

Simultaneous Nitrification-Denitrification of wastewater: Effect of zeolite as a support in sequential batch reactor with step-feed strategy

Simultaneous Nitrification-Denitrification of wastewater: Effect of zeolite in sequential batch reactor with step-feed strategy

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4 **Simultaneous Nitrification-Denitrification of wastewater: Effect of zeolite as a support in**
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6 **sequential batch reactor with step-feed strategy**
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11 *reactor with step-feed strategy*
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14 **Abstract**

15 One of the technologies used for wastewater nitrogen removal consists in simultaneous nitrification-
16 denitrification. The low microbial growth rate and the low availability of organic material for the denitrification
17 stage, makes necessary to study new operational conditions and the use of microbial supports. The aim of this
18 study was to evaluate the operational behavior of a simultaneous nitrification-denitrification process in a
19 sequential batch reactor (SBR) utilizing zeolite as a biomass support and step-feed strategy. Two reactors of 2
20 L were used, one with zeolite and another without zeolite, both operated at constant temperature (31°C), varying
21 nitrogen loading rate (NLR) from 0.041 to 0.113 kg total kjeldahl nitrogen (TKN/m³/day). After 209 days it
22 was obtained removals higher than 86% and 96% in nitrogen compounds and organic matter respectively. There
23 was not accumulation of nitrate and nitrite in any case; this means that there was a simultaneous nitrification-
24 denitrification in the reactors. The incorporation of zeolite in the system held higher concentration of biomass
25 in the reactor; this led to reduce start-up to 21 days and to improve in 11.31% removal kinetic. The use of a
26 step-feed strategy prevents events of inhibition by substrate, even duplicating tolerance to higher NLR for the
27 same operation time.
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43 **Keywords**

44 Sequential batch reactor; simultaneous nitrification-denitrification; step-feed; zeolite
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48 **1. Introduction**

49 The reduction of nitrogen levels from several wastewater plant effluents containing high concentrations of
50 nitrogen compounds is necessary because these compounds can be toxic to aquatic life, causing oxygen
51 depletion and eutrophication in receiving water, and affecting chlorine disinfection efficiency (Haiming et al.
52 2011; Song et al. 2011; Wang et al. 2004). Nitrogen compounds can be removed from wastewaters by a variety
53 of physicochemical and biological processes (Gao et al. 2015; Montalvo et al. 2011; Ozturk and Bal 2015; Sun
54 et al. 2015). Recently, several novel and cost-effective biological nitrogen removal processes have been
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4 developed, including partial nitrification, nitrifier denitrification, anaerobic ammonium oxidation (Anammox),
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6 and its combined systems (completely autotrophic nitrogen removal over nitrite, Canon), among others (Ahn
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8 2006; Chen et al. 2009; Kumar and Lin 2010; Lan et al. 2011; Zhang et al. 2012). However, nitrification –
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10 denitrification process is still the biological nitrogen removal process most used at industrial scale (Kim et al.
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12 2013).

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14 Simultaneous nitrification-denitrification (SND) is a type of technology currently used in ammonium and
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16 nitrate removal. This process has become a versatile and recommended tool in the treatment of wastewater with
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18 a high C/N ratio (Bernet et al. 2000; Guo et al. 2013; Moya et al. 2012). Nitrification is the sequential oxidative
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20 transformation process of ammonium resulting in nitrite (reduced by ammonia-oxidizing bacteria or AOB)
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22 followed by the oxidation of the nitrite to nitrate due to the action of two forced chemolithoautotrophic microbial
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24 groups (Peng and Zhu 2006; Shijian et al. 2014). Meanwhile, heterotrophic microorganisms take the nitrate and
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26 reduces it to N₂ (denitrification), utilizing organic matter as the electron donor (Pepper 2006). Taking into
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28 account this sequential characteristic of the SND process, the sequential batch reactor (SBR) is an attractive
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30 choice to carry out it. The SND process represents a significant advantage over the conventional separated
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32 nitrification and denitrification process. First, SND process eliminates the serial operation of two separated
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34 tanks and, therefore, requires simpler operational procedures. In addition, the SND uses 20-40% less carbon
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36 sources and reduces sludge yield by 30% when compared to conventional biological nitrogen removal (BNR)
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38 systems (Guo et al. 2013).

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

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58 Zeolite is a mineral of hydrated aluminum silicates (AlO₄/SiO₄) (Alejandro et al. 2014; Montalvo et al.
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60 2012) with a tetrahedral structure, with a high cation-exchange capacity (CEC) and high cation selectivity
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4 (Wang and Peng 2010). Specifically, zeolite is a porous material characterized by its ability to 1) lose and gain
5 water reversibly; 2) adsorb molecules of appropriate cross-sectional diameter (adsorption property or acting as
6 molecule sieves), and 3) exchange its constituent cations without a major change in its structure (ion exchange
7 property) (Montalvo et al. 2012). Even though its use has been proposed for nitrification and denitrification
8 separately, little has been studied on its use in SND processes, being the latter one of the objectives of this work.
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15 The temporary distribution of the pulse feeds, known as step-feed, results in an adequate strategy to control
16 parameters in the batch operation such as sedimentability, availability of carbon and resistance against loading
17 shocks (Andreottola et al. 2001; Artan and Orhon 2005; Guihua et al. 2013; Martins et al. 2003). This feeding
18 strategy has been used in the biological elimination process of nitrogen (for example
19 nitrification/denitrification) to avoid partial denitrification and the use of external carbon sources (for example
20 methanol or acetate) and the associated costs (Guihua et al. 2013).
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28 Therefore, the main objective of the present study was to evaluate the operational behavior of Biological
29 Nitrogen Removal (BNR) to optimize the removal of nitrogen compounds through the use of a simultaneous
30 nitrification-denitrification reactor with a strategy of step-feed, utilizing zeolite as a microbial support and/or
31 to improve the ammonium removal process.
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38 **2. Materials and Methods**

39 **2.1. Equipment and inoculum**

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42 Two reactors of 2 L volume were designed for this study. A detailed scheme of the reactor is shown in Figure
43 1. One reactor operated with zeolite (with a concentration of 5 g/L, which was added to the reactor at the
44 beginning of the experiments) and the other without zeolite. From now on, the reactor with zeolite will be
45 referred to as R2 and the reactor without zeolite will be referred to as R1. Both reactors were inoculated with a
46 mixture 70/30 of aerobic/anaerobic sludge, being the total volume of the inoculum 400 mL, to obtain a final
47 concentration of 3.5 g VSS/L inside the system. The characteristics of the inoculum were: 10.07 g TSS/L and
48 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
49 installed and assembled as presented in Figure 2.
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4 The aerobic sludge was obtained from an Activated Sludge Plant located in a poultry industry and the
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6 anaerobic sludge was collected from an anaerobic digester treating wastewaters from a tobacco industry in the
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8 Valparaíso Region, Chile.
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10 **2.2. Characteristics and Pretreatment of Chilean zeolite**

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12 The major mineral contents of the Chilean natural zeolite used were: clinoptilolite (35%), montmorillonite
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14 (30%), mordenite (15%) and other minerals (20%). Its chemical composition was: SiO₂ (66.62%), Al₂O₃
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16 (12.17%), CaO (3.19%), Fe₂O₃ (2.08%) and other chemicals (Wang and Peng, 2010; Montalvo et al., 2012).
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18 Previous to be used, the Chilean zeolite utilized was sieved through a GeyerPrüfsieb (DIN 1170) mesh to obtain
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20 particles of 0.08-0.12 cm diameter applying ASTM C136-01 Method. Afterwards, zeolite was washed with
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22 distilled water and dried in an oven at 110°C for 1 day.
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26 **2.3. Operation in batch cycle**

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28 The operation was done in cycles of 51 h distributed in 3 ramps (step-feed), each one with a 3 h of anoxic feed,
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30 5.5 h of agitated anoxic operation and 8.5 h of agitated aerobic operation.
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33 Both nitrifying and denitrifying substrate were incorporated in both reactors in the anoxic phase of each
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35 step-feed by pulses with a flow of 19 mL/min (9.5 volumes per reactor volume per minute, vvm).
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38 The aeration, 3.4 L/m (1.7 vvm), was carried out in an intermitent form (pulses). At the end of the third
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40 ramp a sedimentation stage was incorporated (3.5 h) and finally with the unloading the cycle (1.5 h) was
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42 finished.
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44 Increasing NLR from 0.031 to 0.112 kg TKN/(m³·day) were used; the carbon/nitrogen ratio was kept
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46 constant during the experiment (C/N=5) and the reactors operated in darkness at 31 °C of temperature.
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49 **2.4. Synthetic wastewater.**

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51 Table 1 shows the synthetic substrates (nitrifying and denitrifying) composition used as feed in the experiments.
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54 **2.5. Operation strategy**

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56 Table 2 shows the duration of each stage as well as the NLR and aeration flows used in the different steps of
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58 the experiments carried out.
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2.6. 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-NO₃⁻, 4500-NO₂⁻ and 4500-ammonium standard methods respectively. pH and oxidation-reduction potential (ORP) were determined by selective electrodes. All determinations were made in triplicate.

2.7. 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 with 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%.

3. Results and Discussion

3.1. 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 to 14). However, close to day 17 the biomass present in the reactor with zeolite (R2) was 7.85% higher than the amount found in the reactor without zeolite (R1). Specifically, during days 35 and 56 there were two peaks of the biomass increase in R2. 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 R2 and R1, respectively. From the previous observation, it is possible to highlight that the biomass in R2 needed less time for the start-up when compared to R1 (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

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4 operating with fluidized anaerobic reactors and UASB, respectively, observed the acceleration of the start-up
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6 stage upon utilizing natural zeolites in both types of reactors. The use of zeolites also accelerated the start-up
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8 of the nitrogen removal processes (Montalvo et al. 2014b).
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10 After day 28, the NLR increased from 0.041 to 0.069 kg TKN/(m³·day). It could be observed a variation in
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12 the biomass (VSS) concentration in both reactors, again being observed a higher growth rate in R2 compared
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14 to R1 at a NLR of 0.069 kg TKN/(m³·day) (59 and 55 mg VSS generated/(L·day), respectively).
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17 By increasing the NLR to 0.089 kg TKN/(m³·day) (day 150), it was observed that the biomass concentration
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19 in R2 was 4000 mg VSS/L, value quite stabilized, while in R1 it drastically decreased to 42% of its initial value
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21 despite it slightly increased after day 150. This suggests that under these conditions, the biomass adhered to the
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23 zeolite was able to maintain itself in the system.
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25 Since the measured mean biomass concentration values were relatively similar to each other, an ANOVA
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27 analysis was done so as to observe if there were significant statistical differences between the VSS values in
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29 both reactors. The results showed that the mean values of VSS-R1 and VSS-R2 were different with a 95%
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31 confidence level. This demonstrates the effect of zeolite avoiding the loss of biomass in a system for the removal
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33 of nitrogen.
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35 It was concluded from these results, that in both reactors existed fluctuations in the biomass, but the use of
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37 zeolite decreased the VSS amounts leaving the reactor, and the mentioned differences in both reactors were
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39 statistically significant.
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42 **3.2. Ammonium removal.**

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44 The average NH₄⁺ removals obtained during the entire study period were of the same order of magnitude, 86.1%
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46 ± 6.6% and 86.9% ± 6.1% for R1 and R2, respectively. Meanwhile, the NH₄⁺ concentrations from the effluent
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48 discharges were 161 ± 72 and 142 ± 79 mg NH₄⁺/L for R1 and R2, respectively (Figure 4). The largest difference
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50 between the values of NH₄⁺-N occurred at higher NLR values (day 202), where the reactor with zeolite (R2)
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52 had a higher removal efficiency, maintaining effluent concentrations lower than 400 mg/L. The above is
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54 consistent with the highest biomass concentration achieved in this system with biomass immobilized on zeolite
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56 (Figure 3).
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4 The higher variance of R2 with respect to R1 can be explained through a comparison of the maximum and
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6 minimum value of each reactor and the implications of the microbial support facing the SND process. Maximum
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8 NH_4^+ removals of 94.9% (day 12) and 93.5% (day 28) were achieved for R1 and R2, respectively, while the
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10 minimum corresponded to 61.8 (day 164) and 61.9% (day 141) for R1 and R2 respectively, observing a slight
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12 difference between R1 and R2.
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15 The best behavior of SND of R2, based on the highest growth of microorganisms and slightly higher
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17 ammonium removal efficiencies, can be a consequence of various causes: a) Formation of anoxic/aerobic
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19 microenvironments due to biofilm thickness, b) Boosting of SND metabolism between the microbial
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21 populations due to the formation of specific microbial communities within the biofilm; c) Higher concentration
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23 of microorganisms by sedimentation and cellular retention and d) Ionic Exchange of NH_4^+ between the zeolite
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25 and its liquid environment.
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28 The physics phenomenon behind the incorporation of zeolite will allow for the creation of anoxic/aerobic zones
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30 due to the increase in the size of the bio-zeolite floccules in R2 when compared to floccules in R1 (Montalvo
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32 et al. 2005; Wilderer et al. 2000). That is to say, the conditions for the establishment of the SND are favored
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34 in R2. At the same time, the zeolite will allow for in R2, on a microenvironmental scale, the adequate microbial
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36 ecological morphology for the concurrent coexistence of nitrifying and denitrifying microorganisms within the
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38 same floccules, allowing for the boosting of SND metabolism in comparison to R1 (Chiu et al. 2007; Zhang et
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40 al. 2009; Jia et al. 2011; Verma et al. 2013). The implications of the symbiont coexistence probably lie in that
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42 both microbial groups would establish a more expedited link for the metabolic pathways of the oxidation of
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44 ammonium and reduction of nitrate/nitrite, favoring the biological process of SND.
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47 On the other hand, the existence of the maximum concentration values in R2 (308 mg NH_4^+ /L at day 141)
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49 versus R1 (304.5 mg NH_4^+ /L at day 164) implies specific events of lesser efficiencies, which can be understood
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51 from three phenomena that imply the operation with support: a) zeolite as a temporary ammonium reservoir, b)
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53 existence of variability in hydrodynamic conditions and substrate concentration and c) presence of diffusional
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55 resistances due to the formation of biofilm over the zeolite.
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58 As was previously viewed, the resistance of the zeolite when facing shock-loads could be due to the
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60 temporary accumulation of ammonium ions within the bio-zeolite floccule structure. In this sense, authors like
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4 Jung et al. (1999) and Wilderer et al. (2001), indicate the possibility of behaving as a temporary reservoir of
5 cations and subsequent cationic release into the medium at low concentrations, to later develop SND.
6 Rigorously, it refers to the comparison of the kinetic limitations product of the nitrification and adsorption-
7 desorption/bioregeneration. Even though it is unknown which of the two velocities exerts a higher impediment
8 over the global process of SND-SBR with microbial support, it is presumed that the nitrification kinetics has a
9 greater influence compared to desorption/bioregeneration. This is a consequence of the fact that the former is
10 generally a slow step and in this manner allows for the increase of specific maximum concentrations of R2
11 compared to R1.
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21 Other possible explanations associated with the above would be the result of the diffusion limitations of the
22 adsorption and desorption/bioregeneration process that occurs once concentrations decrease in the medium and
23 the subsequent ammonium ion liberation of that such as was described in other studies (Cooney et al. 1999;
24 Lahav and Green 1998; Jorgesen 2002).
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30 Lastly, another factor that compliments the explanation is the hydrodynamic conditions to which the bio-
31 zeolite is exposed. In the absence of zeolite support, the operational behavior of R1 is not influenced by the
32 conditions of the mixture, substrate condition and morphological characteristics of the bio-zeolite such as in
33 R2. In this manner, inducing higher variability in the reactor with biomass in the support, product of the ionic
34 exchange factors and adsorption/desorption phenomena.
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40 Along with the observations discussed previously, another implication of using zeolite is the higher
41 concentration of microorganisms that occurs inside of R2 (Figure 3), which was also observed by Lahav and
42 Green (1998) and Jung et al. (1999). After analyzing the immediate effects of the concentration of
43 microorganisms and the existing removal rates, the influence of both variables for R1 and R2 (Figure 5) is clear.
44 Through Figure 5, it is possible to differentiate the highest and lowest correlation between both variables. Even
45 though the adjustment of the experimental points to a polynomial function is lowered as a result of the dispersion
46 of the gathered data, it can solely be used as a qualitative indicator.
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55 **3.3. Nitrate and nitrite accumulation**

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57 According to Figures 6 and 7, the average nitrate and nitrite concentrations obtained during the entire study
58 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 R1 and R2,
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4 respectively. It can be observed in both figures that some specific accumulations of nitrate as well as nitrite
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6 exist, which is an indicator that during most of the operation, nitrification was successful.
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10 There exist different specific events for R1 and R2 where a temporary accumulation of nitrate appears more
11 than nitrite. These events evidence possible shortcomings in denitrification or an imbalance between the
12 velocities of nitrification-denitrification, a behavior similar to that described by Li et al. (2007).
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16 In the case of nitrate, it is possible to explain these events due to the lack of organic material product of the
17 highest growth rates observed in Figure 3 (periods 0-35, 70-71 and 109-117 days), that would evidence the
18 utilization of carbon sources for cellular synthesis before being used for substrate oxidation. Another reason for
19 which temporary accumulation would exist is the high probability of the production of free-hydroxylamine
20 (FH) (Walters et al. 2009; Wang et al. 2008).
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28 On the other hand, it is presumed that the nitrite accumulations in the initial phases are mainly due to the
29 effects of insufficient dissolved oxygen (DO) (0.70 and 0.75 mg O₂/L in R1 and R2) for nitrite-oxidizing
30 bacteria (NOB) (Chiu et al. 2007; Verma et al. 2013) in the process of adapting to the SBR operational
31 conditions (days 0-70). From these values and in accordance with studies done by other authors (Walters et al.
32 2009; Wang et al. 2008), aeration was increased (to 1.45 and 1.28 mg O₂/L for R1 and R2 respectively), leading
33 to the posterior reduction in the existing accumulation of nitrite (from days 73-209). The previous observations
34 are coherent with those described by authors such as Münch et al. (1996) and Breisha (2010), who indicate the
35 existence of the same conditions of “NOB wash-out” at a DO < 1.0 mg O₂/L through pulsed aeration.
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45 Another observation that may be relevant is the existing differences between R1 and R2 in the accumulation
46 of both nitrate and nitrite. In most cases, the accumulations or the elevated nitrate values appeared in instances
47 such as a disturbance or increase in nitrogen load. Therefore, it is possible that the nitrate forming bacteria
48 generated nitrate, however, the nitrite forming bacteria that utilize nitrate as substrate were not capable of taking
49 and converting all the generated nitrate at the same speed as it is produced. In the same manner, the nitrite
50 accumulation is due to a relatively low conversion of nitrite to nitrogen. These accumulations were observed
51 predominantly in the reactor with zeolite considering that the conversion to nitrate may be faster in this case.
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59 **3.4. Removal of total and soluble organic matter**

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4 The average concentrations of total COD exiting the reactors (Figure 8) were 938.0 and 932.7 mg COD total/L,
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6 reaching removal percentages of $96.3\% \pm 2.5\%$ and $95.4\% \pm 4.2\%$ for R1 and R2, respectively. On the other
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8 hand, the soluble COD removal efficiencies were $97.5\% \pm 1.9\%$ and $97.1\% \pm 3.9\%$ for R1 and R2 , respectively,
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10 with the highest variance resulting in R2 over R1 (15.7 and 3.7, respectively).
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13 The small differences in the variances of the removal efficiencies for R1 and R2 can be attributed to the
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15 higher diffusional limitations of both the total and soluble organic matter when it passes to the interior of the
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17 bio-zeolite. In the case of R1, it does not present the diffusional limitation pertaining to the biofilm-zeolite
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19 interphase within the floccule. However, in the case of R2, the presence of this limitation would make difficult
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21 the organic matter to enter the interior of bio-zeolite, reducing removal percentages.
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24 It is interesting to highlight that the points pertaining to the maximum efficiencies of total and soluble COD
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26 correspond to the same points described previously (Figure 3) where each system presents a higher microbial
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28 growth rate.
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31 Due to the very similar values obtained for total and soluble COD, an ANOVA test was carried out so as to
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33 view statistical differences. The results showed that in the case of total COD the differences were not significant,
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35 in spite of overlapping confidence intervals of both series of data. The opposite occurs with soluble COD, where
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37 there existed differences, with a 95% confidence level, therefore, in this case the data revealed different behavior
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39 and performance of the two reactors.
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41 **3.5 Behavior profiles of pH/ORP**

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43 It is observed in Figure 9 that the pH profiles for each NLR studied present behavior tendencies similar to
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45 previous studies done in SND systems and reported in the literature (Ga and Ra 2009; Guo et al 2009; Li et al.
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47 2007; Wang et al. 2008). With respect to the ORP behavior, increments of this parameter were observed during
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49 the anoxic stage, eventually identifying the points where there only existed carbon oxidation (RCMP) and
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51 subsequently the ORP profile drops slowly during the aerobic stage after the elimination of accumulated
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53 nitrates. This can be explained by the manner in which the substrates were introduced into the system and
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55 because the anoxic phase preceded the aerobic phase during the cycle.
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59 Another phenomenon exhibited though the pH/ORP profiles, is the coexistence of the characteristic points
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61 of the nitrifying and denitrifying activity in the anoxic stage as well as in the aerobic stage. In most of the
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4 previous figures, it is possible to observe the presence of the nitrate apex (NA) and RCMP points in the anoxic
5 stage. That is to say, in accordance with Chang and Hao (1996) and Li et al. (2007), that the denitrification of
6 the existing nitrate occurs simultaneously (in the sludge of the previous or current cycle) and at the same time,
7 the nitrification of ammonium is completed. On the other hand, the combination of ammonia valley (AV) along
8 with nitrate break (NK) in the aerobic stage is more frequent. The simultaneous occurrence of these two points
9 indicates that when nitrification occurs the inflexions in the pH profile are formed, while at the same time the
10 generated nitrates are eliminated during this stage, increasing the sulfate reductase activity. These observations
11 agree with other studies reported in the literature (Guo et al. 2009, Li et al. 2007).
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21 Finally, the simultaneous occurrence of NA+RCMP and AV+NK combinations indicate that in the anoxic
22 and aerobic stages existed the joint metabolic activity of nitrificants and dinitrificants.
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25 It was also observed that there are differences between the behaviors of R1 and R2 when analyzing the
26 variances in the pH profiles. As can be appreciated in Table 3, in all the scenarios of NLR variation, the lowest
27 variance was found to be in R2 rather than R1. Comparatively, the characteristics of the ionic exchanger and
28 buffer control when facing changes in the environment, resulting from the use of zeolite, would explain the
29 differences between both reactors, which globally incorporates characteristic improvements for the SND
30 system.
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38 **3.6. SND-SBR Kinetics**

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40 The kinetic study was carried out jointly with the pH/ORP profile shown in Figure 9(f) for a NLR of 0.113 kg
41 NTK/(m³·d) at 3 h intervals between readings.
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45 The ammonium concentration peaks are those pertaining to each stage of feeding of the step-feed process.
46 During the first 3 hours, there exists a rapid decrease in ammonium concentration, although it is necessary to
47 highlight the existence of higher kinetics in R2 as opposed to R1 specially after the 3rd cycle (average values of
48 this step: 253.5 and 282.1 mg NH₄⁺/(L·h) for R1 and R2, respectively). The kinetic analysis showed that the
49 incorporation of zeolite to a SND-SBR reactor improves the reaction rates by 11.31% when compared to the
50 reactor without zeolite. This can be explained through the differences that the support gives in the formation of
51 the bio-zeolite complex, that is to say, in addition to the ammonium removal capacity by microbial metabolism
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4 there is an added adsorption capacity of the zeolite through diffusion within the floc (Fernández et al. 2007;
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6 Montalvo et al. 2014a).

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9 In the anoxic stage there is a minimum concentration of ammonium that according to Al-Ghusain and Hao
10 (1995) represents the complete consumption of nitrate and beginning of anaerobiosis. In light of the fact that
11 there is no evidence of the accumulation of nitrate in the system, it is possible to infer that the nitrification and
12 denitrification rates are found to be in equilibrium in this stage of each reactor. The previous affirmation is valid
13 during the feeding and anoxic stages of each reactor in the 3 step-feed process, but is not in the aerobic stage in
14 the case of the step-feed 1 and 3, for which there are differences in the rates of denitrification and nitrification,
15 in both reactors, which cause a temporary accumulation of nitrate and nitrite in a soft manner in both cases
16 (Figure 10).
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26 During the aerobic stage, the behavior of both reactors was similar, an increase in ammonium concentrations
27 in both SND systems was observed due to that the $\text{NH}_4^+/\text{NH}_3$ equilibrium tends to go towards the formation of
28 ammonium.
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33 From a kinetics point of view, with respect to the organic matter, there exist a rapid soluble COD (CODs)
34 consumption during the feeding stage for each step-feed (3769 and 3742 mg CODs/(L·h) for R1 and R2,
35 respectively). This can be explained by the rapid penetration of CODs in the microbial flocs and/or by its storage
36 in form of slow biodegradability matter such as in the internal structure of polyhydroxybutyrate (PHB) (Chiu
37 et al. 2007; Zhang et al. 2009; Jia et al. 2011; Verma et al. 2013).
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44 According to Figure 11, it was observed an increase in organic material during the aerobic stage. This is
45 coherent with observations made by other authors (Zhang et al. 2009), which stated that during the aerobic
46 stages the storage sources of organic matter such as PHB, are utilized as a carbon source for SND.
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50 The remnants of ammonium existing in both reactors indicate that there exists a deficiency of available
51 organic matter for SND at high NLR values. It is important to indicate that even though in this study a C/N ratio
52 of 5 was used, it would be advisable to have used higher values.
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58 **4. Conclusions**

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4 The removal of nitrogen compounds and organic matter via simultaneous nitrification and denitrification was
5
6 achieved in sequential batch reactors using zeolite as microorganism support as well as with suspended biomass.
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8 It was evidenced, from the simultaneous coexistence of characteristic points in pH/ORP profiles of each aerobic
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10 and anoxic stage, that the major part of the operation consisted in simultaneous nitrification total-denitrification,
11
12 without the accumulation of NO_3^- and NO_2^- . The incorporation of zeolite in a SND-SBR system allowed for
13
14 achieving higher biomass growth rates in the reactor with immobilized biomass compared to the results found
15
16 in the reactor with suspended biomass, in addition, reducing the process start-up time. The kinetic analysis
17
18 showed that the incorporation of zeolite to a SND-SBR reactor improves the reaction rates by 11.31% when
19
20 compared to the reactor without zeolite. The temporary distribution of the feeding through step-feed, decreases
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22 the substrate inhibition events in an immobilized SND-SBR reactor as well as in a conventional reactor, even
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24 duplicating the tolerance for high NLR for the same operation time.
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30
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List of acronyms

| | |
|--|------|
| Ammonia-Oxidizing Bacteria | AOB |
| Biological Nitrogen Removal | BNR |
| Carbon/Nitrogen ratio | C/N |
| Cation-Exchange Capacity | CEC |
| Chemical Oxygen Demand | COD |
| Chemical Oxygen Demand Soluble | CODs |
| Dissolved Oxygen | DO |
| Free-Hydroxylamine | FH |
| Nitrite Oxidizing Bacteria | NOB |
| Nitrogen Loading Rate | NLR |
| Oxidation-Reduction Potential | ORP |
| PolyHydroxyButyrate | PHB |
| Reactor with Chilean Natural Zeolite | R2 |
| Reactor without Chilean Natural Zeolite | R1 |
| Sequential Batch Reactor | SBR |
| Simultaneous Nitrification-Denitrification | SND |
| Total Kjeldahl Nitrogen | TKN |
| Total Suspended Solids | TSS |
| Volatile Suspended Solids | VSS |
| Volumes per reactor Volume per minute | vvm. |

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Table 1.– Nitrifying and Denitrifying substrate components.

| Nitrifying Substrate | | Denitrifying Substrate | |
|---|---------------------|---------------------------------|---------------------|
| Compound | Concentration (g/L) | Compound | Concentration (g/L) |
| (NH ₄) ₂ SO ₄ | 33.533 | NaCH ₃ COO | 5.000 |
| MgSO ₄ ·7H ₂ O | 0.0365 | Peptone | 0.480 |
| K ₂ HPO ₄ | 0.247 | Yeast extract | 0.200 |
| KH ₂ PO ₄ | 0.193 | NaCO ₃ | 1.000 |
| | | K ₂ HPO ₄ | 7.000 |
| | | KH ₂ PO 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 |

Table 3.– Variation of pH in different scenarios of increasing NLR in the SND-SBR system with suspended biomass (R1) and in the reactor with biomass immobilized on zeolite (R2).

| Day | NLR (kg TKN/(m ³ day)) | pH | | Var-pH | |
|-----|--------------------------------------|---------------|---------------|--------|-------|
| | | R1 | R2 | R1 | R2 |
| 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 |

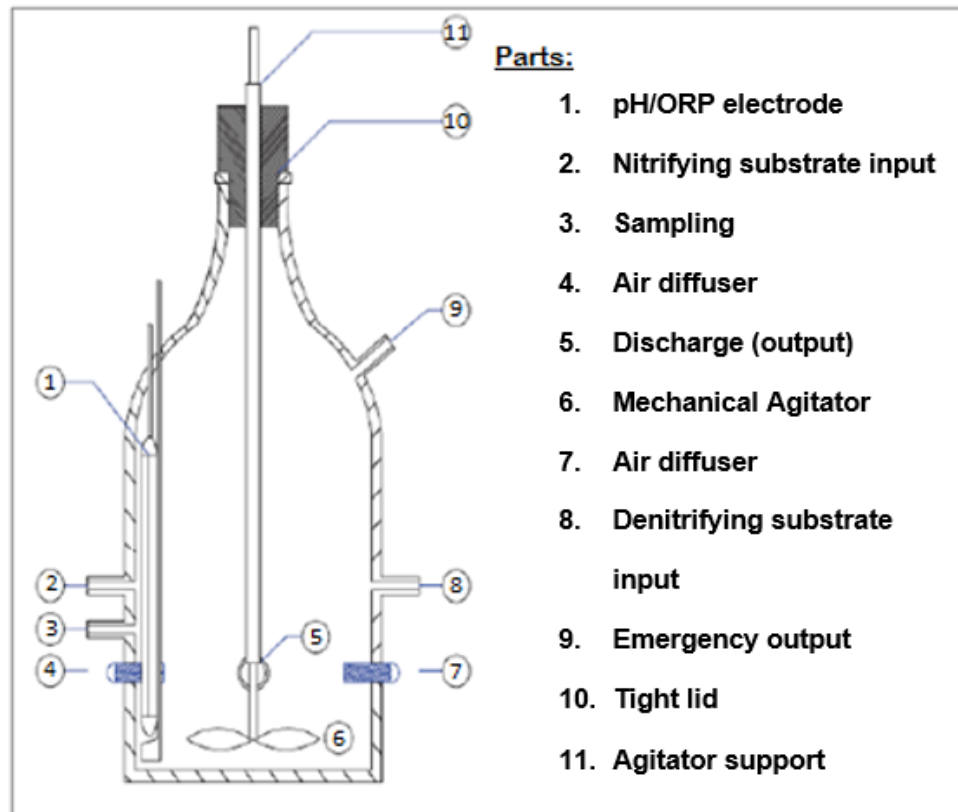


Figure 1 - SBR-SND reactor scheme

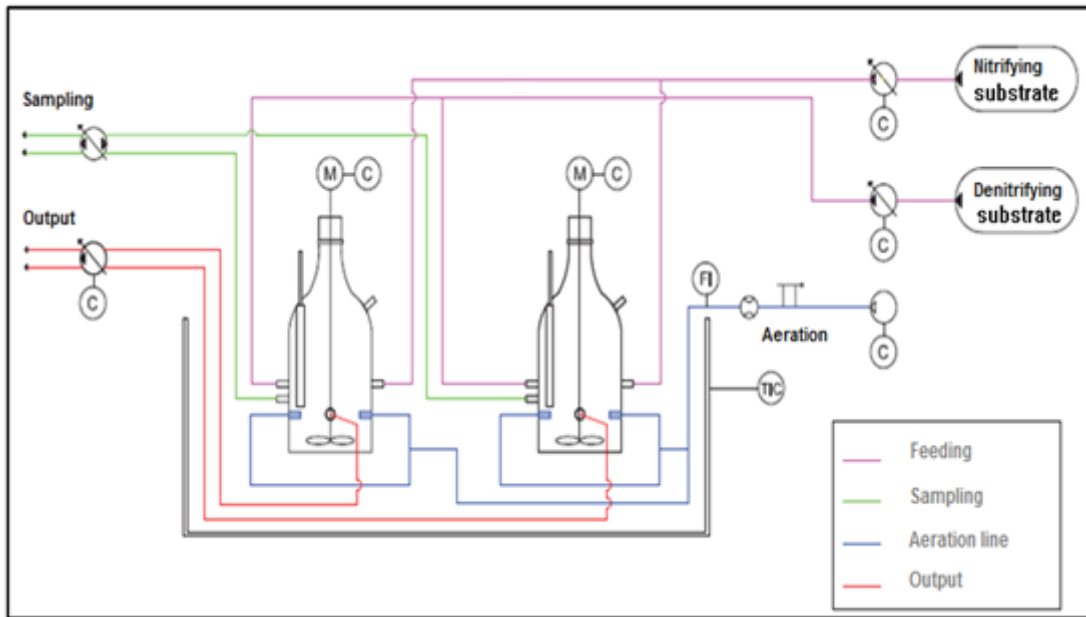


Figure 2. –SND-SBR reactors assembly scheme

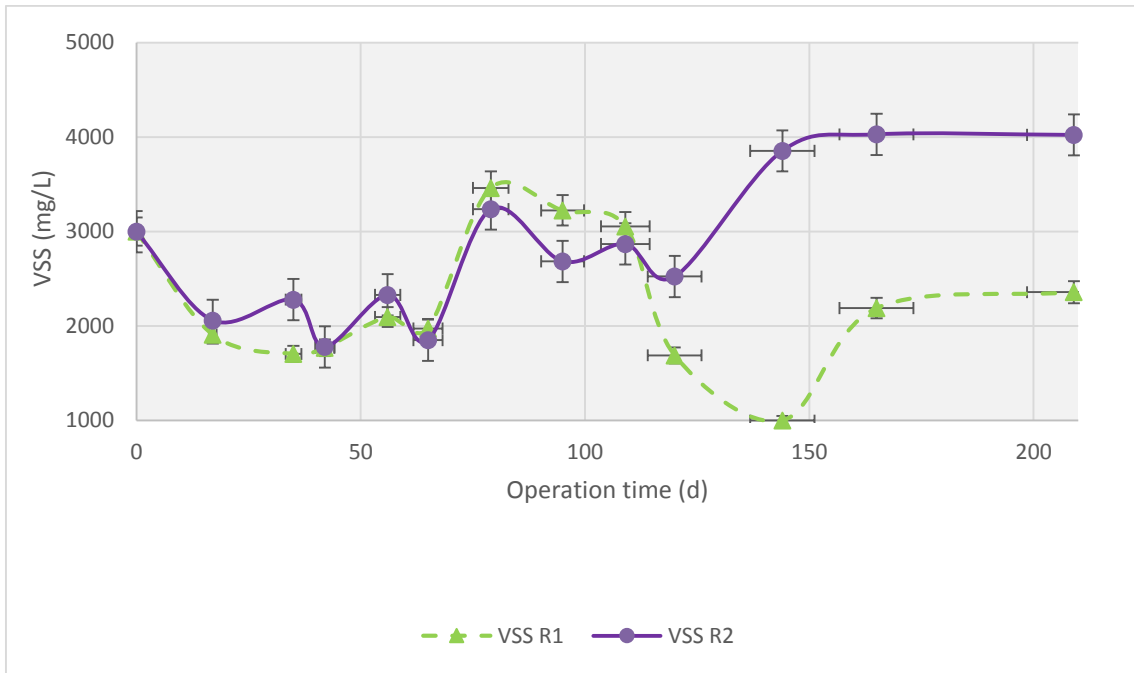


Figure 3. – Microbial growth behavior in the two SND-SBR systems, one with suspended biomass (R1) and another with biomass immobilized on zeolite (R2).

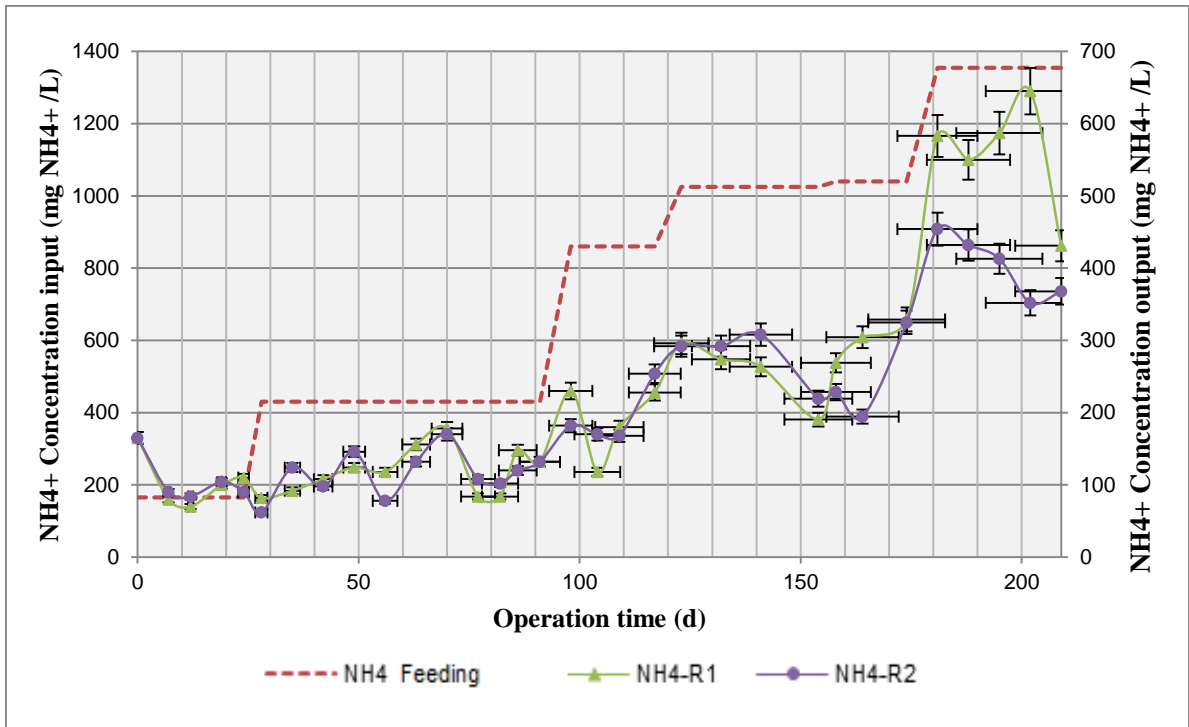


Figure 4. – Effluent NH₄⁺ concentration in the two SND-SBR systems, one with suspended biomass (R1) and another with biomass immobilized on zeolite (R2).

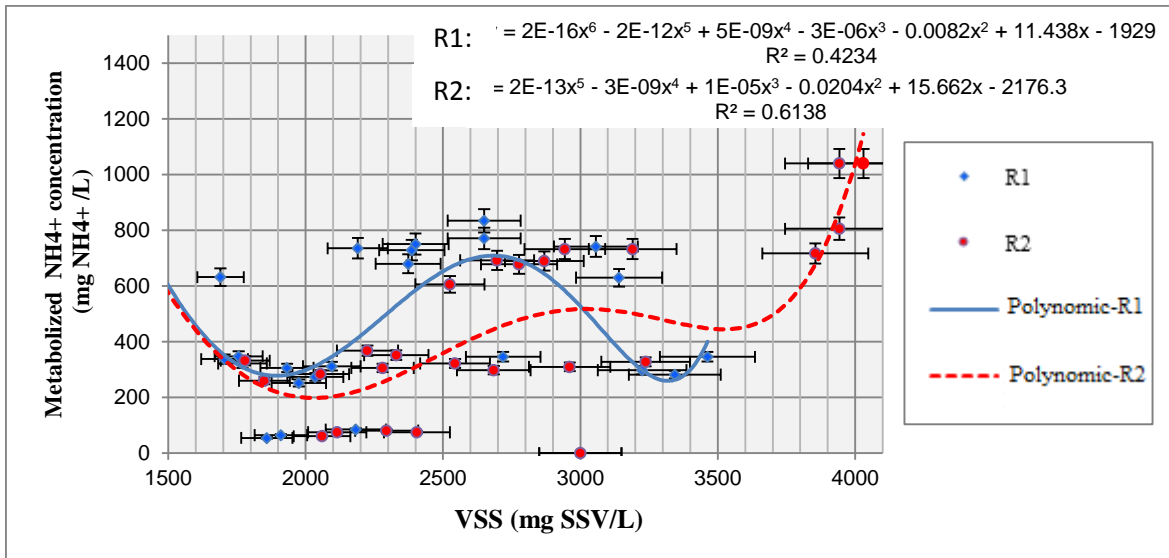


Figure 5.– Correlation between the metabolized ammonium and biomass concentration in the two SND-SBR systems, one with suspended biomass (R1) and another with biomass immobilized on zeolite (R2).

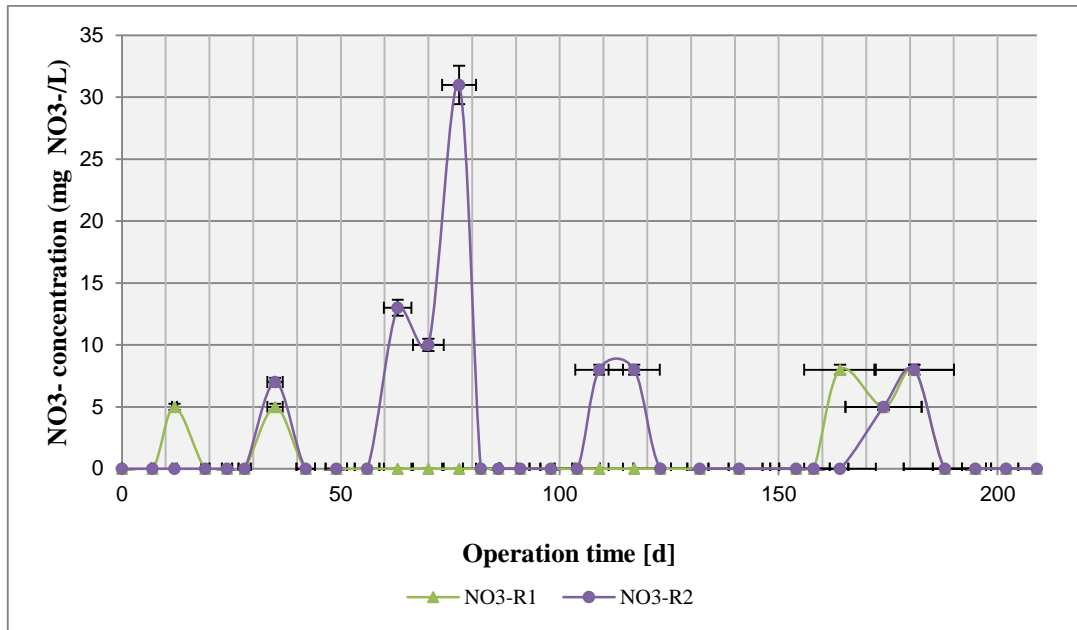


Figure 6.– Nitrate concentration in the two SND-SBR systems, one with suspended biomass (R1) and another with biomass immobilized on zeolite (R2).

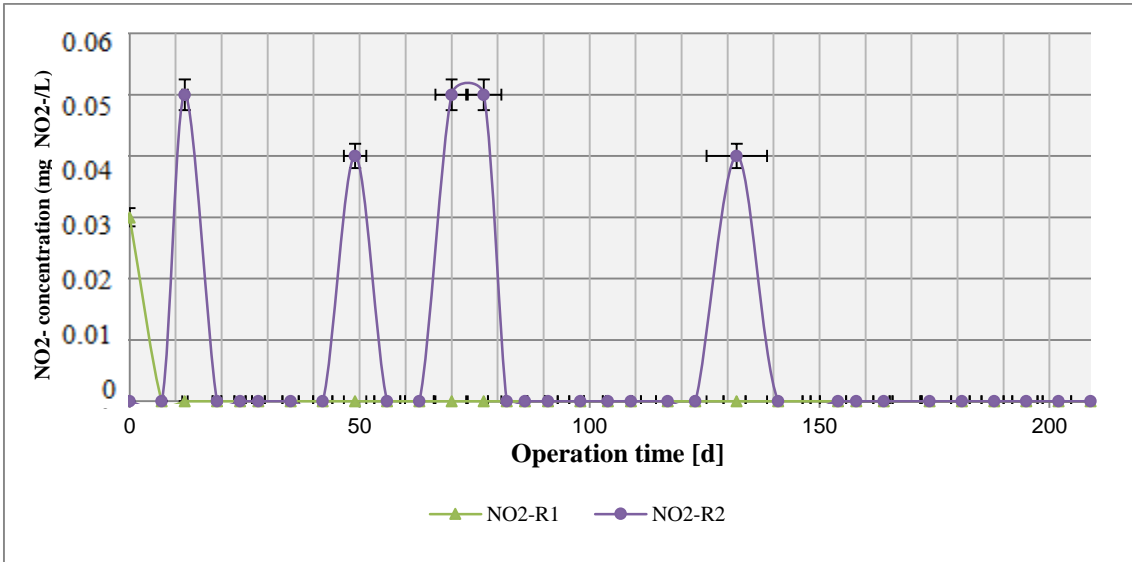


Figure 7.– Concentration of nitrite in in the two SND-SBR systems, one with suspended biomass (R1) and another with biomass immobilized on zeolite (R2).

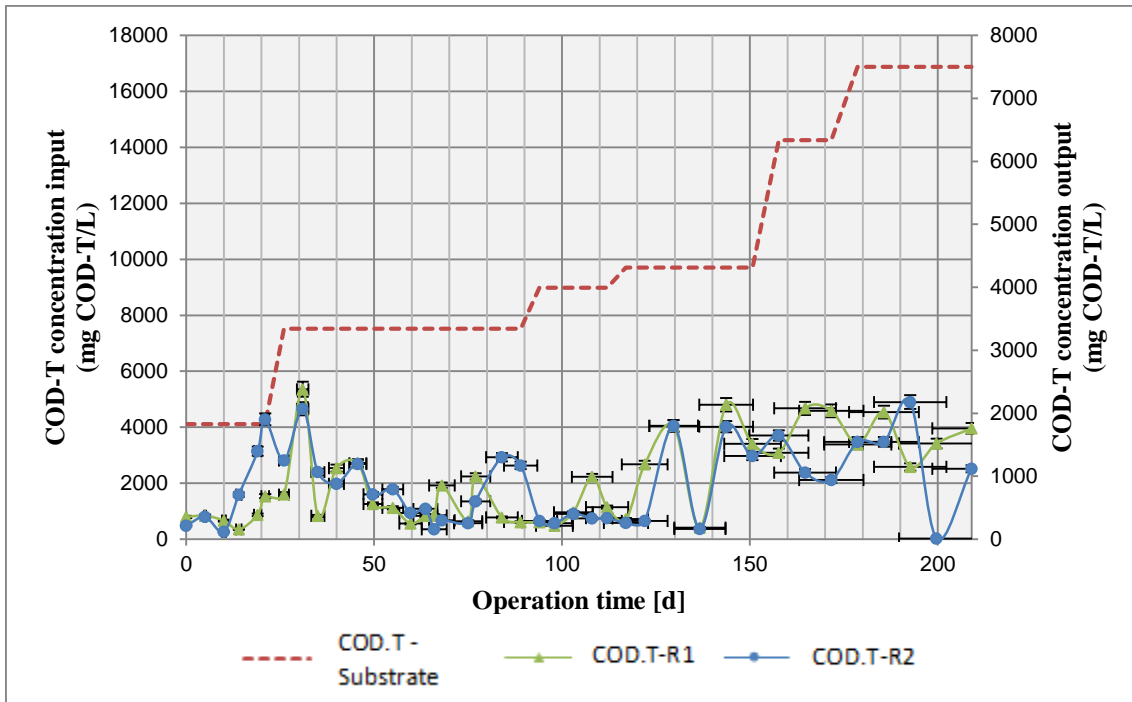
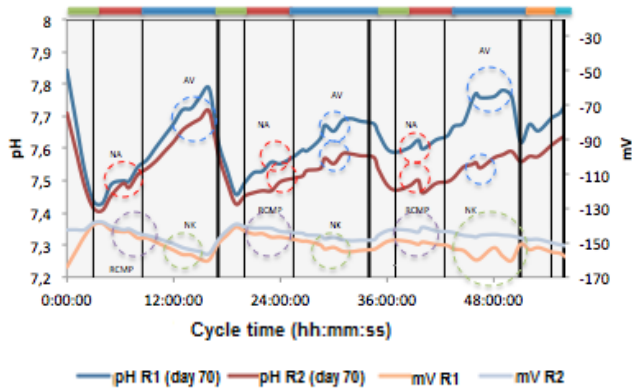
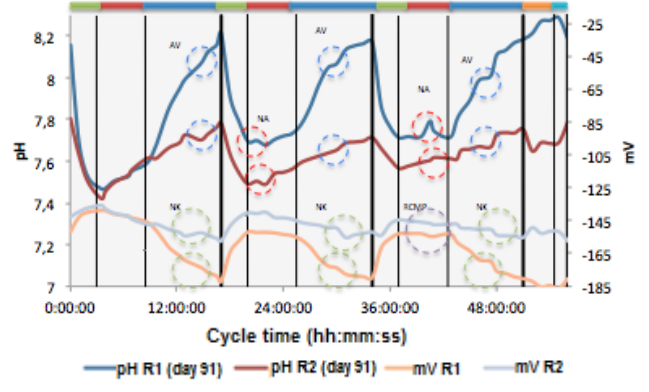


Figure 8.— Total COD concentration entering (C) and exiting in the two SND-SBR systems, one with suspended biomass (R1) and another with biomass immobilized on zeolite (R2).

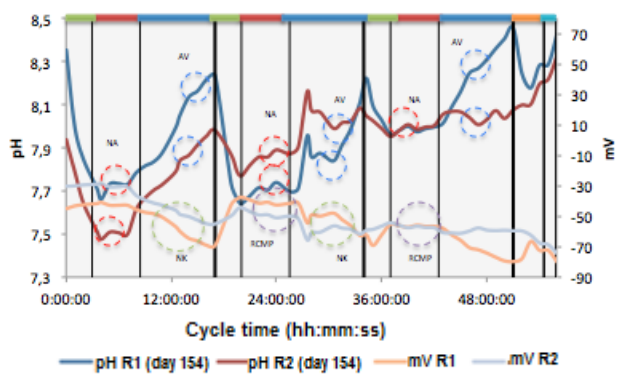
a) NLR = 0.042 (kg TKN/m³·d)



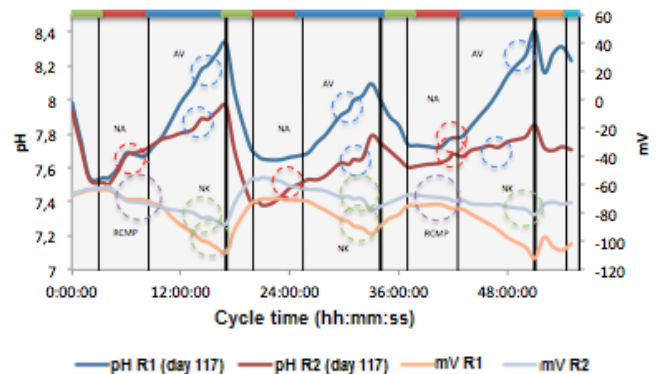
b) NLR = 0.042+ aeration (kg TKN/m³·d)



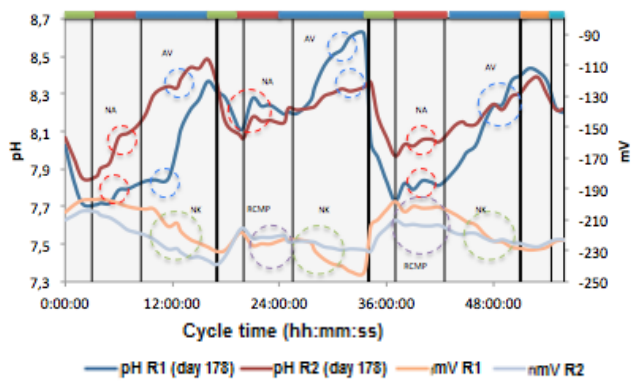
c) NLR = 0.053 (kg TKN/m³·d)



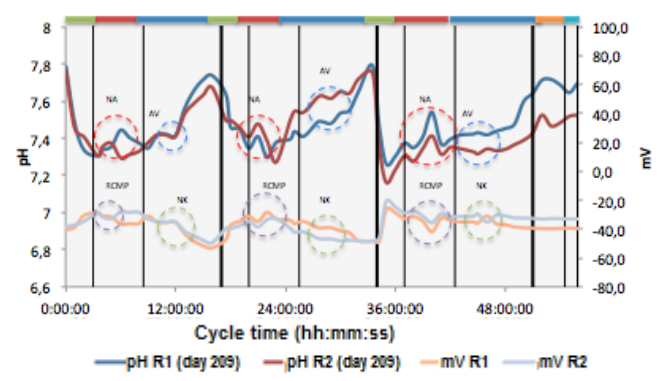
d) NLR = 0.069 (kg TKN/m³·d)



e) NLR = 0.089 (kg TKN/m³·d)



f) NLR = 0.113 (kg TKN/m³·d)



- : Nitrate apex (NA)
- : Ammonia valley (AV)
- : Residual Carbon Manipulation Point (RCMP)
- : Break nitrate (NK)

Figure 9.– pH/ORP profiles at different NLR in the two SND-SBR systems, one with suspended biomass (R1) and another with biomass immobilized on zeolite (R2).

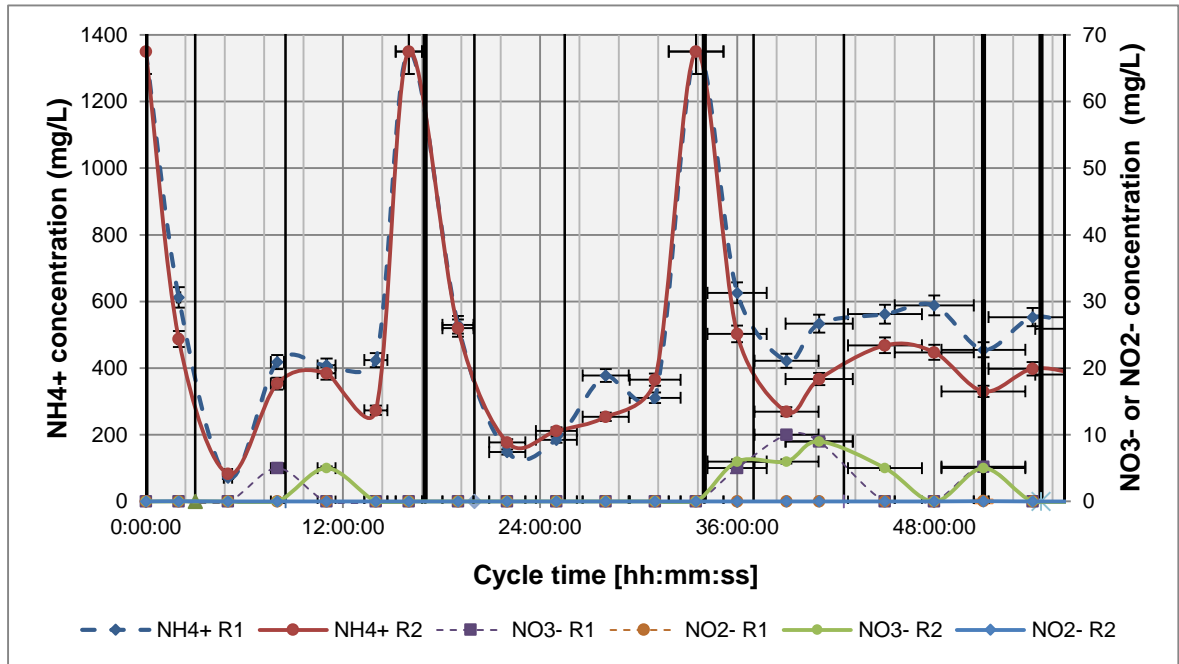


Figure 10.– Kinetics study of the ammonium, nitrate and nitrite removal for both SND-SBR reactors

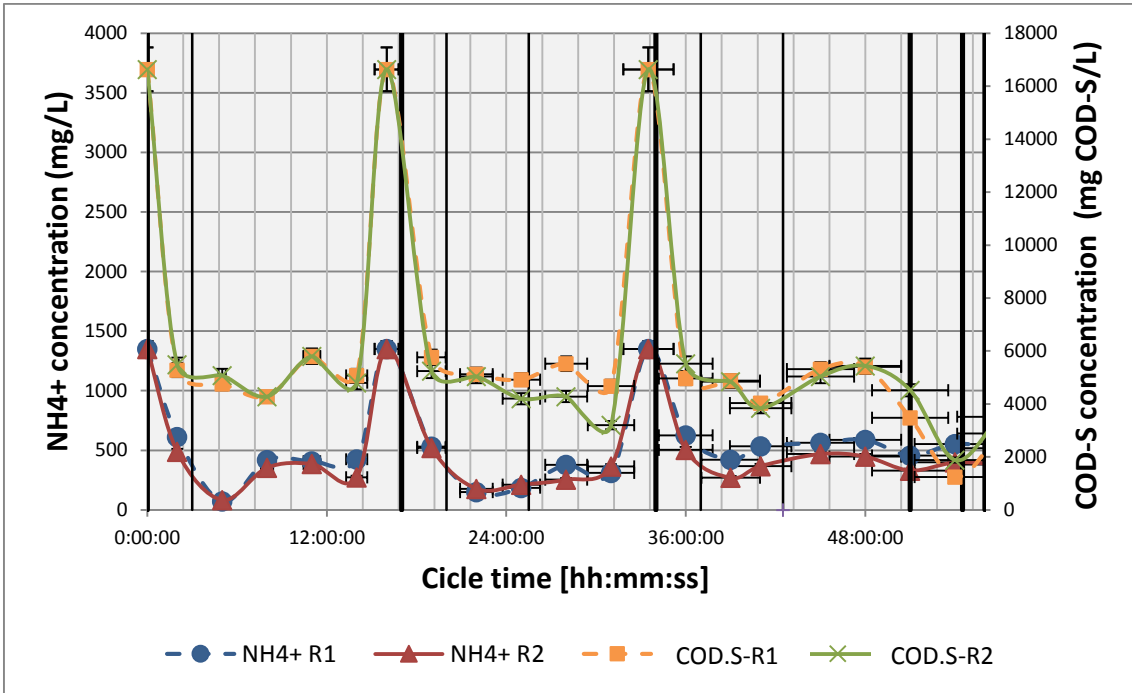


Figure 11.– Kinetic study of the simultaneous removal of nitrogen compounds and organic matter in both SND-SBR reactors.



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