Research Article

Fast start-up of anammox process with hydrazine addition

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Abstract
The anammox process is an economically favourable nitrogen removal process; however, low growth rates of anammox biomass block its more widespread application. As different approaches on techniques for anammox bacteria growth acceleration were tested, this study focused on long-term evaluation of hydrazine addition in the start-up phase. Effect of hydrazine addition (dose 3.7 mgN_2H_4 dm^{-3}) was investigated in a pilot-scale SBR treating ammonia-rich reject water from sludge dewatering after partial nitritation process, and effects were compared to a reference reactor. Hydrazine addition resulted in much higher anammox biomass enrichment observed as higher nitrogen removal rate [0.512 kgN $(m^3\ d)^{-1}$ with hydrazine versus 0.256 kgN $(m^3\ d)^{-1}$ without reagent]. Sludge from chemically stimulated reactor revealed also higher (by 22.6%) specific anammox activity rate than the reference. After the hydrazine addition was stopped, observed process rates remained stable and no regression in the nitrogen removal efficiency could be noticed. In the final phase, nitrogen removal rate was only 48.7% of process rate in the reactor previously stimulated by hydrazine. Results of the experiment confirmed positive effect of hydrazine presence on anammox bacteria enrichment process.

Keywords Anammox · Hydrazine · Start-up · SBR · Side-stream treatment

1 Introduction

Efficient nutrient removal has become one of the most important goals of wastewater treatment in past decades. Higher effluent standards induce constant development of new technologies, inter alia, for efficient nitrogen removal. Among new advances in biological nutrient removal (BNR) technologies, a lot of attention has been attracted to one of the most spectacular discoveries of the end of twentieth century in the wastewater industry—the anammox process.

Anammox is a biochemical process of anaerobic ammonia oxidation using nitrite as final electron acceptor. Process stichometry can be expressed by Eq. 1 [1]:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2 \quad (1)$$

Bacteria able to oxidize ammonia in this process are completely autotrophic micro-organisms phylogenetically related and represented by phylum Planctomycetes [2]. Among other organisms able to oxidize ammonia, the anammox bacteria have very specific construction of cellular wall and specialized organelle called the anammoxosome. As chemolithotrophic metabolism based on ammonium and nitrite conversion to nitrogen gas is their energy source, this group can be also characterized as slow-growing bacteria due to their low maximal growth rate [3]. Reported maximal growth rate of anammox bacteria cultivated in a mixed culture is very low (0.003 h^{-1}), with doubling time in range between 7 and 20 days [4]. Growth rate values obtained in pure cultures were higher: 0.005 h^{-1} for *Ca. Jettenia caeni* and 0.007 h^{-1} for both *Ca. Brocadia sinica* and *Scalindua* sp. resulting in doubling time between 4.1 and 6.3 days [5].

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Anaerobic and completely autotrophic character of the anammox process is a significant advantage compared to the conventional way of nitrogen removal via nitrification–denitrification. No requirements for organic carbon allow to use anammox in wastewater with low C:N ratio (> 2.5) without external carbon source, contrary to traditional denitrification pathway. Another economical advantage of anammox application in N-removal systems is lower oxygen consumption due to only partial nitritation of ~ 50% of ammonium load and thus lower energy costs for aeration [6]. Another important benefit from anammox process use is low sludge production due to slow biomass growth as highlighted before. Unfortunately, low growth rate extends the start-up period, especially considering full-scale applications, when the amount of available inoculum is much lower than biomass required to achieve the facility design nitrogen loading rate (NLR). This vital problem had been studied before using different start-up approaches to shorten this time; however, no crucial breakthrough was made.

1.1 Start-up strategies

As quick and robust anammox start-up is an issue, many researchers investigated this topic with different approaches [7]. Rapid enrichment of anammox bacteria is the key to achieve this goal; thus, most of studies focused on efficient biomass separation from the effluent to preserve maximal amount of bacteria in the reactor. As anammox process can be performed in different reactor configurations, for both suspended and attached growth, tested methods were suitable for certain type of reactor. Use of membrane separation processes seems to provide more efficient biomass separation than the sedimentation process. Tests performed in membrane bioreactor (MBR) shown that the observed doubling time of anammox bacteria could be reduced to 8–11 days [8] or to even 7 days [9]. Other method to improve the process start-up is biomass immobilization, widely used in municipal and industrial wastewater treatment, also popular in anammox-based technologies [10]. In attached growth systems, efficiency of biomass retention was tested by use of different materials as biofilm carriers. Experimental start-up of an anammox process in reactors with different biomass carriers (sponge, volcanic rock, and charcoal) showed no difference in the obtained final nitrogen removal rate (NRR). However, carrier material had impact on time needed to obtain certain NLR; thus, its proper selection can influence the start-up time [11].

Also, addition of chemical agents can stimulate bacterial growth rate improving the start-up of certain process. In case of anammox growth enhancement, several substances were tested, i.e. AHL and hydrazine. AHLs (acyl-homoserinelactones) are one of mediation molecules important for bacterial cell-to-cell interactions, known as quorum sensing. As signal molecule concentration reaches threshold value, certain gene responsible for certain phenotype is activated, resulting, i.e. attachment growth, activity increase, or sludge granulation. Impact of AHL on anammox activity was presented in several reports [12–14]. External addition of AHL in a laboratory-scale CSTR allowed to reduce the start-up period by 17.5% in comparison with reference reactor [15].

Hydrazine (N_2H_4) is considered as a substance responsible for increasing anammox bacteria activity and stimulating their growth. This energy-rich compound is an intermediate in the biochemical pathway of anammox process itself; however, it found application also as a rocket fuel or a corrosion control agent in conventional and nuclear power plant [2]. Hydrazine is also known for its high toxicity for living organisms; nevertheless, some bacteria can use it as an energy source. Anammox micro-organisms can tolerate and convert up to 1 mM of hydrazine [16]. The presence of N_2H_4 increases the rate of ammonia oxidation and nitrite reduction and affects activity of nitrite-oxidizing bacteria simultaneously enhancing growth of the anammox biomass [16, 17]. Positive effect on anammox process performance was observed in a completely autotrophic N-removal over nitrite (CANON) SBR, while hydrazine was added, with optimal concentration at 3.99 mgN_2H_4 dm^{-3}. Higher doses gradually reduced the nitrogen removal rate, probably due to the inhibitory effect [18] Other report revealed that addition of 1 mM of hydrazine resulted in heterotrophic denitrification inhibition, increased anammox growth, higher rate of nitrite depletion, and lower nitrate accumulation [16].

Operational conditions and results of selected approaches to anammox start-up improvements strategies are presented in Table 1.

1.2 Aim of study

In this study, an impact of hydrazine addition on overall anammox start-up performance was investigated. The previous reports investigated hydrazine effect in short-term batch tests; therefore, approach presented in this paper focuses on long-term effects obtained in a SBR treating real reject water from digested sludge dewatering. Such approach gives a good overview on the possibility of enhance anammox growth by simple addition of a chemical agent which can be applicable in all engineering applications of this N-removal process.

Table 1 Different approaches to anammox start-up presented in the literature

Start-up strategy	Type of reactor	Process parameters	Duration of the start-up	Achieved effects	Author
Immobilization of biomass on a carrier	Up-flow column reactors	32 ± 2 °C pH: 7.1–7.8	105 days—sponge + volcanic rock 121 days—sponge + charcoal	Sponge + volcanic rock TN removal: 91.0% NLR: 0.191 kgN $(m^3\ d)^{-1}$ Sponge + charcoal TN removal: 89.9% NLR: 0.18 kgN $(m^3\ d)^{-1}$	Lu et al. [11]
Reagent addition	CSTR	–	66 days—AHL-containing medium 66 days—AHL-containing medium (dilution 1:2) 88 days—tap water	NH_4–NNO_2–N removal 98%—AHL medium 97%—AHL medium (1:2) 96%—tap water	Zhao et al. [15]
Reagent addition	CANON SBR	31 ± 1 °C	Batch test	TN removal 0.370 ± 0.016 kgN·$(m^3\ d)^{-1}$	Yao et al. [18]
Reagent addition	SBR	32 °C	Batch test	–	Ma et al. [16]

2 Materials and methods

2.1 Start-up strategy

Anammox biomass enrichment was performed in two parallel reactors in this experiment. Reactors were operated with the initial NLR for 6 days to obtain stable conditions. During the enrichment period, hydrazine was added to reactor 1 (R1) with a daily dose equal to 3.7 mg (after Phase 1, the dose of hydrazine was set on 4 gN_2H_4/m^3 like in [literature]. During Phase 2, dose was not changed so the final concentration of hydrazine was 3.3 gN_2H_4/m^3. The average concentration (6th–42th day) was 3.7 gN_2H_4/m^3) N_2H_4 dm^{-3} for 36 days. The second reactor (R2) was used as a reference. In both reactors, NLR, solids concentration (MLSS), and specific anammox activity (SAA) were measured throughout the whole test. Inorganic nitrogen forms (NH_4–N, NO_2–N, and NO_3–N), alkalinity, and total/volatile suspended solids (TSS/VSS) were also controlled in the reactors effluent. While ammonium and nitrite concentration in the effluent has dropped, reactors NLR was increased by 5–20%. After 42nd day of the experiment, hydrazine addition and NLR increasement were stopped to observe reactors performance in steady-state conditions.

2.2 Pilot-scale SBR and reactors operation parameters

R1 and R2 were seeded with the same portion of inoculum. Origin of the anammox biomass was SBR operated in stable conditions for over 2 years treating real reject water from digested sludge dewatering. SBRs used in this study had operational volume equal to 150 dm^3 and were equipped with mechanical mixers. Temperature was controlled and kept at 23 °C. pH was in range between 6.6 and 7.5 with a 8% H_2SO_4 solution dosed using a peristaltic pump controlled by a pH meter. To prevent excessive aeration through surface, it was covered with plastic elements reducing the gas–liquid surface area. SBRs were operated in four cycles per day, and each of them consisted of reaction phase with step-feed influent (317 min, six filling phases), sedimentation (25 min), and decantation (18 min). As no excess sludge was withdrawn from the reactors, observed solids retention time (SRT) was the result of amount of solids in the effluent. Average SRT was 39.8 ± 5.5 and 40.3 ± 4.6 days for R1 and R2, respectively.

2.3 Analytical methods

Inorganic nitrogen forms were measured using photometric cuvette tests and a DR3900 spectrophotometer (Hach, Germany): NH_4–N (test LCK303), NO_2–N (test LCK342), and NO_3–N (test LCK340). Before each analysis, collected samples were filtered using syringe filter, pore size 1.2 µm. Alkalinity was determined method according to Standard Methods guideline. Biomass concentration was measured as TSS/VSS according to EN-872—standard direct method for suspended solids on glass fibre filters with 1.2 m pore size.

3 Results

3.1 Preliminary investigation

Both SBRs were fed with the same medium: reject water from digested sludge dewatering pretreated in a partial nitritation SBR. During the test, average anammox influent composition was: 248.1 ± 18.0 gN m^{-3}, 298.3 ± 25.6 gN $m^{-}3$, and 5.5 ± 0.6 gN m^{-3} for ammonium, nitrite, and nitrate,

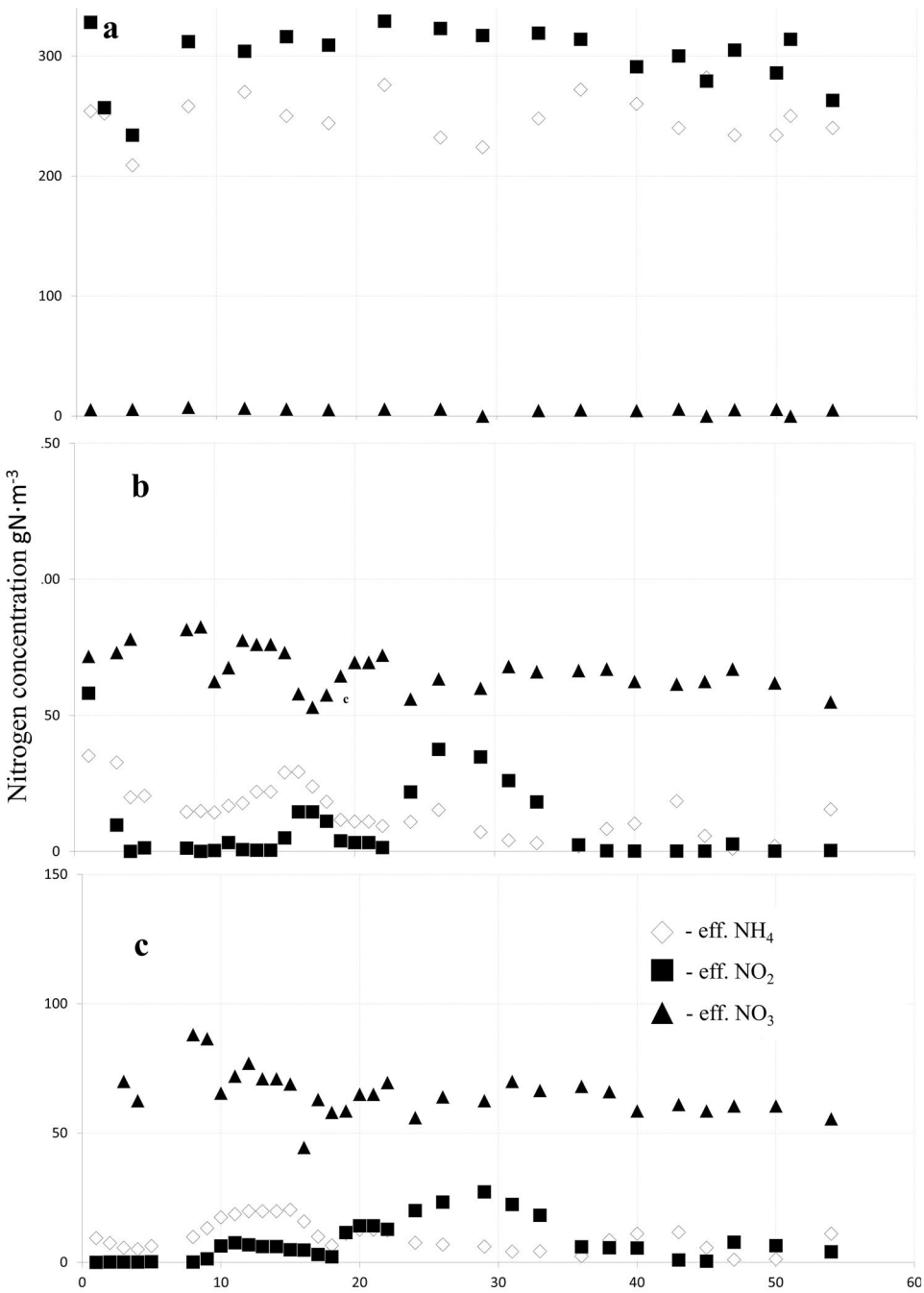

Fig. 1 **a** NH_4–N, NO_2–N, and NO_3–N concentration in the influent medium. **b** NH_4–N, NO_2–N, and NO_3–N concentration in the R1 effluent and **c** NH_4–N, NO_2–N, and NO_3–N concentration in the R2 effluent

respectively. Average NO_2-to-NH_4 ratio was 1.21 ± 0.1 through the experiment. Medium composition is presented in Fig. 1a.

3.2 Technological research

3.2.1 Phase 1: Stabilization #1

The initial NRR during stabilization period (until day 6) was 0.034 ± 0.004 kgN $(m^3\ d)^{-1}$ and 0.053 ± 0.002 kgN $(m^3\ d)^{-1}$ in R1 and R2, respectively. In this phase of experiment, TSS concentration in the reactors was 518 g m^{-3} and 852 g m^{-3} in the R1 and R2, respectively. In both reactors, VSS were between 81 and 89% of the TSS. The initial NLR was 0.051 ± 0.016 kgN $(m^3\ d)^{-1}$ in R1 and 0.059 ± 0.019 kgN $(m^3\ d)^{-1}$ in R2 (Fig. 2). Average effluent composition in R1 was 27.1 ± 6.6 mgNH_4–N dm^{-3}, 17.3 ± 23.4 mgNO_2–N dm^{-3}, and 74.2 ± 3.1 mgNO_3–N dm^{-3}, while in R2 was 6.8 ± 1.3 mgNH_4–N dm^{-3}, 0.13 ± 0.07 mgNO_2–N dm^{-3}, and 66.3 ± 5.2 mgNO_3–N dm^{-3} (daily data shown in Fig. 1b, c). During this phase, in both reactors, average dissolved oxygen concentration was kept

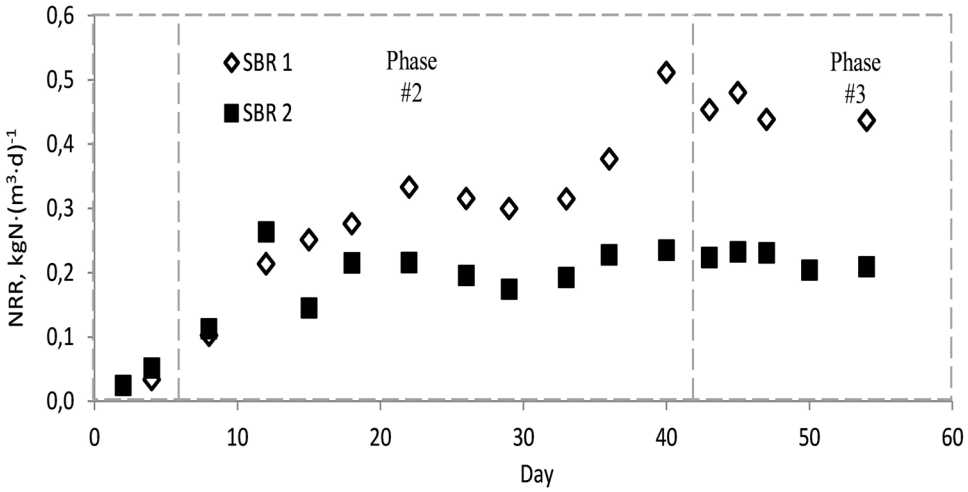

Fig. 2 NLR in both reactors during the experiment

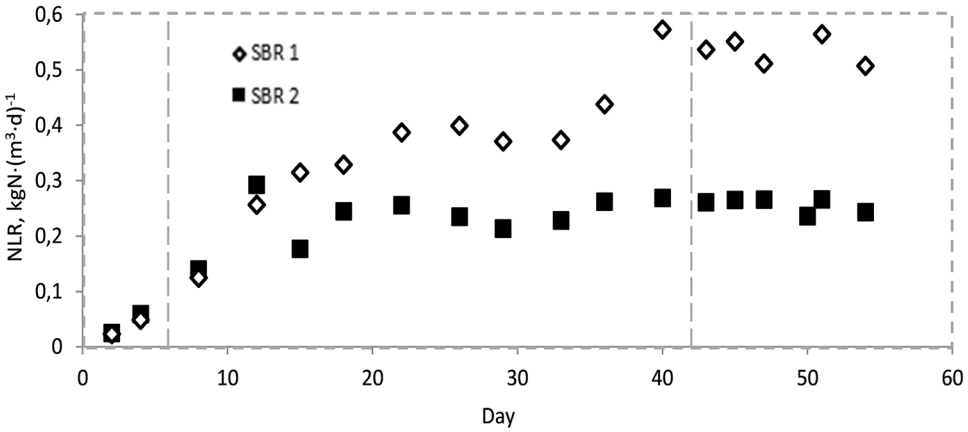

Fig. 3 NRR in both reactors during the experiment

at very low level to prevent potential inhibition: 0.03 ± 0.02 (max. 0.12) $gO_2\ m^{-3}$ and 0.03 ± 0.02 (max. 0.11) $gO_2\ m^{-3}$ for R1 and R2, respectively. pH varied through each SBR cycle and was between 6.69 and 7.46 in R1 and 6.63 and 7.48 in R2. In both reactors, temperature was kept stable at 23 °C in all phases.

3.2.2 Phase 2: NLR increasement

Based on the effluent quality analysis, NLR was increased by higher influent volume. In the stabilization phase, R2 load was increased at following days: 8th, 9th, 18th, and 36th. Hydrazine addition to R1 allowed to increase NLR more frequent than in R2, and influent volume was increased at 8th, 9th, 10th, 12th, 14th, 19th, 23rd, 36th, and 40th day of the experiment. Anammox biomass enrichment could be observed as NRRs increased significantly compared to the stabilization period and at 40th day were 0.511 kgN $(m^3\ d)^{-1}$ and 0.235 kgN $(m^3\ d)^{-1}$ in R1 and R2, respectively (Fig. 3). Higher process efficiency resulted in higher NLR in R1 (Fig. 2) from the 13th day of the experiment, and at the end of Phase 2, NLR in R2 was only 48.7% of the NLR achieved in R1. In both reactors, no significant substrate accumulation in the effluent could be observed (Fig. 1b, c). Average TSS in the reactors were 534 g m^{-3} in the R1 and 461 g m^{-3} in the R2. No change in the VSS/TSS ratio could be noticed. Despite higher influent in this phase, average dissolved oxygen concentration was still kept at very low level: 0.02 ± 0.02 (max 0.32) $gO_2\ m^{-3}$ and 0.02 ± 0.02 (max 0.14) $gO_2\ m^{-3}$ for R1 and R2, respectively. Higher process rate observed in this phase resulted in slightly higher pH which was between 7.04 and 7.43 in R1 and 6.89 and 7.36 in R2.

3.2.3 Phase 3: Stabilization #2

From 43rd day of the experiment, both reactors were operated at stable NLR and hydrazine addition to R1 was stopped. During this period, NRR stabilized at level observed at the end of Phase 2 and was 0.432 ± 0.043 kgN $(m^3\ d)^{-1}$ and 0.220 ± 0.011 kgN $(m^3\ d)^{-1}$ in R1 and R2, respectively (Fig. 3). Also, NLR was similar

to the previous phase: 0.512 ± 0.054 kgN $(m^3\ d)^{-1}$ in R1 and 0.256 ± 0.012 kgN $(m^3\ d)^{-1}$ in R2 (Fig. 2). Effluent quality was stable and comparable in both reactors (R1: 8.6 ± 6.2 $mgNH_4$–N dm^{-3}, 0.7 ± 0.9 $mgNO_2$–N dm^{-3}, and 61.6 ± 3.4 $mgNO_3$–N dm^{-3}, while in R2: 6.1 ± 4.0 $mgNH_4$–N dm^{-3}, 3.9 ± 2.5 $mgNO_2$–N dm^{-3}, and 59.2 ± 1,8 $mgNO_3$–N dm^{-3}) (Fig. 1b, c). Operational conditions in terms of dissolved oxygen, temperature, and pH were similar to Phase 2. Slight drop of NLR observed in R1 between the 47th–50th day was due to mechanical issue with the influent pump, however, did not affected the process efficiency (Fig. 3).

4 Discussion

As suspected, hydrazine addition resulted in higher anammox activity in R1 as in Phase 3, and average NLR was twice higher than the reference process in R2. In the same phase, the SAA (specific anammox activity) in R1 and R2 was 0.504 ± 0.127 gN $(gVSS\ d)^{-1}$ and 0.411 ± 0.037 gN $(gVSS\ d)^{-1}$, respectively. These results correspond with the previous observations where similar dose of hydrazine (3.99 mgN_2H_4/dm^3) was added to reactor with anammox biomass during batch tests [18] Differences in SAA during mentioned tests can be compared to those presented in this study: 0.194 gN $(gVSS\ d)^{-1}$ without hydrazine addition and 0.276 gN $(gVSS\ d)^{-1}$ with hydrazine addition. Higher effect of hydrazine presented in the literature (SAA increase by 42.3%) compared to that obtained in this experiment (SAA increase by 22.6%) can be explained by its continuous character and effect of optimal conditions for non-stimulated anammox biomass growth.

According to the previous reports, hydrazine inhibition threshold for anammox activity was estimated on 32 $mgN_2H_4\ dm^{-3}$ [16]. In the experiment, hydrazine dosage to R1 resulted in more than eight times lower concentration than mentioned the toxicity level (3.7 $mgN_2H_4\ dm^{-3}$). As selected reagent dose was based on other reports, no inhibitory effect was observed neither in this study which confirms the previous results [18]. The presence of hydrazine should also affect the heterotrophic denitrification process [16]. However, in this study, both reactors were fed with reject water from dewatering sludge after mesophilic digestion process. The presence of easily biodegradable organic matter in this medium is very limited; furthermore, anammox process was preceded by partial nitritation reactor where all potential biodegradable compounds were oxidized. As denitrification was not observed before hydrazine addition started, no hydrazine impact on this process could be observed. Some reports suggest that the presence of hydrazine results in changes in anammox process stoichiometry affecting the NO_2-to-NH_4 ratio [18]. In that case, denitrification inhibition can be considered as one of potential explanations of such phenomenon. In this study, where no denitrification occurred, no such change could be observed. Observed NO_2-to-NH_4 ratio was stable and in R1 and R2 was 1.216 ± 0.09 and 1.223 ± 0.09, respectively.

Growth rate of anammox bacteria in laboratory and pilot-scale plants treating non-synthetic medium varies from 0.003 to 0.004 h^{-1} [4, 9]. Similar to other bacterial groups, also in case of anammox, growth rates estimated in pure cultures are higher than in mixed cultures. Reported growth rate values in pure cultures were higher, 0.005 h^{-1} for *Ca. J. caeni* and 0.007 h^{-1} for *Ca. B. sinica* and *Ca. Scalindua* sp. [5]. SAA measured in pure cultures of *Ca. B. sinica* reached 2 gN $gVSS^{-1}$ day^{-1} which is significantly higher than that in this experiment [19]. SAA in R1 and R2 was 34.2% and 23.1% of the mentioned growth rate obtained in pure cultures. Anammox biomass reveals high capacity for attached growth [5]; thus,

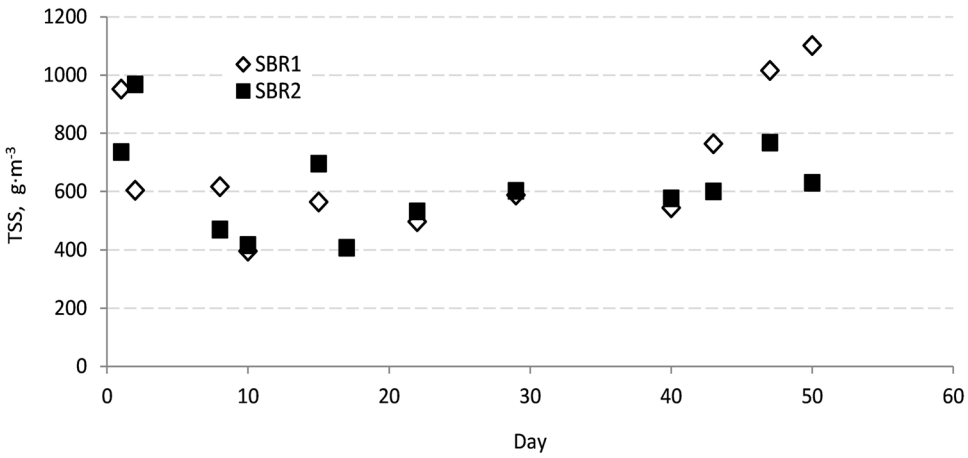

Fig. 4 TSS concentration in both reactors during the experiment

potential estimation of share of anammox biomass in the VSS fraction is impossible due to scale of the experimental reactor where biofilm removal from reactor and used equipment was not possible. That situation explained why NLR and NRR was growing with no change of TSS of biomass (Fig. 4). Participation Anammox biomass in sludge was small and growth of that organism was impossible to measured by regular TSS measurement method. For more accurate evaluation of hydrazine addition effect in a long-term experiment, an alternative method for anammox growth rate estimation must be used, similar to activated sludge procedure used in ASM models [20].

5 Conclusions

Selected hydrazine dose (3.7 mgN_2H_4 dm^{-3}) had positive impact on the anammox process performance. Anammox process kinetics were boosted as reactor with reagent addition allowed to increase NLR from 0.051 ± 0.016 $kgN \cdot (m^3\ d)^{-1}$ to 0.519 ± 0.049kgN $(m^3\ d)^{-1}$ within 36 days. Observed loading increasement was about 190% higher than in the reference reactor which is a spectacular result. At the end of the experiment, NRR in the chemically stimulated biomass was 0.432 ± 0.043 kgN $(m^3\ d)^{-1}$ which was ten times higher than observed during the initial phase. Contrary to the previous reports, no change in the process stoichiometry was observed after hydrazine addition (NO_2-to-NH_4 ratio 1.216 ± 0.09).

Results of the experiment clearly indicate that reagent addition can be a promising method of more efficient process start-up technique as observed NRR increase was much higher than in conventional anammox enrichment.

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Compliance with ethical standards

Conflict of interest Author Stanisław Miodoński is a member of science committee of conference: Interdisciplinary Problems in Environmental Protection and Engineering EKO_DOK. The rest of the authors declare that they have no conflict of interest.

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