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Ecotoxicological and chemical characterization of selected treatment process effluents of municipal sewage treatment plant

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Abstract

The triolein-containing semipermeable membrane devices (SPMDs) were deployed for 4 weeks in a sewage treatment plant in Beijing, China, to sample and concentrate priority hydrophobic organic pollutants in a sewage treatment process. The chemical analyses and ecotoxicities of the residuals of SPMDs dialysate were examined. The data from the chemical analyses by gas chromatography-mass spectrometry selected ion monitoring mode indicated the lower removal for polychlorinated biphenyls (PCB) congeners and polycyclic aromatic hydrocarbons (PAHs) coincided with the persistence of them in the environment. The acute toxicity examined by bioluminescence test with *Vibrio fischeri* revealed approximately only 20% decrease in the overall toxicity of the influent after the activate sludge treatment process. The ethoxy resorufin-*O*-deethylase (EROD) induction with a micro-EROD assay in vitro using H4-IIE rat hepatoma cell cultures demonstrated the presence of persistent organics in influent and sequency effluents. Results obtained suggested that integration of the SPMD technique and chemical analyses and bioassay might be a valuable approach for the risk assessment of hydrophobic organic pollutants in water ecosystem. It revealed the necessity for organic pollutants monitoring and ecotoxicities examining of sewage treatment plants.

Keywords: Sewage treatment process; Priority organic pollutants; Semipermeable membrane devices; Bioluminescence inhibition; EROD induction

1. Introduction

The risk assessment of organic pollutants in aquatic environments is often based on the concentrations detected by analytical chemistry and data on the toxicity of single compounds. However, all pollutants exert their effects as components of complex mixtures. The utility of a battery of biotests is well established for environmental hazard assessment of chemicals and chemical products and used routinely to evaluate the toxicity of complex mixtures such as industrial wastewaters (Baun and Nyholm, 1996). Because concentrations of pollutants in water are usually too low for most short-term bioassays and chemical analysis, samples are often needed to preconcentrate using different extraction techniques. Semipermeable membrane devices (SPMDs) are one of the extraction techniques.

SPMDs can provide a means of determining the dissolved portion of organic chemicals in aquatic environment by mimicking the transfer of organic contaminants through respiratory lamellae of fish (Huckins et al., 1990). It consists of a thin film of neutral lipid (such as triolein) enclosed in a thin-walled lay-flat tubing made of low-density polyethylene which have been used for passive monitoring of hydrophobic organic contaminants in many kinds of water bodies (Huckins et al., 1993). SPMDs were effective for concentrating trace organic pollutants, such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and organochlorinae pesticides (OCPs). (Bennett et al., 1996; Huckins et al., 1997; Sabaliunas and Sodergren, 1997; Sabaliunas et al., 2000).

Chemical pollutants in the aquatic environment come from various sources. Both PAHs and PCBs are present in industrial and urban wastewaters carried along the coastal and estuarine waters. Some organic compounds

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in water are known to be involved in several mechanisms of toxicities. Mixed-function oxygenases (MFOs) are a class of inducible microsomal enzymes capable of a number of modification reactions which can result in the conversion of lipophilic compounds to more water-soluble metabolites (Payne et al., 1987). MFOs can be induced by many organic contaminants, including PCBs, PAHs, chlorinated dibenzodioxinx, and chlorinated dibenzofurans (Hansen and Addisson, 1990; Elskus et al., 1992). Extensive studies have suggested that monooxygenases in liver can serve as biomarkers of exposure to PAH and PCB types of pollutants (Narbonne et al., 1991; Goksoyr and Förlin, 1992; Stegeman and Hahn, 1994) and for assessing the risks of environmental samples in vitro (Pesonen and Andersson, 1992). The bioluminescence inhibition assay with *Vibrio fischeri* is used worldwide to evaluate the toxicological effect of effluents (Riisberg et al., 1996) and wastewaters (Asami et al., 1996). It is not only described as rapid, sensible, and easy to perform, but also as a low-cost bioassay that can be recommended as a valuable test method in water pollution control (Nohava and Vogel, 1995).

The municipal sewage treatment plant studied in Beijing, China, is the largest and most complex treatment plant in Asia. The treatment plant has been designed as a conventional sludge system with secondary biological treatment, with activated sludge process for average aeration detention time of 6 h. It treats annually approximately 50 million mm^3 of wastewater serving a population of about 14 million and receiving both industrial wastewater and domestic wastewaters in the city. It consists of pertreatment (screens, grittraps, preaeration), primary clarifers, aeration tanks, and secondary clarifiers. Since the plant was designed as conventional, effluent standards for settleable solids (SS), total suspended solids, COD and BOD_5 have been set up. The treatment efficiency seems good since BOD, COD, and SS in treated sewage do decrease to 5–10% of the untreated sewage. However, the treatment efficacy for trace priority organic pollutants in sewage and their ecological effects have either never been studied or only detected sporadically, which should be of great value considering their potential toxicity and environmental risk.

Chemical analysis provides only part of the knowledge necessary to evaluate and assess their toxic potential for wildlife and humans. This is because the bioavailability of the compounds is not considered and each of the compounds has different cytosolic binding activities (Piskorska-Pliszczynska et al., 1986) and biological activities (Brunström et al., 1991). Moreover, the complex interactions between different environmental chemicals are not completely understood and considered when hazard assessments and predictions of possible ecotoxicological effects are made based on concentrations alone. A toxicity evaluation is an important parameter in wastewater quality monitoring, as it provides the complete response of test organism to all the compounds in the wastewater. Biotoxicity tests could be used in parallel to detect changes in toxicity of the differently treated wastewater samples. The use of selective bioanalytical tools, particularly in connection with chemical analysis, can circumvent these limitations. Therefore, bioassay is a good complement to chemical analyses and a useful tool to predicating the ecological effects to environment.

In this study, triolein SPMD technology was applied to sample and concentrate the priority organic pollutants in a sewage treatment process. Chemical analyses were performed for PCBs congeners, PAHs, and substituted benzenes. Bioluminescence test and EROD induction bioassay were introduced to assess the potential ecotoxicological effects of the selected effluents from the sewage treatment plant. The objective is to connect the chemical analyses components with their ecotoxicity to provide a basis for an integrated pollutant monitoring and assessment method.

2. Materials and methods

2.1. Assembly of SPMDs and deployment

Semipermeable membrane devices were prepared using lay-flat polyethylene tubing (from Beijing Plastics Company; 2.5 cm wide and 0.05–0.55 mm thick). The tubing was cut into 21-cm-long segments and immersed in cyclohexane for 24 h to remove any contaminants and dried with ultrapure nitrogen. Forty microliters (36.4 mg) of triolein (99% purity, Sigma Chemical, St. Louis, MO) was pipetted into each piece of tubing and coated onto the inner part of the tubing and then sealed the ends.

Prepared SPMDs were stored at 4°C in a low gas permeable and portable cooler, which was solvent-cleaned and cooled with dry ice. SPMDs were deployed in the sewage treatment plant for a 28-day period in March 1999. SPMDs were suspended about 1 m below the water surface. Four sampling sites were selected in the sewage treatment plant, namely as Influent (receives the untreated sewage from Beijing city), FirstP (receives sewage treated by aeration and sedimentation), SecondP (sewage after activated-sludge treatment), and Effluent (treated sewage for discharge after the final sedimentation). There were no major differences in current velocity among the four sites, and the average temperature during the exposure was 12 ± 1.5°C. After a 28-day exposure, the SPMDs were retrieved from each site and placed in the same portable cooler and transported immediately to the laboratory for sample processing and residue enrichment. The field

blank SPMDs were carried out with clean tap water during the whole process.

2.2. Sample processing and residue enrichment

SPMDs were rinsed with tap water, distilled water, and 1 N HCl for 30 s to remove surface-defouled residues. Then they were rinsed with distilled water, dried with clean paper and transferred into a beaker containing 100 mL cyclohexane to dialyze at 13°C for 24 h in the darkness. The dialysates were cleaned up with a column consisted of silica gel (60–80 mess, deactivated, 4 cm long) and anhydrous sodium sulfate (4 cm long), treated with copper wool to remove sulfur, and reduced in volume to about 0.5 mL using high-purity nitrogen blowing. The samples were quantitatively diluted to 1 mL using cyclohexane for chemical analyses. And the samples for bioassays were evaporated to dryness with a stream of pure nitrogen and redissolved in DMSO.

2.3. Chemical analysis

The samples concentrated with SPMDs were analyzed for target chemicals, including 57 PCB congeners, three PAHs, four organochlorine pesticides, two herbicides, and 15 substituted benzenes on a gas chromatography-mass selective detectors (GC-MS, HP Model 5890 II/5971A, HP-5 column, 30 m × 0.25 mm i.d. × 0.25 μm, connected with another HP-5 column, 25 m × 0.25 mm i.d. × 0.33 μm, carrier gas: He). External standards like Clophen A50 were used in quantitative analysis for PCB congeners and other target pollutants. To avoid interference of polyethylene waxes and lipids, selected ion monitoring mode (SIM) was used (Frame, 1997). The recoveries of the PCB congeners (level: 1 μg/ml) and other pollutants (level: 20 μg/ml) were between 60% and 93%, with the relative standard deviation less than 20%. The blank SPMDs were processed and analyzed exactly as the deployed samples. No PCBs and other pollutants were detected in SPMD procedural blanks.

2.4. Bioluminescence test

The bioluminescence test was conducted following the manufacture's procedures (Dr. Lange GmbH, Düsseldorf, Germany) as described previously (Wang et al., 2002). *V. fischeri* (strain NRRL-B-11177) and other reagents used for luminescence test were purchased from Dr. Lange GmbH, (Düsseldorf, Germany). The 15-min standard bioluminescence inhibition assay was carried out according to ISO and DIN 38412 L34/L341. Bioluminescence was measured on a luminometer (LUMISTox 300, Dr. Lange GmbH). Because of the limited quantities of SPMDs concentrates, EC_{50} was not obtained but bioluminescence inhibition (light loss) after a 15-min exposure was determined as in Asami et al. (1996) for the purpose of comparison. Luminescence inhibition was calculated by a comparison of the light with control measured under the same condition. Concentration of the carrier solvent (DMSO) in the test medium, including control, was 0.5%, which was a nontoxic dose to *V. fischeri* as determined in preliminary tests.

2.5. In vitro EROD induction bioassay

The H4-IIE rat hepatoma cell line was obtained from the American Type Culture Collection. Dulbecco's MEM (DMEM, containing 1.0 g/L D-Glucose, 3.7 g/L $NaHCO_3$ and 1.0289 g/L N-acetyl-L-alanyl-L-glutamine, Berlin, Germany) was supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin and used as cell culture medium. The cell culture and EROD assay were based on Donato's method (Donato et al., 1992). Briefly, stock cultures of H4-IIE cells were trypsinized with a trypsin–versene mixture (200 mg versene and 500 mg trypsin per liter in balanced salt solution without Ca^{2+} and Mg^{2+}; Whittaker bioproducts), counted, and diluted into growth medium to achieve the desired number of cells. Cells (2.5×10^3) were seeded into individual wells of a 96-well microtiter tissue culture plate (a Coulter counter was used to count the cell numbers). Cells were grown for 24 h to about 60–70% confluency and exposed with samples for another 24 or 72 h. An 8-well lane was used for replication. The plate was incubated at 37°C, 10% CO_2 incubator (Heraeus instrument).

SPMD samples dissolved in DMSO were added directly to medium. Three concentration levels were chosen for test. The final concentration of DMSO was less than 0.5%, which showed no toxic effect on the cells. However, to eliminate artifactual effects due to DMSO, the same amount of the organic solvent was added to control cultures.

Alkoxyresorufin-*O*-dealkylase assays: EROD enzyme activity was determined directly in intact rat hepatoma cell cultured on 96-well plates as in Donato's method (Donato et al., 1992). Monolayers were washed twice with PBS (phosphate-buffered saline 20 mM, pH 7.2, containing 0.9% NaCl) to remove detached cells. Assay was started by the addition of 200 μl/well of culture medium containing 8 μM 7-ethoxyresorufin as substrate and 20 μM dicumarol to prevent further metabolism of the resorufin formed by the cytosolic enzyme diaphorase. After 30 min incubation at 37°C, a 100-μl aliquot of cell medium was withdrawn from each well and transferred to another 96-well plate. Ethanol of (200 μl) was added to each well to precipitate the protein in the medium. Fluorescence of the product resorufin was recorded directly on Microplate Reader (Labsystems Fluoroskan II, Finland) at 550 nm excitation and 585 nm emission wavelength, which could be

regarded as relative EROD induction value. Otherwise, a standard curve of resorufin could be prepared in culture medium and then the EROD activity could be calculated. In this study, the fluorescence value was used directly to express the relative EROD induction level. 7-Ethoxyresorifin and dicumarol were purchased from Sigma (St. Louis, MO). All other reagents used were of analytical grade.

3. Results and discussions

3.1. GC-MS analysis

PCB congeners, PAHs, and substituted benzenes and other target contaminants identified in SPMD samples in sewage at each deployment site are shown in Tables 1 and 2. It could be seen that 3 PAHs could be detected in all samples; their concentrations in SPMDs ranged from 1 pg/ml triolein to 44 pg/ml. For PCB congeners, 23 congeners could be found in SPMD samples; their concentrations in SPMDs ranged from a low of 0.6 pg/ml triolein to a high of 439 pg/ml. Eight substituted benzenes, including toluene, xylene, and 2,4-dinitrotoluene, were detected in SPMDs; their concentrations in SPMDs ranged from 0.8 μg/ml triolein to 340 μg/ml, while 4 OCPs and other target contaminants were not found in all samples. The higher concentrations of target chemicals in SPMD resulted in easier analysis, improved the detection limits, and increased the accuracy.

The detectable concentrations of target contaminants in sewage at all four sites (Tables 1 and 2) demonstrated the lower removal rates of the priority organic pollutants in the sewage treatment process. The results indicated that the activated-sludge treatment process was not very effective in removing priority organic pollutants studied, which was coincidence with their persistence in the environment.

3.2. Acute toxicity to V. fischeri

Screening of acute toxicity of the SPMD concentrated samples from four sites by a standard bioluminescence test procedure showed an approximately only 20% decrease in the overall toxicity of influent arised from hydrophobic organic pollutants during the treatment process. The acute toxicity variation to *V. fischeri* during the treatment process in the municipal treatment plant is illustrated in Fig. 1. The high acute toxicity examined in influent was not significantly eliminated by the following treatment processes. The effluents after activated sludge process still exhibited 40% luminescence inhibition compared to blank. The acute toxicity to *V. fischeri* decreased slightly along the following treatment steps, which demonstrated the certain effects on the removal of some pollutants.

Table 1
Concentrations of PAHs and PCB congeners in triolein of SPMDs deployed in different processes in the sewage treatment plant (pg/ml triolein)

Contaminants	Influent	FirstP	SecondP	Effluent
PAHs				
Naphthalene	34	29	44	72
Phenanthracene	10	26	7	12
Anthracene	20	7	11	16
PCB congeners				
4, 5, 15, 18, 19, 21	—[a]	—	—	—
28, 31, 37, 40, 41, 44	—	—	—	—
25	46	78	193	439
46, 47, 49, 70, 82	—	—	—	—
45	1	36	13	138
52	—	54	50	21
61	—	4	—	5
83	46	3	3	2
84	4	4	5	78
86	2	2	3	2
87	2.5	8	3	2
95	31	13	387	35
99	0.6	1	1	1
101	1.9	13	3	3
105, 129, 134, 136	—	—	—	—
110	0.6	4	1	1
118	81	136	298	111
120	0.6	12	2	1
128	6.3	12	8	5
132	93	26	248	152
135	29	49	108	40
138,146,149,156	—	—	—	—
174,177,178,179	—	—	—	—
151	40	15	155	32
153	43	12	116	71
170	—	—	298	179
176	6.9	3	4	3
180	26	12	169	102

[a] Undetected.

3.3. EROD induction

EROD is generally regarded as being an early warning signal for the Ah-receptor-related toxic effects of PCBs, PAHs, and related compounds (Brink et al., 2000). Fig. 2 illustrates EROD induction as a function of the sample dosage. It could be seen that all four SPMD samples could induce the activity of EROD during the 24 h incubation with dose–response relationships. A higher concentration of the samples has shown some toxic effects on the cells. Generally most organic chemicals could be metabolized by cultured cells after 72 h incubation (Payne et al., 1987), and then the level of induced EROD activity decrease, except the persistent dioxinlike compounds. The EROD induction of the cells exposure to four SPMD samples after 24 and 72 h incubation is illustrated in Fig. 3. It is obvious that after a 72-h incubation, the high EROD activity still remains, indicating the presence of the persistent contaminants in

Table 2
Concentrations of substituted benzenes and other target contaminants in triolein of SPMDs deployed in different process in the sewage treatment plant (micrograms per milliliter of triolein)

Contaminants	Influent	FirstP	SecondP	Effluent
Toluene	36.3	25	73.8	119
Ylbenzene	—[a]	—	—	—
p-Xylene	2.5	1.3	1.7	25
o-Xylene	3.8	2.5	3.3	31
Phenol	0.8	3.3	1.1	8.7
Aniline	—	—	4.8	2.9
Chlorophenol	—	—	—	—
Nitrobenzene	—	—	—	—
2,4,-Dichlorophenol	—	—	—	—
2,3,-Dichlorophenol	—	—	—	—
2,4,6-Trichlorophenol	—	—	—	—
2,6-Dinitrotoluene		340	6.7	1.6
4-Nitrophenol	1.9	15.6	3.3	2.5
2,4-Dinitrotoluene	105	297.5	30	54
Hexachlorobenzene	—	—	—	—
Atrazine	—	—	—	—
Cetochlor	—	—	—	—
Ecosane	—	—	—	—
Llethrin	—	—	—	—
Hlordane	—	—	—	—
Rans-chlordane	—	—	—	—
Ndosulfan	—	—	—	—

[a] Undetected.

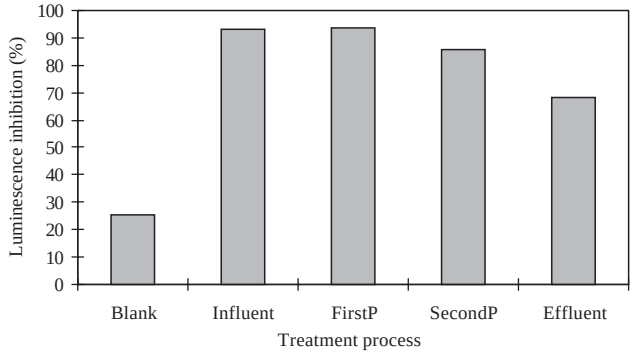

Fig. 1. Luminescence inhibition of SPMD samples from the selected treatment processes of the municipal sewage treatment plant to *V. fischeri*.

all the samples. No distinguish variation of EROD induction levels between the SPMDs samples from four sites indicated the limited removal of persistent pollutants, such as PAHs and PCBs, during the treatment, which was coincident with the chemical analysis results.

Municipal effluents are complex mixtures with possible antagonistic or synergistic effects on the EROD induction, as previously described by Gagne et al. (1993) for the similar induction assays on different treatment processes. Since EROD induction is considered a biomarker of exposure to PAHs, PCBs, and dioxinlike compounds (Stegeman and Hahn, 1994), our results suggest the presence of these pollutants in all the

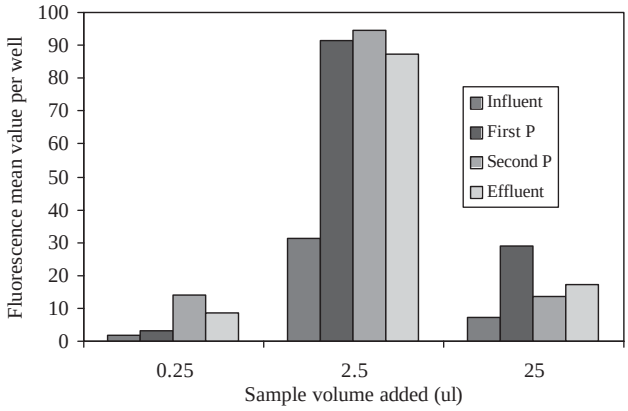

Fig. 2. EROD induction as a function of dosage of SPMD samples from the sewage treatment plant.

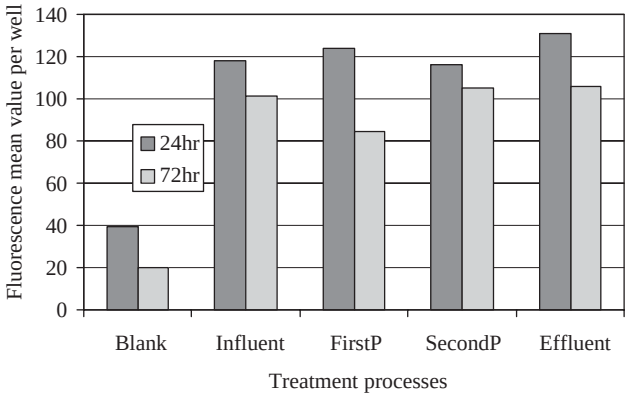

Fig. 3. EROD induction after 24 and 72 h exposure to SPMD samples of sewage treatment plant.

processes selected in the sewage treatment plant. It was inferred that the activated-sludge treatment process was not very effective in removing the priority organic pollutants studied, which might not be biodegraded in such a short-term treatment. Nevertheless, more chemical analysis data are necessary before pointing to a common cause of the EROD induction and the acute toxicity to *V. fischeri*. Further exploration of the dioxinlike compounds in the sewage treatment plant is necessary because of their persistent to activated sludge and impacts to receiving bodies. SPMDs showed great promise for concentrating and estimating trace levels of priority organic contaminants in a sewage treatment process and for supplying the samples for bioassays.

The results obtained indicate that bioassays on water concentrates obtained by means of SPMD have considerable potential as a monitoring tool for organic contaminants in water. Bioassays on aquatic concentrates provide a direct functional response that relates to the overall toxic properties of the mixtures of compounds present in a sample. Bioassays also represent a cost-effective alternative to comprehensive chemical

analysis. Toxicity identification approach allowed to connect the toxic effects observed principally with the compounds detected. This type of information can help to select appropriate treatments or source reduction methodologies. The results from bioluminescence and EROD tests revealed that the municipal sewage treatment plants were contributing to the total biological toxicity emission to receiving water bodies. Preservation of the ecology of rivers and lakes requires regulations in which ecotoxicity is considered.

4. Conclusion

Screening of the municipal wastewater treatment plant for hydrophobic organic compounds and EROD induction revealed the presence of the persistent pollutants in influent and following selected effluents, even in final effluent in the sewage treatment plant. The bioassays could be developed as a promising technique for discharge control of the treated wastewater from municipal sewage treatment plant. Regarding ecotoxicity and chemical analysis result of persistent organics, the treatment efficiency for persistent organics of the municipal sewage treatment plant was limited. The SPMD technique combined with chemical analyses and bioassays might be a valuable monitoring tool for priority organic pollutants in water ecosystem.

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