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Partial Nitrification in a Sequencing Moving Bed Biofilm Reactor (SMBBR) with Zeolite as Biomass Carrier: Effect of Sulfide Pulses and Organic Matter Presence

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Abstract: This work aimed to achieve partial nitrification (PN) in a Sequencing Moving Bed Biofilm Reactor SMBBR with zeolite as a biomass carrier by using sulfide pulses in the presence of organic matter as an inhibitor. Two conditions were evaluated: sulfide (HS^-) = 5 mg S/L and vvm (air volume per liquid volume per minute, L of air L^{-1} of liquid min^{-1}) = 0.1 (condition 1); and a HS^- = 10 mg S/L and a vvm = 0.5 (condition 2). The simultaneous effect of organic matter and sulfide was evaluated at a Chemical Oxygen Demand (COD) = 350 mg/L and HS^- = 5 mg S/L, with a vvm = 0.5. As a result, using the sulfide pulse improved the nitrite accumulation in both systems. However, Total Ammonia Nitrogen (TAN) oxidation in both processes decreased by up to 60%. The simultaneous presence of COD and sulfide significantly reduced the TAN and nitrite oxidation, with a COD removal yield of 80% and sulfide oxidation close to 20%. Thus, the use of a sulfide pulse enabled PN in a SMBBR with zeolite. Organic matter, together with the sulfide pulse, almost completely inhibited the nitrification process despite using zeolite.

Keywords: sulfide doses; NOB inhibition; organic matter; AOB inhibition; zeolite

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1. Introduction

Unless properly treated, wastewater with high nitrogen content causes eutrophication, depletion of dissolved oxygen concentration, and ammonia toxicity in aquatic organisms. The conventional nitrification-denitrification technology is a bioprocess for converting ammonia nitrogen into harmless molecular nitrogen (N_2). Nitrification is an aerobic process that involves, as a first step, the oxidation of NH_3 to NO_2^- by ammonia oxidizing bacteria (AOB), while the second step involves the oxidation of NO_2^- to NO_3^- by nitrite oxidizing bacteria (NOB). Then, denitrifying bacteria reduce the NO_3^- to NO_2^- and finally to N_2 [1].

Biological nitrogen removal is an essential technology to minimize operational costs, particularly in the aerobic nitrification stage. One of these improvements is partial nitrification (PN), which consists of the accumulation of NO_2^- preventing the action of NOB [2]. Several operating factors can limit and inhibit NOB growth during nitrification, such as low dissolved oxygen concentration and pH values higher than 8.5, among others [3].

An alternative procedure to obtain PN consists of providing sulfide pulses during nitrification since sulfide inhibits NOB and AOB at different levels. Erguder et al. [4] added sulfide in the range of 1.3 to 80 mg S^-/L during the anoxic step to reduce the activity of NOB. Ortiz et al. [5] observed that sulfide caused an inhibitory effect on both AOB and

NOB, with nitrite oxidation being the most affected step. Vela et al. [6] also noted that NOB was more affected by sulfide inhibition than AOB.

On the other hand, organic matter (OM) is detrimental to the partial nitrification process. Effluents with high OM concentrations generate competition between heterotrophs and nitrifying bacteria for oxygen [7,8]. Indeed, the Chemical Oxygen Demand (COD)/N ratio is one of the most critical factors affecting nitrification. Heterotrophs display higher growth rates and biomass yields than autotrophic bacteria, enabling them to outcompete AOB and NOB under high readily biodegradable COD loadings [7,9]. Increased competition for oxygen can be an acute event, reducing nitrifying bacteria populations [7]. Furthermore, organic matter, together with sulfide, is expected in anaerobic digester effluents (namely digestate), and therefore, its effects on partial nitrification still need investigation. In this sense, one way to maintain the nitrification performance even in the presence of sulfide and organic matter is using zeolite as a biomass carrier in biofilm systems.

Zeolite has been used in several biological processes, such as anaerobic digestion, denitrification, and nitrification [10]. In this sense, zeolite is an excellent support for enhancing partial nitrification. Yang et al. [11] employed a zeolite biological aerated filter (ZBAF), with natural zeolite and continuous feeding (nitrogen loading rate, NLR, of 0.854–1.200 kg N/m^3 d), that resulted in high nitrite accumulation (>98.0%). Later, Chen et al. [12] used different zeolites as adsorbent media in sequencing biofilm batch reactors (SBBR) to maintain an appropriate free ammonia (FA) range to achieve PN under different alkalinity ratios, demonstrating that the application of zeolite generated higher PN by NH_4^+ adsorption because of the high cation exchange capacity of the zeolite. Wang et al. [13] studied the influence of organics on PN performance in a lab-scale sequencing batch biofilm reactor filled with zeolite. They showed that the higher COD/N ratio, the lower ammonia conversion efficiency (ACE) and nitrite production rate (NPR), indicating that ACE and NPR (average) decreased by 57.3% and 87.5%, with COD/N ratios from 0 to 0.5, respectively. In this sense, a special SBBR is the sequencing moving bed biofilm reactor (SMBBR), which combines the advantages of biofilm and sequencing batch reactors, where the microorganisms inside the biofilm together to the floccular sludge are the main types of microbial aggregates [14]. Even though Huiliñir et al. [15] reported that complete nitrification could be improved by using low concentrations of zeolite as a biomass carrier in an SMBBR, to achieve the PN using a pulse of sulfide doses in a SMBBR using zeolite as a biomass carrier has not been reported yet.

Thus, the goal of this work was to evaluate the performance of the PN process in an SMBBR using zeolite as biomass carriers and adding pulses of sulfide for NOB inhibition. Furthermore, the simultaneous effect of OM and sulfide on NOB as inhibitors was also assessed. Accordingly, there are two main novelties in the present work: using zeolite as a biomass carrier in an SMBBR for partial nitrification and evaluating partial nitrification using sulfide and organic matter simultaneously.

2. Methodology

2.1. Nitrifying Inoculum, Substrate, and Zeolite Characteristics

Nitrifying biomass was obtained from a Continuous Stirred Tank Reactor (CSTR, 15 L liquid volume) with a settler as described in Huiliñir et al. [15]. The main sludge characteristics are shown in Table S1 (supplementary data), which was adapted to nitrifying conditions for three months. The CSTR operated at Solid Retention Time (SRT) = 60 d, Hydraulic Retention Time (HRT) = 8 d at 25 °C with aeration and with no addition of COD or HS^- compounds to the reactor. The volatile suspended solid (VSS) concentration in the inoculum reactor varied around 0.7 g/L. The CSTR reactor was fed with a nitrogen load rate (NLR) of 0.05 kg N/m^3 d, keeping the dissolved oxygen concentration at 4.0 ± 0.5 mg/L by continuous aeration at specific aeration of vvm = 0.8 (air volume per liquid volume per minute, L of air L^{-1} of liquid min^{-1}). The synthetic wastewater for feeding the inoculum reactor was prepared according to Huilinir et al. [15] and it contains (in g/L): KH_2PO_4 (4.5); K_2HPO_4 (3.0); $NaHCO_3$ (3.7); $(NH_4)_2HPO_4$ as TAN source (1.9); trace elements solution supplied at 1.3 mL/L. The trace element solution contained (g/L): EDTA (5), $CaCl_2 \cdot 2H_2O$ (5.54), $MnCl_2$ (5.0), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (1.1).

Regarding the zeolite, it was obtained from a company that commercializes natural zeolite located in Quinamávida, Linares, VII Region, Maule, Chile. Further details of the zeolite characteristics are found in Huiliñir et al. [15] and are shown in Table S2 (supplementary data).

2.2. Experimental Setup and Experimental Design

The experimental setup and reactor operation were similar to the work previously presented by Huiliñir et al. [15]. Two cylindrical glass bioreactors of 2 L liquid volume capacity were used as a sequencing batch reactor (SBR), which were aerated using a mini-compressor (150 L/min, ACO-012, Raoping Xingcheng Mechanical Electricity Aquatic Article Co., Ltd., Guangdong, China) and the air was distributed through a porous ceramic diffuser. The airflow rate was set through a rotameter (1–10 L per minute (lpm), Veto, Santiago, Chile; 4–40 lpm, Veto, Santiago, Chile). The initial VSS concentration in both SBRs was 1 ± 0.1 g/L. In one SBR, 5 g/L of zeolite (particle diameter: 0.25–0.5 mm; Specific area: 24.9 m^2/g zeolite) was added, resulting in 0.2 g VSS/g zeolite. The systems were mechanically agitated (DLAB OSD20-S, Mundolab, Chile) at 400 rpm. They were operated in a cycle of 25 h, consisting of influent (0.1 h), aeration time (24 h), settling (0.7 h), idling, and effluent (0.2 h). The HRT of both systems was 34 h, while the SRT was 49 h. The reactors were operated at a volumetric exchange ratio of 75% at a temperature of 25 ± 0.5 °C.

In order to determine whether the zeolite could improve the partial nitrification performance compared to the system without zeolite, both reactors were operated at vvm = 0.1 to reach low dissolved oxygen (DO) concentrations in both reactors. In this condition, an NLR of 0.141 kg N/m^3 d at T = 25 °C was used. Once the effect of zeolite on partial nitrification was determined, the effect of sulfide on the system was assessed. In this case, the methodology presented by Erguder et al. [4] was implemented. Briefly, aerobic/anoxic (A/An) cycles of 12 h each were established as a reaction time, where the sulfide was added as a pulse at the beginning of the anoxic phase. An initial concentration of HS^- = 5 mg S/L using Na_2S as a source of sulfide was added as the first perturbation (condition 1), using a vvm = 0.1 during this condition. After that, a second perturbation (condition 2) was set at HS^- = 10 mg S/L, using a vvm = 0.5, to maintain the microbial activity. For all these conditions, an NLR = 0.141 kg N/m^3 at T = 25 °C was used.

Finally, the simultaneous effect of sulfide and organic matter was determined. In this condition, a COD = 350 mg/L of organic matter (glucose) was added at the beginning of the aerobic step, while a sulfide concentration of HS^- = 5 mg/L was added at the beginning of the anoxic step. Both conditions were studied at an NLR = 0.141 kg N/m^3 d and T = 25 °C. The initial concentration of Total Ammonia Nitrogen (TAN, 200 mg/L) and COD was calculated at COD/N ratio of 1.75 and was maintained during the entire study.

2.3. Chemical Analyses

Total and suspended solids, VSS, DO and pH were measured according to the Standard Methods [16]. TAN, $NO_2{}^-$-N and $NO_3{}^-$-N were analyzed using flow injection analysis (Lachat's QuikChem® 8500 Series 2 Flow Injection Analysis System, Loveland, CO, USA). Sulfide was determined by potentiometric titration using Titro-Line® 7000 of SI. Sulfate was determined by HACH Method 8051 (turbidimetric method) using the HACH DR3900 spectrophotometer. Soluble COD was measured by a colorimetric method according to APHA (2012), while alkalinity was measured by colorimetry with kits TNT870-LM from HACH. The dissolved oxygen (DO) concentration was measured by a dissolved oxygen meter (WTW, Multi 3620) equipped with a sensor FDO 925 IDS.

2.4. Efficiencies Removal Determination, Production Efficiencies and Statistical Analyses

Partial nitrification was evaluated according to the TAN oxidation (α) and the nitrite accumulation (β). Nitrite accumulation (%) is the fraction of total ammonia nitrogen oxidation to nitrite with regard to the total oxidation to nitrite and nitrate. These parameters were calculated as follows [17]:

$$\alpha = \left(\frac{c_{TAN,0} - c_{TAN}}{c_{TAN,0}} \right) \times 100 \quad (1)$$

$$\beta = \left(\frac{c_{NO_2^-}}{c_{NO_2^-} + c_{NO_3^-}} \right) \times 100 \quad (2)$$

where $c_{TAN,0}$ and c_{TAN} are the initial and final concentration of TAN in each cycle, respectively; $c_{NO_2^-}$ is the concentration of $NO_2{}^-$-N at the end of the cycle and $c_{NO_3^-}$ is the concentration of $NO_3{}^-$-N at the end of the cycle.

The *t*-test was the statistical analysis used to compare data between the systems with and without zeolite. It was performed using GraphPad Prism 7.00© software, with α = 0.05.

3. Results and Discussion

3.1. Partial Nitrification in SBR with and without Zeolite

The results of the partial nitrification process at 0.1 vvm and pH between 7.3 and 7.1 are shown in Figure 1. TAN, nitrite and nitrate concentrations obtained in the system with and without zeolite are shown in Supplementary data (Table S3). The system with zeolite oxidized nearly the entire ammonia content, while the bioreactor without zeolite converted nearly 70% of the ammonia. A possible explanation that we proposed is that the higher AOB activity in the system with zeolite, even at low DO concentrations, can be attributed to the bio-regeneration capacity of zeolite [10]. The microorganisms are prone to be attached to the zeolite surface or entrapped in the powdered zeolite particles, forming a zeolite-floc. This zeolite-floc adsorbs ammonium nitrogen, which is released into the liquid phase due to the chemical equilibrium and later transformed into nitrite and nitrate by AOB and NOB, respectively. Because of these two processes (release of ammonium and biological reaction), the zeolite is completely regenerated [11] and the nitrification capacity of the system improves compared to the reactor without zeolite. Because the VSS concentration was similar in both systems (see Table 1) and DO concentration was even lower in the system with zeolite (see Figure 1C), the bio-regeneration capacity of zeolite appears as the more probable explanation for the observed higher TAN oxidation within the system with zeolite. In addition, the adsorption of $NH_4{}^+$ to the zeolite surface is not likely because the mass balance indicated that TAN was mainly transformed into $NO_2{}^-$ and $NO_3{}^-$ (See Tables S4 and S5, supplementary data).

Table 1. VSS concentrations obtained in the experiments after 20 days of operation.

		Initial VSS Concentration, g/L	**Final VSS Concentration g/L**	**VSS Percentage Change, %**	**Sulfide/VSS Ratio, mg S/g VSS ***
Without sulfide and OM	With zeolite	1.031	1.045	+1.4	-
	Without zeolite	1.102	1.075	−2.5	-
With sulfide (Condition 1: HS^- = 5 mg/L)	With zeolite	1.045	1.435	+37.3	3.48
	Without zeolite	1.075	1.275	+18.6	3.92
With sulfide (Condition 2: HS^- = 10 mg/L)	With zeolite	1.480	0.730	−50.7	13.70
	Without zeolite	1.470	0.700	−52.4	14.30
With sulfide and OM (HS^- = 5 mg/L and COD = 350 mg/L)	With Zeolite	0.820	0.505	−38.4	9.90
	Without zeolite	0.810	0.545	−32.7	9.17

* Ratio calculated with final VSS concentration.

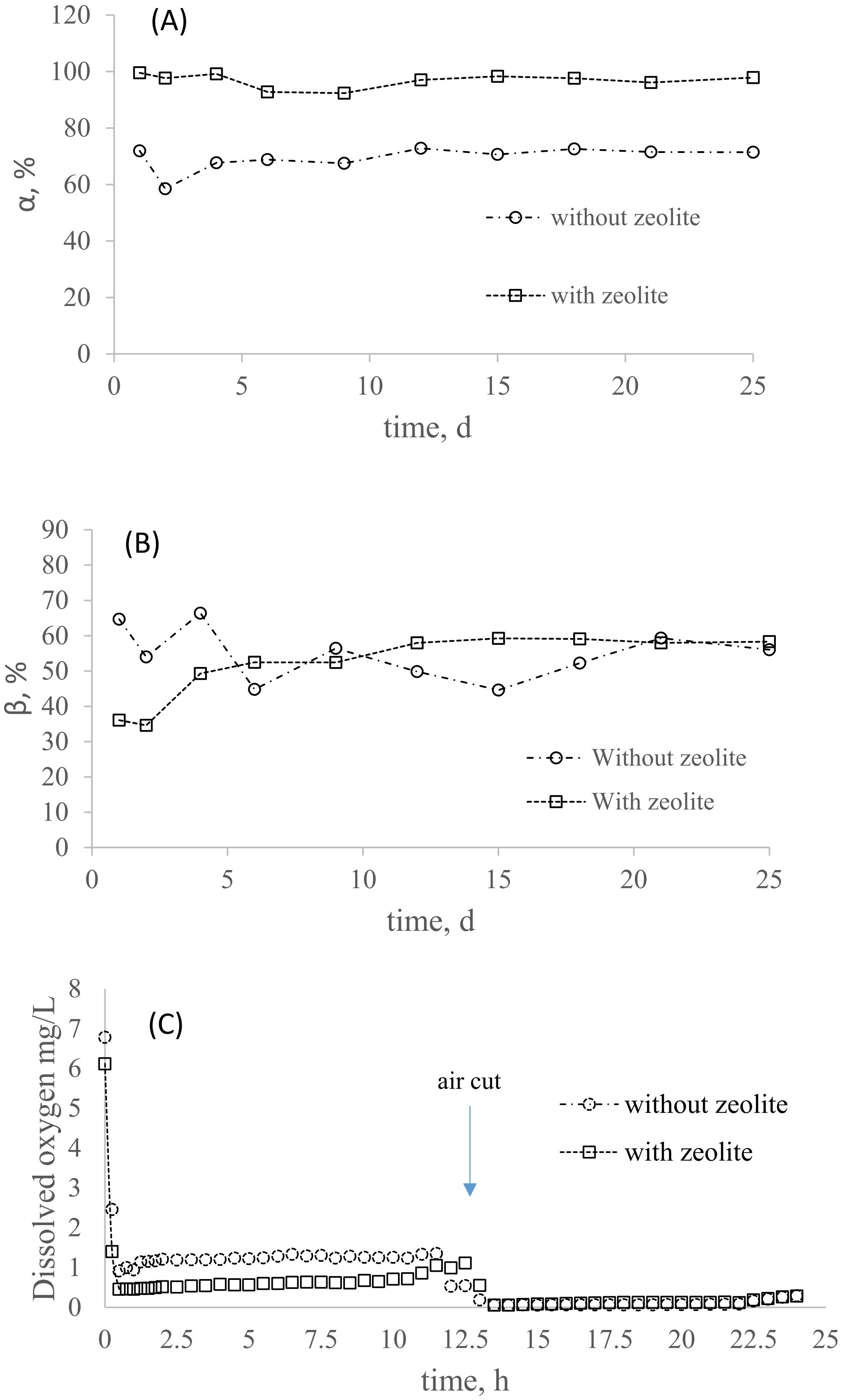


Figure 1. Performance of the systems with and without zeolite at 0.1 vvm, 7.1 < pH < 7.3 and cycles with 12 h/12 h aerobic/anoxic. (**A**) Efficiency in TAN oxidizing; (**B**) nitrite accumulation; (**C**) dissolved oxygen (DO) in a cycle.

Regarding the nitrite accumulation (β), both reactors reached similar values of ~60%. However, the system with zeolite was able to stabilize β much faster than the process with no zeolite, where nitrate fluctuated over time (Figure 1B). It is important to note that, although the β values between both systems were similar, the nitrite concentration was higher in the system with zeolite due to the high ammonia oxidation rate (See supplementary data, Table S3). The nitrite accumulation in both systems was around 60%, even though the pH values varied between 7.3 and 7.1 during the batch process (See Figure S1, supplementary data). Processes carried out with a pH > 8.5 improve the nitrite accumulation at low oxygen levels [18]. In this study, only the low DO concentration might explain the nitrite accumulation in the liquid phase of the SBR (Figure 1C). Further, from a kinetic point of view, AOB exhibit a higher affinity for DO than NOB; therefore, low DO concentrations (below 1.7 mg/L) can deactivate the NOB enzymes due to intermittent aeration accompanied by a stimulated activity of AOB [19–22].

Finally, the concentration of dissolved oxygen (DO) for one batch assay is shown in Figure 1C. The DO profile was similar in both reactors set at 0.1 vvm, without significant differences between the two conditions. It is observed that DO was slightly different between the system with and without zeolite, where the system without zeolite had a DO value of 1 mg/L, while the system with zeolite showed values around 0.5 mg/L at 12 h. At that point, the aeration was stopped, and the oxygen decreased in both systems to values lower than 0.05 mg/L. It is important to note that VSS in both reactors was nearly the same during the process (Table 1). The reactor with zeolite oxidized a higher amount of ammonia, and in turn, required more oxygen. This condition explains the higher DO concentration in the liquid bulk of the system without zeolite compared to the reactor with zeolite. Thus, the system with zeolite maintained the AOB activity at a high level, displaying better performance for partial nitrification.

In this work, the values of α and β were similar to other studies using zeolite as a biomass carrier. Yang et al. [11] used a zeolite biological aerated filter (ZBAF), obtaining a high nitrite accumulation (98%) under optimal operational conditions. However, the ammonia oxidation was lower than the values achieved in this study, most likely due to higher NLR (between 0.760 to 1.200 kg/m^3 d). Finally, Yang et al. [11] also reported ammonia removed around 68% at NLR = 0.760 kg/m^3 d, where high NLR impaired ammonia conversion. Thus, the presence of zeolite even at low concentrations (5 g/L) in an SMBBR system allowed a higher AOB activity at low oxygen levels and pH values close to 7, keeping a nitrite accumulation around 60%. The main difference between the system with zeolite and without zeolite was the ammonia oxidation efficiency, which was 40% higher than the control system (without zeolite), a condition explained by the zeolite bio-regeneration capacity in the process even at low zeolite concentration.

3.2. *Effect of Sulfide Pulse on Partial Nitrification in the Systems with and without Zeolite*

Once the effect of oxygen on the systems with and without zeolite was clarified, we next evaluated the impact of sulfide pulse on the PN process. We provided sulfide during the anoxic step (without aeration) according to the methodology proposed by Erguder et al. [4] to inhibit the NOB activity. The results are shown in Figure 2. TAN, nitrite and nitrate concentrations obtained in the system with and without zeolite are shown in Supplementary data (Table S6).

In both reactors, with and without zeolite, nearly 60% of ammonia was oxidized (Figure 2A). Thus, the presence of zeolite did not keep higher AOB activity than the system without zeolite, although the system's performance with zeolite was more stable. In a previous work [15], we observed that zeolite acting as a biomass carrier sustained the AOB activity in the presence of sulfide and organic matter. The main difference between both processes was how sulfide was added to the system (Table 2). In this work, sulfide was supplied in the anoxic phase. Huiliñir et al. [15] added sulfide at the beginning of the aerobic step, thus oxidized rapidly, avoiding the inhibitory effect to the AOB. As seen in Table 2, the sulfide pulse in the anoxic step exclusively suppresses NOB activity. Meanwhile, sulfide is provided in the aerobic step to the systems aimed at simultaneous sulfide oxidation and nitrification.

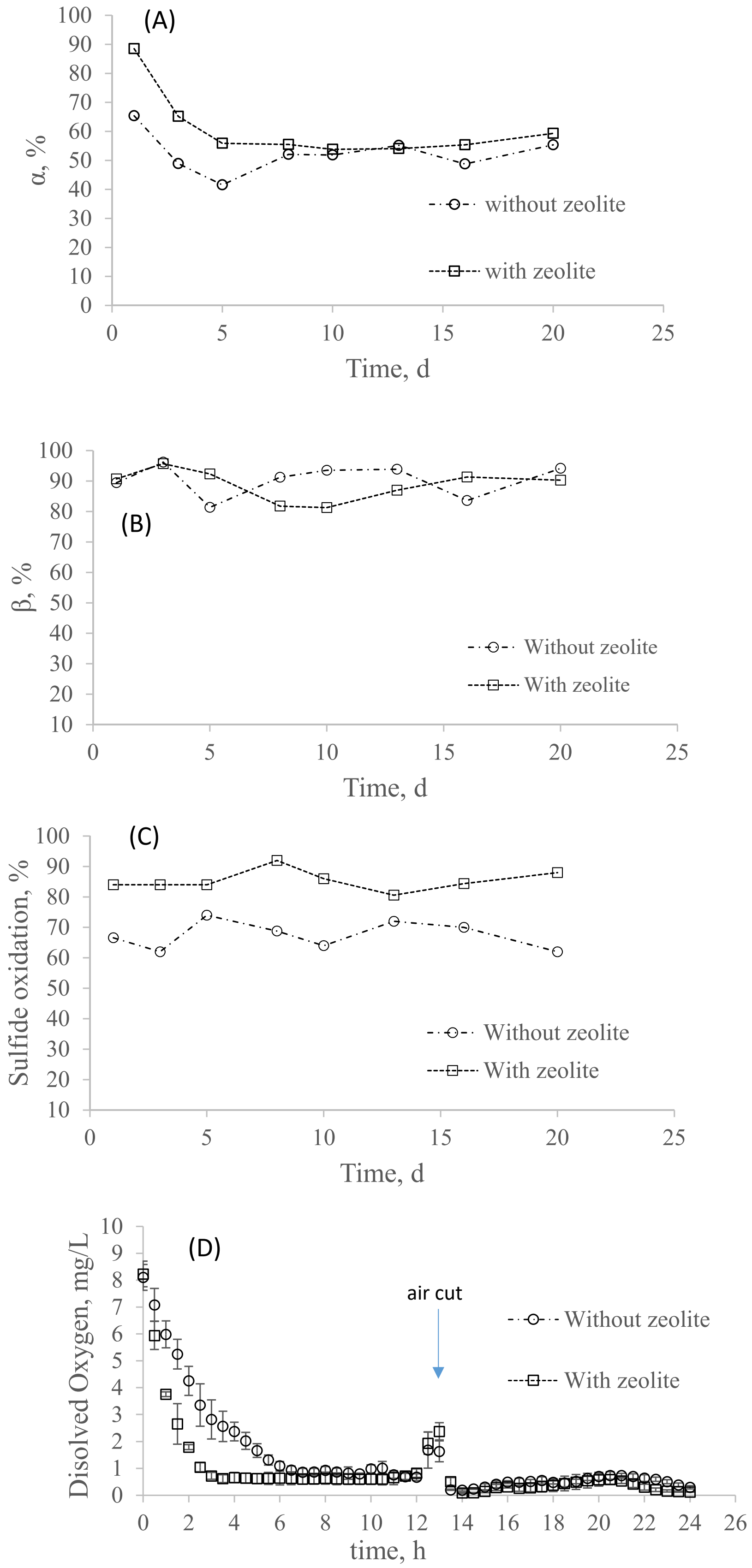


Figure 2. Performance of the systems with and without zeolite at 0.1 vvm, HS^- = 5 mg/L and cycles with 12/12 h aerobic/anoxic. (**A**) Efficiency in TAN oxidizing; (**B**) nitrite accumulation; (**C**) sulfide oxidation efficiency; (**D**) dissolved oxygen (DO) during one cycle.

Another aspect that could explain the strong effect of sulfide on the system, even in the presence of zeolite, is the exposure time. Here, the exposure time (12 h in anoxic conditions) could explain why the AOB was affected by sulfide, despite the possible biofilm formation on the zeolite and higher TAN degradation by the zeolite bio-regeneration capacity as reported in our previous work [15]. Inline, Seuntjens et al. [23] and Zhou et al. [24] indicated that an increase in the anaerobic contact time between microorganisms and sulfide from 1 to 4 h with 10 mg S/L increased the inhibition from 50% to 91%. Furthermore, Seuntjens et al. [23] indicated that the ration mg S^-/g VSS can also affect the performance of the nitrifying biomass. Ratios above 20 mg S^-/g VSS can reduce the AOB and NOB activity by more than 50%. In our case, the ratio mg S^-/g VSS was 3.48 for the system with zeolite and 3.92 for the system without zeolite. These ratios can cause a 30% inhibition on the AOB and NOB activities [23], which agrees with our results where the AOB activity diminished by 40–50%, even in the presence of zeolite.

Another possible reason for the high inhibition of AOB could be the adsorption of HS^- on the zeolite, a situation that was observed by Moradi et al. [25]. This adsorption on the zeolite's surface maintains the sulfide in the system, which could keep its inhibitory effect on the AOB. However, the sulfide oxidation in this system was higher than 80% (see Figure 2C). Thus, a high contact time between AOB and sulfide, together with the sulfide adsorption on the zeolite, could explain the similar behaviors between systems with and without zeolite.

Table 2. Comparison of different HS^- dosage strategies.

Authors	HS^- Concentration, mg/L	Phase of Dosage	Exposition Time, h	Process Studied
This work	5–10	anoxic	12	Partial nitrification
Seuntjenset al. [23]	0–600	anoxic	1 h; 2 days	Partial nitrification
Erguder et al. [4]	1.3–80	anoxic	23	Partial nitrification
Huiliñir et al. [15]	5–10	aerobic	3	Simultaneous nitrification/sulfide oxidation
Ortiz et al. [26]	2.5–20	aerobic	Not reported	Simultaneous nitrification/sulfide oxidation
Cueto et al. [27]	600	aerobic	Not reported	Simultaneous nitrification/sulfide oxidation
Delgado-Vela et al. [6]	2–35	aerobic	Not reported	Simultaneous nitrification/sulfide oxidation
Sekine et al. [28]	96	aerobic	Not reported	Simultaneous nitrification/sulfide oxidation

Accumulation of nitrite is displayed in Figure 2B. A clear improvement in nitrite accumulation was observed in both systems, with and without zeolite, compared to the system without the sulfide pulse (Figure 1B). On average, both systems accumulated nitrite to 85% under simultaneous effects of NOB sulfide inhibition and the low DO concentrations (Figure 2D). Further, the reactor without zeolite had an average pH value of 7.72, while we recorded a pH of 7.56 in the reactor with zeolite (see Figure S1, supplementary data). These values were higher than those obtained in the system without sulfide (Figure 1), which also enhanced nitrite accumulation performance under this alkaline condition. Improvement in nitrite accumulation using sulfide in the anoxic step was also reported by other authors [4,6,23] and it is based on the higher sensitivity of NOB to the sulfide. Furthermore, according to Seuntjens et al. [23], the S^-/VSS ratio established in condition 1 (Table 1) also explains the increased nitrite accumulation because the percentage of inhibition was consistently higher in the NOB under this ratio.

Figure 2C shows the sulfide oxidation to sulfate over 20 days of operation in an SBBR, where higher sulfide oxidation in the system with zeolite is observed, with values around

88%, compared to the system without zeolite (68%). The better performance of the system with zeolite compared to the system with no zeolite could be explained by more significant colonization of Sulfur Oxidizing Bacteria (SOB) in the system with zeolite. Several works have shown a growth of SOB in nitrifying systems with sulfide, even at low dissolved oxygen concentrations. Because SOB has a higher affinity for O_2 than AOB [27], with a $K_S = 0.15$ mg O_2/L [29], SOB can oxidize sulfide during the first period without aeration (DO < 0.5 mg/L).

On the other hand, Kang et al. [30] showed that sulfide oxidation decreases the pH values in the liquid phase. The latter agrees with our experimental results, since the main pH value in the reactor with zeolite (7.55) was lower than the system without zeolite (7.72). Furthermore, several works [23,26,31] have also indicated that sulfur-oxidizing activity in nitrifying sludge could be high because SOB can grow even without sulfide by using organic carbon or ferrous iron as electron donors. Finally, it is essential to note that autotrophic denitrification with sulfide as an electron donor was unlikely because the mass balance was closed among nitrogen species (see supplementary data, Tables S7 and S8).

The profile of dissolved oxygen concentration of an SBBR cycle operated under sulfide dosing is shown in Figure 2D. The DO concentration decreased steeply in the zeolite system, where the oxygen dropped during the first three hours. On the other hand, in the reactor without zeolite, the DO reached values lower than 1 mg/L just after 6 h. The faster depletion of DO in the system with zeolite also can be related to the VSS concentration, as shown in Table 1 (condition 1). The VSS increased by 37.3% during the experiment (20 days) in the system with zeolite, while the system without zeolite, it was only 18.6%. As shown in Figure 2D, after 12 h, there was an increase in DO concentration. This situation can be attributed to the decrease in the oxygen uptake rate (OUR), which depends on the substrate concentration available. In this case, at the end of the aerobic batch step, the concentrations of TAN, sulfide and nitrite were lower than at the beginning of the assays, thus decreasing the oxygen demand and the OUR, which is a situation that is derived from an increase in the oxygen concentration in the liquid bulk. After the aeration was stopped (13 h), the oxygen concentration depleted again, achieving values lower than 0.5 mg/L in both SBBR. This drop in DO concentration can be related to the SOB activity during the anoxic period at low sulfide concentration.

Figure 3 shows the effect of 10 mg/L HS^- on the partial nitrification (condition 2), using a higher vvm (0.5) in comparison to condition 1 (0.1 vvm) in order to prevent a complete inhibition of the system at this level of sulfide. TAN, nitrite and nitrate concentrations obtained in the system with and without zeolite are shown in Supplementary data (Table S9). As shown in Figure 3A, a substantial depletion of ammonia oxidation (α) was observed during the two first days in both systems (significantly different, p-value < 0.022). Afterward, the TAN oxidation was partially recovered, mainly in the system with zeolite, up to $\alpha = 40\%$ between days 3 and 6. The higher depletion of sulfide in the bioreactor with zeolite explained the AOB resistance to the sulfide inhibition in the system with zeolite (Figure 3B). This was also observed in our previous study [15]. However, from the 6th day onward, the high sulfide concentration affected the system with zeolite, with α dropping to values below 1% after 10 days of operation. This decrease of AOB could be related to the drop in the VSS concentration. Because the VSS decreased in both systems (Table 1), the S^-/VSS ratio increased from 6.7 to 13–14 mg S^-/g VSS (Table 1), negatively affecting AOB activity because higher S^-/VSS ratios imply higher inhibition on both AOB and NOB [23].

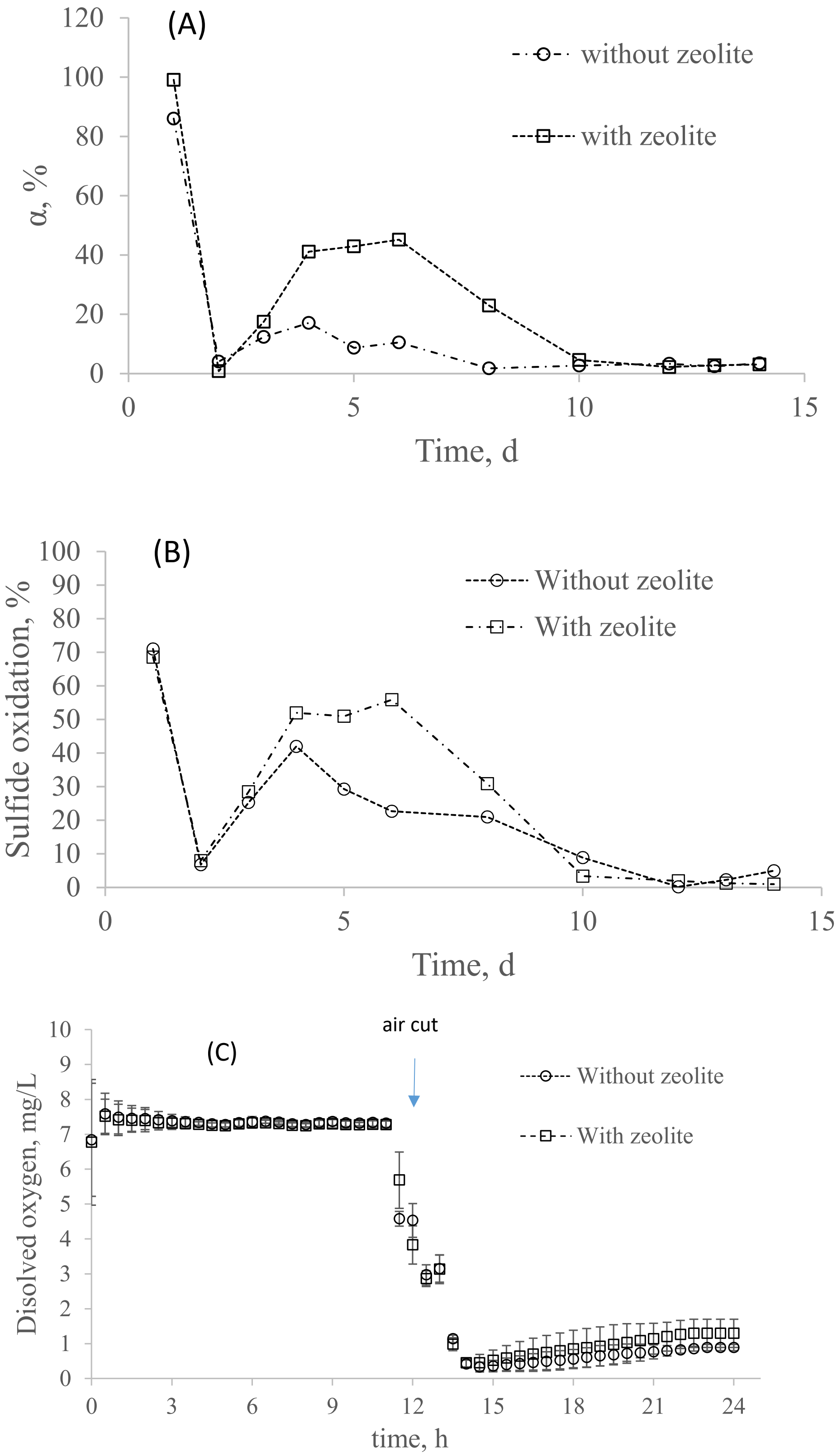


Figure 3. Performance of the systems with and without zeolite at 0.5 vvm, HS^{-} = 10 mg/L and cycles with 12/12 h aerobic/anoxic. (**A**) Efficiency in TAN oxidizing; (**B**) sulfide oxidation; (**C**) dissolved oxygen (DO) during one cycle.

Within the reactor without zeolite, the AOB could only degrade up to 20% of the available TAN, showing a similar trend to the system with zeolite. Both systems led to nitrite accumulation because of sulfide inhibition and a very high pH value in the liquid bulk (around 9.2 in both systems). However, the oxygen concentrations were kept close to 7.5 mg/L in the aerobic step for both bioreactors. High pH values affect the equilibrium NH_3/NH_4^+, increasing the NH_3 presence and causing the inhibition of NOB and AOB by ammonia [32].

Figure 3B presents sulfide oxidation over time, showing similar TAN oxidation rates. The statistical analysis has also shown statistically significant differences between the sulfide oxidation in both systems. The system with zeolite oxidized 50% of sulfide for two days (day 5 to 7), but a sharp decrease in sulfide oxidation was observed. The low sulfide oxidation, even at higher DO concentration values (Figure 3C), can be explained by the sulfide concentration added and the anoxic period of exposure of all microorganisms to the sulfide for 12 h. Indeed, the higher sulfide concentration reduced the VSS in the system, which decreased by almost 50% in both systems (Table 1, condition 2). This behavior was also observed by Sekine et al. [31], describing a decrease in the VSS of around 50% at the threshold sulfide concentration. Thus, we confirm that a high sulfide concentration causes deflocculation of the biomass and a loss of microbial activity [31].

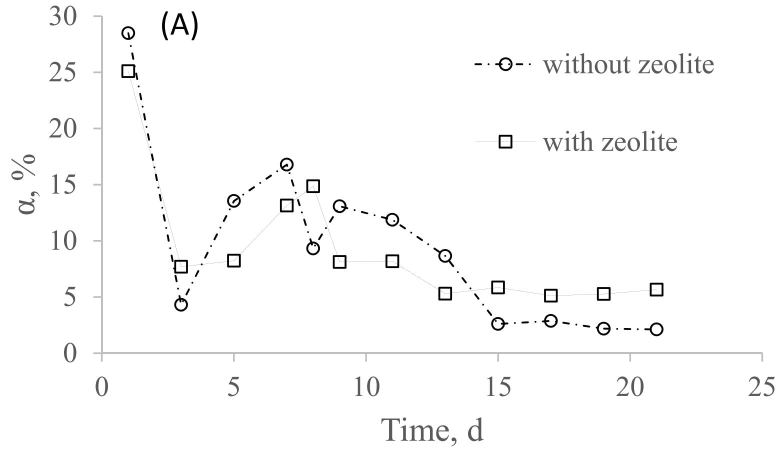
The high oxygen concentrations observed during the aerobic step under this condition (Figure 3C) of operation reflect an inactivation in microorganism activity. After the air-off (anoxic step), the DO concentration decreased to values close to 1 mg/L. Thus, low DO consumption can be attributed to the decrease in VSS concentration in both systems of around 50%.

In general, these results agreed with other studies about nitrification and partial nitrification in the presence of sulfide. Briefly, with an increase of sulfide in the liquid phase, the system cannot remove it and pH increases to values higher than 8.5, inducing a strong inhibition in nitrification [4,5]. Avoiding pH values higher than 9 by implementing a pH control system could help avoid inhibition for nitrifying bacteria and reach better ammonia oxidation.

3.3. Simultaneous Effect of Sulfide and COD on Partial Nitrification in the System with and without Zeolite

After studying sulfide as an enhancing factor for nitrite accumulation, the addition of organic matter as a disturbance, together with the action of sulfide as an inhibitor of NOB, were evaluated. However, before studying this condition, the recovery of microbial activity of the system with and without zeolite was carried out over 25 days (Figures S2 and S3 in supplementary data). The system with zeolite recovered the ammonia oxidation at percentages higher than 80% in 10 days, while the system without zeolite recovered its capacity of ammonia oxidation to values higher than 80% after 20 days. Thus, zeolite enabled a faster recovery of the AOB activity, a condition attributed to the ionic exchange capacity of the zeolite, which adsorbs the ammonia and makes it available for the AOB growth to affect the biofilm on the zeolite surface. This fact was also reported by other researchers in nitrifying systems [10,33–35]. A similar situation was observed with the NOB activity, where the system with zeolite recovered 100% of its capacity of nitrite oxidation after 20 days, while the system without zeolite could not completely recover its nitrite oxidation capacity (70%).

Simultaneous effects of organic matter and sulfide on partial nitrification in both systems (with and without zeolite) are presented in Figure 4. TAN, nitrite and nitrate concentrations obtained in the system with and without zeolite are shown in Supplementary data (Table S10). Regarding the ammonia oxidation (Figure 4A), both systems decreased their AOB activity without statistically significant differences between them, even though the S^-/VSS ratio was around 9.5 mg S^-/g VSS (Table 1). Values of mg S^-/g VSS ratio lower than 20 mg S^-/g VSS should decrease AOB by 20% [15]. However, in our case, the inhibition was much more potent, which could be related to the low biomass concentration and organic matter that activated the heterotrophic biomass. After 25 days of operation, the system with zeolite presented slightly higher ammonia oxidation with an average value of 5.5%. In the case of the system without zeolite, the activity of AOB was even lower than the system with zeolite after 15 days, with α lower than 3%. It is important to mention that nitrite accumulation (β) was higher than 80% in both systems (data not shown), so the effect of sulfide and organic matter also strongly inhibited the NOB activity. The low activity of nitrifying microorganisms can be confirmed by the low oxygen consumption during the aerobic phase (Figure 4B), keeping very high values of DO concentration (6–7 mg/L). When the aeration was cut, the DO concentration decreased to values close to 0.1 mg/L during the entire anoxic phase (from hour 12 onward), a situation that could explain the deficient sulfide oxidation (Figure 4C). The low activity of microorganisms was also observed in the decrease of VSS under this condition (Table 1), whose values fell around 35% in both systems, showing that the nitrifying biomass is very sensitive to sulfide, even though it was subjected to sulfide inhibition earlier (condition 1 and condition 2). It is important to note that the initial VSS concentration in this experiment (in the presence of organic matter and sulfide simultaneously) was lower by 20% than both previous conditions (Table 1). This fact could also affect the rate of biodegradation in the systems. The difficulty of nitrifying biomass to acclimate to conditions with sulfide was observed by Moraes et al. [15]. Furthermore, Sekine et al. [31] indicated that the maximum tolerance of nitrifying biomass to sulfide did not increase with the exposure time of biomass to the sulfide. However, other studies showed that it was possible to simultaneously degrade sulfide and TAN [26], a situation attributed to the different microorganisms presented in those cultures [6] and the step where the sulfide pulse is added, as shown in Table 2. The simultaneous nitrification and sulfide oxidation is attainable when the sulfide pulse is added in the aerobic step, while the addition in the anoxic step produces higher exposition times and, therefore, higher inhibition in nitrifying microorganisms (Table 2).



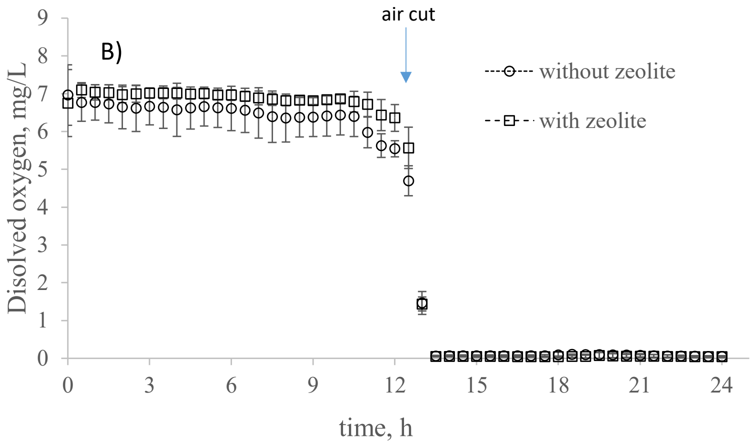


Figure 4. *Cont.*

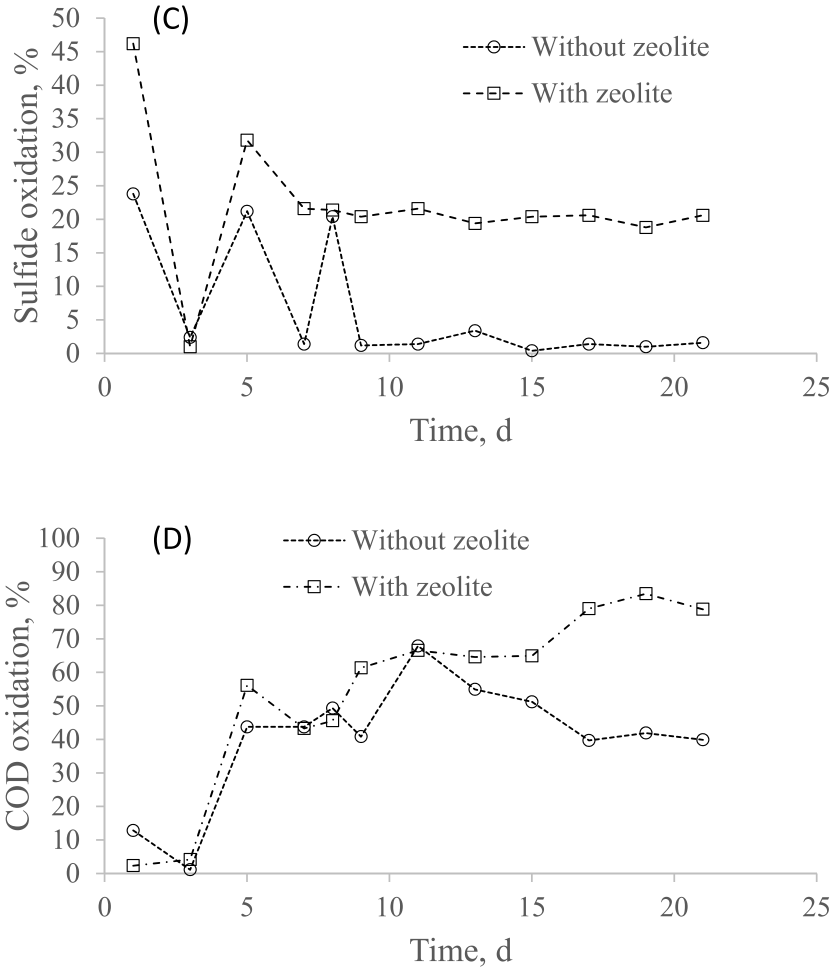


Figure 4. Performance of the systems with and without zeolite at 0.5 vvm, COD concentration = 350 mg/L, HS^- = 5 mg/L and cycles with 12/12 h aerobic/anoxic. (**A**) Efficiency in TAN oxidizing; (**B**) dissolved oxygen (DO) during one cycle; (**C**) sulfide oxidation efficiency; (**D**) organic matter consumption.

Regarding the presence of organic matter, it could also affect the lower TAN oxidation. Wang et al. [13] reported that AOB activity could decrease by low C/N ratios (<2), even at DO higher than 6 mg/L in biofilm systems, because the low autotrophic growth reduces the biofilm thickness and produces detachment of the biofilm. Thus, part of AOB could detach from the biofilm and the TAN capacity can decrease.

Figure 4C shows the different sulfide oxidations obtained in both process at COD concentration = 350 mg/L and HS^- = 5 mg/L. None of the systems could oxidize the sulfide completely; however, the system with zeolite was able to degrade a higher percentage of sulfide (around 20%) in comparison to the system without zeolite, which could not degrade the sulfide after 9 days. The statistical analysis has also shown statistically significant differences between the sulfide oxidation in both systems. As mentioned before, the higher sulfide oxidation in the system with zeolite could be related to the SOB activity. Because the presence of sulfate was observed in the system with zeolite, there was oxidation of sulfide to sulfate. This oxidation should be closely related to SOB activity, even though the VSS concentration was only 0.8 g/L. Furthermore, the lower concentration of nitrite and nitrate could avoid the denitrification process as a possible degrading process of sulfide. The non-oxidation of sulfide in the system also increased the pH values during the operation of the bioreactors, whose values in both systems were around 8.5 on average, which could also affect the equilibrium NH_3/NH_4^+ and decrease the AOB activity due to free ammonia.

The consumption of organic matter is presented in Figure 4D. In general, the system with zeolite obtained higher COD consumption, especially after the 12th day of operation. The *t*-test indicated that there are statistical differences between these two systems (with and without zeolite). However, COD degradation did not increase the VSS concentration in both systems (Table 2). The higher COD degradation in the system with zeolite could be attributed to the biofilm formation on the zeolite, allowing the growth of different types of microorganisms depending on the environmental conditions. According to the literature, biofilm systems with a C/N ratio higher than 0.5 allowed for the growth of heterotrophic biomass [22], while systems with suspended systems allowed for the heterotrophic biomass with a C/N ratio higher than 1.0. Under the condition studied, the C/N ratio was higher than 1.5 in both systems, allowing the growth of heterotrophic biomass at a low oxygen uptake rate [22].

Therefore, according to these results, the bioreactor that used zeolite removed more sulfide and organic matter under this condition; however, no differences were observed in nitrifying activity.

3.4. Recommendations and Shortcomings of the Proposed System

According to this study, the use of zeolite and a pulse of sulfide is recommended to obtain a PN together with the sulfide oxidation during the process. A key aspect that requires more study is the time of exposure of the nitrifying biomass to sulfide to obtain a condition that could inhibit only the NOB and keep the activity of AOB. In our work, we used 12 h of exposure, a situation that decreased both AOB and NOB activities; therefore, a shorter exposure time should be used in further studies. Regarding the simultaneous presence of organic matter and the sulfide pulse, it is clear that this condition decreased the PN strongly, despite the presence of an improver such as the natural zeolite. Therefore, it is recommended to eliminate organic matter before the nitrifying bioreactor to decrease its negative effect on PN with a sulfide pulse.

4. Conclusions

- At low dissolved oxygen concentration (dissolved oxygen < 2.0 mg/L; vvm = 0.1), the system with zeolite was able to have higher ammonia oxidation; however, the nitrite accumulation (β) obtained similar values between the system with and without zeolite;
- The presence of sulfide improved the nitrite accumulation in both systems, with and without zeolite at HS^- = 5 mg/L, where the system with zeolite was able to oxidize a higher percentage of sulfide. At HS^- = 10 mg/L, both systems were strongly inhibited, although the system with zeolite was able to keep its activity longer; and
- The presence of sulfide and organic matter completely inhibited the nitrification process, with low nitrifying bacteria remaining active. Nevertheless, the system with zeolite was able to degrade higher percentages of organic matter and sulfide.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13182484/s1, Figure S1: Outlet pH values of both systems at different conditions; Figure S2: TAN efficiency (%) of both systems during the recovery of nitrifying activity step; Figure S3: Nitrite accumulation (%) in both systems during the recovery of nitrifying activity step; Table S1: Characterization of inoculum from an industrial aerobic activated sludge reactor; Table S2: Chemical and mineral composition of the zeolites used in all the assays; Table S3: TAN, nitrite and nitrate concentrations obtained in the system with and without zeolite (without sulfide and organic matter); Table S4: Mass balance in the partial nitrification process without zeolite; Table S5: Mass balance in the partial nitrification process with zeolite; Table S6: TAN, nitrite and nitrate concentrations obtained in the systems with and without zeolite at 0.1 vvm and HS^- = 5 mg/L; Table S7: Mass balance in the systems with zeolite at 0.1 vvm, HS^- = 5 mg/L and cycles with 12 h/12 h aerobic/anoxic; Table S8: Mass balance in the systems with zeolite at 0.1 vvm, HS^- = 5 mg/L and cycles with 12 h/12 h aerobic/anoxic; Table S9: TAN, nitrite and nitrate concentrations obtained in the systems with and without zeolite at 0.5 vvm and HS^- = 10 mg/L; Table S10: TAN, nitrite and nitrate concentrations obtained in the systems with and without zeolite at 0.5 vvm, COD = 350 mg/L and HS^- = 5 mg/L.

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