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Identification of Sulfate Reducing Bacteria for Abandoned Mine Drainage Bioremediation

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ABSTRACT:

Toxic abandoned mine drainage (AMD) effluent has contaminated many waterways in Pennsylvania, including the Wingfield Pines passive remediation system (PRS) in Pittsburgh, PA, which is an approximately 20-acre passive remediation system with circumneutral pH as well as elevated iron and sulfate concentrations. One effective avenue of bioremediation for sulfate-contaminated waterways utilizes sulfate-reducing properties of bacteria present in the pond slurry to produce sulfide for heavy metal precipitation as metal sulfides. Sulfate-reducing bacteria (SRBs) possess a unique ability to utilize sulfur as a terminal electron acceptor, as opposed to oxygen in aerobic bacteria, which enables them to reduce sulfur-containing compounds (such as sulfate) to sulfide in metabolism. This study investigates the identification and role of anaerobic SRBs in the Wingfield Pines PRS through 16S rRNA gene sequencing. A SIM top agar overlay of bacteria obtained from Wingfield Pines and Boyce PRS slurry revealed many sulfide-producing bacteria in the Wingfield Pines PRS but few in the Boyce PRS. 16S rRNA sequencing of Wingfield Pines Pond #2 SRBs identified *Shewanella baltica*, a facultative anaerobe, as one of the bacteria present. These experiments identify and illustrate the

important roles metabolically diverse anaerobic SRBs play in AMD-afflicted ecosystems through their ability to reduce contaminating sulfates to sulfide for heavy metal precipitation.

KEYWORDS: abandoned mine drainage, sulfate reducing bacteria, bioremediation

INTRODUCTION:

The mining of minerals and heavy metals such as coal and gold are directly associated with long-term organismal and waterway damage through production of abandoned mine drainage (AMD). AMD is produced through mined ores, such as pyrite, contacting oxygen and water, and releasing iron, sulfide, and acid into the environment (Akcil & Koldas, 2006).

In states where mining has played a significant role in the economy, particularly in the Appalachia region, AMD has produced dire consequences for aquatic ecosystems. In just the mid-Atlantic region of the US alone, more than 4500 miles of stream have been affected by AMD and have contaminated water and soil, along with reducing biodiversity (Park et al., 2015). Pennsylvania, in particular, has been one of the most negatively affected states by AMD. According to the U.S. Department of the Interior, Pennsylvania was allocated $53 million dollars in 2019 to repair habitats damaged by AMD, second only to Wyoming (U.S. Department of the Interior, 2019). Not only does AMD pose ecological risks for species diversity, but there are many incurred health risks as a result of proximity of the mines to human populations. A study of metal concentrations in the Dabaoshan mine effluent in South China revealed that waterways affected by AMD contained aquatic organisms with high concentrations of various heavy metals. The dietary intake of affected native fishes, like tilapia and carp, posed a risk for humans, as their consumption would have introduced metals to the body at concentrations exceeding safe thresholds for consumption (Chan et al., 2021).

AMD is primarily produced through a series of biogeochemical reactions in which water first contacts rocks made of iron sulfides, such as pyrite. This encounter causes iron sulfide to be oxidized and dissolved into sulfuric acid (H_2SO_4) and its aqueous ions (Fe^{2+}, SO_4^{2-}, H^+), lowering the pH. Ferrous iron from the previous reaction oxidizes to ferric iron (Fe^{3+}) via oxygen present in the environment. In acidic water (pH between 2.3 and 3.5), the ferric iron precipitates as solid iron(III) hydroxide and more H^+ ions, which further lowers the pH of the water. Any leftover ferric iron from these reactions can then be utilized to oxidize additional pyrite which allows the production of AMD to continue in a recurring cycle by returning iron to its ferrous state (Akcil & Koldas, 2006). This series of reactions leads to AMD affected areas having extremely high levels of acidity from aqueous H^+ production, as well as high levels of contaminants, specifically iron and sulfates.

High levels of acidity and contaminants have disastrous consequences on organismal viability and diversity. Analysis of a 18S rRNA gene library for a community of aquatic eukaryotes in a northern California stream affected by AMD revealed that approximately 68% of the species present in the water were fungi closely related to neutrophils, which suggests that the remaining living species were those who adapted to the stressful environment (Baker et al., 2020). This analysis suggests a loss of biodiversity in prokaryotic communities in AMD-affected waters as well. Declining aquatic species richness in ecosystems affected by AMD has many negative consequences, including lowered ecosystem productivity, health risks from

contaminant exposure, inability to utilize the waterway, and associated cost of remediation to resume use.

Despite the persistent issues AMD has introduced to aquatic ecosystems, there are many biogeochemical and microbiological solutions that have the potential to facilitate bioremediation of contaminated habitats. The addition of limestone and other alkaline materials, as well as uncontaminated topsoil, has helped neutralize acidity at affected sites (Plewniak et al., 2020). Another effective solution has been found in the metabolic capabilities of iron and/or sulfate-reducing bacteria. Gene expression in iron-reducing bacteria has shown that aerobic energy production is coupled to the oxidation of ferrous iron, which makes it convenient for attenuating AMD-affected streams with high levels of iron (Plewniak et al., 2020). Sulfate-reducing bacteria (SRBs) utilize non-organic sulfur compounds as terminal electron acceptors, as opposed to oxygen in solely aerobic bacteria. Many SRBs are anaerobic since they utilize sulfur instead of oxygen in metabolism, and, as a result, oxygen is often either detrimental or has no use to their cells since they do not possess the same uses for oxygen and defenses to oxygen radicals as aerobes. While some SRBs are obligate anaerobes (cannot tolerate O_2 even in low concentrations), there are also sulfate reducing bacteria that are facultative anaerobes, which can survive in both aerobic and anaerobic conditions. At high sulfate concentrations, SRBs readily reduce sulfate to sulfide. The produced sulfide can then react with heavy metal ions present in the water to precipitate toxic heavy metals as metal sulfide (Ayangbenro et al., 2018). The metabolic capabilities of bacteria, such as sulfate

reducing bacteria, to remediate these contaminated environments continually and effectively has become a central component of research in attempting to rehabilitate AMD-affected environments.

In this experiment, methods were developed to assess and identify SRBs inhabiting the Wingfield Pines (circumneutral pH) and Boyce (acidic pH) PRS in Pittsburgh, PA for utilization in bioremediation efforts. Samples from both an acidic and circumneutral PRS were compared to assess differences in bacterial community composition as a product of the pond pH and contaminants present. SIM agar plates with a SIM top agar overlay to induce relative anaerobicity were utilized to select sulfate reducing colonies from the circumneutral pH, high iron and sulfate containing Wingfield Pines PRS from their ability to form ferric sulfide from metabolically synthesized sulfide and iron present in the media. 16S rRNA gene sequencing was utilized to identify sulfate reducing bacteria inhabiting the system for potential use in precipitating heavy metals and reducing sulfate levels. Following identification through 16S rRNA sequencing, bioremediation strategies using identified sulfate-reducing microbes were proposed for exploration and potential use in the Wingfield Pines PRS.

METHODS:

Sample Collection.

Bacterial samples were collected from slurry from two ecologically unique passive remediation systems: Wingfield Pines PRS Ponds 1, 2, and 4 and Boyce PRS Pond 8. Wingfield

Pines is a circumneutral PRS with elevated iron and sulfate concentrations along with noticeable rust and black color across its ponds, which may indicate possible sulfate and iron metabolic activity and contamination. Boyce Park PRS is an acidic remediation system (pH ~3-4) featuring elevated heavy metal concentrations but far lower sulfate concentration than the Wingfield Pines PRS.

SIM Media Soft Agar Overlay.

Sulfur indole motility (SIM) agar, which indicates bacterial sulfide production / sulfate reduction through ferrous sulfide (FeS) formation from sulfide reacting with iron in the agar, was prepared according to the manufacturer's instructions and poured onto plates. Additionally, SIM top agar was prepared using the manufacturer's instructions but only utilizing half of the SIM agar prescribed to create a gelatinous, semi-solid agar for use as an overlay. The top agar overlay was prepared to induce a simulated anaerobic state in which anaerobic sulfur-metabolizing activity was promoted with limited interference from atmospheric oxygen. Three dilutions of slurry (200uL slurry, 100uL slurry, and 10^{-1} in nanopure) from four ponds (WP1, WP2, WP4, B8) from a sulfate-rich source (WP) and a source not rich in sulfate (Boyce) were inoculated in test tubes containing 4mL molten SIM top agar. After pouring the slurry / molten SIM top agar mixture on the SIM agar base, the plates were incubated overnight at 30°C. Following incubation, prospective SRB colonies from the plates underwent serial dilution in saline to isolate single pure colonies. Isolated colonies were

inoculated in LB media in test tubes overnight at 30°C with the caps taped shut to promote anaerobicity.

16S rRNA Sequencing.

Genomic DNA was isolated from pelleted cells from the overnight LB cultures using a Qiagen DNA Extraction Kit (Qiagen, MD, USA) according to the manufacturer's instructions and incubated at -20°C overnight. 16S rRNA gene PCR amplification of extracted gDNA from 18 prospective SRB samples (labeled 1-9A and 1-9B) was performed with 150ng template DNA, 4 mM Mg^{2+}, and 27F (5'-AGAGTTTGATCMTGGCTCAG-3') (Blackwell et al. 2019) and 805R primers (5'-GACTACHVGGGTATCTAATCC-3') (Högfors-Rönnholm et al. 2019). Samples of the same number (such as 1A and 1B) were isolated from the same initial bacterial colony.

Thermocycling parameters were set to those typical for 16S rRNA sequencing procedures. PCR reactions were incubated at -20°C overnight. Agarose gel electrophoresis was performed on a 2% agarose gel for the 18 PCR amplicons and run at 120V for 45 minutes. A sequencing PCR / BigDye reaction was performed following confirmation of PCR amplicons from gel electrophoresis using 300ng PCR product, 2.5x sequencing buffer, BigDye reagent, and 20uM primer 27F. Centri-Sep purification of each sequencing PCR reaction was conducted to remove excess contaminants from the PCR amplicons for Sanger sequencing.

Excess water was removed from the purified amplicons using a centrifuge under vacuum.

Following purification and removal of excess water, the sequencing PCR products were loaded on an ABI Genetic Analyzer for Sanger sequencing, identified on BLAST, and specific genes functioning in sulfate reduction and metabolism were analyzed for use in bioremediation.

RESULTS:

SIM Media Soft Agar Overlay.

All Wingfield Pines slurry dilutions (WP1, WP2, WP4) exhibited strong evidence of bacterial sulfate reduction to sulfide, with black FeS being formed on many individual colonies. Although bacterial sulfide production was robust from Wingfield Pines slurry, the Boyce dilution (B8) had only a few colonies that produced the black precipitate across all dilutions. Additionally, WP1 and WP4 both experienced extensive growth of both sulfide producing and non-sulfide producing bacteria. WP4 exhibited the greatest total bacterial growth and

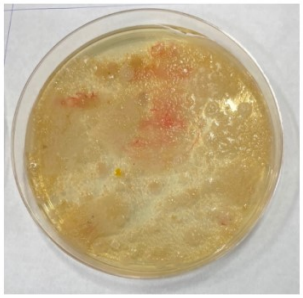


Figure 1. SIM agar plate of 100uL WP1 slurry mixed with 4 mL molten SIM top agar.

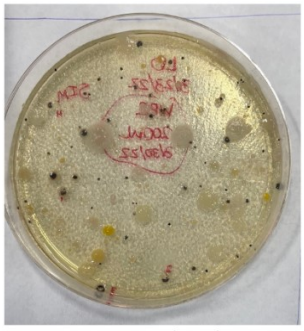


Figure 2. SIM agar plate of 200uL WP2 slurry mixed with 4 mL molten SIM top agar.

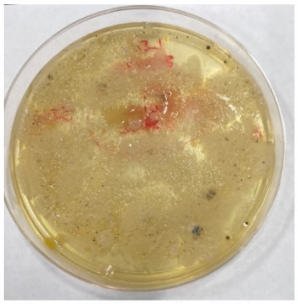


Figure 3. SIM agar plate of 100uL WP4 slurry mixed with 4 mL molten SIM top agar.

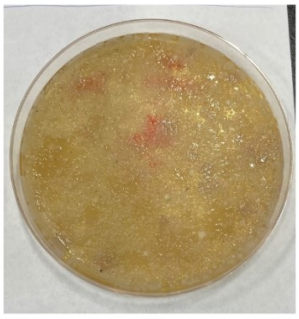


Figure 4. SIM agar plate of a 10^{-1} dilution in nanopure of B8 slurry mixed with 4 mL molten SIM top agar.

Figures 1-4. SIM Agar Plate Depiction of SRB Growth

produced too many sulfide-producing colonies to adequately measure and isolate. WP1 exhibited a moderate amount of bacterial growth but with far more non-sulfide producing bacteria present than those producing sulfide. WP2 exhibited the least total bacterial growth with most of the colonies present being sulfide producers / sulfate reducers. Figures 1-4 depict bacterial growth of both sulfate reducers and non-sulfate reducers for each pond sample.

16S rRNA Sequencing.

2% agarose gel electrophoresis of 16S PCR amplicons was performed to determine if the intended PCR product was adequately synthesized from each gDNA sample. The PCR amplicons were visualized at a length of approximately 500-600bp according to the 100bp DNA ladders labelled as "L" in Figure 5.

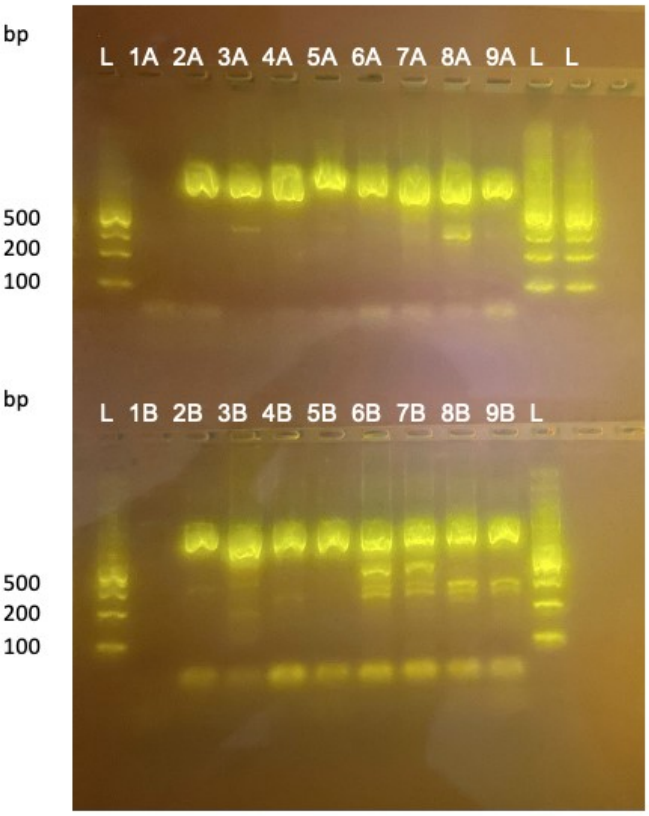


Figure 5.2% agarose gel electrophoresis of 16S PCR amplicons

Lanes L contain a 100bp DNA ladder; All sample lanes contain 3mM $MgCl_2$, 150ng template DNA, and 0.25uM primers 27F and 805R. Lanes are labeled with the corresponding sample from gDNA extraction (1-8A, 1-8B).

Sanger sequencing of the 18 16S rRNA PCR amplicons (27F-805R) resulted in the identification of one sample as Shewanella baltica. The other 17 samples were unable to be identified due to

potential novelty or sequence contamination affecting the accurate reading of nucleotides.

DISCUSSION:

Microbial Community in WP2.

AMD-inhabiting microbes with the ability to perform anaerobic sulfate reduction to sulfide play a significant role in potential bioremediation of elevated iron and sulfate levels in the Wingfield Pines PRS. Not only are these bacteria capable of lowering sulfate levels through their metabolism, but the sulfide (S^{2-}) that is yielded from reducing sulfate has an affinity for reacting with metal cations, forming metal sulfide precipitates. This enables the removal of both contaminating heavy metals and sulfate from the system through collecting the metal sulfide precipitates. The SIM top agar overlay used in this study represents a simulation of the environmental precipitation of metal sulfides through iron present in the SIM media. Iron cations (Fe^{2+}) in the SIM agar react with sulfide (sulfate reduction by bacterium) to produce ferrous sulfide (FeS), similarly to how metal cations in AMD environments react with sulfides to produce solid metal sulfides.

The SIM top agar overlay assay for WP1, WP2, WP4, and B8 revealed an unexpected array of results highlighting the microbial diversity present in each pond. Each of the WP2 slurry dilutions contained far fewer total colonies than the other three ponds and mostly yielded colonies able to reduce sulfate. WP4 had the greatest growth of sulfate-reducers, but

also had prolific growth of non-sulfate-reducing bacteria, which was unexpected due to its distinct black color possibly being evidence of an abundance of sulfide production. WP1 slurry grew far fewer sulfate-reducers compared to WP2 and WP4 but had great total bacterial growth, likely from iron oxidizing and reducing bacteria as supported by a prominent rust color in the slurry. A greater dilution is necessary in order to adequately and confidently isolate bacteria from WP1 and WP4, despite the dilution used in this study being adequate for WP2. Figure 6 depicts the slurry samples from WP1, WP2, and WP4, indicating how their physical differences correspond to differing results in plating on SIM agar plates and likely immensely diverse microbial communities.

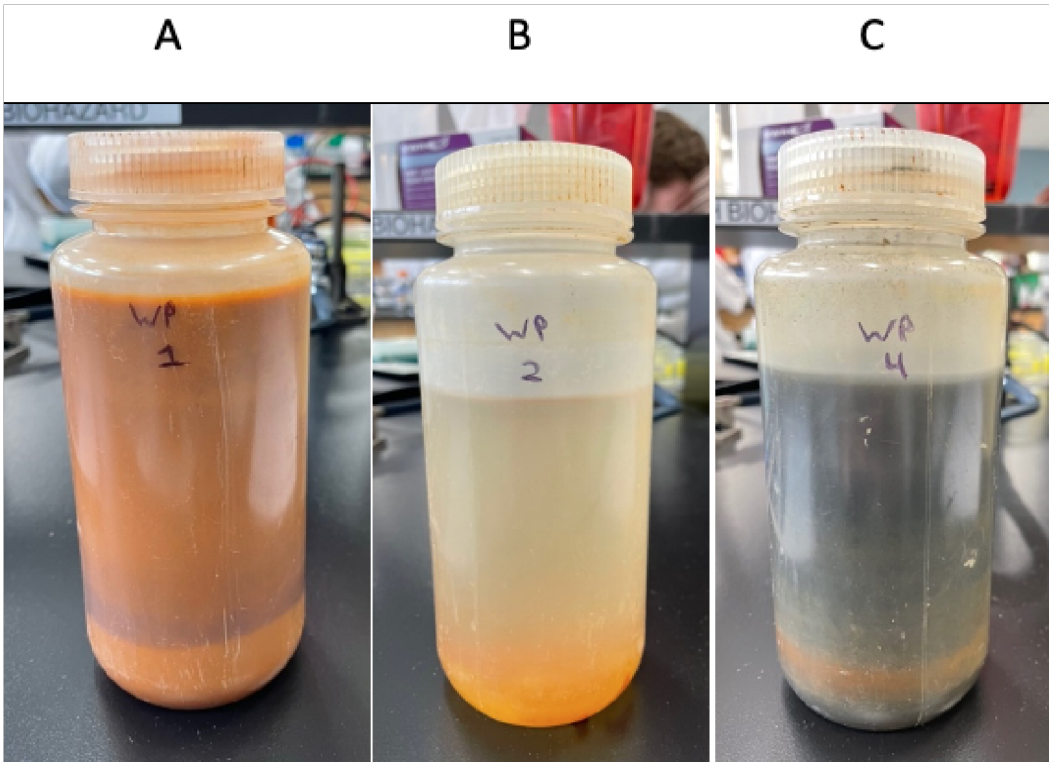


Figure 6. Wingfield Pines Pond Slurry Samples.

A visualization of the unique physical characteristics of each Wingfield Pines pond utilized in this study. Pond 1 (A) has a noticeably deep "rust" color, while pond 2 (B) has a lighter "rust" color. In comparison, pond 4 (C) has a deep black slurry color with some "rust" color present at the base of the slurry.

16S rRNA gene sequencing identified one of the WP2 bacterial samples as *Shewanella baltica*, a gram negative, rod-shaped, facultative anaerobe capable of H_2S production under anaerobic conditions (Vogel et al., 2005). Although anaerobic activity was promoted during the plating of slurry samples through the molten SIM top agar overlay, oxygen was still likely interacting with the agar as well as the pure LB cultures of isolated colonies afterwards. Making a purely anaerobic culture necessitates degassing using N_2 gas and specific anaerobic media to ensure that no oxygen is present. Since these precautions were not taken, the SIM agar assay selected for facultative anaerobes like *Shewanella* that can tolerate oxygen as well as metabolize sulfur under anaerobic conditions. Although facultative anaerobes are typically not as efficient as obligate anaerobes in terms of sulfate reduction, the metabolic diversity and ability of H_2S-producing facultative anaerobes to inhabit the ponds of the Wingfield Pines PRS serves as a potentially viable tool for bioremediating sulfate levels since the resources for maintaining strict anaerobicity are not required for their survival.

Bioremediation of the Wingfield Pines PRS

To effectively bioremediate iron and sulfate contamination in the Wingfield Pines PRS, a thorough understanding of the unique microbial communities inhabiting each pond must

first be understood to effectively remediate the entire system. The stark contrast in appearance between each pond (Figure 6) indicates potential differences in each microbial community, despite all being a part of the same PRS. 16S rRNA sequencing of sulfate-reducing bacteria from many or all Wingfield Pines ponds could help illustrate how differences in the chemical and physical properties of each pond cause predictable differences in microbial composition.

Once communities of SRBs from different Wingfield Pines ponds are identified, a sulfate assay could be utilized to quantify the sulfate-reducing abilities of each culture. Tracking sulfate levels of sterile Wingfield Pines Pond water with an inoculated pure culture would simulate sulfate reduction as it occurs in the PRS, with environmentally relevant concentrations of sulfate. A sulfate assay of pure SRB cultures would give a quantitative comparison of sulfate level changes for each pure culture as well as to a control sulfate solution. From these results, the most efficient sulfate reducing species could be determined and utilized for bioremediation of the system.

One specific passive bioremediation strategy that could be beneficial in the Wingfield Pines PRS involves filtering pond water through an aquatic microbial community of identified sulfate reducing bacteria such as *Shewanella baltica* for an extended period of time. Facultative anaerobes already inhabiting the system like *Shewanella* are a convenient choice for bioremediation of sulfate and iron for multiple reasons. Compared to obligate anaerobes, facultative anaerobes require far less maintenance since obligate anaerobes consistently

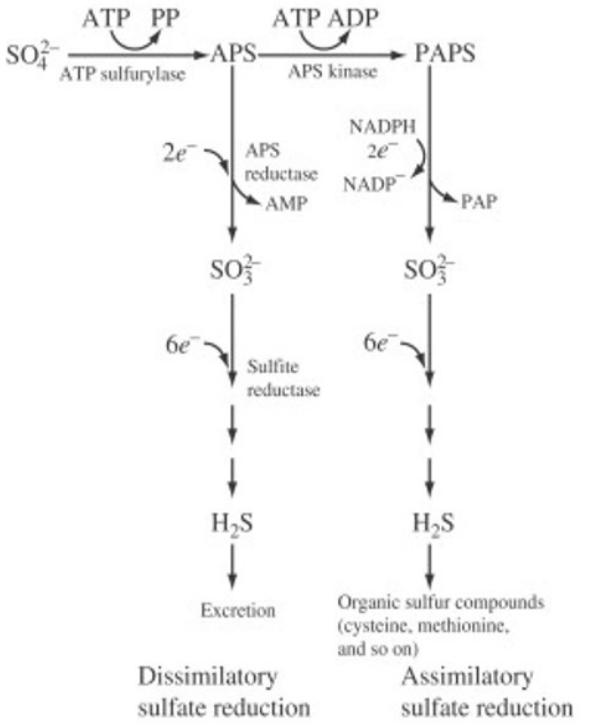
require highly specific nutrients and degassing. Additionally, the identification of *Shewanella baltica* as a H_2S-producing species supports the notion that facultative anaerobes inhabit the Wingfield Pines pond water. Utilizing cultures of SRBs to perform sulfate reduction to sulfide provides a low cost, low maintenance, and natural method to alleviate high sulfate and iron levels. To avoid interference from sulfur oxidizing bacteria, a method of maintaining consistent adequate composition of SRBs in this system must be strictly adhered to. One possibility for this bioremediation strategy involves creating an additional set of ponds at the beginning of the PRS, that is separate from the other ponds in the system. Contaminated water entering the system could be subjected to a high temperature, high pressure pond to kill sulfur oxidizing bacteria that may oxidize any reduced sulfide back to sulfate. Following the sterilization and cooling of the pond water, the pond water would flow into a second pond containing a microbial community of facultative anaerobes identified as inhabiting the passive remediation system. Under these conditions, the anaerobic reduction of sulfate to sulfide would be promoted by high sulfate concentrations, the absence of sulfur oxidizing bacteria, and potential assistance from machinery removing oxygen from the water. Since sulfate reduction is an anaerobic process, lowering the concentration of oxygen (anaerobicity is said to occur at dissolved oxygen concentrations <1 mg/L) would help to promote anaerobicity and thus sulfate reducing metabolic activity (Rolfe et al., 1978). Then, bacterial production of sulfide (S^{2-}) from sulfate reduction would lead to chemical reactions between sulfide anions and iron cations in the system, forming an iron sulfide precipitate that can be removed from the system

(Figure 7). Therefore, this proposed method of bioremediation effectively utilizes the anaerobic sulfate-reducing properties of facultative anaerobes inhabiting the Wingfield Pines PRS to form iron sulfide precipitates, lowering the concentration of both iron and sulfate in the system.

Although this is a potentially viable bioremediation strategy, the EPA places a strong emphasis on reducing contamination and the spread of bacteria in waterways. This policy makes employing the proposed strategy in the previous paragraph a challenge, as introducing *Shewanella baltica* cultures from the lab presents an opportunity for introducing contamination as well as for the cultures to alter the microbial composition of the entire Wingfield Pines PRS and Chartiers Creek downstream. Despite these challenges, this manuscript nonetheless reveals the useful metabolic capabilities of sulfate reducing bacteria and their potential use in bioremediation of waterways with significant sulfate contamination, such as the Wingfield Pines PRS.

Figure 7. Sulfate Reduction Reaction and Metal Sulfide Precipitation

A	B



Rabus et al (2015). "A Post-Genomic View of the Ecophysiology, Catabolism and Biotechnological Relevance of Sulphate-Reducing Prokaryotes". *Advances in Microbial Physiology*.

$$Fe^{2+} + H_2S(s) \rightarrow \mathrm{FeS(s)} + 2H^+$$

Struble, Garret (2021). "Characterizing the dormancy and repair of a circumneutral passive remediation system receiving iron and sulfate-rich AMD". *Duquesne University*.

Figure 7A displays two chemical reduction pathways employed by bacteria in the metabolic reduction of sulfate to sulfide, using various chemical intermediates and proteins to achieve reduction.

Figure 7B displays the chemical reaction between Fe^{2+} cations and sulfide produced from SRBs that yields a ferrous sulfide precipitate. Although this reaction depicts Fe^{2+}, iron in AMD environments can also be found in the oxidized ferric (Fe^{3+}) state as a result of iron reacting with O_2.

CONCLUSION:

These experiments help to illustrate how sulfate-reducing bacteria in the Wingfield Pines PRS identified in this study play a prime role in potential bioremediation of elevated sulfate and iron concentrations in the system. SIM agar plating and isolation of individual

colonies from Wingfield Pines and Boyce pond slurry effectively served as a selective assay for identifying SRBs due to the production of a black, ferrous sulfide precipitate from a reaction between bacterially produced sulfide and iron in the SIM media. 16S rRNA identification of *Shewanella baltica*, an H_2S-producing facultative anaerobe, supports a community of facultative anaerobes capable of sulfate reduction inhabiting the Wingfield Pines PRS. Once isolated, these native SRBs have potential use in the system through the reduction of sulfate present in the pond water. Produced sulfide from the anaerobic sulfate reduction performed by the bacteria would then react with iron cations abundant in the system to form iron sulfide precipitates which could be removed from the pond. Low cost, low maintenance, and effective bioremediation of AMD-affected waterways necessitates further research into identifying specific microbial communities across all ponds as well as fully understanding the ecological niche of SRBs in the high sulfate, high iron, and circumneutral pH environment of the Wingfield Pines PRS. Additionally, quantitative methods of comparatively assessing sulfate reduction across species and ponds such as through a sulfate assay are significant for gaining a complete understanding of the individual SRB species inhabiting the ecosystem. The identification of the H_2S-producing facultative anaerobe, *Shewanella baltica*, and its potential bioremediation applications mentioned in this study,highlights the metabolic diversity of the Wingfield Pines microbial community and provides insight into the future application of SRBs in bioremediation efforts of AMD-affected waterways with sulfate and heavy metal contaminants.

REFERENCES:

(1) Akcil A.; & Koldas S. Acid Mine Drainage (AMD): causes, treatment and case studies. *Journal of Cleaner Production*. **2006**, *14* (12-13): 1139-1145. DOI: 10.1016/j.jclepro.2004.09.006

(2) Ayangbenro A.S.; Olanrewaju O.S.; Babalola, O.O. Sulfate-Reducing Bacteria as an Effective Tool for Sustainable Acid Mine Bioremediation. *Frontiers in Microbiology*. **2018**. DOI: 10.3389/fmicb.2018.01986

(3) Baker B.; Tyson G.; Goosherst L.; Banfield J. Insights into the Diversity of Eukaryotes in Acid Mine Drainage Biofilm Communities. *American Society for Microbiology*. **2020**, *75* (7). DOI: 10.1128/AEM.02500-08

(4) Blackwell N. et al. Seasonal blooms of neutrophilic Betaproteobacterial Fe(II) oxidizers and Chlorobi in iron-rich coal mine drainage sediments. *FEMS Microbiology Ecology*. **2019**, *95*. DOI: 10.1093/femsec/fiz140

(5) Chan W.S.; Routh J.; Luo C.; Dario M.; Miao Y.; Luo D.; Wei L. Metal accumulations in aquatic organisms and health risks in an acid mine-affected site in South China. *Environmental Geochemistry and Health.* **2021**, *43* (11): 4415-4440. DOI: 10.1007/s10653-021-00923-0

(6) Högfors-Rönnholm, E. et al. Metagenomes and metatranscriptomes from boreal potential and actual acid sulfate soil materials. *Nature*. **2019**, *6*: 207. DOI:

10.6084/m9.figshare.9901265

(7) Park D.; Park B.; Mendinsky J.; Paksuchon B.; Suhataikul R.; Dempsey B.; Cho Y. Evaluation of acidity estimation methods for mine drainage, Pennsylvania, USA. *Environmental Monitoring and Assessment*. **2014**, *187*. DOI: 10.1007/s10661-014-4095-9

(8) Plewniak F.; Koechler S.; Le Paslier D.; Hery M.; Bruneel O.; Bertin P. *In situ* metabolic activities of uncultivated *Ferrovum* sp. CARN8 evidenced by metatranscriptomic analysis. *Research in Microbiology*. **2020**, *171* (1): 37-43. DOI: 10.1016/j.resmic.2019.09.008

(9) Rabus et al. "A Post-Genomic View of the Ecophysiology, Catabolism and Biotechnological Relevance of Sulphate-Reducing Prokaryotes". *Advances in Microbial Physiology*. **2015**. DOI: 10.1016/bs.ampbs.2015.05.002

(10) Rolfe R.D.; Hentges, D.J.; Campbell B.J.; Barrett J.T. Factors Related to the Oxygen Tolerance of Anaerobic Bacteria. *Applied and Environmental Microbiology*. **1978**, *36* (2): 306-313. DOI: 10.1128/aem.36.2.306-313.1978

(11) Struble, G. "Characterizing the dormancy and repair of a circumneutral passive remediation system receiving iron and sulfate-rich AMD". *Duquesne University*. **2021**.

(12) U.S. Department of the Interior. Interior Provides More Than $291 Million in Conservation Funding for States and Tribes to Clean Up and Repurpose Abandoned Coal Mines. *U.S. Department of the Interior*. **2019**. https://www.doi.gov/pressreleases/interior-provides-more-291-million-conservation-funding-states-and-tribes-clean-and (accessed 2022-3-18).

(13) Vogel B.F.; Venkateswaran K.; Satomi M.; Gram L. Identification of *Shewanella baltica* as the Most Important H_2S-Producing Species during Iced Storage of Danish Marine Fish. *Applied and Environmental Microbiology*. **2005**, *71* (11): 6689-6697. DOI: 10.1128/AEM.71.11.6689-6697.2005

Daley, L & Trun,N. (2022)Nursing Consideratons for Elderly Adults with Urinary Tract Infections in Nursing Homes. *D.U.Quark*, Volume 7(Issue 1). Retrieved from https://dsc.duq.edu/duquark/vol7/iss1/article11