Revista Ambiente & Água
ISSN: 1980-993X
Instituto de Pesquisas Ambientais em Bacias Hidrográficas

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Revista Ambiente & Água, vol. 13, no. 2, 2018

Instituto de Pesquisas Ambientais em Bacias Hidrográficas

Available in: http://www.redalyc.org/articulo.oa?id=92860561015

DOI: 10.4136/ambi-agua.2174

ARTICLES

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Efeito das diferentes condições operacionais nas rotas de remoção do nitrogênio total e emissões de óxido nitroso em um sistema de lodos ativados em escala de bancada

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Revista Ambiente & Água, vol. 13, no. 2, 2018

Instituto de Pesquisas Ambientais em Bacias Hidrográficas

Received: 23 August 2017
Accepted: 20 January 2018

DOI: 10.4136/ambi-agua.2174

Abstract: This study sought to determine the effects of different operating conditions, such as variable organic loading, different sludge retention times (SRTs) and airflow rates, limited dissolved oxygen (DO) concentrations and ammonium (NH_4^+) shock loading on total nitrogen (TN) removal routes and nitrous oxide (N_2O) emissions in a lab-scale activated sludge system. Short SRT (5 days) combined with very low DO levels (0.5 mg L^{-1}) were responsible for lower TKN oxidation efficiencies and, consequently, negligible NO_2^- accumulation rates. These results suggest that nitrification efficiency was hampered by the oxidation of organic matter, with a large part of TN removed by sludge waste process. As the SRT increased (from 5 to 10 days) and DO was set to 1.0 mg L^{-1}, TKN oxidation rates and NO_2^- accumulation reached their maxima, which are thought to be the optimal conditions for both organic matter oxidation and partial nitrification. Under these conditions, gas transfer to the atmosphere became the preferential route for TN removal instead of incorporation into the sludge waste. However, N_2O contribution is estimated as less than 5.6% (with respect to TN in the influent). Insufficient aeration and stress conditions (such as NH_4^+ shock loading) can cause limited DO conditions and NO_2^- accumulation, leading to higher amounts of emitted N_2O. Therefore, the adequate control of DO concentrations is a key factor to avoid NO_2^- accumulation and consequently high N_2O emissions.

Keywords: activated sludge, dissolved oxygen, nitrite accumulation rate, nitrogen removal routes, nitrous oxide emission.

Resumo: O principal objetivo deste estudo foi determinar os efeitos das diferentes condições operacionais, tais como carga orgânica variável, diferentes idades do lodo e taxas de aeração, limitação das concentrações de oxigênio dissolvido (OD) e choque de carga de amônio (NH_4^+), nas rotas de remoção do nitrogênio total (NT) e nas emissões de óxido nitroso (N_2O) provenientes de um sistema de lodos ativados em escala de bancada. Idades do lodo reduzidas (5 dias) combinadas com baixas concentrações de OD (0,5 mg L^{-1}) foram responsáveis por uma baixa eficiência de oxidação do NT Kjeldahl (NTK) e, consequentemente, negligenciáveis taxas de acúmulo de nitrito (NO_2^-). Esses resultados sugerem uma perda na eficiência da nitrificação completa em razão do maior comprometimento do OD com à oxidação da matéria orgânica, com a maior parcela do N removida pela incorporação ao lodo excedente. O aumento da idade do lodo (de 5 para 10 dias) combinada com o aumento da concentração de OD para 1,0 mg L^{-1}, levaram ao alcance das taxas máximas de oxidação do NTK e de acúmulo de NO_2^-, o que representou uma condição ótima para ambos os processos de oxidação da matéria orgânica e nitrificação incompleta. Sob essas condições, a transferência de gás para a atmosfera tornou-se a rota preferencial de remoção do NT. No entanto, a contribuição do N_2O foi estimada em até 5,6% da carga de NT afluente. Condições de stress (choque de carga de NH_4^+) e aeração insuficiente podem causar períodos de limitação de OD e acúmulo de NO_2^-, podendo levar assim à maiores emissões de N_2O. Portanto, o controle adequado das concentrações de OD é o fator chave para evitar o acúmulo de NO_2^- e, consequentemente, maiores emissões de N_2O.

Palavras-chave: emissão de óxido nitroso, lodo ativado, oxigênio dissolvido, rotas de remoção de nitrogênio, taxa de acúmulo de nitrito.

1. INTRODUCTION

The biological pathway for N removal in WWTPs is nitrification, followed by denitrification processes (Wrage et al., 2001; Law et al., 2012). The activated sludge process can provide optimal conditions for the conversion of ammonium (NH_4^+) to nitrate (NO_3^-) (complete nitrification: $NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$), especially in tropical regions (Wrage et al., 2001). However, to combine both processes, the design and operation of a traditional WWTP must be altered (i.e. an anoxic zone and internal recirculation systems must be added) to allow for the complete denitrification process, the key process for the effective removal of N as molecular nitrogen (N_2) ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) (Wrage et al., 2001). Another route for N removal is by the sludge waste process (Lee et al., 2008; Bernat et al., 2011). Recently, several studies have addressed N removal processes under many controlled operating conditions, such as hydraulic retention time (HRT), sludge retention time (SRT), dissolved oxygen (DO) concentrations and organic loading rates, among others (Bernat et al., 2011; Rasool et al., 2014; Xiang et al., 2014; Lu et al., 2015). However, only some of these studies calculated the total N (TN) balance (Lee et al., 2008; Bernat et al., 2011).

WWTPs with biological N removal (BNR) are an effective way to decrease the discharge of oxidized N forms (as NO_3^-) into water bodies and thus prevent eutrophication. On the other hand, nitrous oxide (N_2O) emissions from WWTPs are an issue of international concern and should also be taken into account (Keller and Hartley, 2003;

IPCC, 2006). Currently, wastewater treatment systems are thought to contribute with about 10% of total anthropogenic N_2O emissions, when including both manure and sewage treatments (Desloover et al., 2012). N_2O is one of the most important substances from an environmental point of view, since it is linked to global warming and climate change. It is a greenhouse gas with a global warming potential 310 times that of carbon dioxide (CO_2), contributing approximately 7% to overall greenhouse gases (IPCC, 2013). In addition, N_2O is indirectly responsible for the depletion of stratospheric ozone (O_3) by its reaction with excited oxygen atoms and nitric oxide (NO) production: $N_2O + O(^1D) \rightarrow 2\ NO$ and $NO + O_3 \rightarrow NO_2 + O_2$ (Crutzen, 1979; Ravishankara et al., 2009). Ravishankara et al., 2009 believe that N_2O will become the major dominant ozone-depleting substance before the end of this century.

During nitrification and denitrification processes, respectively, N_2O is formed as a by-product of NH_4^+ oxidation to nitrite (NO_2^-) and as an intermediate of the reduction of oxidized N forms to N_2 (Wrage et al., 2001). Generally, there is no single N_2O emission mechanism from WWTPs and the pathways related to its production are dependent on the WWTP design and closely related to operating conditions (Ahn et al., 2011; Hu et al., 2013). Nitrification is reported as the main source of N_2O emission under DO limitations and, consequently, high NO_2^- concentrations. However, the denitrification process can contribute significantly to higher N_2O emissions under an insufficient organic carbon source (C/N ratio) with high NO_2^- concentrations (Kampschreur et al., 2009; Law et al., 2012). Thus, the operating conditions that lead to the accumulation of NO_2^- concentrations are subject to high N_2O emissions. Therefore, further studies are required to elucidate the importance of controlling operational conditions in order to reduce NO_2^- concentrations and thus mitigate N_2O emissions (Desloover et al., 2012).

In this context, this study sought to determine the effects of different operating conditions, such as variable organic loading, different SRTs and airflow rates, limited DO concentrations and NH_4^+ shock loading, on TN removal routes and N_2O emissions in a lab-scale activated sludge system.

2. MATERIALS AND METHODS

2.1. Lab-scale system and regular operating condition

A continuous lab-scale reactor (total volume = 20 L) consisting of four adjacent and interconnected chambers (V = 5 L) was monitored over 212 days (Figure 1). For the first 135 days, the lab-scale system functioned with some small variations of load (denoted as regular operating condition) to promote the acclimatization of the biomass to the synthetic wastewater. These some small variations occurred as a function

of the storage conditions of the synthetic sewage used to supply the reactor. The first chamber (C1; average DO < 0.1 mg L^{-1}) was used as a biological selector (with pre-denitrification) and a mixing zone of the synthetic wastewater and biological sludge that returns from the settler (V = 2 L). The other three chambers (V = 15 L) were aerated so as to maintain DO concentrations near 2.0 mg L^{-1}. During this period, HRT and SRT were maintained at 12 hours and 10 days, respectively. The continuous flow rate of synthetic wastewater was 1.7 L h^{-1}. Sludge recycling was performed by a peristaltic pump, pumping the biological sludge from the settler to the anoxic chamber (C1), at a flow of 1.7 L h^{-1}. Internal recirculation was present, in order to allow for the return of NO_2^- and NO_3^- to the reactor, at a flow rate of 3.7 L h^{-1}.

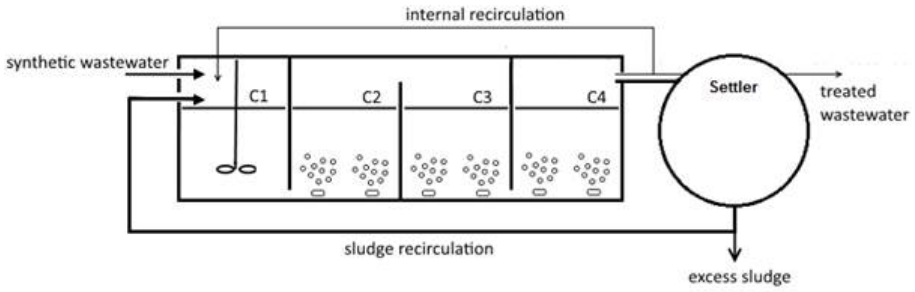


Figure 1.
Schematic design of the lab-scale activated sludge system.

2.2. Operating conditions to determine the distribution of the routes of TN removal

To determine the relative distribution of the TN removal routes (sludge and atmosphere) under different operating conditions, the reactor was submitted to a two-phased experimental condition (Phases 1 and 2), between the 135th and the 212nd monitoring days, with different SRTs, limited DO concentrations (in C2-C4) and variable organic loading. Phase 1 was carried out between the 135th and the 169th day, under a SRT of 5 days and DO concentration of 0.5 mg L^{-1}, and Phase 2, between the 169th and the 212nd day, under a SRT of 10 days and DO concentration of 1.0 mg L^{-1}. The SRT was maintained for 5 or 10 days by controlling sludge wastage and monitoring the concentration of volatile suspended solids (VSS) in the reactor and the sludge recirculation line. In both phases, the HRT was maintained at 12 hours. The first 19 days of Phase 1 were considered an adaptation period (from the 135th to the 154th day), and the same was set for the first 21 days of Phase 2 (from the 170th to the 191st day). The process was performed at room temperature, at approximately 25°C.

2.3. Operating conditions to determine the magnitude of N 2 O emissions

After conducting the experiment described previously (item 2.2.), the system was discontinued due to problems in its three peristaltic pumps used for the input of synthetic wastewater, sludge recirculation and internal recirculation. With the acquisition of new pumps (Masterflex), the continuous lab-scale system was restarted for a new 80-day experiment. The system was operated under the same operating condition as Phase 2 (SRT of 10 days and DO concentrations near 1.0 mg L^{-1}). The purpose was to achieve the accumulation of NO_2^- concentrations, and, after the 72nd monitoring day, to determine the magnitude of N_2O emissions (and the $N_2O/N_{atmosphere}$ ratio) under different operating conditions, such as variable organic loading and different air flow rates applied to promote different DO levels (in C2-C4) (and NO_2^- concentrations) in the reactor. Four experimental conditions (EC) were performed varying the air flow rates, as follows, in chronological order: 200 mL min^{-1} (EC 1: 74th day), 50 mL min^{-1} (EC 2: 76th day), 300 mL min^{-1} (EC 3: 78th day) and 400 mL min^{-1} (EC 4: 80th day). The stabilization period for each EC was of 24 hours.

2.4. Biological sludge and wastewater composition

The biological sludge used in this work was collected from a WWTP and the reactor was fed with the synthetic wastewater. The synthetic wastewater was prepared mixing casein peptone (320 mg L^{-1}), meat extract (220 mg L^{-1}), urea (60 mg L^{-1}), potassium monohydrogen phosphate (56 mg L^{-1}), sodium chloride (14 mg L^{-1}), calcium chloride dihydrate (8 mg L^{-1}) and magnesium sulfate heptahydrate (4 mg L^{-1}) (Holler and Trösch, 2001). The pH of the synthetic wastewater ranged between 7 and 8. Variations in chemical oxygen demand (COD) and TN concentrations were obtained by increasing or decreasing the amount of substances, like casein peptone, meat extract and urea, in the synthetic wastewater.

2.5. Sampling and analytical procedures

Table 1 displays the sampling strategy used for the different operating conditions of the continuous lab-scale system. Liquid samples of synthetic and treated wastewaters were regularly collected to determine COD, TKN and TN concentrations. Liquid and gas samples from the four chambers were collected during the experimental operating conditions to determine the relative distribution of TN removal routes (Phases 1 and 2) and the magnitude of N_2O emissions (ECs 1-4). Liquid samples were filtered through 0.22 μm cellulose acetate membrane filters for the determination of dissolved inorganic nitrogen (DIN = NH_4^+, NO_2^-

and NO_3^-) forms. In addition, VSS and TN sampling and analyses were carried out in the reactor and sludge recycler. The DO concentrations in the reactor were directly measured by a multiparameter Hanna portable meter (Model HI9828).

Gas samples were collected every 15 minutes, totaling 180 minutes per monitoring day (between the 74th and the 80th days), covering the four chambers of the lab-scale reactor for the determination of the N_2O emissions. A modified upturned funnel was used as the gas sampling technique (on a lab-scale). This same technique has been applied by our group in full-scale WWTP studies (Mello et al., 2013; Ribeiro et al., 2015; Brotto et al., 2015). The N_2O emission rate (ER) for the reactor was calculated by multiplying the ΔN_2O concentration, which is the difference between the upturned funnel headspace and the atmospheric N_2O concentrations, by the emerging airflow rate (Q_{air}), as displayed in Equation (1). The latter was measured at the surface of the reactor using the upturned funnel and a digital rotameter, and the result was scaled up for the entire reactor.

(1)

Table 1.
Sampling strategy for the different operating conditions.

Samples	Items 2.1 and 2.2		Item 2.3	
	(0-135th day)[a]	(135-212nd day)[b]	(0-72nd day)[c]	(74-80th day)[d]
Synthetic wastewater	COD and TN	COD, TKN and TN	TKN and TN	TKN and TN
Lab-scale reactor	DO and VSS	DIN, DO and VSS	DIN, DO and VSS	DIN, N_2O, DO and VSS
Sludge excess	VSS	TN and VSS	VSS	TN and VSS
Treated wastewater	COD and TN	COD, TKN and TN	TKN and TN	TKN and TN

aRegular operating condition (no experiments).
bOperating conditions to determine the distribution of the TN removal routes (Phases 1 and 2).
cSame operating condition as Phase 2.
dOperating conditions to determine the magnitude of N2O emissions (EC 1-4).

In the laboratory, N_2O determinations were performed on a Shimadzu gas chromatograph (Model GC-17) equipped with an electron capture detector with ^{63}Ni source. The limits of detection and quantification were of 30 and 300 ppb, respectively. All analytical procedures followed APHA et al. (2012) protocols. COD, TKN and TN were determined using the closed reflux colorimetric, block digestion and direct persulfate digestion methods, respectively. VSS concentrations were determined by the gravimetric method. NH_4^+ concentrations were measured using an ammonia ion-selective electrode method coupled to an Orion pH-meter (Model Star 5). NO_2^- and NO_3^- concentrations were determined by ion chromatography with chemical suppression of eluent conductivity on a Methrohm ion chromatograph (Model 790 Personal). The limits of quantification were of 0.03 mg L^{-1} for NH_4^+, 0.1 mg L^{-1} for NO_2^- and

NO_3^-, and 10 mg N L^{-1} for TN. The analytical precision of the analyses performed in triplicate was within ± 5%.

3. RESULTS AND DISCUSSION

3.1. Regular operating condition

During the first 135 monitoring days, influent COD and TN concentrations (and volumetric COD and TN loads) varied from 447 to 1054 mg L^{-1} (0.5 and 1.3 kg m^{-3} day^{-1}) and from 59 to 130 mg L^{-1} (0.1 and 0.2 kg m^{-3} day^{-1}), respectively. The average COD and TN removal efficiencies (± standard deviation) were 92% (± 1.4%) and 57% (± 6.6%), respectively. Using a lab-scale sequential batch reactor (SBR) system with fixed low volumetric COD load (0.4 kg m^{-3} day^{-1}), Lee et al. (2008) found COD and TN removal efficiencies of 92% and 65%, respectively. Similar values were reported by Vaiopoulou et al. (2007) for COD (74-97%), although their TN removal efficiency was somewhat higher, around 70%. They operated a differential lab-scale activated sludge system with a denitrification cascade and worked with a wide range of influent volumetric COD loads applied to the system (0.3 to 2.0 kg m^{-3} day^{-1}). Vaiopoulou et al. (2007) reported that the higher influent flow rate distribution to the reactor zones (denitrification cascade) explained the higher TN removal rates, in contrast to our study, that operated only with pre-denitrification.

The variability of the organic loading did not have any effect on COD removal efficiency, although lower TN removal efficiencies were related to lower influent TN concentrations. Between the 9th and the 21st monitoring day, influent TN concentrations dropped to about 30%, resulting in a decrease in TN removal efficiency, from 63 to 48% (Figure 2A). The same happened between the 55th and the 76th monitoring day, when the influent TN concentration dropped about 40% and TN removal efficiency decreased from 67 to 41%. These results indicate that significant decreases in influent TN concentrations result in decreasing TN removal efficiencies, given that TN concentrations and its removal efficiencies are positively correlated ($r = 0.68$; $n = 10$; $p < 0.01$) (Figure 2B). Liu et al. (2012) observed losses in TN removal efficiency when applying lower volumetric NH_4^+ loads from a SBR using a completely autotrophic nitrogen-removal via nitrite (CANON) process. On the other hand, Zhang et al. (2014) demonstrated that maximal TN removal efficiency (90%) was obtained in a lab-scale sequencing batch biofilm reactor (SBBR) after 132 days of operation, where the volumetric TN load was gradually increased from 0.08 to 0.6 kg m^{-3} day^{-1}.

3.2. The relative distribution of TN removal routes

The fate of the removed TN was determined based on mass flow rates of the influent wastewater, liquid effluent and sludge waste. TN removal via mass transfer to the atmosphere was calculated by the difference between the influent TN load and TN removal via liquid effluent and sludge waste, as displayed in Equation 2.

(2)

Figure 3 shows the relative distribution of TN loss (and removal) calculated for the three different outlet routes from the 154th to the 169th day (Phase 1) and from the 191st to the 212nd day (Phase 2). The average TN removal efficiency of the system in the Phase 1 was of approximately 50%, of which about 40% was incorporated by the sludge waste and 10% released to the atmosphere. This result can be explained by the short SRT (5 days) and very low DO levels (DO = 0.5 mg L-1). Short SRT can lead to an increased net sludge production rate and, consequently, higher TN removal by biomass production. During this phase, the net sludge production rate increased from 9 to 13 g VSS day-1 under high volumetric COD (on average:1 kg m-3 day-1) and TN (on average: 0.1 kg m-3 day-1) loading conditions. Bernat et al. (2011) reported that a high biomass production was responsible for 16-26% of the TN removed in a SBR at limited DO concentrations (< 0.7 mg L-1) and that these values were the result of a low specific biomass decay rate. In addition, Lee et al. (2008) reported that 50% of the influent TN in a SBR was removed by the sludge waste process.

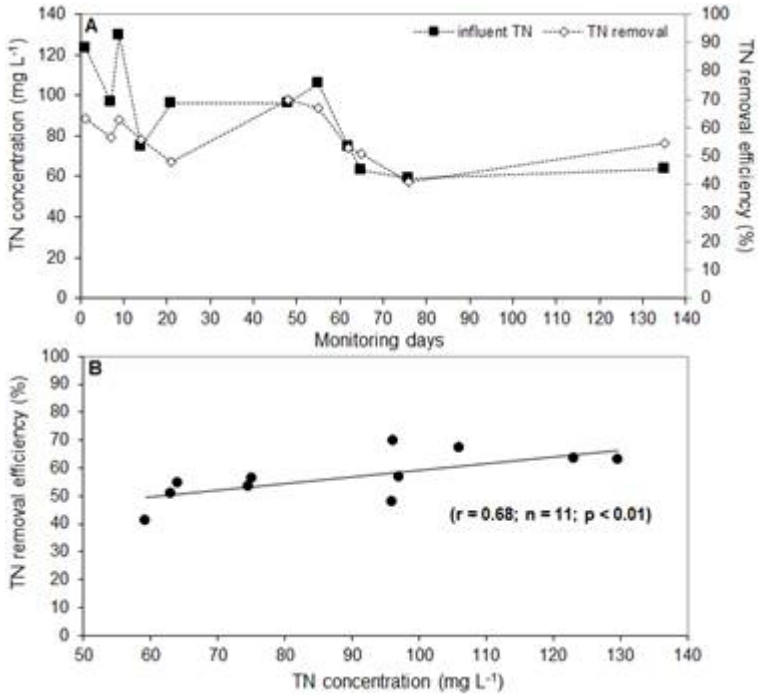


Figure 2.

(A) Influent TN concentrations (mg L^{-1}) and TN removal efficiencies (%) of the lab-scale activated sludge system. (B) Correlation between TN removal efficiencies and influent TN concentrations.

During Phase 2, the average TN removal efficiency was approximately 60% (Figure 3). Throughout this phase, TN removal via mass transfer to the sludge waste decreased from 40 to 15%. Concomitantly, the fraction transferred to the atmosphere increased from 10 to 50%. This can be explained by the increasing SRT (from 5 to 10 days) and DO levels (from 0.5 to 1.0 mg L^{-1}). Longer SRT can lead to decreases in the sludge mass by endogenous metabolism and, consequently, lower TN removal by biomass production. During this period, the net sludge production rate decreased from 13 to 6 g VSS day^{-1} even under the same volumetric COD and TN loadings applied to Phase 1.

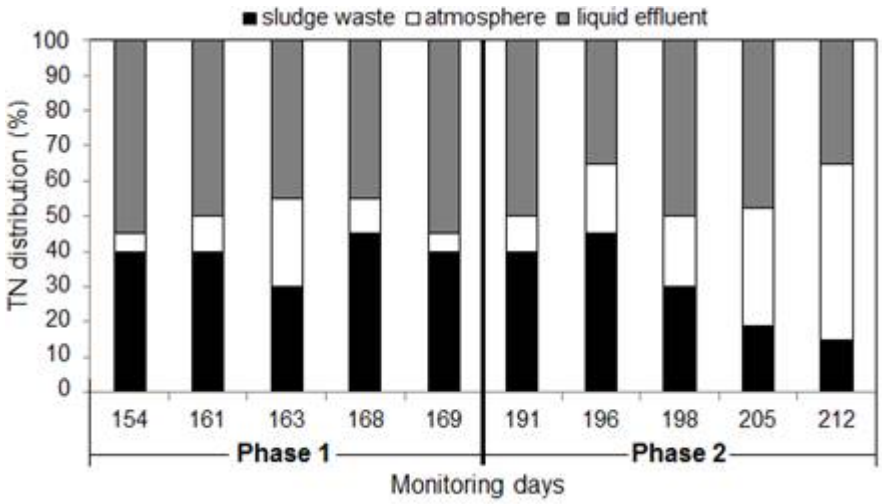


Figure 3.
TN distribution (%) (regarding TN in the influent) in the different system lines (sludge waste, atmosphere and liquid effluent).

Figure 4 displays the effects of DO limitation on DIN concentrations during Phases 1 and 2. NH_4^+ was the predominant form of N in the reactor during Phase 1 (Figure 4A), due to lower TKN oxidation efficiencies (40-60%) (Figure 4B). Consequently, NO_2^- accumulation rates (NAR) were below 15% when the airflow rate was set to maintain DO levels close to 0.5 mg L^{-1}, with a SRT of 5 days. For the calculation of NAR the equation cited by Wei et al. (2014) was used. These results suggest loss of nitrification efficiency with no accumulation of NO_2^-, since DO was restricted by organic matter oxidation. Therefore, the operational condition applied in Phase 1 was characterized by loss of nitrification activity, which explains the highest TN removal efficiencies by the sludge waste (Figure 3).

During Phase 2, NH_4^+ concentrations dropped below 10 mg L^{-1}, with NO_2^- as the major form of DIN after the 200th monitoring day (Figure 4A). The NAR then began to prevail and increased up to 80%, with TKN oxidation reaching efficiencies above 80% (Figure 4B). At DO set near 1.0 mg L^{-1} with a SRT of 10 days, this phase showed optimal conditions for both organic matter oxidation and partial nitrification. Ruiz et al. (2003) reported that around 65% of the influent TN load was converted into NO_2^- in a lab-scale activated sludge reactor, operating at DO around 0.7 mg L-1. These authors reported that when DO was below 0.5 mg L^{-1}, NH_4^+ accumulated in the system. The effect of increasing DO concentrations (from 0.5 to 1.0 mg L^{-1}) seems to explain the accumulation of NO_2^-, since Pollice et al. (2002) reported that NO_2^- accumulation took place regardless of the SRT during oxygen-limiting conditions (DO < 2 mg L^{-1}) in a lab-scale activated sludge reactor. Therefore, in conditions that favor partial nitrification, it is probable that the priority route of nitrogen removal will be through the atmospheric path (Figure 3).

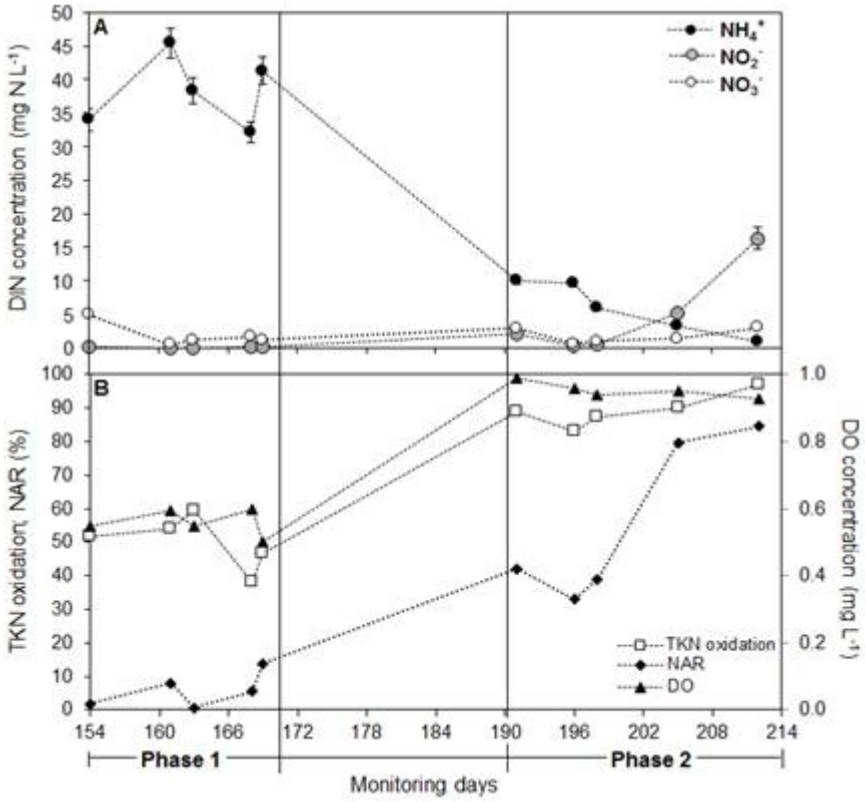


Figure 4.

(A) Average DIN (NH_4^+, NO_2^- and NO_3^-) concentrations (mg N L^{-1}) in the three aerobic chambers, (B) TKN oxidation (%), NO_2^- accumulation rates (NAR) (%) and DO concentrations (mg L^{-1}) during the 154th to 212th monitoring days in the lab-scale reactor.

3.3. Under the same operating condition as Phase 2

In order to achieve NO_2^- accumulation, the system was restarted and operated under the same conditions as Phase 2. During the first 37 days, NH_4^+ concentrations ranged from 5 to10 mg L^{-1} in the reactor (Figure 5A) with high TKN oxidation efficiencies (80-95%) (Figure 5B). These values are similar to those found in Phase 2, displayed in Figure 4. On the other hand, NO_3^- was the predominant form of N in the reactor, with low NO_2^- concentrations (< 0.9 mg N L^{-1}) (Figure 5A). These results suggest favorable conditions for the completion of the nitrification process (and negligible NAR), since DO concentrations were above 1.0 mg L^{-1} (ranging from 0.9 to 1.8 mg L^{-1}) (Figure 5B). However, when DO concentrations dropped to ≤ 1 mg L^{-1} (ranging from 0.7 to 1.1 mg L^{-1}), NO_2^- became the main form of oxidized N in the reactor, without loss of TKN oxidation efficiency. Interestingly, NH_4^+ concentrations were remained constant throughout the last monitoring days, especially between the 37th and the 72nd day. In addition, NAR increased from 0 to 90% and NO_3^- concentrations were low (< 1 mg N L^{-1}). These results indicate the transition from complete to partial nitrification, and, thus,

the greater fraction of N was transferred to the atmosphere, as observed previously in Phase 2 (Figure 3).

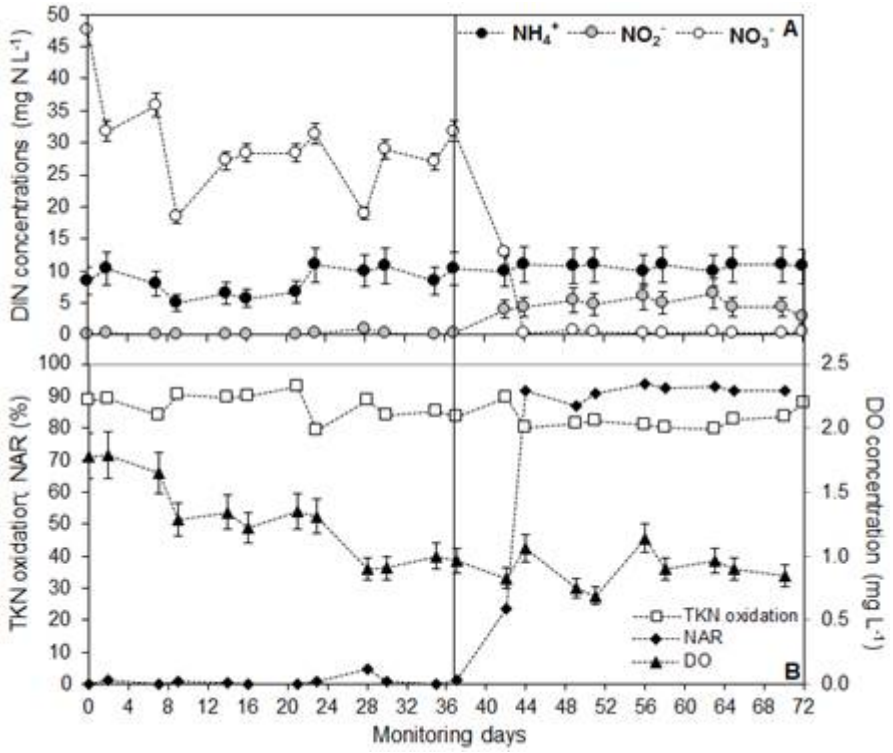


Figure 5.

(A) Average DIN (NH_4^+, NO_2^- and NO_3^-) concentrations in the three aerobic chambers; (B) TKN oxidation (%), NO_2^- accumulation rate (NAR) (%) and DO concentrations (mg L^{-1}) during 72 monitoring days.

3.4. Extent of N 2 O emissions

Figure 6 displays the average DIN concentrations in the three aerobic chambers of the lab-scale reactor (Figure 6A), as well as TKN oxidation and NAR values (Figure 6B) under the different experimental conditions (EC 1-4). In addition, Figure 6C displays the N_2O emission variations and DO concentrations in the four experimental conditions. In EC 1 (Q_{air} = 200 mL min^{-1}), NH_4^+ was the predominant form of N in the reactor, followed by NO_2^- and NO_3^-, respectively, 64% (11 mg N L^{-1}), 30% (5.2 mg N L^{-1}) and 6% (1.1 mg N L^{-1}) (Figure 6A). In addition, TKN oxidation and NAR were 76% and 84%, respectively (Figure 6B). More of the influent TN was converted to NO_2^- (11%) than to NO_3^- (2%). In this experimental condition, DO concentrations varied from 0.9 to 1.1 mg L^{-1} (Figure 6C). These results are similar to those observed by Ruiz et al. (2003), that reported NO_2^- accumulation with DO concentrations close to 0.7 mg L^{-1}. Significant variations in N_2O emissions were observed within the range of 1.4 to 4.1 mg N h^{-1}, representing on average 2.6 mg N h^{-1} and 5.4% when normalized by influent TN load.

Similar $NH4^+$ concentrations (9.6 mg N L^{-1}) and TKN oxidation rates (73%) were observed in EC 2 (Qair = 50 mL min^{-1}) operating at an airflow rate four-fold lower than EC 1 (Figures 6A and 6B). However, NO_3^- (0.6 mg N L^{-1}) and, especially, NO_2^- (0.1 mg N L^{-1}) concentrations were low, and, consequently, with NAR of 14%. In this condition, the DO concentrations were much lower (0.1-0.3 mg L^{-1}) due to the lower airflow rate applied to the system (Figure 6C). Therefore, under anoxic conditions, both NO_2^- and NO_3^- were probably reduced by complete denitrifying activity, without significant N_2O emissions (< 0.5 mg N h^{-1}), which represented, on average, 0.1 mg N h^{-1} and 0.3% of the influent TN load. In a lab-scale system with activated sludge, Wunderlin et al. (2012) reported that N_2O emissions under anoxic conditions are likely to be of minor importance when operated without significant NO2- accumulation (< 2 mg N L^{-1}).

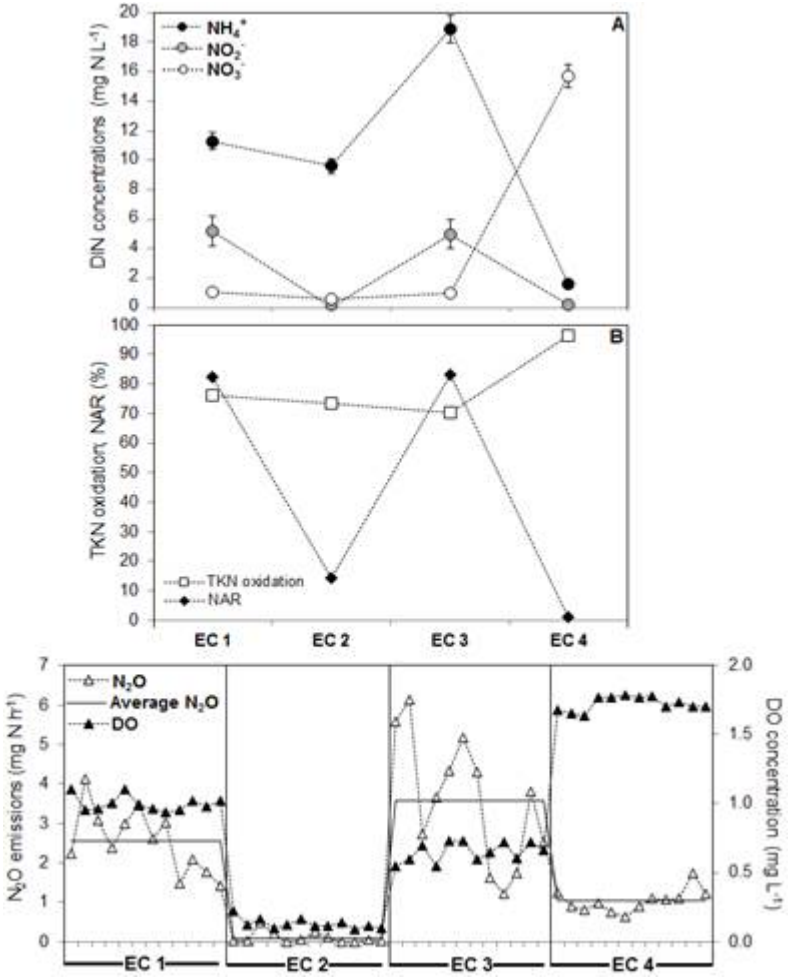


Figure 6.

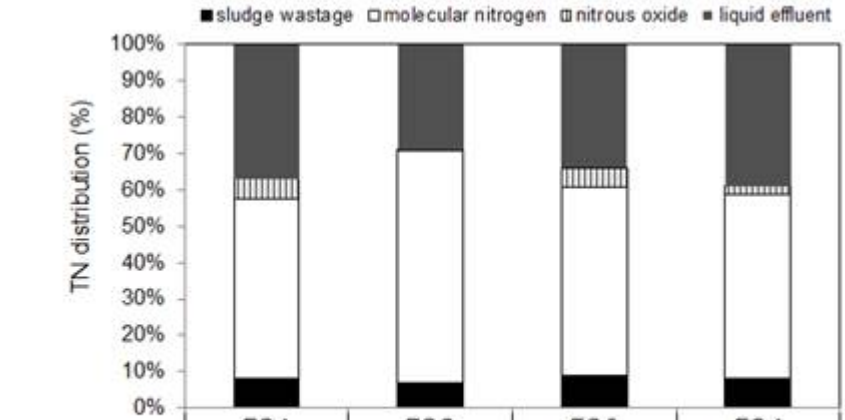
(A) Average DIN (NH_4^+, NO_2^- and NO_3^-) concentrations (mg N L^{-1}) in the three aerobic chambers, (B) TKN oxidation (%) and NO_2^- accumulation rate (NAR) (%) and (C) N_2O emissions (mg N h^{-1}) and DO concentrations (mg L^{-1}) in the lab-scale reactor for each of the four experimental conditions.

In EC 3 (Q_{air} = 300 mL min^{-1}), as in EC 1, more of the influent TN was converted to NO_2^- (8%) than to NO_3^- (2%). Moreover, similar TKN oxidation rates (70%) and NO_2^- accumulation (83%) were observed (Figures 6A and 6B). However, unlike EC 1, the average values of NH_4^+ concentrations for the three aerobic chambers in EC 3 were much higher and linked to higher influent NH_4^+ concentrations (shock loading). Consequently, higher variations in N_2O emissions were observed, within the range of 1.2 to 6.1 mg N h^{-1} (Figure 6C). Nevertheless, this represents an average of 3.6 mg N h^{-1} and 5.6% of the influent TN load, very similar to what was found in EC 1. The NH_4^+ shock loading was responsible for reducing and maintaining DO concentrations close to 0.7 mg L^{-1} (Figure 6C) even under higher air flow rates when compared to EC 1 and EC 2. It could be argued that sudden changes, such as NH_4^+ shock loading, can lead to NO_2^- accumulation (partial nitrification) and higher N_2O emissions, as reported by other authors (Foley et al., 2010; Rodriguez-Caballero et al., 2013; Toor et al., 2015). Ahn et al (2010) suggested that the trigger for N_2O emissions from aerobic zones are simultaneous high NH_4^+ and NO_2^- concentrations, based on studies performed in full-scale BNR and non-BNR processes.

The highest airflow rate to the system was applied in EC 4 (Q_{air} = 400 mL min^{-1}), and a different behavior was observed in relation to the other 3 ECs, since around 35% of the influent TN was converted into NO_3^- (the predominant form of DIN in the reactor). In addition, NH_4^+ (1.6 mg N L^{-1}) and NO_2^- (0.2 mg N L^{-1}) concentrations were low, with 96% TKN oxidation and negligible NAR (Figures 6A and 6B). In this condition, DO concentrations were above 1.5 mg L^{-1} and N_2O emissions varied from 0.6 to 1.8 mg N h^{-1} (on average 1.0 mg N h^{-1} and 2.3% of the influent TN load) (Figure 6C). This low N_2O emission can be attributed to complete nitrification. It could be argued that this experimental condition favors greater DO availability (> 1.5 mg L^{-1}) for organic matter oxidation and complete nitrification and, consequently, low N_2O emissions. In studies performed both in the laboratory and in full-scale processing, Rodriguez-Caballero et al. (2013) and Song et al. (2014), respectively, reported lower N_2O emissions from complete nitrification compared to partial nitrification (with NO_2^- accumulation). Therefore, to reduce N_2O emissions, activated sludge systems should be operated at low NH_4^+ (without shock loading) and NO_2^- concentrations (without build-up) (Ahn et al., 2010; Foley et al., 2010; Wunderlin et al. 2012; Aboobakar et al., 2013). This condition can be achieved through longer SRT, equalization of organic loading and optimal control of airflow rates, depending on the DO concentrations in the reactor.

Figure 7 displays the relative distribution of TN loss (and removal) through three different system routes, (1) sludge waste, (2) atmosphere (via N_2 and N_2O separately) and (3) remaining in the liquid effluent for each of the four experimental conditions. The TN removal efficiencies

of the system were high and ranged from 61 to 71%. A lower fraction of TN was removed by the sludge waste process and was similar in the four evaluated conditions (7-9%). Most of the TN was removed via gas transfer to the atmosphere (53-64%), with the highest efficiency associated to EC 2. Of the amount of TN transferred to the atmosphere, a significant $N_2O/N_{atmosphere}$ ratio was attributed to EC 1 (11%) and EC 3 (10%). On the other hand, the $N_2O/N_{atmosphere}$ ratio decreased from 10 to 5% due to transition from EC 3 (partial nitrification) to EC 4 (complete nitrification), as described previously and reported by other studies (Rodriguez-Caballero et al., 2013; Song et al., 2014). Therefore, the adequate control of DO concentrations is a key factor, in order to avoid the accumulation of NO_2^- and, therefore, achieve lower N_2O emissions.



	EC 1	EC 2	EC 3	EC 4
TN removal (%)	63	71	66	61
$N_2O/N_{atmosphere}$ (%)	11	0,5	10	5

Figure 7.
TN distribution (%) (regarding TN in the influent) through three different system routes: sludge waste, atmosphere (via N_2 and N_2O separately) and liquid effluent for each of the four experimental conditions. In addition, TN removal (%) and $N_2O/N_{atmosphere}$ ratio (%) are also displayed.

4. CONCLUSION

The effects of different operating conditions, such as variable organic loading, different SRTs and airflow rates, limited DO concentrations and NH_4^+ shock loading on TN removal routes and N_2O emissions were studied in a lab-scale activated sludge system and the major conclusions are:

- The variable organic loading did not interfere with COD removal efficiency under the applied operating conditions. However, lower TN removal efficiencies were related to lower TN loads.
- Short SRT (5 days) resulted in a large part of TN removal in sludge waste. As the SRT increased from 5 to 10 days, TN removal decreased in the sludge waste and increased via the atmospheric route.

- Low DO levels (0.5 mg L^{-1}) were responsible for lower TKN oxidation efficiencies, suggesting that nitrification efficiency was hampered by the oxidation of organic matter. For DO set to 1 mg L^{-1}, TKN oxidation rates and NO_2^- accumulation reached their maxima, the best condition for both organic matter oxidation and partial nitrification.
- Part of the N transferred to the atmosphere is attributed to N_2O emissions (reaching a maximum $N_2O/N_{atmosphere}$ ratio of 10%), which varied from 0.3 to 5.6% of the influent TN load.
- The presence of combined remnant NH_4^+ and high NO_2^- concentrations due to partial nitrification strongly triggered N_2O emissions.
- Insufficient aeration and stress conditions (such as NH_4^+ shock loading) can cause limited DO conditions, NO_2^- accumulation and, consequently, higher N_2O emissions.
- The adequate control of DO concentrations is a key factor to avoid NO_2^- accumulation and, consequently, high N_2O emissions.

5. ACKNOWLEDGMENTS

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes).

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