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Version: post-print

How to cite: Valenzuela-Heredia, D., Panatt, C., Belmonte, M., Franchi, O., Crutchik, D., Dumais, J., . . . Campos, J. L. (2022). Performance of a two-stage partial nitritation-anammox system treating the supernatant of a sludge anaerobic digester pretreated by a thermal hydrolysis process. Chemical Engineering Journal (Lausanne, Switzerland : 1996), 429, 131-301. doi:10.1016/j.cej.2021.131301

https://doi.org/10.1016/j.cej.2021.131301

Performance of a two stages partial nitritation-anammox system treating the supernatant of a sludge anaerobic digester pretreated by a thermal hydrolysis process

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Abstract

A two stages system (partial nitritation and anammox processes) was used to remove nitrogen from the dewatering liquor originated from the thermal hydrolysis/anaerobic digestion (THP/AD) of municipal WWTP sludge. Two operating strategies were tested to start-up the PN reactor: 1) Maintain a fixed hydraulic retention time (HRT), feed with diluted dewatering liquor and increase the ammonium loading rate (ALR) applied by decreasing the dilution ratio and 2) feed directly with the undiluted dewatering liquor and gradually decrease the HRT. When the first strategy was applied, the reactor performance had several destabilization episodes. By means of a statistical analysis of the operational data, these episodes were correlated to the application of high specific ammonium (higher than 0.6 g NH_4^+-N/g TSS·d) and organic (higher than 0.7 g COD/g TSS·d) loading rates. The second start-up strategy applied allowed maintaining a stable operation of the PN reactor and treating ammonium loading rates (ALR) up to 4.8 g NH_4^+-N/(L·d) which would demonstrate that dilution of THP/AD effluents, as currently applicable when one single stage N removal systems are used, is not required in this case. The operating conditions promoted the presence of free nitrous acid levels, generally higher than 0.14 mg HNO_2-N/L, inside the PN reactor that avoided themselves the proliferation of nitrite oxidizing bacteria without the need to control both the solids retention time and/or the dissolved oxygen level.

Batch activity tests showed that the inhibitory effects exerted by organic compounds present in the THP/AD dewatering liquor on the ammonia oxidizing bacteria activity can be removed by the PN reactor itself, the implementation of a pretreatment unit being not required when nitrogen removal is carried out by a two stages system. The effluent from the PN reactor was successfully treated by an anammox reactor.

An economic analysis showed that, for treating THP/AD dewatering liquor, using two stages systems instead of one single-stage system is advisable. In the case of applying one single stage systems, the implementation of an aerobic pre-treatment unit is recommended for WWTPs capacities higher than $5 \cdot 10^5$ inhabitants equivalent.

Keywords: Anammox; inhibition; operational stability; partial nitritation; sludge thermal hydrolysis.

1. Introduction

An efficient management of water and energy resources is crucial for human sustainable development given their importance for global economy and life. Wastewater treatment plants (WWTPs) are central to water-energy interactions where the human footprint on the natural water environment is reduced by applying energy to remove pollutants [1]. For this reason, in recent years, efforts made to improve the operation of WWTPs have been focused mainly on promoting their energy efficiency. Some of them focus on the implementation of control systems that allow adjusting the treatment capacity to current demand, while others involved a modification of the WWTP operation, like changes in their configurations or even the installation of new units [2]. To maximize the energy recovery contained in the chemical bonds of organic matter is one of the key aspects to increase the WWTP energy efficiency. In the event that this organic matter is aerobically degraded, this energy simply dissipates in heat and the need for aeration also causes energy consumption. However, this oxygen consumption might be saved by anaerobically degrading the organic matter into methane that might be converted to energy. For this reason, it has been proposed to accumulate a large part of the organic matter in the sludge by improving the efficiency of primary decanters or the use of high-load aerobic systems [3].

Increasing the mass flow of primary and/or secondary sludge would increase the biogas production in the WWTPs but would also cause an increase in the organic load towards the anaerobic digesters that might exceed their design capacity. Several operating strategies have been studied to improve the overall rate of the anaerobic digestion (AD) process [4], and therefore, the treatment capacity of the anaerobic digesters. Among them, the implementation of thermal hydrolysis units previous the anaerobic digestion has been the most successful one applied at the WWTPs sludge line [5]. Nevertheless, promoting sludge hydrolysis caused the increase of the ammonium concentration in the AD dewatering liquor that is returned to the mainstream. This increase entails both the need, in the biological reactor, for more organic matter to carry out the denitrification process (less methane production) and the dissolved oxygen (DO) consumption increase for the nitrification process. To avoid these drawbacks, ammonium from the AD dewatering liquor would have to be removed in the sludge line. Since AD dewatering liquors have a low chemical oxygen demand to nitrogen ratio (COD/N) and their temperature is around 30 °C, the partial nitritation/anammox (PN/A) process is the most suitable nitrogen removal process to treat this stream. PN/A process has been successfully applied in the sludge line of more than 100 WWTPs with the one single stage configuration, where both PN and anammox processes occur under limited DO conditions (0.3-0.6 mg O_2/L) in the same unit [6]. The joint implementation of sludge thermal pre-hydrolysis (THP) and PN/A systems for the AD dewatering liquor treatment seems to be a winning combination to increase the WWTP energy efficiency [7]. However, recent research works have shown that incorporating a THP process can cause operational stability problems during the operation of one single stage PN/A systems [8]. Zhang et al. [9] attributed these problems to both a direct inhibitory effect of the THP/AD dewatering liquor, which caused a decrease in the microbial growth rate, and an indirect one which was related to substrate diffusional limitations due to the presence of particulate and colloidal fractions of COD. Nevertheless, the causes of such operational stability losses are yet not clear [10].

One single stage PN/A systems have a relatively low operational flexibility since they must be operated at low DO concentrations to maintain a balance between the activities of ammonia oxidizing (AOB) (aerobic) and anammox bacteria (anoxic). Moreover, the solids retention time (SRT) need to be fixed in a narrow range that avoid the proliferation of both nitrite oxidizing (NOB) and denitrifying bacteria but allowing the development of AOB and anammox bacteria. Generally, sludge anaerobic digesters operate at high hydraulic residence time (HRT) values that ensure no large fluctuations in the effluent quality. Nevertheless, the implementation of THP units allows shortening the HRT of the sludge anaerobic digesters and causes fluctuations in their effluents. This fact, coupled with the increase in the concentration of solids and organic matter due to the THP process itself, has caused operational problems in the one single stage PN/A systems [8]. In this sense, the application of a two stages PN/A system could be an alternative to treat THP/AD dewatering liquors where ammonium oxidation and anammox processes are carried out in separate units and, therefore, the operating conditions for each process can be independently optimized.

Hence, in this research work, the long-term operational stability of a two stages PN/A system treating THP/AD dewatering liquor was studied. Additionally, batch activity tests were carried out to understand the inhibitory effect of the THP/AD liquor over AOB activity and the benefits of performing an aerobic pre-treatment. Finally, an economic analysis was done to compare the application of one and two stages processes.

2. Materials and methods

2.1. Reactors set-up and operational conditions

2.1.1 Partial nitritation reactor

A sequencing batch reactor (SBR) system, with a working volume of 3 L (total height of 50 cm and diameter of 10 cm), was used to carry out the partial nitritation process. The volumetric exchange ratio was fixed at 50%. The length of the operational cycles was fitted according to the desired HRT and ranged from 3 to 12 h. The cycle consisted of the following series of sequential process phases: simultaneous feeding and reaction under aerobic conditions (120-660 min), aerobic reaction without feeding (30 min), settling (20 min) and withdrawal (10 min). An air pump supplied air through several diffusers located at the bottom of the reactor to promote the oxygen transfer into the bulk liquid and to reach a suitable mixing. The air flowrate was adjusted to maintain always DO concentrations higher than 4 mg O_2/L. A set of two peristaltic pumps was used to introduce the feeding solution (on top of the reactor) and to discharge the effluent (at medium height in the column reactor), respectively. A programmable logic controller (ZELIO model SR2B201FU), programmed using the ZelioSoft2 software, controlled the the pumps and regulated the different phases of the operational cycle. The temperature of the reactor was controlled at 30 °C by means of a thermostatic bath, while the pH value was not controlled and ranged from 6.0 to 8.5.

The PN reactor was fed with the THP/AD dewatering liquor from the municipal WWTP of Mapocho-El Trebal (Santiago, Chile) whose characteristics were: ammonium concentration: 1,420-2,600 mg NH_4^+-N/L; COD concentration: 1,345-3,360 mg COD/L; alkalinity: 4,830-9,360 mg $CaCO_3$/L; and pH: 7.8-8.3. The PN reactor was inoculated with 3.3 g TSS of activated sludge collected from the municipal WWTP of Curacaví (Chile). The HRT of the PN reactor and the dilution applied to the THP/AD liquor fed changed throughout the operational period (778 days), as indicated in Table 1. The HRT was initially fixed at 6 h and diluted THP/AD dewatering liquor was supplied to the PN system. The dilution applied was step wisely decreased but, as destabilization episodes occurred, the HRT was progressively increased up to 24 h. At this HRT, a stable operation was achieved without applying any dilution to the THP/AD dewatering liquor. From this moment, the system was fed with undiluted THP/AD dewatering liquor and the HRT was step wisely decreased down to 9 h (Table 1).

2.1.2 Anammox reactor

An anammox SBR reactor with a useful volume of 1 L (total height of 70 cm and diameter of 5 cm) was used to treat the effluent of the PN reactor collected between 525 and 546 days of its operation period. The volumetric exchange ratio was fixed at 50%. The length of the operational cycles was fitted according to the desired HRT and ranged from 2 to 4 days. The cycle consisted of the following series of sequential process phases: simultaneous feeding and mixing (1,380-2,820 min), mixing without feeding (30 min), settling (20 min) and withdrawal (10 min). A mechanical stirrer (100 rpm) provided a complete mixture inside the system. Control of the operational cycle phases, feeding supply, effluent discharge and temperature control (at 30 °C) were done in a similar way to that described for the PN reactor. The pH value was not controlled and ranged from 7.8 to 8.1. Granular anammox biomass (8.5 g TSS) from an ELAN® full-scale reactor was used as inoculum [11]. The system was initially fed with the effluent of the PN reactor diluted at 50% and operated at an HRT of 3 days (Table 1) and then the effluent of the PN reactor was fed without applying dilution and the HRT was fixed at 2 days.

Table 1. Summary of the operational conditions and influent characteristics.

Partial nitritation reactor				
Period (d)	HRT (h)	Influent dilution ratio (THP/AD effluent: water)	NH_4^+-N concentration (mg NH_4^+-N/L)	COD concentration (mg/L)
1-250	6	1:32.3-1:0.1	46-1581	50-1644
251-350	8	1:2.2-1:0	549-2316	516-3148
351-411	16	1:32.3-1:0	53-1912	1032-1961
412-524	24	1:0.9-1:0	850-1508	905-1480
525-624	20	1:0	1682-1853	1810-2895
625-646	16	1:0	1561-2112	2632-2895
647-688	14	1:0	1703-2105	1905-2632
689-715	11	1:0	1747-2013	1905-2094
716-778	9	1:0	1597-1856	1905-2094
Anammox reactor				

Period (d)	HRT (d)	Influent dilution ratio (THP/AD effluent: water)	NH_4^+-N concentration ($mg\ NH_4^+$-N/L)	NO_2^--N concentration ($mg\ NO_2^-$-N/L)
1-10	2	1:1	438-521	256-455
11-44	4	1:0	611-921	572-758

2.2. Batch respirometric tests

Three series of batch respirometric tests were performed by means of a respirometer BM-T Plus (Surcis S.L., Spain). The first serie of assays was carried out to determine the effect of free ammonia (FA) and free nitrous acid (FNA) on AOB and NOB activities, respectively, following the methodology described in López-Fiuza et al. [12]. The assayed ammonium concentrations ranged from 2.5 to 1,000 mg NH_4^+-N/L while the tested nitrite concentrations ranged from 15 to 700 mg NO_2^--N/L. In the case of AOB activity determination, the assays were done in presence of 24 µM of sodium azide to inhibit NOB activity. For AOB activities, data obtained were fitted to the Haldane kinetic model by means of Solver (Excel) in order to determine the effect of FA concentrations higher than those tested. These assays were carried with biomass taken from the PN reactor on day 404.

In the second series of assays, the possible toxicity of non-ammonium compounds present in the THP/AD dewatering liquor (526, 531, 582 and 594 days of the operational period of the PN reactor) on AOB activity was evaluated. For this, the AOB activity was determined at different concentrations of the THP/AD dewatering liquor (10, 33, 50 and 100%) but maintaining the same ammonium concentration. Since, during the first series of assays, the maximum AOB specific activity was observed at an ammonium concentration around 200 mg NH_4^+-N/L, the samples of the THP/AD dewatering liquor were previously subjected to air stripping process with NaOH addition until achieving this ammonium concentration. The assays were done under not limiting DO conditions. Firstly, the DO consumption rate was determined in the presence of 24 µM of sodium azide to inhibit nitrite oxidation. Then allylthiourea (86 µM) was added to inhibit ammonium oxidation and the new DO consumption rate was determined. Oxygen consumption rate due to ammonium oxidation was calculated as the difference between both oxygen consumption rates. Similar assays were done with the THP/AD dewatering liquor previously treated by activated sludge under aerobic conditions for 24 h. The inhibitory effect was calculated taken as the reference value of the ammonium oxidation activity obtained with a synthetic solution containing 200 mg NH_4^+-N/L. All assays were done at a pH value of 7.0, a temperature of 30 °C and a biomass concentration of 1 g TSS/L. Sludge was taken from the PN reactor the day after the liquor sampling to perform the tests using both raw and pretreated THP/AD dewatering liquor with the same biomass.

The third series of assays were done to determine the possible toxicity of non-ammonium compounds present in the THP/AD dewatering liquor once the PN reactor treated it. For this, two samples of the PN reactor effluent were assayed following the same protocol previously described for the second series of assays. However, in this case, the assays were only done for a concentration of 100%. As a control, similar assays were performed with the THP/AD dewatering liquor supplied to the PN reactor in those days. Both assays were carried with biomass taken from the PN reactor on days 630 and 632.

2.3. Statistical analysis

In order to determine the possible effect of the THP/AD dewatering liquor characteristics (ammonium concentration, COD concentration and inlet COD/N) and operational conditions (specific ammonium loading rate (sALR: g NH_4^+-N/(g TSS·d)) and specific organic loading rate (sOLR: g COD/(g TSS·d)) on the destabilization episodes of the PN reactor, each set of variables obtained during the whole operational period was divided into two groups according to the performance of the reactor (stable and unstable). Since the characterization of periods of instability was not pursued, but rather their cause, data associated with the "unstable" category were not those observed during these periods but those immediately preceding. It was considered that the presence of significant differences (*P*-value less than 0.05) between both groups implies that the analyzed variable plays a significant role in the functional state of the reactor. To determine this, the non-parametric Kruskal Wallis test with Dunn's post-test (with Bonferroni correction) were performed. All Statistical calculations were carried out using Xlstat version 2014 (Addinsoft).

2.4. Capital costs estimation

Capital costs related to implementing different configurations to remove nitrogen from the THP/AD dewatering liquors were estimated. The studied configurations were: a) one single stage system; b) two stages system; c) biological aerobic pretreatment to remove biodegradable organic matter + one single stage system; d) aerobic pretreatment to remove biodegradable organic matter + two stages system. This last case has been considered in order to guarantee the stable performance of the PN reactor during possible unstable operational periods of the sludge AD. Capital costs were determined for WWTPs of different sizes (expressed in terms of inhabitants equivalent). Nitrogen load associated with THP/AD dewatering liquor was calculated as 20% of the N load entering the WWTP, estimated based on a flowrate of 250 L/(inhabitant equivalent·d) and an incoming nitrogen concentration of 50 mg N/L. The flowrate of the THP/AD dewatering liquors stream was determined considering its ammonium concentration was 1,800 mg NH_4^+-N/L. The volumes of the reactors used to remove nitrogen were calculated based on the volumetric nitrogen loading rates that they can manage: one single stage system without aerobic pretreatment 0.6 kg N/m^3·d [8]; one single stage system with aerobic pretreatment 1.7 kg N/m^3·d [13]; for the two stages system the PN reactor a value of 4.8 kg N/m^3·d (this research work); and the anammox reactor a value of 7.5 kg N/m^3·d [14].

The volume of the biological aerobic pretreatment reactor was estimated based on a HRT value of 10 h [13].

The use of two SBR type reactors, operated in parallel, has been considered for implementing the one single stage N removal, biological aerobic pretreatment and partial nitritation units, while to carry out the anammox process, a UASB type reactor has been considered. Equipment costs were adapted from US EPA [15] and Danish Energy Agency [16] since the calculated reactor volumes were within the range of reactor volumes used to obtain these cost correlations. Prices were updated considering the evolution of the Chemical Engineering Plant Cost Index (CEPCI) [17].

2.5. Analytical methods

To monitor the performance of both reactors, the concentrations of ammonium (NH_4^+-N), nitrite (NO_2^--N), nitrate (NO_3^--N), total suspended solids (TSS) and chemical oxygen demand (COD) of their influent and effluent were periodically measured according to the methodology described by "Standard Methods" [18]. Since the PN reactor effluent contained nitrite, a COD measurement calibration was performed to take into account only the part related to the presence of carbonaceous matter. DO concentration, pH, and temperature were also periodically monitored.

3. Results and Discussion

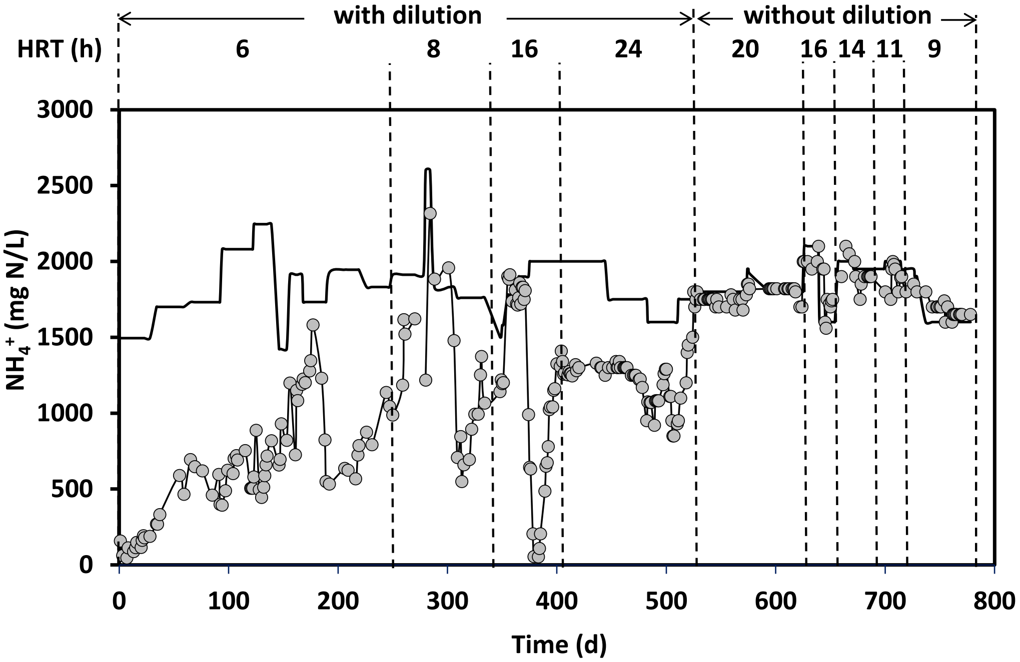
3.1. Partial nitritation reactor performance

3.1.1 Start-up and stabilization

The partial nitritation reactor was initially operated at an HRT of 6 h and was fed with diluted THP/AD dewatering liquor (Figure 1a). The dilution applied to the THP/AD dewatering liquor was gradually decreased as the reactor maintained an ammonium oxidation efficiency of around 50%, which is the maximum efficiency expected according to the available alkalinity (3.5 mg $CaCO_3$/mg NH_4^+-N). During the application of this operating strategy, there were several episodes of operational destabilization, during which the reactor suffered a sharp loss of its ammonium oxidation capacity (Figure 1b). During these destabilization periods, pH value rose from 6.0-6.4 to 8.1-8.5, due to CO_2 stripping from unconsumed bicarbonate. This caused the presence of high concentrations of free ammonia in the system, which inhibited the AOB. In order to recover the operational stability of the system, the TPH/AD dewatering liquor supply to the reactor was stopped for several hours or, even, the reactor was washed with tap water to reduce the FA concentration and, later, the reactor was fed again but with a lower ammonium loading rate (ALR). To gradually decrease the dilution applied to the TPH/AD dewatering liquor and maintain the operational stability, from 251 day on, the HRT was increased to 8 h and later (351 day) to 16 h. However, destabilization periods occurred even at these HRT values, so, finally, on day 412, an HRT of 24 h was imposed. Under these conditions, the system operated stably and the dilution applied to the TPH/AD dewatering liquor could be decreased until it was fed into the PN reactor without applying dilution. From day 525, the reactor was fed with the undiluted TPH/AD dewatering liquor and the HRT was progressively decreased to 9 h. During this stage of the operational period, destabilization episodes were not observed. Biomass was never purged from the reactor and the solids residence time (SRT) was given by the washout of solids that were not able to settle in the time imposed. The average SRT value was 22 days.

The addition of dilution water is an operating strategy generally applied to start-up both one single stage [19] and two stages [20] N removal systems treating THP/AD dewatering liquors. This strategy is also applied during the operation of one single stage systems, even when they have achieved steady state conditions, to improve their stability and increase their nitrogen removal capacity [21]. However, the addition of dilution water implies extra capital and operating costs which could avoided taken into account that, according the results obtained, the start-up of the PN reactor could be directly carried out applying undiluted THP/AD dewatering liquor by decreasing the HRT. In fact, this start-up strategy allowed maintaining a stable performance while that based on decreasing the dilution applied to the THP/AD dewatering liquors caused several destabilization episodes. This could indicate that the stability of the PN reactor is more sensitive to the fluctuations of both inlet ammonium and COD concentrations than to those concentrations themselves (Figure 1a). In this sense, recent research works showed that full scale one single stage N removal systems are able to maintain their properly operation when the dilution ratio applied to the THP/AD dewatering liquor is decreased [13] or, even, when undiluted THP/AD dewatering liquor is fed [22].

a)



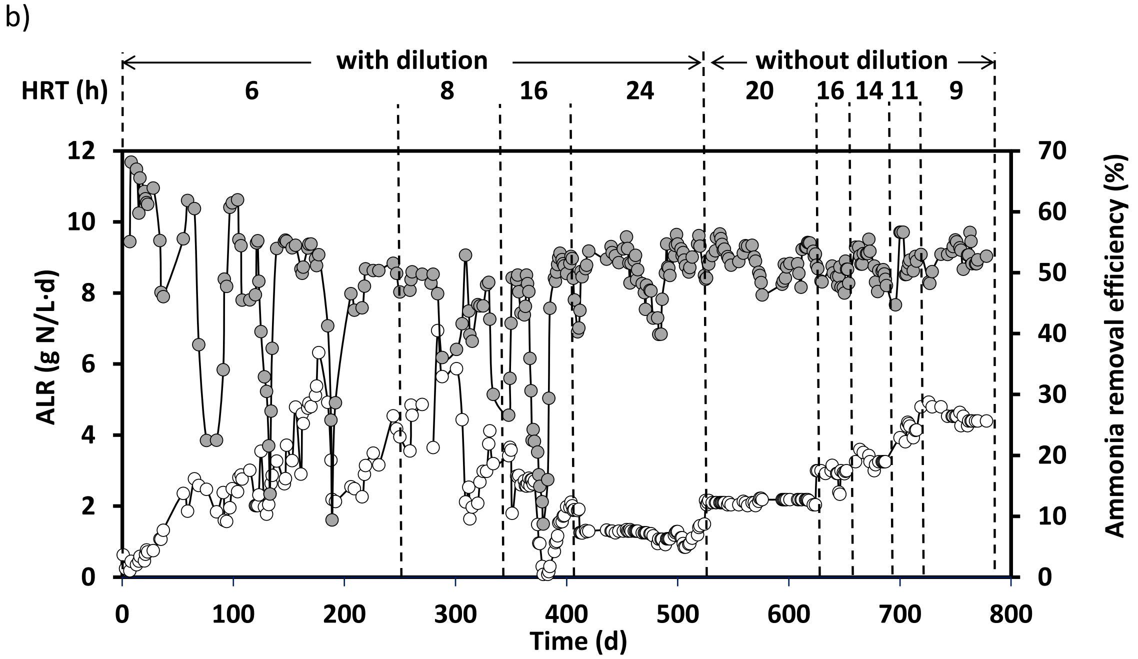
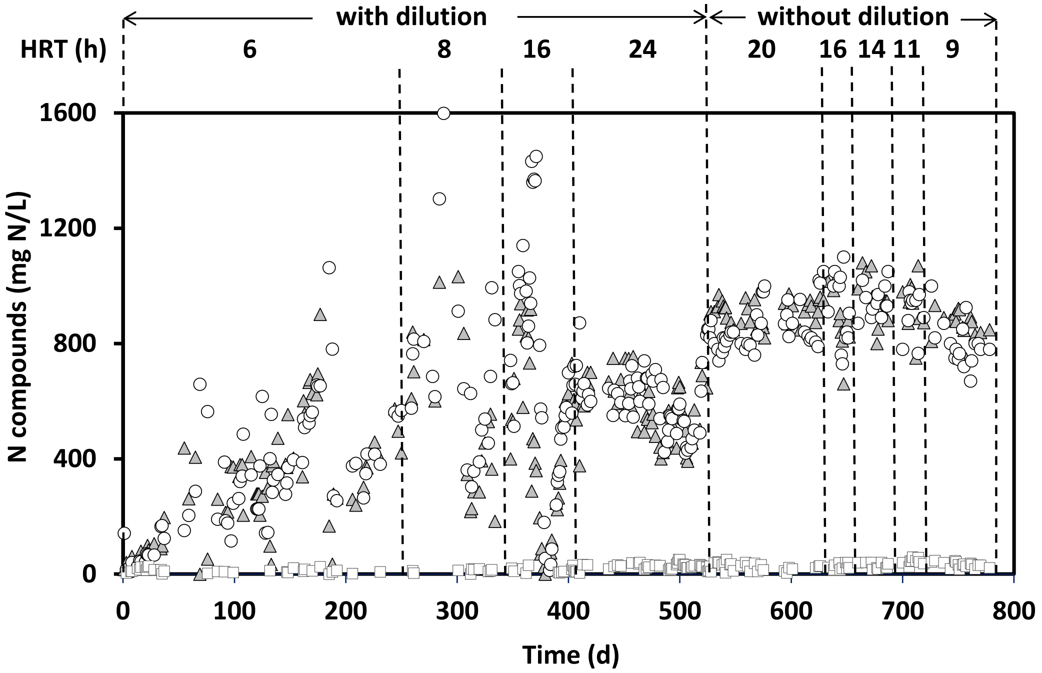


Figure 1. Partial nitritation reactor performance: a) Ammonium concentration of the raw THP/AD dewatering liquor (——) and of the influent supplied (●); b) ALR applied (o) and ammonium removal efficiency (●) during the operational period.

3.1.2 Treated loading rate

The highest ALRs that the system was able to treat in a stable way with both diluted and undiluted TPH/AD dewatering liquor were very similar and around 4.8 g NH_4^+-N/(L·d). From this, it can be inferred that the concentrations of COD and ammonium present in the influent did not significantly affect the treating capacity of the system. This value of ALR is within the range of 2.4 and 5.7 NH_4^+-N/(L·d) obtained by Zhang et al. [23] and Dosta et al. [24], respectively, with PN reactors treating AD dewatering liquors. In the case of treating THP/AD dewatering liquors (without dilution), the achieved ALRs reported for PN reactors ranged from 0.4 g NH_4^+-N/(L·d) [25] and 2.3 g NH_4^+-N/(L·d) [20]. It must be considered that the ALR reported by Zhang et al. [25] was achieved with a pilot scale reactor (65 m^3). This pilot reactor was probably operated under conservative conditions since these authors were able to treat an ALR of 3.8 g NH_4^+-N/(L·d) when treating the same THP/AD dewatering liquor with a PN reactor at lab-scale. Since the ALR achieved, when PN reactors treat both AD and THP/AD dewatering liquors, are in the same range, it could be concluded that the implementation of a thermal hydrolysis unit in the sludge line would not affect the treating capacity of the PN units. This was also supported by the results obtained by Ochs et al. [26] when treated THP/AD dewatering liquors with one single stage N removal systems. Nevertheless, the nitrogen loading rate (NLR) applied to this kind of systems is limited to values between 0.3 and 0.7 g NH_4^+-N/(L·d) [8, 22, 26]. The relatively low treating capacity of the one single stage systems would be associated with the fact that their operating window concerning DO concentration is relatively narrow. Thus, DO concentration should be high enough to maintain a suitable AOB activity and to withstand the greater input of organic matter present in TPH/AD dewatering liquors compared to that of AD dewatering liquors, but low enough to avoid both the proliferation of NOB and the inhibition of the anammox process [8]. In this sense, the removal of the organic matter from TPH/AD dewatering liquors before its entrance to the one single stage [13] or the use of a two stages system, whose PN unit can be operated at high DO concentrations to overcome the decrease of AOB activity due the incoming organic matter [20], would allow increasing the NLR applied.

a)



b)

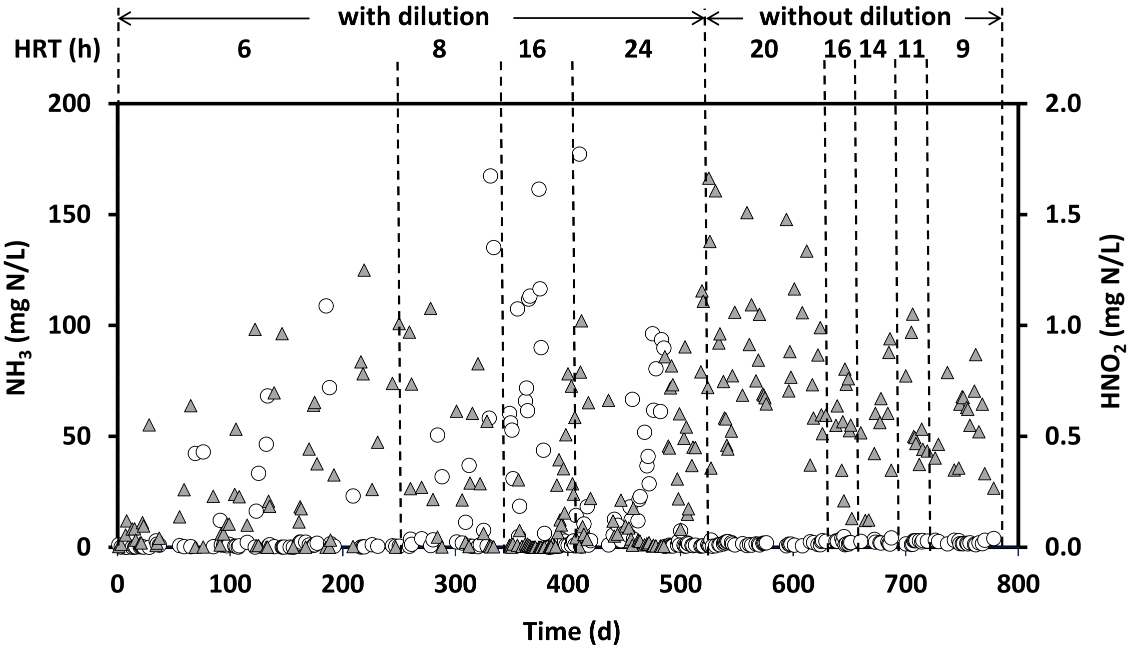


Figure 2. Concentration of N compounds in the effluent of the PN reactor along the operational period: a) NH_4^+-N (o); NO_2^--N (▲); NO_3^--N (□); b) NH_3-N (o); HNO_2-N (▲).

3.1.3 Effect of FA and FNA over the reactor performance

During the stable operational periods, the average NO_2^--N/NH_4^+-N ratio observed in the effluent was of 1.02 g N/g N while nitrate production was hardly observed (3.8-5.6% of ammonium oxidized) (Figure 2a). Since no strategy was applied to promote the selection of AOB over NOB such as operating at low DO levels or controlling the SRT in the PN reactor, it was assumed that the operational conditions themselves were unfavorable for the development of the NOBs. Similarly, Zhang et al. [23] and Dosta et al. [24] observed that nitrate formation did not almost occur during the oxidation of ammonium contained in AD dewatering liquors and attributed the high NO_2^--N/NH_4^+-$N_{oxidized}$ ratio obtained to the inhibitory effects of FA and FNA on NOB. As the ammonium concentration of THP/AD dewatering liquors is higher than that of AD dewatering liquors, stronger inhibitory effects on NOB are expected during the treatment of the first ones [25]. To confirm a possible inhibition on NOB, activity tests were carried out with biomass taken from the PN reactor in the presence of different concentrations of FNA (from 0.003 to 0.140 mg HNO_2/L). The maximum NOB activity was observed at FNA concentrations around of 0.03 mg HNO_2/L. This can be explained by the substrate limitation at the lowest FNA values since FNA concentration is the form of nitrite (substrate of NOB) in acidic medium. Then, as the FNA concentration increase, the NOB activity sharply decrease. Results showed that total inhibition of NOB was practically observed at levels of 0.14 mg HNO_2-N/L (Figure 3a). This value is much lower than those observed generally during the operation of the PN reactor (65% of the samples showed values greater than 0.14 mg HNO_2/L with an average value of 0.44 mg HNO_2/L, Figure 2b), therefore, the inhibition of NOB would be already justified by the only presence of FNA. Except for destabilization episodes, the PN reactor tends to achieve operating conditions that imply the presence of FNA concentrations that do not affect the activity of AOB [27] but that avoid the subsequent oxidation of nitrite to nitrate. This implies that it is not necessary to control the solids retention time at 3-6 d and/or the DO level at 0.3-1.2 mg O_2/L to avoid the proliferation of BON, as occurs in one single stage nitrogen removal systems [26], which allows much larger ALRs to be treated.

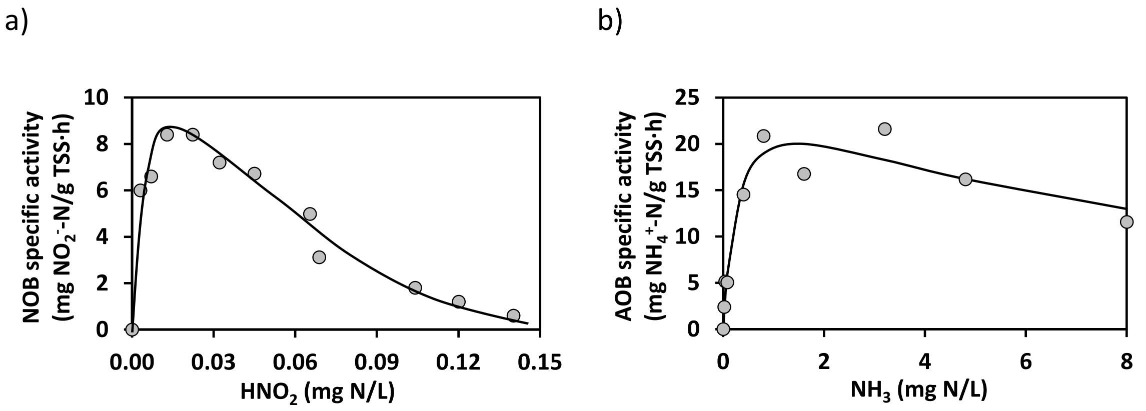


Figure 3. Activity tests carried out with biomass from the PN reactor (sample taken 404 day): a) ammonium oxidizing activity and b) nitrite oxidizing activity.

Regarding FA levels, these were generally low except during the destabilization periods when the combination of pH values of 8.1-8.5 and high ammonium concentrations (357-1,063 mg NH_4^+-N/L) caused the presence of FA concentrations of up to 177 mg NH_3-N/L (Figure 2b). FA is an inhibitory compound for both NOB and AOB and since the loss of ammonium oxidation activity was observed during these destabilization episodes, activity tests were carried out to study the effect of FA on the AOB activity. Similarly to the explained for NOB, at FA concentration below 2 mg NH_3-N/L the AOB activity increase due to substrate limitations at lower FA (and consequently ammonium) values. Data obtained from these tests were fitted to a Haldane kinetic model (Figure 3b) and according to the obtained parameters (affinity constant: 0.31 mg NH_3-N/L; maximum specific activity: 28.48 mg NH_3-N/(g TSS·h); inhibition constant: 6.9 mg NH_3-N/L), the expected activity of AOB would be 12.2, 6.4 and 3.9% of the maximum one at the FA levels around 50, 100 and 170 mg NH_3-N/L, respectively, observed as peaks of the destabilization episodes (days 75/133, 185/475 and 331/374/410 Figure 2b) which agrees with the sharp efficiency decrease observed during these periods (Figure 1b). In these periods of high FA, NOB was also inhibited since their reported inhibition threshold is in the range of 0.1-1.0 mg NH_3-N/L, and they likely cease the growth at above 6 mg NH_3-N/L [28]. Indeed, strategies of maintaining FA levels between 5 -10 mg NH_3-N/L have been proposed to achieve the PN process. However, in this case, due to the alkalinity consumption that caused a pH decrease, NOB were mainly inhibited by the FNA concentration.

3.1.4 Effect of organic matter from THP/AD liquor

Average organic matter removal efficiency over the whole operational period was 44.2±17.7% (Figure 4), while when a biological aerobic batch pretreatment was applied during 1 d to remove organic matter from the THP/AD dewatering liquor (see section 3.2) this efficiency was 39.5±6.4%. The efficiencies observed are in the range of those of 34-50% reported for PN reactors [20, 25], 60% for one single stage N removal systems [9, 22], 60% for a Phospaq reactor [13] and 45% for a biological aerobic batch treatment (1 d) [9], when treated this kind of effluents. On the other hand, despite the increase in the organic matter concentration of the THP/AD dewatering liquor is generally related to unstable episodes of the anaerobic sludge digester and, therefore, to an increase of volatile fatty acids concentration [9], no correlation between the inlet COD concentration and its biodegradability was observed in this work.

Zhang et al. [8] attributed the operational problems observed in a one single stage N removal system, which treated THP/AD dewatering liquor, to the inlet COD concentration. Then in the present research work, a statistical analysis of the data obtained was done (Table 2 in order to discern whether the destabilization episodes that occurred during the operation of the PN reactor were related to the influent characteristics (ammonium and COD concentrations and COD/N ratio) and/or to the operational conditions (sOLR and sALR). This analysis showed that the destabilization episodes would be not related to the influent characteristics but to both sOLR and sALR applied.

To maintain a stable performance of the nitrogen removal system, the sALR applied should be lower than the maximum specific activity of AOB in order to avoid free ammonia accumulation inside the system. As can be seen in Figure 3.b the maximum specific activity of AOB is around 0.6 g NH_4^+-N/g TSS·d which matches with the value of sALR that defines the stable conditions (Table 2). In a similar way, sOLR should be lower than the maximum specific activity heterotrophic bacteria to avoid the accumulation of organic biodegradable compounds causing inhibition on AOB (see section 3.2).

Literature about systems removing nitrogen from THP/AD effluents hardly provides biomass concentration data and in the cases that do, they are for specific stages where the system operates under stable conditions, so that a relationship between unstable periods and application of high specific loads cannot be determined to corroborate the results found in the present work. Stable operation was reported for one single stage systems operated at sALR of 0.08-0.19 g NH_4^+-N/g TSS·d and sOLR of 0.19-0.55 g COD/g TSS·d [8, 21, 29] while, in the case of two stages systems, the PN reactor operated properly at sALR of 0.08 g NH_4^+-N/g TSS·d and sOLR of 0.11 g COD/g TSS·d [25]. As can be seen the reported values are considerably lower than those of 0.8 g NH_4^+-N/g TSS·d and 1 g COD/g TSS·d found in this work as causing unstable operation. Generally, performance of the nitrogen systems treating THP/AD effluents is analyzed based on the volumetric loading rates and biomass concentration is not considered. This parameter should be taken into account since the capability of the system to maintain its stability in presence of inhibitory compounds is proportional to its biomass concentration. In fact, from results obtained by Ochs et al. [10], who tested three different one single stage technologies to treat this kind of effluents, it can be inferred that the technology most prone to destabilization episodes was that which operated at the highest values of sOLR and sALR.

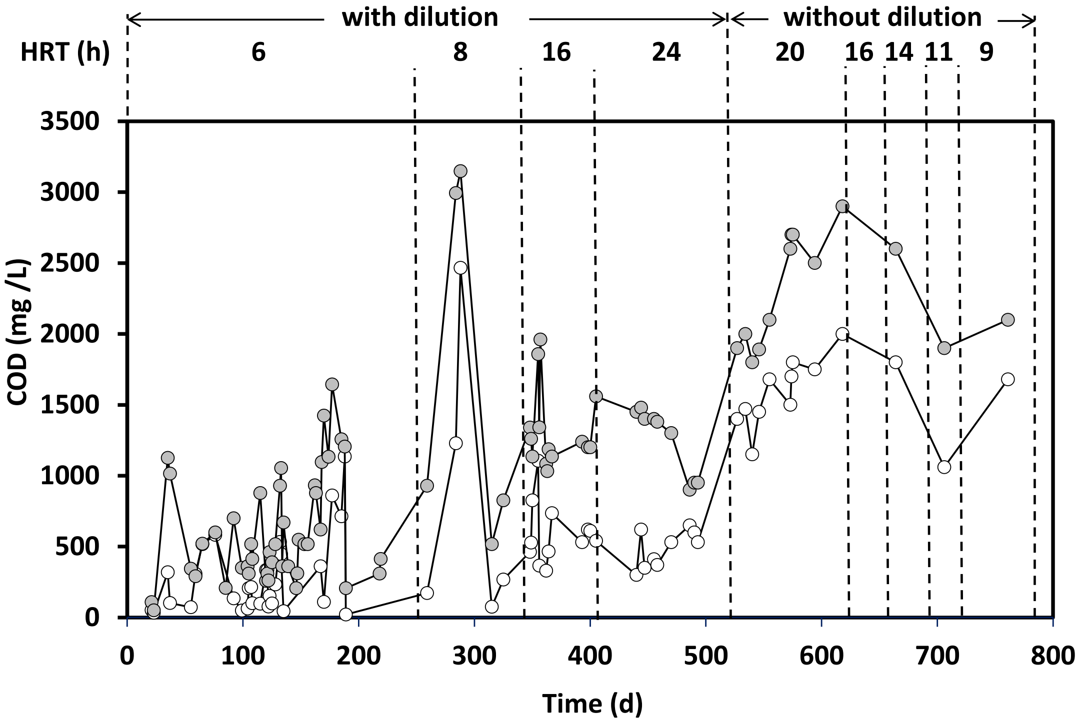


Figure 4. COD concentration along the operational period: influent (•) and effluent (o).

Table 2. Summary statistics of analyzed variables and results of the Kruskal-Wallis test (S: stable periods; U: unstable periods; SD: standard deviation).

Variable (group)	Units	Mean	SD	Range	Kluskal-Wallis test	*P*-value
Inlet COD (S)	mg/L	1119	740	50 - 3148	Not significant	0.8746
Inlet COD (U)	mg/L	1167	844	260 - 2993		
Inlet NH_4^+-N (S)	mg/L	1254	534	47 – 2100	Not significant	0.1039
Inlet NH_4^+-N (U)	mg/L	1093	566	188 - 2315		
COD/N ratio (S)	mg/mg	2.0	1.1	0.6 - 8.1	Not significant	0.3977
COD/N ratio (U)	mg/mg	1.6	0.6	0.8 - 2.5		
sOLR (S)	g COD/g TSS·d	0.7	0.5	0.1 - 3.0	Significant	0.0237
sOLR (U)	g COD/g TSS·d	1.0	0.4	0.5 - 1.7		

sALR (S)	g NH_4^+-N/g TSS·d	0.6	0.3	0.04 - 1.63	Significant	0.0351
sALR (U)	g NH_4^+-N/g TSS·d	0.8	0.4	0.19 - 2.00		

3.2. Toxicity assays

Four samples of THP/AD dewatering liquor feeding to the PN reactor (operational days: 526, 531, 582 and 594) were used to determine its possible toxicity on the AOB activity. Activity tests were done at different THP/AD liquor dilutions, with both the raw sample and the raw sample previously treated by an aerobic biological process to remove the biodegradable organic matter. This treatment was applied during 1 d since soluble COD concentration achieved an almost constant value after this period. In both cases, ammonium concentration was fitted to 200 mg NH_4^+-N/L by air stripping and NaOH addition since this concentration allows achieving the maximum activity of AOB (Figure 3b). In the case of the raw liquor without previous aerobic treatment, an inhibitory effect was observed for all the dilutions tested, and an average decrease of 78% of the AOB specific activity was observed when no dilution was applied (Figure 5). Nevertheless, no inhibitory effect was observed (for all the dilutions tested) when the raw liquor was aerobically pretreated. Therefore, the inhibitory effect of the raw sample over AOB activity could be caused by any compound(s) which was removed during the aerobic treatment. To discard the possible adsorption of this compound(s) on the "fresh" activated sludge used to carry out the aerobic biological pretreatment, new assays were done with different samples of the influent and effluent of the PN reactor (630 and 632 days (HRT of the PN reactor: 16 h)), whose ammonium levels were also fitted to 200 mg NH_4^+-N/L, and no inhibitory effects were observed for the effluent samples while the influent samples always caused a decrease of the AOB activity of 76%. Then, it would be expected that the possible inhibitory compound(s) was probably removed by biodegradation inside the own PN unit (which is aerobic). Deviation values shown in Figure 5 might be ascribed to the use of effluent from different operational days to perform the activity tests, and thus, their composition varied (e.g., COD concentration range between 1,900 and 2,700 mg COD/L in undiluted samples).

Zhang el al. [9] observed, in batch assays, an inhibition of 50% on the AOB activity at a THP/AD dewatering liquor concentration of 46% which agrees with the inhibitory degrees found in this work. Contrary to our results, these authors did not observe a decrease of the inhibitory effect when applied a pretreatment to the effluent based on a short-term (1 d) aerobic biological treatment and only when they applied this pretreatment for 7 days the inhibitory effects disappeared. They also found anaerobic biological treatment applied during 14 d or adsorption by activated carbon was also effective in removing inhibitory effects over AOB. On the basis of their results, these authors concluded that inhibitory effects on AOB activity were related to slowly biodegradable COD, particularly to the large colloidal fraction of COD, which mainly causes a diffusion resistance effect. According to the results obtained by Driessen et al. [13], at a full-scale facility, the previous organic matter removal from the THP/AD dewatering liquor by means of a Phospaq reactor would allow maintaining the stable operation of the subsequent one single stage N removal system which was able to treat a NLR of 2.5 g NH_4^+-N/(L·d) with N removal efficiencies of 90%. This Phospaq reactor was operated at an HRT as low as 10 h indicating that relatively short biological aerobic treatments are enough to remove possible inhibitory effects of THP/AD dewatering liquors.

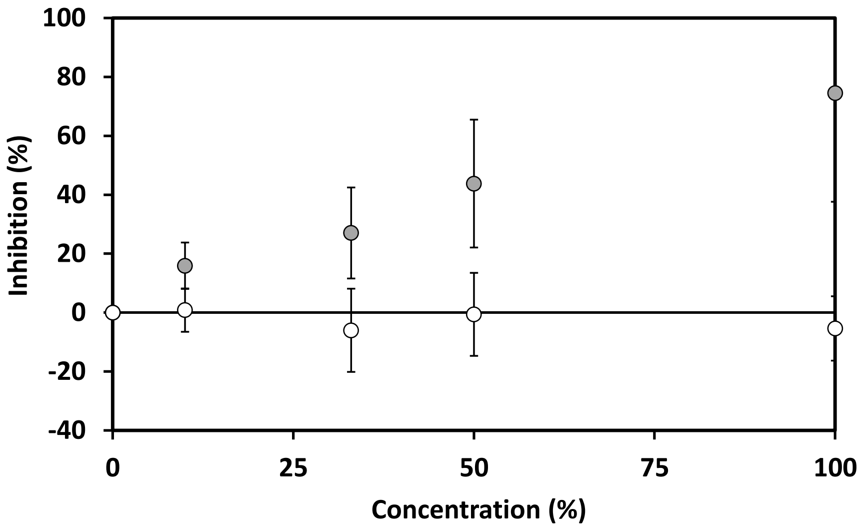


Figure 5. Inhibitory effects of the THP/AD dewatering liquor (with an ammonium concentration adjusted to 200 mg NH_4^+-N/L by air stripping and NaOH addition) at different dilutions: (●) raw liquor without pretreatment; (○) previously treated by activated sludge under aerobic conditions.

Based on the results obtained during the batch tests, it could be concluded that THP/AD dewatering liquors contain compounds that affect the activity of the AOB, but at the same time, they are degraded in the partial nitritation unit (aerobic conditions) and, therefore, the destabilization episodes could be associated with periods of operation in which the inlet loading rate of these compounds to the PN reactor is higher than their degradation rate, causing their accumulation and the subsequent deterioration of the AOB activity. This would be in accordance with the significant correlation found between the sOLR and the destabilization periods during the operation of the PN reactor.

Inhibition of AOB activity due to sludge anaerobic digester effluents is only reported for those effluents in which the sludge was thermal pretreated [30]. Therefore, this inhibition should be associated with effluent characteristics derived from the process of thermal hydrolysis itself. On the one hand, literature shows that the concentration of volatile fatty acids (VFAs) present in the sludge anaerobic digesters increases when sludge is thermally pretreated [31] and, on the other hand, heterocyclic N compounds are present among the dissolved organic compounds generated during the THP and they are not degraded during anaerobic digestion [32]. Then, their entrance into the PN reactor is expected. Literature reports that of both VFAs [33] and heterocyclic N compounds [34] cause a strong decrease in AOB activity but they are biodegradable under aerobic conditions [35] which would conform to the inhibitor(s) profile found in the tests carried out in this work. In this sense, Zhang et al. [9] found that when the operation of an anaerobic digester fed with thermally pretreated sludge became unstable, the inhibitory effect of its filtrated effluent on AOB activity increased. Initially, they attributed this increase to a higher presence of acetate but they found that acetate did not contribute to inhibition and concluded that AOB inhibition was caused by other biodegradable dissolved organic compounds.

To sum up, in this research work it was possible to achieve stable PN system treating undiluted THP/AD with high ALR (4.8 g NH_4^+-N/(L·d)), in the range of the ALR applied in systems for treating the AD dewatering liquor and higher than the reported values for THP/AD dewatering liquors. NOB inhibition was maintained by the operational conditions themselves, mainly by the large FNA concentrations reached in the liquid media, and no control on the SRT and/or DO level was required. Moreover, as a novelty, if a two stage N removal system is used inhibitory effects caused by the THP/AD dewatering liquors can be removed by the PN reactor itself.

3.3. Anammox reactor performance

Zhang et al. [9] found that the thermal pretreated effluent from a sludge anaerobic digester at a concentration of 25% caused a decrease of the specific activity of the anammox bacteria between 54 and 100%. These authors attributed such inhibition to soluble organic compounds associated with the no biodegradable fraction since neither biological treatment nor removal of both particulate and colloidal organic matter fractions were able to decrease this inhibitory effect. To test these possible inhibitory effects, the effluent coming from the PN reactor was treated by an anammox reactor and its operational stability was monitored. The system was operated at an average inlet NLR of 0.45 g N/(L·d) (Figure 6). Initially, the anammox reactor was fed with the effluent from the PN reactor at a concentration of 50% and operated at a HRT of 2 days (1-10 days). Later on (11-44 days), the anammox reactor was fed with undiluted effluent from the PN reactor and a HRT of 4 days was imposed. The system showed a stable performance during the whole operational period and its average N removal efficiency was of 72%. The inlet nitrite mainly limited this efficiency since the NO_2^--N/NH_4^+-N ratio of the PN reactor effluent was of 1.02 g N/g N, lower than the stoichiometric one of 1.32 required for the anammox process. It should be noted that the overall N removal efficiency of the PN/A system could be improved by adding bicarbonate to the PN reactor to increase the alkalinity to nitrogen ratio (from 3.5 to values of 4.0 g $CaCO_3$/g NH_4^+-N) necessary to obtain the desired outlet NO_2^--N/NH_4^+-N ratio [25].

According to the results obtained, once the THP/AD dewatering liquor is treated by means of the PN system, no inhibitory effects on the anammox biomass were observed. This agrees with those results obtained by Zhang et al. [25] and Xu et al. [20], who successfully operated anammox systems, fed with THP/AD dewatering liquors previously treated by a PN reactor, at NLRs of 0.63 and 1.44 g N/(L·d) with N removal efficiencies of 91 and 87%, respectively. The latter authors observed that the entrance of micro-sized suspended organic flocs coming from the PN reactor caused an unstable operational episode due to their adsorption on the surface of the granular anammox biomass, but the operational performance of the anammox reactor was restored by washing these flocs with tap water.

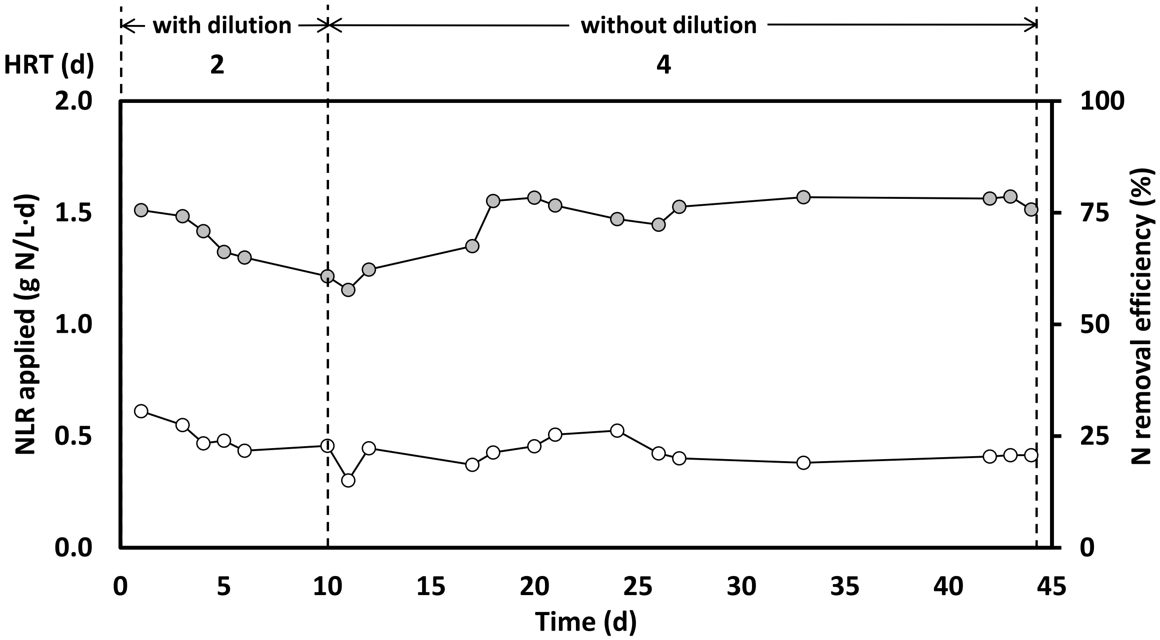


Figure 6. Anammox reactor performance: NLR applied (○) and N removal efficiency (•).

Gu et al. [36] compared the treatment of both AD dewatering liquor and THP/AD dewatering liquor (with added nitrite) by the anammox process. These authors found that, during short-term assays, the effluent at a concentration of 5% caused a 28% decrease of anammox activity in the case of the thermally pretreated effluent, but no effect was found when the no preteated effluent was tested. They observed similar results during long-term assays since the anammox system fed with undiluted AD dewatering liquors was able to maintain its operational stability, while the anammox reactor performance failed when it was fed with 70% diluted THP/AD dewatering liquor. These authors attributed such results to the high content of organic carbon in the thermally pretreated effluent, which caused the overgrowth of denitrifying bacteria, outcompeting anammox bacteria. Of course, if the effluent is previously treated in a PN system the entrance of biodegradable organic matter into the anammox reactor would be very low and, therefore, no inhibitory effects would be expected.

3.4 One single stage or two stages system?

Jaroszynski and Oleszkiewicz [37] showed that one single stage systems were able to treat overall NLRs (0.6 g N/L·d) higher than two stage systems (0.4 g N/L·d) when they were fed with AD dewatering liquors. It should be noted that this study was based on considering the use of a SHARON system, whose treating capacity is around 0.4 g N/(L·d), to carry out the PN process. Such low NLR penalizes the overall treatment capacity of the two stages system, although anammox reactors are able to treat a NLR of 7.5 g N/(L·d) [14]. However, different research works showed that SBR systems are suitable to carry out partial nitritation at NLRs higher than those achieved by the SHARON systems [38]. In the present research work, a NLR of 4.8 g N/(L·d) was treated by the PN reactor under stable conditions without the application of a dilution to the THP/AD dewatering liquor. Taking into account a treating capacity of 7.5 g N/(L·d) for a full scale anammox reactor, the overall NLR of the two stages system would be 2.9 g N/(L·d), which is almost five times the average NLR treated by one single stage systems [8]. This fact supposes that the overall reactors volume requirement in the two stages configuration would be lower, but the use of two units instead of one could negatively influence on the capital costs. For this reason, capital costs needed to implement, at full scale, one single stage and two stages systems, with and without an aerobic pretreatment to remove biodegradable organic matter, were calculated in this research study for WWTPs of different capacities.

As can be seen in Figure 7, when aerobic pretreatment is not applied, capital costs of the one single stage system are always higher than that of the two stages system. According to the results presented by Driessen et al. [13], if the THP/AD dewatering liquor is aerobically pretreated, one single stage systems can be designed to treat a NLR of 1.7 g N/(L·d). Therefore, the application of this aerobic pretreatment would suppose the decrease of the investment costs related to the one single stage system but the necessity of implementing an aerobic system to remove the organic matter. In this sense, Zhang et al. [9] proposed to overcome the inhibitory effects by changing operating parameters of the existing processes, such as optimizing the performance of the dewatering system or increasing the DO level inside the PN/A reactor since the implementation of an additional unit process for pretreating the THP/AD dewatering liquor could not be practically feasible or cost-effective. According to the economic analysis carried in the present research work, the implementation of such pretreatment systems would be only advisable for WWTPs with capacities higher than a population of $5 \cdot 10^5$ inhabitants equivalent (Figure 7). If an aerobic pretreatment is combined with a two stages system, to avoid instability episodes of the PN reactor, the overall capital costs would be similar to those of the treatment train consisting of an aerobic pretreatment and a one single stage system (Figure 7).

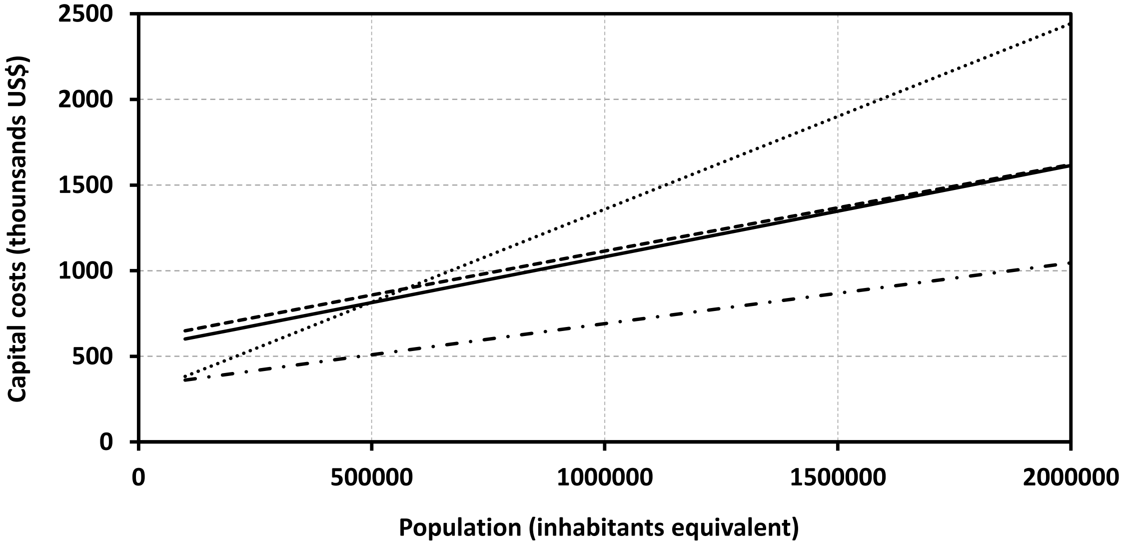


Figure 7. Capital costs of the implementation of one stage and two stages systems to remove nitrogen from the THP/AD dewatering liquors (……… one single stage system; — · — two stages

system; —— aerobic pretreatment + one single stage system; ---- aerobic pretreatment + two stages system).

With regards to the operating costs, generally, when a one stage system is applied to treat effluents of anaerobic sludge digesters, a fraction of the inlet organic matter is removed by denitrification due to the low DO level inside the system. This implies oxygen savings and the improvement of the effluent quality [39]. However, in the case of treating THP/AD dewatering liquors, DO concentration is increased to avoid operating problems and, therefore, inlet organic matter would be mainly oxidized by oxygen instead of nitrate [9]. Taking into account this fact, it would be expected that operating costs are similar in all configurations studied since organic matter would be mainly removed by heterotrophic biomass under aerobic conditions and nitrogen by PN and anammox processes.

In one single stage systems, operational stability is achieved through strict control of the DO level and pH value to prevent the growth of NOB [10]. Sludge thermal pre-hydrolysis brings an increase of the organic matter supplied to the PN/A system, causing the proliferation of heterotrophic bacteria that can compete with AOB for oxygen and with anammox bacteria for nitrite. Since both AOB and anammox bacteria have lower specific growth rates than heterotrophic bacteria, selective biomass purge strategies should be established to allow a balance between the growth of AOB and anammox and the wash-out of unwanted heterotrophs [8]. In this sense, the operational robustness of the two stages systems would be greater than that of the one single stage systems since, on one hand the organic matter is mainly removed in the PN reactor, minimizing heterotrophic denitrification in the anammox reactor and, on the other hand, the possible negative effect of organic matter in the PN reactor could be compensated by operating at high DO levels without taking the risk of promoting the NOB growth or inhibiting the anammox bacteria, as occurs in one single stage systems.

4. Conclusions

The PN reactor start-up strategy based on feeding the undiluted THP/AD effluent and gradually decreasing the HRT allowed to obtain a stable reactor performance. This shows that it would not be necessary to apply dilution water to this type of effluents as is usually done at full scale, which would reduce treatment costs.

Contrary to one single nitrogen removal systems, the PN reactor can be operated under no limited conditions of both solids retention times and DO levels since the proliferation of NOB is avoided by the FNA concentrations achieved inside the reactor themselves. This allowed to treat ALRs up to 4.8 g NH_4^+-N/(L·d).

The destabilization episodes of the PN reactor were related to the application of excessive sALR and sOLR, higher than 0.6 g NH_4^+-N/g TSS·d and 0.7 g COD/ g TSS·d, respectively. This suggests that monitoring of nitrogen removal systems treating THP/AD effluents should carried out in terms of specific loading rates instead of volumetric loading rates as it is generally reported in literature.

The inhibitory effect caused by the organic compounds present in the THP/AD dewatering liquor can be removed by the PN reactor itself and no implementation of a pretreatment unit would be necessary if two stages N removal systems are used.

The economic analysis shows the convenience of using two stages systems instead of one single stage systems to treat THP/AD dewatering liquors. In the case of applying a one single stage system for this purpose, the implementation of an aerobic pretreatment unit is economically advisable for WWTPs capacities higher than $5 \cdot 10^5$ inhabitants equivalent.

The implementation of two stage systems instead of the currently used one single stage systems is an economic viable alternative to maintain stable and efficient N removal at WWTPs sludge line when sludge thermal hydrolysis units are applied to improve their energetic efficiency.

Acknowledgments

This research was funded by the Chilean Government through the Projects ANID/FONDECYT/1200850 and CRHIAM Centre grant number ANID/FONDAP/15130015 and by the Spanish Government through TREASURE [CTQ2017-83225-C2-1-R] and GRANDSEA [CTM2014-55397-JIN] projects. The authors from Universidade de Santiago de Compostela belong to CRETUS Strategic Partnership [ED431E 2018/01] and the Galician Competitive Research Group [GRC ED431C 2017/29]. All the Spanish programs are co-funded by FEDER (EU).

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