Title: Efficient and automated start-up of a pilot reactor for nitritation of reject water: from batch granulation to high rate continuous operation

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Abstract

An automated sequencing batch reactor operation based on online measurement of the ammonium concentration was investigated as a tool for improving the start-up of a nitrifying granular airlift reactor. The effectiveness of this start-up procedure was verified with the characteristics of the developed granular sludge but also the improvement of the start-up was confirmed when comparing with the results achieved with two continuous-mode start-up strategies. Once a stable granular biomass was obtained, the reactor was started to operate in continuous mode during more than 100 days, maintaining the characteristics of the granular biomass and achieving a nitrogen loading rate of 1.75 g N L^{-1} d^{-1}. The intermittent recirculation of small flocs of nitrifying biomass was explored as an alternative to increase the biomass concentration in the reactor and consequently, to increase the treated loading rate.

Keywords: airlift reactor; biofilm; granular sludge; online TAN measurement; partial nitrification.

1 Introduction

Aerobic granular sludge was developed as a way of obtaining high concentration of active biomass together with good settling properties of the sludge, avoiding in turn, the costly supporting material required for biofilm development [1,2]. One of the key applications of aerobic granular reactors was the achievement of COD, nitrogen and phosphorous removal in the same reactor [3]. Aerobic granules are generally developed from activated sludge in sequencing batch reactors [1-4]. The feast-famine regime, the high shear stress, as well as the short settling time applied are accepted as the main driving forces for an effective granulation [2,4-6]. Studies reported the straightforward development of aerobic granulation for the treatment of high-strength organic wastewaters with relatively high concentration of COD [7], whereas the granulation process of activated sludge was recognized to be more difficult when low COD concentration is present in the wastewater although still feasible [3,8].

Once the granular sludge is formed, its stability is thought to be maintained due to the high shear stress and short settling times steadily applied in the granular reactors during the long-term operation. One of the identified challenges of this type of systems is the eventual destabilization of the granules, which is usually an irreversible process [9,10], eventually requiring a new start-up phase. Once the selection pressure applied with the shear stress and the short settling times is relaxed, heterotrophic bacteria could trigger the development of non-compact biomass which leads to a reduction of the settling velocity, which in turn produce the eventual washout of the biomass. Nevertheless, if the granules are colonized by slow-growing bacteria, like for instance nitrifying bacteria, the possibilities of destabilization of the granular sludge are rather reduced and stable operation is usually reported [11-13]. Therefore the operation mode with such granular reactors might be switched to continuous, just after convenient granule formation under sequencing batch mode operation.

Although nitrifying granules were obtained even before the development of aerobic granulation as new technology itself [14], there are only a reduced number of studies focused on the start-up of granular reactors for nitritation of high strength ammonium wastewaters [11,15,16]. During the development of a robust ratio control strategy to achieve and maintain nitritation in granular sludge reactors [12], the start-up was identified as a possible drawback for the scaling-up of the process [15]. Alternatives for the start-up of aerobic granular reactors have been described, as for instance the induction of granulation with small particles (activated carbon [12]), or the use of crushed granules that will serve as nuclei for the later development of the aerobic granules (e.g. anaerobic granules [11]).

A specific procedure for an efficient start-up of a granular reactor for stable full nitritation operating in continuous mode will be presented in this contribution. An airlift reactor (150 L of capacity) was designed to allow sequencing batch and continuous modes of operation. The reactor was placed on site in a municipal wastewater treatment plant. The reactor treated the side water produced by a set of centrifuges after the anaerobic digestion of sludge for the production of biogas (i.e. reject water).

2 Materials and methods

2.1 Reactor description, location, wastewater and inoculum

A schematic diagram of the granular airlift reactor used in this study is presented in Figure 1. The reactor capacity was 150 L. Height to diameter ratio is ca. H/D = 8.4. The temperature of the reactor was kept at 30ºC using an electric heating system. The pH was maintained at 7.5 in the reactor bulk liquid through the addition of solid Na_2CO_3. The dissolved oxygen (DO) concentration was measured by means of an online DO probe (LDO luminescence sensor, Hach-Lange, Düsseldorf, Germany).The total ammonia nitrogen (TAN = $N\text{-}NH_4^+$ + $N\text{-}NH_3$) concentration in the bulk liquid was determined with an online probe (NH4Dsc Ammonium sensor with a Cartrical cartridge, Hach Lange, Düsseldorf, Germany).

The reactor was installed in a municipal WWTP in NE Spain with anaerobic digestion, to be fed in situ with the reject water. Dewatering of digested sludge is performed by a set of centrifuges which operate discontinuously and usually active during the night to use reduced fees for the electricity. The reject water was stored into two tanks of 1 m^3 at room temperature and they were connected alternatively to the reactor inflow pump. Composition of reject water was rather variable with the following concentrations: TAN: 440 – 758 mg N L^{-1}, TOC: 240 – 696 mg C L^{-1}, TIC: 358 – 723 mg C L^{-1}, total nitrite nitrogen (TNN=$N\text{-}NO_2^-$ + HNO_2): 2 – 7 mg N L^{-1}, $N\text{-}NO_3^-$: 0 mg N L^{-1}, Total suspended solids (TSS): 122 – 239 mg L^{-1}, Volatile suspended solids (VSS): 100 – 206 mg L^{-1}; pH: 8.1 – 8.8. The wide range for TAN and TOC concentrations is due to several intensities applied by the ultrasounds treatment of the sludge prior the anaerobic digestion. During the whole period of operation different types of polyelectrolyte at different concentrations were tested by the WWTP operator to improve the efficiency of the liquid-solid separation in the centrifuges used for the dewatering of the digested sludge. The polyelectrolyte used was cationic polyacrylamide (in solution at 0.4%), commercialized as CH82 by Chemipol; to a minor extent also Actipol C-444K (Brenntag) was utilized during the course of the experiments.

The pilot plant was inoculated with activated sludge from the municipal WWTP operated with a modified Ludzack-Ettinger configuration. The inoculum had a VSS/TSS ratio of 0.70 with a mean particle diameter of 0.1 mm and it was composed by

97±2% of heterotrophic biomass, 2±0.5% of ammonia –oxidizing bacteria (AOB) and <1% of nitrite-oxidizing bacteria (NOB).

2.2 Experimental procedure and type of operation

During the start up, the reactor was operated as a SBR to develop a granular sludge. The reactor capacity during SBR operation was reduced to 90 L. Each cycle was divided into a filling phase of 35 min, an aerobic phase (with variable time length), a settling phase of 30 minutes and a draw phase of 2.5 min. The exchange volume in each cycle was 45 L (50% of the total volume). The on-line TAN measurement was used to automatically manipulate the cycle duration. The aerobic phase was finished when the TAN concentration in the bulk liquid decreased to 50 mg N L^{-1} (denoted as TANtriggering). This type of operation was based on the previously developed ratio control strategy, which established that the DO/TAN concentration ratio was governing the nitrite build up in a biofilm reactor [12]. When adapting the strategy for sequencing batch operation aiming to produce granular sludge, a relatively high airflow was applied to assure high shear stress, i.e., superficial air velocity higher than 1.2 cm s^{-1} corresponding to a DO was relatively high: 6-7 mgO_2 L^{-1}. Due to high TAN concentrations in the bulk, DO/TAN concentrations ratio was maintained at very low values during the whole aerobic phase. In that way, strong oxygen limiting conditions were assured along the total length of the cycle to provide the conditions to outcompete NOB just after inoculation of the reactor and along the period of granular sludge development.

Once the granules were developed, the reactor operation mode was switched to continuous. The control strategy applied was very similar to that used to achieve and maintain stable partial nitrification in the biofilm airlift reactors already described in the literature [12]. A low DO/TAN concentration ratio was imposed, manipulating mainly the inflow rate fed to the reactor and the air flow-rate. To show the performance of the feedback control loop applied to maintain the TAN concentration close to the setpoint, both the measured and manipulated variable were plotted (see Figure 2). During the continuous operation mode a TAN concentration setpoint was fixed equal to TANtriggering, i.e. TAN_{sp} = 50 mg N L^{-1}. During the whole period of the continuous mode of operation, the total working volume of the reactor was 150 L.

2.3 Analytical methods

For the off-line measurement of TAN a continuous flow analyzer based on potentiometric determination of ammonia was used. TNN and nitrate were measured with ionic chromatography using a DIONEX ICS-2000 Integrated Reagent-Free IC System with an auto-sampler AS40. VSS, TSS and sludge volumetric index (SVI) were determined according to standard methods [17]. A Malvern Mastersizer 2000 instrument was used to measure the mean granule size (as volume weighted average) and size distribution. The percent of granular sludge was determined as the volumetric fraction of particles with a diameter higher than 0.2 mm. The settling velocity was determined by placing individual granules in a column containing water and measuring the time spent to drop a height of 40 cm.

Fluorescence in situ hybridization (FISH) technique coupled with confocal microscopy was used to investigate the nitrifying population dynamics. A Leica TCS SP2 AOBS confocal laser scanning microscope at a magnification of x63 (objective HCX PL APO ibd.B1 63x1.4 oil) equipped with two HeNe lasers with light emission at 561 and 633 nm was used for biomass quantification. Hybridizations were carried out using at the same time a Cy3-labeled specific probe and Cy5-labeled EUBmix probe (general probe). Specific probe used for AOB detection was Nso190 [18] while for NOB detection was NIT3 [19]. EUBmix probe consisted of the mix of probes EUB338, EUB338 II and EUB338 III [20,21]. Detailed information about FISH quantification can be found in Jubany et al. [22].

3 Results and discussion

3.1 Automated SBR operation: simultaneous development of granular sludge and achievement of stable nitritation

The automated SBR operation applied resulted in a relatively fast development of granular biomass in the reactor (see Figure 3). An initial washout of biomass followed by a slow augment of biomass concentration led, in only few weeks, to a considerable increase of the floc diameter (from 0.1 to 0.35 mm in only 14 days, see Figure 3). Time course size distribution of sludge particles was monitored, and main results were plotted in Figure 4.

At day 64 of operation, the sludge volumetric index (5 minutes) was SVI_5 = 42 mL g^{-1} and the ratio SVI_5/SVI_{30}=1.04. Settling velocity was 43±17 m h^{-1} (day 64). These values demonstrate the achievement of granular sludge in the reactor.

It is a common practice in the dewatering of the sludge to add polyelectrolyte to help flocculation of small particles just before the centrifugation, to improve the efficiency of the dewatering of sludge. A fraction of the polyelectrolyte is always dissolved in the supernatant, being therefore present in the reject water. It is unknown if the presence of the polyelectrolyte added to the digested sludge to improve the centrifugation efficiency, affected the granulation process. Previous research reported that the presence of coagulant-flocculant reagents involved negative effects on the formation process and the physical properties of the aerobic granules [23]. In our study, despite the presence of polyelectrolyte the granule formation progressed acceptably as shown by (i) sludge volumetric indexes and settling velocity (just discussed above), (ii) in terms of size and biomass retention capacity (Figure 3A).

From the beginning of the operation of the reactor, oxidation of ammonium produced mainly nitrite (i.e. full nitritation). Only a weak and short transient state of less than 17 days of operation led to an effluent with only 2 mg N-NO_3^- L^{-1} (see Figure 3). These results showed how the development of NOB in the biofilm is prevented by imposing strong oxygen limiting conditions, due to the great excess of ammonia along the whole cycle, in agreement with previous reports [12], for a deeper discussion on the causes see Pérez et al. [24]; Brockmann and Morgenroth [25] and Bartrolí et al. [12]. During the batch start-up for granule formation, the DO/TAN concentration ratio varied between 0.02 and 0.12 mg O_2 mg^{-1} TAN at the beginning and end of the cycle, respectively. These values are well below 0.25 mg O_2 mg^{-1} TAN, which was the minimal required value found by Bartrolí et al. [12] to assure nitritation in granular reactors operating in continuous mode. It is important to stress that in spite of maintaining the DO concentration in the bulk liquid at high values (ca. 6-7 mg O_2 L^{-1}), oxygen limiting conditions were assured in the reactor because a high excess of ammonium in the bulk liquid is imposed. In those conditions, NOB are outcompeted in the granular sludge due to their lower oxygen affinity (see Pérez et al. [24] for a deeper discussion). Nitrite oxidation is suppressed throughout the whole reaction period in the sequencing batch start-up as demonstrated by the very low values of nitrate concentration measured during this period of operation (0-85 days in Figure 3). This control strategy is feasible due to the on-line measurement of ammonium concentration in the bulk liquid, confirming the convenience of this measurement for the high performance of nitritation in this type of reactors, as recently highlighted in the literature [26]. Quantification of NOB fraction in the sludge at day 85 yielded a percentage <1%(0.8±0.3%), whereas the AOB fraction was 53±9%, demonstrating the efficiency of the control strategy to prevent NOB development in the sludge.

In general, the potential difficulties when switching the reactor operation from batch to continuous mode are related to the stability of the granular sludge [27]. Granulation of conventional activated sludge in SBR is linked to the selection pressure applied when operating at short settling time as already discussed in the introduction. Nevertheless, in the case of autotrophic granular sludge (like for instance, nitrifying granules) the formed granules are very stable [12,15,26-29]. The main characteristics of the granular sludge obtained with the batch start-up (as presented in Table 1) remained rather steady, as expected, once the continuous mode of operation was imposed. Even more, after 100 days of continuous operation, the SVI5 was 36 mL g^{-1} and SVI5/SVI30≅1, granule size was 0.4 mm (see Figures 3 and 4) and settling velocity was 60±20 m h^{-1}. These are typical granular sludge characteristics, demonstrating the feasibility of the operation of granular reactors in continuous mode to perform nitritation, as previously shown with other start-up procedures (Table 1). Additionally, the granular sludge characteristics are also comparable to those reported for autotrophic granular sludge in SBR reactors performing nitrification [28] and nitritation [29], as detailed for a direct comparison in Table 1 (last two rows). The low values of settling velocity determined by Shi et al. [28,29] may indicate poor granular sludge characteristics, since also the SVI_5/SVI_{30} is not reported in their studies. Note how settling velocities for granular sludge should be inside the range (25-70) m h^{-1}, significantly higher than that of flocs (7-10 m h^{-1}) [27]. Overall, it is therefore clear that the switching from batch to continuous is not altering the good granular sludge properties achieved with sequencing batch start-up. For a further discussion on the advantages of continuous operation, see below the last section. The start-up procedure presented in this study (SBR-type start-up) can be directly compared with two different start-up procedures carried out in a similar airlift reactor [15]. Those start-ups were carried out in continuous mode operation, inducing the granules formation through the addition of activated carbon (AC) particles. The only difference among them was the use of bioaugmentation with an AOB population in one of them. The main results achieved in the three different start-ups are summarized in Table 1 (second and third rows). A nitrifying granular sludge was achieved at the end of each of the start-up procedures and the main characteristics of these granular biomasses were similar in terms of SVI_5, settling velocity and granule size (Table 1). Additionally, the total nitrifying granular biomass concentration was similar in all the procedures. The main difference among them was the period required for the start-up. This period was clearly shorter with the SBR-type start-up (Table 1). Another significant difference in favor of the procedure presented in this study is the saving of AC particles and their withdrawal once the granules are formed which represents an unquestionable practical advantage.

Finally, the NOB fraction presented in the granular sludge at the end of the SBR-type start-up was smaller than the achieved with the continuous-type start-ups (Table 1). This fact also represents a practical advantage because facilitates the attainment of a stable partial nitrification process.

3.2 Switching to continuous mode of operation

Once the stable development of aerobic granular sludge was confirmed by both the granule size (0.49 mm, at day 85) and sludge volumetric indexes (SVI_5=41.1 mL g^{-1}, SVI_5/SVI_{30}=1.00, at day 85), the reactor was started to operate in continuous mode (at day 87). As a consequence of the automatic control of the inflow rate based on the online TAN measurement, the nitrogen loading rate (NLR) increased at a stable value of ca. 0.85 g N L^{-1} d^{-1}, with a biomass concentration of ca. 2.0 g VSS L^{-1}. Mean granule size increased in the continuous mode of operation until ca. 0.6 mm.

The NLR achieved together with the measured sludge characteristics demonstrate how the continuous mode of operation does not result in a decrease of the stability of the granules, showing that the dual type of operation is suitable for the nitritation of reject water with granular reactors. For particular advantages of the continuous operation mode see below the last section.

3.3 Intermittent sludge recirculation as a strategy to increase the NLR

Since the NLR was rather steady at 0.85 g N L^{-1} d^{-1}, two recirculation events were used to enhance the increase of granular sludge concentration. Sludge washed out from the reactor has a very small mean floc diameter, measured as ca. 0.1 mm, due to the design of the G-L-S separator with a ratio D_{top} / $D_{downcomer}$ = 2.2 (see Figure 6). Consequently, a very low amount of solids was always measured in the effluent (see Figure 3). The sludge in the effluent was settled in a 20 L settler with an area of only ca. 500 cm^2 and the sludge was recirculated to the reactor twice, in days 146 and 190.

The temporary increase of small flocs due to the sludge recirculation event resulted in an increase of the NLR applied by the control strategy (see Figure 3). This temporary increase of small flocs produced a subsequent increase in the total biomass concentration with a slight decrease in the fraction of granular biomass in the reactor (see Figure 3). The size distribution of the sludge after the recirculation events (day 189 in Figure 4) also confirmed that temporary increase of small flocs consolidated an increase in the granular biomass concentration (see Figures 3 and 4). Consequently, the recirculation events resulted in the achievement of a stable NLR of ca. 1.75 g N L^{-1} d^{-1} and a biomass concentration in the reactor of ca. 6 g VSS L^{-1}.

Quantification of NOB fraction in the sludge at day 188 yielded a percentage <1% (0.3±0.08%), whereas the AOB fraction was 69±9%, demonstrating the efficiency of the control strategy in the continuous operation mode to prevent NOB development in the sludge, even when sludge recirculation events were carried out.

3.4 Why continuous operation may be the preferred choice?

Nitritation has been obtained in continuous mode [11,12,30-34] or batch process [32,35-37]. In general, continuous operation is usually the preferred choice when the reactor has to treat large flow-rates. In the treatment of side streams in WWTP with anaerobic digestion, flow-rate is not too large, and therefore this is not seen as a decisive reason determining the best operational mode. Further, other considerations need to be taken into account. The continuous operation yields steady conditions in terms of conversion. Continuous operation of reactor is advantageous over batch or sequencing mode for efficient full-scale operation [27]. The reduced variation of the main process conditions, such as pH, nitrogen compounds concentrations and DO concentration can be very valuable to decrease the undesired side reactions, like those producing N_2O emissions. Changes in process conditions of those variables are known to cause strong increase of N_2O production [38], and therefore should be avoided when possible.

Moreover, stable nitritation assuring outcompetition of NOB has been identified as the key challenge to apply Anammox to reject water [39], but also to main stream [40]. The development of an efficient continuous nitritation process might contribute to define the best strategy for the application of Anammox to main stream at low temperatures, which would lead to energy sustainable WWTP [41].

4 Conclusions

The applied automated SBR-type strategy for starting-up nitritation systems treating reject water is an attractive alternative for full scale installations, because (i) a short period of operation was required if compared with continuous-type start-up strategies based on AC addition and (ii) the absence of nitrate in the effluent was assured by the control strategy.

The automatic control of the inflow rate based on ammonium concentration measurement allows easily switching from batch start-up operation to continuous mode operation after the stable development of aerobic granular sludge.

The intermittent recirculation of small nitrifying flocs to the airlift reactor results in a significant increase of the granular biomass concentration and consequently, an increase of the treated nitrogen loading rate.

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Figure Captions

Figure 1. Diagram of the granular airlift reactor. (1) TAN probe; (2) DO probe; (3) pH probe; (4) Temperature sensor; (5) Valve for airflow regulation; (6) Na_2CO_3 dispenser; (7) Electric heating system.

Figure 2. Time course inflow rate during the continuous mode of operation. Inflow rate is the manipulated variable of the closed-loop control of TAN concentration. Note how a dead band (± 5 mg O_2 L^{-1}) is used around the TAN concentration setpoint (TAN_{SP}).

Figure 3. **A**: Time course solids concentration, granule size; **B**: time course fraction of granules in the sludge and DO/TAN concentration ratio; **C**: time course nitrogen compounds concentration and nitrogen loading rate (NLR).

Figure 4. Size distribution of the sludge along the whole period of operation. **A**: SBR-type operation; **B**: continuous-type operation.

Figure 5. Figure 5. Granular sludge aspect before (A: day 85) and after (B: day 189) switching from batch start-up to continuous operation.

Figure 6. Reactor used in the experiments. Note the difference in diameter between the top section (G-L-S separation) and the mean diameter of the downcomer (D_{top} / $D_{downcomer}$ = 2.2).

Table 1. Comparing the length of start-up and main features of the sludge obtained with previous studies. *The settling time used for SVI determination is not reported.

Period required for start-up (d)	**SVI_5 (mL g^{-1})**	**Settling velocity (m h^{-1})**	**Granule size (mm)**	**Biomass concentration (g VSS L^{-1})**	**NOB fraction (%)**	**Ref.**
85	41	43	0.5	1.2	<1	This study
125	35	56	0.8	1	3	Bioaugmented reactor [15]
200	40	30	1.0	1.9	7	Non-bioaugmented reactor [15]
120	36*	3	0.3	2.3	15	Nitrifying granules, [28]
90	28*	3	0.5	7.5	<1	Nitritation in granular SBR, [29]

Figure(s)

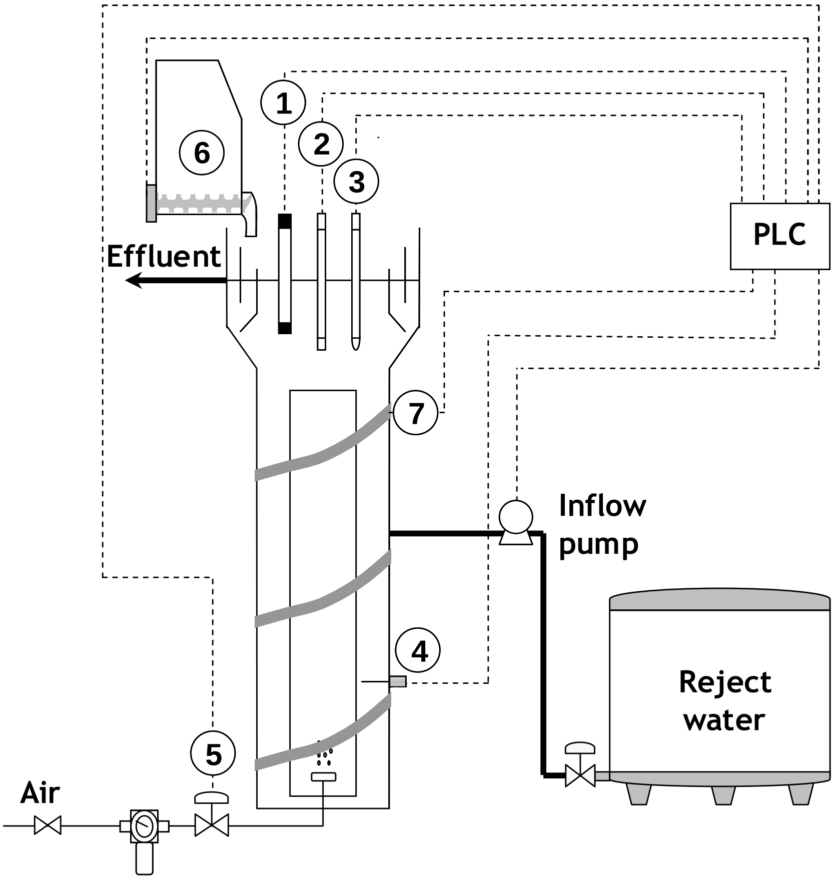


Figure 1.

Figure(s)

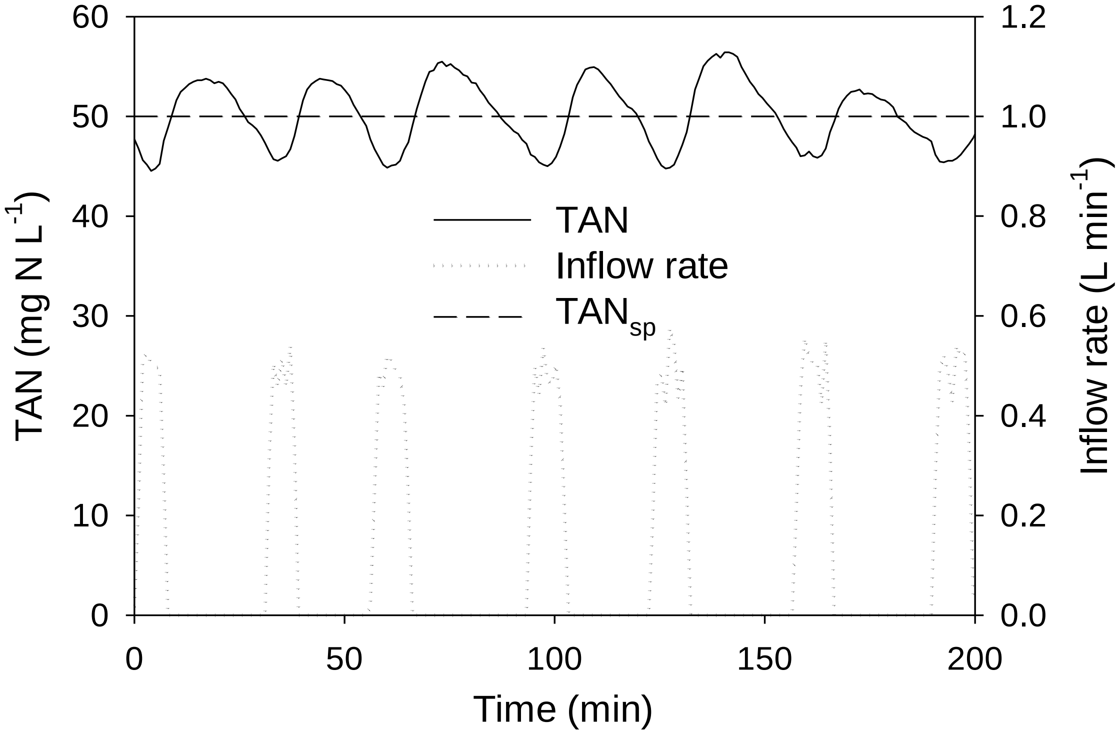
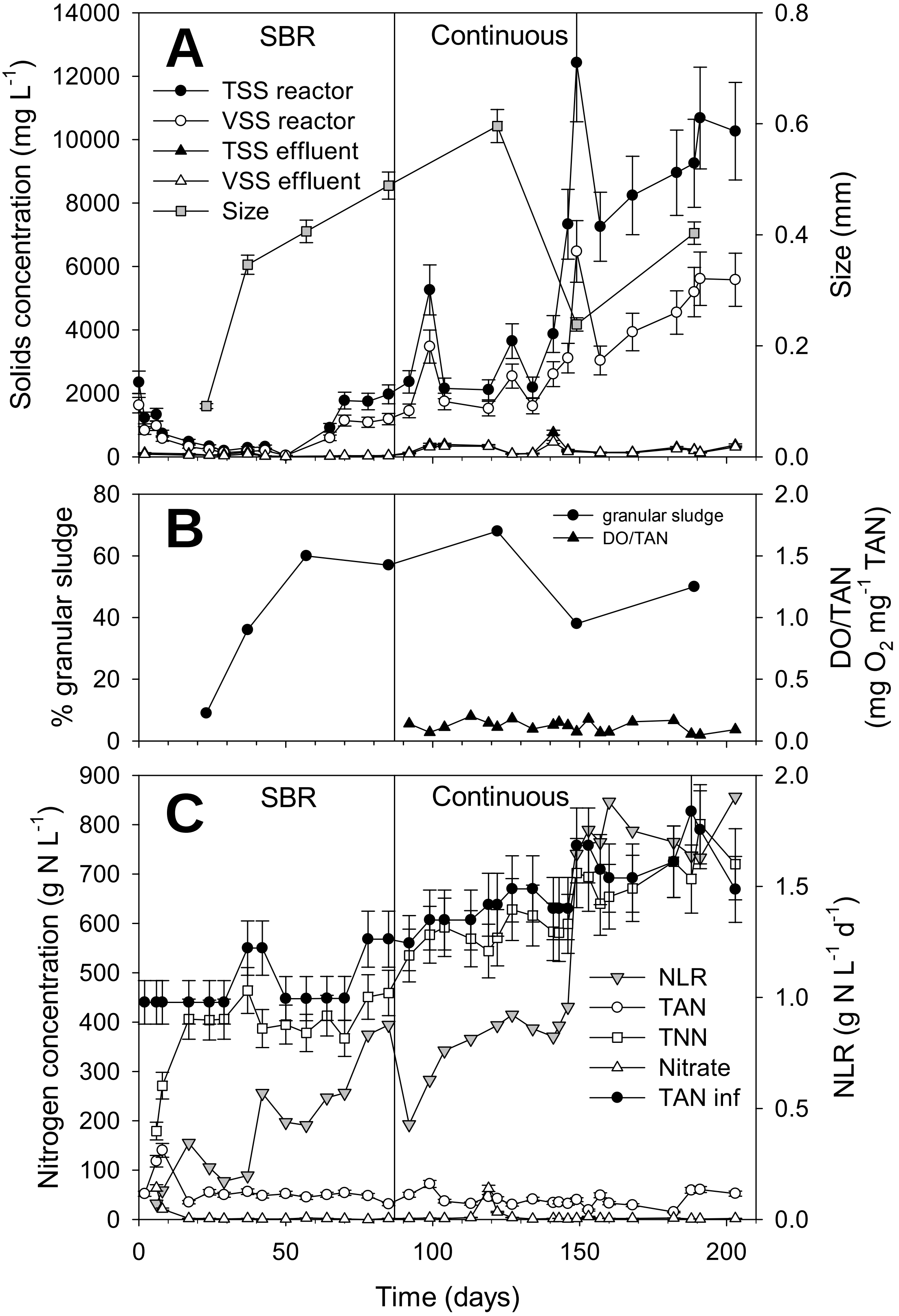


Figure 2.

Figure(s)



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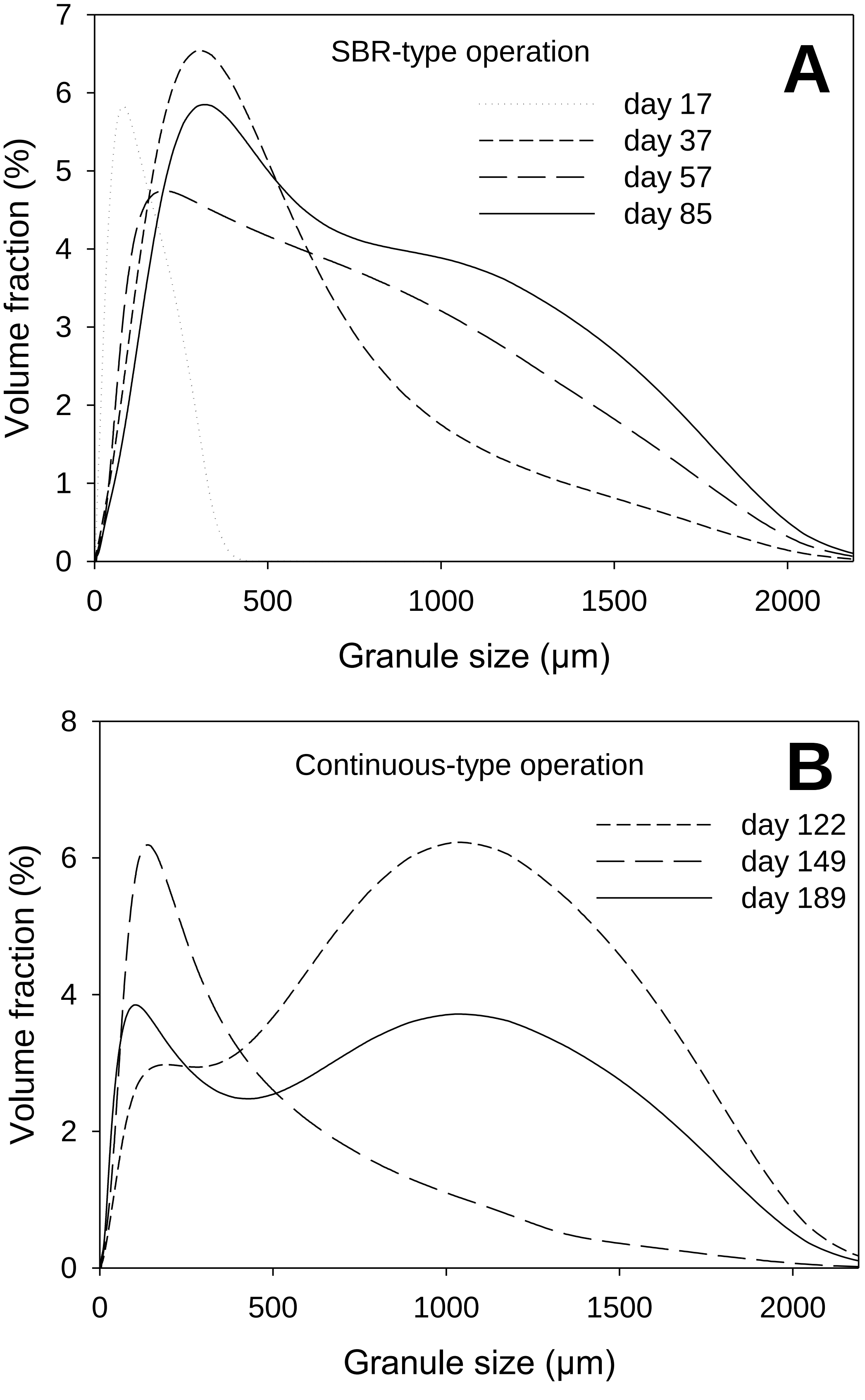
A
SBR-type operation
day 17
day 37
day 57
day 85
Volume fraction (%)
0
1
2
3
4
5
6
7
Granule size (µm)
0
500
1000
1500
2000
B
Continuous-type operation
day 122
day 149
day 189
Volume fraction (%)
0
2
4
6
8
Granule size (µm)
0
500
1000
1500
2000

Figure 4.

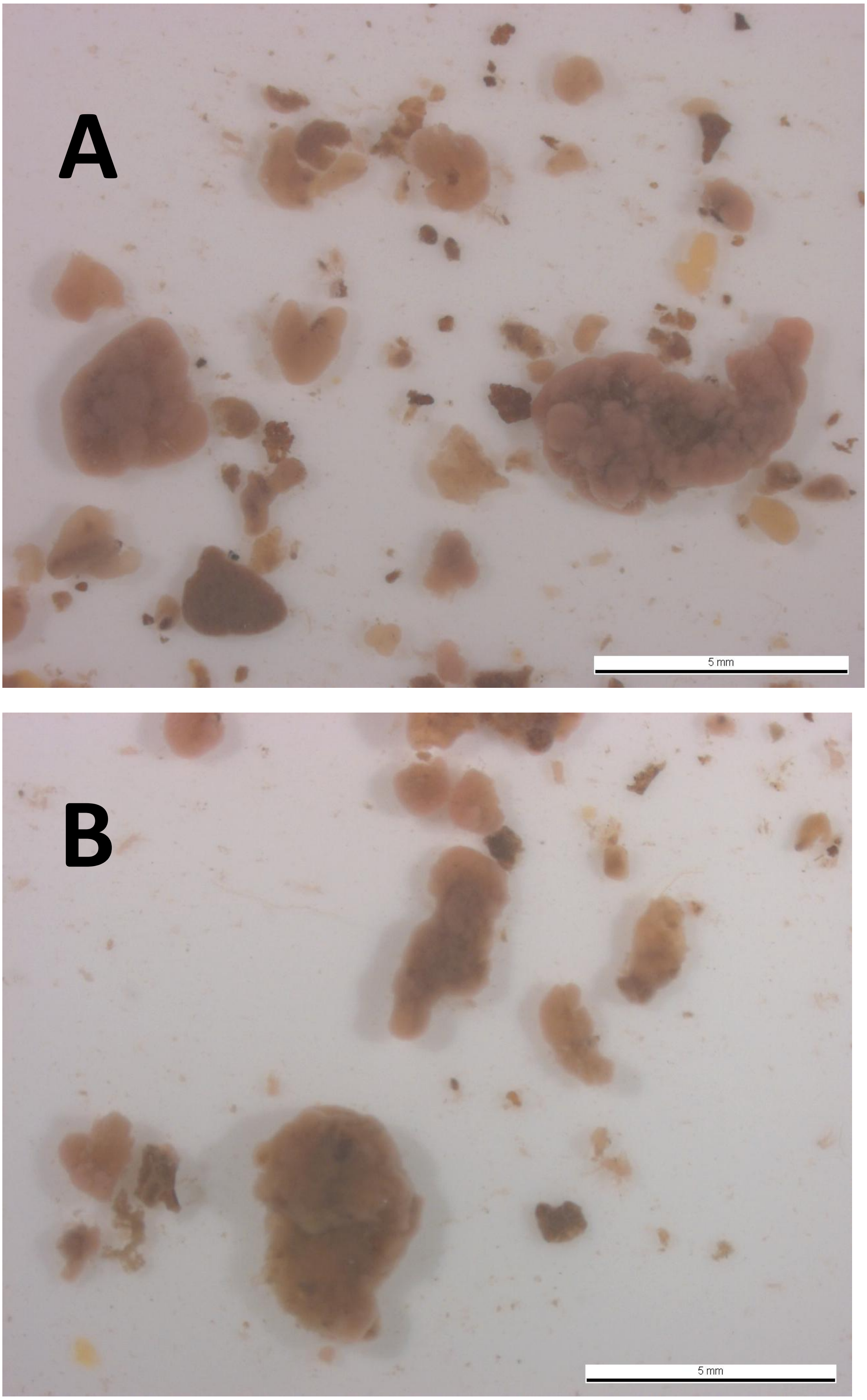

Figure 5.

Figure 6.