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17β-estradiol as precursors of Cl/Br-DBPs in the disinfection process of different water samples☆

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ABSTRACT

During chlorine disinfection process, reactions between the disinfectant and 17β-estradiol (E2) lead to the formation of halogenated disinfection byproducts (DBPs) which can be a risk to both ecosystem and human health. The degradation and transformation products of E2 in sodium hypochlorite (NaClO) disinfection processes of different water samples were investigated. The reaction kinetics research showed that the degradation rates of E2 were considerably dependent on the initial pH value and the types of water samples. In fresh water, synthetic marine aquaculture water and seawater, the reaction rate constant was 0.133 min^{-1}, 2.067 min^{-1} and 2.592 min^{-1}, respectively. The reasons for the above phenomena may be due to the different concentrations of bromide ions (Br^-) in these three water samples which could promote the reaction between NaClO and E2. Furthermore, Br^- could also cause the formation of brominated DBPs (Br-DBPs). The main DBPs, reaction centers and conceivable reaction pathways were explored. Seven halogenated DBPs have been observed including three chlorinated DBPs (Cl-DBPs) and four Br-DBPs. The active sites of E2 were found to be the pentabasic cyclic ring and the ortho position of the phenol moiety as well as C9-C10 position. The identified Cl/Br-DBPs were also confirmed in actual marine aquaculture water from a shrimp pond. The comparison of bio-concentration factors (BCF) values based on calculation of EPI-suite showed that the toxicities of the Br-DBPs were stronger than that of their chloride analogues. The absorbable organic halogens (AOX) analysis also suggested that the DBPs produced in the marine aquaculture water were more toxic than that in the fresh water system.

1. Introduction

For the sake of control aquatic reproduction, monosex aquaculture has emerged as a popular practice in aquaculture systems (Beardmore et al., 2001; Rodgers et al., 2006). A higher growth rate could be achieved through the application of monosex aquaculture. At the same time, it could also reduce variation in harvest size sexual, territorial behaviors and risk of environmental impact resulting from escapes of exotic species (Sagi and Aflalo, 2015). Hormonal induction of sex reversal is usually served as a valuable approach to achieve monosex aquaculture (Pandian and Sheela, 1995). For example, Yamazaki reported that endogenous estrogen could act as an ovarian inducer which leading to protogynous sex change of three-spotted wrasse and saddleback wrasse (Yamazaki, 1983). 17β-estradiol (E2) is an effective feminization hormone because it can induce the sex reversal in aquaculture (Carvalho et al., 2014; Nakamura et al., 2003; Wang and Croll, 2004). However, a number of papers have reported the potentially detrimental effects of E2 on aquatic organism and especially the carcinogenicity on humans (Ahmad et al., 2000; Bradley et al., 2009; Pedram et al., 2006). Through the investigation of the genotoxic effect of E2 on human chromosomes in lymphocytes aquacultures in vitro, E2 was found to associate with chromosomal aberrations and sister chromatid exchanges(Pedram et al., 2006). Estrogens including E2 have been detected in seawater with concentrations about

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8.8 ± 0.7 ng L^{-1} (Heub et al., 2015). For marine aquaculture water, there is no reference reported and about 350 ng L^{-1} of E2 was detected in a shrimp pond in FangChengGang breeding base. Therefore, the residual E2 is still a special pollutant in marine aquaculture water and a matter of continuing concern (Janer et al., 2004). The transformation of E2 in marine aquaculture water should be also researched.

On the other hand, chlorination disinfection is a universal procedure as water quality is very important for the intensive marine aquaculture systems (Boyd, 1996; Sanawar et al., 2017). However, chlorination treatment of water has been shown to generate unintended disinfection byproducts (DBPs) as chlorine-containing disinfectant would react with the natural or artificial organic matter in water (Liu et al., 2012; Teixeira et al., 2011). DBPs, halogenated DBPs in particular, have received a growing concern because of their significant toxicity and environmental risks (Hrudey, 2009; Moudgal et al., 2000; Nikolaou et al., 2004; Zamyadi et al., 2012). Since the 1970's, trihalomethanes (THMs) as the chlorination DBPs were first reported in drinking water, the original halogenated DBPs has been expanded to more than 600 different types (Chu et al., 2011; Liviac et al., 2011). A lot of studies suggested that the halogenated DBPs such as THMs, haloacetic acids (HAAs) and haloacetonitriles (HANs) possess cytotoxicity and genotoxicity (Bekbolet et al., 2005; Sadiq et al., 2004). It is worth noting that brominated DBPs (Br-DBPs) which are substantially more toxic than their chloro-analogues will be generated once the water contains bromide ions (Br^-) such as the marine aquaculture water (Qiang et al., 2014; Abdelwahab et al., 2010; Ged and Boyer, 2014; Parinet et al., 2012; Saidan et al., 2015). Through the studies of the microplate cytotoxicity assay and single-cell gel electrophoresis assay on Chinese hamster, brominated HAAs were found to be 18.4 and 89.8 times more cytotoxic and genotoxic than their chlorinated products (Plewa et al., 2002). The formation of Br-DBPs was due to that the strong oxidizing agent of hypochlorous acid can oxidize the Br^- to hypobromite which is a stronger halogenating agent than hypochloric acid (Sun et al., 2009; Zhang et al., 2017). As reported, under the same condition, the reaction rate constant of hypochlorite with DBPs organic precursors was 0.7–5.0 L $(mol \cdot s)^{-1}$, while the reaction rate constant of hypobromite with DBPs organic precursors could up to 15–167 L $(mol \cdot s)^{-1}$ (Ichihashi et al., 1999).

Researches on the halogenated DBPs were traditionally focused on the reaction product of chlorine-containing disinfectant with natural organic matter such as humic acid presented in water sources, while limited studies has focused on the reaction of disinfectant with other man-made organic compounds (Ichihashi et al., 1999; Jiang et al., 2017; Lu et al., 2009). Some special organic substances are not only pollutants themselves in water sources, but also can react with chlorine-containing disinfectant to form novel halogenated DBPs. Only recently, the related research has aroused concern. For example, an antibiotic oxytetracycline has been reported to react with chlorine disinfectant and six chlorinated DBPs (Cl-DBPs) were detected (Bi et al., 2013). The reaction kinetics, mechanisms and pathways of sulfamethoxazole with free available chlorine were investigated. The results showed that direct reactions were very quickly, two DBPs were found. The formation of DBPs indicated that S-C cleavage, polymerization, S-N hydrolysis, chlorine substitution and desulfonation reactions was occurred (Dodd and Huang, 2004). Hu et al. reported that E2 could react with sodium hypochlorite (NaClO) and seven products including 2,4-dichloro-17β-estradiol, monochloroestrone, 2,4-dichloroestrone, and other four byproducts were identified (Hu et al., 2003). However, compared with fresh water, marine aquaculture water which was a mix of seawater with fresh water with a proportion of 1:2 contains Br^- with a concentration of up to 22 mg L^{-1}, Br-DBPs will be produced consequently (Dorji et al., 2018).

As discussed above, E2 in the marine aquaculture water was not only a pollution but could also act as a special precursor of halogenated DBPs including Br-DBPs. In this paper, the research priority will be mainly focused on the kinetics, pathways and the final products especially the Br-DBPs of the reaction between chlorine-containing disinfectant and E2 in the marine aquaculture water. The fates of E2 in fresh water, marine aquaculture water and seawater samples were analyzed respectively to emphasize the effect of Br^- on the formation of reaction products. NaClO which is often used as an effective disinfectant to control marine aquaculture disease was selected as the chlorine-containing disinfectant. The activity and potential health risk of Br-DBPs brought by E2 during the chlorination disinfection process of marine aquaculture water will be primarily explored.

2. Materials and methods

2.1. Chemicals

Analytical grade E2 was purchased from the Shanghai Yuanye biological technology company (98% purity or higher) and without further purification in use. Both sodium chloride (NaCl) and sodium bromide (NaBr) were of analytical grade and were obtained from Guangzhou Chemical Reagent Company. Sodium sulfite (Na_2SO_3) was purchased from the Chengdu Jinshan Chemical Reagent Company, China. Formic acid was obtained from Chengdu Kelong Chemical Reagent Company, China. Analytical grade NaClO was obtained from Tianjin Damao Chemical Reagent Company at 10% purity. High performance liquid chromatography (HPLC) grade methanol was purchased from Anhui Fulltime Specialized Solvents & Reagents.Co., LTD. The stock solutions of humic acid (HA) which was selected as a surrogate of dissolved organic matter (DOM) were purified by filtration and precipitation and its concentration was measured in mgC L^{-1} (Gao et al., 2016). All reagent solutions were prepared using water from a Millipore Milli-Q Ul-trapure Gradient A10 purification system. Real marine aquaculture water samples of shrimp ponds were obtained from FangChengGang, GuangXi province, China.

2.2. Analytical methods

Liquid chromatography was performed on an ultra-performance liquid chromatography (UPLC) system which equipped with an Acquity UPLC QSM (Waters, USA), a quaternary gradient pump, fluorescence detector, and UV diodearray detector was used to monitor the content of E2. The detection wavelength for E2 was set at 280 nm. The mobile phase used was ultrapure water and acetonitrile at a flow rate of 0.30 ml min^{-1}. All solutions used for UPLC analysis were degassed by sonication for more than 20 min. Adsorbed organic halogen (AOX) was measured with the microcoulometric titration method using a total organic halogen analyzer (XPLORER; TE Instruments B.V.).

2.3. Preparation of synthetic water samples

The synthetic fresh water was obtained by Milli-Q pure water system. The synthetic seawater was prepared by adding NaCl and NaBr to the fresh water (Cl^-: 19 g L^{-1}, Br^-: 65 mg L^{-1}). The inorganic cations such as Na^+, Ca^{2+}, Mg^{2+}, K^+ and inorganic anions including SO_4^{2-} and HCO_3^- which concentrations are high in the seawater were added according to their actual concentration. The marine aquaculture water was a mixture of seawater and fresh water with proportion of 1:2 as in the actual breeding process. The concentration for Cl^- and Br^- in the synthetic marine aquaculture water was about 6.6 g L^{-1} and 22 mg L^{-1}, respectively. The pH values of the synthetic water samples were maintained at 8 and were adjusted by phosphate and borate buffer.

2.4. Batch kinetic experiments

Actually, researches on production identification usually enlarged the substrate concentrations in order to avoid missing part of the transformation products. For example, in the reported paper on tetracycline (TC) oxidation by chloramine (NH_2Cl), the initial concentration of TC was set about as 11 mg L^{-1} that was much larger than it was in the real water (10.3 μg L^{-1}) (Shiqing et al., 2014). In the reported research on oxytetracycline (OTC) oxidation by NH_2Cl, the initial concentration of OTC was set at 2 mg L^{-1} while it is about 340 ng L^{-1} in the actual water (Bi et al., 2013). Therefore, we set a high initial concentration of E2 at 0.1 mM. The chlorine disinfection experiments were conducted in a 50 ml conical flask, each bottle was equipped with 20 ml E2. 1 ml NaClO with concentration of 6 mmol L^{-1} was added to start the reaction. During the experiments, the samples were taken at fixed time intervals and 1 ml Na_2SO_3 with concentration of 12 mmol L^{-1} was used to remove the residual NaClO. All of the kinetic experiments were conducted at room temperature and repeated at least two times. Pseudo-first-order rate constants were obtained from the slopes of fitted linear plots of Ln ([E2]) versus time, R^2 values was ranging from 0.95 to 1.0.

2.5. Product identification

The DBPs were analyze by a Thermo Fisher LC-MS/MS (Q-Exactive) system equipped with a Zorbax SB-C18 column (2.4 mm 150 mm, 5 mm), a diode-array UV/vis detector and a mass spectrometer. The gradient elution consisted of acetonitrile and 0.2% formic acid with a flow rate of 0.30 ml min^{-1}. The mass spectral analysis of products with a mass scan range of 50–1000 *m/z* and under a positive mode electrospray ionization (ESI^+). In the scan mode, the mass resolution was set to 0.0001 *m/z*. Products containing bromine and chlorine elements were analyzed based on the accurate mass and the isotopic ion peaks were found in the mass spectra with non-single isotopic compositions.

3. Results and discussion

3.1. Reaction kinetics of E2 with NaClO

3.1.1. Effect of water composition

To study the disinfection reaction of E2 with NaClO in different water samples, fresh water, synthetic marine aquaculture water and seawater were prepared. Fig. 1(a) presents the time-dependent degradation of E2 by NaClO in all different water samples. As it can be seen, the concentrations of E2 decreased continuously with time. After 5 min disinfection, E2 disappeared from both synthetic marine aquaculture water and seawater. While at this same time, the concentration of E2 remained at 11.53 mgL^{-1} in fresh water and it took about 2 h to consume exhausted. Fig. 1(b) shows a linear regression between the residual concentrations of E2 and times, the rate constants in these three water samples were calculated to be 2.592 min^{-1}, 2.067 min^{-1} and 0.133 min^{-1}, respectively. Thus, the reaction rates of E2 with NaClO in different water samples in the order seawater > synthetic marine aquaculture water > fresh water. The main reason for the mentioned above difference in the reaction rates may be the different concentrations of Br^- in the water samples.

Then, the influence of the Br^- on the oxidation rate of E2 were further investigated. During the experiments, the concentration of Cl^- was set at 6.6 g L^{-1}, the concentration of Br^- increased from 0 mg L^{-1} to 65 mg L^{-1}. It was shown in Fig. 2 that the rate constants were considerably dependent on the concentration of Br^- and the trends were coincided with Fig. 1. In the absence of Br^-, more than 30 min was needed to oxidize E2, while in presence of Br^- with the concentrations greater than 10 mg L^{-1}, E2 were degraded only within 5 min. The oxidation rates of E2 were strongly enhanced by Br^-. The above phenomenon was in general agreement with the reported reference that the reactivity of bromine with organic matter was approximately 10 times faster than that of chlorine (Zhang et al., 2017). The formation of hypobromite which react with organic precursor faster than hypochlorite and the bromine substitution that also faster than chlorine substitution should be in charge of the above phenomenon (Sun et al., 2009).

On the other hand, the activation energy that material requires to transform from the normal state to the active state also affects the rate of reaction. The reaction activation energy can be calculated by the Arrhenius equation as following:

$$k = A \exp\left(\frac{-Ea}{RT}\right) \tag{1}$$

$$\ln k = \ln A - \frac{Ea}{RT} \tag{2}$$

where *k* represent the reaction rate constant, *Ea* is the reaction activation energy and *A* is pre-exponential factors, *R* is the molar gas constant, 8.314 J $(K{\bullet}mol)^{-1}$, *T* is the reaction thermodynamic temperature.

The reaction rates variations on temperature and the activation energies calculation in different water samples were presented in Fig. 3. The energies corresponding to the reaction of NaClO with E2 in these three water samples, fresh water, synthetic marine aquaculture water and seawater were calculated, and the date were 44.649 KJ mol^{-1},33.284 KJ mol^{-1} and 27.939 KJ mol^{-1},respectively. The smaller activation energies in marine aquaculture water and seawater indicated that the reaction should occur more easily in the presence of Br^-. The reason was that Br^- could be oxidized to hypobromous acid (HOBr) which showed stronger halogenating activity than hypochloric acid (HOCl) did (Sun et al., 2009). The high rate constants at high bromide concentrations were resulted from direct reaction between HOBr and E2. The result was consistent with the above conclusions and further proved that the Br^- can promote the reaction of NaClO with E2. It was also found that the Cl^- in seawater can also promote the reaction, but the effect was smaller than that of Br^-.

In addition to the Br^- and Cl^-, there are many other matters in the seawater which may influence the reactions between E2 and NaClO. As shown in Fig. 4, the inorganic cations such as Na^+, Ca^{2+}, Mg^{2+} and K^+ showed no influence on the reaction rates. The effects of inorganic anions SO_4^{2-} and HCO_3^- which concentrations are high in the seawater (SO_4^{2-}: 2.74 g L^{-1}, HCO_3^-: 152 mg L^{-1}) were investigated. The results showed that the adjunction of HCO_3^- negligibly affect the reaction rate of E2 with NaClO. On the contrast, SO_4^{2-} affect the reaction as the rate increased from 5.213 min^{-1} to 5.644 min^{-1} when 914 mg L^{-1} SO_4^{2-} were added. The reason will be further examined.

Besides, the DOM existing in the seawater may act as a competitor for oxidant and exert important impacts on the oxidation of E2. HA as the surrogate of DOM was chosen as the disrupter. The reaction rate decreased from 5.213 min^{-1} to 1.889 min^{-1}, 1.587 min^{-1} and 1.227 min^{-1} when 0.5 mgC L^{-1}, 1 mgC L^{-1} and 2 mgC L^{-1} HA were added to the reaction system as shown in Fig. 4. The similar inhibitory effect was also reported on the research about chlorination of bisphenol S and HA was proved to negligibly affect the products species (Gao et al., 2018).

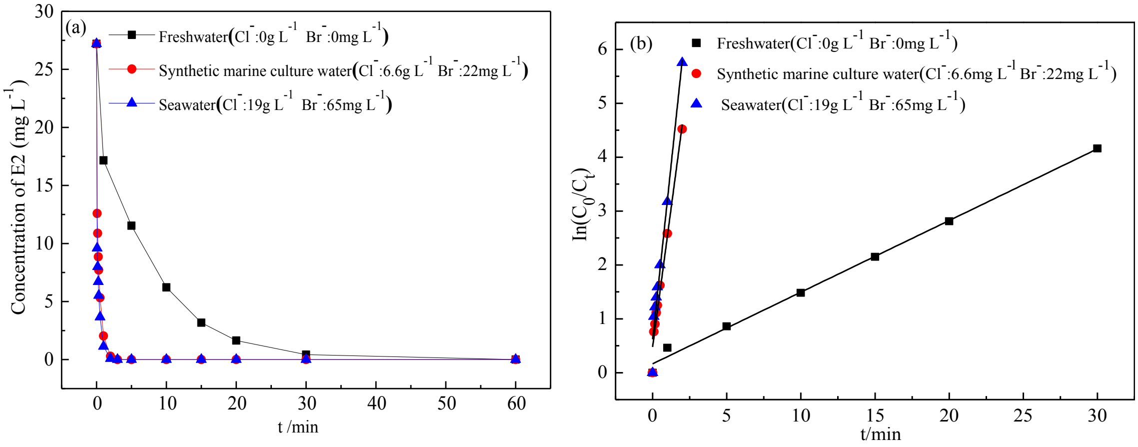


Fig. 1. Oxidation of E2 by NaClO in different water samples and the linear fit of the kinetic data ($[E2]_0 = 0.1$ mM, 20 ml, $[NaClO]_0 = 60$ mM, 1 ml, pH = 7, T = 298.15 K).

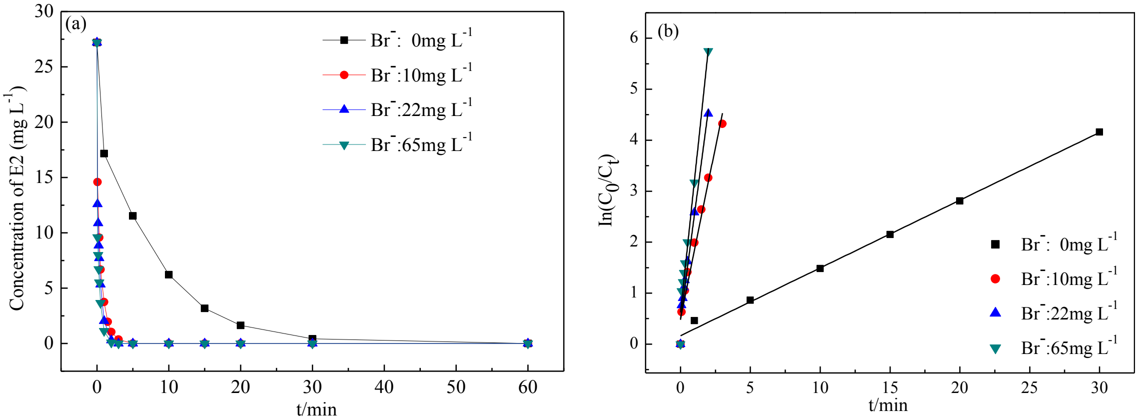


Fig. 2. Oxidation of E2 by NaClO at different concentrations of Br^- and the linear fit of the kinetic data ($[E2]_0 = 0.1$ mM, 20 ml, $[NaClO]_0 = 60$ mM, 1 ml, pH = 7, T = 298.15 K).

3.1.2. *Effect of pH value*

Besides the composition of seawater, the initial pH value as another important water quality parameter can affect the oxidation kinetics and pathways of E2 significantly. Fig. 5 presents that the oxidation rate of E2 in synthetic marine aquaculture water increased as the initial pH value increased. After 0.5 min of oxidation at pH values of 5, 6, 7 and 8, the residual concentration of E2 was 13.917 mg L^{-1}, 5.335 mg L^{-1}, 2.198 mg L^{-1} and 1.108 mg L^{-1}, respectively. The rate constants increased from 0.4429 min^{-1}–5.2131 min^{-1} when the pH value was raised from 5 to 8. The ionization equilibrium constant pKa is the pH value at which a group dissociated in half of the total concentration and can be used to estimate the speciation distribution. In the reaction, NaClO changed to HClO and HBrO, then, HClO and HBrO reacted with E2. pKa for HClO and HBrO was found to be 7.5 and 8.62, respectively. However, as reported by Westerhoff, in the reaction of both aqueous bromine ($HOBr/OBr^-$) and chlorine ($HOCl/OCl^-$) with organic matter during water treatment, the halogen dissociation effect could be precluded (Westerhoff et al., 2004). The effect of initial pH on E2 oxidation by NaClO should mainly be ascribe to the speciation of the organic matter by itself. As reported, E2 has one ionizable proton with a pKa of 10.413 (Deborde et al., 2004). E2 in the non-dissociated form is a weak reducing agent, while at higher pH values, a phenolate anion is transformed from the phenol moiety and the electron density is increased that is more vulnerable to electrophilic attack (Chen et al., 2013; He et al., 2016). Therefore, the reactivity of the dissociated form of E2 was 10^4 to 10^5 times stronger than the neutral form (Westerhoff et al., 2004). For example, the concentration of the deprotonated form of E2 is low at a pH of 8.0, but the rate of the reaction involving electrophilic attack on the phenolate anion is rapid (He et al., 2016). Therefore, the rate of E2 degradation increased with the increase of pH value which influenced by the above mechanism. The higher initial pH value is more favorable for the reaction of E2 with NaClO.

3.2. *Identification of the byproducts and reaction centers*

In order to determine the effects of Br^- on products formation, the final DBPs appeared in fresh water, synthetic marine aquaculture water and seawater were analyzed separately. As discussed above, E2 reacted with NaClO rapidly, the chromatogram of products did not change and the characteristic peaks of E2 disappeared in all water samples after 1 h reaction, suggesting that the fixed final byproducts were formed. The products were inferred by the accurate mass combined with the mass spectra isotope peak clusters. As can be seen in Scheme 1, the final products were denominated p1 - p7, respectively. The mass spectrums of the seven DBPs were given in Fig.S1. p1, p2 and p3 were the products found in the fresh water and they were the same as reported in the literature (Hu et al., 2003). In addition to these three Cl-DBPs, other four Br-DBPs including p4, p5, p6 and p7 were detected in synthetic marine aquaculture water and seawater simultaneously. The chemical

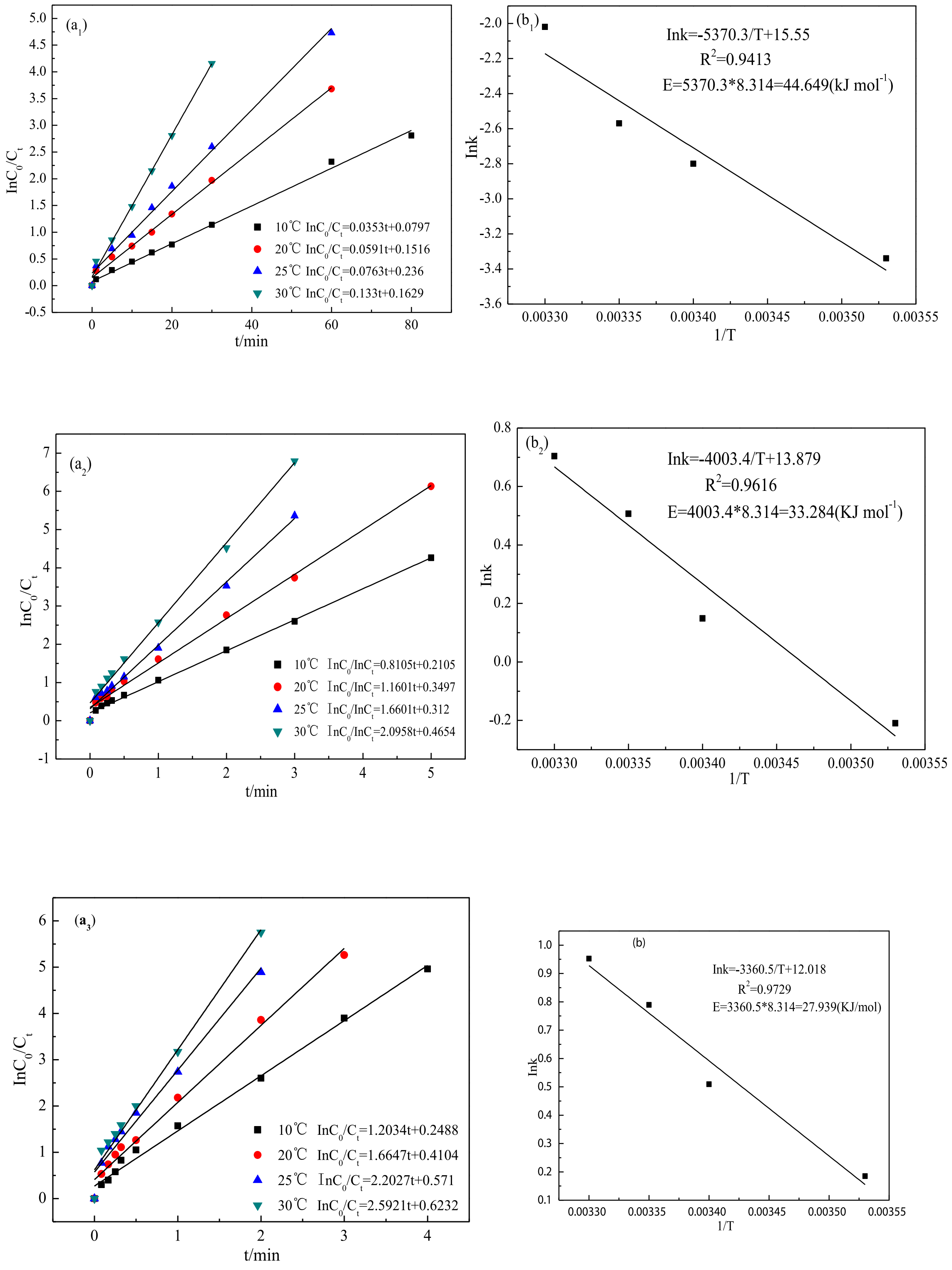


Fig. 3. Reaction activation energy calculation for E2 with NaClO. (a_1) Linear fit of kinetic data at different reaction temperatures in fresh water, (b_1) Linear of Lnk and 1/T in fresh water ($[E2]_0 = 0.1$ mM, 20 ml, $[NaClO]_0 = 60$ mM, 1 ml, pH = 7). (a_2) Linear fit of kinetic data at different reaction temperatures in synthetic marine aquaculture water, (b_2) Linear of Lnk and 1/T in synthetic marine aquaculture water ($[E2]_0 = 0.1$ mM, 20 ml, $[NaClO]_0 = 60$ mM, 1 ml, $[Cl^-]_0 = 6.6$ g L^{-1}, $[Br^-]_0 = 22$ mg L^{-1}, pH = 7). (a_3) Linear fit of kinetic data at different reaction temperatures in seawater, (b_3) Linear of Lnk and 1/T in seawater ($[E2]_0 = 0.1$ mM, 20 ml, $[NaClO]_0 = 60$ mM, 1 ml, $[Cl^-]_0 = 19$ g L^{-1}, $[Br^-]_0 = 65$ mg L^{-1}, pH = 7).

structures of the seven DBPs indicated that the hydroxyl on pentabasic cyclic ring of E2 was oxidized by NaClO in all of the water samples. The atom partial charges of the E2 molecule was presented in Scheme 1, as reported, the chlorination reaction would be facilitated by the negative charge on the phenolic compound (Hu et al., 2002). Therefore, the substitution reaction followed by dehydration will primarily occur at C2 and C4 position on E2 according to the reported chlorination mechanism. Then, the monochloro-compound p1 and dichloro-compound p2 were generated in fresh water. Furthermore, the atom partial charges

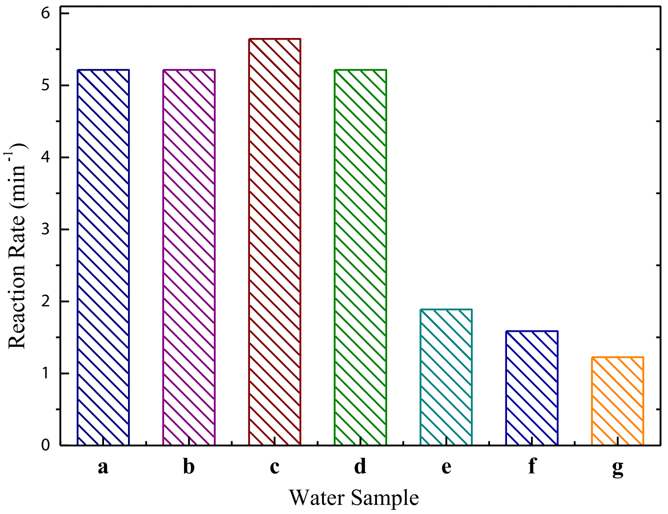


Fig. 4. The rates of E2 and NaClO in different water samples (a: Cl^-: 6.6 g L^{-1}, Br^-: 22 mg L^{-1}; b: Cl^-: 6.6 g L^{-1}, Br^-: 22 mg L^{-1}, Na^+: 3.59 g L^{-1}, Ca^{2+}: 133 mg L^{-1}, Mg^{2+}:430 mg L^{-1}, K^+:126 mg L^{-1}; c:Cl^-: 6.6 g L^{-1}, Br^-: 22 mg L^{-1}, SO_4^{2-}: 914 mg L^{-1}; d:Cl^-: 6.6 g L^{-1}, Br^-: 22 mg L^{-1}, HCO_3^-: 51 mg L^{-1}; e: Cl^-: 6.6 g L^{-1}, Br^-: 22 mg L^{-1}, HA: 0.5 mgC L^{-1}; f: Cl^-: 6.6 g L^{-1}, Br^-: 22 mg L^{-1}, HA: 1.0 mgC L^{-1}; g: Cl^-: 6.6 g L^{-1}, Br^-: 22 mg L^{-1}, HA: 2.0 mgC L^{-1}).

showed that C16 having relatively low negative partial charge and was susceptible to be attacked. The bond between C16 and C17 was breakdown and C17 was oxidized to form carboxyl groups. Then, a new product p3 was generated.

For synthetic marine aquaculture water and seawater in the presence of Br^-, p1 transformed to the monobromo-compound p4, p2 transformed to the dibromo-compound p5. The formation of these two Br-DBPs could be attributed to the faster substitution rate of Br^- as discussed above. Beyond that, oxidation of phenol moiety and bromine substitution reactions led to the transform of p3 to p6. Meanwhile, C10 with low negative partial charge was also attacked and resulted in the cleavage of C9-C10 bond. p7 was then formed combined with the oxidation of phenolic hydroxyl groups.

3.3. *Proposed reaction pathways*

3.3.1. *In fresh water*

All the byproducts of E2 reacting with excessive NaClO were identified to deduce the reaction pathway. Final and intermediate byproducts of E2 were estimated by the molecule mass difference and isotope patterns in the mass spectra. The mass spectral characterized data for these organic products were confirmed by LC/MS which were shown in Fig.S1 and Fig.S2. Based on the organic intermediates, three pathways (A, B and C) for the chloridization of E2 in fresh water are extrapolated as shown in Scheme 2. In pathway A, during the reaction in fresh water, E2 was oxidized to E1 (pf1) firstly, then the C4 atom was substituted by chlorine to formed p1 ($m/z = 304$). For pathway B, the molecular ions at m/z 306 and 340 in the mass spectra were observed, respectively. Based on the molecular and chlorine-substitution mechanism, these two byproducts were postulated to be monochloro-E2 (pf2) and dichloro-E2 (pf3). As the chemical reaction proceeds, the intermediate products pf2 and pf3 disappeared and the final product P2 was formed. For P3, no intermediate product was detected and it was deduced to form through the pathway C directly.

3.3.2. *In synthetic marine aquaculture water and seawater*

The intermediate and final products obtained from the synthetic marine aquaculture water and seawater samples were substantially the same. The ion mass spectra of these byproducts were presented in Fig.S3. The four possible transformation pathways of E2 in the presence of both chlorine and bromine were shown in Scheme 3. In the first 30min, the molecular ion of monochloro product ps1 was recorded at $m/z = 304$, then more stable monobromo-compound p4 was formed based on bromine substitution through the pathway A. The intermediate ps2 with $m/z = 306$ was the same with pf2 in the fresh water reaction system. Then, the chlorine on ps2 was substituted by bromine to form monobromo-E2 ps3 ($m/z = 350$) and p5 was produced through pathway B-1 after the alcohol hydroxyl group on dibromo-E2 ps5 ($m/z = 428$) was oxidized. Another intermediate 2-bromo-4-chloro-E1 ps4 with $m/z = 382$ indicated the possibility of pathway B-2 for p5. On the basis of the above results, two types reactions in pathway A and pathway B have been proposed, which includes (1) the bromine substitution reaction followed by dehydration of the phenol moiety and (2) the reactions of bromine substitute chlorine. In addition, for the compound p6, pathway C was inferred based on the detection of the intermediate ps6 with $m/z = 322$. The molecular ions of p7 being recorded at $m/z = 364$, it has not been detected with intermediate product. Thus, p7 was deduced to form through the pathway D directly.

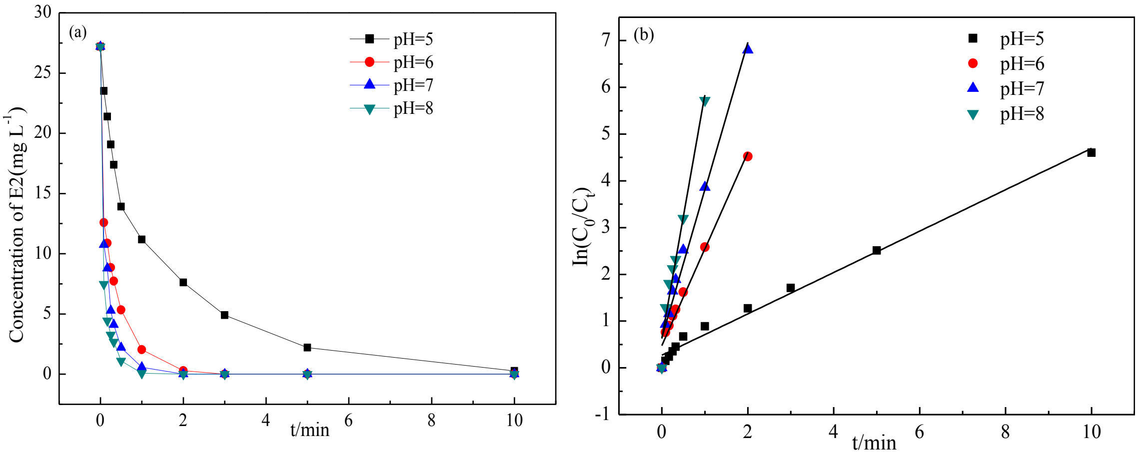
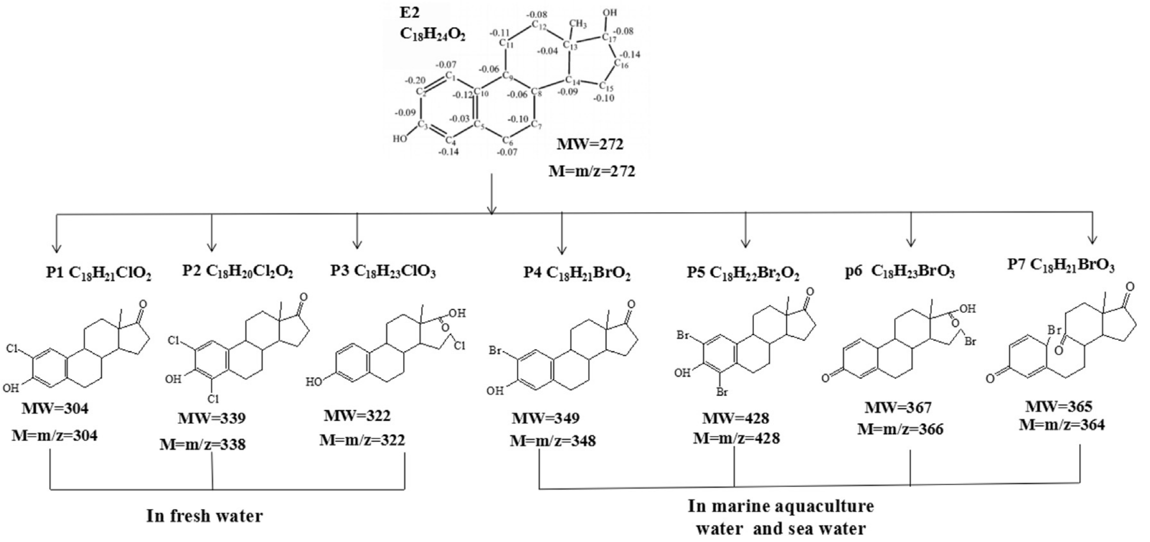
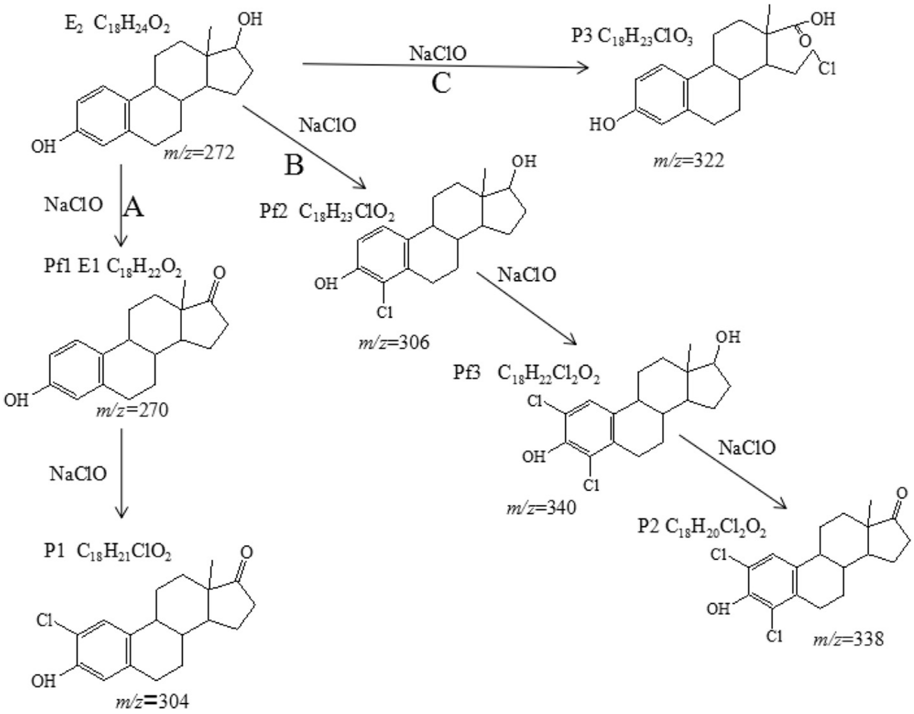


Fig. 5. Oxidation of E2 by NaClO at different pH values and the linear fit of the kinetic data ($[E2]_0 = 0.1$ mM, 20 ml, $[NaClO]_0 = 60$ mM, 1 ml, $[Cl^-]_0 = 6.6$ g L^{-1}, $[Br^-]_0 = 22$ mg L $^{-1}$,$T = 298.15$ K).



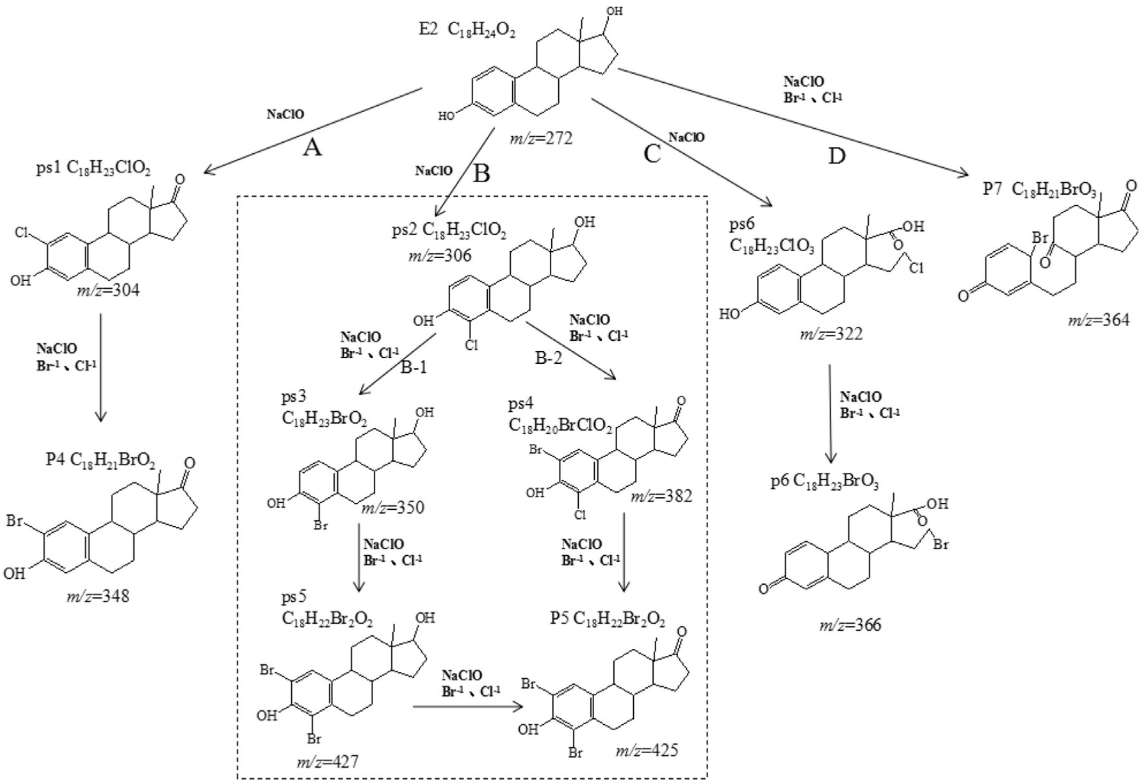
Scheme 1. Atom partial charge of 17β-estradiol and final products of E2 reactions with NaClO in different water samples ($[E2]_0 = 0.1$ mM, 20 ml, $[NaClO]_0 = 60$ mM, 1 ml, pH = 7, T = 298.15 K).



Scheme 2. Products and proposed pathways for E2 reactions with NaClO in fresh water ($[E2]_0 = 0.1$ mM, 20 ml, $[NaClO]_0 = 60$ mM, 1 ml, pH = 7, T = 298.15 K).

3.4. Products identified in actual marine aquaculture water

In the actual marine aquaculture water, besides Br^- and Cl^-, there are many other anions such as carbonate and sulfate. Considering that the anions may affect the formation of DBPs, we added E2 and NaClO to the actual marine aquaculture water. We thus examined the reaction in the water sample which was collected from a shrimp pond. Through LC-MS analysis, although the composition of the actual marine aquaculture water was complex, the four Br-DBPs and three Cl-DBPs were still found as shown in the Fig. 6. The result proved the transformation of the Cl-DBPs to Br-DBPs in the presence of Br^-. Moreover, to reveal the actual state of the DBPs formation during the real marine aquaculture disinfection process, the typical DBPs including THMs and HAAs were also examined. After joining NaClO 2 h later, the concentration of THMs and HAAs were determined to be 154 mg L^{-1} and 109 mg L^{-1}, respectively. The detailed analysis of species composition showed that the content of Br-DBPs was significantly higher than that of Cl-DBPs. Tribromomethane (TBM) and tribromoacetic acid (TBAA) accounted for 77% and 40% of the total five THMs and seven detected HAAs, respectively. While in the fresh water reaction system, almost no brominated products were



Scheme 3. Products and proposed pathways for E2 reactions with NaClO in synthetic marine aquaculture water and seawater ($[E2]_0 = 0.1$ mM, 20 ml, $[NaClO]_0 = 60$ mM, 1 ml, pH = 7, T = 298.15 K).

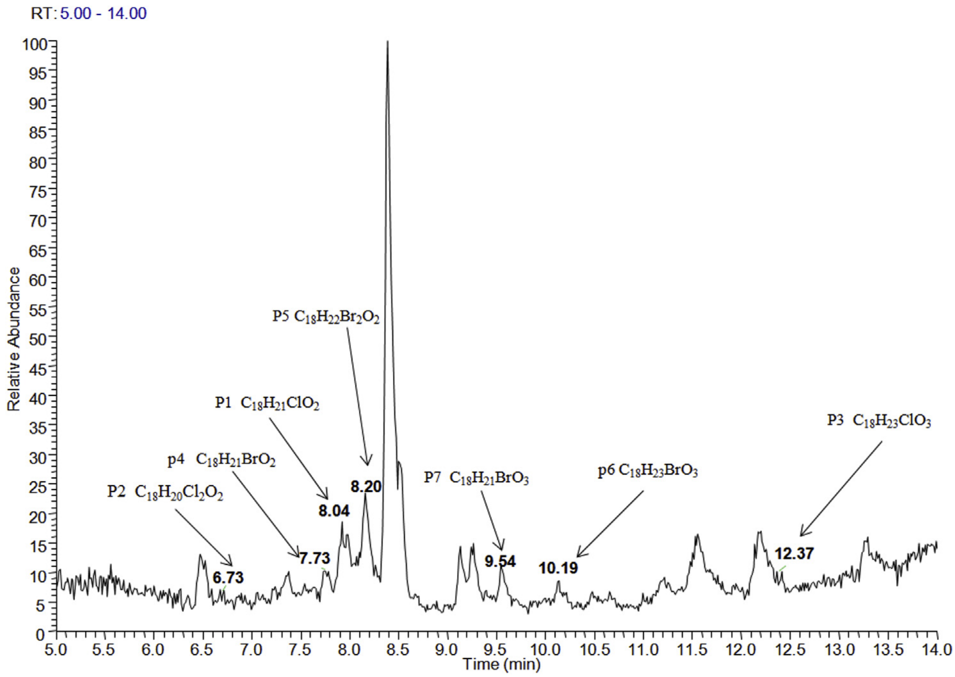


Fig. 6. Chromatogram of products formed in the actual marine aquaculture water.

detected. The results were coincide with the literature reported that the DBPs will transport from their chlorinated species to brominated species when Br^- was contained in the water (Qiang et al., 2014).

3.5. Preliminary analysis of toxicity

Due to the inability to separate the Cl/Br-DBPs and lack of the standard samples, the toxicities of the DBPs could only be predicted based on their structures. EPI-suite was used to make comparison of the toxicity of Cl/Br-DBPs. Bio-concentration factor (BCF) which is an important index to describe the accumulation trend of chemical substances in organisms was selected as the predictive toxic parameter. The value of log BCF was usually adopted. The larger the value of log BCF, the easier it is to accumulate in the organism, and the more toxic the compound is.

In freshwater, the log BCF value of the dichloride p2 (2.782) was greater than that of the monochloride p1 (2.356) and p3 (1.002). In synthetic marine aquaculture water and seawater, the log BCF value of dibromide p5 (3.106) was greater than that of monobromide p4 (2.518), p6 (0.500) and p7 (1.171). The results proved that the biological toxicity increased with the increase of the halogen atoms. Moreover, the log BCF values of Cl/Br-DBPs in the same substituent positions on E2 were compared. The Br-DBPs p4 and p5 obtained in the synthetic marine aquaculture water and seawater were compared to Cl-DBPs p1 and p2, respectively. For P1 and P4, the substituent group on the phenol moiety was Cl^- and Br^-, respectively. Their corresponding calculation results of log BCF were 2.356 and 2.518. Similarly, For P2 and P5, their corresponding calculation result of log BCF was 2.782 and 3.106. The results showed that the Br-DBPs were more toxic than their chloride analogues.

AOX as a reference indicator to measure the water toxicity was also detected. The concentration of AOX obtained from the fresh water, marine aquaculture water and seawater was 1.4878 $mg\,L^{-1}$, 3.2304 $mg\,L^{-1}$ and 6.059 $mg\,L^{-1}$, respectively. Combined with the results of log BCF and AOX, we considered that the DBPs produced in the marine aquaculture water were more toxic than that in the fresh water mainly because some Cl-DBPs transformed to more toxic Br-DBPs which was consistent with the reported researches on toxicity of DBPs (Uyak and Toroz, 2007; Sun et al., 2009).

4. Conclusions

The aim of our work was to investigate of the fate of E2 which could be oxidized by NaClO in fresh water, synthetic marine aquaculture water and seawater. The results of the present study showed that the reaction rate constants increased with the increase of Br^- concentration and the initial pH value. The reaction activation energies corresponding to the reaction of NaClO with E2 in the fresh water, synthetic marine aquaculture water and seawater were calculated to be 44.649 $KJ\,mol^{-1}$, 33.284 $KJ\,mol^{-1}$ and 27.939 $KJ\,mol^{-1}$, respectively. The results indicated that the oxidation of E2 by NaClO was more easily in the presence of Br^-. Moreover, seven DBPs have been detected in the different water disinfection processes. In fresh water, the main reaction was the substitution reaction followed by dehydration at the ortho position of the phenol moiety, the hydroxyl group on pentabasic cyclic ring of E2 was oxidized at the same time. In addition to these reactions, bromine substitution and the oxidation of phenolic hydroxyl groups occurred in synthetic marine aquaculture water and seawater. In the actual marine aquaculture water, besides Cl^- and Br^-, there are some other anions such as carbonate and sulfate, we still find the identified 4 kinds of Br-DBPs mentioned above. The preliminary toxicity analysis by BCF value comparison and AOX value suggested that Br-DBPs were more toxic than the corresponding Cl-DBPs.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.05.035.

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