Original Research

Occurrence and risk assessment of typical PPCPs and biodegradation pathway of ribavirin in wastewater treatment plants

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ARTICLE INFO

ABSTRACT

A large number of pharmaceuticals and personal care products (PPCPs) persist in wastewater, and the consumption of PPCPs for COVID-19 control and prevention has sharply increased during the pandemic. This study investigated the occurrence, removal efficiency, and risk assessment of six typical PPCPs commonly used in China in two wastewater treatment plants (WWTPs). Ribavirin (RBV) is an effective pharmaceutical for severely ill patients with COVID-19, and the possible biodegradation pathway of RBV by activated sludge was discovered. The experimental results showed that PPCPs were detected in two WWTPs with a detection rate of 100% and concentrations ranging between 612 and 2323 ng L^{-1}. The detection frequency and concentrations of RBV were substantially higher, with a maximum concentration of 314 ng L^{-1}. Relatively high pollution loads were found for the following PPCPs from influent: ibuprofen > ranitidine hydrochloride > RBV > ampicillin sodium > clozapine > sulfamethoxazole. The removal efficiency of PPCPs was closely related to adsorption and biodegradation in activated sludge, and the moving bed biofilm reactor (MBBR) had a higher removal capacity than the anoxic-anaerobic-anoxic-oxic (AAAO) process. The removal efficiencies of sulfamethoxazole, ampicillin sodium, ibuprofen, and clozapine ranged from 92.21% to 97.86% in MBBR process and were relatively low, from 61.82% to 97.62% in AAAO process, and the removal of RBV and ranitidine hydrochloride were lower than 42.96% in both MBBR and AAAO processes. The discrepancy in removal efficiency is caused by temperature, hydrophilicity, and hydrophobicity of the compound, and acidity and alkalinity. The transformation products of RBV in activated sludge were detected and identified, and the biodegradation process of RBV could be speculated as follows: first breaks into TCONH$_2$ and an oxygen-containing five-membered heterocyclic ring under the nucleosidase reaction, and then TCONH$_2$ is finally formed into TCOOH through amide hydrolysis. Aquatic ecological risks based on risk quotient (RQ) assessment showed that PPCPs had high and medium risks in the influent, and the RQ values were all reduced after MBBR and AAAO treatment. Ranitidine hydrochloride and clozapine still showed high and medium risks in the effluent, respectively, and thus presented potential risks to the aquatic ecosystem.

1. Introduction

The use of pharmaceuticals and personal care products (PPCPs) increases with the continuous population growth [1,2], and PPCPs cannot be completely metabolized by the human body; therefore, unmetabolized PPCPs are directly discharged into the wastewater and then introduced to wastewater treatment plants (WWTPs) [3–6]. Although activated sludge has certain PPCPs removal

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efficiency, conventional WWTPs have limited PPCPs removal capacity [3,7,8]. Hence, most PPCPs are indirectly discharged into natural water bodies through the effluent of WWTPs [6,9]. At present, PPCP residues have been detected in most WWTPs worldwide (concentrations ranging from ng L^{-1} to μg L^{-1}) and thus pose a huge threat to organisms or ecosystems [9–12]. Hamid et al. found that long-term exposure to a certain concentration of mixed PPCPs can cause the developmental and metabolic dysfunction in male and female fish (zebrafish) [13], and climbazole is toxic to aquatic organisms including algae, aquatic plants, and fish (rainbow trout) [14]. With the rapid degradation of PPCPs, it was found that the characteristics of pseudo persistent and poisonousness to the environment intensified. Therefore, understanding the occurrence, removal efficiency, environmental behavior, and potential ecological risks of PPCPs in WWTPs is urgently necessary.

Since December 2019, the whole world has encountered a serious and challenging period due to the outbreak of coronavirus disease (COVID-19). Owing to its good therapeutic effect for severe patients with COVID-19, antiviral ribavirin (RBV) has received increasing attention to control the pandemic spread [15]. RBV exhibits a conspicuous curative effect against RNA and DNA viruses and is extensively used to treat viral diseases including herpes, hepatitis C, and Lassa fever because of its broad-spectrum characteristics [16,17]. By using quantitative structure-activity relationship (QSAR) modeling, Kuroda et al. predicted that the wastewater from traditional WWTPs under COVID-19 circumstances may contain high concentrations of antiviral pharmaceuticals and their metabolites, with the concentration of total RBV and its metabolite TCONH$_2$ up to 7402 ng L^{-1} [18], and Chen et al. found that the detection frequency and concentrations of RBV increased after the COVID-19 outbreak [19]. Ribavirin has been proven to be an antiviral treatment, whereas there are still risks of hemolysis and congenital malformation. Ye et al. found that ribavirin is detrimental to cardiac differentiation of hiPSCs, which may be associated with DNA damage, upregulated p53, and increased Gas5 [20]. Therefore, the pseudo-persistence of ribavirin may be detrimental to organisms exposed to the environment for a long time. After human metabolism and the biochemical transformation of activated sludge in WWTPs, the excreted PPCPs and their metabolites can be found in measurable quantities in wastewater [21]. The produced metabolites are generally unknown compounds or unpredictable transformation products (TPs) with stronger stability or toxicity than their parent compound and therefore could greatly harm the ecological environment [22]. In addition, systematic investigation into the biodegradation pathway, transportation, and transformation of antiviral pharmaceuticals, including RBV, in WWTPs with different treatment processes is currently scarce. Therefore, it is necessary to explore the occurrence, fate, and biodegradation mechanism of antiviral pharmaceuticals in activated sludge, which is conducive to providing a theoretical basis for the effect of RBV and its metabolites on the ecological environment and humans health. Environmental risk quotient (RQ) was adopted to conduct a preliminary screening-level risk assessment based on PPCP concentrations and predicted no-effect concentration (PNECs) [23] to further evaluate the aquatic ecological risks of the PPCPs within influent and effluent. Kumari and Kumar found that the effluent of WWTPs still contains a large amount of PPCPs [24], which will be discharged directly into the receiving rivers or lakes along with the effluent [21,24]. Therefore, PPCPs have been extensively detected in the receiving natural water systems and may lead to potential ecological risks and adverse effects on aquatic organisms.

This study aimed to investigate the occurrence and removal of six typical PPCPs in two WWTPs located in the Taihu lake basin (China) during winter. Mass balance analysis was applied to estimate the mass loads of PPCPs in different wastewater treatment processes, and the use and emission of PPCPs in the Taihu lake basin were calculated according to the per capita pollution loads in the two WWTPs. Based on the current epidemic situation, there are still tens of thousands of new confirmed cases every day in many countries around the world, and with the acceleration of the virus mutation rate, its infection rate is also rising, ribavirin may continue to be used in the future. Therefore, the biodegradation products of RBV were identified to explore the conversion of RBV, and the biodegradation pathway and mechanism were subsequently proposed. This work is beneficial to understand the occurrence and fate of PPCPs in WWTPs with different treatment processes. The estimated pollution loads will further provide evidence for assessing the potential ecological risks, and the predicted biodegradation mechanism and pathway of RBV can lay the foundation to eliminate the environmental influence of RBV during times of intense use.

2. Materials and methods

2.1. Characteristics of typical PPCPs

Six typical PPCPs were selected according to various criteria: antibiotic pharmaceuticals sulfamethoxazole (SMX), ampicillin sodium (APC), nonsteroidal anti-inflammatory drug ibuprofen (IBU), mental disorder drug clozapine (CZP), digestive system drug ranitidine hydrochloride (RAN), and antiviral drug RBV. Properties of these PPCPs are shown in Supplementary Table 1, and their chemical structures are displayed in Supplementary Fig. 1. A complete list of monitored chemical substances can be found in Supplementary Table 2, including P_{Ka}, Log K_{ow}, and Log k_{oc}. These PPCPs were of high purity grade (above 98%) and purchased from Aladdin Industrial Inc. (Shanghai, China).

2.2. Sampling site and samples collection

PPCP samples from wastewater and activated sludge phases were all collected throughout the wastewater treatment process in two WWTPs, in which the average effluent chemical oxygen demand (COD), ammonia nitrogen, total nitrogen (TN), and total phosphorus (TP) are superior to the issued wastewater discharge standard (DB32/1072−2018, the standard requires COD $\leq$50 mg L^{-1}, NH$_4^+$-N $\leq$ 4 mg L^{-1}, TN $\leq$ 12 mg L^{-1}, TP $\leq$ 0.5 mg L^{-1}). In addition, several large hospitals are located upstream of these WWTPs; therefore, medical wastewater was collected through the drainage network. The basic information and simplified diagram of the two WWTPs are listed in Table 1 and Supplementary Fig. 2, and the main treatment processes are MBBR (WWTP A) and AAAO (WWTP B).

The samples were collected for two consecutive days in a week from December 2020 to March 2021. Each wastewater sample was collected three times in triplicate as a 24-h mixed sample, and the sludge sample was collected once at the oxic tank, with the biomass of 3500 and 2500 mg L^{-1}, respectively. Several 1000 mL brown glass bottles were used as sample containers, methanol and ultrapure water were applied to rinse the bottles three times before sampling. The pH value of wastewater samples was then adjusted to 4.0 by adding 2 mol L^{-1} H$_2$SO$_4$, followed by methanol (5%, v/v) to inhibit microbial activity [9]. All the samples were placed in an incubator and transported to the laboratory within 2 h. The wastewater samples were processed immediately, and the sludge samples were stored at 4 °C after being freeze-dried and ground through a 0.8 mm sieve.

Table 1
Basic information of WWTPs.

Process type	Average flow (m³ d⁻¹)	Population served (10⁴)	Disinfection method	Hydraulic retention time (h)	MLSS (mg L⁻¹)	Excess sludge	
						Daily production (t d⁻¹, wet weight)	Moisture content (%)
MBBR	160,000	43.73	Cl_2	10.19	4000	4.00	60.00
AAAO	140,000	38.27	Cl_2	25.29	3500	40.00	78.00

2.3. Sample extraction and measurement

Six typical PPCPs in wastewater and sludge samples were extracted and analyzed using the optimized method described by Chen et al. [25]. The wastewater samples were first filtered through a 0.45 μm fiber filter and then extracted via solid-phase extraction using oasis hydrophilic-lipophilic balance (HLB) cartridges (200 mg, 6 mL). Subsequently, 10 mL of methanol and 10 mL of Milli-Q water solution were prepared to flush the Oasis HLB cartridges. After nitrogen gas blowing, the extracted samples were diluted using 1 mL of methanol containing 0.025% formic acid.

The sludge samples were extracted via the ultrasonic extraction method [9]. Each homogenized sludge sample (1.0 g) was weighed into a centrifuge tube (15 mL) with 10 mL methanol and placed in an ultrasonic bath for 15 min, followed by centrifugation for 10 min at the speed of 1400 g, then the supernatant was transferred into a brown liquid storage bottle (500 mL). The extraction procedure was repeated twice using 10 mL of methanol and 5 mL of methanol +5 mL of 0.1% (v/v) formic acid solution. The supernatants of each extraction were then mixed and diluted to 300 mL with ultrapure water. The mixture was further purified with an Oasis HLB cartridge (200 mg, 6 mL) in accordance with the procedure of aqueous sample extraction. Finally, the six types of PPCPs were quantified by ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (Waters Acquity UPLC coupled to AB Sciex API 4000) with electrospray ionization under positive and negative ionization mode and equipped with a BEH-C18 column (2.1 mm × 100 mm, 1.7 μm) (Waters, USA). The specific operation condition and method for each target compound are shown in Supplementary Text 1.

2.4. Mass balance and pollution load estimation

Mass balance evaluates the mass load of PPCPs from the influent and effluent of WWTPs and is beneficial to understand the occurrence, fate, transport, and potential removal mechanism of PPCPs. Therefore, mass balance equations were determined using the modified method described by Ren et al. [26], Heidler and Halden [27].

$$M_w = Q_w \times C_w, \tag{1}$$

$$M_s = Q_s \times C_s, \tag{2}$$

Where M_w and M_s (μg d⁻¹) represent the average mass of PPCPs in wastewater and sludge, respectively; Q_w (L d⁻¹) and Q_s (kg d⁻¹) denote the average volume of wastewater and sludge; and C_w and C_s define the average concentration of PPCPs in wastewater and sludge, respectively. The mass balance of PPCPs can be described as follows:

$$M_{Influent} = M_{Effluent} + M_{Sludge} + M_{Loss}, \tag{3}$$

Where $M_{Influent}$, $M_{Effluent}$, and M_{Sludge} (g d⁻¹) represent the mass loads of each PPCP from influent, effluent, and excess sludge of

WWTP, respectively. The estimation methods are detailed in Supplementary Text 2. M_{Loss} (g d⁻¹) defines the loss of mass loads of each PPCP during the whole wastewater treatment process and is mainly attributed to the adsorption and biodegradation of activated sludge. In addition, the loss of mass by adsorption and biodegradation (R_{Loss}, %), and the loss of mass from effluent ($R_{Effluent}$, %) and excess sludge (R_{Sludge}, %) were calculated by using the following equations:

$$R_{Effluent} = M_{Effluent} / M_{Influent} \times 100\% \tag{4}$$

$$R_{Sludge} = M_{Sludge} / M_{Influent} \times 100\% \tag{5}$$

$$R_{Loss} = (M_{Influent} - M_{Effluent} - M_{Sludge}) / M_{Influent} \times 100\% \tag{6}$$

Moreover, the estimated per capita pollution loads of PPCPs from influent, effluent and excess sludge for each WWTP could be calculated through the $M_{Influent}$, $M_{Effluent}$, and M_{sludge} divided by the corresponding population served in Table 1.

2.5. Biodegradation pathway of RBV

The activated sludge (biomass of 2500 mg L⁻¹) system was added with 1 mg L⁻¹ RBV to identify the TPs of RBV and speculate on the biodegradation pathway of RBV. In brief, 1.5 mL of the wastewater sample was collected through a 0.22 μm filter membrane at different reaction time intervals (0, 0.5, 1, 4, and 8 h). The TPs were measured via ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-Q-TOF) equipped with an SB-C18 column (4.6 mm × 150 mm, 5 μm) (Agilent Co. Ltd., USA), and the measurement was implemented in full scan and positive ion modes. MS/MS fragmentation experiments were then conducted to identify the TPs preliminarily, and the detailed instrumental measurement method is the same as expressed in Section 2.3. All data analyses were performed using the Metabolite Pilot 2.0.4 software and SCIEX OS 2.1.6. In addition, the Eawag-BBD Pathway Prediction System database artificial intelligence system for path prediction (Eawag-BBD/PPS, http://eawag-bbd.ethz.ch/predict/) was employed to predict the biodegradation pathway of RBV. Based on microbial metabolic reactions and scientific literature, the unknown TPs could finally be identified.

2.6. Environmental risk assessment

The potential ecological risks of the PPCPs from the two WWTPs were evaluated by using the risk quotient (RQ). RQ was calculated as MEC/PNEC, where MEC is the measure of environmental concentration and PNEC is the predicted no-effect concentration. PNEC values were derived from the European Commission (EC) technical guidance document on risk [28], the WikiPharma database (https://www.mistrapharma.se/), and related values according to Refs. [18,29–32]. In addition, the PNEC value was calculated from the effective concentration (EC50) or lethal concentration (LC50) divided by an assessment factor of 1000 when only short-term or acute toxicity data were available [30]. For long-term or chronic

toxicity data, the PNEC value was calculated from the lowest no observed effect concentration (NOEC) divided by an assessment factor of 100, 50, or 10 for one, two, or three trophic levels, respectively. The derived PNEC values for the six typical PPCPs are shown in Supplementary Table 3. Finally, the criteria of interpreting the RQ values were classified into four levels: minimal risk (RQ < 0.01), low risk (0.01 < RQ < 0.1), medium risk (0.1 < RQ < 1), and high risk (RQ > 1) [23].

3. Results and discussion

3.1. Concentrations of typical PPCPs in WWTPs

Six typical PPCPs were detected at 100% rate in the two WWTPs, and their concentration levels are summarized in Table 2 and Fig. 1. In the two WWTPs, typical PPCPs with the high mean concentrations were IBU (178–2647 ng L^{-1}), RAN (249–6577 ng L^{-1}), and RBV (167–269 ng L^{-1}), and the respective concentrations of IBU, RAN and RBV were 170–3661 ng L^{-1}, 2556–598 ng L^{-1}, and 113–314 ng L^{-1}, which were still the principal PPCPs after fine screen pretreatment. RAN and RBV with high mean concentrations in the secondary sedimentation tank and advanced treatment process in both WWTPs, and the typical dominant PPCPs were RAN (263–780 ng L^{-1}) and RBV (97.3–207 ng L^{-1}) after disinfection process in both WWTPs. Chen et al. found that the detection frequency and concentrations of RBV increased after the COVID-19 outbreak [19], and Golovko et al. measured 164 types of PPCPs in 15 WWTPs in Switzerland and discovered that the detection rate of RAN was substantially high, with mean concentrations of 6400 and 3400 ng L^{-1} in the influent and effluent, respectively [33]. The detection rate of CZP reached 93%, with mean concentrations of 19.5 and 18 ng L^{-1} in the influent and effluent [33], respectively, and these values were close to the concentration measured in the two WWTPs. In addition, considerably high concentrations of PPCPs were discovered in winter, especially for the analgesic and anti-inflammatory pharmaceuticals [34], the possible reason could be the high consumption of IBU and RAN (Fig. 1).

The total concentration levels of the typical PPCPs from the influent (Fig. 1) had certain similarities between the two WWTPs, and the total concentration was as high as 2250 ng L^{-1}. Among

them, IBU, RAN, and RBV were the main compounds. However, the distribution ratios of the PPCPs presented discrepancies in the two WWTPs. The concentration level of APC in MBBR process was significantly higher than that in AAAO process, with the mean influent concentration of 122 ng L^{-1} and mean effluent concentration was 7.72 ng L^{-1} ($p = 0.04 < 0.05$). The concentration level of CZP in AAAO process was higher than that in MBBR process ($p = 0.03 < 0.05$). The mean influent concentrations of CZP in MBBR and AAAO processes were 154 and 6.34 ng L^{-1}, respectively. The mean effluent concentrations were 3.66 and 0.49 ng L^{-1}, respectively. The differences in the above data were mainly due to the discrepancy in the wastewater sources, people served, and wastewater treatment processes [9].

3.2. Typical PPCPs removal efficiency of PPCPs in wastewater

The removal of typical PPCPs is mainly achieved by adsorption and biodegradation in WWTPs [35]. A certain difference was depicted in the removal of the typical PPCPs, with the mean removal efficiencies ranging from −8.29% to 97.68% for each PPCP (Fig. 2), and the total average typical PPCPs removal were 71.23% and 64.18% in MBBR and AAAO processes, respectively. The removal of IBU and CZP displayed excellent removal efficiencies (higher than 92.21%) in both WWTPs, indicating low adverse performance in the wastewater treatment process. The possible reason for the disparity was IBU could be used as the carbon and energy source for activated sludge [36,37], and the disinfection process is conducive to further degrading IBU [38]. SMX and APC showed different removal performances in MBBR and AAAO processes, which the removal efficiencies of SMX and APC were up to 97.68% and 93.65% in MBBR process, and 82.00% and 61.28% in AAAO process, respectively. In addition, significantly low removal of RAN and RBV was discovered in both processes. The removal efficiency of RBV was merely 22.76% and 31.31% in MBBR and AAAO processes, respectively, and RAN even presented a negative removal rate of −8.29% in AAAO process, the possible reason could be attributed to the desorption of PPCPs from activated sludge [39,40]. The concentration of RAN was fluctuating, owing to the refractory property and desorption from activated sludge [41,42]. Furthermore, most PPCPs possess relatively low removal in winter [43,44]

Table 2
Summary of the concentration levels (ng L^{-1}) and the removal rates (R.R, %) of six typical PPCPs in the wastewater phase of each process stage in two WWTPs.

| Process	Compound	Influent	Pretreatment	Secondary sedimentation	Advanced processing	Effluent	Sludge (mg d^{-1})	removal rates
MBBR	RBV	180–250 (220, 217)[a]	124–267 (179, 190)	116–232 (193, 180)	128–220 (196, 182)	98.8–204 (199, 167)	0.690–18.2 (5.81, 3.97)	22.76
	RAN	695–921 (711, 776)	449–598 (577, 542)	581–743 (682, 669)	445–929 (601, 658)	328–512 (488, 443)	11.8–32.7 (19.1, 16.7)	42.95
	CZP	0.400–15.3 (3.94, 6.34)	0.320–9.97 (3.05, 4.10)	0.850–24.4 (5.43, 9.02)	0.480–27.7 (5.15, 9.62)	0.09–1.30 (0.30, 0.49)	1.04–17.8 (7.78, 8.38)	92.21
	SMX	2.56–33.7 (10.1, 15.4)	23.0–56.2 (35.5, 38.2)	1.52–18.7 (13.5, 11.2)	1.88–18.0 (13.4, 11.1)	0–0.66 (0.34, 0.33)	0.368–4.13 (1.55, 0.800)	97.86
	AMP	46.7–257 (60.9, 121.6)	9.41–172 (62.6, 81.2)	24.3–120 (47.6, 64.1)	10.1–77.3 (67.4, 51.6)	0–14.1 (9.11, 7.72)	0.048–1.90 (0.448, 0.192)	93.65
	IBU	231–2551 (301, 1187)	170–3661 (298, 1187)	0–410 (136, 218)	0–616 (110, 290)	0–204 (11.4, 49.9)	0	95.80
AAAO	RBV	167–269 (257, 231)	113–314 (177, 201)	129–279 (147, 185)	168–223 (197, 196)	97.3–207 (172, 159)	6.51–21.5 (12.3, 10.9)	31.31
	RAN	249–6577 (529, 478)	256–446 (442, 381)	175–1835 (337, 782)	183–383 (308, 291)	263–780 (511, 518)	46.9–128 (87.9, 96.2)	−8.29
	CZP	5.62–308 (142, 154)	0.130–337 (4.60, 72.3)	0.520–31.2 (6.69, 13.1)	0–27.2 (1.11, 6.76)	0–16.9 (0.390, 3.66)	15.4–141 (94.5, 125)	97.62
	SMX	2.12–43.0 (24.6, 23.2)	31.8–42.2 (33.0, 35.7)	1.52–10.8 (9.35, 7.21)	1.37–16.9 (14.1, 10.8)	0.05–12.8 (0.210, 4.36)	0.792–18.0 (10.3, 12.2)	81.25
	AMP	23.0–61.8 (42.4, 42.4)	50.6–65.4 (63.2, 59.7)	4.54–30.5 (26.4, 20.5)	33.4–40.8 (34.3, 36.1)	2.21–40.9 (5.53, 16.2)	0.704–3.87 (2.90, 3.52)	61.82
	IBU	178–2647 (348, 1233)	180–2959 (326, 1350)	0–279 (67.0, 88.0)	0–223 (46.3, 71.1)	0–207 (68.7, 73.8)	0	94.02

[a] Range (mean, median).
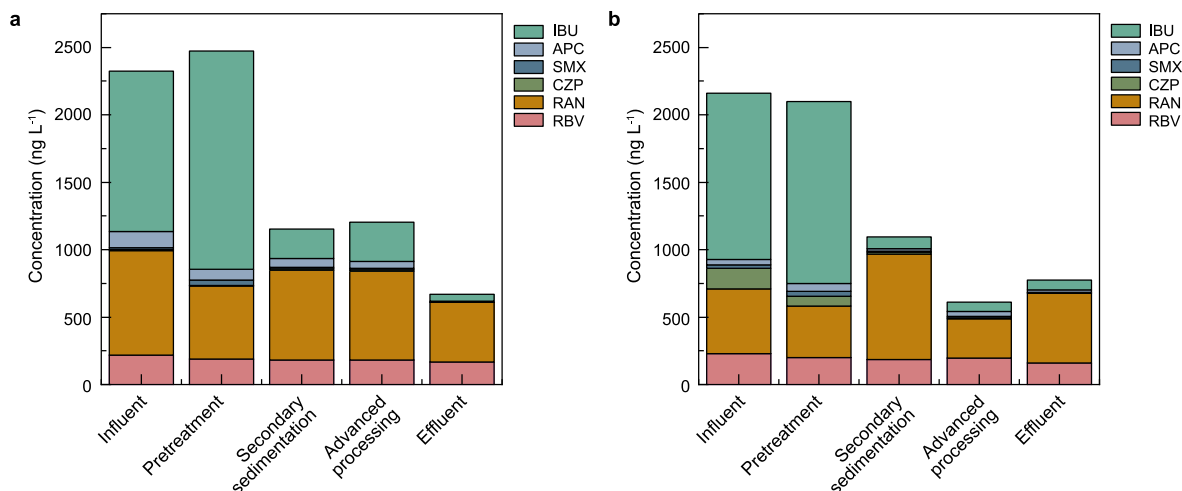
Fig. 1. The concentration distribution of six typical PPCPs in WWTPs. **a**, MBBR process. **b**, AAAO process.

due to the poor microbial activity, reduction of enzyme activity, and loss of biomass under cold conditions [33,45,46]; therefore, relatively high temperature may be in favor of PPCPs removal. In addition, the hydrophilic characteristics of typical PPCPs may lead to low K_{ow} values [26], implying low binding capacity among typical PPCPs with biofilm in MBBR process and activated sludge in AAAO process, and results in poor typical PPCPs removal efficiency.

Through the investigation of other literature, the removal efficiencies of pharmaceuticals in different processes are indeed different. Liu et al. analyzed the occurrence of 19 PPCPs in ten WWTPs and found that the improved anoxic-oxic (AO) process, membrane bioreactor (MBR), and Carrousel oxidation ditch (OD) process had relatively high removal rates of PPCPs, all of which could reach more than 80%. Conversely, reserved anaerobic-anoxic-oxic (AAO) showed only about a 60% removal rate of PPCPs [9]. However, since it is not consistent with the target PPCPs selected in this study, it is impossible to compare the PPCPs removal efficiency of the wastewater treatment process based on the current research. In addition, the molecular structure of compounds can also affect the compounds removal rate in WWTPs. Fe(VI) has a better oxidation effect on secondary amines of acidic drugs and aniline moieties of antibiotics. Compounds with C=C or electron-donating aromatic structures (such as phenol, alkyl, methoxy, or aprotonated amines) are more susceptible to ozonation, and compounds with an amide structure have resistance [47,48]. In this study, the free

carboxyl group of IBU makes it have a certain acidity, and it possessed a high removal effect in different wastewater processes. SMX and APC are acid-base amphoteric and showed different removal effects in different wastewater processes, but in general, the removal effects were both good. RAN and RBV have certain alkalinity due to their structure and showed relatively poor removal effects in WWTPs. Therefore, it is speculated that the discrepancy of compounds' molecular structures presents different acid-base properties and further affects their removal effects in WWTPs.

3.3. Mass balance and pollution load estimation of PPCPs

3.3.1. Mass balance

The mass loads of influent and effluent in both WWTPs were estimated by applying the principle of mass balance (Equation (3)), and the results for mass fractions are depicted in Fig. 3. The distribution of mass fractions displayed slight differences in MBBR and AAAO processes. The mass loads of RBV and RAN were mainly distributed in the effluent, and the loss of RBV and RAN led by adsorption and biodegradation were lower than other PPCPs. The loss of mass fractions was higher than 62% for CZP, SMX, APC, and IBU, indicating that both MBBR and AAAO processes showed excellent removal efficiencies for most of these PPCPs. In particular, the loss of mass fractions of CZP, SMX, APC, and IBU was approximately 100% in MBBR process, implying that the MBBR process is beneficial for the removal of PPCPs due to the relatively high biomass retention [49]. The removal of SMX and APC in MBBR process was higher than that in AAAO process, the possible reason could be attributed to relatively long solid retention time and stable microbial community of biofilm, which could subsequently facilitate good PPCPs removal capacity [50,51]. However, the loss of RAN in MBBR process was only 42.91%, while a negative value of −8.66% was presented in AAAO process. Owing to the refractory property (Supplementary Fig. 1), RAN is difficult to be effectively removed by the biodegradation pathway [42]. In general, most typical PPCPs could be easily removed by the adsorption and biodegradation of activated sludge, especially in MBBR process (Figs. 1 and 2).
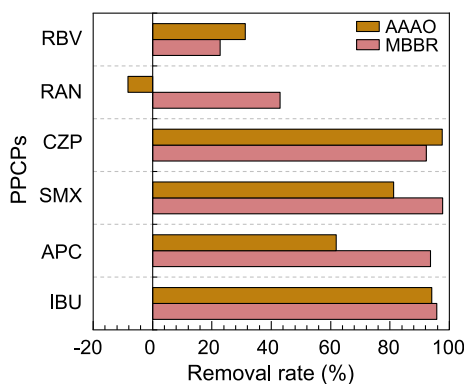
3.3.2. Pollution load estimation

The estimation results of the per capita pollution load for each PPCP in MBBR and AAAO processes are listed in Table 3. The per capita pollution load of influent IBU was the highest, followed by
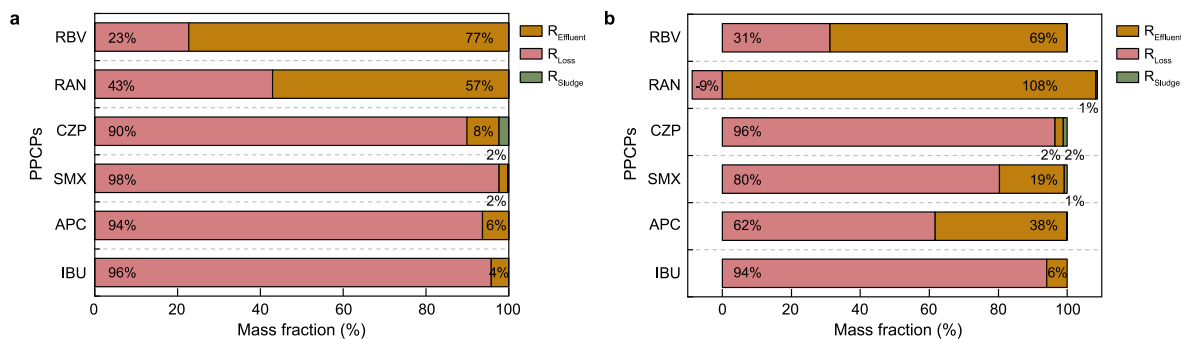

Fig. 2. Removal rates for six typical PPCPs by MBBR and AAAO process.

Fig. 3. Mass balance analysis (mass fractions) for six typical PPCPs in WWTPs. **a**, MBBR process. **b**, AAAO process.

Table 3
The per capita pollution loads (L, ug per d per person) of six PPCPs in the influent, effluent and excess sludge of two WWTPs.

PPCPs	Inflow			Outflow					
	$L_{Influent}$ (ug per d per person)			$L_{Effluent}$ (ug per d per person)			$L_{Excess\ sludge}$ (ug per d per person)		
	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median
IBU	23.19−408.32	175.59	251.33	0.00−32.60	8.81	5.40	0.00	0.00	0.00
APC	3.01−41.17	12.50	7.64	0.00−5.34	1.68	1.09	0.00−0.01	0.00	0.00
SMX	0.28−5.62	2.75	2.42	0.00−1.67	0.31	0.04	0.00−0.05	0.02	0.02
CZP	0.06−40.28	10.56	9.57	0.00−2.20	0.20	0.05	0.00−0.37	0.13	0.17
RAN	32.51−859.38	93.33	91.47	34.3−101.852	69.25	72.44	0.03−0.33	0.14	0.14
RBV	21.83−859.38	32.41	34.42	12.71−32.60	23.75	27.16	0.00−0.06	0.02	0.02

RAN, RBV, APC, CZP, and the lowest for SMX, with the mean values of 176, 93.3, 32.4, 12.5, 10.6, and 2.75 µg per d per person, respectively. Although the per capita pollution load of PPCPs decreased in the effluent, the PPCPs reduction was different. The reduction of IBU was notable, and the per capita pollution loads of APC, SMX, and CZP in the effluent were all less than 10 µg per d per person, whereas the per capita pollution load of RAN and RBV showed relatively high values (higher than 23.75 µg per d per person). This phenomenon was mainly due to the comparatively high per capita pollution loads of RAN and RBV in the influent (Fig. 1) and low removal efficiencies (Fig. 2) from the wastewater phase. The average per capita pollution loads of the typical PPCPs were as low as 0.05 µg per d per person in the sludge phase (activated sludge), which could be attributed to the low K_{ow} values and high hydrophilic property of PPCPs [26]. According to the per capita pollution loads for the six typical PPCPs, up to 327.15 µg per d per person of PPCPs were introduced in the influent, and 104.08 µg per d per person of PPCPs in the effluent were directly discharged to the downstream receiving rivers or lakes. Therefore, PPCPs should be effectively eliminated to reduce the potential ecological risks.

3.4. Biodegradation pathway of RBV in WWTPs

In order to identify the biodegradation pathway and further reduce the potential environmental hazards of RBV, TPs during RBV bioconversion were determined. In mixed standard positive ion mode, two types of TPs (TP-1 and TP-2), with m/z of 113.046 and 114.030, were detected at t_R 7.29 and 7.53 min, respectively. The product ion spectra of intermediate products are illustrated in Supplementary Fig. 3. Most TPs are formed due to the biodegradation process, including hydroxyl derivatives and glucuronic acid conjugates [52]. In addition, TPs are mostly unknown compounds and may be more stable and toxic than their PPCP precursors [22]. During the COVID-19 pandemic, the broad-spectrum and effective antiviral pharmaceutical RBV has been widely used, and the detection frequency and concentration have gradually increased [19].

The retention time of metabolite TP-1 ($m/z = 113.1067$ for MH$^+$) was 7.29 min, and the MS/MS spectra are shown in Supplementary Fig. 3a. Compared with the structure of RBV, the characteristic product ion m/z 113.1067 was exactly a fragment of the hydrogenation ion peak of RBV and was consistent with the fragmentation patterns of the parent compound. Therefore, the transformation product was the triazole ring cleaved [53] between the two rings of the parent compound. In the MS/MS spectra of TP-1, the fragment ion m/z 96.0196 was obtained from the neutral loss of NH$_2$ in the triazole ring. The fragment ion m/z 69.0086 might have been derived from the cleavage of the amide group and the triazole ring [54]. Therefore, the TP-1 with m/z 113.1067 was the metabolite TCONH$_2$ due to the nucleosidase reaction to RBV [55].

TP-2 with m/z of 114.0304 for MH$^+$ was detected at 7.53 min, and the MS/MS spectra are shown in Supplementary Fig. 3b. Compared with TCONH$_2$ (TP-1), the loss of molecular weight for 1 Da suggested that TP-2 may be the product of −OH that is derived from −NH$_2$. In the MS/MS spectra of TP-2, the fragment ion m/z 96.0192 was obtained from the neutral loss of OH in the triazole ring [56]. The fragment ion m/z 69.0083 was possibly derived from the cleavage of the amide group and the triazole ring [54], implying that TP-2 with m/z 114.0304 was TCOOH that is derived from TP-1 with −NH$_2$ and further changed into −OH.

After identifying RBV metabolites, the possible conversion pathway of RBV in activated sludge is illustrated in Fig. 4. RBV was first broken into a triazole ring (TCONH$_2$) and a oxygen-containing five-membered heterocyclic ring under the nucleosidase reaction [55], and TCONH$_2$ finally generated triazole carboxylic acid (TCOOH) by the reaction of amide hydrolysis [57]. The biodegradation process was consistent with that in mammals [58,59]. In addition to TCOOH, other metabolites of RBV possess antiviral activity, and the oxygen-containing five-membered heterocycle may be a pentose derivative based on the chemical structure [60]. As shown by the dotted line in Fig. 4, the possible biodegradation pathway in activated sludge was further deduced. The oxygen-containing five-membered heterocycle may generate oxalate and
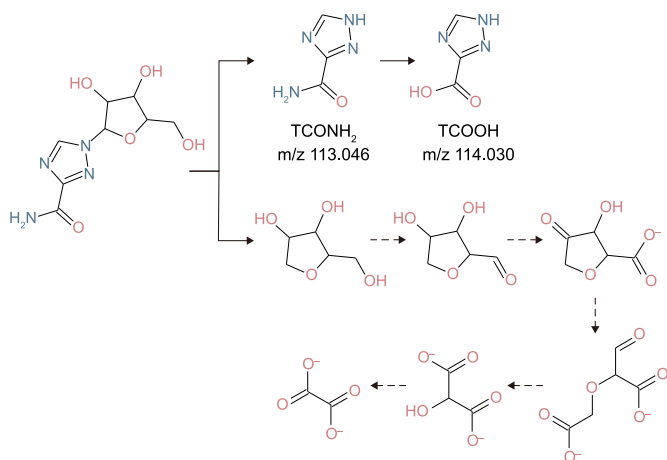
Fig. 4. Possible biodegradation pathway of RBV (the dashed arrow indicates the uncertain pathway).

other substances under the reaction of dehydrogenase, oxygenase, and isomerase [61−63]; however, the metabolic process should be further discovered and verified in detail.

3.5. Risk assessment

As listed in Table 4 and Supplementary Table 4, the RQ of typical PPCPs from the influent was greater than 0.1, indicating that the typical PPCPs pose serious risks to organisms [64,65], and the influent RQ values of RAN and CZP were drastically higher than 1.0 due to the high influent concentration of RAN and the high biological toxicity of CZP. The RQ values of RBV, SMX, APC, and IBU decreased throughout the wastewater treatment process, implying that environmental hazards could be well eliminated [66]. However, the RQ value of RAN showed consistently high risks (RQ > 1) in MBBR and AAAO processes, and the risk level of CZP changed from high risk (RQ > 1) to medium risk (RQ > 0.1). In addition, the effluent RBV, SMX, APC, and IBU presented low risk (RQ < 0.1) in MBBR process, conversely, RAN and CZP still presented high risk (RQ > 1), and SMX showed a medium risk (RQ > 0.1) in AAAO process. The possible reason could be the relatively high concentration of influent and low removal efficiency in AAAO process (Figs. 1 and 2) [9].

RBV is effective on COVID-19 and demonstrated a low risk to the

Table 4
Risk assessment of six PPCPs in influent and effluent of two WWTPs.

		RQ >1		1> RQ >0.1		RQ <0.1		RQ <0.01

PPCPs		RBV	RAN	CZP	SMX	APC	IBU
Influent	MBBR	1>RQ>0.1	RQ>1	RQ>1	1>RQ>0.1	1>RQ>0.1	1>RQ>0.1
	AAAO	1>RQ>0.1	RQ>1	RQ>1	1>RQ>0.1	1>RQ>0.1	1>RQ>0.1
Effluent	MBBR	RQ<0.1	RQ>1	1>RQ>0.1	RQ<0.1	RQ<0.1	RQ<0.01
	AAAO	RQ<0.1	RQ>1	RQ>1	RQ<0.1	RQ<0.1	RQ<0.1

aquatic organism when the effluent was directly discharged to the receiving natural water bodies (Table 4). However, the potential adverse effect of RBV should not be ignored due to its relatively high per capita pollution load (Table 3). In addition, the use of RBV may continuously increase because COVID-19 will last for a long time [67]. Therefore, understanding the bioconversion and transformation pathway of RBV in WWTPs is necessary to develop an effective treatment process. Furthermore, the discharge of typical PPCPs may negatively affect aquatic organisms. Ibuprofen could influence the hatching rate of fish and change their biological morphology [68]. The long-term use of pharmaceuticals and the discharge of metabolites may lead to bioaccumulation in aquatic organisms and ultimately affect the water quality and the safety of aquatic products [69]. At present, residual PPCPs have been detected in the food chain, including fruits and vegetables, and may eventually threaten human health [70]. Therefore, the improvement in the removal efficiency of different wastewater treatment processes and reduction in the usage of PPCPs are significant to reduce the potential ecological risks of PPCPs to aquatic organisms and even humans.

4. Conclusion

The detection rate of PPCPs in WWTPs is relatively high, especially for RBV with substantially increased concentration. The removal efficiency of easily biodegradable CZP and IBU can reach up to 92.21% in MBBR and AAAO processes; however, merely 42.95% and 31.31% of RAN and RBV were removed due to the refractory structure. Comparatively high concentration of effluent RAN and RBV could result in a high pollution load to the receiving natural water bodies; nevertheless, effluent RAN and CZP presented high and medium risks based on RQ values. Although RBV had a low risk according to the RQ value, its ecological risks will gradually increase because of the high consumption of RBV during the COVID-19 pandemic. In addition, the biodegradation pathway of RBV by activated sludge is speculated, and nucleosidase and amide hydrolysis were found to facilitate RBV biodegradation.

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.

Acknowledgement

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ese.2022.100184.

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