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Biodegradation of Pharmaceuticals Substances by a Fixed-Bed Bioreactor

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Abstract: The biodegradation of four pharmaceuticals (naproxen, fenoprofen, ibuprofen and gemfibrozil) was studied by batch experiments. Fixed-bed bioreactor (FBBR) and activated sludge was used under aerobic conditions. In order to elucidate the capability of biomass developed in bioreactor, the kinetic reaction was determined with heterotrophic biomass in the FBBR following the liquid phase concentrations over time. After the biomass acclimation period the removal efficiency for the pharmaceuticals was 100% with respect to the limit of quantification (LOD). The biodegradation of the studied pharmaceuticals followed a first-order kinetics for naproxen and ibuprofen, and for fenoprofren and gemfibrozil a zero order kinetics was determined.

Key words: fixed-bed bioreactor, pharmaceuticals substances, reaction kinetic

1. Introduction

The concern about micropollutants (MPs) has increased over the last years. Micropollutants refer to organic substances occurring in waters at nanograms or micrograms per liter [1, 2]. They include substances such as pharmaceuticals, compounds with biocidal properties, food additives, cosmetic ingredients or detergents [3, 4]. Nowadays, the presence of pharmaceuticals in aquatic environment is becoming widespread and this may involve many environmental impacts [5]. Large amounts of pharmaceutically active compounds (PhACs) are used around the world and can reach the aquatic environment through urinary excretion and improper disposal; as a consequence, these compounds have been found in wastewaters and in surface waters since incomplete elimination occurs in wastewater treatment plants (WWTPs) [6]. Naproxen and fenoprofen are a non-steroidal anti-inflammatory drug used for mild to moderate pain relief and in the treatment of osteoarthritis and rheumatoid arthritis, whereas ibuprofen is used in large quantities for the treatment of pain, dysmenorrhea and inflammation [7, 8]. Gemfibrozil is a blood lipid regulator, used to treat moderate to severe hypertriglyceridemia, clinically prescribed since the early 1980s in patients at high risk of coronary heart disease [9]. The occurrence and distribution of a group of micropollutants in surface and groundwater sources from Mexico City was determined. Results evidenced the occurrence of naproxen, ibuprofen, ketoprofen and gemfibrozil (PhACs) in surface water [10]. Biological wastewater treatment techniques can normally be classified as either suspended or attached growth processes. The suspended activated sludge process is the most frequently used as biological treatment at municipal WWTPs, thus, technical and operational solutions that can improve MPs removal in this process are highly desirable [11]. The attached growth processes having advantages of both attached and suspended growth systems [12]. The biofilm reactor (BRs) are usually filled with plastic biocarriers, on which biomass is attached [13] and circulate in all parts

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of the reactor with the aid of aeration or mechanical stirring. Some of the attached biomass advantages are their ability to cope with high loading conditions, the capacity of treatment of both industrial and municipal wastewater at a relatively low footprint and the avoidance of excess sludge removal [14]. Based on the above paragraph, the main objective of this study was to examine the biodegradation of four PhACs (naproxen, fenoprofen, ibuprofen and gemfibrozil) which were studied through batch experiments, with fixed-bed bioreactor and activated sludge under aerobic conditions. In order to elucidate the capability of biomass developed in the bioreactor, the kinetic reaction was determined with heterotrophic biomass in the FBBR following the liquid phase concentrations over time.

2. Material and Methods

Four PhACs, ibuprofen (IBF) ≥ 98% purity, naproxen (NPX) ≥ 98%, fenoprofen calcium (FNP) and gemfibrozil (GFB) ≥ 98% purity and Nutrient substances [15] , were purchased from Sigma Aldrich.

2.1 Configuration of FBBR System

Activated sludge was obtained from wastewater treatment plant ECCACIV, localizated in Jiutepec, México. The biomass in FBBR was acclimatized to the pharmaceuticals by continuously feeding nutrients [14] and PhACs dissolved in methanol. Inlet concentration of PhACs was about 2 mgL^{-1}.

The adaptation of microorganisms to treat wastewater was carried with cycles of varying reaction to reach the acclimatization of biomass to degradation of drugs using the criteria fixed efficiencies, which consisted in allowing biomass adapted to degradation of 90% of pharmaceuticals.

The experiments were performed in batch. The system consisted of an acrylic reactor with a total volume of 30 L and a useful volume of 22 L. For the control of loading and discharge, 2 peristaltic pumps were used (Master Flex, Cole Palmer). The air inside the reactor was diffused from the bottom of the reactor through a porous diffuser with an air pump. For the FBBR configurations, 50% of the volume of reactor was filled with high density polypropylene plastic carriers. The concentration of suspended solids in the mixed liquor (MLSS) was 2600 ± 400 mgL^{-1}. The biomass attached to the plastic carrier was 0.27±0.07 g/g plastic carrier. Total organic carbon (TOC) was 320-380 mgL^{-1}.

2.2 Batch Experiment for Removal Kinetics

The experimental runs to determine the removal kinetics with attached biomass was conducted in the bioreactor operated them under batch conditions for 30 h.

PhACs were added to the reactor to attain an initial concentration of about 2 mgL^{-1}. Samples of the FBBR were taken over time during 30 h in order to evaluate the pharmaceuticals concentrations, DO, TOC and pH.

2.3 Removal Kinetics Model

For the purpose of quantifying the velocity of the removal of the studied compounds, the zero-order and first-order kinetic model were used.

Zero-order kinetics model

The variation in concentration vs. time is defined by Eq. (1):

$$\frac{dC}{dt} = K_0 \tag{1}$$

First-order kinetics model

In the first-order kinetics model any variation in the concentration of the substance in time is described by Eq. (2):

$$\frac{dC}{dt} = -K_1 \tag{2}$$

where C [mgL^{-1}] is the concentration of each compound in water, k_0, k_1 [mgL^{-1} t^{-1}] is the zero-order and first-order constants respectively and t [t] is time.

2.4 Quantification of General Parameters

Dissolved oxygen (DO), temperature and pH were

determined by APHA Methods 2005. Total organic carbon (TOC) of samples was analyzed using a TOC analyser (Torch TELEDYNE Tekmar). Samples were centrifuged at 3000 rpm for 15 min, and then they were filtered.

Biofilm solids were determined by the difference in weight of dried carriers (105°C for 1 h) before and after removal of biofilm.

2.5 Quantification of Pharmaceuticals

Samples were previously centrifuged at 3000 rpm for 15 min and then filtered through membrane of 45 μm. The analysis was performed by high performance liquid chromatograph (HPLC 1100, Agilent) such was equipped with Zorbax Eclipse XDB C-18 (250 mm × 4.6 mm × 5 μm) column at 35°C, and UV detector with a wavelength 230 nm. The mobile phase, with a flow rate of 0.8 mL/min was composed of water 1%, acetic acid and acetonitrile with a volumetric ratio of 35:65. The limit of Detection (LOD) was 0.1 mgL^{-1}.

3. Results and Discussion

3.1 Performance Evaluation of FBBR

The reactor operated for 11 cycles, during this period the OD was maintained between 5-6 mgL^{-1}, a pH 7.7±0.1 and a temperature of 22°C±2°C. The organic load of wastewater was eliminated with an average removal of 90% which is consistent with a previous reported study. Haribabu and Sivasubramanian [16] studied the efficiency of inverse fluidized bed biofilm reactor for treating wastewater with biocarriers, the results showed a reduction of organic matter of 97.5% at hydraulic retention time of 40 hr.

3.2 Removal of Pharmaceuticals

The degradation capacity of the biomass increased after 30 days of operation of the reactor. The adaptation of microorganism as reached in the cycle 5 was indicated the adaptation of the microorganisms to the degradation of the PhACs, promoting the generation of a new consortium of microorganisms able to degrade the pharmaceuticals. Concentrations of the selected PhACs in the influent and effluent samples for FBBR for cycle of operation are shown in Table 1.

Table 1 Concentrations of the selected PhACs in the influent and effluent samples for FBBR cycle of operation.

Cycle	NPX(mgL^{-1})		FNP (mgL^{-1})		IBP (mgL^{-1})		GFB (mgL^{-1})	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
1	2.53	2.21	2.59	2.44	2.33	> LOD	2.72	2.31
2	2.51	0.31	2.41	2.35	2.09	> LOD	2.68	0.29
3	2.48	0.31	2.33	2.17	2.18	> LOD	1.77	> LOD
4	1.09	0.21	2.19	1.35	2.85	> LOD	2.80	0.38
5	2.49	0.18	2.09	> LOD	1.26	> LOD	1.17	> LOD
6	1.33	0.78	2.45	0.45	1.28	> LOD	1.20	0.10
7	2.49	> LOD	2.09	> LOD	2.12	> LOD	1.94	> LOD
8	2.16	> LOD	1.88	> LOD	2.13	> LOD	2.03	> LOD
9	2.02	> LOD	1.66	0.07	2.45	0.05	2.28	0.25
10	1.88	> LOD	2.95	0.09	1.89	> LOD	1.84	0.14
11	1.93	> LOD	2.29	> LOD	1.93	> LOD	2.02	> LOD

LOD = 0.1 mgL^{-1}

With respect to the hydraulic residence time (HRT), during the first 3 cycles was 168 h, in cycle 4 and 5 of 120 h, in 6 of 72 h and from cycle 7 was 48 h. The removal efficiencies in clycle 1 were 13% NPX, 6% for FNP and 15% for GFB then it increased to 87% for NPX in cycle 2, 38% for FNP in cycle 4 and 89% for GFB in clycle 2. From cycle 5 the removal efficiencies were 100% for all three PhACs. Ibuprofen had been

already 100% degraded in cycle 1 which was consistent with literature reports for this compound [17]. Fig. 1 shows the results. In the first cycles of operation the hydraulic retention times (HRT) were longer and the removal efficiencies were different for each PhACs, which can be attributed to the complexity of the chemical structure of each PhACs. According to Tadkaew [18], the occurrence of electron withdrawing or electron donating functional groups appears to be an important factor governing their removal of pharmaceuticals compounds. The compounds containing electron donating functional groups, such as hydroxyl groups and primary amine groups have high removal efficiency. After 7 cycles operating, the retention time was 48 h with removal efficiencies of 100% with respect to LOD for four PhACs. The reason for the higher removal may be then attributed to the presence of the biofilm, which may lead to different conditions (aerobic–anoxic–anaerobic) along its profile, which increases the degradation possibilities, and also to a higher sludge age of the biofilm, which means a broader variety of bacteria present in the system [19].

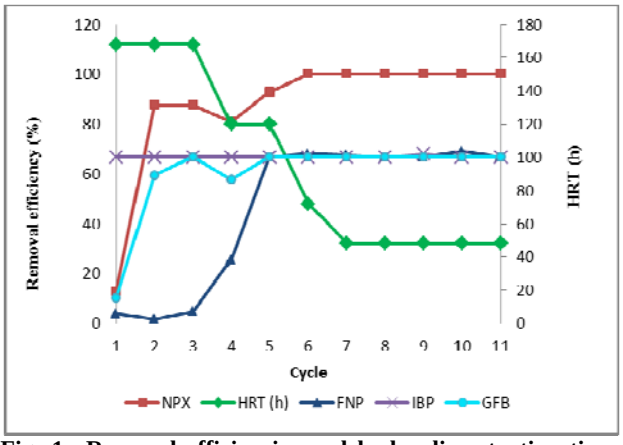


Fig. 1 Removal efficiencies and hydraulic retention time per cycle of operation.

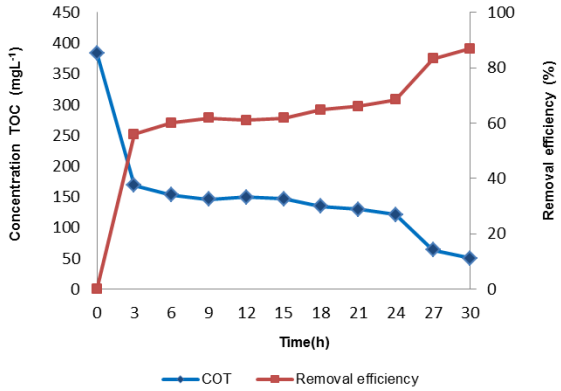


Fig. 2 Changes in concentration and removal efficiencies of TOC.

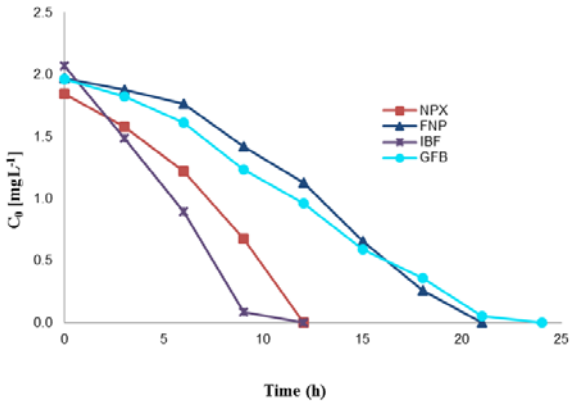
3.3 Removal Kinetics Model

The DO was maintained between 5-6 mgL^{-1} and 7±0.5 pH during kinetics. During the kinetics reaction the removal of organic matter expressed as total organic carbon (TOC), the initial concentration was 384 mgL^{-1} and the removal efficiency was 87%. Changes in concentration and removal efficiencies are shown in Fig. 2.

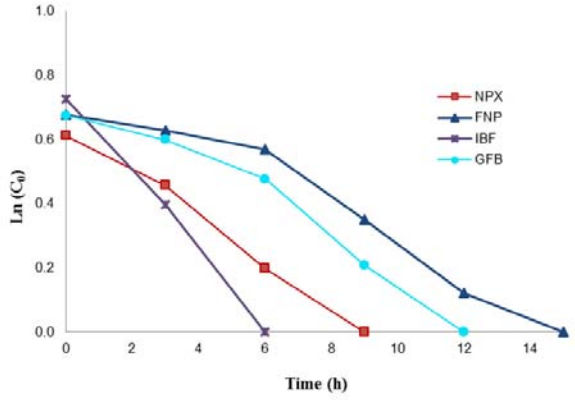
The velocity of the removal and reaction order of the studied pharmaceuticals, the zero-order and first-order kinetic model were used. The linearized form of the zero-order and the first-order models for the degradation of PhACs is shown in figure 3a and 3b, respectively. Fitted parameters and correlation coefficients (R^2) are shown in Table 2.

Table 2 Fitted parameters, kinetic constants and correlation coefficients for the removal kinetics experiment.

Model	Zero-order			First- order		
Parameter	C (mgL^{-1})	K_0 (mgL^{-1} h^{-1})	R^2	C (mgL^{-1})	K_1 (mgL^{-1} h^{-1})	R^2
Naproxen	1.84	0.153	0.97	1.84	0.070	0.99
Fenoprofen	1.97	0.101	0.96	1.97	0.049	0.94
Ibuprofen	2.07	0.184	0.96	2.07	0.121	0.99
Gemfibrozil	1.96	0.091	0.98	1.96	0.058	0.95



(a)



(b)

Fig. 3 (a) Zero-order kinetic model, (b) First-order kinetic model.

The determination of the kinetic constants of studied pharmaceuticals was conducted in the cycle 9 when a complete acclimatization of the biomass to the degradation of the contaminants was observed.

Results show that the zero-order their kinetics was well suited to describe biological degradation for FNP and GFB, while for IBP and NPX the first-order kinetics was suited to describe degradation. Results indicated that the pharmaceutical can be removed by microorganism without causing adverse effects on the biomass, because in biofilm systems, the biomass is attached to the support and, therefore, is better protected against toxic events.

4. Conclusions

The adaptation times of the biomass for each PhACs were different, which depended on the complexity of the chemical structure of the molecule. The removal efficiencies were 100% when operated the reactor at HRT of 48 h.

The organic load (TOC) of wastewater was eliminated with an average removal of 90%. The kinetic model that best describes the biological degradation was the first-order kinetic model for naproxen and ibuprofen while for fenoprofen and gemfibrozil was the zero-order kinetic model.

This research confirms that the employed system can be considered an efficient process for the degradation of the naproxen, fenoprofen, ibuprofen and gemfibrozil.

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