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ORIGINAL PAPER

# Simultaneous nitrification–denitrification of wastewater: effect of zeolite as a support in sequential batch reactor with step-feed strategy

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**Abstract** One of the technologies used for wastewater nitrogen removal consists in simultaneous nitrification–denitrification. The low microbial growth rate and the low availability of organic material for the denitrification stage make it necessary to study new operational conditions and the use of microbial supports. The aim of this study was to evaluate the operational behavior of a simultaneous nitrification–denitrification process in a sequential batch reactor utilizing zeolite as a biomass support and step-feed strategy. Two reactors of 2 L were used, one with zeolite and another without zeolite, both operated at constant temperature (31 °C), varying nitrogen loading rate (NLR) from 0.041 to 0.113 kg total Kjeldahl nitrogen (TKN/m$^3$/day). After 209 days, removals higher than 86 and 96 % in nitrogen compounds and organic matter were obtained, respectively. There was not accumulation of nitrate and nitrite in any case; this means that there was a simultaneous nitrification–denitrification in the reactors. The incorporation of zeolite in the system held higher concentration of biomass in the reactor; this led to reduce start-up to 21 days and to improve 11.31 % removal kinetic. The use of a step-feed strategy prevents events of inhibition by substrate, even duplicating tolerance to higher NLR for the same operation time.

**Keywords** Sequential batch reactor · Simultaneous nitrification–denitrification · Step-feed · Zeolite

## Abbreviations

| | |
|---|---|
| AOB | Ammonia-oxidizing bacteria |
| BNR | Biological nitrogen removal |
| C/N | Carbon/nitrogen ratio |
| CEC | Cation-exchange capacity |
| COD | Chemical oxygen demand |
| CODs | Chemical oxygen demand soluble |
| DO | Dissolved oxygen |
| FH | Free-hydroxylamine |
| NOB | Nitrite oxidizing bacteria |
| NLR | Nitrogen loading rate |
| ORP | Oxidation-reduction potential |
| PHB | PolyHydroxyButyrate |
| R2 | Reactor with chilean natural zeolite |
| R1 | Reactor without chilean natural zeolite |
| SBR | Sequential batch reactor |
| SND | Simultaneous nitrification-denitrification |
| TKN | Total Kjeldahl nitrogen |
| TSS | Total suspended solids |
| VSS | Volatile suspended solids |
| vvm | Volumes per reactor volume per minute |

## Introduction

The reduction of nitrogen levels from several wastewater plant effluents containing high concentrations of nitrogen compounds is necessary because these compounds can be toxic to aquatic life, causing oxygen depletion and

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eutrophication in receiving water and affecting chlorine disinfection efficiency (Haiming et al. 2011; Song et al. 2011; Wang et al. 2004). Nitrogen compounds can be removed from wastewaters by a variety of physicochemical and biological processes (Gao et al. 2015; Montalvo et al. 2011; Ozturk and Bal 2015; Sun et al. 2015). Recently, several novel and cost-effective biological nitrogen removal (BNR) processes have been developed, including partial nitritation, nitrifier denitrification, anaerobic ammonium oxidation (anammox), and its combined systems (completely autotrophic nitrogen removal over nitrite, Canon), among others (Ahn 2006; Chen et al. 2009; Kumar and Lin 2010; Lan et al. 2011; Zhang et al. 2012). However, nitrification–denitrification process is still the BNR process most used at industrial scale (Kim et al. 2013).

Simultaneous nitrification–denitrification (SND) is a type of technology currently used in ammonium and nitrate removal. This process has become a versatile and recommended tool in the treatment of wastewater with a high *C*/*N* ratio (Bernet et al. 2000; Guo et al. 2013; Moya et al. 2012). Nitrification is the sequential oxidative transformation process of ammonium resulting in nitrite (reduced by ammonia-oxidizing bacteria or AOB) followed by the oxidation of the nitrite to nitrate due to the action of two forced chemolithoautotrophic microbial groups (Peng and Zhu 2006; Shijian et al. 2014). Meanwhile, heterotrophic microorganisms take the nitrate and reduces it to $N_2$ (denitrification), utilizing organic matter as the electron donor (Pepper et al. 2006). Taking into account this sequential characteristic of the SND process, the sequential batch reactor (SBR) is an attractive choice to carry out it. The SND process represents a significant advantage over the conventional separated nitrification and denitrification process. First, SND process eliminates the serial operation of two separated tanks and, therefore, requires simpler operational procedures. In addition, the SND uses 20–40 % less carbon sources and reduces sludge yield by 30 % when compared to conventional BNR systems (Guo et al. 2013).

Even though SND has been studied by diverse authors, especially in the latest years (Guo et al. 2013; Scaglione et al. 2013; Zheng et al. 2013), the application of this process has shortcomings that hinder achieving an adequate operational robustness: low growth rate of the microorganisms involved in the process, high sensitivity to moderate concentrations of sulfurs, nitrates and nitrites (Bernet et al. 2001), high energy costs (Cecen 1996) and availability of organic matter (Guo et al. 2009; Martins et al. 2003). That is the reason why the search for improvements of the existing processes has led to the proposal of using zeolite as a microbial support and ammonium ion exchanger (Hedström 2001; Ho and Ho 2012; Mace and Mata-Alvarez 2002; Wei et al. 2010; Wilderer et al. 2000, 2001).

Zeolite is a mineral of hydrated aluminum silicates ($AlO_4/SiO_4$) (Alejandro et al. 2014; Montalvo et al. 2012) with a tetrahedral structure, with a high cation exchange capacity (CEC) and high cation selectivity (Wang and Peng 2010). Specifically, zeolite is a porous material characterized by its ability to (1) lose and gain water reversibly; (2) adsorb molecules of appropriate cross-sectional diameter (adsorption property or acting as molecule sieves); and (3) exchange its constituent cations without a major change in its structure (ion exchange property) (Montalvo et al. 2012). Even though its use has been proposed for nitrification and denitrification separately, little has been studied on its use in SND processes, being the latter one of the objectives of this work.

The temporary distribution of the pulse feeds, known as step-feed, results in an adequate strategy to control parameters in the batch operation such as sediment ability, availability of carbon and resistance against loading shocks (Andreottola et al. 2001; Artan and Orhon 2005; Guihua et al. 2013; Martins et al. 2003). This feeding strategy has been used in the biological elimination process of nitrogen (for example nitrification/denitrification) to avoid partial denitrification and the use of external carbon sources (for example methanol or acetate) and the associated costs (Guihua et al. 2013).

Therefore, the main objective of the present study was to evaluate the operational behavior of BNR to optimize the removal of nitrogen compounds through the use of a simultaneous nitrification–denitrification reactor with a strategy of step-feed, utilizing zeolite as a microbial support and/or to improve the ammonium removal process.

## Materials and methods

### Equipment and inoculum

Two reactors of 2 L volume were designed for this study. A detailed scheme of the reactor is shown in Fig. 1. One reactor operated with zeolite (with a concentration of 5 g/L, which was added to the reactor at the beginning of the experiments) and the other without zeolite. From now on, the reactor with zeolite will be referred to as *R*2 and the reactor without zeolite will be referred to as *R*1. Both reactors were inoculated with a mixture 70/30 of aerobic/anaerobic sludge, being the total volume of the inoculum 400 mL, to obtain a final concentration of 3.5 g VSS/L inside the system. The characteristics of the inoculum were: 10.07 g TSS/L and 7.08 g VSS/L for aerobic sludge; 28.01 g TSS/L and 22.4 g VSS/L for anaerobic sludge. Both reactors were installed and assembled as presented in Fig. 2.

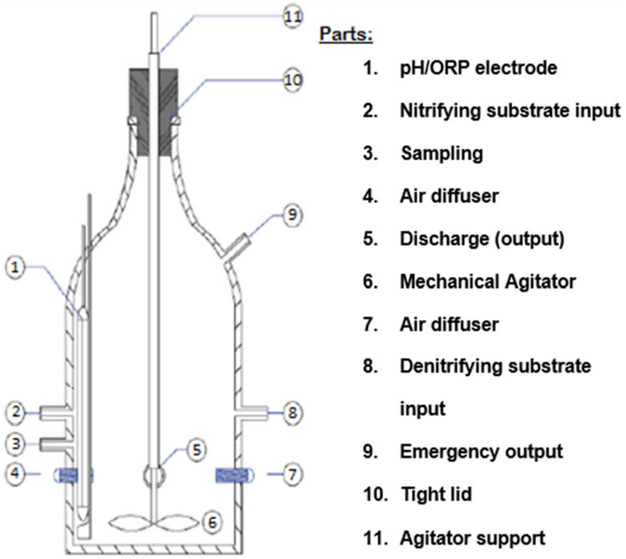


**Fig. 1** SBR-SND reactor scheme

The aerobic sludge was obtained from an activated sludge plant located in a poultry industry, and the anaerobic sludge was collected from an anaerobic digester treating wastewaters from a tobacco industry in the Valparaíso Region, Chile.

### Characteristics and pretreatment of Chilean zeolite

The major mineral contents of the Chilean natural zeolite used were: clinoptilolite (35 %), montmorillonite (30 %), mordenite (15 %) and other minerals (20 %). Its chemical composition was: $SiO_2$ (66.62 %), $Al_2O_3$ (12.17 %), CaO (3.19 %), $Fe_2O_3$ (2.08 %) and other chemicals (Wang and Peng 2010; Montalvo et al. 2012). Previous to be used, the Chilean zeolite utilized was sieved through a GeyerPrüfsieb (DIN 1170) mesh to obtain particles of 0.08–0.12 cm diameter applying ASTM C136-01 Method. Afterward, zeolite was washed with distilled water and dried in an oven at 110 °C for 1 day.

### Operation in batch cycle

The operation was done in cycles of 51 h distributed in 3 ramps (step-feed), each one with a 3 h of anoxic feed, 5.5 h of agitated anoxic operation and 8.5 h of agitated aerobic operation.

Both nitrifying and denitrifying substrates were incorporated in both reactors in the anoxic phase of each step-feed by pulses with a flow of 19 mL/min (9.5 volumes per reactor volume per minute, vvm).

The aeration, 3.4 L/m (1.7 vvm), was carried out in an intermittent form (pulses). At the end of the third ramp, a sedimentation stage was incorporated (3.5 h), and finally with the unloading, the cycle (1.5 h) was finished.

Increasing NLR from 0.031 to 0.112 kg TKN/($m^3$ day) was used; the carbon/nitrogen ratio was kept constant during the experiment ($C/N = 5$), and the reactors operated in darkness at 31 °C of temperature.

### Synthetic wastewater

Table 1 shows the synthetic substrates (nitrifying and denitrifying) composition used as feed in the experiments.

### Operation strategy

Table 2 shows the duration of each stage as well as the NLR and aeration flows used in the different steps of the experiments carried out.

**Fig. 2** SND-SBR reactors assembly scheme

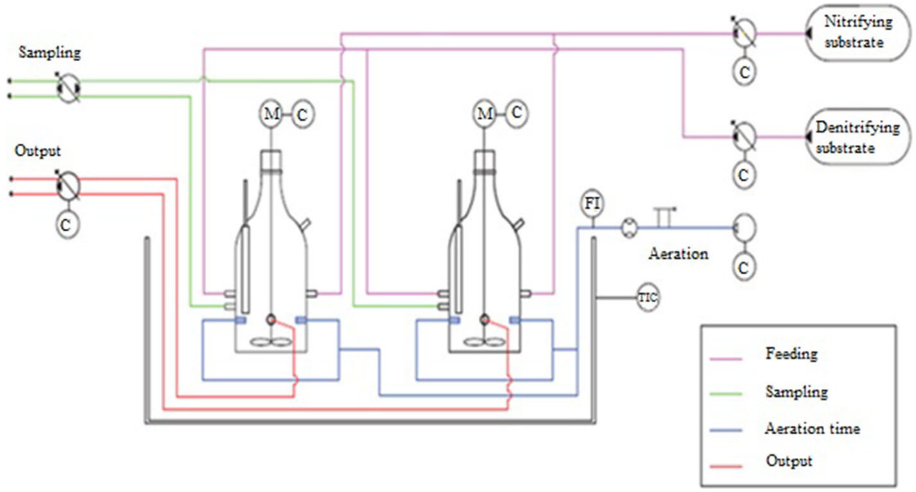


**Table 1** Nitrifying and denitrifying substrate components

| Nitrifying substrate | | Denitrifying substrate | |
|---|---|---|---|
| Compound | Concentration (g/L) | Compound | Concentration (g/L) |
| $(NH_4)_2SO_4$ | 33.533 | $NaCH_3COO$ | 5.000 |
| $MgSO_4 \times 7H_2O$ | 0.0365 | Peptone | 0.480 |
| $K_2HPO_4$ | 0.247 | Yeast extract | 0.200 |
| $KH_2PO_4$ | 0.193 | $NaCO_3$ | 1.000 |
| | | $K_2HPO_4$ | 7.000 |
| | | $KH_2PO_4$ | 5.400 |

**Table 2** Temporary distribution of the increase of NLR during the study period including aeration flows used

| Stage | NLR (kg TKN/(m$^3$ day) | Aeration flow (l/min/vvm) | Operation time (day) |
|---|---|---|---|
| Start-up | 0.031 | 1.54 (0.77) | 0–28 |
| Operation | 0.041 | 1.54 (0.77) | 28–65 |
| | 0.041 | 3.42 (1.71) | 65–100 |
| | 0.053 | 3.42 (1.71) | 100–117 |
| | 0.069 | 3.42 (1.71) | 117–154 |
| | 0.089 | 3.42 (1.71) | 154–178 |
| | 0.113 | 3.42 (1.71) | 178–209 |

### Chemical analysis

Chemical oxygen demand (COD) and solids analyses were carried out according to the 5220D and 2540B methods, respectively, of the Standard Methods for the Examination of Waters and Wastewaters (APHA 2012). Nitrate, nitrite and ammonium nitrogen were determined by spectrophotometry using the 4500-$NO_3^-$, 4500-$NO_2^-$ and 4500-ammonium standard methods, respectively. pH and oxidation–reduction potential (ORP) were determined by selective electrodes. All determinations were made in triplicate.

### Statistic analysis

For the statistic processing and analysis of the data, the software Minitab 8 was utilized. The tests obtained by Minitab 8 were related to descriptive statistics, Anderson–Darling normality tests and ANOVA analysis; all of them were applied in all cases, and a comparison of confidence intervals for mean values was made with a confidence level of 95 %.

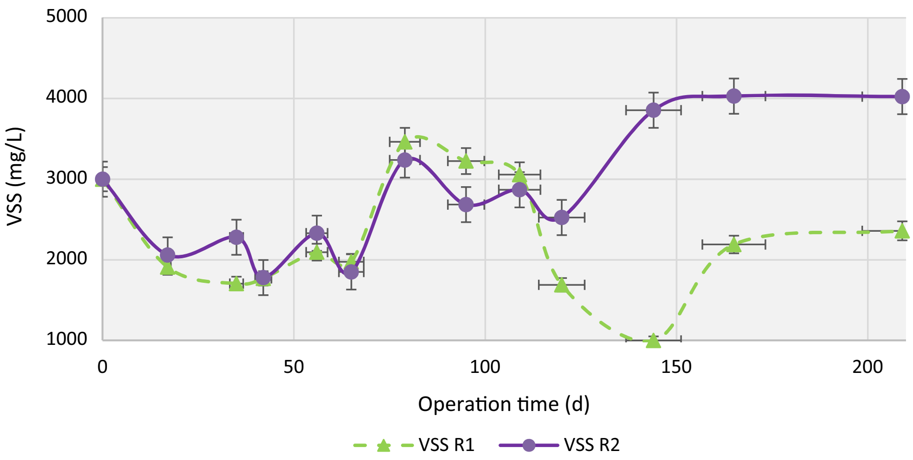
## Results and discussion

### Microbial growth

Figure 3 shows the VSS concentration in the reactors over operation time. During the start-up, there was a decrease in the biomass present in both reactors, reaching values close to 2000 mg VSS/L. This behavior can be attributed to the biomass washed that does not sediment properly (days 0–14). However, close to day 17, the biomass present in the reactor with zeolite (*R*2) was 7.85 % higher than the amount found in the reactor without zeolite (*R*1). Specifically, during days 35 and 56, there were two peaks of the biomass increase in *R*2. On day 39, the biomass growth rates were 12 and 9 mg VSS generated/(L day), and on day 56, the rates were 39 and 23 mg VSS generated/(L day) for *R*2 and *R*1, respectively. From the previous observation, it is possible to highlight that the biomass in *R*2 needed less time for the start-up when compared to *R*1 (35 and 56 days respectively); this may be due to the use of zeolite. Fernández et al. (2007) using scanning electronic microscopy observed, in a fluidised bed anaerobic reactor with natural zeolite as microbial support, a large accumulation of microorganisms in the interior of the ruggedness and on the superficial zones, which were more protected from friction. Therefore, it was demonstrated that natural zeolite possesses excellent physical characteristics as a microorganism support medium. Most recently, Montalvo et al. (2014a) and Guerrero et al. (2014), when operating with fluidized anaerobic reactors and UASB, respectively, observed the acceleration of the start-up stage upon utilizing natural zeolites in both types of reactors. The use of zeolites also accelerated the start-up of the nitrogen removal processes (Montalvo et al. 2014b).

After day 28, the NLR increased from 0.041 to 0.069 kg TKN/(m$^3$ day). A variation in the biomass (VSS) concentration in both reactors could be observed, which is again

**Fig. 3** Microbial growth behavior in the two SND-SBR systems, one with suspended biomass (*R*1) and another with biomass immobilized on zeolite (*R*2)



being observed at a higher growth rate in *R*2 compared to *R*1 at a NLR of 0.069 kg TKN/($m^3$ day) (59 and 55 mg VSS generated/(L day), respectively).

By increasing the NLR to 0.089 kg TKN/($m^3$ day) (day 150), it was observed that the biomass concentration in *R*2 was 4000 mg VSS/L, value quite stabilized, while in *R*1, it drastically decreased to 42 % of its initial value although it slightly increased after day 150. This suggests that under these conditions, the biomass adhered to the zeolite was able to maintain itself in the system.

Since the measured mean biomass concentration values were relatively similar to each other, an ANOVA was done so as to observe whether there were significant statistical differences between the VSS values in both reactors. The results showed that the mean values of VSS-R1 and VSS-R2 were different with a 95 % confidence level. This demonstrates the effect of zeolite avoiding the loss of biomass in a system for the removal of nitrogen.

It was concluded from these results that in both reactors, there existed fluctuations in the biomass, but the use of zeolite decreased the VSS amounts leaving the reactor, and the mentioned differences in both reactors were statistically significant.

## Ammonium removal

The average $NH_4^+$ removals obtained during the entire study period were of the same order of magnitude, 86.1 ± 6.6 and 86.9 ± 6.1 % for *R*1 and *R*2, respectively. Meanwhile, the $NH_4^+$ concentrations from the effluent discharges were 161 ± 72 and 142 ± 79 mg $NH_4^+$/L for *R*1 and *R*2, respectively (Fig. 4). The largest difference between the values of $NH_4^+$-N occurred at higher NLR values (day 202), where the reactor with zeolite (*R*2) had a higher removal efficiency, maintaining effluent concentrations lower than 400 mg/L. The above is consistent with the highest biomass concentration achieved in this system with biomass immobilized on zeolite (Fig. 3).

The higher variance of *R*2 with respect to *R*1 can be explained through a comparison of the maximum and minimum value of each reactor and the implications of the microbial support facing the SND process. Maximum $NH_4^+$ removals of 94.9 % (day 12) and 93.5 % (day 28) were achieved for *R*1 and *R*2, respectively, while the minimum corresponded to 61.8 (day 164) and 61.9 % (day 141) for *R*1 and *R*2, respectively, observing a slight difference between *R*1 and *R*2.

The best behavior of SND of *R*2, based on the highest growth of microorganisms and slightly higher ammonium removal efficiencies, can be a consequence of various causes: (a) formation of anoxic/aerobic microenvironments due to biofilm thickness; (b) boosting of SND metabolism between the microbial populations due to the formation of specific microbial communities within the biofilm; (c) higher concentration of microorganisms by sedimentation and cellular retention; and (d) ionic exchange of $NH_4^+$ between the zeolite and its liquid environment.

The physics phenomenon behind the incorporation of zeolite will allow for the creation of anoxic/aerobic zones due to the increase in the size of the bio-zeolite floccules in *R*2 when compared to floccules in *R*1 (Montalvo et al. 2005; Wilderer et al. 2000). That is to say, the conditions for the establishment of the SND are favored in *R*2. At the same time, the zeolite will allow for in *R*2, on a microenvironmental scale, the adequate microbial ecological morphology for the concurrent coexistence of nitrifying and denitrifying microorganisms within the same floccules, allowing for the boosting of SND metabolism in comparison with *R*1 (Chiu et al. 2007; Zhang et al. 2009; Jia et al. 2011; Verma et al. 2013). The implications of the symbiont coexistence probably lie in that both microbial groups would establish a more expedited link for the metabolic pathways of the oxidation of ammonium and reduction of nitrate/nitrite, favoring the biological process of SND.

On the other hand, the existence of the maximum concentration values in *R*2 (308 mg $NH_4^+$/L at day 141)

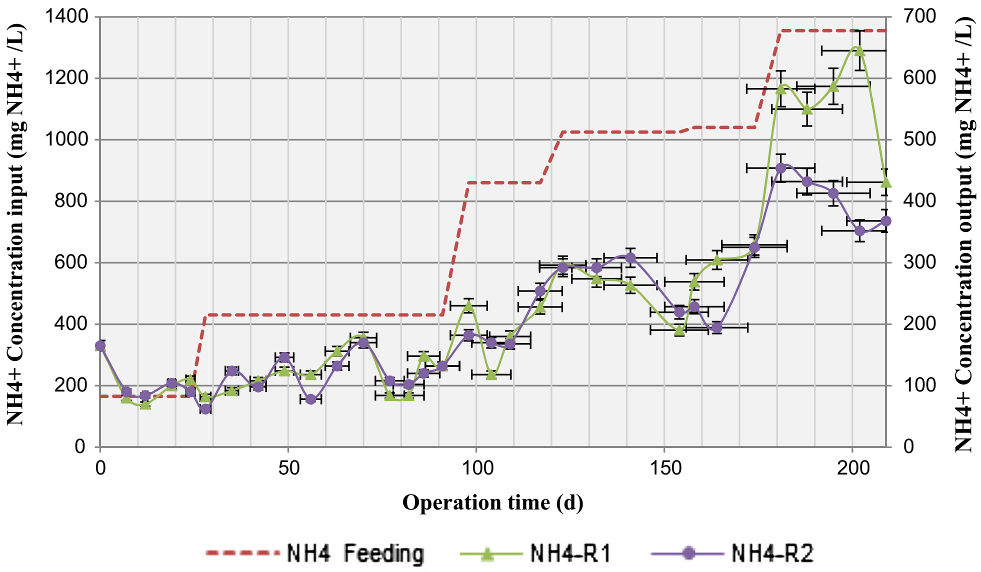


**Fig. 4** Effluent $NH_4^+$ concentration in the two SND-SBR systems, one with suspended biomass (*R*1) and another with biomass immobilized on zeolite (*R*2)

versus *R*1 (304.5 mg $NH_4^+$/L at day 164) implies specific events of lesser efficiencies, which can be understood from three phenomena that imply the operation with support: (a) zeolite as a temporary ammonium reservoir, (b) existence of variability in hydrodynamic conditions and substrate concentration and (c) presence of diffusional resistances due to the formation of biofilm over the zeolite.

As was previously viewed, the resistance of the zeolite when facing shock-loads could be due to the temporary accumulation of ammonium ions within the bio-zeolite floccule structure. In this sense, authors like Jung et al. (1999) and Wilderer et al. (2001) indicate the possibility of behaving as a temporary reservoir of cations and subsequent cationic release into the medium at low concentrations, to later develop SND. Rigorously, it refers to the comparison of the kinetic limitations product of the nitrification and adsorption–desorption/bioregeneration. Even though it is unknown which of the two velocities exert a higher impediment over the global process of SND-SBR with microbial support, it is presumed that the nitritation kinetics has a greater influence compared to desorption/bioregeneration. This is a consequence of the fact that the former is generally a slow step and in this manner allows for the increase of specific maximum concentrations of *R*2 compared to *R*1.

Other possible explanations associated with the above would be the result of the diffusion limitations of the adsorption and desorption/bioregeneration process that occurs once concentrations decrease in the medium and the subsequent ammonium ion liberation of that was described in other studies (Cooney et al. 1999; Lahav and Green 1998; Jorgesen 2002).

Lastly, another factor that compliments the explanation is the hydrodynamic conditions to which the bio-zeolite is exposed. In the absence of zeolite support, the operational behavior of *R*1 is not influenced by the conditions of the mixture, substrate condition and morphological characteristics of the bio-zeolite such as in *R*2. This induces high variability in *R*1 due to the ionic exchange factors and adsorption/desorption phenomena.

Along with the observations discussed previously, another implication of using zeolite is the higher concentration of microorganisms that occurs inside of *R*2 (Fig. 3), which was also observed by Lahav and Green (1998) and Jung et al. (1999). After analyzing the immediate effects of the concentration of microorganisms and the existing removal rates, the influence of both variables for *R*1 and *R*2 (Fig. 5) is clear. Through Fig. 5, it is possible to differentiate the highest and lowest correlation between both variables. Even though the adjustment of the experimental points to a polynomic function is lowered as a result of the dispersion of the gathered data, it can solely be used as a qualitative indicator.

## Nitrate and nitrite accumulation

According to Figs. 6 and 7, the average nitrate and nitrite concentrations obtained during the entire study period were: for $NO_3^-$ 0.69 and 2.96 mg $NO_3^-$/L and for $NO_2^-$ 0.01 and 0.02 mg $NO_2^-$/L for *R*1 and *R*2, respectively. It can be observed in both figures that some specific accumulations of nitrate as well as nitrite exist, which is an indicator that during most of the operation, nitrification was successful.

There exist different specific events for *R*1 and *R*2 where a temporary accumulation of nitrate appears more than nitrite. These events evidence possible shortcomings in

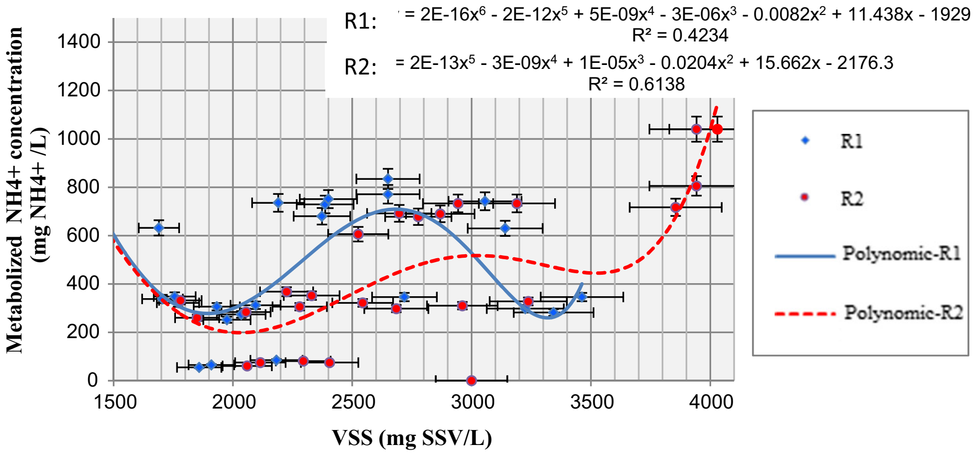
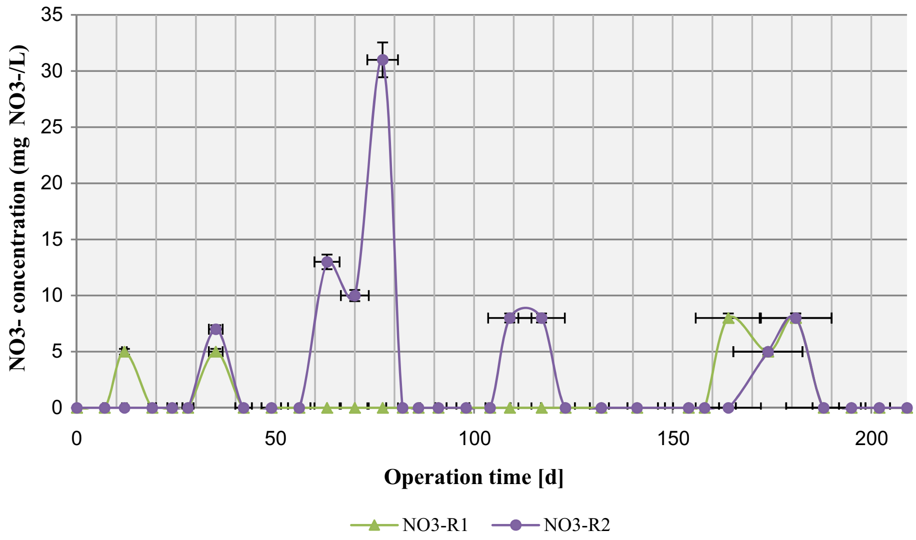


**Fig. 5** Correlation between the metabolized ammonium and biomass concentration in the two SND-SBR systems, one with suspended biomass (*R*1) and another with biomass immobilized on zeolite (*R*2)

**Fig. 6** Nitrate concentration in the two SND-SBR systems, one with suspended biomass (*R*1) and another with biomass immobilized on zeolite (*R*2)



denitrification or an imbalance between the velocities of nitrification–denitrification, a behavior similar to that described by Li et al. (2007).

In the case of nitrate, it is possible to explain these events due to the lack of organic material product of the highest growth rates observed in Fig. 3 (periods 0–35, 70–71 and 109–117 days) that would evidence the utilization of carbon sources for cellular synthesis before being used for substrate oxidation. Another reason for which temporary accumulation would exist is the high probability of the production of free hydroxylamine (FH) (Walters et al. 2009; Wang et al. 2008).

On the other hand, it is presumed that the nitrite accumulations in the initial phases are mainly due to the effects of insufficient dissolved oxygen (DO) (0.70 and 0.75 mg $O_2$/L in *R*1 and *R*2) for nitrite-oxidizing bacteria (NOB) (Chiu et al. 2007; Verma et al. 2013) in the process of adapting to the SBR operational conditions (days 0–70). From these values and in accordance with studies done by other authors (Walters et al. 2009; Wang et al. 2008), aeration was increased (to 1.45 and 1.28 mg $O_2$/L for *R*1 and *R*2 respectively), leading to the posterior reduction in the existing accumulation of nitrite (from days 73–209). The previous observations are coherent with those described by authors such as Münch et al. (1996) and Breisha (2010), who indicate the existence of the same conditions of “NOB washout” at a DO <1.0 mg $O_2$/L through pulsed aeration.

Another observation that may be relevant is the existing differences between *R*1 and *R*2 in the accumulation of both nitrate and nitrite. In most cases, the accumulations or the elevated nitrate values appeared in instances such as a disturbance or increase in nitrogen load. Therefore, it is possible that the nitrate-forming bacteria generated nitrate;

**Fig. 7** Concentration of nitrite in the two SND-SBR systems, one with suspended biomass (*R*1) and another with biomass immobilized on zeolite (*R*2)

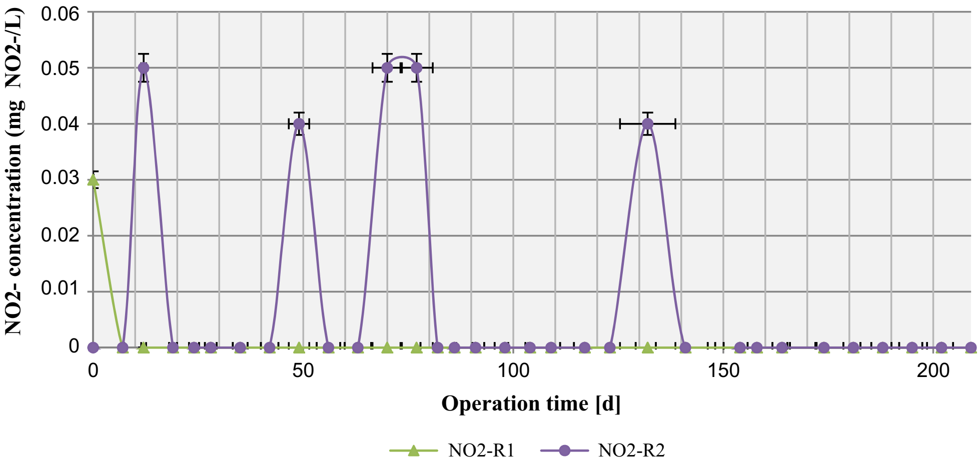
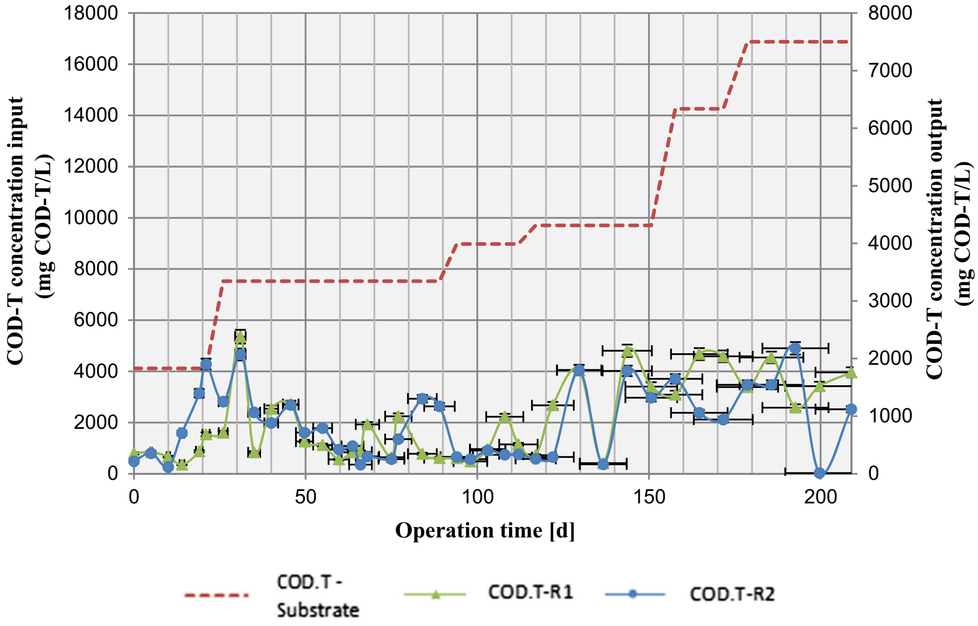


**Fig. 8** Total COD concentration entering (*C*) and exiting in the two SND-SBR systems, one with suspended biomass (*R*1) and another with biomass immobilized on zeolite (*R*2)



however, the nitrite-forming bacteria that utilize nitrate as substrate were not capable of taking and converting all the generated nitrate at the same speed as it is produced. In the same manner, the nitrite accumulation is due to a relatively low conversion of nitrite to nitrogen. These accumulations were observed predominantly in the reactor with zeolite, considering that the conversion to nitrate may be faster in this case.

## Removal of total and soluble organic matter

The average concentrations of total COD exiting the reactors (Fig. 8) were 938.0 and 932.7 mg COD total/L, reaching removal percentages of 96.3 ± 2.5 and 95.4 ± 4.2 % for *R*1 and *R*2, respectively. On the other hand, the soluble COD removal efficiencies were 97.5 ± 1.9 and 97.1 ± 3.9 % for *R*1 and *R*2, respectively, with the highest variance resulting in *R*2 over *R*1 (15.7 and 3.7, respectively).

The small differences in the variances of the removal efficiencies for *R*1 and *R*2 can be attributed to the higher diffusional limitations of both the total and soluble organic matter when it passes to the interior of the bio-zeolite. In the case of *R*1, it does not present the diffusional limitation pertaining to the biofilm–zeolite interphase within the floccule. However, in the case of *R*2, the presence of this limitation would make it difficult for the organic matter to enter the interior of bio-zeolite, reducing removal percentages.

It is interesting to highlight that the points pertaining to the maximum efficiencies of total and soluble COD correspond to the same points described previously (Fig. 3) where each system presents a higher microbial growth rate.

Due to the very similar values obtained for total and soluble COD, an ANOVA test was carried out so as to view statistical differences. The results showed that in the case of total COD the differences were not significant, in spite of overlapping confidence intervals of both series of data. The opposite occurs with soluble COD, where there existed differences, with a 95 % confidence level; therefore, in this case, the data revealed different behavior and performance of the two reactors.

## Behavior profiles of pH/ORP

It is observed in Fig. 9 that the pH profiles for each NLR studied present behavior tendencies similar to previous studies done in SND systems and reported in the literature (Ga and Ra 2009; Guo et al. 2009; Li et al. 2007; Wang et al. 2008). With respect to the ORP behavior, increments of this parameter were observed during the anoxic stage, eventually identifying the points where there only existed carbon oxidation (RCMP), and subsequently, the ORP profile drops slowly during the aerobic stage after the elimination of accumulated nitrates. This can be explained by the manner in which the substrates were introduced into the system and because the anoxic phase preceded the aerobic phase during the cycle.

Another phenomenon exhibited through the pH/ORP profiles is the coexistence of the characteristic points of the nitrifying and denitrifying activity in the anoxic stage as well as in the aerobic stage. In most of the previous figures, it is possible to observe the presence of the nitrate apex (NA) and RCMP points in the anoxic stage. That is to say, in accordance with Chang and Hao (1996) and Li et al. (2007), that the denitrification of the existing nitrate occurs simultaneously (in the sludge of the previous or current cycle), and at the same time, the nitrification of ammonium is completed. On the other hand, the combination of ammonia valley (AV) along with nitrate break (NK) in the aerobic stage is more frequent. The simultaneous occurrence of these two points indicates that when nitrification occurs, the inflexions in the pH profile are formed, while at the same time, the generated nitrates are eliminated during this stage, increasing the sulfate reductase activity. These observations agree with other studies reported in the literature (Guo et al. 2009; Li et al. 2007).

Finally, the simultaneous occurrence of NA + RCMP and AV + NK combinations indicates that in the anoxic and aerobic stages, the joint metabolic activity of nitrificants and denitrificants existed.

It was also observed that there are differences between the behaviors of $R1$ and $R2$ when analyzing the variances in the pH profiles. As can be appreciated in Table 3, in all the scenarios of NLR variation, the lowest variance was found to be in $R2$ rather than $R1$. Comparatively, the characteristics of the ionic exchanger and buffer control when facing changes in the environment, resulting from the use of zeolite, would explain the differences between both reactors, which globally incorporate characteristic improvements for the SND system.

## SND-SBR kinetics

The kinetic study was carried out jointly with the pH/ORP profile shown in Fig. 9f for a NLR of 0.113 kg NTK/($m^3$ day) at 3-h intervals between readings.

The ammonium concentration peaks are those pertaining to each stage of feeding of the step-feed process. During the first 3 h, there exists a rapid decrease in ammonium concentration, although it is necessary to highlight the existence of higher kinetics in $R2$ as opposed to $R1$ specially after the third cycle (average values of this step: 253.5 and 282.1 mg $NH_4^+$/(L h) for $R1$ and $R2$, respectively). The kinetic analysis showed that the incorporation of zeolite to a SND-SBR reactor improves the reaction rates by 11.31 % when compared to the reactor without zeolite. This can be explained through the differences that the support gives in the formation of the bio-zeolite complex, that is to say, in addition to the ammonium removal capacity by microbial metabolism, there is an added adsorption capacity of the zeolite through diffusion within the floc (Fernández et al. 2007; Montalvo et al. 2014a).

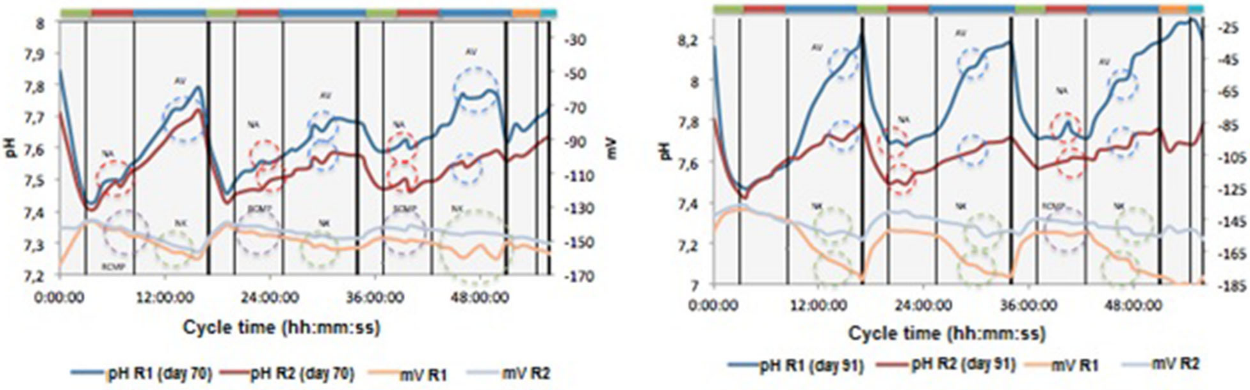
In the anoxic stage, there is a minimum concentration of ammonium that according to Al-Ghusain and Hao (1995) represents the complete consumption of nitrate and beginning of anaerobiosis. In light of the fact that there is no evidence of the accumulation of nitrate in the system, it is possible to infer that the nitrification and denitrification rates are found to be in equilibrium in this stage of each reactor. The previous affirmation is valid during the feeding and anoxic stages of each reactor in the 3 step-feed process, but is not in the aerobic stage in the case of the step-feed 1 and 3, for which there are differences in the rates of denitrification and nitrification, in both reactors, which cause a temporary accumulation of nitrate and nitrite in a soft manner in both cases (Fig. 10).

During the aerobic stage, the behavior of both reactors was similar, and an increase in ammonium concentrations in both SND systems was observed due to that the $NH_4^+/NH_3$ equilibrium tends to go toward the formation of ammonium.

From a kinetics point of view, with respect to the organic matter, there exists a rapid soluble COD (CODs) consumption during the feeding stage for each step-feed (3769 and 3742 mg CODs/(L h) for $R1$ and $R2$, respectively). This can be explained by the rapid penetration of CODs in the microbial flocs and/or by its storage in the

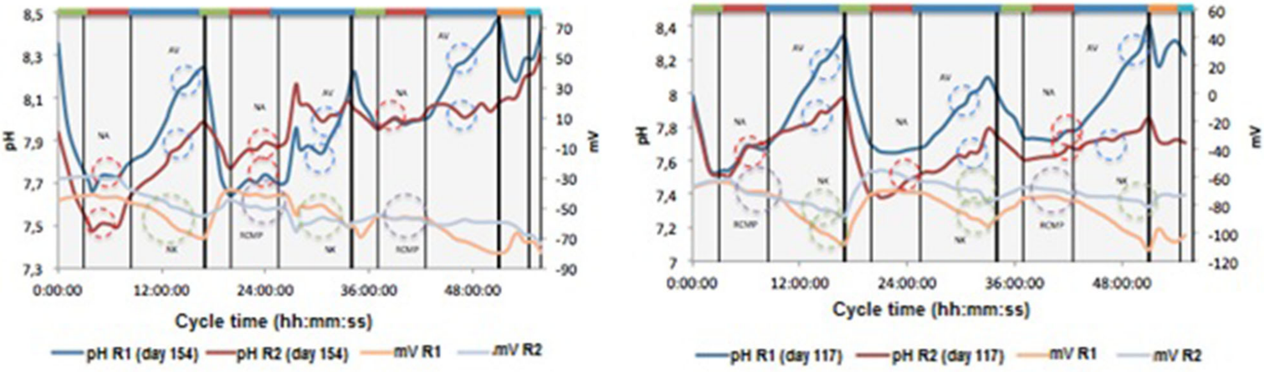
**(a)** NLR = 0.042 (kg TKN/m$^3$·d)

**(b)** NLR = 0.042+ aeration (kg TKN/m$^3$·d)



**(c)** NLR = 0.053 (kg TKN/m$^3$·d)

**(d)** NLR = 0.069 (kg TKN/m$^3$·d)



**(e)** NLR = 0.089 (kg TKN/m$^3$·d)

**(f)** NLR = 0.113 (kg TKN/m$^3$·d)

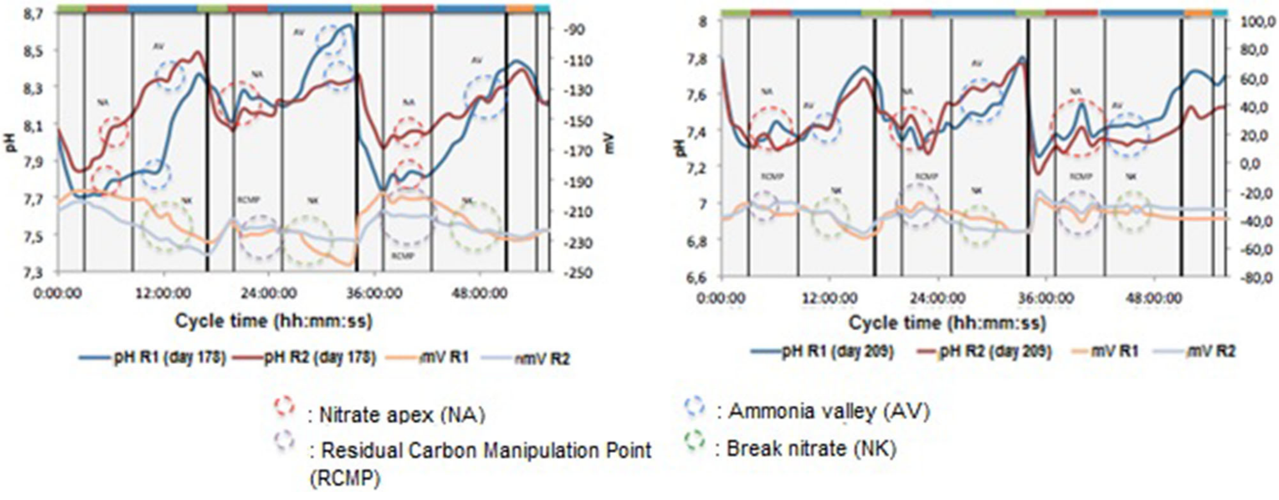


**Fig. 9** pH/ORP profiles at different NLR in the two SND-SBR systems, one with suspended biomass (*R*1) and another with biomass immobilized on zeolite (*R*2)

form of slow biodegradability matter such as in the internal structure of polyhydroxybutyrate (PHB) (Chiu et al. 2007; Zhang et al. 2009; Jia et al. 2011; Verma et al. 2013).

According to Fig. 11, an increase in organic material was observed during the aerobic stage. This is coherent with the observations made by other authors (Zhang et al.

**Table 3** Variation of pH in different scenarios of increasing NLR in the SND-SBR system with suspended biomass (*R*1) and in the reactor with biomass immobilized on zeolite (*R*2)

| Day | NLR (kg TKN/(m$^3$ day) | pH |  | Var-pH |  |
|---|---|---|---|---|---|
|  |  | *R*1 | *R*2 | *R*1 | *R*2 |
| 35 | 0.031 | 7.535 ± 0.111 | 7.296 ± 0.055 | 0.012 | 0.003 |
| 70 | 0.042 | 7.625 ± 0.097 | 7.535 ± 0.071 | 0.009 | 0.005 |
| 91 | 0.042 + air | 7.847 ± 0.210 | 7.623 ± 0.084 | 0.044 | 0.007 |
| 117 | 0.053 | 7.849 ± 0.200 | 7.660 ± 0.142 | 0.040 | 0.020 |
| 154 | 0.069 | 7.948 ± 0.203 | 7.885 ± 0.178 | 0.041 | 0.032 |
| 178 | 0.089 | 8.064 ± 0.253 | 8.170 ± 0.149 | 0.064 | 0.022 |
| 209 | 0.113 | 7.456 ± 0.123 | 7.438 ± 0.142 | 0.015 | 0.020 |

**Fig. 10** Kinetic study of the ammonium, nitrate and nitrite removal for both SND-SBR reactors

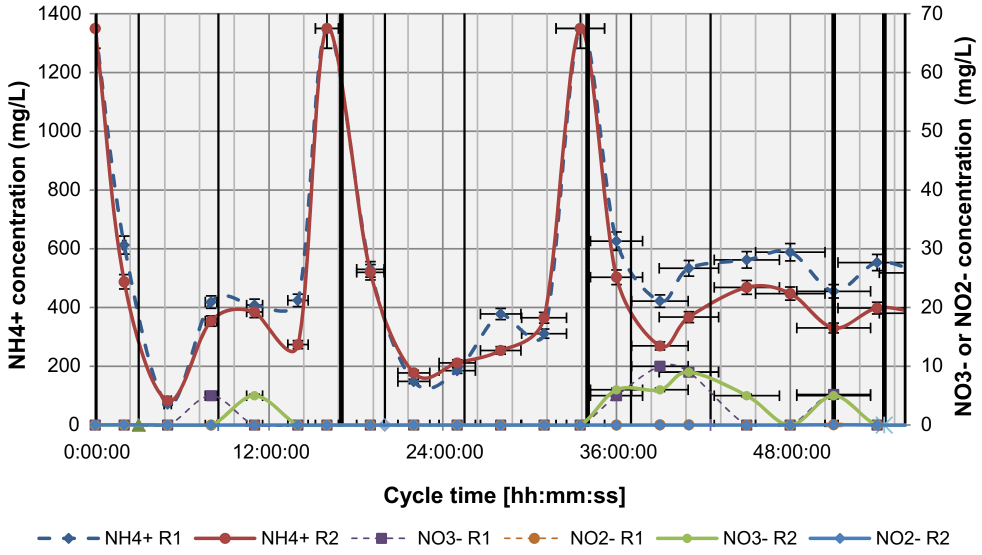
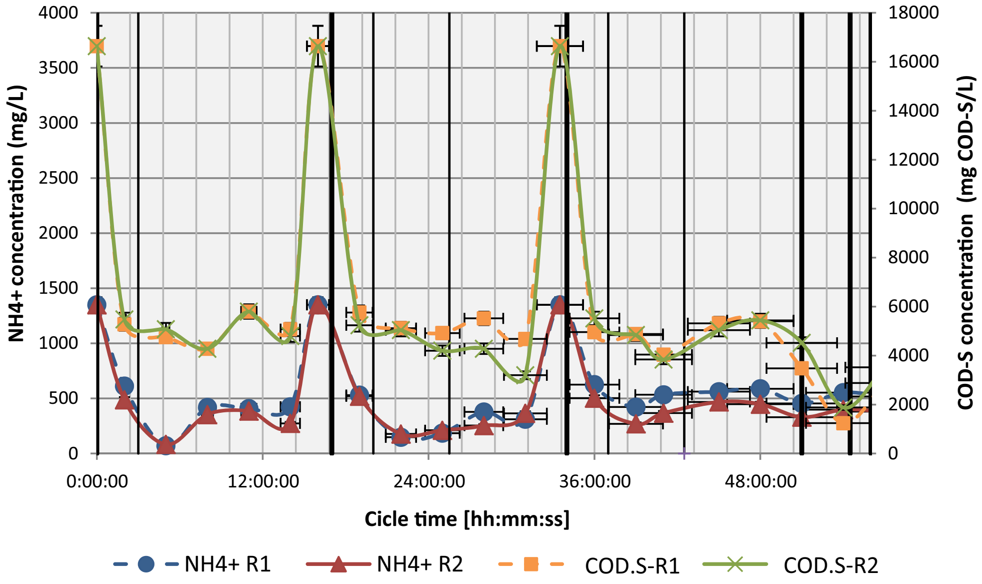


**Fig. 11** Kinetic study of the simultaneous removal of nitrogen compounds and organic matter in both SND-SBR reactors



2009), which stated that during the aerobic stages, the storage sources of organic matter such as PHB are utilized as a carbon source for SND.

The remnants of ammonium existing in both reactors indicate that there exists a deficiency of available organic matter for SND at high NLR values. It is important to indicate that even though in this study a *C*/*N* ratio of 5 was used, it would be advisable to have used higher values.

## Conclusion

The removal of nitrogen compounds and organic matter via simultaneous nitrification and denitrification was achieved in sequential batch reactors using zeolite as microorganism support as well as with suspended biomass. It was evidenced, from the simultaneous coexistence of characteristic points in pH/ORP profiles of each aerobic and anoxic stage, that the major part of the operation consisted in simultaneous nitrification and total denitrification, without the accumulation of $NO_3^-$ and $NO_2^-$. The incorporation of zeolite in a SND-SBR system allowed for achieving higher biomass growth rates in the reactor with immobilized biomass compared to the results found in the reactor with suspended biomass, in addition, reducing the process start-up time. The kinetic analysis showed that the incorporation of zeolite to a SND-SBR reactor improves the reaction rates by 11.31 % when compared to the reactor without zeolite. The temporary distribution of the feeding through step-feed decreases the substrate inhibition events in an immobilized SND-SBR reactor as well as in a conventional reactor, even duplicating the tolerance for high NLR for the same operation time.

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### Abbreviations

| | |
|---|---|
| AOB | Ammonia-oxidizing bacteria |
| BNR | Biological nitrogen removal |
| *C*/*N* | Carbon/nitrogen ratio |
| CEC | Cation exchange capacity |
| COD | Chemical oxygen demand |
| CODs | Chemical oxygen demand soluble |
| DO | Dissolved oxygen |
| FH | Free hydroxylamine |
| NOB | Nitrite-oxidizing bacteria |
| NLR | Nitrogen loading rate |
| ORP | Oxidation–reduction potential |
| PHB | Polyhydroxybutyrate |
| *R*2 | Reactor with Chilean natural zeolite |
| *R*1 | Reactor without Chilean natural zeolite |
| SBR | Sequential batch reactor |
| SND | Simultaneous nitrification–denitrification |
| TKN | Total Kjeldahl nitrogen |
| TSS | Total suspended solids |
| VSS | Volatile suspended solids |
| vvm | Volumes per reactor volume per minute |

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