

Nitrification–denitrification via nitrite accumulation for nitrogen removal from wastewaters

G. Ruiz ^{a,*}, D. Jeison ^b, O. Rubilar ^b, G. Ciudad ^b, R. Chamy ^a

^a School of Biochemical Engineering, Universidad Católica de Valparaíso, General Cruz 34, 234-0950 Valparaíso, Chile

^b Department of Chemical Engineering, Universidad de La Frontera, Av. Francisco Salazar 01145, 481-1230 Temuco, Chile

Received 31 December 2003; received in revised form 7 February 2005; accepted 8 February 2005

Available online 7 April 2005

Abstract

The biological nitrification–denitrification process is used extensively for removal of ammonia nitrogen from wastewaters. Saves in aeration, organic matter (for denitrification) and surplus sludge are achievable if nitrite accumulation is possible in the nitrification step. In this paper, operational parameters were studied for each process for maximum nitrite accumulation in the nitrification step and nitrite adaptation in the denitrification step. Nitrite accumulation during nitrification can be controlled by the dissolved oxygen (DO) concentration, presenting a maximum of 65% at around 0.7 mg DO/L. Denitrification can be adapted to nitrite and the process is stable if nitrite in the reactor is kept low. The performance of a continuous stirred tank reactor (CSTR) and an up flow sludge blanket reactor (USB) were compared. Once the operational parameters were established, a CSTR for nitrification and an USB reactor for denitrification were operated in series for 25 days. The process was stable and a steady state was maintained for 20 days, and 93.5% of overall nitrogen removal was achieved in the nitrification–denitrification via the nitrite process.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Nitrogen removal; Nitrite accumulation, nitrite denitrification; Oxygen concentration; Nitrification–denitrification via nitrite

1. Introduction

Biological nitrification–denitrification is the most studied process for nitrogen removal from wastewaters. The first step, nitrification, is the aerobic oxidation of ammonia to nitrite, and then to nitrate. The process is performed by nitrifying bacteria, which get their energy from the oxidation of these nitrogen compounds (Wiesmann, 1994). Due to the high oxygen demand for ammonia oxidation, aeration is the main cost during this step. The following stage is denitrification, where nitrate (formed in the nitrification step) is anoxically transformed into nitrite, then into nitrous oxide, nitric

oxide, and finally into gaseous nitrogen. Denitrifying microorganisms are heterotrophic, and in anoxic conditions use nitrate or nitrite as the final electron acceptor (Tiedje, 1988; Bliss and Barnes, 1983). Therefore organic matter (electron donor) is needed in this step.

Several efforts have been made in order to optimize biological nitrogen removal. New processes have been developed such as nitrification/denitrification via nitrite accumulation (Ruiz et al., 2003). This process is based on the fact that, since nitrite and nitrate are intermediary compounds in both steps (nitrification and denitrification), a partial nitrification to nitrite and a denitrification from this nitrite, instead from nitrate, would be feasible (Fig. 1). This approach will produce savings in oxygen demands during nitrification, a reduction of the organic matter requirements in the denitrification process, plus a decrease in surplus sludge production.

* Corresponding author. Fax: +56 32 273803.

E-mail addresses: gruiz@usc.es (G. Ruiz), djeison@ufro.cl (D. Jeison).

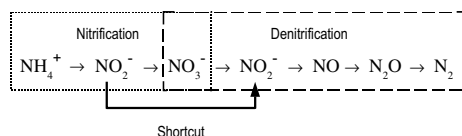


Fig. 1. Nitrification–denitrification with nitrite accumulation.

In order to perform this process, two conditions must be fulfilled: nitrification must be stopped before nitrite oxidation and denitrifying sludge must be adapted to nitrite, which is toxic at low concentrations (Chung and Bae, 2002; Carrera et al., 2004). To achieve partial nitrification it is necessary to selectively reduce the activity of the nitrite oxidizing bacteria without affecting the ammonia oxidizers. Some operational conditions may produce nitrite accumulation during nitrification, such as pH, temperature, and dissolved oxygen (DO) concentration (Suthersan and Ganczarcczyk, 1986; Yoo et al., 1999; Bae et al., 2002). The last operational variable seems to be the most interesting alternative. On the other hand, some researches have proved that denitrifying sludge can be acclimated to nitrite, after an adaptation process (Jones et al., 1990; Chung and Bae, 2002).

Most of the research papers focused on nitrification denitrification through nitrite accumulation are applied to wastewaters with low nitrogen concentrations (Yoo et al., 1999; Bae et al., 2002; Peng et al., 2004). The objective of this research is to study the feasibility of this process under high ammonia concentration conditions, where the eventual savings in energy and operational costs would be more significant. The influence of DO concentration on nitrite accumulation during the nitrification process was studied, as well as the adaptation of denitrifying sludge to a nitrite feed.

2. Methods

2.1. Analytical methods

Nitrate was determined by UV absorption at 220 and 275 nm and nitrite by the sulfanilamide acid reaction. Chemical oxygen demand (COD) was measured by dichromate oxidation. Ammonia was determined using an ion selective electrode (Orion 95-12). All these methods were according to Standard Methods (APHA, 1992).

2.2. Nitrification step

A laboratory activated sludge reactor, composed of a 2.5 L reaction tank and a 1.5 L settler, was used to evaluate the influence of dissolved oxygen (DO) concentration on nitrification (Fig. 2). Aeration was provided by means of a diffuser placed in the bottom of the reaction vessel. The pH was kept constant by an on-off controller, through the addition of an 80 g/L bicarbonate solu-

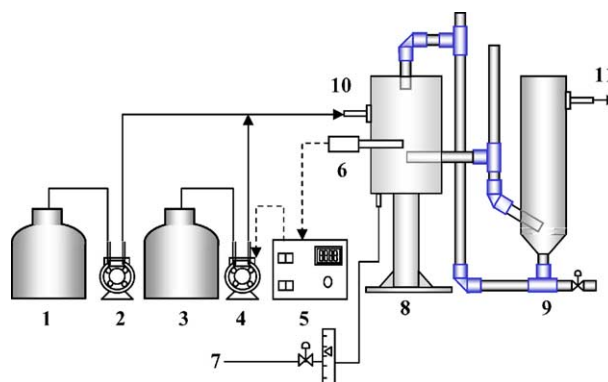


Fig. 2. Schematic diagram of the experimental set-up of the activated sludge unit: (1) feed tank; (2) feed pump; (3) bicarbonate tank; (4) bicarbonate pump; (5) pH controller; (6) pH meter; (7) air line; (8) reactor; (9) settler; (10) reactor inlet and (11) settler outlet.

tion that also served as a carbon source for nitrifying microorganisms. DO concentration was measured with an oxygen electrode (YSI-95, YSI Company) twice a day, and controlled by adjusting the air flow rate manually. The reactor feed was prepared by dilution of a concentrated synthetic wastewater adapted from previously reported medium (Campos et al., 1999) (Table 1) to a final concentration close to 600 mg N-NH₄⁺/L. The biomass recycle from the settler to the reactor was performed by a mammoth pump. The reactor was operated under different DO concentrations in the range 0.5–5.5 mg/L. The reactor was kept at 30 °C, since it has been reported to be the optimal temperature for nitrite accumulation (Jianlong and Ning, 2004).

2.3. Denitrification step

Two laboratory denitrifying reactors were used to conduct this study: a 1.6 L continuous stirred tank

Table 1
Concentrated synthetic wastewater (10 g N-NH₄⁺/L) and composition of the solution of nutrient traces

Compound	Concentration (mg/L)
<i>Concentrated synthetic wastewater</i>	
NH ₄ Cl	19,120
MgSO ₄	625
KH ₂ PO ₄	2500
(NH ₄) ₂ SO ₄	23,560
NaCl	20,000
Solution of traces	5 (mL/L)
<i>Solution of nutrient traces</i>	
EDTAH ₂ Na ₂ × 2H ₂ O	50,000
ZnSO ₄ × 7H ₂ O	2200
CaCl ₂	5540
MnCl ₂ × 4H ₂ O	5060
FeSO ₄ × 7H ₂ O	5000
(NH ₄) ₆ Mo ₇ O ₂₄ × 4H ₂ O	1100
CuSO ₄ × 5H ₂ O	1570
CoSO ₄ × 7H ₂ O	1900
KOH	to pH 6

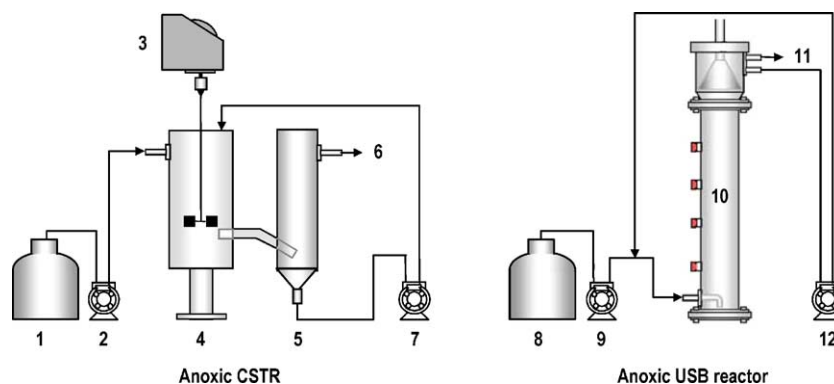


Fig. 3. Schematic diagram of the experimental set-up of the anoxic reactors. CSTR: (1) feed tank; (2) feed pump; (3) stirrer; (4) reactor; (5) settler; (6) settler outlet; (7) sludge recycling pump. USB reactor: (8) feed tank; (9) feed pump; (10) reactor, (11) outlet, (12) effluent recycling pump.

reactor (CSTR) and a 3 L up flow sludge blanket (USB) reactor (Fig. 3). Both were operated individually to compare their performances, and therefore their applicability to a shortcut nitrification–denitrification process. A synthetic wastewater was used to feed both reactors by dilution of a concentrated solution (Table 2), and the addition of nitrite and/or nitrate (sodium salts) up to the desired concentration. The reactors were started up with only nitrate as the electron acceptor, which was gradually replaced by nitrite, keeping the N-NO_x^- ($\text{N-NO}_2^- + \text{N-NO}_3^-$) concentration constant (between 500 and 550 mg N/L). Once all the nitrate was replaced, the reactor was only fed with nitrite as the electron acceptor.

2.4. Nitrification–denitrification

Once the nitrifying conditions for maximum nitrite accumulation were established, and the type of denitrification reactor and its operational conditions were selected, both reactors were operated in a post-denitrification mode to perform the complete process of nitrification–denitrification via nitrite. DO for partial

Table 3

Operational conditions applied to the nitrification–denitrification process during the whole experiment

Parameter	Activated sludge (nitrifying step)	USB (denitrifying step)
Feed flow	25 L/d	25 L/d
N feeding	587 ± 6 mg $\text{N-NH}_4^+/\text{L}$	569 ± 56 mg $\text{N-NO}_x^-/\text{L}$
COD feeding	N.A.	1633 ± 114 mg/L
NLR	5.87 ± 0.06 kg $\text{N-NH}_4^+/\text{m}^3 \text{d}$	4.74 ± 0.46 kg $\text{N-NO}_x^-/\text{m}^3 \text{d}$
COD/N	–	2.87 ± 0.48 mg COD/mg N-NO_x^-
HRT	2.4 h	2.9 h
Biomass	4.6 g VSS/L	10.6 g VSS/L
DO	1.1 mg/L	N.A.
pH	7.85 ± 0.5	7.85 ± 0.5

nitrification was set at 1.1 mg DO/L. A solution of acetate plus micro and macronutrients (Table 2) was added to the nitrifying effluent to reach the desired COD concentration for the denitrifying step. Table 3 presents the operational conditions of the process. These conditions were maintained for 25 days. The overall process was studied and mass balances were carried out in steady state.

Table 2

Concentrated synthetic wastewater composition (40 g COD/L)

Compound	Concentration (g/L)
<i>Concentrated synthetic wastewater</i>	
$\text{NaCH}_3\text{COO} \cdot 3\text{H}_2\text{O}$	82.92
Peptone	4.8
Yeast extract	2
NaHCO_3	8
K_2HPO_4	70
KH_2PO_4	54
Solution of traces	13.5 mL/L
<i>Solution of nutrient traces</i>	
MgSO_4	10.0
FeCl_2	1.0
CaCl_2	1.0
KCl	1.0
CoCl_2	0.2

3. Results and discussion

3.1. Nitrification step

The start up of the activated sludge reactor consisted of the continuous increment of the nitrogen loading rate (NLR) from 0.5 to $3.3 \text{ kg N-NH}_4^+/\text{m}^3 \text{d}$. The pH was automatically controlled between 7.8 and 7.9. A biomass concentration of 6.3 g VSS/L was maintained throughout the experiment by means of a daily mixed liquor purge. Complete nitrification was achieved during the start up period (results not shown).

Fig. 4 presents the reactor behavior during consecutive decreases in dissolved oxygen concentration.

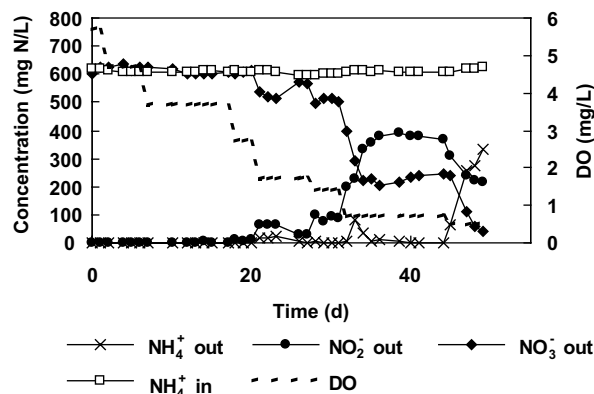


Fig. 4. Time course of the study of the influence of DO concentration (dotted line) on nitrite accumulation.

The results show that nitrification was not affected by DO concentrations in the range 5.7–1.7 mg/L. At a DO concentration of 1.4 mg/L nitrite accumulation took place, and increased as DO concentration decreased. The higher nitrite accumulation, without affecting ammonia removal, took place at 0.7 mg DO/L. At a DO of 0.5 mg/L ammonia conversion was affected. Fig. 5 summarizes these results. Each point represents stable operational behavior for each condition. A 65% nitrite accumulation was feasible with 98% of ammonia conversion. Based on stoichiometry this accumulation implies a 17% reduction in the oxygen needed for nitrification: 1.67 mol of oxygen per mol of ammonia nitrogen compared to 2 for complete nitrification. Furthermore, the operation at low dissolved oxygen concentrations enhances oxygen mass transport, due to an increase of mass transfer driving force (Ciudad et al., 2005). Data from Fig. 5 suggest that maximum nitrite accumulation is between 0.7 and 1.4 mg DO/L, therefore a concentration of 1.1 mg/L was selected as the DO concentration for the activated sludge reactor, during the combined operation of nitrification and denitrification steps.

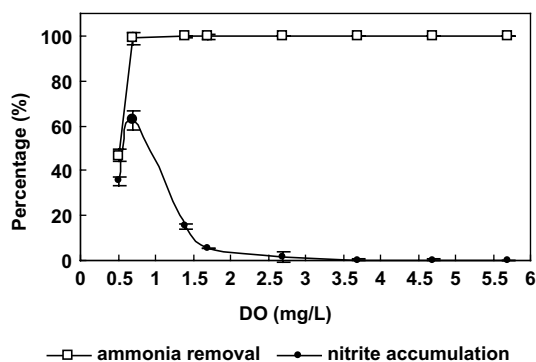


Fig. 5. Influence of DO concentration on ammonia removal and nitrite accumulation state. The bars indicate a standard deviation of the mean of each steady state.

3.2. Denitrification step

The reactors were operated in three steps: start up, nitrate replacement by nitrite, and nitrite operation. Fig. 6 presents the organic and nitrogen loading rates (OLR and NLR) applied to the CSTR and the USB reactor. After 30 days of operation a NLR of 5 kg N/m³d was reached in the USB reactor and was kept constant until the end of the operation. The NLR of the CSTR was increased, up to 1.4 kg N/m³d, which produced an

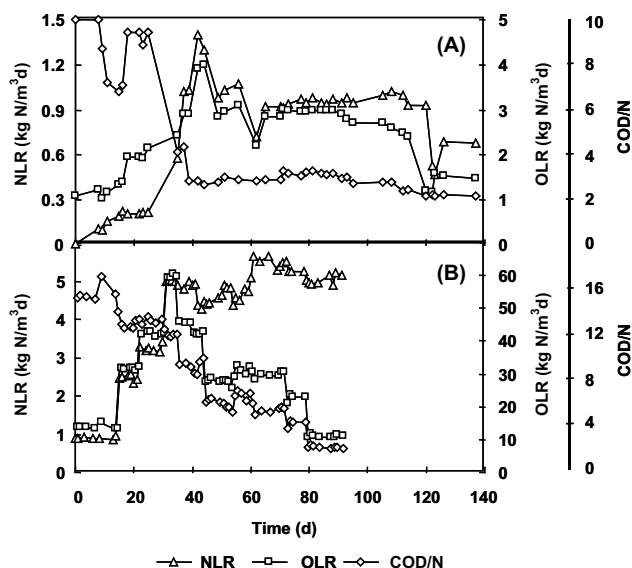


Fig. 6. COD/N ratio and nitrogen and organic loading rates during the operation of CSTR (A) and USB (B) anoxic denitrifying reactors.

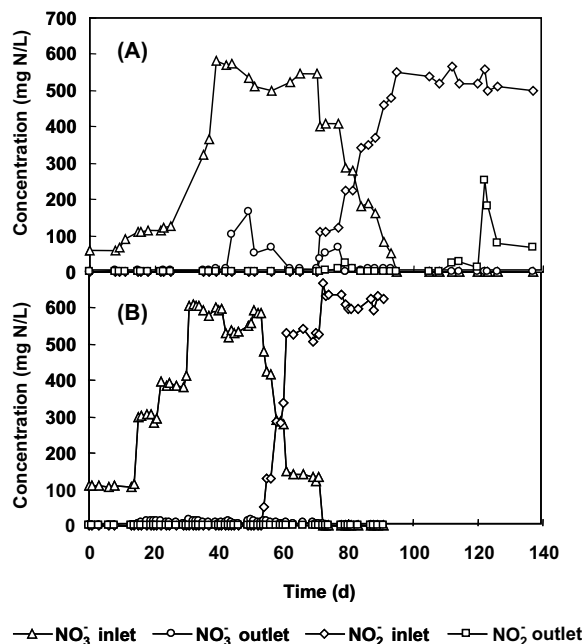


Fig. 7. Nitrogen compounds during the operation of CSTR (A) and USB (B) anoxic denitrifying reactors.

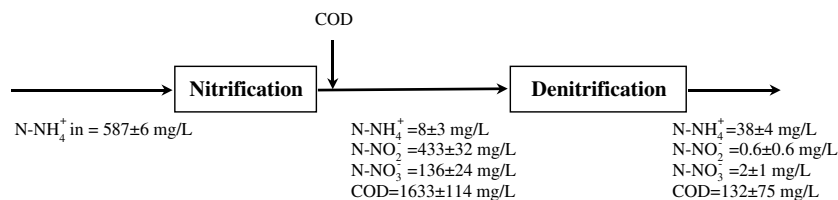


Fig. 8. Mass balance of the overall process. Mean values are indicated with one standard deviation during the 20 days of steady state operation.

increase in the nitrate effluent concentration (see Fig. 7). The NLR was therefore decreased to $1 \text{ kg N/m}^3\text{d}$. The OLR was reduced in both reactors from their original values, decreasing the COD/N ratio, until a value close to 2 by the end of the operation. It is inferred that denitrification was the main pathway for organic matter removal, since no methane formation was detected shortly after the operation of the reactors started (data not shown).

Fig. 7 presents the inlet and the outlet nitrate and nitrite concentrations for both reactors. By a step change from nitrate to nitrite feeding, nitrite adaptation was performed in a short period of time; for instance, in this study it was less than 25 days. During this period the N-NO_x^- inlet concentration ($\text{N-NO}_3^- + \text{N-NO}_2^-$) was kept constant between 500 and 600 mg N/L. Reactors were then operated with nitrite as the electron acceptor.

The USB reactor exhibited a high nitrogen removal and great stability. No inhibitory effect was observed during electron acceptor replacement. Indeed, N-NO_x^- effluent concentration never exceeded 15 mg N/L (Fig. 7).

The CSTR operation was different, mainly due to the development of a sludge which settled poorly. Sludge accumulation in the settler was common, producing temporal reductions of biomass concentration in the reactor. This produced the increases in the effluent N-NO_x^- concentration that can be observed on days 75 and 120. The nitrite accumulation on day 120 was close to 300 mg N/L (Fig. 7), and irreversibly affected the biomass activity. The NLR was reduced to $0.5 \text{ kg N/m}^3\text{d}$, which produced a slight improvement in the performance of the reactor, but the removal never returned to its original levels. Besides, the clarified effluent presented a high concentration of suspended solids, which caused a continuous reduction in the biomass concentration from day 100 (results not shown).

Since poor settleability of the suspended denitrifying sludge can adversely affect biomass retention in the denitrifying CSTR, immobilization is required in order to achieve stable operation. The USB reactor was therefore selected, as a more suitable reactor technology for denitrification.

3.3. Nitrification–denitrification

The complete system (nitrification–denitrification) was operated for a month in the post-denitrification configuration with optimum results. From day 5, the

quality of the effluent remained constant and steady state was maintained until the end of the experiment (data not shown). During the steady state the mean values were: $73.8 \pm 6.2\%$ of nitrite accumulation and $93.5 \pm 0.9\%$ of ammonia nitrogen removal for the complete system.

Fig. 8 presents the mass balance over the process: the nitrite and nitrate are almost completely denitrified, and a small increase in ammonia is observed in the denitrification step. This may be the result of ammonia generation from the degradation of peptone and yeast extract present in the macro and micronutrients fed to the denitrifying reactor (Table 2). Stoichiometric calculations confirm this hypothesis. Considerable savings in aeration are achieved due to operation at low oxygen concentration (1.1 mg DO/L), and a 25% reduction in the COD requirement is accomplished, in comparison with complete nitrification. Moreover, surplus biomass is expected to be reduced, since growth of nitrite oxidizing bacteria is reduced, meaning another save in the process.

It should be considered that a shortcut in the nitrogen removal process may be applied to an existing treatment system when performing a nitrification–denitrification process. No investment costs are needed and only changes in the operational conditions are required (oxygen concentration in the nitrification step), which are the main advantages of this process compared to other technological approaches, such as Sharon, Anammox, Canon, etc. Even though benefits of the nitrification–denitrification process via nitrite are applicable for wastewaters with high and low ammonia concentration, under elevated nitrogen concentration savings would be more relevant. This would be the case of many industrial wastewaters.

4. Conclusions

Results from the present research show that nitrification/denitrification via nitrite is a feasible alternative for the removal of nitrogen from wastewaters. Furthermore, it can significantly enhance the treatment process economical balance through a reduction in the organic matter needs for the denitrification step, a decrease in aeration requirements in nitrification, and a decrease in the surplus sludge generation.

Nitrite accumulation can be performed during the nitrification step restricting the dissolved oxygen

concentration to a low level, around 1 mg DO/L. On the other hand, denitrifying microorganisms can be adapted to a high nitrite feed, as long as the nitrite concentration remains low inside the reactor. Reactors with an immobilized biomass, such as USB technology, are likely to be a more suitable alternative than suspended biomass reactors, due to the poor settleability of the denitrifying suspended sludge obtained in this study.

Nitrification–denitrification via nitrite represents an operational strategy that may also be applied to existing treatment installations with no extra investment costs. This represents an important advantage over other new nitrogen removal technologies such as Sharon, Anamox or Canon processes.

Acknowledgement

The authors wish to thank the financial support provided by the Research Project DIUFRO 110203 of the Dirección de Investigación y Desarrollo from the Universidad de La Frontera. The authors also want to thank J.L. Campos Ph.D. from the Universidade de Santiago de Compostela, for his support.

References

- APHA, 1992. Standard methods for the examination of waters and wastewaters. American Public Health Association, Washington, DC.
- Bae, W., Baek, S., Chung, J., Lee, Y., 2002. Optimal operational factors for nitrite accumulation in batch reactors. *Biodegradation* 12 (5), 359–366.
- Bliss, P., Barnes, D., 1983. *Biological Control of Nitrogen in Wastewater Treatment*, First ed. University Press, London.
- Campos, J., Garrido, J.M., Méndez, R., Lema, J.M., 1999. Nitrification at high ammonia loading rate in an activated sludge unit. *Bioresour. Technol.* 68, 141–148.
- Carrera, J., Jubany, I., Carvallo, L., Chamy, R., Lafuente, J., 2004. Kinetic models for nitrification inhibition by ammonium and nitrite in a suspended and an immobilised biomass systems. *Process Biochem.* 39, 1159–1165.
- Ciudad, G., Rubilar, O., Muñoz, P., Ruiz, G., Chamy, R., Vergara, C., Jeison, D., 2005. Partial nitrification of high ammonia concentration wastewater as a part of a shortcut biological nitrogen removal process. *Process Biochem.* 40 (5), 1715–1719.
- Chung, J., Bae, W., 2002. Nitrite reduction by a mixed culture under conditions relevant to shortcut biological removal. *Biodegradation* 13 (3), 163–170.
- Jianlong, W., Ning, Y., 2004. Partial nitrification under limited dissolved oxygen conditions. *Process Biochem.* 39, 1223–1229.
- Jones, W., Schroeder, E., Wilderer, P., 1990. Denitrification in a batch wastewater treatment system using sequestered organic substances. *J. Water Pollut. Control Fed.* 62 (3), 259–267.
- Peng, Y.Z., Chen, Y., Peng, C.Y., Liu, M., Wang, S.Y., Song, X.Q., Cui, Y.W., 2004. Nitrite accumulation by aeration controlled in batch reactors treating domestic wastewater. *Water Sci. Technol.* 50 (10), 35–43.
- Ruiz, G., Jeison, D., Chamy, R., 2003. Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration. *Water Res.* 37 (6), 1371–1377.
- Suthersan, S., Ganczarczyk, J., 1986. Inhibition of nitrite oxidation during nitrification. Some observations. *Water Pollut. Res. J. Can.* 21 (2), 257–266.
- Tiedje, J.M., 1988. In: *Ecology of Denitrification and Dissimilatory Nitrate Reduction to Ammonium*. Biology of Anaerobic Microorganism. John Wiley & Sons, New York, pp. 179–244.
- Wiesmann, U., 1994. Biological nitrogen removal from wastewater. *Adv. Biochem. Eng.* 51, 113–154.
- Yoo, H., Ahn, K., Lee, H., Lee, K., Kwak, Y., Song, K., 1999. Nitrogen removal from synthetic wastewater by simultaneous nitrification and denitrification (SND) via nitrite in an intermittently aerated reactor. *Water Res.* 33 (1), 146–154.