

**APPENDIX A**

**PERCHLORATE BIOREDUCTION IN LAKE BELTON  
SCOPE OF WORK**

# MEMORANDUM



**MWH**  
MONTGOMERY WATSON HARZA

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**To:** Ronald Hartline **Date:** March 24, 2003  
**CC:** Anthony Magliocchino **File No.:**  
**From:** Thomas Gillogly, Ph.D., Brian Gallagher  
**Subject:** Perchlorate Bioreduction in Lake Belton Bench-Scale Study – Scope of Work

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The Naval Weapons Industrial Reserve Plant (NWIRP) in McGregor, Texas, manufactured, treated, stored, and disposed of solid rocket fuels for nearly 50 years. These rocket fuels contained perchlorate, a highly soluble, highly mobile, and environmentally persistent contaminant. Perchlorate and several other defense-related compounds have contaminated both surface water and groundwater in two watersheds that border the McGregor facility. These watersheds drain to two drinking water reservoirs (Lake Waco and Lake Belton) that serve as the sole-source drinking water supply for 500,000 people in surrounding communities.

In an effort to assist the U.S. Army Corps of Engineers (USACE) in developing a better understanding of the anoxic component of Lake Belton, MWH will perform a series of micro ecology experiments to determine the potential for natural perchlorate bioreduction. These tests will explore this potential in both the water column and the sediment at the water/sediment interface.

## **Biological Reduction of Perchlorate**

The ability for dissimilatory perchlorate and chlorate reduction is widespread among bacteria in the natural environment. Although only a few pure cultures of perchlorate reducing bacteria have been isolated, several general commonalities have been observed:

- All studied perchlorate reducers also reduce chlorate;
- Most studied perchlorate reducers are facultative anaerobes;
- Most studied perchlorate reducers also reduce nitrate;
- Nitrate inhibits perchlorate and chlorate reduction, in most studied perchlorate reducers, and;
- Most studied perchlorate reducers can utilize a variety of electron donors.

Bacteria that practice dissimilatory perchlorate reduction do so to gain energy that they can use for growth and maintenance. In this process, oxygen ions are successively stripped from perchlorate, leaving a chloride ion in solution as an end product and producing carbon dioxide as the main byproduct. The balanced equation for reduction of perchlorate using acetate as the electron donor is:



In the above reaction, perchlorate is the acceptor, gaining eight electrons; and acetate is the donor, losing eight electrons. The steps are perchlorate to chlorate ( $\text{ClO}_3^-$ ), chlorate to chlorite ( $\text{ClO}_2^-$ ), and finally chlorite to a chloride ( $\text{Cl}^-$ ) ion.

In many natural waters that contain perchlorate, oxygen and nitrate normally also are present. If all these electron acceptors are present, there is an order of preference for them:

Oxygen > Nitrate > Perchlorate

In other words, if oxygen is present, the rates of biological reduction of nitrate and perchlorate are reduced. Once the oxygen is depleted, the biological reductions of nitrate (i.e., denitrification) and perchlorate are greatly improved. However, the reduction of perchlorate to chloride is impaired by nitrate present in the water.

### **Experimental Approach**

The microecology experiments have been designed to address several aspects of the potential for perchlorate biodegradation in Lake Belton. These aspects include:

- *Reductive “Medium”*. It is anticipated that biological reduction of perchlorate can occur in both the sediment and in the water column. Consequently, tests will be separately performed on each of these mediums.
- *Spatial Variability*. Due to the inherent variability in nature, it cannot be expected that the microbiological quality and composition is homogeneous throughout the water column, sediment and in different locations around Lake Belton. In order to evaluate how some of this natural variability might impact the perchlorate biodegradation potential, six locations in the lake will be selected. Three of the locations will be selected in the deeper portions of the lake which are likely to have been under anoxic conditions for the longest at the time of sampling. The remaining three locations will be chosen in shallower water where the thermocline is close to the water/sediment interface. Sediment will be collected from each of these locations. Water samples will be collected from one of the deep locations near the bottom, close to (but below) the thermocline, and close to the surface (above the thermocline). One additional water sample will be collected near the bottom of one of the shallower water locations.
- *Nutrients*. While it is not possible to easily determine the limiting nutrient of these biological systems, experiments will be performed to determine if perchlorate degradation in the water column is enhanced by the addition of an electron donor (acetate) and/or minerals. The minerals will be obtained from two sources: 1) sterilized sediment; and 2) laboratory prepared solution. If an enhancement is observed then, the water column has the appropriate microorganisms, but is limited or lacking some nutrient.

- *Oxygen.* Oxygen has been shown to inhibit perchlorate reduction in the facultative reducers. In order to characterize how the oxic/anoxic conditions of the lake can impact perchlorate reduction, an anoxic water sample will be oxygenated and provided sufficient electron donor to reduce the oxygen. This will show if the culture can recover in the time allotted. This will be compared to an unoxygenated system. Additionally, a water sample from above the thermocline will be tested for its ability to reduce perchlorate.
- *Temperature.* The temperature in the anoxic region of the lake can be substantially colder than close to the surface. It is likely that these colder temperatures will reduce the rate of any perchlorate biodegradation. To assess the impact of temperature, a set of microecology experiments will be carried out at both room temperature (~20°C) and at the coldest water temperature recorded at the time of the Lake Belton sample collection.
- *Kinetics.* Determining the rate biodegradation *a priori*, based on the microecology of these environmental mixed cultures and available micronutrients is not possible given the current state of knowledge. Consequently, samples will be collected at regular intervals over the course of 14 days to establish how rapidly biodegradation will take place for selected conditions and establish how different environmental factors may impact this observed rate.

A summary of the experimental matrix is presented in the Appendix. This summary outlines general experimental conditions, matrix modifications, incubation periods, anticipated number of samples, and quality control samples.

### Spatial Variability

To test the influence of spatial variability within the water column and around the lake on the perchlorate bioreduction potential, the following samples will be collected using the protocols set forth in the Field Sampling Plan:

Location	Sample (s)
Deep <sup>†</sup> 1	Sediment, Water near bottom, Water close to (but below) thermocline, Water close to surface (above thermocline)
Deep 2	Sediment
Deep 3	Sediment
Shallow 1 (Close to Thermocline <sup>‡</sup> )	Sediment, Water near bottom
Shallow 2 (Close to Thermocline)	Sediment
Shallow 3 (Close to Thermocline)	Sediment

<sup>†</sup> - The deepest locations of Lake Belton that have presumably been anoxic the longest at the time of sampling.

<sup>‡</sup> - These locations are selected such that the water/sediment interface is close to (but below) the thermocline.

### Sediment Variability

Under temperature controlled conditions, in an anaerobic chamber, a series of autoclaved 100 ml Amber Pyrex Media bottles will be filled with 90 mL of a laboratory prepared stock solution consisting of sterilized water collected from Lake Belton, spiked with 50 µg/L of perchlorate. These bottles will be inoculated with 10 mL aliquots of the six different sediments, nitrogen purged, capped, and agitated (in triplicate). All six core sediment samples will be processed in triplicate to insure representative sampling of the core plug as a whole. The samples will be allowed to react for a 14-day period under constant, low-level agitation, provided by an orbital shaker, in a low-temperature incubator set at ambient conditions. During processing and incubation all sample exposure to light will be minimized to reduce the chance of algal growth that could result in undesirable oxygen generation. For each of two of the sediment samples, an additional five cultures will be prepared so that they may be sacrificed at 1, 3, 5, 7 and 10 days to characterize the rate of perchlorate degradation.

A second set of 10mL aliquots (in triplicate) will be extracted from each of the six sediment samples and allowed to dry to calculate dry weight for reporting purposes. For a control, an additional triplicate series with one of the sediments will be autoclaved to kill the microorganisms prior to its inoculation into the 50 µg/L perchlorate solution. At the end of the contact time, filter-sterilized (0.45 µm) samples will be collected from all of the bottles and submitted to the USACE laboratory for perchlorate analysis. An aliquot of the stock solution will also be sent for analysis.

### Water Column Variability

Under temperature controlled conditions, in an anaerobic chamber, 50 mL aliquots of the three samples from the water column at the “Deep 1” location and the water sample from the “Shallow 1” location, will be volumetrically transferred into amber, 50 mL serum bottles and spiked with 50 µg/L perchlorate. After the perchlorate injection, the samples will nitrogen purged, capped, agitated and allowed to react for a 14-day period in a low-temperature incubator set at ambient conditions. All samples will be carried out in triplicate and exposure to light will be minimized. At the end of the contact time, filter-sterilized (0.45 µm) samples will be collected and submitted to the USACE laboratory for perchlorate analysis.

As the rate of biodegradation is not known, an additional five cultures will be prepared so that they may be individually sacrificed at 1, 3, 5, 7 and 10 days to characterize the rate of perchlorate degradation, for one of the sample locations.

### **Nutrients**

To determine if perchlorate reduction might be inhibited by a nutrient deficiency, samples of the sediment and water column will be amended with an electron donor and/or metals.

### Sediment Nutrients

Portions of the sediment samples collected will be processed as described in the “Sediment Variability” section of this document. These samples will then be spiked to 50 mg/L acetate, nitrogen purged, agitated and allowed to react for a 14-day period. At the end of the contact

time, filter-sterilized (0.45 µm) samples will be collected and submitted to the USACE laboratory for perchlorate analysis. This result will then be compared to the result obtained from the corresponding unamended culture from the “Sediment Variability” tests. To determine if the addition of a readily accessible electron donor will alter the rate of biodegradation an additional 5 cultures will be prepared for two of the sediments. These cultures will be individually sacrificed at 1, 3, 5, 7 and 10 days to characterize any potential impacts on perchlorate degradation.

### Water Column Nutrients

A portion of the water and sediment samples collected will be processed as described in the “Water Column Variability” and “Sediment Variability” sections of this document respectively. Each of these samples will then be amended with the acetate and metal combinations identified in the table below:

Series	Nutrient Amendment	
1	50 mg/L acetate	
2	50 mg/L acetate and 50 mg/L autoclaved and dried “Deep 1” sediment	
3	50 mg/L acetate and 1 mL/L of mineral solution <sup>†</sup>	
<sup>†</sup> Mineral Solution Composition in Deionized Water (g/L)		
	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O	10
	ZnCl <sub>2</sub>	0.05
	H <sub>3</sub> BO <sub>3</sub>	0.3
	FeCl <sub>2</sub> ·4H <sub>2</sub> O	1.5
	CoCl <sub>2</sub> ·6H <sub>2</sub> O	10
	MnCl <sub>2</sub> ·6H <sub>2</sub> O	0.03
	NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.03

Once amended, these triplicate samples will be agitated and allowed to react for a 14-day period. At the end of the contact time, filter-sterilized (0.45 µm) samples will be collected and submitted to the USACE laboratory for perchlorate analysis. The perchlorate reduction in these cultures will then be compared to the corresponding unamended culture from the “Water Column Variability” tests.

For one of the sample locations an additional five cultures will be prepared for each of the nutrient addition conditions. These samples will be individually sacrificed at 1, 3, 5, 7 and 10 days to characterize any potential impacts of nutrient amendment/limitation on the measured rate of perchlorate degradation.

### **Oxygen**

The inhibitory effect of oxygen will only be evaluated in the water column. In addition to the effects of oxygen that can be observed in selected cultures from the “Spatial Variability” tests, these tests will be used to differentiate between the possible impacts of brief oxygen exposure to elevated dissolved oxygen levels. Six aliquots of each of the four water samples will be oxygenated. The of the aliquots for each water will then be subsequently purged with nitrogen and dosed with a small amount of sulfide to remove the available dissolved oxygen and lower the

redox potential. All of the aliquots will then be dosed with 50 µg/L perchlorate and 50 mg/L acetate, sealed, agitated, and returned to the inert environment. The cultures will then be allowed to react for a 14-day period before it is filter-sterilized (0.45 µm) and submitted to the USACE laboratory for perchlorate analysis.

### **Temperature**

It is expected that the lower temperatures at the bottom of the lake will slow the rate of perchlorate reduction. To measure this variability, a portion of the water and sediment samples collected will be processed as described in the “Water Column Variability” and “Sediment Variability” sections of this document respectively. The processed samples will then be agitated, nitrogen purged and stored in a low temperature incubator set at either the coldest temperature measured at the time the samples were initially collected from the lake or at 6°C, based on discussions with USACE at the time of lake sampling. After the respective incubation periods, filter-sterilized (0.45 µm) samples will be collected and submitted to the USACE laboratory for perchlorate analysis.

### **Reporting**

Once the perchlorate results from the USACE have been returned, a technical memorandum will be prepared summarizing the analytical methods, results and conclusions. Copies of the memorandum will be electronically distributed to USACE staff for review and comment. Two weeks after all USACE comments have been received a final memorandum will be presented to USACE. This memorandum will be intended for distribution to the entire project team.

Appendix  
Experimental Matrix Summary

**AQUEOUS SAMPLE MATRIX**

<b>Matrix</b>	<b>Aqueous</b>																
<b>Location ID</b>	<b>Deep 1 (Bottom)</b>					<b>Deep 1 (Thermocline)</b>				<b>Deep 1 (Surface)</b>				<b>Shallow 1 (Bottom)</b>			
<b>Matrix Treatment</b> <sup>†</sup>	-	Autoclaved	Acetate	Sediment	Minerals	-	Acetate	Sediment	Minerals	-	Acetate	Sediment	Minerals	-	-	-	-
<b>Spatial Variability</b>	14 days (x3)*	14 days (x3)*	-	-	-	14 days (x3)	-	-	-	14 days (x3)	-	-	-	14 days (x3)*	-	-	-
spike 50 µg/L ClO <sub>4</sub> <sup>-</sup>	10 days																
N <sub>2</sub> Sparge*	7 days																
	5 days																
	3 days																
	1 day																
Subtotal	8	3	0	0	0	3	0	0	0	3	0	0	0	3	0	0	0
<b>Nutrients</b>	-	-	14 days (x3)*	14 days (x3)*	14 days (x3)*	-	14 days (x3)	14 days (x3)	14 days (x3)	-	14 days (x3)	14 days (x3)	14 days (x3)	-	14 days (x3)*	14 days (x3)*	14 days (x3)*
spike 50 µg/L ClO <sub>4</sub> <sup>-</sup>			10 days	10 days	10 days												
N <sub>2</sub> Sparge*			7 days	7 days	7 days												
			5 days	5 days	5 days												
			3 days	3 days	3 days												
			1 day	1 day	1 day												
Subtotal	0	0	8	8	8	0	3	3	3	0	3	3	3	0	3	3	3
<b>Oxygen</b>	14 days (x3)	-	-	-	-	14 days (x3)	-	-	-	14 days (x3)	-	-	-	14 days (x3)	-	-	-
spike 50 µg/L ClO <sub>4</sub> <sup>-</sup>																	
O <sub>2</sub> Sparge																	
spike 50 µg/L ClO <sub>4</sub> <sup>-</sup>	14 days (x3)	-	-	-	-	14 days (x3)	-	-	-	14 days (x3)	-	-	-	14 days (x3)	-	-	-
O <sub>2</sub> Sparge																	
N <sub>2</sub> Sparge																	
Sulfide addition																	
Subtotal	6	0	0	0	0	6	0	0	0	6	0	0	0	6	0	0	0
<b>Temperature</b>	14 days (x3)*	-	-	-	-	14 days (x3)	-	-	-	14 days (x3)	-	-	-	14 days (x3)*	-	-	-
spike 50 µg/L ClO <sub>4</sub> <sup>-</sup>	10 days																
N <sub>2</sub> Sparge*	7 days																
	5 days																
	3 days																
	1 day																
Subtotal	7	0	0	0	0	3	0	0	0	3	0	0	0	3	0	0	0
<b>SUBTOTAL</b>	21	3	8	8	8	12	3	3	3	12	3	3	3	12	3	3	3

† "-" no treatment of aqueous sample  
 "Autoclaved" sample autoclaved to sterilize solution  
 "Acetate" sample spiked with 50 mg/L acetate  
 "Sediment" sample spiked with 50 mg/L acetate and 50 mg/L autoclaved/dried "Deep 1" sediment  
 "Minerals" sample spiked with 50 mg/L acetate and 1 ml/L of mineral solution (see Scope of Work)

**TOTAL SAMPLES = 111**

**SEDIMENT SAMPLE MATRIX**

<b>Matrix</b>	<b>Sediment</b>						
<b>Location ID</b>	<b>Deep 1</b>		<b>Deep 2</b>	<b>Deep 3</b>	<b>Shallow 1</b>	<b>Shallow 2</b>	<b>Shallow 3</b>
<b>Matrix Treatment</b>	Autocalved	-	-	-	-	-	-
<b>Spatial Variability</b>	14 days (x3)	14 days (x3)	14 days (x3)	14 days (x3)	14 days (x3)	14 days (x3)	14 days (x3)
90 mL - 50 µg/L ClO <sub>4</sub> <sup>-</sup>		10 days			10 days		
10 mL - sediment		7 days			7 days		
N <sub>2</sub> Sparge		5 days			5 days		
		3 days			3 days		
		1 day			1 day		
Subtotal	3	8	3	3	8	3	3
<b>Nutrients</b>	-	14 days (x3)	14 days (x3)	14 days (x3)	14 days (x3)	14 days (x3)	14 days (x3)
90 mL - 50 µg/L ClO <sub>4</sub> <sup>-</sup>		10 days			10 days		
10 mL - sediment		7 days			7 days		
spike 50 mg/L acetate		5 days			5 days		
N <sub>2</sub> Sparge		3 days			3 days		
		1 day			1 day		
Subtotal	0	8	3	3	8	3	3
<b>Oxygen</b>	-	-	-	-	-	-	-
Subtotal	0	0	0	0	0	0	0
<b>Temperature</b>	-	14 days (x3)	14 days (x3)	14 days (x3)	14 days (x3)	14 days (x3)	14 days (x3)
90 mL - 50 µg/L ClO <sub>4</sub> <sup>-</sup>		10 days			10 days		
10 mL - sediment		7 days			7 days		
N <sub>2</sub> Sparge		5 days			5 days		
4°C		3 days			3 days		
		1 day			1 day		
Subtotal	0	8	3	3	8	3	3
<b>SUBTOTAL</b>	3	24	9	9	24	9	9

**TOTAL SAMPLES = 87**

**QA/QC SAMPLE SUBMISSION**

Collected Aqueous Samples

- Deep 1 (Bottom)
- Deep 1 (Thermocline)
- Deep 1 (Surface)
- Shallow 1 (Bottom)

Experimental Solutions

- 50 µg/L ClO<sub>4</sub><sup>-</sup> solution for sediment cultures
- Acetate spike solution for sediment cultures
- ClO<sub>4</sub><sup>-</sup> spike solution for aqueous cultures

**TOTAL SAMPLES = 7**