Comparison of behaviour and fate of tetrabromobisphenol A (TBBPA) in membrane bioreactors and conventional activated sludge process

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ABSTRACT

Research was completed on the behaviour and fate of tetrabromobisphenol A (TBBPA) in pilot-scale membrane bioreactors and conventional activated sludge (CAS) process at the City of Guelph Wastewater Treatment Plant. Emphasis was on the physicochemical processes, biological transformation and sorption kinetics. Measurement of TBBPA at the environmental level can be challenging, so the main focus of this paper was the development of an analytical method for the nano-level determination of TBBPA. The approach used was gas chromatography-negative ion chemical ionization-mass spectrometry in select ion monitoring mode. For the wastewater matrix studied, the developed method had a low method detection level (MDL) of 2.4 ng/L. The matrix effects for influent and activated sludge mixed liquor were determined to be $36.5 \pm 5.9\%$ ($n = 10$) and $79.2 \pm 4.5\%$ ($n = 10$), respectively. Preliminary results show that the CAS system demonstrates better performance for TBBPA removal, with removal at 83%. This was corroborated by the batch experiments that showed that the synergistic effects of biodegradation and biosorption potentially provide more removal of the TBBPA through the activated sludge process.

Key words | biotransformation, matrix effect, membrane bioreactor, method detection level (MDL), tetrabromobisphenol A

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INTRODUCTION

Tetrabromobisphenol A (TBBPA) is one of the most widely used brominated flame retardants in the world with a global consumption of 0.21 Mt (Alaee *et al.* 2003). Distribution of TBBPA in all environmental compartments and biosystems makes it an important candidate for fate investigation. The USEPA (2010) has listed TBBPA as a contaminant of emerging concern due to its adverse effect on human health and the ecosystem. Examples include feminization of fish, alteration of sex in birds and wildlife, birth defects, neurological and developmental problems in humans along with infertility. It has been identified that TBBPA exhibits one of the highest thyroid hormonal activity rates (Kitamura *et al.* 2005), which is most likely due to the structural resemblance of TBBPA with the thyroid hormone tetraidothyroxine, as shown in Figure 1. This resemblance

may provide the impudence for mimicking the endocrine disruption effect.

TBBPA is lipophilic due to the high octanol-water partition coefficient (logKow = 4.5) with potential to bioaccumulate. Bioavailability of TBBPA renders ecotoxicity, neurotoxicity and developmental disorders in humans and wildlife. At high pH values, considerable ground water contamination is likely due to increasing solubility, plus there is increased mobility of TBBPA in soil (Segev *et al.* 2009). The dissociation of TBBPA in the environment is influenced by the pH change, particularly above 7, since pKa_1 and pKa_2 are 7.5 and 8.5, respectively (Kuramochi *et al.* 2008). The negatively charged species of phenolate ion at the high pH range provides for less sorption to the soil surface and sludge matrix. The behaviour and fate of TBBPA in different
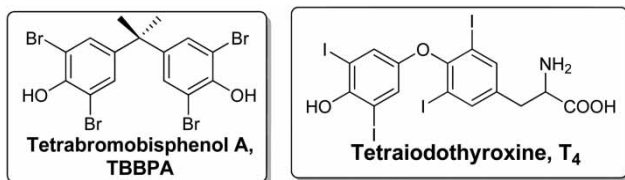
Figure 1 | The chemical structures of TBBPA and thyroid hormone, thyroxine.

treatment processes and landfills depends on the stability, sorption capacity, pH and physicochemical state of the system components.

TBBPA is used mostly as a reactive intermediate in the production of epoxy, phenolic and polycarbonate resins. As a reactive component, the phenolic hydroxyl groups are bonded covalently, resulting in a strong attachment to the polymer structure. However, the additive component is not strongly held by the chemical structure and, consequently, can be readily released to the environment (Birnbaum & Staskal 2004; Grabda *et al.* 2009). The bound portion of TBBPA also has the potential to leach out of the polymer matrix when placed in a landfill.

Dissolved TBBPA in wastewater is relatively high when compared to other brominated flame retardants. Everyday use of polymer and plastic products containing TBBPA as a fire retardant component is the cause of its increasing abundance in wastewater. Migration of dissolved TBBPA in the aqueous phase and sludge matrix can be modelled from its physicochemical behaviour. TBBPA is considered to have a significantly low vapour pressure and, as such, volatilization loss of TBBPA is negligible at typical environmental conditions (Kuramochi *et al.* 2008; Grabda *et al.* 2009).

Much research has been focused on the sludge concentration whereas fate modelling in both sludge and aqueous phase concentration needs to be accomplished for achieving a good understanding on the distribution pathways of TBBPA in the environmental ecosystem. Our research goal is to establish a fate model considering all possible variables of phase transfer and degradation mechanisms. The data for the fate model come from the robust method that has been developed to quantify aqueous phase TBBPA, specifically the gas chromatography-negative ion chemical ionization-mass spectrometry (GC-NCI-MS).

METHODS AND MATERIALS

Chemicals and reagents

All chemicals and reagents were purchased from Sigma-Aldrich Canada unless otherwise specified. Most chemicals were ACS grades and all solvents used were HPLC grades. Whatman GF/B filters (Cat. No 09-874-24A), Whatman GF/F (Cat. No 09-874-71), Whatman 0.2 nylon membrane (Cat. No R02SP04700) and nucleopore polycarbonate filters (Cat. No 0930069) were purchased from Fisher Scientific. Helium (99.9999%) and methane (99.9999%) were supplied by BOC Canada (Guelph, Ontario). Milli-Q water was produced from a Millipore Ultrapure Water System.

GC-MS system

The unit was an Agilent 6890N GC equipped with an auto-sampler injector (model HP 7683) coupled to an Agilent 5975 MSD. A sample volume of $2\,\mu$L was introduced by pulsed splitless injection into a 30 m length and 0.319 mm internal diameter Agilent HP-5 5% phenyl methyl siloxane J & W GC column (Cat # 19091 J-415) coated with a $0.25\,\mu$m film thickness. The oven temperature was held at $100\,°$C for 2 min at initial injection. Thereafter, the temperature was increased by $25\,°$C /min up to $190\,°$C and held for 2 min, then increased by $5\,°$C/min up to $310\,°$C and held for 2 min at this final temperature. Total run time for the oven programme was 33.6 min. Helium was used as the carrier gas, under a constant pressure of 80 kPa. Methane was used as the reagent gas for the negative ion source and was maintained at 40% of flow. The interface, inlet and quadrupole temperatures were maintained at 250, 250 and $150\,°$C, respectively. Select ion monitoring mode was used. The ions monitored were 79 and 81 m/z for TBBPA, 71 and 74 m/z for DDT (dichlorodiphenyltrichloroethane), similar to the approach by Sellstrom & Jansson (1995).

Sampling and analysis

The water samples for analysis were collected from different points of the conventional activated sludge (CAS) treatment process and the three pilot-scale membrane bioreactors

(MBRs) situated at the Guelph Municipal Wastewater Treatment Plant. Amber glass containers were used for sampling, with the water samples placed in an ice cooler system for transport to the laboratory. For both systems, composite sampling was done for the influent to make them representative. Using an auto-sampler over a 24 hour period, samples were collected every half an hour and then combined. After collection, the composite samples were brought immediately to the Environmental Engineering Laboratory for analysis. Physical parameters such as pH, temperature and dissolved oxygen (DO) were measured onsite or in the laboratory. Analysis process flow diagram is shown in Figure 2.

The permeate and the final effluent samples were filtered through Whatman 0.2 μm nucleopore membrane filters and collected in Erlenmeyer flasks. The influent and mixed liquor samples were filtered through 1.0 μm GF/B and 0.7 μm GF/F glass filters followed by 0.2 μm nucleopore membrane filters. After filtration, the samples were acidified (pH ≈ 3) to prepare for the solid phase extraction (SPE). The internal standard DDT was added to all flasks containing the samples, except the blank, before loading the SPE column.
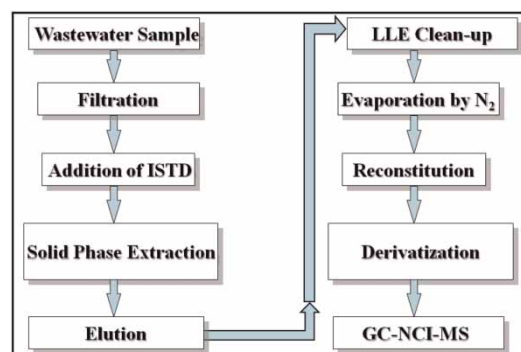
SPE columns (Envi-18, vol. 3 mL, bed wt. 500 mg) were conditioned with 2 mL of dichloromethane (DCM), followed by 2 mL of methanol and 2 mL of Milli-Q water. Precautions were taken to ensure that the SPE cartridges never dried out once conditioned. The acidified samples were then passed through SPE cartridges under vacuum pressure at a rate of 1–2 mL/min to capture the analyte of interest. The SPE cartridges were dried for 30 min under the same vacuum before elution. Gravimetric elution was carried out after soaking the sorbent for 15 min in 2 mL of DCM. Two volumes (each 2 mL) were eluted to ensure elution of all the analyte. At the end of gravimetric elution, forced elution by vacuum was performed to collect all traces of solvent from the column into glass test tubes.

Clean up was done by liquid–liquid extraction. To each test tube, 4 mL of 0.05 N H_2SO_4 was added, followed by gentle shaking. The aqueous phase at the top was removed by using a glass Pasteur pipette. The organic phase was passed over anhydrous sodium sulphate to remove traces of water as the final clean up step. The resulting extract contained in the glass vial was evaporated to complete dryness by a gentle blow of N_2. A multi-flow drying device was used to connect nitrogen flow to each glass vial. For quantitative estimation of analyte concentration, the sample was reconstituted in 450 μL of acetone and transferred to a 2 mL GC vial equipped with 500 μL borosilicate glass insert. TBBPA in the extract was then derivatized by adding 50 μL of acetic anhydride and pyridine (1:1) to each vial insert. Capped vials were heated in an oven for 30 min at 60 °C. After heating for 30 min, the vials were ready for injection into the GC-NCI-MS system for quantification. The derivation reaction scheme is given in Figure 3.
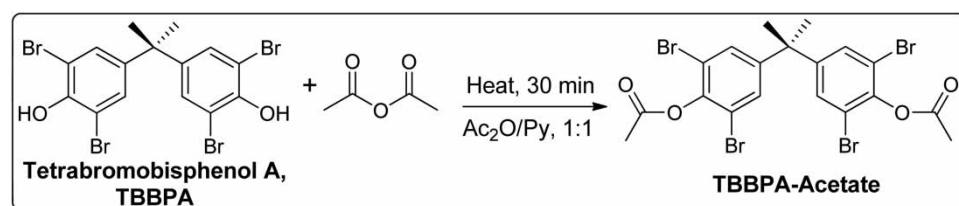

Figure 2 │ Analysis flow diagram.

Figure 3 │ Derivatization reaction.

Membrane bioreactors A–C

The experimental MBR pilot plants were located at the City of Guelph Wastewater Treatment Plant. The MBR pilot plant consists of three parallel full-scale submerged MBR trains, with aeration tanks and membrane tanks housed separately for each train. The three MBR pilot plants are designated as Pilot A–C with three different solids retention times (SRTs) (24, 28, 29 d, respectively) during the sampling period. The hydraulic retention times (HRTs) for all three MBRs were 6 h. The pilot plant receives influent from the headworks after filtering through a 0.75 mm screen to remove large debris. The influent in each MBR pilot train passes through the aeration tanks followed by membrane tanks. The effluent (permeate) from the MBR units are directed to the wastewater treatment plant due to legal obligations to ensure compliance with the Certificate of Approval. A schematic of the MBR pilot plants is presented in Figure 4.

Basic water quality parameters for MBR pilot plants were routinely monitored. Filtered total organic carbon (TOC), chemical oxygen demand (COD) and total nitrogen (TN) were determined for influent and MBR mixed liquor. Unfiltered TOC, COD and TN were measured for the MBR permeate. Dissolved oxygen, pH and temperature were recorded as well.

A Shimadzu TOC-V unit equipped with a solid sample module 5000A was used for TOC measurement. Calibration curves were updated every 2–3 months as specified in *Standard Methods* (APHA 2012). COD measurements were conducted colorimetrically using a Hach DR 2010 spectrophotometer. Analyses for ammonia,

Figure 4 | Schematic of MBR pilot plants.

nitrite and nitrate were all performed using Hach Test N Tube vials. DO, pH and temperature were recorded onsite using a pH/conductivity/DO meter (probe model sensION6, Hach, USA). All tests conformed to *Standard Methods* (APHA 2012).

Conventional activated sludge system

The Guelph Wastewater Treatment Plant (GWWTP) discharges the treated effluent into the Speed River. The GWWTP is owned and operated by the City of Guelph Municipality. An average daily flow of 64 million litres per day (MLD) is the design capacity of the plant. The GWWTP receives domestic, institutional, commercial and industrial wastewater from the city as well as a portion of the village of Rockwood and the Gazer-Mooney subdivision located in the township of Guelph-Eramosa.

All raw wastewater coming to the facility is first pumped into the headworks building using large Archimedes screw pumps. Within the headworks, the wastewater flows through bar racks followed by a step screen to remove both large objects and small particles. The wastewater exits from the headworks to the aerated grit chamber. Following preliminary treatment, the wastewater undergoes multiple stages of treatment consisting of primary, secondary, tertiary and disinfection. The final effluent is discharged to the Speed River. The typical SRT for the CAS system was 12 d, with an HRT of 6–9 h. The schematic of the CAS system is shown in Figure 5.

Matrix effect

The matrix refers to everything in the sample other than the analyte of interest. The extent of interference to the determination of analyte in the sample is the matrix effect. This is normally determined by spiking the target compound into the environmental sample. This process is also known as fortification. A laboratory fortified blank (LFB) is a reagent water sample to which a known concentration of the analyte of interest is spiked. LFB is used to evaluate the method performance or recovery. A laboratory fortified matrix (LFM) is a sample to which a known amount of analyte of interest is added before sample preparation. LFM is used to evaluate the matrix effect. The
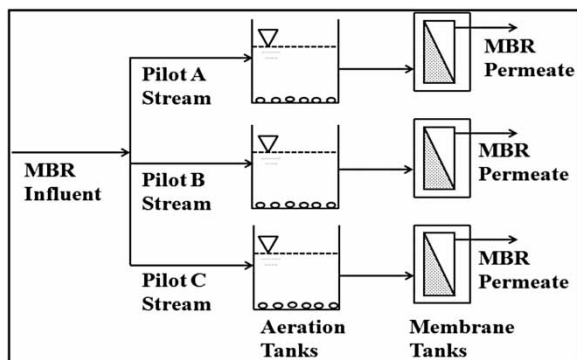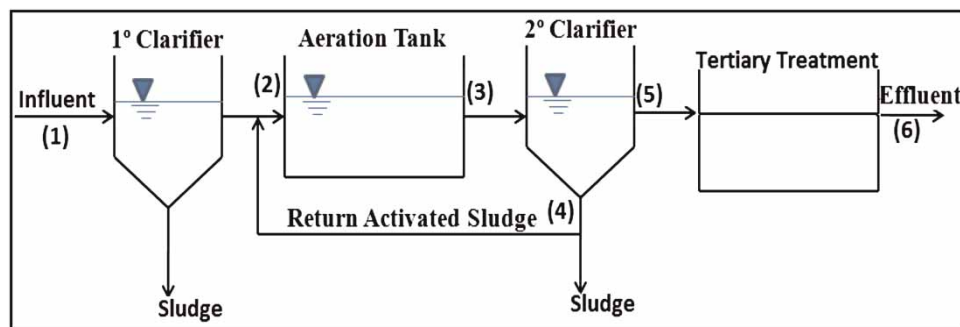
Figure 5 | Schematic of CAS system.

presence of complex matrices in wastewater adversely affects instrumental responses and recovery. The wastewater matrix differs from sample to sample depending on the source and time of sampling. The matrix effect was determined for the influent wastewater and activated sludge mixed liquor. Equation (1) was used to measure the percent matrix effect according to *Standard Methods* (APHA 2012):

$$\% \text{Matrix effect} = \left[1 - \frac{C_{\text{LFB}}}{C_{\text{LFM}}} \right] \times 100 \qquad (1)$$

where C_{LFB} is the concentration measured in the LFB and C_{LFM} is the concentration measured in the LFM.

Researchers have found a matrix effect in the analysis and determination of TBBPA in different environmental samples (Covaci *et al.* 2007, 2009; Mascolo *et al.* 2010; Potvin & Zhou 2011). The physical factors including analytical equipments also contribute to the recovery. Gas chromatographic methods are complicated, time-consuming and sensitive to matrix effect due to extra derivatization step for the analysis of organic compounds with hydroxyl, amine or carboxylic acid functional groups (Frederiksen *et al.* 2007). The presence of two hydroxyl groups in the structure of TBBPA makes it even more challenging for extraction and analysis.

This study reports matrix effect obtained from direct fortification of fresh activated sludge. Erlenmeyer flasks were filled with 500 mL of activated sludge. Spiked concentration was 100 ng/L of TBBPA in each flask. One LFB was included in each batch of the experiment. After thorough mixing, the spike was extracted and quantified according to the method protocol.

Biotransformation batch experiments

Biotransformation was studied by spiking TBBPA (30 ng/L) in 1 mL of activated sludge with growth media and inoculum of heterotrophic bacteria. Polyseed was used as an inoculum. Polyseed is a commercial inoculum approved by the US EPA, containing a blend of broad-spectrum heterotrophic bacteria for biochemical oxygen demand (BOD$_5$) test. The batch experiment was similar to the BOD test. In this case, the reaction mixtures in the bioreactors were stirred continuously placing a magnet bar inside the vessels at 20 °C. During the course of the experiment, the bioreactors were removed from the magnetic stirrer at different time intervals (0, 1, 3 and 5 d) to investigate biodegradation. For each sampling event, duplicates were extracted. Controls were also prepared where TBBPA (30 ng/L) was dissolved in Milli-Q water. Extraction and sample analysis were performed as described earlier in the method protocol. The results showed that there is a potential for biodegradation of TBBPA in the activated sludge along with biosorption.

Sorption kinetics was investigated in a similar manner to compare biosorption with biotransformation. In this case only activated sludge solution containing 30 ng/L of TBBPA was extracted in duplicates at different time intervals. Neither inoculum nor growth medium was introduced for the biosorption kinetic study.

Biosorption batch experiment

Biosorption of TBBPA was studied by using fresh activated sludge. Adsorption isotherms were developed to explain the biosorption behaviour of TBBPA in activated sludge.

Most of the organic compounds in the activated sludge follow the Freundlich isotherm (Dobbs *et al.* 1989; Clara *et al.* 2004). US EPA (1996) guidelines recommend a minimum half an hour of stirring of the mixtures to achieve equilibrium. Therefore, the extraction was carried out after 2 h of spiking, followed by magnetic stirring. The spiked concentration of TBBPA in each reaction vessel was 30 ng/L and the volumes of fresh activated sludge added to the reaction vessels were 0, 1, 2, 4, 6, 8, 10, 12 and 15 mL, respectively. The pH and temperature of the test solution was recorded as 7.1 and 21.5 °C, respectively.

RESULTS AND DISCUSSION

Method development

Method performance and instrument response were measured by processing and injecting the lowest concentration that produced a recognizable signal in the instrument for the analyte, TBBPA. Initial demonstration of the instrument capability was performed by processing and injecting 5 ng/L analyte concentrations in Milli-Q water. A linear calibration curve ($r^2 = 0.9977$) was obtained by injecting standard TBBPA solution in acetone in the range 0–200 μg/L. The calibration curve as seen in Figure 6 was produced from the solvent matrix without the SPE. This range of 0–200 μg/L represents
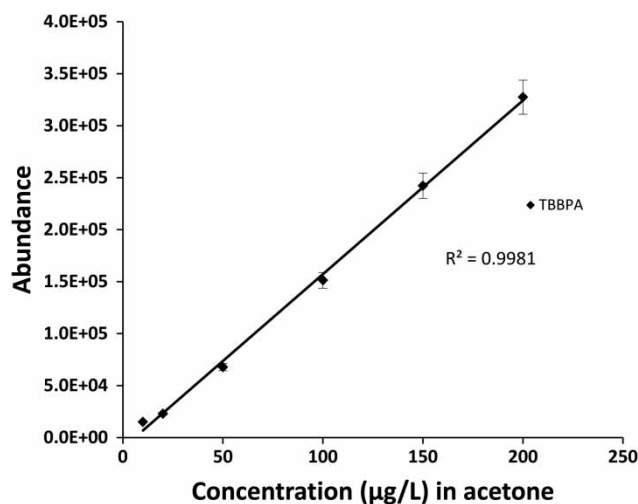
the 0–200 ng/L expected in the real environmental samples, as the injected samples were a thousand-fold more concentrated than the aqueous phase. Through the process, 500 mL of aqueous sample became 500 μL, which was injected after processing through the SPE. Thus, the magnitude of 'μg/L' of TBBPA concentration as detected by the instrumental signal was originally at the magnitude of 'ng/L', which represents the TBBPA concentration in the aqueous phase in the environment. Concentrations above the 200 ng/L range produced a non-linear calibration curve.

The method detection level (MDL) was determined as per *Standard Methods* (APHA 2012). Seven portions of Milli-Q water sample containing 10 ng/L of TBBPA were processed over a period of 3 days. The computation is as follows: $MDL = s \times t_{(n-1, 1-\alpha=0.99)}$ where standard deviation of replicate measurements, $s = \sqrt{\sum_{i=1}^{n}(x_i - \overline{x})^2 / n - 1}$ and $t_{(n-1, 1-\alpha=0.99)} =$ one sided Student's t-value at 99% confidence with $(n-1)$ degrees of freedom. The analytical data for the determination of MDL are presented in Table 1.

The standard deviation of these seven samples was multiplied by 3.14, the Student's t-factor for seven replicates with six degrees of freedom at the 99% confidence. The MDL thus determined was 2.4 ng/L which is reasonable for the environmental level analysis of TBBPA.

Quality assurance, repeatability and reliability of the method was assured by adding method blank, LFB and LFM to each batch according to *Standard Methods*.

Figure 6 | Standard calibration curve for TBBPA in the range 0–200 μg/L.

Table 1 | Experimental data for MDL determination

Sample ID	Date	Abundance	TBBPA (ng/L)
Blank	Jan04, 2013	ND	0
MDL 1	Jan04, 2013	1,217	8.9522
MDL 2	Jan04, 2013	941	7.6156
Blank	Jan06, 2013	ND	0
MDL 1	Jan06, 2013	1,016	7.9788
MDL 2	Jan06, 2013	1,254	9.1314
MDL 3	Jan06, 2013	961	7.7125
Blank	Jan09, 2013	ND	0
MDL 1	Jan09, 2013	1,064	8.2113
MDL 2	Jan09, 2013	807	6.9667

$$\text{Relative error} = \frac{100}{n}\sum_{n=7}\frac{\text{Expected} - \text{Experimental}}{\text{Expected}} = 19\%$$

MBR pilot plants

Wastewater characteristics for influent and reactors effluent were routinely monitored during the study period. The average influent concentrations for the different parameters are as follows: COD at 334 mg/L, influent TOC at 26.5 mg/L, permeate TOC from reactors A–C were on average 5 mg/L, TN at 33 mg/L and pH ranged from 7.2 to 7.6 for all three reactors during this study period.

Influent TBBPA concentrations during the study months were on average (32.7 ± 9.4) ng/L. Permeate TBBPA concentrations from reactors A–C were (8.8 ± 2) ng/L, (6.7 ± 1.4) ng/L and (6.6 ± 1.1) ng/L, respectively. TBBPA concentrations varied from 0 to 11 ng/L for all MBR permeate. Figure 7 shows TBBPA levels in the influent and permeate for reactors A–C. The average TBBPA removal efficiency for MBR was at 76%.

CAS process

Wastewater influent to the CAS system contained TBBPA concentrations in the range of 10–145 ng/L. Corresponding effluent concentrations were in the range of 0–8 ng/L during this study period. Figure 8 shows TBBPA concentrations for the CAS system. The average mixed liquor suspended solids (MLSS) concentration in the aeration basin was 2,500 mg/L. The water content for activated sludge after centrifugation at 2,000 g was 70% on average. Significant variability of TBBPA concentration in the influent was observed due to
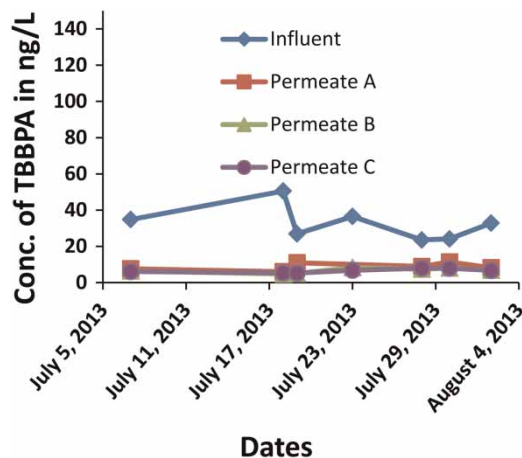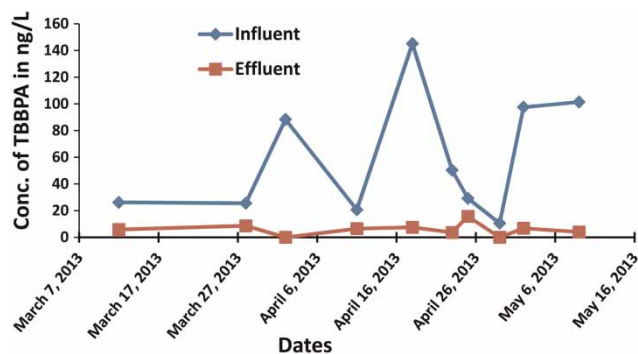

Figure 8 | Influent and effluent concentrations of TBBPA in CAS system.

the daily and seasonal variation. The TBBPA removal efficiency for the CAS system was determined as 83%.

Matrix effect

The percent matrix effect for the influent was 36.5 ± 5.9 ($n = 10$). The influent matrix changes depending on daily variation and other factors related to the source of the wastewater. The percent matrix effect for activated sludge mixed liquor was 79.2 ± 4.5 ($n = 10$). The high matrix effect in the activated sludge was due to the presence of solids and biomass in the fresh mixed liquor. This study investigated matrix effect differently from other reports considering all forms of physical, chemical, biological and instrumental interference due to suppression, sequestration and sorption in the activated sludge. The data for the estimation of matrix effect are presented in Table 2.

Biotransformation and biosorption

The potential for biotransformation of TBBPA by heterotrophic and nitrifying bacteria was investigated. The depletion of TBBPA concentration in the bioreactor can be attributed to the bacterial degradation during incubation. Figure 9 shows the effect of biodegradation over biosorption.

Biotransformation of brominated compounds and organic micropollutants by heterotrophic and nitrifying bacteria has been reported by some researchers (Duddleston et al. 2000; Kosjek et al. 2009; Helbling et al. 2010). Higher depletion of TBBPA in activated sludge solution in

Figure 7 | Influent and permeate concentrations of TBBPA in MBR pilot plants.

Table 2 | Data for matrix effect

	Influent				Activated sludge		
Sample ID	Mass abundance	TBBPA (ng/L)	% Matrix effect		Mass abundance	TBBPA (ng/L)	% Matrix effect
LFB	19,237	96.2			21,306	106.2	
LFM1	12,162	62.0	35.6		4,610	25.4	76.1
LFM2	11,669	59.6	38.1		3,710	21.0	80.2
LFM3	9,897	51.0	47.0		3,150	18.3	82.8
LFM4	12,287	62.6	35.0		3,845	21.7	79.6
LFM5	12,401	63.1	34.4		3,750	21.2	80.0
LFM6	13,220	67.1	30.3		5,419	29.3	72.4
LFM7	12,577	64.0	33.5		2,110	13.3	87.5
LFM8	10,060	51.8	46.2		5,101	27.8	73.9
LFM9	13,430	68.1	29.2		2,940	17.3	83.7
LFM10	12,100	61.7	35.9		4,661	25.6	75.9
Average			36.5				79.2
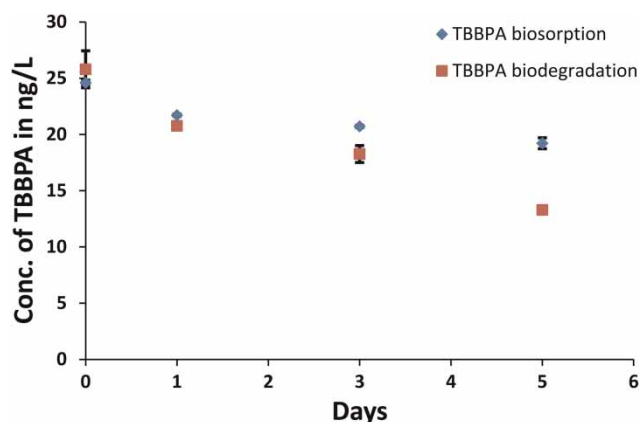
Figure 9 | Comparison of biosorption and biodegradation with time.

Figure 10 | Freundlich isotherm model for TBBPA in activated sludge.

comparison to biosorption clearly demonstrates the potential for biodegradation.

Biosorption isotherms

Biosorption isotherms were developed by using the Freundlich equation. Figure 10 shows the biosorption isotherm fitting for the Freundlich model.

The goodness of fit for the experimental data was reasonable due to the acceptable r^2 value of 0.7406. The fate and behaviour of TBBPA in activated sludge process can be explained from the parametric value of sorbate

intensity. Sorption intensity of TBBPA in activated sludge, 0.92 (1/n = 1.085) is indicative of favourable or higher adsorption to the sludge surface. Therefore, one of the dominant contributors to the removal of TBBPA by activated sludge is biosorption.

SUMMARY

Method development was accomplished successfully for measuring aqueous phase TBBPA. Having this ability was important to allow comparison of the behaviour and fate

between MBR pilot plants and the CAS system. The sensitivity and reproducibility of the method is reasonable. The percent matrix effect suggests that the recovery of the candidate compound in the complex water matrices is challenging. However, this was overcome by the higher sensitivity for the lower level determination of TBBPA. The CAS system showed better removal efficiency at 83% when compared to the MBR pilot system. The higher removal in activated sludge process may be due to the cause of microbial attachment of the TBBPA to the sludge biomass (Subasioglu & Bilkay 2009; Kiser *et al.* 2010). The higher biosorption intensity (0.92) of TBBPA causes higher biosorption to the sludge surface. The MBR sludge has low biological mass which provides only adsorption sites rather than biosorption. The enhanced removal in the CAS system may also be impacted by the biotransformation of TBBPA by heterotrophic and nitrifying bacteria during biological wastewater treatment. Overall, the removal of TBBPA by the aerobic biological wastewater treatment system is due to the synergistic effect of biosorption and biodegradation.

ACKNOWLEDGEMENTS

REFERENCES

Alaee, M., Arias, P., Sjodin, A. & Bergman, A. 2003 An overview of commercially used brominated flame retardants, their application, their use patterns in different countries/regions and possible modes of release. *Environ. Int.* **29**, 683–689.

APHA, AWWA, WEF 2012 *Standard Methods for the Examination of Water and Wastewater*. 22nd edn, American Public Health Association, Washington, DC, USA.

Birnbaum, L. S. & Staskal, D. F. 2004 Brominated flame retardants: cause for concern? *Environ. Health Persp.* **112** (1), 9–17.

Clara, M., Strenn, B., Saracevic, E. & Kreuzinger, N. 2004 Adsorption of bisphenol-A, 17 β-estradiol and 17α-estradiol to sewage sludge. *Chemosphere* **56**, 843–851.

Covaci, A., Voorspoels, S., Ramos, L., Neels, H. & Blust, R. 2007 Recent developments in the analysis of brominated flame retardants and brominated natural compounds. *J. Chromatogr. A* **1153**, 145–171.

Covaci, A., Voorspoels, S., Abdallah, M. A.-E., Greens, T., Harrad, S. & Law, R. J. 2009 Analytical and environmental aspects of the flame retardant tetrabromobisphenol-A and its derivatives. *J. Chromatogr. A* **1216**, 346–363.

Dobbs, R. A., Wang, L. & Govind, R. 1989 Sorption of toxic organic compounds on wastewater solids: correlation with fundamental properties. *Environ. Sci. Technol.* **23**, 1092–1097.

Duddleston, K. L., Bottomley, P. J., Porter, A. J. & Arp, D. J. 2000 New insights into methyl bromide cooxidation by *Nitrosomonas europaea* obtained by experimenting with moderately low density cell suspensions. *Appl. Environ. Microbiol.* **66** (7), 2726–2731.

Frederiksen, M., Vorkamp, K., Bossi, R., Riget, F., Dam, M. & Svensmark, B. 2007 Method development for simultaneous analysis of HBCD, TBBPA, and dimethyl-TBBPA in marine biota from Greenland and the Faroe Islands. *Int. J. Environ. Analyt. Chem.* **87**, 1095–1109.

Grabda, M., Oleszek-Kudlak, S., Rzyman, M., Shibata, E. & Nakamura, T. 2009 Studies on bromination and evaporation of zinc oxide during thermal treatment with TBBPA. *Environ. Sci. Technol.* **43**, 1205–1210.

Helbling, D. E., Hollender, J., Kohler, H. E., Singer, H. & Fenner, K. 2010 High-throughput identification of microbial transformation products of organic micropollutants. *Environ. Sci. Technol.* **44**, 6621–6627.

Kiser, M. A., Ryu, H., Jang, H., Hristovski, K. & Westerhoff, P. 2010 Biosorption of nanoparticles to heterotrophic wastewater biomass. *Water Res.* **44**, 4105–4114.

Kitamura, S., Suzuki, T., Sanoh, S., Kohta, R., Jinno, N., Sugihara, K., Yoshihara, S., Fujimoto, N., Watanabe, H. & Ohta, H. 2005 Comparative study of the endocrine-disrupting activity of bisphenol A and 19 related compounds. *Toxicol. Sci.* **84**, 249–259.

Kosjek, T., Heath, E., Perez, S., Petrovic, M. & Bacelo, D. 2009 Metabolism studies of diclophenac and clofibric acid in activated sludge bioreactors using liquid chromatography with quadruple-time-of-flight mass spectrometry. *J. Hydrol.* **372** (1–4), 109–117.

Kuramochi, H., Kawamoto, K., Miyazaki, K., Nagahama, K., Maeda, K., Li, X. W., Shibata, E., Nakmura, T. & Sakai, S. I. 2008 Determination of physicochemical properties of tetrabromobisphenol A. *Environ. Toxicol. Chem.* **27** (12), 2413–2418.

Mascolo, G., Locaputo, V. & Mininni, G. 2010 New perspective on the determination of flame retardants in sewage sludge by using ultrahigh pressure liquid chromatography–tandem mass spectrometry with different ion sources. *J. Chromatogr. A* **1217**, 4601–4611.

Potvin, C. M. & Zhou, H. 2011 Interference by the activated sludge matrix on the analysis of soluble microbial products in wastewater. *Chemosphere* **85**, 1139–1145.

Segev, O., Kushmaro, A. & Brenner, A. 2009 Environmental impacts of flame retardants (persistence and biodegradability). *Int. J. Environ. Res. Public Health* **6**, 478–491.

Sellstrom, U. & Jansson, B. 1995 Analysis of tetrabromobisphenol A in a product and environmental samples. *Chemosphere* **31**, 3085–3092.

Subasioglu, T. & Bilkay, I. S. 2009 Determination of biosorption conditions of methyl orange by *Humicola fuscoatra*. *J. Sci. Ind. Res.* **68**, 1075–1077.

US EPA 1996 Fate, transport and transformation guidelines – activated sludge sorption isotherm. Prevention, pesticide and toxic substances. EPA-712-C-98-298.

US EPA 2010 *Treating Contaminants of Emerging Concern*. Office of Water Engineering and Analysis Division, Washington, DC, 20460, EPA-820-R-10-002.