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ABSTRACT:

Wastewater source-separation and on-site treatment systems face severe problems in wastewater availability. Therefore, the effect of repeated short-term starvation and reactivation periods on a partial nitritation-anammox (PN/AMX) based process was assessed treating digested blackwater at room temperature. Two sequencing batch reactors (SBR) were operated, one of them during 24 h/day the whole week (SBR-C, which served as control) and the other with repeated starvation/reactivation periods during the nights and the weekends (SBR-D), using simulated blackwater (300 mg N/L and 200 mg COD/L) as substrate. Results showed no remarkable differences in overall process performance between both reactors, achieving total nitrogen removal efficiencies (NRE) around 90 %. Furthermore, no significant variations were measured in specific activities, except for the aerobic heterotrophic one that was lower in SBR-D, presumably due to the exposure to anoxic conditions. Then, the technical feasibility of applying the PN/AMX system to treat real blackwater produced in an office building during working hours was successfully proved in a third reactor (SBR-R), with the same starvation/reactivation periods tested in SBR-D. Despite the low temperature, ranging from 14 to 21 ºC, total NRE up to 95 % and total nitrogen concentration in the effluent lower than 10 mg N/L were achieved. Moreover, the PN/AMX process performance was immediately recovered after a long starvation period of 15 days (simulating holidays). Results proved for the first time the feasibility and long-term stability (100 days) of applying the PN/AMX process for the treatment (and potential reuse) of blackwater in a decentralized system where wastewater is not always available.

Keywords: anammox; blackwater; decentralized systems; starvation; nitritation; wastewater source separation.

1. Introduction

The increasing water scarcity and resources depletion have triggered efforts on the implementation of sustainable water management approaches (European Commission, 2016; WWAP, 2017). Decentralized wastewater treatment systems become an attractive alternative to be applied in small agglomerations enabling the energy and nutrients recovery, ensuring the local water availability by reusing the treated water and decreasing both investment and operational costs (WWAP, 2017). Source-separation systems allow segregating the different streams for a more intensive treatment depending on their characteristics and their final use promoting the water reuse (Malila et al., 2019; WWAP, 2017). Blackwater (i.e., toilet water) is an organic matter and nutrients concentrated stream contributing to approximately 92 % of total nitrogen, 75 % of phosphorus and 52 % of the organic matter contained in mixed domestic sewage (Gottardo Morandi et al., 2018). Moreover, blackwater composition considerably varies according to its origin, infrastructure, toilet flushing systems and user habits (Gao et al., 2019; Ren et al., 2018), as it is summarized in Table 1 blackwater is more concentrated in residential areas whereas the one deriving from workplaces or touristic installations (museums, parks, etc.) is generally more diluted.

Table 1. Summary of blackwater composition depending on the origin and toilets flushing system.

Reference	**Origin**	**tCOD (g/L)**	**sCOD (g/L)**	**pH**	**TN (mg N/L)**	**NH_4^+-N (mg N/L)**	**Toilet flushing (L/flush)**
This study	Office building	2.3 ± 0.1	0.56 ± 0.01	7.5 ± 0.1	115 ± 6	95 ± 5	4.5/ 3
Gallagher and Sharvelle, 2011	Office building	2.0 ± 0.2	0.67 ± 0.16	8.0	145 ± 24 [a]	102 ±8	5 toilet + 1.5 urinal
Ren et al., 2018	Office building	0.4 ± 0.1	0.31 ± 0.03	-	74 ± 6	66 ± 7	-
Zeeman et al., 2008	32 houses	19 ± 3.4	3.2 ± 0.6	8.6	-	1400 ± 300	1
De Graaff et al., 2010	32 houses	7.7 ± 2.5	2.3 ± 0.8	8.6 ± 0.5	1200 ± 180	850 ± 150	1 (7.8 L/p/d)
Palmquist and Hanæus, 2005	44 houses	0.8 - 3.1	-	8.9 - 9.1	130 - 180 [a]	-	-
Knerr et al., 2011	15 inhabitants residential	2.9 ± 0.8	-	9.0 ± 0.1	273 ± 39 [a]	202 ± 32	9
Ren et al., 2018	2 houses	0.7 ± 0.1	0.40 ± 0.06	-	149 ± 19	139 ± 20	-
Zeeman et al., 2008	University	9.5 ± 6.5	1.4 ± 0.5	8.8 ± 0.2	1000 ± 130 [a]	710 ± 10	1
Moges et al., 2018	Student dormitory (48 inhabitants)	5.5 ± 1.3	1.2 ± 0.3	9.0 ± 0.3	-	900 ± 200	1.2
Murat Hocaoglu et al., 2010	Campus lodges	1.1 ± 0.6	0.4 ±1.2	8.0 ± 0.3	180 ± 28 [a]	147 ± 18	9
Todt et al., 2015	Student dormitory (48 inhabitants)	8.9 - 11.4	-	-	1400 - 1700 [a]		1.2
Oarga-Mulec et al., 2017	Tourist park	-	2.2 ± 1.0	8.5 ± 0.6	-	810 ± 240	0.8
Lansing et al., 2017	Hotel	-	1.1 ± 0.3	7.2 ± 0.1	194 ± 24 [a]	164 ±28	4
Lansing et al., 2017	Clinic		6.1 ± 0.8	7.0 ± 0.2	703 ± 267 [a]	161 ± 20	4
Sharma et al., 2016	Residential school	1.7 ± 0.2	0.9 ± 0.2	8.1 ± 0.2	117 ± 28	88 ± 19	5
Ren et al., 2018	Fire Station	0.2 ± 0.1	0.13 ± 0.01	-	62 ± 7	54 ± 9	-
Ren et al., 2018	2 Hotels	0.7 ± 0.2	0.45 ± 0.12	-	69 ± 16	61 ± 14	-

COD: chemical oxygen demand (“t” and “s” refers to total and soluble, respectively); TN: Total nitrogen; [a]TKN: Total Kjeldahl Nitrogen.

The anaerobic digestion of blackwater allows recovering its energy content as biogas (Gao et al., 2019; Moges et al., 2018). In the case of the anaerobic membrane reactors (AnMBR), the high quality and disinfected nitrogen-rich permeate, after the ammonium oxidation to nitrate, may be used as fertilizer while irrigating. However, the irrigation water requirements (quantity and nutrients concentration) vary along the year and the type of crop growing (European Commission, 2016). Thus, a nitrogen removal system needs to be also considered to obtain a clean effluent suitable for other reuse purposes, or ultimately, to discharge, in order to reduce the potential environmental impact of the anaerobic digestion process.

The combination of the partial nitritation and anammox (PN/AMX) processes represents an adequate and intriguing alternative since it allows the completely autotrophic nitrogen removal from wastewater and promotes the recovery of the wastewater energy content. The anaerobic biodegradability of the blackwater range from 40 to 80 %, meaning that residual organic matter is present in the effluent of the anaerobic digester (De Graaff et al., 2010; Gao et al., 2019). Nevertheless, previous studies indicate that it is possible to achieve and maintain stable the PN/AMX processes at moderate nitrogen concentrations and temperature (i.e., the conditions of the anaerobically digested blackwater) when moderate organic matter concentration are present in wastewater (Hoekstra et al., 2019; Pedrouso et al., 2018). Despite the number of research works about blackwater treatment exponentially rose, scarce information is available about its treatment by anammox based process.

Decentralized systems would have to deal with large fluctuation in both flow and composition of wastewaters (European Commission, 2016). This high variability in wastewater production could top at the extreme case of a single office building where wastewater flow rate would be zero during nights, weekends and holidays. Thus, the biological systems are frequently exposed to famine conditions affecting the process robustness (Wang et al., 2018). Modular treatment trains enable to adapt the treatment requirements depending on the final water purpose. In the periods when treated wastewater is reused for irrigation, nitrogen removal process is not required and the corresponding unit would have no water supply. As anammox bacteria are traditionally considered sensitive to environmental changes, the study of short-term starvation effect over the anammox activity is of great interest. However, limited information is available about the response of simultaneous PN/AMX processes under oxygen and nitrogen absence (Reeve et al., 2016) and the influence of repeated starvation on the biomass from a reactor operating in transient conditions, as can occur in a decentralized system treating blackwater, was not studied.

Hence, the aim of this study is to evaluate the operation of a PN/AMX based process (ELAN® process), treating anaerobically digested blackwater at room temperature, and to assess the impact of regular stops and reactivation periods on the biological reactor due to the highly variable influent flow rate in a decentralized system. Additionally, the study of the different microbial populations involved (anammox, ammonium and nitrite oxidizers and heterotrophs) was evaluated in terms of their specific activity, to understand the effect of the starvation/reactivation periods over them.

2. Material and Methods

2.1. Reactors setup and operation

Three one-stage PN/AMX reactors, with a working volume of 4 L and a volume exchange ratio of 20 %, were operated. Reactors were run as sequencing batch reactors (SBR) with a 3-hour cycle configuration (See Table S1 in Supporting Material for details of the phase’s distribution in the cycle). The air flow rate was manually adjusted by means of a gas flow meter (P model, Aalborg). Mechanical stirring (with a velocity of 40 -50 rpm) was provided in order to guarantee the reactor mixture. The SBRs were inoculated with biomass from a full-scale ELAN® reactor (one-stage PN/AMX technology with granular sludge) treating the supernatant from an anaerobic sludge digester of a municipal wastewater treatment plant located in Guillarei (Tui, NW Spain) (Morales et al., 2018). All reactors operated at room temperature and neither pH nor dissolved oxygen (DO) concentration were controlled. The experimental conditions for each reactor are summarized in Table 2.

Firstly, two of the reactors (SBR-C and SBR-D) were fed with synthetic influent (Table S2 in Supporting Material) simulating the moderate nitrogen concentration (300 mg N/L as ammonium chloride) and the residual organic matter (200 mg COD/L as sodium acetate) of the digested blackwater, resulting in an influent COD to nitrogen ratio of 0.67 g COD/g NH_4^+-N. The reactors operated with the SBR cycle configuration named Cycle 1 comprising: 5 min of anoxic mixed feeding, 160 min of aerated reaction, 10 min settling and 5 min of effluent withdrawal. Both reactors were operated in continuous mode for 28 days (7 days/week and 24 h/day). Then, SBR-D was stopped during the night and weekends simulating the lack of wastewater in a decentralized treatment system from an office building. With this change the hydraulic retention time (HRT) in SBR-D increased from 0.63 to 1.75 days (considering that it was only fed 4 cycles/day and 5 days/week), whereas SBR-C served as control and it was operated always without stops with a HRT of 0.63 days. The stops in SBR-D provoked that the temperature inside the reactor varied in a wider range than in SBR-C (Table 2), but this difference was not statistically significant.

Table 2. Summary of the experiments performed.

Reactor	*Media*	*Temperature (ºC)* [a]	*Stages (days)*	*Cycle* [b]	*Stops*
SBR-C	Synthetic	18.6 - 23.7 (21 ± 1)	Start-up (0 - 28) Continuous (29 - 90)	Cycle 1	No
SBR-D	Synthetic	14.4 - 24.4 (21 ± 2)	Start-up (0 - 28)	Cycle 1	No
			Discontinuous (29 - 90)	Cycle 1	Nights and weekends
SBR-R	Pre-treated blackwater	14.0 - 21.3 (19 ± 2)	Stage I (0 - 40)	Cycle 1	Nights and weekends
		14.0 - 20.0 (17 ± 2)	Stage II (41 -58)	Cycle 2	Nights and weekends
		12.8 - 15.6 (15 ± 1)[c]	Starvation (59 - 74)	No	15 days
		14.2-20.3 (19 ± 2)	Stage III (75 - 100)	Cycle 2	Nights and weekends

[a] As temperature was not controlled, the range indicates the minimum and maximum values measured inside the reactors, while the average value with the standard deviation for the corresponding operational period is reported in brackets.

[b] See in supplementary material Table S1 the definition of each cycle.

[c] Low average values due to winter holidays, with no central heating in the laboratory building.

After the operation of the synthetic media fed reactors a third reactor (SBR-R) was fed with anaerobically digested blackwater (Table 3) collected in an office building (where approximately 200 people work) with source-separated sanitation located in Porto do Molle Business Center (Nigrán, NW Spain). The used blackwater was less concentrated that the one used in other studies (Table 1) and that the used previously in the synthetic fed reactors (SBR-C and SBR-D), as it was mainly composed by urine and diluted using regular flushing toilets (3.0 - 4.5 L/flush). The raw blackwater (Table 3) was previously digested in an AnMBR comprising an anaerobic stirred reactor (2.8 m^3) coupled to a membrane tank (1 m^3) equipped with an ultrafiltration flat-sheet membrane module (6.25 m^2). The AnMBR was operated at room temperature (18 - 26 °C) achieving a 90 % of organic matter removal and producing an effluent with a COD/N ratio of 0.9 g COD/g NH_4^+-N. SBR-R was operated for 100 days with different operational stages depending on the cycle configuration applied and the regime of stops (Table 2). It was started-up and operated for 40 days with the same operational cycle and stops as SBR-D (Stage I-Cycle 1). Then, to improve the total nitrogen removal efficiency (NRE), an anoxic reaction phase (20 min) was implemented after the feeding (Stage II-Cycle 2, days 41 - 58). Thus, the aerobic reaction phase was reduced to 140 min. Finally, the reactor was operated from day 75 to 100 (Stage III) to study the reactivation of the PN/AMX process after a 15 days starvation period. During this starvation period (from day 59 to 74) no measurements were done, as the reactor was completely stopped (no feeding, mixing and aeration).

Table 3. Characterization of raw blackwater and anaerobically digested blackwater.

Parameter	*Raw blackwater*	*Anaerobically digested blackwater*
tCOD (mg/L)	2325 ± 58	98 ± 3
sCOD (mg/L)	553 ± 3	98 ± 3
DOC (mg/L)	117 ± 12	32 ± 6
DIC (mg/L)	159 ± 10	124 ± 13
TN (mg/L)	115 ± 6	121 ± 5
NH_4^+-N (mg/L)	95 ± 5	120 ± 12
NO_2^--N (mg/L)	0.02 ± 0.01	0.02 ± 0.01
NO_3^--N (mg/L)	0.3 ± 0.1	0.5 ± 0.1
pH	7.50 ± 0.05	7.25 ± 0.15
Conductivity (mS/cm)	-	1.5 ± 0.2

DOC: Dissolved organic carbon; DIC: dissolved inorganic carbon; sCOD: soluble chemical oxygen demand; tCOD: total chemical oxygen demand; TN: total nitrogen.

2.2. Ex-situ specific activity tests in batch mode

The ex-situ specific activity tests were performed collecting the biomass from the reactors in different operational days and following the corresponding protocol in batch mode. The maximum specific anammox activity (SA_{AMX}) was determined according to the manometric method described by Dapena-Mora et al. (Dapena-Mora et al., 2007) and employing 70 mg N/L of both nitrite and ammonium as substrates. The specific heterotrophic denitrification activity (SA_{HDN}) was assessed by the same procedure but using 200 mg COD/L and 25 mg NO_3^--N/L as substrates. Respirometric assays were conducted to determine the specific aerobic heterotrophic activity (SA_{aerHET}), as well as specific ammonium- and nitrite-oxidizing activities (SA_{AOB} and SA_{NOB}, respectively) (Lopez-Fiuza et al., 2002) using a biological oxygen monitor (BOM, Ysi Inc. model 5300) equipped with oxygen selective probes (YSI 5331). In the SA_{AOB} tests 24 µM of sodium azide was added to selectively inhibit the SA_{NOB} in case it was present (Val del Rio et al., 2019). All these activity tests were performed in triplicate at 20°C, and for SA_{AMX} tests also at 30 °C (temperature of reference).

2.3. Analytical methods

Influent and effluent streams of the three reactors were periodically sampled to follow the process performance. All samples were filtered using a 0.45 µm pore size filters prior to analysis. Spectrophotometric methods were applied to determine the ammonium (Bower and Holm-Hansen, 1980), nitrite and nitrate (APHA et al., 2017) concentrations. Total COD (tCOD) in raw samples and soluble COD (sCOD) in filtered samples were also determined according to Standards Methods (APHA et al., 2017). Total dissolved organic and inorganic carbon concentrations (DOC and DIC, respectively) were measured with a Shimadzu analyzer (TOC-L-CSN). Total nitrogen (TN) was measured in the same Shimadzu analyzer with a TNM-L Unit. The DO concentration and temperature in the bulk liquid were on-line measured using a luminescent DO probe (LDO, Hach Lange). The pH and conductivity values were determined with electrodes (pH1 and EC5, respectively) connected to a Hach Sension+ meter. The concentration of the total suspended solids (TSS), volatile suspended solids (VSS) and sludge volume index (SVI) were determined according to Standard Methods

(APHA et al., 2017). The average diameter of the granules and size distribution were determined (only for SBR-R) utilizing a stereomicroscope (Stemi 2000-C, Zeiss) incorporating a digital camera (Coolsnap, Roper Scientific Photometrics) for image acquisition and then these images were processed using the Image ProPlus® software. The separation of both granular and suspended sludge fractions to further characterize the biomass from the three reactors was performed by means of a 200 μm sieve. The granular biomass density was determined as the mass of the granule per granule volume using the blue dextran method (Beun et al., 2002).

2.4. Calculations

Mass balances and calculations were done considering the stoichiometric reactions and equations described in Supporting Material (Section S1). Statistical analysis was conducted with the software R (version 3.5.2, R Core Team 2015). First, variance homogeneity was confirmed by Levene's test and normal distribution by the Saphiro's test. Then, if the data set met the homogeneity and normal distribution prerequisites, a one-way analysis of variance (ANOVA) was carried out to determine if the values obtained were significantly different at the 95 % confidence level ($p < 0.05$). A post hoc analysis (Tukey's HSD) was applied every time that ANOVA resulted in a significant difference, to find which mean value was significantly different from each other, considering a level of significance of 0.05. If data variance homogeneity and/or normal distribution requirements were not fulfilled, the non-parametric Kruskal-Wallis analysis was applied and then the Wilcoxon post hoc analysis was used.

3. Results

3.1 Comparison between the continuous and the repeated starvation/reactivation modes in the operation of the PN/AMX process

3.1.1 Start-up and performance at the conditions of the digested blackwater

Both SBR-C and SBR-D were inoculated with biomass drawn from a full-scale ELAN® reactor treating the supernatant from a sludge anaerobic digester (Morales et al., 2018), which was acclimated to mesohphilic temperatures (around 30 °C), large nitrogen concentrations (> 500 mg TN/L), as well as by lower COD/N ratios (< 0.5 g sCOD/TN). However, from the start-up, stable PN/AMX process was achieved (Figure 1) by adjusting aeration flow rates between 1.5 - 2.0 L/min (resulting in a DO concentration of 0.1 - 1.5 mg O_2/L). In fact, during the whole experimental period of both reactors, the most influential factor in process performance was the air flow rate. Difficulties in adjusting it, especially during the start-up phase, led to fluctuating process performances (wider in SBR-C, more frequent in SBR-D). However, as air flow rate was properly controlled, process stability was reached and maintained in both reactors. This problem is mostly related to small-scale systems, and it may be easily avoided in case of scale-up, since air-flow rates (i.e., DO concentrations) can be adjusted by using advanced control systems. At the end of start-up (day 28), the observed NRE was around 80 % in both SBRs, at a nitrogen loading rate (NLR) of 480 mg N/(L·d).

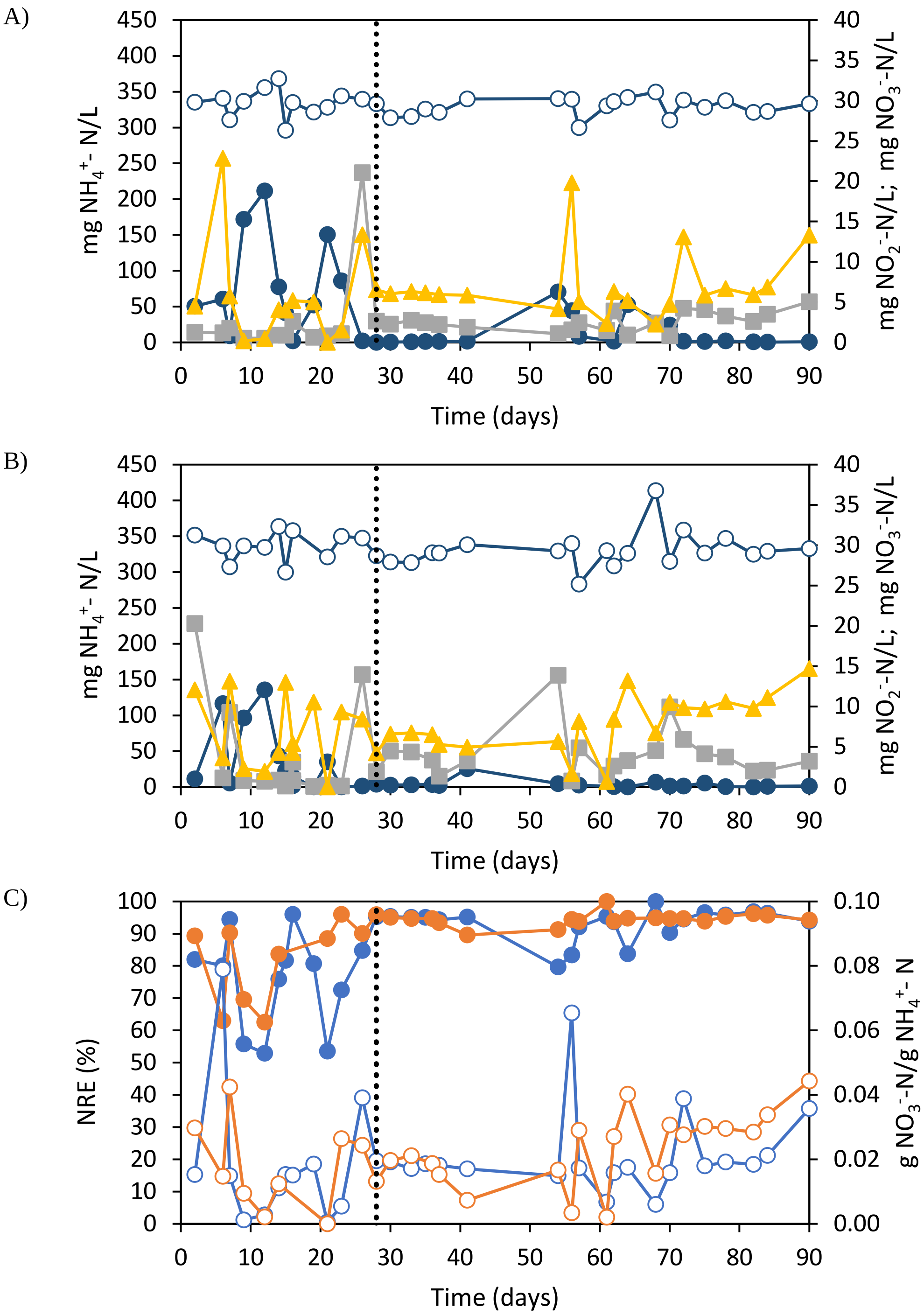

Figure 1. Time profiles of ammonium (○) in the influent, and effluent nitrogen forms as ammonium (●), nitrite (■) and nitrate (▲) in SBR-C (A) and SBR-D (B) whereas C) shows the nitrogen removal efficiency (NRE) (filled dot) and the nitrate produced to ammonium consumed ratio (empty dot) for SBR-C (● and ○) and SBR-D (● and ○). Black dashed line corresponds to day 28 when the start-up of both reactors was completed.

Then, from day 28 onwards, scheduled stops were imposed to SBR-D (Table 2) resulting in a decrease of the applied NLR to 170 mg N/(L·d). The average NRE was 85 % and 89 % in SBR-C and SBR-D, respectively. The overall performance of SBR-D was not significantly affected by the different operating strategy applied (p=0.84) (Table 4). TN concentration in the effluent was 23 ± 17 mg TN/L in SBR-C and 18 ± 6 mg TN/L in SBR-D. The most abundant nitrogen form in the effluent was nitrate as, according to the PN/AMX stoichiometry, the 11 % of the influent ammonium was expected to be converted to nitrate (Burton et al., 2014). However, the observed nitrate produced to ammonium converted ratio in both reactors was 0.02 ± 0.01 g NO_3^--N/g NH_4^+-N (p=0.36), suggesting the presence of heterotrophic denitrifying activity (Figure 1.C).

Table 4. Average values of the mass balances process performance (p<0.05 indicate statiscally differences between mean values). Only data from day 28 onwards in both reactors were used for the calculations.

Removal*	**SBR-C**	**SBR-D**	**p (Kruskal-Wallis)**
% TN	90 ± 12	92 ± 8	0.8392
% N anammox	82 ± 7	85 ± 5	0.3577
% N denitrification	7 ± 3	8 ± 2	0.6359
% N assimilated	11 ± 8	10 ± 8	0.4989
% DOC	82 ± 11	79 ± 20	0.8287
% DOC denitrification	59 ± 39	62 ± 32	0.989
% DOC aerobic heterotrophic	41 ± 39	38 ± 32	0.989

* See section S1 in Supporting Material for calculations

Although approximately 80 % of the influent DOC was also removed, mass balance calculations indicated that the anammox process was the main nitrogen removal pathway in both reactors whereas the heterotrophic denitrification accounted for less than 10 % of the TN removal (p= 0.64) (Table 4). Moreover, an additional 10 % of the nitrogen removed was ascribed to biomass assimilation. Organic matter removal occurred by both aerobic (mineralization) and anoxic routes (nitrate heterotrophic denitrification). High variations were observed regarding the predominant DOC removal pathway, with DO concentration as the key factor. No significant differences between the main DOC removal pathways between SBR-C and SBR-D reactors were observed (Table 4).

3.1.2 Solids concentration and settleability

The ELAN® inoculum consisted of a mixture of granular and suspended biomass. During the start-up (days 0 – 28) a considerable increase of the biomass concentration was observed in both reactors (Figure 2.A) indicating a positive effect of the synthetic feeding and controlled environment counterbalancing the effect of the lower temperature (21 ± 2 °C, Table 2). Furthermore, the suspended biomass growth was promoted due to the presence of readily biodegradable organic matter (200 mg sCOD/L as acetate). Consequently, a progressive depletion in settling capacity occurred, as confirmed by the increase in SVI values. Compared with SBR-C, the development of suspended sludge and fast-growing heterotrophic bacteria was slower in the SBR-D due to the reduced organic load applied (OLR of 110 mg COD/(L·d)), which resulted in lower SVI values (between 80 to 150 mL/g TSS) along the operational period (See Table S3 in Supporting Material).

Despite the decrease of the settling capacity, VSS concentration in the effluent of both reactors remained relatively low (Figure 2.B). Eventually, the concentration of VSS in the effluent of SBR-C increased up to 80 mg VSS/L on day 53 and consequently, sludge was partially washed out from the reactor decreasing the reactor VSS concentration from 7.5 g VSS/L (day 23) to 4.3 g VSS/L (day 54). A possible explanation was the deterioration in the sludge compactness capacity that lead to having the sludge bed close to the level where effluent was discharged. Then, although the biomass continued to show a poor settling capacity, resulting in a slow and constant increase in the effluent VSS concentration, the VSS concentration inside SBR-C was stabilized at 4.4 ± 0.1 g VSS/L (Figure 2). Interestingly, effluent VSS concentration in SBR-D showed the same behaviour than in SBR-C, but it was shifted forward in time (Figure 2.B). Such behaviour may be likely ascribed to the reduced load applied.

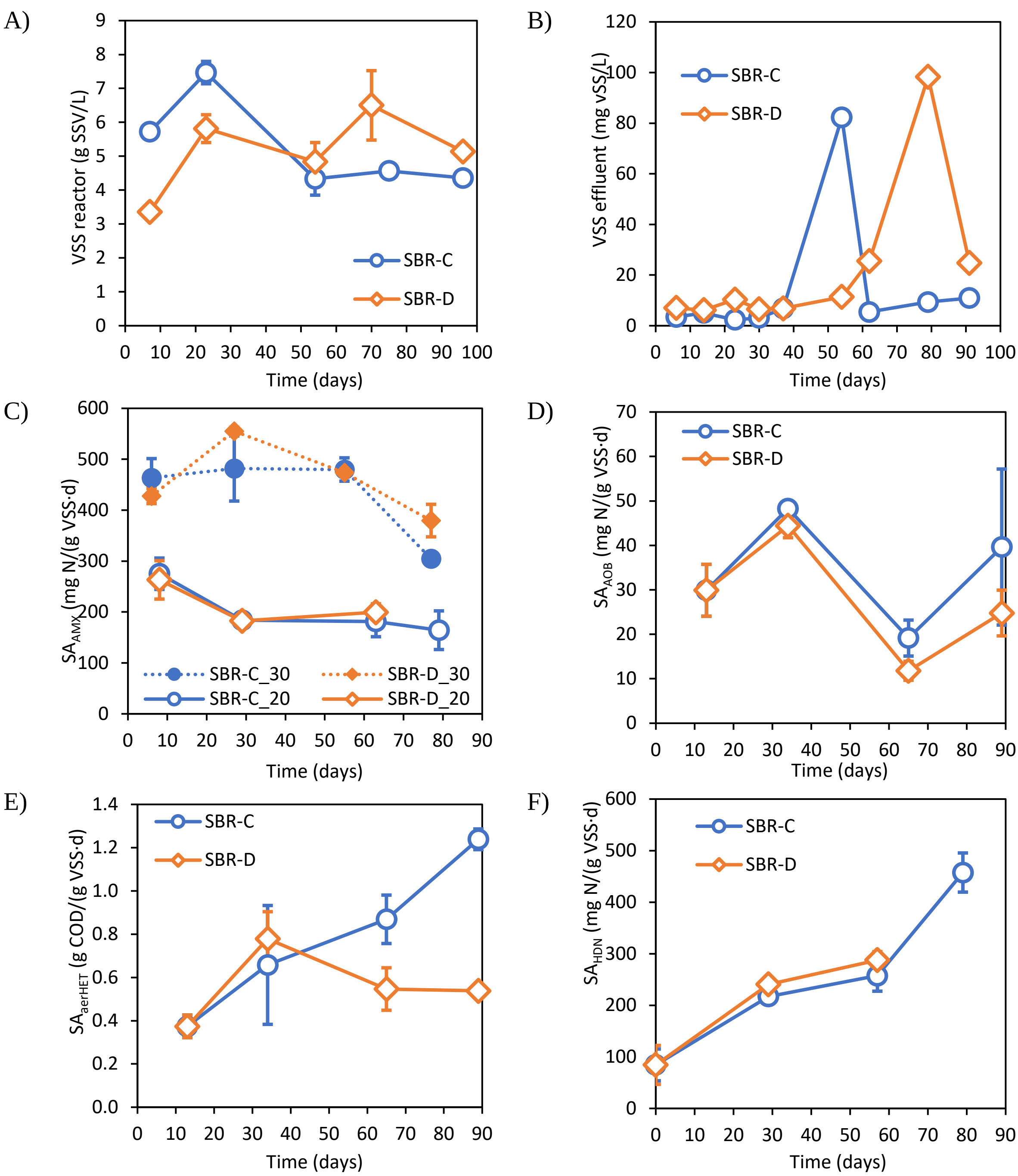

Figure 2. Evolution of the biomass properties for SBR-C (○) and SBR-D (◊). Evolution profile of the volatile suspended solids (VSS) concentration inside the reactors (A) and VSS in the effluent (B). Results of ex-situ maximum specific activity tests: anammox bacteria (C), ammonium oxidizing bacteria (AOB) (D), aerobic heterotrophic bacteria (aerHET) (E) and heterotrophic denitrifying bacteria (HDN) (F) activities. All the activities were determined in triplicate at 20 °C except SA_{AMX} which was also tested at 30 °C.

3.1.3 Specific activities

No significant differences between SBRs were found regarding the SA_{AMX} values (Figure 2.C). During the whole operational period, SA_{AMX} values of 432 ± 86 and 460 ± 74 mg N/(g VSS·d) were obtained for SBR-C and SBR-D at 30 ℃, respectively (p=0.41). The evolution of SA_{AMX} profile over time was different at 30 and 20 ºC. At the end of the experimental period, a decreasing trend was observed for SA_{AMX} at 30 ºC whereas at 20 °C the SA_{AMX} values decreased in the first phase of the experiment, and then they were maintained at approximately 200 mg N/(g VSS·d), for both reactors (p=0.53), along the operational period (Figure 2.C).

The SA_{AOB} values in SBR-D were always slightly lower than in SBR-C, probably due to the repeated oxygen starvation (Figure 2.D), although the difference between the average values was not significant (p=0.19). During the whole experimental period, no SA_{NOB} was detected in batch tests and, in both reactors, nitrate concentration lower than the stoichiometrically expected one for the PN/AMX process was measured in the effluent, confirming this data (Figure 1.C).

In the case of heterotrophic bacteria, both aerobic (SA_{aerHET}) and anoxic (SA_{HDN}) activities increased in SBR-C, whereas the aerobic activity decreased after day 28 in SBR-D (Figure 2.E), being significantly (p=0.01) lower than the one measured in SBR-C. The SA_{HDN} values increased in both reactors with a similar trend.

Despite the decrease of SA_{AOB} or SA_{aerHET} in SBR-D, the theoretical nitrogen and DOC removal capacities determined on their basis, were higher than the corresponding loads applied, therefore reactor performance remained stable.

Finally, on days 80 - 85, the specific microbial activities were also determined on the granular and flocculent biomass fractions (Figure S.1 in Supporting Material). Bacterial segregation was observed being AOB and heterotrophic (both aerobic and anoxic) bacteria more active on flocculent biomass, whereas granules were mainly enriched on anammox bacteria. Significant SA_{AMX} activity was also detected in suspended biomass, probably due to the presence of highly active small anammox granules (< 200 µm) that were difficult to separate from the suspended biomass. It is worth to note that the SA_{AMX} in granular fraction was similar in both reactors, while in the flocculent fraction the SA_{AMX}, SA_{AOB} and SA_{aerHET} were lower in SBR-D than in SBR-C. These results confirmed the lower development of activity in the flocculent biomass in the reactor with repeated starvation/reactivation periods whereas the anammox activity in the granular fraction was maintained.

3.2 Validation of the blackwater treatment in a PN/AMX system under regular starvation and reactivation periods

As the results obtained with the synthetic fed reactors with and without stops showed similar performances, the test with blackwater was performed in a reactor with repeated stops from the start-up, without a control reactor.

3.2.1 Performance of the PN/AMX process

As occurred in the previous operation of SBR-C and SBR-D with synthetic feeding, the control of the aeration flow rate was revealed as the key parameter influencing the PN/AMX process with blackwater (SBR-R). Furthermore, the concentrations of DOC and TN in the digested blackwater were lower than the expected and used previously in the synthetic medium composition making the aeration control even more challenging. During the start-up of SBR-R difficulties on adjusting the aeration flow rate caused highly fluctuating process performance, leading to peaks in effluent nitrite concentration up to 10 mg NO_2^--N/L (Figure 4). Then, air flow rate was maintained at around 1 L/min, resulting in a DO concentration of 0.1 - 0.3 mg O_2/L, and the SBR-R was operated and maintained stable treating anaerobically digested blackwater under regular starvation and reactivation periods (Figure 3), despite temperature fluctuations from 14 to 21 °C (18 ± 3 °C on average) (Table 2). The lower DO concentration needed in SBR-R in comparison with synthetic fed reactors (SBR-C and SBR-D) can be attributed to the lower nitrogen and organic matter concentrations in the feeding.

During Stage I (NLR of 70 ± 6 mg N/(L·d)), the average ammonium removal efficiency was 88 ± 6 % and the NRE was maintained at 79 ± 7 % with effluent TN concentration of 24 ± 7 mg TN/L. The organic matter removal efficiency was approximately 46 % with an effluent concentration of 17 ± 6 mg DOC/L. From day 20 onward, when stable process was achieved, nitrite concentration in the effluent was negligible and low ammonium and nitrate concentrations (close to 10 mg N/L each) were measured (Figure 3). As in the case of SBR-D, the observed nitrate production to ammonium consumption ratio (0.08 ± 0.01 g NO_3^--N/ g NH_4^+-N, days 20-40) was lower than the expected according to the PN/AMX processes stoichiometry. Mass balance calculations indicated that 90 % of the nitrogen removed was due to the anammox process.

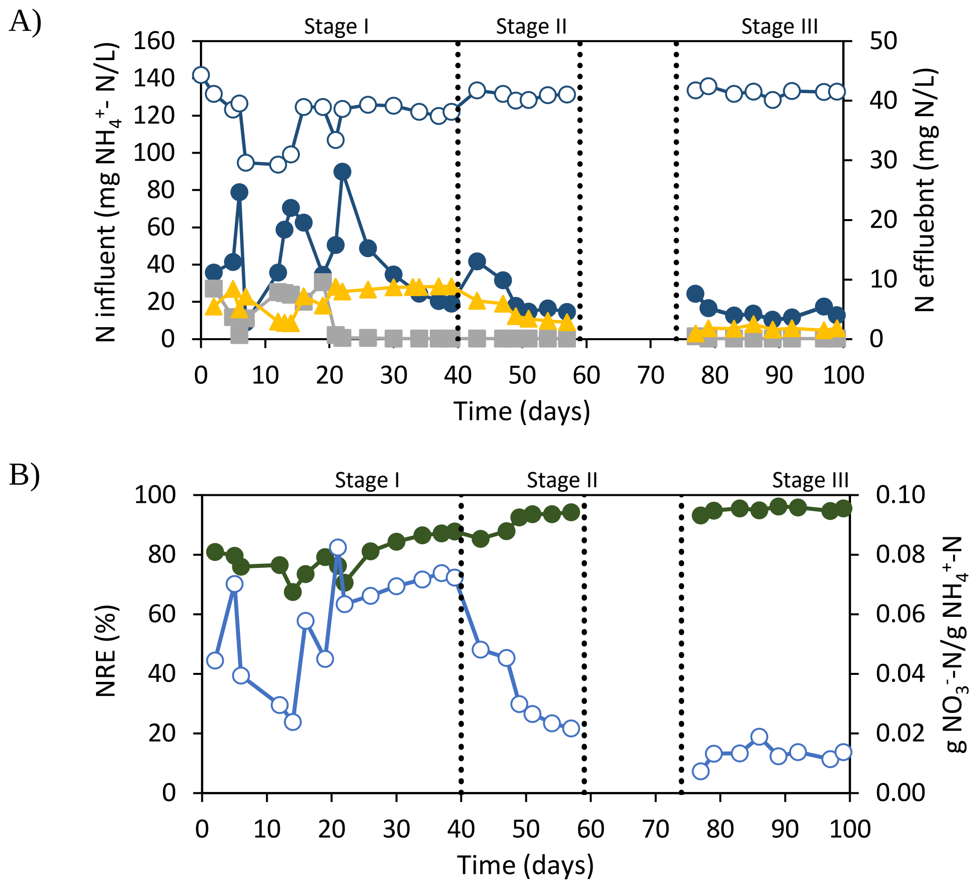

Figure 3. Time profiles in SBR-R treating anaerobically digested blackwater for: A) ammonium (○) in the influent, and effluent nitrogen forms as ammonium (●), nitrite (■) and nitrate (▲) in mg N/L in the effluent and B) nitrogen removal efficiency (NRE) in % (●) and the ratio of nitrate produced to ammonium consumed (○) observed.

Then, with the implementation of the anoxic reaction phase in Stage II, the NRE significantly increased up to 91 ± 4 % ($p<0.05$) due to the occurrence and enhancement of denitrification process. The nitrate production to ammonium consumption ratio decreased from 0.08 ± 0.01 to 0.02 ± 0.01 g NO_3^--N/ g NH_4^+-N ($p=0.03$), and the contribution of denitrification to overall nitrogen removal increased from 5 % to 9 % ($p= 0.015$). At the end of this period, the TN concentration in the effluent was lower than 10 mg TN/L (discharge limit in the EU for sensitive areas).

During Stage III, the reactivation after a long starvation period of 15 days (simulating holiday time) was studied. Once the SBR-R reactor was restarted, the NRE was rapidly recovered and maintained at 95 ± 1 % and the TN concentration in the effluent was 6.5 ± 1.3 mg TN/L, showing the robustness of the system. Thus, the long starvation period did not negatively affect the PN/AMX process performance since the NRE was maintained (if only the last days of Stage II are considered) or slightly increased comparing the complete Stages II and III ($p=0.02$).

3.2.2 Biomass characteristics

During the SBR-R operation, both solids concentration and granular biomass size (i.e. diameter) remained almost constant at 2 g VSS/L and 1 mm (Table 5), respectively. As the COD in the feeding was lower than in SBR-D, the development of aerobic heterotrophic bacteria was limited. A reduction in the flocculent biomass fraction was observed at the beginning of the operation (i.e., from 60 %, day 0, to 45 %, day 30) and then it was maintained at an average value of 42 ± 5 %.

Despite some biomass floatation was observed immediately after the repeated starvation periods, biomass retention was successfully achieved and biomass concentration inside the reactor remained stable. In fact, after only one cycle of operation the biomass settled properly and the VSS concentration in the effluent was 7 - 16 mg VSS/L. The sludge sedimentation capacity was maintained or slightly improved along the operation with a reduction of the SVI_{30} from 70 (inoculum) to 57 mL/g TSS (Stage III). This enhancement was also corroborated by the biomass density (Table 5). Despite SBR-R treated blackwater, in comparison with SBR-D which operated with synthetic feeding, the biomass settleability was better in SBR-R and it did not change significantly along the operational period presumably due to the lower organic matter content.

Table 5. Bacterial specific activities and biomass properties along the operational time of SBR-R.

	Inoculum	**S-I**	**S-II**	**S-III**
Biomass concentration (g VSS/L)	1.91 ± 0.26	1.96 ± 0.15	2.05 ± 0.28	2.09 ± 0.19
SVI_{30} (mL/g TSS)	70	61	60	57
Density (g VSS/$L_{granule}$)	171 ± 2	169 ± 5	175 ± 8	176 ± 3
SA_{AMX} (mg N/(gVSS·d))	210 ± 5	222 ± 8	232 ± 10	230 ± 7
SA_{AOB} (mg N/(gVSS·d))	60 ± 4	71 ± 5	75 ± 6	75 ± 7
SA_{NOB} (mg N/(gVSS·d))	n.d	n.d	n.d	n.d
SA_{aerHET} (mg COD/(gVSS·d))	60 ± 5	55 ± 6	66 ± 4	60 ± 6
SA_{HDN} (mg N/(gVSS·d))	80 ± 8	72 ± 3	81 ± 5	79 ± 8

*n.d: no detected.

3.2.4 Specific activities

Regarding the specific bacterial activities, no significant changes were observed in their respective values throughout the operational stages of SBR-R (Table 5) ($p > 0.45$).

Despite the higher COD/N ratio observed in SBR-R than in SBR-D (0.81 and 0.67 g COD/g TN, respectively), the highest specific bacterial activity measured in SBR-R was the SA_{AMX}, showing a predominant role of this bacteria, whereas in the case of SBR-D the highest potential activity was the SA_{HDN} (in batch tests), doubling the SA_{AMX}. In SBR-R, the maximum heterotrophic bacterial activities were much lower presumably due to the lower OLR applied (56 mg COD/(L·d)), in comparison with SBR-D (110 mg COD/(L·d)). Moreover, the start-up period of SBR-D in continuous mode had favored the heterotrophic bacteria development (as fast-growing microorganisms) by applying even higher loads (320 mg COD/(L·d)).

As SA_{AMX} (210 - 230 mg N/(g VSS·d)) were higher than the ones observed inside the reactor (36 mg N/(g VSS·d)), the NRR of SBR-R might be limited either by the applied NLR or due to the imposed periodic stops. The biomass has the capacity to treat higher NLR. Moreover, specific bacterial activities before and after a weekend stop were measured in order to assess whether the starvation periods affect the bacterial activities or not, and no significant differences were found ($p= 0.82$; data no shown).

4. Discussion

4.1 Effect of starvation/reactivation over the PN/AMX process

Results demonstrated the feasibility of long-term operation of a PN/AMX system under regular starvation and reactivation periods to treat blackwater at room temperature (14 - 21 ºC). To the knowledge of the authors, no previous study investigated all these factors together, as previous literature was focused only on the anammox activity reactivation after storage and/or at higher temperatures. In the present study, both nitritation and anammox activities were re-established immediately after substrate supply was restored as it was also previously reported for anammox enriched biomass (Ye et al., 2018) and for long-term starvation periods in a PN/AMX system at 28 °C (Reeve et al., 2016). A recent investigation of repeated short-term starvation and reactivation cycles was performed by Ye et al. (2018). These authors stated that repeated starvation periods (1 - 4 days) could increase the recovery rate of anammox activity, providing a pathway to enhance the resilience of the starved anammox sludge. They also found that the SA_{AMX} and tolerance of the anammox sludge were enhanced when the same starvation pattern was repeated (Ye et al. (2018). Such results are in good agreement with the findings of the present study, as with repetitive anoxic starvation periods (lasting from 0.5 to 2.5 days) the SA_{AMX} values did not show significantly different behavior compared to the non-starved biomass (Figure 2.C) and any detrimental effect over the process performance was observed during the SBR-R operation (Table 5). These results suggest that anammox biomass might be quickly adapt to regular repeated anoxic starvation periods.

Contrary to the statement of Ye et al. (2018), in the present study, inhibition due to the starvation was not aggravated by prolonging the starvation time as no negative effect was observed after 15 days of stop.

In this study, SBR-D was started-up without repeated starvation/reactivation periods, whereas SBR-R was already started-up under this regime. Despite this fact, in SBR-R high NRE were achieved and lower heterotrophic growth was observed showing that regular stops had no adverse effect on the process performance even when the biomass was not previously adapted to the low operational temperature and blackwater composition.

Regarding other microbial activities, in the present study, only significant differences were found on the SA_{aerHET} which was noticeably lower in the starved reactor (SBR-D) than in the not starved one (SBR-C), likely due to the exposure to prolonged oxygen starvation, more than to the substrate starvation. In the case of the SBR-R, already started-up with starvation periods, the heterotrophic activity was low during the whole operational period. Furthermore, SA_{AOB} in SBR-D was also slightly lower than the one observed in SBR-C. Torá et al. (2011) tested different starvation strategies on an enriched AOB biomass, concluding that fully anaerobic starvation condition was the best alternative to maintain AOB activity, compared to anoxic and aerobic conditions. This might explain the lower effect caused by the oxygen absence over AOB than over the aerobic heterotrophic bacteria.

4.2 Treatment of blackwater with PN/AMX process

Scarce information can be found in the literature about the treatment of blackwater in PN/AMX systems performed in one-stage (Vlaeminck et al., 2009) or two-stages (de Graaff et al., 2011) configurations. These studies were performed in continuous mode, which would be infrequent in a decentralized modular treatment system. Moreover, the blackwater used in the present study (from an office building with regular flushing toilets) was considerably less concentrated than the one treated in previous studies with anammox based processes (de Graaff et al., 2011, Vlaemick et al. 2009) as they used blackwater from a demonstration site with vacuum toilets (de Graaff et al., 2010).

Among them, Vlaeminck et al. (2009) treated concentrated blackwater (1 g N/L) achieving average NRE of 76 %, but at temperatures of 25 °C, higher than in SBR-R. These authors experienced difficulties in managing nitrite oxidizing bacteria (NOB) suppression, and $NaHCO_3$ supply was required to rise the pH and achieve NOB inhibition by free ammonia (FA) (Vlaeminck et al., 2009). At the present research work, satisfactory NOB activity suppression was obtained as confirmed by the negligible SA_{NOB} (Table 5). During the whole SBR-R operational period, the pH fluctuated between 6.5 and 7.4, and both FA and free nitrous acid concentrations were below the NOB inhibition thresholds (Blackburne et al., 2007). Therefore, the low DO concentration during the operational cycles combined with the starvation periods could be the responsible factors for the NOB activity suppression. Ye et al. (2019) found that NOB are much more sensitive to starvation conditions than AOB favoring its suppression. Nevertheless, to confirm the effect of the starvation periods on the NOB suppression and on the PN/AMX process performance, the operation of another reactor in the same conditions than SBR-R but without stops (control) could be of interest.

In the study of de Graaff et al. (2011), a two-stage PN/AMX process was applied to promote the residual organic matter (approximately 400 mg COD/L) oxidation in the partial nitritation unit, avoiding the possible negative effects over anammox bacteria. They reached NRE up to 89 % in the anammox reactor at 35 °C. In the present study, it was demonstrated that the residual organic matter in the blackwater can be removed in the single PN/AMX unit without compromising anammox activity, despite the lower temperature and the repeated starvation/reactivation regime.

The experimental results obtained in this study with SBR-R showed for the first time the feasibility of applying the PN/AMX process (in this case ELAN® technology) to the treatment of anaerobically digested backwater at low temperature (14 - 21 °C) and under a regime of repeated starvation/reactivation periods. With respect to the effluent quality, the procuded effluent contained low COD (≤ 30 mg COD/L), low nitrogen concentration (≤ 10 mg N/L) and low solids concentration (≤ 20 mg VSS/L) accomplishing the discharge limits set on the Urban Wastewater Treatment Directive (91/271/EEC) and the minimum quality requirements for water reuse defined in the European Commission Regulation (TA(2019)0071).

4. Conclusions

Overall, this study demonstrated the technical feasibility of the one-stage PN/AMX process to treat blackwater originated in a decentralized system and operated at room temperatures.

With a synthetic medium simulating blackwater it was proved that the repeated starvation and reactivation periods (nights and weekends) have not adverse effects on the process performance: stable nitrogen removal efficiency (90 %) was achieved with no remarkable difference compared to the process performance from a not starved reactor.

The proof of concept of treating real blackwater (120 mg N/L and 100 mg COD/L) at low temperature (17 ± 2 ºC) in a PN/AMX reactor was also successfully performed, with short (nights and weekends) and long (15 days, holidays) starvation periods, achieving high nitrogen removal efficiencies up to 95 % and total nitrogen concentration in the effluent lower than 10 mg TN/L.

Measurements of the specific activities (anammox, AOB, NOB and heterotrophic aerobic and anoxic) demonstrated that the most affected bacteria were the aerobic heterotrophic one, although their decrease in activity did not compromise the overall removal efficiencies.

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Declarations of interest: None.

References

American Publich Health Association, American Waterworks Association, Water Pollution Cotnrol Federation, 2017. Standard methods for the examination of water and wastewater, 23rd ed. APHA-AWWA-WPCF, Washington.

Beun, J.J., van Loosdrecht, M.C.M., Heijnen, J.J., 2002. Aerobic granulation in a sequencing batch airlift reactor. Water Res. 36, 702-712. doi: 10.1016/S0043-1354(01)00250-0.

Blackburne, R., Vadivelu, V.M., Yuan, Z., Keller, J., 2007. Kinetic characterisation of an enriched Nitrospira culture with comparison to Nitrobacter. Water Res. 41, 3033-3042. doi: 10.1016/j.watres.2007.01.043.

Bower, C.E., Holm-Hansen, T., 1980. A Salicylate–Hypochlorite Method for Determining Ammonia in Seawater. Can. J. Fish. Aquat. Sci. 37, 794-798. doi: 10.1139/f80-106.

Burton, F.L., Tchobanoglous, G., Tsuchihashi, R., Stensel, H.D., Metcalf & Eddy, I., 2014. Wastewater engineering : treatment and resource recovery. 5thed, McGraw-Hill Education, New Yor.

Dapena-Mora, A., Fernández, I., Campos, J.L., Mosquera-Corral, A., Méndez, R., Jetten, M.S.M., 2007. Evaluation of activity and inhibition effects on Anammox process by batch tests based on the nitrogen gas production. Enzyme and Microb. Technol. 40, 859-865. doi: 10.1016/j.enzmictec.2006.06.018.

De Graaff, M.S., Temmink, H., Zeeman, G., Buisman, C.J.N., 2010. Anaerobic Treatment of Concentrated Black Water in a UASB Reactor at a Short HRT. Water 2, 101. doi: 10.3390/w2010101.

de Graaff, M.S., Temmink, H., Zeeman, G., van Loosdrecht, M.C.M., Buisman, C.J.N., 2011. Autotrophic nitrogen removal from black water: Calcium addition as a requirement for settleability. Water Res. 45, 63-74. doi: 10.1016/j.watres.2010.08.010.

European Commision, 1991. Council Directive 91/271/EEC of 21 May 1991 concerning urban waste-water treatment. Official Journal 135/40. Urban Waste Water Treament Directive.

European Commission Report, 2016. Guidelines on Integrating Water Reuse into Water Planning and Management in the context of the WFD. Common implementation strategy for the water Framework Directive and the floods directive.

European Comission, 2019. European Parliament legislative resolution TA/2019/0071 of 12 February 2019 on the proposal for a regulation of the European Parliament and of the Council on minimum requirements for water reuse (COM(2018)0337- C8-0220/2018 - 2018/0169(COD)).

Gallagher, N., Sharvelle, S., 2011. Demonstration of Anaerobic Digestion of Black Water for Methane Capture and Use in an Office Building. Water Practice Technol. 6. doi: 10.2166/wpt.2011.008.

Gao, M., Zhang, L., Florentino, A.P., Liu, Y., 2019. Performance of anaerobic treatment of blackwater collected from different toilet flushing systems: Can we achieve both energy recovery and water conservation? J. Hazard. Mater. 365, 44-52. doi: 10.1016/j.jhazmat.2018.10.055.

Gottardo Morandi, C., Wasielewski, S., Mouarkech, K., Minke, R., Steinmetz, H., 2018. Impact of new sanitation technologies upon conventional wastewater infrastructures. Urban Water J. 15, 526-533. doi: 10.1080/1573062X.2017.1301502.

Hoekstra, M., Geilvoet, S.P., Hendrickx, T.L.G., van Erp Taalman Kip, C.S., Kleerebezem, R., van Loosdrecht, M.C.M., 2019. Towards mainstream anammox: lessons learned from pilot-scale research at WWTP Dokhaven. Environ. Technol., 1721-1733. doi: 10.1080/09593330.2018.1470204.

Knerr, H., Rechenburg, A., Kistemann, T., Schmitt, T.G., 2011. Performance of a MBR for the treatment of blackwater. Water Sci. Technol. 63, 1247-1254. doi: 10.2166/wst.2011.367.

Lansing, S., Maile-Moskowitz, A., Eaton, A., 2017. Waste treatment and energy production from small-scale wastewater digesters. Bioresour. Technol. 245, 801-809. doi: 10.1016/j.biortech.2017.08.215.

Lopez-Fiuza, J., Buys, B., Mosquera-Corral, A., Omil, F., Mendez, R., 2002. Toxic effects exerted on methanogenic, nitrifying and denitrifying bacteria by chemicals used in a milk analysis laboratory. Enzyme Microb. Technol. 31, 976-985. doi: 10.1016/S0141-0229(02)00210-7.

Malila, R., Lehtoranta, S., Viskari, E.L., 2019. The role of source separation in nutrient recovery – Comparison of alternative wastewater treatment systems. J. Cleaner Production 219, 350-358. doi: 10.1016/j.jclepro.2019.02.024.

Moges, M.E., Todt, D., Janka, E., Heistad, A., Bakke, R., 2018. Sludge blanket anaerobic baffled reactor for source-separated blackwater treatment. Water Sci. Technol. 78, 1249-1259. doi: 10.2166/wst.2018.411.

Morales, N., Pedrouso, A., Val del Río, A., Mosquera-Corral, A., Campos, J.L., Vázquez-Padín, J.R., Rogalla, F., 2018. Start-up of the first full scale reactor based on the granular ELAN® technology to remove nitrogen from the co-digester supernatant, Granular Sludge Conference, Delft, The Netherlands.

Murat Hocaoglu, S., Insel, G., Ubay Cokgor, E., Baban, A., Orhon, D., 2010. COD fractionation and biodegradation kinetics of segregated domestic wastewater: black and grey water fractions. J. Chem. Technol. Biotechnol. 85, 1241-1249. doi: 10.1002/jctb.2423.

Oarga-Mulec, A., Jenssen, P.D., Krivograd Klemenčič, A., Uršič, M., Griessler Bulc, T., 2017. Zero-discharge solution for blackwater treatment at remote tourist facilities. J. Clean. Prod. 166, 798-805. doi: 10.1016/j.jclepro.2017.08.002.

Palmquist, H., Hanæus, J., 2005. Hazardous substances in separately collected grey- and blackwater from ordinary Swedish households. Sci. Total Environ. 348, 151-163. doi: 10.1016/j.scitotenv.2004.12.052.

Pedrouso, A., Aiartza, I., Morales, N., Vázquez-Padín, J.R., Rogalla, F., Campos, J.L., Mosquera-Corral, A., Val del Rio, A., 2018. Pilot-scale ELAN® process applied to treat primary settled urban wastewater at low temperature via partial nitritation-anammox processes. Sep. Purif. Technol. 200, 94-101. doi: 10.1016/j.seppur.2018.02.017.

Reeve, P.J., Mouilleron, I., Chuang, H.-P., Thwaites, B., Hyde, K., Dinesh, N., Krampe, J., Lin, T.-F., van den Akker, B., 2016. Effect of feed starvation on side-stream anammox activity and key microbial populations. J. Environ. Manage. 171, 121-127. doi: 10.1016/j.jenvman.2016.01.035.

Ren, X., Chen, H., Cheng, Y., Wu, L., Jiang, H., 2018. Full-scale practice of domestic wastewater source separation and collection in a semicentralized treatment system: a case study. Water Sci. Technol. 78, 2193-2203. doi: 10.2166/wst.2018.505.

Sharma, M.K., Tyagi, V.K., Saini, G., Kazmi, A.A., 2016. On-site treatment of source separated domestic wastewater employing anaerobic package system. J. Environ. Chem. Eng. 4, 1209-1216. doi: 10.1016/j.jece.2016.01.024.

Todt, D., Heistad, A., Jenssen, P.D., 2015. Load and distribution of organic matter and nutrients in a separated household wastewater stream. Environ. Technol. 36, 1584-1593. doi: 10.1080/09593330.2014.997300.

Torà, J.A., Lafuente, J., Baeza, J.A., Carrera, J., 2011. Long-term starvation and subsequent reactivation of a high-rate partial nitrification activated sludge pilot plant. Bioresour. Technol. 102, 9870-9875. doi: 10.1016/j.biortech.2011.08.008.

Val del Rio, A., Campos, J.L., Da Silva, C., Pedrouso, A., Mosquera-Corral, A., 2019. Determination of the intrinsic kinetic parameters of ammonia-oxidizing and nitrite-oxidizing bacteria in granular and flocculent sludge. Sep. Purif. Technol. 213, 571-577. doi: 10.1016/j.seppur.2018.12.048.

Vlaeminck, S.E., Terada, A., Smets, B.F., Linden, D.V.d., Boon, N., Verstraete, W., Carballa, M., 2009. Nitrogen Removal from Digested Black Water by One-Stage Partial Nitritation and Anammox. Environ. Sci. Technol. 43, 5035-5041. doi: 10.1021/es803284y.

Wang, Q., Song, K., Hao, X., Wei, J., Pijuan, M., van Loosdrecht, M.C.M., Zhao, H., 2018. Evaluating death and activity decay of Anammox bacteria during anaerobic and aerobic starvation. Chemosphere 201, 25-31. doi: 10.1016/j.chemosphere.2018.02.171.

World Water Assesment Programme (W.W.A.P.). The United Nations World Water Development Report 2017: Wastewater, The Untapped Resource. UNESCO, Paris, 2017.

Ye, L., Li, D., Zhang, J., Zeng, H., 2018. Resuscitation of starved anaerobic ammonium oxidation sludge system: Impacts of repeated short-term starvation. Bioresour. Technol. 263, 458-466. doi: 10.1016/j.biortech.2018.12.115.

Ye, L., Li, D., Zhang, J., Zeng, H., 2019. Start-up and performance of partial nitritation process using short-term starvation. Bioresour. Technol. 276, 190-198. doi: 10.1016/j.biortech.2018.04.126.

Zeeman, G., Kujawa, K., de Mes, T., Hernandez, L., de Graaff, M., Abu-Ghunmi, L., Mels, A., Meulman, B., Temmink, H., Buisman, C., van Lier, J., Lettinga, G., 2008. Anaerobic treatment as a core technology for energy, nutrients and water recovery from source-separated domestic waste(water). Water Sci. Technol. 57, 1207-1212. doi: 10.2166/wst.2008.101.

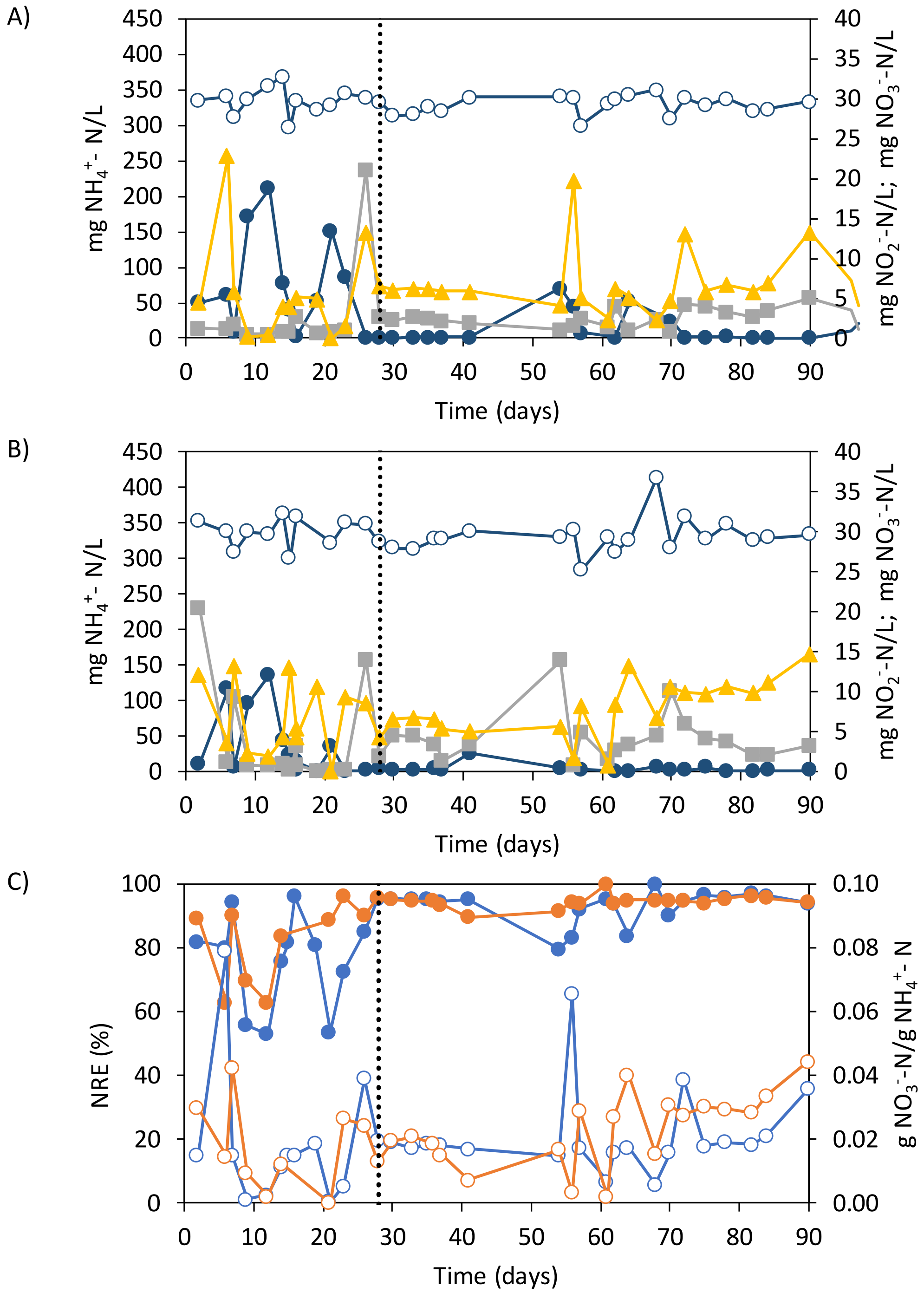

Figure 1. Time profiles of ammonium in the influent (○) and effluent (●), and effluent nitrite (■) and nitrate (▲) concentrations in SBR-C (A) and SBR-D (B) whereas C) shows the nitrogen removal efficiency (NRE) (filled dot) and the nitrate produced to ammonium consumed ratio (empty dot) for SBR-C (● and ○) and SBR-D (● and ○). Black dashed line corresponds to day 28 when the start-up of both reactors was completed.

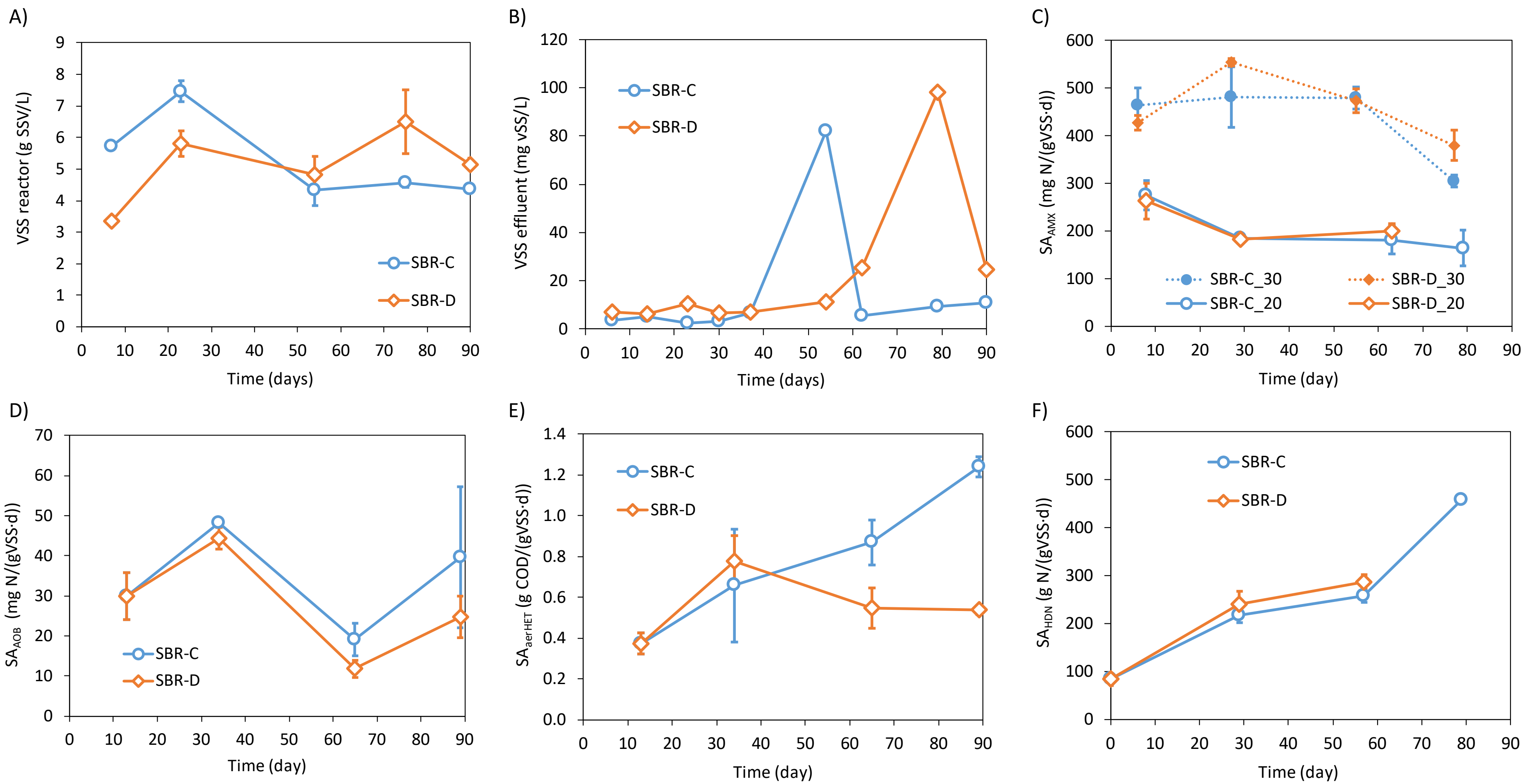

Figure 2. Evolution of the biomass properties for SBR-C (○) and SBR-D (◊). Evolution profile of the volatile suspended solids (VSS) concentration inside the reactors (A) and VSS in the effluent (B). Results of ex-situ maximum specific activity (SA) tests: anammox bacteria (AMX, C), ammonium oxidizing bacteria (AOB, D), aerobic heterotrophic bacteria (aerHET, E) and heterotrophic denitrifying bacteria (HDN, F) activities. SA were determined in triplicate at 20 °C except SA_{AMX} which was also tested at 30 °C.

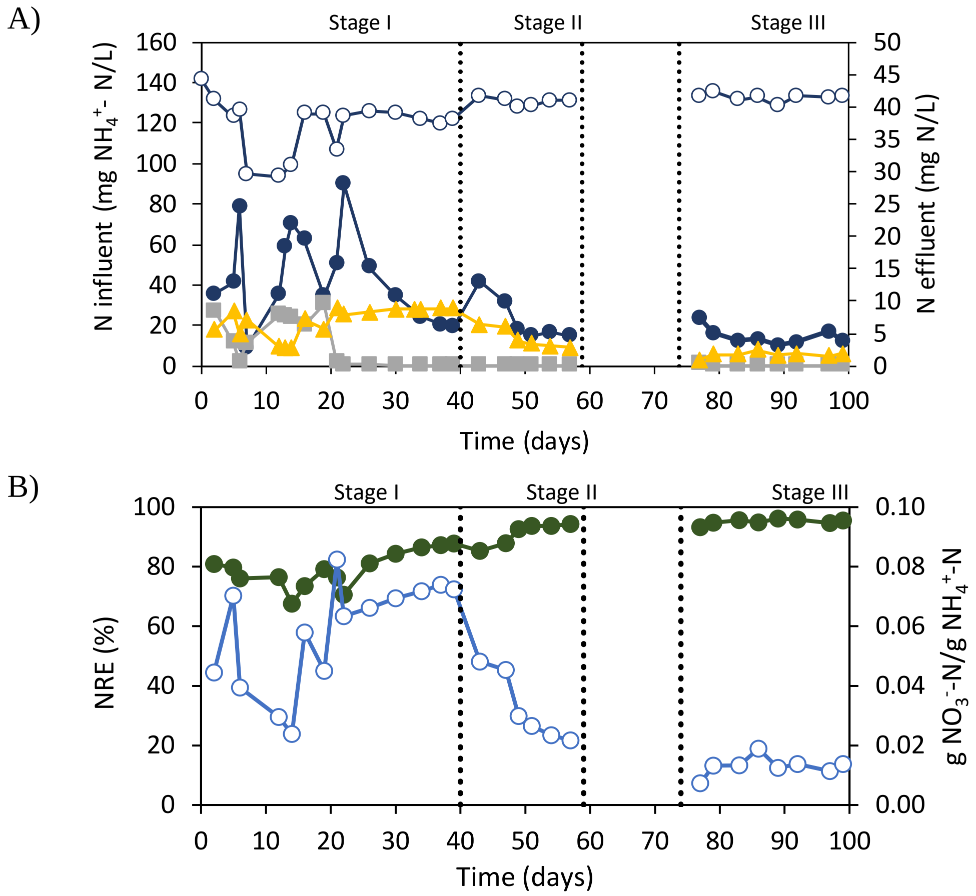

Figure 3. Time profiles in SBR-R treating anaerobically digested blackwater for: A) ammonium (○) in the influent, and effluent nitrogen forms as ammonium (●), nitrite (■) and nitrate (▲) in mg N/L in the effluent and B) nitrogen removal efficiency (NRE) in % (●) and the ratio of nitrate produced to ammonium consumed (○) observed.