Anaerobic biotransformation of estrogens

Cynthia P. Czajka, Kathleen L. Londry *

Department of Microbiology, University of Manitoba, Winnipeg, MB, Canada R3T 2N2

Received 23 August 2005; received in revised form 18 December 2005; accepted 16 January 2006
Available online 17 April 2006

Abstract

Estrogens are important environmental contaminants that disrupt endocrine systems and feminize male fish. We investigated the potential for anaerobic biodegradation of the estrogens 17-α-ethynylestradiol (EE2) and 17-β-estradiol (E2) in order to understand their fate in aquatic and terrestrial environments. Cultures were established using lake water and sediment under methanogenic, sulfate-, iron-, and nitrate-reducing conditions. Anaerobic degradation of EE2 (added at 5 mg/L) was not observed in multiple trials over long incubation periods (over three years). E2 (added at 5 mg/L) was transformed to estrone (E1) under all four anaerobic conditions (99–176 μg L⁻¹ day⁻¹), but the extent of conversion was different for each electron acceptor. The oxidation of E2 to E1 was not inhibited by E1. Under some conditions, reversible inter-conversion of E2 and E1 was observed, and the final steady state concentration of E2 depended on the electron-accepting condition but was independent of the total amount of estrogens added. In addition, racemization occurred and E1 was also transformed to 17-α-estradiol under all but nitrate-reducing conditions. Although E2 could be readily transformed to E1 and in many cases 17-α-estradiol under anaerobic conditions, the complete degradation of estrogens under these conditions was minimal, suggesting that they would accumulate in anoxic environments.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Estrogen; Estradiol; Estrone; Biotransformation; Anaerobic; Bacteria

1. Introduction

Industrial and municipal chemicals that mimic natural hormones regularly enter the environment and disrupt endocrine system functions. Recent scientific reports indicate that two compounds causing adverse effects in both rivers and lakes are the natural 17-β-estradiol (E2) and the synthetic 17-α-ethynylestradiol (EE2) (Fig. 1) — the main active ingredient in birth control pills (Desbrow et al., 1998; Langston et al., 2005). These compounds enter aquatic environments from multiple sources including wastewater treatment plant (WWTP) effluents and run-off from manure application to soils, reaching the receiving waters in minute (<100 ng/L) concentrations (Ying and Kookana, 2003). Aquatic animals such as fish are seriously impacted even at nanogram per liter concentrations of EE2 or E2 (Palace et al., 2002; Purdom et al., 1994). The impact of human- and livestock-derived estrogens on terrestrial fauna is relatively unknown (Boerjan et al., 2002).

The removal of estrogens from water, sediments, and soils is expected to be largely the result of a combination of physical sorption and binding to particles, and biodegradation. Recent studies indicate that sorption of estrogens is significant in aquatic sediments (Holthaus et al., 2002; Jürgens et al., 1999; Lai et al., 2000; Williams et al., 2003; Yamamoto et al., 2003; Yu...
et al., 2004) so accumulation in the sediments is very important in the context of long term fate. River-bed sediments have the potential to be a reservoir for these estrogens, especially when they are anoxic (Peck et al., 2004; Williams et al., 2003, 1999). Estrogens were also found to bind to aquifer material, particularly to organic matter (Ying and Kookana, 2003). Similarly, a variety of soils also bind estrogens (Colucci et al., 2001; Hanselman et al., 2003; Loffredo and Senesi, 2002) and can also become anoxic. Therefore, in order to understand the role of biodegradation in the fate of estrogens it is important to assess the potential for transformation or mineralization of estrogens under both aerobic and anaerobic conditions, and to assess the effects of environmental conditions on biodegradation rates.

Studies in which the biodegradation of estrogens was analyzed in cultures or indirectly by their removal from wastewater treatment plants indicate that E2 and EE2 are degraded aerobically in mixed cultures (D’Ascenzo et al., 2003; Johnson and Sumpter, 2001; Jürgens et al., 1999; Layton et al., 2000; Lee and Liu, 2002; Lee et al., 2003; Matsuoka et al., 2005; Tabak et al., 1981; Tabak and Bunch, 1970; Ternes et al., 1999; Vader et al., 2000; Ying and Kookana, 2003; Ying et al., 2002, 2004). However, under anaerobic conditions, E2 biodegradation is limited and EE2 biodegradation has not been observed at all (Jürgens et al., 1999; Lee and Liu, 2002; Ying and Kookana, 2003). In fact, the loss of E2 was accompanied by a corresponding accumulation of E1 in all cases in which E1 was analyzed (Jürgens et al., 1999; Lee and Liu, 2002; Ying and Kookana, 2003). In fact, the loss of E2 was accompanied by a corresponding accumulation of E1 in all cases in which E1 was analyzed (Jürgens et al., 1999; Lee and Liu, 2002; Ying and Kookana, 2003), such that a loss of total estrogens was not observed. In some studies, a slow and incomplete loss of E2 was noted by chemical analysis, but since E1 was not analyzed any further transformation beyond this initial oxidation is uncertain (Ying et al., 2003, 2004). This recalcitrance of estrogens would have tremendous implications for the environment, because anoxic areas could become repositories for these chemicals, affecting bottom-feeding invertebrates and potentially becoming a future source of estrogens in the water whenever the system is disrupted.

In the environment, different anaerobic conditions may develop, depending on the availability of different electron acceptors such as iron (Fe (III)), nitrate, sulfate, or CO2. These conditions can impact both the rates and mechanisms of organic compound transformations. Using water and sandy sediments from an EE2-amended lake (Palace et al., 2002) we set out to determine whether the microbial populations in these sediments have the capacity for the biodegradation of estrogens.

2. Materials and methods

2.1. Sources of sludge and sediments

Water and sediment samples were collected from Lake 260 of the Experimental Lakes Area near Kenora, Ontario, Canada. Sediment to be used for anaerobic incubations was flushed with N2 gas for 5 min and sealed tightly for transport. The water and sediment were stored on ice or at 4 °C overnight, then thawed gradually to room temperature and used within 48 h. Sludge was collected from an anaerobic digester at the North End Water Pollution Control Centre, Winnipeg, MB, Canada (May 11, 2000) and was stored at room temperature overnight prior to use.

2.2. Anaerobic incubations

To establish cultures under anaerobic conditions, lake sediment or sludge was transferred to an anaerobic chamber and strict anaerobic technique was used throughout. Unless otherwise indicated, sediment (10 ml) was added to 160 ml serum bottles containing 90 ml freshwater mineral medium (Widdel and Bak, 1992), prepared anaerobically with a N2:CO2 (80:20) gas phase, resazurin as a redox indicator, and reduced with 1 mM Na2S immediately prior to inoculation. The media were amended with 20 mM NaNO3 (nitrate-reducing conditions), 20 mM Fe3+-nitritotriacetic acid (NTA) (iron-reducing conditions), 20 mM Na2SO4 (sulfate-reducing conditions), or water (methanogenic conditions). After addition of sediment and water, the
serum bottles were sealed with butyl rubber stoppers and crimp sealed, then the gas phase was aseptically exchanged to N₂:CO₂ (80:20). Sterile controls were autoclaved within 1 h, and again after 24–72 h. Estrogens (E1, E2, EE2) were added from methanolic stock solutions to initial calculated concentrations of 5 mg/L (25 μl or 0.62 mmol methanol) unless otherwise stated. Negative controls did not receive estrogens or methanol; positive controls received estrogens as well as 1 mM benzoate to confirm that actively biodegrading anaerobic populations were present. Cultures were incubated at 28 °C in the dark, without shaking. Cultures prepared with sludge from the WWTP were identical except that 10 ml sludge was added. For large cultures, 150 ml sediment was added to 1350 ml medium in customized 2-L serum bottles with severed anaerobic culture tubes held in place with a butyl-rubber stopper.

2.3. Analytical techniques

For analysis of estrogens concentrations, aqueous samples of cultures were collected using glass syringes and transferred to microcentrifuge tubes containing an equal volume of methanol (HPLC grade, Fisher Scientific). The tubes were centrifuged at 14,000 × g for 5 min to remove sediment and debris. The supernatant was transferred to glass vials and stored frozen until analyzed. E1, E2 and EE2 were quantified by high performance liquid chromatography (HPLC) using a DX 500 HPLC (Dionex Corp.) equipped with an AD20 absorbance detector (at 210 nm) and GP50 gradient pump (Dionex Corp.). The IC was equipped with a PRP®-X100 Ion Column (150×4.1 mm) (Hamilton Company), and ASRS®-Ultrap 4 mm suppressor ( Dionex Corp.). The eluent was composed of 1.8 mM Na₂CO₃, 1.7 mM NaHCO₃ and 2.0 mM phenol, pH 10.0, with a flow rate of 2.0 ml/min.

2.4. Identification of metabolites

Cultures containing lake sediments, as well as water samples (60 ml) amended with 120 μg each of E1, E2, estriol (E3), and EE2, were extracted with 3×20 ml CH₂Cl₂, the extracts were pooled, concentrated, and dried over anhydrous sodium sulfate. Extracts were diluted into 2 ml methylene chloride and 500 μl was saved and analyzed directly, while 1500 μl was derivatized to trimethylsilyl (TMS) derivatives using MSTFA (75 μl) and pyridine (10 μl) at 65 °C for 2 h, dried under nitrogen, then dissolved in 1 ml methylene chloride. Estrogens were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Varian 3800 Gas Chromatograph coupled to a Saturn 2000 mass spectrometer. Columns used were a DB-5ms column (30 m×0.25 mm×0.25 μm) and a 1 m×0.53 mm pre-column. Samples (2 μl) were injected in splitless mode at 80 °C and the injector was heated to 250 °C at 200 °C/min. The oven temperature program was 80 °C for 1.5 min, increased to 180 °C at 50 °C/min, then increased to 300 °C at 20 °C/min and held for 5 min. The MS had a transfer line at 250 °C, EI ion source of 70 eV, and ion trap temperature of 200 °C. The MS-MS was performed for E1 using a precursor ion of 342 and analyzing the daughter ions 244, 245 and 257. The MS-MS for EE2 used a precursor ion of 425 and analyzing the daughter ions 285 and 326. The MS-MS for E2 and 17α-estradiol using a precursor ion of 416 and analyzing the daughter ions 285 and 326. The MS-MS for EE2 used a precursor ion of 425 and analyzing the daughter ions 193, 231, and 407. Peaks detected in the derivatized extracts of the cultures or standards were compared to derivatized authentic standards (10–100 μg) of EE2, E2, E1, E3, 17α-estradiol, 2-hydroxy-17β-estradiol, 4-hydroxy-17β-estradiol and 6-keto-estrone (Sigma Chemical Co), which were all detected, although the latter two were not effectively separated with the temperature program employed.
3. Results

3.1. Transformation of estrogens under different conditions

EE2 was not degraded under anaerobic conditions in any of three independent experiments in which triplicate cultures were established using lake sediment and both E2 and EE2, nor in one experiment in which EE2 was amended to sludge from the anaerobic digestor from a wastewater treatment plant. The cultures were monitored frequently for a total of 725, 594, 383, and 271 days, and again after 35–38 months, and no loss of EE2 was observed, even though reduction of electron acceptors (nitrate, sulfate, iron) and methanogenesis was observed due to the use of endogenous and amended (benzoate, methanol) substrates. For example, Fig. 2A shows the amount of EE2 in cultures established with lake sediment, in which the methanol from the stock solutions of estrogens stimulated methanogenesis (0.7 mmol), iron reduction, sulfate reduction (0.5 mmol), and nitrate reduction (0.6 mmol). The final amount of EE2 in the cultures amended with 5 mg/L EE2 were 3.95±0.39 to 4.41±0.16 mg/L, not significantly different from the sterile controls (4.24±0.28 mg/L).

In contrast, E2 was at least partially removed under all four anaerobic conditions. Both the rate and the extent of transformation varied under the different electron accepting conditions (Fig. 2b). The initial 14 day loss was fastest with iron (325±78 μg L⁻¹ day⁻¹), followed by sulfate (284±31 μg L⁻¹ day⁻¹), carbon dioxide (262±40 μg L⁻¹ day⁻¹), and slowest with nitrate (180±135 μg L⁻¹ day⁻¹). However, the loss of E2 was accompanied by an accumulation of E1. Upon further incubation under iron-reducing conditions, the E2 concentration actually increased from 0.34 mg/L (42 days) to 1.90 mg/L (144 days) and then decreased again to 0.50 mg/L (383 days) as it was re-oxidized back to E1. The total amount of estrogens as E1+E2 ranged from 3.91±0.29 to 4.05±0.58 mg/L, and corresponded to an average of 82% of the sterile controls (Fig. 3), but did not continue to decrease after the initial conversion period. Additional cultures were established under each condition in larger volume (1.5 L), to increase the opportunity for collecting estrogen-degrading anaerobes, yet similar results were obtained. The E2 was oxidized to E1 in all cultures, though the rate of oxidation under nitrate-reducing conditions was relatively faster (Table 1). Trace amounts of E2 remained in the cultures (0.26±0.11 mg/L or 10±4% of the total E1+E2), but most of the estrogen existed as E1 (91±3% of the total E1 E2; 96±16% of the amount of EE2).

3.2. Effects of E1 on E2 oxidation

After the observation that E2 oxidation to E1 was incomplete, a subsequent experiment was conducted in which cultures were established with E2 (4 mg/L) and different initial concentrations of E1 (0.85–3.4 mg/L) to determine whether the end-product E1 inhibited the transformation of E2. Initial rates of E2 transformation to E1 were not affected by the addition of E1 (Fig. 4) except under nitrate-reducing conditions. Under methanogenic conditions, there was an initial loss of E2 over the first two weeks (Fig. 4A) followed by a transient increase in E2 over the subsequent 4 weeks. The amount of E2 observed increased with the amount of E1 added to the cultures, yet 2–3 mg/L E1 remained in the cultures, so the back-conversion to E2 was only partial. E2 was subsequently re-oxidized to E1 with further
incubation, reaching a steady state concentration of E2 of 0.47±0.19 mg/L (80–365 days) regardless of how much E1 was added. Under sulfate-reducing conditions (Fig. 4B), E2 decreased to a steady state of 0.46±0.13 mg/L within the first two weeks as it was oxidized to E1, and this did not change over the course of a year. Under nitrate-reducing conditions (Fig. 4C) the final amount of E2 depended on the amount of E1 added. At the lower estrogen concentrations (4–4.85 mg/L total E1+E2), as used in the previous experiment, E2 was only partially removed to a high steady state value of 1.53±0.19 mg/L. However, with greater amounts of E1 (1.7–3.4 mg/L) the initial oxidation of E2 was rapid. This was followed by a transient accumulation of E2, and a low final steady state concentration of 0.13±0.34 mg/L was reached. Finally, under iron-reducing conditions, the initial oxidation of E2 was rapid regardless of the amount of E1 added. However, E2 was again observed to accumulate transiently, very slowly compared to other conditions (Fig. 4D), then decrease to a final steady-state value of 0.58±

Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Nitrate-reducing</th>
<th>Iron-reducing</th>
<th>Sulfate-reducing</th>
<th>Methanogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ml cultures</td>
<td>21.0±2.3</td>
<td>6.3±2.7</td>
<td>9.1±2.0</td>
<td>15.0±2.2</td>
</tr>
<tr>
<td>100 ml cultures</td>
<td>1.3–21.9</td>
<td>2.5±0.9</td>
<td>5.6±1.0</td>
<td>6.9±1.3</td>
</tr>
<tr>
<td>1.5 L cultures</td>
<td>4.6</td>
<td>12.6</td>
<td>12.1</td>
<td>11.4</td>
</tr>
<tr>
<td>+E1 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+EE2 b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Cultures to which both E2 and E1 were added.
b Single cultures to which both E2 and EE2 were added.
0.39 mg/L. Under all four conditions, recovery of total estrogens as E1+E2 was typically 80–90% of sterile controls after E2 conversion was complete. Reduction of supplied electron acceptors was observed in all cultures, indicating active anaerobic populations.

3.3. Metabolites

Estrone was detected as an accumulated product of E2 biotransformation in all cultures except sterile controls (Fig. 5). E1 was detected in trace amounts (<0.25 mg/L) in some sterile control cultures, even when there was no evidence for contamination (chemical or biological). The presence of estrone was confirmed by GC-MS analysis of culture extracts. In addition, several other peaks were detected by HPLC that corresponded to the loss of E2. One of the additional peaks detected had the same retention time as estriol, under a variety of eluent compositions. It was detected only in some active cultures and not in sterile or negative controls (Fig. 5). This suggested that under anaerobic conditions, as under aerobic conditions, further transformation of E1 may be via E3. However, E3 was not detected by GC-MS analysis of culture extracts, so the identity of this metabolite cannot be confirmed. Another peak detected by HPLC eluted prior to E2, and matched the retention time of 17-α-estradiol. GC-MS analysis also detected the TMS derivative of 17-α-estradiol in active cultures under methanogenic, sulfate-reducing and iron-reducing conditions (Fig. 6), but not under nitrate-reducing conditions. The identity was consistent with an authentic standard in terms of retention time, mass spectrum, and even the secondary MS-MS spectrum (Fig. 6). This peak was not detected in the sterile or negative controls. Based on the GC-MS analysis of the TMS derivatives, the amount of 17-α-estradiol was less than the amount of 17-β-estradiol under methanogenic (0.94±0.29, 2.82±1.24 mg/L), sulfate-reducing (1.32±0.46, 2.41±1.07 mg/L), and iron-reducing conditions (0.93±0.08, 1.88±0.43 mg/L). The total recovery of the two forms of estradiol as a percentage of the amount of EE2 measured (as an internal standard) was 71±10%, 77±19%, 56±15%, and 60±2%.

A

![HPLC chromatogram A](image1)

B

![HPLC chromatogram B](image2)

Fig. 5. HPLC chromatograms showing the disappearance of E2, and appearance of putative metabolites including E1, 17-α-estradiol, and possibly E3, in a sulfate-reducing culture (A) compared to the sterile control (B).
under methanogenic, sulfate-, iron-, and nitrate-reducing conditions, respectively. Therefore, the accumulation of 17-α-estradiol would account for most, but not all, of the “missing” estradiol in cultures where it was detected.

4. Discussion

Biological transformation of estrogens under anaerobic conditions was limited. EE2 degradation was not observed in our experiments, in spite of the presence of viable anaerobic communities as evidenced by the metabolism of endogenous substrates, as well as metabolism of methanol added as a carrier for the EE2, and the benzoate added to positive controls. There was no evidence of inhibition of anaerobic activity due to the addition of estrogens, and the concentrations used (5 mg/L, 17 μM) were just below the aqueous solubility of these compounds, yet barely sufficient for confirmation that enhanced electron-acceptor reduction would...
take place. Some loss of EE2 (up to 10%) was observed in the first few days in all cultures including sterile controls, due to binding to the sediments. Mechanistically, the ethynyl group in the 17-position of the synthetic EE2 blocks the potential formation of a ketone (as observed for the natural estrogen E2) and sterically hinders access to the hydroxyl group in the 17-position, making this compound more recalcitrant than its natural counterpart (Bolt, 1979). However, there is still a hydroxyl group available in the 3-position, on the aromatic ring, which should make this compound more amenable to biodegradation than polycyclic hydrocarbons. Many large organic compounds that were considered recalcitrant under anaerobic conditions have recently been shown to undergo biodegradation, and the same may some day be true for EE2. However, the results of our experiments suggest that the fate of EE2 under anoxic conditions is dominated by sorption rather than biodegradation.

We consistently observed transformation of E2 to E1. This observation has been reported previously for cultures established with sediments (Jürgens et al., 2002; Lee and Liu, 2002; Ying and Kookana, 2003), although strict anaerobic techniques had not been used, so the possibility of transformation due to the presence of lingering oxygen could not be ruled out. However, we have gone much further in demonstrating E2 transformation under four different strictly anaerobic conditions, and comparing both the rate of transformation and final steady-state concentrations. This oxidation probably involves an NADH-dependent hydroxysteroid dehydrogenase, and therefore we expected that the rate and extent of transformation of E2 to E1 under the different electron-accepting conditions would correspond to the redox potential or the amount of free energy thermodynamically available from the use of the different electron acceptors. However, this was not observed, and there was no clear correlation between the use of electron acceptors and the rate or extent of transformation. It is unlikely that the oxidation of E2 to E1 provides energy to the cells, so the reaction probably represents the use of the E2 as an alternative electron acceptor to regenerate cofactors (NAD). The rates of transformation we observed, with an average E2 half life of 12 days, were slower than those observed in a previous study that found that E2 was transformed to E1 with a half life of 0.37 or 0.66 days, in anaerobic cultures established with river water and sediment (Jürgens et al., 2002), yet faster than another study that found a half life of 67 days for E2 transformation under sulfate-reducing conditions (Ying and Kookana, 2003) (both with initial E2 concentrations of 0.5 mg/L).

Further research is needed on transformation rates, particularly the variable rates under nitrate-reducing conditions, as denitrification could potentially be a process to transform estrogens within wastewater treatment plants, before they enter the natural aquatic environment.

The final concentration of E2 remaining under the four anaerobic conditions was relatively high at 0.2 to 2.2 mg/L. The incomplete oxidation of E2 was clearly not due to inhibition by E1. Both the rate of initial oxidation and the final steady-state concentration of E2 were independent of the amount of E1 added, except under nitrate-reducing conditions, in which a bimodal effect was observed. The distribution of total estrogens between E2 and E1 was determined solely by the final steady-state concentration of E2, and not by a proportion — the amount of E1 in each culture simply reflected the rest of the estrogens that were not E2. The exception to this was under nitrate-reducing conditions, in which two different patterns were observed. If the total estrogens were 5 mg/L or less, relatively slow and limited conversion of E2 took place (final 1.55±0.17 mg/L) (Figs. 2B, 4C), whereas if >5 mg/L total estrogens were added, the estrogen concentration decreased quickly to a low final steady-state value of 0.05±0.06 (Fig. 4C). This suggests that multiple enzymes may be involved in the oxidation of E2 under these conditions, and that 5 mg/L is a critical threshold for E2 transformation under the experimental conditions used. More research is needed on the anaerobic transformation of estrogens at lower, environmentally relevant concentrations, but would have to involve a different approach such as the use of 14C-labelled estrogens.

The transformation of E2 to metabolites like estrone occurred under all four anaerobic conditions. The recovery of E1+E2 was 78–81% of the amount added, compared to 97% for sterile controls, indicating that other transformation products were also accumulating. Metabolites were observed by HPLC analysis of the cultures, and preliminary evidence for accumulation of estriol was found. Other peaks detected by HPLC did not correspond to other known metabolites of E2, including 2-hydroxy-17β-estradiol, 4-hydroxy-17β-estradiol and 6-keto-estrone (Bolt, 1979). This suggests that novel E2 metabolites may be generated under anaerobic conditions, and merits further investigation. One metabolite was conclusively identified as 17α-estradiol. This metabolite was detected in comparable amounts in cultures with lake sediment under methanogenic, sulfate-reducing and iron-reducing conditions, regardless of the pattern by which the E2 was transformed to E1 and/or back to E2 again. The
racemization probably occurred during the reduction of the ketone (E1) to the alcohol (17-α or 17-β E2). Further experiments could be done to examine the rates of 17-α-estradiol formation directly from estrone, and the relative proportions of 17-α- and 17-β-estradiol formed. This is the first known report of microbial production of 17-α-estradiol. Further investigation of the biochemistry of this reaction (mechanism, kinetics, etc.) would have potential synthetic applications for pharmaceuticals, as the alpha-form of estrogens are difficult and expensive to make (Zsigmond et al., 2005).

Under all the anaerobic conditions tested, the observed transformations of E2 would only partially reduce the estrogenicity of the water, as the E2 was mostly just converted to estrone or to 17-α-estradiol. The 17-α-estradiol is much less estrogenic than the normal beta-form, but still somewhat estrogenic (Hobe et al., 2002; Sievernich et al., 2004). Concentrations of the estrogens E2 and E1 expected in natural anoxic systems range from up to 100 ng/L in anaerobic digestors in WWTP (Ying et al., 2003), up to 675 ng/kg in soils (Finlay-Moore et al., 2000), to less than 10 ng/kg in lake and river sediments (Williams et al., 1999). Estrogens have a tendency to sorb to sediments (Holthaus et al., 2002; Lai et al., 2000; Ying and Kookana, 2003; Ying et al., 2004; Yu et al., 2004), so local concentrations in anoxic sediments could be higher, and E1 has been found in river sediments at <0.04 to 22.8 ng/g (Kuster et al., 2004). At these concentrations, binding to particles should out-compete anaerobic biodegradation as a fate process for the estrogens, although conducting biodegradation experiments and calculating adsorption isotherms under anaerobic conditions at sub-microgram per liter concentrations would be needed to conclusively confirm this hypothesis. Our results indicate that if the sediments become anoxic, estrogens have the potential to be recalcitrant and to accumulate in the sediments. Understanding the microbiology of estrogen biodegradation will help predict the fate of these compounds when they enter natural ecosystems such as lakes and rivers, and will also help us to improve their removal from wastewater streams prior to their release into the environment.

Acknowledgements

We thank Tess Laidlaw, and Melanie Buss for technical assistance.

This work was supported by grants and scholarships from the University of Manitoba, and the Natural Sciences and Engineering Research Council of Canada.

References


Lee LS, Strock TJ, Sarmah AK, Rao PSC. Sorption and dissipation of testosterone, estrogens, and their primary transformation