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Aqueous photodegradation and toxicity of the polycyclic aromatic hydrocarbons fluorene, dibenzofuran and dibenzothiophene

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Abstract

Decay kinetics resulting from the application of UV and UV/H₂O₂ to the polycyclic aromatic hydrocarbons (PAHs) fluorene, dibenzofuran and dibenzothiophene was studied. Batch experiments were conducted with both low pressure monochromatic (253.7 nm) and medium pressure polychromatic (200–300 nm) UV sources alone or in the presence of up to 25 mg/L hydrogen peroxide, in a quasi-collimated beam apparatus. Degradation of all three PAHs, by both UV and UV/H₂O₂, exhibited pseudo-first order reaction kinetics and low quantum yields ranging from 1.4×10^{-3} to 1.8×10^{-2} mol/E using both UV lamps. Toxicity testing using a bioluminescence inhibition bioassay was correlated to the decay in concentration of the PAHs as analyzed analytically using HPLC. Results demonstrated that treatment efficacy of oxidative PAH degradation measured by following the decay of the target compound is best complemented by also evaluating the toxicity of the treated water due to byproduct formation concerns.

Keywords

Photolysis; UV irradiation; Hydroxyl radicals; Hydrogen peroxide; PAHs

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic substances composed of carbon and hydrogen atoms grouped into at least two condensed aromatic ring structures. PAHs can be introduced to the environment by incomplete combustion of coal, oil, and wood, improper storage or disposal of fuels and oils, and wood treatment processes. Many of the PAHs are toxic, carcinogenic, and tend to bioaccumulate in aquatic organisms. These compounds along with their oxidation products have been identified in environmental samples (Sabaté et al., 2001) such as industrial and municipal wastewater, effluents, rainwater, and drinking water (Maier et al., 2000; Jamroz et al., 2003). In surface water the concentration of PAHs were reported to range from 0.1 to 830 ng/L (Menzie et al., 1992).

Exposure to wildlife and humans can occur in water through advective and/or diffusive transport of contaminants and microbially produced intermediates to the overlying water column, where these chemicals can be subject to photolytic attack. Microbial transformations

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of photo-products can also occur through the activity of suspended or sediment bacteria. The LD₅₀ in aquatic organisms varies considerably, but is generally several orders of magnitude higher than concentrations found in most heavily polluted bodies of water (Arfsten et al., 1996). Growing evidence suggests that the real hazards of PAHs to aquatic life may result from their photo-induced toxicity caused by UV radiation in sunlight (Mekenyan et al., 1994; McConkey et al., 1997; Grote et al., 2005). Photo-induced toxicity of PAHs can be driven from formation of intracellular singlet oxygen and other reactive oxygen species (ROS) that cause oxidative damage in biological systems (El-Alawi et al., 2002), or formation of photo-products, which exert different, often stronger, bioactivity than the parent compound (Grote et al., 2005).

Fluorene (FLU), dibenzofuran (DBF) and dibenzothiophene (DBT) are three ring PAHs largely derived from anthropogenic sources (e.g., petroleum products), although they can also form through natural processes (e.g., forest fires). These compounds share fluorene's basic molecular structure, only differing by one atom in the bridge of the furan ring: C in FLU, O in DBF, and S in DBT (Figure 1). Like many PAHs, these compounds are sparingly soluble with low volatility and high K_{ow}, and in soils and sediments they strongly associate with dissolved or particulate organic matter (McCarthy, 1983; Liu and Amy, 1993). Association with dissolved organic matter may significantly increase apparent solubility (Lassen and Carlsen, 1999) yet often reduce bioavailability and toxicity (Weinstein and Oris, 1999; Steinberg et al., 2000).

Bioremediation is most often the treatment used for the removal of PAHs from contaminated water and soil because it's generally found to have cost and technical advantages (Zeng et al., 2000). Advanced oxidation processes (AOPs) including ozone, hydrogen peroxide, UV radiation, and combinations of these processes were also shown to degrade various PAHs (Zeng et al., 2000; Beltran et al., 1996; Miller and Olejnik, 2001). The highly reactive hydroxyl radicals, generated during AOPs, can lead to complete mineralization of the pollutant but most typically result in the formation of products of higher polarity and solubility in water such as phenols, quinones, and acids (Stucki and Alexander, 1987; Beltran et al., 1996). These by-products are often more bioavailable for microorganisms, increasing overall natural biodegradation (Lehto et al., 2003; Grote et al., 2005).

Commonly the efficiency of AOPs is determined by following kinetics of decay of a target compound. In other cases byproducts, formed during oxidation, are identified. For example, Rivas et al. (2006) proposed a detailed mechanism of oxidation of FLU by hydroxyl radicals at which 9-fluorenone, 9-fluorenol, and DBF were formed in the first stage of the reaction. Further oxidation of these products proceeded via hydroxylation and cleavage of the fluorene structure. Yet, too often the ecological or human health threat is assumed to be relieved upon degradation of a parent compound and the fate of the oxidation byproducts is overlooked. A different approach suggests that chemical analysis should be complemented by the application of bioassays to give an integrated measure of toxicity along with the chemical analysis data. Non target pollutants as well as reduced availability of toxicants, antagonistic or synergistic interactions are all considered when using a biological test system (Loibner et al., 2004). Toxicity bioassays can be used not only to evaluate efficiency of oxidation processes but also to study the fate of the parent compounds as well as the byproducts formed during oxidation. Traditionally, crustaceans, fish and algae are used for aquatic toxicity measurement. Tests based on these organisms require long exposure times and large sample volume. Thus, toxicity measurements based on microorganisms which are rapid, cost effective and reproducible are being used extensively (Parvez et al., 2006). Luminescent bacteria have been found to be particularly useful in evaluating toxicant impacts (El-Alawi et al., 2002) and provide a measure of sub-lethal response for pollutants.

In this research degradation kinetics and quantum yield were established for treating FLU ($C_{13}H_{10}O$), DBF ($C_{13}H_{10}O$) and DBT ($C_{13}H_{10}S$) by UV and UV/ H_2O_2 processes at wavelengths ranging from 200 to 300 nm, applicable for engineered treatment processes rather than naturally occurring sunlight based environmental processes. Toxicity testing using a bioluminescence inhibition bioassay was conducted for each compound and further correlated with the decay of the PAHs as analyzed analytically using HPLC.

2. Materials and methods

2.1. Materials

FLU and DBF (98% purity) were purchased from Alfa Aesar, DBT (98% purity) from Aldrich; molinate from Chem Service Inc; hydrogen peroxide (30% w/w) and HPLC grade acetonitrile from Fisher Chemicals; HPLC grade water from Acros. All chemicals were used as received and all solutions were prepared with de-ionized (DI) water.

2.2. Photolysis experimental set up

Photolysis was carried out with low (LP) Hg vapor germicidal UV lamps (ozone-free, General Electric #G15T8) emitting essentially monochromatic UV light at 254 nm and a polychromatic (200–300 nm) medium-pressure (MP) (Hanovia Co., Union, NJ) Hg vapor lamp in a quasi-collimated beam apparatus. Emission spectrum of the UV lamps is shown elsewhere (Shemer et al., 2006a). In order to overcome the low solubility of the PAHs, methanolic stock solutions of the PAHs were placed in a glass vessel, the methanol was evaporated with a gentle stream of N_2 , after which 20 mM phosphate buffer solution at pH 7 was added and the solution was covered and stirred overnight to dissolve the chemicals. A 100 mL sample was irradiated with gentle stirring in a 70 × 50 mm crystallization dish (34.2 cm² surface area, solution depth approximately 3.3 cm) open to the atmosphere. Exposure times necessary to achieve UV fluences from 0 to 1000 mJ/cm² were determined from the average irradiance as calculated with a spreadsheet program using the lamp spectrum, solution absorbance, the incident irradiance read from a calibrated radiometer (IL1700, SED 240/W, International Light, Peabody, MA) and the measured Petri-factor for the dish (ratio of the average of the incident irradiance over the area of the reaction dish to the irradiance at the center of the dish). A UV spectrophotometer - Cary Bio100 (Varian, Inc., Palo Alto, CA) with a 1 nm slit width, 1 nm step size, 0.3 nm/s average scan rate, deuterium lamp, and quartz cell was used to measure the aqueous solution absorbance and the molar absorption spectra for each of the PAHs.

Hydrogen peroxide assisted PAHs degradation was studied by adding 10 or 25 mg/L H_2O_2 (30%). Rate constant of the reaction between each PAH compound and the hydroxyl radicals was determined using competition kinetic method with molinate as a reference compound, at pH 7 with 25 mg/L hydrogen peroxide and concentration of 2 μ M for each compound. All experiments were conducted in duplicate unless stated differently.

Dark controls were conducted, at identical experimental setup, to ensure that no loss of the PAHs occurred via reactions other than photolysis (i.e. hydrolysis, evaporation, and adsorption to the walls of the reaction vessel).

2.3. Analysis

Concentrations of the PAHs were determined by using C-18 (7.5 × 150 mm) reversed phase Varian Prostar HPLC (Varian, Inc., Palo Alto, CA) equipped with a photodiode array detector. Samples (1 mL) were removed at the desired UV fluences. Isocratic elution was used with a mobile phase of 70:30 HPLC grade acetonitrile and water, at flow rate of 1.5 mL/min. Injection volume was 100 μ L and absorbance detection at 242–252 nm. The retention times were 5.7,

6.2 and 7.1 min for DBF, FLU, and DBT respectively. Concentration of hydrogen peroxide was determined by the I_3^- method (Klassen et al., 1994)

2.4. Toxicity assay

Bioluminescence inhibition assay, based on a marine gram negative bacterium, *Vibrio fischeri* (NRRL B-11177 (ATCC)), was used for acute toxicity estimation. Prior to toxicity testing, cultures of *V. fischeri* were grown in photobacterium broth (Fluka) at 15°C in darkness with continuous mixing. Cultures were harvested after 3 days by centrifuging 17.5 mL at 5000 g for 5 min at 15°C. The pellet was re-suspended in 100 mL 2% NaCl to obtain OD at 600 nm from 0.82 to 0.86 (measured by a UV spectrophotometer). 0.5 mL aliquots of culture suspension were added to a 48-well tissue culture plate (Corning Inc. costar 3548). After 10 min incubation at 15°C in the 48-well culture plate, the luminescence was measured using FLUOstar OPTIMA fluorescence measurement system (BMG Labtech, Inc. Germany) with spectral range of 240–740 nm. Wells were then dosed with 0.5 mL of the tested solution and incubated in darkness for 30 min at 15°C, after which the bacterial luminescence was measured again. Treated solutions were added to the wells in triplicates, and luminescence was measured twice for each plate. Toxicity assays were conducted for DBF, FLU and DBT solutions (4.5 µM) after exposure to direct photolysis at UV fluences from 0 to 2500 mJ/cm² and for UV/H₂O₂ (25 mg/L) treatment at UV fluences from 0 to 1000 mJ/cm². UV and UV/H₂O₂ experiments were conducted in duplicate using both UV lamps. The bioassay was carried out in aqueous solution without solubility-enhancing agents in order to exclude possible interactions with the PAHs. Control samples consisting of bacterial suspension in 2% NaCl with 2 mM phosphate buffer (PBS), the media which the test chemicals were dissolved in, were included along with the test sample. For the UV/H₂O₂ additional controls were conducted for the effect of the enzyme catalase, which was added to the PBS solution in order to destroy residual H₂O₂ prior to HPLC analysis. The decrease in bacterial luminescence (% inhibition) due to addition of the PAHs was determined as follows (McConkey et al., 1997):

$$\% \text{ inhibition} = 100 \times \left(1 - \frac{L_t C_0}{L_0 C_t} \right) \quad (1)$$

where, L_0 and C_0 are the luminescence of test samples and control at $t=0$. L_t and C_t are luminescence values for test samples and control measured after 30 min incubation.

3. Results and discussion

3.1. Absorbance of the PAHs

The molar absorption spectrum of DBF, FLU, and DBT is presented in Figure 1. These compounds share fluorene's basic molecular structure, only differing by one atom in the bridge of the furan ring. These structural differences are reflected by the absorption characteristics of each compound. All three compounds absorb light at wavelength ranging from 200 to 330 nm. Absorption spectrum of the three compounds consists of several bands of various intensities. DBT has maxima absorbance at 230 nm whereas FLU maxima absorbance is at 204, 260 and 300 nm. DBF maxima absorbance is at 206, 216, 248, and 280 nm. Hence, these compounds have the potential to be photolyzed by any UV source at wavelengths below 330 nm.

3.2. Direct photolysis

Pseudo-first order kinetics was observed for degradation of DBF, FLU, and DBT by both LP and MP UV lamps, as indicated by linear relations between $\ln([PAH]/[PAH]_0)$ versus UV fluence (Figure 2). The slope of Fig. 2 gives the UV fluence based pseudo first order rate

constant (k'_d , cm²/mJ). Error bars were derived from standard deviation of PAHs concentration determined experimentally in duplicate.

Very low efficiency of the direct photolysis was obtained for all three compounds studied up to UV fluence of 1000 mJ/cm². Only 6% of the initial 2 µM of DBT and DBF and 15% of FLU were removed by the LP lamp at UV fluence of 1000 mJ/cm². The removal efficiencies θ , were calculated by using Eq. 2. Slightly higher removal was obtained using the MP lamp at the same UV fluence of 1000 mJ/cm², 16% and 18% for DBF and FLU respectively and only 7% of DBT was degraded. For comparison, UV fluence typically used in disinfection applications is between 40 and 200 mJ/cm².

$$\% \theta = \left(1 - \frac{C_t}{C_0} \right) \times 100 \quad (2)$$

where, C_t and C_0 are the concentration (µM) of the PAH at UV fluences of 1000 and 0 mJ/cm² respectively.

Based on the percentage of removal and degradation rates (Figure 3) it can be concluded that the MP lamp was more efficient for direct photolysis of DBF and FLU. While, both lamps appeared to be equally effective for the direct photolysis of DBT. These results can be explained by the substantial overlap of the emission from the MP UV lamp with the absorption band of the three compounds whereas, the LP lamp emits only at 254 nm. Direct photolysis rates of the PAHs, using LP lamp, followed the decreasing order of FLU > DBF > DBT (Figure 3). When applying the MP lamp the difference between the FLU and DBF was insignificant. The low direct photolysis was also reflected by the low quantum yields (Φ) calculated for each compound. The calculation method for the quantum yield is described in detail elsewhere (Sharpless and Linden, 2003). DBT showed the lowest quantum yield using both lamps (2.1×10^{-3} and 1.4×10^{-3} mol/E for LP and MP lamps respectively). The relatively high quantum yield of DBF using the LP lamp is probably due to its low molar absorption ($2018.5 \text{ M}^{-1} \text{ cm}^{-1}$) at 254 nm as compared to 14274.3 and $16713.8 \text{ M}^{-1} \text{ cm}^{-1}$ for DBT and FLU respectively. The molar absorption is taken into account in denominator when calculating the quantum yield hence, its effect on the quantum yield value. Miller and Olejnik (2001) reported $\Phi_{254\text{nm}} 3.8 \times 10^{-3}$ mol/E for FLU, which is the same as the quantum yield obtained in this research. Quantum yield of the same order of magnitude (7.5×10^{-3} mol/E) was reported for FLU by Beltran et al. (1995). No data was found in the literature regarding the other two compounds (DBT and DBF). It is important to note that although the molar absorbance of DBT at 254 nm is fairly high (Figure 1), its direct photolysis rate was found to be the lowest among the three PAHs studied. This demonstrates the fact that a photo-excited species may return to the ground state without being structurally altered by the process.

3.3. UV/H₂O₂

Similar to direct photolysis, pseudo-first order kinetics was observed for the degradation of DBF, FLU, and DBT using UV/H₂O₂. The addition of hydrogen peroxide led to a significant increase in the removal rates and efficiency of all three PAHs studied. For example, the removal rate of DBT in the presence of 10 mg/L hydrogen peroxide was enhanced by 36-fold using the LP lamp (from 6.1×10^{-5} to 2.2×10^{-3} cm²/mJ) and by 109-fold using the MP lamp (from 6.0×10^{-5} to 6.5×10^{-3} cm²/mJ), as compared to direct photolysis removal rates. The enhancement can be attributed to oxidation by hydroxyl radicals, originating from photolysis of the hydrogen peroxide as shown in Eq. 3. The quantum yield for generating hydroxyl radicals using UV/H₂O₂ process is 1.0, while the most efficient hydroxyl radical yields are obtained when short-wave ultraviolet wavelengths (200–280 nm) are used.

Further increase of the hydrogen peroxide concentration from 10 mg/L to 25 mg/L resulted in additional increase in the degradation of the PAHs. These results indicate that oxidation by hydroxyl radicals is the main degradation pathway of the PAHs during AOP treatment. Hydrogen peroxide assisted photolysis by the LP UV lamp followed the decreasing order of $\text{DBT} \geq \text{FLU} > \text{DBF}$ (Figure 4). Whereas, using the MP lamp the degradation of DBF became the fastest followed by DBT and FLU ($\text{DBF} > \text{DBT} > \text{FLU}$; Figure 4). Since it was concluded that the main degradation pathway of the PAHs was oxidation by hydroxyl radicals and in order to explain the different oxidation rates of the three compounds, the rate constants (k_{OH}) for reaction between hydroxyl radicals and the PAHs was determined, using competition kinetics. In this method an organic substrate competes for hydroxyl radicals with a reference compound whose reactivity toward the hydroxyl radicals is known, under identical conditions (Einschlag et al., 2003). k_{OH} was calculated using Eq. 4.

$$k_{\text{OH}}(\text{PAH}) = \frac{k_{\text{PAH}}}{k_{\text{ref}}} \times k_{\text{OH}}(\text{ref}) = \frac{\ln([\text{PAH}] / [\text{PAH}]_0)}{\ln([\text{ref}] / [\text{ref}]_0)} \times k_{\text{OH}}(\text{ref}) \quad (4)$$

where, k_{PAH} is the substrate overall removal rate constant; k_{ref} is the reference compound overall removal rate constant. Molinate was used as a reference compound with k_{OH} of $6.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (Shemer et al., 2006b).

A plot of $\ln([\text{PAH}] / [\text{PAH}]_0)$ versus $\ln([\text{molinate}] / [\text{molinate}]_0)$ resulted in a straight line passing through the origin and whose slope represents the ratio of rate constants $k_{\text{PAH}}/k_{\text{ref}}$ (Figure 5). All three PAHs showed high reactivity toward the hydroxyl radicals, as indicated by rate constants on the order of $10^{10} \text{ M}^{-1} \text{ s}^{-1}$. k_{OH} values followed the decreasing order of $\text{DBT} (1.81 \pm 0.01) \times 10^{10} \text{ M}^{-1} \text{ s}^{-1} > \text{DBF} (1.58 \pm 0.03) \times 10^{10} \text{ M}^{-1} \text{ s}^{-1} > \text{FLU} (1.39 \pm 0.02) \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$. The difference between the reactivity toward the hydroxyl radicals of the three compounds can be attributed to the atom in the bridge of the furan ring: carbon in FLU, oxygen in DBF, and sulfur in DBT. The large and polarizable sulfur atom makes the DBT compound nucleophilic and therefore, the most reactive toward the electrophile hydroxyl radicals. A possible reason for the difference in reactivity between FLU and DBF is the ability of oxygen (in the DBF molecule) to increase the reactivity of the aromatic ring towards electrophilic aromatic substitution reactions by donating electrons. Beltran et al. (1996) reported a value of $(9.9 \pm 0.8) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ for the rate constant of the hydroxyl radical FLU reaction, which is in good agreement with the value obtained in this research.

Once k_{OH} was determined, the steady state concentration of hydroxyl radicals was calculated using Eq. 5. In UV/ H_2O_2 systems degradation of a substance (s) can be described by Eq. 6 where both hydroxyl radicals reactions and direct photolysis contribute to the compound decay. However, because it was found that the PAHs do not undergo significant direct photolysis and their primary reaction is via hydroxyl radicals, Eq. 5, as a simplified form of Eq. 6, can be used to represent contaminant degradation.

$$-\frac{d[\text{PAH}]}{dt} = k_{\text{OH PAH}} [\text{PAH}] [\cdot \text{OH}]_{\text{ss}} \quad (5)$$

where, $k_{\text{OH PAH}}$ is the rate constant for the reaction between PAH and hydroxyl radicals, at ambient temperature and pressure, and $[\cdot \text{OH}]_{\text{ss}}$ is the steady state concentration of hydroxyl radicals

$$-\frac{d[s]}{dt} = k'_d(s)[s] + k_{OH(s)}[\cdot OH][s] \quad (6)$$

Correlation was found between the steady state concentration of hydroxyl radicals and the degradation rate of the PAHs. Higher rates of degradation correlated to higher steady state concentration of hydroxyl radicals (Figure 4). The steady state concentration of hydroxyl radicals was on the order of 10^{-13} – 10^{-12} M in both UV lamps systems. This concentration is a function of the fraction of light absorbed by the hydrogen peroxide ($f_{H_2O_2}$, Eq. 7) and competition between the parent compound (PAH), its degradation intermediates, and hydrogen peroxide for hydroxyl radical. Hydrogen peroxide is a fairly weak absorber of UV light with $\epsilon_{\max}(200\text{ nm}) = 198\text{ M}^{-1}\text{ cm}^{-1}$. While it is photo-reactive over the wavelength range of 200 to 300 nm, short-wave ultraviolet results in the highest hydroxyl radical yields (Glaze et al., 1987). An overlap of the absorbance between the hydrogen peroxide and the organic compound will result in a lower fraction of light absorbed by the hydrogen peroxide therefore lower hydroxyl radicals concentration.

$$f_{H_2O_2} = \frac{\epsilon_{H_2O_2}[H_2O_2]}{\lambda \epsilon_{H_2O_2}[H_2O_2] + \epsilon_{PAH}[PAH]} \quad (7)$$

where, ϵ is the molar absorption coefficient ($\text{M}^{-1}\text{ cm}^{-1}$).

Additionally, hydrogen peroxide reacts with hydroxyl radicals ($\cdot OH$ scavenger) as shown in Eq. 8.

It is expected that an increase in the fraction of light absorbed by the hydrogen peroxide will result in higher steady state concentration of hydroxyl radicals and consequently higher rates of degradation. This assumption was found to be true when comparing the degradation rates and the steady state concentration of hydroxyl radicals for each PAH system, and when comparing the degradation efficiency of the three compounds. The fraction of UV light absorbed by the hydrogen peroxide was higher using 25 mg/L hydrogen peroxide as compared to 10 mg/L and accordingly the steady state concentration of hydroxyl radicals increased (Figure 4). Respectively, an increase of the hydrogen peroxide concentration from 10 mg/L to 25 mg/L resulted in faster degradation of the PAHs. One exception to the rule was found using the LP lamp where the fraction of light absorbed by the hydrogen peroxide in the DBF system was significantly higher than in the other two systems, 0.63, 0.15 and 0.14 for DBF, DBT and FLU respectively (these values represent $f_{H_2O_2}$ using 10 mg/L hydrogen peroxide) yet, the decay of DBF and the steady state concentration of hydroxyl radicals in its system were the lowest. The variation in $f_{H_2O_2}$ became more prominent when 25 mg/L hydrogen peroxide was applied, resulting in 0.81, 0.38 and 0.29 absorbed for DBF, DBT and FLU respectively. As explained previously the molar absorption of DBF was found to be significantly lower at 254 nm, as compared to the molar absorption of FLU and DBT, hence the fraction of light absorbed by the hydrogen peroxide in the DBF system was the highest, whereas the $[OH]_{ss}$ and k'_d followed the decreasing order of $DBT \geq FLU > DBF$. These results might suggest that the recombination reaction between hydroxyl radicals (Eq. 9, (Buxton et al., 1988)), which scavenges the radicals, becomes predominant when their production rate is high. The higher the production of radicals, the probability for reaction 9 to occur increases, such as in the case of the DBF, hence the lower steady state concentration of the hydroxyl radicals.

Similar to direct photolysis higher rates of degradation were found for all three compounds when irradiated using the polychromatic medium pressure UV lamp in the presence of hydrogen peroxide as compared to the LP UV lamp. As explained previously hydrogen peroxide is photo-reactive over the wavelength range of 200–300 nm and therefore the fraction of light absorbed by it and consequently the steady state concentration of hydroxyl radicals is higher when applying the MP lamp (Figure 4).

3.4. Toxicity

Relative toxicity of each PAH compound was determined via a bioluminescence inhibition bioassay using *Vibrio fischeri* bacteria. Light production is directly proportional to the metabolic activity of the bacterial population and any inhibition of enzymatic activity causes a corresponding decrease in bioluminescence (Parvez et al., 2006). A dose response curve was obtained by exposure of *V. fischeri* to a concentration series of DBF, FLU, and DBT. Bacterial response was measured as percentage inhibition of luminescence and plotted versus the logarithmic scale of the chemical concentration (μM) in 2 mM phosphate buffer (Figure 6). Error bars represent the standard deviation of $n=12$. All three compounds caused increasing inhibition of luminescence with increasing pollutant concentration. DBT was the most toxic to *V. fischeri* whereas, no significant difference of toxicity was observed between FLU and DBF. Higher concentration of the PAHs could not be obtained because of water solubility limitation. The lowest observable effect concentration (LOEC) was reached at 1.3, 1.4 and 0.5 μM for DBF, FLU and DBT respectively. Concentrations estimated to give a 10% inhibition of luminescence (EC_{10}) were 1.05 μM for DBT, 5.1 μM for FLU and 5.4 μM for DBF. The calculated EC_{10} values followed the lipophilicity ($\log K_{ow}$) of the compounds (4.1, 4.2 and 4.4 for DBF, FLU, and DBT respectively) hence, DBT which has the higher octanol-water coefficient was the most toxic among the three studied compounds.

Toxicity of each compound as measured by bioluminescence inhibition after exposure to direct photolysis showed a slight increase. The toxicity decreased approximately back to the initial level (Figure 7A and 7B) at UV fluence of 2000 mJ/cm^2 using the LP lamp and 2500 mJ/cm^2 using the MP lamp. Analysis using HPLC showed 17% and 28% decay of DBF parent compound using LP (2000 mJ/cm^2) and MP (2500 mJ/cm^2) UV lamps respectively, despite the fact that there was little to no change in toxicity overall. Similar trends were found for FLU (32% removal) and DBT (15% removal) at a UV fluence of 2000 mJ/cm^2 using UV LP lamp. 32% and 15% of FLU and DBT parent compounds were degraded respectively, by applying 2500 mJ/cm^2 using the MP lamp. These results could be explained by formation of photo-products that are more toxic to the *V. fischeri* bacteria. As the UV irradiation continued these intermediate compounds along with the parent compound were further photolyzed and hence the overall toxicity decreased. During the UV/ H_2O_2 reaction an increase in toxicity was observed after exposure to 50 mJ/cm^2 (Figure 7C and 7D) after which a linear decrease was observed for all three compounds. Again it is expected that formation of oxidation products increased the toxicity of the solution to the *V. fischeri* bacteria and as the UV irradiation continued these intermediates along with the parent compound were degraded. Chemical analysis using HPLC indicated 98%–99% removal of all three compounds using both lamps at a UV fluence of 1000 mJ/cm^2 except for 83% removal of DBF using the LP lamp. Toxicity wise, only DBT showed inhibition at that UV fluence (1000 mJ/cm^2) where only 0.05 μM DBT was measured analytically. This measured concentration is below the LOEC for DBT (Figure 6) indicating that the observed toxicity of the solution was likely due to the oxidation products of DBT rather than the parent compound. It is important to note that the fact the DBF and FLU did not show toxicity after exposure to 1000 mJ/cm^2 did not necessarily mean that there were

no toxic by-products in the irradiated solution. It is possible that some of the by-products are not measurably toxic to the specific bioassay used or the concentrations were below the detection limit of the method.

Unlike most experimental setups described in the literature, at which the bacteria along with the PAH were exposed to light, in this research phosphate buffer solutions of PAHs were irradiated either by UV alone or in the presence of hydrogen peroxide and then mixed with the bacteria. Thus the observed toxicity could be attributed solely to the remaining parent compound and its degradation by-products rather than short-lived reactive species formed via photosensitization processes during exposure to UV light, such as singlet oxygen and ROS that are toxic to the bacteria via membrane, protein, and DNA damage. The half life of these species is only microseconds therefore they can not affect the bacteria using the experimental setup in this research. Often, marked differences in the toxicity are quoted in the literature for the same compound probably due to differences in experimental procedure along with variation in the preparation of the bacteria by the manufacturers and in the way components of the media are used to sustain the bacteria (Jennings et al., 2001) therefore no comparison to other literature values was made.

The results described above emphasize the need for complete evaluation of a destructive treatment not only by following the decay of the target compound or its by-products, analyzed analytically, but also by measuring the toxicity of the treated water.

4. Conclusions

Pseudo-first order kinetics was observed for DBF, FLU, and DBT degradation by direct photolysis and UV/H₂O₂ oxidation. Low rates and efficiencies of direct photolysis were obtained, while high reactivity toward hydroxyl radicals, with k_{OH} values of 1.8×10^{10} , 1.6×10^{10} and $1.4 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ for DBT, DBF and FLU respectively were found. Higher rates of degradation by UV/H₂O₂ were correlated to higher steady state concentration of hydroxyl radicals resulting from a higher fraction of light absorbed by hydrogen peroxide. Slight increase in toxicity, measured using a bioluminescence inhibition assay, occurred during direct photolysis, as the parent compound was degraded after which the toxicity decreased back to the approximately initial level at UV fluence of 2000 mJ/cm². An inhibition of luminescence toxicity assay indicated formation of toxic intermediates generated during UV-based photolysis and oxidation reactions after which oxidative degradation of these by-products along with the parent compounds resulted in reduced toxicity.

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Fig 1.
Molar absorbance of DBF, FLU, and DBT in aqueous solution.

Fig 2.
Direct photolysis of FLU, DBT and DBF using LP and MP UV lamp at pH 7.

Fig 3. Pseudo first order degradation rate constant (k'_d) (A) and quantum yields (Φ) (B) of DBF, FLU, and DBT at 254 nm (LP lamp) and 200–300 nm (MP lamp).

Fig 4.

Fraction of light absorbed by hydrogen peroxide, steady state concentration of hydroxyl radicals ($\times 10^{-13}$ M), and degradation rates ($\times 10^{-3}$ cm²/mJ) of DBF, FLU, and DBT using both UV lamps in the presence of 10 mg/L and 25 mg/L hydrogen peroxide.

Fig 5.
Logarithmic plot of DBF, FLU, and DBT versus molinate used as a reference compound.

Fig 6. Dose-response of *V. fischeri* to DBF, FLU and DBT. Error bars represents the standard deviation of n=12.

Fig 7. Toxicity determined using bioluminescence inhibition assay for DBF (●), FLU(■), and DBT (▲) after exposure to direct photolysis (A and B) and UV/H₂O₂ (C and D). Starting concentrations of PAHs 4.5 μM, error bars represents standard deviation of n=6.