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A Kinetic Study on Chronic Response of Activated Sludge to Diclofenac by Respirometry

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Abstract: The present study investigated the chronic response of activated sludge to the emerging pollutant diclofenac as well as its aerobic biodegradation potential at different sludge retention times (SRTs). The impact of prolonged exposure to diclofenac on microbial process kinetics was explored with respirometric modelling. The long-term operation of lab-scale reactors revealed that continuous feeding of diclofenac at relevant concentrations observed in municipal wastewaters did not affect carbon removal efficiency independentl of SRT. However, in case of diclofenac removal, 34% efficiency could be achieved at a higher SRT of 20 days. Kinetic evaluation showed that the increment in diclofenac dosing resulted in no adverse effect on the microbial growth rate except that high concentrations of diclofenac exposure decreased the growth rate at SRT of 10 days. A significant increase in hydrolysis rate was determined in the diclofenac-acclimated biomass for both SRTs; even at high concentrations of diclofenac exposure, the hydrolysis rate remained unchanged. Long-term acclimation to diclofenac had a progressive impact on the hydrolysis kinetics, which could be attributed to an alteration in the microbial culture profile. Overall, the results suggest that the operation with diclofenac-acclimated biomass at higher SRTs could enrich a microbial culture capable of overcoming the adverse effect of the pollutant and improve the biodegradation potential as well.

Keywords: biodegradation; emerging contaminant; respirometric modelling; non-steroidal anti-inflammatory drugs; sludge retention time

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1. Introduction

Pharmaceuticals have been extensively consumed, and their occurrence in various environmental matrices is causing wide and significant concern. Pharmaceuticals ranging from trace levels of ng/L to µg/L levels have been detected in the environment due to improper discharge by hospitals and pharmaceutical manufacturing facilities and inadequate removal in treatment plants. Indeed, even trace amounts of pharmaceuticals and their residuals or metabolites have adverse effects on the ecosystem [1]. Specifically, non-steroidal anti-inflammatory drugs (NSAIDs) used for pain and inflammation are the most frequently consumed pharmaceuticals. NSAIDs can be obtained easily without prescription worldwide and are listed among the top 10 persistent pollutants [2]. Diclofenac (2-(2-(2,6-dichlorophenylamino) phenyl) acetic acid) is one of the most widely consumed NSAIDs with over 1000 tons of consumption annually [3]. The physico-chemical properties of NSAIDs, including the octanol/water distribution coefficient (K_{ow}), Henry's law constant, and acid dissociation constant (pKa), determine the fate of pollutants in the environment. Diclofenac (DCF) has low solubility (2.37 mg/L), high log K_{ow} (4.98), and low pKa (4.15) values, indicating hydrophobic sorption potential and hydrophilicity, respectively [4]. Pollutants with high log K_{ow} values (≥4.5) are attributed to higher bioaccumulation potential

and are usually identified as high-risk compounds [5]. Diclofenac is reported to have the capability of bioaccumulation in the tissues of various organisms, which has resulted in their extinction due to high acute toxicity rates [6,7]. The European Environmental Agency included diclofenac in the Watch List of the EU (2015/495) and identified it as a contaminant of emerging concern, suggesting ≤ 0.1 μg/L in freshwater [4]; likewise, the UK classified diclofenac as a priority pollutant. The acute and chronic effects of diclofenac on different organisms (i.e., *Daphnia magna*, zebrafish embryos, rainbow trout, invertebrates, insect species, and vultures) were explored and showed that diclofenac exposure resulted in kidney lesions, accumulation in the liver, kidney, and muscle tissues, genetic damage, increase in neurotoxicity and oxidative stress, reduction in mobility, and a significant increase in mortality rates [8–12]. The Asian vulture population was almost exposed to extinction due to diclofenac [13].

Previous studies indicated that diclofenac has an acute and chronic effect on both the aquatic and terrestrial environments and that the appropriate treatment of diclofenac is of the utmost importance [14]. Due to its high polarity and toxicity, conventional systems are not adequate to treat diclofenac, and they have been noticeably detected in aquatic environments and wastewater treatment plants' effluents [15–18]. The occurrence of diclofenac in the influent of WWTPs ranges from 0.75 to 94 μg/L, depending on the configuration of the treatment process [4,19]. Diclofenac treatment efficiencies in conventional activated sludge systems range from 21% to 50% [20,21]. The biodegradability of diclofenac was also investigated in membrane bioreactor (MBR) systems, and low treatment efficiencies (32%) were reported [6,22]. Suspended growth-activated sludge systems are widely used to treat domestic/municipal wastewater, and studies on the biodegradability of diclofenac under aerobic conditions play an important role in figuring out the fate of diclofenac. Diclofenac removal depends on the source of microbial inoculum, operational parameters, and environmental conditions in biological treatment systems. Numerous lab-scale studies on the biological treatability of diclofenac under aerobic conditions reported contradictory results ranging from 0 to 90% of removal efficiencies [23,24]. The biodegradability of micropollutants should be determined together with their chronic effect on biomass, for which respirometric modelling is an effective methodology. The modelling of respirometric data enables the evaluation of the stoichiometry and kinetics of activated sludge systems, which will determine the toxicity of micropollutants in mixed microbial cultures as well as the performance of the biological treatment [25]. Respirometric measurement relies on the monitoring of the oxygen utilization rate (OUR) of the biomass fed with substrate in a batch reactor, and microbial process kinetics are derived from modelling OUR data. OUR is also used for acute/chronic inhibition assessment [26]. The inhibition impact of micropollutants on microbial processes can be estimated through the respirometric modelling approach, which is more reliable compared to conventional methods [27].

In this context, the major objective of this study was to evaluate the chronic response of activated sludge to diclofenac under aerobic conditions. For this purpose, (i) the aerobic biodegradation potential of diclofenac, as well as the effect of chronic diclofenac exposure on carbon removal performance at different sludge retention times (SRTs), was investigated; (ii) the microbial process kinetics of both diclofenac-acclimated and non-diclofenac-acclimated biomass at relevant concentrations observed in full-scale WWTPs and at comparatively higher concentrations were determined to evaluate the chronic impact of diclofenac.

2. Materials and Methods

2.1. Experimental Setup

To assess the impact of sludge retention time on the biodegradability of diclofenac, laboratory-scale fill and draw reactors were operated at 22 °C ± 1 isothermal room temperature. The working volume of the reactors was 6 L, and continuous aerobic conditions were sustained. The reactors at SRTs of 10 and 20 days were operated for 160 and 250 days, respectively. The reactors were inoculated with seed sludge supplied from the aerobic

unit of a municipal WWTP located in Istanbul, Turkey. The seed sludge was analyzed prior to the experiments and suspended solid (SS) and volatile suspended solid (VSS) concentrations were measured as 7700 ± 28 mg/L and 4950 ± 42 mg/L, respectively, and the diclofenac concentration was detected as <5 ng/L. The diclofenac reactors were fed once a day with diclofenac solution and synthetic wastewater (peptone mixture), which resembles domestic wastewater composition. The concentrations of the synthetic wastewater and diclofenac solution were set as 600 mg/L chemical oxygen demand (COD) and 10 µg/L, respectively, to resemble the relevant concentrations of municipal wastewater. The synthetic wastewater was prepared according to ISO 8192 [28], which contains peptone, meat extract, urea, NaCl, and $CaCl_2$ $2H_2O$. In order to sustain microbial growth, macro-nutrients (KH_2PO_4 and K_2HPO_4) and micro-nutrients ($MgSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, $CaCl_2 \cdot 2H_2O$) were also fed into the reactors. Diclofenac salt was supplied from Sigma Aldrich (St. Louis, MO, USA). Diclofenac stock solution of 500 mg/L concentration was prepared freshly in methanol, which was supplied by Merck (Darmstadt, Germany). Diclofenac-free control reactors were fed with peptone mixture and methanol (24 mg/L COD), which was completely biodegradable to ensure the same conditions as the diclofenac reactors. The pH was controlled between 6 and 8. The reactors were magnetically stirred and aerated with a constant air flow. The photo-degradation potential of diclofenac was prevented through packing the reactors with aluminum foil (Figure 1).

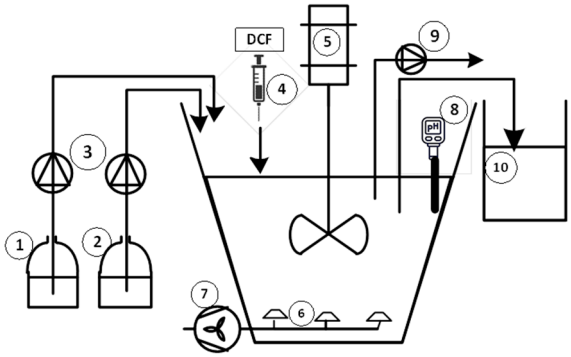


Figure 1. Laboratory-scale fill and draw reactors: (1) nutrient solution, (2) synthetic wastewater, (3) feed pumps, (4) diclofenac feeding, (5) mechanical mixer, (6) diffusers, (7) air pump, (8) pH meter, (9) waste sludge pump, (10) sampling and effluent discharge.

2.2. Respirometric Analyses and Modelling

Respirometric tests were performed with both non-diclofenac-acclimated and diclofenac-acclimated biomass to determine the microbial kinetics through the assessment of the oxygen uptake rate (OUR) profile of the biomass fed with the synthetic wastewater at a determined F/M ratio. Substrate (peptone mixture of 300 mg COD/L) and diclofenac solution were fed to the batch reactors with the same F/M ratio as the main reactors. The chronic effect of diclofenac was tested with different initial concentrations of diclofenac (10 and 1000 µg/L). OUR was monitored with a respirometer (Applitek Ra-Combo). A nitrification inhibitor (2533TM-Hach Company, Loveland, CO, USA) was used to suppress nitrification activity. Control runs were performed with both non-diclofenac- and diclofenac-acclimated biomass, and peptone mixture together with the sole methanol solution was fed in. Diclofenac was not added to the control runs. The COD, SS, and VSS profiles were monitored as well. The respirometric OUR data were interpreted with Modified Activated Sludge Model No.1 (ASM1), which has the structure of ASM1 for organic carbon removal modified for an endogenous decay process [29,30]. The model defines microbial kinetics for the growth process on substrate, the hydrolysis of slowly biodegradable substrate, and the endogenous decay of biomass. In accordance with the microbial processes, ASM1 comprises the major model components of readily biodegradable COD, S_S; rapidly hydrolysable COD, S_{H1}, and slowly hydrolysable COD, S_{H2}; active heterotrophic biomass, X_H, and dissolved

oxygen, S_O. In the ASM1 model, microbial growth is defined by the Monod expression. Hydrolysis rates are expressed in terms of saturation-type surface reaction kinetics, and endogenous decay is defined by the first-degree reaction with respect to X_H. The model kinetic and stoichiometric parameters were estimated according to the estimation method proposed by Insel et al. [31] using the SIMPLEX algorithm. The UNCSIM module was used for the assessment of parameter identification [32]. Model calibration was implemented with an iterative calibration protocol, in which the model components are calibrated in each iteration step and fit the model outputs to real-time data. The model outputs were found to be sensitive to all selected model coefficients. A detailed description of the assessment process is presented in Orhon et al. [30]. Parameter estimation and model calibration were performed with AQUASIM [33]. OUR profiles were simulated by the AQUASIM Version 2.1e simulation program (Swiss Federal Institute for Environmental Science and Technology, Switzerland), which was developed for simulation and data analysis.

2.3. Analytical Measurements

The conventional parameters of pH, suspended solids (SS), volatile suspended solids (VSS), chemical oxygen demand (COD), and diclofenac were measured regularly to assess the reactor performance. SS and VSS measurements were conducted in accordance with the standard methods [34]. Mixed liquor samples were filtered with glass fiber filter papers and further dried in an oven at 105 °C for SS measurement and burned in a furnace at 550 °C for VSS analysis, as described in the SM 2540D and SM 2540C methods [34]. COD measurements were performed according to ISO 6060 [35]. Samples for soluble COD analyses were first filtered with 0.45 µm PVDF filters and determined using the dichromate reflux method. Acidic digestion was performed at 150 °C with potassium dichromate in the presence of a silver-sulfuric acid catalyst, and further titrated with ferrous ammonium sulfate, as described in the protocol [35]. The concentrations of the conventional parameters were measured from duplicate samples, and the relative standard deviations were calculated. Diclofenac measurements were performed for both the aqueous and solid phase samples. Solid phase extraction (SPE) cartridges (Waters Corp., Milford, MA, USA) were used to purify and concentrate the samples. The solvents of acetonitrile and methanol used for conditioning the cartridges were further evaporated at 35 °C and 10 bar of N_2 gas with Turbovap II (Caliper Life Sci., Hopkinton, MA, USA). Diclofenac was quantified by using LC–MS/MS (Thermo Fisher Scientific, Thermo Accela UPLC equipped with Thermo Quantum Access tandem MS, San Jose, CA USA), as explained in Topuz et al. [36]. The diclofenac concentrations reported were the average of the duplicate samples measured.

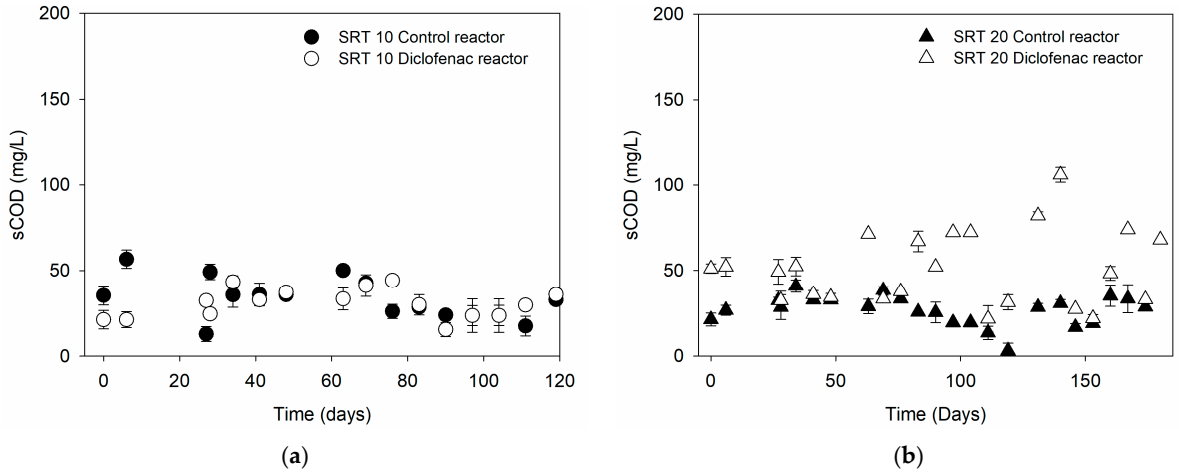
3. Results and Discussion

3.1. Impact of Diclofenac on Reactor Performance

Laboratory-scale reactors were operated to investigate the impact of the SRTs on the aerobic biodegradation potential of diclofenac, as well as the impact of the continuous feeding of diclofenac, on the COD removal performance. Diclofenac, in both liquid and solid phases, and the effluent soluble chemical oxygen demand (sCOD) measurements were performed during the reactor operation to investigate the biodegradability of diclofenac at different SRTs. Under the steady state conditions of the diclofenac-free control reactors, the VSS concentrations were 2170 ± 140 mg/L with a VSS/SS ratio of 0.67 for an SRT of 10 days, and 3400 ± 290 mg/L with a VSS/SS ratio of 0.67 for an SRT of 20 days, respectively. In case of the diclofenac-acclimated reactors, the VSS concentrations were 2270 ± 230 mg/L with a VSS/SS ratio of 0.70 for an SRT of 10 days and 3220 ± 380 mg/L with a VSS/SS ratio of 0.68 for an SRT of 20 days, respectively.

Effluent sCOD concentrations measured in reactors with an SRT of 10 days are given in Figure 2a. Although fluctuations were observed during the acclimation period, the average effluent sCOD concentrations decreased to 33 ± 10 and 32 ± 8 mg/L for the control and diclofenac reactors with an SRT of 10 days, respectively, at steady state. The

removal efficiency was calculated as 95% for both reactors. In case of SRT of 20 days, effluent sCOD concentrations were observed as 25 ± 10 mg/L and 53 ± 18 mg/L for the control and diclofenac reactors on average, respectively (Figure 2b). The overall COD removal efficiencies were 96% and 92% for the control and diclofenac reactors, respectively. Although diclofenac had no effect on the COD removal efficiency at an SRT of 10 days, a slight effect was observed at an SRT of 20 days. The removal mechanism of diclofenac and its effect on COD removal in different reactor configurations, such as MBR and moving bed biofilm reactors (MBBR), were investigated, and a variety of different removal efficiencies of diclofenac were reported. In an aerobic MBR system fed with 100 µg/L diclofenac, an average of 25% diclofenac removal was observed and an average of 97% COD removal efficiency was achieved [37]. In contrast, higher removal efficiencies ranging from 30 to 60% were reported in the MBBR systems [38,39]. However, a slight adverse effect on COD removal performance was observed in the MBBR systems with higher concentrations of the diclofenac dose (10 mg/L). Similar to this study, Zhao et al. [40] reported that COD removal efficiency was decreased to 70% with the increment of diclofenac concentration from 0.01 mg/L to 2 mg/L in a BNR system. All these studies indicate that diclofenac has no adverse effect on COD removal performance of biological systems at environmentally relevant concentrations (1 to 100 µg/L), which is in accordance with the findings of the present study.



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

Figure 2. Effluent sCOD concentration of control and diclofenac reactors for (**a**) SRT of 10 days, (**b**) SRT of 20 days.

3.2. Impact of SRT on Diclofenac Removal Performance

The diclofenac concentrations in liquid phase of the diclofenac reactors operated at SRTs of 10 and 20 days are given in Figure 3, and the theoretical influent diclofenac concentrations in the reactors were 13.3 and 15 µg/L, respectively. The actual effluent diclofenac concentration was measured as 13.4 ± 1.7 µg/L, on average, for SRT of 10 days (Figure 3a), which shows that diclofenac could not be degraded at this sludge age. However, the average diclofenac concentration within the first 50 days of the operation was detected as 15 ± 1.8 µg/L (0 to 50th day of the operation), and then decreased to 10.5 ± 0.6 µg/L (50th to 100th day of the operation) at SRT of 20 days (Figure 3b). The diclofenac removal efficiency increased to 34.3% at higher SRT of 20 days. The fact that diclofenac removal occurred right after the acclimation period draws attention to the importance of acclimation. Contradictory results were reported on the relevance of the SRT on diclofenac removal, but SRT was mainly considered to be an important operational parameter for sustaining efficient diclofenac treatment [41]. The biological removal of micropollutants in various conventional WWTPs was studied, and a variable diclofenac removal efficiency of 0 to 70% was reported. They observed that SRTs higher than 10 days increased the removal efficiency of diclofenac [42]. Contreras et al. [43] also obtained inconsistent removal efficiency, ranging

between 0 and 90%, in an extended aeration system operated at an SRT of 20 days. A longer SRT was thought to favor the diclofenac biodegradation due to adaptation of the activated sludge to diclofenac [44].

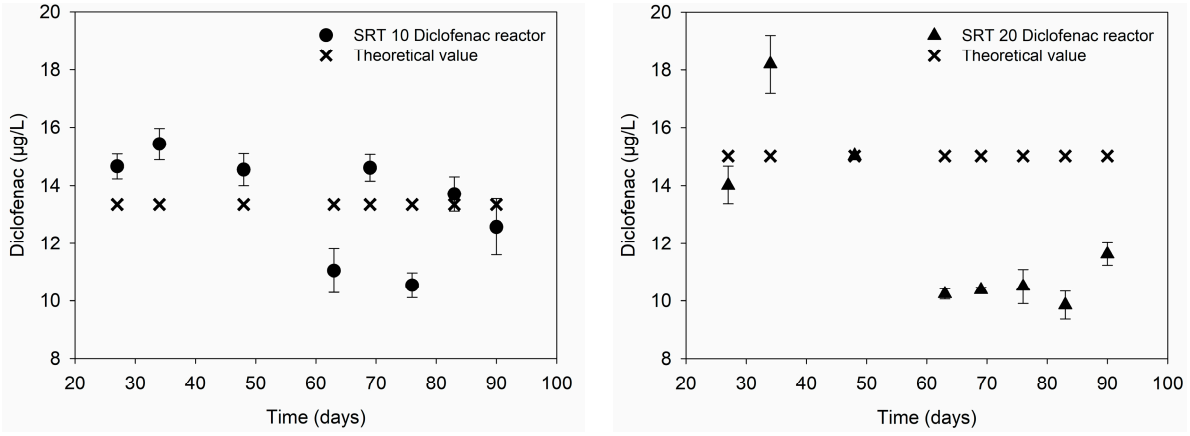


Figure 3. Diclofenac removal efficiency for (**a**) SRT of 10 days and (**b**) SRT of 20 days.

In order to evaluate the biodegradation mechanism of diclofenac, the sorption potential of diclofenac should be also investigated. The sorption of diclofenac in the solid phase depends on its physico-chemical properties as well as the operational conditions of the treatment process. Diclofenac can be observed in both the aqueous and solid phases due to its pH-dependent $\log K_{ow}$ value [4]. The possible sorption potential of diclofenac was investigated, and the results of the diclofenac measurements performed for the sludge samples are given in Table 1. The diclofenac concentration of all sludge samples was found to be <5 ng/g and approximately 2% of the diclofenac adsorbed on the sludge samples. These results indicate that the sorption of diclofenac to biomass was negligible and biodegradation was the major removal mechanism. Similarly, Scheytt et al. [45] reported that the sorption of diclofenac to the solid phase is insignificant and that sorption is not the dominant mechanism of diclofenac removal in biological wastewater treatment systems.

Table 1. Percentage of diclofenac adsorbed onto diclofenac-acclimated biomass.

Time (Days)	SRT 10 Days		SRT 20 Days	
	Before Feeding (%)	After Feeding (%)	Before Feeding (%)	After Feeding (%)
27	1.2	1.1	-	1.8
48	3.0	1.8	2.5	3.3
63	-	1.5	1.5	2.0
69	1.0	1.0	1.3	2.3
76	2.3	1.6	1.3	1.8
83	1.2	1.3	1.5	1.6
90	1.1	1.0	1.8	1.9

3.3. Respirometric Tests

Respirometric measurements were performed to determine the chronic effect of diclofenac on activated sludge with the addition of 10 and 1000 µg/L of diclofenac solution. Control runs were performed solely with peptone mixture and methanol solution without diclofenac addition for both the non-acclimated and diclofenac-acclimated biomass. The OUR and COD versus time profiles for the SRT of 10 days are given in Figure 4. The COD removal was determined as 86% and 94% for the non-acclimated and diclofenac-acclimated biomass without diclofenac addition at SRT of 10 days (Figure 4a,b). The OUR profiles of diclofenac-acclimated biomass obtained from SRT of 10 days' reactor with the addition of 10 and 1000 µg/L diclofenac are shown in Figure 4c,d. Overall, the COD removal efficiencies were determined as 92% and 94% for the diclofenac-acclimated biomass with 10 and 1000 µg/L diclofenac additions, respectively. The impact of various diclofenac

concentrations of 0, 10, and 1000 µg/L on the OUR profiles are explored comparatively (Figure 4e), and it was observed that a low concentration of diclofenac did not affect the OUR levels, while a slight change was observed at higher diclofenac concentrations with a low inhibition effect. The second plateau of OUR was slightly affected by the increasing concentrations of diclofenac, showing the impact on the hydrolysis rate of activated sludge. However, the modelling results give a better insight, as explained below.

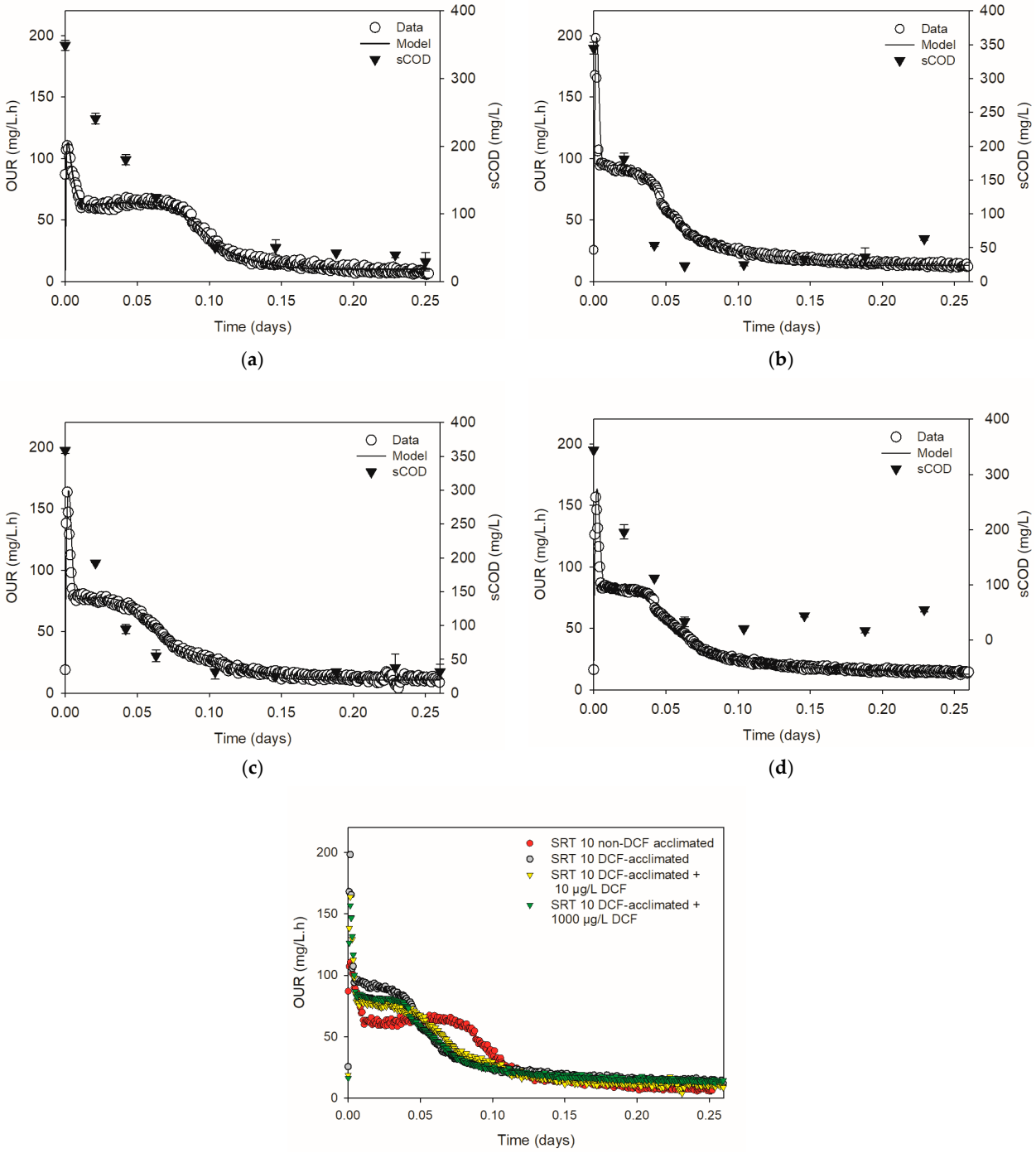


Figure 4. Experimental OUR and COD data and simulation profiles of runs operated at SRT of 10 days: (**a**) non-diclofenac-acclimated biomass, (**b**) diclofenac-acclimated biomass, (**c**) diclofenac-acclimated biomass fed with 10 µg/L diclofenac, (**d**) diclofenac-acclimated biomass fed with 1000 µg/L diclofenac, (**e**) OUR data profiles of all runs.

The OUR and COD profiles of the biomass taken from the non-diclofenac-acclimated and diclofenac-acclimated reactors operated at an SRT of 20 days are given in Figure 5. The COD removals were determined as 92% and 92% for the non-acclimated and diclofenac-acclimated biomass without diclofenac addition at an SRT of 20 days, respectively. The COD removal efficiencies were determined as 95% and 95% for diclofenac-acclimated biomass with 10 and 1000 µg/L diclofenac additions, respectively (Figure 5c,d). Thus, the increment of diclofenac did not have any inhibition effect on the COD removal mechanism. Overall, when the OUR profiles for both 10 and 20 days of SRT were compared (Figures 4e and 5e), diclofenac seemed to affect the growth and hydrolysis processes of the activated sludge, which could be better evaluated through the modelling approach.

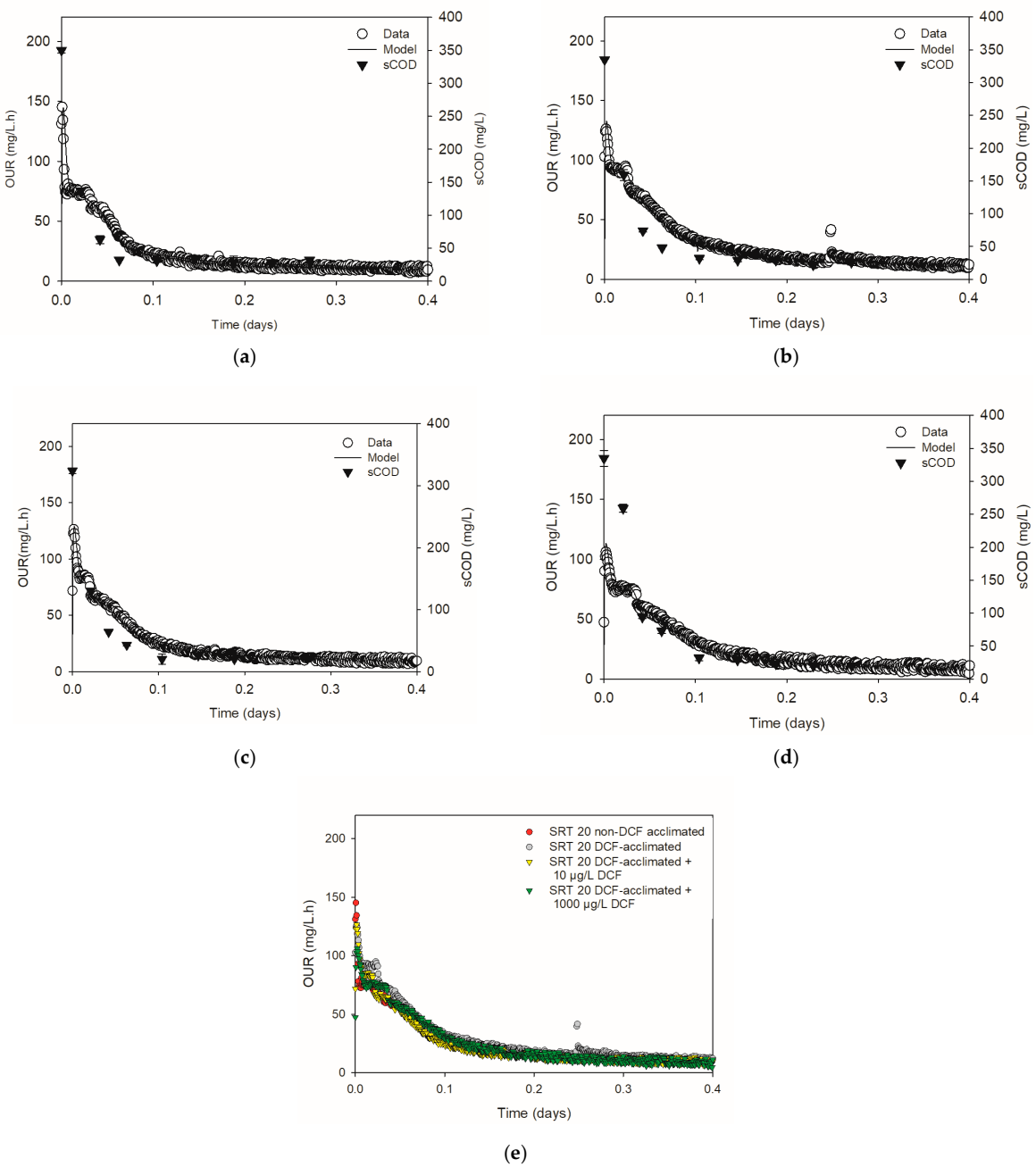


Figure 5. Experimental OUR and COD data and simulation profiles of runs operated at SRT of 20 days: (**a**) non-diclofenac-acclimated biomass, (**b**) diclofenac-acclimated biomass, (**c**) diclofenac-acclimated biomass fed with 10 µg/L diclofenac, (**d**) diclofenac-acclimated biomass fed with 1000 µg/L diclofenac, (**e**) OUR data profiles of all runs.

3.4. Chronic Effect of Diclofenac on Microbial Kinetics

Modelling of respirometric data enables the identification of the acute and chronic inhibition of micropollutants on microbial growth, hydrolysis, and storage kinetics. In this study, the chronic impact of diclofenac on organic carbon degradation and hydrolysis kinetics was evaluated for the diclofenac-acclimated and non-acclimated biomass by parameter estimation with the multi-component modelling approach. The change in process kinetics as a function of SRT was assessed as well. Figures 3 and 4 also shows the modelling simulation profiles for the SRTs of 10 and 20 days. Table 2 summarizes the model state variables and coefficients estimated through the model calibration of the experimental OUR data. The modelling of OUR data depicted that 7.3% of the total COD (C_T) was readily biodegradable COD (S_S), while rapidly hydrolysable (S_{H1}) and slowly hydrolysable (S_{H2}) COD fractions covered 40% and 52.7% of the total COD. The COD composition of the peptone mixture obtained in this study well resembles domestic wastewater and most industrial wastewaters [26]. The stoichiometric coefficients determined in this study were also compatible with the reported studies of domestic wastewater in the literature, as the heterotrophic yield coefficient was 0.58 g cell COD/g COD for all runs. The respirometric tests depicted no adverse effect on the activity of the biomass; indeed, the activity was increased in the diclofenac-acclimated biomass with an SRT of 10 days compared to the non-diclofenac-acclimated biomass. Similar to this study, Dereszewska and Cytawa [46] reported that activity of the activated sludge did not change even at high doses of diclofenac. Moreover, the endogenous decay rate remained unchanged under continuous exposure to diclofenac.

The maximum growth rate and half-saturation constant values are significant for the assessment of the inhibitory effect of pollutants on microbial growth. The heterotrophic maximum specific growth rate and the half-saturation constant remained same for all runs of the diclofenac-acclimated biomass operated at an SRT of 20 days. The maximum specific heterotrophic growth rate ($\hat{\mu}_H$) was 5.5 day^{-1} and the corresponding half-saturation constant for heterotrophic growth (K_S) was 11 mg/L. Thus, the increment in diclofenac concentration resulted in no adverse effect on the microbial growth of the heterotrophs. However, at an SRT of 10 days, the results implied that low concentrations of diclofenac did not have an inhibitory effect on microbial growth, but 1000 µg/L of diclofenac dosing apparently decreased the microbial growth rate, indicating the adverse effect of diclofenac. As expected, a lower SRT selected a microbial culture with a faster microbial growth as the maximum specific heterotrophic growth rate ($\hat{\mu}_H$) for an SRT of 10 days was higher than that of an SRT of 20 days. However, unlike SRT of 20 days, high concentrations of diclofenac exposure (1000 µg/L) negatively affected the growth rate at an SRT of 10 days as $\hat{\mu}_H$ decreased from 7 day^{-1} to 6.2 day^{-1}. The half-saturation constants for growth (K_S) were in the default range (5–30 mg COD/L) for both SRTs. On the other hand, when non-diclofenac-acclimated and diclofenac-acclimated biomass were compared, long-term exposure to diclofenac at an SRT of 10 days surprisingly decreased the half-saturation constant, resulting in an increase in affinity for the substrate, thus positively affecting the half-saturation constant. The determination of the half-saturation constant with the maximum specific growth rate will enable the evaluation of the selection of microbial cultures specific to the substrate; low K_S values usually refer to microbial culture with high affinity for the substrate, resulting in a higher uptake of substrate [47]. The increase in COD removal efficiencies of the non-diclofenac-acclimated and diclofenac-acclimated biomass, as well as at different SRT days, might be attributed to the change in the half-saturation constant values of the aforementioned biomass samples.

Table 2. Model parameters and state variables.

State Variables	Symbol	Unit	SRT 10 Days				SRT 20 Days			
			Non-DCF-Acclimated Biomass	DCF-Acclimated Biomass	DCF-Acclimated Biomass + 10 μg/L DCF	DCF-Acclimated Biomass + 1000 μg/L DCF	Non-DCF-Acclimated Biomass	DCF-Acclimated Biomass	DCF-Acclimated Biomass + 10 μg/L DCF	DCF-Acclimated Biomass + 1000 μg/L DCF
Maximum specific growth rate for X_H	$\hat{\mu}_H$	1/day	7.0	7.0	7.0	6.2	5.5	5.5	5.5	5.5
Half-saturation constant for growth of X_H	K_S	mg COD/L	25	9	9	9	11	11	11	11
Maximum hydrolysis rate for S_{H1}	k_{h1}	1/day	0.92	1.92	1.92	1.92	0.60	0.75	0.75	0.75
Hydrolysis half-saturation constant for S_{H1}	K_X	g COD/g COD	0.01	0.01	0.01	0.01	0.05	0.01	0.01	0.01
Maximum hydrolysis rate for S_{H2}	k_{h2}	1/day	1.6	1.1	1.1	1.1	3.0	2.5	2.5	2.5
Hydrolysis half-saturation constant for S_{H2}	K_{XX}	g COD/g COD	0.01	0.01	0.01	0.01	0.036	0.025	0.025	0.025
Endogenous decay rate for X_H	b_H	1/day	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Yield coefficient for X_H	Y_H	g COD/g COD	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
Volatile suspended solids	X_T	mg COD/L	2160	2380	2075	1980	2145	2960	2500	2250
Initial active heterotrophic biomass	X_H	mg COD/L	1200	1650	1300	1425	1370	1220	1125	975

For dual hydrolysis, a noticeable difference was observed between the diclofenac-acclimated and non-acclimated biomass for both SRTs. A significant increase in the hydrolysis rate for the rapidly hydrolysable COD fraction (k_{h1}) was determined in the diclofenac-acclimated biomass compared to the non-acclimated biomass, and same hydrolysis kinetics were assessed after diclofenac additions for both SRTs. The results might be attributed to a shift in microbial culture after continuous feeding of diclofenac as an apparent difference was detected between the control (diclofenac-free) and diclofenac reactors. The mixed microbial culture seemed to shift to an enriched culture which was more resistant to diclofenac inhibition and had a higher hydrolysis rate for S_{H1} even at higher diclofenac exposure. However, in case of the maximum hydrolysis rate for S_{H2}, a slight decrease was observed for the diclofenac-acclimated biomass at an SRT of 10 days; in contrast, a slight increase was detected for the diclofenac-acclimated biomass at an SRT of 20 days. These results indicated that long-term feeding with diclofenac at a higher SRT increased the slowly hydrolysis rate of the biomass, which was a significant output as the major fraction of the total COD was S_{H2}. On the other hand, the decrease in S_{H2} for the SRT of 10 days might be related to a change in composition of the mixed microbial culture. Generally, the adverse effect of diclofenac was more noticeable for the SRT of 10 days. Indeed, the SRT of 20 days resulted in a change in microbial community profile to overcome the inhibitory effects of diclofenac, which reflected the microbial kinetics. Mixed microbial culture history is supposed to affect microbial process kinetics and substrate affinity as well [48]. The alteration in microbial community profile as a function of culture history (i.e., SRT) resulted in variable process kinetics [49,50]. The enrichment of resistant microbial culture under chronic exposure to antibiotics has previously been reported [51]. In compliance with this finding, Pala-Ozkok et al. [26] experimentally proved that continuous exposure to antibiotics considerably altered the bacterial community profile of the biomass. They also detected antibiotic resistance genes, proving the proliferation of resistance culture as a function of chronic exposure.

Overall, the kinetic evaluation depicted a different kinetic response of acclimated biomass with respect to non-acclimated biomass. The major finding derived from the modelling results was that long-term acclimation to diclofenac had a progressive impact on the hydrolysis kinetics, which could be attributed to an alteration in the microbial culture profile. Moreover, even at high concentrations of diclofenac exposure (100 times that of the initial concentration), the hydrolysis rate of the biomass remained unchanged. Mostly, studies focus on the acute impact of micropollutants, which is critical to determine the response of biomass exposed to unpredicted high dosages of NSAIDs. However, the investigation of the chronic effect of micropollutants is also essential since prolonged exposure might enhance the acclimation of the biomass to micropollutants, which would enrich a microbial culture capable of overcoming the adverse effect of the pollutant and improve the biodegradation potential as well [51,52].

4. Conclusions

The assessment of the chronic impact of diclofenac on the microbial process kinetics revealed that prolonged diclofenac exposure at relevant concentrations observed in municipal wastewater would not adversely affect the carbon removal processes in full-scale WWTPs. However, conventional WWTPs are not practically designed to treat NSAIDs; thus, further studies are required to improve the biodegradation potential of NSAIDs. Furthermore, sustaining an acclimated microbial culture at high SRTs might enhance the biodegradation potential of NSAIDs through the proliferation of NSAID-resistant microorganisms. The modelling of respirometric data enables an understanding of the impact of acute/chronic inhibition on microbial kinetics, which is essential to elucidate the performance of biological treatment systems. Likewise, the assessment of microbial dynamics is of utmost importance since alteration in the microbial population affects the microbial kinetics as well. Thus, further studies of the integration of molecular analyses into respirometric modelling are required to enlighten the inhibition effects of micropollutants on biological systems.

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