Mine Water Environ (2011) 30:175–184
DOI 10.1007/s10230-011-0141-9

TECHNICAL ARTICLE

Microbiological Evidence for the Origin of Acid Mine Drainage at the Green Valley Site, Vigo County, and Friar Tuck Site, Greene County, Indiana, USA

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Received: 29 September 2010 / Accepted: 11 February 2011 / Published online: 13 March 2011

Abstract We examine the origin of acid mine drainage (AMD) that forms within coal refuse (gob) piles at the Green Valley and Friar Tuck sites in Indiana, using microbiology, traditional geochemistry, and oxygen and hydrogen isotopes. Reducing the AMD load from these sites has been an historical priority. Our observations indicate that AMD generation at these sites in Indiana is driven by three complementary factors: elevated populations of chemolithotrophic microbes of the species *Acidithiobacillus ferrooxidans*; a growth substrate that provides 'food' (e.g. pyrite) for these microbes, and a gob pile with geometry and other properties conducive to maintaining the thermal window of 25–40°C for optimal *A. ferrooxidans* growth. In particular, increasing levels of Fe^{+3} and total dissolved solids (TDS), and decreasing pH for gob waters were found to be highly correlated with increasing populations of *A. ferrooxidans*. Furthermore, the chemosythetic bacteria population increase correlates with increasing hydrogen stable isotope shift away from the global meteoric water line for gob waters in this study, though it is unclear if this shift is the result of microbial metabolic processes or a secondary effect due to microbially-mediated pH change or electrolysis.

Keywords AMD · Coal gob · Geochemistry · Chemolithotrophic bacteria · Stable isotopes

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Introduction

Recent efforts have significantly reduced acid mine drainage (AMD) from both the Green Valley and Friar Tuck mine sites in Indiana, though some AMD still occurs and adversely affects local waterways. This study was undertaken to complement earlier studies by the author and others that quantified AMD chemistry at Green Valley. These studies include Brake et al. (2001a, b), who examined protozoan communities living in AMD seeps; Melchiorre et al. (2004), who identified rare minerals that precipitate at Green Valley due to its unusual water chemistry; and Melchiorre et al. (2005), who identified leaky capping materials and highlighted the role of meteoric waters in generating AMD at Green Valley. In this paper, a broad multi-disciplinary approach has been utilized to identify the link between biology, hydrology, and geology with AMD generation from coal refuse piles in southwestern Indiana.

Background

Green Valley Site

The Green Valley site is located in Vigo County about 6.4 km (4 miles) northwest of Terre Haute, Indiana, USA (Fig. 1a, b). The former Green Valley mine and plant covers 64.3 ha (159 acres). Pre-mining topography was low and undulating with a total vertical relief of 18.3 m (60 ft) (Fig. 1b). Reference to the Green Valley site in this paper will specifically indicate the abandoned Green Valley Mine and the adjoining gob pile.

In 1948, the Snow Hill Coal Corporation began underground coal mining operations at the site via shafts sunk in

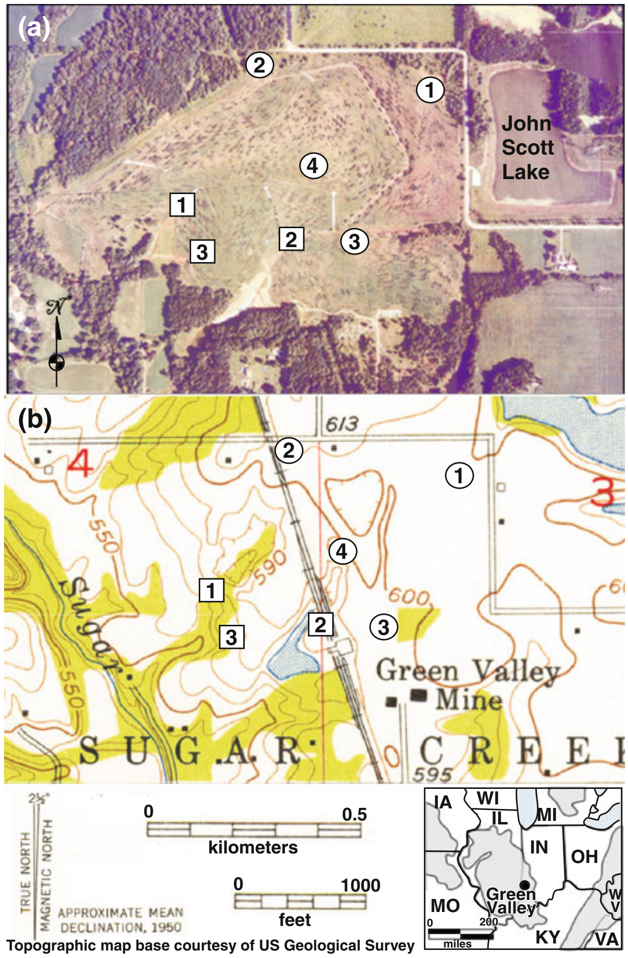

Fig. 1 **a** *Air photograph* of Green Valley in 2002, showing location of AMD seeps (*numbered squares*), and observation wells (*numbered circles*). **b** Topographic map of the Green Valley site in 1950, shown at the same scale as **a**). The *shaded area* in the index map indicates major coalfields of the Midwestern US, and the *black dot* identifies the Green Valley site

the Pennsylvanian-age Springfield (Indiana V) and Seelyville Coal (Indiana III) seams of the Petersburg and Staunton Formations, respectively. Over 12.7 million tonnes (t) (14 million US tons) of coal was extracted during the 15-year life of the mine. By late 1963, the site was abandoned without any reclamation. During the active phase of the mining operations (1948–1963), over 2,064,000 m^3 (2,700,000 $yards^3$) of coal refuse materials (gob and slurry) were deposited on the ground surface at the Green Valley site. The refuse consists of coal fragments, limestone, shale, and pyrite. Scrap steel, rubber, and plastic from the former mining operations were also disposed of in the gob piles.

The site was first studied by Caserotti and Marland (1973), who noted that the polluted sections of West Little Sugar Creek and Sugar Creek were an "orange-rusty color." Caserotti and Marland (1973) reported that the AMD contained dissolved iron concentrations of 56,000 mg/L and dissolved sulfate levels of 200,000 mg/L. In 1976, Green Valley was designated as derelict mine land, and possible solutions with associated costs for dealing with the AMD were evaluated. During late December of 1984, at a time when a light snow blanketed much of the area, the surface of the gob pile was devoid of snow. Some of the areas were warm to the touch and in several places, the gob pile experienced enough heat generation that spontaneous combustion of the gob occurred (Geosciences Research Associates 1985).

In early 1985, the Abandoned Mine Land Section of the Indiana Dept of Natural Resources (IDNR), Division of Reclamation began reclamation efforts, including grading and capping of the pile. The gob pile was successfully vegetated and AMD was significantly reduced, although some AMD still occurs and continues to affect local waterways. Following the initial reclamation in 1985, additional work has been performed to route on-site surface water and AMD towards ditches lined with >20 cm limestone riprap. These reactive channels are presently lined with brown, red, white, and rarely blue and green secondary minerals formed by AMD coming in contact with the limestone and undergoing complex reactions with the atmosphere and organic debris. Microorganisms such as protozoa, diatoms, fungi, and algae have been observed to form iron-rich stromatolite-like communities in the reactive drains (e.g. Brake et al. 2001a, b, 2003). The secondary minerals forming within and adjacent to these reactive channels includes species previously thought to be exceedingly rare, such as xitieshanite ($Fe^{3+}(SO_4)Cl \cdot 6H_2O$), hinting at the unusual chemistry of the AMD (Melchiorre et al. 2004).

During the period of this study, AMD flowed freely from three seeps located along the southern base of the gob pile (Fig. 1). Seeps 1 and 2 have flowed for many years, while seep 3 first appeared in late April 2001, following a large rainfall that produced several subsidence depressions within the gob pile. The water flowing from these seeps often has a pH of between 1.5 and 4, and an electrical conductivity (EC) as high as 25,000 µS/m, reflecting elevated SO_4, Fe (up to 11,800 mg/L), Al (up to 1,840 mg/L), Mn, and Zn. Other metals (Ni, Cr, Cu, Cd, and Pb) are also present but at lower concentrations (Amt et al. 2003; Dale 2001; Eggert et al. 1981; Geosciences Research Associates 1985; Unger et al. 2003). Ground- and surface-water from the site flow northeast to southwest towards Sugar Creek. Four monitoring wells were completed at the site in Nov. 2000 to measure static water levels and collect representative samples of the shallow groundwater and water within the gob material (Fig. 1).

In 2000–2003, frequent sampling of gob seep and well waters at Green Valley permitted collection of a large, multi-season database of geochemical and light stable isotope values. This comprehensive characterization of the Green Valley waters revealed a temporal relationship between rainfall and gob waters, suggesting that precipitation recharges the gob pile waters with a residence time measured in months (Melchiorre et al. 2005). Many studies have used traditional geochemical methods, stable isotopes, or both to address the issue of AMD sourcing and recharge (e.g. Allen and Voormeij 2002; Hazen et al. 2002), setting a precedent for this type of multidisciplinary approach.

Friar Tuck Site

The Friar Tuck site is located in Greene and Sullivan Counties about 3.4 km (2.1 miles) northeast of Dugger, Indiana, USA (Fig. 2a, b). The area included in this study comprises 80.9 ha (200 acres). Pre-mining topography was low and undulating with a total vertical relief of 33.5 m (110 ft) (Fig. 2b). Reference to the Friar Tuck site in this paper specifically refers to the abandoned Friar Tuck and Redbird mine sites and the adjoining gob piles.

The Friar Tuck Mine was opened by the Sherwood Coal Company in Greene County during 1943, and ultimately produced a total of about 2.7 million t (3 million US tons) of coal prior to closure in 1952. The Redbird Coal Company began mining on the west side of Friar Tuck in adjoining Sullivan County during 1945. Redbird closed operations in 1950 and produced a total of about 1.8 million t (2 million US tons) of coal. Early reclamation efforts at Friar Tuck consisted of rough grading and revegetation with grass and pine trees, leaving lakes of AMD and hills of gob. This area was informally known as 'Redbird' in the 1970 s when it became a popular, but illegal destination for off-road vehicular activity. The IDNR completed a report in 1972 on off-road vehicle activity environmental impacts, and the Natural Resources Commission adopted a policy prohibiting these activities on DNR-controlled properties.

Informal and unpublished studies of the Friar Tuck site in the early 1980s revealed a site with significant AMD generation (Indiana Geological Survey, unpublished file data). Formulation of reclamation strategies and characterization of the site was performed in the late 1980s (Harper et al. 1988, 1989). Interest in the off-road recreational potential of the area resumed in 1995, and internal meetings at the IDNR recommended that the Division of Outdoor Recreation develop a strategy for what was now being called the "Redbird site." This area consists of the Friar Tuck site described in this study, as well as a much larger surrounding area. A management group was formed, and over 243 ha (600 acres) was initially purchased. The inaugural riding season at Redbird, the first state owned off-road park in Indiana, was 2003, exactly 60 years after

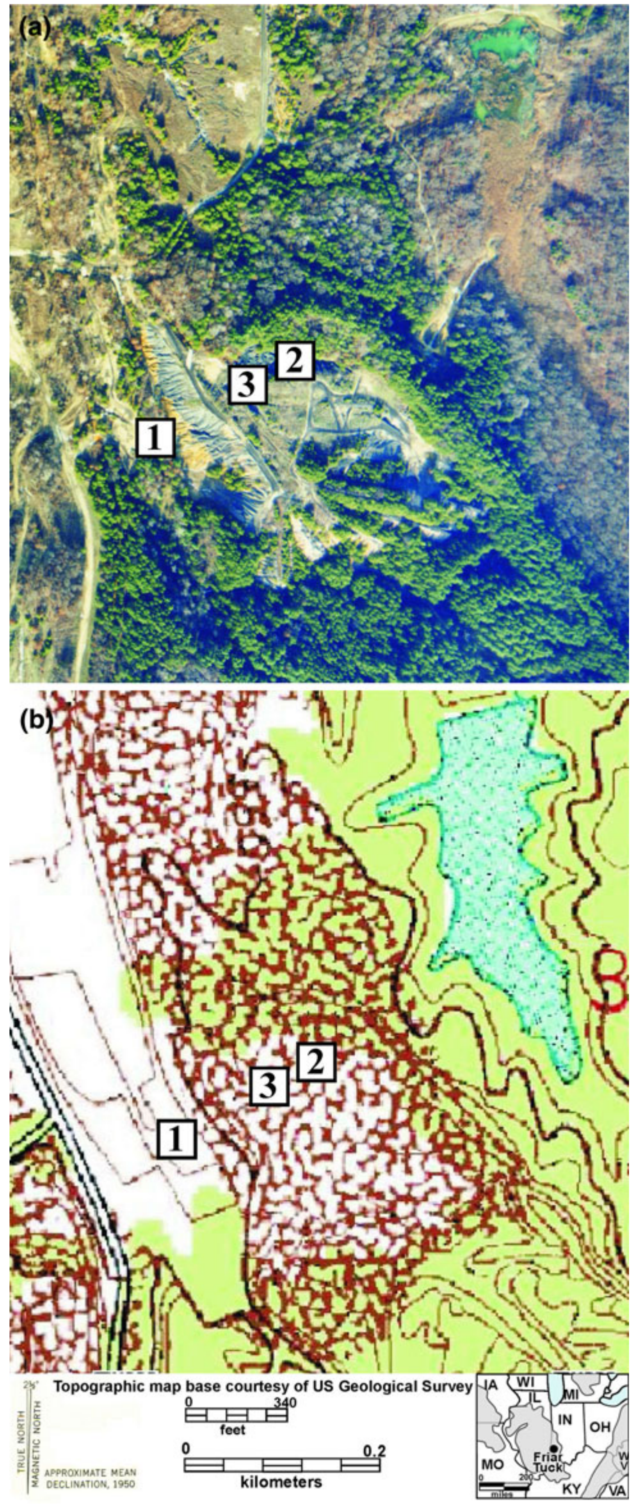

Fig. 2 **a** *Air photograph* of the Friar Tuck site in 2002, and **b** Corresponding topographic map showing location of the main gob pile (*hatched*) and AMD seeps (*numbered squares*)

Friar Tuck first opened for coal mining. As of 2010, Redbird consists of 486 ha (1,200 acres) available for off-road vehicular activities.

AMD generation was clearly significant between 1987 and 1992 when it was studied by Branam and Harper (1994) and Harper et al. (1993) during a program to evaluate the effectiveness of previous reclamation efforts. These efforts consisted of rip-rap check dams to reduce sedimentation, and revegetation with Kentucky fescue and black locust. This test work showed improvement of soil pH and successful revegetation, but only a 10–50% reduction in total AMD generation (Harper et al. 1993).

Remediation of the AMD from this site has been ongoing since the late 1990s, consisting of constructed wetlands (northeast corner of site, Fig. 2a, b) and extensive surface recontouring and capping of AMD-generating wastes. The wetland consists of an old lake that is a natural pre-mining portion of the drainage of the site. This lake was modified to restrict flow to the north into Mud Creek, and treats AMD by dilution with fresh water, and by passing diluted AMD through reactive limestone rip-rap channels into the wetlands, where conditions are optimized for sulfate-reducing bacteria. However, based on sulfur isotope work, dilution appears to be the dominant factor controlling AMD reduction (Flege 2001).

Methods

Samples for this study were obtained during May, 2003 at four monitoring wells and three AMD seeps at the Green Valley site, and at three AMD seeps at the Friar Tuck site. AMD seeps were sampled with a clean bucket directly from the main orifice. Three well volumes of water were hand-bailed using a stainless steel bailer to purge wells prior to sampling and measurements. Wells were allowed to recover to within 10% of the original static water level before 11.4 L (3 gallons) of water were bailed into a clean empty bucket for measurements and sampling. Sampling bailers and buckets were cleaned with distilled water between sampling of the various wells in order to avoid cross-contamination.

Measurements of total dissolved solids (TDS) and pH of the AMD were performed in-situ prior to sampling, using portable meters (i.e. YSI 30 conductivity meter/TDS, Corning-Hanna 98/07 pH meter). Field TDS measurements were only used as qualitative indicators and are not reported in this paper, as field meters estimate TDS using electrical conductance based on a standard relationship that is not likely to be valid in these AMD fluids. TDS measurements reported in this paper are from gravimetry performed in the laboratory. Water was collected in borosilicate glass bottles with a polyseal cap for geochemical and stable isotope analysis, and in sterile bottles for biological samples. Samples were stored on ice prior to elemental analysis.

Samples were analyzed by ICP-MS and gravimetry at licensed laboratories at Test America Inc. and Babcock & Sons of San Bernardino, CA. ICP-MS analyses were performed in accordance with the US Environmental Protection Agency methods 200.8 and 300.0, and used internal laboratory reagent spikes, blank spikes, and matrix spikes. Field replicates were run for one seep and one well for each sampling. Laboratory analysis of pH was performed as a rough check of field pH, and was considered acceptable if within 15%. Water samples were prepared for oxygen isotope analyses using the standard CO_2 equilibration method (Epstein and Mayeda 1953). Preparation of water for hydrogen isotope analysis used zinc shavings (Biogeochemical Laboratories, Indiana University) reacted at 500°C for 30 min with water to yield hydrogen gas by reduction (Coleman et al. 1982). Oxygen and hydrogen isotope values were measured using an automated sample equilibrator interfaced with a Gas Bench II and Delta Plus Advantage isotope ratio mass spectrometer in the stable isotope laboratory at California State University, San Bernardino. Results are reported in the usual "d" notation relative to VSMOW. Laboratory precision is ±0.08‰ for oxygen isotope values and ±0.6‰ for hydrogen isotope values, based on duplicate analyses of internal laboratory standards.

Samples for biological analysis were collected in sterile bottles, iced, and mailed by overnight service to Little Bear Laboratories, Golden, Colorado. Upon arrival, the samples were well mixed and sub-samples were aseptically transferred and serially diluted in acidified 2X nutrient media, with the addition of 5,000 mg/L Fe(II). A standard three-tube most-probable-number (MPN) examination was employed, with the exception that the test was performed in multi-well plates instead of tubes. A laboratory internal reference culture of iron-oxidizing mesophilic acidophiles was used as the positive control. The multi-well plates were incubated at 30°C for 21 days prior to enumeration. Microbial populations in positive samples were examined by phase contrast microscopy.

Results

Geochemical Parameters

Levels of total dissolved solids (TDS) in AMD at the Green Valley site ranged from 890 to 86,000 mg/L for wells, and 25,000 to 140,000 mg/L for AMD seeps (Table 1). At the Friar Tuck site, TDS for AMD seeps ranged from 32,000 to 121,000 mg/L. The pH of AMD at the Green Valley site ranged from 6.9 to 3.0 for wells, and 4.0 to 2.2 for seeps. At the Friar Tuck site, pH for AMD seeps ranged from 3.1 to 2.4.

Table 1 Geochemical and biological data for mine waters sampled May, 2003 at the Green Valley and Friar Tuck sites, Indiana

Description	MPN cells/mL	95% confidence level		$\delta^{18}O$ (VSMOW)	δD (VSMOW)	pH	TDS mg/L	Fe^{3+} mg/L	SO_4 mg/L
		Lower	Upper						
GV Well#1	4.3×10^2	7.0×10^1	2.1×10^3	−6.12	−38.9	4.2	19,000	17.000	600
GV Well#2	<30	*	*	−7.20	−47.1	6.9	890	5.1	450
GV Well#3	<30	*	*	−6.98	−44.8	6.3	5,900	8.5	3,700
GV Well#4	4.3×10^4	7.0×10^3	2.1×10^5	−6.75	−48.3	3.0	86,000	33,000	16,000
GV Seep#1	4.3×10^2	7.0×10^1	2.1×10^3	−7.10	−46.9	4.0	25,000	4,400	15,000
GV Seep#2	9.3×10^3	1.5×10^3	3.8×10^4	−6.42	−42.3	3.4	49,000	7,500	22,000
GV Seep#3	4.3×10^4	7.0×10^3	2.1×10^5	−6.99	−49.8	2.2	140,000	31,000	95,000
FT1 bend	4.3×10^4	7.0×10^3	2.1×10^5	−6.51	−46.2	2.4	98,000	28,000	58,000
FT2 pipe	4.3×10^4	7.0×10^3	2.1×10^5	−6.18	−43.7	2.5	121,000	36,000	41,000
FT3 seep	9.3×10^3	1.5×10^3	3.8×10^4	−6.06	−39.1	3.1	32,000	10,000	17,000

Data for Green Valley and Friar Tuck sampling sites for May, 2003

* Not applicable

Levels of Fe^{3+} in AMD at the Green Valley site ranged from 5.1 to 33,000 mg/L for wells, and 4,400–31,000 mg/L for AMD seeps (Table 1). At the Friar Tuck site, Fe^{3+} for AMD seeps ranged from 10,000 to 36,000 mg/L. For SO_4 concentrations, the Green Valley site ranged from 450 to 16,000 mg/L for wells, and 15,000–95,000 mg/L for AMD seeps. At the Friar Tuck site, SO_4 concentrations for AMD seepage ranged from 17,000 to 58,000 mg/L.

The somewhat different chemistry of the well and seep waters may arise from oxidation reactions in the outer surface of the gob pile versus the more anoxic conditions in the pile core penetrated by the wells. Some of this difference may also result from slightly different sampling flow/recharge rates. The exact mechanism for this difference is not known.

Chemolithotrophic Bacteria Concentrations

Most probable number (MPN) counts of chemolithotrophic bacteria in AMD at the Green Valley site ranged from <30 to 4.3×10^4 cells/mL for wells, and 4.3×10^2 to 4.3×10^4 cells/mL for AMD seeps (Table 1). At the Friar Tuck site, MPN counts of chemolithotrophic bacteria in AMD seeps ranged from 9.3×10^3 to 4.3×10^4 cells/mL (Table 1). Examination of the cell morphologies indicated the presence of *Acidithiobacillus ferrooxidans* and minor Leptospirillum sp. The *A. ferrooxidans* were observed as 1–2 µm colorless rod-shaped cells with a polar flagellum, while the *L. ferrooxidans* cells are distinctively spiral-shaped and averaged 2 µm in length. Both cell morphologies were Gram-negative, and consistent with published descriptions of *A. ferrooxidans* and *L. ferrooxidans* (e.g. Robertson and Kuenen 2005). *Acidithiobacillus ferrooxidans* were formerly called *Thiobacillus ferrooxidans*, before they were reclassified (Kelly and Wood 2000).

Oxygen and Hydrogen Stable Isotope Values

Oxygen isotope values for AMD from wells at the Green valley site ranged from −6.12 to −7.20‰ (VSMOW), while AMD seepage from the same site ranged from −6.42 to −7.10‰ (VSMOW) (Table 1). At the Friar Tuck site, oxygen isotope values for AMD seepage ranged from −6.06 to −6.51‰ (VSMOW) (Table 1). Hydrogen isotope values for AMD from wells at the Green valley site ranged from −38.9 to −47.1‰ (VSMOW), while AMD seepage from the same site ranged from −42.3 to −49.8‰ (VSMOW) (Table 1). At the Friar Tuck site, hydrogen isotope values for AMD seepage ranged from −39.1 to −44.2‰ (VSMOW).

Discussion

This applications-driven study has shown the utility of a broad multi-disciplinary approach to identify the link between biology, isotope hydrology, and geology with AMD generation from coal refuse piles in southwestern Indiana. The generation of AMD in southwestern Indiana has been, and remains, a serious environmental concern. This study has indicated the probable primary drivers for AMD generation in this region and provides direction for future applied research to address AMD issues.

Acidithiobacillus ferrooxidans were found to be the dominant chemolithotrophic proteobacteria in the Green Valley and Friar Tuck AMD samples, with traces of *Leptospirillum ferrooxidans* also present. The optimal temperature for growth of *A. ferrooxidans* occurs between 25 and 40°C (e.g. Brett et al. 2003; Clark and Norris 1996; Ferroni et al. 1986; Silverman and Lundgren 1958), similar to the internal temperatures measured for the gob piles at the Green Valley and Friar Tuck sites. The surface to volume ratio of the gob piles at both sites is small, suggesting internal heat loss is minimized. It is assumed that such favorable gob pile geometries (lower surface to volume ratios) will provide a larger volume of gob growth media within a favorable thermal window. Numerous studies have shown that *A. ferrooxidans* use iron and sulfur oxidization as an energy source (e.g. Kuenen et al. 1992; Rawlings et al. 1999; Robertson and Kuenen 2005). *A. ferrooxidans* have been linked to AMD generation on abandoned mine lands on virtually every continent (e.g. Schrenk et al. 1998).

The level of TDS and chemolithotrophic bacteria population in AMD correlate strongly at the Green Valley and Friar Tuck sites (Fig. 3). Similarly, pH and Fe^{3+} of these AMD correlate with chemolithotrophic bacteria population (Figs. 4, 5). These significant correlations (all with $R > 0.9$) suggest that the chemolithotrophic bacteria in the AMD at the Green Valley and Friar Tuck sites are producing elevated TDS and Fe^{3+}, and lower pH. It is possible that the lower pH and elevated TDS simply provide a favorable environment for elevated populations of chemolithotrophic bacteria. Indeed, low pH does provide favorable conditions for chemolithotrophic bacteria growth (e.g. Schrenk et al. 1998). However, these microorganisms do not use Fe^{3+} or the other dominant ions of the AMD as energy sources, but rather produce them as their waste products. Given the abundance within the Green Valley and Friar Tuck gob piles of pyrite 'food' for *A. ferrooxidans* and *L. ferrooxidans*, the fact that the internal temperatures of the gob piles are optimal for *A. ferrooxidans* growth, that *A. ferrooxidans* and *L. ferrooxidans* 'wastes' are observed at elevated levels in the AMD flowing from the gob piles, and indeed the elevated populations of *A. ferrooxidans* and *L. ferrooxidans* within the AMD waters, it seems extremely likely that the primary driver of AMD generation at both sites is the presence of *A. ferrooxidans* and to a lesser extent *L. ferrooxidans.* Though it has been suggested by some (e.g. Rawlings et al. 1999) that *L. ferrooxidans* may play a much more significant role in AMD generation than *A. ferrooxidans,* this does not appear to be the case at these sites in Indiana. This is most likely due to the specific pH and temperature of the waters within the gob, which are respectively too high and too low to favor *L. ferrooxidans* over *A. ferrooxidans* (e.g. Ferroni et al. 1986; Kuenen et al. 1992).

The SO_4 concentrations in AMD were found to correlate, but much less convincingly ($R = 0.75$), with chemolithotrophic bacteria population of the AMD from the Green Valley and Friar Tuck sites (Fig. 6). This lower SO_4 concentration may be due to the presence of sulfur-reducing bacteria or archaea within portions of the gob pile, or the precipitation of significant quantities of sulfur mineral phases, such as anhydrite or gypsum on the carbonate rocks within the gob. The latter explanation is considered unlikely because these phases were generally found to be absent during remediation-related excavations of the gob piles.

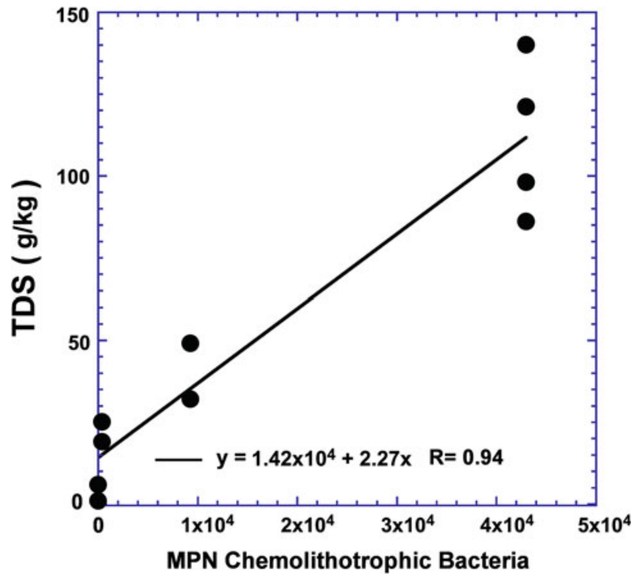

Fig. 3 *Plot* of most probable number (MPN) for chemolithotrophic bacteria population versus total dissolved solids (TDS) for AMD samples from the Friar Tuck and Green Valley sites

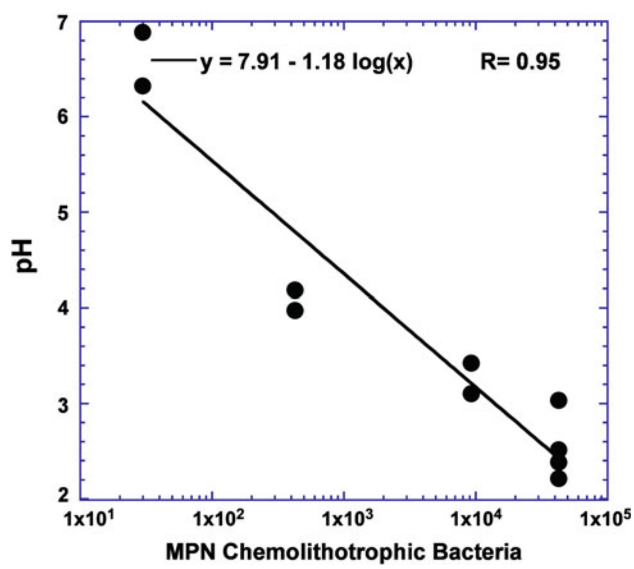

Fig. 4 *Plot* of most probable number (MPN) for chemolithotrophic bacteria population versus pH for AMD samples from the Friar Tuck and Green Valley sites

The oxygen and hydrogen stable isotope values for many AMD samples from the Green Valley and Friar Tuck sites plot close to the meteoric water line of Craig (1961), though deviations are noticeable for some samples (Fig. 7).

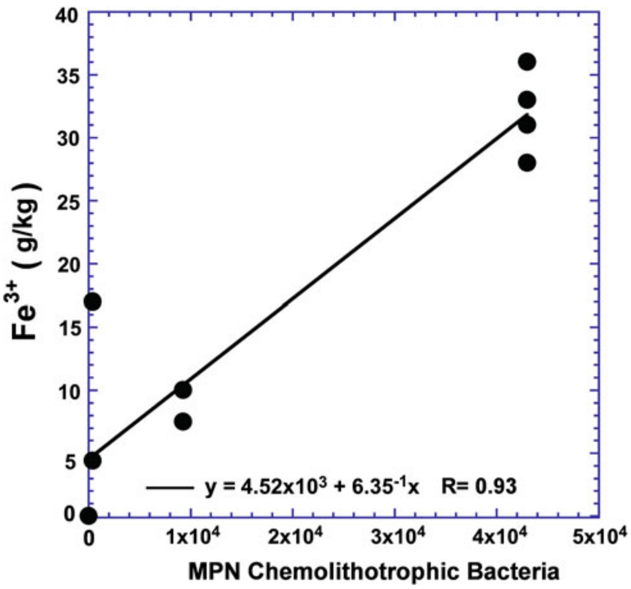

Fig. 5 *Plot* of most probable number (MPN) for chemolithotrophic bacteria population versus trivalent iron (Fe^{3+}) for AMD samples from the Friar Tuck and Green Valley sites

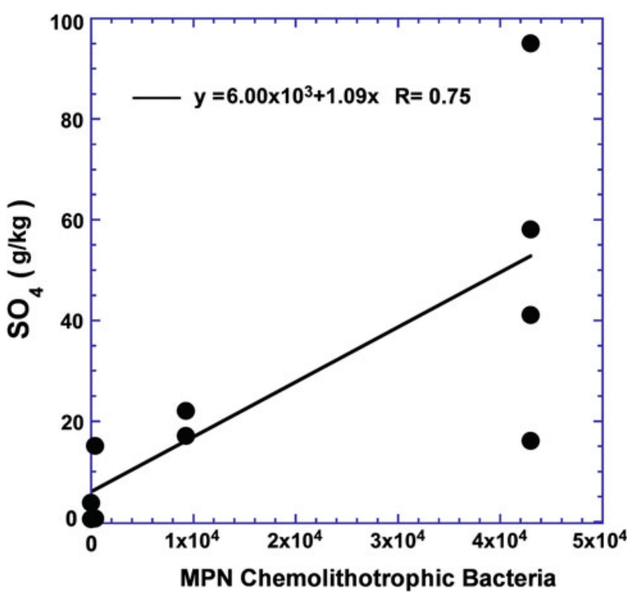

Fig. 6 *Plot* of most probable number (MPN) for chemolithotrophic bacteria population versus sulfate (SO_4) for AMD samples from the Friar Tuck and Green Valley sites

Close examination reveals that relative to the average local meteoric waters at each site, and relative to the total range of hydrogen and oxygen isotope values for all samples, this deviation is more likely dominated by a shift in δD values than δ^{18}O values. Assuming that this shift of AMD away from the meteoric water line is minimally influenced by a shift in δ^{18}O values, and dominated by a shift in the δD values, we calculated the value of this inferred δD shift

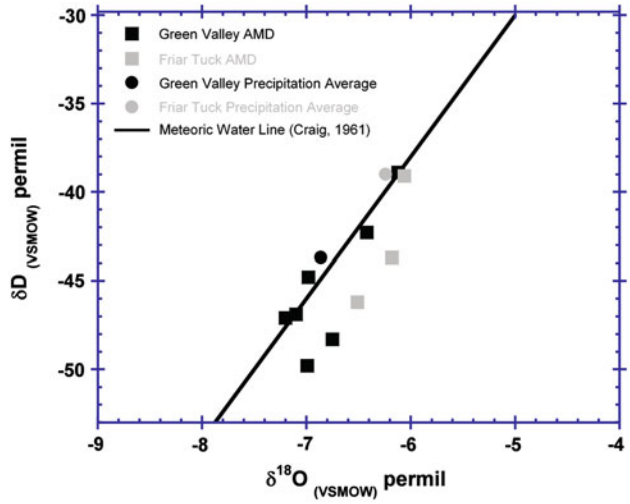

Fig. 7 *Plot* of δ^{18}O versus δD for AMD samples from the Friar Tuck and Green Valley sites; *solid line* shows the meteoric water line of Craig (1961), defined as $\delta D = \delta^{18}O \times 8 + 10$

(expressed as ΔD) relative to meteoric water (Table 2). This inferred δD shift appears to be linked to the chemolithotrophic bacteria population of the AMD from the Green Valley and Friar Tuck sites (Fig. 8).

It is known that sulfur-reducing microbes will produce a measurable fractionation of sulfur and occasionally hydrogen isotopes under a variety of conditions (e.g. Hunkeler et al. 2001; Morasch et al. 2001, 2002). It was initially suspected that bacterial production of H_2S was the mechanism by which large volumes of hydrogen were being fractionated within this system. It was also possible that H_2S production could be abiological, as suggested by some workers, due to the hydrolysis of water caused by elevated concentrations of Al, Mn, and other metals (e.g. Rose and Cravotta 1998). However, no measurable H_2S was recorded at either site. In addition, prior work has shown that δ^{34}S values of AMD at the Friar Tuck site are consistent with a system in which sulfur reduction is not occurring (Flege 2001). The apparent correlation between chemolithotrophic bacteria population and δD does not seem to result from a process that fractionates sulfur, and is a topic that should be addressed by future workers. Hydrogen isotope fractionation may occur due to the presence of complementary methanogenic microorganisms (e.g. Valentine et al. 2004), though it is possible that the relationship exists due to a pH moderated effect.

Future workers may find these insights to be of use in defining the direction for AMD remediation and prevention strategies. For example, this work suggests that a low surface to volume ratio for gob piles may prove more hospitable to growth of AMD-facilitating

Table 2 Stable isotope data for mine waters sampled May, 2003 at the Green Valley and Friar Tuck sites, Indiana, showing calculations of ΔD

Description	$\delta^{18}O$ (VSMOW) measured	δD (VSMOW) measured	δD (VSMOW) calculated[a]	ΔD calculated−measured[b]
GV Well#1	−6.12	−38.9	−39.0	0.06
GV Well#2	−7.20	−47.1	−47.6	0.5
GV Well#3	−6.98	−44.8	−45.8	1.04
GV Well#4	−6.75	−48.3	−44.0	−4.3
GV Seep#1	−7.10	−46.9	−46.8	−0.1
GV Seep#2	−6.42	−42.3	−41.4	−0.94
GV Seep#3	−6.99	−49.8	−45.9	−3.88
FT1 bend	−6.51	−46.2	−42.1	−4.12
FT2 pipe	−6.18	−43.7	−39.4	−4.26
FT3 Seep	−6.06	−39.1	−38.5	−0.62

Data for Green Valley and Friar Tuck sampling sites for May, 2003

[a] Calculated values of δD, using sampled ^{18}O value and meteoric water equation of Craig (1961), $\delta D = \delta^{18}O*8 + 10$

[b] Calculated δD−measured $\delta D = \Delta D$

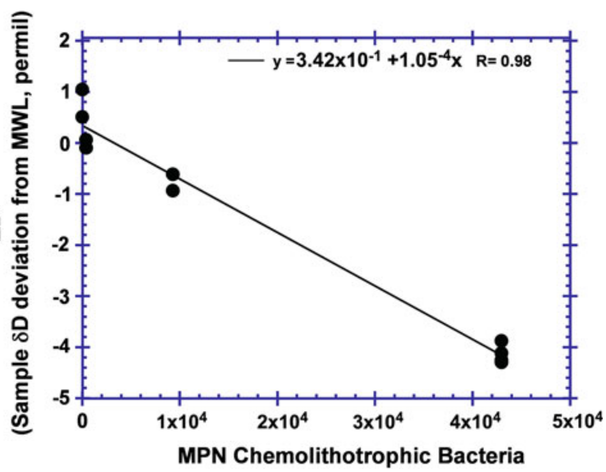

Fig. 8 *Plot* of most probable number (MPN) for chemolithotrophic bacteria population versus ΔD. ΔD is defined as the difference between the δD value for AMD samples from the Friar Tuck and Green Valley sites, and the δD value calculated for a meteoric water having the measured $\delta^{18}O$ using the meteoric *water line* equation of Craig (1961), defined as $\delta D = \delta^{18}O \times 8 + 10$

chemolithotrophic bacteria. This would prove contrary to traditional assumptions that it is best to minimize the surface area of gob piles to reduce their exposure to precipitation and non-biological chemical weathering. It may prove desirable to increase the surface to volume ratio of gob piles to reduce the habitable zone for chemolithotrophic bacteria so that more of the waste is at lower ambient temperature for most of the year. Furthermore, pretreatment or retreatment of gob by amalgamation units that coat waste with a thin phosphate coating or a chemolithotrophic bacteria growth retardant may prove useful.

Conclusions

The combined interdisciplinary methodology of using traditional water chemistry, light stable isotope values, and chemolithotrophic microbial population data provides new insights on the origin of AMD at the Friar Tuck and Green Valley sites, Indiana. At these sites, it was determined that the chemolithotrophic bacteria population of AMD correlates with total dissolved solids, pH, Fe^{3+}, and, to a lesser degree, SO_4. It is suspected that the lesser correlation of SO_4 results from sulfur metabolism by other microbial species known to be living within gob piles and AMD seepages. Similarly, it was observed that light stable isotope values of some AMD have measureable deviation from the global meteoric water line. The magnitude of this deviation was found to correlate with the chemolithotrophic bacteria population. While the exact mechanism driving this alteration of meteoric hydrogen isotope values is not known, it is suspected to result from either microbial metabolism or inorganic hydrolysis. These insights should prove useful in guiding AMD remediation and prevention strategies in southwestern Indiana to minimize the chemolithotrophic bacteria habitable zone within gob piles.

Acknowledgments First, and foremost, I thank Dr. Jim Mills of DePauw University, and Deb Dale of the US Office of Surface Mining for introducing me to the two field locations and thier many valuable discussions. I thank the owner of the Green Valley site and the IDNR for permission to access the properties. The IDNR facilitated access and provided professional expertise on AMD issues. I thank Tracy D. Branam of the Indiana Geological Survey for many valuable discussions on the geochemistry of AMD in Indiana. Jessica Cancilla and Brandon Chapman, students at DePauw University, assisted with water sampling. Drs. Bill and Carolyn Baldwin provided additional field support and historical information on life at the Green Valley site during the period of mining operations. This work was funded by a grant from the Indiana Division of Reclamation, Surface Mining and Reclamation Technology program. Support for mass spectrometry and EDS instrumentation and analyses were provided by the National Science Foundation, EAR-0115884 and EAR-0941106. Acknowledgment is made to the donors of the American Chemical Society Petroleum Research Fund for partial support of this research on some carbonates and pore fluid migration modeling.

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