Nitrogen removal in a sequencing batch biofilm reactor with liquid circulation: Effect of feed strategy and carbon source in the denitrifying step

C.S.A. Canto [1], S.M. Ratusznei [1], J.A.D. Rodrigues [1,*], M. Zaiat [2], E. Foresti [2]

[1]Escola de Engenharia Mauá, Instituto Mauá de Tecnologia (EEM-IMT); Praça Mauá 1; CEP 09580-900; São Caetano do Sul, São Paulo, Brasil. [2]Departamento de Hidráulica e Saneamento, Escola de Engenharia de São Carlos, Universidade de São Paulo (USP); Av. Trabalhador São-Carlense 400; CEP 13.566-590; São Carlos, São Paulo, Brasil

Eliminación de nitrógeno en un reactor biológico secuencial de flujo discontinuo con circulación de líquido: Efecto de la estrategia de alimentación y de la fuente de carbono en la etapa de desnitrificación

Eliminació de nitrogen en un reactor biològic seqüencial de flux discontinu amb circulació de líquid: Efecte de l'estratègia d'alimentació i de la font de carboni a l'etapa de desnitrificació

Recibido: 22 de septiembre de 2009; aceptado: 23 de octubre de 2009

RESUMEN

Se realiza la eliminación del nitrógeno amoniacal de una agua residual sintética mediante procesos de nitrificación y desnitrificación en un reactor biológico secuencial de flujo discontinuo que contiene biomasa inmovilizada sobre espuma de poliuretano con circulación de la fase líquida. Se analiza el efecto de cuatro fuentes externas de carbono (etanol, acetato, medio de carbono sintético y metanol) que actúan como dadores de electrones en el proceso de desnitrificación. Los experimentos se realizan con aireación intermitente y se operan a 30±1°C en ciclos de 8 horas. El agua residual sintética (100 mgCOD/L y 50 mgNH4+-N/L) se adiciona en modo lote, mientras que las fuentes externas de carbono se adicionan en modo lote alimentado durante los periodos en que se suspende la aireación. Las eficiencias de eliminación de nitrógeno amoniacal obtenidas son del 95,7, 94,3 y 97,5% para el etanol, el acetato y el medio de carbono sintético, respectivamente. Por lo que respecta a las concentraciones en el efluente de nitrógeno en forma de nitrito, nitrato y amonio, los resultados obtenidos son, respectivamente: 0,1, 5,7 y 1,4 mg/L para el etanol; 0,2, 4,1 y 1,8 mg/L para el acetato, y 0,2, 6,7 y 0,8 para el medio de carbono sintético. Por otra parte, el uso de metanol, incluso a concentraciones bajas (50% del valor estequiométrico calculado para la desnitrificación completa), comporta un incremento de la acumulación de nitrógeno en forma de nitrato y de amonio al efluente con el transcurso del tiempo.

Palabras clave: Nitrificación, desnitrificación; fuente externa de carbono; biomasa inmovilizada; reactor biológico secuencial de flujo discontinuo, SBBR.

SUMMARY

Ammonium nitrogen removal from a synthetic wastewater by nitrification and denitrification processes were performed in a sequencing batch biofilm reactor containing immobilized biomass on polyurethane foam with circulation of the liquid-phase. It was analyzed the effect of four external carbon sources (ethanol, acetate, carbon synthetic medium and methanol) acting as electron donors in the denitrifying process. The experiments were conducted with intermittent aeration and operated at 30±1°C in 8-h cycles. The synthetic wastewater (100 mgCOD/L and 50 mgNH_4^+-N/L) was added batch-wise, while the external carbon sources were added fed-batch-wise during the periods where aeration was suspended. Ammonium nitrogen removal efficiencies obtained were 95.7, 94.3 and 97.5% for ethanol, acetate and carbon synthetic medium, respectively. As to nitrite, nitrate and ammonium nitrogen effluent concentrations, the results obtained were, respectively: 0.1, 5.7 and 1.4 mg/L for ethanol; 0.2, 4.1 and 1.8 mg/L for acetate and 0.2, 6.7 and 0.8 for carbon synthetic medium. On the other hand using methanol, even at low concentrations (50% of the stoichiometric value calculated for complete denitrification), resulted in increasing accumulation of nitrate and ammonium nitrogen in the effluent over time.

Keywords: Nitrification; denitrification; external carbon source; immobilizedd biomass; sequencing batch biofilm-reactor; SBBR.

RESUM

Es realitza l'eliminació del nitrogen amoniacal d'una aigua residual sintètica mitjançant processos de nitrificació i desnitrificació en un reactor biològic seqüencial de flux discontinu que conté biomassa immobilitzada sobre escuma de poliuretà amb circulació de la fase líquida. S'analitza l'efecte de quatre fonts externes de carboni (etanol, acetat, medi de carboni sintètic i metanol) que actuen com a do-

---

*To whom all correspondence should be addressed: rodrigues@maua.br

nadors d'electrons en el procés de desnitrificació. Els experiments es realitzen amb aeració intermitent i s'operen a 30±1°C en cicles de 8 hores. L'aigua residual sintètica (100 mgCOD/L i 50 mgNH4+-N/L) s'addiciona en mode lot, mentre que les fonts externes de carboni s'addicionen en mode lot alimentat durant els períodes en que es suspèn l'aeració. Les eficiències d'eliminació de nitrogen amoniacal obtingudes són del 95,7, 94,3 i 97,5% per a l'etanol, l'acetat i el medi de carboni sintètic, respectivament. Pel que fa a les concentracions a l'efluent de nitrogen en forma de nitrit, nitrat i amoni, els resultats obtinguts són, respectivament: 0,1, 5,7 i 1,4 mg/L per a l'etanol; 0,2, 4,1 i 1,8 mg/L per a l'acetat, i 0,2, 6,7 i 0,8 per al medi de carboni sintètic. D'altra banda, l'ús de metanol, fins i tot a concentracions baixes (50% del valor estequiomètric calculat per a la desnitrificació completa), porta a un increment de l'acumulació de nitrogen en forma de nitrat i d'amoni a l'efluent en transcorre el temps.

Mots clau: Nitrificació, desnitrificació; font externa de carboni; biomassa immobilitzada; reactor biològic seqüencial de flux discontinu, SBBR.

INTRODUCTION

Nitrification and denitrification are the most widely used large-scale biological processes for organic and ammonium nitrogen removal from wastewaters. Nitrification takes place under autotrophic aerobic means, which oxidize ammonium nitrogen to nitrite and subsequently to nitrate. Denitrification occurs primarily under heterotrophic anoxic conditions, where organic carbon sources act as electron donors and reduce nitrate to nitrogen gas. Since denitrification is generally the last step in wastewater treatment, when the major COD has already been oxidized, a supplemental organic carbon source should frequently be added (Thalasso et al., 1997). An external carbon source reduces reaction time and allows reduction in the hydraulic detention time in the anoxic zones. Several carbon sources, such as methanol, ethanol, methane, acetate and glucose have been used for this purpose (Dinçer and Kargi, 2000; Ilies and Mavinic, 2001; Cervantes et al., 2001; Louzeiro et al., 2002).

Furthermore, controlled addition of these organic compounds to the process may be a means to improve process stability and flexibility since the flow rate of the feeding source can be adjusted as necessary. The denitrification rate depends on the anoxic condition of the mixed liquor, carbon and energy sources used and on the carbon to nitrogen (C/N) ratio (Pambrun et al, 2004). Low C/N ratios may cause nitrite accumulation (Mohseni-Bandpi and Elliot, 1998), while the dissimilative reduction to ammonium may occur at high C/N ratios (Gejlsbjerg et al., 1998) and harm the denitrification process. Residual dissolved oxygen can also shunt the process.

A number of systems configurations developed for nitrification and denitrification processes have been operated successfully, although operating problems can occur, which can result in additional costs, low pollutant removal performance or even wastewater treatment plant upset. As these processes require the use of sequential anoxic and aerobic zones, sequencing batch reactors (SBR) are extremely flexible, very effective treatment systems for small to medium-sized plants and allow appropriated conditions for biological nitrogen removal, which can be achieved alternating aeration and mixing time periods (Louzeiro et al., 2002; Aulenta et al., 2003; Linlin et al., 2005; Obaja et al., 2005).

An immobilized biomass system (biofilm) appears to be advantageous due to the improvement of biofilm growth of species responsible for the nitrification process. When a biofilm is adapted to an SBR, the reactor becomes an SBBR, i.e., Sequencing Batch Biofilm Reactor. Some SBBR configurations still incorporate recirculation of the generated liquid and/or gaseous effluent into the proper reactor to improve mixing as well as contact between biomass and wastewater and to dilute the influent in the case of high-strength wastewaters. Hence SBBR with liquid-phase recirculation seems to be a promising alternative in treating conventional nitrogen-rich wastewaters.

Therefore, the objective of this investigation was to study the effect of fed batch-wise addition of external carbon sources (ethanol, acetate, carbon synthetic medium and methanol) on the removal of ammonium nitrogen from a synthetic wastewater, employing nitrification/denitrification processes and using a sequencing batch biofilm reactor containing immobilized biomass on polyurethane foam and with liquid-phase circulation

MATERIALS AND METHODS

Microorganisms (biomass)

Microorganisms from different wastewater treatment processes were used. For the aerobic nitrification process biomass was taken from an Extended Aeration Activated Sludge System applied to a sanitary wastewater treatment. For the anaerobic/anoxic denitrification process biomass was taken from an Upflow Anaerobic Sludge Blanket (UASB) applied to a poultry slaughterhouse wastewater treatment. The aerobic biomass was added in the first two stages of the reactor (I and II), while the aerobic/anoxic biomass was introduced in the subsequent stages (III and IV). This procedure was utilized to guarantee a dissolved oxygen concentration (DO) supply to the nitrifying microorganisms during the aeration periods. Oxygen was supplied to the microorganisms by circulating the liquid medium (see Figure 1).

For biofilm formation, the microorganisms were previously immobilized on an inert support consisting of 1.0-cm polyurethane foam cubes, according to methodology developed by Zaiat et al. (1994). Later, the supports were placed in the four stages of the reactor (I, II, III and IV) and the wastewater was circulated at low velocity (0.08 cm/s) to avoid biomass detachment from the foam, allowing continuation of the biofilm formation.

Synthetic wastewater and external carbon sources

Synthetic wastewater

The synthetic wastewater containing ammonium nitrogen was added batch-wise. This wastewater, containing approximately 100 mgCOD/L and 50 mgNH_4^+-N/L, was prepared with sucrose (7 mg/L), starch (22.8 mg/L), cellulose (6.8 mg/L), meat extract (41.6 mg/L), soybean oil (10.2 mg/L), NaCl (50 mg/L), $MgCl_2.6H_2O$ (1.4 mg/L), $CaCl_2.2H_2O$ (0.9 mg/L), $NaHCO_3$ (500 mg/L), commercial detergent (3 drops/L) for soybean oil emulsification, and NH_4Cl (195.6 mg/L). The medium (without nitrogen source) was sterilized (121oC, 15 min) in order to maintain its characteristics during the experimental time. It should be mentioned that sterilization did not alter the original characteristics of the influent.

External carbon sources

During the denitrification process, when the reactor was operated fed-batch-wise, the following solutions were used as external carbon sources. It should be pointed out that the external carbon sources solutions were buffered with $NaHCO_3$ (500 mg/L) and all media (synthetic wastewater and external carbon sources solutions) were prepared using tap water.

(a) Ethanol

The concentration of initially used ethanol (117.3 mg/L – 245.2 $mgCOD_{Theoretical}$/L) was calculated from the stoichiometric nitrate reduction reaction using ethanol as the electron donor and assuming all influent ammonium nitrogen was converted to nitrate. The feed volumes during the batch (1.2 L) and fed-batch (0.7 L) were also considered in the calculation. This concentration value was then optimized to increase total nitrogen removal efficiency at reactor operation conditions, resulting in 93% of the stoichiometric value, i.e., 109.1 mg/L (228.0 $mgCOD_{Theoretical}$/L).

(b) Acetate

The acetate solution was prepared initially using 209.9 mg/L of sodium acetate (224.6 $mgCOD_{Theoretical}$/L) whose concentration was determined as in the previous case. This concentration value was then optimized to increase total nitrogen removal efficiency at reactor operation conditions, resulting in 119% of the stoichiometric value, i.e., 249.8 mg/L (267.3 $mgCOD_{Theoretical}$/L).

(c) Carbon synthetic medium

The organic matter concentration of the carbon synthetic medium used in the denitrification step varied between 100 and 200 mg/L (as COD). In order to optimize nitrate reduction some adjustments were made resulting in final substrate concentration (as COD) of 180 mg/L. At these terms, the medium prepared consisted of the following: sucrose (12.6 mg/L), starch (41.0 mg/L), cellulose (12.2 mg/L), meat extract (74.9 mg/L), soybean oil (18.4 mg/L), NaCl (50 mg/L), $MgCl_2.6H_2O$ (1.4 mg/L), $CaCl_2.2H_2O$ (0.9 mg/L), $NaHCO_3$ (500 mg/L) and commercial detergent (3 drops/L) for soybean oil emulsification. All values of substrate concentration (100 to 200 mg/L) were calculated proportionally to the previous composition.

(d) Methanol

Methanol was the fourth carbon source tested. The stoichiometric methanol concentration to promote complete reduction of nitrate to nitrogen gas is 163.2 mg/L (244.9 mgCODTheoretical/L). This value was calculated analogously to those of ethanol and acetate. However, due to suspected toxic effects of methanol on the microorganisms involved in the process (Hallin et al., 1996) its addition was performed with ethanol at the ratios shown in Table 1 in order to minimize such possible effects.

	Methanol		Ethanol	
Phases	(%)	(mL/L)	(%)	(mL/L)
α	25	0.04	75	0.12
β	50	0.08	50	0.08
χ	75	0.12	25	0.04
δ	100	0.16	0	0.00

Table 1: *Percentages and amounts of methanol and ethanol in the medium fed.*

Experimental set-up

The experimental set-up (shown in Figure 1) consisted of an acrylic reactor with a working volume of 2.7 L (total volume 3.7 L), height of 540 mm, external diameter of 100 mm and wall thickness of 3.5 mm. For liquid circulation in the reactor a reservoir, also of acrylic, was attached with a working volume of 0.8 L (total volume 0.9 L), height of 400 mm, external diameter of 60 mm and wall thickness of 3.5 mm. The reactor was divided into 4 (four) levels of 10-cm height each, utilizing stainless steel screens, in order to prevent bed packing. Feed and dewatering were performed by means of diaphragm pumps and circulation by a peristaltic pump. On and off switching of these pumps was performed by an automated system. The chamber in which the reactor was allocated was maintained at 30 ± 1°C by a heating system composed of resistances and fans, as well as a temperature sensor and controller. Eight-hour operation cycles were used.

For measuring the circulation flow rate a 100-mL graduated tube was connected between the reactor and the reservoir. Recirculation flow was set at 20 L/h and fluid flow rate was 0.20 cm/s. These values were obtained from hydrodynamic studies, which showed that under these conditions the reactor-reservoir system behaves as a completely mixed reactor (Camargo et al., 2005).

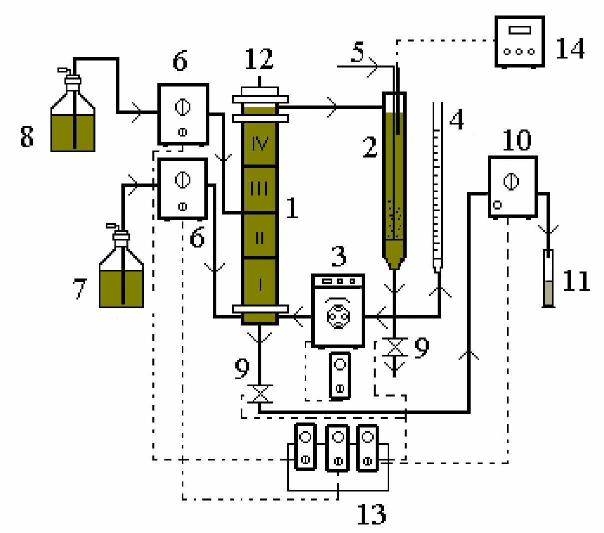

Figure 1: *Schematic representation of the sequencing batch reactor with external circulation of the liquid phase and immobilized biomass [1 – reactor; 2 – circulation reservoir; 3 – circulation pump; 4 – flow rate meter; 5 – aerator; 6 – feed pumps; 7 – synthetic wastewater reservoir; 8 – external carbon source reservoir; 9 – discharge valve; 10 – dewatering pump; 11 – effluent outlet; 12 – biogas outlet; 13 – control unit; 14 - dissolved oxygen concentration meter; ——— hydraulic connections; ——— electric connections].*

Orifices in the circulation reservoir cover (see Figure 1) allowed introduction of an aerator for intermittent aeration of the medium and a dissolved oxygen (DO) electrode for concentration measurement. Redox potential (RP) was also measured by means of an electrode inserted through the reactor top.

Reactor operation

Reactor feeding was batch-wise followed by fed-batch-wise in eight-hour operation cycles (Table 2). In the batch mode a synthetic wastewater containing ammonium nitrogen was added during 10 (ten) minutes. On the other hand, when the external carbon source was added, the reactor was operated fed batch-wise. Batch feeding was through the reactor bottom and fed-batch feeding between the second and third level of the reactor (see Figure 1). The objective of this procedure was to add the external carbon source at the third stage of the bed where the anaerobic/anoxic microorganisms were confined. It should be mentioned that fed-batch feeding of the external carbon source was carried out during the periods when aeration was interrupted. Experimental conditions used are summarized in Table 2.

Table 2: *Operational conditions during the cycle period.*

Characteristics of the process	t' (min)	V' (L)	Subs-trate	Aeration	Recircu-lation
Batch-wise	10	1.20	SW	No	No
No feed	120	0	-	Yes	Yes
Fed-batch-wise	60	0.19	ECS	No	Yes
No feed	120	0	-	Yes	Yes
Fed-batch-wise	160	0.51	ECS	No	Yes
Discharge	10	-1.90	-	No	No

Analytical methods

System monitoring was done according to Standard Methods for the Examination of Water and Wastewater (1995). The following were quantified: substrate as COD for non-filtered (C_T) and filtered (C_S) samples; total solids (TS), total suspended solids (TSS), total volatile solids (TVS) and volatile suspended solids (VSS); bicarbonate alkalinity (BA); total volatile acids (TVA); pH; ammonium nitrogen (NH_4^+-N); organic nitrogen (Org-N); nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N). It should be mentioned that concentrations of total solids (TS) and total volatile solids (TVS) in the reactor were calculated based on the support mass (polyurethane foam cubes) with and without immobilized biomass and the solids mass after detachment from the foam. After stabilization the following concentration profiles were obtained during the cycle: C_S, BA, TVA, IVA, pH, NO_2^--N, NO_3^--N, NH_4^+-N, Org-N, DO and RP.

Intermediate volatile acids (IVA – acetic, propionic, isobutyric, butyric, isovaleric, valeric and caproic) samples were analyzed by gas chromatography, using a Gas Chromatograph HP6890, Series CG System, with flame ionization detector (FID) at 300°C and an HP-INNOWAX column (length: 30 m; internal diameter: 0.25 mm; film: 0.25 µm). 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 to 180°C, and held at that temperature for 5 min. H_2 and N_2 were used as carrier gas and make-up gas, respectively.

Mathematical equations

Reactor performance was analyzed by Equations (1) to (16) (Canto et al., 2008a and b):

$$\mathrm{VOL_B} = \frac{3 \cdot \mathrm{C_B} \cdot \mathrm{V_B}}{\mathrm{V_D}} \quad (1)$$

$$\mathrm{VOL_{FB}} = \frac{3 \cdot \mathrm{C_{FB}} \cdot \mathrm{V_{FB}}}{\mathrm{V_D}} \quad (2)$$

$$\mathrm{C'_B} = \mathrm{C_B} \cdot \mathrm{V_B} \quad (3)$$

$$\mathrm{C'_{FB}} = \mathrm{C_{FB}} \cdot \mathrm{V_{FB}} \quad (4)$$

$$\mathrm{C'_S} = \mathrm{C_S} \cdot \mathrm{V_D} \quad (5)$$

$$\mathrm{C'_T} = \mathrm{C_T} \cdot \mathrm{V_D} \quad (6)$$

$$\frac{\mathrm{C}}{\mathrm{N}} = \frac{\mathrm{C_B} \cdot \mathrm{V_B} + \mathrm{C_{FB}} \cdot \mathrm{V_{FB}}}{(\mathrm{NH_4^+} - \mathrm{N})_\mathrm{B} \cdot \mathrm{V_B}} \quad (7)$$

$$\mathrm{VANL} = \frac{3 \cdot (\mathrm{NH_4^+} - \mathrm{N})_\mathrm{B} \cdot \mathrm{V_B}}{\mathrm{V_D}} \quad (8)$$

$$\mathrm{VTNL} = \frac{3 \cdot (\mathrm{Total} - \mathrm{N})_\mathrm{B} \cdot \mathrm{V_B}}{\mathrm{V_D}} \quad (9)$$

$$\%\left(\mathrm{NO_2^-} - \mathrm{N}\right)_\mathrm{P} = \frac{\left(\mathrm{NO_2^-} - \mathrm{N}\right)_\mathrm{D} \cdot \mathrm{V_D} - \left(\mathrm{NO_2^-} - \mathrm{N}\right)_\mathrm{B} \cdot \mathrm{V_B}}{\left(\mathrm{NH_4^+} - \mathrm{N}\right)_\mathrm{B} \cdot \mathrm{V_B}} \cdot 100$$

(10)

$$\%\left(\mathrm{NO_3^-} - \mathrm{N}\right)_\mathrm{P} = \frac{\left(\mathrm{NO_3^-} - \mathrm{N}\right)_\mathrm{D} \cdot \mathrm{V_D} - \left(\mathrm{NO_3^-} - \mathrm{N}\right)_\mathrm{B} \cdot \mathrm{V_B}}{\left(\mathrm{NH_4^+} - \mathrm{N}\right)_\mathrm{B} \cdot \mathrm{V_B}} \cdot 100$$

(11)

$$\%\left(\mathrm{NH_4^+} - \mathrm{N}\right)_\mathrm{R} = \frac{\left(\mathrm{NH_4^+} - \mathrm{N}\right)_\mathrm{B} \cdot \mathrm{V_B} - \left(\mathrm{NH_4^+} - \mathrm{N}\right)_\mathrm{D} \cdot \mathrm{V_D}}{\left(\mathrm{NH_4^+} - \mathrm{N}\right)_\mathrm{B} \cdot \mathrm{V_B}} \cdot 100$$

(12)

$$\%\left(\mathrm{Org} - \mathrm{N}\right)_\mathrm{R} = \frac{\left(\mathrm{Org} - \mathrm{N}\right)_\mathrm{B} \cdot \mathrm{V_B} - \left(\mathrm{Org} - \mathrm{N}\right)_\mathrm{D} \cdot \mathrm{V_D}}{\left(\mathrm{Org} - \mathrm{N}\right)_\mathrm{B} \cdot \mathrm{V_B}} \cdot 100$$

(13)

$$\%\left(\mathrm{Total} - \mathrm{N}\right)_\mathrm{R} = \frac{\left(\mathrm{Total} - \mathrm{N}\right)_\mathrm{B} \cdot \mathrm{V_B} - \left(\mathrm{Total} - \mathrm{N}\right)_\mathrm{D} \cdot \mathrm{V_D}}{\left(\mathrm{Total} - \mathrm{N}\right)_\mathrm{B} \cdot \mathrm{V_B}} \cdot 100$$

(14)

$$\left(\frac{\mathrm{BA}}{\mathrm{NH_4^+} - \mathrm{N}}\right)_\mathrm{R} = \frac{(\mathrm{BA_B} \cdot \mathrm{V_B} + \mathrm{BA_{FB}} \cdot \mathrm{V_{FB}}) - (\mathrm{BA_D} \cdot \mathrm{V_D})}{\left(\mathrm{NH_4^+} - \mathrm{N}\right)_\mathrm{B} \cdot \mathrm{V_B} - \left(\mathrm{NH_4^+} - \mathrm{N}\right)_\mathrm{D} \cdot \mathrm{V_D}}$$

(15)

$$\mathrm{R} = 3 \cdot \frac{\left(\mathrm{NH_4^+} - \mathrm{N}\right)_\mathrm{B} \cdot \mathrm{V_B} - \left(\mathrm{NH_4^+} - \mathrm{N}\right)_\mathrm{D} \cdot \mathrm{V_D}}{\mathrm{V_D}} \quad (16)$$

It should be pointed out that in Equations (1), (2), (8), (9) and (16), the value "3" refers to the number of cycles a day. Influent ammonium concentrations were considered

Table 3: *COD, VOL and percent COD removed when ethanol, acetate and carbon synthetic medium were used as external carbons sources.*

Phase	Parameter	Unit	Values		
			Ethanol	Acetate	CSM
Batch-wise	CB	mgCOD/L	114.0 ± 5.2	110.3 ± 6.9	121.0 ± 25.6
	C'B	mgCOD/cycle (c)	136.8	132.4	145.2
	VOLB (a)	gCOD/L.d	0.22	0.21	0.23
Fed-batch-wise	CFB	mgCOD/L	238.3 ± 18.3	231.3 ± 4.4	182.1 ± 9.2
	C'FB	mgCOD/cycle (d)	166.8	161.9	127.5
	VOLFB (b)	gCOD/L.d	0.26	0.26	0.20
Discharge	CS	mgCOD/L	12.8 ± 7.9	9.8 ± 4.2	14.4 ± 6.9
	C'S	mgCOD/cycle (e)	24.3	18.6	27.4
	CT	mgCOD/L	10.6 ± 6.4	20.7 ± 5.4	25.8 ± 6.7
	C'T	mgCOD/cycle (f)	20.1	39.3	49.0
Percent removed	CS	%	92.0 ± 5.0	93.7 ± 2.7	90.1 ± 4.4
	CT	%	93.3 ± 4.0	86.7 ± 3.2	82.0 ± 4.4

Notes: CSM: Carbon synthetic medium; (a) Equation 1; (b) Equation 2; (c) Equation 3; (d) Equation 4; (e) Equation 5; (f) Equation 6.

and not total nitrogen in calculating the produced amounts of nitrite and nitrate (Equations 10 and 11) and ammonium nitrogen removal efficiency for the three carbon sources used. Volumes fed in batch mode (V_B = 1.2 L) and discharged (V_D = 1.9L) were also considered, as shown in the equations. On the other hand, calculation of organic nitrogen removal (Equation 13) involved the amounts of influent and effluent organic nitrogen as well as volumes V_B and V_D. Finally, determination of total nitrogen removal efficiency (Equation 14) considered, besides inlet and outlet volumes, total amount of influent nitrogen, i.e., the sum of nitrite, nitrate, ammonium nitrogen and organic nitrogen.

RESULTS AND DISCUSSION

Ethanol, acetate and carbon synthetic medium as external carbon sources

Tables 3 to 5 show average values of the main monitored parameters and Figures 2 to 7 show the results obtained from the concentration profiles. In the graphs, (a) and (c) represent the phases with aeration and (b) and (d) represent the anoxic phase with fed-batch addition of the external carbon source. Assays duration varied according to the period necessary for stabilization of the concentrations of nitrite, nitrate and ammonium nitrogen in the effluent. Hence, assays using ethanol, acetate and synthetic medium lasted 97, 25 and 45 days, respectively, with stability periods at the best performance condition of approximately 10 days. The assay in which methanol and ethanol mixtures was used lasted 58 days. During this period stability could not be attained for the 75/25 methanol/ethanol mixture (Table 1), due to progressive decrease in total nitrogen removal efficiency.

Figure 2 shows the filtered organic carbon concentration (C_S) of the three tested carbon sources. The lower values of the initial samples (t = 0) in relation to the influent concentrations (Table 3) are due to dilution caused by moisture in the foam retained in the reactor after discharge. Higher substrate consumption was seen during the first two hours of the cycle, probably due to metabolization by the aerobic heterotrophic microorganisms. After this stage carbon source levels remained approximately constant (at about 20 mgCOD/L) up to the end of the cycle, which shows that the carbon sources used were readily consumed during denitrification. It can be seen from Figure 3 that bicarbonate alkalinity (BA) was consumed when the reactor was aerated, i.e., when nitrification was more effective. Without aeration a slight BA accumulation can be seen, which is more evident for the system fed with acetate. Curiously, this behavior was not observed during the first anoxic phase (phase b) of the assay using carbon synthetic medium.

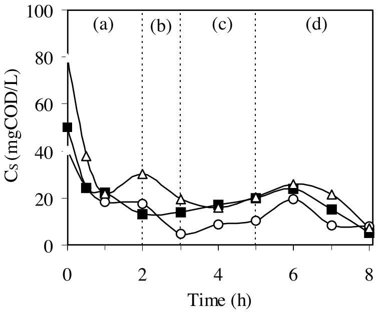

Figure 2: *Effluent organic matter concentration (CS) for ethanol (o), acetate (■) and carbon synthetic medium (Δ) [(a) and (c) with aeration, (b) and (d) without aeration].*

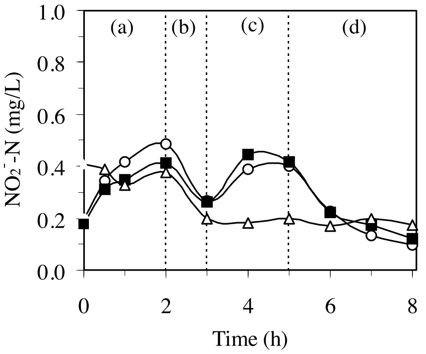

Figure 3: *Bicarbonate alkalinity (BA) for ethanol (o), acetate (■) and carbon synthetic medium (Δ) [(a) and (c) with aeration, (b) and (d) without aeration].*

Despite alkalinity consumption during nitrification, the (BA/NH4+-N)R ratios seen in Table 4 presented much lower values (2.2 mgCaCO$_3$/mgNH$_4^+$-N for ethanol, 1.4 mgCaCO$_3$/mgNH$_4^+$-N for acetate and 3.5 mgCaCO$_3$/mgNH$_4^+$-N for the carbon synthetic medium) than that observed by Kim and Hao (2001), which amounted to 8.5 mgCaCO$_3$/mgNH$_4^+$-N when they used an alternating aerobic/anoxic system. According to these authors the theoretical value for this ratio is 7.1 mgCaCO$_3$/mgNH$_4^+$-N. These low values of (BA/NH$_4^+$-N)R might be a result of excessive buffering of the liquid medium, since the synthetic wastewater as well as the external carbon sources were buffered with 500 mgNaHCO$_3$/L.

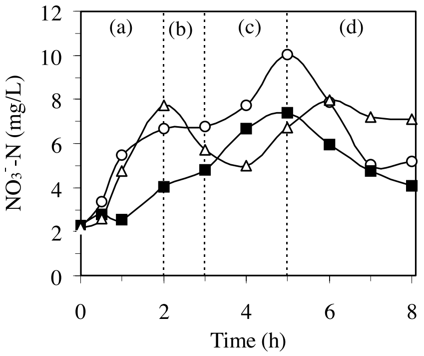

Figure 4: *Concentration of nitrite nitrogen (NO2 --N) for ethanol (o), acetate (■) and carbon synthetic medium (Δ) [(a) and (c) with aeration, (b) and (d) without aeration].*

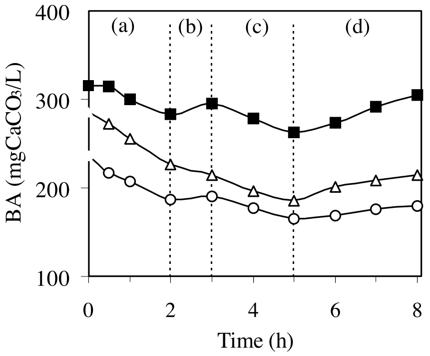

Figure 5: *Concentration of nitrate nitrogen (NO3 --N) for ethanol (o), acetate (■) and carbon synthetic medium (Δ) [(a) and (c) with aeration, (b) and (d) without aeration].*

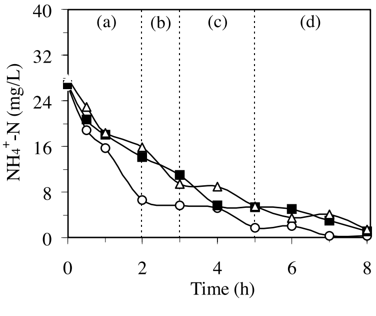

Figure 6: *Concentration of ammonium nitrogen (NH4 +-N) for ethanol (o), acetate (■) and carbon synthetic medium (Δ) [(a) and (c) with aeration, (b) and (d) without aeration].*

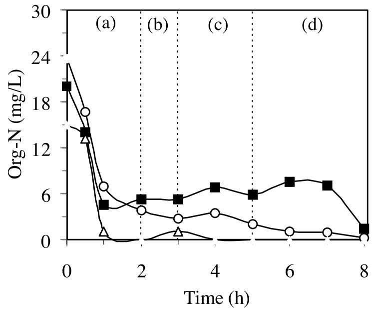

Figure 7: *Concentration of organic nitrogen (Org-N) for ethanol (o), acetate (■) and carbon synthetic medium (Δ) [(a) and (c) with aeration, (b) and (d) without aeration].*

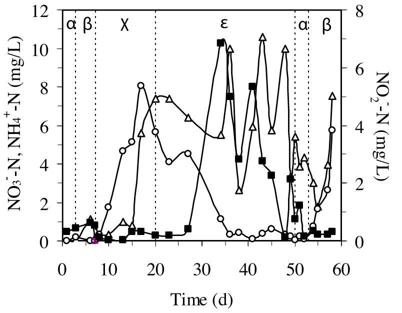

Figure 8: *Concentrations of nitrite (■), nitrate (Δ) and ammonium nitrogen (○) obtained during system monitoring, when the methanol and ethanol mixtures was used as external carbon source [phase ɑ - 25% methanol and 75% ethanol, phase β - 50% methanol and 50% ethanol, phase χ - 75% methanol and 25% ethanol, phase ε – re-inoculation and stabilization of the system with 80% of the stoichiometric ethanol concentration for complete denitrification].*

Regarding total volatile acids, no accumulation was seen for any condition. Moreover, in the system containing acetate as external carbon source, with an influent concentration of about 185 mgHAc/L, the acids were readily consumed along the cycle. It should be mentioned that no intermediate volatile acids (acetic, propionic, isobutyric, butyric, isovaleric, valeric and caproic) have been detected by gas chromatography analysis during the cycle in any conditions. Acid concentrations might have been below the detection limit of the analysis methodology used. Concentration profiles of nitrite, nitrate, ammonium and organic nitrogen are shown in Figures 4 to 7. Nitrite (NO$_2$--N) formed during the aerobic stages was rapidly oxidized to nitrate (NO$_3$--N) in the assays with ethanol and acetate, avoiding its accumulation and reducing its concentration during the stages without aeration. On the other hand, NO3--N reached a maximum at the fifth hour, being then reduced up to the end of the cycle and at a higher rate in the ethanol assay, as shown in Figure 5. In the assay with carbon synthetic medium, the nitrite concentration showed a different behavior. A slight reduction was observed in the first hour, certainly due to rapid formation of NO$_3$--N (Figure 5). On the other hand, during the second aerobic phase (phase c), nitrite concentration remained practically constant (at about 0.2 mgNO$_2$--N/L)

Table 4: *Influent and effluent concentrations of nitrogen forms, percentages of produced and removed nitrogen forms, C/N, VANL, VTNL, BA/NH4+-N, and R when ethanol, acetate and carbon synthetic medium were used as external carbons sources.*

Phase	Parameter	Unit	Values		
			Ethanol	Acetate	CSM
Batch-wise (influent)	(NO2--N)B	mg/L	0	0	0
	(NO3--N)B		0	0	0
	(NH4+-N)B		51.9 ± 0.6	50.4 ± 0.2	50.8 ± 0.8
	(Org-N)B		3.8 ± 0.5	4.3 ± 0.6	6.1 ± 0.4
	(NO2--N)'B	mg/cycle (a)	0	0	0
	(NO3--N)'B		0	0	0
	(NH4+-N)'B		62.3	60.5	61.0
	(Org-N)'B		4.6	5.2	7.3
	C/N (b)	mgCOD/mgNH4+-N	4.9	4.9	4.5
	VANL	mgNH4+-N/L.d (c)	98	95	96
	VTNL	mgN/L.d (d)	106	104	108
Discharge (effluent)	(NO2--N)D	mg/L	0.1 ± 0.05	0.2 ± 0.05	0.2 ± 0.02
	(NO3--N)D		5.7 ± 1.3	4.1 ± 1.5	6.7 ± 0.4
	(NH4+-N)D		1.4 ± 0.8	1.8 ± 1.7	0.8 ± 0.6
	(Org-N)D		0.4 ± 0.7	1.5 ± 1.5	0.8 ± 0.4
	(NO2--N)'D	mg/cycle (e)	0.2	0.4	0.4
	(NO3--N)'D		10.8	7.8	12.7
	(NH4+-N)'D		2.7	3.4	1.5
	(Org-N)'D		0.8	2.9	1.5
Produced amount (P) (f)	%(NO2--N)P	%	0.3	0.6	0.6
	%(NO3--N)P		17.4	12.9	20.9
Removal efficiency (R) (f)	%(NH4+-N)R	%	95.7	94.3	97.5
	%(Org-N)R		83.3	44.8	79.2
	%(Total-N)R		78.4	78.0	76.3
(BA/NH4+-N)R (g)	-	(mgCaCO3/mgNH4+-N)	2.2	1.4	3.5
R (h)	-	(mgNH4+-N/L.d)	94.1	90.1	93.9

Notes: CSM: Carbon synthetic medium; (a) (NO2--N)'B = (NO2--N)B . VB, etc; (b) Equation 7; (c) Equation 8; (d) Equation 9; (e) (NO2--N)'D = (NO2--N)D . VD, etc; (f) Equations 10 to 14; (g) Equation 15; (h) Equation 16.

up to the end of the cycle. At this phase nitrate concentration increased slightly and remained around 7.0 mgNO3--N/L up to the end of the cycle. This higher residual nitrate concentration might be due to oxidation of nitrogen compounds present in the carbon synthetic medium (e.g. nitrogen compounds in the meat extract), which were not part of the ethanol and acetate solutions. Despite the fact that approximately 20.9% of the produced nitrate remained in the reactor total nitrogen removal efficiency was 79.2%, a value close to those obtained with ethanol and acetate.

From Table 4 it can be seen that the three carbon sources showed ammonium nitrogen (NH_4^+-N) efficiency exceeding 94%. This indicates that ethanol, acetate and the carbon synthetic medium might be efficiently used in the proposed reactor. Finally, the organic nitrogen (Org-N) removal profiles indicate low residual values and efficiency of around 83 and 79% for ethanol and carbon synthetic medium, respectively, but only 44.8% for acetate carbon source (Table 4).

Methanol as external carbon source

Methanol, due to its inhibitory effect, was added gradually along with ethanol to increase the concentration of organic carbon necessary for denitrification. The negative effect of methanol on the nitrifying and denitrifying microorganisms can be verified in Figure 8 from the increase in effluent ammonium and nitrate nitrogen for the following phases: α (25% methanol and 75% ethanol), β (50% methanol and 50% ethanol) and χ (75% methanol and 25% ethanol). After this last phase, the reactor was re-inoculated (phase ε) with the two previously mentioned biomasses and fed with ethanol (80% of the stoichiometric concentration), during the fed-batch operation up to system stabilization. After stabilization, the methanol and ethanol mixture was added again during denitrification. Once more, the negative effect of methanol on the nitrifying microorganisms could be seen already in phase β (50% methanol and 50% ethanol), since the effluent ammonium nitrogen concentration increased again. So, the experiment was suspended.

Some authors have reported on the need of an adaptation phase of the microorganisms to methanol. Adaptation to carbon source may involve both enzyme induction and synthesis in the existing microflora, as well as genetic modifications in population (Hallin et al., 1996). In our case, a very long adaptation period would be required until attainment of maximum denitrification rate, which would make the process unfeasible.

The C/N ratio is also a determining factor for process efficiency, as it affects microbial activity and determines the reduction of nitrate either to ammonium nitrogen (NH4+-N) or nitrogen gas. In the proposed experiments C/N ratios were 4.9, 4.9 and 4.5 when ethanol, acetate and carbon synthetic medium were used as electrons donors, respectively (Table 4). These values agree with values in the literature (Henze et al., 1997).

Profiles of DO concentration for the three carbon sources (ethanol, acetate and carbon synthetic medium) showed that the reservoir remained at aerobic conditions during the whole cycle, including the periods during which aeration was suspended. Concentrations varied between 0.8 and 4.7 mg O_2/L, 0.2 and 3.7 mg O_2/L and 0.2 and 2.9 mg O_2/L when ethanol, acetate and the carbon synthetic medium were used as external carbon sources, respectively. This behavior was also corroborated by the RP profiles, which were positive throughout the cycles. Values var-

Table 5: *Bicarbonate alkalinity (BA), total volatile acids (TVA), pH and volume treated per cycle (V) in the influent and effluent.*

Phase	Parameter	Unit	Values		
			Ethanol	Acetate	CSM
Batch-wise (influent)	BAB	mgCaCO3/L	296.5 ± 21.9	315.9 ± 6.4	286.4 ± 1.5
	TVAB	mgHAc/L	16.4 ± 2.5	16.4 ± 1.3	28.8 ± 8.2
	pHB	-	9.5 ± 0.3	8.7 ± 0.04	9.0 ± 0.5
	VB	L	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
	TSB	mg/L	701 ± 63	684 ± 30	693 ± 27
	TVSB	mg/L	201 ± 32	180 ± 12	193 ± 15
	TSSB	mg/L	13 ± 18	34 ± 9	22 ± 6
	VSSB	mg/L	12 ± 9	10 ± 5	15 ± 6
Fed-batch-wise (influent)	BAFB	mgCaCO3/L	307 ± 7	311 ± 6	297 ± 10
	TVAFB	mgHAc/L	17.3 ± 4.5	184.6 ± 8.2	22.8 ± 5.3
	pHFB	-	8.7 ± 0.2	8.7 ± 0.1	9.1 ± 0.3
	VFB	L	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
	TSFB	mg/L	-	-	712 ± 21
	TVSFB	mg/L	-	-	173 ± 10
	TSSFB	mg/L	-	-	42 ± 8
	VSSFB	mg/L	-	-	12 ± 6
Discharge (effluent)	BAD	mgCaCO3/L	231.1 ± 19.9	271.4 ± 15.6	181.3 ± 9.9
	TVAD	mgHAc/L	18.8 ± 5.9	18.9 ± 3.5	17.3 ± 5.9
	pHD	-	7.9 ± 0.1	8.2 ± 0.1	7.8 ± 0.1
	VD	L	1.9 ± 0.1	1.9 ± 0.1	1.9± 0.1
	TSD	mg/L	638 ± 26	616 ± 15	680 ± 12
	TVSD	mg/L	183 ± 21	164 ± 26	191 ± 18
	TSSD	mg/L	12 ± 12	12 ± 7	19 ± 5
	VSSD	mg/L	7 ± 7	42 ± 13	15 ± 4

Notes: CSM: Carbon synthetic medium.

ied between 14 and 104 mV, 34 and 97 mV and -62 and 105 mV when ethanol, acetate and the carbon synthetic medium were used as external carbon sources, respectively. Negative RP values were only seen in the carbon synthetic medium assay after 7 hours from the beginning of the cycle, when residual nitrate concentration was practically already constant. However, it should be mentioned that these values were measured in the liquid phase and efficiency of the ammonium nitrogen removal process might arise from the fact that the denitrifying microorganisms were at anoxic conditions inside the foam particles, in which the biomass was immobilized, when the three sources were used.

As to solids concentration in the reactor, total solids and total volatile solids amounted to 71.4 g and 59.4 g, respectively. Considering the volume discharged by the reactor (1.9 L), this resulted in 37.6 g/L and 31.3 g/L, respectively. It should be mentioned that, despite initial separation of the aerobic and anoxic biomass in the reactor (aerobic biomass – stages I and II; anoxic biomass – stages III and IV), at the end of the experiment they were visually distributed throughout the whole bed.

In general, the concentration profiles show that the three external carbon sources present similar behavior for the proposed system. Thus, none showed improved efficiency over the other. According to the literature, depending on the treatment system used one or other carbon source excels in the denitrification process, i.e., each carbon source has a specific role in the biological nitrogen removal, which varies according to the treatment system used (Tam et al., 1992). However, since the carbon synthetic medium is similar in composition to domestic wastewater, these results show two advantages regarding treatment plants when using carbon sources from the proper wastewater as electron donors during denitrification: reduction in operational costs, since purchase of synthetic organic compounds will not be necessary, as well as reduction in volume of wastewater to be treated during organic carbon removal.

CONCLUSIONS

In general, the implemented operation conditions (immobilized biomass, recirculation of the liquid phase, batch-wise followed by fed-batch-wise feeding and intermittent aeration) resulted in satisfactory ammonium nitrogen removal efficiencies. The reactor showed high removal efficiency of influent ammonium nitrogen, attaining an efficiency of around 95% when ethanol, acetate and synthetic medium were used as external carbon source. Total nitrogen removal percentages for the three external carbon sources were 78.4% for ethanol, 78.0% for acetate, and 76.3% for carbon synthetic medium, showing that these compounds may be successfully implemented. On the other hand, when methanol and ethanol mixtures were used an inhibitory effect was observed through accumulation of ammonium and nitrate nitrogen in the effluent, even at the lower methanol contents in the mixture.

ACKNOWLEDGMENTS

This study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP, processes numbers 01/05489-0 and 02/07504-0. We gratefully acknowledge Dr. Baltus C. Bonse for the revision of this paper.

NOMENCLATURE

Abbreviations
CSM – Carbon synthetic medium
COD – Chemical oxygen demand
ECS – External carbon source
FID - Flame ionization detector
SBR – Sequencing batch reactor
SBBR – Sequencing batch biofilm reactor
SW – Synthetic wastewater
UASB – Up flow anaerobic sludge blanket
Symbols
BA – bicarbonate alkalinity, mg$CaCO_3$/L
(BA/NH_4+-N)R – bicarbonate alkalinity consumed per ammonium nitrogen removed from the process, mg$CaCO_3$/L
C/N – carbon to nitrogen ratio
C – organic matter concentration, mgCOD/L
C’ – organic matter concentration, mgCOD/cycle
DO – dissolved oxygen concentration, mgO_2/L
IVA – intermediate volatile acids, mg/L
NH_4^+-N – concentration of ammonium nitrogen, mg/L
%(NH_4^+-N)R – percent ammonium nitrogen removed from the process, %
NO_2^--N – concentration of nitrite nitrogen, mg/L
%(NO_2--N)P – percent nitrite nitrogen produced by the process, %
NO_3--N – concentration of nitrate nitrogen, mg/L
%(NO_3--N)P – percent nitrate nitrogen produced by the process, %
Org-N – concentration of organic nitrogen, mg/L
%(Org-N)R – percent organic nitrogen removed from the process, %
R – removal rate of ammonium nitrogen, mgNH_4^+-N/L.d
RP – redox potential, mV
(Total-N) – concentration of total nitrogen (= (NO2$^-$-N) + (NO_3^--N) + (NH_4^+-N) + (Org-N)), mg/L
%(Total-N)R – percent total nitrogen removed from the process, %
t – time, min
t’ – time of each stage of the process during the cycle, min
TS – total solids concentration, mg/L
TSS – total suspended solids concentration, mg/L
TVA – total volatile acids concentration, mgHAc/L
TVS – total volatile solids concentration, mg/L
VANL – volumetric ammonium nitrogen load, mgNH_4^+-N/L.d
V – volume, L
V’ – volume fed during batch-wise or fed-batch-wise, L
VOL –volumetric organic load, gCOD/L.d
VSS – volatile suspended solids concentration, mg/L
VTNL – volumetric total nitrogen load, mgTotal-N/L.d
Subscript
B – batch-wise, parameter relative to synthetic wastewater
D – discharge, parameter relative to effluent
FB – fed-batch-wise, parameter relative to external carbon source
P – produced by the process
R – removed from the process
S – filtered sample
T – non-filtered sample

BIBLIOGRAPHY

1. Aulenta, F.; Dionisi, D.; Majone, M.; Parisi, A.; Ramadori, R.; Tandoi, V. (2003). Effect of periodic feeding in sequencing batch reactor on substrate uptake and storage rates by a pure culture of Amaricocus kaplicensis. Water Research, 37, 2764-2772.
2. Camargo, E.F.M.; Canto, C.S.A.; Ratusznei, S.M.; Rodrigues, J.A.D.; Zaiat, M.; Borzani, W. (2005). Hydrodynamic analysis of an anaerobic sequencing batch biofilm reactor with liquid-phase external circulation. Journal of Science and Technology of the Americas - INTERCIÊNCIA, 30, 188-194.
3. Canto, C.S.A.; Ratusznei, S.M; Rodrigues, J.A.D.; Zaiat, M.; Foresti, E. (2008a). Effect of Ammonia Load on Efficiency of Nitrogen Removal in an SBBR with Liquid-Phase Circulation. Brazilian Journal of Chemical Engineering, 25, 275-289.
4. Canto, C.S.A.; Rodrigues, J.A.D.; Ratusznei, S.M.; Zaiat, M.; Foresti, E. (2008b). Feasibility of Nitrification/Denitrification in a Sequential Batch Biofilm Reactor with Liquid Circulation Applied to Post-Treatment. Bioresource Technology, 99, 644-654.
5. Cervantes, F.J.; De la Rosa, D.A.; Goméz, J. (2001). Nitrogen removal from wastewaters at low C/N ratios with ammonium and acetate as electron donors. Bioresource Technology, 79, 165-170.
6. Dinçer, A.R.; Kargi, F. (2000). Kinetics of sequential nitrification and denitrification processes. Enzyme and Microbial Technology, 27, 37-42.
7. Hallin, S.; Rothman, M.; Pell, M. (1996). Adaptation of denitrifying bacteria to acetate and methanol in activated sludge. Water Research, 30, 1445-1450.
8. Henze, M.; Harremöes, P.; Jansen, J.L.C.; Arvin, E. (1997). Wastewater Treatment: Biological and Chemical Processes, 2rd ed., Springer Verlag, Germany.
9. Ilies, P.; Mavinic, D.S. (2001). The effect of decreased ambient temperature on the biological nitrification and denitrification of a high ammonia landfill leachate. Water Research, 35, 2065-2072.
10. Kim H. and Hao O.J. (2001). pH and oxidation-reduction potential control strategy for optimization of nitrogen removal in an alternating aerobic-anoxic system. Water Environmental Research, 73, 95-102.
11. Linlin, H.; Jianlong, W.; Hua, W.X.; Yi, Q. (2005). Study on performance characteristics of SBR under limited dissolved oxygen. Process Biochemistry, 40, 293-296.
12. Louzeiro N.R.; Mavinic D.S.; Oldham W.K.; Meisen A.; Gardner I.S. (2002). Methanol-induced biological nutrient removal kinetics in a full-scale sequencing batch reactor. Water Research, 36, 2721-2732.
13. Mohseni-Bandpi, A.; Elliot, D.J. (1998). Groundwater denitrification with alternative carbon source. Water Science and Technology, 38, 237-243.
14. Obaja, D.; Macé, S.; Mata-Alvarez, J. (2005). Biological nutrient removal by a sequencing batch reactor (SBR) using an internal organic carbon source in digested piggery wastewater. Bioresource Technology, 96, 7-14.
15. Pambrun, V.; Paul, E.; Spérandio, M. (2004). Treatment of nitrogen and phosphorus in highly concentrated effluent in SBR and SBBR processes. Water Science and Technology, 50, 269-276.

16. Standard Methods for the Examination of Water and Wastewater (1995). 19th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.
17. Tam N.F.Y.; Wong Y.S.; Leung, G. (1992). Significance of external carbon sources on simultaneous removal of nutrients from wastewater. Water Science and Technology, 26, 1047-1055.
18. Thalasso, F.; Vallecillo, A.; García-Encina, P.; Fdz-Polanco, F. (1997). The use of methane as a sole carbon source for wastewater denitrification. Water Research, 31, 55-60.
19. Zaiat M.; Cabral A.K.A.; Foresti E. (1994). Horizontal-flow anaerobic immobilized sludge reactor for wastewater treatment: conception and performance evaluation. Brazilian Journal of Chemical Engineering, 11, 33-42.