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2 **IMPROVEMENT IN NITRIFICATION THROUGH THE USE OF NATURAL**  
3 **ZEOLITE: INFLUENCE OF THE BIOMASS CONCENTRATION AND INOCULUM**  
4 **SOURCE**

5 **Silvio J. Montalvo<sup>1</sup>, Lorna E. Guerrero<sup>2</sup>, Rafael Borja<sup>3\*</sup>**

6  
7 <sup>1</sup> Departamento de Ingeniería Química, Universidad de Santiago de Chile, Avenida Libertador  
8 Bernardo O'Higgins 3363 – Santiago de Chile, Chile.

9 <sup>2</sup> Departamento de Ingeniería Química y Ambiental, Universidad Técnica Federico Santa María,  
10 Valparaíso, Chile.

11 <sup>3</sup> Instituto de la Grasa (C.S.I.C.), Avda. Padre García Tejero, 4. 41012-Sevilla, Spain.

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13 \* Corresponding author: **Dr. Rafael Borja, PhD, Research Scientist, Department of “Industrial**  
14 **Processes and Environment”, Instituto de la Grasa (C.S.I.C.), Avda. Padre García Tejero, 4.**  
15 **41012-Sevilla, Spain (Telephone: +34 95 4692516, Ext. 152; Fax: +34 95 4691262; E-mail:**  
16 **[rborja@cica.es](mailto:rborja@cica.es)).**

17  
18 **ABSTRACT**

19  
20 A batch nitrification process was studied using synthetic wastewater as substrate and  
21 Chilean natural zeolite as biomass carrier at ambient temperatures (20 °C). Three groups of  
22 experiments were carried out: a first experimental set (I) with and without added zeolite using  
23 initial biomass concentrations of 1,000 and 2,000 mg VSS/L; a second set of experiments (II)  
24 with added zeolite and at the same initial biomass concentrations. In these two experimental  
25 sets, biomass from an activated sludge process located in an urban wastewater treatment plant  
26 (WWTP) at *La Farfana*, Santiago de Chile, was used as inoculum (1). Finally, a third set of  
27 experiments (III) was carried out with zeolite at an initial biomass concentration of 1,000 mg  
28 VSS/L using an inoculum derived from an activated sludge process treating wastewater from a  
29 paper mill (inoculum 2). Nitrifying biomass concentration values in the range of 13,000-18,800  
30 mg VSS/L were achieved when initial biomass concentrations varied between 1000-2000 mg  
31 VSS/L. Inoculum (1) generated higher biomass concentrations than inoculum (2). Ammonium  
32 N removals higher than 70% were obtained in experimental sets II and III when zeolite was  
33 used. For both initial biomass concentrations tested, an exponential biomass growth was  
34 observed up to the second day of operation, and a slight decrease was evident afterwards,  
35 achieving stationary values after 10-12 days of operation. The third experimental set (III)  
36 revealed that the highest N consumption took place between days 11 and 16 of digestion.

37

38 **Key words:** batch mode, inoculum **type**, nitrifying biomass carrier, zeolite.

39

## 40 **INTRODUCTION**

41

42 Because of its increased use as an artificial addition, one of the most problematic and well-  
43 known pollutants is nitrogen in its diverse forms, a phenomenon already considered to be a new  
44 environmental global change of unforeseeable consequences (Tortosa *et al.*, 2011). The main  
45 sources of nitrogen are chemical fertilizers (nitrate, ammonia and urea), agricultural and animal  
46 wastes (nitrite and ammonia) and industrial liquid effluents (nitrite, nitrate and ammonia)  
47 (Nemerow, 1991). In the literature, different negative effects have been described thoroughly,  
48 among which eutrophication (fertilization in excess) should be highlighted, as it causes the  
49 excessive growth of algae and aquatic plants (Guo *et al.*, 2010; Zaman, 2010). In 2008, it  
50 affected 54% of Asian lakes, 53% of European lakes, 48% of North American lakes, 41% of  
51 South American and 28% of African lakes.

52

53 In addition, the direct effects of nitrogen compounds on the aquatic systems, indirect  
54 effects resulting from the nitrogen gases that are generated from the liquid wastes, such as NO<sub>x</sub>  
55 substances, also contribute either to the greenhouse effect or to acid rain (Wang *et al.*, 2008).  
56 There are two main methods for removing nitrogen from wastewaters: physico-chemical and  
57 biological processes (Guo *et al.*, 2008). The physico-chemical processes such as air and stream  
58 stripping are sometimes used for the control of nitrogen in strong nitrogen wastewaters.  
59 However, from environmental and economic viewpoints, it would be more interesting to use  
60 biological nitrogen removal for treating high ammonium strength wastewaters. Its removal  
61 process has been widely adopted in preference to the physico-chemical processes because of its  
62 higher effectiveness and relatively low cost, especially in the field of urban wastewater  
63 treatment (Guo *et al.*, 2008). Among the biological methods, the nitrification-denitrification  
64 system is the most widely used alternative (Leu *et al.*, 2010; Liu *et al.*, 2007; Pagga *et al.*,  
65 2006).

66

67 The efficiency of the biological processes, such as nitrification-denitrification, can be  
68 improved by increasing the microorganism retention time, which is usually independent from  
69 the wastewater retention time. In most cases this is achieved by the immobilization of  
70 microorganisms (Yan and Hu, 2009). Biofilm reactors are especially useful when slow growing  
71 organisms like nitrifiers have to be kept in a wastewater treatment process. Due to the efficient  
72 biomass retention, long sludge ages and more compact reactors can easily be achieved  
(Hooshyari *et al.*, 2009).

73

74 However, a restrictive factor for the suitability of this technological alternative (nitrification  
– denitrification) is that the microorganism support medium or bacterial carrier have to fulfill  
the following main characteristics: its surface should favour the adherence and colonization of

75 the microorganisms, it should be physically and chemically resistant and relatively inert (Liu *et*  
76 *al.*, 2007; Rostron *et al.*, 2001). Several previous research works have demonstrated that natural  
77 zeolites meet these characteristics (Fernández *et al.*, 2007; Nikolaeva *et al.*, 2009). However,  
78 the specific behaviour of the zeolites in each process is different depending on the type of  
79 microorganisms involved in each case. As a consequence, the need to assess the nitrification  
80 process using natural zeolite as microorganism carrier for this process (Liu *et al.*, 2007; Rostron  
81 *et al.*, 2001) has emerged.

82 Natural zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth  
83 cations, consisting of three-dimensional frameworks of  $\text{SiO}_4^{4-}$  and  $\text{AlO}_4^{5-}$  tetrahedra linked  
84 through shared oxygen atoms (Tashauoei *et al.*, 2010). They are porous materials characterized  
85 by their ability to 1) lose and gain water reversibly, 2) adsorb molecules of appropriate cross-  
86 sectional diameter (adsorption property or acting as molecule sieves) and 3) exchange their  
87 constituent cations without a major change in their structure (ion-exchange property). The  
88 exploitation of these properties underlies the use of zeolites in a wide range of industrial,  
89 agricultural and contamination prevention applications (Milán *et al.*, 2001a, b and c; Tashauoei  
90 *et al.*, 2010). The structure and physical properties of natural zeolite [channel and pore cavities,  
91 minimum diameter of pores in the range of 3 to 10 Angstroms, average surface area of 24.9  
92  $\text{m}^2/\text{g}$ , low bulk density, high exchange (CEC) and adsorption capacities] make it ideal for use in  
93 biological purification wastewater processes (Carretero and Pozo, 2009). Consequently, the use  
94 of natural zeolite in different wastewater biological treatment processes has increased  
95 significantly over the past few years.

96 Therefore, taking previous works into account, the aim of this paper was to make a  
97 comparative study of the nitrification process with and without natural zeolites as  
98 microorganism immobilization support or carrier while simultaneously assessing the influence  
99 of the initial biomass concentration and the inoculum source. All this research was carried out in  
100 the laboratories of the Department of Chemical Engineering of the University of Santiago de  
101 Chile and of the Department of Chemical and Environmental Engineering of the “Federico  
102 Santa María” Technical University of Valparaiso (Chile). These experiments were made within  
103 the period November 2010-July 2011.

## 104 105 106 **MATERIALS AND METHODS**

### 107 108 *Experimental design*

109 Three different sets or groups of experiments were carried out:

- 110 I) A first set of experiments comparing a batch nitrification process with and without  
111 added zeolite, using two different initial biomass concentrations (1,000 and 2,000

112 mg VSS/L). Biomass from an activated sludge process belonging to an urban  
113 wastewater treatment plant (UWTP) called *La Farfana* located in Santiago de Chile  
114 (Chile), was used as inoculum in the reactors. The evolution of biomass  
115 concentration over time was assessed.

116 II) A second set of batch nitrification experiments with added zeolite and initial  
117 biomass concentrations of 1,000 and 2,000 mg VSS/L. The same inoculum was  
118 used. The bacterial growth, the decrease in ammonium concentration and formation  
119 of nitrate over time were evaluated in this case.

120 III) A third set of batch nitrification experiments with added zeolite and an initial  
121 biomass concentration of 1,000 mg VSS/L. An inoculum from an activated sludge  
122 process treating wastewater from a paper mill was used in this case. The bacterial  
123 growth, variation of pH, decrease in ammonium concentration and formation of  
124 nitrate over time were followed up in this third set of experiments.

125

#### 126 ***Zeolite used***

127 Chilean natural zeolite supplied by “Minera Formas”, Chile (named “ZeoClean R”) was  
128 used in the experiments. Table 1 shows the main chemical composition of this zeolite. The  
129 phase composition (% w/w) of the zeolite was: 35% Clinoptilolite, 15% Mordenite, 30%  
130 Montmorillonite, and 20% others (calcite, feldespate and quartz).

131

#### 132 **Chemical analyses**

133 Chemical oxygen demand (COD), solids and total phosphorus analyses were carried out  
134 according to Standard Methods for the Examination of Waters and Wastewaters (APHA, 1995).  
135 Nitrate, ammonium nitrogen, pH and dissolved oxygen (DO) were determined by selective  
136 electrodes.

137

#### 138 **Experimental procedure**

##### 139 ***Set of experiments I and II***

140 Experimental sets I and II were carried out at ambient temperature (an average of 20 °C)  
141 in glass reactors of 200 mL working volume, in which synthetic wastewater was added. Table 2  
142 shows the composition and main characteristics of the synthetic wastewater used as substrate in  
143 these experiments. Aerobic biomass from a full-scale activated sludge process located in the  
144 Urban Wastewater Treatment Plant (UWTP) at *La Farfana* was used as inoculum in both  
145 groups of experiments. The characteristics of this biomass were: total suspended solids (TSS),  
146 8,950 mg/L; volatile suspended solids (VSS), 7,300 mg/L; pH, 7.2; and sludge volume index  
147 (SVI), 150 mL/g. Air was supplied to these reactors with a flow rate of 20 L/min. The air was  
148 injected through the bottom of the reactor using a porous ceramic diffuser. In addition, each

149 reactor had a detachable grille located in the upper part to avoid microorganisms being lost in  
150 the reactor effluents as a consequence of the breaking of the air bubbles in the interface gas-  
151 liquid.

152 At the beginning of the experiments and every 2 days thereafter, two reactors were selected  
153 and sampled with the aim of obtaining duplicate results. Once these two reactors were finished  
154 with, 30 mL of synthetic wastewater was added to the rest of reactors to balance the evaporation  
155 losses.

156 During the first set of experiments (I) the behaviour of the nitrification process with and  
157 without added zeolite was evaluated in batch mode and in parallel by using initial biomass  
158 concentrations of 1,000 and 2,000 mg VSS/L. A total of 32 reactors was used: 16 with zeolite  
159 and 16 without zeolite. During this first set of experiments, the variation of biomass  
160 concentration over time was measured.

161 During the second set of experiments (II) the batch nitrification process was evaluated using  
162 only reactors with added zeolite and two different initial biomass concentrations (1,000 and  
163 2,000 mg VSS/L). 32 reactors were used for each of these concentrations. For the reactors with  
164 an initial biomass concentration of 1,000 mg VSS/L, 6 mL of inoculum were added, while for  
165 those with 2,000 mg VSS/L, 12 mL of inoculum were added, completing the remaining volume  
166 with synthetic wastewater in both cases. The variation of biomass concentration, ammonium  
167 removal and nitrate formation over time were assessed during this second set of experiments.

168 In all cases the amount of zeolite added (with the characteristics shown in Table 1)  
169 corresponded to a ratio of 40 mg VSS/g zeolite. The duration of the set of experiments I and II  
170 ranged from 8 to 15 days.

171

### 172 ***Set of experiments III***

173 This set of experiments was carried out in triplicate at ambient or room temperature  
174 (average of 20 °C) using reactors of 350 mL working volume with the same synthetic substrate  
175 as described in Table 2.

176 This group of batch nitrification experiments was carried out with added zeolite and an  
177 initial biomass concentration of 1,000 mg VSS/L. An inoculum from an activated sludge  
178 process treating wastewater from a paper manufacture factory was used in this case. The  
179 characteristics of this biomass were: TSS, 7,500 mg/L; VSS, 6,400 mg/L; pH, 7.1; and SVI, 300  
180 mL/g. The bacterial growth, pH variation, decrease in ammonium concentration and formation  
181 of nitrate over time were assessed in this third set of experiments. The duration of the three  
182 experiments carried out in parallel was 16 days.

183

184

## 185 **RESULTS AND DISCUSSION**

186

### 187 ***Set of experiments I***

#### 188 *Experiments with an initial biomass concentration of 1,000 mg VSS/L*

189 Figures 1 and 2 show the variation of the biomass concentration (mg VSS/L) over time for  
190 the experiments with and without added zeolite, respectively. As can be seen in the first case,  
191 there was an increase in the biomass concentration until a maximum value of 18,500 mg VSS/L  
192 after two days of digestion time was achieved, which afterwards decreased slightly to a stable  
193 value of 15,300 mg VSS/L on the 15<sup>th</sup> day of operation. By contrast, in the reactors without  
194 zeolite, there was a continuous decrease in the biomass concentration over time achieving a  
195 minimum concentration of 600 mg/L after 9 days of operation. This behavior can be explained  
196 not only by the fact that zeolite acted as a microorganism carrier but also because the reactors  
197 without bacterial support are more prone to ammonia volatility (the main substrate of the  
198 process) and to the destabilization caused by the high flow of air used in the experiments.

199 Similar initial biomass concentrations (1,700 mg VSS/L) were used in experiments of  
200 partial nitrification carried out in sequencing batch reactors (SBR) under aeration rates in the  
201 range of 0.1-1.6 L/min (Wu *et al.*, 2009).

202

#### 203 *Experiments with an initial biomass concentration of 2,000 mg VSS/L*

204 Figures 3 and 4 illustrate the evolution of the biomass concentration (mg VSS/L) over time  
205 for the experiments with and without added zeolite respectively, when the initial biomass  
206 concentration was 2,000 mg VSS/L. As can be observed when zeolite was used as a biomass  
207 carrier, there was an increase in the biomass during the first two days of operation achieving a  
208 maximum value of 18,900 mg VSS/L, while decreasing slightly and stabilizing after the 4<sup>th</sup> day  
209 of operation, at a value of 15,000 mg VSS/L. On the other hand, for the reactors without zeolite  
210 a decrease in the VSS concentration from 2,000 to 650 mg/L was detected after 8 days of  
211 operation time.

212 The same biomass evolution as that observed in the present work with added zeolite was  
213 also detected in an activated sludge system with fireclay (excess sludge from ceramic and tile  
214 manufacturing plants) as the biomass carrier operating in batch mode with an initial biomass  
215 concentration of 2,400 mg/L (Tilaki, 2011). When the amount of fireclay was increased (to  
216 values higher than 2,250 mg/L) the total biomass concentration was also increased.

217

### 218 ***Set of experiments II***

219 This set of experiments was carried out with initial biomass concentrations of 1,000 and  
220 2,000 mg VSS/L, the initial ammonium concentration in the synthetic wastewater and inoculum  
221 at 148 and 73 ppm, respectively. The inoculum was derived from an activated sludge system  
222 installed in the Urban WWTP at *La Farfana* (Santiago de Chile, Chile).

223

224 *Experiments with an initial biomass concentration of 1,000 mg VSS/L*

225 Figure 5 shows the variation of the biomass concentration over time. As can be seen, an  
226 exponential growth was observed during the first two days of operation achieving a maximum  
227 value of 18,550 mg/L. From day 4 onward, the microorganism concentration remained virtually  
228 constant with a value of about 15,000 mg VSS/L. Therefore, the addition of 30 mL of synthetic  
229 wastewater to the reactors every two days to compensate for the evaporation losses meant that  
230 the biomass concentration remained constant over time.

231 **On the other hand**, no lag phase was observed because the inoculum used was derived from  
232 a previous aerobic process and, therefore, an adaptation or acclimation period was not  
233 necessary. The exponential phase or step was clearly evident during the first two days of  
234 operation, a time lapse for which there was no nutrient restriction reaching a maximum VSS  
235 concentration of 18,550 mg/L. From day 2 onward, a stationary phase was started due to the  
236 depletion of some of the nutrients, although the addition of the above-mentioned volume of  
237 fresh wastewater every two days determined that the dead stage of the microorganisms cannot  
238 be clearly observed.

239 Finally, **Figure 6** shows the variation of the ammonium concentration over time. As can be  
240 seen, a gradual decrease in the ammonium concentration over time was observed up to a  
241 constant value after the 12<sup>th</sup> day of digestion. Simultaneously a gradual increase in nitrate  
242 concentration was observed over time (**Figure 7**), the nitrate being the final product of the  
243 nitrification process. It is worth noting that the ammonium that did not transform into nitrate can  
244 be found as nitrite although this is unlikely as a consequence of the high DO concentrations  
245 (2.8-4.9 mg/L) measured in the reactors. In addition, part of the initial ammonium could have  
246 been evaporated due to the high aeration levels and another part could have been adhered to the  
247 zeolite or added to the microorganism cells present in the medium.

248

249 *Experiments with an initial biomass concentration of 2,000 mg VSS/L*

250 The variation of the biomass concentration over time is illustrated in **Figure 8** and showed  
251 similar behaviour to that observed when the initial biomass concentration was 1,000 mg VSS/L.  
252 An exponential growth was observed up to the 2<sup>nd</sup> day of digestion, in which a maximum  
253 concentration of 18,800 mg VSS/L was achieved. From the 4<sup>th</sup> day onward, the increase in the  
254 VSS concentration tended to be constant despite the previously mentioned synthetic wastewater  
255 being added every 2 days.

256 **In addition**, there was no lag phase because the inoculum used came from an urban WWTP  
257 and no acclimation stage was necessary. The stationary stage occurred after the 4<sup>th</sup> day of  
258 operation and was reached despite the reactors being fed with 30 mL of synthetic wastewater  
259 every 2 days. As a consequence of this batch feed system, the microorganism dead phase was

260 not observed. Similar initial biomass concentrations (1,750 mg VSS/L) also behaved well in  
261 nitrifying SBR systems operating in continuous mode at an HRT of 1 day treating reject water  
262 (Pérez et al., 2007).

263 A gradual decrease in the ammonium concentration over time was observed until a  
264 relatively constant value after the 10<sup>th</sup> of digestion was reached (Figure 9). The maximum  
265 reduction of ammonium in the liquid medium was approximately 70%. Figure 10 confirms that  
266 nitrate was obtained as a final product of the process. Effective nitrification was also reported in  
267 SBR systems treating reject water (supernatant of an anaerobic sludge digestion) with initial  
268 biomass concentrations of 3,500 mg/L and initial ammonium concentrations of up to 1,200  
269 mg/L (Galí et al., 2006).

270 Therefore, it can be concluded that the inoculum from an Urban WWTP was very effective  
271 in the batch nitrification process described in the present work. Similar inoculum sources were  
272 shown to be interesting and efficient in other nitrification processes reported in the literature  
273 using batch reactors treating synthetic and sludge liquors mixed with wastewater from diesel  
274 production (Canto et al., 2008; Malá and Maly, 2010).

275

### 276 ***Set of experiments III***

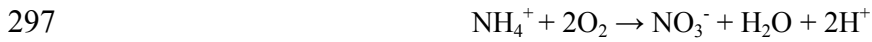
277 For this group of experiments, the assays were carried out in triplicate with the aim of  
278 obtaining higher representative results. This group of batch nitrification experiments was carried  
279 out using zeolite and an initial biomass concentration of 1,000 mg VSS/L. An inoculum from an  
280 activated sludge process treating wastewater from a paper factory was used in this case.

281 Given that the biomass concentration is directly related to the ammonium concentration, the  
282 evolution of the biomass over time was assessed during the three assays made within this  
283 experimental set. Table 3 shows the variation of the average VSS concentration for these three  
284 assays over time. As can be seen, a considerable increase in the biomass concentration with  
285 operation time was observed, achieving maximum VSS concentrations higher than 13,000 mg/L  
286 on the 12<sup>th</sup> day of operation. It is worth considering this when a full-scale nitrification process is  
287 started. Once the maximum biomass concentration value was reached, a slight decrease was  
288 observed. This may be due to the presence of insufficient amounts of substrate available for the  
289 microorganisms at these high biomass concentration values.

290 A rapid growth of nitrifying bacteria also took place after 12 days of operation during the  
291 nitrification process of poultry slaughterhouse wastewater in a lab-scale aerobic fixed film  
292 reactor (Del Pozo et al., 2004). However, lower biomass concentrations (5.45 g VSS/L) were  
293 achieved in an aerobic fixed-bed bioreactor operating in continuous mode at an HRT of 3.5 h  
294 using an acclimated municipal biosludge and 4-nitroaniline as carbon sources (Saupe, 1999).

295 The pH has a tendency to decrease due to the fact that the conversion of  $\text{NH}_4^+$  to  $\text{NO}_3^-$   
296 involves the transformation of an alkaline ion into an acid ion as follows:





298 **Table 3** also shows the variation of the **average** pH with the digestion time. As can be  
299 observed, the lower pH values were found between days 10 and 12, which is when the  
300 maximum amount of biomass was produced.

301 **Table 4** summarizes the evolution of ammonium and nitrate concentrations (average values  
302 of the three experimental runs and expressed in molar concentration) with operation time. **It can**  
303 **be observed a slight increase in the  $\text{NH}_4^+$  concentration with time throughout the 16 days of the**  
304 **assay. By contrast, the  $\text{NO}_3^-$  concentration increased slightly during the first 8 days, showing a**  
305 **considerable increase between the 10<sup>th</sup> and 12<sup>th</sup> day, for which a maximum concentration of 0.02**  
306 **(molar) was achieved.**

307 A previous nitrification study carried out with a special biomass carrier made of a mixture  
308 of zeolite and pellets of sodium alginate (1-2 mm diameter), revealed that the physical air-  
309 stripping effect was stronger than both chemical ion exchange and biological nitrification effects  
310 occurring in the system for initial ammonium concentration levels of 10-20 mg N/L (Yan,  
311 1997).

312 When comparing the biomass production with this inoculum and with the previously  
313 studied inoculum derived from an activated sludge process located in an urban WWTP (set of  
314 experiments I and II) by using the same initial biomass concentrations (1,000 mg VSS/L), it can  
315 be concluded that lower maximum biomass generation (13,000 mg VSS/L) was obtained with  
316 the sludge derived from the activated sludge process treating wastewater from a paper factory as  
317 compared with the first biomass used (18,800 mg VSS/L).

318 Nitrogen consumption was calculated from the measurements made during the different  
319 time periods taking into account that 30 mL of synthetic wastewater containing ammonium  
320 were added every two days. The nitrogenous chemical species that were considered for this  
321 analysis were: ammonia (liquid), ammonia (gas),  $\text{NO}_3^-$ , all of them measured with selective  
322 electrodes,  $\text{N}_{\text{biomass}}$  measured in the biomass adhered to zeolite and  $\text{N}_{\text{zeolite}}$  calculated from the  
323 ionic exchange capacity of the zeolite and data provided by the zeolite supplier (ZeoClean R).  
324 The results **obtained in this N balance** were gathered together in three time intervals: from 1 to 6  
325 days (**period 1**); from 7 to 10 days (**period 2**); and from 11 to 16 days (**period 3**) for a better  
326 analysis. **The average N consumption of the three experimental runs carried out within the set of**  
327 **experiments III were found to be 14%, 23% and 46% for the time periods 1, 2 and 3,**  
328 **respectively, amounting the N losses an average value of 17%.**

329 **Therefore**, the higher N consumption took place between days 11 and 16, which coincided  
330 with the maximum biomass concentration generated and maximum ammonium removal. As  
331 expected, N consumption is directly related to the amount of biomass generated. Of 100% of N  
332 added, an average of **46%** was consumed in the time interval between 11-16 days. It was  
333 noteworthy that the loss of nitrogen was high (average of **17%**), which was due to the

334 experimental conditions used with a high air flow, which determined DO concentration values  
335 in the range of 4.7-6.2 ppm contributing to a high level of ammonia stripping.

336 It has also been reported in another batch nitrification process with zeolite (with the dual  
337 purpose of ion exchanger and physical carrier for nitrifying bacteria) that nitrite and oxygen  
338 concentrations were determined as the major parameters responsible for the formation of  
339 gaseous N ( $N_2$  and  $N_2O$ ) and, therefore, for nitrogen losses (Green *et al.*, 2002).

340

341

## 342 **CONCLUSIONS**

343 It can be concluded from these studies that the use of natural zeolite as a nitrifying  
344 microorganisms carrier offers clear advantages over nitrification systems without added zeolite.  
345 Despite using a batch feed system rather than a continuous one, high ammonium concentration  
346 removals were obtained (higher than 70%). The growth of nitrifying biomass achieved high  
347 values ranging between 13,000 and 18,800 mg VSS/L starting from inocula with 1,000-2,000  
348 mg VSS/L. The two inocula assayed were found to be very effective, generating higher biomass  
349 concentrations from the sludge derived from an activated sludge process located in an Urban  
350 WWTP. An increase in the VSS concentration brought about a decrease in the ammonium  
351 concentration and an increase in the nitrate concentration, which is also a consequence of the  
352 nitrogenous biomass formation.

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354

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**Table 1:** Composition and main features of Chilean natural zeolite (Clinoptilolite type) used in the three sets of experiments carried out.\*

Component	Composition (%)
SiO <sub>2</sub>	67.00
Al <sub>2</sub> O <sub>3</sub>	13.01
Fe <sub>2</sub> O <sub>3</sub>	3.60
CaO	3.46
Na <sub>2</sub> O	1.32
TiO <sub>2</sub>	0.28
MgO	0.78
K <sub>2</sub> O	0.53

\* Particle size: 1 mm; SiO<sub>2</sub>/Al<sub>2</sub>O<sub>3</sub> ratio: 5.15; average diameter of pores: 170.7 Å or 0.017 µm

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**Table 2:** Composition of the synthetic wastewater used.

	Units	Concentration
COD	mg O <sub>2</sub> /L	360
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	mg N/L	707.1
MgSO <sub>4</sub> · 7H <sub>2</sub> O	mg Mg/L	3.6
K <sub>2</sub> HPO <sub>4</sub>	mg P/L	43.9
KH <sub>2</sub> PO <sub>4</sub>	mg P/L	43.9

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**Table 3:** Variation of the average biomass concentration and pH values (with their respective standard deviations) with time in the set of experiments III.

Time (days)	Average VSS concentration (ppm)	pH
0	1,000 ± 40	7.8 ± 0.4
2	7,800 ± 390	7.1 ± 0.4
4	6,700 ± 340	6.5 ± 0.3
6	11,300 ± 450	6.5 ± 0.1
8	11,550 ± 530	6.3 ± 0.1
10	11,700 ± 480	6.2 ± 0.2
12	13,900 ± 520	6.1 ± 0.3
14	12,200 ± 450	6.3 ± 0.4
16	12,100 ± 490	6.4 ± 0.3

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528 **Table 4:** Variation of the average nitrogenous compounds ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) with their  
529 respective standard deviations with time in the set of experiments III.

Time (days)	$\text{NH}_4^+$ (molar concentration)	$\text{NO}_3^-$ (molar concentration)
2	$0.0020 \pm 0.0001$	$0.0022 \pm 0.0001$
4	$0.0035 \pm 0.0001$	$0.0040 \pm 0.0001$
6	$0.0037 \pm 0.0002$	$0.0048 \pm 0.0002$
8	$0.0040 \pm 0.0002$	$0.0059 \pm 0.0001$
10	$0.0042 \pm 0.0001$	$0.0075 \pm 0.0002$
12	$0.0049 \pm 0.0002$	$0.0200 \pm 0.0008$
14	$0.0050 \pm 0.0002$	$0.0185 \pm 0.0007$
16	$0.0049 \pm 0.0002$	$0.0189 \pm 0.0008$

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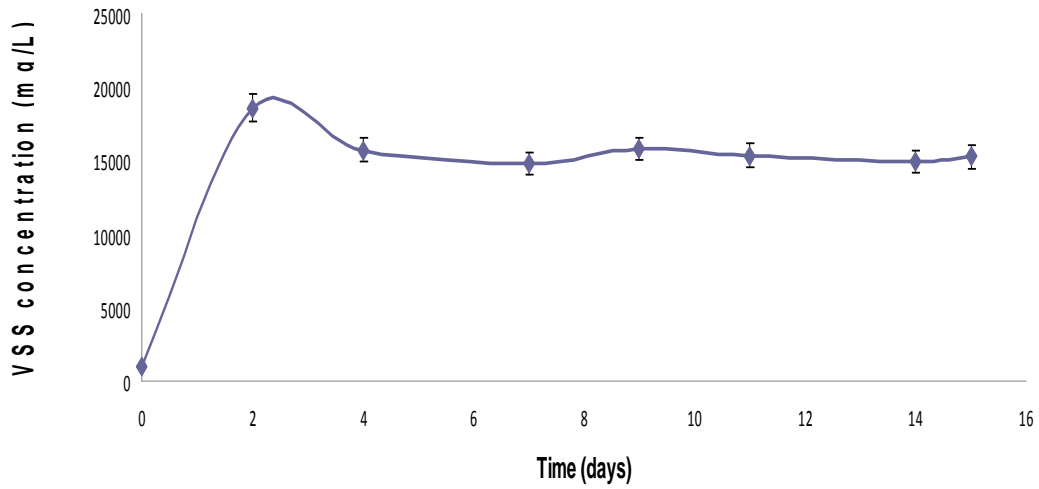
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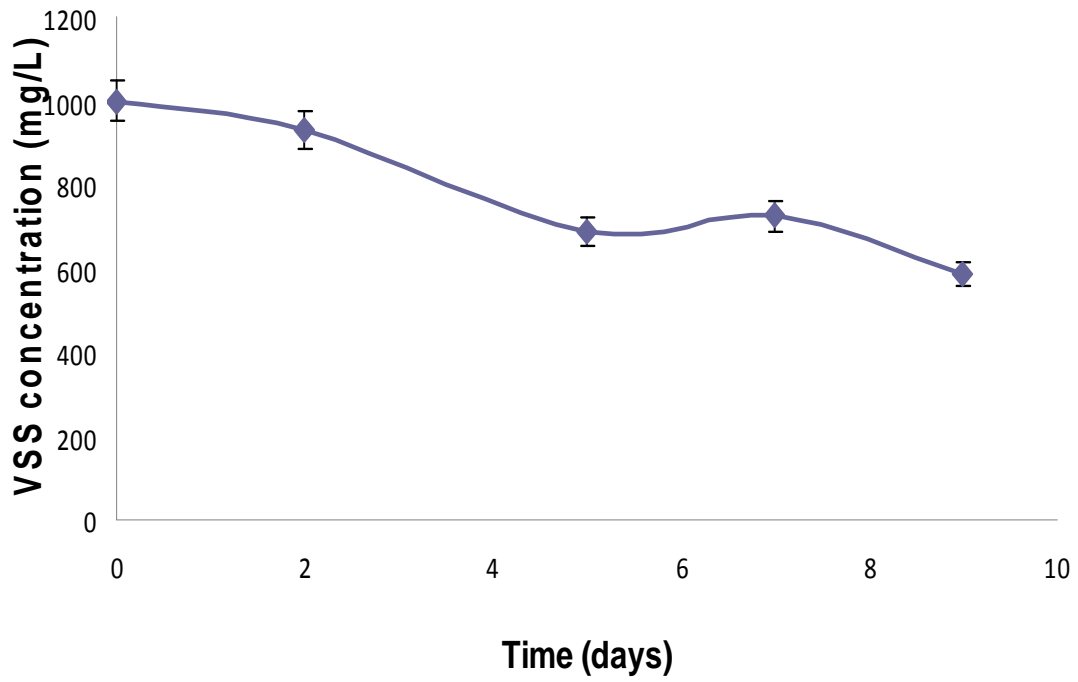
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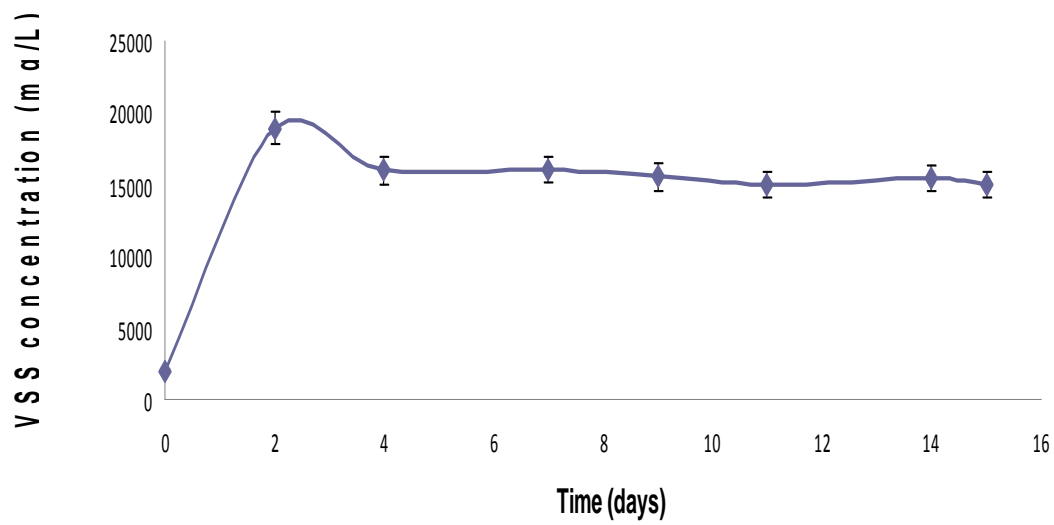
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**Figure 1:** Variation of the biomass concentration (mg VSS/L) with time in the reactors with zeolite (initial biomass concentration of 1000 mg VSS/L) in the set of experiments I.



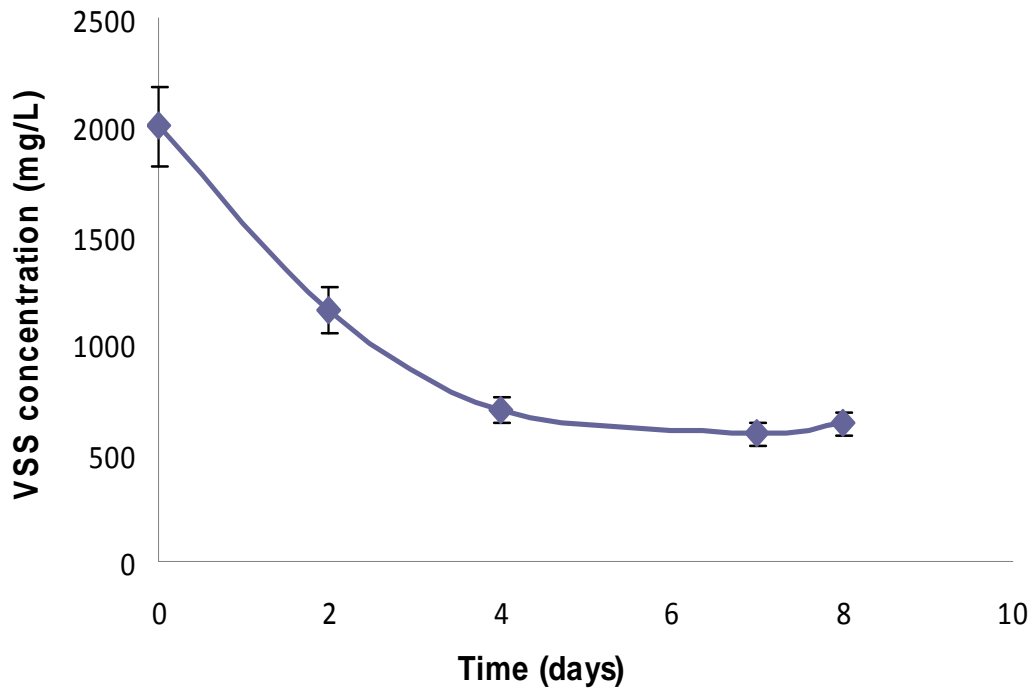
**Figure 2:** Variation of the biomass concentration (mg VSS/L) with time in the reactors without zeolite (initial biomass concentration of 1000 mg VSS/L) in the set of experiments I.

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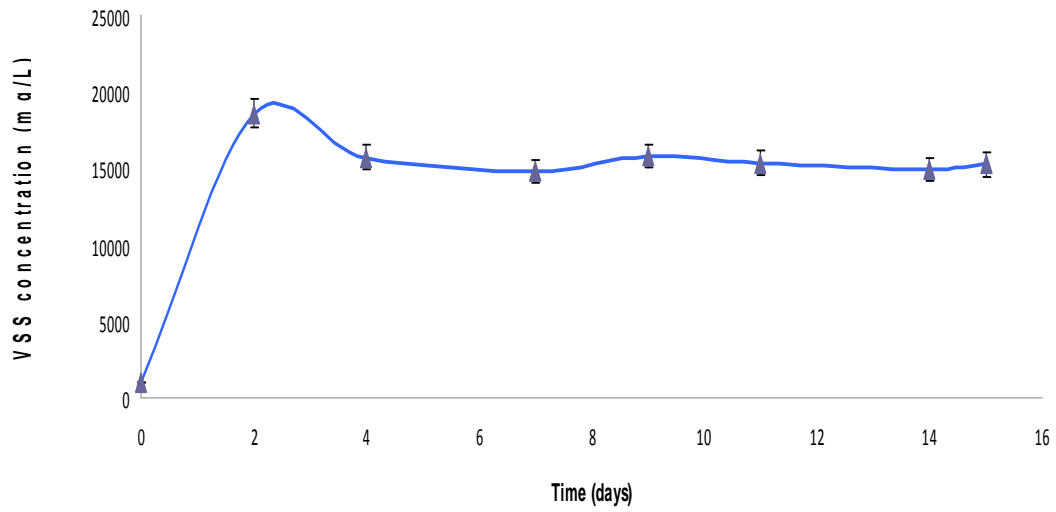
604 **Figure 3:** Variation of the biomass concentration (mg VSS/L) with time in the reactors  
 605 with zeolite (initial biomass concentration of 2000 mg VSS/L) in the set of  
 606 experiments I.  
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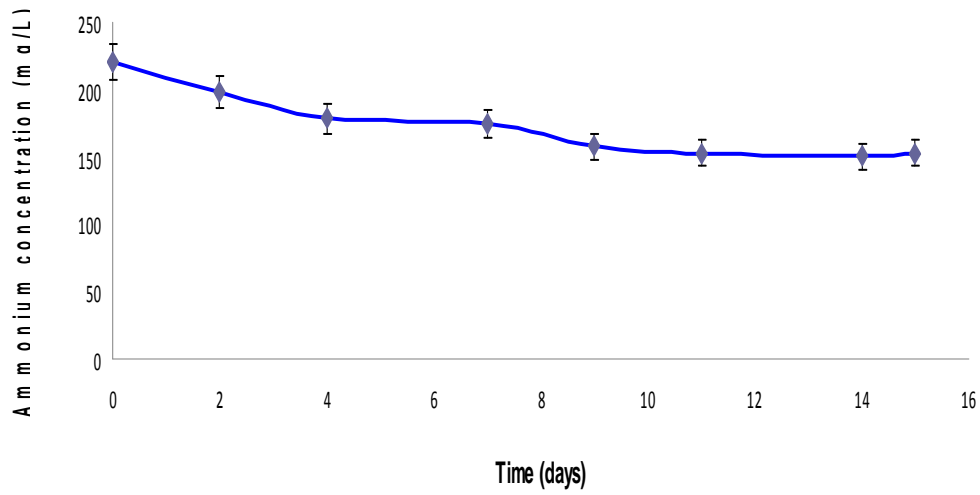
**Figure 4:** Variation of the biomass concentration (mg VSS/L) with time in the reactors without zeolite (initial biomass concentration of 2000 mg VSS/L) in the set of experiments I.

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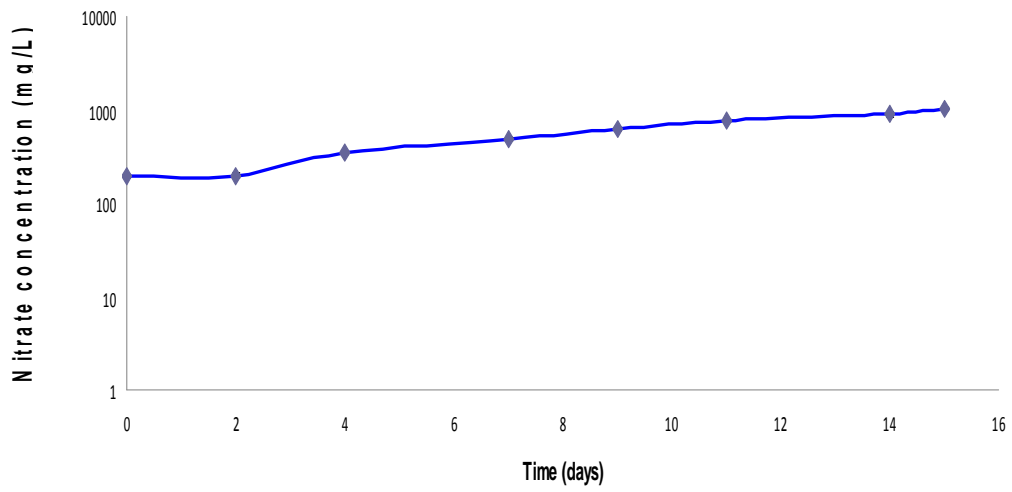
**Figure 5:** Variation of the biomass concentration with time in the set of experiments II (initial biomass concentration: 1000 mg VSS/L).

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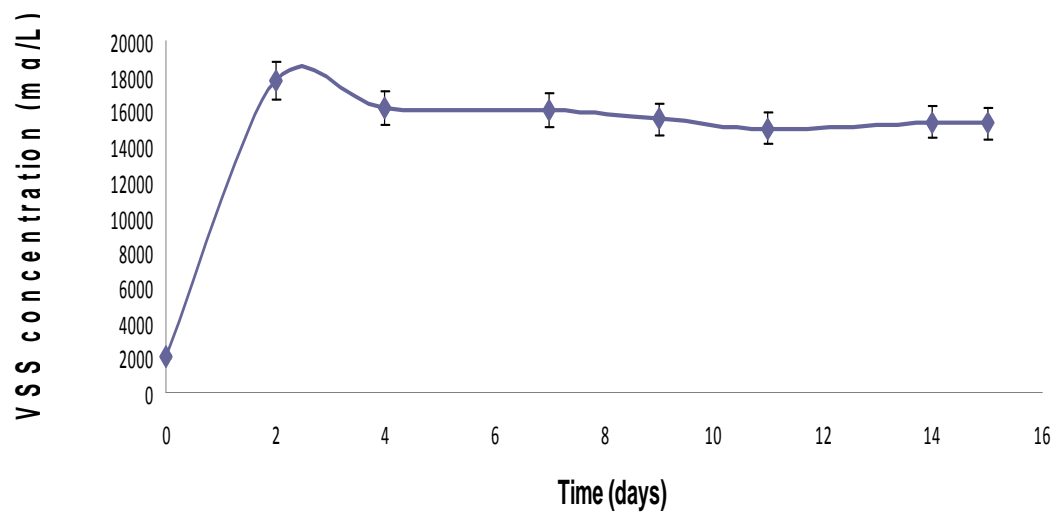
**Figure 6:** Variation of the ammonium concentration with time in the set of experiments II (initial biomass concentration: 1000 mg VSS/L).

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**Figure 7:** Variation of the nitrate concentration with time in the set of experiments II (initial biomass concentration: 1000 mg VSS/L).

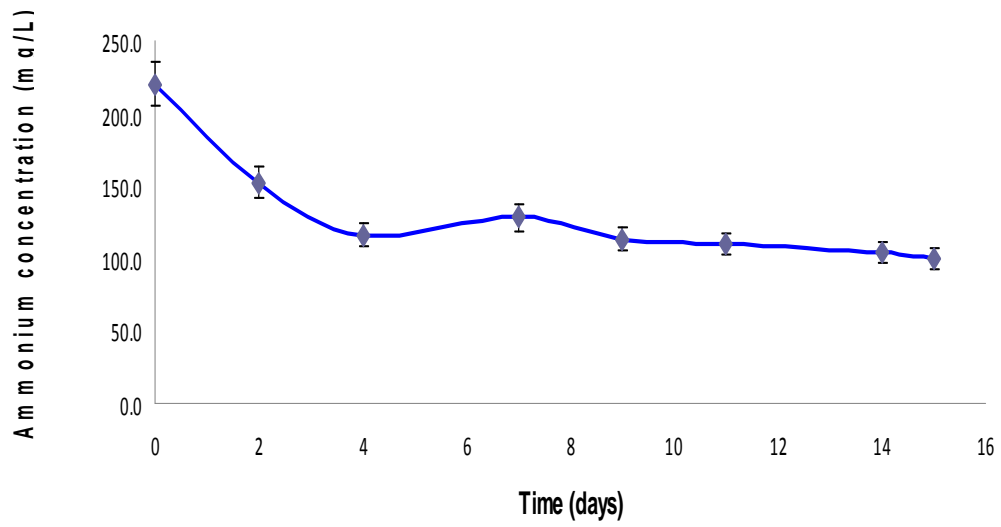
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**Figure 8:** Variation of the biomass concentration with time in the set of experiments II (initial biomass concentration: 2000 mg VSS/L).

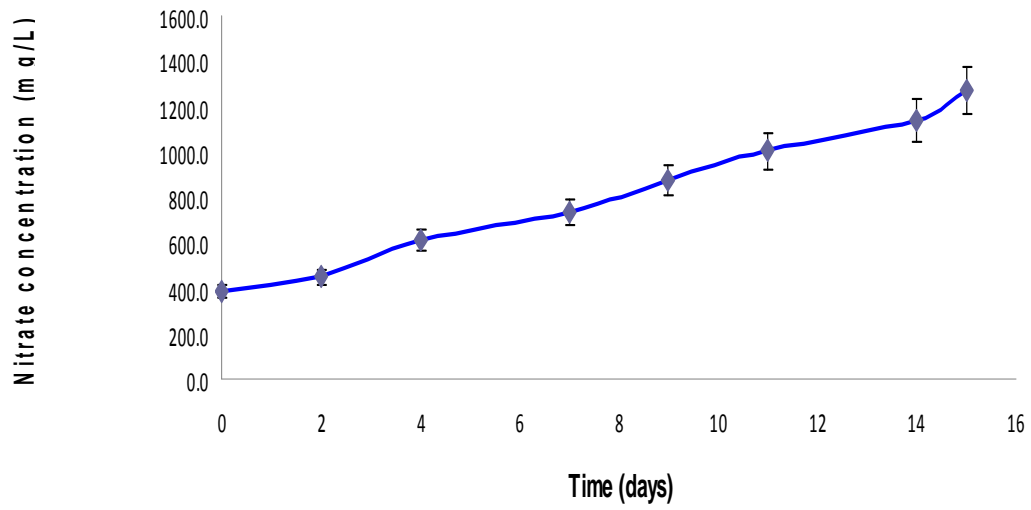
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**Figure 9:** Variation of the ammonium concentration with time in the set of experiments II (initial biomass concentration: 2000 mg VSS/L).

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**Figure 10:** Variation of the nitrate concentration with time in the set of experiments II (initial biomass concentration: 2000 mg VSS/L).

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