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Heterotrophic Kinetic Study and Nitrogen Removal of a Membrane Bioreactor System Treating Real Urban Wastewater under a Pharmaceutical Compounds Shock: Effect of the Operative Variables

Antonio Monteoliva-García [1,2], Juan Carlos Leyva-Díaz [3,*], Cristina López-López [1,2], José Manuel Poyatos [1,2], María del Mar Muñío [4] and Jaime Martín-Pascual [1,2]

1 Department of Civil Engineering, University of Granada, 18071 Granada, Spain
2 Institute of Water Research, University of Granada, 18071 Granada, Spain
3 Department of Chemical and Environmental Engineering, University of Oviedo, 33006 Oviedo, Spain
4 Department of Chemical Engineering, University of Granada, 18071 Granada, Spain
* Correspondence: jcleyvadiaz@uniovi.es; Tel.: +34-985103509

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Abstract: Numerous studies have analyzed the viability of the biodegradation and removal of different compounds of emerging concern in biological systems for wastewater treatment. However, the effect on the heterotrophic biomass of organic matter removal is sometimes missed. The aim of the present research was to study the effect of the addition of a mix of three pharmaceuticals (carbamazepine, ciprofloxacin, and ibuprofen) on the behavior of the biomass in two different membrane-based biological systems treating urban wastewater. The present research studied a membrane bioreactor (MBR) pilot plant operating at a similar mixed liquor suspended solids (MLSS) concentration (about 5.5 g/L). This system works as an MBR and is combined with a moving bed biofilm reactor (MBBR-MBR) to treat real urban wastewater at 6 and 10 h of hydraulic retention time (HRT) under three different shocks of pharmaceuticals with increasing concentrations. In all cases, the organic matter removal was, in average terms, higher than about 92% of biochemical oxygen demand on the fifth day (BOD_5), 79% of chemical oxygen demand (COD), and 85% of total organic carbon (TOC). Nevertheless, the removal is higher in the MBBR-MBR technology under the same HRT and the MLSS is similar. Moreover, the removal increased during the shock of pharmaceutical compounds, especially in the MBR technology. From a kinetic perspective, MBBR-MBR is more suitable for low HRT (6 h) and MBR is more effective for high HRT (10 h). This could be due to the fact that biofilm systems are less sensitive to hostile environments than the MBR systems. The removal of $N\text{-}NH_4^+$ decreased considerably when the pharmaceutical compounds mix was introduced into the system until no removal was detected in cycle 1, even when biofilm was present.

Keywords: heterotrophic kinetics; membrane bioreactor; nitrogen; pharmaceutical shock

1. Introduction

Increasing attention has recently been paid to the presence of micropollutants in the aquatic environment and wastewater treatment plants [1]. Recent studies have identified the presence of contaminants of emerging concern (CECs) in effluents and in receiving waters, where CECs can potentially affect aquatic organisms [2]. The presence of CECs, such as pharmaceuticals, personal care products, or pesticides in the body of water, produces a major challenge to human health and ecosystems [3]. CECs are not completely removed during wastewater treatments, and as a result, they are discharged into the receiving streams and can end up in soils when the sewage sludge

generated is employed as a fertilizer [4]. CECs are discharged into the environment throughout effluents from wastewater treatment plants (WWTPs) that are not specifically designed for their removal, and their impact is of particular relevance to wastewater disposal and re-use in agricultural settings due to CEC uptake and the accumulation in food crops and their consequent diffusion into the food-chain [5].

Numerous studies have analyzed the viability of the biodegradation and removal of different CECs in biological systems for wastewater treatment, such as activated sludge (AS) or membrane bioreactors (MBR). However, the effect on the heterotrophic biomass of organic matter removal is sometimes missed. Pharmaceuticals in municipal WWTPs represent an important focus for reducing micro-pollutant emissions because these compounds are used throughout the year and are designed to produce specific biological effects on organisms or living tissue; therefore, they are also likely to cause unwanted effects in the environment [1]. In biological WWTPs, the removal of organic matter relies on the activity of a mixed community of heterotrophic microorganisms [6].

In the last decades, the MBR process has become an alternative to AS processes for the removal of CECs and conventional pollutants (organic matter and nutrients) during wastewater treatment [7]. MBR technology has advantages such as absolute control of solid retention times and smaller volume requirements [8], which improve, with an exceptional quality, its effluent so that it is capable of meeting the most stringent water quality requirements [9].

The AS technology is based on the growth of biomass as floc; nevertheless, biomass can also attach and grow on small carrier elements as biofilm in other technologies such as moving bed biofilm reactors (MBBR) [10]. MBBR is a compact technology based on a mixed tank in which carriers, which move freely around the bioreactor, are immersed and gradually colonize on the protected surface, growing as biofilm [11].

The aim of the present research was to study the effect of the addition, at increasing concentrations, of a mix of three pharmaceuticals (carbamazepine, ciprofloxacin, and ibuprofen) on the behavior of the biomass in a conventional MBR and a hybrid MBBR-MBR when treating urban wastewater at 6 h and 10 h of hydraulic retention time (HRT). The concentrations of carbamazepine, ciprofloxacin, and ibuprofen were 100, 10, and 100 μg/L, 1000, 100, and 1000 μg/L, and 5000, 500, and 5000 μg/L in the doping cycles 1, 2, and 3, respectively. The lowest mixing ratio between the different chemicals was based on the average values found in previous studies [12]; once the initial values were chosen, increasing concentrations were used to check the bioreactor by keeping the initial ratio constant. Moreover, this work was also designed to analyze the capacity of the bioreactor to adapt when different concentrations of pharmaceutical mixes were introduced into the biological process.

2. Materials and Methods

2.1. Experimental Set-Up

The pilot plant used in this study was located in the WWTP Oeste of Granada (Spain) and was continuously fed with real urban wastewater (Figure 1). It had a total treatment volume of 350 L divided into two compartments, with a 272 L cylindrical tank in which the biological degradation of organic matter and oxidation of ammonium were carried out by fine bubble aeration. Urban wastewater was subsequently led by gravity to a second 78 L rectangular tank where the treated water was separated from the mixed liquor by means of 4 submerged hollow fiber ultrafiltration membranes with 0.04 μm nominal pore size and a 0.97 m^2 unit surface area (ZW-10, ZENON®). To keep the system well mixed and to maintain the working mixed liquor suspended solids (MLSS) concentration (in mg/L), a pump recirculated the MLSS from the rectangular tank to the cylindrical tank.

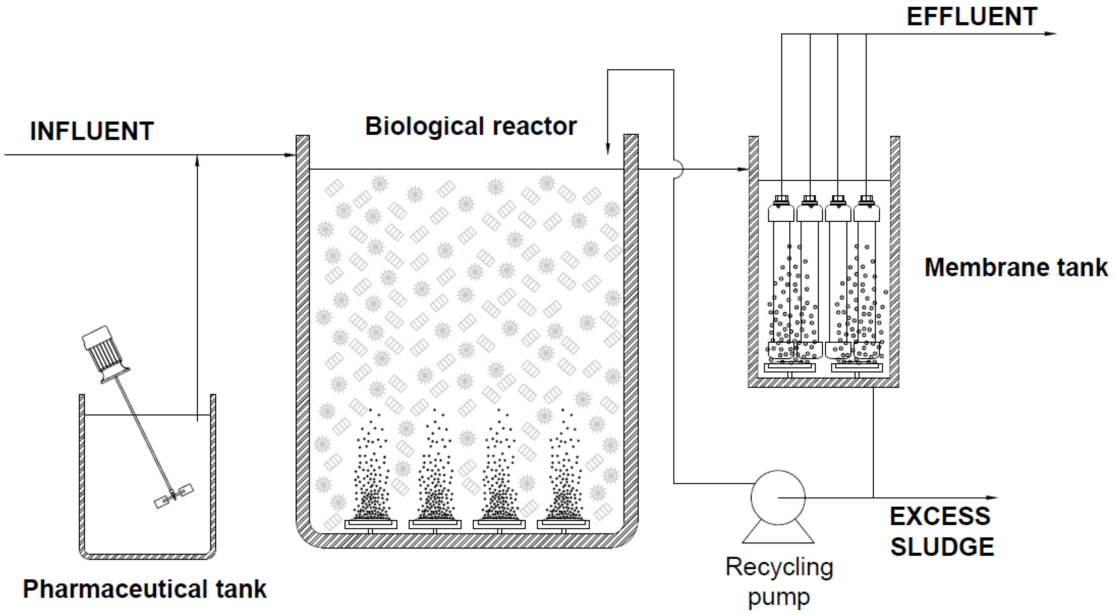

Figure 1. Schematic diagram of the pilot plant used in the research.

2.2. Operation Conditions

To achieve the objectives of the research, four experimental cycles were conducted at different operating conditions (Table 1).

Table 1. Operation variables during the research.

Cycle	MLSS (mg/L)	HRT (h)	Temperature (°C)	Filling Ratio (%)	SRT (Days)
1	5643 ± 578	6	21.5 ± 3.1	-	11.2
2	5333 ± 304	10	12.6 ± 1.8	-	21.7
3	5773 ± 492	6	28.1 ± 2.7	35	6.0
4	5285 ± 280	10	17.6 ± 3.1	35	23.9

During cycles 1 and 2, the pilot plant was working as a submerged MBR for a period of 3 months per cycle, during which samples were taken daily from influent, effluent, bioreactor, and excess sludge. In cycle 1, the MBR was operated for 6 h of HRT, while in cycle 2, the MBR was working for 10 h of HRT.

To operate the pilot plant as a hybrid MBBR-MBR treatment, in cycles 3 and 4, the cylindrical bioreactor was filled with AnoxKaldnes™ K1 carriers with a 35% filling ratio, corresponding to a 175 m^2/m^3 net surface area in the bioreactor. In cycles 3 and 4, the hybrid MBBR-MBR system was tested at similar concentrations of MLSS and identical values of HRT with respect to the MBR system in cycles 1 and 2, respectively.

The temperature in each cycle was imposed by environmental conditions due to the outdoor location of this plant, and the operation conditions determined the given sludge retention times (SRTs). Once the performance of the pilot plant was assessed at the different conditions, the addition of carbamazepine, ciprofloxacin, and ibuprofen into the influent made it possible to determine how the presence of these pharmaceuticals affected organic matter and ammonium removal. For this purpose, three different mixtures at increasing concentrations were added to the influent: The concentrations of carbamazepine, ciprofloxacin, and ibuprofen were 100, 10, and 100 µg/L, 1000, 100, and 1000 µg/L, and 5000, 500, and 5000 µg/L in dopings 1, 2, and 3, respectively. In light of this, the time necessary to ensure that the selected concentration of pharmaceutical was available for the microorganisms,

as a result of the dilution effect in the bioreactor, is an important variable to consider for the assay. Consequently, a non-steady compound balance allowed for the prediction of the evolution of the pharmaceutical in the bioreactor. From this balance, the time required to ensure the working concentration of pharmaceutical was assessed according to Calero–Díaz et al. [12]. To simulate punctual exposures to these pharmaceuticals, once the concentration of pharmaceutical was achieved, the pilot plant was run for 3 days under each doping.

2.3. Physical and Chemical Determinations

Mixed liquor suspended solids (MLSS), chemical oxygen demand (COD), and biochemical oxygen demand on the fifth day (BOD_5) measurements were carried out according to procedures established in the Standard Methods [13]. Total organic carbon (TOC) was determined by the difference between total carbon and inorganic carbon in a Formacs HT TOC/TN analyzer by oxidative combustion at 950 °C. Ammonium was measured by ionic chromatography with a Metrosep C4 column (Metrohm) using a solution of dipicolinic acid as the eluent and distilled water as the regenerate. Conductivity and pH were determined using a conductivity meter (Crison CM 35® Hach Lange Spain, S.L.U., L´Hospitalet de Llobregat, Barcelona, Spain) and a pH meter (Crison pH 25®, Hach Lange Spain, S.L.U., L´Hospitalet de Llobregat, Barcelona, Spain), respectively; the calibration of pH was carried out by using three buffers, i.e., 4.01, 7.00, and 9.21, at 25 °C, while the calibration of conductivity was conducted by using three buffers, i.e., 147 µS/cm, 1413 µS/cm, and 12.88 mS/cm, at 25 °C. The environmental temperature was taken continuously in the pilot plant with a thermometer (LANGE LDOTM/sc100, Hach Lange Spain, S.L.U., L´Hospitalet de Llobregat, Barcelona, Spain).

2.4. Kinetic Study

The influence of carbamazepine, ciprofloxacin, and ibuprofen on the heterotrophic biomass was assessed during the steady state of the four operation cycles of the MBR and MBBR-MBR systems. For this, biomass samples were taken from the previous biological systems and analyzed by respirometry in a BM-Advance respirometer [14]. In light of this, the following kinetic parameters were determined through exogenous and endogenous respirometric tests. On the one hand, the exogenous respiration experiment allowed for the assessment of the maximum specific growth rate for heterotrophic biomass ($\mu_{m,H}$), half-saturation coefficient for organic matter (K_M), and yield coefficient for heterotrophic biomass (Y_H). On the other hand, the endogenous respiration experiment made it possible to determine the decay coefficient for heterotrophic biomass (b_H) [15]. Apart from the kinetic parameters for heterotrophic bacteria, the substrate degradation rate for organic matter removal ($r_{su,H}$) was evaluated for the control cycle (in the absence of pharmaceuticals) and three doping cycles under these pharmaceuticals, according to Leyva–Díaz et al. [16], as shown in Equation (1):

$$r_{su,\,H} = \frac{\mu_{m,H} \times S \times X_H}{Y_H \times (K_M + S)} \quad (1)$$

where S is the substrate concentration ($mgO_2\ L^{-1}$) and X_H is the concentration of heterotrophic biomass. X_H was estimated by applying the heterotrophic fraction (f_H) to the mixed liquor volatile suspended solids (MLVSS) concentration [17], as indicated in Equation (2):

$$X_H = f_H \times MLVSS \quad (2)$$

2.5. Statistical Analysis

The results obtained from the analyses were treated using statistical software SPSS 20 for Windows. A least significant differences (LSD) test was used to measure the differences between the results obtained for each cycle. An analysis of variance (ANOVA) was used to assess the homogeneity of the variance, with a confidence interval of 95%.

3. Results and Discussion

3.1. Influent Characterization

The pilot plant was fed with real urban wastewater from the WWTP Oeste of Granada where the pilot plant was located. The average values of the physical–chemical characteristics of the influent during the four cycles represented in the present investigation are shown in Table 2.

Table 2. Average values of pH, conductivity, total suspended solids (TSS), and organic matter concentration (Total organic carbon (TOC), chemical oxygen demand (COD), and BOD_5) of the influent during the experiments.

Cycle	pH	Conductivity (μS/cm)	TSS (mg/L)	TOC (mg/L)	COD (mg/L)	BOD_5 (mg/L)
1	8.04 ± 0.49	1394 ± 392	156 ± 49	208 ± 54	618 ± 123	344 ± 92
2	7.69 ± 0.29	1032 ± 164	144 ± 37	170 ± 36	495 ± 132	295 ± 87
3	7.53 ± 0.19	1267 ± 118	124 ± 26	210 ± 32	603 ± 99	367 ± 53
4	7.76 ± 0.40	1551 ± 751	139 ± 31	191 ± 36	597 ± 89	376 ± 75

The pH of the influent was relatively stable during the experimental period, it changed between 7.53 (cycle 3) and 8.54 (cycle 1), and presented the typical variation of real urban wastewater. Such values are similar to those observed by other authors studying urban wastewater [18,19]. The conductivity ranged between 1032 μS/cm (cycle 2) and 1550 μS/cm (cycle 4); in a unitary drainage network, the lowest conductivity of the wastewater is related to a period of rain or particularly rainy days [20] throughout the cycle, with minimum values of 580 μS/cm in cycle 4 and 700 μS/cm in cycle 2.

The average total suspended solids (TSS) of the influent changed from 124 to 156 mg/L throughout four cycles. The organic matter was measured as TOC, COD, and BOD_5 (Table 2) because it is water with the presence of organic pollutants and it is necessary to know what the removal of organic matter is in the effluent by relating the three ways of measuring it. The TOC provides the amount of carbon in the sample and the BOD_5/COD ratio shows the biodegradability of the organic carbon in the sample. Considering the average data of the organic matter, some fluctuations were observed in the four cycles studied because it is real urban wastewater.

The ANOVA test of the influent data did not show statistically significant differences, which suggests that the variations in the results are due to the different operating variables of each cycle (HRT, environmental temperature, and operating system).

3.2. Biological System Performance

The introduction of the pharmaceutical mix in the influent effected the biomass of the bioreactor (Table 3).

Table 3. Average Mixed liquor suspended solids (MLSS) (mg/L) in steady state and the three dopings for the four cycles studied.

Cycle	Steady State	Doping 1	Doping 2	Doping 3
1	5643 ± 578	5473 ± 501	5284 ± 369	4147 ± 963
2	5333 ± 304	4807 ± 326	4707 ± 792	-
3	5773 ± 492	5281 ± 540	5302 ± 104	4616 ± 139
4	5285 ± 280	4482 ± 587	4483 ± 398	3460 ± 541

In cycle 1, with the MBR system, the MLSS in the bioreactor was 5643 ± 578 mg/L, and with doping at the highest concentration of pharmaceutical mix, almost 1500 mg/L of biomass was lost. The MLSS

decrease with pharmaceuticals could be due to the shock in the system caused by the complexity of these compounds the effected the different microorganisms [21]. In cycle 3, which is the analogue of cycle 1, but with an MBBR-MBR system, observed a loss of 492 and 471 mg/L of biomass, respectively, during the first two dopings, while for doping 3, a large decrease of the MLSS concentration was observed in the hybrid MBBR-MBR system, although it was less pronounced compared to MBR. This could be explained by the average temperature of 28.1 °C favoring a reduction of the shock of the pharmaceutical mix on the biomass. This is due to the higher temperature having greater microbial activity, which means that the biomass is adapted faster to the medium [22]. In cycle 2, with an 8.9 °C lower temperature than in cycle 1, the loss of biomass was high, and the cycle with the highest concentration of doping could not be completed; this is because the low temperatures caused a shock in the reactor, causing, in turn, the anomalous growth of bacteria. This leads to sludge bulking at temperatures below 12–15 °C [23]. Gur–Reznik et al. [24] found, in their study with an MBR system, that when the temperature is low, the metabolic rate undergoes changes affecting the degradation of organic matter. Finally, cycle 4 lost more than 1800 mg/L during the three dopings, and was the cycle that lost the largest amount of biomass. Something similar to cycle 2 occurred, so that low temperatures effected the system when it was doped with the mix of pharmaceutical compounds. In summary, the incorporation of the pharmaceuticals produced a shock in the microorganisms and biomass decreased. In general terms, the reduction of biomass is more significant when the temperature is lower and the reduction of biomass decreases at temperatures between 21–28 °C.

The organic matter removal by the systems is shown in Table 4 for each condition tested.

Table 4. Organic matter removal efficiency of the process measured as BOD_5, COD, and TOC during the experiments.

Cycle	BOD_5				COD				TOC			
	Steady State	**Doping 1**	**Doping 2**	**Doping 3**	**Steady State**	**Doping 1**	**Doping 2**	**Doping 3**	**Steady State**	**Doping 1**	**Doping 2**	**Doping 3**
1	92.07 ± 7.17	97.87 ± 0.89	95.83 ± 2.77	94.46 ± 6.05	87.63 ± 3.87	86.72 ± 2.56	85.87 ± 3.88	83.95 ± 0.58	87.81 ± 1.40	87.54 ± 1.22	86.44 ± 1.86	85.31 ± 2.32
2	94.46 ± 6.34	98.16 ± 0.89	90.98 ± 10.82	-	86.87 ± 2.17	79.62 ± 14.69	87.89 ± 5.40	-	88.64 ± 1.04	86.56 ± 8.33	88.17 ± 4.69	-
3	97.48 ± 2.78	98.81 ± 0.71	99.34 ± 0.50	99.27 ± 0.46	89.86 ± 4.58	90.53 ± 4.63	90.89 ± 4.44	87.30 ± 2.15	87.98 ± 1.80	90.42 ± 1.34	90.61 ± 0.69	90.41 ± 0.32
4	97.90 ± 1.17	98.42 ± 0.21	97.87 ± 1.54	96.04 ± 1.48	91.19 ± 1.09	93.60 ± 3.07	92.07 ± 4.4	88.55 ± 6.36	86.98 ± 1.46	90.43 ± 0.95	85.03 ± 2.15	86.39 ± 3.48

In general, the values of BOD_5 in the four cycles were above 92% in the steady state and above 90% in the dopings. In cycle 3, with an HRT of 6 h and a temperature of 28 °C, the removal of BOD_5 was higher than in other cycles, with values of about 98% in the steady state to about 99% in doping 2. The COD had a removal performance somewhat lower than BOD_5. The values in the steady state varied from about 87% in cycle 2 to about 91% in cycle 4. The values in the different dopings ranged from about 80% in cycle 2 to about 94% in cycle 4. Cycles 3 and 4 with 6 and 10 h of HRT, respectively, and temperatures of 28 °C and 17 °C, respectively, had very close values of COD removal. Organic matter was also measured with the TOC, which is intimately related to the COD, so the values are very similar. The highest performances were in cycles 3 and 4, ranging from about 85% in doping 3 of cycle 1 to about 91% in doping 2 of cycle 3.

The ANOVA test did not show statistically significant differences between the steady state and the dopings in the different cycles. The organic matter consumption, independently of the decrease of MLSS in the bioreactor, remained constant. Shariati et al. [25] used an MBR system for the removal of pharmaceutical compounds from wastewater and demonstrated that the most important parameters for the removal of these compounds are COD and MLSS; increasing the COD in the medium decreased the pharmaceutical compound removal, while increasing the MLSS favored it.

On the one hand, in Table 4, it can be seen that the removal of BOD_5 in the three dopings in cycle 1 was slightly higher than in the steady state. The same occurs with cycle 2. Cycles 3 and 4 with the MBBR-MBR system removed practically the same amount during the three dopings as in the stationary state. The BOD_5 performances were high in the four cycles, with values above 92% and up to about 99%. Leyva–Díaz et al. [14] achieved a removal of about 98.9% with an MBBR-MBR system working for 26 h of HRT and at 99.1% with an MBR system, although the biomass was lower in the system. Furthermore, 91% and 92% of COD were removed with the MBBR-MBR and MBR systems, respectively. Therefore, in both systems, high percentages were achieved in the removal of organic matter, but both had lower HRTs. Cycles with biofilm removed slightly more BOD_5 than cycles with only suspended biomass, which could be due to a higher biomass concentration as a consequence of its growth as biofilm onto the carriers.

On the other hand, COD removal is also shown in Table 4. With the MBBR-MBR system, slightly more organic matter is removed during the different dopings than with the MBR system. Furthermore, either less COD is removed by adding the different concentrations of pharmaceutical mix or the removal in the different dopings is kept constant with respect to the steady state. In cycle 1 with the MBR system, the removal is lower in the three dopings than in the steady state; it decreased with an increased concentration of the pharmaceutical mix. Cycle 2 started removing less (about 80%), and in doping 2, it removed the same as in the stationary state (about 88%). In cycles 3 and 4, the removal remained practically constant even when the pharmaceutical mix was introduced, which means that the COD removal in the hybrid MBBR-MBR system was less affected by inhibitory substances. This could be due to the presence of attached biomass as biofilm, which entails a protected form of growth.

No relevant differences were observed in relation to the TOC. With the MBR system (cycles 1 and 2), regardless of temperature and HRT, TOC removal was slightly lower than in the steady state. However, for cycle 3 (MBBR-MBR system), removal was slightly higher than in the steady state, and for cycle 4 (MBBR-MBR system), the observed removal is comparable to that of the steady state, as is the case in dopings 2 and 3 in cycle 4, corresponding to the lowest value of temperature for this system. In general, the removal of COD and TOC in the system is not as high as BOD_5, ranging from about 80% to about 94% in COD removal and from about 85% to about 91% in TOC removal.

3.3. Kinetic Modeling

Table 5 shows the kinetic parameters for heterotrophic bacteria, as well as values of $r_{su,H}$ for cycles 1, 2, 3, and 4 for MBR and MBBR-MBR.

Table 5. Kinetic parameters for heterotrophic biomass in the absence (control) and presence (doping) of carbamazepine, ciprofloxacin, and ibuprofen for the four operation cycles of membrane bioreactor (MBR) and bed biofilm reactors–membrane bioreactor (MBBR-MBR).

Parameter	Operation Phase			
	Control	Doping 1	Doping 2	Doping 3
MBR				
Cycle 1				
Y_H (mgVSS mgCOD^{-1})	0.6344	0.6156	0.6231	0.6205
$\mu_{m,H}$ (h^{-1})	0.0170	0.0054	0.0158	0.0358
K_M (mgO_2 L^{-1})	8.0550	1.6285	9.2261	14.9804
b_H (day^{-1})	0.0557	0.0606	0.0564	0.0728
$r_{su,H}$ (mgO_2 L^{-1} h^{-1})	107.84	38.98	91.56	125.05
Cycle 2				
Y_H (mgVSS mgCOD^{-1})	0.6090	0.6232	0.6290	-
$\mu_{m,H}$ (h^{-1})	0.0239	0.1835	0.2259	-
K_M (mgO_2 L^{-1})	19.8920	129.6460	173.0295	-
b_H (day^{-1})	0.0540	0.0426	0.0529	-
$r_{su,H}$ (mgO_2 L^{-1} h^{-1})	150.86	699.94	1024.48	-
MBBR-MBR				
Cycle 3				
Y_H (mgVSS mgCOD^{-1})	0.6462	0.6007	0.6446	0.6093
$\mu_{m,H}$ (h^{-1})	0.0200	0.0152	0.0199	0.0141
K_M (mgO_2 L^{-1})	17.4453	17.9443	17.5387	13.0614
b_H (day^{-1})	0.0726	0.0435	0.0962	0.0760
$r_{su,H}$ (mgO_2 L^{-1} h^{-1})	72.37	106.48	116.34	76.12
Cycle 4				
Y_H (mgVSS mgCOD^{-1})	0.6058	0.5824	0.5845	0.5975
$\mu_{m,H}$ (h^{-1})	0.0252	0.1025	0.1079	0.0175
K_M (mgO_2 L^{-1})	13.2984	78.5140	81.3655	4.9995
b_H (day^{-1})	0.0769	0.0654	0.0702	0.1427
$r_{su,H}$ (mgO_2 L^{-1} h^{-1})	146.14	206.04	222.92	69.35

The yield coefficient for heterotrophic biomass (Y_H) was slightly lower in the presence of pharmaceuticals, with the exception of cycle 2, which corresponded to the MBR working for 10 h of HRT. In general, $\mu_{m,H}$ and K_M increased with the progressive dopings, with the exception of cycle 3, which corresponded to the MBBR-MBR working for 6 h of HRT. The influence of these variations on kinetic parameters of heterotrophic biomass is included in the values of $r_{su,H}$ for the different cycles analyzed.

Regarding the effect of the mix of carbamazepine, ciprofloxacin, and ibuprofen in increasing concentrations on the heterotrophic biomass within the MBR system, it must be highlighted that the $r_{su,H}$ decreased in the presence of the pharmaceuticals for doping 1 and doping 2 (about 64% and 15%, respectively) and increased for doping 3 (about 16%) in cycle 1. These results could indicate the existence of an adaptation period for heterotrophic biomass. However, $r_{su,H}$ significantly increased for dopings 1 and 2 (about 364% and 579%, respectively) in cycle 2. This difference in behavior between

the two cycles could be due to the most favorable operation conditions for cycle 2, as the MBR worked for 10 h of HRT and 21.7 days of SRT, compared with the MBR from cycle 1 (HRT = 6 h, SRT = 11.2 day).

In relation to the influence of the mix of pharmaceuticals on the heterotrophic biomass within the MBBR-MBR, it must be pointed out that the $r_{su,H}$ showed a higher increase for doping 1 and doping 2 (about 47% and 61%, respectively) than for doping 3 (about 5%) in cycle 3. This could indicate that the adaptation response of the heterotrophic biomass of MBBR-MBR was better than that corresponding to an MBR under the same HRT (cycle 1). This could be due to the higher temperature for the MBBR-MBR (28.1 ºC) than that obtained for the MBR (21.5 ºC). Moreover, the attached biomass from the MBBR-MBR constitutes a protected form of growth, which is generally considered less sensitive to toxic influents and hostile environments [26]. In light of this, the behavior of the MBBR-MBR was similar in cycle 4, with the exception of doping 3. The $r_{su,H}$ increased for doping 1 and doping 2 (about 41% and 53%, respectively), although $r_{su,H}$ lessened for doping 3 (about 53%). While $r_{su,H}$ decreased for doping 3 in relation to doping 1 and doping 2 in cycles 3 and 4, the reduction was more significant in cycle 4. This could be due to the lower temperature (17.6 °C) compared to the temperature of cycle 3 (28.1 °C), though working at higher values of HRT and SRT (Table 1). The comparison between cycles 2 and 4 shows that the MBR system had more favorable substrate degradation rates than those observed for the MBBR-MBR at similar operation conditions.

In general, the decay coefficient increased under the different dopings in relation to the control phase with the exception of cycle 2, which corresponded to the MBR working for 10 h of HRT, 21.7 days of SRT, and 12.6 °C. This suggests that the cell decay rate also increased in the presence of these chemicals, which could explain the reduction of MLSS, as indicated in Table 3.

The combined effect of b_H and $r_{su,H}$ made similar removal efficiencies of organic matter in both the control and doping phases possible (Table 4). Aubenneau et al. [27] analyzed the effect of carbamazepine on an MBR system and did not observe any variation of organic matter removal under exogenous respiration; this explained the increase of b_H as a consequence of higher maintenance requirements. Kraigher et al. [28] explained this by the presence of a different bacterial community, whereas Wang et al. [29] indicated a change in the metabolic pathways of the substrate. Vasiliadou et al. [30] analyzed the influence of pharmaceuticals on an AS system and also indicated modifications of the microbial community as the reason for the adaptation of microorganisms in the presence of these chemicals.

Calero–Díaz et al. [12] investigated an identical mix of pharmaceuticals and obtained similar results for an MBR working at HRT for 6 h, SRT for 7.5 days, and average value for MLSS of 4551 mg L^{-1}. These authors determined the increases in the values of $r_{su,H}$ and b_H in relation to the control phase, proposing that these results were caused by chemical stress once the pharmaceuticals were introduced. Leyva–Díaz et al. [31] analyzed the effect of nalidixic acid (antibiotic) on a NIPHO activated sludge reactor working at HRT values of 2.8–3.8 h, temperatures of 12.6–14.8 °C, SRT values of 11.0–12.6 days, and biomass concentrations of 1400–1700 mgVSS L^{-1}. These authors also found an increase in the values of $r_{su,H}$ and argued that the heterotrophic biomass counteracted a possible physiological stress by increasing the $r_{su,H}$ to favor its acclimatization. In light of this, Bouki et al. [32] stated that the environmental conditions in wastewater treatment plants are suitable for the acquisition and proliferation of antibiotic-resistant bacteria, which may transfer resistant genes to resident bacteria.

3.4. Nitrogen Removal

The extent of the removal of nitrogen in the form of ammonium ($N\text{-}NH_4^+$) in both the MBR and the hybrid MBBR-MBR system is shown in Table 6.

Table 6. $N\text{-}NH_4^+$ removal rate (%).

Cycle	Steady State	Doping 1	Doping 2	Doping 3
1	9.00 ± 2.08	-	-	-
2	6.36 ± 1.81	3.48 ± 0.62	0.15 ± 0.21	-
3	22.61 ± 5.07	9.84 ± 6.02	6.00 ± 1.02	1.06 ± 1.84
4	6.24 ± 3.32	0.66 ± 0.28	0.04 ± 0.02	1.42 ± 1.03

Unlike the organic matter, the introduction of the pharmaceutical compounds mix in the system affects the removal of $N\text{-}NH_4^+$ considerably.

A statistical study on the removal of $N\text{-}NH_4^+$ was carried out with the ANOVA test. In this case, the nitrogen removal was compared between the steady state and each doping concentration for each of the cycles. The MBBR-MBR system did not show statistically significant differences, while the MBR system did. This is because the biofilm systems are more stable and soften the shocks suffered by the system when the pharmaceutical compound mix is introduced [33].

With warmer temperatures in the steady state, as in cycles 1 and 3, the removal of $N\text{-}NH_4^+$ is higher than when the temperature is colder (around 6% for cycles 2 and 4), and especially in cycle 3, this removal is above 22% with a temperature of 28 °C. Regardless of the system used, the temperature is the operational variable that most affects the behavior of the reactor, and in the steady state, more $N\text{-}NH_4^+$ is removed. However, when the pharmaceutical compound mix is introduced into the system, the removal decreases considerably until no more removal was detected in cycle 1.

The hybrid MBBR-MBR system had a better behavior in the removal of $N\text{-}NH_4^+$, although with very low removal values, especially in cycle 4. The removal decreased in the three dopings with respect to the stationary state, by about 13%, 17%, and 22%, respectively, in cycle 3, and for cycle 4, the difference was about 6%, 6%, and 5% less, respectively.

The $N\text{-}NH_4^+$ removal in the MBR system was greatly affected by doping. In cycle 2 with 10 h of HRT and about 13 °C, $N\text{-}NH_4^+$ removal was detected, but with a very low performance. These removal performances differed with respect to the steady state by about 3% and 6% for dopings 1 and 2, respectively. In doping 3 of cycle 2, there is no $N\text{-}NH_4^+$ data because the system suffered a shock when introducing the pharmaceuticals compound, proliferating filamentous bacteria that caused sludge bulking of the system, resulting in the total loss of the biomass.

The COD and nitrogen removal goes in parallel with the removal of pharmaceutical compounds, especially in biofilm systems in which it is involved in co-metabolic activities [34].

4. Conclusions

Given the results obtained in a membrane bioreactor pilot plant operating at similar MLSS concentration (about 5.5 g/L) considered whether or not, with a moving bed biofilm reactor for treating real urban wastewater for 6 and 10 h of HRT under three different shocks of pharmaceuticals (carbamazepine, ciprofloxacin, and ibuprofen) with increasing concentrations, the following conclusions were drawn:

- Organic matter removal was higher in the MBBR-MBR technology for the same HRT and similar MLSS.
- From a kinetic perspective, MBBR-MBR is more suitable for low HRT (6 h) and MBR is more effective for high HRT (10 h). This might be due to the fact that biofilm systems are less sensitive to more unfavorable operating conditions than the MBR systems.
- The removal of $N\text{-}NH_4^+$ considerably decreased in both MBR and MBBR-MBR when the pharmaceutical compounds mix was introduced into the system, until no removal was detected in cycle 1.

Considering the above, MBR and MBBR-MBR are adequate technologies to treat real urban wastewater exposed to the impact of pharmaceutical compounds; however, the MBBR-MBR technology reduces the negative effect of a pharmaceutical shock in the organic matter removal and in the heterotrophic biomass behavior.

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