

DETAILED STATEMENT OF WORK

Attached to and made a Part of contract No. DACW57-97-D-0004

Task Order No. DY01
Modification No. 006

Lake Belton Anoxic Study Field Sampling Plan
Lake Belton and Lake Waco, Texas

June 09, 2003

1.0 General. This modification addresses activities associated with the Bosque and Leon River Watersheds Perchlorate project. Task Order No. DY01, is being modified (Modification No. 6) to address the performance of a series of micro-ecology experiments to determine the potential for natural perchlorate bioreduction in both the water column and the sediment at the water/sediment interface. All sections of the original task order and previous modifications shall remain valid except where discussed below.

1.3 Period of Performance. This modification extends the period of performance to December 31, 2004.

2.0 Scope. As stated above, this modification continues efforts that were begun under previous authorizations. The numbering for the tasks of this mod continues the sequence of those authorizations.

2.4 Task 4 – PROJECT REPORTING AND PAYMENT REQUESTS.

The A-E shall continue to submit Monthly Progress Reports and payment invoices through December 2004.

2.11 Revise Task 11 – EVALUATE THE IMPORTANCE OF ANOXIC COMPONENT OF LAKE BELTON ON PERCHLORATE REDUCTIVE METABOLISM – to include the following additional work: 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. In many natural waters that contain perchlorate, oxygen and nitrate normally are also present and the order of preference is oxygen > nitrate > perchlorate. Once the oxygen is depleted, the biological reductions of nitrate and perchlorate are greatly improved. However, the reduction of perchlorate to chloride is impaired if nitrate is present in the water. The Contractor shall perform micro-ecology experiments designed to address several aspects to determine the potential for natural perchlorate biodegradation in Lake Belton. These aspects include experiments for reductive medium, spatial variability, nutrients, oxygen, temperature, and kinetics.

Subtask 11.4 – Reductive Medium. It is anticipated that biological reduction of perchlorate can occur in both the sediment and in the water column. The Contractor shall perform separate tests on each of these mediums.

Subtask 11.5 – Spatial Variability. Due to 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 shall be selected. Three of the locations shall be selected in the deepest 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 shall be chosen in shallower water where the thermocline is close to the water/sediment interface. Sediment shall be collected from each of these locations. Water samples shall 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 shall be collected near the bottom of one of the shallower water locations.

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

Location	Sample (s)
Deep [†] 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 [‡])	Sediment, Water near bottom
Shallow 2 (Close to Thermocline)	Sediment
Shallow 3 (Close to Thermocline)	Sediment

[†] - The deepest locations of Lake Belton that have presumably been anoxic the longest at the time of sampling.

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

Subtask 11.5.1 - Sediment Variability. Under temperature controlled conditions, in an anaerobic chamber, a series of autoclaved 100 ml Amber Pyrex Media bottles shall 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 shall be inoculated with 10 mL aliquots of the six different sediments, nitrogen purged, capped, and agitated (in triplicate). All six-core sediment samples shall be processed in triplicate to insure representative sampling of the core plug as a whole. The samples shall 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 shall be minimized to reduce the chance of algal growth that could result in undesirable oxygen generation. For each of the two sediment samples, an additional five cultures shall 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) shall 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 shall 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 shall be collected from all of the bottles and submitted to the USACE laboratory for perchlorate analysis. An aliquot of the stock solution shall also be sent for analysis.

Subtask 11.5.2 - 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, shall be volumetrically transferred into amber, 50 mL serum bottles and spiked with 50 µg/L perchlorate. After the perchlorate injection, the samples shall be nitrogen purged, capped, agitated and allowed to react for a 14-day period in a low-temperature incubator set at ambient conditions. All samples shall be carried out in triplicate and exposure to light shall be minimized. At the end of the contact time, filter-sterilized (0.45 µm) samples shall be collected and submitted to the USACE laboratory for perchlorate analysis. As the rate of biodegradation is not known, an additional five cultures shall 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.

Subtask 11.6 – Nutrients. While it is not possible to easily determine the limiting nutrient of these biological systems, experiments shall 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 shall 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. To determine if perchlorate reduction might be inhibited by a nutrient deficiency, samples of the sediment and water column shall be amended with an electron donor and/or metals.

Subtask 11.6.1 - Sediment Nutrients. Portions of the sediment samples collected shall be processed as described in Subtask 11.5.1 “Sediment Variability” section of this document. These samples shall 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 shall be collected and submitted to the USACE laboratory for perchlorate analysis. This result shall 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 shall be prepared for two of the sediments. These cultures shall be individually sacrificed at 1, 3, 5, 7, and 10 days to characterize any potential impacts on perchlorate degradation.

Subtask 11.6.2 - Water Column Nutrients. A portion of the water and sediment samples collected will be processed as described in Subtask 11.5.2 “Water Column Variability” and Subtask 11.5.1 “Sediment Variability” sections of this modification respectively. Each of these samples shall then be amended with the acetate and metal combinations identified in the table below:

Culture	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 [†]	
† Mineral Solution Composition in Deionized Water (g/L)		
	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	10
	ZnCl ₂	0.05
	H ₃ BO ₃	0.3
	FeCl ₂ ·4H ₂ O	1.5
	CoCl ₂ ·6H ₂ O	10
	MnCl ₂ ·6H ₂ O	0.03
	NiCl ₂ ·6H ₂ O	0.03

Once amended, the samples shall be agitated and allowed to react for a 14-day period. At the end of the contact time, filter-sterilized (0.45 µm) samples shall be collected and submitted to the USACE laboratory for perchlorate analysis. The perchlorate reduction in these cultures shall then be compared to the corresponding un-amended culture from the “Water Column Variability” tests. For one of the sample locations an additional five cultures shall be prepared for each of the nutrient addition conditions. These samples shall 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.

Subtask 11.7 – 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 shall be oxygenated and provided sufficient electron donor to reduce the oxygen. This will show if the culture can recover in the time allotted. This shall be compared to an unoxygenated system. Additionally, a water sample from above the thermocline shall be tested for its ability to reduce perchlorate. The inhibitory effect of oxygen shall 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 shall be used to differentiate between the possible impacts of brief oxygen exposure to elevated dissolved oxygen levels. Six aliquots for each of the four water samples shall be oxygenated. Two of the aliquots for each water shall 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 shall then be dosed with 50µg/L perchlorate and 50mg/L acetate, sealed, agitated, and returned to the inert environment. The cultures shall then be allowed to react for a fourteen (14) day period before it is filter-sterilized (0.45 µm) and submitted to the USACE laboratory for perchlorate analysis.

Subtask 11.8 – 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 micro-ecology experiments shall be carried out at both room temperature (~ 20° C) and at the coldest water temperature recorded at the time of Lake Belton sample collection. 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 shall be processed as described in the “water column variability” and “sediment variability” sections of this document respectively.

The processed samples shall 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 6 ° C, based on discussions with USACE at the time of lake sampling. After the respective incubation periods, filter-sterilized (0.45 µm) samples shall be collected and submitted to the USACE laboratory for perchlorate analysis.

Subtask 11.9 – Kinetics. Determining the rate biodegradation *a priori*, based on the micro-ecology of these environmental mixed cultures and available micronutrients is not possible given the current state of knowledge. Consequently, samples shall be collected at regular intervals over the course of fourteen (14) days to establish how rapidly biodegradation will take place for selected conditions and establish how different environment factors may impact this observed rate.

3.0 Delivery Schedule.

3.8 Task 11 – Project Reporting. Shall change the project reporting requirements to include the following:

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