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Research Article

Removal of Diclofenac in Wastewater by Activated Sludge in Batch and Moving-bed Biofilm Reactor Experiments

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Abstract

This study aimed to determine the diclofenac removal in wastewater by sludge taken from an operating wastewater treatment facility. Laboratory experiments were conducted in two parts: batch and moving-bed biofilm reactor (MBBR) experiments. Results from batch experiments showed that 0.1–2 mg L^{-1} of diclofenac could be removed more than 80% within 72 h, and the removal efficiency reduced to less than 60% for higher concentrations. The increase in the removal rate from 0.00058 to 0.16527 mg L^{-1} h^{-1} was observed when the initial diclofenac concentration increased from 0.1 to 10 mg L^{-1}, respectively. The average first-order rate constants of 24-h and 72-h degradation were calculated as 4.71×10^{-2} and 1.99×10^{-2} h^{-1}, respectively. The removal of diclofenac by sludge was mainly from biodegradation by microorganisms in sludge, followed by the adsorption onto the sludge biomass. The addition of various metal ions in the studied range did not significantly increase the diclofenac removal; however, the addition of Ca^{2+}, Co^{2+}, Fe^{3+}, Mn^{2+}, and Zn^{2+} tended to increase both diclofenac removal rate and efficiency. This positive effect was reduced when the metal ion concentrations were increased up to 0.75 ppm. Lastly, results from an initial phase of continuous MBBR showed that sludge addition during the start-up also extended the diclofenac removal efficiency to one week compared with 3 days in the experiment without sludge addition. In conclusion, the findings show the capability of using activated sludge in diclofenac wastewater treatment by the traditional or alternative systems.

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Introduction

The increase of emerging pollutants in wastewater such as pharmaceuticals brings a concern of the effectiveness of wastewater treatment systems in removing these micropollutants [1]. Various pharmaceuticals have been found in the effluent from a municipal wastewater treatment plant (WWTP) [2–3], which suggests that the conventional treatment system might not be effective enough in removing pharmaceuticals, leading to their transport from the effluent into aquatic environment. Among various pharmaceuticals, diclofenac is a non-steroidal anti-inflammatory drug (NSAID) widely used worldwide with the global consumption more than 900 t a^{-1} [4]. Diclofenac has been detected in the effluent from wastewater treatment facilities in many countries [2–3, 5]. The maximum concentrations of diclofenac found in residential and hospital wastewater can be up to 0.1 and 0.03 mg L^{-1}, respectively [6]. It was found that the removal of diclofenac by WWTP varied between more than 70% to no removal for the concentrations of 0.1 to 1.5 μg L^{-1} [7]. Incomplete removal of diclofenac by municipal WWTPs resulted in contamination of diclo-

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fenac in soil and aquatic environment [8], where it can show acute toxicity as low as 1 mg L^{-1} of the EC50 [9]. It has been shown that diclofenac does not readily biodegrade and will continue to exist in the environment, which brings about aquatic ecotoxicity and health problems even in low concentrations [10]. Adverse effects of diclofenac on organisms including birds, fish, and mussels range from endocrine disruptions, reduced hatching success, bioconcentration, tissue damage to death [11]. Therefore, the development of conventional wastewater treatment system to be able to biodegrade diclofenac might be the key to reduce this pharmaceutical release and toxicity in environment.

Removal of diclofenac from wastewater relies on biodegradation by microorganisms in the wastewater treatment system. The effectiveness of biodegradation depends on various factors including temperature, oxygen conditions, carbon source and nutrients, and the presence of other constituents that can help in metabolic pathways [12–13]. Metal ions are normally found in wastewater such as Ca^{2+}, Cu^{2+}, Mn^{2+}, and Fe^{3+} [14]. Based on the literatures, various metal ions play a role in bacterial growth as well as bacterial enzyme activation [15–19]. For emerging contaminants, a study found that the addition of manganese led to the generation of biogenic manganese oxides by microorganisms, leading to better removal of pharmaceuticals in wastewater [1]. Therefore, the presence of some metal ions in wastewater could be a key in diclofenac biodegradation in the wastewater treatment plant.

Moving-bed biofilm reactor (MBBR) is a wastewater treatment system that relies on the removal of pollutants by the biomass of biofilms forming on the suspended carriers [1]. The system has been proved to be effective in the removal of more than 20 pharmaceutical compounds compared with the conventional activated sludge system (CAS) [20]. For this reason, modification of MBBR for treatment of wastewater containing pharmaceuticals has been continuously studied [21–22], but the complete removal was not always achieved [23–24]. Also, the disadvantages of MBBR needs to be considered for successful operation, which include the oxygen conditions, long start-up time, and the effects from other toxic pollutants [1, 21, 25]. Thus, bio-augmentation of specific microbes that can grow and tolerate those conditions in the reactor could be a way to obtain higher removal efficiencies of pharmaceuticals in wastewater by MBBR [26]. However, bioaugmentation with pure cultures for wastewater treatment is challenging as the cultures may not be dominating the environment, so the application is moving toward the use of microbial communities [20]. Even though CAS is a system that may not provide the diclofenac removal efficiency as high as MBBR [25], the biomass grown in CAS could provide various pharmaceutical-degrading microorganisms useful for diclofenac biodegradation in wastewater [27]. For this reason, the development of MBBR could be based on the use of AS as a source of microorganisms on the development of effective biomass in MBBR to remove a pharmaceutical like diclofenac from wastewater.

This study aims to observe the diclofenac removal by sludge obtained from a municipal wastewater treatment facility. Sludge from the activated sludge system was used as sources of active microorganisms and biomass for the diclofenac removal via biodegradation and adsorption, respectively. After that, to enhance the diclofenac biodegradation by sludge, the effect of metal ion addition was observed in batch experiments. Lastly, the application of sludge in an initial phase of a lab-scale MBBR for diclofenac removal in wastewater was tested to study the potential of sludge application with an alternative wastewater treatment system.

Materials and methods

1) Preparation of diclofenac, wastewater, and sludge samples

Diclofenac used in this study was purchased in powder form as 2-[2-(2,6-dichlorphenyl)amino] benzene acetic acid sodium salt (HPLC grade, purity > 98.5%, Sigma-Aldrich). Diclofenac was dissolved in sterile deionized (DI) water to the concentration of 1,000 mg L^{-1}, then it was kept as a stock solution in dark at 4 °C. Before use in an experiment, the stock solution was diluted with sterile DI water to the required concentrations.

Both wastewater and sludge samples were taken from a wastewater treatment facility in Amphoe Meuang, Chiang Mai Province, Thailand. The facility receives wastewater from the nearby community, which includes educational institutions, residential area, restaurants, and a hospital. The schematic diagram of the wastewater treatment system is shown in Figure 1. Wastewater was taken from an influent collection tank while sludge was collected after dewatering process. The water quality parameters of the influent were obtained from the facility as background information (Table 1). The methods used for determination of some water quality parameters are: hexane extraction method for FOG, titrimetric for NH_3-N, macro Kjeldahl method for TKN and Org-N, and ascorbic acid method for total P. Both wastewater and sludge samples were kept at 4 °C to maintain the water condition and microbial activity before used within 48 h in experiments. To control the quality of sludge and wastewater throughout the experiments, both of them were collected in a single time to be enough for the experiments in this study.

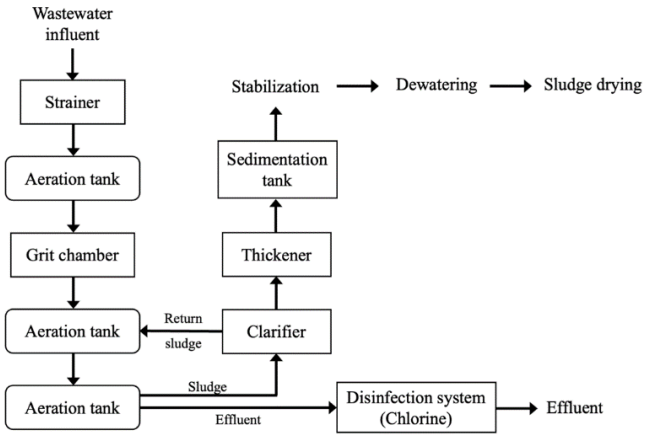

Figure 1 Simplified diagram of the wastewater treatment process in the wastewater treatment facility used in this study.

Table 1 Water quality parameters of the influent from the wastewater treatment facility used in this study

Parameter	Unit	Mean ± SD
MLSS	mg L^{-1}	146.00 ± 29.70
MLVSS	mg L^{-1}	129.00 ± 11.31
%MLVSS	%	89.42 ± 10.44
FOG	mg L^{-1}	4.13 ± 0.18
pH	-	5.62 ± 0.12
Temp	°C	25.70 ± 0.28
BOD	mg L^{-1}	206.25 ± 31.82
COD	mg L^{-1}	273.00 ± 38.18
NH_3-N	mg L^{-1}	17.13 ± 0.39
TKN	mg L^{-1}	24.04 ± 0.65
Org-N	mg L^{-1}	6.92 ± 0.27
Total P	mg L^{-1}	0.33 ± 0.13

2) Batch experiments

Batch experiments were conducted to observe diclofenac removal in wastewater and wastewater added with sludge. Each experiment was conducted in 250-mL Erlenmeyer flasks wrapped with aluminum foil to avoid diclofenac photodegradation during the experimental time. Each flask consists of 100 mL of wastewater added with 10 mL of the diluted diclofenac solution to the final concentrations of 0.1, 0.2, 0.5, 1, 2, 5, and 10 mg L^{-1}. Sludge was added as a wet weight of around 3 g into the flasks requiring both wastewater and sludge. The amount of sludge added in the experiment was determined from preliminary experiments varying the sludge amount of 1 to 5 g, in which the lowest amount of sludge that could yield high removal efficiency was selected. The control experiment for self-degradation of diclofenac was conducted using 100 mL of DI water instead of the wastewater. All flasks were then incubated at 100 rpm of shaking and room conditions (25 to 33 °C) for 72 h. A sample of 2 mL was taken from each flask at 0, 24, 48, and 72 h. Each sample was analyzed for diclofenac by a high-performance liquid chromatography (HPLC) described in further subsection. To determine the diclofenac removal by adsorption on the biomass of the sludge, sludge and wastewater were autoclaved twice (121 °C, 30 min, 15 psi), and both were used in the batch experiments conducted as described above. All experiments were conducted in triplicate using separated flasks.

3) Diclofenac analysis by HPLC

The samples taken from experiments were filtered through a nylon syringe filter (0.2 µm of pore size). The filtered samples were analyzed for diclofenac immediately or kept in dark at 4 °C before being analyzed to prevent any photodegradation. An HPLC (Agilent 1100 VWD) with a HypersilTM ODS C18 reverse phase column (125 x 4.0 mm, 5 µm) and a UV detector was used for diclofenac analysis. The mobile phase was acetonitrile: DI water:formic acid (25 mM) at the ratio of 55:40:5 (%v/v) with the flow rate of 0.5 mL min^{-1}. Both acetonitrile and formic acid were HPLC grade. Diclofenac was detected at the wavelength of 276 nm [28]. The stock solution of diclofenac prepared as described above was diluted to various concentrations before the analysis by HPLC, and the data were used to construct a standard curve with the lowest concentration of 50 µg L^{-1} as the detection limit. The concentration of diclofenac was then determined from the standard curve. The data were reported as a C_t/C_0 curve, where C_t is the diclofenac concentration at each sampling time and C_0 is the diclofenac concentration at 0 h. All data were plotted as the mean and SD (presented as error bars) using GraphPad Prism® software version 10.0.0 (131) (GraphPad Software, La Jolla, CA).

4) Effects of metal ions on diclofenac biodegradation

The metal ions used this experiment were: Ca^{2+} (from $CaCl_2$), Co^{2+} (from $CoCl_2{\cdot}6H_2O$), Cu^{2+} (from $CuSO_4$), Fe^{3+} (from $FeCl_3$), Mn^{2+} (from $MnSO_4$), Ni^{2+} (from $NiCl_2$), and Zn^{2+} (from $ZnCl_2$). All metals were selected based on the role in bacterial growth, enzymes' active sites, and metallic enzyme activators [12,15–19, 29–30]. Stock metal solutions were prepared from commercial grade chemicals in sterile DI water. Each metal solution was diluted, and a total volume of 1 mL of the diluted solution was added into the flask to the final concentrations of 0.25, 0.50, and 0.75 ppm. The diclofenac initial concentration was 1 mg L^{-1}. The biodegradation experiment was conducted for 72 h in the batch experiments as described earlier.

5) MBBR experiments

A lab-scale MBBR was used to observe diclofenac removal in a continuous-mode wastewater treatment system. An MBBR reactor was made of a 2-L glass bottle with the working volume of 1 L according to the outlet position of the reactor (Figure 2). The selected volume

was based on the 10-time increase in the volume from the batch experiment, and the materials of the reactor should be glass as same as experimental flasks. The inlet was supplied with a feeding tank containing wastewater mixing with diclofenac at a desired concentration (0.5 and 1 mg L^{-1}). Plastic carriers (polyethylene, size of 12 mm x 10 mm) were added into the reactor with the volume of 40% of the reaction volume. An air pump was used to suspend the carriers and supply aerobic condition. The start-up of the reactor was conducted by filling the reactor with 1 L of wastewater and the carriers, then it was allowed to run in batch mode for 72 h. After that, the wastewater containing diclofenac was fed into the reactor through the inlet. A peristaltic pump was used to control a constant flowrate of 1 mL min^{-1}. Since the working volume of the reactor is 1 L, the hydraulic retention time was calculated as 16.67 h, which is similar to 15 h of a typical HRT applied in a wastewater treatment plant [31]. Every 24 h, 2 mL of sample was taken from the outlet of the reactor, and two samples were taken for the analysis of diclofenac by HPLC as described above. The reactor was operated for 21 days or until the constant diclofenac removal was achieved. To observe the effect of sludge addition on the initial MBBR performance, 30 g of sludge was added at the 3-day start-up of the reactor before running the experiment with the same procedure.

Results and discussion

1) Removal of diclofenac in wastewater by sludge from a wastewater treatment facility: Role of biodegradation and adsorption

The removal of diclofenac was observed in batch experiments with various concentrations (0.1-10 mg L^{-1}) of diclofenac (Figure 3(a)-(d)). Diclofenac in sterile DI water showed no to very low reduction, suggesting a very small degree of diclofenac self-degradation (Figure 1(a)). Therefore, any reduction of diclofenac observed in the following results should be mainly from the removal processes of diclofenac by wastewater constituents, sludge biomass, and/or microorganisms. In wastewater, diclofenac was not effectively removed (Figure 3(b)), showing the removal efficiencies of 18.52±9.20%, 7.56± 1.60%, and 0% for 0.1, 1, and 10 mg L^{-1} of diclofenac within 72 h, respectively. The increase in diclofenac concentration resulted in lower removal efficiency. Whether this reduction of diclofenac in wastewater was from biodegradation or interactions with constituents in wastewater, the results still showed that diclofenac contaminated in municipal wastewater should not be effectively removed over 72 h.

The municipal wastewater treatment facility uses the activated sludge system as the main wastewater treatment process. Therefore, the experiments with sludge addition into wastewater were conducted to determine a potential for biodegradation. The removal of diclofenac in wastewater by sludge addition was observed in Figure 3(c). The results showed high removal efficiency (more than 80% within 72 h) for diclofenac concentrations of 0.1-2 mg L^{-1}. Most of the removal occurred within the first 24 h of the experiments. When the concentrations were increased to 5 and 10 mg L^{-1}, the removal efficiency reduced to 56.46±1.19% and 43.03±6.31%, respectively. The removal of diclofenac by sludge should be mainly from biodegradation by microorganisms in sludge. Previous studies showed that activated sludge from a wastewater treatment system contains multidrug resistant bacteria important to diclofenac removal [32].

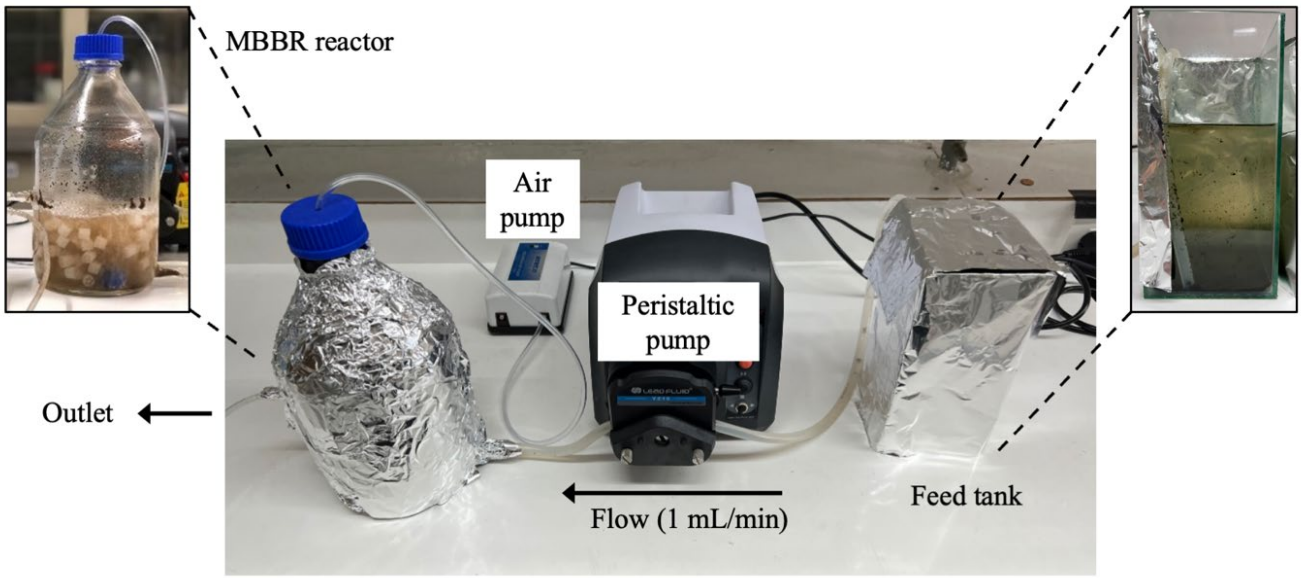

Figure 2 An MBBR reactor used in this study.

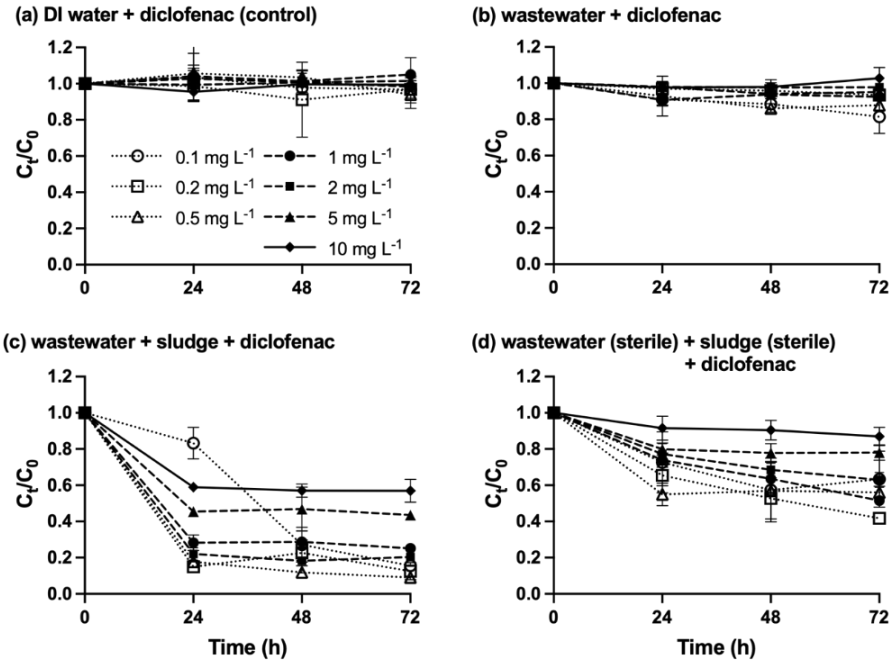

Figure 3 Removal of diclofenac at different concentrations (0.1-10 mg L^{-1}) in batch conditions: (a) DI water (control), (b) wastewater, (c) wastewater and sludge, and (d) sterile wastewater and sludge. The removal efficiency was represented by the ratio of remaining concentration (C_t)/initial concentration (C_0) (x-axis) over 72 h of experimental time (y-axis).

In a wastewater treatment system using biomass such as biofilter, biosorption plays a key role in pharmaceutical removal from wastewater [33]. In this study, diclofenac might not only be biodegraded but adsorbed onto the biomass of sludge, causing the results of removal. To observe the diclofenac removal by adsorption, the experiments were then conducted using sterile sludge and wastewater (Figure 3(d)). The results showed that high diclofenac adsorption onto sludge was observed at low concentrations of diclofenac, ranging between 36.62± 15.51% to 10.03±5.01% when the concentration was increased from 0.1 to 10 mg L^{-1}. Elshikh et al. [32] observed higher adsorption capacity of diclofenac by activated sludge, showing more than 80% of removal for 1 mg L^{-1} of diclofenac with the increased adsorption at the concentration up to 100 mg L^{-1}. Because the amount of sludge was fixed and the adsorption capacity was not observed in this study, it cannot be confirmed that the yielding of diclofenac removal in higher concentrations in Figure 3(d) was due to the reached adsorption capacity or not. However, another explanation for low biosorption of diclofenac at higher concentrations might be the repulsion between negatively charged biomass and diclofenac in wastewater [33]. Since diclofenac was removed rapidly in 24 h, the initial removal rate in 24 h (r_{24h}) was calculated (Table 2). The results showed an increase in the removal rate from 0.00058 to 0.16527 mg L^{-1} h^{-1} when the initial diclofenac concentration increased from 0.1 to 10 mg L^{-1}, respectively. This increasing rate with concentration suggested a first-order reaction; therefore, the first-order rate constants were obtained from a slope of the graph plotted between ln C_t and time. The average first-order rate constants of 24-h degradation and 72-h degradation were calculated as 4.71 x 10^{-2} and 1.99 x 10^{-2} h^{-1}, respectively. The lower rate constant for 72-h degradation was observed because it included the data with low degradation rate after 24 h. It should be noted that the second-order model was also considered but the R^2 values were lower than the first-order model (0.71, 0.65, 0.60, 0.82, 0.77, 0.59, and 0.38 of the 72-h second-order rate constants compared with 0.95, 0.95, 0.82, 0.83, 0.73, 0.62, and 0.40 of the 72-h first-order rate constants for diclofenac at 0.1, 0.2, 0.5, 1, 2, 5, and 10 mg L^{-1}), proving that the first-order model was better fitted with the experiments.

Table 2 Initial rates and first-order rate constants of diclofenac removal by sludge in wastewater

Initial diclofenac concentration (mg L^{-1})	24-h initial removal rate (r_{24h}) (mg L^{-1} h^{-1})	24-h first-order rate constant (k_{24h}) (h^{-1})	72-h first-order rate constant (k_{72h}) (h^{-1})
0.1	0.00058	7.70×10^{-3}	2.80×10^{-2}
0.2	0.00454	7.93×10^{-2}	2.45×10^{-2}
0.5	0.01568	7.19×10^{-2}	3.18×10^{-2}
1	0.02734	5.28×10^{-2}	1.72×10^{-2}
2	0.06409	6.30×10^{-2}	2.06×10^{-2}
5	0.10420	3.29×10^{-2}	1.03×10^{-2}
10	0.16527	2.20×10^{-2}	7.20×10^{-3}
Average	0.05453	4.71×10^{-2}	1.99×10^{-2}

It should be noted that the diclofenac concentrations used in this study were high compared to the concentrations found in wastewater, which can be lower than 2 μg L^{-1} [22]. The use of high diclofenac concentrations

was to clearly exhibit the mechanisms of diclofenac removal by the activated sludge system, which should be mainly from biodegradation followed by adsorption onto biomass in wastewater. According to previous research, pharmaceuticals influenced 48.13% of the diversity in the bacterial community structure where Proteobacteria, Gemmantimonadales, and Actinobacteria were more abundant during the diclofenac exposure and biodegradation process [34]. The increase in abundance suggested that some bacteria could benefit from diclofenac removal. The families of Xanthomonadaceae, Rhizobiacea, Gemmantimonadaceae, and Ilumatobacteraceae were among the bacteria that were promoted in the presence of diclofenac [34]. Other studies have isolated bacterial strains capable of degrading diclofenac from activated sludge and from a municipal WWTP such as *Brevibacterium* sp. D4 [35], which could degrade 90% of 10 mg L^{-1} of diclofenac, and *Enterobacter hormaechei* D15 isolated from sludge, which could remove 52.8% of diclofenac when it was a sole carbon course, and the efficiency increased to 82% in a co-metabolic system with glucose [36].

2) Effect of metal ions on diclofenac biodegradation

Various metals can act as a source of micronutrients for microorganisms, playing a role in cell transport systems and bacterial enzyme activation, which leads to more biodegradation of organic substances [37]. Effect of metal ions including Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, and Zn^{2+} on diclofenac degradation by sludge was observed in batch experiments. The removal of 1 mg L^{-1} of diclofenac was determined for 72 h, and the results are described in Figure 4(a)-(g). Addition of different metal ions resulted in different efficiencies of diclofenac degradation in wastewater by sludge. After 24 h, the addition of 0.25 ppm of Ni^{2+} and Zn^{2+} showed higher removal efficiency of diclofenac than the control (0 ppm). When the metal concentrations were increased to 0.5 ppm, Ca^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} showed almost 20% higher in the removal efficiency. However, when the metal concentrations were increased to 0.75 ppm, the positive effect of metal ions on diclofenac removal was reduced except Co^{2+}, Fe^{3+}, and Zn^{2+}.

Apart from the total removal efficiency, the removal rate is also important to the diclofenac degradation. According to Figure 4, the removal efficiencies rapidly and differently increased until 48 h where they reached a plateau of 90 to 100% efficiencies, and they did not significantly increase ant further until the end of the experiment at 72 h. Therefore, only the initial removal rates of diclofenac in 24 h (r_{24h}) and 48 h (r_{48h}) were calculated for better comparison of the effect of metal ion addition (Table 3). In terms of the removal rates, increasing the concentrations of Ca^{2+}, Co^{2+}, Fe^{3+}, Mn^{2+}, and Zn^{2+} from 0.25 to 0.75 ppm could increase r_{24h} of diclofenac removal. However, considering the r_{48h}, the higher removal rate was achieved only when increasing the concentration of Co^{2+} from 0.25 to 0.75 ppm.

The enhancement of diclofenac removal by various concentrations of metal ions can possibly be explained by several mechanisms. First, trace metal ions can interact with microbial cells such as the incorporation into catalytic enzymes for degradation and transformation of substances in wastewater [38]. Secondly, some metal ions such as Fe can form with other ions to be solid particles such as FeS, which can enhance the removal of pharmaceuticals by adsorption [39]. Another mechanism is the formation of metal ion nanoparticles by metal-oxidizing bacteria in biofilms such as manganese oxides, which showed high redox potential to induce oxidation of pharmaceuticals to occur [1, 40]. The results from this study suggested that the presence of trace amount of some metal ions as pollutants pose a positive effect to the diclofenac removal efficiency in wastewater treatment.

Table 3 Effect of metal ions on diclofenac removal rates by sludge in wastewater (initial diclofenac concentration = 1 mg L^{-1})

Initial removal rate (r)	Metal ion Concentration (ppm)	Control	Ca^{2+}	Co^{2+}	Cu^{2+}	Fe^{3+}	Mn^{2+}	Ni^{2+}	Zn^{2+}
r_{24h}	0	1.851	-	-	-	-	-	-	-
($\times10^{-2}$ mg L^{-1} h^{-1})	0.25	-	1.577	1.737	2.184	1.968	1.710	2.900	2.735
	0.50	-	2.887	2.386	3.005	1.847	1.888	2.561	2.493
	0.75	-	2.228	2.754	2.895	2.693	2.495	1.439	2.877
r_{48h}	0	1.511	-	-	-	-	-	-	-
($\times10^{-2}$ mg L^{-1} h^{-1})	0.25	-	1.300	2.965	1.452	1.522	1.299	2.735	1.675
	0.50	-	1.749	3.381	1.564	1.760	1.338	2.493	1.431
	0.75	-	1.640	3.529	1.669	1.735	1.734	2.877	1.707

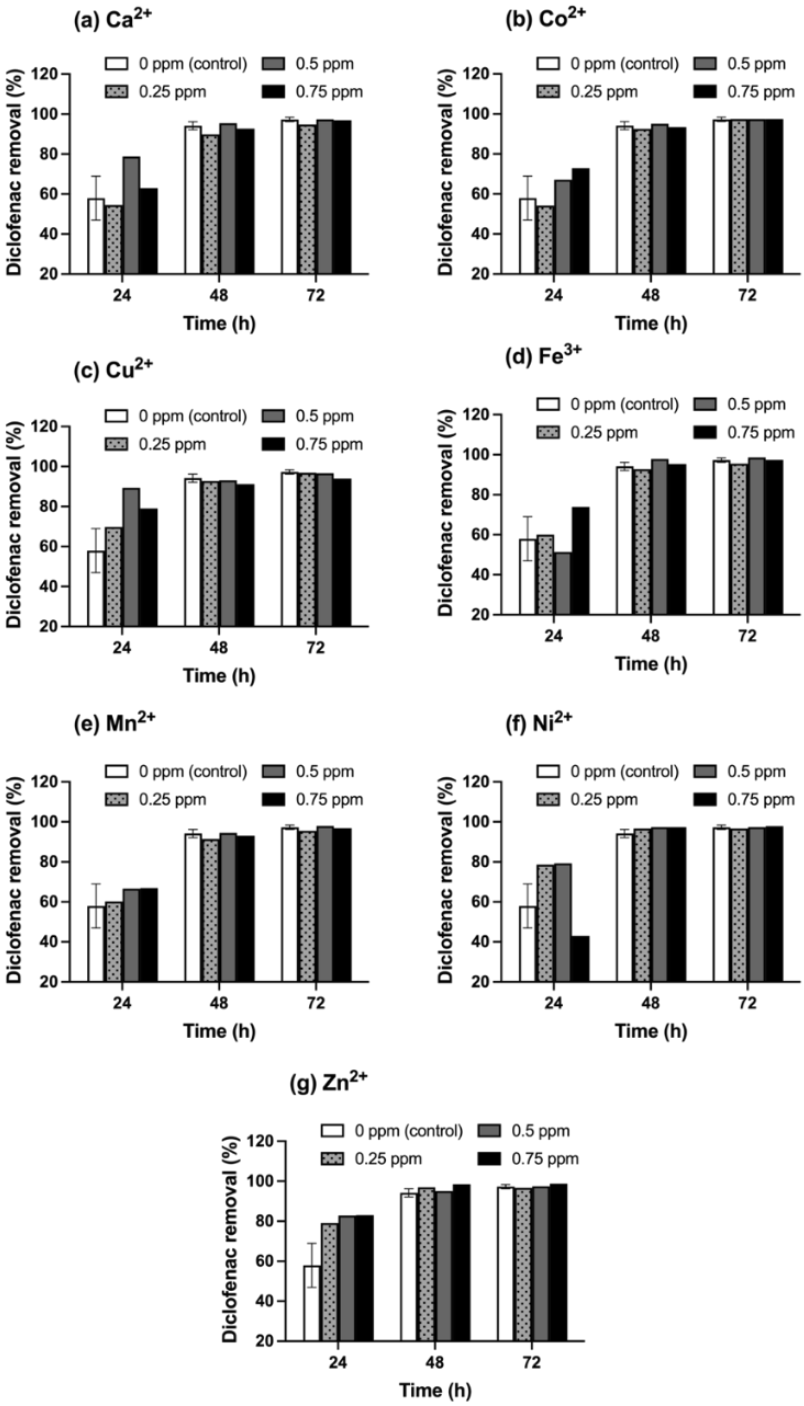

Figure 4 Effect of various metal ions on diclofenac removal by sludge in wastewater: (a) calcium (Ca^{2+}), (b) cobalt (Co^{2+}), (c) copper (Cu^{2+}), (d) iron (Fe^{3+}), (e) manganese (Mn^{2+}), (f) nickel (Ni^{2+}), and (g) zinc (Zn^{2+}). The batch experiments were conducted for 72 h with 1 mg L^{-1} of diclofenac.

3) Diclofenac removal by an MBBR experiment with sludge addition

A lab-scale MBBR was tested for the diclofenac removal in wastewater in a continuous-mode operation, and the results are shown in Figure 5. From the results, the MBBR with only wastewater during the start-up could not sustain high removal of 1 mg L^{-1} of diclofenac as suggested by the rapid increase in C_{eff}/C_{inf} ratio (diclofenac concentration in the effluent/influent) from 0.21 to 0.99 within 3 days. The removal observed in the reactor should be mainly from the biomass on the carriers since diclofenac could not be degraded effectively in wastewater (Figure 3(b)). The fast reduction in the removal efficiency in MBBR could result from low biomass formation of the effective microorganisms on the carriers during the start-up even though the municipal wastewater has considerable amount of carbon and nutrient sources according to its BOD, COD, TKN, and TP (Table 1). However, when sludge was added during the start-up, the reactor was able to extend its removal efficiency. The removal ratio (C_{eff}/C_{inf}) of 1 mg L^{-1} of diclofenac was still at 0.61 at day 3 of the experiment before the removal

efficiency disappeared at day 8 (C_{eff}/C_{inf} = 0.99), and the MBBR reactor could not remove diclofenac further until the end of the experiment at day 21. The concentration of diclofenac was then reduced to 0.5 mg L^{-1} to observe the efficiency; however, the removal efficiency also reached the plateau at day 8 (C_{eff}/C_{inf} = 0.97) so that the experiment was stopped at day 10 (Figure 5). Wang et al. [1] used an MBBR to remove various pharmaceuticals including diclofenac, and the results showed that only MBBR could not remove diclofenac within 25 h of operation, while in this study the removal of diclofenac was observed longer. This could be due to the use of mixture of pharmaceuticals in the study, which could deter the removal by MBBR. However, when the biogenic manganese oxides were generated in the MBBR, the complete removal of diclofenac was observed, which was less than one day. This also suggested the importance of the presence of trace metal elements in wastewater that can help with enzymatic biodegradation.

It was found that the mechanisms of diclofenac biodegradation by biofilms in the attached growth system and biomass in activated sludge system are similar even though the removal rates might be varied in different systems [41]. In this study, it can be estimated that the lower removal efficiency of diclofenac in MBBR with time was from low growth or detachment of biomass from the carriers. Various parameters play a role in biofilm formation in MBBR and its performance to remove diclofenac in wastewater; for example, longer HRT to provide the attached growth of slow-growing bacteria and to enrich the microbial community [42], COD and ammonium loads for the attached growth [24], and dissolved oxygen amount and hydrodynamic conditions [43].

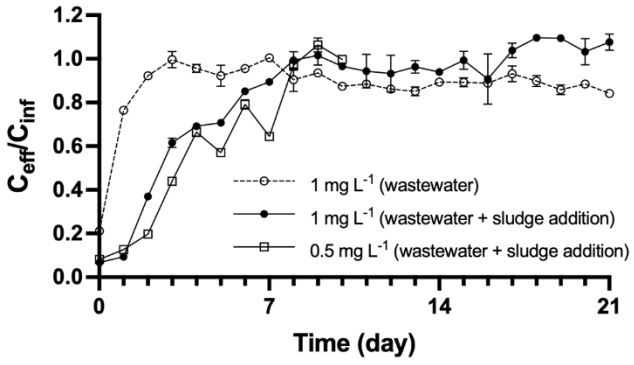

Figure 5 Removal of diclofenac by an MBBR reactor. The removal efficiency was represented by the ratio of effluent concentration (C_{eff})/influent concentration (C_{inf}) (y-axis) over 21 days of experimental time (x-axis).

In summary, the effect of sludge addition into the start-up phase of the reactor could help prolonging the efficiency of diclofenac removal in wastewater, which proves a useful application of sludge in enhancing the diclofenac biodegradation in wastewater treatment. The removal process was mainly caused by the biomass of biofilms growing on the carriers during the start-up of the reactor. To achieve higher efficiency, other environmental conditions should be optimized for biofilm formation, e.g., longer HRT [1], additional substrates [21], and redox conditions [22]. Apart from the reactor operation, the limitations of this study should also be addressed in future research including the measurement of water quality parameters along with diclofenac removal, the presence of other pollutants that interfere with the removal, and the characteristics of sludge and microorganisms that play a role in the adsorption and biodegradation of diclofenac. These limitations can be overcome by a thorough analysis of wastewater parameters, the use of synthetic wastewater, the chemical and biological characterization of sludge.

Conclusions

In this study, diclofenac in wastewater was effectively removed by sludge addition. The removal mechanism was mainly from biodegradation followed by adsorption. A certain amount of diclofenac was only degraded in wastewater while only small amount was self-degraded. Most of diclofenac degradation was observed within 24 h of the batch experiments, and initial removal rates along with first-order degradation rate constants were determined. The addition of some metal ions especially Ca^{2+}, Co^{2+}, Fe^{3+}, Mn^{2+}, and Zn^{2+} could increase both diclofenac removal rate and efficiency. The effect of sludge addition was also observed in the continuous-mode MBBR where the removal efficiency was retained longer in the reactor with sludge addition during the start-up. In conclusion, it is implied that diclofenac released from anthropogenic sources is not degraded in municipal wastewater but can be removed effectively in an activated sludge system. However, the remaining diclofenac concentrations in effluent and sludge biomass must be considered for further studies and improvements of the wastewater treatment system.

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