Use of a Biological Polishing Step to Improve Bioassay Test Results

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Abstract The Clean Water Act requires point source dischargers to determine toxicity of facility effluent using Whole Effluent Toxicity (WET) tests. At a permitted facility in Missouri where the primary contaminants are metals, wastewater treatment plant effluent meets stringent numerical discharge limits but periodically fails WET tests. Previous investigations indicated low alkalinity in the WWTP effluent was causing WET toxicity. Use of a biological polishing treatment step to increase alkalinity and improve WET results was examined. Effluent from an anaerobic biocell and aerobic limestone tank were submitted to a laboratory for chronic WET testing. Only effluent from the anaerobic biocell improved alkalinity and passed a chronic WET test.

Keywords Whole Effluent Toxicity, WET, biotreatment, alkalinity, wastewater treatment, Missouri

Introduction The Clean Water Act requires point source dischargers to determine toxicity of facility effluent using Whole Effluent Toxicity (WET) tests. At a permitted facility in Missouri where the primary contaminants are metals, effluent from the facility’s wastewater treatment plant (WWTP) meets stringent numerical discharge limits for metals and semi-metals, typically by large margins, but periodically fails WET tests. Previous toxicity investigations indicated that low alkalinity and subsequently poor buffering capacity in the WWTP effluent is the source of the toxicity.

Anaerobic biotreatment technology was selected in the pilot program. Anaerobic biotreatment utilizes sulfate-reducing bacteria in an anaerobic organic substrate to remove metals from wastewater via sulfide precipitation (Gusek 2009). A by-product of the sulfate reduction reaction is the generation of bicarbonate which increases an effluent’s alkalinity. Sulfate-reducing bacteria (SRB) are obligate anaerobes that use sulfate to decompose simple organic compounds. The dominant species of SRB are in the genera Desulfotomaculum and Desulfovibrio. The reactions as presented by Gusek (2009) are as follows:

\[
\begin{align*}
2 \text{CH}_2\text{O} + \text{SO}_4^{2-} & \rightarrow \text{S}^{2-} + 2 \text{CO}_2 + 2 \text{H}_2\text{O} & (1) \\
\text{S}^{2-} + 2 \text{CO}_2 + 2 \text{H}_2\text{O} & \rightarrow \text{H}_2\text{S} + 2 \text{HCO}_3^- & (2)
\end{align*}
\]

The goal of this project was to obtain site-specific pilot test data demonstrating that an anaerobic biotreatment polishing step could increase alkalinity at the facility’s WWTP effluent sufficiently enough to consistently pass chronic WET tests (USEPA 2002). Anaerobic biotreatment is a low-cost, low-energy and low-maintenance technology relative to traditional chemical water treatment. If shown to be viable, the addition of an anaerobic biotreatment system to generate alkalinity in WWTP effluent could enable the operator to consistently pass chronic WET tests and potentially result in significant cost savings to the operator compared to modification of the WWTP.

Methods and Materials
An anaerobic biotreatment pilot cell (anaerobic biocell) was constructed using a 5.7 m³ tank (Fig. 1). An organic substrate consisting of
2.3 m³ of sawdust was installed in the anaerobic biocell. The organic substrate was underlain by a 30 cm sand layer and 15 cm gravel plenum for drainage. Effluent from the anaerobic biocell flowed to an aerating cascade constructed from a series of 19 L buckets. The aerating cascade oxidized residual sulfide present in the anaerobic biocell effluent and restored dissolved oxygen concentrations. Water flowed through the anaerobic biocell in a downflow configuration and water level was controlled with a P-trap. Following construction, the anaerobic biocell was filled with water, inoculated with sulfate-reducing bacteria, and allowed to incubate for two weeks prior to the start of operation. Following the incubation period, the anaerobic biocell was operated at a flow rate of approximately 2 L/min.

In addition to the anaerobic biocell, an aerobic limestone tank was installed to test if simple dissolution of limestone could generate sufficient alkalinity to pass chronic WET tests. This was considered unlikely because WWTP effluent pH is required to be >7.5 and limestone solubility is poor at pH >7.0 (Oates 1998). However, it was deemed worth investigating due to ease and low cost of set up and would avoid the secondary aeration step required with an anaerobic system to remove residual sulfide and restore dissolved oxygen. The aerobic limestone tank was constructed from a 380 L tank filled with approximately 57 L of limestone gravel and a flow rate of approximately 2 L/min (Fig. 2). Water flowed through the aerobic limestone tank in a downflow configuration and water level was controlled with a P-trap.

The anaerobic biocell and aerobic limestone tank began operating in late September 2012. A maturation period of approximately six weeks was required for the anaerobic biocell to reach optimum operating conditions. Chronic WET tests were not conducted until early December 2012 due to scheduling conflicts with WWTP operations and the analytical laboratory. A total of three chronic WET tests were conducted using effluent from the WWTP, the anaerobic biocell and the aerobic limestone tank.

The following analytical parameters were analyzed during the pilot test.

**Laboratory Parameters**

- Total cadmium;
- Total lead;
- Total thallium;
- Sulfide;
- Alkalinity;

The following analytical parameters were analyzed during the pilot test.
Acidity; Total suspended solids (TSS); and Chronic Whole Effluent Toxicity

Field Parameters

- pH;
- Temperature;
- Conductivity;
- Oxidation-reduction potential;
- Dissolved oxygen;
- Flow; and
- Alkalinity (field reagent kit).

Results

Total cadmium, total lead and total thallium were not detected in WWTP effluent. Total cadmium, total lead and total thallium were not detected in effluent from the aerobic limestone tank. Total cadmium and total thallium were not detected in effluent from the anaerobic biocell. Total lead was detected in the effluent from the anaerobic biocell at very low concentrations, with a mean concentration of 5.0 µg L⁻¹. This was below the WWTP effluent’s monthly average NPDES limit for lead.

Total suspended solids were non-detect in all effluent samples. Sulfide concentrations in effluent from the anaerobic biocell ranged from 0.5–1.1 mg L⁻¹ following the maturation period. This range was considered ideal because it confirmed that sulfate reduction and alkalinity generation were occurring, but removal of excess sulfide was not problematic. Sulfide was non-detect in WWTP effluent, effluent from the anaerobic biocell’s aerating cascade and the effluent from the aerobic limestone tank.

Full laboratory alkalinity and acidity results are presented in Table 1, Table 2 and Table 3. Mean net alkalinity results are presented in Fig. 3. Mean net alkalinity for WWTP effluent, effluent from the aerobic limestone tank and effluent from the anaerobic biocell was -8.9 mg L⁻¹, -8.2 mg L⁻¹ and 22.5 mg L⁻¹, respectively. The anaerobic biocell increased mean WWTP effluent net alkalinity by 353%.

In the Ceriodaphnia dubia (water flea) portion of the chronic WET tests only the effluent from the anaerobic biocell was considered as passing. The WWTP effluent and effluent from the aerobic limestone tank tests would have failed. The No Observable Effect Concentration (NOEC) for survival and reproduction, Lethal Concentration 50 (LC50) and Toxic Units are summarized in Table 4.

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Alkalinity mg L⁻¹</th>
<th>Acidity mg L⁻¹</th>
<th>Net Alkalinity mg L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec. 3, 2012</td>
<td>7.1</td>
<td>25.0</td>
<td>-17.9</td>
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<tr>
<td>Dec. 5, 2012</td>
<td>8.8</td>
<td>0.0</td>
<td>8.8</td>
</tr>
<tr>
<td>Dec. 7, 2012</td>
<td>7.5</td>
<td>25.0</td>
<td>-17.5</td>
</tr>
</tbody>
</table>

Table 1 WWTP Effluent Alkalinity and Acidity Results

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Alkalinity mg L⁻¹</th>
<th>Acidity mg L⁻¹</th>
<th>Net Alkalinity mg L⁻¹</th>
</tr>
</thead>
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<tr>
<td>Dec. 3, 2012</td>
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<td>0.0</td>
<td>7.0</td>
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<tr>
<td>Dec. 5, 2012</td>
<td>10.4</td>
<td>25.0</td>
<td>-14.6</td>
</tr>
<tr>
<td>Dec. 7, 2012</td>
<td>8.1</td>
<td>25.0</td>
<td>-16.9</td>
</tr>
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Table 2 Aerobic Limestone Tank Effluent Alkalinity and Acidity Results

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Alkalinity mg L⁻¹</th>
<th>Acidity mg L⁻¹</th>
<th>Net Alkalinity mg L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec. 3, 2012</td>
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<td>0.0</td>
<td>20.2</td>
</tr>
<tr>
<td>Dec. 5, 2012</td>
<td>25.0</td>
<td>0.0</td>
<td>25.0</td>
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<tr>
<td>Dec. 7, 2012</td>
<td>22.2</td>
<td>0.0</td>
<td>22.2</td>
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</tbody>
</table>

Table 3 Anaerobic Biocell Effluent Alkalinity and Acidity Result

<table>
<thead>
<tr>
<th>Sample Source</th>
<th>NOEC Survival</th>
<th>NOEC Reproduction</th>
<th>LC50</th>
<th>Toxic Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWTP Effluent</td>
<td>100%</td>
<td>6.25%</td>
<td>&gt;100%</td>
<td>2.94</td>
</tr>
<tr>
<td>Aerobic Limestone Tank</td>
<td>100%</td>
<td>6.25%</td>
<td>&gt;100%</td>
<td>3.24</td>
</tr>
<tr>
<td>Anaerobic Biocell Effluent</td>
<td>100%</td>
<td>100%</td>
<td>&gt;100%</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Table 4. Ceriodaphnia dubia Chronic WET Test Results
In the Pimephales promelas (fathead minnow) portion of the chronic WET tests the WWTP effluent, effluent from the aerobic limestone tank and effluent from the anaerobic biocell were all considered as passing. For all three tests the NOEC for survival and growth was 100 %, the LC50 was >100 % and the Toxic Units were <1.

Conclusions
Pilot test data demonstrates that anaerobic biotreatment generated sufficient alkalinity to move WWTP effluent from net-acid to net-alkaline and increased the likelihood that WWTP effluent will consistently pass chronic WET tests. Pilot test data does not indicate that aerobic limestone dissolution can generate sufficient alkalinity to move WWTP effluent from net-acid to net-alkaline and increase the likelihood that WWTP effluent will consistently pass chronic WET tests. Additional chronic WET testing has been recommended to confirm these initial positive results.

References