Abiotic degradation of triphenylborane pyridine (TPBP) antifouling agent in water

Xiaojian Zhou,[a] Hideo Okamura,[b*] and Shinichi Nagata [a]

[a] Environmental Biochemistry Division, Research Center for Inland Seas, Kobe University, Fukaeminami 5-1-1, Higashinada, Kobe 658-0022, Japan.

[b] Laboratory of Marine Environmental Management, Faculty of Maritime Sciences, Kobe University, Fukaeminami 5-1-1, Higashinada, Kobe 658-0022, Japan.

* Corresponding author: Hideo Okamura

Tel./Fax: +81-78-431-6272

E-mail: okamurah@maritime.kobe-u.ac.jp

Abstract

The abiotic degradation of the new antifouling agent, triphenylborane pyridine (TPBP), was investigated in buffer solutions having different pH values (pH = 5, 7, and 9), and in artificial and natural seawater to estimate environmental fate of TPBP. The TPBP in these waters was decomposed by a seven-day hydrolysis process at 50 °C both in the dark and a photolysis process under UV-A irradiation using a high-pressure mercury lamp for periods up to 24 h. TPBP hydrolysis was significantly enhanced by acidic pH solutions. The photolysis rate of TPBP was higher in acidic pH solutions than in neutral or basic pH solutions, and was highest in natural seawater, which could have contained naturally dissolved organic matter.

Two degradation products, phenol and an unknown substance (Peak #1), were observed during the hydrolysis and photolysis studies of TPBP. The concentration of these substances after a one-day photolysis treatment was higher than after a seven-day hydrolysis treatment. The degradation rate of TPBP in the five test water samples was related to the simultaneous photolysis formation of phenol and Peak #1. However, the degradation rate of TPBP was not related to the formation of the hydrolysis products. Therefore, it is suggested that photodegradation of TPBP follows a different pathway to the hydrolysis degradation of TPBP. Our results indicate the chemical and photochemical reaction of TPBP in water occurs in natural aquatic environments.

Keywords: Biocide, Buffer solution, Environmental fate, Hydrolysis, Photodegradation, Seawater, UV-A.

Introduction

Antifouling chemicals are used as biocides to deter the growth of organisms on the surface of submerged structures, such as ship's hulls and fishing nets. Organotin (OT) compounds, which have been used as major antifouling agents for several decades, have been regulated internationally since 1990 due to their severe impact on aquatic environments (Tanabe et al., 1998; Yamada and Kakuno, 2003). Many tin-free co-biocides have been developed and utilized in commercial antifouling paints as substitute compounds for OT complexes since the use of OT compounds was regulated (Voulvoulis et al., 1999). Much research has been devoted to Irgarol 1051 and diuron as representative biocides among the substitute OT compounds. One of the reasons for this is that these compounds are not difficult to be analyzed in environmental matrices, and another reason is their relatively high persistence in aquatic environments. Therefore, their environmental fate and impact on marine environments has been reported in several literature references (Okamura et al., 2000; Thomas et al., 2002). However, there is little information available on other biocides because of the lack of suitable established analytical methods.

Triphenylborane pyridine (TPBP) is used as a biocide in ship hull antifouling paints in Japan (Okamura and Mieno, 2006). Amey and Waldron (2004) noted that TPBP is an efficient biocide against both soft and hard fouling organisms, with extremely low water solubility and a long lasting antifouling efficacy. In addition, TPBP is most frequently used in two-biocide mixtures, which are present in 40 antifouling products in Japan (Okamura and Mieno, 2006). There have been some reports on the ecotoxicity of TPBP towards marine bacteria (Zhou et al., 2006), cultured fish cells and rainbow trout (Okamura et al., 2002), sea urchin eggs

(Kobayashi and Okamura, 2002), and towards nontarget plant species (Okamura et al., 2003). All the data indicate a relatively high toxicity of TPBP to aquatic and terrestrial organisms. However, it is difficult to evaluate the environmental risk of TPBP, since residue concentrations of TPBP in environmental matrices are not available. Recently, Takahashi et al. (2004) have developed an analytical method for TPBP using an ion-pair HPLC and have reported the detection limit of TPBP as 0.5 μg l^{-1} in 100 ml of seawater obtained by solid phase extraction. Amey and Waldron (2004) proposed a degradation pathway for TPBP by hydrolysis, thermolysis, and photolysis using a low-pressure mercury lamp, which generates UV-C radiation. They identified some degradation products from the degradation of TPBP in water. However, there are no available data on the photodegradation of TPBP by solar radiation, i.e., UV-A. Therefore, it is necessary to clarify the fate of TPBP under these conditions to evaluate its environmental risk.

In this study, the abiotic degradation of TPBP in water was investigated under hydrolysis and photolysis using UV-A irradiation. The pH and ionic strength of the test waters was focused on as the main factors affecting the degradation of TPBP. The degradation of TPBP, as well as degradation products, was analyzed using ion-pair HPLC with both ultraviolet and fluorescence detection.

Materials and Methods

Chemicals

The triphenylborane pyridine (TPBP), and the estimated degradation products diphenylborane monohydroxide (DPB) and monophenylborane dihydroxide (MPB) were donated by the Hokko Chemical Industry Co. Ltd., Japan. The phenol, benzene, pyridine, acetonitrile (HPLC grade), and methanol (HPLC grade) were purchased from Wako Pure Chemical Industries, Ltd., Japan. The tetrabutylammonium phosphate (TBAP, 0.5 mol L^{-1}) used as the ion pair reagent was purchased from the Tokyo Kasei Kogyo Co. Ltd., Japan. The water used to prepare all the aqueous solutions was obtained using an Aquarios RFU554CA ultrapure water system (Advantec, Japan).

Preparation of the test solutions

Buffer solutions were prepared consisting of 20 mM of sodium acetate adjusted to pH = 5, 7, and 9. ASPM media with an Artoxkit M (Microbiotest Inc., Belgium) toxicity testing kit using marine crustaceans was used as the artificial seawater (ASW) sample. Natural seawater (NSW) samples were collected from the port at the Faculty of Maritime Sciences, Kobe University, Japan during April 2006, and were filtered through a 0.45 μm pore size membrane filter. The pH of the ASW and NSW solutions were pH = 7.9 and 8.2, respectively. One milliliter of a 1,000 mg l^{-1} TPBP in acetonitrile solution freshly prepared before each degradation treatment was added to 1,000 ml of each aqueous solution that had been filtered through a 0.45 μm pore size membrane filter to remove any microorganisms and small particles. Thus, the test solutions containing 1 mg l^{-1} of TPBP were prepared as

five different aqueous solutions.

Hydrolysis experiments

Each of five different test solutions containing 1 mg l^{-1} of TPBP (200 ml) was divided into four test glass tubes (50 ml each), and then the tubes were tightly capped. One tube was subjected to HPLC analysis to determine the initial TPBP concentration immediately after preparation of the test solutions. The remaining three tubes were kept at 50 °C in the dark in an incubator for a period of seven days according to ASTM Procedure E895-89 (ASTM, 1993a). After their seven-day incubation the three test samples were subjected to HPLC analysis to determine the concentration of TPBP and its degradation products. Each sample was analyzed in triplicate using HPLC.

Photodegradation experiments

A test solution (950 ml) in a glass bottle of a photochemical device (UVL-100HA, Riko Kagaku Sangyo, Co., Japan) was irradiated using a high-pressure mercury lamp (P = 100 W) inside of the device. The lamp emitted UV-A radiation, with a wavelength from 312 to 577 nm, with a maximum wavelength at λ = 365 nm. The UV-A intensity was measured using a digital UV meter attached to a UV-A detector (Model UV-103, Macam, USA). The maximum UV-A intensity on the lamp surface was ca. 300 Wm^{-2}, and the maximum intensity at the outer surface of the glass bottle was ca. 50 Wm^{-2}. Therefore, it was estimated that the test solution was irradiated with a UV-A intensity of 50–300 Wm^{-2}, which is more than double the value observed on fine summer days in west Japan. A water cooling unit was installed to cool the mercury lamp and the reaction mixture, which was homogenized using a

magnetic stirrer. A volume of 50 ml of the test solution sampled at 0, 0.25, 0.5, 1, 2, 3, 4, 8, 12, and 24 h intervals was subjected to HPLC analysis to determine the concentration of TPBP and its degradation products. Each sample was analyzed in triplicate using HPLC.

Concentration of the test solutions and HPLC analysis

Pyridine (0.5 ml) was added to the test solutions (50 ml) immediately after collection (pyridine concentration = 1 %) to stabilize TPBP in water, and the samples were concentrated using solid phase extraction and analyzed using ion pair HPLC according to the procedure of Takahashi et al. (2004). The test samples were loaded onto a Sep-Pak tC18 ENV column (Waters, USA), preconditioned for use by eluting it with methanol and water under reduced pressure. The column after loading the test sample was eluted with water to remove any salts in the test samples, and the column was then eluted with 2.5 ml of acetonitrile containing 1 % pyridine. Thus, the eluate was 20 times more concentrated than the sample in the original test solution, and this was subjected to HPLC analysis. The TPBP, DPB, MPB, phenol, and benzene in the eluates were analyzed using a semi-micro HPLC system (LaChrom Elite, Hitachi, Japan) equipped with a photodiode array detector (PDA, L-2450, Hitachi, Japan) and a fluorescence detector (FLD, L-2480, Hitachi, Japan), installed in tandem after outlet of the column (Develosil ODS UG5, 2.0 mm x 150 mm, particle size = 5 μm, pore size = 14 nm, endcapped two-times by trimethyl silylation, Nomura Chemical, Japan), and mounted with an ODS precolumn. The solvents used were: (A) 5 mM of TBAP in water, and (B) acetonitrile at a flow rate of 0.2 ml min^{-1} at 40 °C. A linear gradient elution was employed with programming from an initial 20 % of B to 100 % B in a 20 min run, and a constant flow of 20 % of B was achieved from 20.1 min to 40 min to

equilibrate the column for further analysis. A volume of 2 μl of the sample was injected into the column using an autosampler (L-2200, Hitachi, Japan). The UV absorption at λ = 210 nm and the fluorescence intensity at the excitation wavelength (Ex = 275 nm) and the emission wavelength (Em = 295 nm) were used for quantification. All the data were analyzed using the EZChrom Elite software package. The analytical data are shown as the average value with the standard deviation of triplicate analyses.

Results and Discussion

HPLC chromatograms

Standard compounds of TPBP, DPB, MPB, phenol, and benzene mixed in acetonitrile solutions containing 1 % pyridine were detected by both the photodiode array detector and the fluorescence detector (Figs. 1-A and 1-C). The pyridine in the acetonitrile solution was eluted at a retention time of 3.4 min. The TPBP, phenol, and benzene samples were well separated, but the DPB and MPB samples were eluted at the same retention time (6.3 min) under the HPLC conditions used in this study. The separation of the DPB and MPB samples was also not accomplished using other HPLC columns and other mobile phases. Phenol had a low absorption rate at λ = 210 nm, but a high fluorescence intensity (retention time = 8.2 min). Therefore, this fluorescence intensity was used to quantify the phenol concentration in the test samples.

The sample shown in Figs. 1-B and 1-D is the 20 times-concentrated one in acetonitrile after a 24 h irradiation period in a sodium acetate buffer at pH = 5, when analyzed using the same HPLC conditions as for the standard solutions. It can be seen

that the TPBP residue was detected by the ultraviolet detection (Fig. 1-B), and phenol at 8.2 min and an unknown Peak #1 at 21.0 min appeared in the fluorescence detection (Fig. 1-C). There were also some other peaks observed in the samples' chromatograms, but most of these peaks were also detected in the standard chromatograms. Therefore, we targeted these five peaks: DPB plus MPB, phenol, benzene, TPBP, along with the unknown Peak #1 in all the samples during the hydrolysis and photolysis experiments.

Hydrolysis of TPBP

The concentration of TPBP in the tested solutions after the hydrolysis experiments is shown in Table 1. The initial concentration of TPBP was calculated to be in the range 0.63 to 0.97 mg l^{-1}. Thus, the recovery rate of TPBP from water using solid phase extraction varied from 63 % for natural seawater to 97 % for a buffered solution at pH = 9. The recovery from seawater was apparently lower than that from a buffered solution, regardless of the pH. The TPBP concentration in all the test solutions significantly decreased after hydrolysis at 50 °C after 7 d in the dark. Hydrolysis at 50 ºC for 7 d is equivalent to hydrolysis at 25 ºC for six months according to the ASTM protocol (ASTM 1993a). The degradation rate of TPBP after a 7 d hydrolysis treatment (the ratio of the degraded concentration after 7 d compared to the initial concentration) was 72 % in a buffered solution at pH = 5, and was ranged from 24 % to 30 % in the other test waters. Thus, TPBP was hydrolyzed in all the waters tested, and its degree of hydrolysis increased in solutions with acidic pH.

Photodegradation of TPBP and the formation of photoproducts

The concentration of TPBP in buffered solutions having different pH showed the formation of phenol and an unknown Peak #1 (Fig. 2). The concentration of TPBP decreased with irradiation time up to a period of 24 h. In a buffered solution at pH = 5 (Fig. 2-A), more than half of the TPBP was degraded in the first four hours. In the case of pH = 7 (Fig. 2-B), half of the TPBP was degraded after 12 h. However, for pH = 9 (Fig. 2-C), only 40 % of the TPBP was degraded after 24 h irradiation.

Since the photolysis reaction rate was assumed to be first-order, a plot of the natural log concentration versus time should produce a straight line (ASTM, 1993b). As shown in Table 2, the first-order rate constant (K) of TPBP calculated using the data in the period 0–12 h was: 0.096, 0.054, and 0.023 h^{-1} for the buffered solutions at pH = 5, 7, and 9, respectively. From the value of K, the half-life was: 7.2, 13, and 30 h for the buffered solutions at pH = 5, 7, and 9, respectively. It can be seen that acidic pH enhances the photolysis of TPBP in a buffered solution.

Accompanying TPBP photodegradation, the concentrations of two compounds, phenol and Peak #1, increased with increasing irradiation time (Fig. 2). Peaks corresponding to DPB plus MPB and benzene were not observed in all the chromatograms of the photolyzed samples. The phenol concentration after 24 h irradiation was 0.06 mg l^{-1} in a buffered solution at pH = 5, 0.02 mg l^{-1} in a buffered solution at pH = 7, and 0.01 mg l^{-1} in a buffered solution at pH = 9. The peak areas of Peak #1 were: 1.5×10^7, 0.51×10^7, and 0.32×10^7 in buffered solutions at pH = 5, 7, and 9, respectively. Thus, the production of phenol and Peak #1 in the photoreaction mixtures was related to the decrease in TPBP concentration.

The concentration of TPBP in the seawater samples (ASW and NSW) also decreased under photolysis (Fig. 3). Except for DPB, MPB, and benzene, two photoproducts (phenol and Peak #1) simultaneously formed with increasing

irradiation time. The initial TPBP concentration in the ASW and NSW samples was 0.82 and 0.83 mg l^{-1}, respectively, which decreased to 0.40 and 0.11 mg l^{-1}, respectively, after 24 h irradiation. As shown in Table 2, the photolysis rate constant for the first 12 h was calculated to be 0.047 h^{-1} for the ASW sample, and 0.11 h^{-1} for the NSW sample. The half-life was estimated to be 15 h for the ASW sample, and 6.5 h for the NSW sample. High values of the correlation coefficients of each test water sample indicate that the photolysis of TPBP follows first order kinetics in the initial stage of photolysis. The phenol concentration after 24 h irradiation was 0.04 mg l^{-1} for the ASW sample, and 0.1 mg l^{-1} for the NSW sample. The peak areas of Peak #1 were 1.0×10^7 for the ASW sample, and 2.2×10^7 for the NSW sample. Similar to the buffer solutions, the production of phenol and Peak #1 in seawater was also related with the decrease in TPBP concentration.

A degradation pathway of TPBP in aqueous solution has been proposed (Amey and Waldron, 2004), in which DPB, MPB, benzene, phenol, and boric acid were the degradation products of TPBP under hydrolysis, thermolysis, and photolysis. Three compounds, DPB, MPB and benzene, were not found in the hydrolysis and photolysis experiments in this study. Beside this, the unknown Peak #1, which has a high fluorescence intensity, was produced during the abiotic degradation of TPBP in water, although it has not yet been identified chemically.

Factors affecting the abiotic degradation of TPBP

To clarify the factors influencing the hydrolysis and photolysis of TPBP, the degradation rates of hydrolysis and photolysis are shown in Fig. 4. After a 7 d hydrolysis period, about 70 % of the TPBP was degraded in a buffered solution at

pH = 5. In the other four solutions used in this study, all the TPBP degradation rates were below 30 %. TPBP in water is more stable in neutral and alkaline pH solutions than in acidic pH solutions, regardless of the ionic strength of the test solutions. In our photolysis experiments, the degradation rate of TPBP after a 24 h irradiation period were 75 %, 60 %, and 38 % in buffered solutions at pH = 5, 7, and 9, respectively. In the ASW sample at pH = 7.9, the photodegradation rate was 51 %, which was a mid-value of the 38 % photodegradation rate in a buffered solution at pH = 9 and the 60 % photodegradation rate in a buffered solution at pH = 7. For comparison, the photodegradation rate of TPBP in the NSW sample at pH = 8.2 was 85 %, which was the highest rate among all the test solutions. This apparent difference in degradation rates between the ASW and NSW samples suggests that factors other than pH can accelerate the photolysis of TPBP, because the pH values of the seawater samples were of the same magnitude.

It has been reported that the photolysis of bisphenol and Irgarol 1051 is enhanced by humic substances acting as photosensitizers in natural waters (Okamura et al., 1999; Sakkas et al., 2002; Okamura and Sugiyama, 2004; Zhan et al., 2006), and there is the possibility that dissolved organic matter in the NSW sample could enhance the photodegradation of TPBP.

Two compounds, phenol and Peak #1, were formed in most of the test solutions during the hydrolysis and photolysis of TPBP (Fig. 5). Less than 0.02 mg l^{-1} of phenol was detected after a 7 d hydrolysis treatment in the test water samples, except for the NSW sample, in which 0.05 mg l^{-1} of phenol was observed (Fig. 5-A). The highest degradation rate of TPBP in the hydrolysis experiments was observed in buffered solutions at pH = 5. However, the phenol concentration produced was the lowest among the test solutions. The concentration of phenol produced during

photolysis was higher than that produced during hydrolysis, except for the buffered solution at pH = 9. The maximum phenol concentration (0.11 mg l^{-1}) produced by photolysis was observed in the NSW sample. It is obvious that higher photodegradation rates result in a higher production of phenol.

The rate of production of the unknown Peak #1 is shown in Fig. 5-B. The rate of production of Peak #1 during photolysis was higher than that during hydrolysis. The peak area of Peak #1 during hydrolysis was less than 6×10^5, and its appearance in a buffered solution at pH = 9 was negligible. It is also speculated that a higher photodegradation rate resulted in a higher rate of production of Peak #1. Thus, the photodegradation of TPBP in water led to the simultaneous formation of phenol and Peak #1, as shown in Figs. 4 and 5. These results suggest that phenol and Peak #1 are possible photoproducts of TPBP in natural aquatic environments.

The photodegradation of TPBP in five different waters showed similar behavior, with the production of phenol and Peak #1, but this was not so for hydrolysis. This observation suggests the possibility that the photodegradation pathway of TPBP is different during hydrolysis. Further study is required to clarify the degradation pathway of TPBP in natural environments, as well as identifying the unknown Peak #1.

Conclusions

The abiotic degradation of TPBP in water was investigated under hydrolysis and photolysis to estimate the fate of TPBP in aquatic environments. Our conclusions are as follows:

1. TPBP was recovered from five different water samples using solid phase extraction

with recovery rates ranging from 63 % to 97 %.

2. A concentration of 1 mg l^{-1} of TPBP in water significantly degraded after 7 d hydrolysis at 50 °C in the dark.

3. Acidic pH values enhanced the hydrolysis and photolysis of TPBP.

4. TPBP in water was significantly photodegraded by UV-A irradiation using a high-pressure mercury lamp.

5. The photolysis rate constant of TPBP ranged from 0.023 h^{-1} in a buffer solution at pH = 9 to 0.11 h^{-1} in natural seawater.

6. The abiotic degradation of TPBP was accompanied by the formation of phenol and an unknown Peak #1. There may be different degradation pathways for TPBP in water under hydrolysis and photolysis.

7. The degradation of TPBP and the simultaneous production of phenol and Peak #1 in natural seawater was the highest among the water samples tested. This suggests that the chemical and photochemical reactions of TPBP in water can occur in natural aquatic environments.

Acknowledgments

The triphenylborane pyridine, diphenylborane monohydroxide, and phenylborane dihydroxide were kindly donated by the Hokko Chemical Industry Co. Ltd., Japan. This work was partly supported by a Grant-in-Aid for Scientific Research (B16310026) and the Shipbuilders' Association of Japan.

References

Amey, R.L. and Waldron, C., 2004. Efficacy and chemistry of BOROCIDE™ *P*-Triphenylboron-Pyridine, a non-metal antifouling biocide. In: Proceeding of International symposium on antifouling paint and marine environment. Tokyo, Japan, pp. 234-243.

ASTM. 1993a. Practice for determination of hydrolysis rate constants of organic chemicals in aqueous solutions. E895-89, ASTM standards on aquatic toxicology and hazard evaluation, pp. 336-340.

ASTM. 1993b. Standard test method for conducting aqueous direct photolysis tests. E896-92, ASTM standards on aquatic toxicology and hazard evaluation. 341-347.

Kobayashi, N. and Okamura, H. 2002. Effects of new antifouling compounds on the development of sea urchin. Mar. Pollut. Bull. 44, 748-751.

Okamura, H., and Mieno, H., 2006. Present status of antifouling systems in Japan: Tributyltin substitutes in Japan. Eds Konstantinou, I. Antifouling paint biocides. Volume 5 Part O “The handbook of environmental chemistry” Springer, New York, pp. 201- 212.

Okamura, H. and Sugiyama, Y., 2004. Photosensitized degradation of Irgarol 1051 in water. Chemosphere 57, 739-743.

Okamura, H., Nishida, T., Ono, Y., and Shim, W.J. 2003. Phytotoxic effects of antifouling compounds on non-target plant species. Bull. Environ. Contam. Toxicol. 71, 881-886.

Okamura, H., Watanabe, T., Aoyama I., Hasobe, M., 2002. Toxicity evaluation of new antifouling compounds using suspension-cultured fish cells. Chemosphere 46, 945-951.

Okamura, H., Aoyama, I., Takami, T., Maruyama, T., Suzuki, Y., Matsumoto, M., Katsuyama, I., Hamada, J., Beppu, T., Tanaka, O., Maguire, R.J., Liu, D., Lau, Y.L., and Pacepavicius, G.J., 2000. Phytotoxicity of the antifouling compound Irgarol 1051 and a major degradation product. Mar. Pollut. Bull. 40, 743-752.

Okamura, H., Aoyama, L., Liu, D., Maguire, R.J., Pacepavicius, G.J., Lau, Y.L., 1999. Photodegradation of Irgarol 1051 in water. J. Environ. Sci. Health B34, 225-238.

Sakkas, V.A., Lambropoulou, D.A., Albanis, T.A., 2002. Photochemical degradation study of Irgarol 1051 in natural waters: influence of humic and fulvic substances on the reaction. J. Photochem. Photobiol. A147, 135-141.

Takahashi, K., Yoshikawa, E., Akiyama, M., Kitaori, K., Masuoka, S., 2004. Determination of release rate of pyridine-triphenylborane (PK) from copper-free antifouling paints. Proceeding of International symposium on antifouling paint and marine environment. Tokyo, Japan, pp. 196-203.

Tanabe, S., Prudente, M., Mizuno, T., Hasegawa, J., Iwata, H., Miyazaki, N. 1998. Butyltin contamination in marine mammals from North Pacific and Asian coastal waters. Environ. Sci. Technol. 32, 193-198.

Thomas, K.V., McHugh, M., Waldock, M., 2002. Antifouling paint booster biocides in UK coastal waters: inputs, occurrence and environmental fate. Sci. Total Environ. 293, 117-127.

Voulvoulis, N., Scrimshaw, M.D., Lester, J.N., 1999. Alternative antifouling biocides. Appl. Organometal. Chem. 13, 135-143.

Yamada, H. and Kakuno, A., 2003. Present status on the development of alternative tributyltin-free antifouling paints and toxicity of new biocides to aquatic organisms: Review. Bull. Fish. Res. Agen. 6, 56-72 (in Japanese).

Zhan, M.J., Yang, X., Xian, Q.M., X., Kong, L.R., 2006. Photosensitized degradation of bisphenol A involving reactive oxygen species in the presence of humic substances. Chemosphere 63, 378-386.

Zhou, X.J., Okamura, H., Nagata, S., 2006. Remarkable synergistic effects in antifouling chemicals against *Vibrio fischeri* by bioluminescent assay, J. Health Sci. 52, 243-251.

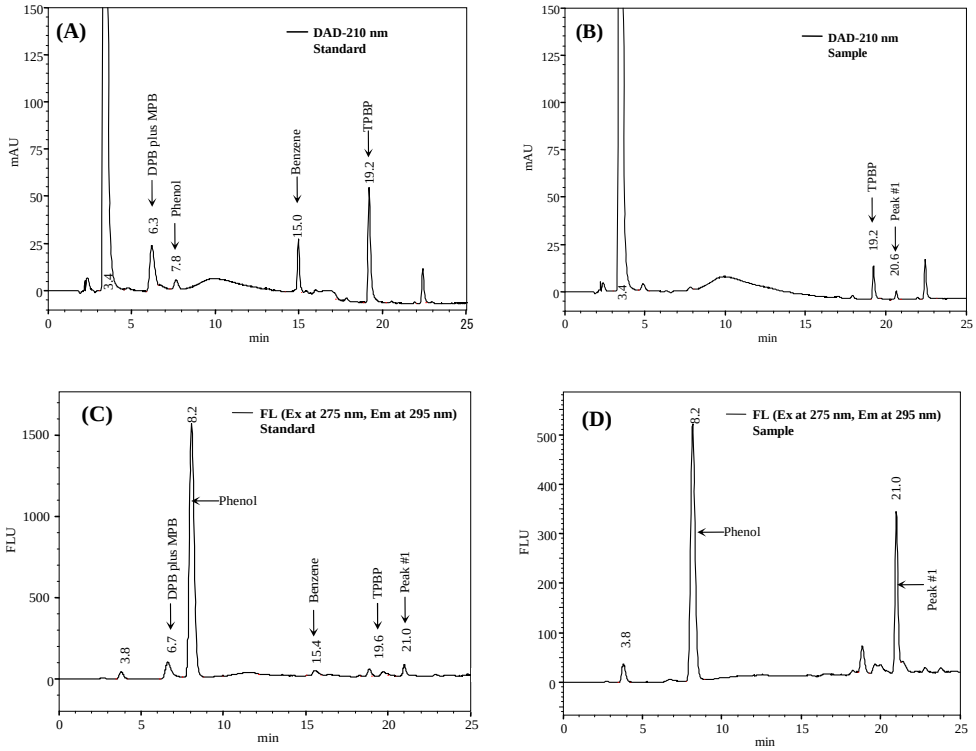

Fig. 1. HPLC chromatograms of a standard solution and a sample.

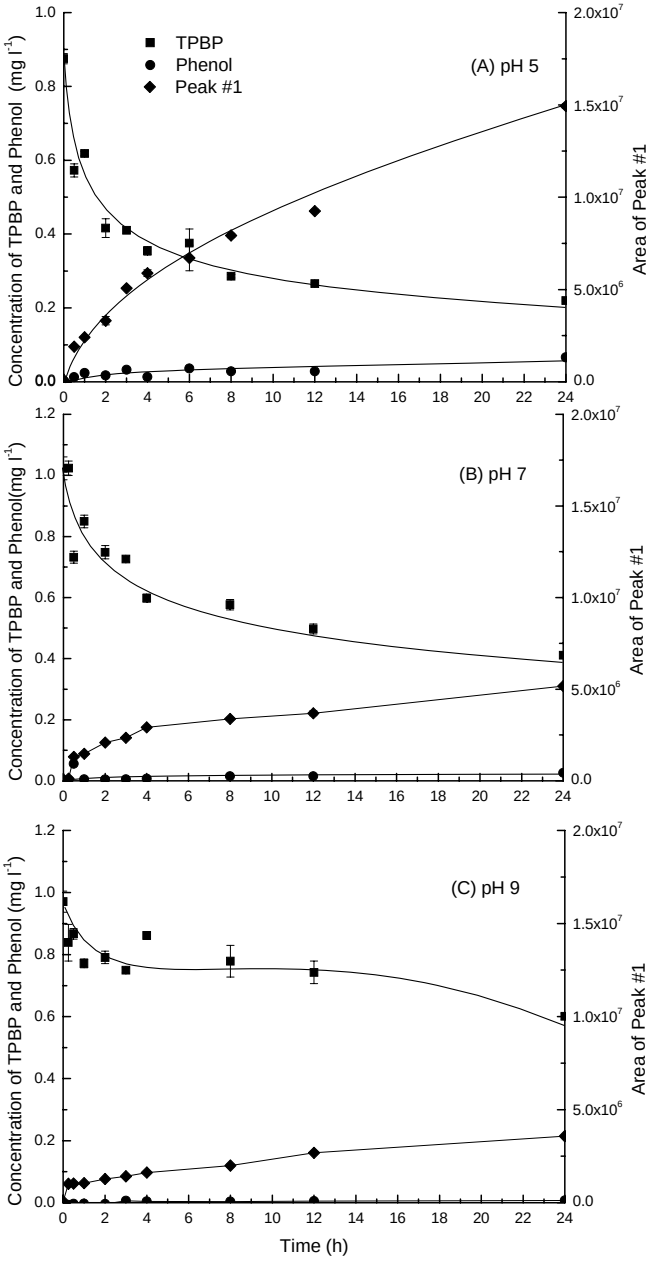

Fig. 2. Photodegradation of TPBP and the formation of phenol and the unknown Peak #1 in a sodium acetate buffer solution at: (A) pH = 5, (B) pH = 7, and (C) pH = 9.

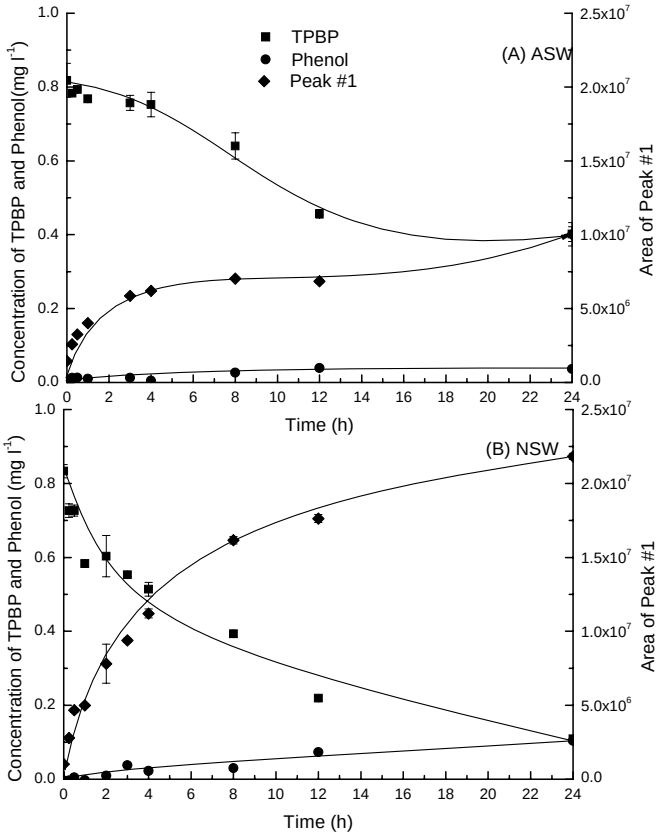

Fig. 3. Photodegradation of TPBP and the formation of phenol and the unknown Peak #1 in: (A) artificial seawater and (B) natural seawater.

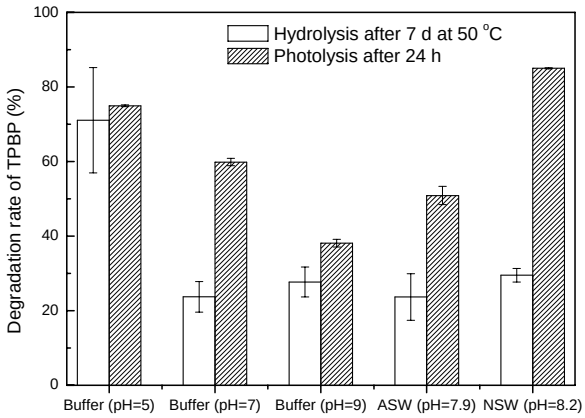

Fig. 4. Degradation rate of TPBP in the five aqueous solutions after hydrolysis and photolysis.

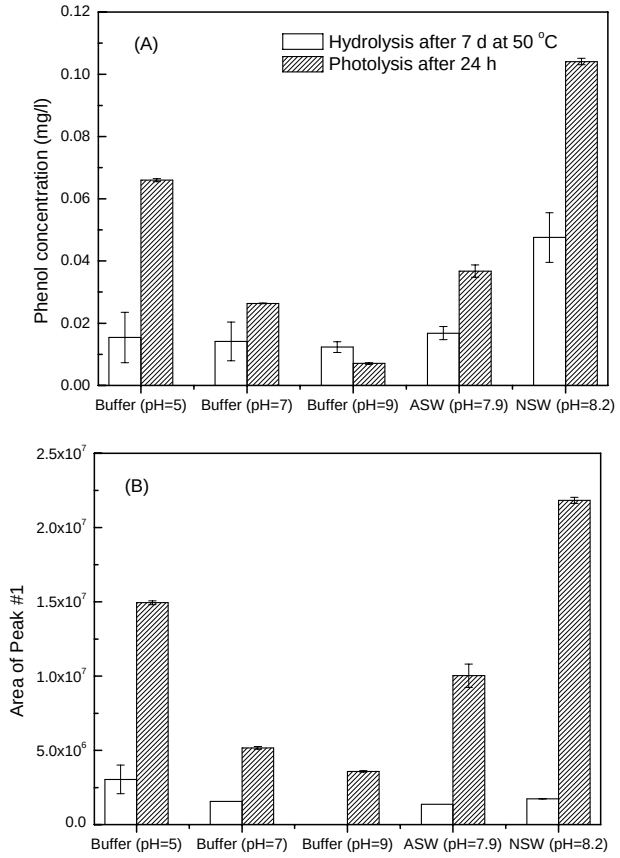

Fig. 5. Production of: (A) phenol and (B) unknown Peak #1 in the five aqueous solutions after hydrolysis and photolysis.

Table 1. Hydrolysis of TPBP in the five aqueous solutions after 7 d at 50 °C in the dark.

Aqueous solution	Initial concentration	Concentration after 7-day at 50°C
Buffer at pH 5	0.88±0.01	0.25±0.12 *
Buffer at pH 7	0.78±0.03	0.59±0.03 *
Buffer at pH 9	0.97±0.01	0.70±0.04 **
artificial seawater	0.69±0.01	0.53±0.04 *
natural seawater	0.63±0.01	0.44±0.01 **

TPBP concentrations are denoted as the means ± standard deviation (for n =3). The statistical difference is indicated at the significance level of: * = $p<0.05$ and ** = $p<0.01$, calculated using Student's paired *t* -test.

Table 2 Photolysis of TPBP in water (0-12 h) [a]

Test Solution	Equation of linear regression [b]			$t_{1/2}$ (h) [c]
	K (h^{-1})	b	r^2	
Buffer of pH 5.0	0.096	-0.36	0.80	7.2
Buffer of pH 7.0	0.054	-0.11	0.81	12.8
Buffer of pH 9.0	0.023	-0.05	0.96	30.0
ASW (7.9)	0.047	-0.24	0.92	14.6
NSW (8.2)	0.110	-0.19	0.98	6.6

[a]: The data of 24 h of incubation was not included due to the long interval from the former sampling time (12 h).

[b]: Natural logarithm of TPBP concentration (ln(C)) as a function of irradiation time (t) was linearly regressed for TPBP photolysis as: $\ln(C) = -Kt + b$, correlation coefficent was shown as r^2.

[c]: Half life of TPBP calculated based on the equation of linear correlation