

# Conditions of dissolved oxygen concentration for effective removal of natural and estrogens in wastewater treatment process

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**Abstract** Pilot plant simulating the activated sludge processes was operated to study relationship between conditions of dissolved oxygen (DO) concentration and estrogens removal efficiencies. Results from DO controlled batch experiments, showed that decrease of estrone (E1) under aerobic condition depended on the DO level. And it was found that 17 $\beta$ -estradiol (E2) and E1 increased under anoxic or anaerobic conditions where easily degradable organic matters had been already removed. From the results, it is advisable that DO concentrations in the latter part of the aeration tank and secondary sedimentation tank should be kept high for effective estrogen removal in the activated sludge process.

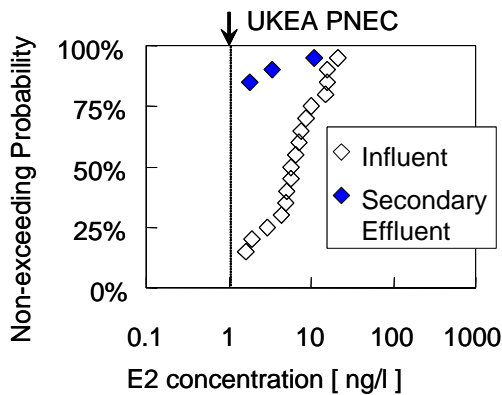
**Keywords** 17 $\beta$ -estradiol, estrone, activated sludge process, dissolved oxygen concentration

## INTRODUCTION

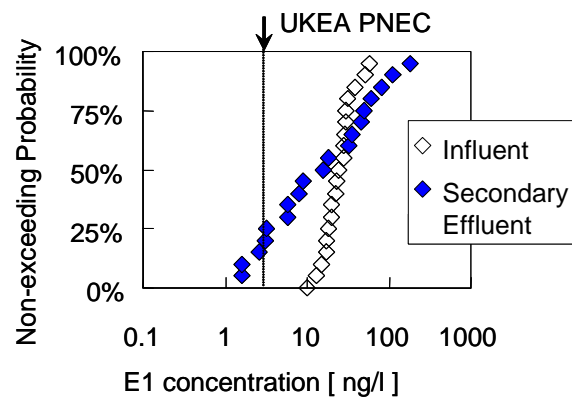
During the past ten years feminization of male fish has been detected in several European countries, USA and Japan. These surveys have shown a high incidence of sexual disruption in populations of wild fish living downstream of wastewater treatment works, and the degree of intersex (gonads containing both male and female structural characteristics) has been correlated with the concentration of effluent in those rivers. The theory of estrogenic compounds in wastewater treatment plant (WWTP) effluent as the cause of the feminizing effects, which have been found among male fish, has also been supported by a number of studies using cell culture assays designed to detect estrogenic activity. These have demonstrated the estrogenic activity of WWTP effluent, and also have quantified the estrogenic potential in relation to the potency of 17 $\beta$ -estradiol. Further, chemical analysis of the composition of WWTP effluent and determinations of concentrations of estrogens and estrogenic compounds in the effluent have in a large number of cases demonstrated the natural estrogens (17 $\beta$ -estradiol (E2) and estrone (E1)) and the synthetic estrogen (17 $\alpha$ -ethinylestradiol (EE2)) used in contraceptives) as likely candidates for the observed disturbances in fish species in the sewage effluent receiving rivers. In 2002, a report produced under the UK Environment Agency proposes Predicted – No – Effect – Concentrations (PNECs) for steroid estrogens with the aim of protecting fresh and saltwater life, removal of estrogens is a subject of current concern in wastewater treatment engineering.

In Japan, a study by Komori *et al.*, (2004) revealed the occurrence and the behavior of E2, E1 and EE2 in 20 WWTPs adopting an activated sludge process. Results from this study showed that E2 concentration in the effluent (Figure 1) was eliminated during the process, E1 concentration in the effluent (Figure 2) ranged from n.d. to 180 ng/l, and EE2 was not detected in any effluent. Additionally, results (Figure 3) from a pilot plant study by Suzuki *et al.*, (2007) indicate that condition of dissolved oxygen concentration in the latter part of aeration tank has a great effect on E1 removal efficiency during wastewater treatment process, but limited research data (MLIT 2001; Nasu *et al.*, 2001; Tanaka *et al.*, 2001) are available regarding operational conditions for effective removal of estrogens.

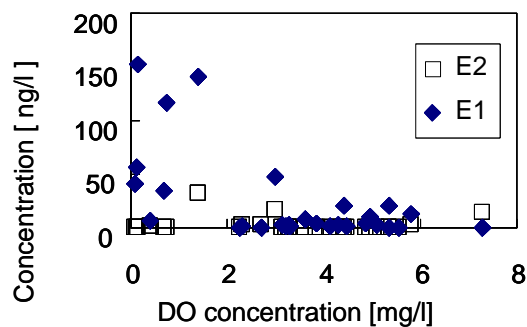
Then, the purpose of this study is to find out proper operational conditions which can constantly remove E2 and E1 during the conventional activated sludge process, which are used mostly in municipal wastewater treatment plants in Japan. In this study we focused on relationship between the condition of dissolved oxygen concentration in aeration tank and the estrogen (E2, E1 and EE2) removal efficiency using the pilot plant and batch experiments.



**Figure 1** Distribution of 17 $\beta$ -estradiol (E2) surveyed in the 20 WWTPs in Japan



**Figure 2** Distribution of estrone (E1) surveyed in the 20 WWTPs in Japan



**Figure 3** Relationship between dissolved oxygen (DO) concentration in the latter part of aeration tank and dissolved 17 $\beta$ -estradiol (E2) and estrone (E1) concentrations in the secondary effluent

## MATERIALS AND METHODS

### *LC/MS/MS Method for natural estrogens and synthetic estrogen in Wastewater*

In this study, the analytical method by Komori *et al.*, (2004) was used for the analysis of natural estrogens; E2 and E1, and the synthetic estrogen, EE2. Sample preparation of the method consists of solid-phase extraction with an Oasis HLB cartridge and cleaning with Sep-Pak Plus Florisil and Sep-Pak Plus NH<sub>2</sub>. The prepared sample was analyzed using a LC/MS/MS. Analytical conditions for the LC/MS/MS were the same as reported by Komori *et al.*, (2004).

An effluent sample was filtered through a glass fiber filter (GF/B Whatman) at the pilot plant site. A mixed liquor sample was settled for about 20 minutes and separated into the supernatant liquid and the sludge, and the supernatant liquid was taken and filtered as well as the effluent sample. One gram of *L*-ascorbic acid was added to 1 liter of sample for estrogens in order to prevent oxidation of estrogens. All filtrate samples were collected in one-liter glass bottles, refrigerated, and transported to the laboratory within one day. And sample preparation was conducted at the laboratory within three days. In this study, the samples were not analyzed for estrogens adsorbed on the suspended matter, while the samples were analyzed for dissolved organic carbon (DOC), ammonium (NH<sub>4</sub><sup>+</sup>-N), nitrite (NO<sub>2</sub><sup>-</sup>-N) and nitrate (NO<sub>3</sub><sup>-</sup>-N).

### *Batch experiments simulating behavior of estrogens in an aeration tank*

Three parallel aerobic batch experiments were conducted in order to simulate the transformation of E2, E1 and EE2 in an aeration tank in a WWTP. Three 150-liter stainless steel cylinders equipped with a stirrer were used for the experiment. At the beginning of the experiment, 55 liters of primary effluent and 25 liters of return sludge were added into the each cylinder and stirred. The primary effluent and the return sludge were obtained from a pilot plant in Kohoku WWTP in Ibaraki Prefecture in Japan, adopting a conventional activated sludge process. The aeration tank of the pilot plant was divided into 4 complete mixing cells (AT-1, AT-2, AT-3 and AT-4) in series for modeling plug flow. The solid retention time (SRT) and the hydraulic retention time of the

pilot plant were 10 days and 8 hours, respectively, and almost complete nitrification was achieved in the aeration tank. In one cylinder, the air was not supplied into the mixed liquor. In other two cylinders, the air was supplied into the mixed liquor through porous stones by a oil-free compressor, and mixed liquor dissolved oxygen (MLDO) concentrations were controlled below 0.5, 3.0 mg/l using a DO controller, respectively. In each system, initial and final mixed liquor were analyzed for MLSS and MLVSS. Further, the mixed liquors were taken every 2 hours and the supernatant filtrates were analyzed for E2, E1, EE2, DOC,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and  $\text{NO}_3^-\text{-N}$ . The experiment was conducted in August. Water temperature during the experiment period ranged from 27 to 29 degrees centigrade. No rainfall was observed for previous 3 days.

#### ***Batch experiments simulating behaviors of estrogens in a final sedimentation tank and in return sludge***

In the activated sludge process, after the sewage has received biological treatment with activated sludge, mixed liquor is transferred to a secondary sedimentation tank and is separated into the supernatant liquid and the sludge. DO concentration decreases in the secondary sedimentation tank because the air is not supplied while DO is consumed by organisms living in activated sludge.

Anoxic and anaerobic batch experiments were conducted in order to simulate the transformation of E2, E1 and EE2 in a secondary sedimentation tank using activated sludge. A 150-liter stainless steel cylinder equipped with a stirrer was used for the experiment. At the beginning of the experiment, 80 liters of mixed liquor from an aeration tank was added to the cylinder and stirred without aeration. The mixed liquor was obtained from the pilot plant in Kohoku WWTP previously mentioned. Initial and final mixed liquor were analyzed for MLSS and MLVSS. Further, the mixed liquors were taken every 1.5 hours and the supernatant filtrates were analyzed for E2, E1, EE2, DOC,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and  $\text{NO}_3^-\text{-N}$ . The anoxic experiment was conducted in November when almost complete nitrification was achieved in the aeration tank of the pilot plant. Water temperature during the experiment period ranged from 19 to 22 degrees centigrade. No rainfall was observed for previous 3 days. The anaerobic experiment was conducted in February when nitrification was not observed in the aeration tank of the pilot plant. Water temperature during the experiment period ranged from 12 to 14 degrees centigrade. No rainfall was observed for previous 5 days.

#### ***Pilot plant experiments with an anaerobic-oxic process or an aerobic-anoxic process***

To examine estrogen removal efficiencies under anaerobic or anoxic conditions, the pilot plant in Kohoku was operated with an anaerobic-oxic process or an aerobic-anoxic process, and behavior and removal efficiencies of E2, E1 and EE2 in the pilot plant were observed. The anaerobic-oxic operation was conducted for three weeks in summer season, and water temperature during the experiment period ranged from 25 to 27 degrees centigrade. The aerobic-anoxic operation was conducted for three months from winter to spring season, and water temperature during the experiment period ranged from 16 to 19 degrees centigrade.

## **RESULTS AND DISCUSSION**

### **A batch experiment simulating behavior of estrogens in an aeration tank**

#### ***Anaerobic System***

Figure 4 shows the conditions of pH and DO concentration, and Figure 5 shows the change of DOC,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$  and  $\text{NO}_3^-\text{-N}$  during the experiment. E2 and E1 were detected at the beginning of the experiment, and then, E2 concentrations decreased to less than the detection limits (2.2ng/l) within 2 hours, while E1 concentrations did not decrease but increased gradually (Figure 6).

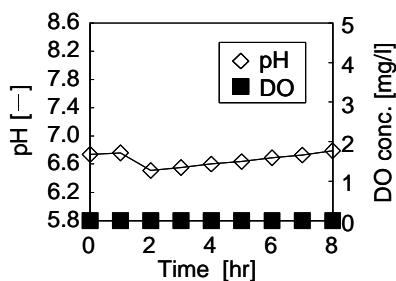
#### ***Aerobic (MLDO<0.5mg/l) System***

Figure 7 shows the conditions of pH and DO concentration. In this system, though the air compressor was fully loaded, very low DO was observed for 1 hour because the supplied oxygen was rapidly consumed. DO concentration became higher after 1 hour aeration and reached 0.5mg/l

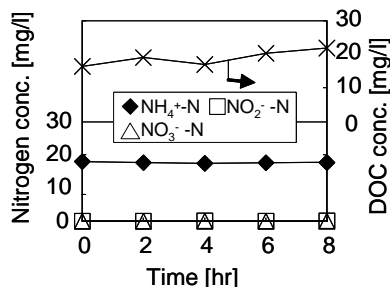
after 6 hours. Since then, DO concentrations were controlled so as to be about 0.5mg/l. Figure 8 shows that though DO concentrations were below 0.5 mg/l, nitrification proceeded successfully. E2 concentrations, as shown in Figure 9, decreased to less than the detection limits (2.2ng/l) within 2 hours under the semi anaerobic condition. On the other hand, E1 concentrations were almost constant over the experiment period.

### Aerobic (MLDO<3.0mg/l) System

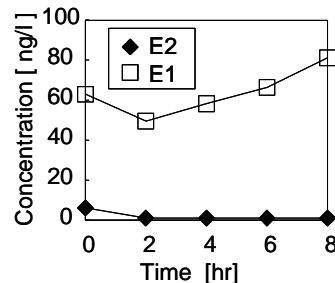
Figure 10 shows the conditions of pH and DO concentration. The same tendency as Figure 7 of DO concentration was observed until 6 hours, then the DO concentration suddenly jumped to 3mg/l at 7 hour aeration, and after that, DO concentrations were controlled so as to be about 3.0mg/l. Figure 11 shows that nitrification proceeded more rapidly than the aerobic (MLDO<0.5mg/l) system. E2 concentrations, as shown in Figure 12, decreased to less than the detection limits (2.2ng/l) within 4 hours under the semi anaerobic condition. On the other hand, E1 concentrations were almost constant until 6 hours, and then they decreased drastically as DO concentrations became higher. EE2 was not detected in any samples.



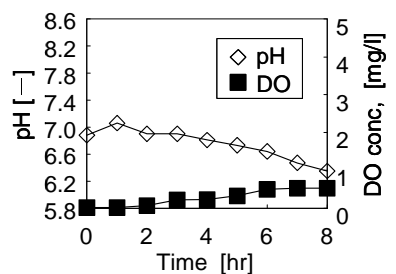
**Figure 4** Variation of pH and dissolved oxygen (DO) concentration in the anaerobic system



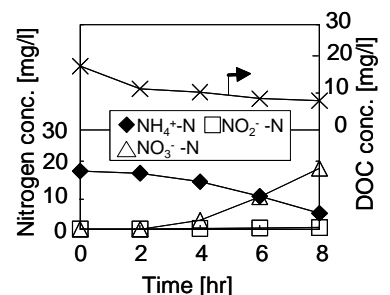
**Figure 5** Variation of dissolved organic carbon (DOC), ammonium, nitrite and nitrate in the anaerobic system



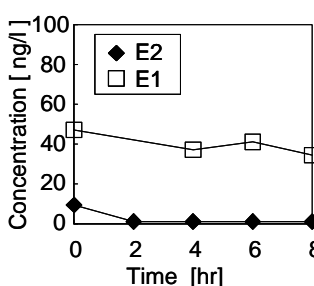
**Figure 6** Variation of dissolved 17β-estradiol (E2) and estrone (E1) in the anaerobic system



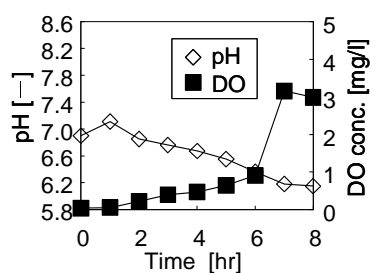
**Figure 7** Variation of pH and dissolved oxygen (DO) concentration in the aerobic (MLDO<0.5mg/l) system



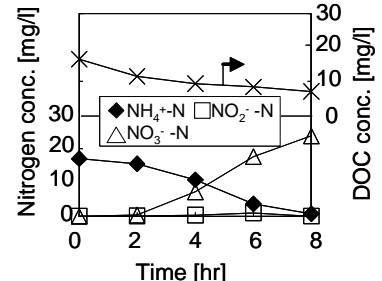
**Figure 8** Variation of dissolved organic carbon (DOC), ammonium, nitrite and nitrate in the aerobic (MLDO<0.5mg/l) system



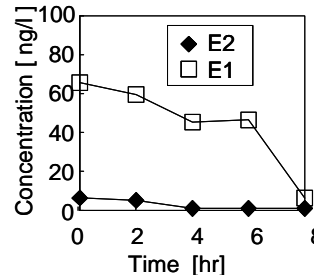
**Figure 9** Variation of dissolved 17β-estradiol (E2) and estrone (E1) in the aerobic (MLDO<0.5mg/l) system



**Figure 10** Variation of pH and dissolved oxygen (DO) concentration in the aerobic (MLDO<3.0mg/l) system



**Figure 11** Variation of dissolved organic carbon (DOC), ammonium, nitrite and nitrate in the aerobic (MLDO<3.0mg/l) system



**Figure 12** Variation of dissolved 17β-estradiol (E2) and estrone (E1) in the aerobic (MLDO<3.0mg/l) system

In summary, E2 concentrations decreased in an aeration tank regardless of the DO concentrations within short time, on the other hand, E1 concentrations decreased if DO concentrations were sufficiently high, while E1 concentrations were almost constant or increased slightly under anaerobic and semi-anaerobic conditions regardless of nitrification proceed.

### **Batch experiments simulating behavior of estrogens in a final sedimentation tank or in return sludge**

#### ***Anoxic batch experiment using mixed liquor***

Table 1 and Figure 13 show the pilot plant monitoring data on the day of the anoxic batch experiment. Grab samples were collected at the pilot plant sites for water quality analysis. Complete nitrification proceeded in the aeration tank, and nitrate amounted to almost all dissolved nitrogen in the excess mixed liquor. SS and  $\text{NH}_4^+$ -N concentrations in secondary effluent were slightly high since the secondary effluent was contaminated by scum. E2 and E1 were detected in AT-1, AT-2 and AT-3, but not detected in AT-4.

Figure 14 shows the change of E2 and E1 in the anoxic batch experiment. E2 was not detected a half hour after the beginning of the experiment, but E2 concentration increased steadily after 2 hours, and finally reached 246 ng/l after 6.5 hours operation. E1 was always detected over the experiment, and E1 concentration went on increasing, finally reaching 5572ng/l. E1 concentration of high value may not be so correct because E1 level exceeded the normal range of the calibration curve and E1 concentration was calculated by extrapolation method. EE2 is not detected in any sample, and  $\text{NO}_3^-$ -N concentration decreased and endogenous de-nitrification was observed. DO was not detected in any time.

#### ***Aerobic batch experiment using excess mixed liquor***

Table 2 and Figure 15 show the pilot plant monitoring data on the day of the anaerobic batch experiment. Nitrification did not proceed in the aeration tank, and nitrite and nitrate were scarcely produced.

Figure 16 shows the change of E2 and E1 in the anaerobic batch experiment. E2 was always detected over the experiment, and reached the peak (166 ng/l) after 3 hours. E1 was also always detected over the experiment, and reached 609ng/l after 3 hours.

### **Pilot plant experiments with an anaerobic-oxic process or an aerobic-anoxic process**

#### ***Anaerobic-oxic process***

Aeration in AT-1 was reduced to the minimum necessary to keep activated sludge mixed. Average MLSS and MLVSS concentration were 1303 and 1073 mg/l, respectively, during the experiment. Figure 17 shows the distribution of concentrations of  $\text{NH}_4^+$ -N,  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N in the aeration tank, and nitrification was not observed in the aeration tank. E1 concentrations (Figure 19) tended to be high under anaerobic condition (AT-1) and decrease under aerobic condition (AT-3 and AT-4). E2 was not detected in any sample.

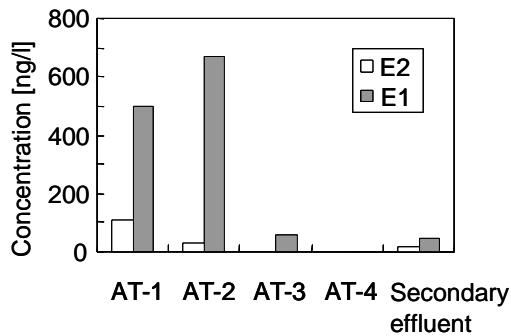
#### ***Aerobic-anoxic process***

In this study, aeration in AT-4 was reduced to the minimum necessary to keep activated sludge mixed. Average MLSS and MLVSS concentration were 1575 and 1230 mg/l, respectively during the experiment. Figure 20 shows the distribution of concentrations of  $\text{NH}_4^+$ -N,  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N in the aeration tank. Almost complete nitrification proceeded in the aeration tank, and decrease of nitrate, endogenous de-nitrification, was observed in AT-4. E1 concentrations (Figure 22) tended to decrease under aerobic condition (AT-1, AT-2 and AT-3) and increase under anoxic condition (AT-4 and secondary sedimentation tank). E2 was not detected in any sample.

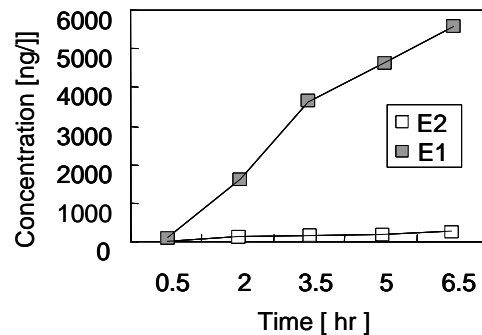
Table 3 summarizes these results from batch experiments and pilot plant studies. These results indicate that decrease of E1 concentration in aeration tank depends on DO concentration and E1 concentration increase under anaerobic or anoxic conditions even where easily degradable organic matters are already removed.

**Table 1** Pilot plant monitoring data on the day of the anoxic batch experiment

	pH	MLDO mg/l	SS mg/l	VSS mg/l	DOC mg/l	NH <sub>4</sub> <sup>+</sup> -N mgN/l	NO <sub>2</sub> <sup>-</sup> -N mgN/l	NO <sub>3</sub> <sup>-</sup> -N mgN/l
Influent	-	-	243	207	44.6	28.53	n.d.	0.00
Primary Effluent	6.72	-	157	132	41.1	26.74	n.d.	0.00
Aeration Tank								
AT-1	6.68	0.00	-	-	7.9	11.32	n.d.	0.06
AT-2	6.41	0.10	-	-	7.0	5.38	0.15	3.75
AT-3	6.26	0.26	-	-	6.7	n.d.	n.d.	8.63
AT-4	6.00	3.00	1740	1480	9.3	n.d.	n.d.	9.57
Secondary Effluent	6.07	0.00	25	21	9.6	3.14	n.d.	8.73
Excess Sludge	-	-	4280	3560	-	-	-	-



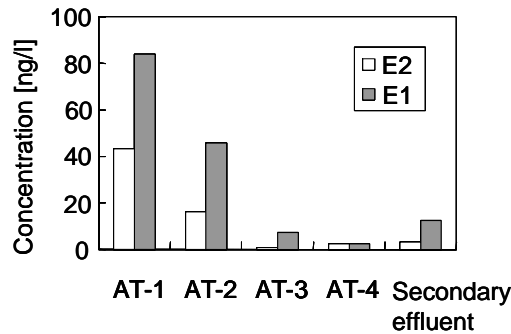
**Figure 13** Distribution of dissolved 17β-estradiol (E2) and estrone (E1) in the pilot plant



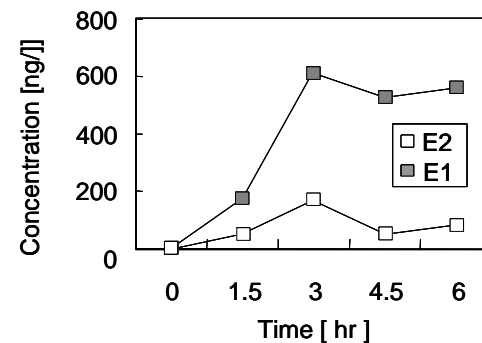
**Figure 14** Variation of dissolved 17β-estradiol (E2) and estrone (E1) in the anoxic batch experiment

**Table 2** Pilot plant monitoring data on the day of the anaerobic batch experiment

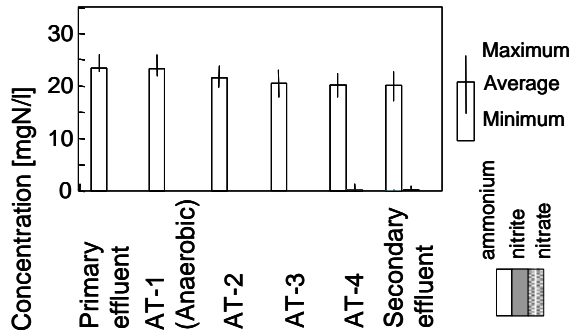
	pH	MLDO mg/l	SS mg/l	VSS mg/l	DOC mg/l	NH <sub>4</sub> <sup>+</sup> -N mgN/l	NO <sub>2</sub> <sup>-</sup> -N mgN/l	NO <sub>3</sub> <sup>-</sup> -N mgN/l	D-PO <sub>4</sub> <sup>3+</sup> -P mgP/l
Influent	-	-	150	133	48.2	24.82	n.d.	n.d.	1.89
Primary Effluent	6.72	-	133	115	43.8	25.92	n.d.	n.d.	1.98
Aeration Tank									
AT-1	6.62	0.14	-	-	17.7	26.11	n.d.	n.d.	6.24
AT-2	6.63	0.23	-	-	16.7	23.83	n.d.	n.d.	0.46
AT-3	6.61	2.40	-	-	15.5	22.97	n.d.	n.d.	0.14
AT-4	6.67	5.80	1080	940	14.4	22.24	n.d.	n.d.	0.29
Secondary Effluent	6.52	0.00	2	1	17.0	22.91	n.d.	0.02	0.01
Excess Sludge	-	-	3060	2660	-	-	-	-	-



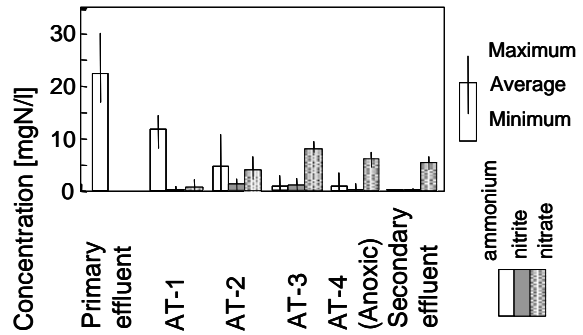
**Figure 15** Distribution of dissolved 17β-estradiol (E2) and estrone (E1) in the pilot plant



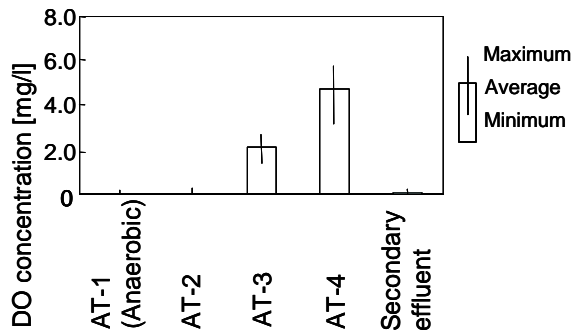
**Figure 16** Variation of dissolved 17β-estradiol (E2) and estrone (E1) in the anaerobic batch experiment



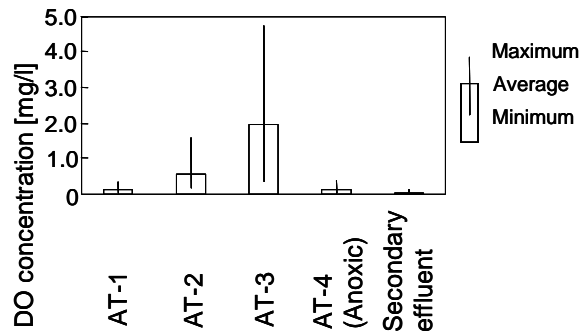
**Figure 17** Distribution of ammonium, nitrite and nitrate in the anaerobic-oxic process



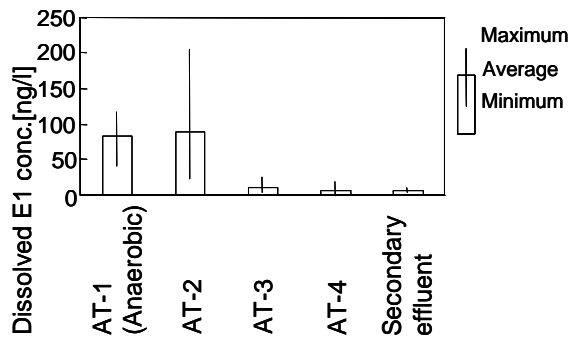
**Figure 20** Distribution of ammonium, nitrite and nitrate in the aerobic-anoxic process



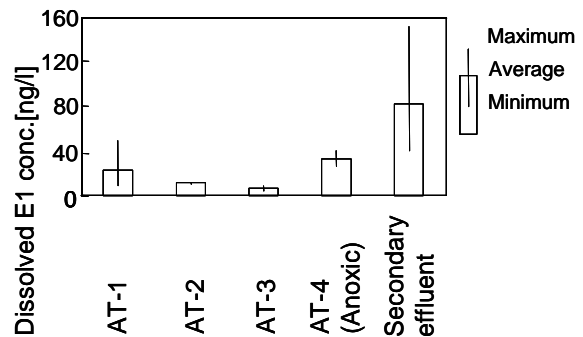
**Figure 18** Distribution of dissolved oxygen (DO) concentration in the anaerobic-oxic process



**Figure 21** Distribution of dissolved oxygen (DO) concentration in the aerobic-anoxic process



**Figure 19** Distribution of dissolved estrone (E1) in the anaerobic-oxic process



**Figure 22** Distribution of dissolved estrone (E1) in the aerobic-anoxic process

**Table 3** Characteristics of transformation of dissolved 17 $\beta$ -estradiol (E2), estrone (E1) under various conditions in activated sludge processes

			Dissolved oxygen concentration		
			no	low	high
Easily degradable organic matters	exist	E2	↘	↘	
		E1	↗	→	↘
	no	E2	↗	※	※
		E1	↑	→	↓

Flow of activated sludge in wastewater treatment process  
 increase rapidly increase gradually decrease gradually decrease rapidly not detected

## SUMMARY AND CONCLUSIONS

In this study, we investigated relationship between conditions of dissolved oxygen concentration in aeration tank and estrogen removal efficiency in the conventional activated sludge processes.

1) A batch experiment was conducted for simulating behavior of estrogens in an aeration tank. In an anaerobic system, E1 concentration increased slightly. In an aerobic system in which DO concentration was controlled below 0.5mg/l, nitrification proceeded, but E1 concentration unchanged. In an aerobic system in which DO concentration was controlled below 3.0mg/l, nitrification proceeded, and when DO concentration reached 3.0mg/l E1 concentration decreased rapidly.

2) Batch experiments were conducted under anoxic and anaerobic conditions using mixed liquor from the aeration tank for simulating behavior of estrogens in a secondary sedimentation tank or in return sludge. E2 and E1, in particular E1, concentrations increased drastically both under anoxic and anaerobic conditions.

3) A pilot plant simulating an anaerobic-oxic process or an aerobic-oxic process was operated. In the anaerobic-oxic process, E1 concentration was high under anaerobic condition and decrease under aerobic condition in the aeration tank. In the aerobic-anoxic process, E1 concentration tended to decrease under aerobic condition, but it increased again under anoxic condition in the latter part of the aeration tank.

These results suggest that condition of dissolved oxygen concentration in an aeration tank and a secondary sedimentation tank has a great effect on E1 removal efficiency in wastewater treatment processes. For the sake of effective removal of E1 in an activated sludge process, it is advisable that DO concentrations in the latter part of an aeration tank should be kept high. Moreover, these results also suggested that considerable source of estrogens (E2, E1) still remain in effluent and/or activated sludge and some fermentation reactions under anoxic and anaerobic conditions are related to estrogen transformation. Therefore, much further research is needed to understand whole behavior of estrogens including adsorbed matter and their metabolites.

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