; Schmidt *et al.* 2002), difficulties in sustaining them in the system and in the control of the partial nitrification process (Ma *et al.* 2016) are basic problems hindering a wide application of this approach. The partial nitrification can be maintained by keeping the oxygen concentration below 1 mg/L (Ma *et al.* 2016). The above problems can be addressed by immobilizing the bacteria in a gel. This approach has been applied, e.g. by Isaka *et al.* (2013), who immobilized a mixed culture of the nitrification and anammox bacteria in a cube-shaped gel. Also, Hitachi company (<http://www.hitachi.com>) has a registered trademark on polyethylene glycol Bio-N-cubes with immobilized nitrifying bacteria for supporting nitrification in

nitrification/denitrification process Pegasus. This method enables to form bacterial biofilm on surface of cubes.

The present work used the Lentikats Biotechnology (<http://www.lentikats.eu>), which has been used with success in many nitrogen removal applications. Both nitrification bacteria (Vacková *et al.* 2012) and denitrification bacteria (Trögl *et al.* 2011a, 2011b, 2012; Vacková *et al.* 2011) were encapsulated.

The Lentikats Biotechnology is based on bacteria immobilization by encapsulation in a porous PVA gel pellet (patents DE 19827552 and WO/2007/104268). This approach to immobilize bacteria inside, not on surface, possesses many advantages against other immobilization methods: the specific lenticular shape facilitates diffusion of the substrate and separation of the pure wastewater from the pellets, and the matrix material is biologically non-degradable, non-toxic and mechanically resistant (Schlieker & Vorlop 2006). The pores in the matrix are large enough to support bacterial growth inside the pellets and small enough to prevent other bacteria in the surrounding medium from penetrating into the lentil material. This ensures a species-constant composition of the biocatalyst during its entire service time. Height of pellets was 200–400 µm and width was 3–4 mm.

The immobilization of the bacterial cultures into the PVA matrix contributes to an adequate stability of the process, which is frequently a problem in the anammox process where the biomass is usually present as spontaneously formed granules. The used method of immobilization by encapsulation in porous PVA gel pellets allows anammox bacteria to be protected behind the pellet pores against short-term stresses, resulting in system stability. The biomass in pellets is a component of the system throughout the process; it is not washed out, e.g. when temperature decreases, as is typically the case with granulated sludge (Ma *et al.* 2016). Factors that must be maintained during the anammox process include the appropriate ammonia-to-nitrite nitrogen ratio, optimum temperature, optimum pH and the anoxic conditions while ammonia and nitrite nitrogen are converted to nitrogen gas. The optimum temperature at which the anammox bacteria are highly active has been reported to be 20–43 °C (Schmidt *et al.* 2002) or 30–37 °C (Jetten *et al.* 2001). Also, the new approach for adaptation of anammox bacteria to 10 °C was shown (Kouba *et al.* 2017). The optimum pH range has been reported to be 6.4–8.3 (Schmidt *et al.* 2002) or 7.0–8.5 (Jetten *et al.* 2001).

Apart from the stability of the process, the financial benefits are also important: much less oxygen is used up

compared to the conventional methods and no organic substrate needs to be added. Also, this technology occupies a lower built-up area and sludge production is minimal. In comparison with conventional nitrification/denitrification process, the investigated process represents saving of operation costs. There is no need to supply substrate for denitrification and supply of necessary oxygen represents 50% saving of costs (Fux *et al.* 2002). The saving of pollution charges also represents an economic benefit.

The aim of the present work was to verify the long-term stability of the two-step nitrification/anammox process in laboratory conditions when setting the key parameters such as dissolved oxygen concentration and ammonia nitrogen load. The solution presented is novel in the fact that the well-known procedure of immobilization is modified by applying the Lentikats technology for cultivation of anammox bacteria inside the pellets to enhance the stability of an economically advantageous and environmentally significant process because it has also a positive impact on the environment by reducing excess ammonia nitrogen contamination in the discharged wastewater.

MATERIAL AND METHOD

Experiment design

The two-stage nitrification/anammox reactor consisted of two separate rectangular stirred reactors equipped with sensors for process monitoring and control, pumps and a blower to establish the required conditions in the reactors (Figure 1). The process was controlled by using two microprocessor-controlled units, products of Saturn Holešov, Ltd Company. The units were used for data collection from sensors and control of blowers, pumps and heating elements (also supplied by Saturn Holešov, Ltd).

Nitrification reactor – first stage

The effective volume of the first reactor was 16.25 L. The nitrification biocatalyst content of the reactor was 1.45 kg, which is equivalent to 90 g/L, i.e. roughly 9% reactor filling. Since the maximum usable reactor filling was 20%, the actual filling was less than 50% of the maximum. The nitrification biomass consisted of a pure culture of the *Nitrosomonas europaea* bacteria (NCIMB 118 50, NCIMB Ltd, UK), cultured and immobilized in PVA by Lentikat's a.s. company. The reactor flow rate was 16.7 L/d, which implies a hydraulic retention time of 23.4 h for the particular

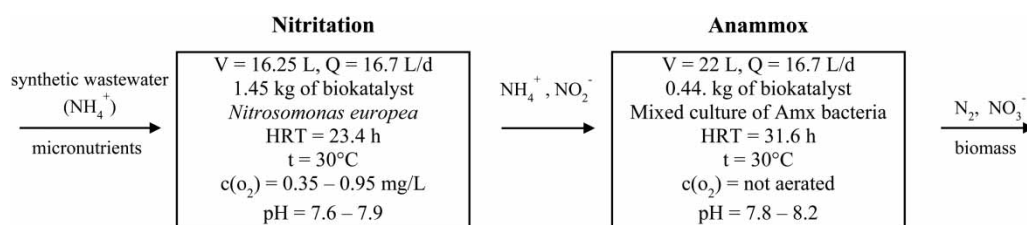


Figure 1 | Arrangement of the two-stage nitritation/anammox reactor (V = reactor volume, HRT = hydraulic retention time).

reactor volume. The reactor was fed with synthetic wastewater where the ammonia nitrogen source included $(\text{NH}_4)_2\text{SO}_4$ and reject water from dewatering of anaerobically digested sludge which represented 4 volumetric percent in the inflow. The reject water has been added to simulate a real substrate. Micronutrients were also added to the wastewater (0.375 mL of solution per liter of reactor). Solution of micronutrients contained per liter 2.768 g of $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$, 1.5 g of $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$, 1 g of Na_2MoO_4 , 0.245 g of CuSO_4 , 0.367 g of $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$, 0.2 g of $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$, 0.3 g of H_3BO_3 , 1 mL of HCl). The composition of the micronutrients solution was prepared according to the recommendation of the Lentikat's a.s. company. The reject water does not support denitrification because only poorly biodegradable organic substrates are present. The ammonia nitrogen load of the reactor was 370 mg/(L·d), to be increased to over 400 mg/(L·d) at a later stage. The flow through the reactor was maintained by means of inlet and outlet pumps. The flow control system was based on the continuously run outlet pump in the second anammox stage. The other pumps were switched automatically based on signals from the level sensors (control of height of the reaction mixture in reactor) in both stages. Temperature in the reactor was maintained near 30 °C by using an aquarium heating element. The pH level was maintained at 7.6 to 7.9 by the control system involving a pH probe activating/deactivating the Na_2CO_3 solution dosing pump. The dissolved oxygen concentration was held within 0.35 to 0.95 mg/L by the control system, which activated/deactivated the blower based on the oxygen probe signal.

Anammox reactor – second stage

The anammox reactor was larger in size (22 L) than the nitritation reactor, and so the hydraulic retention time was 31.6 h. The reactor flow rate was 16.7 L/d. The anammox reactor biomass (mixed culture), taken from a reactor operated previously, was also immobilized in PVA pellets. The

amount of the anammox biocatalyst in the reactor was 0.44 kg, which was equivalent to 20 g/L, i.e. roughly 2% of the reactor filling. The flow rate control was based on the continuously run pump at the anammox reactor outlet. The liquid heights in the nitritation and anammox reactors were levelled based on signals from the level indication sensors, whereby the pumps were controlled – the nitritation feed pump and the nitritation-to-anammox reactor transfer pump. The nitritation reactor outflow was used as feed for anammox stage. A little bit less than half of the incoming ammonia nitrogen was converted to nitrite nitrogen in the nitritation reactor. The mixture was then fed to the anammox reactor, where both the ammonia nitrogen and nitrite nitrogen were converted to nitrogen gas. Temperature of the system in the anammox reactor was held at 30 °C by an aquarium heating element. The pH level in the reactor was held at 7.8 to 8.2 automatically by the control system, viz. by activating/deactivating the Na_2CO_3 solution dosing pump based on signals received from the pH probe. The dissolved oxygen concentration in the anammox reactor was not controlled because the anammox process requires anoxic conditions. The conditions were only monitored by means of the oxygen probe.

Inorganic nitrogen species measurement

The inorganic nitrogen species were determined spectrophotometrically on a PhotoLab 6100 VIS (WTW) spectral photometer (Rice et al. 2012).

Ammonia nitrogen was determined by using the Nessler method (reagents for Nessler solution: 10 g of HgI_2 , Lachner; 7 f of KI, Lachner and 16 g of NaOH, Penta in 100 mL of distilled water, reagents for Seignet salt: 2.5 g of sodium potassium tartrate, Penta in 50 mL of distilled water). 100 μL of Seignet salt and 100 μL of Nessler solution were added to 5 mL of sample and mixed. Absorbance at 425 nm was measured after 10 min.

Nitrite nitrogen was determined by using the SANED reagent. 125 μL of SANED (25 mL of phosphoric acid,

Penta; 10 g of sulphanilamide, avocado and 0.5 g *N*-(1-naphthyl)-1,2-ethylenediamine dihydrochloride, Merck in 250 mL of distilled water) and 1 100 μ L of distilled water were added to 5 mL of sample and mixed. Absorbance at 540 nm was measured after 20 min.

Nitrate nitrogen was determined by the method with 2,6-dimethylphenol. 50 μ L of amidosulfuric acid (0.8 g in 100 mL distilled water, Alpha Aesar), 3.5 mL mixture of acids (1 L of sulfuric acid, Sigma Aldrich and 1 L of phosphoric acid, Penta), 500 μ L of DMP (0.24 g of 2,6-dimethylphenol in 100 mL distilled water, Alpha Aesar) were added to 500 μ L of sample and mixed. Absorbance at 360 nm was measured after 10 min.

Organic nitrogen has not been the subject of the research because it is converted to ammonia nitrogen prior to nitrification.

RESULTS AND DISCUSSION

Influence of oxygen concentration on nitrification

In the first stage of the experiment, dissolved oxygen concentration in the nitrification process had to be set so that the nitrification biomass should be able, while preserving its activity, to remove increased ammonia nitrogen concentrations in conditions that do not support the growth of nitrification bacteria (Bernet et al. 2001). Initially, the dissolved oxygen concentration was held within 0.35 to 0.8 mg/L and later increased to 0.5 to 0.95 mg/L. This brought about an increased rate of ammonia nitrogen conversion to nitrite nitrogen, whereupon the inlet ammonia nitrogen concentration had to be increased. The results obtained during the long-term operation of the nitrification reactor at various oxygen concentrations are shown in Figure 2.

The left-hand part of the graph shows the results for dissolved oxygen concentrations held within 0.35 to 0.8 mg/L while the right-hand part of the graph (starting at week 13) shows the results for dissolved oxygen concentrations held within 0.5 to 0.95 mg/L. When using the former dissolved oxygen concentration range (0.35 to 0.8 mg/L), the nitrite nitrogen concentration increased steadily while the ammonia nitrogen concentration decreased in parallel. The volumetric and specific nitrite nitrogen concentration increase rates were approximately 145 mg/(L-d) and 67 mg/(kg-h), respectively, while the volumetric and specific ammonia nitrogen concentration decrease rates were about 191 mg/(L-d) and 89 mg/(kg-h), respectively. The specific

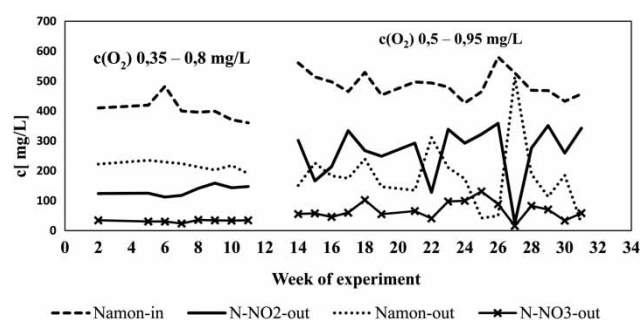


Figure 2 | Concentrations of nitrogen compounds at the inlet into and outlet from the nitrification reactor at different oxygen concentration range settings ($N_{\text{amon-in}}$, $N_{\text{amon-out}}$ = input and output concentration of ammonia nitrogen, $\text{NO}_2\text{-out}$ = output nitrite nitrogen concentration, $\text{NO}_3\text{-out}$ = output nitrate nitrogen concentration).

rate is related to the wet biocatalyst weight. The removed ammonia nitrogen fraction was 46% at the lower oxygen concentrations.

Dissolved oxygen concentration increase to 0.5–0.95 mg/L brought about an increase in the rate of ammonia nitrogen conversion to nitrite nitrogen, whereupon the inlet ammonia nitrogen concentration had to be increased. This change is apparent from the right-hand part of the plot in Figure 2 (starting week 13), demonstrating a higher difference between the ammonia nitrogen concentrations in the inlet and outlet solutions. The volumetric and specific nitrite nitrogen production rates increased to 276 mg/(L-d) and 129 mg/(kg-h), respectively, while the volumetric and specific ammonia nitrogen concentration decrease rate increased to 304 mg/(L-d) and 142 mg/(kg-h), respectively. The ammonia nitrogen removal efficiency increased to 64%.

Certain amount of nitrate nitrogen was produced during the experiment. The volumetric and specific nitrate nitrogen production rates were 27 mg/(L-d) and 13 mg/(kg-h), respectively, at the lower oxygen concentrations, and 52 mg/(L-d) and 24 mg/(kg-h), respectively, at the higher oxygen concentrations. As to the proportions, the nitrate nitrogen to oxidized ammonia nitrogen ratio was about 13%. According to published data (Ma et al. 2016), nitrate at concentrations below 500 mg/L does not inhibit the anammox process. This limit was not exceeded in our experiments. Presumably, the nitrate production was due to contamination of the pure *Nitrosomonas europaea* culture by a small amount of the nitrification culture, which could take place during the culture or immobilization processes. Wang et al. (2016) were able, by using the PCR method within the shortened nitrification process, to detect bacteria of the *Nitrospira* and *Nitrobacter* genera; their amounts relative to the amount of the ammonia-oxidizing bacteria,

however, were very low. The volumetric rates of increase/degradation of nitrogen compounds, together with the volumetric ammonia nitrogen rate median, are shown in Figure 3.

Just before the oxygen concentration was increased in week 12 (23 June), oxygen concentration decreased below 0.6 mg/L for a certain period of time due to sensor failure, whereupon the ammonia oxidation rate decreased significantly, as can be seen in Figure 3. The inlet wastewater supply was interrupted in early September due to clogged tubing. This unfavorable fact was also mirrored by the process kinetics (Figure 3).

The volumetric rates of nitrogen compound concentration increase/degradation, including the median of the volumetric rate of nitrogen removal as the ammonia plus nitrite nitrogen sum, are displayed in Figure 4.

Influence of nitrification step on anammox process

The solution leaving the nitrite step was fed to the anammox step, which implies that the anammox process was affected appreciably by the nitrification step. The development of the anammox process mirrors the activity and load changes in the nitrification reactor. This concerns, in particular, experiment week 13 (after 23 June). The anammox biomass in the second step was exposed to variable concentrations of the incoming nitrite nitrogen and ammonia nitrogen.

Figure 4 demonstrates that the abrupt oxygen concentration decrease below 0.6 mg/L, or the abrupt nitrite concentration decrease, adversely affected the anammox process rate. Joss *et al.* (2011) have shown that the anammox biomass ceases to exhibit any activity if the oxygen concentration is lowered to 0.2 mg/L. The increase of volumetric rate of ammonia nitrogen was observed from 23 June. This could be caused by nitrification/denitrification processes or by sorption of ammonia in biomass. The nitrogen degradation rate change accompanying increase in the ammonia nitrogen inlet concentration for the nitrification step was not so pronounced in the anammox steps as it was in the nitrification step. In contrast, a slight efficiency decrease was observed. The volumetric and specific rates of ammonia and nitrite nitrogen removal were about 162 mg/(L·d) and 338 mg/(kg·h), respectively, and the nitrogen removal fraction was about 58%, before increasing the inlet ammonia nitrogen concentration. The figures changed roughly to 153 mg/(L·d), 319 mg/(kg·h), and 44%, respectively, when the inlet ammonia nitrogen concentration was increased. Some nitrates were also formed during the anammox treatment, which is natural in this process. The non-sterility of the anammox biomass, which could contain, e.g. some nitrification and denitrification bacteria in the consortium, also played a part. The nitrate formation rate was 0.1 kg/(m³·d). Isaka *et al.* (2013) studied the

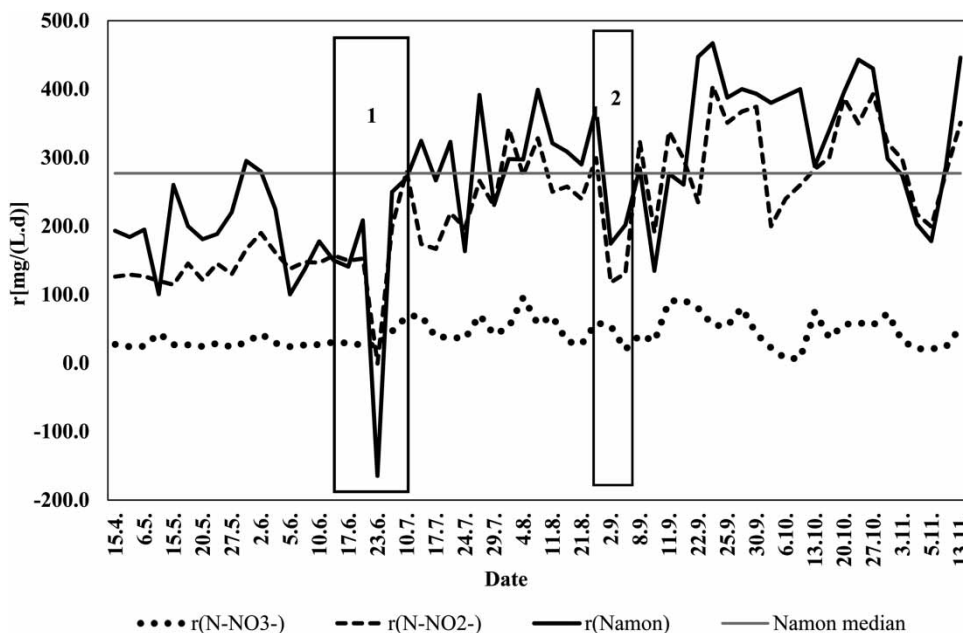


Figure 3 | Volumetric rates of ammonia nitrogen degradation and nitrite/nitrate nitrogen growth in the nitrification reactor compared to the ammonia nitrogen degradation rate median; effect of accident on the process kinetics – 1 = $c(\text{O}_2)$ drops below 0.6 mg/L, 2 = substrate dosing/aeration failure ($r(\text{N-NO}_3^-)$, $r(\text{N-NO}_2^-)$, $r(\text{N}_{\text{amon}})$) = volumetric rates of nitrate/nitrite nitrogen growth, volumetric rates of ammonia nitrogen degradation, N_{amon} median = volumetric ammonia nitrogen rate median).

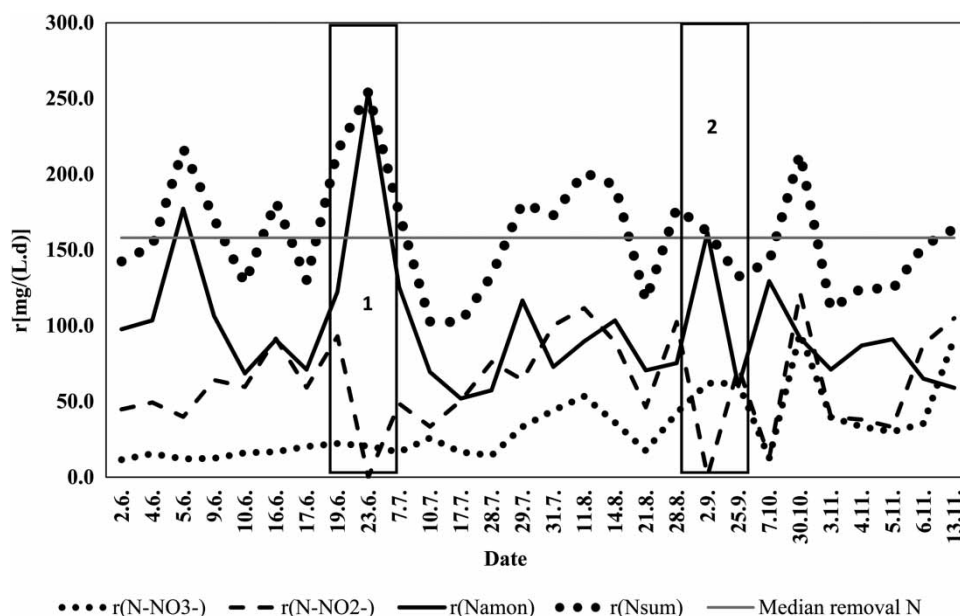


Figure 4 | Volumetric rates of ammonia nitrogen and nitrite nitrogen degradation, volumetric rates of nitrate nitrogen increase in the anammox reactor compared to the nitrogen degradation rate median; effect of accident on the process kinetics – 1 = $c(O_2)$ nitritation failure, 2 = nitritation problems, low nitrite inlet concentration ($r(N-NO_3^-)$, $r(N-NO_2^-)$), $r(N_{amon})$ = volumetric rates of nitrate nitrogen increase, volumetric rates of nitrite nitrogen and ammonia nitrogen and degradation, $r(N_{sum})$ = volumetric rate of nitrogen removal as the ammonia and nitrite nitrogen sum, median removal N = median of the volumetric rate of nitrogen removal as the ammonia and nitrite nitrogen sum).

single-step nitrification/anammox process with the biomass anchored on gel supports. Nitrates were produced during this process at a rate of $0.092 \text{ kg}/(\text{m}^3 \cdot \text{d})$, which is consistent with our results. Li & Sung (2015) demonstrated nitrate formation in granulated sludge at $36.4 \pm 16.5 \text{ mg/L}$ ($N-NO_3^-$), due to the activity of the anammox bacteria solely.

CONCLUSION

The systems using anammox cultures grown in natural aggregates often suffer from the instability of these aggregates, especially at highly varying conditions in cultivation reactor. Experiments have shown that the nitrification and anammox biomass can be immobilized in PVA pellets while preserving their activity also in the event of abrupt changes in the system (oxygen, substrate feed). Significant dependence of the nitrification kinetics on the dissolved oxygen concentration was identified. The anammox biomass activity in the second stage was fairly constant despite the fluctuating input load. The overall volumetric nitrogen removal rate was $158 \text{ mg}/(\text{L} \cdot \text{d})$. The experimental data suggest that the volumetric conversion rates can be readily modified in each of the two steps through dissolved oxygen concentration.

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