Aspects of microbial sulfur cycle activity at a western coal strip mine

by Gregory James Olson

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Microbiology
Montana State University

Abstract:
The activity of certain groups of sulfur cycle bacteria associated with waters, sediments, and the coal bearing strata of a coal strip mine at Decker, Montana, was studied. Other mining areas of southeastern Montana and northeastern Wyoming were examined to a lesser degree.

Thiobacillus ferrooxidans, one of the major contributors to acid mine drainage, was consistently detected in the mining environment. Physiological studies of T. ferrooxidans isolates indicated that these acidophilic iron and sulfur oxidizing organisms were typical of the species in their preference for low pH and ability to oxidize pyrite. Since 1) acidic conditions were never observed at Decker, 2) the isolates died off in mine water environments, and 3) no acid could be formed from coal samples inoculated with a Th ferrooxidans isolate, it was thought that their activity was limited to microzones in the coal bearing strata where they oxidized sulfuritic material. Any acid formed was quickly neutralized by bicarbonate in the groundwaters.

Sulfate reducing bacteria also were common in the mine waters and sediments. These organisms were particularly active in the settling pond sediments as was evidenced by the rapid rate of conversion of radiolabeled sulfate to sulfide. The hydrogen sulfide produced by these organisms contributed to heavy metal precipitation in the settling pond.

Well waters sampled over a wide area of southeastern Montana contained hydrogen sulfide and sulfate reducing bacteria were detected in all but one well. Activity of these organisms in the groundwater could not be demonstrated by radioisotope experiments, however, a comparison of stable sulfur isotope ratios between groundwater sulfates and sulfides showed the sulfide was likely produced by sulfate reducing bacteria.

ASPECTS OF MICROBIAL SULFUR CYCLE ACTIVITY AT A
WESTERN COAL STRIP MINE

by

GREGORY JAMES OLSON

A thesis submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Microbiology

Approved:

Chairperson, Graduate Committee

Head, Major Department

Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

June, 1978

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Dr. Gordon A. McFeters for his assistance, guidance, and encouragement throughout the course of his study. Sincere thanks are also due to the rest of the committee members, Drs. David G. Stuart, Nels M. Nelson, Samuel J. Rogers, Douglas Bishop, and, especially, Kenneth L. Temple, for their advice and assistance in the course of the author's graduate study.

I especially thank my wife, Susan, and son, Joey, for their understanding, encouragement and tolerance throughout the past months.

The author is also grateful to Ms. Susan Turbak for many helpful discussions and assistance throughout this study, and to Dr. David M. Ward for his advice and encouragement.

Special thanks are due to Dr. Richard W. Gregory of the Montana Cooperative Fishery Research Unit for his varied and unending assistance without which this project would have been much more difficult.

Thanks also go to Marie Martin for cleaning unending piles of glassware, and to Dana Baham, Department of Civil Engineering, William Brady, Department of Chemistry, Bill Dockins, Department of Microbiology, and Paul Garrison, Department of Biology, for their assistance in chemical and microbiological studies.

The author is also grateful to the Decker Coal Company for their cooperation and interest in the project.

The assistance of Jerrie Beyrodt in all aspects of office and administrative work is thankfully acknowledged.

This project was supported by funds from the U.S. Environmental Protection Agency (EPA-WQO research grant number R803950), the U.S. Department of the Interior authorized under the Water Resources Research Act of 1964, Public Law 88-379, and administered through the Montana University Joint Water Resources Research Center (grant number A-108-Mont), the U.S. Geological Survey (grant number 14-08-0001-G-497), and the Department of Microbiology, Montana State University.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The activity of certain groups of sulfur cycle bacteria associated with waters, sediments, and the coal bearing strata of a coal strip mine at Decker, Montana, was studied. Other mining areas of southeastern Montana and northeastern Wyoming were examined to a lesser degree.

Thiobacillus ferrooxidans, one of the major contributors to acid mine drainage, was consistently detected in the mining environment. Physiological studies of *T. ferrooxidans* isolates indicated that these acidophilic iron and sulfur oxidizing organisms were typical of the species in their preference for low pH and ability to oxidize pyrite. Since 1) acidic conditions were never observed at Decker, 2) the isolates died off in mine water environments, and 3) no acid could be formed from coal samples inoculated with a *T. ferrooxidans* isolate, it was thought that their activity was limited to microzones in the coal bearing strata where they oxidized sulfuritic material. Any acid formed was quickly neutralized by bicarbonate in the groundwaters.

Sulfate reducing bacteria also were common in the mine waters and sediments. These organisms were particularly active in the settling pond sediments as was evidenced by the rapid rate of conversion of radiolabeled sulfate to sulfide. The hydrogen sulfide produced by these organisms contributed to heavy metal precipitation in the settling pond.

Well waters sampled over a wide area of southeastern Montana contained hydrogen sulfide and sulfate reducing bacteria were detected in all but one well. Activity of these organisms in the groundwater could not be demonstrated by radioisotope experiments, however, a comparison of stable sulfur isotope ratios between groundwater sulfates and sulfides showed the sulfide was likely produced by sulfate reducing bacteria.

INTRODUCTION

The Fort Union coal formation in southeastern Montana contains large deposits of low-sulfur sub-bituminous coal which occurs in seams up to 25 m thick (36). Mining operations in this area have been expanding and, with the depletion of readily available sources of low-sulfur coal elsewhere, removal of the abundant and easily accessible coal reserves in portions of the western United States by surface extraction methods has accelerated, and will likely continue.

The water resources of southeastern Montana are scant. Except for Rosebud Creek and the Tongue and Powder Rivers, little surface water is available (36). Protection of surface and groundwater quality in the face of increasing mining activity is of major importance to this region.

Microorganisms catalyzing sulfur transformations can have significant effects on surface and groundwater quality, especially in connection with coal mining. The exposure of sulfuritic minerals (chiefly pyrite and marcasite) found in association with coal deposits results in the formation of sulfuric acid and the solubilization of heavy metals (46). This process has resulted in serious water pollution problems in certain areas of the United States, adversely affecting thousands of miles of rivers and streams (32). The Ohio River alone receives the equivalent of three million tons of concentrated sulfuric acid annually from mine effluents (35). The acidophilic iron and sulfur oxidizing bacterium <u>Thiobacillus</u> <u>ferrooxidans</u> is a major contributor to acid mine drainage (58). The oxidation of pyrite, and the resultant formation of sulfuric acid, can be accelerated several hundredfold over over the non-microbial chemical rate as a result of the activities of this organism (8). Heavy metals may be leached into groundwaters as a result of movement of these waters through mine tailings where sulfide minerals are oxidized by thiobacilli to form acid (12). Certainly, not all mines produce acidic drainage. The amount of pyrite available, its distribution and particle size, and the buffering capacity of waters coming in contact with the pyrite affect the amount of acid produced (55). Even in the Ohio-West Virginia-Pennsylvania area where acid mine drainage is most common, mine drainage is often not highly acidic (60). Acid mine drainage has not been reported in southeastern Montana but does exist elsewhere in the state (37).

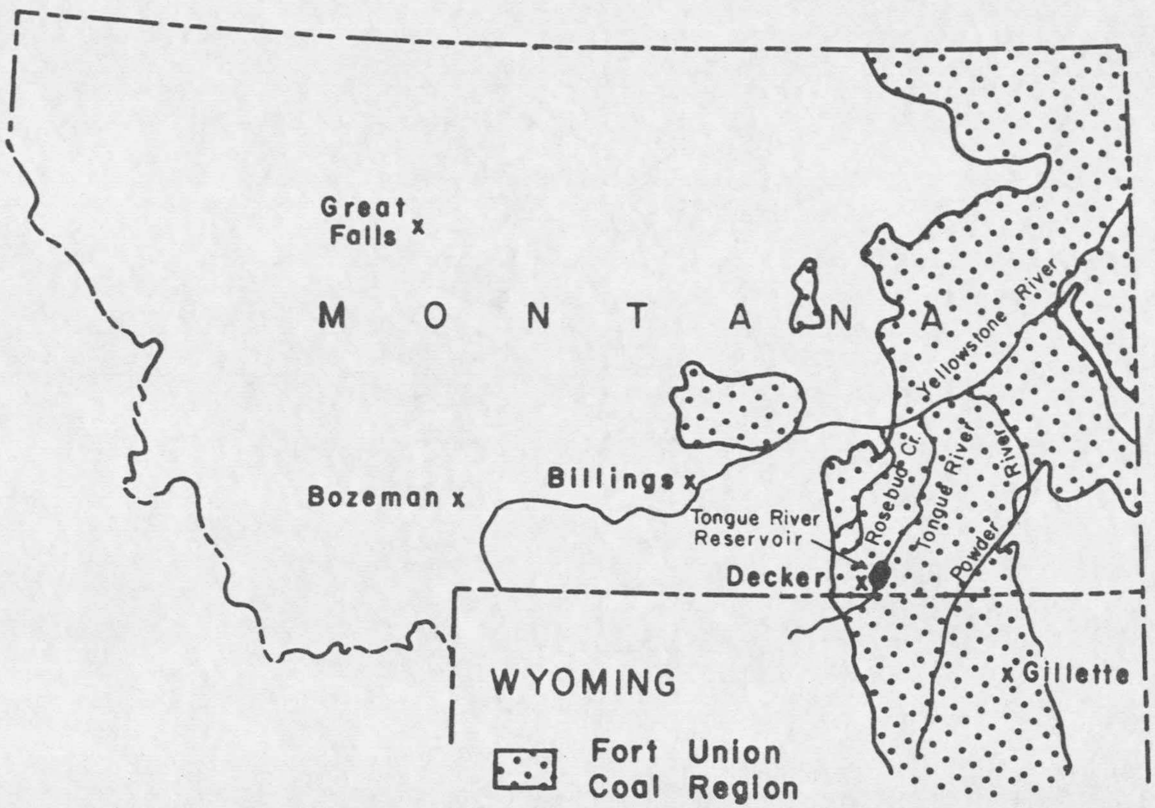
Another group of bacteria important in sulfur transformations, the sulfate reducers, has been suggested by Tuttle, et al., and King, et al., as a means of combatting acid mine drainage due to the ability of this group to raise the pH of waters and precipitate heavy metals (26,65). These authors have suggested that addition of organic matter to acidic mine settling ponds or lakes will accelerate sulfate reduction and thus improve water quality in regard to pH and heavy metal content. Even in non-acidic environments, sulfate reducing bacteria have been used as a means of trapping heavy metals in settling ponds

(18). Sulfate reducing bacteria are strict anaerobes which use sulfate as a terminal electron acceptor producing hydrogen sulfide (43). The sulfide formed reacts rapidly with heavy metals that may be present, creating highly insoluble metal sulfides (42).

Sulfate reducing bacteria have also been implicated in contamination of certain groundwaters with hydrogen sulfide (15,20,30). Groundwaters over a large area of southeastern Montana have varying levels of hydrogen sulfide (29). Oftentimes these groundwaters flow through coal beds of the region which serve as important sources of groundwater (69). It is not known if mining affects hydrogen sulfide formations in these waters.

In contrast to the eastern part of the United States, microbial sulfur cycle processes important in western coal mining environments have not been well studied. The microbiology of alkaline mine drainage, in general, is poorly understood (32). This study was undertaken to describe the possible effects sulfur transformations could have on surface and groundwater quality which result from coal strip mining in the Decker area of southeastern Montana (Figure 1). Here mining operations have interrupted the normal flow of groundwater. The most important aquifers to be interrupted are the coal seams themselves (69). As a result, groundwater flows into the mine pit and is pumped out so that mining activities may continue. This altered groundwater is collected in a sump pit from which it is pumped into the mine settling

Figure 1. Map showing the Decker area and the Fort Union coal region. Adapted from VanVoast and Hedges (69).

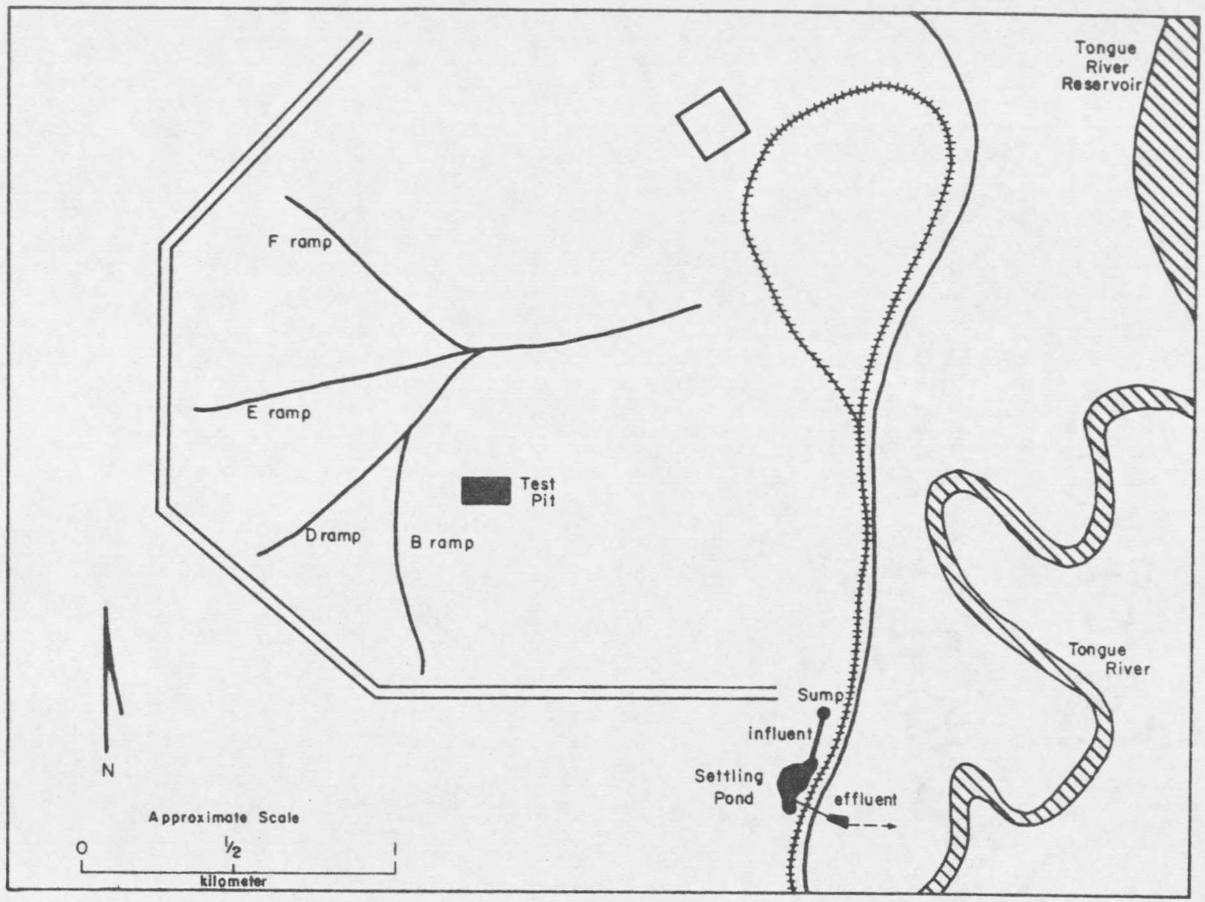

Great Falls
MONTANA
Yellowstone River
Billings
Bozeman
Rosebud Cr.
Tongue River
Powder River
Tongue River Reservoir
Decker
WYOMING
Gillette
Fort Union Coal Region

pond. Overflow water (the mine effluent) is discharged on to the Tongue River flood plain (Figure 2). When mining is completed, spoils will be replaced in the mined out area. As groundwater accumulates in the replaced spoil, significant changes in groundwater quality may result due to leaching of soluble minerals (68).

This study involves an investigation of microbial sulfur cycle processes that may be important in the aquatic environment within the mine and of the effects these transformations may have on the quality of mine discharge. This study attempts to answer the following questions: 1) are acidophilic sulfur bacteria found in connection with the Decker mine environment, and, if so, are these the same organisms implicated in the production of acid mine drainage in other areas of the country, and are they active in the coal-bearing strata at Decker; 2) are sulfate reducing bacteria found in the settling pond sediments at the Decker mine and, if so, are they active and contributing to heavy metal precipitation; and 3) are sulfate reducing bacteria present in the groundwater of the coal deposit areas and are they responsible for formation of the hydrogen sulfide which occurs there?

The answers to these questions should contribute to a better understanding of the important microbial sulfur cycle processes occurring in alkaline mine drainage of the western United States as well as in the groundwaters of mining areas in southeastern Montana.

Figure 2. Map of the Decker Mine. The double line represents the mine pit.


Tongue
River
Reservoir
F ramp
E ramp
Test
Pit
D ramp
B ramp
Tongue
River
Sump
influent
Settling
Pond
effluent
N
Approximate Scale
0
1/2
1
kilometer