Chinese Chemical Letters 34 (2023) 107433

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Unraveling pharmaceuticals removal in a sulfur-driven autotrophic denitrification process: Performance, kinetics and mechanisms

Jiying Huang[a], Yujian Yan[b], Yuyi Ou[b], Yanyan Jia[b,*], Lianpeng Sun[a,c], Qing Zhao[d], Hui Lu[a,c,*]

[a] School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou 510006, China
[b] School of Ecology, Sun Yat-sen University, Guangzhou 510006, China
[c] Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology (Sun Yat-sen University), Guangzhou 510006, China
[d] School of Civil Engineering, Guangzhou University, Guangzhou 510006, China

ARTICLE INFO

Article history:
Received 7 January 2022
Revised 4 March 2022
Accepted 12 April 2022
Available online 15 April 2022

Keywords:
Pharmaceuticals (PhACs)
Ibuprofen (IBU)
Ketoprofen (KET)
Biodegradation
Sulfur-driven autotrophic denitrification process

ABSTRACT

The removal of eight typical pharmaceuticals (PhACs) (*i.e.*, ibuprofen (IBU), ketoprofen (KET), diclofenac (DIC), sulfadiazine (SD), sulfamethoxazole (SMX), trimethoprim (TMP), ciprofloxacin (CIP) and enoxacin (ENO)) in sulfur-driven autotrophic denitrification (SdAD) process were firstly investigated *via* long-term operation of bioreactor coupled with batch tests. The results indicated that IBU and KET can be effectively removed (removal efficiency > 50%) compared to other six PhACs in SdAD bioreactor. Biodegradation was the primary removal route for IBU and KET with the specific biodegradation rates of 5.3±0.7∼18.1±1.8 μg g^{-1}-VSS d^{-1} at initial concentrations of 25-200 μg/L. The biotransformation intermediates of IBU and KET were examined, and the results indicated that IBU was biotransformed to three intermediates *via* hydroxylation and carboxylation. KET biotransformation could be initiated from the reduction of the keto group following with a series of oxidation/reduction reactions, and five intermediates of KET were observed in this study. The microbial community composition in the system was markedly shifted when long-term exposure to PhACs. However, the functional microbes (*e.g.*, genus *Thiobacillus*) showed high tolerance to PhACs, resulting in the high efficiency for PhACs, N and S removal during long-term SdAD reactor operation. The findings provide better insight into PhACs removal in SdAD process, especially IBU and KET, and open up an innovative opportunity for the treatment of PhACs-laden wastewater using sulfur-mediated biological process.

Pharmaceuticals (PhACs), as one class of emerging contaminants, were frequently detected in surface water, sewage, groundwater and even drinking water due to extensively used by humans and animals [1–4]. The presence of PhACs in the environments could pose a serious risk to the ecosystem and humans [5–8]. PhACs commonly enter environments *via* the effluent discharge of wastewater treatment plants (WWTPs). Thus, the fate and removal of PhACs in WWTPs have widely attracted attention. However, conventional wastewater treatment process, *i.e.*, activated sludge process, is with the disadvantages of poor removal on PhACs and high energy consumption and huge amount of sludge generation [9]. Therefore, it is necessary to develop an energy-efficient biological process to achieve effective removal of PhACs and other pollutants (including organic matter and nutrients).

Recently, a series of energy-efficient sulfur-mediated biological technologies, including sulfate-reducing bacteria (SRB) mediated and sulfur-oxidizing bacteria (SOB) mediated biotechnologies, have widely attracted attention, which integrating S, C, and N removal [10]. For example, Sulfate reduction Autotrophic denitrification and Nitrification Integrated (SANI) [11], Flue Gas Desulfurization-SANI (FGD-SANI) [12], and Denitrifying Sulfur-assisted Enhanced Biological Phosphorous Removal (DS-EBPR) biotechnologies [13] were applied for industrial and municipal wastewaters treatment. Among these, the SRB mediated biotechnology has been applied to treat wastewater containing PhACs (*e.g.*, antibiotics) due to several advantages, *e.g.*, less sludge production and energy consumption, and high efficiency for PhACs, C and S removals [14,15]. However, there were few studies on PhACs removal in the SOB mediated bioprocess, *i.e.*, sulfur-driven autotrophic denitrification (SdAD).

Thus, the objectives of this study were to investigate the PhACs removal *via* long-term running of SdAD bioreactor, and unravel the removal mechanisms of PhACs removal *via* kinetics and

* Corresponding authors.
E-mail addresses: jiayy5@mail2.sysu.edu.cn (Y. Jia), lvhui3@mail.sysu.edu.cn, luhui.sysu@qq.com (H. Lu).

https://doi.org/10.1016/j.cclet.2022.04.031
1001-8417/

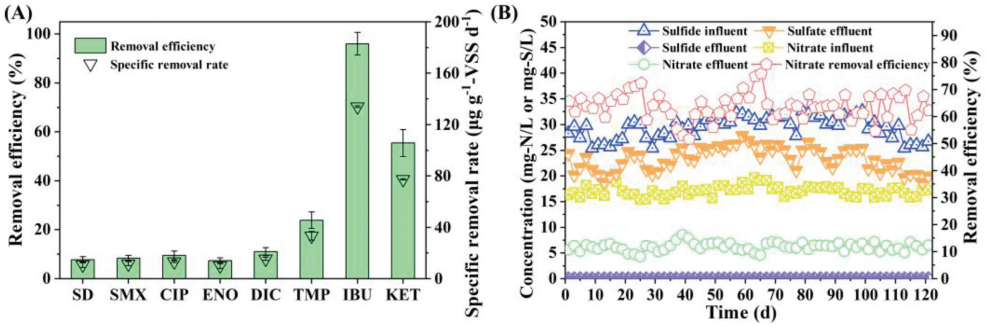

Fig. 1. The performance of SdAD bioreactor on PhACs removal (A), sulfur and nitrate removal (B).

biodegradation intermediates analyses. This study would provide better insight into PhACs removal in SdAD process, and offer an innovative sulfur-mediated biotechnology for the treatment of PhACs-laden wastewater.

In this study, a lab-scale of SdAD bioreactor with working volume of 1.0 L (Fig. S1 in Supporting information) was conducted and inoculated with SOB sludge, which was taken from the mother SdAD reactor operated for two years in our laboratory. The SdAD bioreactor was fed with synthetic wastewater containing eight PhACs (*i.e.*, sulfadiazine (SD), sulfamethoxazole (SMX), ciprofloxacin (CIP), enoxacin (ENO), trimethoprim (TMP), ibuprofen (IBU), ketoprofen (KET) and diclofenac (DIC), which were widely used by humans and animals and frequently detected in the environments), and continuously operated for around 120 days to examine PhACs removal (Tables S1 and S2 in Supporting information for the eight PhACs physical-chemical properties and synthetic wastewater characteristics). The concentration of each PhAC added in the influent was 100 μg/L, respectively. The samples (including influent, effluent and sludge) were regularly collected from SdAD bioreactor for routine chemical analyses (Pages S1 and S2 in Supporting information for more details). The contents of PhACs were determined by UPLC (Pages S2 and S3 in Supporting information for more details), the removal efficiencies and specific removal rates of PhACs were calculated by Eqs. S1 and S2 in Supporting information. Parallel three sludge samples collected at Day 0, Day 40, Day 80 and Day 120 named as S-0, S-1, S-2 and S-3, were extracted DNA and sequenced by Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA). The microbial community was further analyzed as detailed in Supporting information.

Based on the performance of the SdAD bioreactor, two PhACs, *i.e.*, KET and IBU, were effectively removed during long-term operation (Fig. 1A). To unravel the removal mechanisms of KET and IBU in SdAD process, we conducted a series of batch experiments. The batch experiments can be classified into three Groups (details were shown in Pages S1 and S2 and Table S3 in Supporting information): Group I (Control) was conducted to examine the abiotic removal of PhACs without SOB sludge addition; Group II and Group III were designed to examine the adsorption and biodegradation of PhACs in SdAD process at different each PhACs concentrations of 25, 50, 100, 150 and 200 μg/L, respectively. Three replicates were conducted for each group test. To investigate the removal kinetic and mechanism of each single pharmaceutical in SOB sludge system, KET and IBU were added individually in each group of batch test. The concentrations of nitrate, nitrite, sulfide, sulfite, thiosulfate, sulfate and PhACs in liquid phase were regularly analyzed (details of routine chemical analyses in Supporting information). The kinetics of adsorption and biodegradation of IBU and KET were investigated using the *pseudo*-first-order and *pseudo*-second-order adsorption models (Eqs. S4 and S5 in Supporting information, respectively), and first and second-order biodegradation models (Eqs. S6 and S7 in Supporting information, respectively).

The pollutants (*i.e.*, nitrate and sulfide) and PhACs removal in SdAD bioreactor during a long-term operation were examined as shown in Fig. 1. Over 60% of nitrate were effectively removed along with sulfide oxidized to sulfate during the long-term operation, suggesting that PhACs had no inhibitory effect on functional microorganisms in SOB sludge system (Fig. 1B). The performance of SdAD bioreactor in PhACs removal was illustrated in Fig. 1A. It is worth noting that IBU and KET were effectively removed (removal efficiency of 96.0%±4.6% and 55.4%±0.7%) during the long-term operation, and the specific removal rates of IBU and KET were 133.9±0.9 and 77.3±1.1 μg g^{-1}-VSS d^{-1}, (VSS, volatile suspended solids concentration) respectively. In comparison to IBU and KET, TMP was moderately removed, removal efficiency and specific removal rate were 23.9%±3.4% and 33.3±0.5 μg g^{-1}-VSS d^{-1}, respectively. Several studies reported that IBU, KET and TMP can be effectively removed in aerobic sludge (AS) system with removal efficiency ranging from 20% to 95% [16–18]. However, the specific removal rates of those PhACs in AS system (2.2 ∼ 23.8 μg g^{-1}-VSS d^{-1}) were much lower than that obtained in this study (33.3 ∼ 133.9 μg g^{-1}-VSS d^{-1}). Differently from IBU and KET, SdAD bioreactor showed low removal capacity for DIC and three antibiotics (SMX, CIP and ENO) during the long-term operation (removal efficiency < 10%). The results suggested that functional microbes in SOB sludge system showed high tolerance to PhACs, which was consistent with the microbial analyses results. Moreover, the SdAD bioprocess showed significant potential in treating wastewater containing IBU and KET. However, the removal mechanisms of IBU and KET in SOB sludge system of SdAD process were not clear. To further reveal the removal mechanisms of IBU and KET in SOB sludge system, batch experiments were conducted.

In the control test, the results suggested that the removal of IBU and KET *via* volatilization and hydrolysis can be ignored (removal efficiency < 1%) (Fig. S2 in Supporting information). Based on the adsorption results, SOB sludge showed limited adsorption capacity for IBU and KET (lower adsorption efficiency < 10%) at different initial concentrations (*i.e.*, 25, 50, 100, 150 and 200 μg/L) during 24 h operation (Fig. 2). The adsorption coefficients (K_d, which represented the adsorption capacity, was calculated using Eq. S3 in Supporting information) of IBU and KET were 2.15×10^{-2} ∼ 3.58×10^{-2} L/g-SS (SS, suspended solids) and 3.94×10^{-2} ∼ 5.54×10^{-2} L/g-SS (Table S4 in Supporting information for details), respectively, which further proved that the absorption contributed little for IBU and KET removal [19]. Similar studies also found that poor adsorption of IBU and KET by AS and anaerobic methanogenic (AnM) sludge [20–22], which could be due to electrostatic repulsion between IBU/KET and biological sludge (that were both negative charged) [23,24].

Compared to adsorption, biodegradation played an important role in IBU and KET removal during SdAD process. As shown in Fig. 3, IBU at lower initial concentrations (*i.e.*, 25, 50 and 100 μg/L) can be effectively biodegraded (biodegradation efficiency >

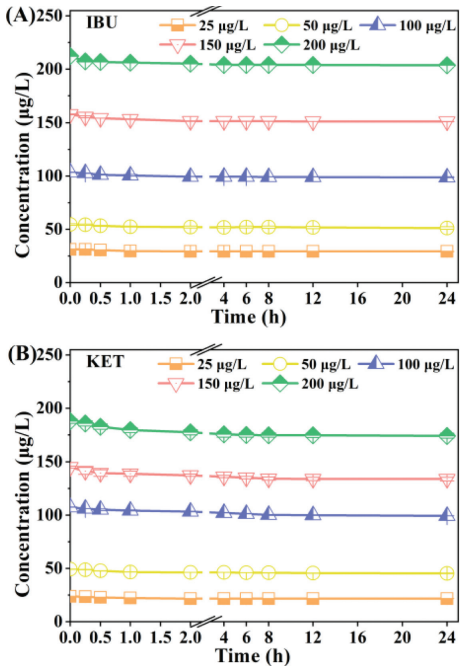

Fig. 2. The concentrations of IBU (A) and KET (B) in aqueous phase at adsorption batch tests at initial concentrations of 25, 50, 100, 150 and 200 μg/L.

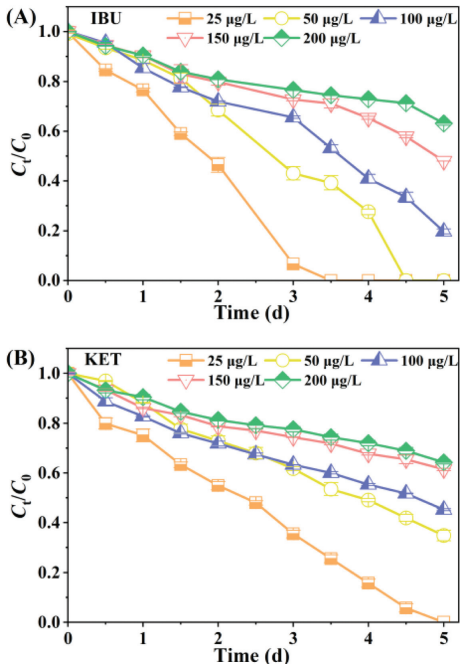

Fig. 3. Biodegradation of IBU (A) and KET (B) by SOB sludge in batch tests at initial concentrations of 25, 50, 100, 150 and 200 μg/L.

90%) within 5 days. Although IBU biodegradation showed a decreasing trend along with IBU concentrations increasing from 100 to 200 μg/L, the specific biodegradation rates were 11.6±2.2 ~ 18.1±1.8 μg g^{-1}-VSS d^{-1}, much higher than that obtained in AS (1.8 μg g^{-1}-VSS d^{-1}) and AnM systems (1.1 μg g^{-1}-VSS d^{-1}) [17,22]. Similar trends were observed during KET biodegradation, KET at lower initial concentrations (*i.e.*, 25 and 50 μg/L) can be completely degraded within 5 days. However, KET biodegradation was blocked at higher initial concentrations (*i.e.*, 100, 150 and 200 μg/L). The specific biodegradation rates of KET at different initial concentrations were 5.3±0.7 ~ 11.8±2.4 μg g^{-1}-VSS d^{-1}.

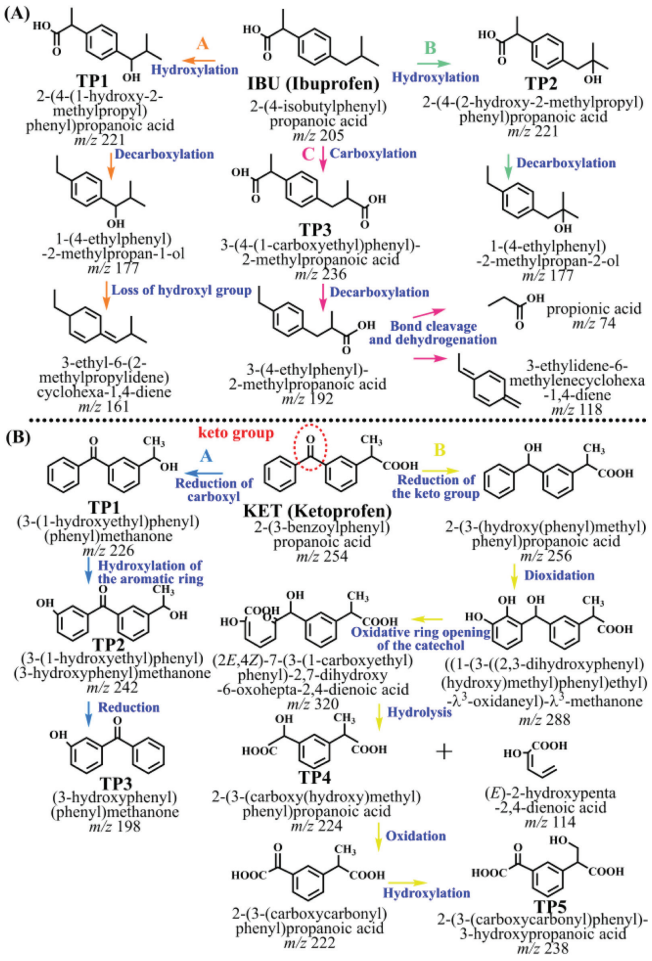

Fig. 4. The biodegradation intermediates and pathways of IBU (A) and KET (B).

The biodegradation kinetics of IBU and KET were also examined, and the first-order kinetic model provided better fit for IBU and KET biodegradation with correlation coefficient (r^2) of 0.85 to 0.99 (Table S5 in Supporting information). The results indicated higher concentrations of IBU and KET, the slower biodegradation rate [25], which was in consistent with the biodegradation rates obtained at different concentrations of IBU and KET (Table S5). The biodegradation rates (K_1', calculated using the first-order kinetic model) of IBU and KET were 0.078±0.027 ~ 0.38±0.016 d^{-1} and 0.088±0.027 ~ 0.53±0.037 d^{-1}, respectively, at different initial concentrations. Those above results suggested that KET could be harder degraded than IBU in SOB sludge system, which could be due to refractory double benzene ring chemical structure of KET [26].

To further underlying the biodegradation pathways of IBU and KET, the biodegradation products during batch tests were examined by HPLC-MS/MS (Pages S2 and S3 in Supporting information for details). IBU and KET biodegradation pathways and intermediates were illustrated in Fig. 4. IBU was firstly transformed to two isomers of hydroxy-ibuprofen *via* hydroxylation, *i.e.*, 2-(4-(1-hydroxy-2-methylpropyl)phenyl)propanoic acid (**TP1**, in pathway A) and 2-(4-(2-hydroxy-2-methylpropyl)phenyl)propanoic acid (**TP2**, in pathway B) (Table S6 in Supporting information for intermediates molecular anion and ion spectra). Then, both the intermediates were further decomposed *via* decarboxylation (Fig. 4A). Additionally, IBU can be transformed to 3-(4-(1-carboxyethyl)phenyl)-2-methylpropanoic acid (**TP3**, in pathway C) *via* carboxylation, then, it could be further decarboxylated (Fig. 4A).

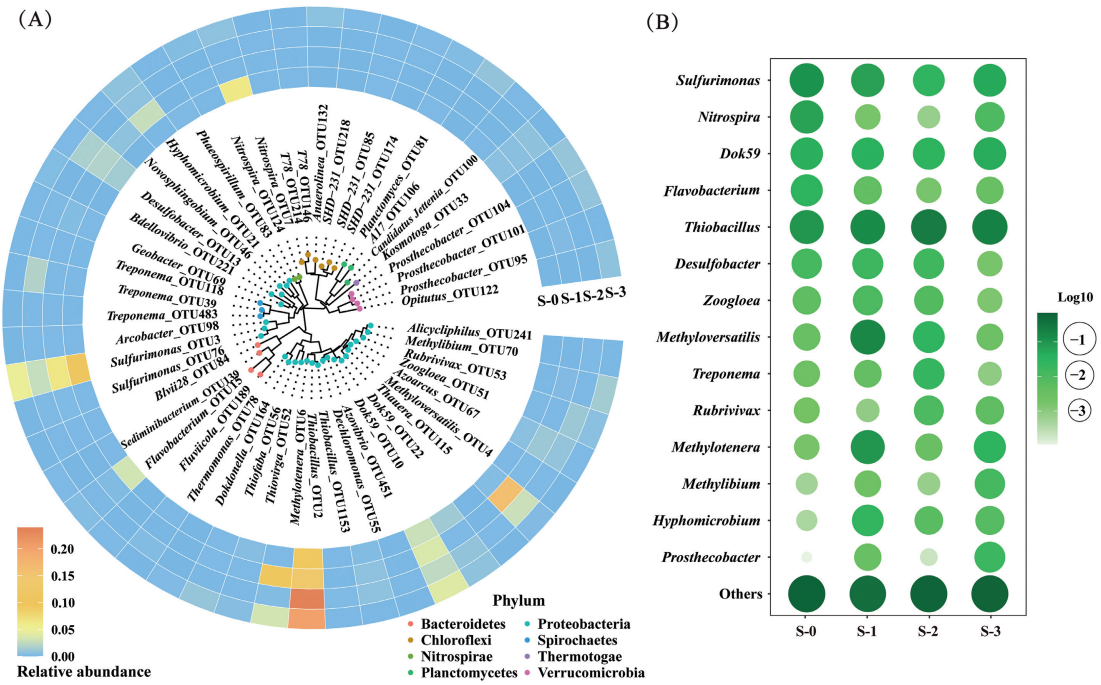

Fig. 5. The microbial composition in SOB sludge collected from SdAD bioreactor at different time. (A) Phylogenetic tree of dominant species. (B) Relative abundance of top 14 genera.

These three intermediates were also observed in AS system under aerobic condition [27,28].

In this study, KET was possibly biotransformed to five intermediates through two pathways *via* a series of oxidation/reduction and hydroxylation reactions, *i.e.*, KET-**TP1**∼**TP5**, as shown in Fig. 4B. In pathway A, KET was transformed into **TP1** *via* reduction of carboxyl, and **TP1** was further transformed to **TP2** *via* hydroxylation of the aromatic ring followed by the formation of **TP3** *via* the reduction [29]. In pathway B, KET biotransformation was possibly initiated from the keto group reduction, and the intermediate (*i.e.*, 2-(3-(hydroxy(phenyl)methyl)phenyl)propanoic acid) underwent dioxygenation and formation of the respective catechol, which was subsequently ring-opened by oxidation. Subsequently, **TP4** was formed *via* hydrolysis. Then, **TP4** was further oxidized at the secondary alcohol followed by the formation of **TP5** *via* hydroxylation. KET transformation *via* pathway B was observed in the AS system [30], however, **TP5** was firstly reported in biological sludge systems.

The microbial community variation of SOB sludge system when exposed to PhACs was evaluated during the long-term operation of SdAD bioreactor. The microbial community shifted significantly after exposure to PhACs based on Principal Component Analysis (PCA) analysis as shown in Fig. S3 (Supporting information), *i.e.*, the data point for Day 0 was far from other data points (*i.e.*, Day 40, Day 80 and Day 120). The composition of the microbial community in SOB sludge at different time (*i.e.*, Day 0, Day 40, Day 80 and Day 120) was shown in Fig. 5. *Proteobacteria*, *Bacteroidetes* and *Chloroflexi* were dominant phyla and occupied for over 80% of microbes in all sludge samples (Fig. S4 in Supporting information). It was worth noticing that the relative abundance of *Proteobacteria* increased from 44.8% to 58.3%-80.7% after PhACs addition, suggesting that PhACs had little inhibitory effect on microbes belong to *Proteobacteria* (*e.g.*, SOB) (Fig. S4) [31,32]. Compared to *Proteobacteria*, the phyla of *Bacteroidetes* and *Chloroflexi* were significantly inhibited and the relative abundance decreased markedly during long-term PhACs exposure. At genus level, top 14 genera (relative abundance $>$ 0.1%) were identified as shown in Fig. 5B. It was worth noticing that denitrifying genera were predominant and accounted for top 30% at all sludge samples (*e.g.*, *Sulfurimonas*, *Thiobacillus*, *Nitrospira*, *Methyloversatilis*, *Methylotenera*) [33–36]. It was consistent with the result that the denitrification of SdAD bioreactor was not suppressed with long-term feeding PhACs-laden wastewater. *Sulfurimonas*, an autotrophic sulfur oxidative and denitrification genus, which uses reduced sulfur as electron donor and nitrate as electron acceptor, was sensitive to PhACs with relative abundance significantly decreased during long-term PhACs exposure [37]. However, *Thiobacillus*, another autotrophic sulfur oxidative and denitrification genus, was gradually becoming the dominant species during long-term PhACs exposure, the relative abundance increased from 9.4% (Day 0) to 20.4% (Day 120), suggesting that *Thiobacillus* showed higher tolerance to PhACs [38]. Additionally, the genera of *Dok59*, *Methyloversatilis*, *Methylotenera* and *Hyphomicrobium* were also dominant species in SdAD bioreactor during long-term PhACs exposure, and potentially played important roles in IBU and KET biotransformation [39].

The removal of eight typical PhACs removal were evaluated during SdAD process *via* a long-term operation of bioreactor. The results indicated that IBU and KET compared to other six PhACs were effectively removed, and the specific removal rates of IBU and KET were 133.9±0.9 and 77.3±1.1 μg g^{-1}-VSS d^{-1}, respectively. IBU and KET removal *via* adsorption by SOB sludge were less effective, and biodegradation was the main removal route (specific biodegradation rates of 5.3±0.7 ∼ 18.1±1.8 μg g^{-1}-VSS d^{-1}) according to the batch experiments results. IBU was biotransformed to three intermediates *via* hydroxylation and carboxylation, and KET was biotransformed to five intermediates *via* a series of oxidation/reduction and hydroxylation reactions. The genus of *Thiobacillus* along with other denitrifying genera becoming dominant bacteria during long-term PhACs-laden wastewater treatment, which could play important roles in IBU and KET biodegradation. This study gave an insight into removal mechanisms of IBU and KET in SdAD process, which further implicated the engineering significance of sulfur-mediated biological technology for PhACs-laden wastewater treatment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The study was supported by the National Natural Science Foundation of China (Nos. 52000186, 51922110 and 52131001).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2022.04.031.

References

[1] A. Sengar, A. Vijayanandan, Sci. Total Environ. 807 (2022) 150677.
[2] M. Huerta-Fontela, M.T. Galceran, F. Ventura, Water Res. 45 (2011) 1432–1442.
[3] R. Loos, G. Locoro, S. Comero, et al., Water Res. 44 (2010) 4115–4126.
[4] A.S. Adeleye, J. Xue, Y. Zhao, et al., J. Hazard. Mater. 424 (2022) 127284.
[5] M. Ashfaq, K. Nawaz Khan, M. Saif Ur Rehman, et al., Ecotox. Environ. Safe. 136 (2017) 31–39.
[6] A.Y. Lin, T. Yu, C. Lin, Chemosphere 74 (2008) 131–141.
[7] S.A. Ortiz De García, G.Pinto Pinto, P.A. García-Encina, et al., Ecotoxicology 23 (2014) 1517–1533.
[8] B. Ren, X. Shi, X. Jin, et al., Chem. Eng. J. 404 (2021) 127024.
[9] G. Wang, D. Wang, Y. Xu, et al., Sci. Total Environ. 739 (2020) 140166.
[10] J. Wang, H. Lu, G. Chen, et al., Water Res. 43 (2009) 2363–2372.
[11] H. Lu, D. Wu, D.T.W. Tang, et al., Water Sci. Technol. 63 (2011) 2149–2154.
[12] J. Qian, R. Liu, L. Wei, et al., Water Res. 80 (2015) 189–199.
[13] D. Wu, G.A. Ekama, H. Wang, et al., Water Res. 49 (2014) 251–264.
[14] Y. Jia, S.K. Khanal, H. Zhang, et al., Water Res. 119 (2017) 12–20.
[15] Y. Jia, S.K. Khanal, H. Shu, et al., Water Res. 136 (2018) 64–74.
[16] T. Alvarino, S. Suarez, J.M. Lema, et al., J. Hazard. Mater. 278 (2014) 506–513.
[17] S. Suarez, J.M. Lema, F. Omil, Water Res. 44 (2010) 3214–3224.
[18] N.H. Tran, T. Urase, O. Kusakabe, J. Hazard. Mater. 171 (2009) 1051–1057.
[19] T.A. Ternes, N. Herrmann, M. Bonerz, et al., Water Res. 38 (2004) 4075–4084.
[20] Y. Jia, L. Yin, S.K. Khanal, et al., Water Res. 170 (2020) 115303.
[21] T. Alvarino, S. Suarez, J. Lema, et al., Sci. Total Environ. 615 (2018) 297–306.
[22] T. Alvarino, P. Nastold, S. Suarez, et al., Sci. Total Environ. 542 (2016) 706–715.
[23] E. Ferrer-Polonio, J. Fernández-Navarro, M. Iborra-Clar, et al., J. Environ. Manage. 263 (2020) 110368.
[24] I. Martínez-Alcalá, J.M. Guillén-Navarro, C. Fernández-López, Chem. Eng. J. 316 (2017) 332–340.
[25] Y. Tang, X.M. Li, Z.C. Xu, et al., Biotechnol. Appl. Biochem. 61 (2014) 333–341.
[26] B. Almeida, A. Oehmen, R. Marques, et al., Bioresour. Technol. 133 (2013) 31–37.
[27] C. Boix, M. Ibáñez, J.V. Sancho, et al., J. Hazard. Mater. 302 (2016) 175–187.
[28] L. Ferrando-Climent, N. Collado, G. Buttiglieri, et al., Sci. Total Environ. 438 (2012) 404–413.
[29] M.M. Ismail, T.M. Essam, Y.M. Ragab, et al., Biotechnol. Lett. 38 (2016) 1493–1502.
[30] J. Quintana, S. Weiss, T. Reemtsma, Water Res. 39 (2005) 2654–2664.
[31] Q. Yan, J. Min, Y. Yu, et al., Chemosphere 186 (2017) 823–831.
[32] D. Pokorna, J. Zabranska, Biotechnol. Adv. 33 (2015) 1246–1259.
[33] N. Jakus, N. Blackwell, D. Straub, et al., FEMS Microbiol. Ecol. 97 (2021) fiab145.
[34] C. Juncher Jørgensen, O.S. Jacobsen, B. Elberling, et al., Environ. Sci. Technol. 43 (2009) 4851–4857.
[35] P. Charoensuk, W. Thongnueakhaeng, P. Chaiprasert, Int. J. Environ. Sci. Technol. 16 (2019) 5767–5782.
[36] I. Mustakhimov, M.G. Kalyuzhnaya, M.E. Lidstrom, et al., J. Bacteriol. 195 (2013) 2207–2211.
[37] K. Takai, M. Suzuki, S. Nakagawa, et al., Int. J. Syst. Evol. Micr. 56 (2006) 1725–1733.
[38] M. Zhu, M. Zhang, Y. Yuan, et al., J. Environ. Manage. 289 (2021) 112473.
[39] Y. Feng, Z. Li, Y. Long, et al., Environ. Pollut. 292 (2022) 118357.