(75) Aerobic and anaerobic biotransformation of 17β-estradiol in sediment cores

Fusheng Li[1*], Reni Desmiarti[2], Akira Yuasa[3] and Yutaka Suzuki[4]

[1] Department of Civil Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
[2] Graduate School of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
[3] River Basin Research Center, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
[4] Water Environment Research Group, Public Works Research Institute, Tsukuba, Ibaraki 305-8516, Japan
*E-mail: *lifs@gifu-u.ac.jp*

Four sediment cores consisted of 30cm of undisturbed sediment and 60 cm of overlying water collected from two sites within a natural reservoir were designed and the behavior of 17β-estradiol (E2) spiked into the overlying water of the sediment cores was investigated. Besides, with the addition of glucose, the likely effect of easily degradable organic substrates upon the behavior of E2 and its biotransformation intermediate of estrone (E1) was also assessed. The results showed that the spiked E2 (at 16.5 μg/L) disappeared faster in aerobic sediment cores than in anaerobic ones, and under both aerobic and anaerobic conditions, E1 was formed as the biotransformation by-product of E2 and then disappeared from the water phase in a pace considerably slower than E2. The half-lives of E2 without and with the coexistence of glucose (added at three different concentration levels) varied in 0.4~7.5 hours for runs of the aerobic cores, while those for runs of the anaerobic cores in 2.7~15.1 hours. The removal of glucose quantified using the index of TOC was also found faster in aerobic sediment cores than in anaerobic ones.

Keywords : Biotransformation, Sediment, Estrogens, Half-life, Reservoir

1. INTRODUCTION

Natural and synthetic steroid estrogens, such as 17-β estradiol (E2), estrone (E1), estradiol (E3) and 17α-ethinylestradiol (EE2), are frequently detected in natural aquatic environment systems (Johnson *et al.*, 2000; Heberer, 2002; Andersen *et al.*, 2003), and the sediments of closed water bodies (Lopez de Alda & Barcelo, 2001; Koh *et al.*, 2005; Isobe *et al.*, 2006). Their likely estrogenic impacts on humans and wild lives are greatly concerned by water quality regulators and drinking water production units (Routledge *et al.*, 1998). Most endocrine disrupting chemicals (EDCs) are man-made organic compounds being introduced to the environment through a variety of anthropogenic activities. These chemicals include such species as surfactants, pesticides, antifouling compounds, pharmaceuticals and personal care products. In addition, EDCs can be naturally occurring in the environment. For example, E1 and E2 are natural female hormones excreted from humans and animals, being thus ubiquitous for natural water sources that receive effluents from associated sewage treatment plants (STPs). The likely adversary health effects of estrogens upon aquatic organisms are also documented (Christianshen *et al.*, 2002).

Estrogenic compounds from humans and animals are excreted in urine and feces as conjugated forms (such as glucuronide and sulfated conjugates) and un-conjugated forms (such as E1 and E2). Upon entering the STPs, the large densities of microorganisms in activated sludge can disassociate the conjugated estrogens into un-conjugated ones and then biodegrade them (Lai *et al.*, 2002; Fujii *et al.*, 2002), thus lowering their presence in natural water sources. It is conceivable that the disappearance of estrogens from water, sediments and soils is largely attributed to the combination of physical sorption and biodegradation. Recent studies have indicated that sorption of estrogens is significant in aquatic sediments (Holthaus *et al.*, 2002 & Yu *et al.*, 2004). However, the contribution of sorption may change greatly according to the

physicochemical features of sediments, and the types and densities of microbes populated (Li *et al.*, 2004). Sediments of natural water bodies, especially those in anoxic states, have the potential to be a reservoir for estrogenic compounds.

Biological degradation of estrogens under aerobic conditions has been examined through batch and field studies in many STPs (Ternes *et al.*, 1999; D'Ascenzo *et al.*, 2003; Johnson & Williams, 2004; Joss *et a.l*, 2004; Li *et al.*, 2005). It was reported that E2 is converted rapidly to E1, the removal of E1 is slower than E2 (Ternes *et al.*, 1999). Significant differences on the removal efficiency of E2 and E1 among different STPs were observed, for instance: 19-98% for E1 and 62-98% for E2 (Johnson & Sumpter, 2001; Johnson & William, 2004). Similarly, Tanaka *et al.* (2003) investigated the concentrations of E2 in 47 municipal STPs in Japan using either enzyme-linked immunosorbent assay (ELISA) or LC-MS/MS. The ELISA-based removals for E2 varied in the range of 15–99% (a mean value of 79%), while, the removals based upon the LC-MS/MS measurements led to a mean removal value of 96%. The marked differences in removal are considerable since the monitored STPs differ with one another in many aspects, including the type and age of plants, bacterial population, operational mode and operation conditions (such as the temperature, the sludge retention time), as well as the composition of influent wastewater. Under anaerobic conditions, the biodegradation of E2 is very limited (Ying & Kookana, 2003; Joss, *et al.*, 2004). However, little is known about how E2 is disappeared or accumulated in the sediment of natural water bodies, a complex natural ecosystem that could change its physicochemical and biological features regarding such parameters as ORP, DO, N and P, and the species and densities of microbes (both aerobic ones and anaerobic ones).

The aim of this study was to examine the behavior of E2 in the overlying water of static sediment cores under well controlled aerobic and anaerobic conditions of the water phase. Besides E2, for several experimental conditions, glucose, a representative readily biodegradable organic substrate, was also added into the overlying water for varied concentrations (30, 500 and 1000 mg/L) to assess how and to what extent the coexistent organic compound affects the behavior of E2 and its transformed intermediate product of E1 under both aerobic and anaerobic conditions.

2. MATERIALS AND METHODS

(1) Stock solution of E2

A stock solution of E2 (about 0.9 mg/L) was prepared by dissolving a weighted amount of E2 (Wako Pure Chemical Co., Osaka, Japan) in Milli-Q water. Organic solvent was not used in order to eliminate its effect on the biotransformation of the targeted E2. To obtain the stock solution, a pre-weighted amount of E2 powders was added to a glass water reservoir filled with about 5 L of Milli-Q water to make an initial suspension at about 2 mg/L. After stirring the suspension for more than 24 hours, filtration was performed using a pre-washed 0.2 μm PTFE membrane filter to separate the fraction of E2 not yet dissolved, and the filtrate was then used as the stock solution and was refrigerated at dark at 5°C untill use.

(2) Sediment and water samples

Sediment and overlying water samples collected on 15 July 2004 from two sites (referred hereafter as St. A and St. B, **Fig 1.**) within the Ushikubiri Reservoir, one of all five pre-reservoirs of the Miharu Dam at the Miharu-town of Fukushima Prefecture, were used in the study. Compared to St. A that was located in the downstream being about 50m away from the overflow weir that separates the reservoir from the main dam, St. B was located in the midstream of the reservoir. Having a storage capacity of about 214,000m^3, an average water depth of 6.5m and a detention time of about 22.5 days, this pre-reservoir is constructed to cut the inflow levels of pollutants to the main reservoir. Detailed information on water quality of the reservoir could be found elsewhere (Tsumori *et al.*, 2004).

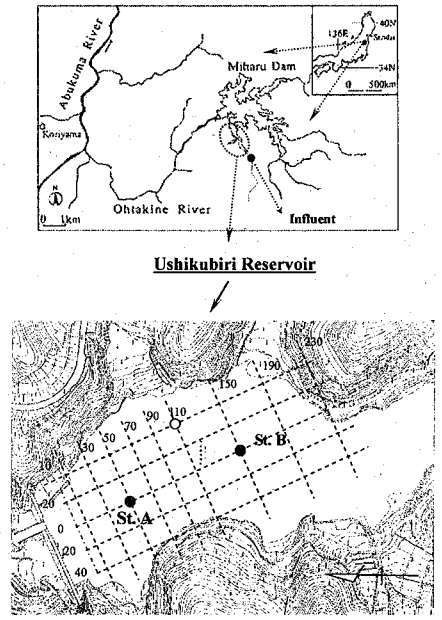

Fig. 1. Location map of the Ushikubiri reservoir of the Miharu Dam and sampling sites

Sediment samples were collected using a gravity core sampler that enables easy installation the sampling cores having a diameter of 4cm and a length of 50cm. All collected core samples were transported to the laboratory using a core container supplied consistently with nitrogen to prevent the sediment samples from contact with air. After reaching the laboratory, the overlying water (with a depth of about 15 cm for most cores) above the sediment mud surface was suctioned away from the cores and the sediment layer with a depth of 30 cm from the surface was then transplanted carefully into columns with the same internal diameter as the cores used during sampling, to ensure that the sediment layer was not disturbed. This process was also performed under an environment that could prevent the sediment from being oxidized by air. The columns used here are 100 cm in length, thus allowing the addition of collected water from the sampling site to reach a level with the overlying water depth being 60 cm. Several important physicochemical features of the sediment (including the ignition loss, total carbon, total phosphorus and total nitrogen) are summarized in **Table 1**. Additional information on sampling and sediment slicing is also available (Li *et al.*, 2004; Tsumori *et al.*, 2004).

Table 1 The characteristics of sampled cores of sediment assessed on dry weight basis (%, wt/wt)

Location	VSS	total C	total N	total P
St. A	10.11-14.0 (11.15)	1.74-2.73 (2.19)	0.23-0.39 (0.298)	0.103-0.17 (0.138)
St. B	8.97-12.16 (10.16)	1.62-2.88 (2.16)	0.164-0.356 (0.254)	0.094-0.163 (0.125)

All values represent the measurements for all 15 sediment layers sliced from the top of each core sediment, with the thickness of each layer being 2 cm. The values in parentheses are the average values for the whole 30 cm core sediment depth.

(3) Experiments

The schematic diagram of the static core experiments is displayed in **Fig. 2.** Columns referred as OC-A and OC-B in this figure received the sediment cores from St. A and St. B, respectively, and the overlying water within them is always saturated with oxygen as a result of consistent supplying of air to the level close to the sediment-water interface with air diffusers. Columns AC-A and AC-B shown in this figure corresponded also to the sediment cores from St. A and St. B, respectively. However, since wetted-nitrogen was continuously supplied to the overlying water from a depth close to the sediment-water interface, the penetration of air to column was well prevented. These two columns are referred hereafter as anaerobic ones as against the above-mentioned aerobic ones.

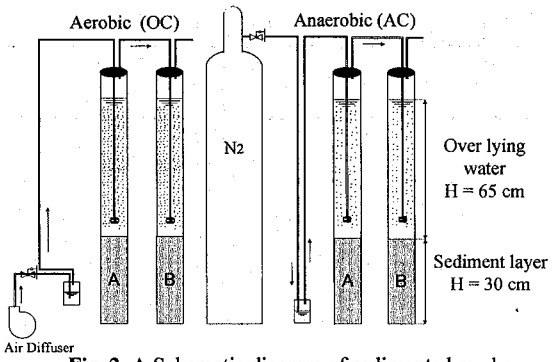

Fig. 2 A Schematic diagram of sedimented mud core

Since the set up of the experimental system, the overlying water in all columns has been replaced with fresh water from the sampling sites for several times (about one for every two or three months). And, water quality indices including DO, ORP and pH have been continuously monitored, together with nitrogen (NH_4^+, NO_2^- and NO_3^-), PO_4^{3-}-P and organic carbon. Due to the continuous supplying of wetted-nitrogen for AC-A and AC-B, the sediment in the anaerobic columns has kept its reductive state not changed, as the vertical profiles of the oxidative-reductive potentials (ORP) measured for the sediment phase showed minor variations at about -300mV. For the aerobic columns OC-A and OC-B supplied continuously with air, however, a sharp change of the ORP trend occurred at the level about 4cm from the sediment layer surface, i.e., the water-sediment interface. For the top 4 cm layer, the values of ORP have converted to positive (0-450mV), indicating that this layer has already been oxidized as expected by the authors. This could be easily judged from the apparent changes in the sediment color (from dark to yellow). For the remaining sediment (about 26 cm in depth) below the oxidative layer, the ORP values are still negative (-120~-300mV).

Two series of experiments were performed based on the conditions shown in **Table 2**. The first series was devised for investigation of the behavior of added E2 under aerobic conditions, while, the second one for investigation under anaerobic conditions. The behavior of E1, an intermediate product of E2, in the experimental systems was also traced and the likely impacts of the easily biodegradable compound (glucose) on the behavior of E2 and E1 were evaluated. For the latter purpose, glucose was added to the column at different initial concentrations as 30, 500 and 1000 mg/L, respectively, and its concentrations were measured using the index of total organic carbon (TOC). The addition of E2 was made by adding weighted volumes of the stock solution of E2 to the overlying water of each column. A gentle stirring was followed in order to achieve the same initial E2

Table 2 Aerobic and anaerobic sediment core experiment conditions

Series no.	Run no.	Conditions	Column	Added E2 (μg/L)	Glucose added (mg/L)	Initial TOC (mg/L)	Note
1	1-a	Aerobic	OC-A	16.5	0	5.3	without glucose
	1-b		OC- B		0	3.9	without glucose
	2-a		OC-A		30	16.6	with low content of glucose
	2-b		OC-B		30	15.4	with low content of glucose
	3		OC-A		500	216	with high content of glucose
	4		OC-B		1000	410.4	with high content of glucose
2	5-a	Anaerobic	AC-A	16.5	0	6.8	without glucose
	5-b		AC- B		0	6.7	without glucose
	6-a		AC-A		30	18	with low content of glucose
	6 -b		AC-B		30	18.3	with low content of glucose
	7		AC-A		500	212.7	with high content of glucose
	8		AC-B		1000	418	with high content of glucose

(The values of initial TOC represent the whole organic carbons in the overlaying water after addition of glucose)

concentration in the vertical direction of the overlying water using a special self-made mixer that could prevent the suspension of sediment from occurring.

The experiments were performed within a temperature-controlled laboratory (20 °C) and intermittent monitoring of dissolved oxygen (DO) in the overlying water of all columns showed a small DO fluctuation at around 8 mg/L for aerobic columns, and about zero for anaerobic ones. To generate data that could better describe E2's behavior, the sampling time intervals were carefully designed. For each sampling, about 10 mL of water was taken into a 10 mL glass tube for centrifugation (3500 rpm for 2 minutes) and the resultant supernatant was then filtered through a pre-washed 0.45 μm PTFE membrane filter.

E2 and E1 in the filtrate were analyzed using an Agilent 1100 series LC/MSD system. The internal standards 17β-estradiol-C_4 and estrone-C_4 were applied and the detection limits for both E2 and E1 were confirmed as about 0.01 μg/L.

3. RESULTS AND DISCUSSION

(1) Concentration profiles of E2 and E1 under aerobic condition

The concentration profiles of E2 and its by-product E1 under the aerobic condition for varying initial glucose concentrations (0, 30, 500, and 1000 mg/L) are illustrated in **Fig. 3 (a-f)**. For the OC-A column, indicating rapid decreases at the initial period, E2 dropped to 0.51, 3.19, and 0.57μg/L after being spiked for 17 hours with glucose at 0, 30 and 500 mg/L, respectively **(Fig. 3a, 3c & 3e)**. This represented a reduction of E2 by about 97, 81, and 97%, respectively. The decreasing trend of E2 continued after this run time even if its extent was getting less apparent. For samples collected after running for 54 hours, the residual E2 decreased to 0.04 μg/L and 0.06 μg/L for runs 2a and 3, respectively, and was not detectable for run-1a. In confirmation of the rapid transformation of E2 shown above, the results obtained through the parallel column experiments using the sediment from the midstream of the reservoir (OC-B) revealed similar trends as those for OC-A **(Fig. 3b, 3d & 3f).** The concentrations of E2 after 17 hours dropped to 1.89, 3.5 and 1.57 μg/L, which represented a removal of E2 by about 89, 79 and 90% in relation to the initial glucose concentrations of 0, 30 and 1000 mg/L, respectively.

The removals for E2 differed with OC-A and OC-B, however, the difference was less significant as the sampling sites for the sediments used in these two cores are not too far from each other. The similar physicochemical features for the cores of sediment at both St. A and St. B, as shown earlier in **Table 1,** may imply that their overall microbe composition in regarding both densities and species is also similar. The results may also imply that the disappearance of E2 from the overlying water phase is less dependent of the initial glucose concentrations under the aerobic condition. These results supported previous results obtained by Suzuki & Maruyama (2006) using activated sludge that the removal of E2 under aerobic condition more affected by the microbe activity than by the presence levels of the biodegradable glucose. In another literature, Li *et al.* (2005) reported that the removal efficiency of E2 is markedly dependent on the bacterial population in aerobic biodegradation reactors using MLVSS at different levels. The concentration of E1 was generated as a by-product

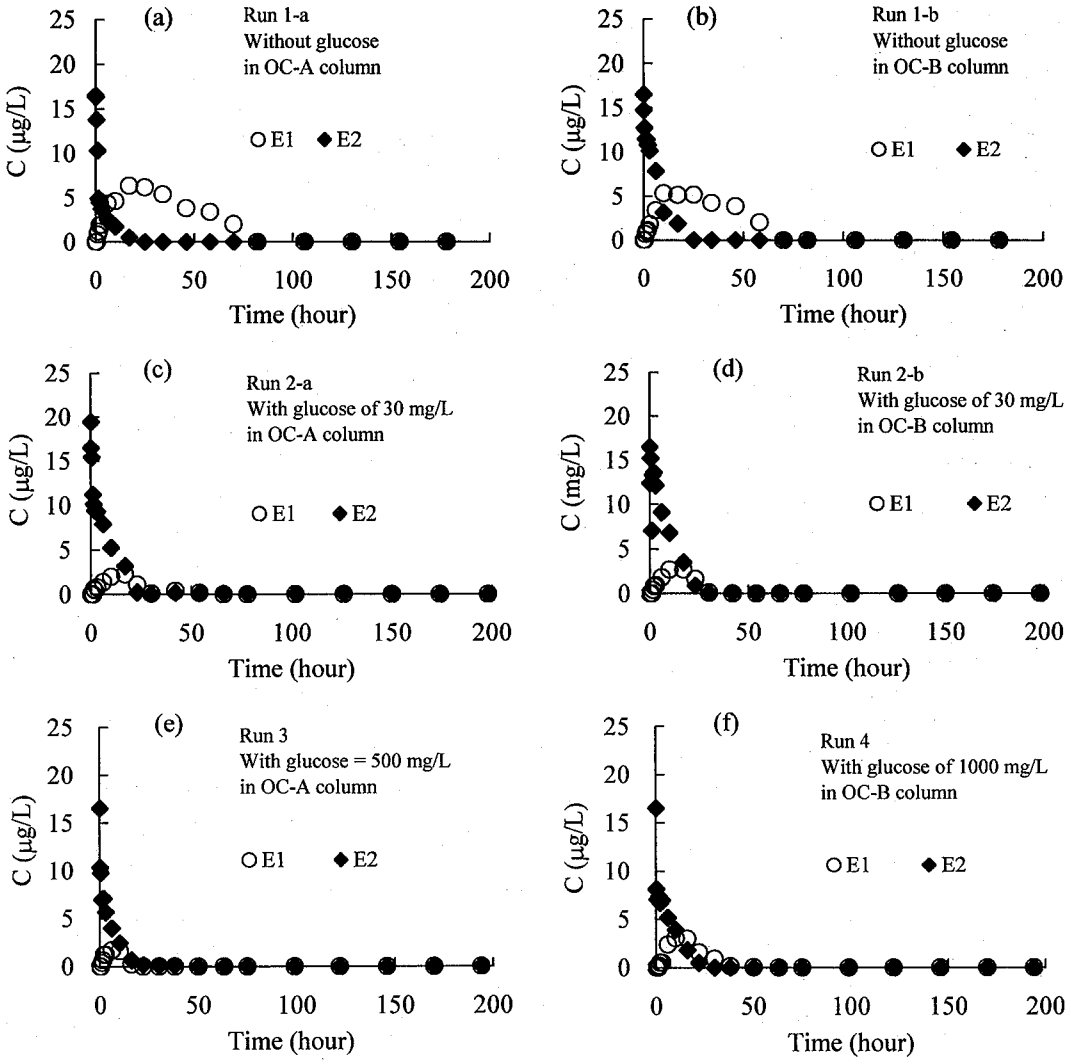

Fig. 3. Decreasing profiles of E2 and its by-product E1 in sediment cores under aerobic conditions

of E2 as could be see from its accumulation at the initial stage of each run. The apparent decreases in the concentration of E1 occurred after E2 was entirely disappeared from the reactor, with its decreasing rate being considerably slow. For instance, for run 1-a, E1 reached the maximum level (6.35μg/L) after 17 hours and was then reduced to 2.02 μg/L after another 41 hours. Based upon the apparent maximum accumulation concentration (C_{max}) of E1 and the time (t_{max}) needed for E1 to reach C_{max}, the *apparent* biotransformation rate of E1 from E2 at the initial stage was computed using $r_{E1}=C_{max}/t_{max}$. As displayed in **Fig.4**, the computed r_{E1} values were 0.37, 0.13, and 0.16 (μg/L)/hr for OC-A runs 1-a, 2-a and 3 added with 0, 30 and 500 mg/L of glucose, respectively. Similar results were also obtained for

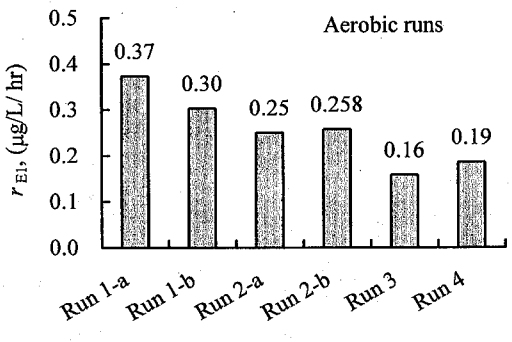

Fig. 4 Apparent biotransformation rate from E2 to E1

runs 1-b, 2-b and 4 performed by use of the OC-B column, with the computed r_{E1} values being 0.3, 0.16, and 0.19 (μg/L)/hr for runs with 0, 30 and 1000 mg/L of added glucose, respectively. The real biotransformation rate of E1 from E2 was probably higher if the decreased fraction of E1 in the apparent accumulation stage of E1 (having the time length t_{max}) could be taken into consideration, i.e., be added to the observed C_{max} values.

The results indicated that in comparison to the disappearance rate of E1, the transformation rate of E1 from E2 was faster, which thus led to apparent accumulation of the former at the initial stage of E2 addition. It was also indicated that the presence of easily biodegradable substrate such as glucose seemed to be capable of lowering the apparent E1 accumulation level, thus leading to faster disappearance of E1 from the sediment/water system. The results obtained above are significant, by taking into account the fact that experiments using cores installed with undisturbed sediment from the field are very limited, and that the findings obtained are probably more applicable than those obtained through batch experiments using suspended sediment liquors (Li *et al.*, 2004). Using activated sludge from a STP, Ternes *et al.* (1999) studied the degradation behavior of E2 and found that E2 was converted to E1 rapidly, and the removal of E1 was slower than the oxidation of E2 to E1. Even if the microbes targeted in their study (activated sludge) may incomparably deviate from the natural ones we used in this study (sediment), the findings in regarding the pathway of E2 under aerobic conditions and the relative disappearance

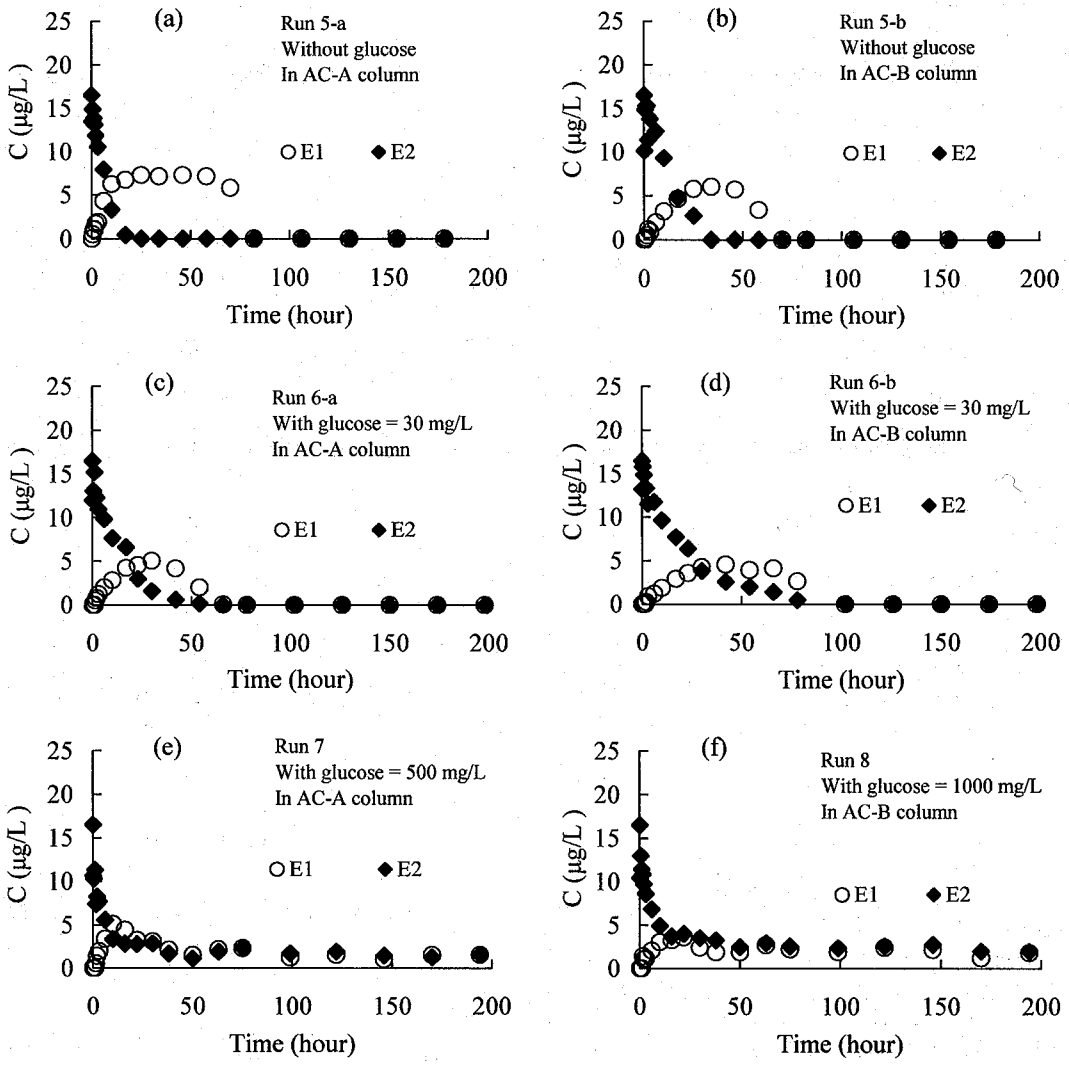

Fig. 5. Decreasing profiles of E2 and its by-product E1in sediment cores under anaerobic conditions

rate of E2 to E1 are similar, and they could thus be considered as general features when E2 is exposed to aqueous microorganisms. Further experimental work is planned for quantification the transformation from E2 to E1 under a variety of conditions and for investigation of the behavior of E2 and E1 under continuous flow mode by also altering the retention time and the composition for coexistent organic substrates and nutrients.

(2) Concentration profiles of E2 and E1 under anaerobic condition

The concentration profiles of E2 under anaerobic conditions are shown in **Fig. 5**. Within the AC-A column, the spiked E2 dropped to 0.43, 0.17, and 1.28μg/L after 17, 54 and 170 hours for the run conditions at glucose concentrations of 0, 30 and 500 mg/L, respectively. These represented the reduction of E2 by about 97, 99, and 92%, respectively. The decreasing trend of E2 continued even if its extent was getting less apparent. For instance, the residual E2 in samples collected after 78 hours decreased to 0.02μg/L for runs 6-a and was not detected at all for run-5a. Similar results were also observed for the parallel runs 5-b, 6-b and 8 that used the AC-B column filled with the sediment from St. B. For these runs, the spiked E2 decreased with time and dropped to 4.78, 2.0, and 1.99μg/L after 17, 54 and 170 hours, respectively. The removals of E2 were 71, 88 and 88% in relevance to the initial glucose concentration of 0, 30 and 1000 mg/L, respectively, which were lower than the removals obtained with the AC-A column

Besides, for both AC-A and AC-B, it was interesting to see that the organic species E1, which is generally considered as a biotransformation product of E2 under only aerobic conditions, was also produced within the anaerobic sediment/water system, where free oxygen was inexistent. Based on the detected levels of E1, the *apparent* biotransformation rate parameters (r_{E1}) were also computed. As displayed in **Fig. 6**, the values of r_{E1} were 0.21, 0.17 and 0.30 (μg/L)/hr for runs with the glucose concentrations of 0, 30 and 500 mg/L, respectively.

(3) Concentration profiles of glucose under aerobic and anaerobic conditions

The concentration profiles of glucose added to the sediment columns simultaneously with E2 are shown in **Fig. 7**. The concentration of glucose decreased with time under both aerobic and anaerobic conditions. The decreasing rate varied with the sediments and the initial glucose concentrations. For runs added with 30 mg/L of glucose **(Fig.7a)**, the value of TOC decreased to 6.39 mg/L after 30 hours for the run 2-a of the aerobic column OC-A. For the run 6-a with the anaerobic column AC-A, however, 54 hours were needed for TOC reach the steady level at about 8.39 mg/L. This indicated that glucose was degraded more readily under aerobic conditions than under anaerobic ones. The TOC concentration at the steady level reflected the background organic species in the relative overlying water, which was a summation of the organics presented originally in the reservoir water with those released from the sediment under both aerobic and anaerobic conditions. For OC-B and AC-B that used the sediment collected from St. B, similar results were revealed **(Fig. 7b)**. For runs added with high concentrations of glucose, as shown in **Fig. 7c and 7d**, the reduction of TOC also proceeded faster in the aerobic columns (OC-A an OC-B) than in the anaerobic ones (AC-A and AC-B). Based on observed results, the half-lives ($t_{1/2}$) were computed by linear interpolation between two neighboring data that had TOC values closest to the TOC residual of 50%. The values of $t_{1/2}$ shown in **Table 3** indicated that, for either the aerobic columns or the

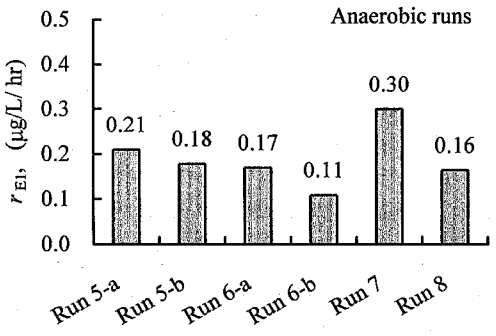

Fig. 6 Apparent biotransformation rate from E2 to E1

Table 3 Half-lives of E2 and glucose for all runs.

Run no.	Column	E2	Glucose
		$t_{1/2}$ (hour)	
1-a	OC-A	1.2	-
1-b	OC- B	5.5	-
2-a	OC-A	5.3	27.8
2-b	OC-B	7.5	29.4
3	OC-A	0.8	70.7
4	OC-B	0.4	159.5
5-a	AC-A	5.6	-
5-b	AC- B	11.7	-
6-a	AC-A	8.9	48.0
6 -b	AC-B	15.1	46.7
7	AC-A	2.7	118.5
8	AC-B	3.6	271.2

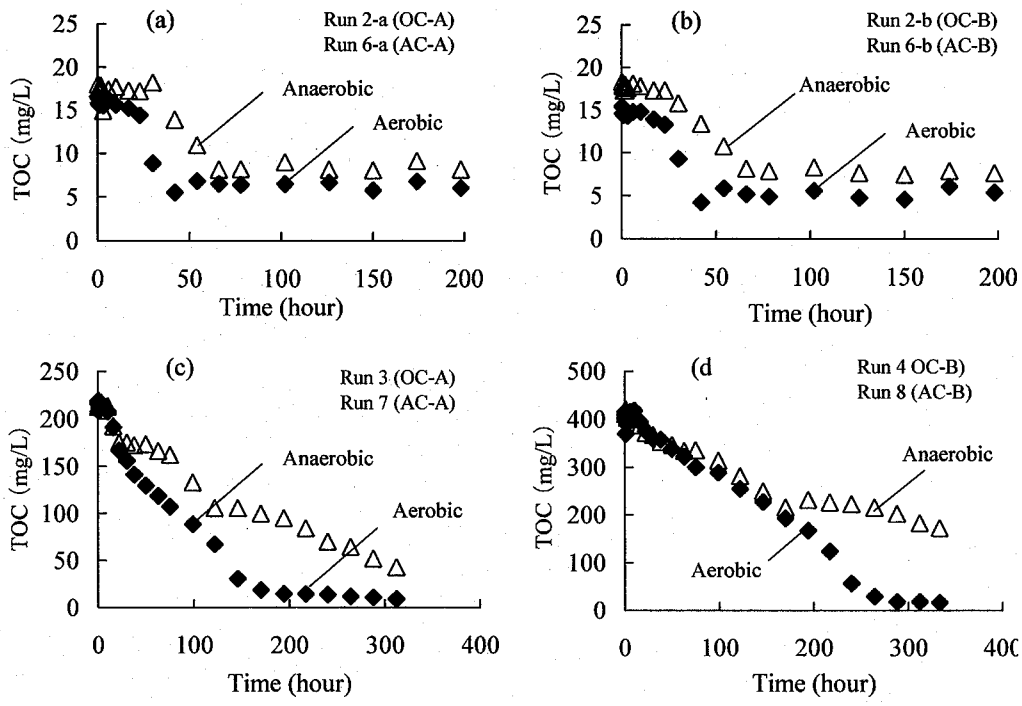

Fig. 7. Decreasing profiles of glucose under aerobic and anaerobic conditions under intial glucose concs. of 30 mg/L for (a) and (b), 500 mg/L for (c) and 1000 mg/L for (d)

anaerobic columns, the time needed for elimination of glucose from the water phase was extended as the initial glucose concentration increased. Besides, In comparison with the half-lives obtained for all runs with the anaerobic columns, the half-lives obtained for runs with the aerobic columns were significantly smaller. This thus indicates that aerobic condition is better for microbes populated within the sediment to eliminate organic substances in the water phase, and that anaerobic condition is a major factor that contributes to the persistency of natural and synthetic hydrophobic organic compounds such as nonylphenol, estrone and polychlorinated biphenyl in sediment ecosystems (Holthaus *et al.*, 2002; Isobe *et al.*, 2006).

(4) Half-lives of E2 under aerobic and anaerobic conditions

To further assess the disappearance rate of E2, the half-lives of E2 under all experimental conditions were obtained based on the concentration profiles shown above in relative figures. As summarized in **Table 3,** the half-lives of E2 under the aerobic condition fluctuated in the range 0.4-7.5 hours, while those under the anaerobic conditions in the range of 2.7-15.1 hours. These values were close to the results of a previous study that reported the half-lives for E2 to be transformed into E1 falling in the range of 8.8 -15.84 hours in aerobic cultures established with river water and sediment (Jurgen *et al.*, 2002), and were distinctly smaller than the half-life of 67 days reported for a system under sulfate-reducing conditions (Ying & Kookana, 2003).

In regarding the effect of glucose on the biotransformation rate of E2, a general trend of either increasing or decreasing of the half-lives of E2 with the increases of glucose concentration was not revealed. It could be seen from **Table 3** that, compared to the $t_{1/2}$ values for the aerobic runs 1-a and 1-b, and the anaerobic runs 5-a and 5-b, all conducted without the addition of glucose, the $t_{1/2}$ values for the aerobic runs of 2-a and 2-b, and the anaerobic runs of 6-a and 6-b became larger, indicating the biotransformation rate of E2 was inhibited with the presence of glucose. However, for the aerobic runs 3 and 4, and the anaerobic runs 7 and 8, where the added glucose concentrations were several folds higher, the half-lives were getting shorter, indicating enhancement in the biotransformation of E2.

4. CONCLUSIONS

The behavior of 17β-estradiol in sediment columns was examined under well-controlled aerobic and anaerobic conditions. The results clearly indicated that E2 was readily disappeared from the overlying water of the columns under aerobic conditions, as compared to those under

anaerobic ones. With the decrease of E2, the organic species E1, a by-product of E2 generally considered to be transformed under only aerobic conditions was also detected in the overlying water of the anaerobic sediment columns, where free oxygen was not existent. The disappearance rate for E1 converted from E2 was also faster for aerobic sediment columns than for anaerobic ones.

Glucose added to the sediment columns was well degraded by microbes populated in the sediment, with the degradation rate being also faster for aerobic columns than anaerobic ones. The coexistence of glucose did not lead to a set of data that supported the initial assumption of the authors that the easily degradable organic substrate may competitively inhibit the biotransformation rate of E2. Batch studies using suspended slurries of sediment and water are probably effective and will be performed as well in coming studies.

ACKNOWLEDGEMENTS: This work was supported in part by Grants-in-Aid for Scientific Research (Kiban-B, No. 17360261) from the Japan Society for the Promotion of Science, and by the River Fund in charge of the Foundation of River and Watershed Environment Management (FOREM), Japan. The authors also thank Shinsuke Makino, a former student of the laboratory for his great contribution to the experiment.

REFERENCES

1) Andersen, H., Siegrist, H., Halling-S, B., and Ternes, T.A.: Fate Estrogens in a municipal sewage treatement plant, *Env. Sci. Technol.* Vol. 37, pp. 4021-4026, 2003.
2) Chistianshen, B.L., Winther-Nielsen, and M., Helweg, C. : Feminisation of fish: The effect of estrogenic compounds and their fate in sewage treatment plants and nature, *Enviromental Project* No. 729, Danish Environmental Protection Agency, Denmark, 2002.
3) D'Ascenzo,G., Corcia,A., Gentili,A., Mancini,R., Mastropasqua,R. and Nazzari, M.: Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities, *Sci. Total Environ.*, Vol. 302, pp. 199–209, 2003.
4) Fujii, K., Kikuchi, S., Satomi, M., Ushio-sata, N., Morita, N.: Degradation of 17β-estradiol by a gram-negative bacterium isolated from activated sludge in a sewage treatment plant in Tokyo, Japan, *Appl. Environ. Microbiol,* Vol. 68, pp. 2057-2060, 2002.
5) Heberer, T.: Tracking persistent pharmaceutical residues from municipal sewage to drinking water, *J. Hydrol.* Vol. 266 (2–3), p. 175–189, 2002.
6) Holthaus, K.I.E., Johnson, A.C., Jurgens, M. D., Williams, R. J., Smith, J. J. L. and Carter, J. E.: The potential for estradiol and ethinylestradiol to sorb to suspended and bed sediments in some English rivers, *Environ Toxicol Chem,* Vol. 21, pp. 2526–2535, 2002.
7) Isobe.T., Serizawa.S., Horiguchi.T., Shibata.Y., Managaki.S., Takada. H., Morita. M., and Shiraishi. H.: Horizontal distribution of steroid estrogens in surface sediments in Tokyo Bay, Enviromental Pollution, *in Press.*
8) Johnson,AC.and Sumpter, J.P.: Removal of endocrine -disrupting chemicals in activated sludge treatment works, *Env. Sci Tech,* Vol. **35**, pp. 4697–4703, 2001.
9) Johson, A.C. and William, R.J.: A. model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works, *Environ. Sci. Technol.*, Vol. 38, pp. 3047-3055, 2004.
10) Johnson, A.C., Belfroid. A., and Di Corcia, A. : Estimating stereoid estrogens inputs into activated sludge treatment works and observations on their removal from the effluent, *The Sci. of Tot. Environment.* Vol. 256, pp. 163-173, 2000.
11) Joss, A., Andersen, H., Ternes, T., Richle, P.R., and Siegrist, H. : Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: consequences for plant optimization, *Environ. Sci. Technol,* Vol. 38, pp. 3047 – 3055, 2004.
12) Jürgens, M. D., Holthaus. K. I. E., Johnson, Smith, A. C., Hetheridge, J. J. L. M. and. Williams, R.J.: The potential for estradiol and ethinylestradiol degradation in English rivers, *Environ Toxicol Chem,* Vol. 21, pp. 480–488, 2002.
13) Koh. C-H., Khim. J.S., Villeneuve. D. L., Kannan. K., Johnson.B.G, and Giesy.J.P.: Rapid communication: Instrumental and bioanalytical measures of dioxin-like and estrogenic compounds and activities associated with sediment from the Korean coast, *Ecotoxicology and Environ. Safety,* Vol. 61, pp. 366-379, 2005.
14) Lai, K.M., Scrimshaw and M.D., Lester, J.N.: Biotransformation and bioconcentration of steroid estrogens by *Chlorella vulgaris, Appl. Environ. Microbiol.,* Vol. 68, pp. 859-864, 2002.
15) Li, F.S., Tsumori,J., Yamashita,N., Tanaka,H., and Suzuki Y.: Biodegradation of Biotransformation pathways of 17 β-estradiol by reservoir sediment under aerobic and anaerobic conditions, *Env. Eng. Research,* Vol. 41, pp. 447-458, 2004.
16) Li, F.S., Yuasa .A., Obara.A, and Matheuws.P. A.: Aerobic batch degradation of 17β-estradiol (E2) by activated sludge: Effect of spiking E2 concentrations, MLVSS and Temp, Water Research, Vol. 39, pp. 2065 – 2075, 2005.
17) L. Alda.M.J. and Barcelo.D.: Use of solid-phase extraction in various of its modalities for sample preparation in the determination of estrogens and progestogens in sediment and water, *J. Chromatogr. A.,* Vol. 938, pp. 145-153, 2001.
18) Routledge, E.J., Sheahan, Desbrow.D., Beighty, C., Waldock, M., Sumpter, J. P.: Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach, *Env. Sci. Technol,* Vol. 32, pp. 1559-1565,1998.
19) Suzuki,Y. and Maruyama,T.: Fate natural estrogens in batch mixing experiments using municipal sewage and activated sludge, *Water Res,* Vol. 40, pp. 1061-1069, 2006.
20) Tanaka,H., Sato,C., Komori,K., Yakou,Y., Tamamoto,H., Miyamoto,N., and Higashitani,T.: Occurrence of endocrine disruptors in sewage and their behavior in sewage treatment plants in Japan. Environ. Sci, Vol. 10, pp. 1-24, 2003.
21) Ternes,T.A., Stumpf,M., Mueller,J., Haberer,K., and Wilken, R.D.: Behaviour and occurrence of estrogens in municipal sewage treatment plants-I. Investigation in Germany, Canada and Brazil.: *Sci. Total Environ,* Vol. 225, pp. 81-90, 1999.
22) Tsumori, J., Tanaka, H., Suzuki, Y., Li, J., Wada J. and Fukui S.: Continuous monitoring of water quality in supplying high concentration dissolved oxygen water into hypolimnion of a dam reservoir, Environ. Eng. Research, Vol. 41, pp. 625-634, 2004.
23) Ying,G.G and Kookana,R.S.: Degradation of five selected endocrine-disrupting chemicals in seawater and marine sediment, *Env. Sci. Tech* , Vol. 37, pp. 1256–1260, 2003.
24) Yu, Z.Q., Xiao, B.H., Huang, W. L. and Peng, P.: Sorption of steroid estrogens to soils and sediments, *Env. Toxicol Chem,* Vol. 23, pp. 531–539, 2004.

(Received May 26, 2006)