Fe(III) and Fe(II) induced photodegradation of nonylphenol polyethoxylate (NPEO) oligomer in aqueous solution and toxicity evaluation of the irradiated solution

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Abstract

Photodegradation of nonylphenol tri-ethoxylate (NPEO₃) in aqueous solution, and the effects of Fe(III) or Fe(II) were studied. The increasing degradation kinetics of NPEO₃ were observed when 500 μ M Fe(III) or Fe(II) was present in the solutions. Altered formation of NPEO oligomers with shorter EO chains, including nonylphenol (NP), NPEO₁ and NPEO₂, was observed in water and in solutions containing Fe(III) or Fe(II). The molar percentage yields of NP and NPEO_{1,2} production from NPEO₃ photodegradation were approximately 20% in NPEO₃ solution, while NPEO₃ solution with Fe(III), this percentage increased to approximately 50%. In solution with Fe(II), the molar balance between the photodegradation of NPEO₃ and the production of NP and NPEO_{1,2} was observed. A luminescent bacterium, *Vibrio fischeri*, was used to identify changes in the toxicity of NPEO₃ solutions during the photodegradation process under different conditions, while dose addition (DA) model was used to estimate the toxicity of products. Toxicity of NPEO₃/water solution increased significantly following the irradiation of UVA/UVB mixture. In contrast, obviously decreasing toxicity was observed when NPEO₃ underwent photodegradation in the presence of Fe(III).

Keywords Photodegradation; Nonylphenol polyethoxylate (NPEO) oligomer; Ferric/ferrous iron (Fe(III)/Fe(II)); Degradation pathways; Toxicity to luminescent bacteria

1. Introduction

Nonylphenol polyethoxylates (NPEOs) used to be the most commonly used non-ionic surfactants for industrial, agricultural and domestic applications. The annual world-wide production of NPEOs was estimated to be hundreds of thousands of tonnes to several million tonnes (Ohlson, 1998). Approximately 60% of the NPEOs produced were estimated to enter the aquatic environment finally, via various pathways such as municipal and industrial wastewater discharges and sewage treatment plant (STP) effluents (Ying et al., 2002). Even if they have been prohibited in the European Union due to their adverse effects on human health and the environments, NPEOs are still important pollutants in many water environments. In STPs or natural aqueous environments, NPEOs can be biodegraded to their shorter chain homologues, e.g. NP and mono-, di-, and tri-ethoxylates (NPEO_{1, 2, and 3}), as well as to nonylphenoxy ethoxy acetic acids (e.g. NPEC_{1,2}) (Field and Reed, 1996; Potter et al., 1999; Staples et al., 1999; Jonkers et al., 2003; Samaras et al., 2013; Liu et al., 2017). As important pathways for the abiotic degradation of many organic contaminants, photodegradation and photocatalytical degradation of NPEOs in different aquatic solutions have been studied (Brand et al., 1998; Castillo et al., 2001; Chen et al., 2007; Goto et al., 2004; Karci et al., 2013; Karci et al., 2014; Wang et al., 2009). In these studies, NPEOs were shown to be degradable under UV irradiation, while TiO₂ and ferric iron (Fe(III)) were identified to be effective catalysts for the photo-induced degradation of NPEOs.

Multiple studies have reported bio- and abio- degradation of NPEOs (Castillo et al., 2001; Chen et al., 2007; da Silva et al., 2015; Gori et al., 2010; Karci et al., 2013; Karci et al., 2014; Lu et al., 2007; McAdam et al., 2011; Ömeroglu and Sanin, 2014; Ying et al., 2002; Zhang et al., 2008). However, knowledge of the degradation pathways and the ecological risk of the intermediates and products formed during the NPEOs photodegradation is still insufficient. Moreover, a mixture of NPEO polymers was used in the majority of the previous studies,

which resulted in difficulty in most cases to distinguish the parent NPEO from an intermediate. Although many different intermediates and products have been proposed (Brand et al., 1998; Castillo et al., 2001; Chen et al., 2007; Wang et al., 2009), the proportion of NPEO photodegradation following different pathways under various conditions have not been qualified. Besides, all available results on NPEO degradation focused on NPEOs with relatively high degrees of polymerization (i.e. with long EO chain). In contrast, little is known on the photochemical degradation of NPEO oligomers with low polymerization degrees, even if it has been well documented that the ecological risk of NPEO homologues increases with a decreasing degree of polymerization (Goto et al., 2004; Lu et al., 2007; Ying et al., 2002). Different degradation pathways, such as EO shortening and cleavage of the aromatic ring (Chen et al., 2007; Róźalska et al., 2010; Zhang et al., 2008), will lead to different toxic changes compared with the parent compounds. Fe species are natural water substances with photochemical activity (Wu and Deng, 2000). The degradation of NPEOs photoinduced by Fe(III) aquacomplexes has been identified (Brand et al., 1998). In the photoinduced reaction of Fe(III), formation of Fe(II) was documented (Bajt et al., 2001; Faust and Hoigné, 1990). It also involved in degradation process of NPEOs, as a reducing agent (Brand et al., 1998). However, studies on the direct effect of Fe(II) presence on NPEOs degradation are still scarce. In addition, quality balance between NPEO degradation and the formation of products through different pathways is far from being established. Therefore, it is necessary to assess the ecological risk resulting from different degradation processes.

In the present study, effects of Fe(III) or Fe(II) presence in aqueous solutions on the degradation of NPEO₃ and the formation of NPEO_{0~2} under two types of UV irradiations were determined. In addition, the toxicity of NPEO₃ solutions during photoinduced degradation was tested using a luminescent bacterium. The aim of the study is to evaluate the contribution of an EO shortening pathway to the photodegradation of short chain NPEO

oligomers and the effects of iron species on photoinduced degradation, and to assess the toxicity of the intermediates and products formed during NPEO₃ photodegradation under different conditions.

2. Experimental Methods

2.1. Chemicals

Nonylphenol (NP, technical grade) standard was purchased from Tokyo Chemical Synthesis Ind. Company, Japan. The individual standards of NPEO₁, ₂, and ₃ were purchased from Hayashi Pure Chemical Ind. Company, Japan. Stock solutions of Fe(III) and Fe(II) were made by dissolving FeCl₃•6H₂O (A.R.) and FeCl₂ (A.R.) in water. Water used in this study was prepared using a Milli-Q system (Millipore, Bedford, MA).

2.2. Photodegradation experiments

Experiments were carried out in 150 mL open pyrex glass vessels, containing 100 mL of 2.0 μmol L⁻¹ NPEO₃. To study the effects of Fe(III) and Fe(II), 500 μmol L⁻¹ of Fe(III) or Fe(II) was added originally in some cases. The initial pH was adjusted to 3.1±0.1 with 0.1 M HCl before the experiments. Experiments were conducted in water bath shakers equipped with a UV lamp. Vessels were placed symmetrically under the lamp to receive direct irradiation, and shaken at 150 rpm. The vertical distance from the lamp to the liquid surface was approximately 7.0 cm, and the light intensities on the solution surface of each beaker were tested with a radiometer (UV-A/UV-B types, Photoelectric Instrument Factory of Beijing Normal University, China) to ensure the consistency and stability of irradiation throughout the experiment. Two types of UV irradiation, namely UV-1 and UV-2, were used in this study. A 30-W UVA lamp (Nanjing Electronic Vacuum Devices Company, China) was used as UV-1 for 48 h irradiation. The light intensity of the main wavelength of UVA irradiation on the solution surface was measured to be approximately 0.67 mW cm⁻² (365 nm). Milli-Q water was added at interval of 12 h to maintain a volume of 100 mL. A 400-W

medium pressure mercury lamp (Tianjin Yingze Technology Company, China) was used as UV-2 in the 60 min irradiation experiment, with light intensities of 3.97 (at 302 nm), 4.20 (at 313 nm) and 9.82 mW cm⁻² (at 365 nm).

The solution temperature under UV-1 irradiation was 20 °C controlled by the water bath. In UV-2 irradiation experiment, the samples were placed in an ice-water bath due to the higher power of the medium pressure mercury lamp used. The solution temperature was determined to be 7.5±2.5 °C during the 60 min experiment. Vessels covered by aluminum foil were used as a dark control, while the loss of NPEO₃ after 48 h was less than 2% in water solutions as well as in solutions containing Fe(III) or Fe(II).

At the desired time, i.e. 0 h, 4 h, 12 h, 24 h, 48 h for UV-1 irradiation and 0 min, 10 min, 20 min, 30 min, 60 min for UV-2 irradiation, ten milliliters of the solution in each vessel was taken out to test the toxicity to luminescent bacteria, according to the process described below. The residual 90 mL of solution were concentrated by solid phase extraction (SPE) using a Waters OasisTM HLB cartridge (3cc) and then analyzed by high-performance liquid chromatography coupled with a fluorescence detector (HPLC-FL). The detailed procedure for SPE as well as the extraction recoveries is described in Text S1 in the Supplementary Material. Experiments were carried out in triplicate to determine the experimental variability (RSD < 15% in each triplicates). Average values of the triplicates were applied in the statistical analysis.

2.3. Chemical analysis and Quality Assurance/Quality Control

NPEO_{*n*} (n=0-3) was separated by a Waters 1525 HPLC system using a Waters W2108N007 NH₂ normal phase column (μBondapakTM 3.9 mm i.d. × 300 mm × 5 μm, Waters Corp., Ireland), and detected with a Waters 2475 fluorescence detector (Waters Company, USA). *n*-hexane/isopropanol (98/2, v/v) and isopropanol/water (98/2, v/v), named

as solvent A and B, respectively, were used as the mobile phase. The mobile phase was ramped linearly from 95% A to 80% A in 10 min, and the flow rate was 1.0 mL min⁻¹, with detailed chromatogram shown in our previous work (Wang et al., 2009). Excitation and emission wavelengths of the fluorescence detector were 233 and 302 nm, respectively. The method detection limits for NPEO₀₋₃ were 0.48 (for NP), 0.80 (for NPEO₁), 0.68 (for NPEO₂), and 0.60 nmol L⁻¹ (for NPEO₃), which were calculated based on 3 times the valid lowest acceptable calibration standard and a water sample volume of 90 mL. Duplicate analyses were conducted for each sample and the average response was calculated. Instrumental calibration was verified by injection of 10 calibration standards with regression coefficients (*R*) higher than 0.99 for all calibration curves.

Potential sample contamination by NPEO_{*n*} during experiments and extraction was evaluated by running solvent blanks and procedure blanks. No NPEO analytes were detected in these blank samples.

2.4. Toxicity test with *Vibrio fischeri*

Different biomarkers have been used in assessing eco-toxicity, i.e., *Allium cepa* (Iqbal, et al. 2015a; Iqbal, et al. 2015b; Iqbal and Nisar, 2015; Iqbal et al., 2017), *Vicia faba* (Iqbal, M., 2016), and luminescent bacterium (Lechuga et al., 2016). Luminescent bacterium test has been applied for a long time to evaluate the toxicity of aquatic environments (Bulich et al., 1981; Lechuga et al., 2016; Parvez et al., 2006; Zhang et al., 2015). According to a standard method recommended by the Chinese EPA (Water quality-determination of the acute toxicity-luminescent bacteria test. GB/T 15441-1995), *Vibrio fischeri* freeze-dried powder (the Institute of Soil Science, Chinese Academy Sciences (ISSCAS), Nanjing, China) was selected as the test luminescent bacteria. Before the test, 0.5 g of the bacteria were reactivated in 1 mL 3% NaCl solution for 30 min and stored in an ice water bath. Fixed amounts of NaH₂PO₄•H₂O (1.21 g) and Na₂HPO₄ (1.75×10⁻¹ g) were added into 10 mL of a standard

solution or samples from the photodegradation experiments, to adjust pH to 6.0. Two milliliters of the water solution were transferred into a test tube and then 10 μL of the reactivated bacteria were added. Bioluminescence was measured by a Model Toxicity Analyzer (DXY-2, ISSCAS, Nanjing, China) after an exact 15 min contact of bacteria and test solution. The percentage of luminescence inhibition ($x\%$) was calculated as follows,

$$x = \left(1 - \frac{I}{I_0}\right) \times 100 \quad (1)$$

where I_0 and I were the luminescence intensities of the blank control solution (containing only $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$) and the sample solution, respectively. Quadruplicate tests were carried out for each sample, and the average $x\%$ was used to evaluate the degree of the effects of NP, NPEO_{1, 2, and 3} and the degradation products on *Vibrio fischeri*.

3. Results and Discussion

3.1. Photodegradation of NPEO₃ in aqueous solution and effects of Fe(II)/Fe(III)

Different concentrations of NPEO₃ (C_{NPEO_3}) in aqueous solutions under UV-1 or UV-2 irradiation were measured (Fig.1). In the NPEO₃/water solution, C_{NPEO_3} decreased slowly under both types of UV irradiations. At the end of the experiments, C_{NPEO_3} decreased from the initial $2.00 \mu\text{mol L}^{-1}$ to $1.63 \mu\text{mol L}^{-1}$ under 48 h UV-1 irradiation and $1.72 \mu\text{mol L}^{-1}$ under 60 min (UV-2). The pseudo-first order kinetics model, i.e. $C = C_0 \times e^{-kt}$, was used to describe the photodegradation process (Table 1). The kinetics coefficient of NPEO₃ removal (k_{NPEO_3}) under UV-2 irradiation was 0.16 h^{-1} , while that under UV-1 was 0.005 h^{-1} . Photodegradation of NPEO₃ under UV-2 irradiation (composed of high intensity UVB and UVA) was observed in this study. UV absorption of NPEO₃ was found to be low at wavelength $>300\text{nm}$ (Fig. S1 in the Supplementary Material). Therefore, photodegradation of NPEO_n in pure water solution was primarily attributed to reactive oxygen species (ROS)

(Goto et al., 2004), which can form efficiently under UVB irradiation (Masaki et al., 1995). In addition, cleavage of the benzene ring induced by UV irradiation might also present (Chen et al., 2007).

The presence of 500 μM Fe(III) significantly increased k_{NPEO_3} in solution. Under UV-1 irradiation, 60% of the NPEO₃ disappeared in 48 h, with a k_{NPEO_3} value of 0.018 h⁻¹. Under UV-2 irradiation, 41% of the NPEO₃ was degraded in 60 min, while a k_{NPEO_3} of 0.57 h⁻¹ was determined. It has been well documented that Fe(III) aquacomplexes undergo a photo-Fenton process under UV irradiation, which induces formation of •OH radicals and Fe(II) through an internal electron transfer (equation (2)) (Bajt et al., 2001; Faust and Hoigné, 1990). Moreover, the formation of photocatalytically active ferric-organo complexes (Fe-carboxylates) and organic radicals due to radical chain reactions (Arslan-Alaton et al., 2010) also speculatively explained the increasing photodegradation of NPEO₃ in solutions with Fe(III), as carboxylated products of NPEOs might form during photodegradation (Castillo et al., 2001; Chen et al., 2007).

Fe(II) generated in the photoreduction process can be reoxidized to Fe(III) by oxidants such as dissolved oxygen (Faust and Hoigné, 1990). Therefore, Fe(III) species can act as a powerful catalyst in the photodegradation of organic contaminants, since •OH is an effective oxidant, which can non-selectively attack the structures of most organic chemicals. Except for NPEO oligomers with shorter EO chains and nonylphenol, aldehyde ethoxylates (Brand et al., 1998), formate ethoxylates (Brand et al., 1998), NPECs (Castillo et al., 2001) and dicarboxylated formate ethoxylates (Wang et al., 2009) have been identified as products of Fe(III) induced NPEO photodegradation.

The presence of Fe(II) also accelerated photodegradation of NPEO₃, and the k_{NPEO_3} almost double to 0.010 h⁻¹ (under UV-1) and 0.36 h⁻¹ (under UV-2), compared with those in NPEO₃/water solution (Table 1). As described in equation (2), Fe(II) can be oxidized to Fe(III) and then result in formation of •OH radicals. However, it seems difficult for NPEO₃ to be oxidized by •OH radicals under these conditions because the concentration of Fe(II) is much higher than C_{NPEO_3} and thus inhibits the oxidation of target organic compounds (Arslan-alaton et al., 2009). Therefore, mechanisms other than the •OH radical reaction might contribute to the NPEO_n degradation in the presence of Fe(II), which will be further discussed below.

3.2. Formation of NPEO_{0,1,2}

Formation of NPEO₂, NPEO₁, and NP under UV irradiation could be observed at significantly different concentrations in water solution and solutions containing Fe(II) or Fe(III) (Fig. 1&2). On the contrary, there is no NP or NPEO_{1,2} detected in the dark control experiments. In NPEO₃/water solutions, NPEO_{0,1,2} were detected at relatively low levels, with a final total $C_{\text{NPEO}_{0\sim 2}}$ of 0.075 μmol L⁻¹ (after 48 h of UV-1 irradiation) and 0.066 μmol L⁻¹ (after 60 min of UV-2 irradiation), respectively (Fig. 1). Compared to the corresponding decrease in C_{NPEO_3} , i.e. 0.37 μmol L⁻¹ (after 48 h of UV-1 irradiation) and 0.28 μmol L⁻¹ (after 60 min of UV-2 irradiation), the molar percentages yields of NPEO_{0,1,2} from NPEO₃ photodegradation only accounted for approximately 20% (Fig. 1&2). NPEO_{0,1,2} were not the major products of NPEO₃ photodegradation in these conditions.

$C_{\text{NPEO}_{0,1,\text{and } 2}}$ increased quickly in solutions containing 500 μmol L⁻¹ Fe(III) undergoing UV-1 and UV-2 irradiation (Fig. 1&2). Following 48 h of UV-1 irradiation, C_{NPEO_2} , C_{NPEO_1} , and C_{NP} increased to 0.42, 0.20, and 0.02 μmol L⁻¹, respectively (Fig. 1). Following 60 min of UV-2 irradiation, concentrations of NPEO₂, NPEO₁, and NP were 0.10, 0.22, and 0.08

$\mu\text{mol L}^{-1}$, respectively (Fig. 1). The total $C_{\text{NPEO}_{0\sim 2}}$ at the end of the experiments were 0.65 $\mu\text{mol L}^{-1}$ (48 h of UV-1 irradiation) and 0.40 $\mu\text{mol L}^{-1}$ (60 min of UV-2 irradiation), which accounted for 54.3% and 49.4% of the disappearance of NPEO₃ (Fig. 2), respectively.

Formation of NPEO_{0,1, and 2} also increased significantly in the presence of Fe(II) (Fig. 1&2). In solutions with 500 μM of Fe(II), $C_{\text{NPEO}_{0,1,\text{and } 2}}$ were 0.48 (NPEO₂), 0.26 (NPEO₁), and 0.13 $\mu\text{mol L}^{-1}$ (NP), following 48 h of UV-1 irradiation; while those following 60 min of UV-2 irradiation were 0.25 (NPEO₂), 0.18 (NPEO₁), and 0.09 $\mu\text{mol L}^{-1}$ (NP), respectively. The sum of NPEO_{0,1,2} production was near to the amount of disappeared NPEO₃ observed in Fe(II) solutions, especially for that under UV-1 irradiation (Fig. 1), indicating an approximate molar balance between the photodegradation of NPEO₃ and the production of NP and NPEO_{1,2} in Fe(II) solutions. This observation can explain the low degradation kinetics coefficients observed for total NPEO_{0~3} in solutions containing Fe(II) (Table 1). Compared to the formation of NPEO_{0~2} in solutions with Fe(III), the amount of NPEO_{0,1,2} produced in solutions with Fe(II) was similar or even higher.

Formation of NPEO_{0,1,2} is likely not attributable to the Fe induced radical reaction described above, since production of $\bullet\text{OH}$ radicals is limited when Fe(II) is dominant (equation 2). In solutions containing Fe(III) or Fe(II), it is hypothesized that formation of NPEO_{*n-1*} from NPEO_{*n*} is due to the photolysis of an Fe-NPEO_{*n*} complex. Fe(III) species tend to complex with the hydroxyl group of organic chemicals (Banerjee and Haldar, 1950). It is difficult to confirm the structure of these complexes. However, complex of crowns and Fe(III) has been identified before (Koshima and Onishi, 1986). Similar forms of NPEO₃-Fe(III) complex might exist, since NPEO₃ and 12-C-4 crown ether have very similar structure (Fig. S2 (a, b)). Compared with the UV spectra of NPEO₃ solution, a weak red shift of NPEO₃ peak can be observed (Fig. S2 (c)). Additional evidence for this mechanism is the previous

observation that polyethylene glycol was the most abundant degradation product of NPEO₉ in the presence of Fe(III) (Castillo et al., 2001).

High photochemical activity of ferric complexes has been confirmed (Ou et al., 2008; Wu and Deng, 2000). With reference to the previous studies (Ou et al., 2008; Wu and Deng, 2000), a photodegradation pathway for NPEO_n which results in shortening of the EO chain is proposed in present study (as shown in Scheme (1)). In solutions containing Fe(II), photo-induced formation of Fe(III) was observed, especially under the UV-2 irradiation (Fig. S3). This might explain the beginning of NPEO₃ degradation according to Scheme (1) in Fe(II) solutions

Shortening of the EO chain was proposed as one of the major routes for NPEO_n photodegradation (Brand et al., 1998; Castillo et al., 2001; Goto et al., 2004). However, mixtures of NPEOs with different polymerization length of the EO chains were used as target compounds in most studies. Therefore, the proposed formation of NPEO_{n-1} from NPEO_n following EO shortening mechanism was based on the shift of the distribution of homologues of NPEO_n to oligomers with shorter EO chains. However, the change in distribution of the NPEO_n homologues could also be attributed to higher degradation rates of NPEO_n homologues with longer EO chains (Chen et al., 2007). Only one previous photochemical study used a single substance (NPEO₉) as the target compound and indicated the direct formation of NPEOs with shorter EO chain (Castillo et al., 2001). Even though, the contribution of EO shortening to the degradation of NPEO₉ was still not characterized in that study.

The degradation pathway presented in Scheme (1) does not result in a decrease of total $C_{NPEO_{0-3}}$. Therefore, the decrease of total NPEO₀₋₃ observed in water and Fe(III) solutions should be mainly due to other mechanisms, e.g. oxidation induced by ROS.

3.3. Toxicity of NPEO_n to *Vibrio fischeri*

Photooxidation of NPEOs was proposed to occur on the EO chain (Brand et al., 1998; Goto et al., 2004), the alkyl chain (Chen et al., 2007), and the benzene ring (Chen et al., 2007), with different products formed for each reaction. However, the mass balances of NPEO_n during photodegradation in different systems have not been established until now and there should be other products or intermediates that were not observed. Therefore, identifying any changes in the toxicity of the solutions during the photodegradation process will be helpful to assess the ecological risks resulting from photodegradation, and to speculate on the products and intermediates formed. The ability of water solutions containing different concentrations of NPEO_n ($n = 0-3$) to inhibit the luminescence of *Vibrio fischeri* was tested as a measure of the toxicity of the solutions. Usually the dose-response curves show a sigmoidal shape, and a linear relationship will be present in the middle portion of the curve. In the present study, the

$x\%$ was found to be linearly related to the concentration of NPEO_{*n*} in the $x\%$ range between 20~80% (Fig. S4). The fitted linear correlations were shown in Table 2, which indicated that the toxicity of NPEO_{*n*} to *Vibrio fischeri* decreased with the increasing degree of EO polymerization. For example, an increase of one $\mu\text{mol L}^{-1}$ of NP (in the selected concentration range) resulted in a decrease of 6.26% in luminescent intensity, while the decreases in luminescent intensity caused by one $\mu\text{mol L}^{-1}$ increase of $C_{\text{NPEO}_{1,2,\text{and }3}}$ were 5.75%, 5.62%, and 5.34%, respectively (Table 2). This relationship is in accordance with the effects of NPEO_{0~3} on other organisms (Talmage, 1994). Therefore, the EC₅₀ to *Vibrio fischeri* were calculated to be 4.64 (NP), 5.33 (NPEO₁), 5.42 (NPEO₂), and 5.89 $\mu\text{mol/L}$ (NPEO₃) (Table 2).

The toxicity of phenolic compounds to luminescent bacteria has been reported in previous studies (Calza et al., 2008; Strotmann and Eglsaer, 1995). However, little is known of the effects of alkylphenols and their polyethoxylate derivatives on luminescent bacteria. Compared with the reported toxicity of phenolic or other substituted benzene compounds on luminescent bacteria, the EC₅₀ for NP (1020 $\mu\text{g L}^{-1}$ in this study) is similar to that determined for phenol on *Vibrio fischeri*, which was reported to be 1000 $\mu\text{g L}^{-1}$ (Strotmann and Eglsaer, 1995). For chlorophenols and dichlorophenols, much lower EC₅₀ of 90~900 $\mu\text{g L}^{-1}$ have been reported (Strotmann and Eglsaer, 1995). Besides, the microtox EC₅₀ of benzene (Bulich et al., 1981), phenoxyphenol and benzoquinone (Calza et al., 2008) were reported to be much lower than phenol, which indicates a higher toxicity for these chemicals.

3.4. Changes in toxicity during the photochemical degradation process

Many products and intermediates, e.g. dicarboxylated formate ethoxylates (Wang et al., 2009), products with carboxylated or carbonylated ethoxylate chains (Chen et al., 2007), as well as phenol, dihydroxylbenzene, and benzoquinone (Neamțu and Frimmel, 2006) might be

involved in the UV photochemical degradation of NPEO_n. It is difficult to quantify the amount and toxicity of all these products. The toxicity of NPEO₃ solution during photolysis was determined by measuring its effect on the luminescence of *Vibrio fischeri* and the results were shown in Fig. 3. Photodegradation in NPEO₃/water solution resulted in a slight decrease in luminescence intensity (I), with the relative luminescence intensity (I/I_0) of the NPEO₃ solution decreasing from 68.7% to 60.4% after 48 h of UV-1 irradiation (Fig. 3). Following UV-2 irradiation, a significant increase in the toxicity of the solution toward *Vibrio fischeri* was observed, and the I/I_0 decreased from 69.3% to 25.0% at 10 min, and then to 10.2% at 60 min.

The presence of Fe(III) led to a “detoxification” of the NPEO₃ solution, since the I/I_0 increased from 68.5% to 72.0% (after 48 h of UV-1 irradiation) and 89.0% (after 60 min of UV-2 irradiation) (Fig. 3). The net detoxification may be due to •OH radical reactions in this system, which potentially breaks the benzene ring and thus greatly decreases the toxicity of the molecules (Iqbal and Bhatti, 2015).

For the NPEO₃ solution containing 500 μM Fe(II), the I/I_0 decreased slightly under the two types of UV irradiations, which changed from 69.4% to 67.7% after 48 h of UV-1 irradiation, and to 61.8% after 60 min of UV-2 irradiation (Fig. 3). For experiments following UV-1 irradiation, the molar balance between NPEO₃ reduction and NPEO_{0~2} production was observed during the photodegradation (Fig. 1&2). Therefore, the slight decrease of I/I_0 can be completely attributed to the formation of NPEO_{0~2} after irradiation, which is more toxic to *Vibrio fischeri* than NPEO₃ (Fig. S4). Under UV-2 irradiation, the difference between NPEO₃ reduction and NPEO_{0~2} production in NPEO₃/Fe(II) solution was also small (0.03 μmol L⁻¹).

As mentioned above, many products except NPEO_n homologues might present during NPEO₃ photodegradation. When summarizing all products and intermediates as one model compound, namely "Product", and assuming (i) the concentration of "Product" ($C_{Prod.}$) can be calculated as " $C_{0,NPEO_3} - C_{t,NPEO_{0\sim3}}$ " (ii) photodegradation products and NPEO_n homologues (n=0~3) cause toxicity to *Vibrio fischeri* by a similar mechanism, while the estimated concentration of "Product" related to toxic effect can be calculated and compared with those of NPEO_n according to the luminescence inhibition percentage (x%) after photodegradation using a dose addition (DA) model (equation (3)) (Faust et al., 2001),

$$\frac{1}{ECx_{mix}} = \sum_{i=1}^n \frac{p_i}{ECx_i} = \frac{PNPEO_3}{ECx_{NPEO_3}} + \frac{PNPEO_2}{ECx_{NPEO_2}} + \frac{PNPEO_1}{ECx_{NPEO_1}} + \frac{PNP}{ECx_{NP}} + \frac{P_{prod}}{ECx_{Prod}} \quad (3)$$

where x is the luminescence inhibition percentage in different experiments; ECx_{mix} (2.0 μmol L⁻¹) is defined as the total concentration of NPEO_n and products which induces an effect of x%; p_i is the relative proportion of constituent *i* (i.e. NP, NPEO_{1,2,3} and Product) in the total mixture concentration ($p_i = C_i/2.0$), and ECx_i is the concentration of constituent *i* which can induce an effect of x%. ECx_i (*i*= NP and NPEO_{1,2,3}) can be calculated according to the linear correlations shown in Table S1 in the Supplementary Material.

Significant differences in toxicity were found for the water solution following UV irradiation. According to the above DA model (equation 3), the calculated $ECx_{Prod.}$ was 0.52 μmol L⁻¹, which is lower than those of NP and NPEO₁₋₃ (2.97~3.94 μmol L⁻¹). Therefore, formation of products with higher toxicity under this irradiation can be estimated. Additionally, the toxicity of NPEO₃/water solution after 60 min of UV-2 irradiation indicated an $ECx_{Prod.}$ of 0.25 μmol L⁻¹, which was greatly lower than the corresponding $ECx_{NPEO_{0\sim3}}$ (11.00-13.35 μmol L⁻¹). These results indicate that under the irradiation of UV-2, products with toxicity, e.g. benzoquinone species, might form. As a photodegradation product of NPEO_n (Neamtu and Frimmel, 2006), EC₅₀ of benzoquinone for luminescent bacteria was

2000 times lower than that of phenol (Calza et al., 2008). Considering the similar NPEO₃ disappearance and similar NPEO₀₋₂ production following 48 h of UV-1 and 60 min of UV-2, it is hypothesized that the UVB irradiation of UV-2 likely leads to the formation of products with higher toxicity.

Based on the DA model (equation 3), $ECx_{prod.}$ in NPEO₃/Fe(III) and NPEO₃/Fe(II) solution under UV-1 or UV-2 irradiation were calculated (Table S1). Only for NPEO₃/Fe(II) solution under UV-2 irradiation, products with higher toxicity ($ECx_{prod.}=0.41 \mu\text{mol L}^{-1}$) were estimated, but at relatively low concentration ($C_{prod.}=0.03\mu\text{mol L}^{-1}$) (Table S1).

Overall, a significant increase in the NPEO₃/water solution's toxicity toward luminescent bacteria was observed following photodegradation induced by UV irradiation, which was hypothesized to be due to the formation of products with higher toxicity, e.g. quinones. The presence of Fe(III) resulted in a net decrease in toxicity of an aqueous solution of NPEO₃ that underwent photodegradation. For NPEO₃ solutions containing Fe(II), the toxicity slightly increased during the photodegradation period, which might be mainly due to the production of NPEO oligomers with a shorter EO chain.

4. Conclusions

Photodegradation of the NPEO oligomer, NPEO₃, can occur in water under UV irradiation. An increased removal of NPEO₃ was observed in solutions containing Fe(III), where EO shortening, as well as other oxidation mechanisms, may have contributed to the photodegradation of NPEO₃. The presence of Fe(II) also enhanced the photodegradation of NPEO₃, which mainly followed the EO shortening pathway. Obvious detoxification was observed for NPEO₃/Fe(III) water solutions undergoing UV irradiation. Photodegradation of NPEO₃ in an Fe(II) solution led to a slight increase in toxicity to luminescent bacteria, which is attributed to the formation of NPEO oligomers with shorter EO chains, i.e. NPEO_{0,1,2}.

Notably, significant increase in toxicity was observed in NPEO₃/water solution undergoing UV irradiation. This indicates that unknown products with toxicity are formed during the photodegradation of NPEO₃.

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Table 1. Pseudo-first order kinetics equation of NPEO₃ and NPEO_{0~3} degradation in different solutions under two UV irradiations.

	NPEO ₃	NPEO _{0~3}
UV-1 irradiation		
Milli-Q	$-\ln(C/C_0) = 0.005t \quad R = 0.96$	$-\ln(C/C_0) = 0.0043t \quad R = 0.92$
Fe(III) 500μM	$-\ln(C/C_0) = 0.018t \quad R = 0.99$	$-\ln(C/C_0) = 0.007t \quad R = 0.96$
Fe(II) 500μM	$-\ln(C/C_0) = 0.010t \quad R = 0.97$	NA
UV-2 irradiation		
Milli-Q	$-\ln(C/C_0) = 0.16t \quad R = 0.99$	$-\ln(C/C_0) = 0.12t \quad R = 0.98$
Fe(III) 500μM	$-\ln(C/C_0) = 0.57t \quad R = 0.96$	$-\ln(C/C_0) = 0.24t \quad R = 0.98$
Fe(II) 500μM	$-\ln(C/C_0) = 0.36t \quad R = 0.95$	NA

NA: not analyzed because no changes on the concentrations of NPEO_{0~3} were observed during the experimental period under 48 h UV-1 or 60 min UV-2 irradiation.

Table 2. Linear correlations between the luminescence inhibition percentage ($x\%$) and the aqueous concentration of NP and NPEO_{1,2,3} standards.

	Linear correlation	R	EC ₅₀ (μmol/L)
NPEO ₃	$x = 5.34 \times C_{\text{NPEO}_3} + 18.53$	0.99	5.89
NPEO ₂	$x = 5.62 \times C_{\text{NPEO}_2} + 19.78$	1.00	5.42
NPEO ₁	$x = 5.75 \times C_{\text{NPEO}_1} + 19.34$	0.97	5.33
NP	$x = 6.26 \times C_{\text{NP}} + 20.98$	0.96	4.64
Range of $x\%$: 20~80%			

Figure legends

Figure 1. Concentration changes of NPEO₃ and NPEO_{0,1,2} in Milli-Q water solutions and solutions containing 500 µM of Fe(III) or Fe(II) during photodegradation within 48 h under UV-1 irradiation or 60 min under UV-2 irradiation.

Figure 2. Composition distribution of NPEO_{0~2} and other products of NPEO₃ photodegradation in Milli-Q water solutions, 500 µM Fe(III) solutions, and 500 µM Fe(II) solutions, after 48 h UV-1 irradiation or 60 min UV-2 irradiation. Amount of "other products" was calculated as the difference of the disappearance of NPEO₃ and the production of NPEO_{0~2}.

Figure 3. Changes of relative luminescence intensity (I/I_0) of NPEO₃ solution with/without Fe(II) or Fe(III) within 48 h under UV-1 irradiation or 60 min under UV-2 irradiation. The diagram represents average value of the triplicate, while the error bar represents SD.

Figure 1.

Figure 2.

Figure 3.