

# Removal efficiencies of natural and synthetic progesterones in hospital wastewater treated by different disinfection processes

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## HIGHLIGHTS

- The concentrations of 61 progesterones in HWW, PFTE, SBTE were evaluated.
- The removal efficiencies of progesterones by PFT and SBT were identified.
- Compared the removal efficiencies of progesterones in five disinfection processes.

## ARTICLE INFO

### Article history:

Received 6 November 2021

Revised 15 December 2021

Accepted 10 January 2022

Available online 4 March 2022

### Keywords:

Progesterones

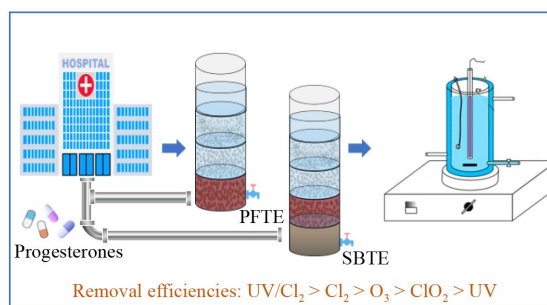
Hospital wastewater

Primary filtration treatment effluent

Secondary biological treatment effluent

Disinfection process

## GRAPHIC ABSTRACT



## ABSTRACT

Progesterones are ubiquitous in hospital wastewater (HWW) with concentrations much higher than those of estrogens and androgens. To ensure that these water systems are safe to use, disinfection is crucial during HWW treatment by providing “front line” defense against biological contaminations. Here, five disinfection processes, namely, chlorine ( $Cl_2$ ), chlorine dioxide ( $ClO_2$ ), ozone ( $O_3$ ), ultraviolet (UV), and UV/chlorine ( $UV/Cl_2$ ), were selected to investigate their removal efficiencies for progesterones in primary filtration and secondary biological treatment effluents. There were 61 natural and synthetic progesterones detected in HWW, with the natural progesterones being the main components with a concentration of 845.51 ng/L and contributing to 75.08% of the total progesterones. The primary filtration treatment presented insignificant removal effects on the progesterones, while the secondary biological treatment significantly reduced the progesterone content by biodegradation. The order of removal efficiencies of total progesterones by different disinfection processes was  $UV/Cl_2 > Cl_2 > O_3 > ClO_2 > UV$ .  $UV/Cl_2$  showed the highest removal efficiency against progesterones mainly due to the activation of  $Cl_2$  by ultraviolet (UV) photolysis, which helps open the heterocyclic, aromatic, and phenolic rings, thus accelerating progesterone degradation. In addition, the removal efficiencies of natural progesterones in the five disinfection processes were higher than those of synthetic progesterones (progesterone derivatives, 19-nortestosterone derivatives, and 17 $\alpha$ -hydroxyprogesterone derivatives).

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## 1 Introduction

Hospital wastewater (HWW) contains plenty of persistent compounds, dangerous substances, and pathogenic microorganisms (Sdiri-Loulizi et al., 2010; Nasri et al., 2017a; Nasri et al., 2017b; Souza et al., 2018), such as antibiotics,

psychiatric drugs,  $\beta$ -receptor blockers, anesthetics, analgesics, anti-inflammatory drugs, and X-ray contrast agents (Maa et al., 2020). Endocrine disrupting chemicals (EDCs) are among the high-risk substances in HWW. Progesterones, as typical representative compounds of steroid hormone EDCs, are capable of entering and accumulating in the human body through the food chain (Zhang et al., 2017). Due to their incomplete removal, natural and synthetic progesterones can enter surface waters continuously with

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the discharge of the effluent from HWW treatment stations, which may cause adverse effects on the aquatic environment and organisms (Jobling et al., 2002; Sumpter and Johnson, 2005; Phillips and Foster, 2008; Fent, 2015; Perez-Alvarez et al., 2018). In addition, progesterones can react with albumin and corticosteroid protein in the human body, causing damage to multiple systems, such as the endocrine system, immune system, reproductive system, and nervous system (Perez-Alvarez et al., 2018).

Natural and synthetic progesterones and their metabolites were widespread in various water bodies. In a surface water environment, the concentrations of progesterones were usually in the range of nanogram per liter, while in wastewater or livestock farm runoff, the concentrations of progesterones were up to microgram per liter (Fent, 2015; Kumar et al., 2015). For example, the progesterone (P) and nortestosterone derivative (NTD) concentrations of 1.6 and 2 ng/L in surface water in the Rhone Alps of France were determined, respectively (Vulliet and Cren-Olive, 2011). The concentrations of P, medroxyprogesterone-17-acetate (MPA), and NTD in a river in Beijing were 35, 18, and 16 ng/L, respectively. Moreover, the concentrations of these three progesterones in the influent of a municipal wastewater treatment plant (MWTP) were (108±89), (58±17), and (12±0.6) ng/L, respectively (Chang et al., 2009; Chang et al., 2011). In addition, progesterones were also found in the water plants of Belgium, the United States, and Switzerland (Kolodziej et al., 2003; Pauwels et al., 2008; Zhang et al., 2017). The concentrations of progesterones detected in Belgian HWW ranged from 0.2 to 100 ng/L (Pauwels et al., 2008). For livestock farm wastewater, previous studies showed that the concentration of progesterones in the runoff of dairy farms was as high as 2.47 µg/L and the concentration of progesterones in sow excreta could reach up to 2.64 µg/L (Shan et al., 2012; Yost et al., 2014).

Two methods are often used to remove the pollutants and pathogenic microorganisms in HWW treatment. The first method combines primary filtration treatment (PFT) with disinfection and the treated effluent is discharged into the urban pipe network and mixed with other urban sewage for further treatment in the MWTP. In the second method, HWW was treated by PFT, secondary biological treatment (SBT), and disinfection in that order, and the effluent can be directly discharged into the receiving water body (Verlicchi et al., 2012; Verlicchi et al., 2015). Disinfection is an essential wastewater treatment unit that also exhibits a certain removal effect on trace organic pollutants. Considering the different principles and oxidation capabilities of different disinfectants, the removal efficiency of progesterones in each disinfection process is also different. The disinfectants commonly used in HWW disinfection are chlorine (Cl<sub>2</sub>), chlorine dioxide (ClO<sub>2</sub>), ozone (O<sub>3</sub>), ultraviolet (UV), and UV/chlorine (UV/Cl<sub>2</sub>) (Hijnen et al., 2006; Navalon et al., 2008; Dong et al., 2017). However, the removal efficiencies of natural and synthetic progesterones in HWW using these disin-

fection processes have not been reported yet. Moreover, the potential risk of HWW in various disinfection processes has not been evaluated.

The objectives of the current study are (i) to determine the exposure concentrations of natural and synthetic progesterones in HWW, primary filtration treatment effluent (PFTE), and secondary biological treatment effluent (SBTE) and (ii) to determine the removal efficiencies of progesterones using Cl<sub>2</sub>, ClO<sub>2</sub>, UV, O<sub>3</sub>, and UV/Cl<sub>2</sub> disinfection processes.

## 2 Materials and methods

### 2.1 Chemicals and materials

Tables S1 and S2 list the names and abbreviations of 19 natural and 42 synthetic progesterones, respectively. These progesterones and isotope-labeled standards, including mifepristone-d<sub>3</sub>, cyproterone acetate-d<sub>3</sub>, melengestrol acetate-d<sub>3</sub>, norgestrel-d<sub>6</sub>, progesterone-d<sub>6</sub>, and ethisterone-<sup>13</sup>C<sub>2</sub>, were obtained from TRC (Toronto, ON, Canada) and Steraloids (Newport, RI, USA). Acetonitrile (ACN), methanol, and ethyl acetate (EtOAc) were purchased from Meryer Chemicals (Shanghai, China) and were of high performance liquid chromatography grade. Glass fiber pads (GF/F, 0.7 mm) and oasis HLB (500 mg, 6 mL) cartridges were procured from Whatman (Maidstone, UK) and Waters (Milford, MA, USA), respectively. Sodium hypochlorite (NaClO) was purchased from Aladdin Chemical. ClO<sub>2</sub> solution was prepared according to the method proposed by Jia et al. and stored at 4 °C (Feng et al., 2018). O<sub>3</sub> was produced by an ozone generator (3S-T3, Beijing Tonglin Technology Co., Ltd., China), and the concentration of O<sub>3</sub> was measured by UV spectrophotometry (Hua and Reckhow, 2013). All other reagents were analytical grade and procured from Beijing Chemicals.

### 2.2 Sample collection

HWW was collected from a septic tank of a sewage treatment station in a general hospital in Beijing and stored at 4 °C. A glass cylinder containing ceramsite, zeolite, and quartz sand was used as a PFT unit for the HWW treatment. PFTE was obtained by filtering HWW raw water through the PFT unit at a flow rate of 5 L/h. Activated sludge taken from the aeration tank of a sewage treatment plant in Beijing was domesticated in the laboratory and then inoculated to an SBT unit. The activated sludge concentration in the reactor was controlled at about 4000 mg/L, and the aeration reaction time was 4 h. The effluent from the PFTE unit was used as feed water in SBT, and the supernatant after 1 h of precipitation was collected as SBTE.

### 2.3 Disinfection experiment

The optimal dosage of the disinfectant in each disinfection process was determined according to the water quality and the requirements of the Technical Specifications for Hospital Sewage Treatment (HJ2029-2013) and Discharge Standard for Water Pollutants in Medical Institutions (i.e., for PFTE, the number of fecal coliforms was <5000 MPN/L. For SBTE, the number of fecal coliforms was <500 MPN/L). The number of fecal coliforms in PFTE and SBTE in each disinfection process was detected by multitube fermentation. The disinfectant dosages for PFTE and SBTE were preliminarily determined according to Table S3, and the fecal coliform concentrations obtained in the disinfection processes are shown in Fig. S1. The optimal dosages of the disinfectants for PFTE and SBTE in the disinfection processes are shown in Text S1.

All disinfection experiments were performed in a glass cylinder wrapped with tin foil paper, and the outer layer was equipped with condensate water circulation to control the temperature, and then an ultraviolet lamp tube was set in the center (Fig. 1). In the  $\text{Cl}_2$  and  $\text{ClO}_2$  disinfection experiments, a specific dose of  $\text{NaClO}$  and  $\text{ClO}_2$  stock solution was added to 500 mL of HWW, and then the samples were taken out at the set time. In the  $\text{O}_3$  disinfection experiment,  $\text{O}_3$  was injected into 500 mL of HWW to start the  $\text{O}_3$  disinfection process. An ozone dosage of 10–90 mg/L was obtained according to the operating time of 50–450 s of the ozone generator. Prior to the UV and UV/ $\text{Cl}_2$  experiments, the UV lamp was preheated for at least 30 min, then HWW was added into the disinfection device to start the disinfection reaction, and samples were taken out at the set time. In the UV/ $\text{Cl}_2$  experiment, HWW was injected and then  $\text{NaClO}$  was added immediately. The light intensity of the UV lamp at 254 nm determined by a light irradiation meter was 0.20 mW/cm<sup>2</sup> (UV-B, China).

### 2.4 Analytical methods

Before solid-phase extraction (SPE), HWW, PFTE, and

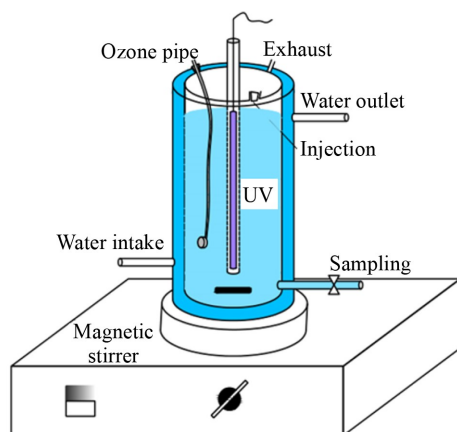


Fig. 1 Device used in the disinfection experiment.

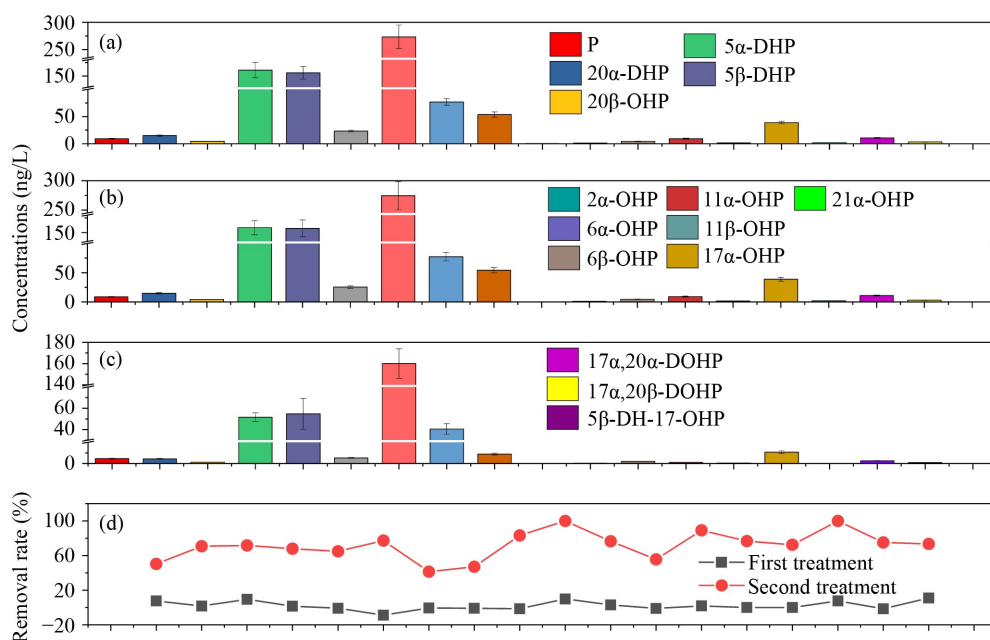
SBTE were filtered using a 0.7 mm glass fiber pad to remove the suspended solids, and then 100 ng/L of six isotope-labeled standards (i.e., CPA- $\text{d}_3$ , MGA- $\text{d}_3$ , MFP- $\text{d}_3$ , MGA- $\text{d}_3$ , P- $\text{d}_9$ , and ETH- $^{13}\text{C}_2$ ) were injected into the samples. OASIS HLB cartridges were pretreated with a 1:1:2 volume ratio of ETOAC, ACN, and MilliQ water. The samples were then pumped into the cartridges to preserve the organic matters on the cartridges. Finally, the residual organic matters in the column were eluted with 6 mL of ethyl acetate/acetonitrile (50:50, V/V) and dried under a stable nitrogen flow. The gathered progesterones were detected by ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS).

Methanol (A) and ultrapure water with 0.1% formic acid (B) were used as the mobile phases in UPLC-MS/MS detection. The temperature of the HSS T3 column, injection volume, and flow rate were 40 °C, 2 mL, and 0.3 mL/min, respectively. The initial gradient of mobile phase A was set to 60% and gradually increased to 70% in 8 min, 85% in 5 min, and 100% in 1 min and held for 3 min. Then, mobile phase A was reduced to 60% within 0.5 min and maintained for 3 min. The source and desolvation temperatures were 150 °C and 450 °C, respectively. Capillary voltage was 3.00 kV, and desolvation and cone gas flows were 900 and 150 L/h, respectively. Multiple reaction monitoring in UPLC-MS/MS was used for the quantitative analysis of the target progesterones. The interscan and interchannel delay times were 3 and 5 ms, respectively.

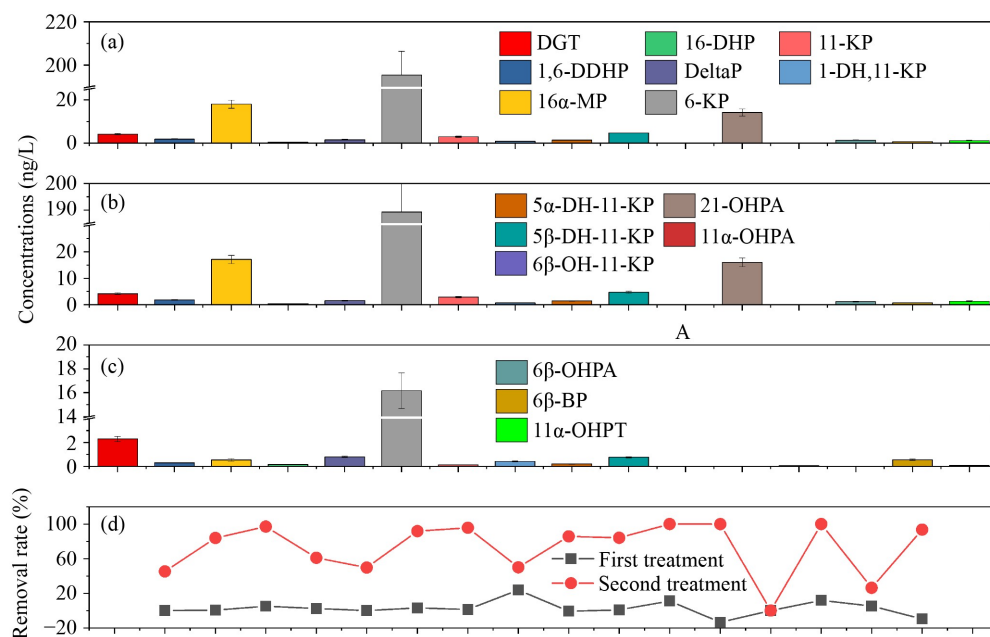
## 3 Results and discussion

### 3.1 The concentrations of natural and synthetic progesterones in HWW

Figures 2(a), 3(a), 4(a) and 5(a) show the original concentrations of progesterones in HWW. A large proportion of progesterones was detected in HWW with the concentrations at the ng/L level. However, some progesterones were not detected, such as 5 $\beta$ -dihydro-17-hydroxyprogesterone (5 $\beta$ -DH-17-OHP), 11 $\alpha$ -hydroxyprogesterone acetate (11 $\alpha$ -OHPA), DH-norgestrel (NG), 17-caproxypregesterone (17-CP), and flugestone 17-acetate (FGA). The total concentration of the four types of progesterones (namely, natural progesterones, progesterone derivatives, 19-nortestosterone derivatives, and 17 $\alpha$ -hydroxyprogesterone derivatives) in HWW was 1126.18 ng/L. Natural progesterones with a concentration of 845.51 ng/L contributed up to 75.08% of the total progesterones, indicating the higher exposure concentration of natural progesterones in HWW than that of synthetic progesterones. The main components of natural progesterones were 3 $\beta$ -hydroxy-5 $\beta$ -tetrahydroprogesterone (3 $\beta$ ,5 $\beta$ -THP), 5 $\alpha$ -dihydroprogesterone (5 $\alpha$ -DHP), and 5 $\beta$ -dihydroprogesterone (5 $\beta$ -DHP), accounting for 32.33%, 19.05%, and 18.46% of the total natural progesterones, respectively. High levels of progesterone derivatives probably resulted from microbial conversion. Estrogens and androgens can undergo



**Fig. 2** The concentrations of natural progesterones in (a) hospital wastewater (HWW), (b) primary filtration treatment effluent (PFTE), and (c) secondary biological treatment effluent (SBTE) and (d) the removal efficiencies of progesterones in primary and secondary treatments.



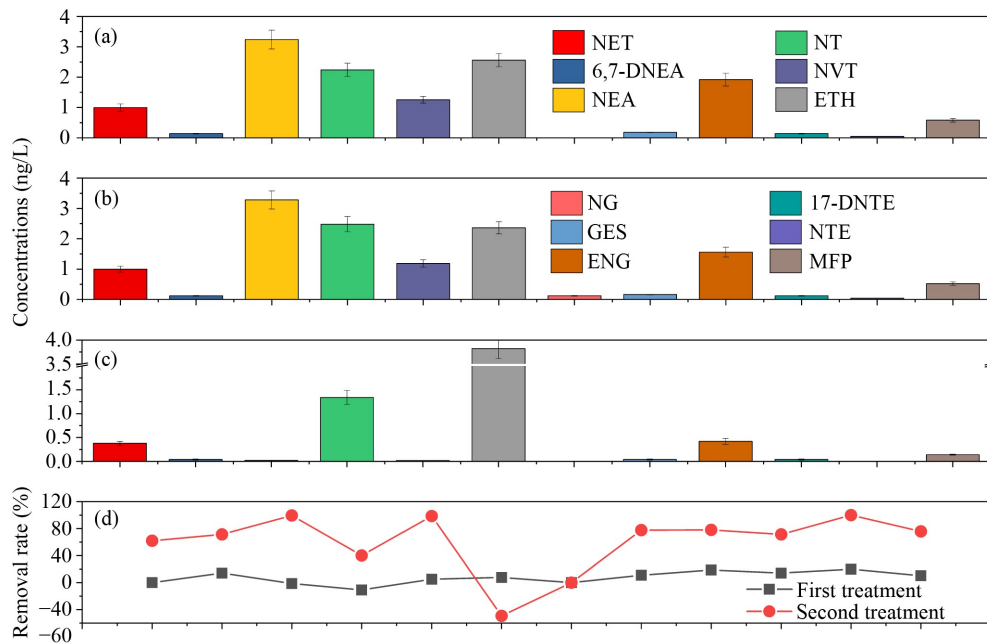
**Fig. 3** The concentrations of progesterone derivatives in (a) HWW, (b) PFTE, and (c) SBTE and (d) the removal efficiencies of progesterones in primary and secondary treatments.

mutual hydroxy-ketone interconversion and dehydrogenation in both natural and engineering systems (Samir et al., 2006; Khan et al., 2008; Yang et al., 2011). 6-keto-progesterone (6-KP), 16 $\alpha$ -methylprogesterone (16 $\alpha$ -MP), and 21-hydroxyprogesterone acetate (21-OHPA) were the main components of progesterone derivatives, accounting for 78.47%, 7.24%, and 5.67%, respectively. 19-norethisterone acetate (NEA), ethisterone (ETH), 19-nortestosterone (NT), and etonogestrel (ENG) were the main

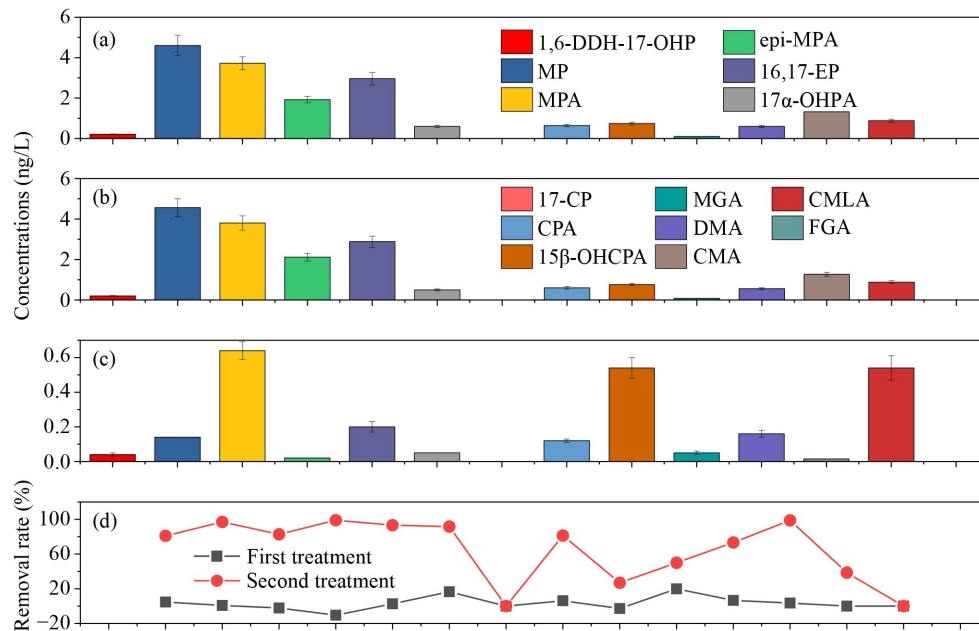
components of 19-nortestosterone, accounting for 24.34%, 19.23%, 16.83%, and 14.42%, respectively. The main components of 17 $\alpha$ -hydroxyprogesterone derivatives were medroxyprogesterone (MP), MPA, 6-epi-medroxy progesterone 17-acetate (epi-MPA), and 16,17-epoxyprogesterone (16,17-EP), accounting for 25.15%, 20.34%, 10.50%, and 16.18%, respectively.

As shown in Figs. 2(b), 3(b), 4(b) and 5(b), the total content of progesterones in PFTE was 1121.35 ng/L, which





**Fig. 4** The concentrations of 19-nortestosterone derivatives in (a) HWW, (b) PFTE, and (c) SBTE and (d) the removal efficiencies of progesterones in primary and secondary treatments.



**Fig. 5** The concentrations of 17 $\alpha$ -hydroxyprogesterone derivatives in (a) HWW, (b) PFTE, and (c) SBTE and (d) the removal efficiencies of progesterones in primary and secondary treatments.

was similar to that in HWW, indicating the insignificant removal effect of progesterones by PFT (Shen et al., 2019). The total progesterone concentration in SBTE was 380.78 ng/L (Figs. 2(c), 3(c), 4(c) and 5(c)), and some progesterone contents decreased dramatically or were even removed completely. The concentrations of natural progesterones and progesterone derivatives decreased to 349.52 and 31.26 ng/L, respectively. The proportion of natural progesterones in the total progesterones in SBTE

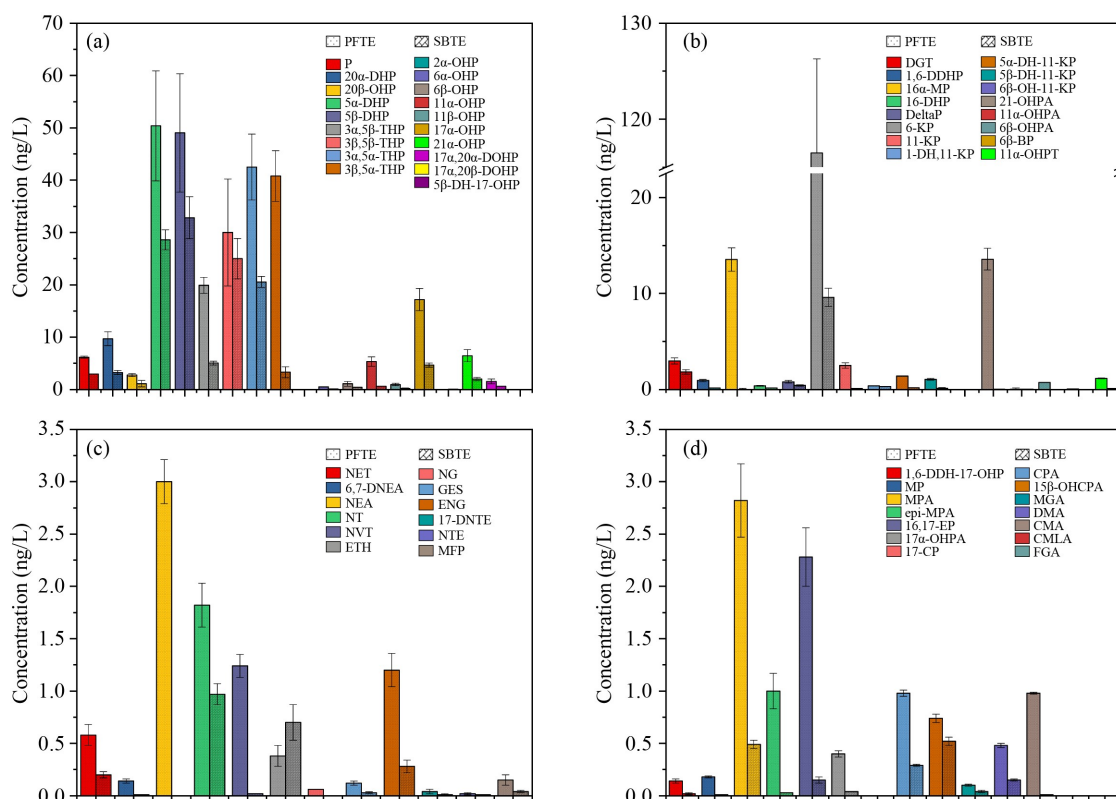
increased to 91.79%, and the progesterone derivatives accounted for 71.94% of the synthetic progesterones. After SBT, the removal rates of natural progesterones and progesterone derivatives were 58.67% and 88.86%, respectively, indicating the higher removal efficiency of SBT for progesterone derivatives. However, it also demonstrated that the proportion of natural progesterones increased after SBT. In Figs. 2(d), 3(d), 4(d) and 5(d), SBT had high removal efficiencies for most progesterones, and the

removal rates of some progesterones (21 $\alpha$ -hydroxyprogesterone (21 $\alpha$ -OHP), 6 $\beta$ -hydroxy-11-ketoprogesterone (6 $\beta$ -OH-11-KP), 21-OHPA, 6 $\beta$ -hydroxyprogesterone acetate (6 $\beta$ -OHPA), and 19-norethindrone (NET)) can even reach 100%. Surprisingly, the concentration of ETH in 17 $\alpha$ -hydroxyprogesterone derivatives increased from 2.56 to 3.82 ng/L after SBT, which may be due to the presence of a small amount of ETH in the inoculated activated sludge. In addition, most progesterones were highly active due to a ketone group on carbon 3 and a double bond between carbon 4 and carbon 5 ( $\Delta$ 4-3-ketone structure). For example, the reduction of NET could lead to the formation of dihydro- and tetrahydro-derivatives (such as ETH) (Stanczyk, 2003; Azzouni et al., 2012). Testosterone was found to be capable of replacing a hydrogen on the progesterone containing a 17-carbon acetyl group and converting it into ETH (Sobey, 2008; Giatti et al., 2016). Compared with PFT, SBT can effectively reduce the content of progesterones through the biodegradation of microorganisms (Shen et al., 2019). Previous studies showed that the microbial transformation of progesterones can generate dehydrogenation and hydroxylation products. Furthermore, 20 $\beta$ -hydroxyprogesterone (20 $\beta$ -OHP), 1,6-didehydroprogesterone (1,6-DDHP), and 16-dehydroprogesterone (16-DHP) were the microbial transformation products of progesterones in biological treatment (Alawadi et al., 2002; Donova and Egorova, 2012; Liu et al., 2013; Ojogboro et al., 2017).

### 3.2 Effect of different disinfection processes on the removal of progesterones

#### 3.2.1 Effect of Cl<sub>2</sub> disinfection on the removal of progesterones

In Fig. 6, the concentrations of the total progesterones in PFTE and SBTE after Cl<sub>2</sub> disinfection were 459.10 and 148.16 ng/L, with removal rates of 59.05% and 61.00%, respectively. In addition, compared with the removal efficiencies of other three types of progesterones (progesterone derivatives, 19-nortestosterone derivatives, and 17 $\alpha$ -hydroxyprogesterone derivatives), the removal efficiencies of natural progesterones in PFTE (66.42%) and SBTE (62.42%) were significantly higher. The removal rates of 3 $\beta$ ,5 $\beta$ -THP in both PFTE and SBTE were the highest in natural progesterones, up to 89.07% and 84.38%, respectively. These experimental results showed that Cl<sub>2</sub> disinfection can effectively remove progesterones. Studies showed that compounds containing unsaturated carbon bonds could be well oxidized in the presence of hypochlorite (Hu et al., 2003; Moriyama et al., 2004; Deborde and Gunten, 2008). When the compounds with unsaturated carbon bonds were oxidized by hypochlorite, they usually promoted the electrophilic substitution and electrophilic addition of Cl<sup>+</sup> to other oxygen-containing functional groups (Mash et al., 2010). However, in PFTE and SBTE, the removal rates of P were 29.13% and 36.97%, respectively, attributable to P (the



**Fig. 6** The effect of Cl<sub>2</sub> disinfection on progesterone concentrations in PFTE and SBTE: (a) natural progesterones, (b) progesterone derivatives, (c) 19-nortestosterone derivatives, and (d) 17 $\alpha$ -hydroxyprogesterone derivatives.

chemical formula of P is shown in Fig. S2), lacking a phenol ring, and having a weak reactivity with chlorine (De Bor De et al., 2004). Consistent with previous studies, P showed negligible observable reactivity toward free chlorine (Mash et al., 2010). Due to the different removal efficiencies of  $\text{Cl}_2$  disinfection on the different progesterones, the main components of the four kinds of progesterones in the effluent also changed, and  $5\alpha$ -DHP,  $5\beta$ -DHP,  $3\alpha$ -hydroxy- $5\alpha$ -tetrahydroprogesterone ( $3\alpha,5\alpha$ -THP), and  $3\beta$ -hydroxy- $5\alpha$ -tetrahydroprogesterone ( $3\beta,5\alpha$ -THP) were the main components of natural progesterones after  $\text{Cl}_2$  disinfection. The main components of progesterone derivatives were unchanged but their contributions decreased. In PFTE, the proportions of 6-KP,  $16\alpha$ -MP, and 21-OHPA of progesterone derivatives decreased from 74.70%, 7.05%, and 6.69% to 69.73%, 2.42%, and 5.39%, respectively, while in SBTE, their proportions decreased from 71.85%, 2.40%, and 0.89% to 63.40%, 0.00%, and 0.66%, respectively. In 19-demethyltestosterone derivatives, the main components were NEA, NT, ENG, and norvinisterone (NVT). In  $17\alpha$ -hydroxyprogesterone derivatives, MPA and 16, 17-EP were the main components.

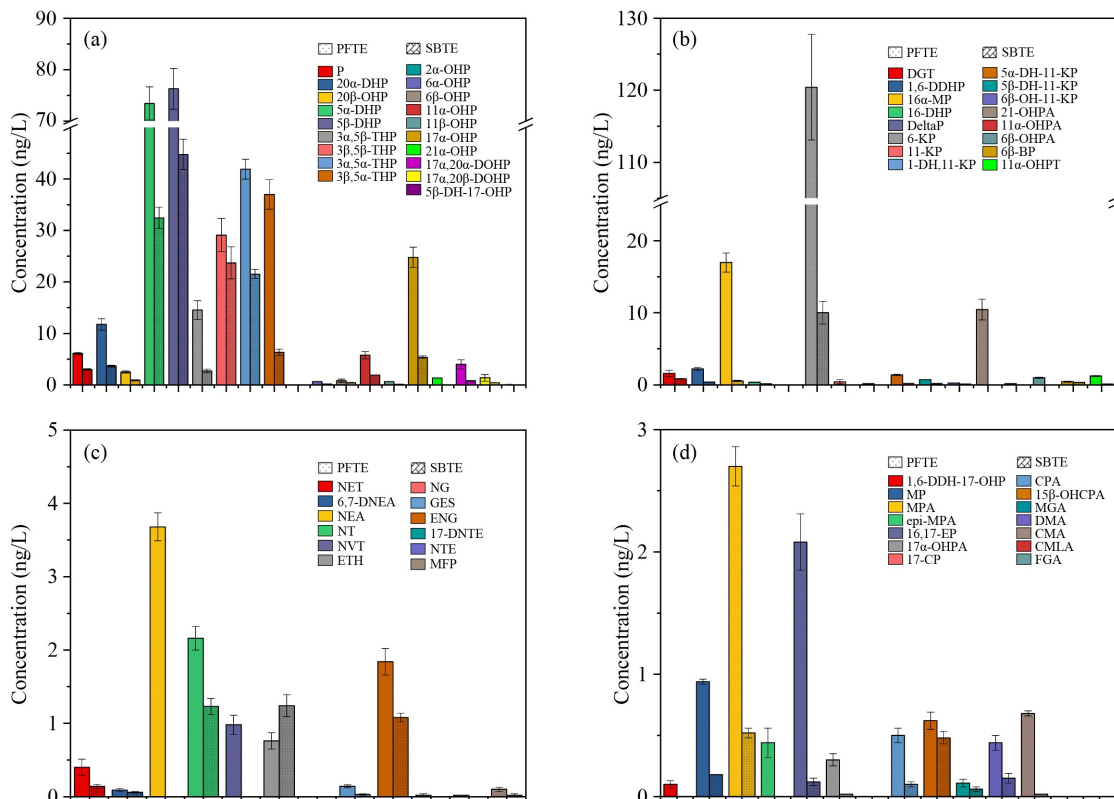
### 3.2.2 Effect of $\text{ClO}_2$ disinfection on the removal of progesterones

In Fig. 7, the total progesterones in PFTE and SBTE after  $\text{ClO}_2$  disinfection have concentrations of 507.24 and

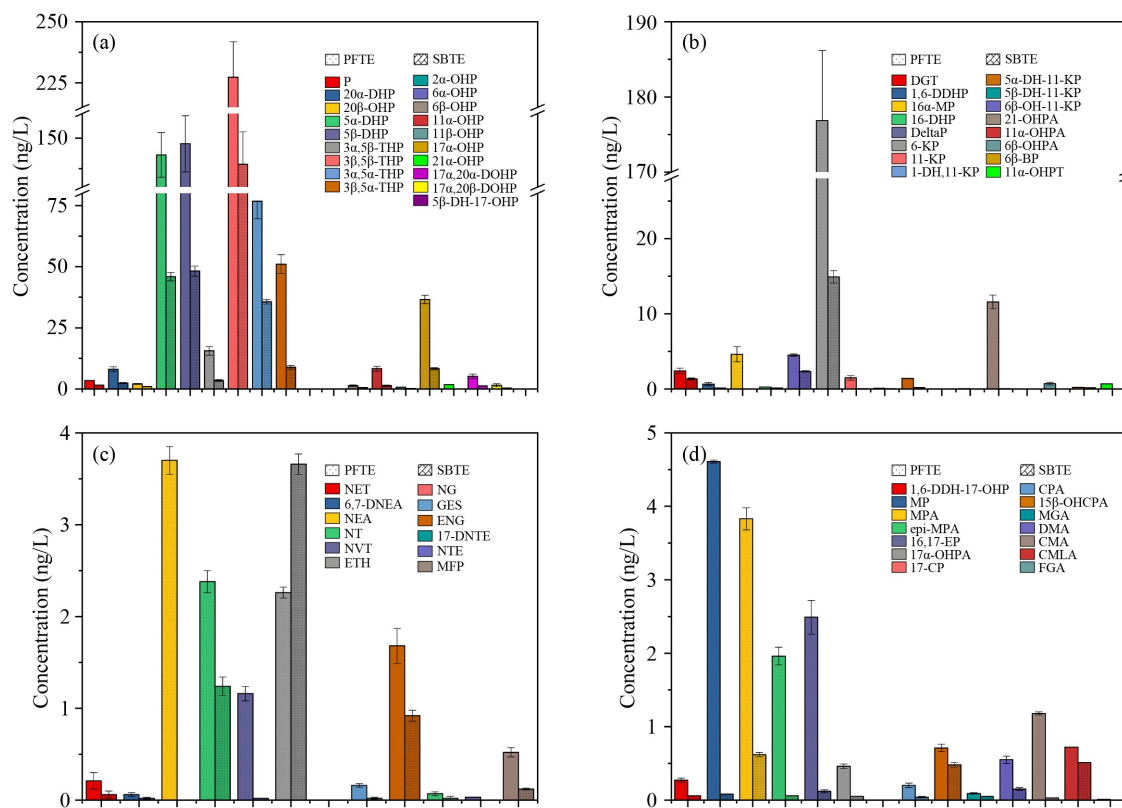
161.99 ng/L, with removal rates of 54.75% and 57.45%, respectively. Similar to  $\text{Cl}_2$  disinfection, among the four types of progesterones (natural progesterones, progesterone derivatives, 19-nortestosterone derivatives, and  $17\alpha$ -hydroxyprogesterone derivatives),  $\text{ClO}_2$  showed the highest removal efficiencies for natural progesterones, with removal rates of 60.97% and 58.88% for PFTE and SBTE, respectively. In addition, the removal efficiency of  $17\alpha$ -hydroxyprogesterone derivatives by  $\text{ClO}_2$  disinfection was higher than that by  $\text{Cl}_2$  disinfection, while others were lower than that by  $\text{Cl}_2$  disinfection. These results showed that although  $\text{ClO}_2$  had stronger oxidation intensity than  $\text{Cl}_2$ , its removal performance for progesterones was not as effective as that of  $\text{Cl}_2$  disinfection. It could be due to different oxidation pathways between  $\text{Cl}_2$  and  $\text{ClO}_2$ . Compared with  $\text{ClO}_2$ , progesterones were more likely to react with free chlorine in water, and chlorine could be directly hydrolyzed to  $\text{OCl}^-$  in water, while  $\text{ClO}_2$  needed to generate  $\text{OCl}^-$  by in situ reaction before progesterones (Acero et al., 2010; Valentin et al., 2018). Another probable explanation was the lower dosage of  $\text{ClO}_2$  than  $\text{Cl}_2$  in this experiment.

### 3.2.3 Effect of UV disinfection on the removal of progesterones

In Fig. 8, after UV disinfection, the total concentrations of progesterones in PFTE and SBTE were 964.87 and



**Fig. 7** The effect of  $\text{ClO}_2$  disinfection on progesterone concentrations in PFTE and SBTE: (a) natural progesterones, (b) progesterone derivatives, (c) 19-nortestosterone derivatives, and (d)  $17\alpha$ -hydroxyprogesterone derivatives.



**Fig. 8** The effect of UV disinfection on progesterone concentrations in PFTE and SBTE: (a) natural progesterones, (b) progesterone derivatives, (c) 19-nortestosterone derivatives, and (d) 17 $\alpha$ -hydroxyprogesterone derivatives.

331.49 ng/L, respectively, which were slightly lower than those in PFTE and SBTE without disinfection treatment (1121.35 and 380.78 ng/L, respectively). Moreover, the removal rates of the total progesterones in PFTE and SBTE by UV disinfection were 13.93% and 12.93%, respectively. The 19-demethyltestosterone and 17 $\alpha$ -hydroxyprogesterone derivatives showed a marginal decrease after UV disinfection. These results indicated that short-time UV irradiation was incapable of removing progesterones from HWW, which could be due to the reluctance of the ring opening of heterocycles, aromatic rings, and phenolic rings by UV (Qin et al., 2014).

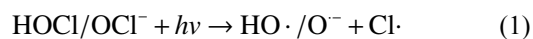
### 3.2.4 Effect of O<sub>3</sub> disinfection on the removal of progesterones

In Fig. 9, the total concentrations of progesterones in PFTE and SBTE after O<sub>3</sub> disinfection were 499.46 and 157.52 ng/L, and their removal rates were 55.45% and 58.62%, respectively. It was noteworthy that the removal efficiencies of progesterone derivatives, 19-demethyltestosterone derivatives, and 17 $\alpha$ -hydroxyprogesterone derivatives in PFTE and SBTE after O<sub>3</sub> disinfection were significantly higher than those after Cl<sub>2</sub> disinfection. For natural progesterones, except 5 $\alpha$ -DHP and 5 $\beta$ -DHP, the removal efficiencies of other progesterones were also higher than that in Cl<sub>2</sub> disinfection because the molecular

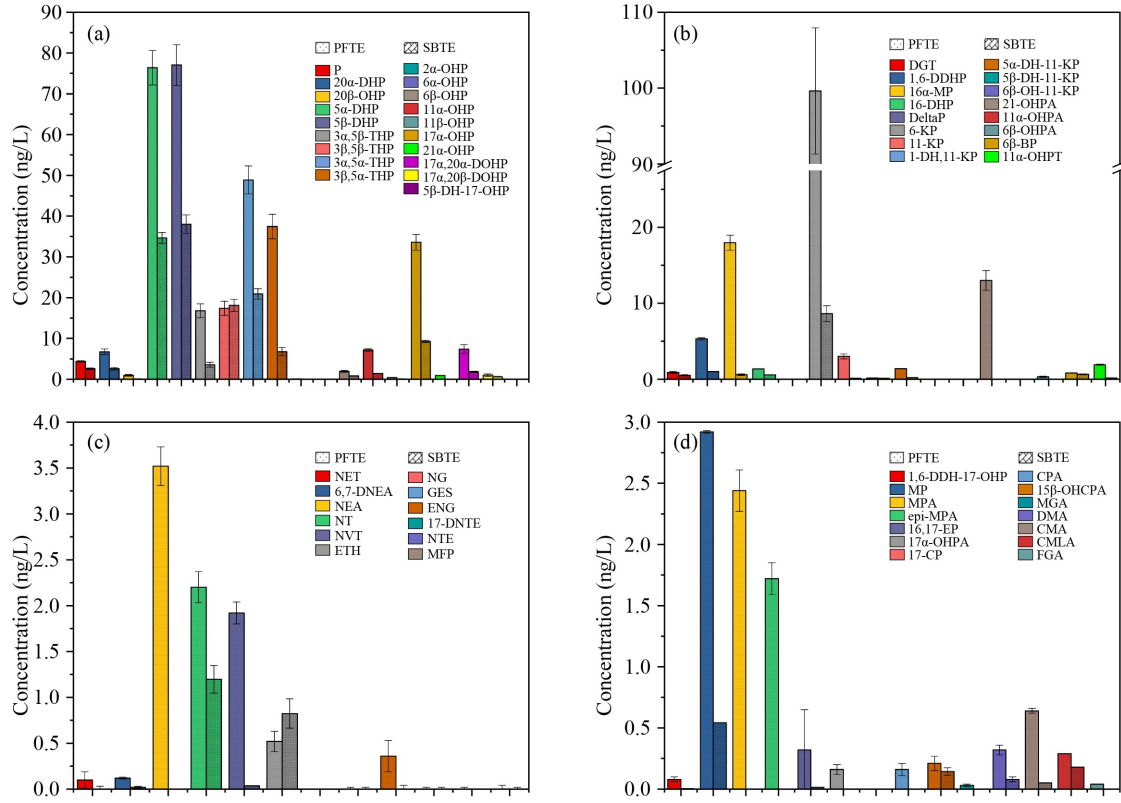
structure of progesterones was easy to react with O<sub>3</sub>, which can destroy the conjugated  $\pi$ -electron and ring system of progesterones (Pflug et al., 2017). Conversely, 5 $\alpha$ -DHP and 5 $\beta$ -DHP had reaction sites prone to chlorine substitution (Hu et al., 2003; Pflug et al., 2017), leading to a better removal performance by Cl<sub>2</sub> disinfection than by O<sub>3</sub> disinfection.

### 3.2.5 Effect of UV/Cl<sub>2</sub> disinfection on the removal of progesterones

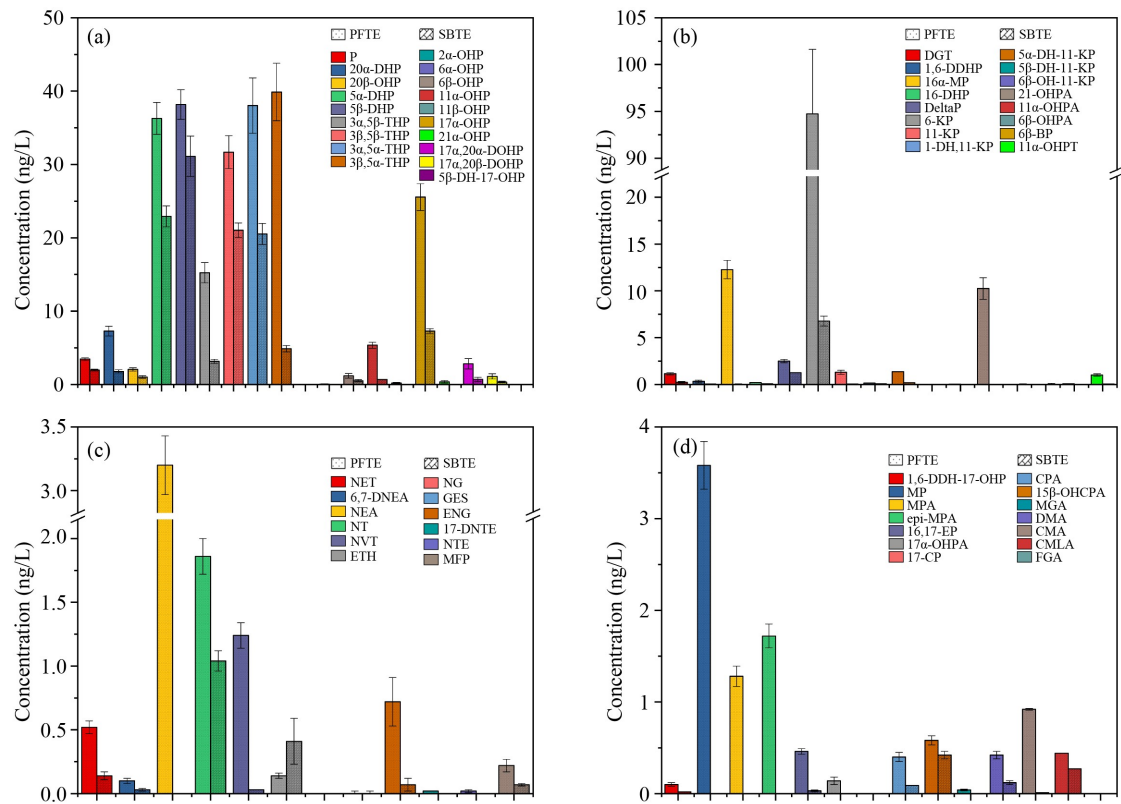
In Fig. 10, after UV/Cl<sub>2</sub> disinfection, the total concentrations of progesterones in PFTE and SBTE were 390.76 and 134.69 ng/L, respectively, lower than those after Cl<sub>2</sub> disinfection. The removal rates of the total progesterones in the two types of effluents after UV/Cl<sub>2</sub> disinfection were 65.14% and 64.62%, respectively, higher than those after Cl<sub>2</sub> disinfection. The reason was that in the UV/Cl<sub>2</sub> process, Cl<sub>2</sub> was activated by UV photolysis to produce active substances such as hydroxyl radicals (HO $\cdot$ ) and active chlorides (RCS, such as Cl $\cdot$  and Cl<sub>2</sub> $^{\cdot-}$ ) (reactions 1 and 2) (Tang et al., 2018), which were conducive to the ring opening of heterocyclic, aromatic, and phenolic rings and accelerated the degradation of progesterones (Saggiaro et al., 2019).



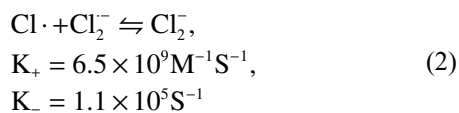




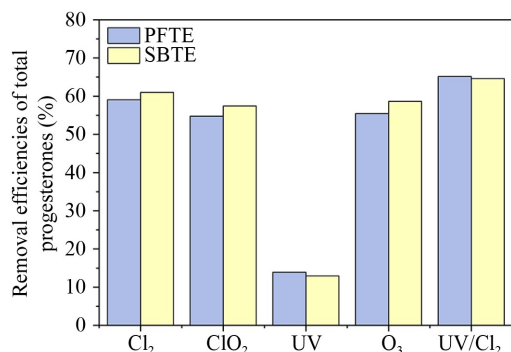
**Fig. 9** The effect of  $O_3$  disinfection on progesterone concentrations in PFTE and SBTE: (a) natural progesterones, (b) progesterone derivatives, (c) 19-nortestosterone derivatives, (d) 17 $\alpha$ -hydroxyprogesterone derivatives.



**Fig. 10** The effect of  $UV/Cl_2$  disinfection on progesterone concentrations in PFTE and SBTE: (a) natural progesterones, (b) progesterone derivatives, (c) 19-nortestosterone derivatives, and (d) 17 $\alpha$ -hydroxyprogesterone derivatives.



The removal efficiencies of the total progesterones by five different disinfection processes are summarized in Fig. 11. The order of the removal efficiencies of total progesterones was  $\text{UV}/\text{Cl}_2 > \text{Cl}_2 > \text{O}_3 > \text{ClO}_2 > \text{UV}$ . The removal efficiency of natural progesterones in each disinfection process was higher than those of the other three types of progesterones. Due to the different removal efficiency of each kind of progesterone, the main components of the four types of progesterones in the effluent also changed. Future work regarding the specific reasons needs to be performed.



**Fig. 11** The effect of the five disinfection processes on progesterone concentrations in PFTE and SBTE.

## 4 Conclusions

There were 61 types of natural and synthetic progesterones detected in HWW in the range of nanogram per liter, with natural progesterones as the main components. PFT had almost no progesterones removal efficiencies and SBT can effectively reduce the contents of progesterones by biodegradation.

After different disinfection treatments, the concentrations of total progesterones in PFTE and SBTE were in the order of  $\text{UV}/\text{Cl}_2 < \text{Cl}_2 < \text{O}_3 < \text{ClO}_2 < \text{UV}$  and the removal efficiencies of total progesterones were in the order of  $\text{UV}/\text{Cl}_2 > \text{Cl}_2 > \text{O}_3 > \text{ClO}_2 > \text{UV}$ . The removal efficiencies of natural progesterones by the five disinfection processes were better than those of synthetic progesterones (progesterone derivatives, 19-nortestosterone derivatives, and 17 $\alpha$ -hydroxyprogesterone derivatives).

## 5 Prospect

It should be noted that the results of this study may be unique since the water samples were only collected from one local wastewater treatment station. Therefore, further investigation is needed on various water samples. Evalu-

ation of disinfection in terms of the removal efficiencies of progesterones, the time required for disinfection, stability, cost of the disinfectant, and toxicity of disinfection byproducts should also be conducted.

**Acknowledgements** We gratefully acknowledge the funding from the National Natural Science Foundation of China (Grant Nos. 42177051 and 41977317).

**Electronic Supplementary Material** Supplementary material is available in the online version of this article at <https://doi.org/10.1007/s11783-022-1558-z> and is accessible for authorized users.

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