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Start-up of a One-stage Biofilm Reactor for the Removal of Nitrogen from Digester Supernatant in the Partial Nitrification-Anammox Process

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1. Introduction

The digester supernatant that comes from sludge handling facilities is one of a range of difficult-to-treat wastewaters with high nitrogen and low organics concentrations. It is difficult to remove nitrogen from this wastewater by sequential autotrophic nitrification and heterotrophic denitrification due to its low concentration of organic compounds. One promising alternative process is the co-operation of aerobic and anaerobic ammonium-oxidizing bacteria and heterotrophic denitrifying bacteria (Langone et al. 2014). This alternative is the most attractive when the treatment is performed in a single reactor in which partial nitrification followed by Anammox can successfully support heterotrophic denitrification for the removal of high nitrogen loads without external carbon sources. The advantages of this solution over conventional technologies for nitrogen removal include lower energy consumption, reduced need for additional carbon sources, minimal sludge production (Jetten et al. 2009) and lower emissions of the greenhouse gases NO and N_2O (Kampschreur et al. 2009).

In the Anammox reaction, performed under anaerobic conditions by autotrophic bacteria, ammonium and nitrite contribute in equimolar amounts to the formation of dinitrogen gas (Strous et al. 1999). It is possible to obtain simultaneous partial nitrification and Anammox in one-stage reactors with immobilized biomass (Dreissen & Reitsma 2011) but in such systems, nitrite limitation is a problem (van der Star et al. 2007). Dissolved oxygen, temperature, pH, free ammonia, and free nitrous acid are important factors for encouraging the growth of I-phase nitrifiers and inhibiting the growth of II-phase nitrifiers in the reactor (Volcke et al. 2006). Our experience has shown that partial nitrification, without external control of factors like pH or temperature, is possible in reactors with immobilized biomass. In these reactors, high concentrations of ammonium can be effectively oxidized to nitrite as a result of the accumulation of free ammonia at levels high enough to inhibit II-phase nitrifiers (Cydzik-Kwiatkowska et al. 2014).

Although Anammox bacteria are able to remove nitrogen effectively, their growth rate is very low (generation time 10–12 days), their sludge yield is low, and they are vulnerable to changes in environmental conditions (Monballiu et al. 2013). For these reasons, the start-up periods of Anammox reactors are longer than those of other nitrogen removal technologies. Anammox biomass easily attaches to solid surfaces (Monballiu et al. 2013). Since the physical retention of slow-growing Anammox bacteria in the reactor is crucial, these have been retained in membranes (Tao et al. 2012), immobilized in PVA cryogel carriers (Magri et al. 2012) or in moving-bed biofilm reactors (Szatkowska et al. 2007). These practices have allowed nitrogen removal with over 93% efficiency due to the higher resistance of the immobilized bacteria to inhibition by nitrites. The start-up period can be shortened by using the biomass from existing Anammox reactors for inoculation (van der Star et al. 2007). Without large amounts of active biomass, this period is much longer, up to several months.

Until recently, Anammox bacteria were considered obligate autotrophs that are negatively affected by organic carbon. Ni et al. (2012) reported that the number of Anammox bacteria decreased and the number of denitrifiers increased when the influent contained 400 mg COD/L. Tang et al. (2013) compared two SBRs: one operated under inorganic conditions with efficient and stable Anammox performance and another

under organic conditions with up to 800 mg COD/L in the influent. The authors reported that there was a risk of elimination of Anammox bacteria from the SBRs due to preferential metabolism of nitrite by denitrification, which starved the Anammox bacteria. However, although organic load has an inhibitory effect on Anammox activity, Anammox bacteria have the capacity to oxidize volatile fatty acids with nitrate as an electron acceptor, while forming ammonium with nitrite as the intermediate (Guven et al. 2005, Kartal et al. 2008). Anammox bacteria do not incorporate fatty acids into biomass, but completely oxidize them to CO_2, thus maintaining low biomass yield (Kartal et al. 2007). Winkler et al. (2012) have suggested that Anammox bacteria use their organotrophic capability to successfully compete with heterotrophs for organic carbon, which reduces sludge production. Anammox bacteria can outcompete heterotrophic denitrifying bacteria at ambient temperatures when the ratio of C/N in the influent is less than 0.5 g COD/g N (Tang et al. 2010).

The present study investigated the possibility of initiating efficient nitrogen removal from digester supernatant in a one-stage biofilm reactor for nitritation-Anammox process. The biofilm reactor was seeded with aerobic granular sludge collected from full-scale reactors that were not operated with the Anammox process. Because inorganic carbon (IC) limitation may be the main reason for the decrease in growth and in the activity of nitrifying bacteria at acidic pH (Wett & Rauch 2003) and of the Anammox bacteria (Liao et al. 2008, Yang et al. 2010), IC concentration in the wastewater may be important for the enrichment of Anammox microorganisms and can influence the length of the period of the reactor start-up. Therefore, the goal of the study was to determine the effect of bicarbonate addition on overall reactor performance, species composition of biofilm and changes in nitrogen forms in the effluent during the transformation of non-Anammox biomass to nitritation/Anammox biomass when treating nitrogen-rich wastewater under low oxygen concentration.

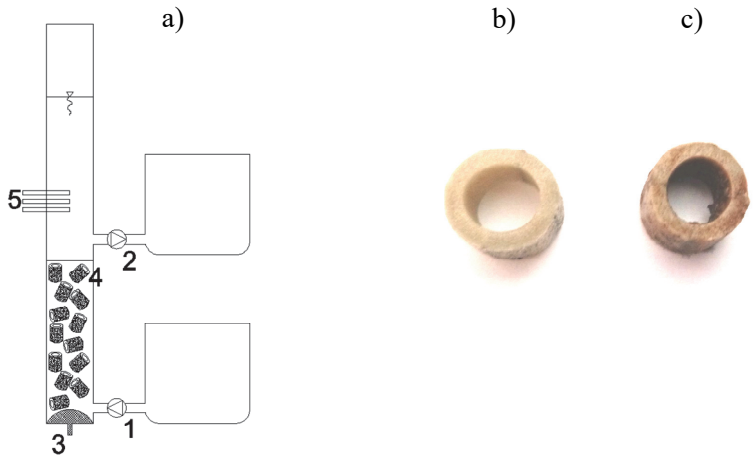
2. Materials and methods

In this study, a one-stage biofilm batch reactor with a working volume of 3 L treated digester supernatant from a sludge dewatering station in a full-scale municipal wastewater treatment plant (Fig. 1). In the supernatant, the average concentrations of pollutants were as follows:

320±52 mg COD/L, 413±78 mg TN/L, 328±46 mg NH_4-N/L and 97±19 mg TP/L, 7.5±0.1 pH, and alkalinity 31.0±2.5 mval/L. As inoculum (seed sludge, SS), aerobic granular sludge from a full-scale municipal wastewater treatment plant operated with simultaneous nitrification and denitrification was used. The experimental reactor was operated with a volumetric exchange rate of 50% and at a hydraulic retention time of 16 h. The operation of the reactor was automated to maintain the appropriate cycle length. The cycle length was 8 h, and it consisted of 5 min of filling, a 460 min reaction phase, 10 min of settling, and 5 min of decantation. Air was supplied continuously through a fine-bubble diffuser. To decrease the activity of nitrite-oxidizing bacteria (NOB) in the one-stage reactor, selection pressure was employed by maintaining a low dissolved oxygen (DO) concentration of about 0.5 mg/L at the end of the cycle, and a high temperature of about 35ºC. After the filling stage in the reactor cycle, the DO concentration was almost zero, and it increased to 0.5 mg/L after 3 h of aeration, therefore, in fact, the reactor worked under intermittent anoxic/oxic conditions. The reactor was protected from sunlight, which negatively affects Anammox activity (Yang et al. 2010). To stimulate nitrification and Anammox, external doses of bicarbonate solution were added into the digester supernatant. The experiment was conducted in phases that differed in the bicarbonate/TN ratio in the influent: Phase 1 (ratio of 2.5), Phase 2 (ratio of 0), Phase 3 (ratio of 1.5), Phase 4 (ratio of 2.5), and Phase 5 (ratio of 3.5).

In the reactor, the biomass inhabited porous supports, which were cylinders with an external diameter of 15 mm, an internal diameter of 10 mm and a height of 10 mm. These supports were made of plasticized PVC modified with a blowing agent, which allows for a highly porous structure. The physical characteristics of the supports were as follows: density 892.73±3.43 kg/m^3, porosity 39.66±1.59%, hardness 23.9±0.96 °Sh and tensile strength 138.97±2.41 N.

During the adaptation of the reactor to the treatment of digester supernatant, the influents and effluents were sampled to measure the concentrations of COD, total nitrogen (TN), ammonium, nitrites and nitrates, phosphate, pH and alkalinity (Hermanowicz 1999, HACH). DO was measured using an YSI ProODO^{TM} probe.



Rys. 1. Schemat bioreaktora; (a): 1 – dopływ, 2 – odpływ, 3 – napowietrzanie, 4 – kształtki, 5 – pomiar tlenu, temperatury i pH, (b): fotografia kształtki, (c): fotografia kształtki z błoną biologiczną
Fig. 1. Scheme of the bioreactor; (a): 1 – influent, 2 – effluent, 3 – aeration, 4 – supports, 5 – control of DO, temperature and pH, (b): photo of the support (c): photo of the support with biofilm

To indicate changes in biofilm composition, fluorescence *in situ* hybridization (FISH) was used as an indicator of adaptation of the microorganisms to high influent nitrogen concentrations. A sample of inoculum and samples of biomass collected from the reactor at the end of each phase were fixed and microorganisms were identified as described by Nielsen (2009). The molecular probes used in this study are listed in Table 1; the conditions used for applying these probes can be found in ProbeBase (www.microbial-ecology.net/probebase). The probes were labeled with Cy3 or Fluos fluorochromes. Vectashield (Vector laboratories, USA) was used to mount the samples prior to visualization with a Nikon Eclipse (Nikon, Japan) epifluorescence microscope. The FISH-defined populations were quantified by image analysis using ImageJ software (http://rsb.info.nih.gov/ij/) and the ratio of the bio-area fraction of the targeted microbial population (stained by the specific probe) relative to that of the total microbial community (stained by the universal probe) was expressed as the percentage abundance. This analysis was

based on examination of at least 20 fields of view for each probe. For FISH quantification, three replicates per inoculum and each biomass sample were analyzed. The standard deviations of all values obtained with each probe were 15-20% of the mean value for that probe. Only means are shown in Figure 3.

Tabela 1. Sondy oligonukleotydowe 16S rRNA do identyfikacji AOB, NOB I bakterii Anammox (EUBmix była znakowana Fluos, pozostałe sondy – Cy3)
Table 1. 16S rRNA oligonucleotide probes for the identification of AOB, NOB and Anammox bacteria (EUBmix was labeled by Fluos, the rest probes – by Cy3)

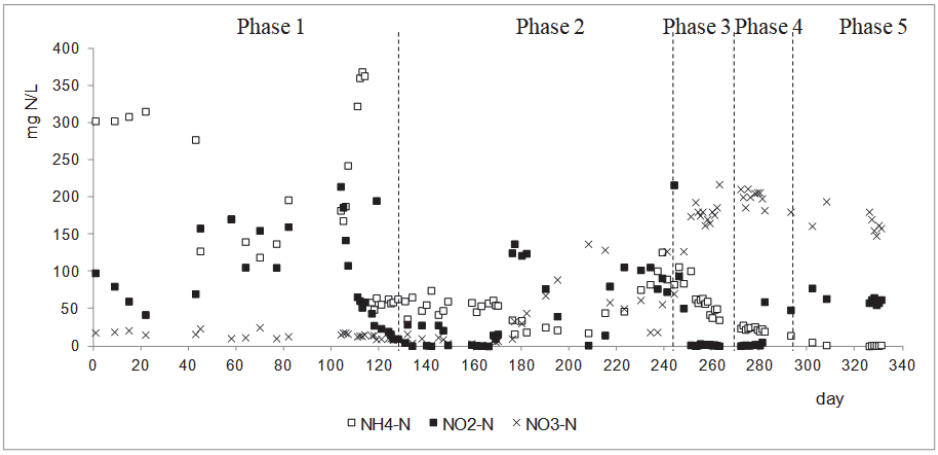
Probe	Sequence 5'-3'	Target	FA* (%)	Reference
EUBmix	EUB338-I (GCTGCCTCCCGTA-GGAGT), EUB338-II (GCA GCC ACC CGT AGG TGT), EUB338-III (GCT GCC ACC CGT AGG TGT)	Bacteria	20	Amann et al. (1990), Daims et al. (1999)
Nso190	CGATCCCCTGCTTTT CTCC	Betaproteobacterial ammonium-oxidizing bacteria	55	Mobarry et al. (1996)
NIT3	CCTGTGCTC-CATGCTCCG	*Nitrobacter* sp.	40	Wagner et al. (1996)
Ntspa662	GGAATTC-CGCGCTCCTCT	*Nitrospira* sp.	35	Daims et al. (2001)
Amx368	CCTTTCGGGCAT-TGCGAA	All Anammox microorganisms	15	Schmid et al. (2005)

*FA – formamide

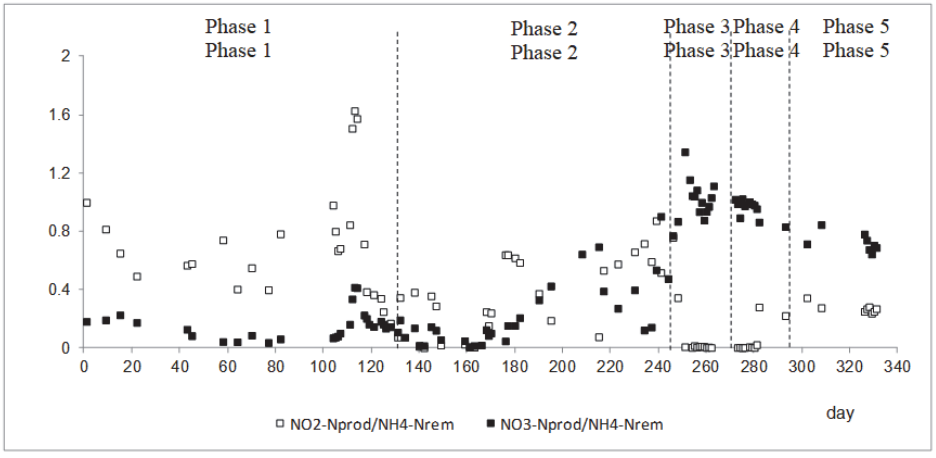
3. Results and discussion

In this study, the adaptation of biomass, taken from the conventional municipal wastewater treatment plant with simultaneous nitrification and denitrification, to nitrogen-rich digester supernatant was investigated in terms of overall reactor performance, changes in nitrogen forms in the effluent and changes in microbial composition of the biofilm. During the whole period of the study, the suspended part of biomass was characterized by good settling abilities, with the sludge volume index of about 50 mL/g MLSS.

In the first period of biomass adaptation (Phase 1), ammonium was the main nitrogen form in the effluent (Fig. 2) and the average efficiency of ammonium removal was 44±19%. In this Phase 1, bicarbonates were added to obtain the bicarbonate/TN ratio in the influent of 2.5. The biomass yield was minimal in the reactor (yield coefficient below 0.2 g MLSS/g COD); therefore, the ammonium use for biomass synthesis can be neglected. In addition, loss of ammonium by stripping was not taken into account under all experimental conditions because a significant ammonium removal by stripping was observed at pH of 10.5 and 11 (Guštin & Marinšek-Logar 2011) and at pH of above 11.5 (Bonmati & Flotats 2003) that was not observed in the present experiment. Apart from the fact that it was a period of a start-up of the reactor, the reason for inefficient ammonium removal was the presence of free ammonia (FA). The FA inhibition threshold is 10-150 mg NH_3-N/L and 0.1-4.0 mg NH_3-N/L for nitritation and nitratation, respectively (Anthonishen et al. 1976, Bae et al. 2001). In the present experiment, FA concentration was 18 mg NH_3-N/L at the beginning of the reactor cycle that could cause a low ammonium loss and, in addition, accumulation of nitrites. After about 10 days, ammonium concentration started to decrease to about 50 mg NH_4-N/L and it corresponded to the increase in nitrite concentration. Excluding the initial period of nitritation inhibition, the effluent consisted of similar concentrations of ammonium and nitrites, whereas nitrate concentration was about 10-20 mg NO_3-N/L. In one-stage reactors, the main problem in cultivating Anammox bacteria is the maintenance of the most important parameter required for Anammox process, which is the nitrite/ammonium ratio that should be of about 1:1. The insufficient alkalinity of wastewater may limit nitrification. However, in this study, the addition of bicarbonate could have balanced the acidifying effect of nitrification (van Dongen et al. 2001). The alkalinity of the effluent was about 21 mval/L, and pH 8.3. The calculated ratio between nitrite produced (NO_2-Nprod) and ammonium removed (NH_4-Nrem) in the reactor cycle (Fig. 3) in Phase 1 was 0.66, which indicates that nitrite produced could have been removed either in the Anammox process or in denitrification.



Rys. 2. Stężenia związków azotu w ściekach oczyszczonych w fazach eksperymentalnych
Fig. 2. Concentrations of nitrogen compounds in the effluents in the experimental phases



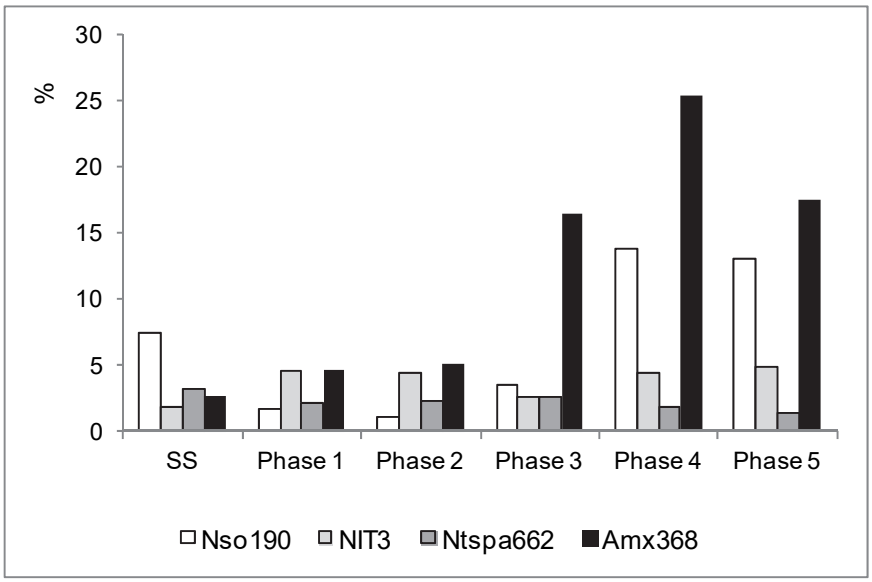
Rys. 3. Stosunki powstałego azotu azotanowego(III) i usuniętego azotu amonowego oraz powstałego azotu azotanowego(V) i usuniętego azotu amonowego w fazach eksperymentalnych
Fig. 3. The ratios between nitrite produced and ammonium removed and between nitrate produced and ammonium removed in the experimental phases

In Phase 2, nitrogen changes in the reactor were tested when there was no additional bicarbonate in the influent. The average ammonium loss was 64±15%. During the first half of this phase, the alkalinity in the bioreactor was quite high (10-15 mval/L, pH 8.0), as the effect of alkalinity that was present in raw digester supernatant. In this period, ammonium concentration in the effluent was about 50-60 mg NH_4-N/L and nitrite and nitrate concentrations were low (Fig. 2). In the second half of Phase 2, the acidifying effect of nitrification started to be observed resulting in the alkalinity of the effluent of about 0.5-3.6 mval/L. As a result, ammonium concentration increased to about 100 mg NH_4-N/L and simultaneously the nitrite concentration increased resulting in the ammonium/nitrite ratio of about 1. These could have created favorable conditions for the Anammox process or denitrification because the observed increase in nitrite concentration was not stoichiometric, which is indicated by the NO_2-Nprod/NH_4-Nrem ratio of 0.36 (Fig. 3). At the second half of the Pase 2, the increase in the NO_3-Nprod/NH_4-Nrem ratio was observed to about 0.4, indicating the nitratation.

In Phases 3, 4, and 5, the bicarbonate/TN ratio in the influent was increased to 1.5, 2.5 and 3.5, respectively. Bicarbonate addition increased the alkalinity of the effluent to above 7 mval/L, pH was 8.4. As a result, ammonium concentration in the effluents gradually decreased to achieve 0–10 mg NH_4-N/L in the Phase 5 (Fig. 2). The efficiency of ammonium removal was 77±4%, 89±1% and 98±2% in Phase 3, 4 and 5, respectively. In Phases 3 and 4, nitrite concentration in the effluent did not exceed 5 mg/L. Efficient nitrite conversions are indicated by the NO_2-Nprod/NH_4-Nrem ratio of 0.01-0.04 (Fig. 3). Nitrate concentrations of about 170-210 mg NO_3-N/L indicated good activity of nitrite-oxidizing bacteria. It was also confirmed by high values of NO_3-Nprod/NH_4-Nrem ratio of about 0.8. According to van der Star et al. (2007), nitrate production is an indicator of Anammox activity. In the Phase 5, in which the bicarbonate/TN ratio was 3.5, the nitratation was inhibited. This resulted in the accumulation of nitrite in the effluent to about 65-77 mg NO_2-N/L and a decrease in the nitrate concentration to about 150-160 mg NO_3-N/L. The NO_2-Nprod/NH_4-Nrem ratio increased to 0.27. The obtained results point out that the start-up of the reactor during the treatment of nitrogen-rich wastewater is difficult and time-consuming when the inoculum taken from Anammox reactors is not

available. As the main problems during start-up, apart from the limitation of nitrite as a substrate for the Anammox process, incidental nitrite toxicity is reported, which is caused by a too high loading rate (van der Star et al. 2007). The ranges of the nitrite concentrations that adversely affect the Anammox process are wide, from 100 mg NO_2-N/L (Strous et al. 1999) to 400 mg NO_2-N/L (Lotti et al. 2012).

According to van der Star et al. (2007), in initial phase of reactor operation, Anammox conversions could not be identified by traditional methods such as following the nitrogen profiles. As reliable indicators of the Anammox bacteria growth, molecular methods are reported. In the present experiment, in Phases 1, 2 and 3, the abundance of aerobic AOB in the biomass was lower than in the inoculum (1.1-3.5%) (Fig. 4).



Rys. 4. Udział w biomasie AOB (Nso190), NOB (NIT3 i Ntspa662) i bakterii Anammox (Amx368) w fazach eksperymentalnych
Fig. 4. The abundance of AOB (Nso190), NOB (NIT3 and Ntspa662) and Anammox bacteria (Amx368) in the biomass in the experimental phases

In Phases 4 and 5, AOB accounted for 13% of the biomass, which resulted in the ammonium concentration in the effluent lower than 28 NH_4-N/L. Regarding NOB, the abundance of *Nitrobacter* sp. was slightly

higher than in the inoculum and in all phases was about 4.2%. The *Nitrospira* sp. abundance in all phases was similar to that in the inoculum. *Nitrospira*-like bacteria are usually more numerous NOB in wastewater treatment systems than *Nitrobacter*-like bacteria (Daims et al. 2011), however our studies indicated higher abundance of *Nitrobacter* sp. than *Nitrospira* sp. Such a proportion between NOB is also reported as typical for the nitritation-Anammox biomass (Langone et al. 2014). Significant numbers of *Nitrobacter* sp. was reported to be an indicator for high-loaded wastewater treatment plants (Gieseke et al. 2003). In addition, *Nitrobacter* sp. is more resistant to free nitrous acid than *Nitrospira* sp. (Blackburne et al. 2007).

In this study, the presence of low abundance (2.7%) of Anammox bacteria was observed already in the inoculum (Fig. 4). In Phases 1 and 2, the abundance of Anammox bacteria was slightly higher than in the inoculum. Starting from Phase 3, their abundance highly increased to 25.4% in Phase 4. According to Duan et al. (2012), Anammox bacteria can account even for 50% of all the bacteria in the reactor in which Anammox process was started-up from conventional activated sludge with ammonium concentration in the effluent elevated from 50 to 270 mg NH_4-N/L. To conclude, in the final phases of the experiment, FISH tests confirmed high abundance of microorganisms responsible for ammonium removal: AOB and Anammox bacteria. This could have been the result of the nitrifier presence in the outer parts of the biomass particles and biofilm, which protected Anammox bacteria located in the internal zones from low oxygen concentration in the reactor.

4. Conclusions

In this study, the effect of bicarbonate addition on the nitrogen compound conversions during the start-up of the one-stage biofilm reactor treating digester supernatant was investigated with the bicarbonate/TN ratio in the influent from 0 to 3.5. Only with the highest ratio in the influent of about 3.5 the final ammonium concentration in the effluent was below 10 mg NH_4-N/L. Under these conditions, nitrates were the predominant form of nitrogen in the effluent. Aerobic AOB and Anammox bacteria were major components of the biomass (13.0% and 17.5%, respectively) that ensured efficient ammonium removal.

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References

Amann, R.I., Binder, B.J., Olson, R.J., Chisholm, S.W., Devereux, R., Stahl, D.A. (1990). Combination of 16S rRNA-targeted Oligonucleotide Probes with Flow Cytometry for Analyzing Mixed Microbial Populations. *Applied and Environmental Microbiology*, *56*, 1919-1925.

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., Srinath, E.G. (1976). Inhibition of Nitrification by Ammonia and Nitric Acid. *Journal of Water Pollution Control Federation*, *48*, 835-852.

Bae, W., Baek, S., Chung, J., Lee, Y. (2001). Optimal Operational Factors for Nitrite Accumulation in Batch Reactors. *Biodegradation*, *12*, 359-366.

Blackburne, R., Vadivelu, V.M., Yuan, Z., Keller, J. (2007). Determination of Growth Rate and Yield of Nitrifying Bacteria by Measuring Carbon Dioxide Uptake Rate. *Water Environment Research*, *79*, 2437-2445.

Bonmati, A., & Flotats, X., (2003). Air Stripping of Ammonia from Pig Slurry: Characterisation and Feasibility as Pre- or Post-treatment to Mesophilic Anaerobic Digestion. *Waste Management*, *23*, 261-272.

Cydzik-Kwiatkowska, A., Rusanowska, P., Zielińska, M., Bernat, K., Wojnowska-Baryła, I. (2014). Structure of Nitrogen-converting Communities Induced by Hydraulic Retention Time and COD/N Ratio in Constantly Aerated Granular Sludge Reactors Treating Digester Supernatant. *Bioresource Technology*, *154*, 162-170.

Daims, H., Bruhl, A., Amann, R., Schleifer, K.H., Wagner, M. (1999). The Domain-specific Probe EUB338 is Insufficient for the Detection of all Bacteria: Development and Evaluation of a More Comprehensive Probe Set. *Systematic and Applied Microbiology*, 22*,* 434-444.

Daims, H., Nielsen, J.L., Nielsen, P.H., Schleifer, K.H., Wagner, M. (2001). In Situ Characterization of Nitrospira-like Nitrite-oxidizing Bacteria Active in Wastewater Treatment Plants. *Applied and Environmental Microbiology*, *67*, 5273-5284.

Daims, H., Lucker, S., Le Paslier, D., Wagner, M. (2011). Diversity, Environmental Genomics, and Ecophysiology of Nitrite-oxidizing Bacteria in Nitrification. eds Ward, B.B., Arp, D.J., Klotz M.G. (Washington, DC: ASM press), 295-322.

Dreissen, W., & Reitsma, G. (2011). One-step Anammox Process a Sustainable Way to Remove Ammoniacal Nitrogen. *UK Water Projects*, 101-102.

Duan, X., Zhou, J., Qiao, S., Yin, X., Tian, T., Xu, F. (2012). Start-up of the Anammox Process from the Conventional Activated Sludge in a Hybrid Bioreactor. *Journal of Environmental Sciences*, *24*, 1083-1090.

Gieseke, A., Bjerrum, L., Wagner, M., Amann, R. (2003). Structure and Activity of Multiple Nitrifying Bacterial Populations Co-existing in a Biofilm. *Environmental Microbiology*, *5*, 355-369.

Guštin, S., & Marinšek-Logar, R. (2011). Effect of pH, Temperature and Air Flow Rate on the Continuous Ammonia Stripping of the Anaerobic Digestion Effluent. *Process Safety and Environmental Protection*, *89*, 61-66.

Guven, D., Dapena, A., Kartal, B., Schmid, M.C., Maas, B., van de Pas-Schoonen, K., Sozen, S., Mendez, R., Op den Camp, H.J.M., Jetten, M.S.M., Strous, M., Schmidt, I. (2005). Propionate Oxidation by and Methanol Inhibition on Anaerobic Ammonium-oxidizing Bacteria. *Applied and Environmental Microbiology*, *71*, 1066-1071.

Hermanowicz, W., Dojlido, J., Dożańska, W., Koziorowski, B., Zerbe, J. (1999). Fizyczno-chemiczne badanie wody i ścieków. Arkady, Warszawa.

Jetten, M.S.M., van Niftrik, L., Strous, M., Kartal, B., Keltjens, J.T., Op den Camp, H.J.M. (2009). Biochemistry and Molecular Biology of Anammox Bacteria. *Critical Reviews in Biochemistry and Molecular Biology*, *44*, 65-84.

Kampschreur, M.J., Poldermans, R., Kleerebezem, R., van der Star, W.R.I., Haarhuis, R., Abma, W.R., Jetten, M.S.M., van Loosdrecht, M.C.M. (2009). Emission of Nitrous Oxide and Nitric Oxide from a Full-scale Singlestage Nitritation-Anammox Reactor. *Water Science and Technology*, *60*, 3211-3217.

Kartal, B., Kuypers, M.M.M., Lavik, G., Schalk, J., den Camp, H., Jetten, M.S.M., Strous, M. (2007). Anammox Bacteria Disguised as Denitrifiers: Nitrate Reduction to Dinitrogen Gas via Nitrite and Ammonium. *Environmental Microbiology*, *9*, 635-642.

Kartal, B., van Niftrik, L., Rattray, J., de Vossenberg, J., Schmid, M.C., Damste, J.S.S., Jetten, M.S.M., Strous, M. (2008). Candidatus "Brocardia fulgida": an Autofluorescent Anaerobic Ammonium Oxidizing Bacterium. *FEMS Microbiology Ecology*, *63*, 46-55.

Langone, M., Yan, J., Haaijer, S.C.M., Op den Camp, H.J.M., Jetten, M.S.M., Andreottola, G. (2014). Coexistence of Nitrifying, Anammox and Denitrifying Bacteria in a Sequencing Batch Reactor. *Frontiers in Microbiology*, *5*, 1-12.

Liao, D., Li, X., Yang, Q., Zeng, G., Guo, L., Yue, X. (2008). Effect of Inorganic Carbon on Anaerobic Ammonium Oxidation Enriched in Sequencing Batch Reactor. *Journal of Environmental Sciences*, *20*, 940-944.

Lotti, T., van der Star, W.R.L., Kleerebezem, R., Lubello, C., van Loosdrecht, M.C.M. (2012). The Effect of Nitrite Inhibition on the Anammox Process. *Water Research*, *46*, 2559-2569.

Magri, A., Vanotti, M.B., Szogi, A.A. (2012). Anammox Sludge Immobilized in Polyvinyl Alcohol (PVA) Cryogel Carriers. *Bioresource Technology*, *114*, 231-240.

Mobarry, B.K., Wagner, M., Urbain, V., Rittmann, B.E., Stahl, D.A. (1996). Phylogenetic Probes for Analyzing Abundance and Spatial Organization of Nitrifying Bacteria. *Applied and Environmental Microbiology*, *62*, 2156-2162.

Monballiu, A., Desmidt, E., Ghyselbrecht, K., De Clippeleir, H., Van Hulle, S.W.H., Verstraete, W., Meesschaert, B. (2013). Enrichment of Anaerobic Ammonium Oxidizing (Anammox) Bacteria from OLAND and Conventional Sludge: Features and Limitations. *Separation and Purification Technology*, *104*, 130-137.

Ni, S.Q., Ni, J.Y., Hu, D.I., Sung, S.H. (2012). Effect of Organic Matter on the Performance of Granular Anammox Process. *Bioresource Technology*, *110*, 701-705.

Nielsen, J.L. 2009. Protocol for Fluorescence in Situ Hybridization (FISH) with rRNA-targeted Oligonucleotides. In: Nielsen, P.H., Daims, H., Lemmer, H. (Eds.), FISH Handbook for Biological Wastewater Treatment: Identification and Quantification of Microorganisms in Activated Sludge and Biofilms by FISH. 1. ed. IWA Publishing Company, London, UK, pp. 73-84.

Schmid, M.C., Maas, B., Dapena, A. van de Pas-Schoonen, K., van de Vossenberg, J., Kartal, B., van Niftrik, L., Schmidt, I., Cirpus, I., Gijs Kuenen, J., Wagner, M., Damsté, J.S.S., Kuypers, M., Revsbech, N.P., Mendez, R., Jetten, M.S.M., Strous, M. (2005). Biomarkers for in Situ Detection of Anaerobic Ammonium-oxidizing (Anammox) Bacteria. *Applied and Environmental Microbiology*, *71*, 1677-1684.

Strous, M., Kuenen, G., Jetten, M. (1999). Key physiology of anaerobic ammonium oxidation. *Applied and Environmental Microbiology*, *65*, 3248-3250.

Szatkowska, B., Cema, G., Plaza, E., Trela, J., Hultman, B. (2007). A One-stage System with Partial Nitritation and Anammox Processes in the Moving-bed Biofilm Reactor. *Water Science and Technology*, *55*, 19-26.

Tang, C.J., Zheng, P., Wang, C.H., Mahmood, Q. (2010). Suppression of Anaerobic Ammonium Oxidizers under High Organic Content in High-rate Anammox UASB Reactor. *Bioresource Technology*, *101*, 1762-1768.

Tang, C.J., Zheng, P., Chai, L.Y., Min, X.B. (2013). Thermodynamic and Kinetic Investigation of Anaerobic Bioprocesses on ANAMMOX under High Organic Conditions. *Chemical Engineering Journal*, *230*, 149-157.

Tao, Y., Gao, D.W., Fu, Y., Wu, W.M., Ren, N.Q. (2012). Impact of Reactor Configuration on Anammox Process Start-up: MBR versus SBR, *Bioresource Technology*, *104*, 73-80.

Van der Star, W.R.L., Abma, W.R., Blommers, D., Mulder, J.W., Tokutomi, T., Strous, M., Picioreanu, C., van Loosdrecht, M.C.M. (2007). Startup of Reactors for Anoxic Ammonium Oxidation: Experiences from the First Full-scale Anammox Reactor in Rotterdam. *Water Research*, *41*, 4149-4163.

Van Dongen, U., Jetten, M.S.M., van Loosdrecht, M.C.M. (2001). The SHARONs-Anammoxs-process for Treatment of Ammonium Rich Wastewater. *Water Science and Technology*, *44*, 153-160.

Volcke, E., van Loosdrecht, M., Vanrolleghem, P. (2006). Controlling the Nitrite: Ammonium Ratio in a SHARON Reactor in View of its Coupling with an Anammox Process. *Water Science and Technology*, *53*, 45-54.

Wagner, M., Rath, G., Koops, H.P., Flood, J., Amann, R. (1996). In situ Analysis of Nitrifying Bacteria in Sewage Treatment Plants. *Water Science and Technology*, *34*, 237-244.

Wett, B., & Rauch, W. (2003). The Role of Inorganic Carbon Limitation in Biological Nitrogen Removal of Extremely Ammonia Concentrated Wastewater. *Water Research*, *37*, 1100-1110.

Winkler, M.K.H., Yang, J., Kleerebezem, R., Plaza, E., Trela, J., Hultman, B., van Loosdrecht, M.C.M. (2012). Nitrate Reduction by Organotrophic Anammox Bacteria in a Nitritation/Anammox Granular Sludge and a Moving Bed Biofilm Reactor. *Bioresource Technology*, *114*, 217-223.

Yang, J., Zhang, L., Fukuzaki, Y., Hira, D., Furukawa, K. (2010). High-rate Nitrogen Removal by the Anammox Process with a Sufficient Inorganic Carbon Source. *Bioresource Technology*, *101*, 9471-9478.

Wpracowanie jednostopniowego reaktora z błoną biologiczną do usuwania azotu z wód nadosadowych w procesie częściowej nitryfikacji-Anammox

Streszczenie

Proces częściowej nitryfikacji-Anammox jest obiecującą alternatywą wobec konwencjonalnej nitryfikacji-denitryfikacji do usuwania azotu ze ścieków o wysokim stężeniu związków azotowych i niskim stosunku ChZT/N, skutkującą oszczędnością energii i dodatkowych źródeł węgla organicznego. W procesie Anammox, prowadzonym w warunkach anoksycznych przez bakterie autotroficzne, azot amonowy i azot azotanowy(III) uczestniczą w równoważnych ilościach w powstawaniu azotu cząsteczkowego.

Wpracowanie jednostopniowych reaktorów, w których planowane jest prowadzenie częściowej nitryfikacji i Anammox, jest trudne. Z uwagi na niską szybkość autotroficznego wzrostu (czas generacji 10-12 dni), biomasa bakterii Anammox charakteryzuje się niskim przyrostem. Ponadto, azot azotanowy(III) jest substratem do procesu Anammox, jednak w pewnych stężeniach inhibuje proces. Dodatkowo, niedostatek nieorganicznych związków węglowych jest czynnikiem limitującym wzrost tlenowych bakterii utleniających azot amonowy

oraz bakterii Anammox. Celem badań było określenie wpływu dozowania do reaktora jednostopniowego wodorowęglanów na występowanie poszczególnych form związków azotowych w ściekach oczyszczonych oraz skład biomasy podczas wpracowania reaktora do oczyszczania ścieków bogatych w azot z wykorzystaniem procesu Anammox.

W prezentowanych badaniach, jednostopniowy reaktor porcjowy z błoną biologiczną był wykorzystywany do oczyszczania wód nadosadowych pochodzących z oczyszczalni ścieków komunalnych pracującej w skali technicznej. Stężenia zanieczyszczeń w wodach nadosadowych były następujące: 320±52 mg ChZT/dm^3, 413±78 mg N_{og}/dm^3, 328±46 mg NH_4-N/dm^3 i 97±19 mg P_{og}/dm^3. Reaktor został zaszczepiony tlenowym osadem granulowanym z oczyszczalni ścieków komunalnych pracującej w skali technicznej eksploatowanej z jednoczesną nitryfikacją i denitryfikacją. Parametry eksploatacyjne reaktora badawczego były następujące: objętość robocza 3 dm^3, długość cyklu 8 h, stopień wymiany objętościowej 50%. W reaktorach jednostopniowych bakterie utleniające azot azotanowy(III) muszą być poddawane selektywnej presji w celu ograniczenia ich aktywności. Z tego względu w reaktorze eksperymentalnym utrzymywano niskie stężenie tlenu (około 0,5 mg/dm^3), pH około 8,0 i wysoką temperaturę (około 35ºC). Wskaźnikiem adaptacji mikroorganizmów do wysokich stężeń azotu w ściekach dopływających były zmiany w profilu związków azotowych w odpływie oraz wyniki uzyskane techniką fluorescencyjnej hybrydyzacji *in situ*, pozwalającą na określanie liczebności poszczególnych grup mikroorganizmów w biomasie.

Stabilną pracę reaktora uzyskano przy stosunku wodorowęglanów do azotu ogólnego w dopływie równym około 3,5; stężenie azotu amonowego w ściekach oczyszczonych nie przekraczało 10 mg/dm^3. Główną formą azotu w odpływie był azot azotanowy(V). W okresie wpracowania proporcje między liczebnością w biomasie tlenowych bakterii utleniających azot amonowy i azot azotanowy(III) oraz bakterii Anammox podlegały dynamicznym zmianom. Część biomasy pozostająca w zawieszeniu charakteryzowała się dobrymi właściwościami sedymentacyjnymi; indeks objętościowy osadu wynosił poniżej 50 cm^3/g s.m.

Abstract

For nitrogen-rich wastewater with a low COD/N ratio, the partial nitrification-Anammox process is considered a promising alternative to conventional nitrification-denitrification, saving energy and additional carbon source. In the Anammox reaction, performed under anoxic conditions by autotrophic bacteria, ammonium and nitrite contribute in equimolar amounts to the formation of dinitrogen gas.

Anammox bacteria are characterized by low biomass yield because of their autotrophic growth mode and their high maintenance requirement due to their slow growth rate (doubling time of 10-12 days). In addition, nitrite is a substrate for Anammox on one hand and an inhibitor of Anammox microorganisms at some concentrations on the other hand. Next, inorganic carbon limitation is the limiting factor in the growth of nitrifiers and Anammox bacteria. These are the reasons that one-stage reactors are extremely difficult to start-up. The goal of this study was to determine the effect of bicarbonate addition on the changes in nitrogen forms in the one-stage reactor, biofilm composition and overall reactor performance during the adaptation of non-Anammox biomass to nitrogen-rich wastewater.

In this study, a one-stage biofilm batch reactor treated the digester supernatant from the full-scale municipal wastewater treatment plant. In the supernatant, the average concentrations of pollutants were as follows: 320±52 mg COD/L, 413±78 mg TN/L, 328±46 mg NH_4-N/L and 97±19 mg TP/L. Aerobic granular sludge from the full-scale municipal wastewater treatment plant operated with simultaneous nitrification and denitrification was used as inoculum. The operational parameters of the reactor were: working volume 3 L, 8-hour cycle, volumetric exchange ratio 50%/cycle. In one-stage reactors, nitrite-oxidizing bacteria (NOB) must be selectively pressured to decrease their activity. Therefore, in this reactor, low dissolved oxygen, about 0.5 mg/L, a pH of about 8.0, and a high temperature, about 35ºC, were maintained. Apart from determining changes in nitrogen profile, fluorescence *in situ* hybridization technique indicating the changes in the biofilm composition was used as an indicator of adaptation of the microorganisms to high influent nitrogen concentrations.

With the bicarbonate/TN ratio in the influent of about 3.5, stable reactor performance was obtained with the final ammonium concentration in the effluent below 10 mg N-NH_4/L. Nitrate was the predominant form of nitrogen in the effluent. In this period, the abundance proportion between Anammox bacteria, ammonium-oxidizing bacteria (AOB) and NOB dynamically changed in the biomass. This part of biomass that was suspended in the reactor was characterized by good settling abilities, with the sludge volume index below 50 mL/g MLSS.

Słowa kluczowe:
Anammox, częściowa nitryfikacja, wody nadosadowe, bakterie utleniające azot amonowy, bakterie utleniające azot azotanowy(III)

Keywords:
Anammox, partial nitrification, digester supernatant, AOB, NOB