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ORIGINAL PAPER

Improvement in nitrification through the use of natural zeolite: influence of the biomass concentration and inoculum source

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

Keywords Batch mode · Inoculum type · Nitrifying biomass carrier · Zeolite

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Introduction

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

In addition, the direct effects of nitrogen compounds on the aquatic systems and the indirect effects resulting from the nitrogen gases that are generated from the liquid wastes, such as NO_x substances, also contribute either to the greenhouse effect or to acid rain (Wang et al. 2008). There are two main methods for removing nitrogen from wastewaters: physicochemical and biological processes (Guo et al. 2008). The physicochemical processes such as air and stream stripping are sometimes used for the control of nitrogen in strong nitrogen wastewaters. However, from environmental and economic viewpoints, it would be more interesting to use biological nitrogen removal for treating high ammonium strength wastewaters. Its removal process has been widely adopted in preference to the physicochemical processes because of its higher effectiveness and relatively low cost, especially in the field of urban wastewater treatment (Guo et al. 2008). Among the biological methods, the nitrification–denitrification system is the most widely used alternative (Leu et al. 2010; Liu et al. 2007; Pagga et al. 2006).

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

However, a restrictive factor for the suitability of this technological alternative (nitrification–denitrification) is that the microorganism support medium or bacterial carrier, has to fulfil the following main characteristics: its surface should favour the adherence and colonization of the microorganisms, it should be physically and chemically resistant and relatively inert (Liu et al. 2007; Rostron et al. 2001). Several previous research works have demonstrated that natural zeolites meet these characteristics (Fernández et al. 2007; Nikolaeva et al. 2009). However, the specific behaviour of the zeolites in each process is different depending on the type of microorganisms involved in each case. As a consequence, the need to assess the nitrification process using natural zeolite as microorganism carrier for this process (Liu et al. 2007; Rostron et al. 2001) has emerged.

Natural zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, consisting of three-dimensional frameworks of SiO_4^{4-} and AlO_4^{5-} tetrahedra linked through shared oxygen atoms (Tashauoei et al. 2010). They are porous materials characterized by their 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 their constituent cations without a major change in their structure (ion-exchange property). The exploitation of these properties underlies the use of zeolites in a wide range of industrial, agricultural and contamination prevention applications (Milán et al. 2001a, b, c; Tashauoei et al. 2010). The structure and physical properties of natural zeolite [channel and pore cavities, minimum diameter of pores in the range of 3–10 Å, average surface area of 24.9 m^2/g, low bulk density, high exchange (CEC) and adsorption capacities] make it ideal for use in biological purification wastewater processes (Carretero and Pozo 2009). Consequently, the use of natural zeolite in different wastewater biological treatment processes has increased significantly over the past few years.

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

Materials and methods

Experimental design

Three different sets or groups of experiments were carried out:

I. A first set of experiments comparing a batch nitrification process with and without added zeolite, using two different initial biomass concentrations (1,000 and 2,000 mg VSS/L). Biomass from an activated sludge process belonging to an urban wastewater treatment plant (UWTP), called *La Farfana* located in Santiago de Chile (Chile), was used as inoculum in the reactors. The evolution of biomass concentration over time was assessed.
II. A second set of batch nitrification experiments with added zeolite and initial biomass concentrations of 1,000 and 2,000 mg VSS/L. The same inoculum was used. The bacterial growth, the decrease in ammonium concentration and formation of nitrate over time were evaluated in this case.
III. A third set of batch nitrification experiments with added zeolite and an initial biomass concentration of 1,000 mg VSS/L. An inoculum from an activated sludge process treating wastewater from a paper mill was used in this case. The bacterial growth, variation of pH, decrease in ammonium concentration and formation of nitrate over time were followed up in this third set of experiments.

Zeolite used

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

Chemical analyses

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

Experimental procedure

Set of experiments I and II

Experimental sets I and II were carried out at ambient temperature (an average of 20 °C) in glass reactors of 200 mL working volume, in which synthetic wastewater was added. Table 2 shows the composition and main characteristics of the synthetic wastewater used as substrate in these experiments. Aerobic biomass from a full-scale activated sludge process located in the UWTP at *La Farfana* was used as inoculum in both groups of experiments. The characteristics of this biomass were as follows: total suspended solids (TSS), 8,950 mg/L; volatile suspended solids (VSS), 7,300 mg/L; pH, 7.2; and sludge volume index (SVI), 150 mL/g. Air was supplied to these reactors with a flow rate of 20 L/min. The air was injected through the bottom of the reactor using a porous ceramic diffuser. In addition, each reactor had a detachable grille located in the upper part to avoid microorganisms being lost in the reactor effluents as a consequence of the breaking of the air bubbles in the interface gas–liquid.

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

Components	Composition (%)
SiO_2	67.00
Al_2O_3	13.01
Fe_2O_3	3.60
CaO	3.46
Na_2O	1.32
TiO_2	0.28
MgO	0.78
K_2O	0.53

Particle size: 1 mm, SiO_2/Al_2O_3 ratio: 5.15, average diameter of pores: 170.7 Å or 0.017 μm

Table 2 Composition of the synthetic wastewater used

	Units	Concentration
COD	mg O_2/L	360
$(NH_4)_2$ SO_4	mg N/L	707.1
$MgSO_4·7H_2O$	mg Mg/L	3.6
K_2HPO_4	mg P/L	43.9
KH_2PO_4	mg P/L	43.9

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

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

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

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

Set of experiments III

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

This group of batch nitrification experiments was carried out with added zeolite and an initial biomass concentration of 1,000 mg VSS/L. An inoculum from an activated sludge process treating wastewater from a paper manufacture factory was used in this case. The characteristics of this

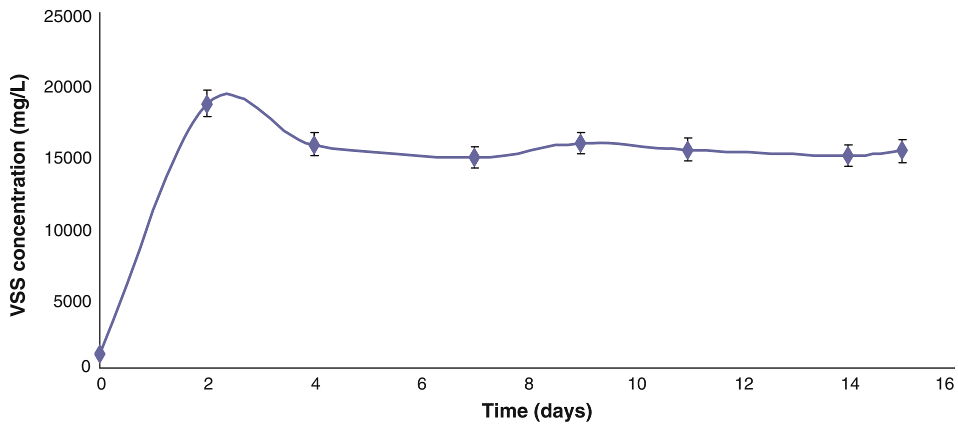


Fig. 1 Variation of the biomass concentration (mg VSS/L) with time in the reactors with zeolite (initial biomass concentration of 1,000 mg VSS/L) in the set of experiments I

biomass were as follows: TSS 7,500 mg/L; VSS 6,400 mg/L; pH 7.1; and SVI 300 mL/g. The bacterial growth, pH variation, decrease in ammonium concentration and formation of nitrate over time were assessed in this third set of experiments. The duration of the three experiments carried out in parallel was 16 days.

Results and discussion

Set of experiments I

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

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

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

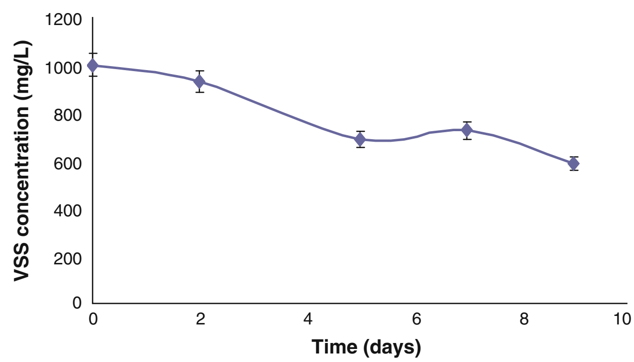


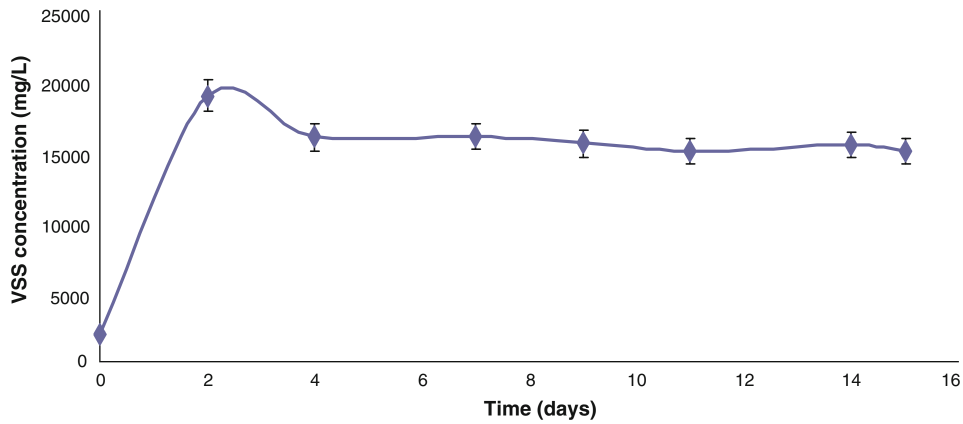
Fig. 2 Variation of the biomass concentration (mg VSS/L) with time in the reactors without zeolite (initial biomass concentration of 1,000 mg VSS/L) in the set of experiments I

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

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

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

Fig. 3 Variation of the biomass concentration (mg VSS/L) with time in the reactors with zeolite (initial biomass concentration of 2,000 mg VSS/L) in the set of experiments I



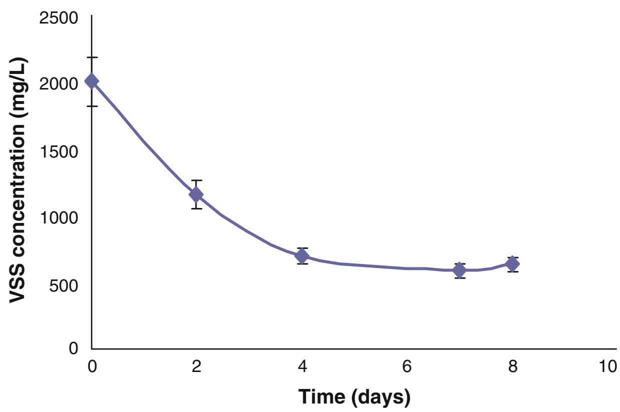


Fig. 4 Variation of the biomass concentration (mg VSS/L) with time in the reactors without zeolite (initial biomass concentration of 2,000 mg VSS/L) in the set of experiments I

2,250 mg/L), the total biomass concentration was also increased.

Set of experiments II

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

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

Figure 5 shows the variation of the biomass concentration over time. As can be seen, an exponential growth was observed during the first 2 days of operation achieving a maximum value of 18,550 mg/L. From day 4 onward, the microorganism concentration remained virtually constant with a value of about 15,000 mg VSS/L. Therefore, the addition of 30 mL of synthetic wastewater to the reactors every 2 days to compensate for the evaporation losses meant that the biomass concentration remained constant over time.

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

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

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

The variation of the biomass concentration over time is illustrated in Fig. 8 and showed similar behaviour to that observed when the initial biomass concentration was 1,000 mg VSS/L. An exponential growth was observed up to the 2nd day of digestion, in which a maximum concentration of 18,800 mg VSS/L was achieved. From the 4th

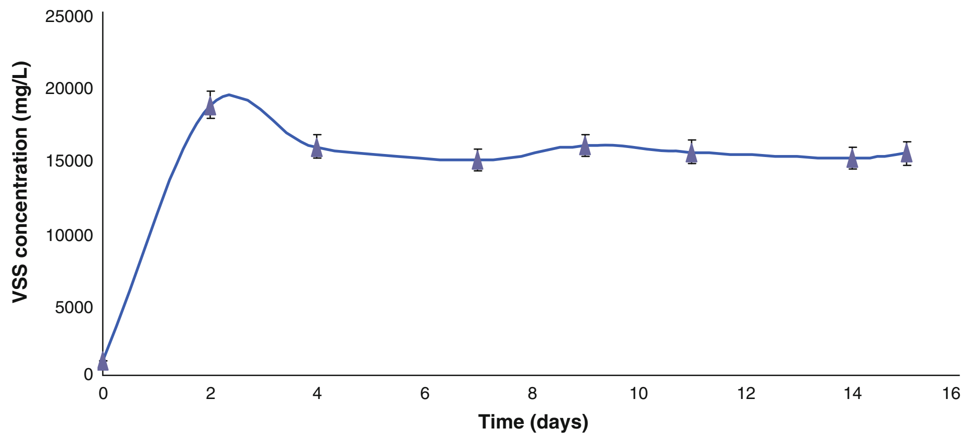


Fig. 5 Variation of the biomass concentration with time in the set of experiments II (initial biomass concentration: 1,000 mg VSS/L)

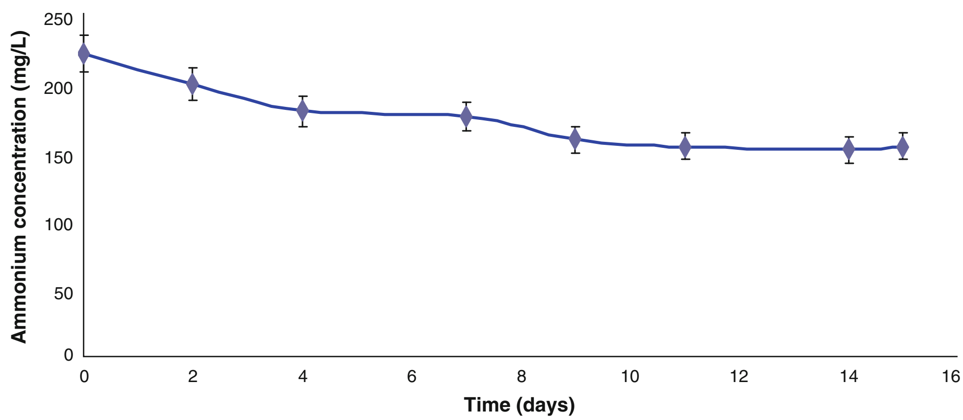


Fig. 6 Variation of the ammonium concentration with time in the set of experiments II (initial biomass concentration: 1,000 mg VSS/L)

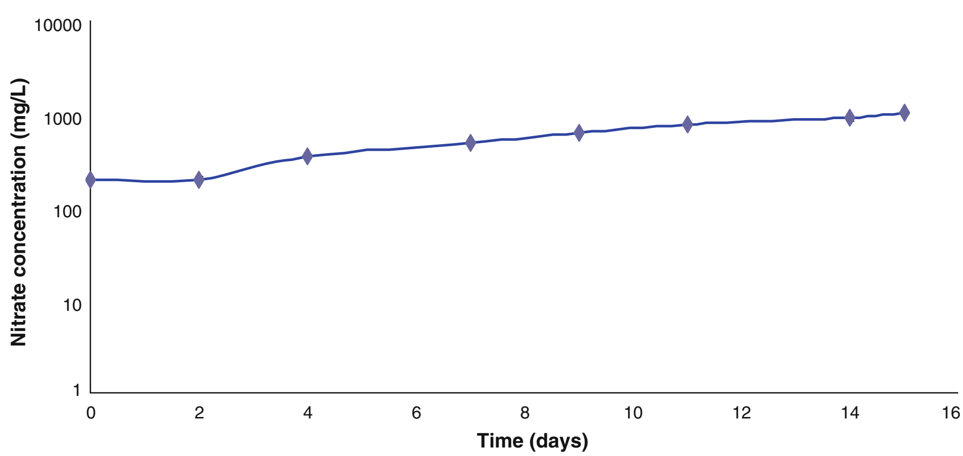


Fig. 7 Variation of the nitrate concentration with time in the set of experiments II (initial biomass concentration: 1,000 mg VSS/L)

day onward, the increase in the VSS concentration tended to be constant despite the previously mentioned synthetic wastewater being added every 2 days.

In addition, there was no lag phase because the inoculum used came from an urban WWTP and no acclimation stage was necessary. The stationary stage occurred after the 4th day of operation and was reached despite the reactors being fed with 30 mL of synthetic wastewater every 2 days. As a consequence of this batch feed system, the microorganism dead phase was not observed. Similar initial biomass concentrations (1,750 mg VSS/L) also behaved well in nitrifying SBR systems operating in continuous mode at an HRT of 1 day treating reject water (Perez et al. 2007).

A gradual decrease in the ammonium concentration over time was observed until a relatively constant value after the 10th of digestion was reached (Fig. 9). The maximum reduction in ammonium in the liquid medium was approximately 70 %. Figure 10 confirms that nitrate was obtained as a final product of the process. Effective

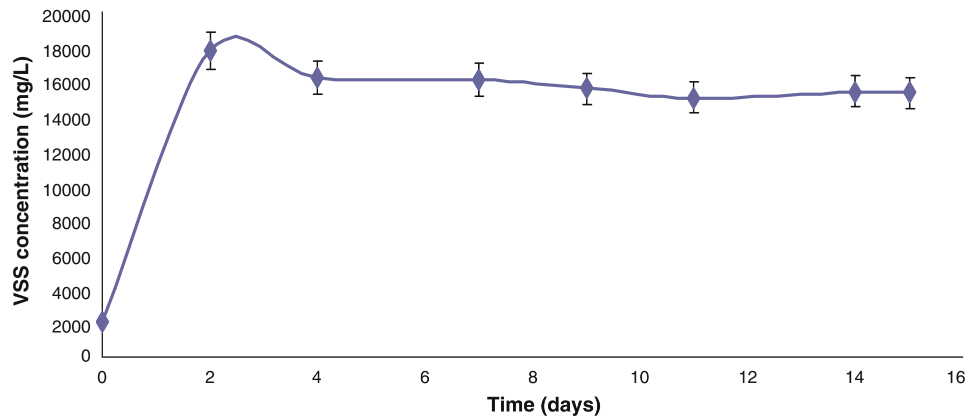


Fig. 8 Variation of the biomass concentration with time in the set of experiments II (initial biomass concentration: 2,000 mg VSS/L)

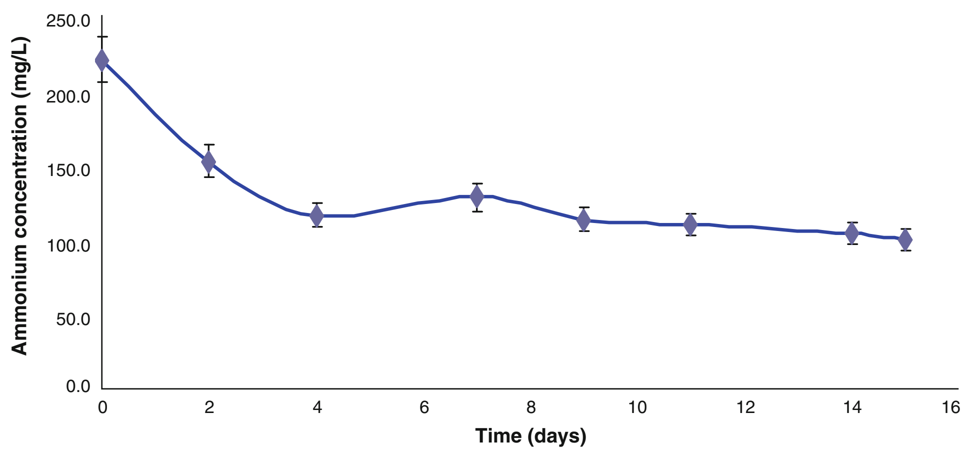


Fig. 9 Variation of the ammonium concentration with time in the set of experiments II (initial biomass concentration: 2,000 mg VSS/L)

nitrification was also reported in SBR systems treating reject water (supernatant of an anaerobic sludge digestion) with initial biomass concentrations of 3,500 mg/L and initial ammonium concentrations of up to 1,200 mg/L (Galí et al. 2006).

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

Set of experiments III

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

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

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

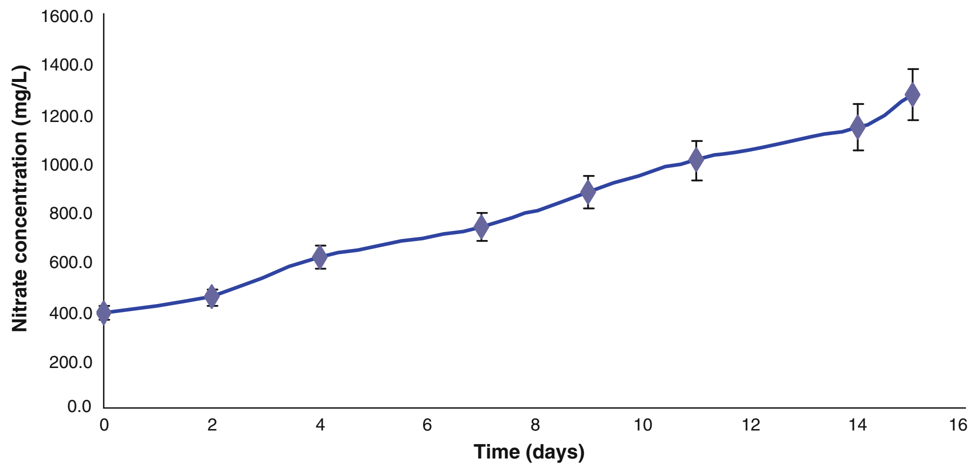


Fig. 10 Variation of the nitrate concentration with time in the set of experiments II (initial biomass concentration: 2,000 mg VSS/L)

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

Table 4 Variation of the average nitrogenous compounds (NH_4^+ and NO_3^-) with their respective standard deviations with time in the set of experiments III

Time (days)	NH_4^+ (molar concentration)	NO_3^- (molar concentration)
2	0.0020 ± 0.0001	0.0022 ± 0.0001
4	0.0035 ± 0.0001	0.0040 ± 0.0001
6	0.0037 ± 0.0002	0.0048 ± 0.0002
8	0.0040 ± 0.0002	0.0059 ± 0.0001
10	0.0042 ± 0.0001	0.0075 ± 0.0002
12	0.0049 ± 0.0002	0.0200 ± 0.0008
14	0.0050 ± 0.0002	0.0185 ± 0.0007
16	0.0049 ± 0.0002	0.0189 ± 0.0008

The pH has a tendency to decrease due to the fact that the conversion of NH_4^+ to NO_3^- involves the transformation of an alkaline ion into an acid ion as follows:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$$

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

Table 4 summarizes the evolution of ammonium and nitrate concentrations (average values of the three experimental runs and expressed in molar concentration) with operation time. It can be observed a slight increase in the NH_4^+ concentration with time throughout the 16 days of the assay. By contrast, the NO_3^- concentration increased slightly during the first 8 days, showing a considerable increase between the 10th and 12th day, for which a maximum concentration of 0.02 (molar) was achieved.

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

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

Nitrogen consumption was calculated from the measurements made during the different time periods taking into account that 30 mL of synthetic wastewater containing ammonium was added every 2 days. The nitrogenous chemical species that were considered for this analysis were as follows: ammonia (liquid), ammonia (gas), NO_3^-, all of them measured with selective electrodes, $N_{biomass}$ measured in the biomass adhered to zeolite and $N_{zeolite}$ calculated from the ionic exchange capacity of the zeolite and data provided by the zeolite supplier (ZeoClean R). The results obtained in this N balance were gathered together in three time intervals: from 1 to 6 days (period 1); from 7 to 10 days (period 2); and from 11 to 16 days (period 3) for a better analysis. The average N consumption of the three experimental runs carried out within the set of experiments III was found to be 14, 23 and 46 % for the time periods 1, 2 and 3, respectively, amounting the N losses an average value of 17 %.

Therefore, the higher N consumption took place between days 11 and 16, which coincided with the maximum biomass concentration generated and maximum ammonium removal. As expected, N consumption is directly related to the amount of biomass generated. Of 100 % of N added, an average of 46 % was consumed in the time interval between 11 and 16 days. It was noteworthy that the loss of nitrogen was high (average of 17 %), which was due to the experimental conditions used with a high air flow, which determined DO concentration values in the range of 4.7–6.2 ppm contributing to a high level of ammonia stripping.

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

Conclusion

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

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