Ferrate(VI) and freeze-thaw treatment for oxidation of hormones and inactivation of fecal coliforms in sludge

James Diak and Banu Örmeci

ABSTRACT

This study examined the individual and combined effects of potassium ferrate(VI) additions and freeze-thaw conditioning for the treatment and dewatering of wastewater sludge in cold climates, with particular focus on the inactivation of fecal coliforms and oxidation of estrogens, androgens, and progestogens. The first phase of the study evaluated the effects of potassium ferrate(VI) pre-treatment followed by freeze-thaw at \(-20\) °C using a low (0.5 g/L) and high (5.0 g/L) dose of potassium ferrate(VI). The results showed that pre-treatment of anaerobically digested sludge with 5 g/L of potassium ferrate(VI) reduced the concentration of fecal coliforms in the sludge cake to below 100 MPN/g DS. The second phase evaluated the ability of ferrate(VI) to oxidise selected hormones in sludge. Anaerobically digested sludge samples were spiked with 10 different hormones: estrone (E1), \(17\alpha\)-estradiol, \(17\beta\)-estradiol (E2), estriol (E3), \(17\alpha\)-ethinylestradiol (EE2), equilin, mestranol, testosterone, norethindrone and norgestrel in two groups of low (3–75 ng/mL) and high (12–300 ng/L) concentration ranges of hormones. The samples were treated with either 0.5 or 1.0 g/L of potassium ferrate(VI), and hormone concentrations were measured again after treatment. Potassium ferrate(VI) additions as low as 1.0 g/L reduced the concentration of estrogens in sludge. Potassium ferrate(VI) additions of 0.5 and 1.0 g/L were less effective at reducing the concentrations of androgens and progestogens. Increasing ferrate(VI) dose would likely result in more substantial decreases in the concentrations of fecal coliforms and hormones. The results of this study indicate that the combined use of freeze-thaw and ferrate(VI) has the potential to provide a complete sludge treatment solution in cold regions.

Key words | coliform bacteria, endocrine disrupting compounds (EDCs), ferrate, freeze-thaw, hormones, sludge

INTRODUCTION

There has been considerable interest in freeze-thaw technology to help solve the sludge treatment problems in cold climates. When sludge freezes, ice crystals grow, which consolidate sludge solids and create a continuous network of ice. When the sludge thaws, the meltwater drains freely, leaving a dewatered cake. Freeze-thaw can be used to dewater sludge (Parker et al. 1998; Northcott et al. 2005; Diak et al. 2011; Hu et al. 2011) and reduce pathogens and indicator bacteria (Sanin et al. 1994; Kato et al. 2002; Gao et al. 2006, 2009) to some level, but not enough for beneficial use.

The ferrate(VI) ion, \(\text{FeO}_4^{2-}\), is a very strong oxidant (oxidation reduction potential, \(E^0 = 2.2\) V in acidic conditions, 0.72 V in alkaline), in which iron exists at a +6 oxidation state (Li et al. 2005; Mácová et al. 2009). It is an attractive chemical oxidant for environmental remediation, since the spent ferrate(VI) is reduced to environmentally benign Fe(III) species (Jiang 2007; Yang et al. 2022a). Ferrate(VI) is capable of oxidising odorous compounds, pathogens and indicator organisms (Schink & Waite 1980; Read et al. 2003; Schuck et al. 2006; He et al. 2009; Ding et al. 2012), and furthermore, once the ferrate ion has been reduced, ferric hydroxide is generated, which acts as a coagulant (Gombos et al. 2013). It is also capable of oxidising a variety of pharmaceuticals and personal care products (Zhu et al. 2006; Jiang 2007; Li et al. 2008; Yang et al. 2012b; Jiang & Zho 2013). Therefore, the combined use of freeze-thaw and ferrate(VI) has the potential to provide a complete sludge treatment solution in cold regions.

Although several studies have demonstrated that ferrate(VI) is capable of oxidising a variety of endocrine
disrupting compounds (EDCs) in water and wastewater (Read et al. 2003; Sharma & Mishra 2006; Jiang 2007; Li et al. 2008; Yang et al. 2012b; Jiang & Zhoo 2013), very few studies have investigated the ability of ferrate(VI) to oxidise EDCs in wastewater sludges. There are a variety of oxidisable components in sludge, meaning that there are countless competing reactions with the highly reactive (and short-lived) ferrate(VI) ions. As a result, the ferrate(VI) may be quickly consumed by the other sludge constituents, leaving very little or no oxidising strength for the EDCs in the sludge. Thus, it is important to determine the dose range that would be required for sludge treatment and whether this dose would be feasible.

The goal of this study was to investigate the individual and combined effects of potassium ferrate(VI) and freeze-thaw conditioning for the inactivation of coliform bacteria, and the oxidation of hormones in wastewater sludge to provide an alternative sludge treatment and dewatering method in cold climate regions.

MATERIALS AND METHODS

Hormone stock solutions

The hormones used in these experiments were seven estrogens: estrone (E1) (>99), 17α-estradiol (>98), 17β-estradiol (E2) (>98%), 17α-ethinylestradiol (EE2) (>98%), estriol (E3) (>97%), mestranol (99%), and equilin (>99), the progestins (synthetic progestogens): norethindrone (>98%) and norgestrel (98%), and the natural androgen: testosterone (99%). All hormones were purchased from Sigma-Aldrich, Co. (St Louis, MO, USA). To prepare the hormone stock solutions, 0.5 mg of each hormone was weighed using a Sartorius MC21S digital balance (Sartorius Canada Inc., Mississauga, ON), and placed in 0.5 mL sample vials. 0.5 mL of methanol was added to each vial, and the resulting 1 mg/mL solutions were mixed using a vortex mixer (Baxter Diagnostics Inc., IL, USA). To prepare the hormone stock solutions, 0.5 mg of each hormone was weighed using a Sartorius MC21S digital balance (Sartorius Canada Inc., Mississauga, ON), and placed in 0.5 mL sample vials. 0.5 mL of methanol was added to each vial, and the resulting 1 mg/mL solutions were mixed using a vortex mixer (Baxter Diagnostics Inc., IL, USA). Most of the hormone solutions were diluted a second time. For these samples, 0.05 mL portions of the 1 mg/mL solution were added to 0.45 mL of methanol to create the 100,000 ng/mL stock solutions. All stock solutions were stored in the freezer until required.

Sludge sample and hormone spiking

Anaerobically digested sludge obtained from a wastewater treatment plant in Ontario, Canada, was used in the experiments. The initial total solids (TS) and volatile solids (VS) concentrations of the sludge were 20.3 and 11.7 g/L, respectively, and were measured according to Standard Methods 2540 B and 2540 E (APHA 2005). Two 100 mL sludge samples were placed in 250 mL Erlenmeyer flasks, and stirred using a magnetic stirrer. Individually prepared stock solutions containing the hormones were removed from the freezer and thawed at room temperature. Once the vials of stock solutions were completely thawed and warmed to room temperature, a vortex mixer was used to thoroughly mix the stock solutions. The concentration of each hormone stock solution was 10³ ng/mL, except for norgestrel, which was 10⁶ ng/mL. The spiked initial concentrations of each hormone in the low concentration and the high concentration sludge samples are presented in Table 1. Following the addition of the selected hormones, the sludge samples were stirred using a magnetic stirrer for 4 hours. Three subsets from each of the two sludge samples were removed. The first subset was used to measure the initial concentration of hormones, and the other two were used for subsequent ferrate(VI) treatments.

Ferrate(VI) treatment

Ferrate(VI) treatments were conducted using Ferratec Brand™ >90% pure potassium ferrate salt (K₂FeO₄) from Sigma-Aldrich Canada Ltd, in Oakville, ON, Canada. For the fecal coliform inactivation experiments, anaerobically

<p>| Spiked concentrations of the selected hormones in the low concentration and high concentration sludge samples used for subsequent tests with potassium ferrate(VI) |</p>
<table>
<thead>
<tr>
<th>Hormone</th>
<th>Spiked concentration (ng/mL)</th>
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<tbody>
<tr>
<td></td>
<td>Low concentration range</td>
</tr>
<tr>
<td>Equilin</td>
<td>30</td>
</tr>
<tr>
<td>Estrone (E1)</td>
<td>15</td>
</tr>
<tr>
<td>17β-Estradiol (E2)</td>
<td>15</td>
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<tr>
<td>17α-Estradiol</td>
<td>15</td>
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<tr>
<td>17α-Ethinylestradiol (EE2)</td>
<td>15</td>
</tr>
<tr>
<td>Estradiol (E3)</td>
<td>60</td>
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<tr>
<td>Mestranol</td>
<td>75</td>
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<td>Norethindrone</td>
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<td>Norgestrel</td>
<td>15</td>
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<td>Testosterone</td>
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</table>

*a ng/mL is equivalent to parts per billion (ppb).*
digested sludge samples were treated at a low dose (LD = 1.0 g/L) or a high dose (HD = 5.0 g/L) of potassium ferrate(VI). Following the addition of the potassium ferrate(VI), the samples were stirred using a magnetic stirrer for 15 minutes, and a sub-sample was removed to measure fecal coliform inactivation in the sludge following the ferrate(VI) treatments. 200 mL samples of anaerobically digested sludge (untreated, LD and HD samples) were then placed in 500 mL bottles. The bottles were sealed, inverted and placed in a freezer set to –20 °C. The freezing rate generated was approximately 6 mm/hr (4.5 hrs to travel 25 mm). Once the samples were completely frozen, they were kept frozen at –20 °C for an additional 1, 8 or 15 days. After the designated time in the freezer, the samples were removed, and the solid lid was replaced by a perforated lid lined with drainage fabric. The frozen samples were then placed upside down in a funnel, thawed at room temperature (22–25 °C), and the meltwater was collected via gravity drainage. The concentration of fecal coliforms in the remaining sludge cake was measured using multiple tube fermentation (MTF) with A1 medium (EMD Chemicals Inc., Gibbstown, NJ, USA) according to US EPA Method 1681 (US EPA 2005). All MTF tests were carried out in duplicate using a minimum of four dilutions, with five replicates per dilution.

For the hormone oxidation experiments, potassium ferrate(VI) was used to oxidise the spiked hormones in the anaerobiocally digested sludge samples, without any freeze-thaw treatments. Two levels of ferrate(VI) treatment were used: an LD of 0.5 g/L and an HD of 1.0 g/L of potassium ferrate(VI). The equivalent ferrate(VI) ion added was approximately 0.3 (>0.27) and 0.6 (>0.55) mg/L of FeO4²⁻. After spiking the anaerobiocally digested sludge samples with the hormones, 40 mL sludge samples were treated with 20 and 40 mg of potassium ferrate(VI) salt, and then mixed using a vortex mixer for 5 minutes.

**Hormone analysis**

Axys Analytical Services Ltd in Sidney, BC, Canada, performed the extraction of hormones from the liquid sludge samples (TS = 2%) and measured the concentrations of hormones using AXYS Method MLA-072 (AXYS 2012) on a high performance liquid chromatograph coupled to a triple quadrupole mass spectrophotometer (LC-MS/MS). All information related to the analysis of hormones, including hormone extraction and clean-up, has been taken from the Method Summary of AXYS Method MLA-072 Revision 03 Version 02 (AXYS 2012). Prior to hormone extraction and clean-up procedures, the pH of each sample was adjusted to 2.0. The aqueous phase of each sample was obtained through filtration, to remove all particulates. The solid phase of each sample was extracted by sonication with aqueous buffered acetonitrile and pure acetonitrile, then concentrated using rotary evaporation, and diluted to a volume of 200 mL with ultra-pure water. The aqueous samples and the diluted extracts from the solid phase were cleaned up using solid phase extraction, and the recovery standards (13C6-13C3-atrazine, 2,4,5-trichlorophenoxyacetic acid) were added to the extracts. After the addition of the recovery standards, the samples were filtered, then analysed via LC-MS/MS. The reporting limits for the Method MLA-072 range from 0.3 to 150 ng/g based on a 1 g solid sample, and from 0.3 to 150 ng/L for a 1 L aqueous sample. The results for each of the samples were reported on a dry weight (ng/g dry solids) basis, and the results were converted to a wet basis using the TS concentrations of the representative samples. All figures show the concentrations of hormones in the anaerobiocally digested (liquid) sludge samples, expressed in units of ng/mL.

**Statistical analysis**

For the fecal coliform inactivation experiments, all MTF tests were carried out in duplicate, using a minimum of four dilutions, with five replicates per dilution. Error bars on all figures show the 95% confidence interval, which is built in to the MTF test. A two-tailed t-test was used to determine the p values and statistical significance (p < 0.05) of the fecal coliform inactivation following freeze-thaw and ferrate(VI) treatments.

For the hormone oxidation experiments, an independent samples t-test was used to estimate the magnitude of the HD (1.0 g/L) potassium ferrate(VI) treatments on the concentrations of estrogens in the sludge samples. All samples were analysed by AXYS via LC-MS/MS, however due to the high cost of analysis, only one measurement was performed per sample. These individual measurements were considered to be the mean estimates for each of the untreated and treated samples. To estimate the error for each of the sample measurements, calibration data for each analyte (five to seven replicates each) were used. For each calibration test, a percent recovery was determined, which varied greater or lesser than the targeted recovery (i.e. 100%). The residuals of each replicate were then squared, summed, and averaged to obtain what was considered the variance of the calibration tests, and the square root of these variances were assumed to be the standard deviations (SD). Because the target value of each calibration test was 100%, the standard deviations of these calibration tests was a percent error, which was used...
to estimate the SD of the individual analyte concentration measurements (measurement error). The analyte concentration of the untreated sludge sample \((X_1)\), the analyte concentration of the ferrate(VI)-treated sample \((X_2)\), and the estimated error of both samples \((SD_1, SD_2)\) were then used to calculate the Cohen’s \(d\) effect size, and estimate the magnitude of the ferrate(VI) treatment effects. Cohen (1988) defined the effect sizes as small when \(d = 0.2\), medium when \(d = 0.5\) and large when \(d = 0.8\), whereas Plonsky & Oswald (2014) suggest \(d = 0.4\) as small, \(d = 0.7\) as medium and \(d = 1.0\) as large effect sizes. For this study, we used the more conservative Plonsky and Oswald’s guidelines.

**RESULTS AND DISCUSSION**

**Fecal coliforms**

Figure 1 shows the effect of LD and HD ferrate(VI) treatments on the concentration of fecal coliforms in the sludge when added prior to freeze-thaw with meltwater separation. The initial concentration of fecal coliforms in the anaerobically digested sludge was approximately \(3.9 \times 10^2\) MPN/g DS. Following a 15-minute contact time, HD resulted in a 2.1-log fecal coliform inactivation, whereas LD did not have a significant effect \((p = 0.3910)\). When LD was used as a pre-
treatment to freeze-thaw, overall fecal coliform inactivation in the sludge cake ranged from 0.9 to 2.0-log. However, stand-alone freeze-thaw resulted in 1.4 to 2.1-log inactivation, suggesting that LD pre-treatment did not improve the fecal coliform inactivation caused by stand-alone freeze-thaw \((p = 0.1354)\). The length of time that the samples were kept frozen \((1, 8\) or \(15\) days at \(-20\) °C) did not have a significant effect on fecal coliform inactivation in the sludge cake \((p > 0.05)\). These results are similar to the results from previous studies by Diak & Örmeci (2016) and Sanin et al. (1994), when samples were kept frozen for <15 days. Similarly, in the experiments by Gao et al. (2006), it was demonstrated that 95% inactivation of Escherichia coli occurred during the first freezing cycle, therefore subsequent freezing cycles did not have a large contribution to the overall E. coli inactivation. These results suggest that fecal coliform inactivation is predominantly the result of the freezing process, and less to do with the duration of the frozen conditions.

When HD was used as a pre-treatment with freeze-thaw, overall fecal coliform inactivation ranged from 3.6 to 3.9-log, which is 1.8 to 2.2-log greater than stand-alone freeze-thaw, indicating that pre-treatment using 5 g/L of potassium ferrate(VI) prior to freeze-thaw increases the fecal coliform inactivation caused by stand-alone freeze-thaw \((p = 0.0016)\).

**Estrogens**

The estrogens used in this study were selected due to their general persistence and adverse effects in the environment. Furthermore, the log KOW values for all spiked estrogens range from 2.45 to 4.68, which means they have a high affinity for the organic phase, and are likely to bind with the organic solids in wastewater sludges. Other estrogens, which were not spiked but present in the sludge, were also measured. Two different sludge samples were used in this study: a sample that was spiked with a low concentration range of estrogens \((15–75\) ng/mL), and a sample that was spiked with a high concentration range of estrogens \((60–300\) ng/mL). Figure 2(a) shows the spiked and measured initial concentrations of selected estrogens in the low concentration sludge sample, and the effects of LD and HD ferrate(VI) treatments on the selected estrogens, and Figure 2(b) shows the spiked and measured initial concentrations of selected estrogens in the high concentration sludge sample, and the effects of LD and HD treatments on the measured estrogens.

In most cases, the measured concentration of a particular hormone was lower than the spiked concentration. This was likely due to adsorption onto the walls of the sample container. It could also be due to biotransformations to
other (measured and not measured) compounds. Ternes et al. (1993) demonstrated that spiked E2 biodegraded to E1 in the presence of activated sludge, and others have confirmed that E2 biodegrades under both aerobic and anaerobic conditions (Hamid & Eskicioglu 2012). This may explain why the measured concentrations of E2 were consistently below the specific detection limits (SDLs), and why E1 concentrations in the untreated samples were greater than the intended (spiked) concentrations, unlike the other compounds. Another source of error is potentially due to variability in the sampling and measurements. It is very difficult to extract and quantify low concentrations of hormones in a complex matrix such as sludge. During the LC-MS/MS calibration tests, the percent recovery of some analytes varied from as low as 65% and up to 136%, and during the LC-MS/MS sample analysis runs, the percent recovery of the labelled surrogates varied from 68 to 114% (average = 90%, SD = 17%) over the 12 runs.

For all comparisons, the measured initial concentrations were used to evaluate the effects of the ferrate(VI) treatments.

Table 2 shows the percent change in the concentrations of estrogens in the low concentration and the high concentration sludge samples following the addition of 0.5 g/L (LD) and 1.0 g/L (HD) of potassium ferrate(VI). The measured initial concentrations of E1 were reduced by 15 and 24% following HD treatment on the low concentration and high concentration sludge samples respectively. Similarly, HD treatment on the low and high concentration sludge samples reduced E3 by 36 and 42%, EE2 by 11 and 7%, equilin by 20 and 25%, and equilenin by 1 and 24%, respectively. 17α-dihydroequilin was consistently detected at very small concentrations (<0.9 ng/mL) in all samples; however, the measured values were all less than the SDL, as indicated by the downwards arrows in the figures. Excluding the readings for E2 and 17α-dihydroequilin, which were below the SDL, HD decreased the concentrations of measured estrogens (E1, E3, EE2, 17α-estradiol, mestranol, equilin and equilenin) by an average of 16% and 22% in the low hormone concentration and high hormone concentration sludge samples, respectively. The increases observed in some hormones might be due to the biotransformation process.

An independent samples t-test was used to estimate the magnitude of the HD (1.0 g/L) potassium ferrate(VI) treatments on the concentration of estrogens in the sludge samples. The analyte concentrations of the untreated and the ferrate(VI)-treated samples, and the estimated error of both samples were used to calculate the Cohen’s d effect

| Table 2 | Percent change in the concentrations of the measured estrogens in the low estrogen concentration range (15–75 ng/mL) and the high estrogen concentration range (60–300 ng/mL) sludge samples following the addition of 0.5 g/L (LD) and 1.0 g/L (HD) of potassium ferrate(VI) |
|------------------------------------------------|
| **Estrogen** | **Low concentration range** | **High concentration range** |
| | **LD** | **HD** | **LD** | **HD** |
| Estrone (E1) | 3.0 | −15.2 | 1.7 | −23.4 |
| 17β-Estradiol (E2) | 1.2<sup>a</sup> | −0.5<sup>a</sup> | 1.4<sup>a</sup> | 0.1<sup>a</sup> |
| 17α-Estradiol | 5.8 | −15.2 | −10.1 | −23.5 |
| Estradiol (E3) | −14.2 | −36.2 | −15.4 | −42.1 |
| 17α-Ethinylestradiol (EE2) | 2.9 | −11.4 | −0.6 | −13.3 |
| Mestranol | −28.7 | −11.0 | −7.9 | −7.1 |
| Equilin | −2.5 | −20.2 | 1.4 | −23.1 |
| Equilenin | −1.0 | −1.4 | −3.1 | −24.1 |
| 17α-Dihydroequilin | −142<sup>a</sup> | −15.6<sup>a</sup> | 1.4<sup>a</sup> | 0.1<sup>a</sup> |

<sup>a</sup>Indicates concentration measurements that were below the reporting limit.
size for each of the estrogens that were measured above the SDs. Table 3 presents the data for the untreated (X1, SD1) and HD ferrate(VI)-treated (X2, SD2) samples used to calculate the Cohen’s d effect sizes (listed in Table 3) for the low and high hormone concentration range sludge samples.

The Cohen’s d effect size for the comparisons of the E1, E2, 17α-estradiol, E3, EE2 and equilin concentrations in the untreated (X1, SD1) and HD ferrate(VI)-treated (X2, SD2) samples were large (Cohen’s d ≥ 1.0), in both the low and high hormone concentration sludge samples. This suggests that the HD ferrate(VI) treatments had a large impact on the concentrations of these estrogens. On the other hand, mestranol showed a small to medium effect size (Cohen’s d = 0.7) in the low and high hormone concentration sludge samples, while equilenin had a small effect size (Cohen’s d = 0.1) in the low hormone concentration sample, and a large effect size (Cohen’s d = 2.6) in the high hormone concentration sample.

LD treatment with 0.5 g/L of potassium ferrate(VI) had mixed effects on the measured estrogens. Overall, LD resulted in a 5% average reduction in the concentration of estrogens in both the low concentration and the high concentration sludge samples; however, generally the change in concentration following LD treatment was negligible. Estriol (E3) and mestranol, on the other hand, were reduced by 14 and 29%, respectively, in the low concentration sludge sample, and by 15 and 8%, respectively, in the high concentration sludge sample. The Cohen’s d effect sizes for E3 were 0.6 (X1 = 24.2, SD1 = 5.9, X2 = 20.8, SD2 = 5.1) and 0.7 (X1 = 150.9, SD1 = 36.9, X2 = 127.7, SD2 = 31.2) for the low and high hormone concentration range samples, respectively, which is a small to medium effect size. The Cohen’s d effect size for mestranol was 1.6 (X1 = 80.6, SD1 = 17.0, X2 = 57.5, SD2 = 12.1) for the low hormone concentration range, suggesting a large effect. However, the Cohen’s d effect size for mestranol was only 0.4 (X1 = 197.1, SD1 = 41.6, X2 = 181.5, SD2 = 38.3) for the high hormone concentration range, suggesting a small effect. In most cases however, LD ferrate(VI) treatment did not result in a large effect size.

### Androgens and progestogens

In addition to the seven estrogens (natural and synthetic) that were added to the sludge, a natural androgen (testosterone), and two synthetic progestogens or progestins (northindrone and norgestrel) were also added.

Figure 3(a) shows the spiked and measured initial concentrations of androgens and progestogens in the low concentration (3–15 ng/mL) sludge sample, and the effects of LD and HD treatments on the selected hormones. Figure 3(b) shows the spiked and measured initial concentrations of androgens and progestogens in the high concentration (12–60 ng/mL) sludge sample, and the effects of LD and HD treatments on the selected hormones. In addition to the three hormones that were spiked

<table>
<thead>
<tr>
<th>Estrogen</th>
<th>Low concentration range</th>
<th>High concentration range</th>
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<tbody>
<tr>
<td></td>
<td>X1 (ng/mL)</td>
<td>SD1 (ng/mL)</td>
</tr>
<tr>
<td>Estrone (E1)</td>
<td>18.8</td>
<td>3.0</td>
</tr>
<tr>
<td>17β-Estradiol (E2)</td>
<td>0.8a</td>
<td>0.1</td>
</tr>
<tr>
<td>17α-Estradiol</td>
<td>12.7</td>
<td>1.2</td>
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<tr>
<td>Estriol (E3)</td>
<td>24.3</td>
<td>5.9</td>
</tr>
<tr>
<td>17α-Ethinylestradiol (EE2)</td>
<td>9.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Mestranol</td>
<td>80.6</td>
<td>17.0</td>
</tr>
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<td>Equilin</td>
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<td>Equilenin</td>
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<tr>
<td>17α-Dihydroequilin</td>
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</table>

*HD = 1.0 g/L potassium ferrate used for all ferrate-treated samples.
*aIndicates concentration measurements that were below the reporting limit.
(testosterone, nortindrone and norgestrel), androstenedione and androsterone (androgens), progesterone and allyl trenbolone (progestogens) and desogestrel (progestin) were also measured due to the potential for transformations among the hormones. Similar to the analysis of estrogens, the measured initial concentrations of testosterone, nortindrone and norgestrel were significantly lower than the spiked concentrations in both the low concentration and the high concentration sludge samples likely due to adsorption onto the sample container, variability in the sampling and measurements, or due to biotransformations in the presence of the sludge biomass.

In general, the LD (0.5 g/L) and HD (1.0 g/L) treatments with potassium ferrate(VI) did not have a large effect on the concentration of androgens and progestogens in sludge. This is likely due to the competing reactions taking place in the sludge, and to the relatively LDs of potassium ferrate(VI) doses used. The percent change in the concentrations of all measured androgens and progestogens following the addition of LD and HD ferrate(VI) treatments are presented in Table 4. The shaded values with an asterisk (*) are values that were below the individual SDLs for the target compounds.

<table>
<thead>
<tr>
<th>Androgen/Progestogen</th>
<th>Change in concentration (%)</th>
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<tr>
<td></td>
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<tr>
<td></td>
<td>LD</td>
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<td>Testosterone</td>
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<td>Androstenedione</td>
<td>1.7</td>
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<tr>
<td>Androsterone</td>
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<tr>
<td>Progesterone</td>
<td>−2.2</td>
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<tr>
<td>Allyl Trenbolone</td>
<td>1.7a</td>
</tr>
<tr>
<td>Norethindrone</td>
<td>−3.0</td>
</tr>
<tr>
<td>Norgestrel</td>
<td>−0.8a</td>
</tr>
<tr>
<td>Desogestrel</td>
<td>−3.0a</td>
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</tbody>
</table>

*Indicates concentration measurements that were below the reporting limit.

**CONCLUSION**

The results of this study showed that pre-treatment of anaerobically digested sludge treated with 5 g/L of potassium ferrate(VI), followed by freeze-thaw with meltwater drainage, reduced the concentration of fecal coliforms in the sludge cake to below 100 MPN/g DS, representing >3.5-log inactivation. The study also demonstrated that potassium ferrate(VI) additions as low as 1.0 g/L can reduce the concentration of estrogens in sludge. A 1.0 g/L dose of potassium ferrate(VI) reduced the concentration of E1 by 15 and 23%, E3 by 36 and 42%, and EE2 by 11 and 13% in the low concentration and high concentration sludge samples, respectively. Potassium ferrate(VI) additions of 0.5 and 1.0 g/L were less effective at reducing the concentrations of measured androgens and progestogens; however, most of these hormones were measured at concentrations that were near or below the SDL for the specific target compound.

While these experiments aimed to keep the dose and cost of the ferrate(VI) treatment low, higher doses of ferrate(VI) would likely result in more substantial decreases in the concentrations of fecal coliforms, pathogens, hormones, other emerging contaminants and odour causing compounds. Ferrate(VI) offers several advantages including its very
strong oxidizing capacity and no by-product formation, and recent developments in the on-line generation of ferrate have brought down the cost substantially and increased its applicability for wastewater and sludge treatment.

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Ver 02: Analysis of Hormones in Solid and Aqueous Samples by LC-MS/MS. (proprietary).


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