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Performance and Kinectics Aspects of Nitrogen Removal in a Biofilm Sequencing Batch Reactor

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ABSTRACT: A biofilm sequencing batch reactor with a volume of 1.42 m^3, nylon nets providing a 4,140 m^2/m^3 support area for biofilms and an automated operation with 8 hour cycles was studied. The duration of the experiment was 135 days. Removal efficiencies e≥ 80% were obtained for carbonaceous matter, producing an effluent with 31±26.8 mg/L of filtered COD, 7±3.6 mg/L of BOD_5 and 12±3.2 mg/L of TOC. The average removal efficiency of ammonium was 77 ± 16.6%, with a mean concentration in the effluent of 14 ± 10.2 mg NH_4-N/L. The denitrification efficiency was 80±14.7%. The effluent characteristics met the requirements of Brazilian environmental standard for discharge to receiving water bodies. A kinetic study of nitrification and denitrification showed that during the aerobic phase the specific rate of ammonium consumption was 0.057 g NH_4-N/g VSS.d and the production of NOx-N was 0.074 g NOx-N/g VSS.d, while the specific rate of NOx-N consumption was 0.05 g NOx-N/g VSS.d during the anoxic phase. The suspended and fixed biomass was composed of 50% ammonium-oxidizing bacteria (AOB).

Key words: Biological nitrogen removal, hybrid reactor, wastewater treatment, nitrification, denitrification, kinetics parameters

INTRODUCTION

One technology that has been successfully used in wastewater treatment with excessive amounts of nitrogen and phosphorus involves the use of cyclically operated reactors known as sequencing batch reactors (SBR). Due to its simplicity and low capital and operating costs, the SBR provides an alternative for advanced wastewater treatment facilities (Jin Lim *et al.*, 2000, Artan & Orhon, 2005).

Many studies have been carried out to investigate processes using combinations of both suspended cultures and fixed biomass that are called *Hybrid Reactor Systems* (HRS). The systems have been studied for both domestic wastewater treatment (Andreottola *et al.*, 2005, Al-Sharekh & Hamoda, 2001,Wolff *et al.*, 2005, ∅degaard, 2006) and industrial wastewater treatment (Wessman *et al.*, 2004). A hybrid system can have separate units for suspended and for fixed biomass, or it can include both suspended and fixed biomass in the same reactor (Paul *et al.*, 2007, Gapes & Keller, 2009). By providing biofilm carriers, the hybrid reactor configuration has an enhanced nitrification capacity compared to other configurations because nitrifiers can undergo attached growth in a biofilm (Watts & Münch, 2000, Uemura *et al.*, 2011), while organic matter is metabolized by suspended microorganisms (Oyanedel *et al.*, 2002, Ochoa *et al.*, 2002). When calculated on reactor volume, the typical biomass concentration is on the order of 2-5 kg SS/m3 (Rusten et al. 1998 cited by Ødegaard, 2006). The research of Paul et al. (2007) showed that adding carriers to conventional activated sludge systems allowed them to stably attain nearly complete nitrification with a sludge retention time of 3 days at 16 °C. This was attributed to the high percentage (95 %) of nitrifying activity associated with the biomass fixed on the supports. Nitrifying bacteria have a prolonged residence time in hybrid reactors due to

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their being trapped on the support material, which contributes to nitrification and increases ammonia nitrogen removal (Zhang *et al.*, 2012). Consequently, hybrid reactors can be operated at sludge ages shorter than the critical value, which is the minimum sludge age required for nitrification to occur. The reactors can also be operated with lower volatile suspended solids (VSS) concentrations than are required in conventional activated sludge (CAS). According to Helness & Ødegaard (2000), the HRS configuration offers many advantages compared to those offered in CASS configurations, including a more compact footprint (smaller process volume), less dependence on clarification due to a large fraction of the biomass being attached to the support material and lower VSS concentrations. For Ødegaard (2006), the primary advantages of the process are its compactness and the fact that there is no need for sludge recirculation. The advantage of the HRS configuration over other biofilm processes is its flexibility. One may use almost any reactor shape, and one may choose different operational loadings in a given reactor volume simply by adjusting the fraction of the reactor that is filled with biofilm carrier material. The residence time in HRS configurations will be quite low: 15-90 min, depending on the organic load and the strength of the wastewater. When studying a moving bed biofilm reactor (MBBR), Aygun et al. (2008) showed that by changing the wastewater composition, the organic removal efficiency decreased. The efficiency ranged between of 95.1% and 45.2% as the organic loading rate was increased from 6 to 96 g COD/m^2.d. The biofilm reached an average concentration of 3.28 kg TSS/m^3 at the highest organic loading rate. The ratio between the TSS production and the total COD removal was 0.12 kg TSS/kg total COD at an influent total COD of 500 mg/L. Ødegaard (2006) found that attached biomass, i.e., having a higher concentration of relevant organisms than suspended biomass, is more specialized. This holds true at any given point in the process train because there is no biomass return. For example, Ødegaard found that the order of colonization seems to be reversed in a moving bed compared with CAS. High surface loading rates, approximately 30 g COD/m^2.d, produce compact bacterial biofilms, with protozoan populations either absent or limited to small free-swimming protozoa and *Vorticella* spp. Moderate loading rates of approximately 10-15 g COD/m^2.d promote a more "fluffy" biofilm with a rich variety of ciliated protozoa. Low loading rates of < 5 g COD/m^2.d promote very "fluffy" biofilm generally dominated by stalked ciliates. Molecular biology techniques, such as fluorescence *in situ* hybridization (FISH) (Park *et al.*,2008, Jan *et al.*,2002), have been used to characterize the microbial diversity in biofilm reactors. The goal of this paper is to study a hybrid (suspended biomass + biofilm) sequencing batch reactor (HSBR) for the treatment of sewage. The work includes a kinetic study of the nitrification and denitrification processes occurring in the reactor.

MATERIALS & METHODS

A hybrid sequencing batch reactor (HSBR) was used. The reactor was cylindrical, had a total height of 2.20 m (effective height of 2 m) and was 0.95 m in diameter, with a total volume of 1.42 m^3. Aeration was performed through two 0.2 m diameter membrane-type circular air diffusers installed at the base of the reactor that produced small bubbles. These diffusers were fed by an air compressor with a capacity of 100 L/min (Fig.1). The wastewater came from a public sewage collection system. The wastewater was captured by a pump and sent to a 5 m^3 storage tank with a hydraulic retention time (HRT) of 1-2 days. From this tank, the sewage was pumped directly into the HSBR. Treated effluent was removed from the reactor through a set of pumps similar to the feed pumps and returned to the public sewage collection system. The operation of the reactor was automated and managed by WinDosPs software. This program allowed the desired parameter values to be entered for the reactor's operation, which were sent to a central PLC (Siemens) located in a control panel. The PLC performed the required control functions. Nylon nets that served as a support material for the growth of fixed biomass were installed inside the HSBR. These nets were set in stainless steel frames that were 85 cm in height and a diameter of 75 cm, forming rectangles parallel to each other. The nets provided a specific surface area of 4,140 m^2/m^3 to support biofilm development. The reactor was seeded with activated sludge from a sequencing batch reactor used in the wastewater treatment system at a residential condominium.

The reactor was operated cyclically. Each complete cycle lasted a total of eight hours, and consisted of filling, anoxic, aerobic, settling and withdrawal periods. Each cycle included three step-feed periods, with anoxic and aerobic periods between feed periods. Setting and effluent discharge followed the final aerobic period. Table 1 shows the reactor's operational conditions.

The reactor's performance was evaluated by physical-chemical and biological analyses of the wastewater (influent) and the treated sewage (effluent) after each cycle. The following variables were analyzed using the indicated means: pH, dissolved oxygen (DO) and temperature (T °C) using a multiparameter probe YSI 556 (YSI, Yellow Springs, Ohio, USA), biochemical oxygen demand (BOD_5) using a manometric method (HACH, BOD Trak, Loveland, Colorado, USA), total

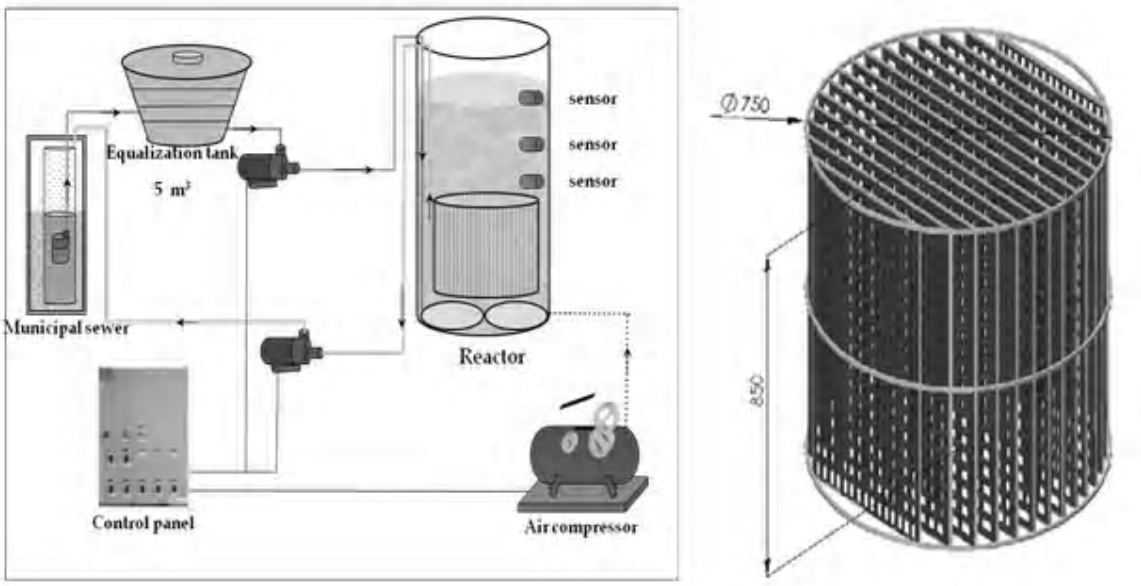

Fig. 1. Schematic diagram of the treatment system and the nylon nets system

Table 1. Operational conditions applied to the reactor

	Condition
Number of cycles per day	3
Total time for each cycle (h)	8
Sewage volume (L) per filling	150
Number of fillings per cycle	3
Treated effluent volume (L) per day	1350
Time of each anoxic phase (min)	50
Total time of the anoxic phases per cycle (min)	150
Time of each aerobic phase (min)	90
Total time of the aerobic phases per cycle (min)	270
Time of the withdrawal phase + idle per cycle (min)	90
Applied volumetric organic loading rate (kg COD/m^3.d)	0.51 ± 0.01
Applied nitrogen loading rate (kg NH_4-N/m^3.d)	0.06 ± 0.01

and filtered chemical oxygen demand (TCOD and FCOD) using a closed reflux colorimetric method (HACH DR-4000, Loveland, Colorado, USA), total organic carbon (TOC) using Shimadzu equipment (TOC - 5000A, Tokyo, Japan), total Kjeldahl nitrogen (TKN) using an acidic digestion method with distillation (Velp - UDK-130, Usmate, Italy), ammonia nitrogen (NH_4-N) using the Nessler method, nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N) and phosphate (PO_4-P) using ion chromatography (Dionex - DX-120, Sunnyvale, CA, USA), alkalinity using a potentiometric method, and total suspended and volatile suspended solids (TSS, VSS) using a gravimetric method. All analyses were performed following Standard Methods (APHA, 2005). The HSBR experiment lasted 135 days. After the experiment was completed, a kinetics study of ammonium consumption and the production and consumption of nitrogen oxides (NOx-N) was performed. The kinetics of nitrification (aerobic phase) and denitrification (anoxic phase) were studied in the

HSBR at operational conditions specifically selected to attain the maximum consumption rate of ammonium during nitrification (N) and maximum reduction rates of nitrite and nitrate during denitrification (DN). For each sample collection, the pH, DO, temperature and alkalinity were monitored, and analyses of TCOD, FCOD, NH_4-N, PO_4-P, N NO_2-and NO_3-N were performed. To keep the pH above 7 and thus maintain good conditions for nitrification,, lime (CaO) was added when necessary. Table 2 shows the step-by-step procedure used in the kinetics study.

To calculate the maximum consumption rate of ammonium ($r_{NH4\text{-}N}$), the production rate of nitrogen oxides ($r_{NOx\text{-}N}$) during the aerobic period, and the maximum consumption rate of nitrogen oxides ($r_{NOx\text{-}DN}$) during the anoxic period, equations 1 to 3 were used:

$$r_{NH4\text{-}N} = \frac{C\,NH4{-}N\,init - C\,NH4{-}N\,fnit}{tfnit - tinit} \quad (1)$$

$$r_{NOx\text{-}N} = \frac{C\,NOx{-}N\,init - C\,NOx{-}N\,fnit}{t\,fnit - t\,init} \quad (2)$$

$$r_{NOx\text{-}DN} = \frac{C\,NOX{-}N\,idenit - C\,NOX{-}N\,fdenit}{t\,fdenit - t\,idenit} \quad (3)$$

The initial time (tinit) of nitrification was t_1 = 30 min and the final (tfnit) was t_6 = 330 min in the aerobic phase. During the anoxic phase, the initial time of denitrification was t idenit = 360 min and final t f denit = 480 min. The values were correlated with the biomass in the reactor expressed as VSS and the rates calculated for each specific case (g/g VSS.d). Olympus microscope model BX40 FISH analyses ("Fluorescence *in situ* Hybridization") were performed for suspended sludge and biofilm samples from the reactor. Samples were fixed in a 4% paraformaldehyde-phosphate-buffered saline solution and placed on 0.6% gelatin and 0.06% $KCr(SO_4)_2$ gelatin-coated glass slides (Amann, 1995, Wagner & Amann, 1997). For bacteria identification, 8 different probes containing specific sequences were used as shown in Table 3.

Table 2. Procedure used in the nitrification and denitrification kinetics

Stage	Time(min)	Sample collected	Remarks
Aerobic	0	S_0	Endogenous phase
Aerobic	30	S_{1N}	Addition of NH_4Cl
Aerobic	90	S_{2N}	
Aerobic	150	S_{3N}	
Aerobic	210	S_{4N}	
Aerobic	270	S_{5N}	
Aerobic	330	S_{6N}	Addition of $C_6H_{12}O_6$
Anoxic	360	S_{1DN}	
Anoxic	420	S_{2DN}	
Anoxic	480	S_{3DN}	

N= nitrification, DN= denitrification

Table 3. Probe sequences used for fluorescent *in situ* hybridization

Probe	Sequence	Reference
EUB mix (EUB338 I + EUB338 II + EUB338 III)	5´- CTG CCT CCC GTA GCA - 3´ 5´- CAG CCA CCC GTA GGT GT - 3´ 5´- CTG CCA CCC GTA GGT GT - 3´	Amann et al. (1990)
Nso190	5-CGATCCCCTGCTTTTCTCC-3	Mobarry et al. (1996)
NIT3 (alfa)	5-CCTGTGCTCCATGCTCCG-3	Wagner et al. (1996)
Competitor	*5-CCTGTGCTCCATGCTCCG-3	
NEU	5'-CCCCTCTGCTGCACTCTA-3'	Wagner et al. (1995)
Competitor	*5'-TTCCATCCCCCTCTGCCG-3'	
Nmv	5-TCCTCAGAGACTACTACGCGG-3	Juretschko et al. (1998)
Ntspn693	5-TTCCCAATATCAACGCATTT-3	Juretschko (2000)
Ntspa 662	5'-GGAATTCCGCGCTCCTCT-3' * 5'-GGAATTCCGCTCTCCTCT-3'	Daims et al. (2001)
AMX820	5'- AAA ACC CCT CTA CTT AGT GCC C -3'	Schmid et al. (2001)

The slides were examined with an Olympus BX 40 (Melville, NY. USA) microscope and the abundance of hybridized cells was estimated by randomly selecting 10 microscope fields with the magnification increased to 1000X. Individual cells in those selected fields that had been stained with DAPI (4,6 - diamino -2 phenyl-indole) were then counted. Sludge and biofilm in the HSBR were also analyzed directly through optical microscopy (Microscope Olympus BX 40).

RESULTS & DISCUSSION

Because the influent was real wastewater from a collection system, the ratio of TCOD: NH_4-N: PO_4-P in the influent was 150:16:7. This nutrient ratio is higher than 150:5:1, which is recommended by Metcalf & Eddy (2003) for maintaining a proper balance between organic matter and nutrients for biological treatment. The pH values ranged between 6.5 and 7.28 at the beginning of the cycles and between 6.40 and 7.81 at the end of the cycles. The temperature and the DO concentrations averages were, respectively, 19.52 ± 1.84 °C and 0.41 ± 0.5 mg O_2/L at the beginning of the anoxic phases and 19.49 ± 2.52 °C and 2.27 ± 1.29 mg O_2 / L at the end of the cycles. Several authors report efficient nitrification in hybrid reactors operating at temperatures between 16 and 20 °C (Maas *et al.*, 2008, Paul *et al.*, 2007). The average DO values during the aerobic phases were close to 2.0 mg O_2/L, which is the minimum recommended by Rittmann & McCarty (2001) for the occurrence of nitrification. Table 4 presents results for COD (total and filtered), BOD_5 and TOC during the experimental period. More results for the same reactor operated with different loading rates were previously presented by Costa et al. (2008).

The removal efficiencies were e" 80% for variables representative of carbonaceous matter. The correspondingly low effluent concentrations of COD, BOD_5 and TOC meet Brazilian regulation requirements for effluent discharge into receiving water bodies (CONAMA, 2011). The efficiency values are greater than those reported by Al-Sharekh & Hamoda (2001), who studied an aerated hybrid reactor with a biofilm fixed on a submerged ceramic material to treat urban sewage. At the same organic loading rate that was used in this study (0.5 kg COD/m^3.d) Al-Sharekh & Hamoda achieved an average COD removal efficiency of 75%.

The sludge in the HSBR had VSS concentrations between 1,040 and 3,460 mg VSS/L. The ratio of VSS/TSS ranged between 0.6 and 0.9, which is close to that normally found in conventional activated sludge systems (0.7 to 0.85), as reported by Metcalf & Eddy (2003). The treated effluent had TSS average concentrations of 16 ± 10.6 mg/L, while the average TSS removal efficiency was 84 ± 12%.

As presented in Table 5, concerning the removal of total nitrogen, the reactor showed an average efficiency of 61 ± 15.4% for the removal of total nitrogen, with 48.8 % of the removal by denitrification. In an HSBR treating synthetic wastewater with an organic loading rate of 1 kg COD/m^3.day, Li et al (2003) found an average total nitrogen removal efficiency of 57%, slightly lower than those found in the present work.

Table 4. Mean values and removal efficiencies of COD, BOD_5 and TOC (n= 42)

Variable	Influent (mg/L)	Effluent (mg/L)	Removal Efficiency (%)
TCOD	538 ± 94.2	59 ± 36.8	89 ± 6.1
FCOD	395 ± 74.4	31 ± 26.8	92 ± 5.6
BOD_5	223 ± 49.9	7 ± 3.6	97 ± 1.2
TOC	61 ± 16.5	12 ± 3.2	80 ± 7.0

Table 5. Mean values results for alkalinity, nitrogen and phosphorus (n= 40)

Variable	**Influent (mg/L)**	**Effluent (mg/L)**	**Removal Efficiency (%)**
Alkalinity (mg$CaCO_3$/L)	325 ± 51.1	136 ± 2.5	-
NH_4-N (mg/L)	59 ± 8.9	14 ± 10.2	77 ± 16.6
NO_2-N (mg/L)	-	0.4 ± 0.9	-
NO_3-N (mg/L)	-	8.5 ± 7.3	-
PO_4-P (mg/L)	24 ± 4.1	17 ± 7.5	29 ± 14.2
Removal efficiency of total nitrogen (%): 61 ± 15.4			

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The reactor achieved ammonium removal efficiencies between 52 to 99%, with a mean efficiency of 77 ± 16.6% (Fig. 2). The effluent concentrations of ammonium at the end of cycles varied between 0.5 and 27 mg NH_4-N/L, with an average of 14 ± 10.2 mg NH_4-N/L. By comparison, Brazilian environmental standard (CONAMA, 2011) established a maximum of 20 mg NH_4-N/L for effluent discharge into receiving water bodies. Wolff et al. (2005) reported a total nitrogen removal efficiency of 95% in a moving bed biofilm reactor with an applied load of 0.16 kg TKN/m^3.day. Step-feed filling and biological reactions in an anoxic/aerobic SBR were also studied by Lin & Jing (2001). In that work, the treatment of synthetic wastewater was improved compared to a reactor with a single fill per cycle. They found that it was possible to accomplish both nitrification and denitrification during aerobic and anoxic phases, respectively, resulting in an average total nitrogen removal of 90%. A residual concentration of NO_2-N (0.4 mg/L in average) and NO_3-N (8.5 mg/L in average), indicating incomplete denitrification, was observed.

At the start of the aerobic phase, the average temperature was 24.7 ± 0.51°C and the pH was 6.2. At the end of the anoxic phase, the temperature increased to 25.9°C. After lime addition, the pH increased to 7.16, and remained above 7.0 until the end of the aerobic phase. The alkalinity was 30 mg $CaCO_3$/L, but increased to 60 mg $CaCO_3$/L following lime addition, that value was maintained until the end of this phase. Due to the denitrification process, the pH increased from 7.20 to 7.35 (t = 360 min, S_{1DN}), reaching 7.48 at the end of the phase (t = 480 min, S_{3DN}). There was a significant increase in alkalinity over time, reaching a value of 106 mg $CaCO_3$/L at the end of the anoxic phase. This behavior of reduction and recovery of alkalinity is usual in nitrification-denitrification processes, as reported by Hoffmann et al. (2007) and Andreottola et al. (2005). The DO concentration was 7.94 mg O_2/L after the endogenous phase, dropped to 4.41 mg O_2/L 30 minutes after the addition of ammonium due to bacteria consumption, and reached 7.8 mg O_2/L at the end of aerobic phase. During the anoxic phase, the DO values dropped to approximately zero.

Fig. 3 presents the results obtained for COD during the kinetic study. Residual concentrations of total and filtered COD were observed at time zero (after the endogenous phase). During the anoxic phase, COD

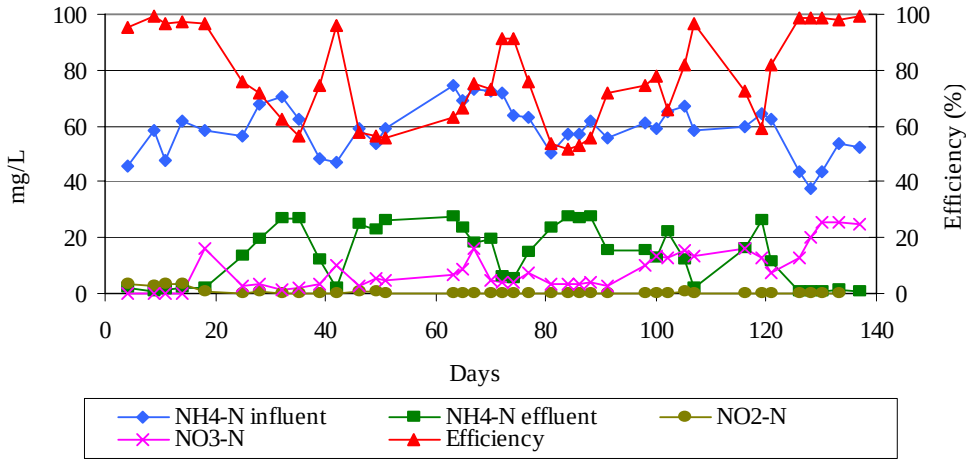

Fig. 2. NH_4-N influent and effluent, NO_2-N and NO_3-N effluent and ammonium removal efficiency during the experimental period

reductions were observed, with removals of 27% TCOD and 31% FCOD, between the beginning and the end of the phase.

Fig. 4 shows the behavior of NH_4-N, NO_2-N, NO_3-N and PO_4-P during the kinetic study. A period of 330 minutes was required to achieve almost total oxidation of ammonium. During the aerobic phase, the ammonia introduced into the reactor was reduced from 29 mg/L, representing an efficiency of 99%. During the anoxic phase the nitrate concentration decreased from 73 m/L to 69 mg/L 30 minutes after the addition of glucose. This indicated the beginning of biological denitrification. As the phase continued, there was a release of N_2 in the atmosphere, which led to flotation of the sludge. Thus, the anoxic phase had to be stopped 2 hours after it started (t = 480 min). At that time, the concentrations of ammonium, nitrite and nitrate were 3.8 mg NH_4-N/L, 0.74 mg NO_2-N/L and 59.2 mg NO_3-N/L, respectively.

The rates of ammonium consumption and nitrogen oxide (nitrite and nitrate) production during the aerobic phase were 5.94 mg NH_4-N/L.h and 7.8 mg NOx-N/ L.h, respectively. Because the VSS concentration in the sludge was 2,500 mg/L, the specific rates for ammonium consumption and nitrogen oxide production were 0.057 g NH_4-N/g VSS.day and 0.074 g NOx-N/g VSS.day, respectively. The nitrogen oxide (nitrite and nitrate) consumption rate was 5.22 mg NOx-DN/L.h, and the specific rate was 0.05 g NOx-DN/g VSS.day. Chowdhury et al.(2008), using fluidized bed reactors for wastewater treatment, observed nitrification rates between 13.75 and 25.4 mg N/L.h and specific rates of 0.09 ± 0.02 g NH_4-N/g VSS.day for nitrification and 0.15 ± 0.02 g NOx-DN/g VSS.day for denitrification. Sen & Dentel (1998) reported rates ranging between 10.42 and 26.67 mg N/L.h for denitrification in fluidized bed reactors, where bacterial activity is known to be higher than in activated sludge reactors.

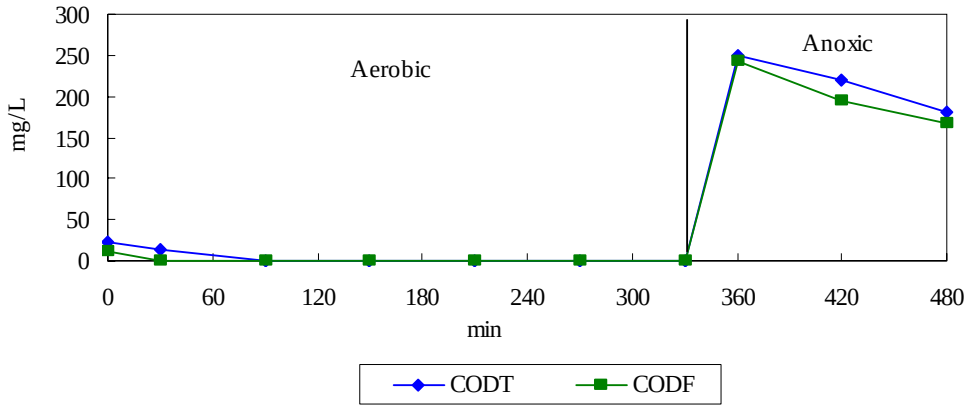

Fig. 3. Behavior of TCOD and FCOD during kinetic study

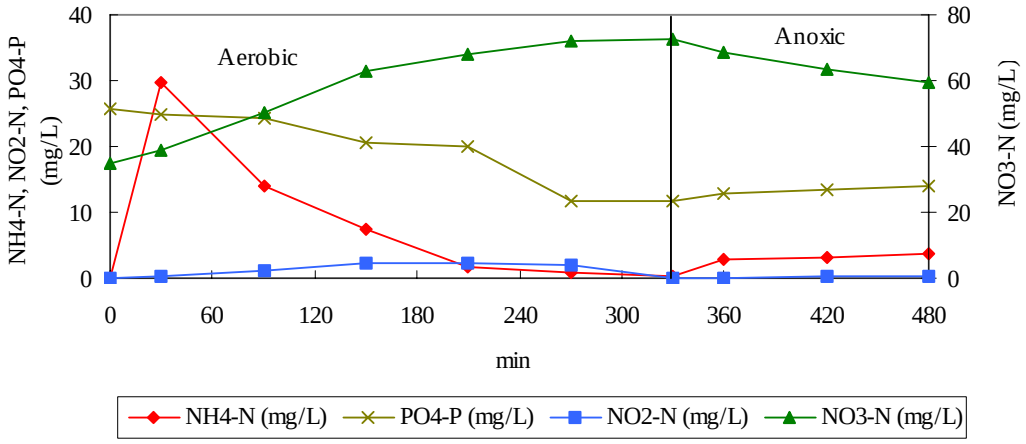

Fig. 4. Behavior of NH_4-N, NO_2-N, NO_3-N and PO_4-P during the kinetic

For phosphate, it was observed that there was phosphate assimilation by bacteria during the aerobic phase. The average concentration decreased from 26 mg PO_4-P/L at the beginning of the phase to 12 mg PO_4-P/L at the end, representing a removal of 54%. In the anoxic phase, the TCOD was consumed, and phosphate was released by the bacteria. The concentration increased from 12 mg PO_4-P/L to 14 mg PO_4-P/L from the beginning to the end of the phase due to low DO conditions. This behavior was also observed in the study of Machnicka et al. (2008).

Observations of the suspended sludge through an optical microscope showed the presence of many microorganisms, which was indicative of good treatment performance (Jenkins *et al.*, 1993). In the last week of reactor operation, the sludge exhibited a variety of microorganisms, including tecamoebas, Rotifers sp and Vorticellas sp (Fig.5). This indicated that the reactor operation remained stable (Warren *et al.*, 2010).

The fixed biomass was observed to be a dense biofilm and was seen to include large numbers of *Rotatoria sp*, Rotifers and Zooglea sp (Fig. 6). Fried & Lemmer, (2003) suggested that peritrichous ciliates can protect against biofilm abrasion, either by attachment to the lower (basal) part of the biofilm e.g., Vorticella sp, or by the formation of large colonies consisting of up to several hundred zooids, which adhere to the support material. The occurrence of numerous Rotifers in the sludge of biological reactors treating sewage, was characteristic of high sludge ages (Canler *et al.*, 1999, Warren *et al.*, 2010) and reactors with high removal efficiencies for carbonaceous matter and for nitrification (>90%).

Working with different biofilm reactors (lab and large scale), Fried et al. (2000) showed that ciliates and metazoans are able to rapidly change their communities as a reaction to changed plant operating conditions. Nematodes and rotifers seem to compete with peritrichous ciliates and are also able to rapidly increase their population.

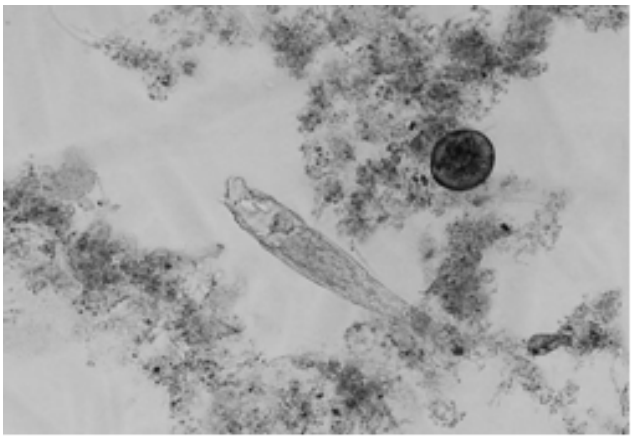

Fig. 5. Tecamoebas, Vorticellas sp e Rotífer sp in sludge (100X) (T=133 days)

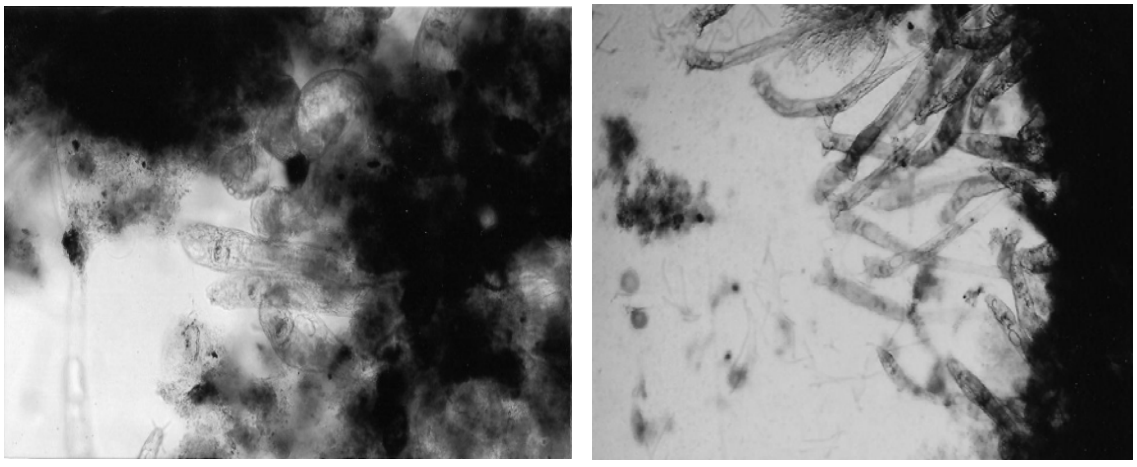

Fig. 6. Optical image of biofilm (100X) (T = 133 days)

The results of FISH analysis for both the sludge samples and the biofilm showed that 50% of the bacteria in both media were ammonium-oxidizing bacteria (AOB), such as *Nitrosomonas sp* and *Nitrosococcus mobilis*. Nitrite-oxidizing bacteria (NOB) such as *Nitrobacter sp* were also found in the sludge (15%) and the biofilm (5%).The growth of the autotrophic biomass was made possible by suitable pH and DO conditions, and enabled an ammonium removal of 77%. Park et al. (2008) observed in biofilm reactors with different DO concentrations (1 to 7 mg/L) an increase in the ratio of AOB from 9.5 to 34.6% with increasing DO. In the case of NOB consisting of *Nitrospira* genus and *Nitrobacter* sp, higher DO levels resulted in a higher distribution ratio.

CONCLUSION

The reactor was able to treat loads of 0.51 kg COD/m^3.day and 0.06 kg NH_4-N/m^3.day, with average removal efficiencies of 92% for FCOD, 29% for PO_4-P, 77% for NH_4-N and 61% for total nitrogen. The effluent characteristics met the requirements of Brazilian environmental standard for release to receiving water bodies. The kinetic study of nitrification and denitrification showed specific ammonium consumption rates of 0.057 g NH_4-N/g VSS.day and 0.074 g NOx-N/g VSS.day of NOx-N production. The specific consumption rate of NOx-N was 0.05 g NOx-DN/g VSS.day. The suspended and fixed biomass was composed of 50% ammonium-oxidizing bacteria (AOB) and 20% nitrite-oxidizing bacteria (NOB). Optical microscopy revealed protozoans and metazoans in the reactor, indicating a stable treatment situation.

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REFERENCES

Al-Sharekh, H. and Hamoda, M. F. (2001). Removal of organics from wastewater using a movel biological hybrid system. Water Science and Technology, **43** (**1**), 321-326.

Amann, R. I. (1995) In situ identification of microorganisms by whole cell hybridization with rRNA-targeted nucleic acide probes. In: A.D.L. Akkermans, J.D. van Elsas and F.J. de Bruijn, Editors, Molecular Microbial Ecology Manual, Kluwer Academic Publications, London,p.MMEM-3.3.6/1-MMEM-3.3.6/15.

Amann, R. I., Blinder, B. J., Olson, R. J., Chisholm, S. W., Devereux, R. and Stahl, D. A. (1990). Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol. **56**: 1919-1925.

Andreottola, G., Foladori, P., Nardelli, P. and Denicolo, A. (2005). Treatment of winery wastewater in a full-scale fixed bed biofilm reactor. Water Science and Technology, **51** (**1**), 71-79.

Andreottola, G., Foladori, P., Ragazzi, M. andTatano, F. (2000). Experimental comparison between MBBR and activated sludge system for the treatment of municipal wastewater. Water Science and Technology, **41** (**4-5**), 383-391.

APHA, (2005). Standard Methods for the Examination of Water and Wastewater. 21th ed. Washington DC: APHA, AWWA, WEF.

Artan, N. and Orhon, D. (2005). Mechanism and design of sequencing batch reactors for nutrient removal. London: IWA Publishing. Scientific and Technical Report Series, 19.

Canler, J. P., Cotteux, E. and Duchène, P. (1999) Aide ou diágnostic des stations d'épuration par l'observation microscopique des boues activées. France: CEMAGREF.

Chowdhury, N., Nakhla, G. and Zhu, J. (2008). Load maximization of a liquid-solid circulating fluidized bed bioreactor for nitrogen removal from synthetic municipal wastewater. Chemosphere, **71**, 807-815.

CONAMA, (2011). Conselho Nacional do Meio Ambiente. 'Resolução CONAMA nº 430, de 13 de maio de 2011'. Dispõe sobre as condições e padrões de lançamento de efluentes, complementa e altera a Resolução no 357, de 17 de março de 2005, do Conselho Nacional do Meio Ambiente-CONAMA.

Costa, R. H. R. , Souto, V. S. , Prelhaz, A. T. S. , Neto, L. G. L. and Wolff, D. B. (2008). Utilization of a hybrid sequencing batch reactor (HSBR) as a decentralized system of domestic wastewater treatment. Water Science and Technology, **57** (**12**), 1951-1956.

Daims, H., Nielsen, J. L., Nielsen, P. H., Schleifer, K. H. and Wagner, M. (2001). In situ characterization of Nitrospira-like nitrite-oxidizing bacteria active in wastewater treatment plants. Appl. Environ. Microbiol., **67**, 5273-5284.

Fried, J. and Lemmer, H. (2003) On the dynamics and function of ciliates in sequencing batch biofilm reactors. Water Science & Technology, **47** (**5**), 189-196.

Fried, J., Mayr, G., Berger, H., Traunspurger, W., Psenner, R. and Lemmer, H. (2000). Monitoring protozoa and metazoan biofilm communities for assessing wastewater quality impact and reactor up-scaling effects. Water Science & Technology, **41** (**4-5**), 309-316.

Gapes, D. J. and Keller, J. (2009). Impact of oxygen mass transfer on nitrification reactions in suspended carrier reactor biofilms. Process Biochemistry, **44**, 43-53.

Jenkins, D., Richard, M. G. and Daigger, G. T. (1993). Manual on the causes and control of activated sludge bulking and foaming**.** 2ªEd.Michigan: Lewis Publishers.

Juretschkos, S. (2000). Mikrobielle Populationsstruktur und -dynamik in einer nitrifizierenden/denitrifizierenden Belebtschlammanlage. Doctoral thesis (Technische Universität München).

Juretschko, S., Timmermann, G. Shimid, M., Schleifer, K-H., Pommerening-Roser, A., Koops, H. P. and Wagner, M. (1998). Combined molecular and conventional analyses of nitrifying bacterium diversity in activated sludge: *Nitrosococcus mobilis* and *Nitrospira*-like bacteria as dominant populations. Appl. Environ. Microbiol., **64**, 3042-3051.

Helness, H. and Ødegaard, H. (2000). Biological phosphorus and nitrogen removal in a sequencing batch moving bed biofilm reactor. Proceedings of the 1st World Water Congress of the International Water Association (IWA), Paris.

Hoffmann, H, Costa, T. B. Wolff, D. B., Platzer, C. and Costa, R. H. R. (2007). The potential of denitrification for the stabilization of activated sludge processes affected by low alkalinity problems. Brazilian Archives of Biology and Technology, **50 (2),** 329-337.

Jang, A., Bishop, P. L., Okabe, S., Lee, S. G. and Kim, I. S. (2002) Effect of dissolved oxygen concentration on the biofilm and *in situ* analysis by fluorescence *in situ* hybridization (FISH) and microelectrodes. Water Science and Technology, **47** (**1**), 49-57.

Jin Lim, S., Moon, R. K., Woo, G. L., Kwon, S., Park, B. G. and Chang, H. N. (2000). Operation and Modeling of Bench-Scale SBR for Simultaneous Removal of Nitrogen and Phosphorus Using Real Wastewater. Biotechnol. Bioprocess Eng., **5**, 441-448.

Li, J., Xing, X. H. and Wang, B. Z. (2003). Characteristics of phosphorus removal from wastewater by biofilm sequencing batch reactor (SBR). Biochemical Engineering Journal**, 16**, 279-285.

Lin, Y. F. and Jing, S. R. (2001). Characterization of denitrification and nitrification in a step-feed alternating anoxic-oxic sequencing batch reactor. Water Environment Research, **73** (**5**), 526-533.

Machnicka, A., Grübel, K. and Suschka, J. (2008). Enhanced biological phosphorus removal and recovery. Water Environment Research, **80** (**7**), 617-623.

Maas, C. L., Parker, W. J. and Legge, R. L. (2008). Detachment of solids and nitrifiers in integrated, fixed-film activated sludge systems. Water Environment Research, **80** (**12**), 2202-2208.

Metcalf & Eddy. (2003). Wastewater Engineering: Treatment and Reuse. 4ª Ed. Mc Graw Hill, Inc. New York, USA.

Mobarry, B. K., Wagner, R. M., Urbain, V., Rittmann, B. E. and Stahl, D. A. (1996). Phylogenetic probes for analyzing abundance and spatial organization of nitrifying bacteria. Appl. Environ. Microbiol., **62**, 2156-2162.

Ochoa, J. C., Colprim, J., Palacios, B. Paul, E. and Chatellier, P. (2002). Active heterotrophic and autotrophic biomass distribution between fixed and suspended systems in a hybrid biological reactor. Water Science and Technology, **46** (**1-2**), 397-404.

degaard, H. (2006). Innovations in wastewater treatment: the moving bed biofilm process. Water Science and Technology, **53** (**9**), 17-33.

Oyanedel, V., Garrido, J. M., Colprim, J., Lazarova, V. and Mendez, R. (2002) Comparison and modelling of the growth of heterotrophs and nitrifiers in biofilm and hybrid airlift reactors. International Specialised Conference on Biofilm Monitoring, Porto, Portugal, 241-244.

Park, J. J., Byun, I. G., Yu, J. C., Park, S. R., Ju, D. J., Hur, S. H. and Park, T. J. (2008) Analysis of nitrifying bacterial communities in aerobic biofilm reactors with different DO conditions using molecular techniques. Water Science and Technology, **57** (**12**), 1889-1899.

Paul, E, Wolff, D. B., Ochoa, J. C. and Costa, R. H. R. (2007). Recycled and virgin plastic carriers in hybrid reactors for wastewater treatment. Water Environmental Federation**, 79** (**7**), 765-774.

Rittmann, B. E. and McCarty, P. L. (2001). Environmental Biotechnology: Principles and Applications. McGraw-Hill, New York.

Sen, P. and Dentel, S. K. (1998). Simultaneous nitrification-denitrification in a fluidized-bed reactor. Water Science and Technology, **38** (**1**), 247-254.

Uemura, S., Suzuki, S., Abe, K., Ohashi, A., Harada, H., Ito, M., Imachi, H. and Tokutomi, T. (2011) Partial Nitrification in na Airlift Activated Sludge Reactor with Experimental and Theoretical Assessments of the pH Gradient Inside the Sponge Support Medium. Int. J. Environ. Res., **5** (**1**), 33-40.

Wagner, M. and Amann, R. (1997). Molecular techniques for determining microbial community structures inactivated sludge. In: IAWQ. Microbial community analysis: the key to the design of biological wastewater treatment systems. pp 61-71.

Wagner M., Rath G., Amann R., Koops H. P. and Schleifer, K. H. (1995). In situ identification ofammonia-oxidizing bacteria. Syst. Appl. Microbiol., **18**, 251-264.

Wagner, M., Rath, G., Koops, H. P., Flood, J. and Amann R. (1996). In situ analysis of nitrifyingbacteria in sewage treatment plants. Water Science Technology, **34**, 237-244.

Warren, A., Salvadó, H., Curds, C. R. and Roberts, D. (2010). Protozoa in activated sludge processes. In: Microbial Ecology of Activated Sludge. Ed. Seviour & Nielsen, IWA Publishing, London, UK.

Watts, K. and Münch, B. (2000). Upgrading activated sludge plants for nitrogen and phosphorus removal by process intensification using suspended plastic carries. Proceedings: WaterTech, Sydney, AWA.

Wessman, F. G., Tuegen, E. Y., Zheng, Q., He, G., Welander, T. and Rusten B. (2004). Increasing the capacity for treatment of chemical plant wastewater by replacing existing suspended carrier media with Kaldnes Moving Bed™ media at a plant in Singapore. Water Science and Technology, **49** (**11-12**), 199-205.

Wolff, D. B., Ochoa, J. C., Paul, E. and Costa, R. H. R. (2005). Nitrification in hybrid reactor with a recycled plastic support material. Brazilian Archives of Biology and Technology, **48**, special issue, 243-248.

Zhang, L., Sun, K. and Hu, N. (2012). Degradation of organic matter from domestic wastewater with loofah sponge biofilm reactor. Water Science and Technology, **65 (1),** 190-195.