(40) Behavior of 17β-estradiol in the completely mixed overlying water phase of sedimented mud cores

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Four sediment cores (two aerobic cores and two anaerobic ones) consisted of 30 cm of undisturbed sediment and 60 cm of overlying water collected from two sites within a natural reservoir were designed and the behavior of the spiked 17β-estradiol (E2) and its byproduct E1 in the overlying water phase of all cores operated under batch and continuous flow conditions was investigated. By comparing the E2 levels remaining in the effluent from all columns operated for different hydraulic retention times (HRT), the impact of HRT on the fate and behavior of E2 within the sedimented mud columns was assessed, and the differences in the effluent E2 and E1 levels under aerobic and anaerobic conditions were discussed.

Keywords : Sediment, Estrogens, biodegradation, microorganism

1. INTRODUCTION

Natural estrogens including estrone (E1), 17β-estradiol (E2), estradiol (E3) are secreted by human and animals in conjugated forms (such as glucuronides or sulfates ones) and un-conjugated ones (Spengler et al., 2001). The estrogenic activity of E2 is much stronger than E1 and E3 as shown from documented results of yeast assay (Matsui et al., 2000) and feminization of male fishes (Christianshen et al., 2002).

The presence of natural estrogens in the final effluent of conventional sewage treatment plants (STPs) was measured in levels from tens to hundreds nanograms per liter (Ternes et al., 1999a; Johnson et al., 2000; Andersen et al., 2003; A. Svensons et al., 2003; Tanaka et al., 2003). Due to the merging of the final wastewater effluent into rivers and lakes, their presence has been also confirmed in water and sedimented mud phases of natural water environment systems, especially in relatively closed water bodies (Lopez de Alda & Barcelo, 2001; Koh et al., 2005; Isobe et al., 2006).

Natural estrogens may change their forms and features in natural water environment due to various biological interactions through suspended and sedimented microorganisms. Physicochemical interactions in water environment may also cause sorption of estrogens onto sedimented mud particles, thus leading to changes in the fate and behavior of estrogens (Holthaus et al., 2002 & Yu et al., 2004). Sediments accumulated in natural water bodies, especially those in anoxic states, may serve as a reservoir for natural estrogens having hydrophobic property. Using sedimented mud from a freshwater reservoir, Li et al. (2006) investigated the biotransformation behavior of E2 under batch aerobic and anaerobic conditions. It was found that the addition of the readily biodegradable substrate (glucose) seemed to be capable of inhibiting the disappearance rate of E2 and E1 from packed sediment cores. To obtain more information related to the fate and behavior of estrogens in relatively closed water environment systems, batch and continuous flow experimental approaches using water and sedimented mud from real river and lake environments are still necessary.

The major aim of this study was to examine the behavior of E2 and its transformed intermediate product of E1 in relatively enclosed reservoir water environment. For this, batch and continuous flow experiments using four sedimented mud cores, which were consisted of undisturbed sedimented mud phase and an overlying water phase, were

performed under well controlled 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 bottle filled with about 5 L of Milli-Q water to make an initial suspension at about 2 mg/L. After stirring the suspension for about 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 obtained was then used as the stock solution and refrigerated stored at dark at 5°C for use.

(2) Sampling sites for sediment

Ushikubiri Reservoir, a natural pre-reservoir of the Miharu Dam located in the northeastern prefecture of Fukushima, Japan was chosen as the site for study. As shown in **Fig. 1**, having a storage capacity of about 214,000 m^3, an average water depth of 6.5 m and the hydraulic retention time (HRT) of 22 days, this pre-reservoir is separated from Miharu Dam through an overflow weir and positioned in order to cut the influent pollutant

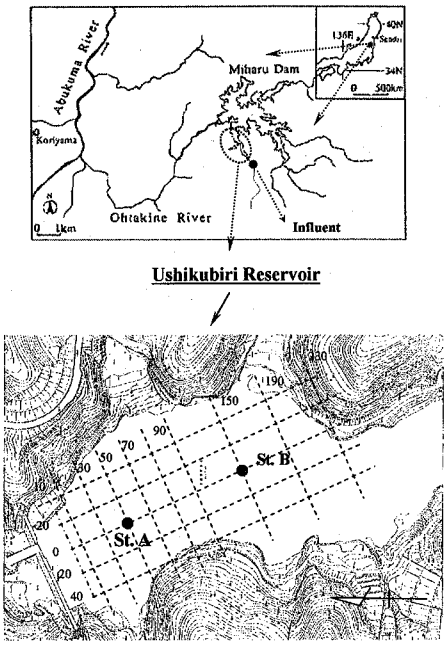

Fig. 1. Location map of the Ushikubiri reservoir of the Miharu Dam and sampling sites

levels into the main dam reservoir. Under normal weather conditions, a small river stream, namely the Ushikubiri River, which has a yearly mean flow rate of about 0.11 m^3/sec, is the sole surface water source to the pre-reservoir.

Two sampling sites, referred hereafter as St. A and St. B, were designated along the flow direction

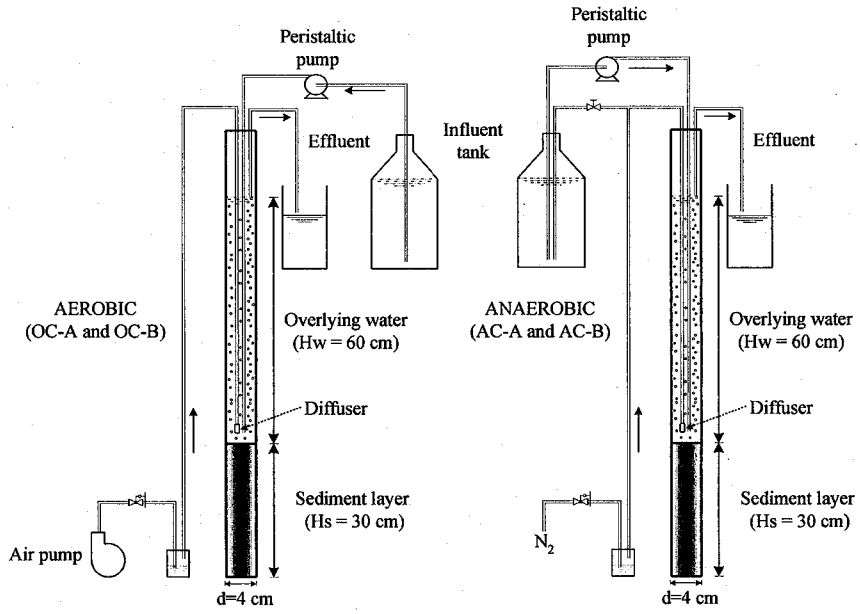

Fig. 2. A schematic diagram of the continuous flow process of sedimented mud cores

of the Ushikubiri reservoir. St. A was located in the downstream, which was about 100 m away from the overflow weir separating the reservoir from the main dam, and St. B was located in the midstream of the reservoir. The distance between these two sites was about 100 m.

(3) Sediment sampling

Sediment samples were collected using a gravity core sampler that enables easy installation of the sampling cores, each having a diameter of 4 cm and a length of 50 cm. All collected core samples were transported to the laboratory using a core container supplied consistently with nitrogen to prevent sediment samples from contact with air. 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 carefully transplanted 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. All columns used here are 100 cm in length, thus allowing the addition of collected water from the sampling site (or Nagara River for several cases) to reach a level of 60 cm thickness for the overlying water column. Additional information on sampling and sediment slicing is also available (Li et al., 2004; Tsumori et al., 2004). Nagara River water was also used because its quality resembled the quality of the Ushikubiri River, the sole surface inflow to the pre-reservoir under normal weather conditions.

(4) Experiments

Batch experiments were performed to investigate the behavior of E2 and its byproduct of E1 when E2 was spiked once into the overlying water phase of all columns. After spiking, a gentle stirring was followed in order to achieve the same initial E2 concentration in the vertical direction of the overlying water using a special self-made mixer that could prevent suspension of surface sediment from occurring. The schematic diagram of the continuous flow experimental setup is displayed in **Fig. 2.** Experimental runs, where the influent rate and the effluent rate were all given as zero, corresponded to batch experimental runs. Columns, referred as OC-A and OC-B in this figure, received sediment cores from St. A and St. B, respectively, and the overlying water within both columns was saturated with oxygen as a result of consistent supply of wetted air via air diffusers lowed to a level close to the sediment-water interface. For columns AC-A and AC-B, which corresponded also to sediment cores from St. A and St. B, respectively, since wetted nitrogen was continuously supplied to the overlying water through diffusers lowered to a level close to the sediment-water interface; the penetration of air to these two columns was fully prevented. These two columns were thus referred as anaerobic ones.

In regard of the continuous flow experiments, the first set of experimental runs were carried out by varying the influent E2 concentrations at 5, 15 and 50 μg/L, respectively, to examine the likely effects of influent E2 load on the effluent E2 and its byproduct E1 levels. For this set of runs, the hydraulic retention time (HRT) was controlled identical as HRT=24 hours. With this HRT, the influent E2 was nearly all disappeared, with the effluent E2 and E1 showing insignificant changes with influent E2 levels for all four aerobic and

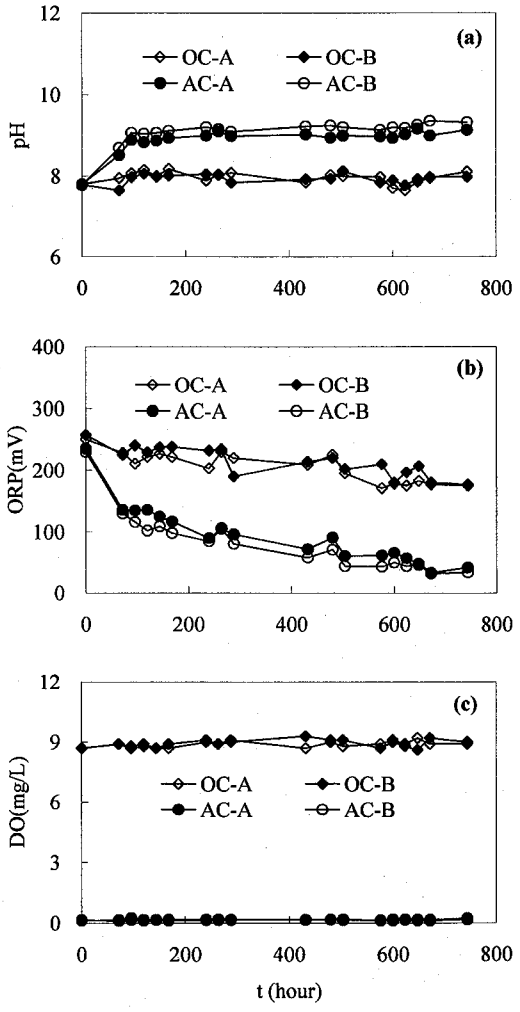

Fig 3. Time profiles of pH, ORP and DO in the overlying water of the aerobic and anaerobic sedimented mud cores.

anaerobic columns. In the following set of experimental runs, the flow rates of water into each column were changed sequentially to obtain HRT at 24, 8, 3, and 2 hours, respectively. By doing so, the likely effects of the retention time upon the levels of E2 and E1 in each column effluent were evaluated. The influent E2 for this set of experimental runs was controlled identical as 50 μg/L. The addition of E2 was made by adding weighted volume of E2's stock solution into the influent water tank.

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 filtered through a pre-washed 0.45 μm PTFE membrane filter and the obtained filtrate was subjected to quality analysis. The experiments were performed within a temperature-controlled laboratory at 20°C. Monitoring of pH, dissolved oxygen (DO), and the oxidative and reductive potential (ORP) in the overlying water phase of all columns was also performed. As examples, a part of the time profiles of pH, DO and ORP observed in the overlying water phase of all four columns during a set of batch experimental runs are displayed in **Fig. 3**.

(5) Analysis

E2 and E1 were analyzed using an Agilent 1100 series LC/MSD system (HP1100MSD). For every commencing of the analysis, calibration was performed using 10 and 50 μg/L of E2 and E1 standard solutions with a methanol/water ratio of 20/80 in v/v. To minimize measurement errors, two internal standards, namely 17β-estradiol-C_4 and estrone-C_4 were added to all samples and quantifications were thus made following the well-used internal standard methodology. Besides E2 and E1, total dissolved organic carbon (DOC) in the effluent was also analyzed using the TOC analyzer (TOC-Vws, Shimadzu Co., Japan).

3. RESULTS AND DISCUSSION

(1) Behaviors of E2 and E1 in batch experiments

The concentration profiles of E2 and its byproduct E1 under the aerobic and anaerobic conditions are illustrated in **Fig. 4**. Under the aerobic condition (**Fig. 4a and 4b**), indicating rapid decreases at the initial period, E2 dropped to 3.67 and 3.27 μg/L after being spiked for 14 hours in OC-A and OC-B column, respectively. This represented reductions of E2 by about 75% and 78 %, respectively.

Over the whole running time of this set of experimental runs, even if the levels of E2 and E1 differed with both aerobic columns (OC-A and OC-B), the extent was less significant. This was probably a result reflecting the fact that the sampling sites for the sediment cores used in these two columns were not too far from each other. The

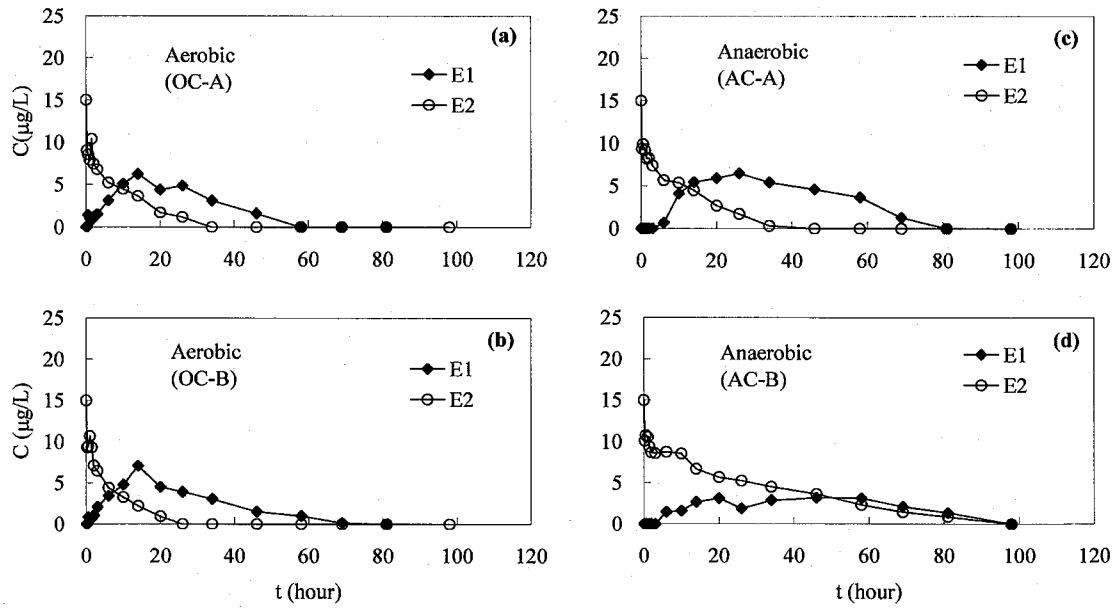

Fig. 4. Concentration profiles of E2 and its byproduct E1 from batch sedimented mud core experiments under aerobic and anaerobic conditions spiked with 15 μg/L of E2.

similarity in the physicochemical features of sediments at St. A and St. B, and the similarity in the overall microbe composition relating to both densities and species were possible reasons behind (Li et al., 2006). Following the concentration decreases of E2, its intermediate byproduct E1 emerged and apparent disappearance of E1 was observed to occur after E2 was nearly fully eliminated from the columns.

Compared to E2, the degradation of E1 took place in a manner slower. Estimation by assuming a first-order reaction for both E2 and E1 led to the generation of the disappearance rates of E2 in both OC-A and OC-B as 0.078 and 0.116 h^{-1}, and that of E1 in OC-A and OC-B as 0.032 and 0.037 h^{-1}, respectively. In comparison with the disappearance rates of E1, the degradation rates of E2 were two to three folds larger. The results obtained above are significant, by taking into account the fact that experiments using cores installed with undisturbed sediments from dam reservoirs 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. (1999b) 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 disappearance pathway of E2 under aerobic conditions seemed to be similar.

Under anaerobic conditions, as shown in **Fig. 4c and 4d**, the concentrations of E2 after 14 hours dropped to 4.49 and 6.71 μg/L, which represented reductions of E2 by about 70 and 62 % in relation

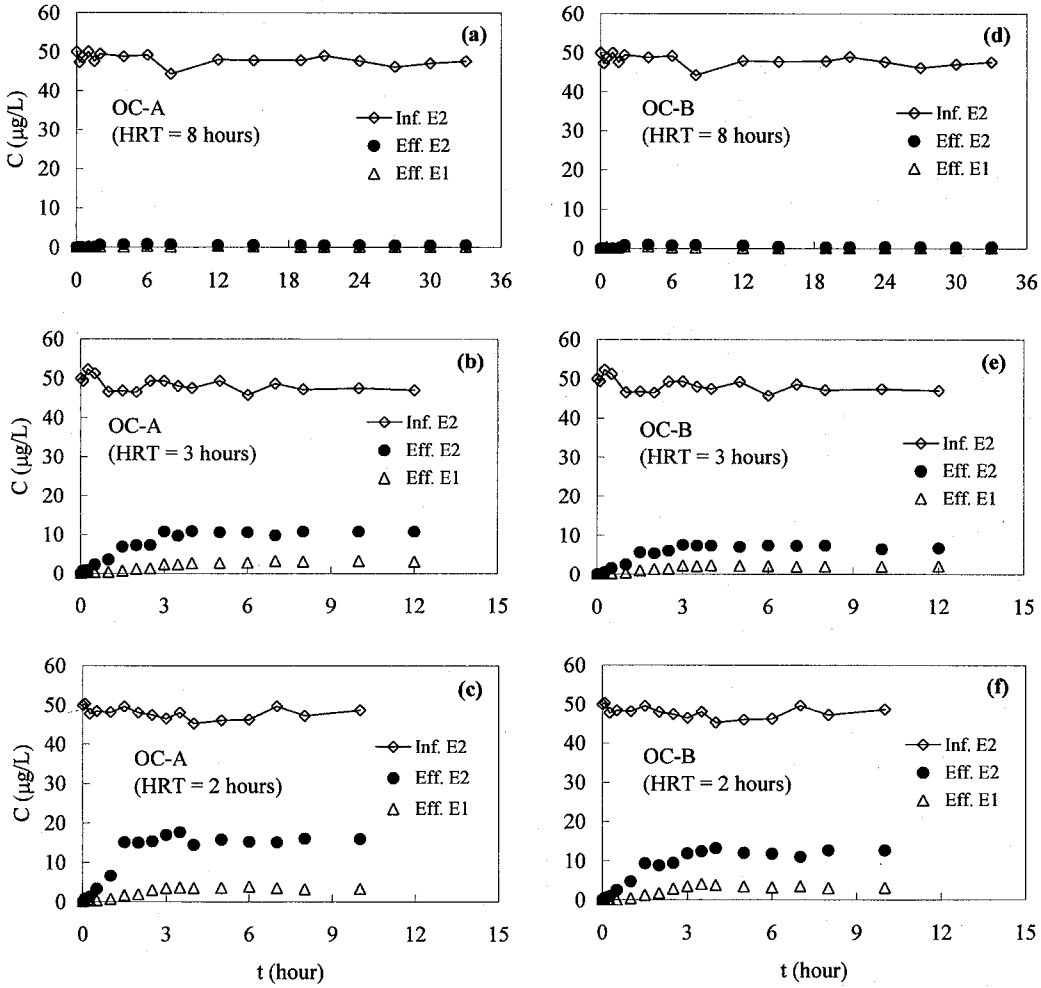

Fig 5. Concentration profiles of E2 and its byproduct E1 from continuous flow sedimented mud core experiments performed for different HRT under aerobic condition.

to the anaerobic columns AC-A and AC-B, respectively. Besides, for both these columns, it was important to see that the organic species E1, which is generally considered as an oxidized product of E2 under aerobic conditions, was also produced within the anaerobic sediment/water system where free oxygen was inexistent. The apparent first-order reaction constants estimated for E2 in AC-A and AC-B were 0.0631 and 0.0231; and those for E1 were 0.0174 and 0.0128 hr^{-1}, respectively.

From the results of batch experiments, it is clear that E2 and its byproduct E1 could be eliminated from the overlying water in contact with sedimented mud; and is also inferable that accumulation or storage of E1 in closed water sources is more likely than E2.

(2) Behaviors of E2 and E1 in continuous flow experiments

The concentration profiles of E2 and its byproduct E1 in continuous flow sedimented mud cores operated under aerobic condition (referenced to the columns OC-A and OC-B) with different HRT are displayed in **Fig. 5**. Similarly, the concentration profiles of E2 and E1 under anaerobic conditions (referenced to columns AC-A and AC-B) are displayed in **Fig. 6**. The influent E2 loading to all columns was controlled to a level about 50 μg/L.

As shown in **Fig. 5**, for the experimental runs related to the HRT of 3 and 2 hours, the effluent E2 increased with time over the initial running period, with the period lengths being found to be equivalent to the designated HRT values. After the initial

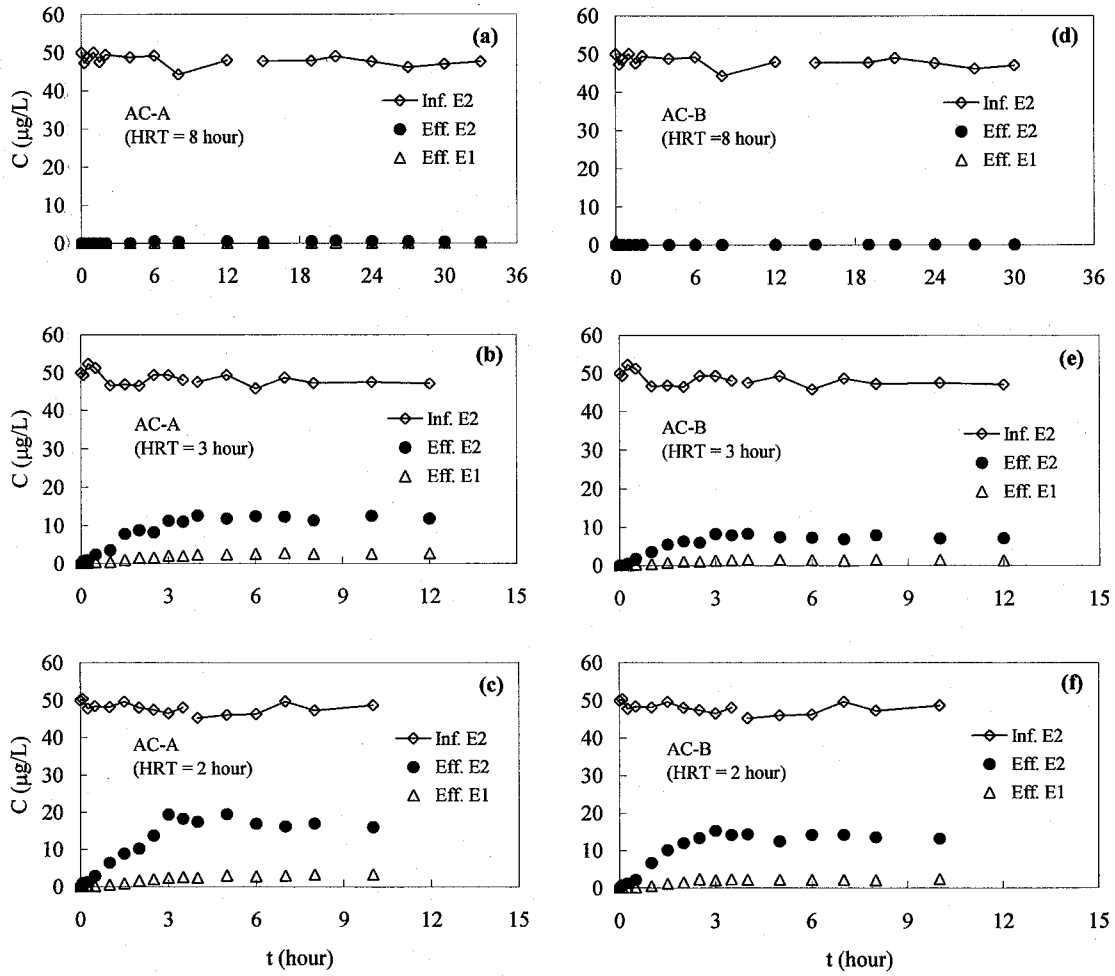

Fig. 6. Concentration profiles of E2 and its byproduct E1 from continuous flow sedimented mud core experiments performed for different HRT under anaerobic condition.

period, apparent changes in the effluent concentration of E2 were not appeared, indicating that the biological reaction had reached the steady state, which could be expected from completely mixed continuous flow reactors. Besides, in reflectance of the retention time effect, for both aerobic columns investigated the effluent E2 differed with HRT. The average E2 concentrations after reaching the steady state were 0.56, 10.63 and 15.40 μg/L for OC-A, and 0.47, 7.02 and 12.02 μg/L for OC-B relating to the HRT of 8, 3 and 2 hours, respectively. The results indicated that the longer HRT caused the lower the effluent E2.

Under anaerobic conditions the concentration profiles of E2 with both AC-A and AC-B showed effluent trends in close similarity with those mentioned above for aerobic columns. This was obvious by comparing the results shown in **Fig. 6** with those in **Fig. 5**. In the column of AC-A, the effluent E2 reached 19.5 and 11.25 μg/L with the HRT of 2 and 3 hours, respectively. Extending of the retention time from 2 to 3 hours caused significant decreases in the effluent E2. Similar results were also revealed for in the column of AC-B, as shown from the computed average effluent E2 values summarized in **Table 1**. From this table, a trend of slightly higher effluent E2 in anaerobic columns than aerobic ones was also revealed, which was supported by the batch sedimented core experimental results shown earlier in **Fig. 4**.

To further assess the behavior of influent E2 within the continuous flow sedimented mud columns, the residual E2 percentages computed based on the results shown in **Table 1** were plotted against the applied HRT values. As shown in **Fig. 7**, a general trend of decreases in the residual percentage of E2 with increases in the HRT was confirmed existent. The residual percentages of E2 for two aerobic columns fell in the range of 32.2-0.9 % over the investigated HRT range of 2-24 hours and those for anaerobic columns in the range

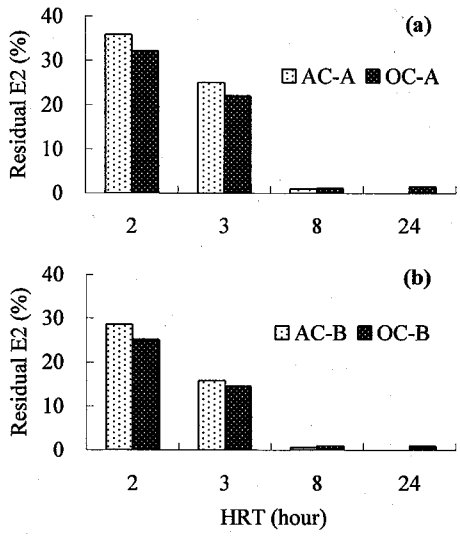

Fig. 7. Comparisons of the residuals of E2 with different HRT in continuous flow sedimented mud cores operated under aerobic and anaerobic

Table 1

Influent and effluent concentrations with continuous experiments

Source of influent	Condition	Column	HRT	Influent*		Effluent**	
				E2	E1	E2	E1
			hour	μg/L			
Miharu Dam	Aerobic	OC-A	24	51.99±0.82	0.36±0.10	0.76±0.07	3.36±0.99
		OC-B				0.47±0.06	0.72±0.08
	Anaerobic	AC-A				Not detected	
		AC-B				Not detected	
Nagara River	Aerobic	OC-A	8	47.90±1.37	0.51±0.23	0.56±0.04	0.25±0.04
		OC-B				0.47±0.07	0.72±0.08
	Anaerobic	AC-A				0.50±0.10	0.21±0.01
		AC-B				0.32±0.04	0.11±0.01
	Aerobic	OC-A	3	48.31±1.78	0.65±0.25	10.63±0.36	2.97±0.22
		OC-B				7.04±0.38	2.10±0.07
	Anaerobic	AC-A				12.11±0.47	2.60±0.15
		AC-B				7.63±0.51	1.48±0.09
	Aerobic	OC-A	2	47.85±1.42	0.62±0.17	15.40±0.62	3.48±0.22
		OC-B				12.02±0.71	3.25±0.20
	Anaerobic	AC-A				17.19±1.25	2.97±0.33
		AC-B				13.65±0.73	2.22±0.12

* the measured values are given in the form of the mean value±the standard deviation over the whole running period

** the measured values are given in the form of the mean value±the standard deviation at steady state (7 data)

of 35.90 to 0 %. Further experimental runs are necessary in order to generate relation curves that could used for estimation of the fate of E2 under any given HRT and influent E2 levels.

4. CONCLUSIONS

Batch and continuous flow experiments using sedimented mud cores were performed to investigate the behavior of E2 and its byproduct E1 under well controlled aerobic and anaerobic conditions. The results obtained indicated that compared to E2 spiked into the overlying water of anaerobic columns, that spiked into the aerobic columns disappeared relatively faster. In both aerobic and anaerobic columns, the formation and followed degradation of E1 were confirmed even if the apparent maximum E1 concentration levels emerged in all columns tended to be lower when operated in the continuous flow mode than the batch mode. The effluent E2 concentration from all columns operated in the continuous flow mode increased with time over the initial running period and then reached respective steady state after running for the time lengths equivalent to the designated HRT values. The effluent E2 concentration at the steady state showed a decreasing trend as the HRT was extended.

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