

- 1. Title:** Development and Characterization of Microbial Inocula for High-Performance Passive Treatment of Acid Mine Drainage
- 2. Investigators:** Dr. Kenneth F. Reardon; Dr. Amy Pruden
- 3. Institutions:** Colorado State University, Fort Collins, CO 80523
- 4. Project Period:** 1 November 2003 – 31 October 2005
- 5. Project Cost:** \$256,945 (\$195,433 Rocky Mountain Regional HSRC; \$61,512 Cost Share)
- 6. Project Summary:** Mine drainage (MD) is well known for the threat that it poses to human health and the environment. Sulfate-reducing permeable reactive zones (SR-PRZs) are one promising means of treating acid mine drainage. The approach is particularly attractive because it is low-cost, is carried out in-situ, and because of its passive design (low required maintenance). However, there is a lack of consensus with respect to optimal design criteria. Maintaining consistent and reliable performance is particularly challenging, especially during cold or freezing weather. One critical aspect of SR-PRZ design that can address these issues and that deserves further attention is the role of the microbial inoculum. Current inoculum sources are poorly suited for the SR-PRZ environment and may be one of the primary factors in the short lifetime, low performance, or long startup times that are often observed.
 - a. Objectives:** The objectives of this study are to identify and develop microbial inocula that optimize SR-PRZ performance and reliability for MD remediation and to produce a protocol for developing site-specific inocula.
 - b. Approach:** In particular, this study will (1) screen and develop a variety of inocula both from environmental sources (such as manure and optimally functioning SR-PRZs) and ones that are “constructed” from microorganisms known to play a key role in SR-PRZ function. These inocula will be enriched in anaerobic serum bottles and (2) select inocula will be further tested in column studies. The inocula will be evaluated specifically for the required start-up time, active lifetime, and response to cold temperatures of the SR-PRZs they seed. In addition, site-specific factors will be investigated. (3) Inocula will be further improved and tested by combining inocula that are identified to have key capabilities. Analysis of microbial community structure and function will be a critical for accomplishing this. We have developed a suite of molecular techniques for meeting this goal, including: denaturing gradient gel electrophoresis (DGGE) of both rDNA and rRNA, and slot-blot analysis of SR-PRZ consortia based on 16S rDNA and functional genes.
 - c. Expected Results:** Current approaches to SR-PRZ inoculation generally involve the application of materials, such as manure, which are not well suited for the actual conditions present in the SR-PRZ. Therefore we expect that developing inocula that are specifically suited for the SR-PRZ environment will greatly enhance the function of these passive barriers. Specifically, improving the inoculum is a promising and low-cost means of improving SR-PRZ performance. The research will yield a protocol that can be used to produce site-specific inocula for SR-PRZs, facilitating the installation of PRZs that become active sooner, have longer lifetimes, and are resistant to site-specific challenges such as low temperatures or high metal levels.