Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.
This journal is © The Royal Society of Chemistry 2017

Supplementary Data

Fate and transformation of tetrabromobisphenol A in natural waters by oxidoreductase enzymes

Yiping Feng,[1,2] Kun Lu,[1] Shixiang Gao,[*,1] and Liang Mao[*,1]

[1] *State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, Jiang su 210023, P.R. China*

2 Institute of Environmental Health and Pollution Control, School of Environmental Science and Engineering, Guangdong University of Technology, Guangzhou, 510006, P.R. China

*Corresponding author. Tel(Fax): +86 25-89680359; ecsxg@nju.edu.cn (S.X. Gao)

*Corresponding author. Tel(Fax): +86 25-89680393; lmao@nju.edu.cn (L. Mao)

2. Materials and methods

Sample collection and preparation

The water samples used in this study were obtained from four water sources: (1) Two natural lake waters were collected from Taihu Lake, a typical shallow freshwater lake with an area of 2338 km^2 and average depth of 1.9 m, located in one of the most populated and developed areas of China. In the current study, two representative sampling sites were chosen. Site 1 is close to Meiliang Bay, located at the northwest part of the lake. Meiliang Bay serves as a principal water resource for the industrial city (Wuxi City, Jiangsu Province) and also receives industrial wastewater and domestic sewage from the city. This bay is identified to be one of the most seriously eutrophicated water bodies in China. Site 2 is in East Taihu Lake, located at the southeastern corner of Taihu Lake. This part of the lake is a large and shallow bay with abundant aquatic plants and good water quality. (2) A river water was collected from Yangtze River at Shibuqiao village in Qixia district of Nanjing. This river water is characterized with good quality and used as a source of drinking water for Nanjing city. (3) A municipal wastewater effluent, which was obtained from an activated sludge sewage treatment plant (STP) in Nanjing, China. The collected water samples were taken back to laboratory within 12 hours and stored at 4 °C for further use. Prior to analysis and use, all collected waters were filtered through a 0.45-μm filter to remove particulate matters. (4) The synthetic water, which was prepared by 10 mM phosphate buffer solution (PBS) in laboratory. The corresponding physicochemical parameters of the municipal wastewater and natural surface waters were characterized and listed in Table S1-2.

Michaelis–Menten kinetics study

The reactions for Michaelis–Menten kinetics study were performed in 8-mL glass test tubes at room temperature. The reaction solutions initially contained various concentrations of TBBPA and a consistent dosage of laccase or HRP at 0.2 U mL^{-1}. 10 μM H_2O_2 was used for HRP-catalyzed process. Then the reaction solution was mixed through a miniature whirlpool mixture apparatus for 30 s in laccase-catalyzed process or 20 s in HRP-

catalyzed process before adding 2 mL methanol to terminate the reaction. Three replicate experiments were carried out for each initial TBBPA concentration. The parameters K_M and V_{max} of the Michaelis–Menten reaction kinetics were estimated using the initial reaction rate method[1-3]. According to the method, the initial reaction rate (V_0) was calculated based on the formulation $V_0 = (S_0 - S_t)/\Delta t$ through measuring the substrate concentration in both the blank (S_0) and reaction tubes (S_t). The reaction time, Δt, was determined in preliminary tests to enable capturing the pseudo-first-order rate behavior of the enzymatic reactions and to ensure reproducible handling of the reactors. The obtained initial rate data were then fitted to Michaelis-Menten equation $V_0 = V_{max} \times S_0 / (S_0 + K_M)$ to obtain the maximum rate of enzymatic reaction V_{max} and the substrate's Michaelis constant K_M. Generally, the enzyme activity ([E]) can be assumed to be same to its initial activity ($[E_0]$) because the reaction time is very short (30 s and 20 s). We thus calculated the initial reaction rate constant according to $k_{CAT} = V_{max} / [E_0]$.

Michaelis–Menten constants were obtained by curve fitting the plot of reaction rate versus substrate concentrations to the Michaelis-Menten equation (1) with Sigma Plot 12.5 software (SPSS Inc., Chicago, IL). The enzyme molecular weight was used to evaluate the k_{CAT} value. For HRP the molecular weight was equal to 44 kDa, and the molecular weight of laccase was estimated to be 65 kDa [2].

$$V = \frac{V_{max}[S]}{K_M + [S]} \qquad (1)$$

$$k_{CAT} = \frac{V_{max}}{[E_0]} \qquad (2)$$

Quantification

An Agilent 1200 series high performance liquid chromatography (HPLC) equipped with a variable wavelength detector (VWD) was used to analyze the concentration of TBBPA. The separation was performed on a Thermo Syncronis C18 reversed phase column (250 × 4 mm, 5 μm, Thermo, USA). The mobile phase was comprised by methanol/water (with 0.5‰ acetic acid) = 85:15 (v/v) with a flow rate of 0.8 mL min^{-1}. The injection volume was 20 μL, and the detection wavelength was set at 230 nm.

A Shimadzu 5000A total organic carbon (TOC, mgC L^{-1}) analyzer was used to determine the TOC of the collected municipal wastewater effluent and natural surface waters. UV_{254} measurement was performed on a Varian Cary 50 spectrophotometer.

An Agilent (Palo Alto, CA) 1200 series HPLC equipped with an Agilent C18 reversed phase column (150 × 2.1 mm, 5 μm, Agilent, USA) coupled to the a TOF 5600 MS (AB Sciex, Foster City, CA) (HPLC–MS) was used for the products separation and identification. The mobile phase was comprised by methanol/water = 85:15 (v/v) at a flow rate of 0.2 mL min^{-1}. The sample injection volume was 5 μL by an autosampler. The TOF MS scan type was operated in negative electrospray ionization (ESI−) mode over a mass range of 50–1200 m/z. The optimized mass parameters were: ion source temperature 550 °C, ion spray voltage −4500 V, nebulizer gas 55 psi, auxiliary gas 55 psi, curtain gas 35 psi.

3. Results and discussion

Table S1. Water qualities (pH, TOC and UV_{254}) of the collected water samples.

Water samples	pH	TOC (mg L^{-1})	UV_{254} (cm^{-1})
Municipal wastewater	7.38	8.99±3.74	0.254±0.055
Meiliang Bay	8.41	7.67±2.19	0.121±0.011
East Taihu Lake	8.18	6.90±2.68	0.060±0.007
Yangtze River	8.21	3.00±0.38	0.049±0.002

Table S2. Metal ions concentrations in collected water samples after filtered by 0.45 μm filters.

	Metal ions (ppm)[a]									
	Ca	Mg	K	Na	Al	Fe	Cu	Zn	Mn	Pb
Municipal wastewater	38.2	8.09	8.03	30.2	<0.01	<0.01	<0.005	0.004	0.012	0.11
Meiliang Bay	29.8	7.79	4.59	36.4	<0.01	<0.01	<0.005	0.005	0.002	<0.05
East Taihu lake	18.5	3.60	2.13	17.3	0.012	0.016	0.008	0.046	0.022	<0.05
Yangtze river	39.6	8.22	2.87	8.93	<0.01	0.006	<0.005	0.002	0.009	<0.05

[a] Metal ions concentrations were determined by ICP-OES (Optima 5300DV, PerkinElmer, USA).

Table S3.

The recovery of SPE process for 10 nM of TBBPA in in synthetic water, wastewater and natural lake waters systems.

Water sample	TBBPA recovery rate (%)[a]		
	Raw	7.0	6.0
Municipal wastewater	97±3.3	94±2.5	93±3.4
Meiliang Bay	101±3.7	99±5.1	91±1.5
East Taihu lake	103±1.1	93±4.3	92±3.7
Yangtze river	98±2.7	107±5.4	95±3.9
PBS	-	98±4.1	93±3.5

[a] Each reaction condition was performed in duplicate.

Table S4.

Experimental and predicted TBBPA removal based on TOC values (Fig. 3a) via laccase- and HRP-catalyzed reaction from the collected waters (pH 7.0).

Water sample	TOC	Laccse			HRP/H_2O_2		
	(mg L^{-1})	Exp.	Pre.	RPD(%)	Exp.	Pre.	RPD(%)
Municipal water	8.99	47.9±5.5	45.7±19.0	4.7	87.9±4.4	78.8±32.7	10.4
Meiliang Bay	7.67	77.4±4.2	48.6±13.9	37.2	93.2±5.0	80.4±23.0	13.7
East Taihu lake	6.90	84.0±1.0	50.5±19.6	39.9	95.2±2.7	81.4±31.6	14.4
Yangtze river	3.00	84.1±2.1	62.9±8.4	25.2	97.5±9.3	87.0±11.5	10.8
SRHA	4.97	45.7±1.5	56.0±0.9	-22.5	79.9±5.2	84.1±1.4	-5.2
SRFA	4.73	53.3±1.2	56.72±6.0	-6.3	81.1±4.7	84.4±8.9	-4.1
Glucose	19.43	84.9±3.7	30.9±1.1	63.6	100±0.9	67.6±2.4	32.5
BSA	22.97	69.6±3.4	27.9±1.2	59.9	88.1±1.3	64.5±2.8	26.7
PBS	0.33	84.3±3.5	75.8±50.5	10.1	99.6±0.4	50.5±19.6	8.4

RPD: relative percent difference

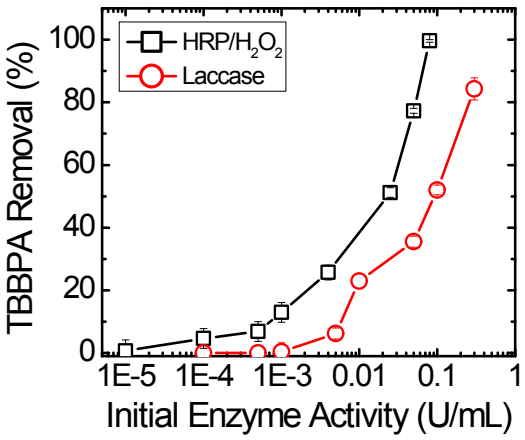

Fig. S1 TBBPA removal under various concentrations of enzymes after 4-hour treatment. Reaction conditions: [TBBPA]=10 nM, [laccase]=0.0001-0.3 U/mL, [HRP]=0.00001-0.08 U/mL.

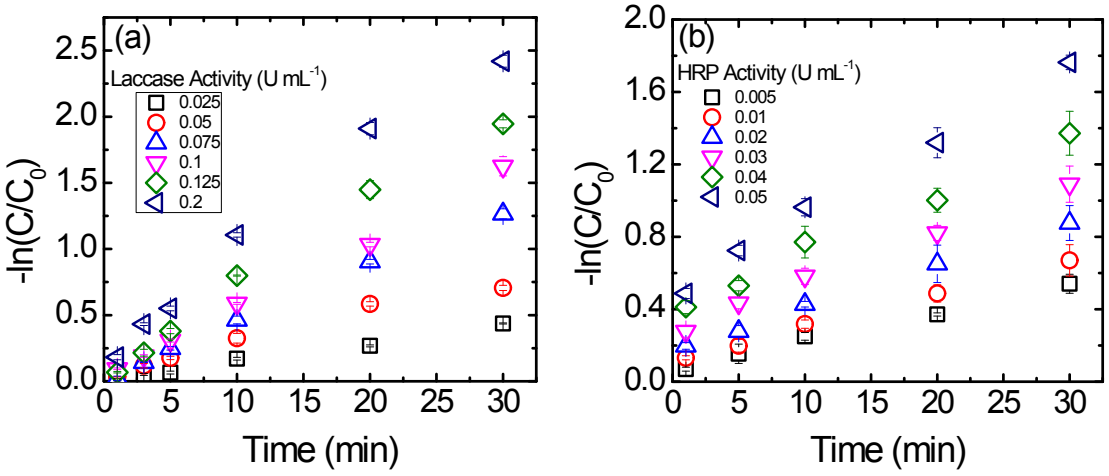

Fig. S2. Pseudo first-order rate plots for TBBPA removal at different laccase (a) and HRP (b) dosages, Experimental conditions: $[TBBPA]_0$ = 10 μM, $[H_2O_2]$ = 10 μM for (b), pH 7.0, 25 °C. Error bars present standard deviations (n = 3). Laccase data are reproduced from [4] with permission from the American Chemical Society.

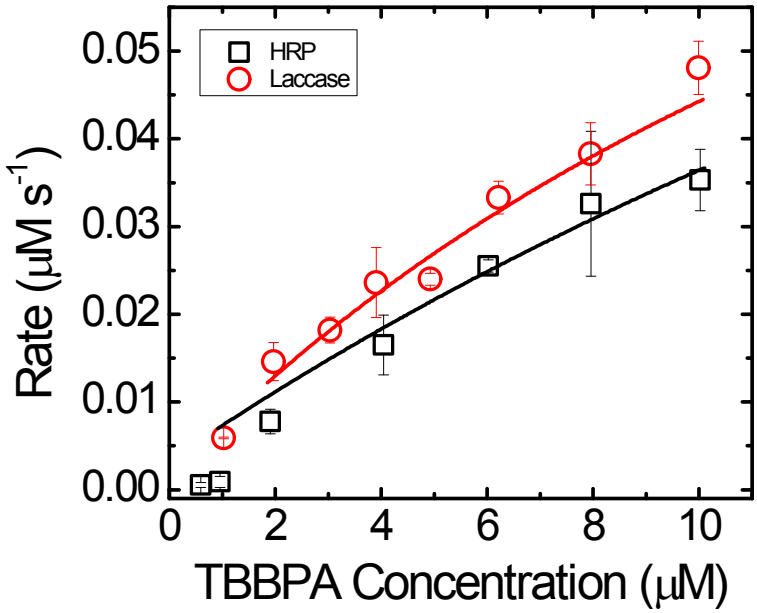

Fig. S3. Initial reaction rate of TBBPA at various initial concentrations.

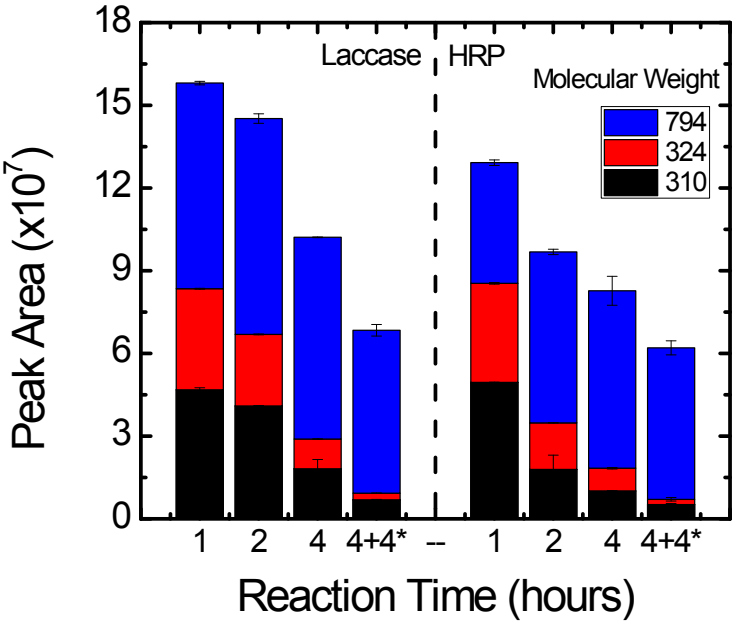

Fig. S4. Time-dependent evolution of TBBPA transformation products during enzymatic reaction process. Initial TBBPA concentration was 10 μM, laccase concentration was 0.3 U mL-1, HRP concentration was 0.05 U mL^{-1}, H_2O_2 was 50 μM, pH was 7.0. Asterisks mean that the additional dose of laccase (0.3 U mL-1), or HRP (0.05 U mL-1) and 50 μM H_2O_2 were added and prolonged the enzymatic reaction for another 4 h. Error bars represent standard deviations (n = 3).

Reference:

1. L. M. Colosi, Q. G. Huang and W. J. Weber, *J Am Chem Soc*, 2006, 128, 4041-4047.
2. M. Auriol, Y. Filali-Meknassi, C. D. Adams, R. D. Tyagi, T. N. Noguerol and B. Pina, *Chemosphere*, 2008, 70, 445-452.
3. W. Zheng and L. M. Colosi, *Chemosphere*, 2011, 85, 553-557.
4. Y. P. Feng, L. M. Colosi, S. X. Gao, Q. G. Huang and L. Mao, *Environ Sci Technol*, 2013, 47, 1001-1008.