Steroid Hormone Levels and Biological Removal Strategies.

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ABSTRACT

Endocrine disrupting compounds (EDCs) are chemicals that interfere with normal hormone function, often at extremely small doses. This disruption can be through synthetic chemicals that act or block normal hormonal activity or through the exposure to high doses of naturally produced body hormones, such as those in this study. These compounds are important because of increasing evidence that excess doses affect the endocrine system in humans and wildlife. Any effect on this system could cause reproductive and/or health effects. A potential sources of EDCs in the aquatic environment is wastewater treatment plant (WWTP) discharges. The discharges from WWTPs that contain EDCs can have an effect on the receiving wildlife, such as vitellogenin production in male or juvenile fish.

The steroid hormones included in this study are 17β-estradiol (E2), Progesterone (P), and Testosterone (T). This study examined the biological treatability of these compounds, using conventional suspended growth methodologies. In this study, the operating parameter of food-to-microbe ratio (F/M) was varied, ranging from 0.05-0.5 in four bench-scale biological reactors, with a constant biosolids concentration. This achieved varying solids residence times (SRT)—range 3 to 25 days—to test the degradation of hormones. Typical SRTs are 3-15 days, for conventional processes, to 20-40 days, for extended aeration. Twelve samples, taken at 12 hour intervals from each reactor, were used to assess the impact of varying F/M on removal. The mean influent hormone levels were 20.24 ng/L E2, 50.94 ng/L P, and 32.22 ng/L T. Hormone removals ranged from 60-93% with removal increasing with decreasing F/M. The removal of the steroid hormones using conventional biological treatment may not be adequate to meet future regulations.

INTRODUCTION

Endocrine disrupting compounds (EDCs)—chemicals that interfere with normal hormone activity—are emerging environmental concerns. Endocrine disruption can be through synthetic chemicals that act or block normal hormonal activity or through the exposure to high doses of naturally produced body hormones. These compounds may cause adverse reproductive and health effects in humans and wildlife.

The endocrine system controls metabolism, reproduction, behavior, growth, and development by secreting hormones which travel via the bloodstream to affect other cells of the body. Hormones circulate in the body at very low concentrations, ranging from as little as 1 picogram per milliliter of blood to a few micrograms per milliliter
of blood (Guyton 1991). The available receptors in target cells also vary; receptors on
the cell must be occupied for a response to occur. The level of circulating hormones
and amount of receptors available dictate cellular responses to endocrine stimuli.
Endocrine disruptors can alter this natural control and lead to detrimental effects.

The steroid hormones included in this study are 17β-estradiol (E2), Progesterone (P),
and Testosterone (T). These hormones are primarily produced in the gonads. Females
predominately produce estrogen and progesterone, while males predominately
produce testosterone. All three hormones are in each sex but at different ratios.
Estrogen and testosterone are produced to regulate the reproductive functions,
behavior, and development of secondary sexual characteristics. Progesterone is
produced in females to prepare the body for pregnancy. E2 in the plasma circulates at
25 to 300 pg/mL in females and 20 to 90 pg/mL in males (Harvey et al. 1988). Also,
between 10 to 100 µg of estrogens are excreted by cycling women daily, while
pregnant women can excrete up to 30 mg of estrogen a day and 100 mg progesterone
per day in late pregnancy (Baronti et al. 2000; Huang and Sedlak 2001). P levels in
plasma range 237-2425 ng/dl (luteal) in females and 25-45 ng/dl in males (Harvey et
al. 1988). T is produced primarily in the testes and levels in plasma range 15-95 ng/dl
in females and 260-1120 ng/dl in males (Harvey et al. 1988).

Some researchers propose links between endocrine disruptors and human health, but
the results are not definitive. Exposure to EDCs at key stages of pregnancy may lead
to abnormal genitalia in children and lowered IQs (Guo et al. 1995; Mendes 2002).
Presumably healthy babies may have lowered infertility as adults due to reproductive
tract malformations (Mendes 2002). In females, exposure to endocrine disruptors has
been theorized to lead to breast cancer and endometriosis (Mendes 2002). In males,
exposure may be associated with prostate cancer, lower sperm count, and testicular
cancer (Giwercman et al. 1993; Mendes 2002; Toppari et al. 1996).

In contrast, studies in wildlife are much more persuasive. It has been reported that
fish exposed to waters receiving wastewater treatment plant (WWTP) effluents have
induced vitellogenin (VTG) synthesis. VTG is an egg yolk precursor that is produced
in adult females in response to estrogen. Adult males and juveniles do not normally
produce VTG due to their low levels of E2; therefore, VTG has been used as a
biomarker of fish exposure to estrogenic compounds. Studies have shown VTG
induction in male fish downstream of numerous WWTPs (Rodgers-Gray et al. 2000;
Solé et al. 2001).

EDCs enter surface waters through a variety of pathways such as WWTP effluents.
Human hormone excretions enter WWTPs, are treated, and the remaining hormones
are discharged into the receiving water. Studies addressing fate and transport of EDCs
through the WWTP, specifically sex hormones, are limited.

Research has been conducted to examine EDC removal in WWTPs. In addition,
studies have been conducted to determine the concentrations of endocrine disruptors
in the effluents of various WWTPs. These studies have been conducted on both sex
hormones and a variety of synthetic compounds. Estrogen removal rates from 50-95%
have been reported in conventional activated sludge plants (Baronti et al. 2000; Fujii et al. 2002; Williams et al. 2003). Other studies, on conventional process plants, have provided average discharge levels of estrogens as 0.2-4.1 ng/L. Application of the advanced reverse osmosis process at one plant produced discharges of less than 0.4 ng/L (Huang and Sedlak 2001).

Research on the fate of hormones through the activated sludge process has speculated that removal occurs from sorption onto the biofloc particles and degradation by microorganisms (Birkett and Lester 2003; Fujii et al. 2002; Sedlak et al. 2000). Most research indicates sorption onto suspended biofloc in the mixed liquor is the primary removal mechanism. Biological degradation contributes, but to a lesser extent. Biodegradation can be influenced in the activated sludge reactor by Solids Residence Times (SRT). With low SRT, biodegradation is minimal since there is little time for interaction between the target compounds and the microbes—high wasting rates and loss of specific degraders (Jacobsen et al. 1993). In contrast, high SRTs can allow for more influence by biodegradation—little wasting and an accumulation of specific degraders (Birkett and Lester 2003). Overall, perhaps up to 10% of hormones will be biodegraded, while the remainder will be removed by adsorption to sludge (Sedlak et al. 2000; Shäfer and Waite 2002).

Regarding municipal sewage discharges, this study evaluates the potential for optimizing conventional AS treatment for hormone removal by evaluating the performance of bench-scale biological reactors under different operating conditions.

**MATERIALS AND METHODS**

**Assay**

Samples were filtered through glass fiber discs and 0.2 μm pore size cellulose acetate and cellulose nitrate (MCE) filters, then extracted using C18 discs and eluted with methanol. The methanol was dried down under filtered air and the extracts were re-suspended in Enzyme Immunoassay (EIA) buffer. The samples are diluted with EIA buffer until within the standard curve. Then 5-50 microliter samples were used to detect hormones via EIA kit and method (Cayman Chemical, Ann Arbor, MI). Figure 1 shows a sketch of the basic method theory.
Hormone Sample Collection

Hormone samples were collected in glass beakers every 12 hours at approximately 7:00 am and 7:00 pm from influent and effluent sample points. Approximately 50 mL of influent samples were collected from the influent pump discharge tube before entering into the reactors and approximately 100 mL of effluent samples were collected from the clarifier overflow weir discharge tubes. Samples were filtered through glass fiber discs and filtered with a 0.2 µm pore size cellulose acetate and cellulose nitrate (MCE) general filter using a Millipore filtration apparatus. Samples possible containing hormones were stored in a refrigerator at 4°C until hormone extraction.

Statistics

Normality tests were conducted on the data sets collected in each reactor for each hormone to test if they fit normal distributions. For each of the three hormones, the effluent data sets passed normality with the exception of P data in reactor 1 T data in reactor 4. Because of small sample sizes, normality can not be determined with full confidence. One way ANOVAs were conducted assuming normality and repeated using one way ANOVAs on ranks, a nonparametric test in case the normality assumption was violated (as it was in two cases). Both techniques were used to
compare the reactors effluent concentrations and to determine if each effluent data set was statistically different from the other. The statistical procedure is outlined in Error! Reference source not found.. Mean standard deviations and coefficients of variance (COV) were calculated to describe the variation in the data. All statistical analyses were performed using SigmaStat (3.10)(Systat Software Inc., Richmond, CA), SigmaPlot (9.0)( Systat Software Inc., Richmond, CA and Excel (10.0)(Microsoft Corporation, Redmond, WA).

Bench-Scale Biological Treatment Process

Four identical reactors were installed, each with a total volume of approximately 8 L. The AS reactors were constructed from a typical design (Eckenfelder et al. 1969; Qasim 2004) and were custom fabricated by a local Dallas plastics company as shown in Figure 2. The reactors were seeded with mixed liquor from the Dallas Southside WWTP. The organic substrate, containing hormones, was effluent collected from the primary clarifier at the City of Dallas Central WWTP. The reactors were operated at different Food-to-Microbe (F/M) ratios, ranging from 0.05-0.5 with a constant biosolids concentration.

Figure 2. Activated sludge reactor.

The F/M ratios were used to achieve a typical range of solids residence times (SRT) to study the degradation of hormones. Typical SRTs are 3-15 days for conventional completely mixed processes to 20-40 days for extended aeration. The influent pump rates were adjusted to achieve the four different F/M ratios and aeration was used for mixing. The partition wall was adjusted in each reactor to allow for biosolids settling and recirculation back into the aeration chamber to occur. The units were operated
for 10 days to achieve steady-state operation followed by six days of hormone data collection.

Constant influent flowed into each reactor where the biosolids degraded the waste, and then treated effluent discharged from an overflow weir in the clarifier. The supernatant then flowed down a tube to the effluent sampling location. The influent pumps were continuously monitored and adjusted to maintain a constant flow rate. The temperature was also kept at room temperature (constant 23°C) via submersible heating units.

The primary clarifier effluent, from the Central WWTP, was collected every Monday, Tuesday, and Wednesday into two 55-gallon drums and transported back to the research laboratory. The sample was then transferred using a self-priming jet pump with a garden hose to two plastic 55-gallon drums placed in a chromatography refrigerator set at 4°C. The wastewater was rapidly cooled using dry ice placed into large 13-gallon plastic bags suspended from the lids of the drums down into the sample to not allow for the dry ice to come in contact with the sewage, thus minimizing any characteristic changes that could be caused by the dry ice, e.g., lowering the pH. The rapid cooling was conducted to minimize the biodegradation of the organic waste by microbes already present in the sample, to maintain a relatively constant and high substrate concentration to pump into the reactors.

The wastewater stored in two 55 gallon drums was constantly re-circulated, at a turnover rate of approximately 10 times an hour, using two Beckett submersible pumps purchased at a local home improvement store. A pump was placed in each 55 gallon drum which pumped the wastewater from each drum into the 5 gallon bucket sitting on top. The 5 gallon bucket had two overflow weirs of 1.5 inch plastic PVC pipe that emptied back into the 55 gallon drums. Each drum was connected by a 5/8 inch rubber tube to a stopcock with a hose barb at the bottom to allow for a steady volume in each. This apparatus is shown in Figure 3.
Two different pump types were used for the operation of the AS reactors. Air pumps, from a local aquarium supply store, were used with 6 inch air diffuser stones to supply the oxygen for the AS reactors. One pump was used for each reactor to allow for enough oxygen transfer for microbe metabolism as well as for proper mixing. LMI electronic chemical metering pumps (series AA) were purchased from a local distributor to supply the steady flow of influent wastewater from the two 55-gallon drum holding tanks into each reactor. Each reactor had a pump that was set at a different cycle rate to allow for different flow rates entering into the reactors. This allowed for different F/M ratios in each reactor to be maintained.

Total Suspended Solids (TSS), Biochemical Oxidation Demand (BOD), and Chemical Oxidation Demand (COD) tests (Error! Reference source not found.-l) were conducted daily in order to make adjustments and maintain steady-state operation. Once the biosolids concentration was determined from the TSS data, appropriate daily wasting at approximately 5:00 pm would be applied in each reactor to maintain a constant level of biosolids.
Kinetic Constants

The microbial kinetics for the treatment of the domestic sewage was determined from steady-state data gathered prior to hormone sampling. This data set has BOD data that is inline with results seen previously based on the COD data. The results during the sampling week were unreliable possibly due to a faulty dissolved oxygen (DO) meter. The BOD/COD ratio is not similar to that seen in previous reactor studies prior to hormone sample collection. Hence, COD data, rather than BOD, was used to adjust operation of the reactors during the period of hormone data collection.

Results

The AS reactors were operated in Fall 2004. Table 1 is a summary of the operations data acquired from each of the four reactors. Prior to hormone sampling, the reactors were operated until steady-state conditions were present. At steady-state, reactors 1 and 2 have operational values seen in extended aeration AS reactors while reactors 3 and 4 have values seen in completely mixed AS (CMAS) reactors. Extended aeration reactors have F/M ratios of .04-.10 and SRTs of 20-40 days. Typical design parameters for CMAS reactors have F/M ratios of 0.2-0.6 and SRTs of 3-15 days (Tchobanoglous et al. 2003).

Table 1. Operational results for bench-scale CMAS reactors.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>$F_0$</th>
<th>$S_0$</th>
<th>$S_s$</th>
<th>Removal</th>
<th>$X_B$</th>
<th>$W_T$</th>
<th>F/M</th>
<th>SRT</th>
<th>HRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>56.4</td>
<td>1.38</td>
<td>97.6%</td>
<td>1200</td>
<td>49</td>
<td>0.05</td>
<td>24.48</td>
<td>22.67</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>56.4</td>
<td>1.94</td>
<td>96.6%</td>
<td>1500</td>
<td>69</td>
<td>0.08</td>
<td>21.89</td>
<td>11.18</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>56.4</td>
<td>4.80</td>
<td>91.5%</td>
<td>1200</td>
<td>152</td>
<td>0.19</td>
<td>7.91</td>
<td>5.48</td>
</tr>
<tr>
<td>4</td>
<td>71</td>
<td>56.4</td>
<td>7.61</td>
<td>86.5%</td>
<td>1200</td>
<td>373</td>
<td>0.35</td>
<td>3.22</td>
<td>2.83</td>
</tr>
</tbody>
</table>

$F_0$ = Influent flow rate (L/day), $S_0$ = Influent substrate concentration (mg/L BOD), $S_s$ = Effluent substrate concentration (mg/L BOD), $X_B$ = Biosolids concentration maintained (mg/L MLSS), $W_T$ = Biosolids wasting (mg/day TSS), F/M = Food-to-microbe ratio, SRT = Solids retention time (days), HRT = Hydraulic retention time (hours).

Table 2 shows a summary of the hormone concentration data acquired in the study. The mean influent levels were 20.24 ng/L E2, 50.98 ng/L P, and 32.22 ng/L T. The removal rates presented in the last column in Table 2 are given for the three hormones in each reactor running at different F/M ratios. The removal rates are based on the mean hormone concentrations (column 3, Table 2). The removal range was 60-93%
across the F/M range of 0.05-.035. Reactors 1 and 2—running at the lower F/M—achieved the highest removal rates, up to 93%. For E2 and T, significant increases in removal occurred when the F/M ratio decreased from 0.19 to 0.08. For P, the significant differences were seen between the extreme F/Ms of 0.35 and 0.05. In general, hormone removal increased with decreasing F/M.

Table 2. Reactor mean influent and effluent hormone levels (ng/L).

<table>
<thead>
<tr>
<th>Hormone Sample</th>
<th>Mean</th>
<th>SD</th>
<th>COV</th>
<th>Min</th>
<th>Max</th>
<th>F/M</th>
<th>REM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influent</td>
<td>20.24</td>
<td>5.20</td>
<td>26%</td>
<td>12.29</td>
<td>28.27</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1</td>
<td>4.09</td>
<td>1.02</td>
<td>25%</td>
<td>2.43</td>
<td>6.47</td>
<td>0.05</td>
<td>79.8%</td>
</tr>
<tr>
<td>2</td>
<td>5.10</td>
<td>1.22</td>
<td>24%</td>
<td>3.12</td>
<td>7.38</td>
<td>0.08</td>
<td>74.8%</td>
</tr>
<tr>
<td>3</td>
<td>8.05</td>
<td>2.10</td>
<td>26%</td>
<td>5.37</td>
<td>13.04</td>
<td>0.19</td>
<td>60.2%</td>
</tr>
<tr>
<td>4</td>
<td>7.79</td>
<td>1.96</td>
<td>25%</td>
<td>4.14</td>
<td>11.01</td>
<td>0.35</td>
<td>61.5%</td>
</tr>
<tr>
<td>E2</td>
<td>4.09</td>
<td>1.02</td>
<td>25%</td>
<td>2.43</td>
<td>6.47</td>
<td>0.05</td>
<td>79.8%</td>
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<td>11.01</td>
<td>0.35</td>
<td>61.5%</td>
</tr>
<tr>
<td>P</td>
<td>4.09</td>
<td>1.02</td>
<td>25%</td>
<td>2.43</td>
<td>6.47</td>
<td>0.05</td>
<td>79.8%</td>
</tr>
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<td>1.22</td>
<td>24%</td>
<td>3.12</td>
<td>7.38</td>
<td>0.08</td>
<td>74.8%</td>
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<tr>
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<td>2.10</td>
<td>26%</td>
<td>5.37</td>
<td>13.04</td>
<td>0.19</td>
<td>60.2%</td>
</tr>
<tr>
<td>4</td>
<td>7.79</td>
<td>1.96</td>
<td>25%</td>
<td>4.14</td>
<td>11.01</td>
<td>0.35</td>
<td>61.5%</td>
</tr>
<tr>
<td>Influent</td>
<td>50.98</td>
<td>23.45</td>
<td>46%</td>
<td>13.78</td>
<td>75.72</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1</td>
<td>9.25</td>
<td>4.67</td>
<td>50%</td>
<td>4.32</td>
<td>18.30</td>
<td>0.05</td>
<td>81.9%</td>
</tr>
<tr>
<td>2</td>
<td>12.39</td>
<td>5.18</td>
<td>42%</td>
<td>6.03</td>
<td>23.56</td>
<td>0.08</td>
<td>75.7%</td>
</tr>
<tr>
<td>3</td>
<td>13.15</td>
<td>4.75</td>
<td>36%</td>
<td>6.40</td>
<td>22.63</td>
<td>0.19</td>
<td>74.2%</td>
</tr>
<tr>
<td>4</td>
<td>16.83</td>
<td>5.50</td>
<td>33%</td>
<td>9.34</td>
<td>26.77</td>
<td>0.35</td>
<td>67.0%</td>
</tr>
<tr>
<td>T</td>
<td>4.09</td>
<td>1.02</td>
<td>25%</td>
<td>2.43</td>
<td>6.47</td>
<td>0.05</td>
<td>79.8%</td>
</tr>
<tr>
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<td>1.22</td>
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<td>25%</td>
<td>4.14</td>
<td>11.01</td>
<td>0.35</td>
<td>61.5%</td>
</tr>
</tbody>
</table>

SD = Standard deviation.
COV = Coefficient of variation.
REM = Percent hormone removal

ANOVA compares whether there is a statistically significant difference (p-value <0.05) in the mean values among different groups. One way ANOVA and ANOVA on ranks were conducted on the effluent concentrations to quantify the differences, if any, among the reactors. These results are presented in Table 3. For E2, there is no significant difference in the effluent concentrations measured in reactors 1 and 2, and also 3 and 4. For T, effluent concentrations in 1 & 2 are not statistically different, whereas 3 & 4 was found to be statistically different using one way ANOVA and not different using ANOVA on ranks. All other groups are statistically different, i.e., 1 & 3, 1 & 4, 2 & 3, and 2 & 4. For P, only reactors 1 and 4 showed statistically significant differences in the mean effluent concentrations.
Table 3. ANOVA reactor comparison summary.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>E2</th>
<th>P</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs. 4</td>
<td>0.000*</td>
<td>0.001*</td>
<td>0.000*</td>
</tr>
<tr>
<td>2 vs. 4</td>
<td>0.000*</td>
<td>0.036</td>
<td>0.000*</td>
</tr>
<tr>
<td>1 vs. 3</td>
<td>0.000*</td>
<td>0.065</td>
<td>0.003*</td>
</tr>
<tr>
<td>2 vs. 3</td>
<td>0.000*</td>
<td>0.714</td>
<td>0.004*</td>
</tr>
<tr>
<td>3 vs. 4</td>
<td>0.697</td>
<td>0.080</td>
<td>0.023</td>
</tr>
<tr>
<td>1 vs. 2</td>
<td>0.139</td>
<td>0.134</td>
<td>0.935</td>
</tr>
</tbody>
</table>

*Statistically significant difference when P < critical level

The microbial kinetics for the treatment of the waste was determined on steady-state conditions observed the week before hormone collection data are presented in Table 4. The kinetic constants calculated from the reactors were transformed from 23°C to 20°C using typical theta (θ) values. These values are compared with typical kinetic coefficients for the degradation of domestic sewage (Tchobanoglous and Burton 1991).

Table 4. Kinetic constants summary.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Notation</th>
<th>Experimental</th>
<th>Reactorsa</th>
<th>Typical Rangea</th>
<th>θb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>mg TSS/mg BOD5</td>
<td>1.0</td>
<td>0.4-0.8c</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>d⁻¹</td>
<td>0.011</td>
<td>0.025-0.075</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
<td>Ks</td>
<td>mg/L BOD5</td>
<td>29</td>
<td>25-100</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>k</td>
<td>d⁻¹</td>
<td>2</td>
<td>2-10</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

Y = True Growth Yield.
b = Endogenous Decay Coefficient.
Ks = Half-Saturation Constant.
k = Maximum Specific Substrate Removal Rate.
aValues reported for 20°C.
bθ Typical Temperature Correction Values.
cTypical Range is reported in mg VSS/mg BOD5.

The true growth yield (Y) is above the typical range. This could result from the reporting the coefficient Y using TSS data while the typical range is reported using volatile suspended solids (VSS) data. Typical MLVSS/MLSS ratios range from 50-
90%. Therefore, the small inorganic portion could be pushing the value above the typical range. The endogenous decay coefficient (b) is also outside typical range, while the remaining coefficients are within range. The higher value of Y and lower value of b indicates a higher observed yield of biosolids is typically observed.

Conclusions

The hormone levels found in the effluent may be sufficient to cause detectable toxicological effect, e.g., vitellogenin production in male or juvenile fish. Other studies have shown a response to similar hormone levels using long exposure periods (months) in several aquatic species (Cheek et al. 2001; Rodgers-Gray et al. 2000). In addition to the concern regarding ecological risks to aquatic species, there is some concern regarding possible human health risk to downstream users, e.g., drinking water consumers. However, it is likely that the risk to human health is small considering dilution in the river, detention time, and treatment at drinking water plants prior to consumption.

Activated sludge biological treatment operating at the F/M ratios of 0.05-0.35 was used to look at the removal of hormones. The microbial kinetics calculated were approximately within the typical ranges, which indicates the reactors performed characteristically for the degradation of domestic wastewater. Hormone removal rates range from 60%-80% (E2), 67%-82% (P), and 79%-93% (T). In general, removal increased with decreasing F/M. ANOVAs using parametric and nonparametric techniques applied to the treatment study indicate a significant improvement in hormone removal, perhaps up to an additional 15-20% gain, across the range of typical F/M ratios and SRTs. However, operation at the limits for activated sludge processes is unlikely to provide enough additional removal to comply with future regulations designed to protect sensitive aquatic species. If the EPA were to regulate the release of hormones, biological treatment may not be the answer. Municipal WWTP operators would likely have to employ additional advanced treatment prior to discharge.

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