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

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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]. Pharmaceutical 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]. 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
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 an estrogen-conjugated 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 integrant of oral-contraceptive pill. Both case, initial concentration of female hormones was fitted with stirrer not to accumulate the activated sludge. Process of aerobic treatment was conducted to separate water-phase and sludge-phase that 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 adsorption 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-d₄ and 2,3-dichlorophenoxyacetic acid were used as surrogate standards and chrysene-d₁₂ as internal standard. These analytical methods were referred to the literature [18-20].

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

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

<table>
<thead>
<tr>
<th>S/N</th>
<th>Chemical Name</th>
<th>Abbrev.</th>
<th>Mol. Weight</th>
<th>Water Solubility [mg/L]</th>
<th>Henry's Law Constant [atm·m³/mol]</th>
<th>LogKᵣw</th>
<th>pKa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17β-estradiol</td>
<td>E2</td>
<td>272.39</td>
<td>3.60</td>
<td>3.64×10⁻¹⁰</td>
<td>4.01</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Estrone</td>
<td>E1</td>
<td>270.39</td>
<td>30</td>
<td>3.80×10⁻¹⁰</td>
<td>3.13</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17α-ethynylestradiol</td>
<td>EE2</td>
<td>296.41</td>
<td>11.3</td>
<td>7.94×10⁻¹²</td>
<td>3.67</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Bisphenol A</td>
<td>BPA</td>
<td>228.29</td>
<td>120</td>
<td>1.00×10⁻¹⁰</td>
<td>3.32</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Benzoephoneone</td>
<td>BZP</td>
<td>182.22</td>
<td>137</td>
<td>1.94×10⁻⁶</td>
<td>3.18</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Clofibric acid</td>
<td>CA</td>
<td>214.65</td>
<td>583</td>
<td>2.19×10⁻⁸</td>
<td>2.57</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Gemfibroizol</td>
<td>GFZ</td>
<td>250.34</td>
<td>N/A</td>
<td>N/A</td>
<td>4.77</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Ibuprofen</td>
<td>IBP</td>
<td>206.29</td>
<td>21</td>
<td>1.50×10⁻⁷</td>
<td>3.97</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Fenoprofen</td>
<td>FEP</td>
<td>242.28</td>
<td>N/A</td>
<td>N/A</td>
<td>3.9</td>
<td></td>
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<tr>
<td>10</td>
<td>Ketoprofen</td>
<td>KEP</td>
<td>254.29</td>
<td>51</td>
<td>2.12×10⁻¹¹</td>
<td>3.12</td>
<td></td>
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<tr>
<td>11</td>
<td>Naproxen</td>
<td>NPX</td>
<td>230.27</td>
<td>15.9</td>
<td>3.39×10⁻¹⁰</td>
<td>3.18</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Diclofenac</td>
<td>DCF</td>
<td>296.16</td>
<td>2.37</td>
<td>4.73×10⁻¹²</td>
<td>4.51</td>
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<tr>
<td>13</td>
<td>Indomethacin</td>
<td>IDM</td>
<td>357.80</td>
<td>0.94</td>
<td>3.13×10⁻¹⁴</td>
<td>4.27</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Propyphenazone</td>
<td>PPZ</td>
<td>230.31</td>
<td>3×10⁶</td>
<td>1.84×10⁻⁹</td>
<td>1.94</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Carbamazepine</td>
<td>CBZ</td>
<td>236.28</td>
<td>17.7</td>
<td>1.08×10⁻¹⁰</td>
<td>2.45</td>
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</table>

### Table 2. Conditions of the experiments

<table>
<thead>
<tr>
<th>pH</th>
<th>6.7</th>
</tr>
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<tbody>
<tr>
<td>Temperature</td>
<td>20°C</td>
</tr>
<tr>
<td>Initial concentration of DOC</td>
<td>150mg/L</td>
</tr>
<tr>
<td>Initial concentration of target compounds</td>
<td>100µg/L</td>
</tr>
<tr>
<td>Operation time</td>
<td>96h</td>
</tr>
</tbody>
</table>
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. Equation (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.

$$\frac{d(βC_W)}{dt} = -k_b(k_bC_W - C_S)X$$

$$\frac{d(C_S X)}{dt} = k_b(k_bC_W - C_S)X - k_1C_S X$$

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.

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.
4 Results and discussion

4.1 The removal characteristics 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 0 µg/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

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>MLSS [gMLSS/L]</th>
<th>Keeping duration by artificial wastewater</th>
<th>1 day</th>
<th>5 months</th>
<th>1 day</th>
<th>1 year</th>
<th>1 day</th>
<th>1 day</th>
<th>1 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>EE2</td>
<td>4.508</td>
<td>3.381</td>
<td>3.823</td>
<td>0.097</td>
<td>0.013</td>
<td>0.167</td>
<td>0.098</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>BPA</td>
<td>4.263</td>
<td>1.678</td>
<td>1.130</td>
<td>&gt;10</td>
<td>2.278</td>
<td>2.118</td>
<td>&gt;10</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>BZP</td>
<td>4.270</td>
<td>0.085</td>
<td>0.823</td>
<td>0.013</td>
<td>0.217</td>
<td>0.028</td>
<td>0.252</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>IBP</td>
<td>4.263</td>
<td>0.111</td>
<td>0.200</td>
<td>0.097</td>
<td>0.167</td>
<td>0.167</td>
<td>0.098</td>
<td>0.043</td>
<td></td>
</tr>
<tr>
<td>CA</td>
<td>2.663</td>
<td>0.077</td>
<td>0.599</td>
<td>0.087</td>
<td>0.117</td>
<td>0.087</td>
<td>0.252</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>PPZ</td>
<td>2.115</td>
<td>0.077</td>
<td>0.059</td>
<td>0.087</td>
<td>0.117</td>
<td>0.087</td>
<td>0.252</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>CBZ</td>
<td>2.797</td>
<td>0.931</td>
<td>0.022</td>
<td>0.087</td>
<td>0.117</td>
<td>0.087</td>
<td>0.252</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>FEP</td>
<td>5.969</td>
<td>0.077</td>
<td>0.664</td>
<td>0.243</td>
<td>0.161</td>
<td>0.331</td>
<td>0.305</td>
<td>0.881</td>
<td></td>
</tr>
</tbody>
</table>

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 disposal site are different from the other cases.

Figure 4 shows the comparison of k₁ values among different types of WWTPs. Figure 5 shows the comparison between conventional WWTP and pure oxygen aeration WWTP. Figure 6 shows the effect of keeping activated sludge in laboratory by artificial wastewater for female hormones.
The activated sludge taken from pure oxygen aeration WWTP are shown in Figure 5. The biodegradation rates of female hormones under aerobic 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 oxidation 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 kaolinite 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'.
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
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 LogKow 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 \( k_p' \) 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 \( k_p' \) 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 leachate 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 $k'$ 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 kaolinite clay. When the pH was lowered, the amount of adsorbed female hormones was increased. However, the ratio of female hormones associated with kaolinite was lower than that associated with activated sludge. The degradation capacity of female hormones was declined by activated sludge under anaerobic condition.

References


