Type or print clearly:

NAME: __Bill Burgos___________________________________________________________
AGENCY/COMPANY: _Penn State________________________________________________
ADDRESS: _Department of Civil and Environmental Engineering, 212 Sackett Bldg____
CITY, STATE, ZIP: __University Park, PA 16802____________________________________
TELEPHONE: __814-836-0578_____________ FAX: __814-863-7304_____________
E-MAIL : __wdb3@psu.edu_______________________________

Type of presentation: Oral _X_ Poster ___

**Technical Division or Session:**
Ecology ___ Forestry and Wildlife ____ Geotechnical Engineering ____
Refuse and Tailings Management & Reclamation ___ Land Use and Design ____
Soils and Overburden ___ Water Management _X__ Other _____ Case Study ___

Other interests for those persons who may or may not be presenting a paper:
Field Tour _______ Workshop ______ Send Hotel and Conference Registration information
______

Exhibitor __________ Attendee ______
Sponsor (Gold, Silver, Bronze or Break) ____________
Importance of fungi in biological Mn(II) oxidation in limestone treatment beds

William D. Burgos¹, Hui Tan¹, Cara M. Santelli² and Colleen M. Hansel²

¹Department of Civil and Environmental Engineering, The Pennsylvania State University, University Park, PA, USA. ²Harvard School of Engineering and Applied Sciences, Cambridge, MA

ABSTRACT

Coal mine drainage (CMD) is the single greatest threat to the environment of Appalachia in the United States, releasing large volumes of acid and metal contaminants (e.g., Mn, Fe). Passive limestone-based treatment systems are the most cost-effective method for manganese(II) removal from CMD. The success of passive Mn(II)-removal systems has been variable due to a poor understanding of the mechanisms of Mn removal and the microbial communities involved. We selected one Mn-removal system from northwestern Pennsylvania that treats an exceptionally high concentration of 150 mg/L Mn(II), and conducted laboratory experiments to evaluate the relative importance of abiotic versus biotic processes responsible for Mn removal, and to evaluate the relative importance of bacteria versus fungi on biological Mn(II) oxidation. We found that while abiotic processes such as Mn(II) sorption and heterogeneous oxidation contribute to Mn removal, biological Mn(II) oxidation is the most important process to ensure effective, long-term Mn removal. We also found, that fungal activity accounted for over 80 % of Mn(II) oxidation in this Mn-removal bed. We also selected four additional Mn-removal systems from western Pennsylvania for an extensive culture enrichment survey of bacteria and fungi. From this survey, we found that Mn(II)-oxidizing fungi were isolated more readily than Mn(II)-oxidizing bacteria – fungal isolates outnumbered bacterial isolates 84:10 in 3 of the 4 systems, and that fungi were extremely tolerant to elevated concentrations of Mn(II).

Key Words: acid mine drainage, metal oxidation, manganese removal
INTRODUCTION

The removal of elevated concentrations of dissolved manganese, Mn(II), from mine drainage is a significant problem for many regions in the United States and throughout the world. In Appalachia, centuries of coal mining have left thousands of abandoned mines that are discharging metal-contaminated effluent, with Mn concentrations as high as 150 mg/L, and severely degrading water resources (Cravotta III, 2007 and references therein; Herlihy et al., 1990). Manganese, while not considered to be acutely toxic to humans (although this is poorly studied), can be damaging to ecosystems and water distribution networks. Furthermore, Mn(II) oxidation reactions produce protons, which perpetuates the generation of acid mine drainage (AMD). In the eastern United States, one of the most common methods to remediate high concentrations of dissolved Mn(II) in coal mine drainage (CMD) is the use of biologically-active limestone treatment beds, where oxidation and subsequent precipitation of Mn(III/IV) oxides is catalyzed by abiotic and biotic processes (Hallberg and Johnson, 2005; Johnson et al., 2005). In essence, Mn is immobilized via the precipitation of sparingly soluble minerals, which subsequently remove other metal contaminants (e.g., Cu, Co, Zn) through both co-precipitation and surface adsorption reactions. To date, the overall success of Mn removal within passive treatment systems is widely variable due to a poor understanding of the processes and mechanisms that govern Mn(II) oxidation at near-neutral pH in these systems.

Microorganisms accelerate Mn(II) oxidation rates up to several orders of magnitude faster than abiotic catalysis, thus it is believed that the precipitation of Mn(III/IV) oxide minerals in the environment is largely driven by microbiological activity (Nealson et al., 1988; Tebo, 1991; Tebo et al., 2004). Mn(II)-oxidizing bacteria are ubiquitously distributed in the environment and much research has recently been devoted to understanding the mechanisms,
pathways, and products of Mn(II) oxidation by bacteria (Bargar et al., 2005; Dick et al., 2008b; Francis et al., 2001; Hansel and Francis, 2006; Johnson and Tebo, 2007; Ridge et al., 2007; Webb et al., 2005a; Webb et al., 2005b). Although much less studied, Mn(II)-oxidizing fungi have also been recovered from a wide variety of ecosystems, such as agricultural soil (Pedler et al., 1996), deep sea sediments (Shao and Sun, 2007), building stone, (de la Torre and Gomez-Alarcon, 1994), desert varnish (Grote and Krumbein, 1992; Krumbein and Jens, 1981), streambeds (Miyata et al., 2006a; Miyata et al., 2004; Takano et al., 2006), and an artificial wetland (Takano et al., 2006).

The contribution of microbial activity in the remediation of Mn-contaminated waters has frequently been observed (Bamforth et al., 2006; Haack and Warren, 2003; Hallberg and Johnson, 2005; Johnson et al., 2005; Johnson and Younger, 2005). Several different strains of Mn(II)-oxidizing bacteria have even been used in a patented bioremediation method, the “Pyrolusite Process”, for treating manganiferous mine waters (Vail and Riley, 2000). A recent study by Mariner et al. (2008) identified Mn(II)-oxidizing fungi, in addition to bacteria, that successfully grow in a Mn(II) attenuation bioreactor for treating mine waters. To our knowledge, this is the first study to document the role of fungi in these bioremediation technologies. In general, however, the identities and growth characteristics of the Mn(II)-oxidizing community contributing to Mn remediation remains largely unresolved.

The purpose of this research was to improve our understanding and design capability of passive manganese(II)-removal systems for the treatment of coal mine drainage. The objectives of this research were to: 1) Measure Mn(II)-removal kinetics in controlled laboratory experiments; and 2) Characterize the microbial communities that promote Mn(II) oxidation in these Mn-removal systems using a culture-based approach.
MATERIALS AND METHODS

Site description and sampling techniques

One passive Mn-removal system, referred to as Fairview, was characterized in this study. The system is located in northwestern Pennsylvania (Elk County, 41°21'05" N, 78°39'16" W) and was constructed in 2004 to treat CMD from a surface coal mine with an influent Mn(II) concentration of 130 – 150 mg/L. CMD is conveyed through an underground limestone drain before discharging into a limestone-laden constructed wetland and then enters the limestone Mn-removal bed. Discharge into the wetland comes from a pipe elevated several feet above the ground that provides aeration as the water enters the wetland. Iron is completely removed before entering the Mn-removal bed (Table 1). The rectangular bed (10-cm limestone gravel, 30-m long, 15-m wide, 1-m deep) contains five ditches (perpendicular to bed flow, ~½ the depth of the bed) that were added to promote passive aeration and served as our water and sediment sampling locations. Straw and corncobs were worked into the Mn-removal bed after initial construction when the ditches were added. The flow rate into the Mn-removal bed is ca. 10 gallons/min and the bed provides a hydraulic residence time of ca. 100 hrs.

Water and sediment samples were collected in July, September, and November of 2005, April, June, September and December of 2006, and May of 2007 to monitor system performance during all seasons and over an extended period of time. Samples were collected from the influent and effluent pipes, the wetland and from the five ditches in the Mn-removal bed. Water samples were filtered (0.2 µm) in the field and chemically preserved (dependent on analyte). Sediment “crust” samples were collected with sterile spatulas and placed in sterile endotoxin-free centrifuge tubes or whirl-pak bags. Sediments were scraped from the top 1-cm of black precipitates that armored the limestone cobbles. All samples were stored on ice for transport to
the laboratory, except for samples intended for nucleic acid-based microbial community analysis, which were transported on dry ice. Dissolved oxygen (DO) concentrations, temperature, pH and conductivity were determined in the field using portable meters.

**Sediment incubation experiments**

Laboratory sediment incubation experiments were conducted to determine the relative importance of biological activity on Mn(II) loss from solution. Experiments were conducted with a single, large quantity of sediment collected from the middle ditches in the Mn-removal bed. Sediment crusts were collected with sterile spatulas, homogenized, and wet sieved (<2-mm sieve fraction), using sterile tools in the field, into sterile mason jars. Experiments were conducted with 1.0 g moist “live” sediments (0.60 g dry mass) mixed with 50 mL of filter sterilized influent water in 120 mL serum bottles with air or 100 % N₂ in the headspace and sealed with thin Teflon-coated stoppers and Al crimp tops. Abiotic controls were conducted with “killed” sediments that been exposed to 100 kilogram of $^{60}$Co γ irradiation and maintained under a 100 % N₂ headspace. No loss of Mn(II) was measured in sediment-free controls under an air headspace. All reactors were kept in the dark and shaken at 100 rpm at room temperature (25 °C). Water and sediment suspension samples were periodically collected from the reactors using sterile needle and syringe to measure soluble Mn(II) and pH. Samples were centrifuged at 11,000 g for 10 minutes to separate liquid and sediments. Mn(II) concentration in the supernatants were measured using the PAN method (Goto et al., 1976). pH was measured using a combination electrode (Thermo Scientific, Waltham, MA). When the soluble Mn(II) concentration decreased to below detection limit (<0.1 mg/L) in the “live” reactors, MnCl₂ was re-spiked into the reactors to re-establish a Mn(II) concentration of ca. 150 mg/L and MnCl₂ was spiked into the reactors three times.
Culture enrichments and isolations

Samples for culture enrichments were collected in October 2007 from four different treatment systems near Fairview: Saxman Run (Westmoreland County, PA), De Sale Phase 1, De Sale Phase II, and De Sale Phase III (Butler County, PA) as described previously (Santelli et al., 2010). Rock and sediment samples were lightly crushed and homogenized using a mortar and pestle. All samples were diluted in sterile, artificial freshwater (AFW; 34.22 mM NaCl, 1.62 mM MgSO$_4$$\cdot$7H$_2$O, 1.36 mM CaCl$_2$$\cdot$2H$_2$O, 0.44 mM K$_2$HPO$_4$$\cdot$3H$_2$O, 20 mM HEPES buffer at pH 7) using serial dilutions to 1/10^4. Dilutions were plated onto 7 different types of agar-solidified media with 20 mM HEPES buffer (pH 7) and 200 µM MnCl$_2$. The culture media were described previously: HEPES-buffered AY medium (Miyata et al., 2004); K, Leptothrix, and M media with 0.2 µm filter-sterilized natural site water (Templeton et al., 2005); J and J+acetate (JAC) media with AFW (Hansel and Francis, 2006); and Medium 3 (M3; de la Torre and Gomez-Alarcon, 1994). Mn(II)-oxidizing bacteria and fungi were putatively identified by the presence of brown/black precipitates, Mn(III/IV) oxides, and transferred to fresh media a minimum of 5 times until cultures were deemed axenic. Mn(III/IV) was confirmed using the leucoberbelin blue (LBB) colorimetric assay – LBB reacts specifically with Mn(III) and Mn(IV) and turns deep blue (Krumbein and Altman, 1973).

Growth experiments

Fungal isolates were tested for their ability to grow and oxidize Mn(II) in the presence of varying metal concentrations. Fungi were grown in dark conditions on AY media supplemented with Mn$^{2+}$ (added as MnCl$_2$) at the following concentrations: 0 µM, 250 µM, 500 µM, 750 µM, 1 mM, 5 mM, and 10 mM. Fungi were inoculated with a toothpick in the center of petri dishes and allowed to grow radially outward until the diameter of mycelia reached the plate edges (typically
less than 10 days). Colony growth diameter was evaluated approximately every other day. The effects of light/dark and pH (5.5, 6.0, 6.5, 7.0, 7.6, and 8.0) on growth were also tested using the same protocol. MES buffer (20 mM) was used to maintain a solution pH of 5.5, 6.0, and 6.5, and HEPES buffer (20 mM) was used for pH values ≥ 7.0.

RESULTS AND DISCUSSION

Mn(II) removal in the field

Limestone beds designed for Mn removal from CMD create a unique geochemical and physical environment for promoting biological Mn(II) oxidation. To function most effectively, Fe, Al and some of the influent acidity are removed first through limestone-based systems before entering the Mn-removal system. In a survey of 140 abandoned coal mine discharges in Pennsylvania, the median Mn concentration was 2.35 mg/L with a range from 0.019 to 74.0 mg/L (Cravotta, 2008). Most of these discharges were from underground mines. For the surface coal mine at the Fairview site, the exceptionally high Mn concentration (130 – 150 mg/L) is believed to originate from reactions with the overburden (e.g., MnCO_3 inclusions in limestone). Influent CMD to the Fairview Mn-removal system contains essentially no Fe, Ca and Mg are elevated from upstream neutralization with limestone, and the elevated concentrations of Mn and Al make the water net acidic (Table 1). Sulfate is the primary anion and nutrient concentrations (i.e. TOC, N, P) are relatively low. We have sampled this system on several occasions between December 2005 and May 2007 to capture seasonal effects and have found that Mn is never completely removed through the limestone bed (Tan et al., 2010). Greater removal of Mn(II) occurs in summer months (e.g., 35 mg/L effluent Mn), as compared to the winter sampling event (e.g., 70 mg/L).
Mn(II) removal via biotic versus abiotic processes

Reactors containing wet-sieved (<2-mm), MnOx-rich sediment crusts and their associated natural microbial communities were operated in a fed-batch mode with respect to dissolved Mn(II). MnCl₂ was periodically re-spiked into the reactors to re-establish the initial, influent Mn(II) concentration of ca. 2.5 mM. This fed-batch approach was required to overcome abiotic Mn(II) removal processes such as non-oxidative sorption and heterogeneous oxidation so that the contribution of biological Mn(II) oxidation on Mn removal could be better ascertained. Control reactors that had been sterilized by ⁶⁰Co γ-irradiation and/or maintained under a N₂ atmosphere were compared to “live” (i.e. non-sterile) reactors. Our simplified, operational interpretations were that Mn(II) could be removed only by non-reductive sorption in sterilized reactors maintained under N₂, while both heterogeneous oxidative precipitation of Mn(II) (as MnOₓ) and non-reductive sorption could occur in sterilized reactors maintained under air.

As seen with the live reactors maintained under air or under N₂ (black and white squares, respectively, in Figure 1), the difference between Mn(II) removal through each fed-batch cycle increased as the experiment proceeded. Our interpretation of these results is that the non-reductive Mn(II) sorption capacity of the sediments was essentially saturated after four cycles. The growing difference between the live reactors maintained under air versus the live reactors under N₂ was presumably caused by biological Mn(II) oxidation and the subsequent precipitation of MnOₓ.

A series of similar, fed-batch experiments were conducted to determine the effects of dissolved oxygen on Mn(II) removal (Figure 2). A significant difference in Mn(II) removal was observed when the headspace P₀₂ was maintained at 1 % (v/v)-as compared to 10 and 21 %. The difference became more pronounced after several cycles as the abiotic removal mechanisms
contributed less to Mn(II) removal (due to saturation). The rate of biological Mn(II) oxidation by *Leptothrix discophora* SS1 has been shown to be directly proportional to dissolved oxygen (Zhang et al., 2002). Our results suggest a different response for the whole, natural microbial community in this Mn-removal bed as compared to *Leptothrix*. Specifically, our results suggest that dissolved oxygen had more of a saturation-type effect on Mn(II) removal where the rate and extent of Mn(II) removal were not different above a P_{O2} level of 10 %.

Fed-batch experiments were also conducted to determine the effects of organic carbon addition on Mn(II) removal (Figure 3). In practice, Mn-removal beds are often designed with an upstream wetland. Dissolved organic carbon (DOC) released via primary production in the wetland into the Mn-removal bed presumably will increase microbial Mn(II) oxidation. Glucose and carboxymethylcellulose (CMC) were selected to represent labile and recalcitrant DOC, respectively. CMC was also selected to represent DOC that might be generated from wood dissolution with the thought that wetlands could possibly be replaced with a solid-phase carbon source emplaced within the bed. The addition of glucose was shown to slightly improve Mn(II) removal while the addition of CMC was shown to significantly inhibit Mn(II) removal. Our interpretation is that glucose stimulated general heterotrophic activity that in turn fortuitously stimulated biological Mn(II) oxidation. We are unsure about the inhibitory effect of CMC, however, we speculate that CMC may complex Mn(II) to form soluble species that are difficult to oxidize.

**Mn(II) oxidation via bacterial versus fungal activity**

A series of fed-batch experiments were conducted in the presence/absence of fungicides (0.2 g/L cyclohexamide plus 0.2 g/L pentachloronitrobenzene) to operationally assess the relative importance of Mn(II) oxidation by fungi as compared to other microbial community members.
A somewhat surprising and important finding from these experiments was that the fungicides knocked out the majority of the sediment’s ability to remove Mn(II). Reactors that contained sterile sediments and/or were maintained under N₂ all yielded results similar to those reactors that contained fungicides. Our interpretation of these results is that, e.g., the difference between the live reactors with fungicides (black circles in Figure 4) and the live reactors without fungicides (black squares) was due to fungal activity. Furthermore, we believe that fungi were the most important bio-catalysts of Mn(II) oxidation in this treatment system.

Based on enumerations of culturable heterotrophic bacteria measured at the end of these experiments, the fungicides did not non-specifically harm the bacterial population in the sediments (Figure 5). We actually observed an increase in culturable bacterial numbers and speculate that this occurred because of the absence of fungi competing for space on the plates. Another possible explanation for increased numbers of culturable bacterial in the presence of fungicides is that fungi themselves may produce antibiotics to suppress bacterial activity.

**Mn(II) oxidation by pure cultures of fungi**

We conducted an extensive culture enrichment survey of several Mn(II)-treatment systems in western Pennsylvania. An interesting and unexpected result from this survey was that Mn(II)-oxidizing fungi were isolated more readily than Mn(II)-oxidizing bacteria, where fungal isolates outnumbered bacterial isolates 84 to 10. In other words, in these Mn(II)-treatment systems we found that fungi constituted 89% of the Mn(II)-oxidizing cultures while bacteria constituted just 11%. The most commonly recovered species, in terms of number of treatment systems and number of times obtained, was representative isolate DS2psM2a2 (Figure 6, left). Based on phylogenetic analysis and morphological characterization, DS2psM2a2 was identified as *Plectosphaerella cucumerina* belonging to the class Sordariomycetes (Santelli et al., 2010).
We initially hypothesized that Mn(II) oxidation was predominantly mediated by bacteria; however, our results suggest that fungi are also important mediators of Mn(II) oxidation. Also of great practical importance, we found that most of these fungi were highly tolerant to elevated Mn(II) concentrations. As shown in Figure 6, two representative fungal isolates from these Mn-removal limestone beds can tolerate astonishingly high levels of Mn(II) (e.g., 1,000 to >10,000 μM). It is generally believed that fungi are more tolerant than bacteria to high concentrations of heavy metals leading to a prevalence of fungi in heavy metal-contaminated soil (Chander et al., 2001a; Chander et al., 2001b; Kelly et al., 1999; Rajapaksha et al., 2004). Considering that Mn(II) concentrations in surface coal mine drainage in Appalachia often exceed 1,000 μM (= 55 mg/L) and can even exceed 3,000 μM, microbial catalysts in these Mn(II)-removal systems must be tolerant of high Mn(II) concentrations – further demonstrating that Mn(II)-oxidizing fungi likely play an important role in the treatment process. Consistent with this conclusion, a recent study (Mariner et al., 2008) suggested that Mn(II)-oxidizing fungi are more abundant than bacteria in bioreactors treating mine water with elevated Mn(II) concentrations.

Acknowledgements:
This research was supported by the National Science Foundation through Grant No. CHE-0431328 and Grant No. EAR07-45374, and by the Office of Surface Mining under Cooperative Agreement S07AP12478.
References


Figure 1. Mn(II) loss from solution in laboratory sediment incubation experiments. Black squares (■) – live sediments under air; white squares (□) – live sediments under N2; black circles (●) – killed sediments (γ-irradiated) under air; and, white circles (○) – killed sediments under N2. For live sediments Mn(II) was repeatedly added (as MnCl₂) at 120, 460 and 600 h, while killed reactors were never re-spiked with Mn(II). Experiments were conducted with 1.0 g moist sediment (0.82 g dry) and 50 mL filter sterilized influent site water. Figure from Tan et al. (2010).
Figure 2. Mn(II) loss from solution under variable partial pressures of oxygen (P\textsubscript{O2}). White circles (○) – incubated under 21% P\textsubscript{O2} (i.e. air); black squares (■) – incubated under 10% P\textsubscript{O2}; 90% N\textsubscript{2}; and, black triangles (▲) – incubated under 10% P\textsubscript{O2}; 99% N\textsubscript{2}. Mn(II) was repeatedly added (as MnCl\textsubscript{2}) at 120, 460 and 600 h. Experiments were conducted with 1.0 g moist sediment (0.82 g dry) and 50 mL filter sterilized influent site water. Corresponding controls using killed sediments (γ-irradiated) are not shown.
Figure 3. Mn(II) loss from solution with and without supplemental organic carbon source. White circles (○) – incubated with 10 mg C/L of glucose; black squares (■) – incubated with no supplemental carbon; and, black triangles (▲) – incubated with 10 mg C/L of carboxymethylcellulose. All incubations maintained under air. Mn(II) was repeatedly added (as MnCl₂) at 120, 460 and 600 h. Experiments were conducted with 1.0 g moist sediment (0.82 g dry) and 50 mL filter sterilized influent site water. Corresponding controls using killed sediments (γ-irradiated) are not shown.
Figure 4. Mn(II) loss from solution in the presence and absence of fungicides. Black squares (■) – live sediments incubated with no fungicides; white squares (□) – killed sediments (γ-irradiated) incubated with no fungicides; black circles (●) – live sediments incubated with 0.2 g/L cyclohexamide and 0.2 g/L pentachloronitrobenzene; and, white circles (○) – killed sediments incubated with 0.2 g/L cyclohexamide and 0.2 g/L pentachloronitrobenzene. All incubations maintained under air. Mn(II) was repeatedly added (as MnCl₂) at 120, 460 and 600 h. Experiments were conducted with 1.0 g moist sediment (0.82 g dry) and 50 mL filter sterilized influent site water.
Figure 5. Culture-based bacterial enumerations at the end of select sediment incubations.

Numbers of culturable heterotrophic bacteria were determined with K media.
Figure 6. Tolerance of fungal isolates to elevated Mn(II) concentrations. Images show two different Mn(II)-oxidizing fungi isolates grown in petri dishes on agar-based media with increasing (0 to 10,000 μM) concentrations of dissolved Mn(II). Fungal isolate 1 is *Plectosphaerella cucumerina* and fungal isolate 2 is *Microdochium bolleyi*. Isolates were inoculated by “stabbing” the center of the petri dish, allowing the fungal hyphae to grow radially outwards. The brown color is due to the Mn oxide minerals precipitated on the fungi during growth.
Table 1. Aqueous geochemistry of the Fairview Mn-removal system for samples collected in December 2006. Front, middle and rear refer to locations within the limestone treatment bed with the front location nearest the influent. All dissolved concentrations reported in mg/L.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Influent</th>
<th>Front</th>
<th>Middle</th>
<th>Rear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn</td>
<td>140</td>
<td>137</td>
<td>107</td>
<td>70</td>
</tr>
<tr>
<td>Fe</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Al</td>
<td>5.1</td>
<td>3.0</td>
<td>0.62</td>
<td>0.07</td>
</tr>
<tr>
<td>Ca</td>
<td>230</td>
<td>223</td>
<td>248</td>
<td>260</td>
</tr>
<tr>
<td>Mg</td>
<td>245</td>
<td>240</td>
<td>228</td>
<td>208</td>
</tr>
<tr>
<td>Si</td>
<td>6.5</td>
<td>7.7</td>
<td>5.6</td>
<td>5.7</td>
</tr>
<tr>
<td>Na</td>
<td>50</td>
<td>47.5</td>
<td>45</td>
<td>42.5</td>
</tr>
<tr>
<td>K</td>
<td>6.6</td>
<td>6.3</td>
<td>6.15</td>
<td>5.65</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>2010</td>
<td>2010</td>
<td>2010</td>
<td>2010</td>
</tr>
<tr>
<td>Cl</td>
<td>3.65</td>
<td>2.32</td>
<td>2.85</td>
<td>2.57</td>
</tr>
<tr>
<td>pH (standard units)</td>
<td>5.43</td>
<td>5.65</td>
<td>6.11</td>
<td>6.34</td>
</tr>
<tr>
<td>Alkalinity (mg CaCO$_3$/L)</td>
<td>32.5</td>
<td>25.6</td>
<td>77.5</td>
<td>100</td>
</tr>
<tr>
<td>Hot peroxide acidity (mg CaCO$_3$/L)</td>
<td>373</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>TOC</td>
<td>2.22</td>
<td>2.02</td>
<td>1.96</td>
<td>1.77</td>
</tr>
<tr>
<td>TN</td>
<td>0.30</td>
<td>0.18</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>NO$_3$-N</td>
<td>0.18</td>
<td>0.10</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>PO$_4$-P (mg P/L)</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Dissolved oxygen (mg O$_2$/L)</td>
<td>5.5</td>
<td>7.2</td>
<td>6.1</td>
<td>4.1</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>10.4</td>
<td>7.1</td>
<td>5.2</td>
<td>4.3</td>
</tr>
</tbody>
</table>