Effect of the difference in activated sludge origin for degradation of female hormones and pharmaceuticals

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活性汚泥の性状が女性ホルモン様物質および医薬品の除去へ及ぼす影響

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本研究では下水処理で広く用いられている活性汚泥の性状が女性ホルモン様物質および医薬品の除去にどのような影響を与えるかを調べるために，異なる処理場から採取した活性汚泥を用いて室内分解実験を行った．分解速度を比較するため，女性ホルモン様物質については二相挙動モデルを適用し，医薬品については水相からのみの除去速度を考えた．得られた分解速度の統計処理を行い，各処理場の活性汚泥の微量物質分解特性を明らかにした．

1 Introduction

Estrogens are female hormones which are important for maintaining the health of the reproductive tissues, breast, skin and brain [1]. But the presence of these compounds in our environment has become a major subject of world-wide growing concern, because these compounds may interfere with the normal endocrine system [2]. Ternes reported that estrogens were detected in sewage effluent wastewater at the level of ng/L [3]. After this research, concentrations of estrogens in water environment were measured in many countries including Japan [4-7]. Laboratory experiments were also performed. The concentration of 17β-estradiol (E2) was immediately reduced, whereas the concentration of estrone (E1) increased up to 95% with regard to the initial concentration of E1 [8]. Vader et al performed a biodegradation experiment of estrogens by using activated sludge with nitrification to investigate the effect of nitrifying bacteria, and came to a conclusion that nitrifying bacteria play an important role in degrading of 17α-ethynylestradiol (EE2) which is known as a persistent compound [9].

BPA is one of the most commonly used chemicals and known endocrine as a potential endocrine disrupter. There are a few literatures in the treatment in activated sludge process. Adsorption experiments with activated sludge were performed focusing on BPA, E2 and EE2. According to this research, a high adsorption potential to sewage sludge could be observed in spite of very high initial concentrations for all substances [10].

The occurrence and fate of pharmaceutically active compounds in the aquatic environment has been recognized as one of the emerging issues in recent years [11]. Most of pharmaceuticals are expected causing physiologic activity for human body. Possible side effects for human body are recognized to some extent because of the enforcing of drug legislation. However, the effect of pharmaceuticals on aquatic organisms is not known, especially in the case of long term exposure in low concentration level [12]. Pharmaceuticals are originated from not only human activity but livestock production [13].

Though a lot of pharmaceuticals are used in Japan, few surveys were conducted in water environment. Some investigations were carried out in other countries, such as Germany and Switzerland. Removal rate of six pharmaceuticals which are the target compounds in this research was estimated in surface water. CBZ and CA were fairly persistent and phototransformation was identified as the main elimination process of DCF [14]. C.Zeiener described that applying advanced oxidation processes is effective for eliminating CA, IBP and DCF [15]. The biodegradation of these pharmaceuticals was investigated in short-term tests with a pilot sewage plant. CA and DCF were not eliminated and reached a level of approximately 95% of their initial concentration, whereas the concentration of IBP was decreased approximately 40% [16].

Female hormones and pharmaceuticals are released into water environment through sewage system. So, it is important to treat these compounds in waste treatment plant appropriately. Different activated sludges were taken to investigate the behaviors of biodegradation in this research. Degradation rate was compared with each other considering two-phase fate model and first order equation. In addition, the cluster analysis was applied to characterize of the results.

2 Experiments

2.1 Target compounds and activated sludge

Target compounds are three kinds of estrogen, 17β-estradiol (E2), Estrone (E1) and 17α-ethynylestradiol (EE2), two kinds of synthetic chemicals, Bisphenol A (BPA) and Benzophenone (BZP) and ten kinds of pharmaceuticals, Clofibric acid (CA), Gemfibrozil (GFZ), Ibuprofen (IBP), Fenoprofen (FEP), Ketoprofen (KEP), Naproxen (NPX), Diclofenac (DCF), Indomethacin (IDM), Propyphenazone (PPZ) and Carbamazepine (CBZ). E2

Table 1. The chemical and physical properties of the target compounds

S/N	Chemical Name	Abbrev.	Mol. Weight	Water Solubility [mg/L]	Henry's Law Constant [atm-m^3/mol]	LogK_{ow}	pKa
1	17β-estradiol	E2	272.39	3.60	3.64×10^{-11}	4.01	
2	Estrone	E1	270.39	30	3.80×10^{-10}	3.13	
3	17α-ethynylestradiol	EE2	296.41	11.3	7.94×10^{-12}	3.67	
4	Bisphenol A	BPA	228.29	120	1.00×10^{-11}	3.32	
5	Benzophenone	BZP	182.22	137	1.94×10^{-6}	3.18	
6	Clofibric acid	CA	214.65	583	2.19×10^{-8}	2.57	
7	Gemfibrozil	GFZ	250.34	N/A	N/A	4.77	
8	Ibuprofen	IBP	206.29	21	1.50×10^{-7}	3.97	4.91
9	Fenoprofen	FEP	242.28	N/A	N/A	3.9	7.3
10	Ketoprofen	KEP	254.29	51	2.12×10^{-11}	3.12	4.45
11	Naproxen	NPX	230.27	15.9	3.39×10^{-10}	3.18	4.15
12	Diclofenac	DCF	296.16	2.37	4.73×10^{-12}	4.51	4.15
13	Indomethacin	IDM	357.80	0.94	3.13×10^{-14}	4.27	4.50
14	Propyphenazone	PPZ	230.31	3×10^{6}	1.84×10^{-9}	1.94	
15	Carbamazepine	CBZ	236.28	17.7	1.08×10^{-10}	2.45	

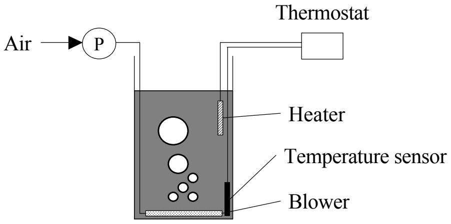

Figure 1. Experimental apparatus

Table 2. Conditions of the experiments

pH	6.7
Temperature	20°C
Initial concentration of DOC	150mg/L
Initial concentration of target compounds	100μg/L
Operation time	96h

is produced in ovary that has the strongest endocrine activity. E1 is the intermediate compounds produced in the course of biodegradation of E2 [17]. EE2 is integrant of oral-contraceptive pill. Both BPA and BZP are white solid substances. BPA is used for production of polycarbonate plastic, while BZP is used for basic gradient of drugs, ultraviolet absorber and so on. CA and GFZ are antilipemic agent, IBP, FEP, KEP, NPX, DCF, IDM and PPZ are categorized nonsteroidal antiinflammatory drug (NSAID) and CBZ is antiepileptic agent. Estrogens and synthetic chemicals are female hormones. The chemical and physical properties of the target compounds are shown in Table 1.

Activated sludge was taken from 5 wastewater treatment plants (two conventional WWTPs, solid waste disposal site, pure-oxygen aerobic WWTP, human-waste treatment plant). Activated sludge of conventional WWTP was taken from 2 plants (Shibaura and Morigasaki) and in the case of activated sludge of Shibaura WWTP, the effect of keeping it by artificial wastewater in the laboratory was investigated.

2.2 Method of biodegradation experiment and adsorption experiment by kaorinite clay

Biodegradation experiments were performed in the reactor whose volume is 2L. The target compounds and artificial wastewater were added in the reactor at t=0 where the target compounds of initial concentrations were 100μg/L and time dependent changes in the concentrations of the target compounds and DOC were measured. Artificial wastewater was composed of glucose, peptone, and other nutrient. During the experiments, pH and temperature were also adjusted where pH=6.7 and temperature=20°C. Blower was put on the bottom of the reactor to keep aerobic condition. Experimental apparatus and conditions are shown in Figure 1 and Table 2 respectively.

At the same time, biodegradation experiment under anaerobic condition and adosorption experiment by kaorinite clay for female hormones were also investigated. The blower was removed to put the reactor into anaerobic condition, but the reactor was fitted with stirrer not to accumulate the activated sludge. Both case, initial concentration of female hormones were adjusted 100μg/L. Concentration of karinite clay was 10g/L and operation time was two hours.

2.3 Analytical Method

80mL of mixed liquor was taken from the reactor and centrifugation was conducted to separate water-phase and sludge-phase that were measured separately. After filtration for water-phase and methanol extraction for sludge-phase, solid-phase extraction was carried out by using C18 disk. Then the extracts were derivatized for the detection by GC/MS. 17β-estradiol-d_4 and 2,3-dichlorophenoxyacetic acid were used as surrogate standards and chrysene-d_{12} as internal standard. These analytical methods were referred to the literature [18-20].

3 Modeling and statistical analysis

3.1 Two-phase fate model

Two-phase fate model was suggested to evaluate the adsorption-biodegradation procedure in the reactor quantitatively [21]. Equation (1) describes the behavior in water-phase and equation (2) describes the behavior in sludge-phase. A.I. Schäfer reported that amount of trace compounds adsorbed to activated sludge is independent of its concentration and the amount is proportional to the MLSS [22]. For these reasons, it is hypothecated that adsorption phenomenon follow liner isotherm. Biodegradation in only water phase was not occurred in the preparatory experiment. So the degradation is thought to occur after adsorption.

$$\frac{d(\beta C_W)}{dt} = -k_b(k_p C_W - C_S)X \tag{1}$$

$$\frac{d(C_S X)}{dt} = k_b(k_p C_W - C_S)X - k_1 C_S X \tag{2}$$

where C_W is concentration in water phase [µg/L], C_S is concentration in activated sludge phase [µg/gMLSS], X is mixed liquor suspended solid (MLSS) [gMLSS/L], k_p is water-sludge partition coefficient [L/gMLSS], k_b is water-sludge mass-transfer rate constant [hr^{-1}], k_1 is first order biodegradation rate constant [hr^{-1}]. β is volume correction factor because C_W is defined as concentration in water phase and not as that in mixed liquor. A conceptual diagram of the two-phase fate model is shown in Figure 2.

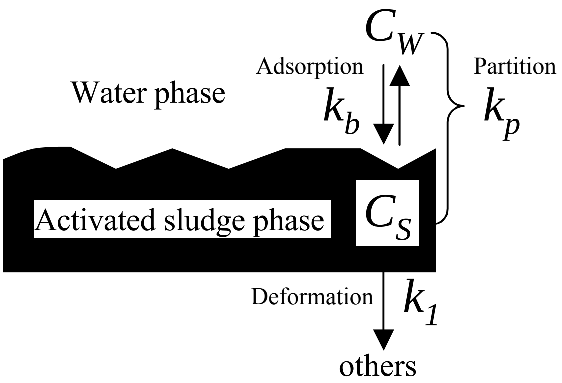

Figure 2. A conceptual diagram of the two-phase fate model.

3.2 Definition of k' and kp'

In the case of pharmaceuticals, two-phase fate models cannot be applied because of the low concentrations of pharmaceuticals in sludge-phase. Therefore, *k'* and *kp'* were defined as describe below.

$$\frac{dC_W}{dt} = -k' C_W \qquad k'_p = \frac{C_S}{C_W} \tag{3}$$

where *k'* is first order biodegradation rate constant considering only water-phase [hr^{-1}]. C_W and C_S is the average of three measurements at 0.5h, 3h and 6h on ground that the sludge retention time in WWTP is about 6-8 hours.

3.3 Cluster statistics

Cluster statistics is in order to extract similar characteristics out of wide variety of deta-set. Cluster statistics was used to grasp the tendency of the biodegradation. Results of cluster statistics were expressed by tree diagram. The combination of the branch in the earlier stage implies that these things have similar characteristic. There are lots of ways to calculate the distance of the each thing, but scaling Euclidean geometry was chosen because of its generality.

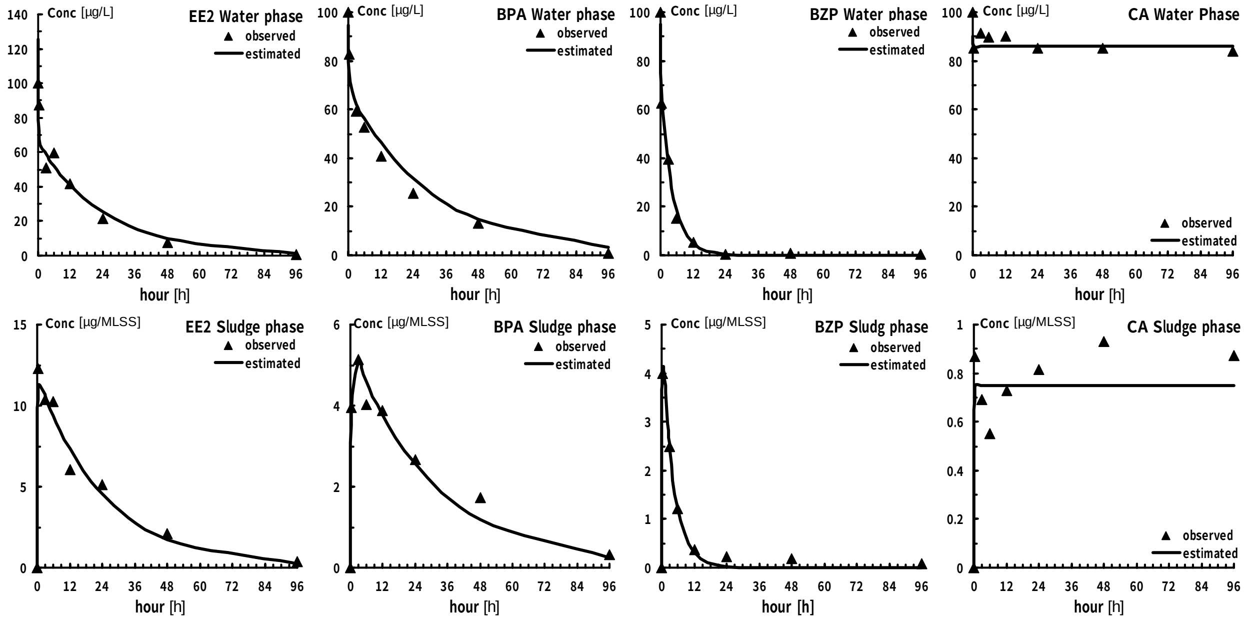

Figure 3. The plots show experimental results on time dependent change in concentrations by using activated sludge taken Shibaura WWTP. Solid lines represent most probable fitting-curves. Upper figures show concentrations in water phase. Bottom figures show concentrations in sludge phase.

Table 3. The values of each parameter identified by two-phase fate model

Activated sludge		**Shibaura**	**Shibaura**	**Morigasaki**	**Morigasaki**	**human-waste**	**pure-oxigen**	**waste disposal**
MLSS [gMLSS/L]		4.508	4.263	4.270	2.663	2.115	2.797	5.969
Keeping duaration by artificial wastewater		1 day	5 months	1 day	1 year	1 day	1 day	1 day
EE2	k_b [hr^{-1}]	3.381	1.782	2.635	2.000	2.264	0.341	1.324
	k_p [L/gMLSS]	0.182	0.823	0.097	0.438	0.823	1.048	0.649
	k_1 [hr^{-1}]	0.085	0.200	0.167	0.013	0.167	0.098	0.043
BPA	k_b [hr^{-1}]	1.678	1.130	>10	2.278	2.118	>10	--
	k_p [L/gMLSS]	0.085	0.377	0.124	0.217	0.245	0.259	--
	k_1 [hr^{-1}]	0.111	0.589	0.117	0.028	0.252	0.035	--
BZP	k_b [hr^{-1}]	3.699	1.048	>10	3.542	1.691	1.326	1.657
	k_p [L/gMLSS]	0.077	0.664	0.243	0.161	0.331	0.305	0.881
	k_1 [hr^{-1}]	0.931	0.022	1.406	0.363	0.051	0.058	0.116

4 Results and discussion

4.1 The removal chrateristics of the target compounds

The activated sludge degraded E2 and oxidized to E1 immediately and generated E1 was also degraded with high biodegradation rate. Concentration of E1 decreased almost 0μg/L after 12h experimental operation. BZP was degraded completely after 24h and BZP was degraded easier than BPA and EE2 which persist until 96h experimental operation. These tendencies were also observed in the case of activated sludge taken from Morigasaki WWTP. However, EE2 was degraded easier than BPA by the activated sludge taken from human-waste and pure oxygen aeration WWTP. Degradation rate of the IBP was the highest and the concentration of IBP was less than 1μg/L after 12h experimental operation, while CA, PPZ and CBZ were persistent until 96h experimental operation except for the results by using activated sludge taken from waste disposal site. Biodegradation of FEP and NPX was completed at t=48h whereas FEP and NPX needed 96h experimental operation to degraded less than blank levels respectively.

4.2 Application of two phase fate model

Figure 3 shows the typical results by using activated sludge taken from Shibaura WWTP and fitting curves by the two-phase fate model. All target compounds were seemed to be adsorbed immediately because the concentrations at t=0.5h in sludge phase were the highest for all target compounds and the concentrations decreased gradually.

The degradation of EE2, BPA and BZP followed the two-phase fate model, whose parameters were identified and shown in Table 3. It is assumed that concentrations of these compounds in sludge phase were relatively high, so pore water in the sludge phase could be neglected. This is an important point to apply the two-phase fate model. Only parameters of BPA in the case of activated sludge taken from the waste

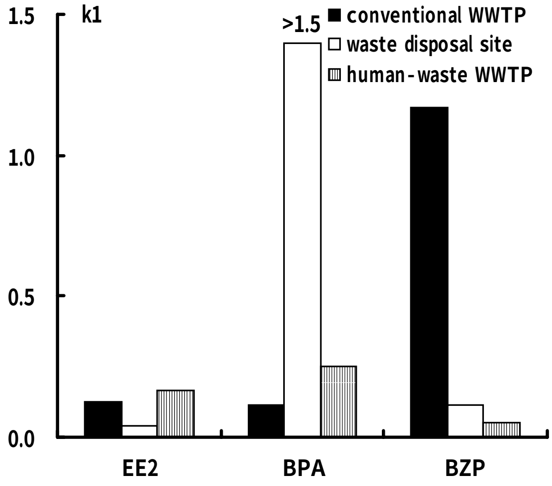

Figure 4. Comparison of k_1 values among different types of WWTPs.

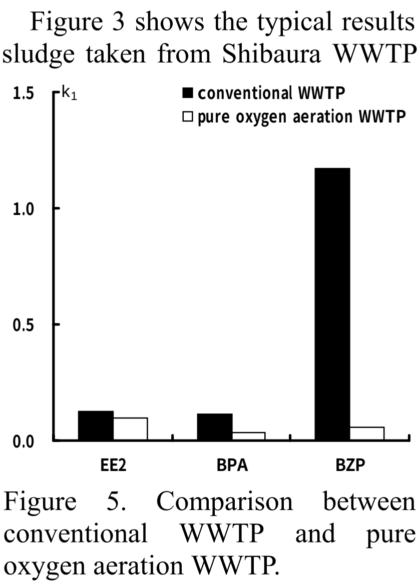

Figure 5. Comparison between conventional WWTP and pure oxygen aeration WWTP.

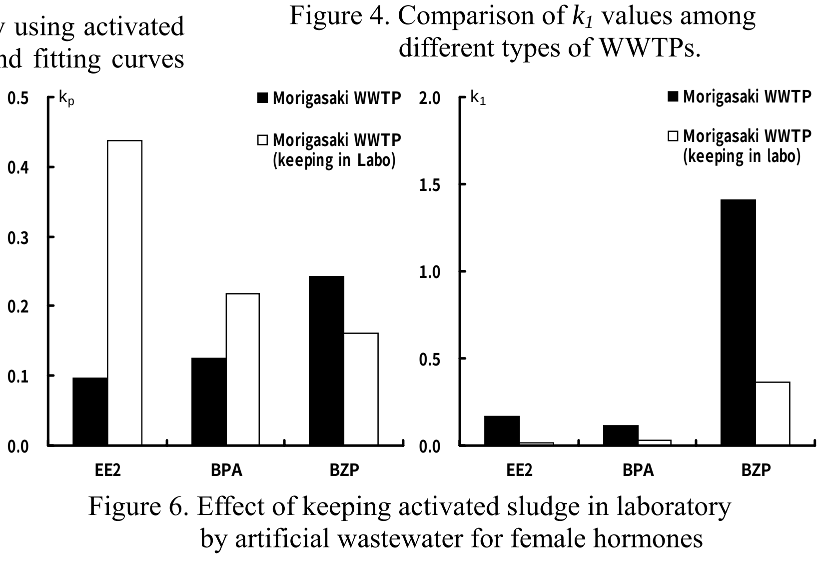

Figure 6. Effect of keeping activated sludge in laboratory by artificial wastewater for female hormones

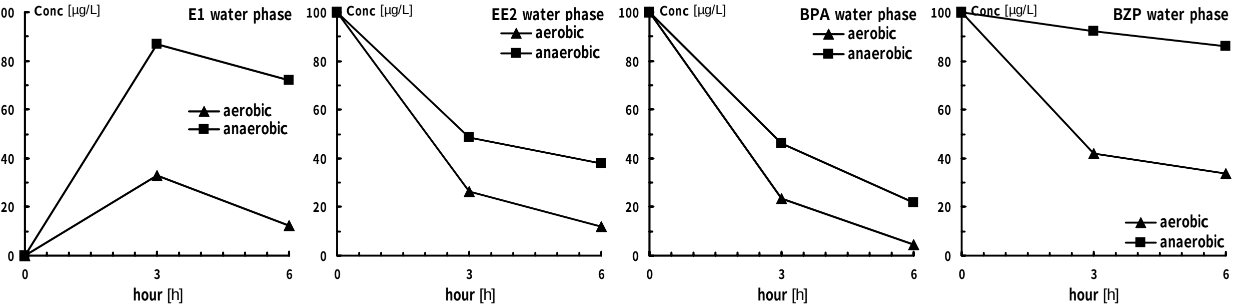

Figure 7. The plot shows the time dependent change in concentrations under aerobic and anaerobic condition

Table 4. The k'/X values of each experiments

Activated sludge	**Shibaura**	**Shibaura**	**Morigasaki**	**Morigasaki**	**human-waste**	**pure-oxigen**	**waste disposal**
MLSS [gMLSS/L]	4.508	4.263	4.270	2.663	2.115	2.797	5.969
Keeping duaration by artificial wastewate	1 day	5 months	1 day	1 year	1 day	1 day	1 day
E2+E1 k'/X [L/(hr·gMLSS)]	0.0456	0.0729	0.0113	0.0270	0.7441	0.6895	0.0523
EE2 k'/X [L/(hr·gMLSS)]	0.0108	0.0545	0.0085	0.0050	0.0738	0.0405	0.0077
BPA k'/X [L/(hr·gMLSS)]	0.0081	0.1242	0.0073	0.0068	0.0543	0.0084	2.0150
BZP k'/X [L/(hr·gMLSS)]	0.0477	0.0066	0.2665	0.0183	0.0133	0.0110	0.0208
CA k'/X [L/(hr·gMLSS)]	0.0001	0.0000	0.0000	0.0005	0.0005	0.0006	0.0047
GFZ k'/X [L/(hr·gMLSS)]	0.0087	0.0068	0.0129	0.0026	0.0088	0.0213	0.0160
IBP k'/X [L/(hr·gMLSS)]	0.0872	0.0324	0.2485	0.0087	0.0783	0.3257	0.0838
FEP k'/X [L/(hr·gMLSS)]	0.0166	0.0071	0.0446	0.0046	0.0207	0.0562	0.0658
KEP k'/X [L/(hr·gMLSS)]	0.0095	0.0008	0.0096	0.0013	0.0046	0.0124	0.0955
NPX k'/X [L/(hr·gMLSS)]	0.0172	0.0049	0.0052	0.0002	0.0017	0.0971	0.0023
DCF k'/X [L/(hr·gMLSS)]	0.0045	0.0031	0.0036	0.0004	0.0001	0.0060	0.0015
IDM k'/X [L/(hr·gMLSS)]	0.0025	0.0020	0.0037	0.0185	0.0028	0.0097	0.0067
PPZ k'/X [L/(hr·gMLSS)]	0.0005	0.0008	0.0000	0.0010	0.0004	0.0001	0.0039
CBZ k'/X [L/(hr·gMLSS)]	0.0007	0.0002	0.0003	0.0020	0.0010	0.0001	0.0026
DOC k'/X [L/(hr·gMLSS)]	0.0042	0.0037	0.0044	0.0094	0.0085	0.0086	0.0027

disposal site could not be calculated because the biodegradation rate of BPA under the activated sludge taken from there was very high. The concentration of BPA under activated sludge taken from waste disposal site was less than 1μg/L at t=0.5h. However, it was difficult to apply two phase fate model because of low concentration in the sludge phase.

4.3 The comparison of obtained parameters with different activated sludges

Figure 4 shows the comparison of k_1 values among different types of WWTPs. Conventional WWTPs stands for Shibaura and Morigasaki WWTP, k_1 value shown in this figure is the average of these WWTPs. Under the activated sludge taken from the waste disposal site, k_1 value of BPA was much higher than that of others. The biodegradation of BPA by using activated sludge taken from waste disposal site was promoted probably because lechate contained high concentration of BPA (date is not shown). Comparison of the k_1 and k_p values between conventional WWTP and pure oxygen aeration WWTP are shown in Figure 5. The activated sludge taken from pure oxygen aeration WWTP has lower biodegradation rate degradation of female hormones. Effect of keeping activated sludge in laboratory by artificial wastewater for female hormones is shown in Figure 6. Adsorption ability of activated sludge was increased by keeping it with artificial wastewater, while the biodegradation ability was deteriorated.

4.4 Results with anaerobic condition

Figure 7 shows the time dependent change in concentrations under aerobic and anaerobic condition. The biodegradation rates of female hormones under anaerobic condition were lower than those under aerobic condition. The concentration of E1 was increased up to approximately 85μg/L under the anaerobic condition, so the oxidization rate of E2 into E1 was also lower.

4.5 Adsorption onto kaolinite clay

Adsorption efficiency of kaorinite clay which was used as a representation of inorganic matter was also observed. The adsorbed amount in unit weight of kaorinite clay was in the order of 1/100 of that in the case of activated sludge. Female hormones considered to be remain in the water phase when it is mixed with clat particles.

4.6 The removal of pharmaceuticals by conventional activated sludge

It was difficult in the case of pharmaceuticals to apply the two-phase fate model because of the low concentration in the sludge phase. So k' and kp' was simply defined to evaluate the results of pharmaceuticals. The values of k' was corrected by dividing by MLSS and the each values of k'/X and kp' were shown in Table 4 and Table 5 respectively. Figure 8 and Figure 9 show the comparison of k'/X and kp'

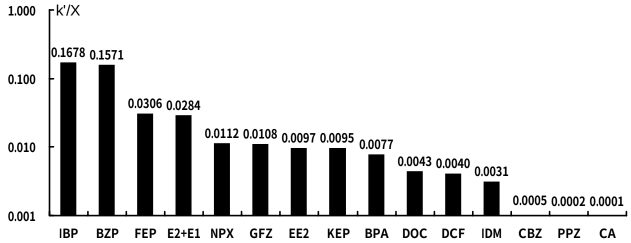

Figure 8. Comparison of *k'/X* values among all of the target compounds

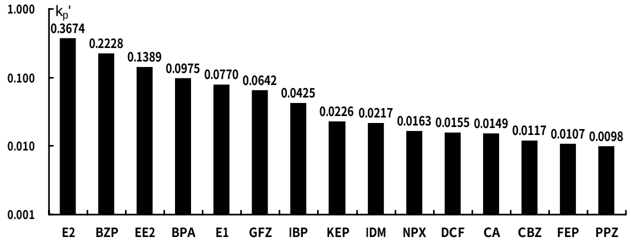

Figure 9. Comparison of *kp'* values among all of the target compounds

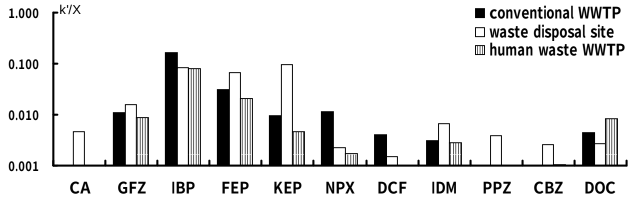

Figure 10. Comparison of *k'/X* values between among defferent types of WWTPs

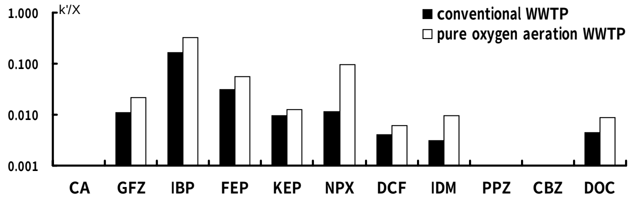

Figure 11. Comparisons of *k'/X* values between Shibaura WWTP and the pure oxygen aeration WWTP

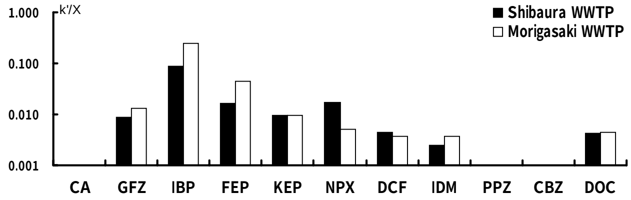

Figure 12. Comparison of *k'/X* values between Shibaura WWTP and Morigasaki WWTP

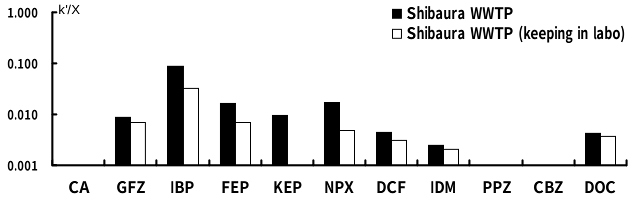

Figure 13. Effect of keeping activated sludge in laboratory by artificial wastewater on pharmaceuticals

values among all target compounds under the activated sludge taken from conventional WWTP. IBP gives the highest *k'/X* value and the *kp'* value of IBP was also relatively high. The average *kp'* value of pharmaceutical was approximately one tenth of that of female hormones. DCF, IDM, CBZ, PPZ and CA have low *kp'* value as well as low *k'/X* value, so these compounds are considered not to be treated appropriately by activated process in WWTP.

4.7 The removal of pharmaceuticals by activated sludge with different origins

Figure 10 shows the comparison of *k'/X* values among different types of WWTPs. Only the activated sludge taken from waste disposal site degraded CA, PPZ and CBZ. According to the *k'/X* values of activated sludge taken from human-waste WWTP, DCF was not degraded as well as CA, CBZ and PPZ. The degradation rate in the case of the activated sludge taken from human-waste WWTP was lower than that of the activated sludge taken from conventional WWTPs. Comparisons of *k'/X* values between the activated sludge taken from Shibaura WWTP and that of pure oxygen aeration WWTP are shown in Figure 11. The *k'/X* values under the activated sludge taken from pure oxygen aeration WWTP were higher than those under the activated sludge taken from conventional WWTP. So the methods of pure oxygen aeration seem to be effective in degrading of pharmaceuticals. But the

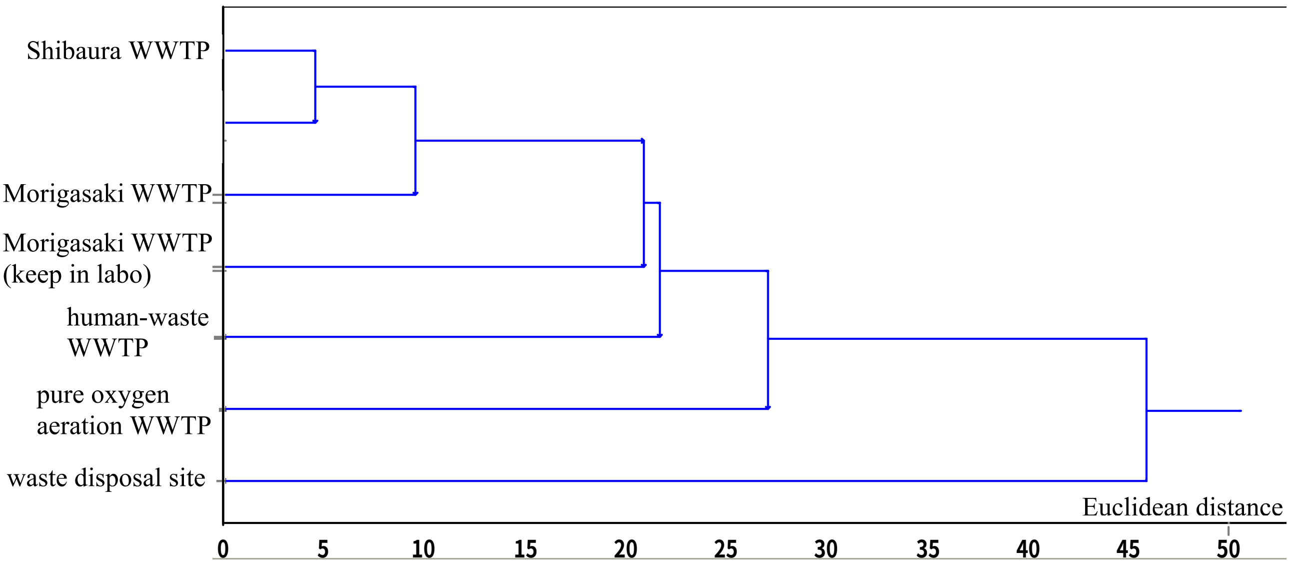

Figure 14. Tree diagram obtained by cluster statistics

method had a disadvantage for biodegradation of female hormones as described in the previous section.

Figure 12 shows the comparison of the *k'/X* values between activated sludge taken from Shibaura WWTP and that taken from Morigasaki WWTP. IBP has the highest *k'/X* value and the persistent compounds in the experiment were the same. DCF and IDM were degraded relatively slow. These tendencies were the same in the both cases of the activated sludges taken from conventional WWTPs. So experimental blurring caused by the type of activated within conventional plants was thought to be slight. Effect of keeping activated sludge in laboratory by artificial wastewater on the removal of pharmaceuticals is shown in Figure 13. In the similar way with the case of female hormones, the biodegradation rates were decreased by keeping the activated sludge in laboratory.

4.8 Obtained tree diagram by cluster statistics

The tree diagram which was obtained by cluster statistics based on *k'/X* values was shown in Figure 14. The branch of Shibaura WWTP and Morigasaki WWTP were combined in the early stages including the activated sludge keeping in laboratory by artificial wastewater. The branch of pure-oxygen aeration WWTP was combined relatively at the late stage. This reason is that activated sludge of pure oxygen aeration WWTP could degrade pharmaceuticals efficiently however that did not have better biodegradation capacity of female hormones. The biodegradation spectrum seem to be similar with each other. The branch of waste disposal site was combined at the latest. Only the activated sludge taken from the waste disposal site was capable of degrading CA, PPZ and CBZ and, in addition, the biodegradation rate of BPA was extremely higher than that in the other sludges.

5 Conclusions

The characteristics of biodegradation of natural hormones and pharmaceuticals in activated sludges with different properties were investigated. Laboratory experiments were carried out by using activated sludges which were taken from 5 WWTPs. The conclusions were summarized as follows;

1) In the case of the activated sludge taken from conventional WWTPs, E2 is oxidized to E1 immediately and the order of biodegradation rates among female hormones were BZP > E2+E1 > EE2 > BPA. Biodegradation rate of IBP is the highest among pharmaceuticals that while CA, PPZ, CBZ were identified as persistent substances. These persistent compounds have low Log*Kow* of lower than 3, and the k_p*'* values of these compounds are also relatively lower.
2) The k_p*'* value of E2 was the highest among female hormones while that of GFZ was the highest among pharmaceuticals. Overall, the concentrations of female hormones in sludge-phase were higher than those of pharmaceuticals. The average *kp'* value of female hormones was 10 times higher than that of pharmaceuticals.
3) The results of the experiments by using activated sludge of two different conventional WWTPs agreed with each other approximately. The persistent compounds which have low *kp'* value and low *k'* value were DCF, IDM, CBZ, PPZ and CA.
4) CA, PPZ and CBZ were persistent in the degradation by activated sludge taken from conventional WWTPs but those compounds were degraded by activated sludge taken from waste disposal site. And *k'* value of BPA was extremely high and that value was 240 times higher than that under the activated sludge of conventional WWTP. BPA was degraded very quickly possibly because BPA was included in lechate with high concentration.

5) Activated sludge taken from the human-waste treatment plant had lower rates for degradation of pharmaceuticals. The k' values of female hormones under the activated sludge taken from pure-oxygen aerobic WWTP were lower, but that of pharmaceuticals were higher compared with the results of conventional WWTPs.
6) The tree diagram obtained from the cluster statistics showed that biodegradation tendency under the activated sludge taken from the waste disposal site differed substantially from others. This reason is that activated sludge taken from the waste disposal site showed high biodegradation properties of target compounds.
7) Two-phase fate model was applied well to EE2, BPA and BZP whose kp' value is relatively high. Activated sludge fed with artificial wastewater for a certain period showed high adsorption tendencies, which enabled the analysis with two-phase fate model.
8) Female hormones were not adsorbed to kaorinite clay. When the pH was lowered, the amount of adsorbed female hormones was increased. However, the ratio of female hormones associated with karinite was lower than that associated with activated sludge. The degradation capacity of female hormones was declined by activated sludge under anaerobic condition.

References

[1] Guang-Guo Ying, Rai S. Kookana, Ying-Jun Ru (2002): Occurrence and fate of hormone steroids in the environment, *Environment International*, **28**, 545-551.

[2] A. C. Belfroid, A. Van der Horst, A. D. Vethaak, A. J. Schäfer, G.B.J. Rijs, J. Wegener, W. P. Cofino (1999): Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands, *The Science of the Total Environment*, **225**, 101-108.3.

[3] T. A. Ternes, M. Stumpf, J. Mueller, K. haberer, R.-D, Wilken, M. Servos (1999): Behavior and occurrence of estrogens in municipal sewage treatment plant – I. Investigation in Germany, Canada and Brazil, *The Science of the Total Environment*, **225**, 81-90.

[4] Chiara Baronti, Roberta Curini, Giuseppe D'Ascenzo, Antonio Di Corcia, Alessandra Gentili, Roberto Samperi (2000): Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water, *Environmental Science and Technology*, **34**, 24, 5059-5066.

[5] A. C. Johnson, A. Belfroid, A. Di Corcia (2000): Estimating steroid oestrogen inputs into activated sludge treatment works and observations on their removal from the effluent, *The Science of the Total Environment*, **256**, 163-173.

[6] Anders Svenson, Ann-Sofie Allard, Mats EK (2003): Removal of estrogenicity in Swedish muicipal sewage treatment plants, *water research*, **37** 4433-4443.

[7] Nasu M, Goto M, Kato H, Oshima Y, Tanaka H (2000): Study on endocrine disrupting chemicals in wastewater treatment plant, *Water Science Technology*, **43**, 101-108.

[8] T. A. Ternes, P.Kreckel J. Mueller, (1999): Behavior and occurrence of estrogens in municipal sewage treatment plant –Aerobic batch experiments with activated sludge, *The Science of the Total Environment*, **225**, 91-99.

[9] J. S. Vader, C. G. van Ginkel, F. M. G. M. Sperling, J. de Jong, W. de Boer, J.S. de Graaf, M. van der Most, P. G. W. Stokman (2000): Degradation of ethynil estradiol by nitrifying activated sludge, *Chemosphere*, **41**, 1239-1243.

[10] Manfred Clara, Birgit Strenn, Ernis Saracevic, Norbert Kreuzinger (2004): Adsorption of bisphenol-A, 17β-estradiole and 17α-ethinyleestradiole to sewage sludge, *Chemosphere*.

[11] Thomas Heberer (2002):Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data, *Toxicology Letters*, **131**, 5-17.

[12] 岩根泰蔵 (2003): 水環境中の医薬品化学物質

[13] S.E. Jørgensen, B. Halling-Sørensen (2000): Drugs in the environment, *Chemosphere*, **40**, 691-699.

[14] Céline Tixier, Heinz P. Singer, Sjef Oellers, Stephan R. Müller (2003): Occurrence and Fate of Carbamazepine, Clofibric Acid, Diclofenac, Ibuprofen, Ketoprofen, and Naproxen in Surface Waters, *Environmental Science and Technology*, **37**, 6, 1061-1068.

[15] C.Zwiener and F.H.Frimmel (2000): Oxidative treatment of pharmaceuticals in water, *Water Research*, **34**, 6, 1881-1885

[16] C. Zwiener, F. H. Frimmel (2003): Short-term tests with a pilot sewage plant and biofilm reactors for the biological degradation of the pharmaceutical compounds clofibric acid, ibuprofen, and diclofenac, *The Science of the Total Environment*, **309**, 201-211.

[17] H. B. Lee, D. Liu (2002): Degradation of 17β-estradiol and its metabolites by sewage bacteria, *Water, Air, and Soil Pollution*, **134**, 353-368.

[18] 環境庁水質保全局水質管理課 (1999): エストラジオール類の分析法(メチル誘導体化・GC/MS-SIM 法), 要調査項目等調査マニュアル, 47-62.

[19] V. Koutsouba, Th. Heberer, B. Fuhrmann, K. Schmidt-Baumler, D. Tsipi, A. Hiskia (2003): Determination of polar pharmaceuticals in sewage water of Greece by gas chromatography-mass spectrometry, *Chemosphere*, **51**, 69-75.

[20] Frank Sacher, Frank Thomas Lange, Heinz-Jürgen Brauch, Iris Blankenhorn (2001): Pharmaceuticals in groundwaters, Analytical methods and results of a monitoring program in Baden-Württemberg, Germany, *Journal of Chromatography A*, **938**, 199-210.

[21] 菊田友哉, 浦瀬太郎 (2004): 固液間の化学物質の分配に着目した微量有害物質の活性汚泥法での分解モデルの構築, 東京工業大学大学院土木工学専攻修士論文.

[22] A. I. Schäfer, M. Mastrup, R. Lund Jensen (2002): Particle interactions and removal of trace contaminants from water and wastewaters, *Desalination*, **147**, 243-250.