Degradation behavior of 17α-ethinylestradiol by ozonation in the synthetic secondary effluent

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Abstract

Endocrine disrupting chemicals (EDCs) in the secondary effluent discharged from wastewater treatment plants (WWTPs) are of great concern in the process of water reuse. Ozonation has been reported as a powerful oxidation technology to eliminate micropollutants in water treatment. Due to the complexity of the wastewater matrix, orthogonal experiments and single factor experiments were conducted to study the influence of operational parameters on the degradation of 17α-ethinylestradiol (EE2) in the synthetic secondary effluent. The results of the orthogonal experiments indicated that the initial ozone and natural organic matter (NOM) concentration significantly affected EE2 degradation efficiency, which was further validated by the single factor confirmation experiments. EE2 was shown to be effectively degraded by ozonation in the conditions of low pH (6), NOM (10 mg/L), carbonate (50 mg/L), but high suspended solid (20 mg/L) and initial ozone concentration (9 mg/L). The study firstly revealed that the lower pH resulted in higher degradation of EE2 in the synthetic secondary effluent, which differed from EDCs ozonation behavior in pure water. EE2 degradation by ozone molecule instead of hydroxyl radical was proposed to play a key role in the degradation of EDCs by ozonation in the secondary effluent. The ratio between O_3 and TOC was identified as an appropriate index to assess the degradation of EE2 by ozonation in the synthetic secondary effluent.

Key words: ozonation; EE2; pH; natural organic matter; secondary effluent

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Introduction

Endocrine disrupting chemicals (EDCs) have been widely detected in a variety of aquatic environment recently, posing potential threat and risk to human beings and wildlife. Feminization of male fish caused by EDCs has been demonstrated by *in vitro* assay (Brian et al., 2007). The exposure of fishes to 1–10 ng/L of 17β-estradiol (E2) (Routledge et al., 1998) and 0.1 ng/L of the synthetic contraceptive 17α-ethinylestradiol (EE2) (Zacharewski, 1997; Caldwell et al., 2008) can provoke feminization in some species of wild male fishes.

The effluent discharged from wastewater treatment plants (WWTPs), generally with its estrogenic compound concentrations of ng/L–μg/L (Ternes et al., 1999; Baronti et al., 2000; Rodgers-Gray et al., 2000; Fawell et al., 2001; Johnson and Sumpter, 2001; Bruchet et al., 2002), has been reported as a major source of EDCs (Desbrow et al., 1998; Jobling et al., 1998; Matsui et al., 2000). Since water shortage has been a globally pressing issue, there is a growing interest in reclamation of the secondary effluent from domestic wastewater treatment plants. Much

attention must be paid to the degradation of EDCs in the secondary effluent to ensure the safety of wastewater reuse. EE2 has been mostly detected in the secondary effluent and was shown to be approximately 11–27 times more estrogenic potent than E2 through *in vivo* tests (Lee et al., 2008). Therefore, EE2 was selected as a representative of EDCs in this study.

Ozonation has been considered as a promising treatment technology for EDCs degradation in both drinking water (Ternes et al., 2002; Broséus et al., 2009) and wastewater (Ternes et al., 2003; Kamiya et al., 2005). The apparent ozonation kinetic parameters of six endocrine disruptors (EDs: 4-*n*-nonylphenol, bisphenol A, 17α-ethinylestradiol, 17β-estradiol, estrone, and estriol) are in the range of 10^4–10^5 L/(mol·sec) for neutral EDCs and 10^9 L/(mol·sec) for ionized EDCs in the aqueous solution (Deborde et al., 2005), showing a very high degree of reactivity between ozone and phenolic moieties. Until now, a great number of studies have focused on the effect of single factor on the degradation of EDCs by ozonation in pure water (Lee et al., 2003; Maniero et al., 2008; Garoma and Matsumoto, 2009). However, the secondary effluent is relatively complex and changeable in composition. Results from single factor experiments might be insufficient for

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evaluation and prediction of EDCs ozonation behavior in the secondary effluent. It was reported that the effluent organic matters strongly reacted with ozone in competition with EE2 during the wastewater ozonation process, leading to low oxidation efficiency of EE2 (Lee and von Gunten, 2010). Hence, a comprehensive investigation of the effects of multiple factors on EDCs ozonation in the secondary effluent is necessary to further our understanding of the process and guide the design of the treatment process.

Previous studies showed that initial ozone concentration, EE2 concentration, pH value, natural organic matter (NOM), suspended solid (SS) and carbonate concentration exerted great influence on the degradation efficiency of ozonation and were critically crucial to the practical application of ozonation technology (Huber et al., 2005; Snyder et al., 2006). To the best of our knowledge, the relationship between the degradation of EDCs and the complex matrix of the secondary effluent has not yet been elucidated so far. Thus, in the present work, all six factors mentioned above were simultaneously taken into consideration to investigate the degradation behavior of EE2 by ozonation in the synthetic secondary effluent. The overall objective is to study and validate the comprehensive influence of operational parameters on the degradation of EE2 in the secondary effluent, and to find out an appropriate index to assess the degradation of EE2 by ozonation.

1 Materials and methods

1.1 Chemicals and reagents

17α-Ethinylestradiol (EE2) (> 99%) was obtained from the Laboratory of Dr. Ehrenstorfer (Germany) and used without further purification. NOM (mainly humic acid (HA)) was purchased from Fluka (Swiss). Bovine serum albumin (BSA) and sodium alginate (NaAg) were obtained from Sigma-Aldrich (USA). Suspended solid (SS) was prepared by freezing-drying activated sludge taken from a wastewater treatment plant in Beijing, China. Other chemical reagents were all analytical grade. Ultrapure water produced by Barnstead system (Thermo, USA) was used throughout the experiments.

Ozone was generated with an ozone generator (DHX-SS-1G, Jiujiu Co., China) by using pure oxygen as feed gas. The flow rate of feed gas was regulated to 1 L/min. The ozone stock solution was prepared by sparging ozone/oxygen mixed gas into ultrapure water, which was cooled at 4°C (Bader and Hoigné, 1981).

1.2 Experimental procedures

The synthetic secondary effluent was prepared according to our previous study (Zhu et al., 2008). It was mainly composed of 20 mg/L NOM (about 10 mg/L equivalent DOC), 10 mg/L SS and 100 mg/L carbonate (including carbonic acid, carbonate and bicarbonate in various pH). The initial added EE2 concentrations were at the level of μg/L, which were higher than those normally detected in the effluent of WWTPs (ng/L) and often used in batch studies (Kim et al., 2004; Bila et al., 2007). The water

samples were prepared by dissolving EE2 to be a given concentration (50, 100, 200 μg/L). The solution pH was adjusted to the desired values by adding phosphate buffer (pH < 8) or borate saline buffer (pH > 8) and then trimmed by 1 mol/L HCl or NaOH. NOM, SS and carbonate were also added to the samples to be a given concentration to study their effects.

To study the comprehensive influence of operational parameters on the degradation of EE2 in the synthetic secondary effluent, a set of six-factor three-level fractional factorial orthogonal experiments were firstly designed (Table S1). Ozonation experiments were conducted with synthetic water samples by injecting different amount (2, 4, 9 mg/L) of ozone stock solution into the continuous stirred batch reactors with volume of 40 mL. The reactor was left overnight to remove the residual ozone and radicals prior to analysis of EE2 concentration. A set of blank controls without injecting ozone stock solution were also tested. All the experiments were repeated in triplicate at room temperature.

At the second stage of experiments, single factor confirmation experiments were conducted to validate the influence of main factors (the initial ozone and NOM concentration) on the degradation efficiency of EE2 based on the results of orthogonal experiments. Ozonation experiments were conducted with synthetic water samples by injecting certain amounts (0–9 mg/L) of ozone stock solution into the continuous stirred batch reactors with volume of 100 mL. The initial EE2 concentration was spiked to be 100 μg/L, and pH was adjusted to about 8 with the addition of phosphate buffer. The NOM concentrations were set at a range of 2.5 mg/L equivalent DOC to 40 mg/L equivalent DOC to study the influence of NOM on EE2 degradation.

Thirdly, to validate the conclusion that EE2 was shown to be effectively removed by ozonation at lower pH, lower NOM and higher ozone concentration in the synthetic secondary effluent, confirmation ozonation experiments were conducted with 5 mmol/L phosphate buffer or borate saline buffer at different pH (6–10), 100 μg/L EE2, 4 mg/L initial ozone concentration and 10 mg/L DOC supplied by different natural organic matter compositions (HA, BSA and NaAg).

Finally, since both the initial ozone and NOM concentration significantly affected EE2 degradation, the ratio of ozone/NOM, which could be used as an operational parameter of ozonation for effective degradation of EE2, was tested. The experiments were conducted with 5 mmol/L phosphate buffer (pH 8), 100 μg/L EE2, different initial ozone concentrations (2–10 mg/L) and 10 mg/L DOC.

1.3 Analytical methods

About 4 mL of each sample was transferred to a 4-mL glass vial and its EE2 concentration was determined by high-performance liquid chromatography (1200 series HPLC, Agilent, USA). The mobile phase consisted of 60% acetonitrile and 40% ultrapure water with 1% acetic acid at a flow rate of 1 mL/min. Eluted substances were analyzed with a fluorescence detector at 209 nm (excitation

wavelength) and 317 nm (emission wavelength). Under the experimental condition, EE2 was linearly quantified in the range of 5–2000 μg/L ($R^2 = 0.9999$, data not shown). The error of triplicate samples was <5%. Ozone concentration was measured by iodometric method (Boyd et al., 1970). TOC was determined using a TOC analyzer (TOC-V CPH, Shimadzu, Japan).

1.4 Data analysis

Statistical analysis was performed using Minitab 15.0 for Windows (SPSS Co., USA). Analysis of variance (ANOVA) for the ratios of signal to noise (S/N ratios) and means was discussed to reveal the main factors and their interactions under default parameters. Note that besides means, the S/N ratios were calculated to illustrate the robustness of ANOVA. The level of significance was set at $\alpha = 0.05$ throughout this study.

2 Results and discussion

2.1 Orthogonal experiments

The results of orthogonal experiments (Table 1) showed that EE2 degradation efficiency ranged from $(20.80 \pm 1.12)\%$ to $(94.73 \pm 0.19)\%$. The minimum degradation efficiency occurred in the conditions of 2 mg/L initial ozone concentration, 200 μg/L EE2, pH 10, 20 mg/L NOM, 10 mg/L SS and 50 mg/L carbonate, while the maximum degradation efficiency occurred in the conditions of 9 mg/L initial ozone concentration, 100 μg/L EE2, pH 6, 10 mg/L NOM, 20 mg/L SS and 50 mg/L carbonate. EE2 degradation efficiency increased from 26.78% to 76.98% when the initial ozone concentration increased from 2 to 9 mg/L, suggesting that the initial ozone concentration is a key parameter in the ozonation of EE2, which could be supported by the previously reported results (Kim et al., 2007).

The response table for S/N ratios is shown in Table S2. The degree of influence on degradation efficiency of EE2 by six factors was ranked in the order of initial ozone concentration, NOM concentration, pH, EE2 concentration, SS and carbonate. The analysis of variance (ANOVA) for S/N ratios of EE2 ozonation further revealed that the initial ozone and NOM concentration significantly affected the degradation efficiency of EE2 (at $p < 0.05$) (Table S3), which was also consistent with previous reported results (Kim et al., 2004; Paraskeva and Graham, 2005; Petala et al., 2008; Tsuno et al., 2008). Three dimensional schematic diagram of EE2 degradation affected by NOM and initial ozone concentration was generated in Fig. 1. The visualized 3D response curve could be roughly divided into three parts represented by three colors (blue, green and yellow). They were approximately coincided with three gradient of initial ozone concentration. It was observed that the initial ozone concentrations was notably more influential than NOM. As the initial ozone concentration increased, the degree of influence by NOM concentration became more obvious. EE2 was removed to a lower extent as NOM concentration was higher, possibly because NOM

Table 1 Results matrix of orthogonal array designed experiments

| Sample number	EE2 degradation percentage (%)			Mean (%)	STD (%)
	Exp. 1	Exp. 2	Exp. 3		
1	29.19	29.03	26.27	28.16	1.64
2	24.23	26.95	23.66	24.95	1.76
3	23.04	24.93	23.53	23.83	0.98
4	21.07	29.73	23.59	24.80	4.45
5	37.34	34.33	35.65	35.77	1.51
6	27.15	26.73	27.01	26.96	0.21
7	30.39	33.85	32.92	32.39	1.79
8	23.24	23.16	23.61	23.34	0.24
9	20.86	21.89	19.65	20.80	1.12
10	51.25	48.22	50.28	49.92	1.55
11	58.44	59.88	59.92	59.41	0.84
12	37.23	37.11	35.87	36.74	0.75
13	53.12	52.81	54.40	53.44	0.84
14	58.63	58.25	59.18	58.69	0.47
15	40.53	44.57	45.23	43.44	2.54
16	37.86	37.24	39.94	38.35	1.41
17	42.47	42.97	44.12	43.19	0.85
18	52.45	54.05	52.76	53.09	0.85
19	69.06	70.58	70.44	70.03	0.84
20	76.44	77.61	78.00	77.35	0.81
21	90.82	94.82	93.94	93.19	2.10
22	94.78	94.89	94.52	94.73	0.19
23	59.37	59.45	60.01	59.61	0.35
24	68.39	68.89	69.54	68.94	0.58
25	79.96	82.25	79.58	80.60	1.44
26	67.17	67.11	67.21	67.16	0.05
27	80.82	82.09	80.84	81.25	0.73
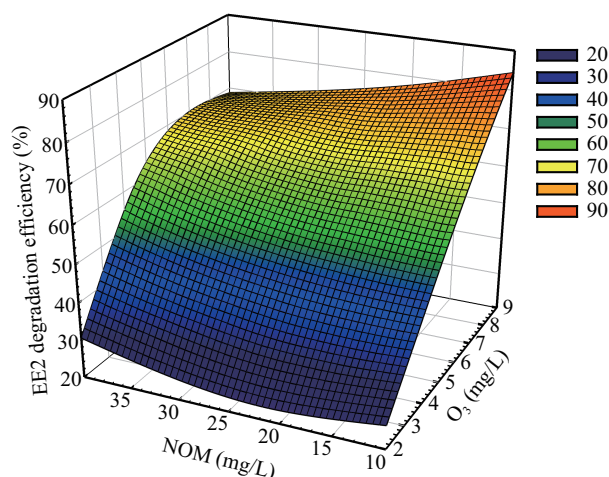
Fig. 1 Three dimensional schematic diagram of EE2 degradation efficiency affected by NOM and initial ozone concentration.

facilitated the decomposition of dissolved ozone and the consumption of hydroxyl radical. The higher initial ozone concentration and lower NOM concentration resulted in greater EE2 degradation efficiency, which was further validated by the single factor confirmation experiments (Fig. 2). On the other hand, based on the AONVA for means of EE2 degradation efficiency, EE2 degradation by ozonation was most significantly affected by the initial ozone concentration (at $p < 0.05$) (Table S3), which demonstrated the key role of the initial ozone concentration to control estrogenic compounds in the ozonation process again.

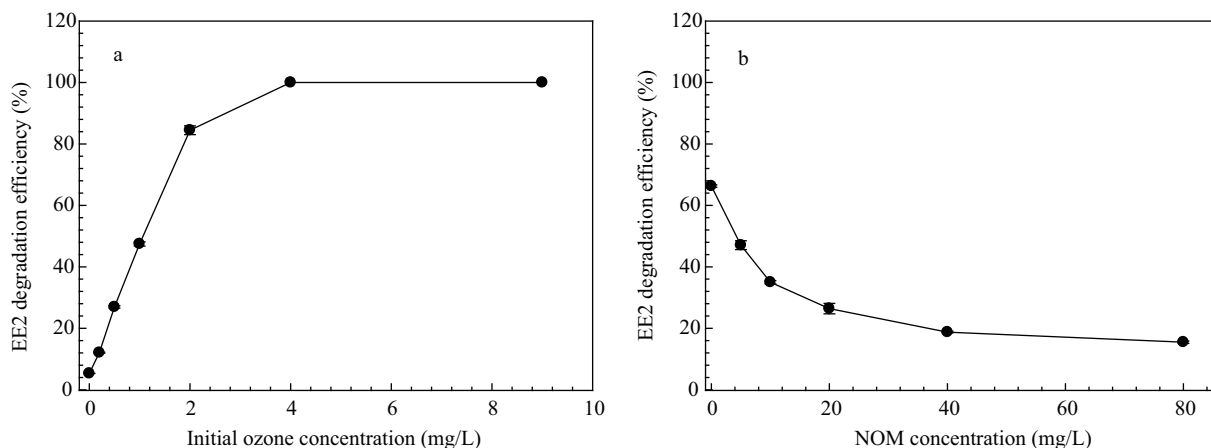In principle, the optimal operation parameters are

Fig. 2   Influence of initial ozone concentration (a) and NOM (b) on EE2 degradation efficiency.

determined by both main factors and their statistically significant interactions. If an interaction between two variables is statistically significant, it must contain at least one significant variable. Taking the results of AONVA for S/N ratios and means of EE2 degradation efficiency into account (Table S3), the initial ozone concentration was the only significant variable. The *p* values of O_3 and EE2, O_3 and pH, O_3 and NOM, O_3 and SS, O_3 and carbonate were estimated as 0.890, 0.198, 0.095, 0.365 and 0.351, respectively. Besides, the relationship of interactions among six factors was tabulated in Fig. 3. The curves of EE2 degradation efficiency were almost parallel to the different gradient of the initial ozone concentration in combination with other factors, suggesting no significant interactions between the initial ozone concentration and other factors.

2.2 Influence of pH and NOM on EE2 degradation efficiency

Generally, higher pH facilitates higher degradation of organic compounds by ozonation in pure water. However, it was interesting to find that the maximum degradation efficiency of EE2 occurred in the conditions of the lowest pH (6), NOM (10 mg/L) and carbonate (50 mg/L), but the highest SS (20 mg/L). To further validate the experiment results, a series of EDCs ozonation experiments were conducted in different pH and different NOM concentrations. The results demonstrated a similar trend of EE2 ozonation behavior (Fig. 4) to that of the orthogonal experiments, suggesting that lower pH is advantageous for EDCs ozonation in the synthetic secondary effluent. It could be explained that, in general, it is easier for ozone molecule
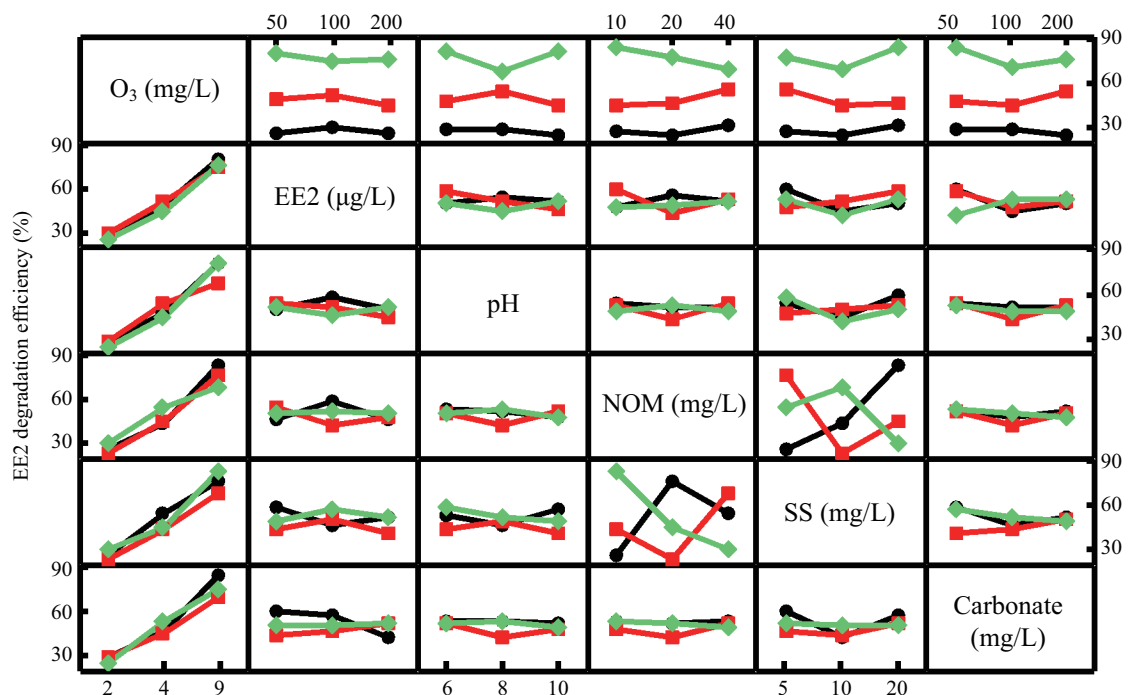

Fig. 3   Full interaction plot matrix for means of EE2 degradation efficiency affected by six factors.
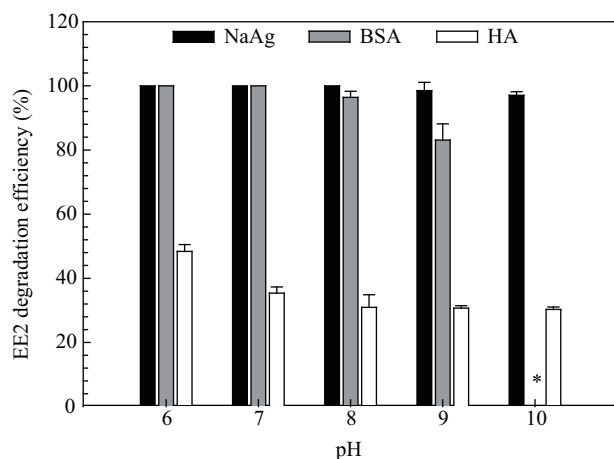
Fig. 4　Influence of pH and NOM on EE2 degradation efficiency. *: missing value.

Fig. 5　Influence of O_3:TOC on EE2 degradation efficiency.

to convert to a stronger oxidant (hydroxyl radical) at higher pH, which results in a higher degradation of pollutants by ozonation in pure water (von Gunten, 2003; Tsuno et al., 2008). However, in the secondary effluent, there are diverse inorganic and organic matters with high concentrations at the level of mg/L, acting as hydroxyl radical scavengers and ozone consumption promoters. Consequently, the hydroxyl radical and the ozone molecule could be more quickly consumed at higher pH in the secondary effluent, losing their chances to degrade EDCs. Thus, it could be proposed that ozone molecule instead of hydroxyl radical plays a key role in the degradation of EDCs by ozonation in the secondary effluent. In addition, the degradation efficiency of EE2 was inhibited by NOM in the order of HA > BSA > NaAg, which are the main constituents of organic matter in the secondary effluent. This indicated that the degradation of EE2 by ozonation was greatly influenced not only by NOM concentration, but also by NOM composition and structure.

2.3 Influence of O3:TOC on EE2 degradation efficiency

The influence of O_3:TOC on EE2 degradation efficiency was investigated. As shown in Fig. 5, EE2 degradation efficiency was significantly linear to O_3:TOC ($p < 0.05$, R^2 = 0.90 and 0.99 for BSA and HA, respectively), indicating that O_3:TOC could be an appropriate index to assess the degradation of EE2 in the secondary effluent by ozonation. Thus, the degradation of EDCs in the secondary effluent by ozonation can be elevated by raising initial ozone concentration or reducing NOM through membrane pretreatment before ozonation (Schlichter et al., 2003; von Gunten, 2003; Oh et al., 2007).

3 Conclusions

The results of the orthogonal array designed experiments showed that the initial ozone and NOM concentration exerted remarkable influence on the EE2 degradation efficiency in the secondary effluent, and the initial ozone concentration was the most influential factor. No significant interaction existed among factors tested in the degradation of EE2 by ozonation in the synthetic sec-
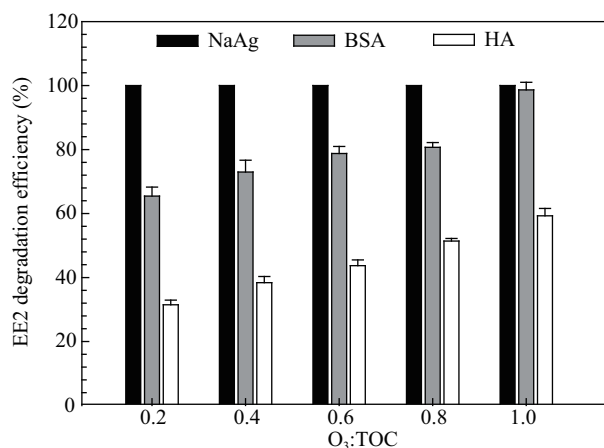
ondary effluent. It was for the first time to report and validate that lower pH facilitated higher degradation of EE2 in the synthetic secondary effluent, which differed from EDCs ozonation behavior in pure water. The ozone molecule instead of hydroxyl radical played a key role in the degradation of EDCs by ozonation in the secondary effluent. O_3:TOC was identified as an appropriate index to assess the degradation of EE2 by ozonation in the synthetic secondary effluent.

Acknowledgments

Supporting materials

Supplementary data associated with this article can be found in the online version.

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Supporting materials

Table S1 Design matrix of orthogonal array designed experiments

Sample number	C_{0,O_3} (mg/L)	$C_{0,EE2}$ (μg/L)	pH	NOM (mg/L)	SS (mg/L)	HCO_3^- (mg/L)
1	2	50	6	10	5	50
2	2	50	8	20	10	100
3	2	50	10	40	20	200
4	2	100	6	20	10	200
5	2	100	8	40	20	50
6	2	100	10	10	5	100
7	2	200	6	40	20	100
8	2	200	8	10	5	200
9	2	200	10	20	10	50
10	4	50	6	20	20	200
11	4	50	8	40	5	50
12	4	50	10	10	10	100
13	4	100	6	40	5	100
14	4	100	8	10	10	200
15	4	100	10	20	20	50
16	4	200	6	10	10	50
17	4	200	8	20	20	100
18	4	200	10	40	5	200
19	9	50	6	40	10	100
20	9	50	8	10	20	200
21	9	50	10	20	5	50
22	9	100	6	10	20	50
23	9	100	8	20	5	100
24	9	100	10	40	10	200
25	9	200	6	20	5	200
26	9	200	8	40	10	50
27	9	200	10	10	20	100

C_{0,O_3}: initial ozone concentration, $C_{0,EE2}$ concentration, SS: suspended solid concentration, HCO_3^-: carbonate concentration.

Table S2 Response table for S/N ratios

Level	C_{0,O_3} (mg/L)	$C_{0,EE2}$ (μg/L)	pH	NOM (mg/L)	SS (mg/L)	HCO_3^- (mg/L)
1	27.79	31.92	31.88	38.37	36.43	35.25
2	33.59	36.36	38.9	29.42	34.47	35.36
3	43.28	36.38	33.88	36.86	33.76	34.06
Delta	15.48	4.46	7.02	8.95	2.68	1.3
Rank	1	4	3	2	5	6

Table S3 Analysis of variance for S/N ratios and mean values

Source		S/N ratio					Mean				
	DF	Seq SS	Adj SS	Adj MS	F	p	Seq SS	Adj SS	Adj MS	F	p
O_3	2	1101.69	1101.69	550.85	11.65	0.00*	11412.9	11412.9	5706.43	65.5	0.00*
EE2	2	118.88	118.88	59.44	1.26	0.32	46.1	46.1	23.05	0.26	0.77
pH	2	235.65	235.65	117.83	2.49	0.12	41.2	41.2	20.58	0.24	0.79
NOM	2	413	413	206.5	4.37	0.03*	44	44	21.98	0.25	0.78
SS	2	34.59	34.59	17.3	0.37	0.7	357.6	357.6	178.82	2.05	0.17
HCO_3^-	2	9.34	9.34	4.67	0.1	0.91	155.4	155.4	77.72	0.89	0.43
Residual error	14	662.2	662.2	47.3			1219.6	1219.6	87.12		
Total	26	2575.36					13276.8				

* $p < 0.05$