in Batch and Fed-batch Mode Applied to Nitrification and Denitrification Processes

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Viabilidad de un reactor operado en lotes secuenciales aplicado a procesos de nitrificación y desnitrificación

Viabilitat d'un reactor operat en lots seqüencials aplicat a processos de nitrificació i desnitrificació

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RESUMEN

El objetivo de este trabajo fue el estudio de la viabilidad operacional de los procesos de nitrificación y desnitrificación en un reactor de lotes secuenciados (operación en lote y lote alimentado). El reactor era agitado mecánicamente y tenía un tubo alrededor del propulsor (*draft tube*) para facilitar la transferencia de materia de la fase liquida para la biomasa dispersa (aerobia) y granulada (anaerobia). Las variables ajustadas fueron: tiempo de aeración en la etapa de nitrificación; concentración de oxígeno disuelto; tiempo de alimentación para definición de las fases de operación por lotes y por lotes alimentados; concentración de la fuente exógena de materia orgánica utilizada como donador de electrones durante la etapa de desnitrificación y la carga volumétrica de nitrógeno amoniacal. El reactor (volumen de 5 L) fue mantenido a 30±1 °C y trataba 1,0 o 1,5 L de agua residual en lotes de 8 h. Las concentraciones de nitrógeno amoniacal en la alimentación fueron 50 (condición 1) y 100 mgN-NH_4^+·L^{-1} (condición 2), resultando en 29 y 67 mgN-NH_4^+·L-1·d^{-1}, respectivamente. Agua residual sintética y etanol fueron utilizados como fuente exógena de carbón. Las remociones de nitrógeno total fueron 94,4 y 95,9 % cuando el reactor fue operado bajo las condiciones 1 y 2, respectivamente. Bajas concentraciones de nitrito (0,2 y 0,3 mgN-NO_2^-·L^{-1}, respectivamente) y nitrato (0,01 y 0,3 mgN-NO_3^-·L^{-1}, respectivamente) fueron detectadas en el efluente del reactor. Las remociones de nitrógeno amoniacal fueron 97,6 % y 99,6 % bajo las condiciones 1 y 2, respectivamente.

Palabras clave: Nitrificación/desnitrificación. Operación en lote. Operación en lote alimentado. Agua residual sintética. Fuente exógena de materia orgánica.

SUMMARY

The objective of this work was to study the operational feasibility of nitrification and denitrification processes in a mechanically stirred sequencing batch reactor (SBR) operated in batch and fed-batch mode. The reactor was equipped with a draft-tube to improve mass transfer and contained dispersed (aerobic) and granulated (anaerobic) biomass. The following reactor variables were adjusted: aeration time during the nitrification step; dissolved oxygen concentration, feed time defining batch and fed-batch phases, concentration of external carbon source used as electron donor during the denitrification stage and volumetric ammonium nitrogen load in the influent. The reactor (5 L volume) was maintained at 30±1°C and treated either 1.0 or 1.5 L wastewater in 8-h cycles. Ammonium nitrogen concentrations assessed were: 50 (condition 1) and 100 mgN-NH_4^+·L^{-1} (condition 2), resulting in 29 and 67 mgN-NH_4^+·L-1·d^{-1}, respectively. A synthetic medium and ethanol were used as external carbon sources (ECS). Total nitrogen removal efficiencies were 94.4 and 95.9% when the reactor was operated under conditions 1 and 2, respectively. Low nitrite (0.2 and 0.3 mgN-NO_2^-·L^{-1}, respectively) and nitrate (0.01 and 0.3 mgN-NO_3^-·L^{-1}, respectively) concentrations were detected in the effluent and ammonium nitrogen removal efficiencies were 97.6% and 99.6% under conditions 1 and 2, respectively.

Key words: Nitrification/denitrification. Batch and fed-batch reactor. Feasibility operation. Synthetic wastewater. External carbon source.

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RESUM

L'objectiu d'aquest treball és l'estudi de la viabilitat operacional dels processos de nitrificació i desnitrificació en un reactor de lots seqüencials (operació en lot i lot alimentat). El reactor s'agita mecànicament i té un tub envoltant el propulsor (*draft tube*) per facilitar la transferència de matèria de la fase líquida per la biomassa dispersa (aeròbia) y granulada (anaeròbia). Les variables ajustades són: temps d'aeració en l'etapa de nitrificació; concentració d'oxigen dissolt; temps d'alimentació per a definició de les fases d'operació per lots i per lots alimentats; concentració de la font exògena de matèria orgànica utilitzada com a donador d'electrons durant l'etapa de desnitrificació i la càrrega volumètrica de nitrogen amoniacal. El reactor (volum de 5 L) es manté a 30±1ºC i tracta 1,0 o 1,5 L d'aigua residual en lots de 8 h. Les concentracions de nitrogen amoniacal en l'alimentació són 50 (condició 1) i 100 100 mgN-NH_4^+·L^{-1} (condició 2), resultant en 29 i mgN-NH_4^+·L-1·d^{-1}, respectivament. S'empren aigua residual sintètica i etanol com a font exògena de carboni. Les eliminacions de nitrogen total són del 94,4 i 95,9% quan el reactor es fa operar sota les condicions 1 i 2, respectivament. Es detecten baixes concentracions de nitrit (0,2 i 0,3 mgN-NO_2^-·L^{-1}, respectivament) i nitrat (0,01 i 0,3 mgN-NO_3^-·L^{-1}, respectivament) a l'efluent del reactor. Les eliminacions de nitrogen amoniacal són del 97,6% i 99,6% sota les condicions 1 i 2, respectivament.

Mots clau: **Nitrificació/desnitrificació. Operació en lot. Operació en lot alimentat. Aigua residual sintètica. Font exògena de matèria orgànica.**

INTRODUCTION

Biological nitrification and denitrification processes have been widely used in the removal of nitrogen from municipal and industrial wastewaters, employing different reactor configurations. Particularly, sequencing batch reactors (SBR) have shown to be extremely flexible, relatively cheap and very efficient in the treatment of these wastewaters, as nitrification and denitrification processes require the existence of several sequencing zones, which include anaerobic (organic matter removal), aerobic (autotrophic ammonium removal by nitrification processes) and anoxic (heterotrophic nitrate removal by denitrification processes) zones(1, 2).

Denitrification is heterotrophic and therefore requires a carbon source. This carbon source is used as an electron donor (organic compound that is oxidized) in the reduction of nitrate to nitrogen gas. Utilization of carbon sources already present in the influent results in economy in the final process costs. However, low reaction rates may result as these sources are usually long-chain compounds(3). Therefore, many investigations have been carried out in which short carbon chain compounds are added to the wastewater to reduce reaction and hydraulic detention times in the anoxic zones. These so-called «External Carbon Sources» (ECS) include: acetic acid, acetates, ethanol, methanol, glucose and methane, among others(4).

Canto *et al*.(5, 6) investigated the biological removal of ammonium nitrogen from synthetic wastewater in a sequencing batch biofilm reactor (SBBR) with recirculation of the liquid phase. The SBBR, maintained at 30 ± 1°C, had a working volume of 1.8 L and contained immobilized biomass on polyurethane foam. The effect of biomass type, as well as feed (batch and/or fed-batch operation modes) and aeration (continuous or intermittent) strategies were analyzed. The presence of an autotrophic aerobic sludge showed to be essential for nitrification startup, despite publications stating the existence of heterotrophic organisms capable of nitrifying organic and inorganic nitrogen compounds at low dissolved oxygen concentrations(7). With regard to feed strategy, batch operation (wastewater) followed by fed-batch (carbon source) seemed to be the most adequate. Regarding aeration strategy, intermittent aeration presented the best results. Under these optimal conditions of feed and aeration strategy, the effect of four external carbon sources was analyzed: ethanol, acetate, synthetic carbon medium and methanol which acted as electron donors in the denitrifying process. Total nitrogen removal efficiencies obtained were 78.4, 78.0 and 76.3% for ethanol, acetate and synthetic carbon medium, respectively. Nitrite, nitrate and ammonium nitrogen concentrations in the effluent were, respectively: 0.1, 5.7 and 1.4 mg.L^{-1} for ethanol; 0.2, 4.1 and 1.8 mg.L^{-1} for acetate and 0.2, 6.7 and 0.8 mg.L^{-1} for synthetic carbon medium. On the other hand, methanol, even at low concentrations (50% of the calculated stoichiometric value for complete denitrification), caused increased accumulation of nitrate and ammonium nitrogen in the effluent.

The main objective of this work was to study the operational feasibility of biological removal of ammonium nitrogen from a synthetic wastewater employing a mechanically stirred sequencing reactor containing a mixed sludge and equipped with a draft-tube. Stirring and draft-tube were used to improve mass transfer between the substrate in the wastewater and the microorganisms in the sludge. The reactor was operated in sequencing batch and fed-batch mode to improve the nitrification (aerobic autotrophic process) and denitrification (anoxic heterotrophic process) phases. A mixed sludge was used with dispersed (aerobic) and granulated (anaerobic) biomass to perform both biological phases (nitrification and denitrification) in a same tank. The results obtained were compared to those from an SBBR (Sequencing Batch Biofilm Reactor) operated under similar conditions, and indicated that the investigated system has potential for large-scale use.

MATERIALS AND METHODS

Inoculum

Two types of biomass were used: *(i)* an aerobic autotrophic biomass from a prolonged aeration activated sludge system treating domestic wastewater, and *(ii)* an anaerobic heterotrophic biomass from an up-flow anaerobic sludge blanket reactor (UASB) treating wastewater from a poultry slaughterhouse. It should be pointed out that the aerobic microorganisms were in a dispersed state and the anaerobic ones in granulated state. The reason for using these two types of microorganism was to improve the operation feasibility of the two stages involved in the removal of ammonium nitrogen, i.e. nitrification (aerobic and autotrophic) and denitrification (anoxic and heterotrophic), in a same tank.

The reactor was inoculated with 1 L of each microorganism type. Inoculum concentrations from the UASB in terms of total solids (TS_I) and total volatile solids (TVS_I) were about 62.0 g.L^{-1} and 51.0 g.L^{-1}, respectively, whereas those from the activated sludge reactor with continued aeration were 2.3 gTS_I.L^{-1} and 1.6 gTVS_I.L^{-1}, respectively.

Synthetic wastewater (SWN)

The synthetic wastewater (SWN), fed during the batch stage and with concentration around 100 mgCOD/L, was prepared with sucrose (7 mg.L^{-1}), starch (22.8 mg.L^{-1}), cellulose (6.8 mg.L^{-1}), meat extract (41.6 mg.L^{-1}), soybean oil (10.2 mg.L^{-1}), NaCl (50 mg.L^{-1}), $MgCl_2.6H_2O$ (1.4 mg.L^{-1}),

$CaCl_2.2H_2O$ (0.9 mg.L^{-1}), $NaHCO_3$ (initially 500 mg.L^{-1}), commercial detergent for soybean oil emulsification (3 drops.L^{-1}) and NH_4Cl as ammonium nitrogen source.

Two influent ammonium concentrations were analyzed: 50 (condition 1) and 100 mgN-NH_4^+.L^{-1} (condition 2). NH_4Cl concentrations were about 195.6 and 391.2 mg.L^{-1}, respectively. Concentrations of the remaining components were maintained under both conditions.

The substrate (without nitrogen source) was sterilized (121°C, 15 min) in order to maintain the original physicochemical properties of the synthetic wastewater and to prevent natural degradation due to contamination by microorganisms during the assays.

The low concentration of organic matter in the SWN was used to favor aerobic autotrophic nitrification. The synthetic wastewater used in this study, with composition (in terms of carbohydrates, proteins and lipids) similar to domestic sewage, was formulated to simulate the effluent of an anaerobic reactor(8). The ammonium concentration levels used varied between medium and high strength (50 and 100 mg.L^{-1}, respectively), as suggested by Metcalf and Eddy(22), for untreated domestic wastewater.

The purpose of using synthetic feed instead of real field wastewater was to work with a reference influent, i.e., a wastewater previously used in other works(9), of which the anaerobic biodegradability is already known. This enables a more reliable analysis of the effect of operation mode (feed strategy, inoculum, aeration periods, external carbon source etc.).

External carbon sources (ECS)

During the experiments, an assessment was made of the performance of two different carbon sources used as electron donors in the reduction of nitrate to nitrogen gas in the denitrification stage:

(i) External carbon source 1 (ECS 1): with composition equal to the previously described synthetic wastewater, except for the ammonium nitrogen source, and with concentration varying between 100 and 500 mgCOD.L^{-1} during the experiment.

(ii) External carbon source 2 (ECS 2): ethanol solution buffered with $NaHCO_3$ (approximately 370 mg.L^{-1}), with concentration varying between 300 and 700 mgCOD.L^{-1} during the experiment.

Organic matter concentration of the two investigated carbon sources was varied, and consequently the C/N ratio, to discover the optimum operation conditions, i.e., those that would result in highest nitrogen removal efficiencies. Optimum ethanol concentration for concentrations of 50 and 100 mgN-NH_4^+.L^{-1} were 99.0 mgC_2H_5OH.L^{-1} (approximately 300 mgCOD.L^{-1}) and 182.0 mgC_2H_5OH.L^{-1} (approximately 550 mgCOD.L^{-1}), respectively.

Experimental set-up

A scheme of the system used in the experiments is shown in Figure 1. The reactor (BIOFLO III®, New Brunswick Scientific Co.) had a work volume of 5.0 L (total capacity of 6.0 L). Mechanical stirring at 150 rpm was provided through a three-blade (ϕ_I = 5.7 cm) helix type impeller located 13.5 cm from the reactor bottom.

To improve circulation of the medium a draft-tube (ϕ_{DT} = 7.6 cm; L = 15.0 cm) was placed in the reactor at 6.0 cm from the reactor bottom. The stirring frequency adopted was based on previous studies(10), where the same reactor was employed to assess the removal efficiency of carbonaceous organic matter from the previously described synthetic wastewater. It should be mentioned that the liquid level in the reactor was always maintained above the draft-tube to ensure perfect homogenization of the medium. If this condition were not met the external liquid would not flow into the draft-tube and mixing would only be effective inside the tube.

Experimental procedure

The reactor was operated in sequencing batch and fed-batch mode. In the batch mode the reactor was fed with the synthetic wastewater containing the ammonium nitrogen source, whereas in the fed-batch mode the external carbon source was fed. Feeding and discharge were accomplished by diaphragm pumps.

An automation system, consisting of timers, provided for on/off switching of the pumps and the stirrer to execute the feeding stages (batch and fed-batch), stirring, settling and discharge. Aeration was performed intermittently, i.e., during fed-batch operation aeration was interrupted. System temperature was controlled at 30 ± 1°C and each cycle lasted 8 hours.

The operational conditions studied were *condition 1* where the influent concentration was 50 mgN-NH_4^+.L^{-1} for 80 days and *condition 2* where the influent concentration was 100 mgN-NH_4^+.L^{-1} for 30 days.

The study of the biological ammonium nitrogen removal process started by varying aeration time (t_A) and dissolved oxygen concentration (DO), which was controlled by means of the air flow applied during the nitrification step. The t_A varied from 1.5 to 4.0 h during the 8-h cycle and air flow rate from 1.5 to 4.0 L.min^{-1}.

After improvement of the nitrification stage, the fed-batch feed time (t_{FB}) and external carbon source concentration (C_{FB}) were varied. The t_{FB} ranged from 1.0 to 4.0 h and C_{FB} from 100 to 700 mgCOD.L^{-1}. It should be mentioned that the best system operation conditions were first defined during application of a load of 29 mgN-NH_4^+.L^{-1}.d^{-1} (50 mgN-NH_4^+.L^{-1}). After process improvement, influent organic ammonium nitrogen load was increased to 67 mgN-NH_4^+.L^{-1}.d^{-1} (100 mgN-NH_4^+.L^{-1}) in order to assess system performance after this perturbation. The time distribution of the implemented experimental conditions is summarized in Table 1.

Analytical methods

System monitoring was done according to *Standard Methods for the Examination of Water and Wastewater*(23) and the following were quantified: filtered and unfiltered substrate concentration in the effluent as COD (C_S, C_T) – method 5220 D; non-volatile and volatile total solids and

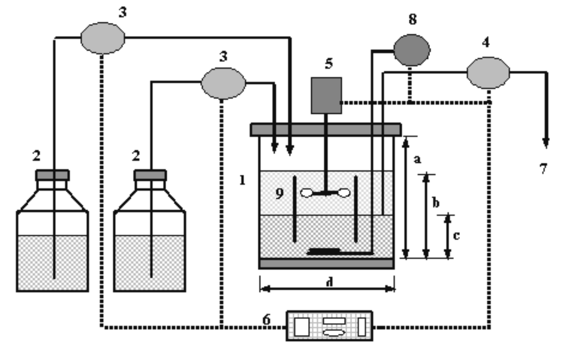


Figure 1. Schematic representation of the mechanically stirred sequencing batch/fed-batch reactor equipped with a draft-tube [1 – reactor (a = 26 cm; b = 20 cm; c = 16 cm; d = 18 cm); 2 – influent reservoirs (SWN and ECS); 3 – feed pumps; 4 – dewatering pump; 5 – agitation system with helix impeller; 6 – pumps control unit; 7 – effluent outlet; 8 – aeration system; 9 – draft-tube; ææ hydraulic lines; – power lines].

TABLE I

Time distribution of the operational conditions implemented during the cycles.

	Time (min)										
0 - 480	10	60	120	180	240	300	360	420	440	470	
SWN feeding	■										
Aeration		■	■		■						
ECS feeding				■		■	■				
Agitation		■	■	■	■	■	■	■	■		
Sedimentation										■	
Discharge											■

Notes:
Assay period (improvement/stabilization): 80 and 30 days for conditions 1 and 2, respectively; sedimentation period: 30 min for both conditions; air flow: 2 and 4 $l.min^{-1}$ for conditions 1 and 2, respectively; organic matter concentration in fed-batch mode: 300 and 550 $mgCOD.l^{-1}$ for conditions 1 and 2, respectively.

total suspended solids (TS, TSS, TVS, VSS) – methods 2540 B, 2540 D and 2540 E; bicarbonate alkalinity (BA) – method 2320 B modified by Ripley *et al*.(11); total volatile acids (TVA) – method 2310 B modified by Dilallo and Albertson(12); pH – method 4500-H^+ B; ammonium nitrogen ($N\text{-}NH_4^+$) – methods 4500-NH_3 B and 4500-NH_3 C; organic nitrogen (N-Org) – method 4500-N_{org} B; nitrite nitrogen ($N\text{-}NO_2^-$) – method 4500-NO_2^- B; and nitrate nitrogen ($N\text{-}NO_3^-$) – method 4500-NO_3^- B.

The concentrations of total solids (TS_I) and total volatile solids (TVS_I) in the inoculum were measured as follows. The mixed liquor in the reactor was left to stand for about 6 h for solids settling. Afterwards, the supernatant was discarded and the settled solids were weighed. A sample was taken from the previously weighed settled portion and analyzed as to total solids and total volatile solids according to *Standard Methods for the Examination of Water and Wastewater*(23) (Methods 2540 B and 2540 E). Concentrations of TS_I and TVS_I in the reactor were determined from the values relative to the sample, given the proportionality between the masses of the sample and the mixed liquor. To determine biomass concentration in the reactor per total volume of liquid (C_X), the previously encountered value of TVS_I was divided by the work volume of the reactor (5.0 L).

After stabilization of the monitored variables at the final cycle condition, the following concentration profiles were run to accompany reactor behavior during a cycle: C_S; BA; TVA; pH; $N\text{-}NO_2^-$; $N\text{-}NO_3^-$; $N\text{-}NH_4^+$; N-Org; DO and ORP. During profile runs, intermediate volatile acids (IVA) samples (1.0 μL) were analyzed by gas chromatography, using a gas chromatograph HP6890 with flame ionization detector (FID) at 300°C and an HP-INNOWAX column (length 30 m; column ID 0.25 mm; film thickness 0.25 μm; phase ratio 250). The injector temperature was kept at 250°C; the oven was held at 100°C for 3 min, after which it was heated at a rate of 5°C.min^{-1} to 180°C, and held at that temperature for 5 min. H_2 (30 mL.min^{-1}), N_2 (33 mL.min^{-1}) and synthetic air (300 mL.min^{-1}) were used as carrier/flame gas, make-up gas and flame gas, respectively. The split/splitless ratio was 1:20. Dissolved oxygen concentration (DO) and redox potential (ORP) were measured using specific probes.

The experimental data obtained during monitoring were statistically analyzed by Microsoft Excel® software, where the standard deviations of the monitored parameters were calculated. The number of samples used for estimation is specified in Table 2. Nitrogen time profiles, because of the sample volume required for analytical methods (about 250 mL), were run only once, i.e., they were not run in duplicate. It should be mentioned that the maximum volume of wastewater that could be sampled per cycle without affecting bioreactor behavior was 10% of the total work volume (5 L). Hence, only two samples were taken during the second cycle of the day. For the entire 8-h cycle 10 cycles (or 5 days) were required to analyze the different forms of nitrogen. COD and alkalinity profiles were run in one cycle each, usually after nitrogen profiles. Profiles were run in a way to perturb system behavior as little as possible.

Mathematical reactor performance calculation

Reactor performance was analyzed through equations (1) to (15). In these calculations it is important to take into account the periods of batch and fed-batch operation(6):

$$\%\,(N-NO_2^-)_{Produced} = \frac{(N-NO_2^-)_{Ef}\cdot V_{Ef} - (N-NO_2^-)_B\cdot V_B}{(N-NH_4^+)_B\cdot V_B}\cdot 100 \qquad (1)$$

$$\%\,(N-NO_3^-)_{Produced} = \frac{(N-NO_3^-)_{Ef}\cdot V_{Ef} - (N-NO_3^-)_B\cdot V_B}{(N-NH_4^+)_B\cdot V_B}\cdot 100 \qquad (2)$$

$$\%\,(N-NH_4^+)_{Removed} = \frac{(N-NH_4^+)_B\cdot V_B - (N-NH_4^+)_{Ef}\cdot V_{Ef}}{(N-NH_4^+)_B\cdot V_B}\cdot 100 \qquad (3)$$

$$\%\,(N-Org)_{Removed} = \frac{(N-Org)_B\cdot V_B - (N-Org)_{Ef}\cdot V_{Ef}}{(N-Org)_B\cdot V_B}\cdot 100 \qquad (4)$$

$$\%\,(N-Total)_{Removed} = \frac{(N-Total)_B\cdot V_B - (N-Total)_{Ef}\cdot V_{Ef}}{(N-Total)_B\cdot V_B}\cdot 100 \qquad (5)$$

$$\left(\frac{BA}{N-NH_4^+}\right) = \frac{(BA_B\cdot V_B + BA_{FB}\cdot V_{FB}) - (BA_{Ef}\cdot V_{Ef})}{(N-Org)_B\cdot V_B} \qquad (6)$$

$$VNLF = n\cdot\frac{(N-NH_4^+)_B\cdot V_B}{V_R} \qquad (7)$$

$$SNLF = \frac{VNLF}{C_X} \qquad (8)$$

TABLE II

Average values of the monitored parameters in the influent and effluent during development of conditions 1 (50 mgN-NH$_4^+$.L^{-1}) and 2 (100 mgN-NH$_4^+$.L^{-1}).

	Condition 1		Condition 2	
Parameters	**Influent**	**Effluent**	**Influent**	**Effluent**
C_B (mg.L^{-1})	98.7 ± 5.6 (4)	–	104.3 ± 11.8 (9)	–
C_{FB} (mg.L^{-1})	300.2 ± 10.1 (4)	–	528.5 ± 50.5 (9)	–
C_S (mg.L^{-1})	–	44.3 ± 13.9 (4)	–	20.7 ± 5.0 (9)
C_T (mg.L^{-1})	–	115.2 ± 21.2 (4)	–	17.8 ± 6.5 (9)
ε_S (%)	–	73.3 ± 8.3 (4)	–	91.5 ± 2.1 (9)
ε_T (%)	–	30.7 ± 10.7 (4)	–	92.7 ± 2.7 (9)
VOL_B (mgCOD.L^{-1}.d^{-1})	59.2	–	62.6	–
VOL_{FB} (mgCOD.L^{-1}.d^{-1})	90.1	–	158.6	–
BA (mgCaCO$_3$.L^{-1}) Batch	425.0 ± 6.9 (4)	313.2 ± 24.3 (4)	346.9 ± 70.1 (4)	194.8 ± 9.5 (7)
Fed-batch	348.7 ± 13.2 (4)		353.3 ± 9.3 (4)	
TVA (mgHAc.L^{-1}) Batch	40.4 ± 11.4 (4)	21.7 ± 5.1 (4)	15.5 ± 2.6 (4)	14.0 ± 4.8 (7)
Fed-batch	30.7 ± 4.9 (4)		26.7 ± 3.2 (4)	
pH	8.5 ± 0.1 (4)	8.0 ± 0.02 (4)	8.6 ± 0.1 (4)	8.2 ± 0.1 (7)
V_{Ef} (L)	–	1.5 ± 0.02 (6)	–	1.5 ± 0.01 (6)
VSS (mg.L^{-1})	10.0 (1)	22.0 (1)	13.2 (1)	20.1 (1)
N-NO$_2^-$ (mg.L^{-1})	0.0 (1)	0.2 ± 0.1 (4)	0.0 (1)	0.3 ± 0.2 (6)
N-NO$_3^-$ (mg.L^{-1})	0.0 (1)	0.01 ± 0.03 (4)	0.0 (1)	0.3 ± 0.4 (5)
N-NH$_4^+$ (mg.L^{-1})	48.3 ± 1.0 (8)	0.8 ± 0.2 (7)	111.5 ± 3.2 (7)	0.3 ± 0.2 (9)
N-Org (mg.L^{-1})	5.8 ± 2.6 (7)	1.0 ± 0.8 (7)	13.1 ± 2.1 (5)	2.5 ± 1.8 (5)

Note: number in brackets refers to the number of data used to calculate averages.

$$VNLR = n \cdot \frac{(N-NH_4^+)_B \cdot V_B - (N-NH_4^+)_{Ef} \cdot V_{Ef}}{V_R} \qquad (9)$$

$$SNLR = \frac{VNLR}{C_X} \qquad (10)$$

$$\frac{C}{N} = \frac{C_B \cdot V_B + C_{FB} \cdot V_{FB}}{(N-NH_4^+)_B \cdot V_B} \qquad (11)$$

$$VOL_B = n \cdot \frac{C_B \cdot V_B}{V_R} \qquad (12)$$

$$VOL_{FB} = n \cdot \frac{C_{FB} \cdot V_{FB}}{V_R} \qquad (13)$$

$$\varepsilon_S = \frac{(C_B \cdot V_B + C_{FB} \cdot V_{FB}) - (C_{ES} \cdot V_{Ef})}{(C_B \cdot V_B + C_{FB} \cdot V_{FB}} \qquad (14)$$

$$\varepsilon_T = \frac{(C_B \cdot V_B + C_{FB} \cdot V_{FB}) - (C_{ET} \cdot V_{Ef})}{(C_B \cdot V_B + C_{FB} \cdot V_{FB}} \qquad (15)$$

RESULTS AND DISCUSSIONS

The monitoring results obtained when the system was operated under condition 1 (50 mgN-NH$_4^+$.L^{-1}) for 80 days and under condition 2 (100 mgN-NH$_4^+$.L^{-1}) for 30 days are shown in Tables 2 and 3, and Figures 2 to 4. The profiles along the cycle, obtained as soon as stability was reached, are shown in Figures 5 to 7. In Table 2 and Figures 2 to 4 the numbers in brackets and the time axis refer to the number of data and the time used to calculate the average values of each variable, respectively. It should be mentioned that only values obtained during reactor stability were used to this end, resulting in average values with low standard deviation. In Figures 2 to 4, numbers 1 and 2 refer to conditions 1 and 2, respectively.

With regard to the inoculum, 30.7 g of total solids (ST_I) and 24.6 g of total volatile solids (SVT_I) were detected in the reactor. Considering the reaction medium in the reactor (5.0 L), these concentrations resulted in 6.1 gST$_I$.L^{-1} and 4.9 gSVT$_I$.L^{-1}, respectively. These values were used to calculate the specific variables in Table 3.

Condition 1:50 mgN-NH$_4^+$.L^{-1}

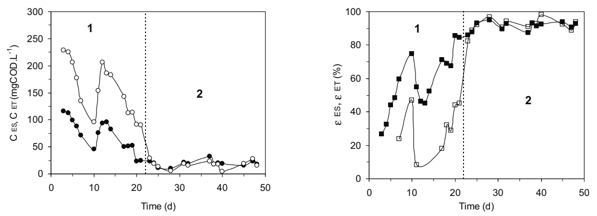
From Figures 2a and 2b and Table 2 it can be seen that after 14 days of operation organic matter concentrations were less than 55 mgCOD/L, resulting in an average organic matter removal efficiency of 73.3% (filtered samples). Regarding unfiltered samples, organic matter removal effi-

TABLE III

Performance parameters of the process.

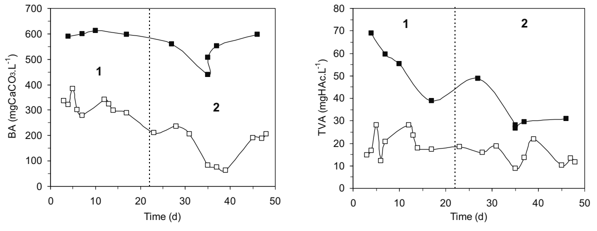
Parameter	50 mgN-NH$_4^+$.L^{-1}	100 mgN-NH$_4^+$.L^{-1}
%(N-NO$_2^-$)$_{Produced}$	0.7	0.4
%(N-NO$_3^-$)$_{Produced}$	0.0	0.4
%(N-NH$_4^+$)$_{Removed}$	97.6	99.6
%(N-Org)$_{Removed}$	74.1	71.4
%(N-Total)$_{Removed}$	94.4	95.9
BA/N-NH$_4^+$ (mgCaCO$_3$.(mgN-NH$_4^+$)$^{-1}$)	2.8	2.1
C/N	5.2	3.3
VNLF (mgN-NH$_4^+$.L^{-1}.d^{-1})	29.0	67.0
SNLF (mgN-NH$_4^+$.g^{-1}.d^{-1})	6.0	13.8
VNLR (mgN-NH$_4^+$.L^{-1}.d^{-1})	28.3	66.7
SNLR (mgN-NH$_4^+$.g^{-1}.d^{-1})	5.8	13.7

Note: C_X = 4.9 gTVS.L^{-1}.



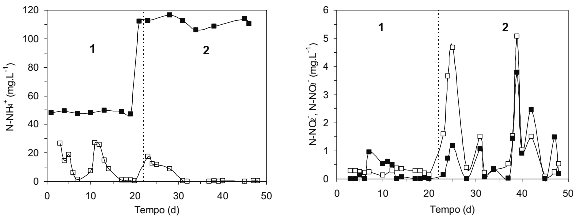
(a) (b)

Figure 2. (a) Organic matter concentration in the filtered effluent C_S (•) and in the unfiltered effluent C_T (○), and (b) organic matter removal efficiency in the filtered effluent ε_S (■) and in the unfiltered effluent ε_T (□) (50 mgN-NH$_4^+$.L^{-1} and (□) 100 mgN-NH$_4^+$.L^{-1}).



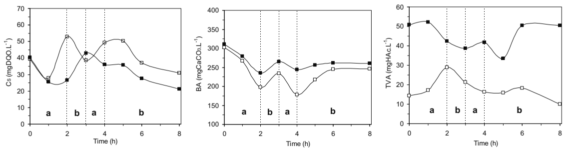
(a) (b)

Figure 3. (a) Bicarbonate alkalinity (BA) and total volatile acids (TVA) in the influent (■) and in the effluent (□) (50 mgN-NH$_4^+$.L^{-1} and (□) 100 mgN-NH$_4^+$.L^{-1}).



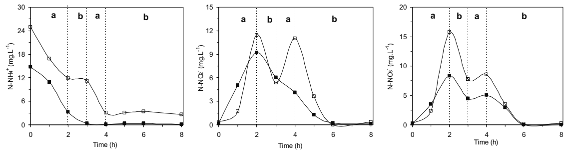
(a) (b)

Figure 4. (a) Influent (■) and effluent (□) ammonium nitrogen concentrations (N-NH$_4^+$); (b) effluent concentration of nitrite - N-NO$_2^-$ (□) and nitrate -N-NO$_3^-$ (■) (**1:** 50 mgN-NH$_4^+$.L^{-1}; **2:** 100 mgN-NH$_4^+$.L^{-1}).
Notation in the graphs: a - aeration period and b - anoxic period.



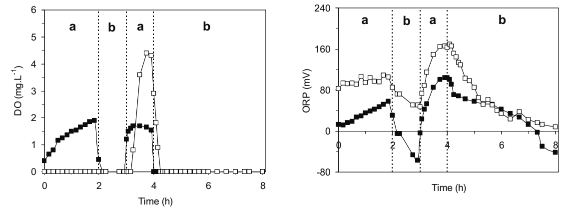
(a) (b) (c)

Figure 5. Effluent profiles for (a) organic matter concentration (C_S), (b) bicarbonate alkalinity (BA) and (c) total volatile acids (TVA): (■) 50 mgN-NH$_4^+$.L^{-1} and (□) 100 mgN-NH$_4^+$.L^{-1}.
Notation in the graphs: a - aeration period and b - anoxic period.



(a) (b) (c)

Figure 6. Effluent concentration profiles for (a) ammonium (N-NH$_4^+$), (b) nitrite (N-NO$_2^-$), and (c) nitrate (N-NO$_3^-$): (■) 50 mgN-NH$_4^+$.L^{-1} and (□) 100 mgN-NH$_4^+$.L^{-1}.
Notation in the graphs: a - aeration period and b - anoxic period.



(a) (b)

Figure 7. Profiles for (a) dissolved oxygen concentrations (DO) and (b) oxidation-reduction potential (ORP): (■) 50 mgN-NH$_4^+$.L^{-1} and (□) 100 mgN-NH$_4^+$.L^{-1}.
Notation in the graphs: a - aeration period and b - anoxic period.

ciency was lower (30.7%) because of a large amount of solids in the reactor (mainly fragments from granulated and dispersed biomass). It should be mentioned that on calculating the efficiency the concentrations of influent organic matter fed during the batch operation (1.0 L containing 98.7 ± 5.6 mgCOD.L^{-1}, i.e. 59.2 mgCOD.L^{-1}.d^{-1}), as well as the ECS added during fed-batch (0.5 L containing 300.2 ± 10.1 mgCOD.L^{-1}, i.e., 90.1 mgCOD.L^{-1}.d^{-1}) were considered, as shown in Equations 14 and 15.

According to Pambrum *et al.*(13) the denitrification rate depends on the carbon and energy sources used and on the carbon to nitrogen (C/N) ratio. Low C/N ratios may cause nitrite or nitrate accumulation(14), whereas the dissimilative reduction to ammonium may occur at high C/N ratios(15) and harm the denitrification process. Therefore, during the monitoring period the external carbon source concentration (and consequently C/N ratio) was varied to encounter the optimum operation conditions, i.e., those that result in the highest total nitrogen removal efficiencies. The average value obtained during system stabilization was 300.2 ± 10.1 mgCOD.L^{-1} (99.0 mgC$_2$H$_5$OH.L^{-1}), which resulted in a C/N ratio of 5.2 (Table 3), considering concentrations of organic matter fed during batch and fed-batch operation, as shown in Equation (11).

It should be mentioned that under condition 1, during the denitrification stage, two different carbon sources acting as electron donors in the reduction reaction of nitrate to nitrogen gas, were used. The assay was initiated using the synthetic carbon medium, i.e., «external carbon source 1» (ECS 1), which presented a composition similar to that of the SWN but without the ammonium nitrogen source. This procedure was based on a work of Canto *et al.*(6), who assessed the performance of four different carbon sources (synthetic carbon medium, acetate, ethanol and methanol) during the denitrification stage in an SBBR with recirculation of the liquid phase. The authors observed that, except for methanol, all sources showed good performance during the denitrification stage with total nitrogen removal efficiencies exceeding 75%. However, after the experimental period of 50 days using the mechanically stirred SBR containing a draft-tube, the denitrification process was unsatisfactory and nitrate concentrations in the effluent remained around 30 mgN-NH$_4^+$.L^{-1} (only 40% ammonium nitrogen removal efficiency). This was likely due to the fact that the synthetic carbon medium contained difficult to degrade substances. In addition the environment encountered by the auto-immobilized denitrifying microorganisms was not favorable for their metabolic activities. The synthetic medium was, therefore, replaced by the more readily degradable ethanol, which showed good efficiency already during the first 15 operation days.

With regard to bicarbonate alkalinity, in Table 2 it can be observed that alkalinity was consumed during the cycle. However, influent alkalinity was insufficient to maintain the system buffered during the cycle, which was confirmed by the effluent pH value (8.0 ± 0.02). It can be seen from Figure 3a that BA$_{Ef}$ varied between 280 and 380 mgCaCO$_3$.L^{-1}, remaining around 313.2 ± 24.3 mgCaCO$_3$.L^{-1} up to the end of the experiment (stabilization period). Influent and effluent total volatile acids concentrations (measured by the titration method) varied during the assay, but maximum effluent values did not exceed 30 mgHAc.L^{-1} (Figure 3b and Table 2). This indicates that the system requires a «reserve» of influent alkalinity to maintain stability, even with no significant volatile acids production. It should be mentioned that both wastewaters used in the assays (SW and ECS) supplied alkalinity to the system, as exposed in Table 2.

According to Fujii *et al.*(16) for every oxidized N-NH$_4^+$ molecule, two alkaline molecules are consumed and for every reduced N-NO$_3^-$ molecule, only one is formed. During the monitoring period, 2.8 mgCaCO$_3$ were consumed per mgN-NH$_4^+$, value below the theoretical one (7.1 mgCaCO$_3$ per mgN-NH$_4^+$) described by Kim and Hao(17). These authors obtained a BA/NH$_4^+$ ratio of 8.5 mgCaCO$_3$ per mgN-NH$_4^+$, in a step-feed alternated anoxic-oxic system, where the environmental conditions required for aerobic nitrification and anoxic denitrification are created. This value probably depends on the particularities of each system defining the process direction. In this case, buffering both influents (synthetic wastewater and external carbon source) possibly contributed to maintaining BA$_{EF}$ at high levels and consequently the BA/N-NH$_4^+$ ratio.

Figures 4a and 4b show the concentrations of ammonium nitrogen, nitrite and nitrate throughout the experiment. It can be seen that only at day 15 N-NH$_4^+$ concentrations in the effluent attained values below 1.0 mg.L^{-1}, resulting in ammonium nitrogen removal efficiency of 97.6% (Table 2). Nitrite concentrations (Figure 4b and Table 2) remained below 0.4 mgN-NO$_2^-$.L^{-1} during the entire assay, whereas nitrate concentrations reached almost 1.0 mgN-NO$_3^-$.L^{-1} at day 7 and at day 13 this ion was no longer detected in the medium. The ammonification process (transformation of organic nitrogen into ammonium nitrogen) also showed to be effective. In Table 3 an effluent organic nitrogen removal efficiency of 74.1% can be seen. These results show that implementing the experimental protocol, defined after adjustment of some process variables (aeration time, dissolved oxygen concentration, feed time and concentration of the ECS, and influent ammonium nitrogen volumetric load), improved the biological nitrification and denitrification processes in the mechanically stirred SBR provided with a draft tube. Moreover, the microorganisms involved in these processes encountered favorable environmental conditions for developing the conventional metabolic route of ammonium nitrogen removal, i.e., complete nitrification with subsequent reduction of the formed nitrate. The difference between organic matter removal efficiency of filtered and unfiltered samples, due to low solids retention of the fine solids and/or of the dispersed biomass also did not affect system performance.

In general, during the stabilization period the average effluent concentrations were 0.2, 0.01, 0.8 and 1.0 mg.L^{-1} for nitrite, nitrate, ammonium nitrogen and organic nitrogen, respectively (Table 2). Total and ammonium nitrogen removal efficiencies were found to be 94.4 and 97.6%, respectively. The removal rate of ammonium nitrogen was 28.3 mgN-NH$_4^+$.L^{-1}.d^{-1} (i.e., 5.8 mgN-NH$_4^+$.g^{-1}.d^{-1}), confirming the high ammonium nitrogen removal efficiency of the process when compared to the applied rate of 29.0 mgN-NH$_4^+$.L^{-1}.d^{-1} (i.e., 6.0 mgN-NH$_4^+$.g^{-1}.d^{-1}). Additionally, only 0.7% of produced nitrite and no nitrate remained in the system (Table 3).

Condition 2: 100 mgN-NH$_4^+$.L^{-1}

Similar to condition 1, the external carbon source (ECS 2) concentration in the fed-batch mode was varied (between 300 and 700 mgCOD.L^{-1}) during the assay in order to obtain an optimal concentration for maximizing total nitrogen removal. The average value found was 528.5 ± 50.5 mgCOD.L^{-1} (i.e. 158.6 mgCOD.L^{-1}.d^{-1}). COD values obtained for unfiltered influent samples (17.8 ± 6.5 mgCOD.L^{-1}) were close to those obtained for the filtered samples (20.7 ± 5.0 mgCOD.L^{-1}), indicating good solids retention, which was not observed when the reactor was operated at the previous condition (Figure 2a and Table 2). At these terms organic matter removal efficiencies for filtered and unfiltered samples were 91.5 ± 2.1% and 92.7 ± 2.7%, respectively, which allows using the system as a residual COD treatment system.

Theoretically, even after doubling influent ammonium nitrogen load, the best C/N ratio under condition 1 should have been maintained. However, this behavior was not observed and the calculated C/N ratio (Equation 11) was 3.3 (Table 3). This likely occurred because of the operation mode of the reactor, i.e., batch followed by fed-batch. Therefore, concentrations of the organic matter fed in both operation modes were considered in the calculation and a lower ratio was obtained. Moreover, it was observed that organic matter concentrations above 550 mgCOD.L^{-1} led to a probable dissimilative reduction of nitrate to ammonium nitrogen, which was minimized by maintaining the organic matter concentration at 550 mgCOD.L^{-1} in the fed-batch mode. It is therefore necessary to assess to what extent the C/N ratio may be considered constant when the ammonium load varies. This parameter should thus be used with care in treatment plant designs. Also, it should be pointed out that at both conditions C/N ratio was varied to encounter the best operation conditions, i.e., those that would result in the highest total nitrogen removal efficiencies.

Figures 3a and 3b and Table 2 contain the results of bicarbonate alkalinity and total volatile acids obtained during reactor monitoring. Higher alkalinity consumption is seen relative to condition 1, likely due to the increase in influent ammonium nitrogen concentration and the high nitrification efficiency (Figures 4a and 4b and Tables 2 and 3). Despite this fact, there was no need to double the value of the influent alkalinity relative to condition 1, i.e., influent alkalinity remained around 524 mg mgCaCO$_3$.L^{-1} (considering both batch and fed-batch operations), whereas under condition 1 total influent alkalinity was approximately 600 mgCaCO$_3$.L^{-1}. At these results, influent and effluent pH values were 8.6 ± 0.1 and 8.2 ± 0.1, respectively, demonstrating the excellent buffering capacity of the system at the implemented operation conditions.

On comparing conditions 1 and 2, variation in bicarbonate alkalinity between influent (considering batch and fed-batch feeding) and effluent was 129.6 and 231.4 mgCaCO$_3$.L^{-1}, respectively, indicating an almost proportional increase. This information is useful for future applications of this system to actual wastewater treatment plants, which often present varying concentrations. With regard to BA/N-NH_4^+ ratio, 2.1 mgCaCO$_3$ per mgN-NH_4^+ were consumed. This value was also below the theoretical value described by Kim and Hao(7) of 7.1 mgCaCO$_3$ per mgN-NH_4^+. As already mentioned this value probably depends on the particularities of each system that define the process direction. Influent and effluent total volatile acids concentrations (measured by titration method) also varied, but their maximum values did not exceed 70 mgHAc.L^{-1} during the assay and the average effluent concentration was 14.0 ± 4.8 mgHAc.L^{-1} (Table 2).

Increase in influent ammonium load from 29.0 mgN-NH_4^+.L^{-1}.d^{-1} (6.0 mgN-NH_4^+.g^{-1}.d^{-1}) to 67.0 mgN-NH_4^+.L^{-1}.d^{-1} (13.8 mgN-NH_4^+.g^{-1}.d^{-1}) obviously led to higher ammonium and organic nitrogen concentrations in the influent. Consequently, larger amounts of nitrite and nitrate were formed during the cycle, when compared to the values of condition 1. In Figures 4a and 4b the behavior of the concentrations of ammonium nitrogen, nitrite and nitrate during system monitoring can be observed. Within a week of operation under condition 2, effluent ammonium nitrogen concentration already attained minimum values (< 0.4 mg.l^{-1}), ratifying the high ammonium nitrogen removal rate of the system (66.7 mgN-NH_4^+.L^{-1}.d^{-1} or 13.7 mgN-NH_4^+.g^{-1}.d^{-1}).

Nitrite and nitrate concentrations, on their turn, varied over time. However, the maximum values attained were 5.0 and 3.7 mg.L^{-1} nitrite and nitrate, respectively. In an attempt to reduce these values both the concentration of the external carbon source fed during the denitrification stage and the air flow rate (from 2 to 4 L.min^{-1}) were increased, thus increasing the C/N ratio. Increase in air flow rate and, consequently, the dissolved oxygen concentration favored oxidation of accumulated nitrite to nitrate, which was readily reduced because of the appropriate applied C/N ratio. It should be mentioned that increase in C/N did not imply dissimilative reduction of nitrate to ammonium nitrogen, and so the latter remained at minimum levels.

Under stable system the following results were obtained: only 0.4% of nitrite and nitrate remained in the system and about 99.6, 71.4 and 95.9% of ammonium, organic and total nitrogen were removed, respectively (Table 3). These results show that even on doubling influent ammonium nitrogen concentration, the microorganisms involved in the process encountered an appropriate environment for the development of their metabolic functions, because of the optimal defined operation conditions implemented during the experiment. Moreover, the reactor maintained robustness and presents a promising alternative to treat wastewaters containing ammonium nitrogen concentrations between medium and high strength wastewaters (50 and 100 mg.L^{-1}, respectively), as suggested by Metcalf and Eddy(23), for untreated domestic wastewater.

Operating variable profiles under conditions 1 and 2

After stabilization (considering the monitored variables at cycle values) of the reactor during development of conditions 1 (C-1) and 2 (C-2), operating variable profiles were run and results are shown in Figures 5 to 7. These Figures aid in the understanding of the kind of biotransformation that occurs in the sequencing processes. The letter «a» in the Figures refers to the aeration period, that is, when the nitrification step took place. The letter «b» refers to the periods at which aeration was interrupted (anoxic phase), i.e., where the denitrification process took place (Table 1).

Figure 5a shows the organic matter concentration profiles relative to the two investigated conditions. In both cases rapid consumption can be seen during the first hour of the cycle, probably by the facultative heterotrophic microorganisms, as the system was aerated during the first two hours to favor nitrification. Under condition 1 organic matter concentration (about 16 mgCOD.L^{-1}) slightly increased between hour 2 and 3, when the rector was fed in the fed-batch mode with external carbon source. Despite this increase in organic matter concentration and interruption of aeration, Figure 6a shows that the nitrification process (with subsequent decrease in formed nitrate) was not harmed. COD was then gradually consumed until the end of the cycle despite operation conditions (aeration or fed-batch feeding). Under condition 2 the organic matter concentration behavior was better defined, that is, higher concentrations at the beginning of the fed-batch operation and gradual consumption throughout the period were obtained. Residual organic matter concentrations were 21.2 and 30.9 mgCOD.L^{-1} for conditions 1 and 2, respectively.

As previously mentioned, for every oxidized N-NH_4^+ molecule, two alkaline molecules are consumed and for every reduced N-NO_3^- molecule, one is formed(16). This behavior can be verified in Figure 5b, where alkalinity is seen to be consumed during the periods where the system was aerated (represented by the letter «a») and where the nitrification process was more intense. On the other hand, in the periods where aeration was interrupted (represented by the letter «b») accumulation of bicarbonate alkalinity occurred. The equilibrium between the periods with and without aeration assured buffering of the medium. This is confirmed by the pH values throughout the cycles (7.5 to 8.0).

Total volatile acids (titration method varied throughout the cycle under the two investigated conditions. However, TVA concentrations under condition 1 were about 30% higher than those under condition 2 (Figure 5c). This behavior might be a result of the higher amount of carbonaceous organic matter required to reduce all the nitrate formed during operation under condition 2, where influent ammonium nitrogen concentration was twice as high and obviously generated a higher amount of nitrate. In other words, a higher amount of organic matter was diverted to the denitrification process during development of condition 2, causing less generation of acids in relation to condition 1, as VNLF (from 29.0 to 67.0 mgN-NH$_4^+$.L^{-1}.d^{-1}) was doubled and VOL$_{FB}$ (from 90.1 to 158.6 mgCOD.L^{-1}.d^{-1}) did not increase at the same proportion.

It should be mentioned that no intermediate volatile acids (acetic, propionic, isobutyric, isovaleric, valeric and caproic) were detected by gas chromatography analysis during the cycle under any of the conditions. The chromatographic method measures the individual concentrations of some acids, such as: acetic, propionic, butyric, valeric and isovaleric acid. On the other hand, formic and lactic acids were only detected by titration. The concentrations of the acids that could be measured by chromatography were well below the detection limits and were therefore measured by the titration mesthod.

Figures 6a to 6c contain the concentration profiles for ammonium nitrogen, nitrite and nitrate for conditions 1 and 2. Under condition 1, all ammonium nitrogen was practically oxidized at hour three of the cycle (Figure 6a). Under condition 2, despite residual ammonium nitrogen (2.7 mgN-NH$_4^+$.L^{-1}) at the end of the cycle, removal efficiency was 99.3%. This shows that the implemented operation conditions improved the ammonium nitrogen removal process.

In Figure 6b it can be observed that under condition 1, nitrite (N-NO$_2^-$) concentration reached 9.2 mg.L^{-1} at hour two of the cycle. Nitrite was then slowly oxidized up to hour six when concentration reached almost zero. Similar behavior was observed for nitrate (N-NO$_3^-$), which attained maximum concentration of 8.3 mg.L^{-1}, also at hour two and was completely reduced at the end of hour six (Figure 6c).

Under condition 2, nitrite and nitrate concentrations presented similar behavior to that observed at the previous condition. N-NO$_2^-$ showed concentrations of about 11.0 mg.L^{-1} during the periods of aeration. Nitrate attained maximum value (15.8 mg.L^{-1}) at hour two of the cycle. Both nitrogen forms disappeared from the system up to hour six, demonstrating the good efficiency of the reactor in removing ammonium nitrogen from liquid effluents. It was therefore concluded that in both cases reactor operation could be reduced from 8 to 6 hours, implying an increase in cycles and consequently the ability to treat larger volumes of wastewater a day.

Figures 7a and 7b contain profiles for dissolved oxygen concentration (DO) and redox potential (ORP), respectively. Under condition 1, DO concentration reached 2.0 mg.L^{-1} during periods of aeration. Under condition 2, DO was detected only at hour three, i.e., in the second aeration period. However, DO was rapidly consumed and disappeared completely when aeration ceased. This might have occurred because of the high ammonium nitrogen concentration in the influent, which allowed the N-NH$_4^+$ oxidizing microorganisms to rapidly consume the oxygen dissolved in the medium.

With regard to ORP, during condition 1 the system presented typical behavior of aerobic/anoxic systems, that is, ORP was positive in the aerobic zones and negative in the anoxic ones. On the other hand, during condition 2, ORP

TABLE IV

Comparison between reactors R-1 (SBR with disperse and granulated biomass and mechanical agitation) and R-2 (SBR with immobilized biomass and liquid-phase circulation).

	Reactor 1 (R-1)		Reactor 2 (R-2)(5, 6)	
	C-1	C-2	C-1	C-2
VNLF (mgN-NH$_4^+$.L^{-1}.d^{-1})	29.0	67.0	58.4	118.5
SNLF (mgN-NH$_4^+$.g^{-1}.d^{-1})	6.0	13.8	1.8	3.7
VNLR (mgN-NH$_4^+$.L^{-1}.d^{-1})	28.3	66.7	54.8	115.7
SNLR (mgN-NH$_4^+$.g^{-1}.d^{-1})	5.8	13.7	1.7	3.6
N-NO$_2^-$ - effluent (mg.L^{-1})	0.2 ± 0.1	0.3 ± 0.2	0.07 ± 0.01	0.17 ± 0.03
N-NO$_3^-$ - effluent (mg.L^{-1})	0.01 ± 0.03	0.3 ± 0.4	6.4 ± 1.9	20.4 ± 0.8
N-NH$_4^+$ - effluent (mg.L^{-1})	0.8 ± 0.2	0.3 ± 0.2	1.8 ± 0.7	1.4 ± 0.7
N-NH$_4^+$ - influent (mg.L^{-1})	48.3 ± 1.0	111.5 ± 3.2	51.1 ± 1.1	103.7 ± 3.3
C$_T$ (mgDQO.L^{-1})	115.2 ± 21.2	17.8 ± 6.5	26.8 ± 8.2	25.1 ± 5.8
C/N	5.2	3.3	5.2	2.4
TS$_I$ (mg.L^{-1})	6.1		37.6	
TVS$_I$ (mg.L^{-1})	4.9		31.3	
V$_R$ (L)	5.0		2.1	

Individual characteristics of the systems:
R-1 – Mechanically stirred SBR containing granulated (anaerobic/anoxic) and dispersed biomass (aerobic), draft-tube, 8-h cycle, ethanol as ECS;
R-2 - SBR with immobilized biomass, circulation of the liquid phase, 12-h cycle, synthetic wastewater as ECS.
Other characteristics were common to both systems, like: operation mode, temperature, composition of the wastewater containing nitrogen source and ammonium nitrogen source. C-1 and C-2 refer to conditions 1 and 2, respectively.

presented positive values throughout the entire cycle. The conclusion thus reached is that due to complexity of the system ORP can not be considered a process control parameter, as in other systems(17, 18, 19, 20, 21).

In general, the experimental protocol allows understanding the main steps of this process and can be used to make changes in order to establish the optimal operation conditions for maximum nitrogen removal efficiencies.

Canto *et al*.(6) employed a 1.9-L SBR (R-2) containing immobilized biomass, with recirculation of the liquid phase, operated with 12-h cycles and used synthetic carbon medium as ECS. The remaining operation conditions were similar to those employed in the current investigation. The authors assessed reactor behavior during ammonium nitrogen removal from a synthetic wastewater containing 50 mgN-NH_4^+.L^{-1} (C-1) and, afterwards 100 mgN-NH_4^+.L^{-1} (C-2). They verified that when the reactor was fed with 50 mgN-NH_4^+.L^{-1}, ammonium and total nitrogen removal efficiencies were 93.8 and 72.2%, respectively. Effluent nitrite and nitrate concentrations were 0.07 and 6.4 mg.L^{-1}, respectively. On the other hand, when ammonium nitrogen concentration was twice as high, a residual of 20.4 mgN-NO_3^-.L^{-1} in the effluent resulted (34.4% of the produced nitrate). At these terms 97.6% of ammonium nitrogen and 66.7% of total nitrogen were removed.

Table 4 contains a comparison between the reactor developed in the current investigation (designated R-1) and that developed by Canto *et al*.(6) (designated R-2). The volumetric feed load of ammonium nitrogen was higher in R-2. Analogously, in this system a higher nitrogen volumetric removal load was observed. The ammonium nitrogen removal efficiency was high at C-1 and C-2, however, at C-2 a relatively high amount of nitrate accumulated in the effluent, which did not decrease even at a longer cycle length.

Regarding specific loads (feed and removed), R-1 presented the highest values because of the smaller amount of TVS_I, despite the larger work volume. The higher amount of TVS_I (about 6 times higher) in R-2 was a result of biomass immobilization which reduced solids retention in the reactor. On the other hand, despite the smaller amount of biomass, R-1 showed improved denitrification ability at the two investigated conditions. R-1 also presented a higher C/N ratio. However, as already mentioned the C/N ratio was adjusted to prevent either accumulation of nitrite and nitrate in the effluent or dissimilative reduction of nitrate to ammonium nitrogen. Hence, the residual nitrate in R-2 (C-2) was generated even when the system operated at optimum conditions. Despite the differences, both reactors show great potential for application in the removal of ammonium nitrogen from wastewaters.

CONCLUSIONS

The mechanically stirred reactor with a draft-tube operated in batch and fed-batch sequencing mode containing a mix of dispersed (aerobic) and granulated (anaerobic) biomass showed high ammonium nitrogen removal efficiencies, indicating its potential in the post treatment of liquid effluents. When the reactor was fed with 50 mgN-NH_4^+.L^{-1}, total nitrogen removal efficiency amounted to 94.4%. When ammonium nitrogen concentration was twice as high, total nitrogen removal efficiency equaled 95.9%. These results are ratified by the low concentrations of nitrite (0.2 and 0.3 mg.L^{-1}), nitrate (0.01 and 0.3 mg.L^{-1}), ammonium nitrogen (0.8 and 0.3 mg.L^{-1}) and organic nitrogen (1.0 and 2.5 mg.L^{-1}) in the effluent and by the high ammonium nitrogen removal efficiencies, which amounted to 97.6 and 99.6%, respectively. The buffering capacity of the system was demonstrated by the high effluent bicarbonate alkalinity values and by the pH values around 8.0 at both investigated conditions. Moreover, production of total volatile acids was relatively low in the cycles (< 70 mgHAc.L^{-1}).

Under both conditions, based on the concentrations profiles for nitrite and nitrate, aeration and feeding time in the fed-batch mode may be readjusted to reduce cycle length from 8 to 6 hours. These profiles allow insight into the main stages of the processes (nitrification/denitrification) and can be used to make changes in the reactor to establish the best operation conditions for possible increases in the applied ammonium load. From ORP profiles it became clear that ORP can not be considered a process control parameter, as in other systems, probably due to the complexity of the system.

Finally, the denitrification stage showed to be strongly affected by the C/N ratio. An increase in this ratio implied a probable dissimilative reduction of nitrate to ammonium nitrogen, with increase in ammonium nitrogen concentration in the final effluent. On the other hand, lower C/N ratios led to lower total nitrogen removal efficiency. For both investigated conditions, C/N ratios were 5.2 and 3.3, respectively. These results restrict the use of the C/N ratio as a design parameter, indicating this ratio should be used with care in designing treatment plants.

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NOTATION

Symbols

BA – Bicarbonate alkalinity (mg$CaCO_3$.L^{-1})

BA/N-NH_4^+ – Bicarbonate alkalinity consumed per ammonium nitrogen removed from the system (mg$CaCO_3$.L^{-1}.(N-NH_4^+)$^{-1}$)

BA_B – Bicarbonate alkalinity in the batch mode (mg$CaCO_3$.L^{-1})

BA_{Ef} – Bicarbonate alkalinity in the effluent (mg$CaCO_3$.L^{-1})

BA_{FB} – Bicarbonate alkalinity in the fed-batch mode (mg$CaCO_3$.L^{-1})

C/N – Ratio of the total influent organic matter by the ammonium nitrogen

C_B – Influent organic matter concentration in the batch mode (mgCOD.L^{-1})

C_{FB} – Influent organic matter concentration in the fed-batch mode (mgCOD.L^{-1})

C_S – Filtered organic matter concentration in the effluent (mgCOD.L^{-1})

C_T – Unfiltered organic matter concentration in the effluent (mgCOD.L^{-1})

C_X – Biomass concentration in the reactor per total volume of liquid (mgTVS.L^{-1})

DO – Dissolved oxygen concentration (mg.L^{-1})

IVA – Intermediate volatile acids (mg.L^{-1})

L – Draft-tube height (cm)

n – Number of cycles per day

(N-NO2-)$_B$ – Concentration of nitrite nitrogen in the batch mode (mg.L^{-1})

$(N\text{-}NO_2^-)_{Ef}$ – Concentration of nitrite nitrogen in the effluent ($mg.L^{-1}$)

$\%(N\text{-}NO_2^-)$Produced – Percent nitrite nitrogen produced by the system (%)

$(N\text{-}NO_3^-)_B$ – Concentration of nitrate nitrogen in the batch mode ($mg.L^{-1}$)

$(N\text{-}NO_3^-)_{Ef}$ – Concentration of nitrate nitrogen in the effluent ($mg.L^{-1}$)

$\%(N\text{-}NO_3^-)_{Produced}$ – Percent nitrate nitrogen produced by the system (%)

$(N\text{-}NH_4^+)_B$ – Concentration of ammonium nitrogen in the batch mode ($mg.L^{-1}$)

$(N\text{-}NH_4^+)_{Ef}$ – Concentration of ammonium nitrogen in the effluent ($mg.L^{-1}$)

$\%(N\text{-}NH_4^+)_{Removed}$ – Percent ammonium nitrogen removed from the system (%)

$(N\text{-}Org)_B$ – Concentration of organic nitrogen in the batch mode ($mg.L^{-1}$)

$(N\text{-}Org)_{Ef}$ – Concentration of organic nitrogen in the effluent ($mg.L^{-1}$)

$\%(N\text{-}Org)_{Removed}$ – Percent organic nitrogen removed from the system (%)

$(N\text{-}Total) – (N\text{-}NO_2^-) + (N\text{-}NO_3^-) + (N\text{-}NH_4^+) + (N\text{-}Org)$ ($mg.L^{-1}$)

$(N\text{-}Total)_B$ – Concentration of total nitrogen in the batch mode ($mg.L^{-1}$)

$(N\text{-}Total)_{Ef}$ – Concentration of total nitrogen in the effluent ($mg.L^{-1}$)

$\%(N\text{-}Total)_{Removed}$ – Percent total nitrogen removed from the system (%)

ORP – Oxidation-reduction potential (mV)

SNLF – Specific ammonium nitrogen loading fed to the system ($mgN\text{-}NH_4^+.g^{-1}.d^{-1}$)

SNLR – Specific ammonium nitrogen loading removed from the system ($mgN\text{-}NH_4^+.g^{-1}.d^{-1}$)

t_A – Aeration time (h)

t_{FB} – Fed-batch feeding time (h)

TS – Total solids in influent or effluent ($mg.L^{-1}$)

TS_I – Total solids in inoculum ($mg.L^{-1}$)

TSS – Total suspended solids in influent or effluent ($mg.L^{-1}$)

TVA – Total volatile acids ($mgHAc.L^{-1}$)

TVS – Total volatile solids in influent or effluent ($mg.L^{-1}$)

TVS_I – Total volatile solids in inoculum ($mg.L^{-1}$)

V_B – Synthetic wastewater (containing ammonium nitrogen) volume fed in the batch mode (1.0 L)

V_{Ef} – Dewatered volume (1.5 L)

V_{FB} – External carbon source volume fed in the fed-batch mode (0.5 L)

VNLF – Volumetric ammonium nitrogen loading fed to the system ($mgN\text{-}NH_4^+.L^{-1}.d^{-1}$)

VNLR – Volumetric ammonium nitrogen loading removed from the system ($mgN\text{-}NH_4^+.L^{-1}.d^{-1}$)

VOL_B – Volumetric organic loading in the batch mode

VOL_{FB} – Volumetric organic loading in the fed-batch mode

V_R – Work volume (5.0 L)

VSS – Volatile suspended solids in influent or effluent ($mg.L^{-1}$)
Greek letters

ε_S – Filtered organic matter removal efficiency (%)

ε_T – Unfiltered organic matter removal efficiency (%)

ϕ_I - Impeller diameter (cm)

ϕ_{DT} - Draft-tube diameter (cm)

Abbreviations

C-1 – condition 1, i.e., ammonium nitrogen concentration equal to 50 $mgN\text{-}NH_4^+.L^{-1}$

C-2 – condition 2, i.e., ammonium nitrogen concentration equal to 100 $mgN\text{-}NH_4^+.L^{-1}$

COD – Chemical oxygen demand

ECS – External carbon source

FID – Flame ionization detector

R-1 – Mechanically stirred reactor with granulated (anoxic) and dispersed biomass (aerobic)

R-2 – Reactor with immobilized biomass and recirculation of the liquid phase

SBR – Sequencing batch reactor

SBBR – Sequencing batch biofilm reactor

SWN – Synthetic wastewater

UASB – Up-flow anaerobic sludge blanket

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