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Removal of Ibuprofen and Diclofenac in Batch Nitrifying Reactors: Effect of Natural Zeolite on the Process

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Abstract: Ibuprofen (IBP) and diclofenac (DFC) are two of the most commonly used non-steroidal anti-inflammatory drugs (NSAIDs) to treat inflammation and pain. However, they can impact the environment if not treated adequately before discharge into waterways. Biodegradation through the nitrification process is an alternative to reducing the concentration of these micropollutants (MPs) in wastewater. Thus, this work aimed to evaluate the effect of natural zeolite on IBP and DFC removal in a nitrifying batch reactor. Mini-reactors were set up with 90 mL of inoculum and 110 mL of synthetic wastewater with a concentration of 25 mg total ammonia nitrogen TAN/L, at 25 °C and 1 vvm (volume of air/volume liquid·min) of aeration. Two conditions were tested: high concentrations (IBP = 700 µg/L, DFC = 100 µg/L) and low concentrations (IBP = 30 µg/L, DFC = 20 µg/L). The research used a concentration of 5 g/L of the natural zeolite. Results indicated that the zeolite negatively affected the nitrification rate. At high MPs concentration, the natural zeolite negatively affects the removal of IBP and DFC, where biodegradation and sorption are the mechanisms that eliminate both NSAIDs. Conversely, at low DFC and IBP concentrations, the natural zeolite improves the removal of IBP and DFC, wherein biodegradation is the primary removal mechanism.

Keywords: diclofenac; ibuprofen; kinetic; nitrification; zeolite

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1. Introduction

In recent decades, non-regulated emerging micro-pollutants (MPs) in aquatic systems have raised increasing international concerns because of their persistent, low biodegradability, bioaccumulative and toxic nature [1]. Society uses MPs non-steroidal anti-inflammatory drugs (NSAIDs) as inflammatory and pain killers worldwide [2,3]. Among the most widely used therapeutic agents worldwide, it highlights ibuprofen (IBP) and diclofenac (DFC) [3]. In wastewater and drinking water sources, IBP concentrations range from 3.7 to 603.0 µg/L [3,4]. Meanwhile, DFC has one of the highest acute toxicity rates [3], with concentrations ranging from 47.1 µg/L to mg/L level [5]. Pharmaceutical compounds can reach various watercourses, including drinking water, through different ways, starting with human and animal excretions [6]. Although wastewater plants can eliminate some pharmaceutical compounds, they can enter the environment through wastewater plant discharges, where most wastewater plants have not been designed to eliminate these contaminants. The accumulation of micropollutants can damage the environment and the health of people and various species, and pharmaceutical products in aquatic systems can cause ecotoxicity through synergistic interactions [7]. Ibuprofen is potentially risky for aquatic ecosystems because it can bioaccumulate, has an organic matter adsorption capacity, and has high water solubility [8]. Also, some toxic effects can provoke trace concentrations of ibuprofen in plants; for example, researchers have reported adverse effects on plant growth, considerable reductions in shoots and roots, reduced mineral uptake, and cell damage in various species [9]. On the other hand, diclofenac exerts some adverse effects in plants associated with this micropollutant. For instance, exposure to this micropollutant for *Solanum lycopersicum* (tomato plant) provokes an increment in sugar level and a reduction in the shoot size [9]. Moreover, for *Lactuca sativa* (lettuce), researchers observed a negative effect on gene expression with two days of diclofenac exposure, where this micropollutant generates significant stress in the plant [10]. The two plants presented above are highly consumed in the human diet worldwide; therefore, it is an example of how relevant it is to study methods to ensure an adequate micropollutant efficiency elimination in wastewater discharges to avoid a negative impact on ecosystems and human health.

Biodegradation remains one of the best options to remove MPs because of its low cost and the potential for complete destruction [1]. Due to nitrifying bacteria's broad metabolic activity, e.g., ammonia-oxidizing bacteria (AOB), MPs removal is positively associated with high ammonium oxidizing activities reached during nitrification. Its non-specific enzyme ammonia monooxygenase (AMO) can degrade various MPs compounds through cometabolic biodegradation [11]. IBP is known for its high biodegradability in aerobic mixed cultures, such as nitrifying cultures in activated sludges or biofilm systems. In contrast, DFC has low or medium biodegradability depending on the system used. Several investigations have studied the IBP and DFC together or separately in nitrifying reactors. For instance, the fate of IBP in systems with suspended biomass indicated that biodegradation was the primary removal route of IBP in the activated sludge (AS) process [12,13], with a small amount of IBP removed via adsorption onto the biological sludge [14]. Conversely, conventional activated sludge processes showed that DFC was more resistant to biodegradation, although certain situations under aerobic nitrifying conditions enabled biodegradation to be observed [15]. The sorption of DCF to sludge is negligible in most studies [15,16]; however, it can still be an effective mechanism of DCF removal in some situations [17], especially using sorbent materials.

Due to the MP destruction depending on the ammonia oxidation rate performed by AOB, it is essential to use a system that allows a good performance of the AOB metabolism. Using immobilized biomass on an inert support such as zeolite may help to retain the nitrifying biomass and improve the complete nitrification, increasing the activity of the AMO enzyme. Several studies have reported that natural zeolite improves the microbial activity of anaerobic digestion, denitrification, and nitrification processes [18]. Furthermore, natural zeolite is effective in adsorbing MPs, where researchers have applied it to the sorption of DFC, IBP, and other types of MPs [19,20]. Using natural Jordanian zeolite, Al-rimawi et al. [21] removed ibuprofen and diclofenac, among other MPs, from aqueous solutions at high concentrations (order of mg/L). Subsequently, Karthik and Philip [22] used natural zeolite from India to investigate the sorption of carbamazepine (CBZ), DFC, IBP, and nutrients, in wastewater. They concluded that the presence of organics and nutrients adversely affected the sorption of MPs on zeolite, showing that DFC has more affinity to sorption, followed by IBP and CBZ, based on their hydrophobicity [22]. Thus, zeolite has the potential not only for improving microbial activity but also for the sorption of MPs. However, despite these advantages, there are no studies regarding the effect of zeolite on the nitrification process for MPs degradation. Therefore, the present research aims to investigate the degradation of two MPs, specifically ibuprofen (IBP) and diclofenac (DCF), by nitrification in the presence of natural zeolite.

2. Materials and Methods

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 previously described in our studies [23,24]. This CSTR with settler has been operated for three years. The operational conditions are solid retention time (SRT) = 60 d, hydraulic retention time (HRT) = 8 d, and temperature 25 °C, with an aeration rate of 0.5 vvm (air volume per liquid volume per minute, L of air L^{-1} of liquid min^{-1}). We obtained the zeolite from a company commercializing natural zeolite in Quinamávida, Linares, VII Region, Maule, Chile. Its main chemical composition was [24]: SiO_2, 64.19%; Al_2O_3, 11.65%; Fe_2O_3, 2.53%; CaO, 3.42%; Na_2O, 0.75%; TiO_2, 0.51%; MgO, 0.66%; K_2O_2, 1.60%; and P_2O_5, 0.03%.

Determination of physicochemical parameters such as BET surface area, pore volume, and average pore diameter of natural zeolite was performed according to Ghampson et al. [25]. Briefly, the nitrogen adsorption–desorption isotherms were obtained at −196 °C using a Micromeritics 3 Flex instrument (Norcross, GA, USA). Before the measurements, the samples were degassed under a vacuum at 200 °C for 3 h. The BET-specific surface area was calculated from the adsorption using Rouquerol criteria [26], and the total pore volume was recorded at $P/P_0 = 0.99$. The distribution pore diameter was estimated via the desorption branch using the BJH method and average pore diameters were calculated from the equation $d_{pore} = 4V/A$.

2.2. Experimental Setup and Experimental Design

Eight mini-reactors were set up to investigate the degradation of DFC and IBP. Each reactor had 90 mL of inoculum and 110 mL of synthetic wastewater with a concentration of 25 mg TAN/L, in a thermoregulated bath at 25 °C and at 1 vvm (volume of air/volume liquid·min) of aeration. Mixing was performed through aeration, maintaining the substrates and solids homogeneously distributed inside the bioreactors. Bioreactors were sealed with rubber stoppers, each as an independent experimental unit sacrificed after sampling the liquid phase. The VSS (volatile suspended solid) concentration in each bioreactor varied between 0.9 and 1.1 g VSS/L. Huiliñir et al. [27] present more details about the mini-reactors. The IBP concentrations were 30 and 700 µg/L, while DCF concentrations were 10 and 100 µg/L, generating two experimental runs: 30 IBP-10 DFC (low MPs concentrations); 700 IBP-100 DFC (high MPs concentrations). The concentrations of DFC and IBP were obtained from the literature [28]. To investigate the effect that natural zeolite has on the biodegradation of IBP and DFC, we conducted experiments with a zeolite concentration of 5 [g/L], using five controls for each condition:

- Control 1: without zeolite and without biomass;
- Control 2: with zeolite and without biomass;
- Control 3: with nitrifying biomass and without zeolite;
- Control 4: with allylthiourea (ATU, 98%), nitrifying biomass, and without zeolite;
- Control 5: with ATU, nitrifying biomass, and zeolite.

Control 2 was used to study the sorption phenomena on zeolite, while controls 4 and 5 were used to study the sorption phenomena on biomass and zeolite.

2.3. Determination of Reaction Rates and Removal Efficiencies

The analysis of batch experimental data was performed by comparing the maximum specific substrate consumption rates, $\hat{q}$. All nitrogen species (NO_3^--N, NO_2^--N, and TAN) were expressed as total nitrogen. We obtained the TAN removal rate (r_{TAN}) from the maximum slope of the TAN vs. time plot. The maximum slope was obtained using the Microsoft Excel 2023 software, version 2211 (Microsoft® Excel® for Microsoft 365 MSO). To obtain the maximum specific rates ($\hat{q}$), the value of each maximum rate was divided by the average VSS concentration (X) in the reactor:

$$\hat{q}_{TAN} = \frac{r_{TAN}}{X} \tag{1}$$

The kinetic of DFC and IBP degradation was studied by pseudo-first-order kinetic, given by [29]:

$$\frac{dC}{dt} = -k_T \cdot C \cdot X \tag{2}$$

Equation (2) involves all the processes associated with MPs transformation, i.e., biodegradation and sorption. In addition, the kinetic (Equation (2)) was linearized by:

$$ln\left(\frac{C}{C_0}\right) = -k_T \cdot t \cdot X = -K \cdot t \tag{3}$$

where

$$K = k_T \cdot X \tag{4}$$

The parameters were obtained by Microsoft Excel 2023.

The removal efficiencies of TAN, IBP, and DFC were determined by:

$$Efficiency\ (\%) = \left(\frac{C_{initial} - C_{final}}{C_{initial}}\right) \cdot 100 \tag{5}$$

where $C_{initial}$ is the initial concentration of TAN, IBP, or DFC, and C_{final} is the concentration of TAN, IBP, or DFC at the end of the batch assay.

2.4. Chemical Analyses

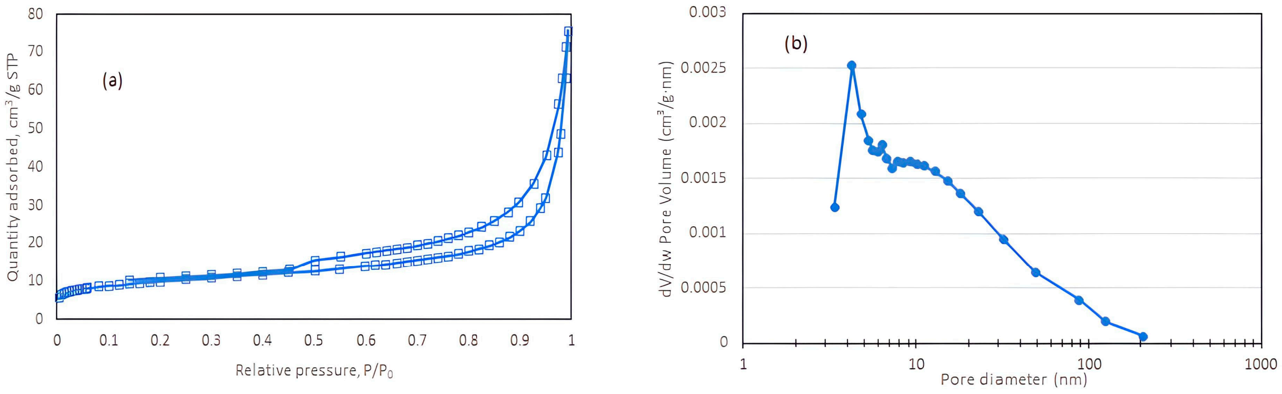
Ibuprofen (IBP), diclofenac (DFC), and allylthiourea (ATU) were purchased from Sigma-Aldrich company (San Luis, MO, USA). Total and suspended solids, VSS, DO, and pH were measured using standard methods [30]. TAN, NO_2^--N, and NO_3^--N were analyzed using flow injection analysis (Lachat's QuikChem® 8500 Series 2 Flow Injection Analysis System, Loveland, CO, USA). Total alkalinity was measured with the TitroLine® 7000 equipment from SI Analytics (Mainz, Germany). The dissolved oxygen (DO) concentration was measured by a dissolved oxygen meter (WTW, Multi 3620) equipped with a sensor FDO 925 IDS.

Diclofenac (DFC) and ibuprofen (IBP) were measured by high-resolution liquid chromatography coupled to a diode array detector (HPLC-DAD) using the Shimadzu Prominence equipment with a Purospher® STAR RP-18 column. Before using the HPLC, the concentration of the sample was increased 100 times using cartridges solid phase extraction (SPE) Chromabond® HLB. More details in Candia-Onfray et al. [31].

3. Results

3.1. Characteristics of Natural Zeolites (NZ) and Its Effect on Batch Nitrification

Figure 1 shows the N_2 adsorption–desorption isotherms and the BJH pore size distribution of the natural zeolite (NT) used in the study. According to Figure 1a, the N_2 isotherms are type IV according to IUPAC classifications [26]. It is interpreted as the NZ is mainly mesoporous with a small contribution of macroporosity (i.e., pores with sizes greater than 50 nm), such as shown in Figure 1b. At low P/P_0, there is a gradual curvature which indicates a significant overlap of monolayer coverage and the onset of multilayer adsorption [26]. From P/P_0 onward, the adsorption and desorption isotherms are separated, existing hysteresis at P/P_0 around 0.45. The NZ displayed a type H3 hysteresis [26], corresponding to the existence of non-rigid aggregates of plate-like particles.



(**a**) (**b**)

Figure 1. Morphology of samples under study: (**a**) N_2 adsorption–desorption isotherms of natural zeolites and (**b**) BJH pore size distributions of natural zeolite.

Figure 1b shows that NZ has an extensive pore size distribution, with a higher distribution around 10 nm. Indeed, the average pore diameter obtained was 12 nm (Table 1), which agrees with Figure 1b. The average pore diameter is very similar to the value presented by Karthik and Philip [22], who obtained an average pore diameter of 11 nm. Table 1 also shows the BET surface area and total pore volume obtained for NZ, these values being similar to the value presented by Karthik and Philip [22], who obtained a BET surface area of 33 m^2/g and a total pore volume of 0.0995 cm^3/g.

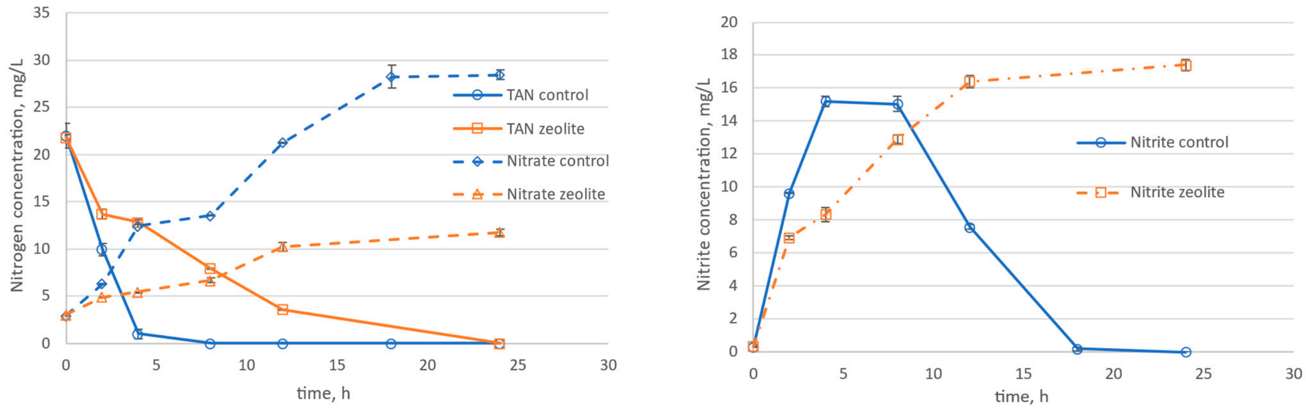
Table 1. Physicochemical characteristics of natural zeolites.

Properties	Value
BET surface area (m^2/g)	35
Total pore volume (cm^3/g)	0.11
Average pore diameter (4V/A by BET) (nm)	12

Thus, NZ has the typical characteristic of a mesoporous material. The possible adsorption can be attributed to the mesoporous, where capillary condensation (hysteresis) and multilayer/monolayer adsorption can be the adsorption mechanism.

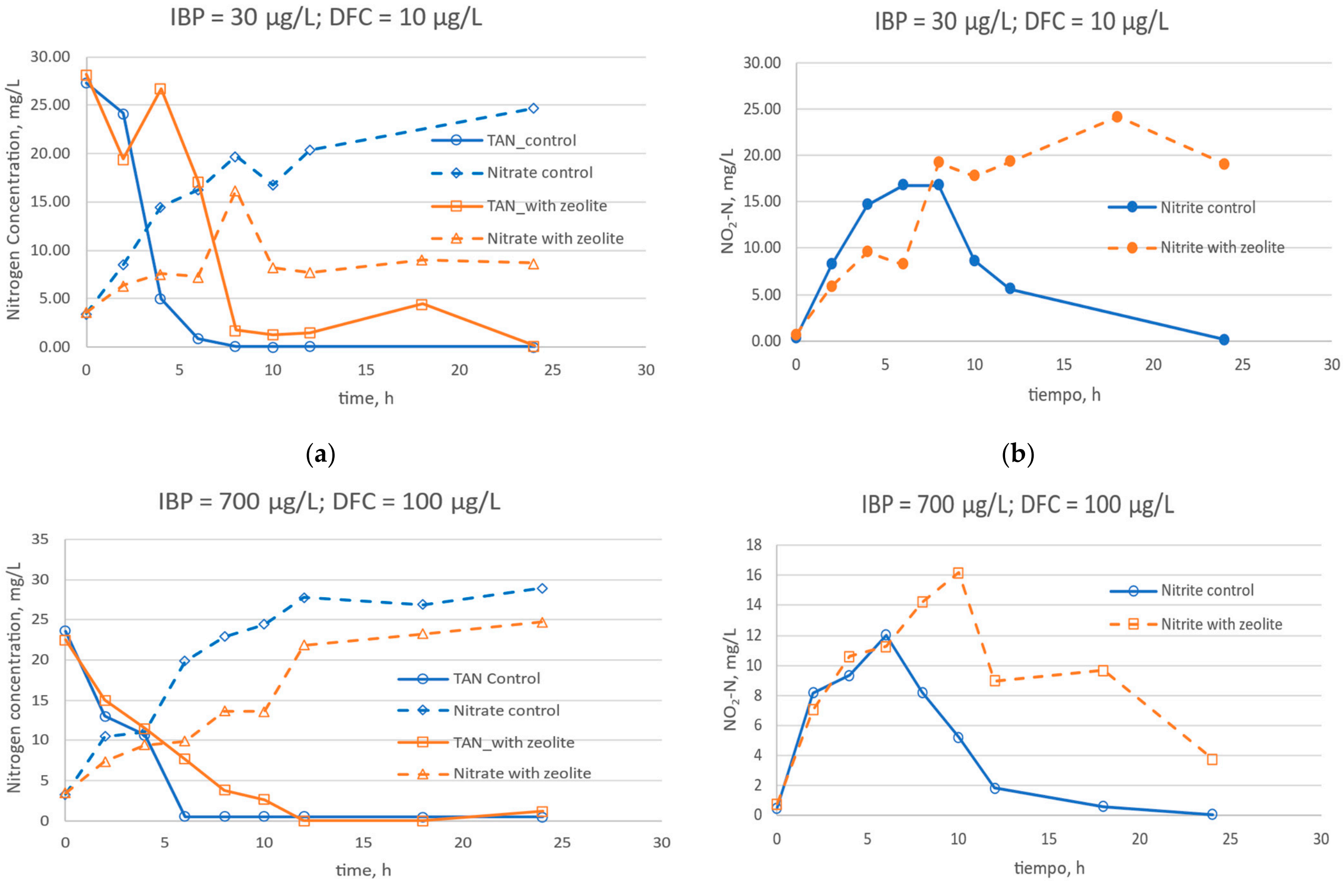
Figure 2 shows the effect of zeolite on the nitrification process without IBP and DFC. Using natural zeolite decreases the TAN removal rate in the system, generating a slower nitrite production rate (Figure 2b) and slower nitrogen transformation to nitrate (Figure 2a). In the control experiment, TAN concentration decreases quickly during the first 5 h of experiments, while the system with zeolite exhibited a slower removal rate, achieving only 87% removal after a 12 h assay period. This behavior was similar for nitrite, as illustrated in Figure 2b. Specifically, the absence of zeolite led to a rapid increase in nitrite concentration, followed by a swift removal that resulted in the total elimination of NO_2^- after 18 h. In contrast, nitrite in the presence of zeolite was considerably less degraded, giving rise to an accumulation of the compound in the bioreactor, with concentrations as high as 17 mg/L. So, zeolite affects AOB and NOB, decreasing the removal kinetic and efficiency. In addition, the slow rate of ammonia and nitrite in the system with zeolite affects the nitrate concentration (Figure 2a), getting a much higher concentration in the system without zeolite.

It was unexpected that zeolite had a detrimental effect on the nitrification process. This finding contradicts the conclusions drawn by earlier studies that investigated the impact of zeolite on this process. Indeed, Huiliñir et al. [27] demonstrated that zeolite improved the nitrification rate; however, this behavior was under inhibitory conditions, i.e., in the presence of sulfide and organic matter. One explanation can be related to the adsorption process on zeolite's surface, which can be faster than the biological reaction rate. This explanation was also indicated by Semmens and Goodrich [32], who identified that the adsorption process on the zeolite surface might be faster than the biological reaction rate, which may explain the phenomenon. Nevertheless, Figure 3 illustrates that zeolite did not sorb TAN because the TAN removal was lower than 20% in both controls (Figure 3f). Thus, we propose that, under these conditions (batch system, first interaction zeolite-biomass), zeolite prevented the biomass/substrate interaction, working as a barrier that slowed down the biological reaction.



(**a**) (**b**)

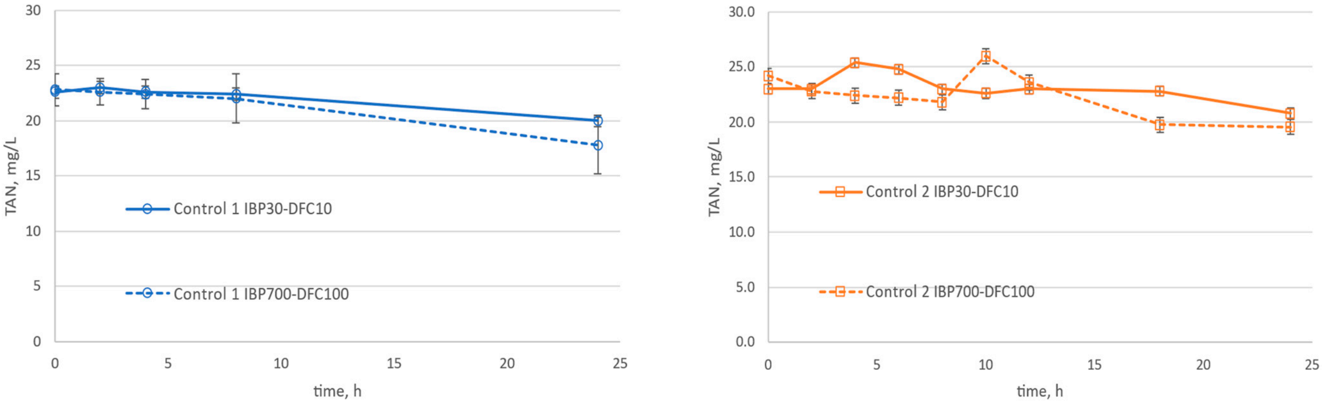
Figure 2. Nitrification in the presence of zeolite. (**a**) Variation of TAN and NO_3^-. (**b**) Variation of NO_2^-.



(**a**) (**b**)

(**c**) (**d**)

Figure 3. *Cont.*



(**e**) (**f**)

Figure 3. Nitrification in the presence of low and high IBP and DFC concentrations. (**a**) Variation of TAN and NO_3^- at IBP = 30 µg/L and DFC = 10 µg/L; (**b**) variation of NO_2^- at IBP = 50 µg/L and DFC = 10 µg/L; (**c**) variation of TAN and NO_3^- at IBP = 700 µg/L and DFC = 100 µg/L; (**d**) variation of NO_2^- at IBP = 700 µg/L and DFC = 100 µg/L; (**e**) TAN profile in control 1, i.e., without biomass and without zeolite; and (**f**) TAN profile in control 2, i.e., without biomass and with zeolite, for both MPs conditions.

3.2. Nitrification in the Presence of DFC, IBP, and Zeolite

Figure 3 shows the TAN, NO_2^-, and NO_3^- profiles obtained at different IBP and DFC concentrations, including 5 g/L of zeolite. The removal of TAN during the first 8 h in both systems, with and without zeolite, occurred at low IBP and DFC concentrations (Figure 3a,b). In the presence of zeolite, TAN removal started after 5 h of the experiment, which is later than the system without zeolite. The fast TAN removal generated a NO_2^- accumulation in both systems; however, the system with zeolite could not remove nitrite, with a low nitrate production. Instead, nitrite is accumulated and consumed in the bioreactor without zeolite, completing the nitrification and generating mainly NO_3^- as a product after 24 h. It is important to note that TAN was not removed in a control experiment in the presence of zeolite without biomass (Figure 3e), which dismisses that it can act as an adsorbing material for TAN under this condition (zeolite concentration of 5 g/L; TAN concentration of 25 mg/L), even though it has good characteristics for adsorption (see Figure 1b). According to these results, the NOB activity was the most affected by the presence of zeolite. As was mentioned before, under this condition, zeolite acts as a barrier to the contact between biomass and substrate. Thus, the contact between NO_2 and NOB could be diminished by zeolite presence, decreasing the nitrite removal rate. Other explanations could be the inhibition of NOB by free nitrous acid (FNA, HNO_2) [33]. The inhibitory concentrations of HNO_2 ranged between 0.22 and 2.8 mg HNO_2-N/L. However, in this research, the values are between $2.45 \cdot 10^{-7}$ and $1.8 \cdot 10^{-4}$ mg HNO_2-N/L, according to the chemical equilibrium [33]. Therefore, we discarded the inhibition of NOB by FNA. Finally, the inhibitory effect of MPs (IBP and DFC) on nitritation is discarded since, in the assay without zeolite, the removed rate of nitrite was like the control (see Table 2).

Table 2. Values of $\hat{q}$ at different conditions.

Condition	$\hat{q}$ (mg TAN/g VSS h)
Nitrification without MP and without zeolite	4.78 ± 0.404
Nitrification without MP and with zeolite	1.48 ± 0.414
Control 3, IBP30-DFC10	4.55 ± 1.105
Assay with zeolite IBP30-DFC10	2.81 ± 1.083
Control 3, IBP700-DFC100	3.69 ± 0.883
Assay with zeolite IBP700-DFC100	2.12 ± 0.523

The situation differed at high MP concentrations (Figure 3c,d). In both systems, with and without zeolite, the TAN removal rate was slower than the control (nitrifying reactors without MPs). In addition, there was a lower nitrite accumulation in both systems and a higher NO_2^- degradation in the bioreactor with zeolite compared to the control and the system with lower MPs concentrations (Figure 3b). Although nitrification was almost complete in the system with zeolite, the TAN removal rate was slower than in the system without zeolite, with the nitrite consumption starting a couple of hours later. This behavior confirms that zeolite could be a barrier that slows the biological reaction.

Regarding the effect of IBP and DFC on nitrification rates, Table 2 shows that IBP and DFC decrease the TAN removal rate, especially at high concentrations (IBP = 700 μg/L; DFC = 100 μg/L). Further, the zeolite decreases the TAN removal rate compared to a system without zeolite at both MPs concentrations studied. High concentrations of MPs affect the nitrification process; in the systems without zeolite, there is a 4.81% of decreasing in $\hat{q}$ at low MPs concerning the control and a 22.8% decrease at high MPs concentration. Several studies [28,34] reported a reduction of $\hat{q}$ at high MPs concentrations, which can be attributed to the competition between MPs and ammonia for binding with AMO enzyme [35,36]. Indeed, Melo et al. [37], working with a nitrifying SBR, showed that IBP concentration between 10,000 and 5000 μg/L inhibits SBR biomass activity, accumulating NO_2^- and decreasing NO_3^-, demonstrating that very high IBP concentrations disturb the biomass in the nitrification process. On the other hand, a DFC concentration of 100 μg/L has also shown a negative effect on nitrifying activity [38]; however, Zhao et al. [5] indicated that a DFC concentration of 0.01 mg/L (100 μg/L) does not affect nitrification efficiency. Therefore, we conclude that under the studied concentration of MPs (IBP 700 μg/L and DFC 100 μg/L), only IBP negatively affects nitrifying activity.

Table 2 also shows that the zeolite presence in the nitrification process with IBP and DFC decreases the $\hat{q}$ respect to a system without zeolite, 38.24% at low MPs concentration and 42.5% at high MPs concentration. This behavior can be attributed to the slower contact between microorganisms and substrates because of the inorganic material (zeolite) in the system.

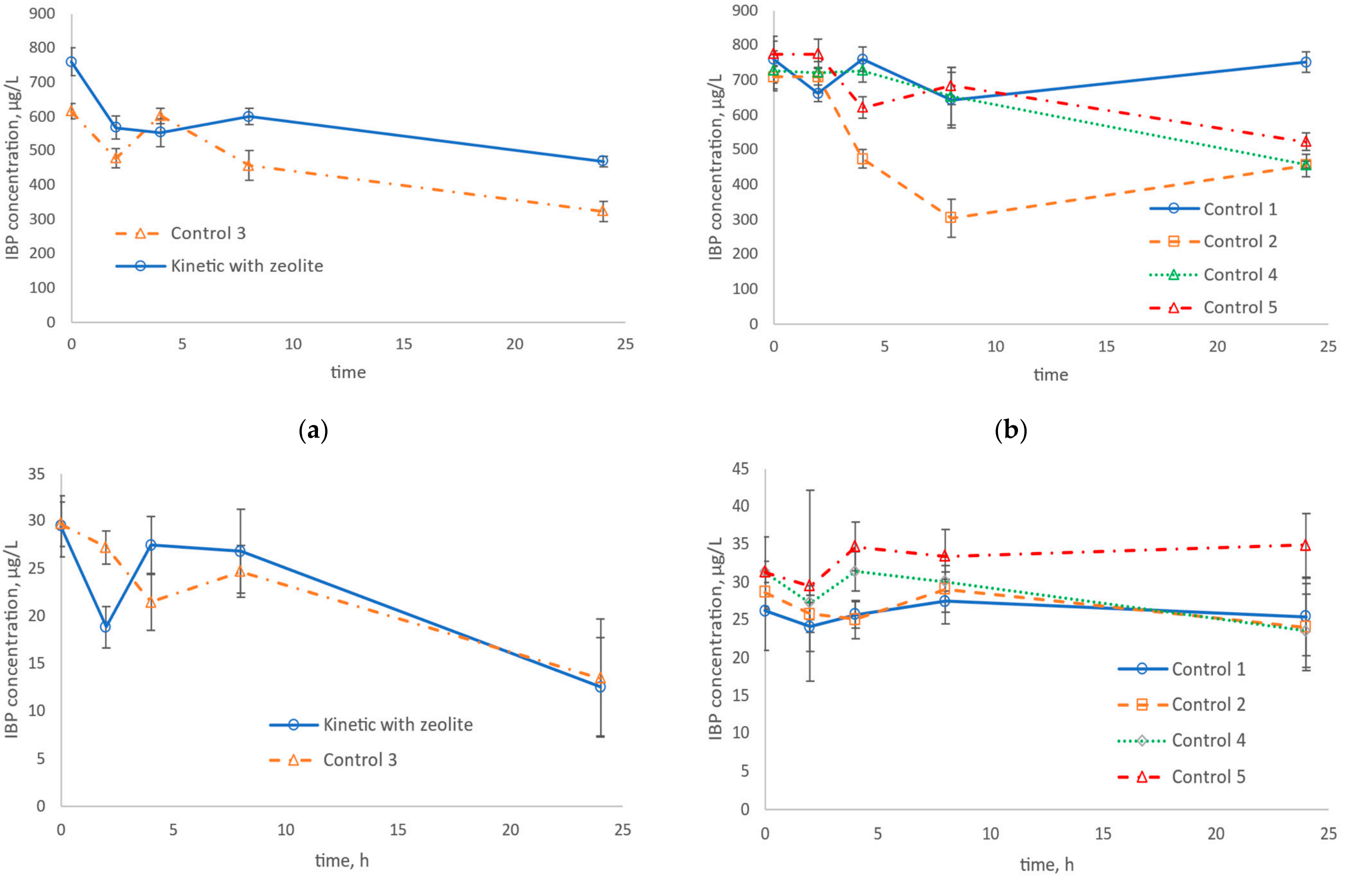
In particular, at high MPs concentrations (IBP = 700 μg/L and DFC = 100 μg/L), nitrification was almost complete, even in the presence of zeolite. The decrease in $\hat{q}$ produces a lower amount of nitrite, which can explain it. This lower concentration allows NOB to remove nitrite and generate the nitrate, as shown in Figure 3c,d, even though the high IBP and DFC concentration can inhibit NOB activity, as mentioned by other researchers [5,36,37].

3.3. IBP and DFC Removal in Nitrifying Assays

Figures 4 and 5, and Table 3, show the results related to the removal kinetic of IBP and DFC. Supplementary Materials show the linearization of MPs concentration at different conditions. The presence of zeolite decreased the IBP removal rate and efficiency at high IBP concentrations (Figure 4a,b). Indeed, IBP removal at high MPs concentrations was 47.39% for the system without zeolite; in contrast, the removal efficiency was only 38.3% in the presence of zeolite. It is interesting to note that controls with nitrifying activity inhibitor (ATU) have a removal close to the removal present by the system with nitrifying activity. Indeed, in the control experiment without zeolite and ATU, IBP removal was 37.14%, while the removal in the control with zeolite and ATU was 32.57%. So, at high IBP concentrations, the removal of IBP can be attributed to two mechanisms: sorption on the biomass and biodegradation.

Biodegradation was one IBP removal mechanism at high IBP concentrations, as Table 3 shows. The k_T of IBP of the system with ATU was 61.9% lower than the k_T of the system with nitrifying activity and 68.35% lower than the k_T of the system with nitrifying activity and zeolite. Interestingly, the control assay with zeolite and without biomass suggests that zeolite can sorb IBP during the first 8 h (Figure 3b) at high IBP concentration; however, the experiment did not repeat this behavior with the presence of biomass (active and inhibited by ATU). Further, there is evidence that zeolite can sorb IBP with high concentrations, as Karthik and Phillip showed [22], and according to the characteristics observed in Figure 1 and Table 1. Natural zeolites' most dominant adsorption mechanisms are ion exchange, hydrophobic interaction, and physisorption [19]. Other authors suggest that pharmaceutical compounds positively charged led to the experience of an electrostatic attraction by the zeolite external surface charged negatively, allowing for moving to the pores quickly [39]. Moreover, researchers concluded that the adsorption mechanism is affected by the physicochemical properties of micropollutants, such as size, charge, and hydrophobicity, as well as the zeolite characteristics [39]. However, it is unclear why the sorption phenomenon is absent when there are zeolite and biomass simultaneously.

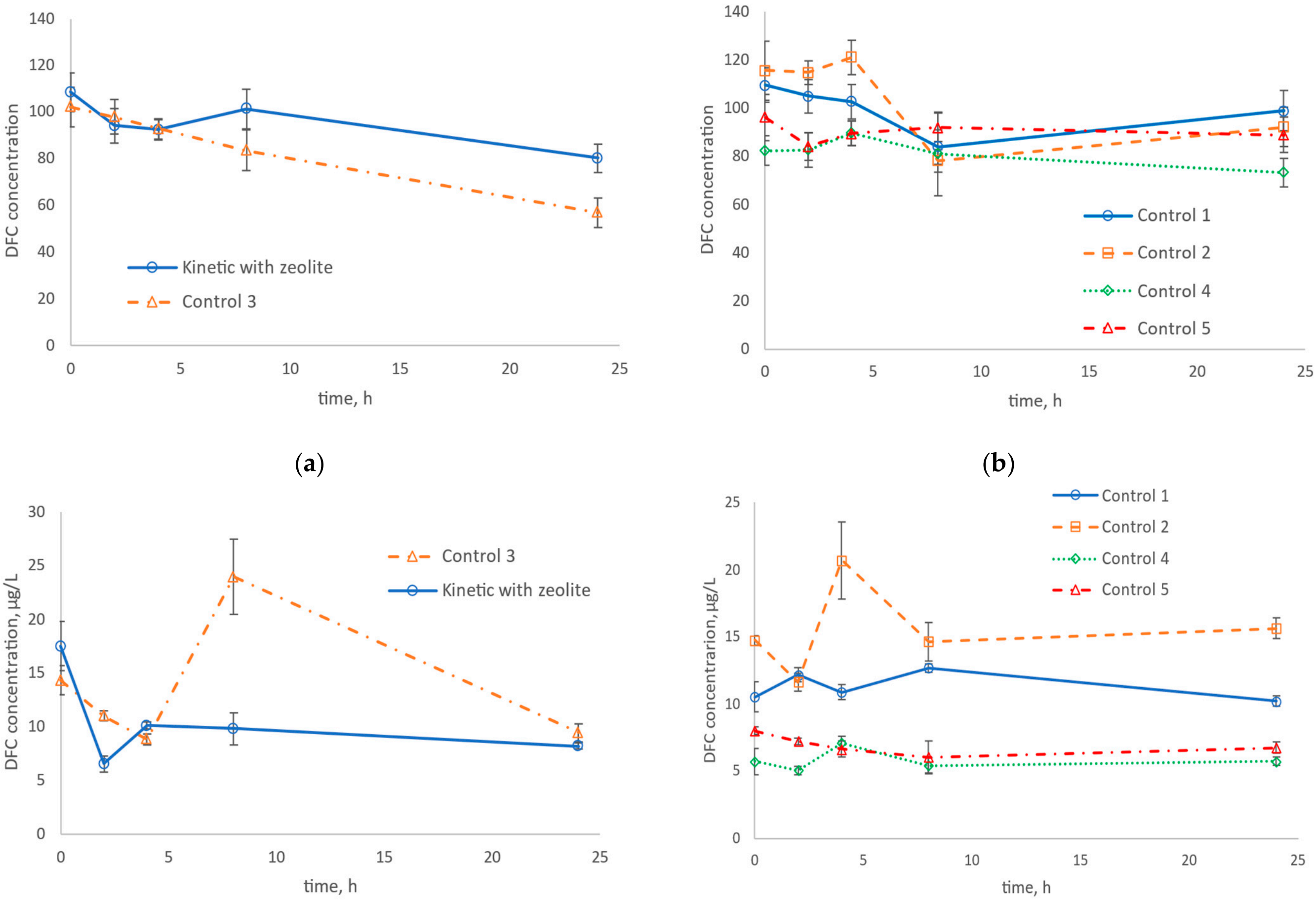
Another aspect that appears at high MPs concentration is the negative effect of zeolite on IBP kinetic removal. Indeed, the kinetic parameter k_T for the system without zeolite was 14.24% higher than the system with zeolite. Kinetic assays with biomass and ATU show that sorption phenomena obey a first-order kinetic, with values of 0.0214 and 0.0172 L/g VSS h for experiments without zeolite and with zeolite, respectively. These values are between 25.7 and 40.3% lower than experiments with nitrifying biomass. We obtained the lowest kinetic for experiments with biomass, ATU, and zeolite, again showing that zeolite exerts a barrier that avoids the interaction between biomass-substrate or sorbate.



(**a**) (**b**)

(**c**) (**d**)

Figure 4. Ibuprofen (IBP) profiles in a nitrifying culture with and without zeolite. (**a**) IBP concentration profiles with and without zeolite at high MP concentrations; (**b**) controls of IBP at high MP concentrations; (**c**) IBP concentration profile with and without zeolite at low MP concentrations; (**d**) controls of IBP at low MP concentrations.



(**a**) (**b**)

(**c**) (**d**)

Figure 5. Profiles of diclofenac (DFC) in a nitrifying culture with and without zeolite. (**a**) DFC concentration profiles with and without zeolite at high MP concentrations; (**b**) controls of DFC at high MP concentrations; (**c**) DFC concentration profile with and without zeolite at low MP concentrations; (**d**) controls of DFC at low MP concentrations.

Table 3. Kinetic parameters obtained at different MPs concentrations and in the presence or absence of zeolite.

Condition	K DFC (h^{-1})	k_T DFC (L/g VSS h)	R^2 DFC	K IBP (h^{-1})	k_T IBP (L/g VSS h)	R^2 IBP
Control 4, IBP30-DFC10	---	---		$0.0113 \pm 2.64 \times 10^{-3}$	$0.0113 \pm 2.64 \times 10^{-3}$	0.8219
Control 5, IBP30-DFC10	---	---		---	---	
Control 3, IBP30-DFC10	0.0208 ± 0.011	0.0193 ± 0.0102	0.543	$0.0332 \pm 4.09 \times 10^{-3}$	$0.0307 \pm 3.79 \times 10^{-3}$	0.9429
Assay with Zeolite IBP30-DFC10	0.0382 ± 0.0115	0.0402 ± 0.0116	0.7871	$0.034 \pm 8.29 \times 10^{-3}$	$0.0357 \pm 8.73 \times 10^{-3}$	0.8082
Control 4, IBP700-DFC100	---	---		$0.0182 \pm 1.78 \times 10^{-3}$	$0.0214 \pm 2.09 \times 10^{-3}$	0.963
Control 5, IBP700-DFC100	---	---		$0.0172 \pm 3.08 \times 10^{-3}$	$0.0172 \pm 3.08 \times 10^{-3}$	0.8861
Control 3, IBP700-DFC100	$0.0245 \pm 1.59 \times 10^{-4}$	$0.0252 \pm 1.64 \times 10^{-4}$	0.9998	$0.0279 \pm 4.47 \times 10^{-3}$	$0.0288 \pm 4.61 \times 10^{-3}$	0.9068
Assay with Zeolite IBP700-DFC100	$0.0132 \pm 3.16 \times 10^{-3}$	$0.0140 \pm 3.36 \times 10^{-3}$	0.8144	$0.0232 \pm 6.69 \times 10^{-3}$	$0.0247 \pm 7.12 \times 10^{-3}$	0.7508

The results differ at low MPs concentrations (Figure 4c,d). The zeolite positively affects the IBP degradation; in fact, it has a slightly increasing removal rate and removal efficiency. Figure 4c shows that removal efficiency was practically the same (54.62% for the system without zeolite; 57.52% for the system with zeolite) and superior to all the controls (Figure 4d). Certainly, the highest IBP removal efficiency among the control was for the system with biomass, ATU, and without zeolite, with an efficiency of 24.89%. Thus, the sorption phenomenon of IBP at low MPs concentration was only on biomass, contrary to the observed at high IBP concentrations; the sorption was present in both biomass and zeolite. Fernandez-Fontaina et al. [40] reported the sorption of IBP on biomass was very low or negligible (<1%). Other studies reported IBP sorbed onto biomass in a percentage close to what we observed in our work [41,42]. Indeed, Yu et al. [42] indicated that sorption on bio-carriers was 28% for IBP. It is essential to note that we did not obtain the sorption equilibrium in our experiments because that process occurs after 3–4 days.

Regarding the kinetic removal rate at low MPs concentration, zeolite positively affected the process. The k_T was 14% superior to the value obtained in the experiment without zeolite and 68.3% higher than the control with ATU and without zeolite. The results agree with the behavior observed for the nitrogen compounds (Figure 3a), where the system with zeolite has no difference from the control regarding TAN degradation, showing that under this condition, there was sufficient AMO enzyme for IBP degradation [37]. Further, another relevant aspect is the higher k_T values obtained at lower MPs concentrations than those obtained at high MPs concentrations. For instance, the system without zeolite at low MPs concentration has a k_T value of 6.18%, superior to the same experiment but with a higher MPs concentration. In the presence of zeolite, the observed behavior was similar, achieving 30.81% higher than the system at high MPs concentrations. The explanation for that, as we mentioned early, can be the inhibitory effect that exerts both MPs at high concentrations. Competition between MPs (IBU and DFC) and ammonia for binding with the AMO enzyme produced by nitrifying biomass decreases nitrifying and MPs' degradation [35–37].

For IBP, results show that at low concentrations, the bioreaction was the primary mechanism of IBP transformation, even though there was sorption on biomass but not on zeolite. Instead, at high IBP concentrations, removal was associated with both sorption phenomena on biomass and bioreaction. At low IBP concentrations, zeolite positively affects both removal rate and efficiency, while at high IBP concentrations, zeolite exerts a negative effect on the system.

For the case of DFC, Figure 5 presents the results obtained for control and nitrifying conditions at high and low MPs concentrations. At high DFC concentrations (Figure 5a,b), it is clear that zeolite does not positively affect DFC removal, with lower removal efficiency and lower removal rate. For instance, removal efficiency decreased from 44.44% in the system without zeolite to 26.16% in the system with zeolite. Further, it highlights the removal efficiency in the control experiment with zeolite (without biomass) with 20.19% removal. The rest of the control experiments achieved removals lower than 11%. This result shows that removal was mainly by bioreaction, without sorption on biomass and low sorption on the zeolite surface. The low sorption percentage on zeolite disagrees with previous research, where the natural zeolite adsorbed the DFC [22]. In our research, the lower concentration compared to other studies (DFC concentrations between 0.01 and 0.1 mg/L) can explain these differences. Concerning the sorption of DFC on biomass, removal through this mechanism was approximately 10% in the study presented by Wu et al. [17] and was negligible in other studies [15,16]. Thus, our results confirm that behavior.

For the removal rate, zeolite exerts a negative effect under high MPs concentrations, as Figure 5a and Table 3 show. The zeolite decreases the contact between biomass and substrate, so AOB activity in the presence of zeolite is lower than in the assays without zeolite. As a result, it declined the AMO enzyme activity and the DFC removal rate. Indeed, the kinetic constant k_T for the experiment with zeolite is 44% lower than k_T for the system without zeolite. The kinetic for the control using only zeolite does not follow a first-order kinetic; then, the research did not analyze it.

At low DFC concentrations (Figure 5c,d), zeolite has a positive effect on the DFC removal rate and DFC removal efficiency. For this last parameter, there is an increment in removal efficiency of 57.9% in the assay with zeolite compared to the experiment without zeolite. In controls, the removal efficiency was lower than 6%, except for the control with inhibited biomass and zeolite. Here, the removal efficiency was 15.63%, although the low concentration can imply a high error in analysis, as He et al. [43] mentioned. For the kinetic removal rate (Table 3), k_T for the assay with zeolite is 108.3% higher than the value obtained for the system without zeolite, even though the nitrifying activity was similar. With the DFC, it did not observe the sorption phenomenon, and removal was mainly by nitrifying biomass action. In fact, at high DFC concentrations, we observed a similar situation.

Values of k_T are between the values reported in the literature for IBP and DFC [28,29,44]. For IBP, values of k_T are lower than those presented by Dawas-Massalha et al. [28] and Fernandez-Fontaina et al. [29], but higher (almost ten times) than the values presented by Peng et al. [44]. For DFC, the situation was different, with k_T values lower than reported in other works [29,44]. In addition, the kinetic coefficient values for DFC are much lower than those obtained for ibuprofen, which shows the low biodegradability of this drug. The AMO enzyme action can attribute to this phenomenon because it has a high affinity for low-polarity substrates such as ibuprofen. Likewise, some chemical groups, such as chlorine, make it difficult for diclofenac to bind to this enzyme.

According to the results, zeolite addition is recommended only for treating wastewater with low MPs concentrations. In this case, biodegradation is the primary mechanism for reducing the MPs concentration. It is important to note that the possible saturation of zeolite with MPs can be problematic when it is discarded from bioreactors. Indeed, Karthik and Philip [22] indicate that DFC and IBP have desorption percentages from natural zeolites between 9.39–18.07% and 11.29 and 20.67%, respectively. Nevertheless, when zeolites are used as carriers in bioreactors, they are scarcely discarded, and the possibility of secondary pollution on soils is difficult.

Another point to consider is the cost of a possible zeolite application in bioreactors. According to the "Mineral Commodity Summaries 2020" (https://pubs.usgs.gov/periodicals/mcs2020/mcs2020-zeolites.pdf, accessed on 1 July 2023), the maximum cost of a zeolite ton is USD 300. Because the recommended dose of zeolite used in nitrifying bioreactors is five g/L [24] and this concentration was used in this study, the application of it gives a price of USD 1.5/m^3 bioreactor. For instance, a WWTP that treats eight m^3/s (La Farfana, Santiago de Chile) of wastewater is necessary for 16 bioreactors of 11.300 m^3. In that case, applying zeolite as an improver costs USD 271,200. It is important to note that zeolite should be applied just once because it is a solid mainly kept in the bioreactor.

4. Conclusions

Nitrifying culture can remove IBP and DFC in the presence of zeolite, although the improvement depends on the IBP and DFC concentrations. At low DFC and IBP concentrations, zeolite improves the removal rate and elimination efficiency of IBP and DFC. In this case, biodegradation is the primary removal mechanism. On the contrary, at high MPs concentration, zeolite negatively affects the removal rate and removal efficiency of IBP and DFC, as biodegradation and sorption of the mechanisms through MPs are removed. The possible adsorption can be attributed to the mesoporous, where capillary condensation (hysteresis) and multilayer/monolayer adsorption can be the adsorption mechanism. Finally, zeolite negatively affects the nitrification process under the conditions studied (batch system, first interaction zeolite–biomass) because zeolite prevents biomass/substrate interaction, working as a barrier that slows down the biological reaction. Even though the use of natural zeolite in nitrifying bioprocess shows good performance in removing ibuprofen, it could be interesting the study synthetic zeolites that can help to improve the adsorption of hardly biodegradable emerging contaminants such as diclofenac.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15142665/s1; Figures S1–S11: linearization of MPs concentration at different conditions. Figure S1: Linearization of DFC concentration. Experiment without zeolite. Initial DFC concentration = 100 µg/L; Figure S2: Linearization of DFC concentration. Experiment with zeolite. Initial DFC concentration = 100 µg/L; Figure S3: Linearization of DFC concentration. Experiment without zeolite. Initial DFC concentration = 10 µg/L; Figure S4: Linearization of DFC concentration. Experiment with zeolite. Initial DFC concentration = 10 µg/L; Figure S5: Linearization of IBP concentration. Experiment without zeolite. Initial DFC concentration = 700 µg/L; Figure S6: Linearization of IBP concentration. Experiment with zeolite. Initial DFC concentration = 700 µg/L; Figure S7: Linearization of IBP concentration. Control experiment without zeolite, with biomass and with ATU. Initial DFC concentration = 700 µg/L; Figure S8: Linearization of IBP concentration. Control experiment with zeolite, with biomass and with ATU. Initial DFC concentration = 700 µg/L; Figure S9: Linearization of IBP concentration. Experiment without zeolite. Initial DFC concentration = 30 µg/L; Figure S10: Linearization of IBP concentration. Experiment with zeolite. Initial DFC concentration = 30 µg/L; Figure S11: Linearization of IBP concentration. Control experiment without zeolite, with biomass and with ATU. Initial DFC concentration = 30 µg/L.

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