Available online at www.sciencedirect.com
ScienceDirect
Journal of Hazardous Materials 143 (2007) 271–276
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Phototransformation of 2,4,6-trinitrotoluene: Sensitized by riboflavin under different irradiation spectral range

Xin Yang [a,b], Xueheng Zhao [a], Huey-Min Hwang [a,*]

[a] *Department of Biology, Jackson State University, Jackson, MS 39217, USA*
[b] *Beijing Institute of Pharmacology and Toxicology, Beijing 100850, People's Republic of China*

Received 23 June 2006; received in revised form 5 September 2006; accepted 7 September 2006
Available online 10 September 2006

Abstract

Riboflavin-sensitized phototransformation of 2,4,6-trinitrotoluene (TNT) under natural sunlight was investigated with reverse-phase high performance liquid chromatography/mass spectrometry (HPLC/MS) and gas chromatography/mass spectrometry (GC/MS). The effect of different spectral region of sunlight on TNT phototransformation in the absence or presence of riboflavin was also investigated by using optical filters with cut-off at 400 or 455 nm. The concentration of riboflavin in the phototransformation of TNT was optimized. Concentration of riboflavin and TNT was 1.0 and 50 μM, respectively. The rates of phototransformation of TNT under natural sunlight in the presence or absence of riboflavin were conformed to initial pseudo-first-order rate equation. The photolysis half life of TNT in the presence of riboflavin was 21.87 min, compared to 39 min in the absence of riboflavin under natural sunlight. Two major phototransformation products of TNT, 3,5-dinitroaniline (3,5-DNA) and 1,3,5-trinitrobenzene (1,3,5-TNB), were detected in the samples in the presence of riboflavin receiving irradiation at full wavelength or wavelength >400 nm. The results indicate that riboflavin mediates TNT sensitized-phototransfomation under natural sunlight or near-UV–vis light.
© 2006 Elsevier B.V. All rights reserved.

Keywords: 2,4,6-Trinitrotoluene; Riboflavin; Phototransformation

1. Introduction

2,4,6-Trinitrotoluene (TNT) is a nitroaromatic compound and the most widely used military explosive. Contamination of soils by TNT, generated as waste from the munitions and defense industries, is a significant worldwide environmental problem. Many of the explosive compounds are mutagenic and toxic and have the tendency to persist in the environment [1,2]. There have been several attempts to degrade TNT via phototransformation [3,4]. Exposure of TNT under sunlight or near-UV irradiation in soil and water was reported to proceed via a variety of transformation pathways [5–7]. Studies of TNT photodegradation pathway under specified experimental conditions will help predicting the photochemical fate of TNT under known environmental conditions. Riboflavin is a natural photosensitizer secreted by phytoplanktons [8]. Riboflavin was found to facilitate TNT phototransformation in a freshwater environment and selected TNT phototransformation products were identified [9,10]. Recently, the effect of riboflavin on the phototransformation of benzo[a]pyrene under natural sunlight and UVA irradiation was reported, and the major products in the photolysis of benzo[a]pyrene were identified [11]. In addition, the kinetics and mechanism of the riboflavin-promoted photochemical degradation with visible light of the herbicide norflurazon was studied with time-resolved and stationary techniques [12]. However, TNT phototransformation in the absence or presence of riboflavin under different spectral range was not investigated.

The aim of the present work was to investigate the contribution of different spectral region of sunlight to TNT phototransformation in the absence or presence of riboflavin. Two optical filters, cut-off at 400 and 455 nm, were used. Phototransformation of TNT in an aqueous solution with riboflavin as the photosensitizer was conducted. The influence of riboflavin concentration and light spectral quality was investigated. In addition, selected phototransformation products of TNT were identified to compare the phototransformation pathway under different spectral conditions.

* Corresponding author. Tel.: +1 601 979 2595; fax: +1 601 979 2778.
E-mail address: hwang@jsums.edu (H.-M. Hwang).

0304-3894/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2006.09.020

2. Materials and methods

2.1. Chemicals

2,4,6-Trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), 1,3,5-Trinitrobenzene (1,3,5-TNB), and 2,4,6-trinitrobenzoic acid (2,4,6-TNBA) were purchased from Chemservice (West Chester, USA). Riboflavin, lumichrome, lumiflavin, 3,5-dinitroaniline (3,5-DNA), and 2,4-dinitrotoluene (2,4-DNT) were purchased from Sigma Chemical Co. (Milwaukee, Wisconsin). TNT Stock solutions were prepared in acetonitrile at the concentration of 5 mM and stored at 4 °C when unused. All solvents were of HPLC grade and other chemicals were of analytical reagent grade.

2.2. Phototransformation of TNT

Phototransformation of TNT (50 μM) with the photosensitizer riboflavin in distilled water solution at the final concentration of 1 μM was carried out in 150 mL quartz flasks (GM Associates, Inc., Oakland, CA). The reaction solutions were composed of a certain amounts TNT in acetonitrile stock solution. The mixed solution was prepared for incubating in triplicate, including dark control, TNT only, and TNT with riboflavin. The flasks were filled with 25 mL solution and irradiated under natural sunlight outdoors between 10 a.m. and 2 p.m. in May and June. The intensity of sunlight irradiation was measured with a research radiometer with UVA or visible light detectors (PMA 2100, Solar Light Co., Philadelphia, PA). The average UVA intensity was 4.561 mW/cm^2 (0.287 J/cm^2 min) and average visible photonic intensity was 150.4 W/m^2 (9.044 kJ/m^2 min) during the irradiation period. To investigate the contribution of different spectral region of sunlight to TNT photolysis, two cut-off optical filters at 400 and 455 nm (Andover Corp., Salem, NH) were used. This is to ensure only visible light was absorbed by the relevant treated samples. The concentration of TNT in the reaction solution was detected by HPLC with a photodiode array detector. All the results, which are reported in this paper, were analyzed in triplicate flasks for each measurement. In the process of irradiation, all quartz flasks were capped with silicon stoppers. Dark control samples, flasks being wrapped in aluminum foil, were used for contrasting in the phototransformation experiments.

2.3. HPLC analysis

2.3.1. Apparatus

HPLC analyses were carried out on a Waters system equipped with two model 515 quaternary pumps, a 717 plus autosampler and a 996 photodiode array detector operating at 254 nm (Waters Corporation, Milford, MA). The Empower pro software was utilized for instrument control, data acquisition, and analysis. A 250 mm × 4.6 mm SUPELCO reversed phase Discovery C18 (Supelco, Bellefonte, PA) column with 5 μm particle size, and a RP-C18 guard column were used for the separation of chemical species. The injection volume was 10 μL each time with autosampler temperature set at 15 °C.

2.3.2. Gradient

Two mobile phases were utilized to establish the gradient system. The organic mobile phase was HPLC-grade acetonitrile. The aqueous mobile phase was distilled water. The flow rate of the mobile phase was 1.0 mL min^{-1}. The mobile phase initially consisted of 90% aqueous phase and 10% acetonitrile. The gradient was changed to 100% acetonitrile at 15 min, and held constant for 5 min. The solvent ratio was returned to the initial condition for 3 min and held for an additional 2 min before injection of the next sample. The total run time of each sample was 25 min.

2.4. Gas chromatograph/mass spectrometry (GC/MS) analysis

The analysis was also performed with a Hewlett-Packard (Palo Alto, CA, USA) HP 5890 series II gas chromatography coupled to a HP 5972 series mass selective detector via a heated transfer capillary line (300 °C). Samples were separated on a 30 m × 0.25 mm i.d. DB-5 MS fused-silica capillary column with film thickness of 0.25 μm. The oven temperature program was as follows: 50 °C for 2 min, from 50 to 100 °C at a rate of 25 °C/min, from 100 to 290 °C at a rate of 10 °C/min, and kept at 290 °C for 5 min. The injector temperature was 250 °C. The mass spectrometer was operated in the full-scan mode, scanning from 50 to 550 m/z at a rate of 1.53 scans/s.

2.5. HPLC/mass spectrometry (HPLC/MS) analysis

HPLC/MS analyses were carried out on a Waters system equipped with two model 515 quaternary pumps, a 717 plus autosampler and EMD 1000 mass spectrometer of Waters Corp. (Milford, MA) to confirm the identity of major degradation products of photolysis. A 150 mm × 2.1 mm reversed phase Nova-Pak C18 (Waters, Milford, MA) column with 4 μm particle size, and a RP-C18 guard column were used for the separation of chemical species. The injection volume was 10 μL each time with autosampler temperature set at 15 °C. The concentrated photolysis sample (natural sunlight, 240 min) was infused directly into the mass detector with full scan APCI negative mode. Operation parameters of mass spectrometry have been optimized for the best sensitivity: corona 20 μA, cone voltage 15 V, source extractor voltages 4 V, RF lens voltages 0.4 V, source temperature 150 °C, desolvation temperature 200 °C, desolvation gas flow 300 L/h. Sample solution was introduced through continuous infusion by means of a syringe pump.

2.6. Measurement of TNT concentration and identification of TNT phototransformation products

Performance assessment was based on calibration standards of authentic compounds and multipoint standard calibration curves, in the linear range of 1–500 μmol L^{-1}, with a $r^2 = 0.999$. TNT and its phototransformation products were separated, quantified and identified by HPLC. Generally, the samples were analyzed immediately after irradiation for different time periods.

In order to identify the products of TNT phototransformation, the sample solution was concentrated with a rotary evaporator (Bűchi Waterbath B-480 and Bűchi Rotavapor R-114) at 40 °C. The extracted samples were dissolved in 1 mL acetonitrile solution and subjected to HPLC, HPLC/MS and GC/MS analyses by comparison of both retention time and spectrum with authentic compounds.

3. Results and discussion

3.1. Effect of concentration of riboflavin on TNT phototransformation

The effect of different concentrations of riboflavin, 0, 0.1, 1, 10, 25, 50, 100 μM on TNT phototransformation was examined. The results indicated that 1 μMof riboflavin could adequately accelerate TNT phototransformation; therefore, in the process of subsequent irradiations 1 μM riboflavin was taken as the optimum concentration for TNT phototransformation. TNT was photolyzed in solution with a half life of about 39 min in the absence of riboflavin under natural sunlight; however, in the presence of riboflavin, the rate of phototransformation of TNT was enhanced significantly (Table 1). Fig. 1 shows that phototransformation of TNT under natural sunlight in the presence or absence of riboflavin was conformed to initial pseudo-first-order rate equation with $r^2 = 0.9843$ and 0.9889, respectively. Initial pseudo-first-order rate constants and the half lives were calculated based on linear regression of $\ln (C_0/C_t)$ *versus* time, where C_0 is initial TNT concentration and C_t is TNT concentration at time t.

In the presence of riboflavin, the irradiation process of TNT under natural sunlight involved phototransformation of both TNT and riboflavin. The literature reported that riboflavin was easily photolyzed [13]. It was firstly transformed into an intermediate compound named 7,8-dimethyl-10-(formylmethyl)isoalloxzaine(formylmethylflavin), and further hydrolyzed to lumichrome and lumiflavin, with lumichrome regarded as the major product. In the process of identifying TNT phototransformation products, lumichrome indeed was detected in the mixture of the intermediates.

The mechanism of riboflavin photosensitized reactions is an active area of recent researches and the photolysis enhancement is suggested to result from redox reactions, in which free radicals and singlet oxygen may be involved [12,14]. It is generally believed that photo-excited riboflavin sensitizes oxidation predominantly by a type-I photoreaction, i.e., by direct one-electron or hydrogen transfer [15,16]. Furthermore, two major photoproducts of riboflavin, i.e. lumichrome and lumiflavin, were demonstrated to further sensitize phototransformation of chemicals such as benzo[a]pyrene after riboflavin was degraded [11]. Therefore, we speculate that photolysis enhancement by riboflavin was mainly mediated through type-I photoreaction and photosensitization was further carried out by its photoproducts.

Table 1
Initial pseudo-first-order rate constants and half life of TNT under natural sunlight without and with riboflavin

Riboflavin concentration (μmol L^{-1})	Natural sunlight	
	Rate constant (s^{-1})	Half life (s)
0	3.0E−4 ± 0.3E−4	2340
1	5.4E−4 ± 0.7E−4	1312

Rate constant: mean ± S.D. ($n = 3$).

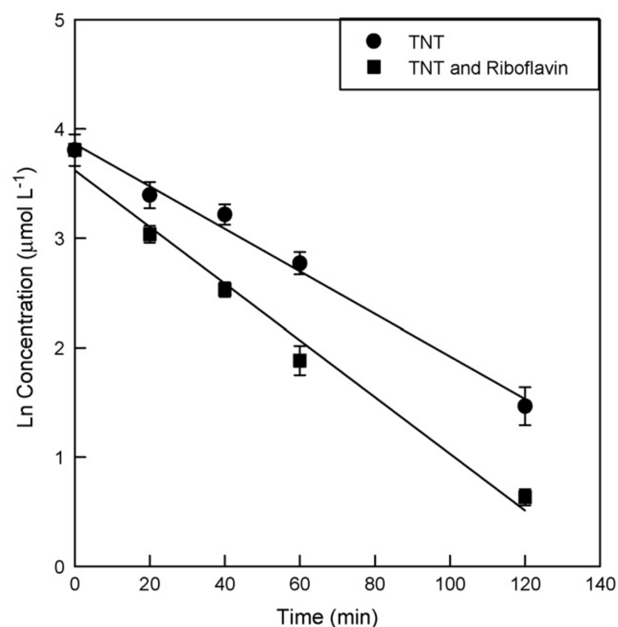

Fig. 1. Photolysis of TNT without or with riboflavin in distilled water under natural sunlight. Concentration of TNT and riboflavin were 50 and 1 μM, respectively. Transformation equation in the absence and presence of riboflavin was $\ln C = 3.86 - 0.019t$ and $3.62 - 0.026t$, respectively.

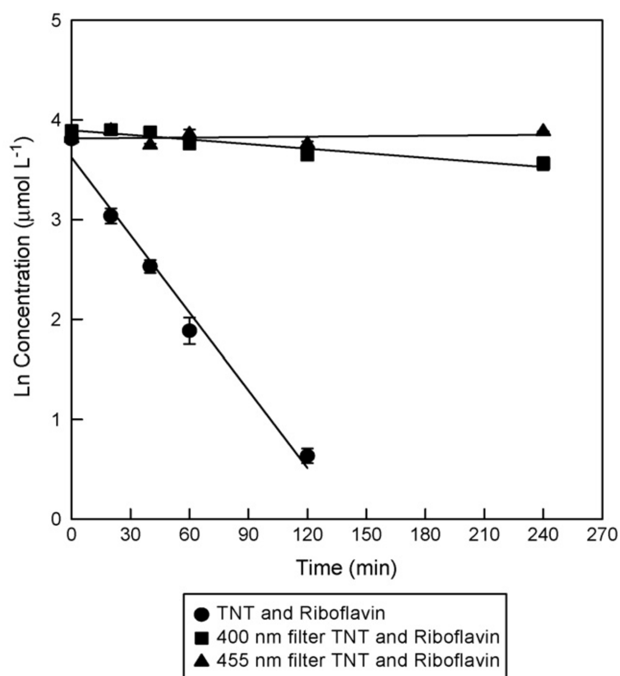

Fig. 2. Photolysis of TNT with riboflavin as the photosensitizer under natural sunlight, with 400 or 455 nm optical filter.

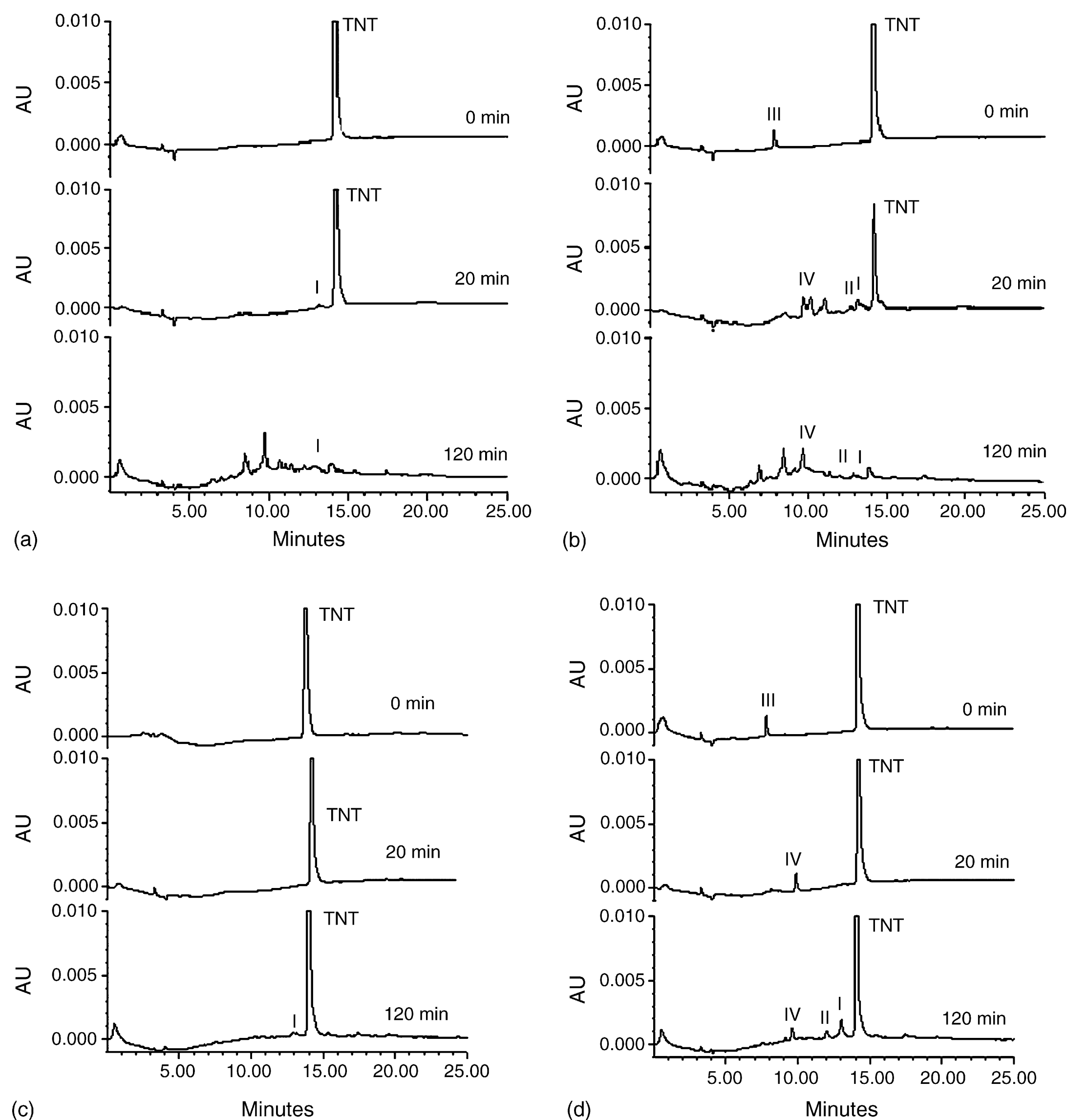

Fig. 3. HPLC chromatograms of phototransformation samples of TNT: (a) direct photolysis of TNT by natural sunlight, (b) riboflavin photosensitized transformation, (c) photolysis with 400 nm optical filter, (d) riboflavin photosensitized transformation with 400 nm optical filter. Compound I: 1,3,5-trinitrobenzene; compound II: 3,5-dinitroaniline; compound III: riboflavin; compound IV: lumichrome.

3.2. Effect of spectral range of irradiation

In order to investigate the effect of natural sunlight at different spectral range on the process of TNT phototransformation, phototransformation of TNT was performed under optically filtered irradiation at cut-off wavelength 400 or 455 nm. Fig. 2 shows that phototransformation of TNT under natural light in the presence of riboflavin was significantly faster than that with cut-off 400 or 455 nm filter. Phototransformation of TNT under natural sunlight in the presence or absence of riboflavin was conformed to initial pseudo-first-order rate equation. By comparison, TNT riboflavin-sensitized phototransformation under irradiation with cut-off 400 or 455 nm filter yields $r^2 = 0.8974$ and 0.4405, respectively. Analysis of the chromatograms indicates that most of the added TNT in the process of phototransformation under natural sunlight was completely transformed into intermediate products. Selected products of TNT phototransformation with cut-off 400 nm filter were detected. Nevertheless, no TNT phototransformation product was detected in the samples treated with cut-off 455 nm filter. It was reported that sunlight reaching the earth surface is composed of 91% visible, 8.7% UVA (320–400 nm), and 0.3% UVB (280–320 nm) [17]. Therefore, realistically solar irradiation on the earth surface consists of UVA and visible lights. Since the UV–vis absorption spectra of riboflavin have major bands at 224.9, 268.8, 359.2, 442.1 nm, respectively [11], the results match with the expectation that riboflavin-sensitized reaction occurs in the range of near-visible UV and visible light. When the cut-off 400 nm filter was used, all of light of wavelength shorter than 400 nm was blocked. Therefore, only the visible light was passed and promoted the photosensitized reaction of riboflavin. Since cut-off 455 nm filter can block all UV light and the visible light of wavelength shorter than 455 nm, the photosensitized of riboflavin ceases to function in this study.

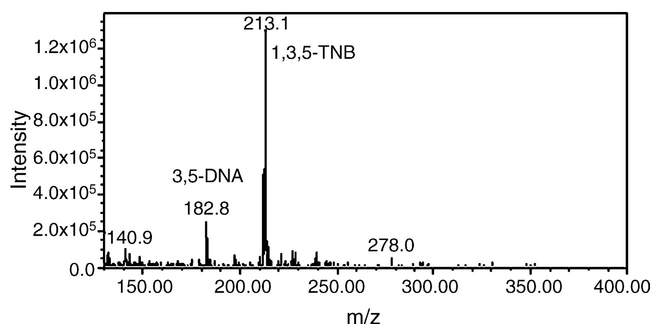

Fig. 4. Identification spectrum of the concentrated sample of TNT in the presence of riboflavin being irradiated under natural sunlight for 240 min (APCI negative ion) by HPLC/MS.

3.3. Identification of TNT phototransformation products

The phototransformation products of TNT under different light wavelength and exposure time were analyzed with HPLC with a photodiode array detector, GC/MS and HPLC/MS. The transformation samples of different treatment groups, i.e. dark controls, irradiations under natural sunlight in the absence or presence of riboflavin, and irradiation under natural sunlight with cut-off 400 or 455 nm filter were concentrated with a rotary evaporator under vacuum to dryness and redissolved in acetonitrile. In the presence of riboflavin, after 120 min irradiation under natural sunlight and using cut-off 400 nm filter, the samples contained two product peaks at 12.00 and 12.95 min, which correspond to 3,5-dinitroaniline (3,5-DNA) and 1,3,5-trinitrobenzene (1,3,5-TNB), respectively (Fig. 3). These two compounds were also identified by GC/MS (data not shown). In addition, after 120 min irradiation using cut-off 455 nm filter, there was no other peaks besides TNT appearing in the chromatogram. The same result of the identification of phototransformation products was observed from the HPLC/MS spectrum (Fig. 4).

TNT solution was quickly turned to pink in the absence of riboflavin under natural sunlight. Numerous organic compounds such as 1,3,5-trinitrobenzene, 2,4,6-trinitrobenzaldehyde were reported to be present in aqueous solutions as the result of phototransformation process [18]. In the present work, only 1,3,5-TNB could be found in the HPLC chromatogram of the irradiated samples in the absence of riboflavin under natural sunlight and using cut-off 400 nm filter. However, both 1,3,5-TNB and 3,5-DNA were detected as photoproducts of TNT in the irradiated samples in the presence of riboflavin (Fig. 3). Therefore, it is likely that TNT phototransformation undergoes different pathways in the presence or absence of riboflavin. The cytotoxicity of phototransformation products of TNT including 3,5-dinitroaniline, 2-amino-4,6-dinitrotoluene, 4-amino-2,6-dinitrotoluene on freshwater microbial assemblages was determined in our previous research [19]. It has been found that TNT was more phototoxic than its photoproducts and photosensitized transformation decreased TNT cytotoxicity to microbial assemblages in natural water. For example, after exposure to 3,5-dinitroaniline, the viability count of heterotrophic bacterial assemblages was enhanced by 148.7% and these phototransformation products were speculated to become growth substrates for beacterial assemblages in natural water.

This study showed that 1,3,5-TNB was the only product of TNT phototransformation when riboflavin was absent. This is in agreement with a previous report of TNT photolysis in freshwater environment [10]. In that report, two unidentified intermediates were found in the fresh river water sample when riboflavin was present and they disappeared after 60 min. In our study with nanopure water, 1,3,5-TNB and 3,5-DNA were identified after 120 min, indicating that TNT phototransformation products were more stable in nanopure water than that in the fresh river water. The results also suggest that TNT phototransformation may undergo different pathways in the nanopure water and fresh river water in the presence of riboflavin.

4. Conclusion

1,3,5-TNB and 3,5-DNA, the two major TNT phototransfomation products were identified in the samples in the presence of riboflavin receiving irradiation at full wavelength or wavelength >400 nm. The results confirm that riboflavin mediates TNT sensitized-phototransfomation under natural sunlight or near-UV–vis light. Therefore, riboflavin sensitized photolysis can be a practical technique for TNT remediation in the field. The results also suggest that TNT phototransformation may undergo different pathways in the nanopure water and fresh river water in the presence of riboflavin.

Acknowledgements

This research was supported by the following grants: (1) NIH-SCORE S06GM08047 (to JSU) and (2) US Department of the Army #W912H2-04-2-0002 and #W911NF-04-1-0327 to JSU.

References

[1] J.E. Walker, D.L. Kaplan, Biological degradation of explosives and chemical agents, Biodegradation 3 (1992) 369–385.
[2] K. Zeng, H.M. Hwang, Y. Zhang, S. Cook, Assessing cytotoxicity of photosensitized transformation products of 2,4,6-trinitrotoluene (TNT) and atrazine with freshwater microbial assemblages, Environ. Toxicol. 19 (2004) 490–496.
[3] S.J. Lee, H.S. Son, H.K. Lee, K.D. Zoh, Photocatalytic degradation of explosives contaminated water, Water Sci. Technol. 46 (2002) 11–12.
[4] H.S. Son, S.J. Lee, I.H. Cho, K.D. Zoh, Kinetics and mechanism of TNT degradation in TiO_2 photocatalysis, Chemosphere 57 (2004) 309–317.
[5] D.C. Schmelling, K.A. Gray, Photocatalytic transformation and mineralization of 2,4,6-trinitrotoluene (TNT) in TiO_2 slurries, Water Res. 29 (1995) 2651–2662.
[6] D.G. Crosby, Environmental Toxicology and Chemistry, Oxford University Press, Oxford, UK, 1998, pp. 287–288.
[7] P.S. Lang, W.-K. Ching, D.M. Willberge, M.R. Hoffmann, Oxidative degradation of 2,4,6-trinitrotoluene by ozone in an electrohydraulic discharge reactor, Environ. Sci. Technol. 32 (1998) 3142–3148.
[8] K. Mopper, R.G. Zika, Natural photosensitizers in sea water: riboflavin and its breakdown products, Photochemistry of environmental aquatic systems, in: ACS Symposium series, vol. 327, 1985.
[9] H.M. Hwang, L.F. Slaughter, S.M. Cook, H. Cui, Photochemical and microbial degradation of 2,4,6-trinitrotoluene (TNT) in a freshwater environment, Bull. Environ. Contam. Toxicol. 65 (2000) 228–235.

[10] H. Cui, H.M. Hwang, S. Cook, K. Zeng, Effect of photosensitizer riboflavin on the fate of 2,4,6-trinitrotoluene in a freshwater environment, Chemosphere 44 (2001) 621–625.
[11] X.H. Zhao, X.K. Hu, H.M. Hwang, Effects of riboflavin on the phototransformation of benzo[a]pyrene, Chemosphere 63 (2006) 1116–1123.
[12] W. Massad, S. Criado, S. Bertolotti, A. Pajares, J. Gianotti, J.P. Escalada, F. Amat-Guerri, N.A. García, Photodegradation of the herbicide Norflurazon sensitised by riboflavin. A kinetic and mechanistic study, Chemosphere 57 (2004) 455–461.
[13] I. Ahmad, O. Fasihullah, A. Noor, I.A. Ansari, Q. Ali, Photolysis of riboflavin in aqueous solution: a kinetic study, Int. J. Pharm. 280 (2004) 199–208.
[14] R.A. Larson, P.L. Stackhouse, T.O. Crowley, Riboflavin tetraacetate: a potential photosensitizing agent for the treatment of contaminated waters, Environ. Sci. Technol. 26 (1992) 1792–1798.
[15] I. Schulz, H.C. Mahler, S. Boiteux, B. Epe, Oxidative 3,5-DNA base damage induced by singlet oxygen and photosensitization: recognition by repair endonucleases and mutagenicity, Mutat. Res. 461 (2000) 145–156.
[16] K. Zeng, H. Hwang, Y. Zhang, H.T. Yu, Identification of 6-aminochrysene photoproducts and study of the effect of a humic acid and riboflavin on its photolysis, J. Photochem. Photobiol. B: Biol. 72 (2003) 95–100.
[17] International Agency for Research on Cancer (IARC), Sunscreens, Lyon, France, International Agency for Research on Cancer, 2001.
[18] L.A. Kaplan, N.E. Burlinson, M.E. Sitzmann, Photochemistry of TNT: investigation of the "pink water" problem. Part 2, Rep. No. NSWC/WOL/75-152 (AD-A020072), Naval Surface Weapons Center, White Oaks Laboratory, Silver Spring, MD, 1975.
[19] K. Zeng, H. Hwang, Y. Zhang, S. Cook, Assessing cytotoxicity of photosensitized transformation products of 2,4,6-trinitrotoluene (TNT) and atrazine with freshwater microbial assemblages, Environ Toxicol. 19 (2004) 490–496.