Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2018.23203

129 (2018) 345–350
October

Transformation of clofibric acid in sequencing batch reactor and microbial characteristics

Xin Jin[a], Xiurong Zhu[a,b], Pengkang Jin[a,*], Xiaochang C. Wang[a]

[a]*Key Laboratory of Environmental Engineering, School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology, Xi'an, Shaanxi province 710055, China, Tel. +86 13379217572; emails: pkjin@hotmail.com (P. Jin), jinxinjason2006@163.com (X. Jin), zz552288@126.com (X. Zhu), xcwang@xauat.edu.cn (X.C. Wang)*
[b]*Environmental Protection Bureau of Shaanxi Province, Xi'an, Shaanxi province 710054, China*

Received 20 September 2017; Accepted 16 May 2018

ABSTRACT

The removal and transformation of clofibric acid (CA), which is a metabolite of clofibrate drugs, in aerobic sequencing batch reactor (SBR) was studied in this paper. By the analysis of CA concentration in the sewage and sludge phase, the biological removal mechanism was shown to be important, achieving 10%–12% of removal efficiency. However, the adsorption effect was relatively weak. High performance liquid chromatography (HPLC) analysis showed that CA mainly produced three biodegradation metabolites: α-hydroxyisobutyric acid, lactic acid and 4-chlorophenol. The α-hydroxyisobutyric acid was the major metabolite, and the second most important was lactic acid. The two substances accumulated for a period, and were then gradually utilised by microorganisms. These results indicated that CA can be transformed into other products during biological treatment although it is refractory. Moreover, the concentration of 4-chlorophenol was very low. Microbial community distribution was analysed by high-throughput sequencing. The dominant phyla were *Proteobacteria*, *Actinobacteria* and *Bacteroidetes*. After addition of CA, the component of both *Actinobacteria* and *Bacteroidetes* dropped. For genus level analysis, a new *Porphyromonadaceae* emerged in the SBR with CA.

Keywords: Clofibric acid; Metabolites; Microbial community distribution

1. Introduction

Clofibric acid (CA) is the pharmaceutically active metabolite of lipid regulators, and it is considered both environmentally persistent and refractory [1,2]. It can be discharged into natural waters through domestic wastewater, hospital wastewater and industrial wastewater [3]. Several studies have detected CA even in groundwater and drinking water [4–6]. The concentration of CA in waters is typically between ng/L and μg/L levels [7]. The daily dose for hyperlipidemia patients can reach 1–2 g/d, which can be released into water environment to cause ecological and health risk [8]. As an emerging micropollutant, the behaviour, effect and fate of CA in water environment received extensive attention in recent years.

Some studies indicated that there was little difference in CA concentration between influent and effluent of wastewater treatment plant (WWTP), which indicates biological persistence [9,10]. Other studies revealed that the CA removal efficiency was around 28% in conventional activated sludge treatment [11,12]. Some studies investigated the biological degradation of ibuprofen, ketoprofen, naproxen, CA, etc. during activated sludge process [13,14]. The results indicated that the removal efficiency of CA was the lowest among these PPCPs (49%–59%), and the removal efficiency of other PPCPs was higher than 87%. In addition, hydraulic retention time

* Corresponding author.

Presented at 2017 Qingdao International Water Congress, June 27–30, 2017, Qingdao, China.

(HRT) can affect the removal efficiency of PPCPs. Higher HRT can increase the removal efficiency of CA [15]. Only 5% removal efficiency was obtained in the conventional activated sludge process with short HRT [16]. Furthermore, the long sludge retention time (SRT) also resulted in increased removal efficiency of CA, which attributed to the enrichment of microbial cultures that are suitable towards xenobiotic biodegradation [15,17]. Based on the above studies, there is still a large amount of CA being discharged into the environment after biological treatment. In addition to slow biodegradation, CA was also photostable, which implied that the CA direct photolysis is not important in the natural environment [18].

It is suggested that the presence of chlorine in the molecular structure and a relatively complex aromatic structure of CA are the reasons for the low degradation rate in conventional biological processes [19]. The removal of CA in activated sludge process can be due to adsorption by sludge and biological degradation. Previous research indicated that CA occurs in water in ionic form, and it is difficult to be adsorbed by activated sludge. Furthermore, the adsorption ability increases with decreasing pH [20]. However, the distribution of CA between sludge and water in biological treatment processes is still poorly known, and there is little information related to the mechanism and pathway of CA degradation.

The microorganism community is of great importance in activated sludge processes. The variation of species, quantities and structures can affect the removal efficiency of the treatment system. Therefore, the study of the microbial community in the wastewater treatment system can contribute to the investigation of pollutants transformation. In addition, high-throughput sequencing techniques were widely used in microorganism analysis during activated sludge process. However, there is little experimental data about the effect of CA on microbial community.

The aim of this study was to investigate the transformation and distribution of CA in sequencing batch reactor (SBR) in both water and sludge phase. Furthermore, the metabolites of CA in SBR were detected to analyse the effect of biological processes on CA degradation. In this study, microbial community distribution was compared as well in SBR with and without CA by the high-throughput pyrosequencing.

2. Materials and methods

2.1. Experimental setup

Two identical SBR reactors were used in this study. Each reactor was fed by synthetic wastewater. The effective volume of the reactor was 6 L. The seed activated sludge came from the return sludge from a local municipal WWTP in Xi'an, China, with A^2O process with treatment capacity of 500,000 m^3/d. During the starting period, the seed sludge taken from the WWTP was aerated for 24 h before use. Then, the 4 L of seed sludge with mixed liquor suspended solids (MLSS) of 6,000 mg/L was transformed to the reactor, and mixed with 2 L of synthetic wastewater to start cultivation until the effluent water quality reaches stable. The composition of the simulated wastewater is shown in Table 1. The operation cycle was 12 h, that is, 5 min inflow, 11 h aeration, 50 min sedimentation and 5 min outflow. SRT was controlled at around 15 d. The MLSS was around 4,000 mg/L, and dissolved oxygen was controlled at 2–5 mg/L. Both reactors were operated at ambient temperature. A total of 1 mg/L CA was added in one of the SBR reactors in the steady state to analyse the transformation of CA. The average running temperature of the experiments was 20°C ± 2°C.

Table 1
Composition of the simulated wastewater

Composition	Concentration (mg/L)
Glucose (COD)	500
NH_4Cl (N)	25
KH_2PO_4 (P)	5
$NaHCO_3$	80
$MgSO_4$	40
$FeCl_3$	1.94
$CaCl_2$	3

COD, Chemical oxygen demand.

2.2. Sample preparation

To determine CA in the water phase, 10 mL supernatant of the reactor was taken, and pH was adjusted to 3. In addition, samples were centrifuged at 8,000 rpm for 5 min. After filtration with 0.22 μm filters, samples were stored at 4°C for HPLC analysis. A total of 30 mL of mixed liquor was sampled for CA extraction. The CA in the sludge phase was extracted using ultrasonic solvent extraction method with water and methanol (9/1, v/v) at pH 11 for 15 min [3,21], and the extracted samples were stored at 4°C for HPLC analysis.

2.3. Analytical methods

HPLC (LC-2010A HT, Shimadzu, Japan) was used to measure the concentration of CA and its metabolites. The HPLC column was Hypersil BDS C18 (250 mm × 4.6 mm × 5 μm). The HPLC analysis parameters are shown in Table 2. Total nitrogen, ammonia, nitrate and nitrite were measured according to standard methods [22]. Each test was repeated at least three times. Mass balance of CA in the SBR was obtained based on the determination of CA concentration in water and sludge phase.

2.4. Analysis of microbial community

Sludge samples of SBR reactors with and without CA were taken from the system for DNA extraction using the Power Soil DNA Isolation Kit (MP Biomedicals, USA). The above DNA mixture was pooled and amplified by polymerase chain reaction (PCR) using primer 357F (5′-CCTACGGGAGGCAGCAG-3′) and 926R (5′-CCGTCAATTCMTTTRAGT-3′) [23] targeting the V3–V5 regions. A 25 mL of the PCR mixture contained 2.5 μL of 10 × Ex Taq Buffer, 2 μL of dNTP mixture, 16.50 μL of sterilisation ultrapure water, 1 μM aliquots of each primer (10 μM), 0.125 μL of ExTaq (TaKaRa, Dalian, China) and 2 μL DNA (2 ng/μL). The thermocycling steps were as follows: 93°C for 3 min followed by 27 cycles at 94°C for 30 s, 55°C for 45 s, 72°C for 1 min and a final extension step at 72°C for 10 min. The composition of the PCR products of the 16S rRNA gene's V3–V5 region was determined by pyrosequencing using the Roche 454 GS FLX+ Titanium sequencer.

3. Results and discussions

3.1. Removal performance of CA in SBR

The removal performance of CA can be seen in Fig. 1. It can be seen from Fig. 1 that the removal efficiency keeps decreasing in the first 15 d. This is because, on one hand, the added CA had adverse effect on microbial community in SBR, and the microbial community needed to adapt. On the other hand, the adsorption of CA by activated sludge occurred during the initial 15 d. After 15 d, the effluent concentration of CA became stable at around 0.82–0.84 mg/L due to adsorption equilibrium. The removal efficiency remained between 16% and 18%. These results are consistent with other studies [9,11] and suggest that CA is refractory compound. The effluent water quality of two SBR reactors during steady state operation can be seen in Table 3. According to Table 3, the addition of 1 mg/L CA had little effect on effluent water quality. It can be inferred that although CA is refractory, it will not inhibit the performance of SBR.

There are several reasons for which it is difficult to remove CA in wastewater activated sludge treatment process. CA itself and its metabolites are toxic. Some studies evaluated the toxicity of CA its metabolites [24,25]. The results showed that the toxicity of metabolites was much higher than that of CA itself. The accumulation of the metabolites of CA inhibited the microbial activity and resulted in low removal efficiency of CA. On the other hand, some refractory organic compounds containing hydroxyl, carboxyl, aromatic structure and chloride groups can be removed along with nitrification in activated sludge. Fig. 2 shows the relationship between CA degradation and the variation of nitrogen-containing substances from the average values from 10 cycles during Day 50 to Day 60. It can be seen that the concentration of NH_3-N decreased after aeration. After 6 h of aeration, the NH_3-N concentration was very low. It was reported that the enriched culture of nitrifiers exhibited high removal efficiencies of pharmaceuticals (CA, diclofenac, carbamazepine and propyphenazone) [13]. Cometabolic oxidation by the ammonium monooxygenase enzyme is probably initiating the biotransformation of many micropollutants [26]. In this study, NH_3-N-limiting condition occurred because the concentration of NH_3-N became quite low after 6 h within one cycle, which resulted in the limited activity of nitrifier bacteria and the decreased removal efficiency of CA.

3.2. CA distribution in SBR

In order to analyse the removal performance of CA, CA distribution in water and sludge phase was investigated. The average values from 10 cycles during Day 50 to Day 60 at

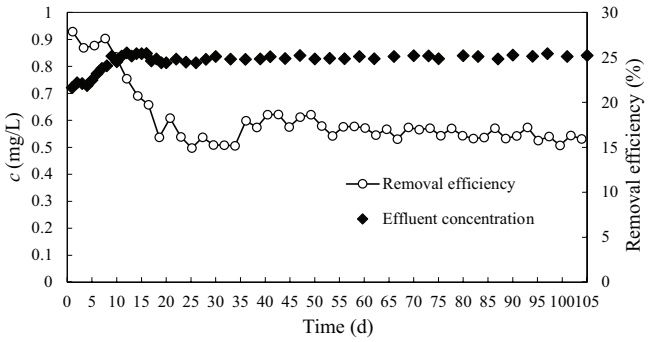

Fig. 1. CA removal performance in SBR.

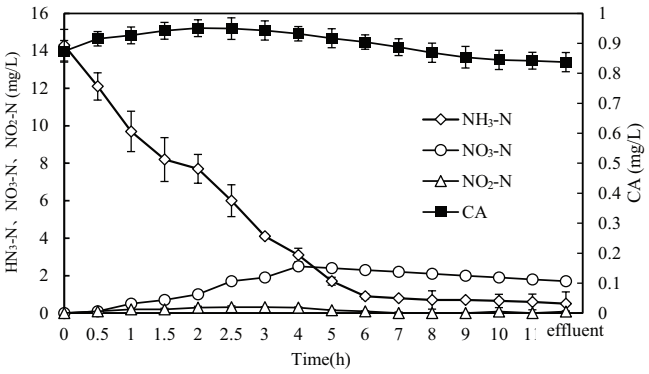

Fig. 2. The relationship between clofibric acid degradation and the variation of nitrogen substances.

Table 2
HPLC analysis of clofibric acid and its metabolites

Chemicals	Mobile phase Methanol:H_2O (pH = 3)	Velocity (mL/min)	Column temperature (°C)	Wavelength (nm)	Inject volume (μL)	Retention time (min)
CA	65:35	0.9	30	230	20	8.3
AHIBA	20:80	0.6	35	212	20	6.7
LA	10:90	0.7	35	205	20	4.9
4-CP	70:30	0.8	35	290	20	5.3

Table 3
Water quality of the SBR effluent

	COD (mg/L)	TN (mg/L)	NH_3-N (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	TP (mg/L)
SBR 1	47.11 ± 0.79	1.98 ± 0.08	0.33 ± 0.05	1.63 ± 0.06	0.02 ± 0.01	0.28 ± 0.04
SBR 2	46.90 ± 0.70	1.95 ± 0.07	0.33 ± 0.04	1.60 ± 0.06	0.02 ± 0.01	0.29 ± 0.04
SBR 1 with CA	48.34 ± 0.83	1.92 ± 0.06	0.30 ± 0.05	1.68 ± 0.04	0.02 ± 0.01	0.29 ± 0.03

COD, Chemical oxygen demand; TP, total phosphorus.

steady state are shown in Fig. 3. After feeding water at 0 h, CA concentration in sludge phase was the highest because of the adsorption effect of sludge, while the CA concentration was the lowest in water phase. About 15% of CA in the sludge phase was released into water phase after aeration in 0–0.5 h. Although CA in the sludge phase was still being released into water phase in 0.5–2 h, the release rate became slower. With ongoing aeration, CA concentration in the water phase reached the highest values. In addition, the total CA concentration changed little before 2 h. It can be seen from Fig. 3 that CA concentration in water phase decreased, which suggests that CA started to be removed by microbial degradation. Furthermore, the microbial degradation effect was significant at 2–6 h because of the decrease of CA in water phase. The total removal efficiency during one cycle was only around 10%–12%. During the whole cycle of SBR, the CA concentration in the sludge phase was very low. Activated sludge had very weak adsorption capability towards CA. On the one hand, K_{ow} of CA is very low, and hydrophobicity is weak. Therefore, CA is difficult to be removed by adsorption. On the other hand, CA has very low pKa because of the carboxyl group. Therefore, CA is very easy to ionise and has a negative charge, thus, it is difficult to be adsorbed [24].

As shown in Fig. 3, because of the transformation and distribution of CA between water and sludge phase, mass balance calculation was conducted in this study. Besides the CA in effluent of SBR, CA in SBR can be removed by biological process or transferred into sludge phase. Therefore, the mass balance of CA in SBR can be obtained in this study. About 82%–84% of CA was discharged with the effluent. In addition, there was very poor CA removal performance in SBR. About 4%–8% of CA was discharged with excess sludge, and only 10%–12% of CA was decomposed by microorganisms in activated sludge. Based on the above results, if there is no further treatment of the WWTP effluent and excess sludge, CA could cause adverse effects (directly or through its metabolites) on aquatic environments, the food webs and also human health.

3.3. CA metabolites analysis

Three kinds of CA metabolites were monitored in this study. The concentration trend of CA and its metabolites can be seen in Fig. 4. In general, the main metabolite was α-hydroxyisobutyric acid (α-HIBA). Moreover, 4-chlorophenol (4-CP) exhibited the lowest concentration among the three metabolites. At the beginning of aeration (0–3 h), the concentration of the three metabolites was very low indicating weak microbial effects. The α-HIBA concentration increased to 147 μg/L gradually during 3–6 h. This implies that biodegradation starts to be effective. However, lactic acid (LA) concentration remained almost the same as within 3 h. During 6–9 h, the concentration of α-HIBA was still increasing with lower increasing rate. A maximum α-HIBA concentration of 200 μg/L was achieved at 9 h. In addition, LA concentration kept increasing and reached the highest concentration (73 μg/L) at 10 h. This indicates that CA was first degraded into α-HIBA, which led to the increase of α-HIBA between 3–6 h. Furthermore, although α-HIBA still increased in 6–9 h, it started to transform into LA, which resulted in increasing LA concentration. After 10 h, LA started decreasing as well. In addition, 4-CP concentration remained very low throughout the entire cycle.

Based on the CA metabolites concentration variation and their structures, the pathway of CA decomposition can be speculated as shown in Fig. 5. The branch of CA is ruptured first and transformed to α-HIBA and 4-CP. Furthermore, 4-CP would not accumulate in this case. However, α-HIBA is transformed into LA at longer reaction times. These results indicate that CA can be transformed into other products during biological treatment despite its refractory nature. In other words, although CA can be hardly removed by biological decomposition, its structure can be changed by transformation into other products through biological processes.

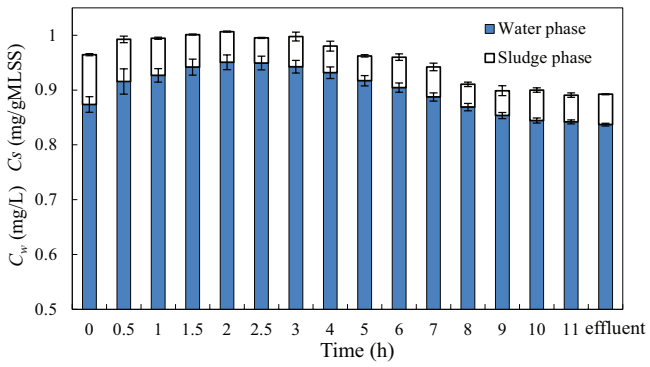

Fig. 3. The distribution and transformation rule of clofibric acid in the sewage and sludge phase.

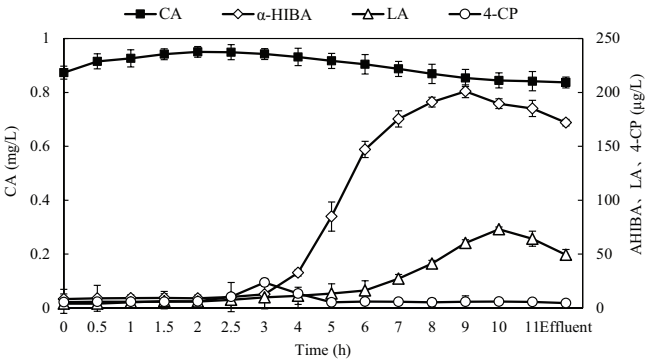

Fig. 4. The production and transformation of clofibric acid metabolites in the wastewater treatment process.

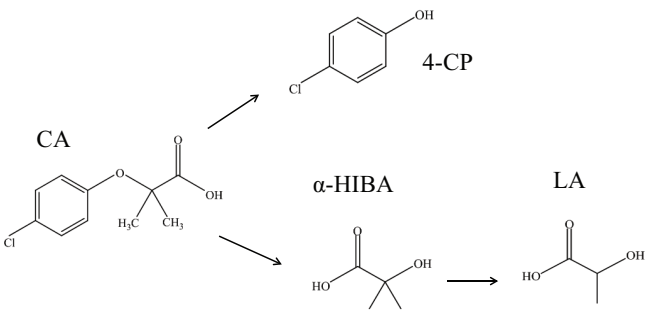

Fig. 5. The proposed transformation pathway of clofibric acid in the wastewater treatment process.

3.4. Microbial community distribution

Fig. 6 shows the bacterial community composition at phylum level in the reactor with and without the addition of CA as revealed by pyrosequencing. The phyla with relative abundance higher than 1% are presented. As shown in Fig. 6, 10 phyla were detected in the SBR either with or without CA. *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* were the three most abundant phyla in the two reactors.

Proteobacteria was the most abundant phylum with relative abundance of 33.11% and 32.74%, respectively, in SBR with and without CA. *Proteobacteria* contains a number of bacteria which are mainly responsible for organic matter removal in wastewater treatment [27]. After the addition of CA, the relative abundance of *Proteobacteria* slightly decreased. The relative abundance of *Actinobacteria* was 25.07% and 30.57%, respectively, in SBR with and without CA. In addition, the relative abundance of *Bacteroidetes* was 24.28% and 28.01%, respectively, in SBR with and without CA. According to the results above, CA may inhibit *Actinobacteria* and *Bacteroidetes* growth and results in relative abundance decrease. Generally, the relative abundance of the dominant bacterial community

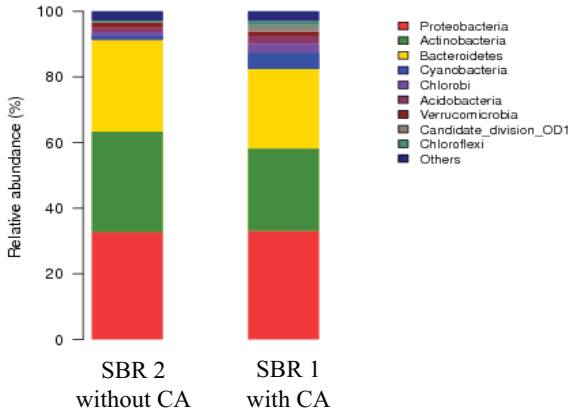

Fig. 6. Bacterial community composition at phylum level revealed by pyrosequencing.

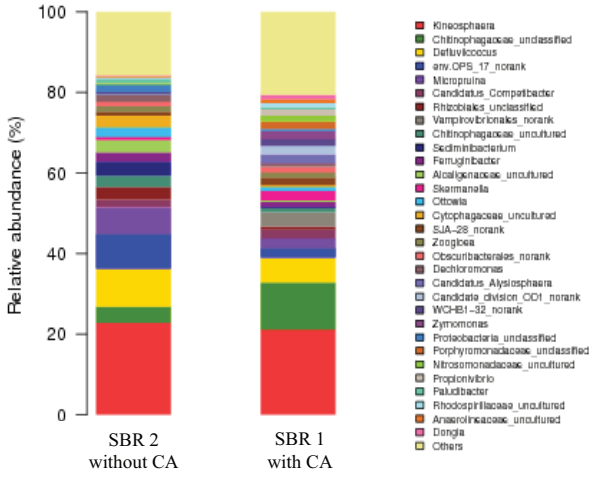

Fig. 7. Bacterial community composition at genus level revealed by pyrosequencing.

decreased from 91.32% to 82.46%. Besides the three most abundant phyla, the relative abundance of other phyla was different in the two SBR reactors as well, among which the relative abundance of *Cyanobacteria* had the maximum difference (4.91% and 1.16% in SBR with and without CA, respectively). After the addition of CA, the relative abundance of *Cyanobacteria* increased.

Fig. 7 shows the bacterial community composition from the reactor with and without the addition of CA at genus level as revealed by pyrosequencing. As shown in Fig. 6, the most abundant genus was *Kineosphaera* which account for 21.14% and 22.77% in SBR with and without CA, respectively. For *Chitinophagaceae*, *Defluviicoccus*, *env.OPS_17* and *Micropruina*, the relative abundance increased. For the SBR with CA, *Porphyromonadaceae* was detected. Nonetheless, *Porphyromonadaceae* was not discovered in the SBR without CA. Therefore, *Porphyromonadaceae* may be related to CA decomposition.

4. Conclusions

CA removal efficiency was around 16%–18% in SBR, and 10%–12% removal of CA was due to biological decomposition. The analysis of CA transformation pathway indicated that the branch of CA is ruptured first and transformed to α-HIBA and 4-CP. Furthermore, 4-CP did not accumulate in this case and α-HIBA was further transformed into LA. Although CA is refractory in wastewater treatment, its structure can still be changed, and transformed into other products. *Proteobacteria, Actinobacteria* and *Bacteroidetes* were the three most abundant phyla. The relative abundance of *Actinobacteria* and *Bacteroidetes* was lower in the presence of CA. For the bacterial community composition at genus level, *Porphyromonadaceae* may be related to the removal of CA.

Acknowledgment

This study was financially supported by National Key Technology Support Program (Grant No. 2014BAC13B06).

References

[1] A.V. Dordio, C. Duarte, M. Barreiros, A.J. Carvalho, A.P. Pinto, C.T. da Costa, Toxicity and removal efficiency of pharmaceutical metabolite clofibric acid by *Typha* spp. – potential use for phytoremediation?, Bioresour. Technol., 100 (2009) 1156–1161.

[2] S.K. Khetan, T.J. Collins, Human pharmaceuticals in the aquatic environment: a challenge to Green Chemistry, Chem. Rev., 38 (2007) 2319–2364.

[3] T.A. Ternes, A. Joss, H. Siegrist, Scrutinizing pharmaceuticals and personal care products in wastewater treatment, Environ. Sci. Technol., 38 (2004) 392A–399A.

[4] K. Joonwoo, J. Hyosang, K. Jonggu, H. Ishibashi, M. Hirano, K. Nasu, N. Ichikawa, Y. Takao, R. Shinohara, K. Arizono, Occurrence of pharmaceutical and personal care products (PPCPs) in surface water from Mankyung River, South Korea, J. Health Sci., 55 (2009) 249–258.

[5] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK, Water Res., 42 (2008) 3498–3518.

[6] P. Verlicchi, A.M. Al, E. Zambello, Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment—a review, Sci. Total Environ., 429 (2012) 123–155.

[7] N. Nakada, K. Komori, Y. Suzuki, C. Konishi, I. Houwa, H. Tanaka, Occurrence of 70 pharmaceutical and personal care products in Tone River basin in Japan, Water Sci. Technol., 56 (2007) 133–140.
[8] H.R. Buser, A. Thomas Poiger, M.D. Müller, Occurrence and fate of the pharmaceutical drug diclofenac in surface waters: rapid photodegradation in a lake, Environ. Sci. Technol., 32 (1998) 3449–3456.
[9] D. Ashton, M. Hilton, K.V. Thomas, Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom, Sci. Total Environ., 333 (2004) 167–184.
[10] J.B. Quintana, S. Weiss, T. Reemtsma, Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor, Water Res., 39 (2005) 2654–2664.
[11] M. Petrovic, M.J. Lopez d.A, S. Diaz-Cruz, C. Postigo, J. Radjenovic, M. Gros, D. Barcelo, M.R. Templeton, N. Graham, N. Voulvoulis, Fate and removal of pharmaceuticals and illicit drugs in conventional and membrane bioreactor wastewater treatment plants and by riverbank filtration, Philos. Trans. R. Soc. London, Ser. A, 367 (2009) 3979–4003.
[12] J. Radjenovic, M. Petrovic, D. Barceló, Fate and distribution ofpharmaceuticals in wastewater and sewage sludge of the conventionalactivated sludge (CAS) and advanced membrane bioreactor (MBR) treatment, Water Res., 43 (2009) 831–841.
[13] N.H. Tran, T. Urase, O. Kusakabe, The characteristics of enriched nitrifier culture in the degradation of selected pharmaceutically active compounds, J. Hazard. Mater., 171 (2009) 1051–1057.
[14] B. Alpaslan Kocamemi, F. Çeçen, Cometabolic degradation of TCE in enriched nitrifying batch systems, J. Hazard. Mater., 125 (2005) 260–265.
[15] K. Kimura, H. Hara, Y. Watanabe, Elimination of selected acidic pharmaceuticals from municipal wastewater by an activated sludge system and membrane bioreactors, Environ. Sci. Technol., 41 (2007) 3705–3714.
[16] C. Zwiener, F.H. Frimmel, Short-term tests with a pilot sewage plant and biofilm reactors for the biological degradation of the pharmaceutical compounds clofibric acid, ibuprofen,and diclofenac, Sci Total Environ., 309 (2003) 201–211.
[17] R. Salgado, A. Oehmen, G. Carvalho, J.P. Noronha, M.A.M. Reis, Biodegradation of clofibric acid and identification of its metabolites, J. Hazard. Mater., 241–242 (2012) 182–189.
[18] P. Avetta, D. Fabbri, M. Minella, M. Brigante, V. Maurino, C. Minero, M. Pazzi, D. Vione, Assessing the phototransformation of diclofenac, clofibric acid and naproxen in surface waters: model predictions and comparison with field data, Water Res., 105 (2016) 383–394.
[19] K. Kimura, H. Hara, Y. Watanabe, Removal of pharmaceutical compounds by submerged membrane bioreactors (MBRs), Desalination, 178 (2005) 135–140.
[20] T. Urase, T. Kikuta, Separate estimation of adsorption and degradation of pharmaceutical substances and estrogens in the activated sludge process, Water Res., 39 (2005) 1289–1300.
[21] T. Okuda, N. Yamashita, H. Tanaka, H. Matsukawa, K. Tanabe, Development of extraction method of pharmaceuticals and their occurrences found in Japanese wastewater treatment plants, Environ. Int., 35 (2009) 815–820.
[22] APHA, Standard Methods for the Examination of Water and Wastewater, American Public Health Association/American Water Works Association/Water Environment Federation, Washington, D.C., 1998.
[23] D. Shu, Y. He, H. Yue, Q. Wang, Microbial structures and community functions of anaerobic sludge in six full-scale wastewater treatment plants as revealed by 454 high-throughput pyrosequencing, Bioresour. Technol., 186 (2005) 163–172.
[24] T. Kosjek, E. Heath, Metabolism studies of diclofenac and clofibric acid in activated sludge bioreactors using liquid chromatography with quadrupole – time-of-flight mass spectrometry, J. Hydrol., 372 (2009) 109–117.
[25] R. Rosal, M.S. Gonzalo, K. Boltes, P. Letón, J.J. Vaquero, E. García-Calvo, Identification of intermediates and assessment of ecotoxicity in the oxidation products generated during the ozonation of clofibric acid, J. Hazard. Mater., 172 (2009) 1061–1068.
[26] E. Fernandez-Fontaina, F. Omil, J.M. Lema, M. Carballa, Influence of nitrifying conditions on the biodegradation and sorption of emerging micropollutants, Water Res., 46 (2012) 5434–5444.
[27] Y. Miura, M.N. Hiraiwa, T. Ito, T. Itonaga, Y. Watanabe, S. Okabe, Bacterial community structures in MBRs treating municipal wastewater: relationship between community stability and reactor performance, Water Res., 41 (2007) 627–637.