



Effect of water composition on TiO₂ photocatalytic removal of endocrine disrupting compounds (EDCs) and estrogenic activity from secondary effluent

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ABSTRACT

The effect of inorganic ions and dissolved organic matter (DOM) on the TiO₂ photocatalytic removal of estrogenic activity from secondary effluents of municipal wastewater treatment plants was investigated. The presence of HPO₄²⁻, NH₄⁺, and HCO₃⁻ resulted in a significantly negative impact on the photocatalytic removal of estrogenic activity from synthetic water due to their strong adsorption on the surface of TiO₂. However, only a weak impact was noted during photocatalytic removal of estrogenic activity from secondary effluent with these ions added, since the presence of DOM in real wastewater played a more important role in inhibiting photocatalytic removal of estrogenic activity than inorganic ions. By investigating the effect of different DOM fractions on photocatalytic removal of estrogenic activity, polar compounds (PC) were found to cause a temporary increase in estrogenic activity during TiO₂ photocatalysis. Fluorescence spectroscopy and molecular weight (MW) analysis on secondary effluent spiked with PC during TiO₂ photocatalysis suggest that large MW organic matter (>4.5 kDa) in secondary effluent, such as humic/fulvic acid, not only could play an important role in inhibiting photocatalytic removal of estrogenic activity but also is responsible for the temporary increase in estrogenic activity during the same process.

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1. Introduction

Water demand has been increasing with population growth, industrialization, and expansion of irrigated agriculture in recent years. Therefore, wastewater reclamation and reuse have become essential for conserving and extending available water supplies [1]. Conventional municipal wastewater treatment plants (MWWTPs), typically based on biological processes, are capable of removing some ordinary substances, whereas nonbiodegradable compounds may escape the treatment and be released into the environment [1]. Recently, many types of micropollution have been documented in the effluents of MWWTPs [2–5]. Among them, endocrine disrupting chemicals (EDCs), which may cause adverse effects on aquatic organisms and humans via interactions with the endocrine system, have been found at mean concentrations ranging from ng/L to µg/L [6–8]. EDCs can induce a number of reproductive and sexual abnormalities in wildlife even at trace levels [9]. Therefore, effective advanced treatments are required to remove these substances and ensure the quality of the reused water.

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Among the advanced treatments capable of degrading EDCs, TiO₂ photocatalytic oxidation has been one of the most promising technologies since the first report of the use of TiO₂ photocatalysis for the degradation of estrogens in water [10]. In recent years, many studies have been conducted on the removal of single EDCs or EDC mixtures from deionized water or surface water during TiO₂ photocatalysis. For example, Ohko et al. [11] reported the degradation of 17β-estradiol (E2, 1 µM) in TiO₂ water suspensions by photocatalysis. Results show that more than 99% of the E2 was degraded within 30 min and the estrogenic activity was almost concurrently lost with photocatalytic degradation. Coleman et al. [12] investigated the removal of estrogenic activity of several potent and environmentally relevant steroid estrogens [estrone (E1), E2, and 17α-ethynylestradiol (EE2), 10 µg/L] by photocatalysis over an immobilized TiO₂ catalyst. Their results show the three estrogens were decomposed and all estrogenic activity was removed within 55 min. Benotti et al. [13] assessed 32 pharmaceuticals and EDCs removed from surface water in a TiO₂ photocatalytic reactor membrane pilot system. Their results indicate that no estrogenically active transformation products were formed during treatment.

However, the initial concentrations of EDCs used in the foregoing studies were in the range of mg/L to g/L, much higher than those in the secondary effluent of MWWTPs (ng/L to mg/L). Related research concerning photocatalytic removal of EDCs from secondary effluent

using TiO_2 is limited [14–16]. It has been reported in our previous research that TiO_2 photocatalysis is an effective method for the rapid removal of certain EDCs [E1, E2, estriol (E3), nonylphenol (NP), and bisphenol A (BPA)] and estrogenic activity from secondary effluents of MWTPs [16]. It was also found that the photocatalytic reduction rates of EDCs and estrogenic activity were depressed by the inorganic ions and dissolved organic matter (DOM) in the secondary effluent [16].

Several researches focusing on the influence of inorganic ions and organic substances on TiO_2 photocatalysis have recently been published [17–20]. Karpova et al. [17,18] investigated the influence of urea, saccharose, and ethanol on photocatalytic oxidation of steroid estrogens. They found that the influence depended on solutions pH and the structures of steroid estrogens. Other authors investigated the influence of inorganic ions on TiO_2 photocatalytic degradation of azo dyes, dichloroethane, naphthalene and so on [19,20]. There seems to be a general consensus that inorganic ions affect these photocatalytic processes by their adsorption onto the surface of TiO_2 and trapping positive hole and hydroxyl radicals during photocatalysis.

However, most of the researches focusing on the influence of water composition on TiO_2 photocatalysis were conducted by means of bench-scale tests, using model solutions of commercially available inorganic salt and certain organic matter with limited comparability to secondary effluents of MWTPs. Moreover, in secondary effluents of MWTPs, the presence of EDCs at low levels is accompanied by lots of other substances at high levels, such as NH_4^+ , NO_3^- , HPO_4^{2-} , microbial products, humic acids and so on [21]. Therefore, these various co-pollutant substances may cause an unknown influence on photocatalytic removal of EDCs and estrogenic activity. To our knowledge, no study has investigated the effect of secondary effluent composition on TiO_2 photocatalytic removal of EDCs and estrogenic activity. In order to better understand the removal mechanism of EDCs and estrogenic activity in secondary effluent by TiO_2 photocatalysis, it is important to clarify this issue.

Therefore, the aim of this study is to evaluate the impact of secondary effluent composition (inorganic ions and DOM) on TiO_2 photocatalytic removal of EDCs and estrogenic activity. In this work, high-performance size exclusion chromatography (HPSEC) and fluorescence excitation–emission matrix (EEM) spectroscopy were used to provide an assessment of DOM removal during TiO_2 photocatalysis and to investigate the detailed effect of DOM composition on the process. The experimental protocol was designed to study photocatalytic removal of estrogenic activity in a relatively uncomplicated aqueous matrix (synthetic water) first, before gradually increasing complexity by studying secondary effluent, to increase understanding of the reaction mechanism in secondary effluent of a MWTP and acquire information on the process operation.

2. Materials and methods

2.1. Reagents

Titanium dioxide powder P25 (greater than 99.5% purity, average primary particle size of 21 nm, BET surface area of $50 \pm 15 \text{ m}^2/\text{g}$) used in this study was purchased from Degussa (Germany). The seven EDCs [E1, E2, E3, EE2, octylphenol (OP), NP, and BPA] were purchased from Sigma–Aldrich and used without further purification. The inorganic salts used in the synthetic water were Na_2HPO_4 , NaHCO_3 , NaNO_3 , Na_2SO_4 , NaCl , and NH_4Cl . All reagents used for chromatographic analyses were of HPLC grade. Analytical standards for chromatography analyses were purchased from Sigma–Aldrich.

2.2. Water samples

Milli-Q water from a Milli-Q purification unit spiked with 100 ng/L of each EDC was used in the experiments. The salts HPO_4^{2-} , HCO_3^- , SO_4^{2-} , Cl^- , NO_3^- , and NH_4^+ were separately added to the above-mentioned synthetic water. The concentration of each ion was 0.2 mM, equivalent to 19 mg/L of HPO_4^{2-} , 12 mg/L of HCO_3^- , 19 mg/L of SO_4^{2-} , 7 mg/L of Cl^- , 12.3 mg/L of NO_3^- , and 3.6 mg/L of NH_4^+ .

The secondary effluents used in this study were taken from a municipal wastewater treatment plant (Suzhou, China), in which the anaerobic–anoxic–oxic process was used as a secondary treatment. All samples were stored in acid-washed amber bottles, and stored in ice for transport to the laboratory, where they were immediately processed for later extraction and analysis. The effluent characteristics are summarized in Table S1.

NH_4Cl , Na_2HPO_4 , and NaNO_3 were separately added to the secondary effluent to obtain wastewater samples containing 8 mg N/L, 1 mg P/L, and 12 mg N/L.

2.3. Concentration of wastewater samples

Secondary effluent samples (105 mL) were acidified to pH 2.0 with 2 M H_2SO_4 , and passed through Oasis HLB resin cartridges (Waters Corporation, America) previously washed with 10 mL of methanol and 10 mL of ultrapure water at a flow rate of 1–2 mL/min. Retained organics on the cartridge were eluted with 10 mL methanol to obtain polar compounds (PC), followed by 10 mL dichloromethane to yield midpolar compounds (MPC), and finally, by 10 mL hexane to obtain nonpolar compounds (NPC). The three fractions were dried under nitrogen flow. Thereafter, the dry residues were dissolved in 105 μL dimethylsulfoxide to obtain a 1000-fold concentration for estrogenic activity assay.

2.4. Photocatalytic experiments

The photocatalytic experiments were conducted in a cylindrical glass reactor (60 cm height, 18 cm outer diameter, and 15 cm inner diameter). The double-walled glass reservoir was equipped with a thermostatically controlled water flow between the outer walls. The TiO_2 powder was added into the 8 L solution to give a loading of 0.8 g/L. The aqueous solutions were stirred with a magnetic stirrer for 40 min after TiO_2 was added. The suspension was then irradiated with a Philips TL 6-W black light lamp which was positioned vertically in the middle of the reactor. The lamp provided a nominal UVA radiation of 0.7 W, with a maximum emission peak centered at 365 nm. The solution was sampled for analysis from the reactor cell using a glass syringe at specified time intervals during degradation.

2.5. Analytical methods

Inorganic ions were quantified using ion chromatography (IC, Waters 2690, USA) coupled with an IC-PAK anion HR column (4.6 mm \times 75 mm, Waters, USA) and a 432 conductivity detector (Waters, USA).

To determine the EDCs in secondary effluent, solid phase extraction (SPE) method was applied to concentrate the compounds from the wastewater sample using Oasis HLB resin cartridges (Waters Corporation, America) previously washed with 10 mL of ethyl acetate, 10 mL methanol, and 15 mL ultrapure water. Then, water samples were extracted at a flow rate lower than 5 mL/min. After washing the cartridges with 10 mL ultrapure water:methanol (9:1), they were dried under vacuum for 30 min and eluted with 15 mL of ethyl acetate at a flow rate of 1 mL/min. Extracts were reduced to 1 mL under a gentle nitrogen flow at 40 °C and further

evaporated to dryness under nitrogen at room temperature. The dried eluate post SPE was derivatised with 100 μ L pyridine and 50 μ L *N,O*-bis(trimethylsilyl) trifluoroacetamide solution containing 1% trimethylchlorosilane (Sigma) at 70 °C for 60 min, followed by cooling down to room temperature. A gas chromatography tandem mass spectrometry system (GC: 7890 Series; MS: 5975c Network, Agilent) with a DB-5MS capillary column (30 m \times 0.25 mm \times 0.25 μ m) for organic separation was used to analyze the EDCs. The estrogenic activity of the concentrated samples was evaluated with a two-hybrid assay based on yeast cells (*Saccharomyces cerevisiae* Y190) containing the rat estrogen receptor ER α and the coactivator TIF2. In the assay, the expression of β -galactosidase was used to monitor estrogenic activity of the estrogenic chemicals. The ability of the concentrated sample to inhibit β -galactosidase activity of E2 was measured to determine the anti-estrogenic activity of the concentrated sample according to the yeast two-hybrid assay. In the assay, tamoxifen (TAM) was used as a standard anti-estrogen. Detailed procedures of EDC extraction, estrogenic activity and anti-estrogenic activity assay can be found in the Supporting Information (Methods S1 and S2).

The dissolved organic carbon (DOC) was analyzed by a TOC Analyzer (Phoenix 8000 system, Tekmar-Dohrman Co., USA) using the method of sodium peroxydisulphate/orthophosphoric acid wet oxidation and UV radiation after filtration through a 0.45 μ m membrane. HPSEC (Shimadzu, Corp., Japan) with a SEC column (Protein-pak125, Waters, Milford, USA) was used to determine the molecular weight (MW) distributions of the wastewater samples. Polyethylene glycol standards of various MW (330, 700, 1050, 5050, and 10,225 Da) were used to calibrate the equipment. Fluorescence spectra of wastewater sample before and after TiO₂ photocatalysis were recorded on a fluorescence spectrophotometer (model F-7000, Hitachi, Japan). Three-dimensional spectra were obtained as previously described [22]. All contour maps were plotted using the same scale range of fluorescence intensities and number of contours.

3. Results and discussion

3.1. Effect of inorganic ions on estrogenic activity removal from synthetic water

Results of the TiO₂ photocatalytic removal of estrogenic activity (measured by the yeast cell test) from synthetic water [Milli-Q water spiked with 100 ng/L of each EDC (E1, E2, E3, EE2, OP, NP, and BPA)] are shown in Fig. 1. The estrogenic activity was almost totally removed by TiO₂ photocatalysis in 50 min with 0.2 mM SO₄²⁻, Cl⁻, and NO₃⁻ added. Comparatively, only 48.2%, 39.5%, and 78.3% reduction occurred during the same process with NH₄⁺, HPO₄²⁻, and HCO₃⁻ added. The inhibition of photocatalytic removal of estrogenic activity by ions is in the following decreasing order: HPO₄²⁻ > NH₄⁺ > HCO₃⁻ > SO₄²⁻ > NO₃⁻ > Cl⁻. Generally, photocatalysis can be divided into three main steps: the preliminary adsorption of the substrates on the surface of the photocatalyst, the generation of holes (h⁺) and free radicals [principally hydroxyl radicals (HO[•])] by the irradiated photocatalyst, and the oxidation reactions between the h⁺/HO[•] and the substrates on the surface of photocatalyst. The photocatalytic reaction rate strongly depends on the amount of the substrates adsorbed on the photocatalyst surface and the reaction rate between the h⁺/HO[•] and the adsorbed substrates [23,24]. Therefore, the process is affected by two interactions, i.e., ion-EDCs-TiO₂ adsorption and ion-EDCs-h⁺/HO[•] reaction. Which interaction is the rate-controlling step must be understood to optimize the process.

In the first stage, before the photocatalytic reaction starts, the inorganic ions compete with the adsorption of EDCs for

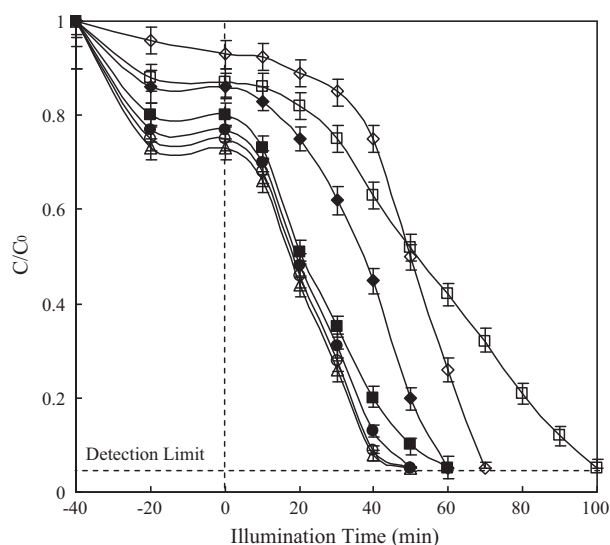


Fig. 1. Reduction of estrogenic activity of synthetic water with some ions added during the photocatalysis process: TiO₂ (0.8 g/L), NH₄⁺ (◇), HCO₃⁻ (◆), HPO₄²⁻ (□), Cl⁻ (○), NO₃⁻ (●); SO₄²⁻ (■), without addition of ions (△). Initial estrogenic activity: 178.7 ng-E2 equivalents/L; the incident photon flux: 2.8×10^{-4} E m⁻² s⁻¹. Error bars represent the standard deviation on triplicate analyses.

the surface site of TiO₂ in the dark. The inorganic ions are ranked according to their effect on the competitive adsorption: NH₄⁺ > HPO₄²⁻ > HCO₃⁻ > SO₄²⁻ > NO₃⁻ > Cl⁻ (as shown in Fig. 1). The pH has an effect on the electrostatic charge of the TiO₂ surface, which determines the density of TiOH²⁺ groups [25]. Consequently, the adsorption performance and the photocatalytic reactivity of TiO₂ could be modified by pH changes. For pH values higher than the point of zero charge (PZC, 6.5) of titania, the surface becomes negatively charged. In contrast, it is positive for pH lower than PZC. The pH values of the synthetic water samples were 8.2, 7.3, 7.0, 7.0, 7.0, and 6.6, respectively, with HPO₄²⁻, HCO₃⁻, SO₄²⁻, Cl⁻, NO₃⁻, and NH₄⁺ added. At these working pH values, TiO₂ is negatively charged (>PZC), resulting in an enhanced adsorption for NH₄⁺ and a depressed adsorption for HPO₄²⁻, HCO₃⁻, SO₄²⁻, Cl⁻, and NO₃⁻.

The TiO₂ surface strongly adsorbs HPO₄²⁻ ions. The adsorption of phosphate takes place as a ligand attaches to a surface Ti⁴⁺ ion by an exchange reaction between the surface hydroxyl groups of TiO₂ and HPO₄²⁻ [26]. The bridging bidentate complex formed is adsorbed on the TiO₂ surface and is essentially invariable with pH change [26]. Therefore, the photocatalytic reactivity of the photocatalyst is inhibited in a long-lasting manner by the adsorbed phosphate. This is the reason for the lower rate of estrogenic activity abatement in the system with HPO₄²⁻ compared to NH₄⁺ (Fig. 1). Similar results can also be found by measuring the concentrations of inorganic ions in the systems (Fig. S1). Within the dark adsorption phase, the amount of SO₄²⁻, Cl⁻, and NO₃⁻ remained almost constant in the solution, whereas 73.4%, 62.5%, and 49.6% of NH₄⁺, HPO₄²⁻, and HCO₃⁻ were absorbed.

After photo-generation of holes and free radicals, the adsorbed ions compete for them with the adsorbed organic compounds. In principle, ion-radicals are also capable of oxidizing pollutants, but at rates lower than that of h⁺/HO[•], due to their lower oxidation power [27]. Therefore, the inhibition order can be explained by the rates of scavenging of h⁺/HO[•] by inorganic ions. It has been reported in previous studies that NO₃⁻ and SO₄²⁻ were almost unreactive toward h⁺/HO[•] [28,29]. Also it is noted that Cl⁻ scavenges h⁺/HO[•] only in acidic solution, where it forms Cl[•]/Cl₂^{•-}. However, in neutral conditions, Cl⁻ cannot scavenge h⁺/HO[•] without protonation [30]. NH₄⁺, HCO₃⁻ and HPO₄²⁻ quench h⁺/HO[•]

more efficiently than the above three inorganic ions. The rate constants for quenching of hydroxyl radicals by NH_4^+ , HCO_3^- and HPO_4^{2-} are 1.0×10^8 , 5.7×10^6 and $6.0 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, respectively [30–32]. Therefore, the ions are ranked according to their reaction rates with $\text{h}^+/\text{HO}^\bullet$: $\text{NH}_4^+ > \text{HCO}_3^- > \text{HPO}_4^{2-} > \text{SO}_4^{2-} \approx \text{NO}_3^- \approx \text{Cl}^-$. Similar result was also found in this study (as shown in Fig. 1). By comparison of adsorption of ions on the photocatalyst, of their ability to scavenge HO^\bullet and of their effect on EDC removal, it can be concluded that the rate-controlling step of the systems is not ion-EDCs- $\text{h}^+/\text{HO}^\bullet$ reaction but the adsorption of ion-EDCs- TiO_2 . Moreover, NH_4^+ and HPO_4^{2-} show a significant negative effect on the process due to their strong competition for adsorption sites with the targeted EDCs on the TiO_2 surface.

3.2. Effect of inorganic ions on estrogenic activity removal from secondary effluent

Results of the TiO_2 photocatalytic removal of estrogenic activity from the secondary effluent samples spiked with a given amount of NH_4Cl , Na_2HPO_4 , and NaNO_3 are shown in Fig. S2. The estrogenic activities (original 52.4 ng-E2 equivalents/L) of all these wastewater samples were almost totally removed by TiO_2 photocatalysis within 180 min. The inhibition order of photocatalytic removal of estrogenic activity by ions in wastewater was similar to that in the synthetic water. However, the rates of the photocatalytic reduction of estrogenic activity in wastewater samples with different inorganic ions showed less variation (0.0072 – 0.0085 min^{-1} , 15.3%) than in synthetic water (0.0211 – 0.0567 min^{-1} , 62.7%). These findings suggest that the inhibition of the removal of estrogenic activity by these ions was weaker in secondary effluent than that in synthetic water.

The difference in the rate variation can be explained by the fact that the DOM in secondary effluent could compete for adsorption sites on the TiO_2 surface and scavenge hydroxyl radicals [33,34]. DOM in the secondary effluent could play a more important role in inhibiting photocatalytic removal of estrogenic activity than the inorganic ions do. DOM in secondary effluent is a complex heterogeneous mixture composed of humic acids, fulvic acids, low-molecular weight organic acids, carbohydrates, proteins, and other compound classes [21]. Moreover, some of the DOM in wastewater is made up of estrogenic substances. Therefore, further studies on the evolution of estrogenic activity of different DOM fractions and their effect on photocatalytic removal of estrogenic activity in secondary effluent are necessary.

3.3. Evolution of estrogenic activity of different DOM fractions

DOM fractions of the secondary effluent exhibited different proportions of DOC distribution (Fig. 2). The MPC fraction was found to be the most abundant, constituting about 57% of DOC. PC was the second most dominant fraction, accounting for about 30% of DOC. The NPC fraction was the smallest among all the fractions, constituting about 13% of DOC. For the three fractions, PC and MPC exhibited significant estrogenic activity, whereas that of NPC was below the detection limit (1 ng E2 equivalents/L). However, the sum of estrogenic activity of the three fractions was higher than that of the original sample. All the fractions did not inhibit the growth of yeast cells (data not shown). Some compounds in the secondary effluent may have anti-estrogenic activity [35]. Some chemicals may adsorb the estrogenic chemicals and mask the estrogenic activity in the yeast bioassay analysis, resulting in a false-positive result [36]. All these compounds are responsible for approximately a 42.7% reduction in estrogenic activity of the secondary effluent sample.

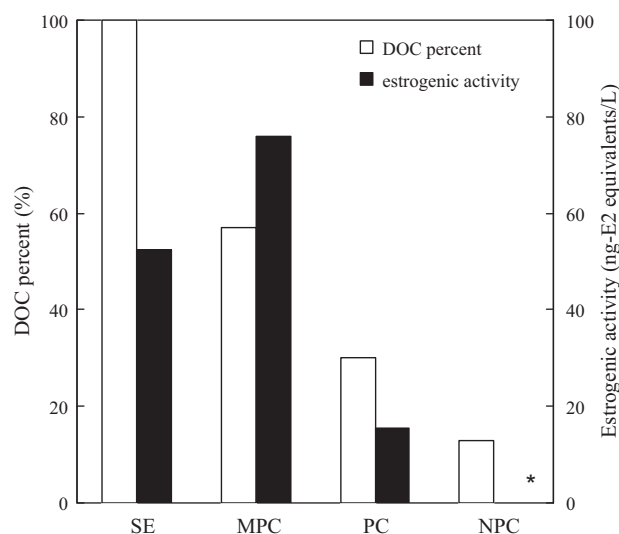


Fig. 2. Estrogenic activity and percent of DOC of secondary effluent sample (SE) and its fractions from municipal wastewater treatment plant. Asterisks represent the estrogenic activity of samples which was below method detection limit (1 ng-E2 equivalents/L).

3.4. Effect of DOM fractions on the photocatalytic removal of estrogenic activity from secondary effluent

To investigate the effect of the three fractions on estrogenic activity removal by TiO_2 photocatalysis, three fractions were firstly concentrated from the 8 L secondary effluent (collected from the MWTP in Suzhou). Then they were separately added to three secondary effluent samples collected from the same MWTP. Thereafter, the estrogenic activities of the samples were measured during TiO_2 photocatalysis (Fig. 3). The estrogenic activities of samples with both PC and NPC noticeably decreased. However, in the sample with the PC fraction added, the estrogenic activity increased from 31 to 78 ng E2 equivalents/L, and then decreased to the value below the detected limit within 200 min.

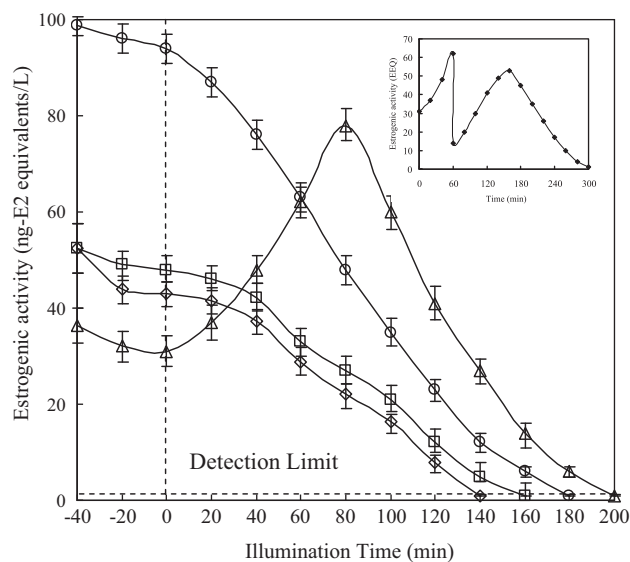


Fig. 3. Temporal profile of estrogenic activity of wastewater sample (\diamond) and the samples spiked with PC (\triangle), NPC (\square) and MPC (\circ) each during the TiO_2 photocatalysis. TiO_2 : 0.8 g/L, the incident photon flux: $2.8 \times 10^{-4} \text{ E m}^{-2} \text{ s}^{-1}$. Error bars represent the standard deviation on triplicate analyses. The inset contains estrogenic activity removal during TiO_2 photocatalysis with a amount of commercial humic acids (4.6 mg/L, 8 L) added.

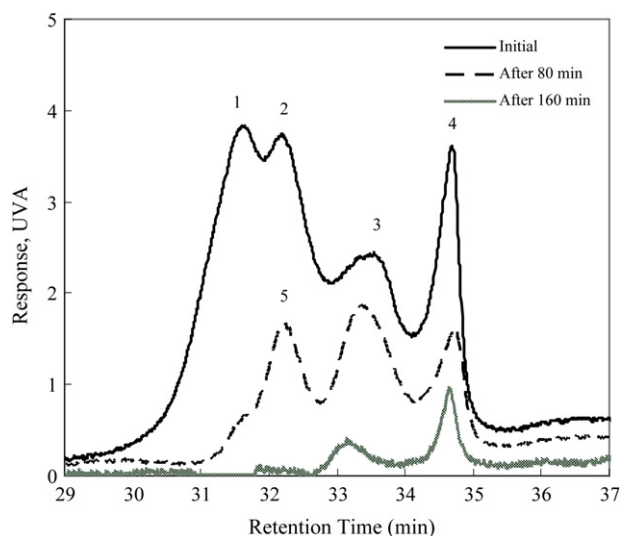


Fig. 4. Molecular weight (MW) distribution of secondary effluent sample spiked with PC during TiO₂ photocatalysis: (1) MW: 5593 Da, (2) MW: 4552 Da, (3) MW: 2837 Da, (4) MW: 1902 Da, (5) MW: 4525 Da. TiO₂: 0.8 g/L, the incident photon flux: $2.8 \times 10^{-4} \text{ E m}^{-2} \text{ s}^{-1}$.

There may be two main explanations for the phenomenon of temporary increase in estrogenic activity during TiO₂ photocatalytic removal of EDCs from the wastewater sample with added PC. Different estrogens interact in synergy, resulting in an inhibition of the expression of the yeast-based estrogen assay at the initial stage of reaction. The yeast-based enzyme expression then changes at a suitable concentration of estrogenic compounds [37]. The estrogenic activity of the wastewater sample could be inhibited by the presence of some compounds with anti-estrogenic activity and/or some macromolecules, which may mask the estrogenic activity in the yeast bioassay analysis at the outset of the reaction. The estrogenic activity of the wastewater sample increased with the degradation of these compounds. Detailed discussions of the two possible explanations are as follows.

First, five EDCs (E1, E2, E3, NP, and BPA) considered priority pollutants for their high estrogenic activity and wide occurrence in wastewater [38,39] have been measured by GC–MS during TiO₂ photocatalysis in the secondary effluent with PC added, as shown in Fig. S3. All photocatalytic degradations of the five EDCs exhibited pseudo first-order reaction kinetics, such as E1 (0.0272 min^{-1} , $R^2 = 0.969$), E2 (0.0269 min^{-1} , $R^2 = 0.962$), E3 (0.0242 min^{-1} , $R^2 = 0.956$), NP (0.0356 min^{-1} , $R^2 = 0.953$), and BPA (0.026 min^{-1} , $R^2 = 0.983$). The fact that all EDC photodegradations followed pseudo first-order reaction kinetics suggests that EDCs undergo almost the same photodegradation mechanism, and that no polymerization reaction occurs during the TiO₂ photocatalysis. This confirms that the temporary increase of the estrogenic activity during TiO₂ photocatalysis is caused by the presence of anti-estrogenic chemicals and/or macromolecules in the secondary effluent.

Second, the MW of the typical anti-estrogenic chemicals in wastewater, such as phenylalanine, 4-hydroxytamoxifen, and benzotriazole, are lower than 1 kDa, whereas the macromolecules that may adsorb the estrogenic chemicals have a MW higher than 1 kDa [40,41]. Therefore, to investigate which compounds are accountable for the temporary increase in estrogenic activity in the secondary effluent with PC fraction, a detailed MW distribution analysis on the wastewater sample with PC added during TiO₂ photocatalysis was performed (Fig. 4). The HPLC analysis was carried out at 260 nm to assess the photodegradation of aromatic rings of the organic substances [42]. The results depict

consecutive degradation of large-MW organic matter into small-MW compounds, followed by potential mineralization into innocuous substances during TiO₂ photocatalysis. In the initial 80 min after photocatalytic reactions started, the organic compounds with MW larger than 4.5 kDa were continually photocatalyzed into compounds with lower MW. Meanwhile, the estrogenic activities of the wastewater samples increased and the macromolecules decomposed at the same time. Therefore, it can be found that macromolecules degradation (larger than 4.5 kDa) could have a role in the observed increase of estrogenic activity. Moreover, the anti-estrogenic activities of the samples at 0 and 80 min were measured. It was found that the anti-estrogenic activities of the two samples were approximately equal ($0.44 \pm 0.004 \mu\text{mol-TAML}^{-1}$ at 0 min, $0.43 \pm 0.006 \mu\text{mol-TAML}^{-1}$ at 80 min). These results demonstrated that the macromolecules that can adsorb the estrogenic chemicals and mask the estrogenic activity in the yeast bioassay analysis play more important roles in the increase in estrogenic activity than the anti-estrogenic chemicals do.

To confirm the experimental result, some commercial humic acids (Sigma–Aldrich, 4.6 mg/L, MW: 5.2 kDa) were added into the wastewater sample after 80 min of photocatalytic degradation (Fig. 3 inset). The estrogenic activity of the wastewater sample decreased (even below the original value) when the commercial humic acids were added. This demonstrates that the macromolecules are responsible for the reduction in estrogenic activity of the secondary effluent sample.

The findings of Fig. 3 could also be used to explain the phenomenon in Fig. 2 that the sum of estrogenic activity of the three fractions (PC, NPC, and MPC) was higher than that of the original sample. Fig. 3 suggested that the masking compounds (macromolecules) could be largely found in the PC fraction. They can adsorb the estrogenic chemicals and mask the estrogenic activity not only in PC fraction but also in MPC fraction. Therefore, the estrogenic activity of the original sample is lower than the sum of that of the three fractions.

3.5. Fluorescence spectroscopy of the wastewater sample during TiO₂ photocatalysis

The EEM fluorescence spectroscopy of the wastewater sample with PC fraction added before and after TiO₂ photocatalysis are shown in Fig. 5. Five EEM peaks were observed for the original wastewater sample. The peaks for $\text{Ex}_{\text{max}}/\text{Em}_{\text{max}}$ and their intensities are shown in Table S3. These EEM peaks are associated with humic/fulvic acid-like, tyrosine-like, or soluble microbial byproduct-like organic compounds, according to a location of EEM peaks of many typical chemicals in wastewater or surface water [43]. The wastewater sample spiked with PC contained abundant humic/fulvic acid with MW larger than 4.5 kDa (refer to HPLC results). However, most of the humic/fulvic acid appear to be decomposed by TiO₂ photocatalysis in the initial 80 min. Within the same phase, the estrogenic activity of the wastewater sample increased. After 80 min, the estrogenic activity gradually decreased. This means that TiO₂ photocatalysis degrades large MW organic compounds, such as humic/fulvic acid-like compounds, into small MW organic compounds, and then mineralizes the small-MW organic compounds. The lower are the concentrations of humic/fulvic acid-like compounds, the weaker is the inhibition of estrogenic activity removal during TiO₂ photocatalysis. Therefore, it can be found that the organic matter of the humic/fulvic acid fraction, which has a large MW (>4.5 kDa), not only plays an important role in inhibiting photocatalytic removal of estrogenic activity from secondary effluent but also is responsible for the temporary increase in estrogenic activity during the same process.

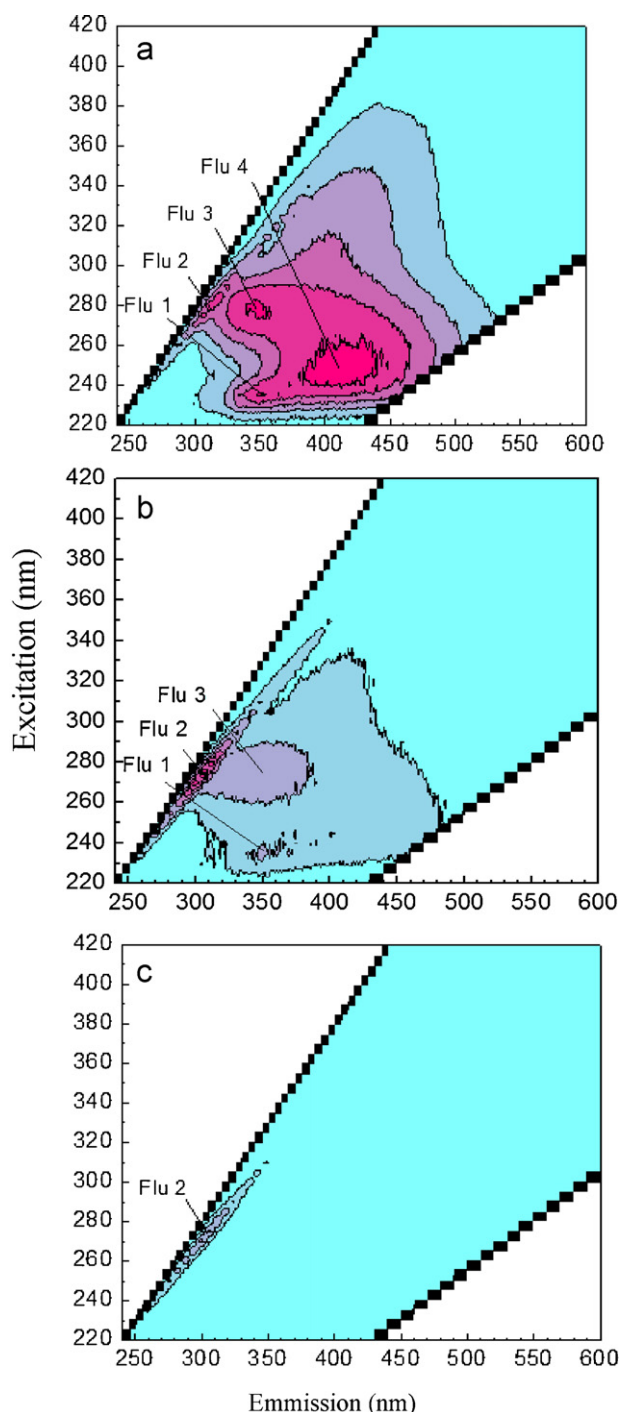


Fig. 5. Fluorescence spectroscopy of wastewater sample spiked with PC during TiO_2 photocatalysis at (a) 0 min, (b) 80 min, and (c) 200 min. TiO_2 : 0.8 g/L, the incident photon flux: $2.8 \times 10^{-4} \text{ Em}^{-2} \text{ s}^{-1}$.

4. Conclusions

The inhibition by inorganic ions of TiO_2 photocatalytic removal of EDCs and estrogenic activity from synthetic water and secondary effluent depends on the adsorption of inorganic ions on the surface of TiO_2 . The stronger adsorption was, the more significant inhibition exhibited. NH_4^+ and HPO_4^{2-} show a significant negative effect on the process due to their strong competition for adsorption sites with the targeted EDCs on the TiO_2 surface. DOM in secondary effluent plays a more important role in inhibiting photocatalytic removal of estrogenic activity than inorganic ions. The humic/fulvic

acid fraction in DOM, which has a large MW (>4.5 kDa), not only plays an important role in inhibiting photocatalytic removal of estrogenic activity from secondary effluent but also is responsible for the temporary increase in estrogenic activity during the same process. Therefore, in order to increase the efficiency of photocatalytic removal of EDCs and estrogenic activity from secondary effluent and ensure the security of the reclaimed water, a pre-treatment technology which could effectively remove NH_4^+ , HPO_4^{2-} and humic/fulvic acid from secondary effluent should be promoted.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2012.02.060.

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