THEORETICAL ANALYSIS OF *THIOBACILLUS FERROOXIDANS* FOR ENHANCED, NON-INVASIVE BIOREMEDIATION OF DIMETHYL SULFIDE AND DIMETHYL DISULFIDE IN SUBAQUEOUS SOILS USING ORGANIC REMEDIATION CELL TECHNOLOGY

by

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DEDICATION

I want to dedicate this paper to my family, especially my better half, for her unwavering love, support, and encouragement throughout this endeavor. Your belief in me has been my constant motivation. I would also like to dedicate the completion of this paper to my father. Despite his medical issues and recent passing, every night when I left the hospital, he would say, “Go get your homework done.” Thank you, Dad.

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LIST OF ABBREVIATIONS

Abbreviation	Full Terminology
AQUASIM	Data analysis model program
ATP	Adenosine triphosphate
BGC	Bacterial growth chamber
DMDS	Dimethyl disulfide
DMS	Dimethyl sulfide
DMSO	Dimethyl sulfoxide
MFC	Microbial fuel cell
MS	Mass spectrometer
OM	Organic matter
ORC	Organic remediation cell
rRNA	ribosomal ribonucleic acid
RxC	Reaction chamber
T. ferrooxidans	*Thiobacillus ferrooxidans*
XRF	X-ray fluorescence

ABSTRACT

Dimethyl sulfide (DMS, $(CH_3)_2S$) is a volatile, colorless liquid with a characteristic sulfurous odor, and dimethyl disulfide (DMDS, $(CH_3S)_2$) is a colorless to pale yellow liquid with a pungent odor. Both are organic sulfur compounds from pulp mill black liquor waste, pose a significant threat to subaqueous soil environments. They are often released into these environments through industrial activities like spills and discharge that have been untreated. Subaqueous soils, permanently submerged underwater in areas like wetlands and estuaries, are vital components of aquatic ecosystems, serving as natural filters, nutrient sinks, and habitats. The introduction of DMS and DMDS disrupts these critical environments. Traditional remediation methods, such as dredging and aeration, are challenging due to the unique conditions of subaqueous soils and the exorbitant costs. Innovative and sustainable remediation strategies are crucial for the health and stability of these vital ecosystems. My paper explores the potential of *Thiobacillus ferrooxidans*, a chemoautolithotrophic bacterium, coupled with organic remediation cell technology (ORC), as a novel bioremediation approach for DMS/DMDS-contaminated subaqueous soils. *T. ferrooxidans* is a rod-shaped bacterium that oxidizes iron and sulfur through metabolic processes. *T. ferrooxidans* can grow anaerobically on sulfur substrates using ferric iron as an electron acceptor and exhibits a broad temperature tolerance, making it an ideal candidate for this application. ORC technology offers a promising synergistic approach. A single-cell microbial fuel cell (MFC) design has demonstrated the ability of anaerobic microbes to use petroleum contaminants for power generation while simultaneously facilitating bioremediation. This technology reduces the need for conventional aeration methods, a significant advantage in oxygen-limited subaqueous environments. Theoretically, an ORC

chamber, potentially housing *T. ferrooxidans*, could be inserted into a subaqueous environment to break down DMS and DMDS safely and cost-effectively. My paper will delve into the theoretical framework of this combined approach, using existing research, chemical processes, and bacterial methods to assess its feasibility and potential efficacy in remediating DMS/DMDS-contaminated subaqueous soils. My paper aims to contribute to this emerging field by exploring a non-invasive, cost-effective, and potentially transformative solution for remediating these contaminated environments.

INTRODUCTION

ORGANIC BIOREMEDIATION WITH BACTERIA

Bioremediation offers a promising approach to mitigate the environmental impact of dimethyl sulfide and dimethyl disulfide, both volatile organic sulfur compounds emitted from various industrial processes (Zhang *et al.*, 2018). Traditional methods (Rodrigues *et al.*, 2020)often fall short in terms of cost-effectiveness and environmental sustainability, thus driving the exploration of biological alternatives. This professional paper introduces a theoretical bioremediation strategy leveraging the oxidation capabilities of *Thiobacillus ferrooxidans* in conjunction with organic remediation cell technology. *T. ferrooxidans* is well-known for its ability to oxidize sulfur compounds (Suzuki *et al.*, 1990). Theoretically, ORCs may offer the potential to simultaneously remove DMS and DMDS during the bioremediation process. By integrating these two technologies, my research aims to develop a sustainable and efficient method for DMS and DMDS removal, potentially transforming harmful pollutants into valuable energy resources (Nemati and Webb, 1999). This study will investigate the synergistic effects of *T. ferrooxidans* and ORCs.

CHEMICAL PROCESSES WITH DMS/DMDS AND *T. FERROOXIDANS*

Bioremediation of dimethyl sulfide and dimethyl disulfide using *T. ferrooxidans* in conjunction with iron exploits the bacterium's capacity to oxidize both iron and sulfur under acidic conditions (Kiragosyan *et al.*, 2020). This approach offers a promising strategy for mitigating the negative impacts of these compounds on subaqueous soil environments (Kucera *et al.*, 2016). *T. ferrooxidans*, a chemolithoautotrophic bacterium, derives energy from oxidizing organic compounds like iron and sulfur, utilizing carbon dioxide as its carbon source (Suzuki *et al.*, 1990). In this bioremediation strategy, the process unfolds in two main stages.

1. Iron Oxidation: *T. ferrooxidans* oxidizes ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) (Zhang et al., 2018). This reaction is crucial because ferric iron acts as a strong oxidizing agent.

$$4Fe^{2+} + O_2 + 4H^+ \ -> 4Fe^{3+} + 2H_2O$$

The reaction also requires protons (H^+) from the acidic environment that *T. ferrooxidans* thrives in. *T. ferrooxidans* catalyzes this reaction through a series of complex biochemical steps involving several enzymes. These enzymes, located in the cell membrane of the bacterium, facilitate the transfer of electrons from ferrous iron to oxygen, ultimately generating energy for the bacterium's metabolic processes (Zhang et al., 2018). The resulting ferric iron is a crucial component in bioleaching and other bioremediation strategies, where it acts as an oxidizing agent for other compounds, such as sulfur-containing pollutants like DMS and DMDS.

2. DMS Degradation: The generated ferric iron (Fe^{3+}) chemically oxidizes DMS and DMDS, breaking them down into less harmful products, primarily sulfate. A simplified representation of the oxidation of DMS by ferric iron can be written as:

$$DMS + 4Fe^{3+} + 2H_2O \rightarrow DMSO + 4Fe^{2+} + 4$$

$$\downarrow$$

followed by further oxidation to dimethyl sulfone ($DMSO_2$):

$$DMSO + 2Fe^{3+} + H_2O \rightarrow DMSO_2 + 2Fe^{2+} + 2H^+$$

$$\downarrow$$

and ultimately to sulfate:

$$DMSO_2 + \text{other oxidants} \rightarrow SO_4^{2-} + \text{other products}$$

3. DMDS Degradation: The generated ferric iron (Fe^{3+}) chemically oxidizes DMS and DMDS, breaking them down into less harmful products, primarily sulfate. The oxidation of DMDS is more complex, often involving the cleavage of the disulfide bond. A simplified and generalized pathway could be represented as:

$$DMDS + Fe^{3+} + H_2O \rightarrow DMSO + \text{other sulfur compounds} + Fe^{2+} + H^+$$

with DMSO and other sulfur intermediates subsequently oxidized further to sulfate. (Leduc and Ferroni, 1994) It is important to note that these are simplified representations of complex processes, and the exact reaction pathways and product distributions can vary depending on the specific conditions of the subaqueous soil environment. More detailed studies are needed to fully elucidate the chemical mechanisms involved in the oxidation of DMS and DMDS by ferric iron under environmentally relevant conditions.

BACTERIUM PROCESSES

There are two oxidation pathways for *T. ferrooxidans*:

1. Direct Sulfur Oxidation: *T. ferrooxidans* can directly oxidize elemental sulfur (S^0) to sulfate (SO_4^{2-}). This process involves several enzymatic steps, starting with sulfur dioxygenase, which oxidizes elemental sulfur to sulfite (SO_3^{2-}). Sulfite is then further oxidized to sulfate by sulfite oxidase. The oxidation of these compounds releases electrons that the bacteria use for energy and allows them to thrive in sulfur-rich environments. The electrons released from sulfur oxidation enter the electron

transport chain. This chain involves various redox proteins and ultimately leads to the reduction of oxygen to water. This then generates a proton gradient that drives ATP synthesis (Drobner, Huber, and Stetter, 1990).

2. Indirect Sulfur Oxidation Coupled with Iron Oxidation: In the presence of both ferrous iron (Fe^{2+}) and sulfur, *T. ferrooxidans* prioritizes iron oxidation. The ferric iron (Fe^{3+}) generated can then chemically oxidize sulfur compounds, including reduced sulfur species like sulfide (S^{2-}) and potentially other sulfur intermediates, ultimately producing sulfate (Karavaiko et al., 1998). This indirect sulfur oxidation mechanism contributes to the overall bioleaching process in sulfide ore environments. The ferric iron acts as an electron acceptor, generating ferrous iron, which *T. ferrooxidans* can again oxidize, creating a cyclical process. *T. ferrooxidans* can utilize sulfur in a few different ways, primarily through direct oxidation and as an indirect energy source coupled with iron oxidation. The following stoichiometry equation shows the basic anaerobically oxidized S^0 with Fe^{3+} (Kucera *et al.*, 2016).

$$8Fe^{3+} + 5Fe_2(OH)_2^{4+} + 3S^0 + 11SO_4^{2-} + 2H_2O \rightarrow 10Fe^{2+} + 8FeSO_4^0 + 6HSO_4^- + 8H^+$$

T. ferrooxidans can directly oxidize the sulfur component of DMS and DMDS, contributing further to their degradation. This dual oxidation pathway, combining both direct microbial action and indirect chemical oxidation mediated by ferric iron, amplifies the efficiency of DMS and DMDS removal (Zhan *et al.*, 2019). This is particularly relevant in subaqueous soils where oxygen availability is limited. *T. ferrooxidans* can effectively operate under anaerobic or aerobic conditions (Schafer, Myronova and Boden, 2010).

Several factors influence the efficacy of this bioremediation strategy:

1. pH and Temperature. *T. ferrooxidans* thrives in acidic environments and has an optimal temperature range. Maintaining these conditions is essential for maximizing bacterial activity (Suzuki *et al.*, 1990). This bacterium has a broad temperature tolerance, suggesting its adaptability within a reasonable range.
2. Oxygen Availability: Although *T. ferrooxidans* can function under anaerobic conditions, some level of oxygen may be beneficial for optimal growth and activity. This needs further investigation specific to DMS and DMDS degradation (Antizar-Ladislao, 2010). The anaerobic capability of *T. ferrooxidans* is a key advantage in subaqueous soils, where traditional aeration methods are challenging (Reichenauer and Germida, 2008).
3. Presence of Other Compounds: Other electron donors or acceptors present in the soil can impact the efficiency of DMS and DMDS degradation. the presence of other sulfur compounds might compete for oxidation by *T. ferrooxidans*, potentially reducing the degradation rate of DMS and DMDS. the disruptive nature of DMS and DMDS on microbial communities, suggesting that careful consideration of the existing soil microbiome is crucial for successful bioremediation. The following flow chart is a model of the electron transport pathways (Kucera et al., 2016)

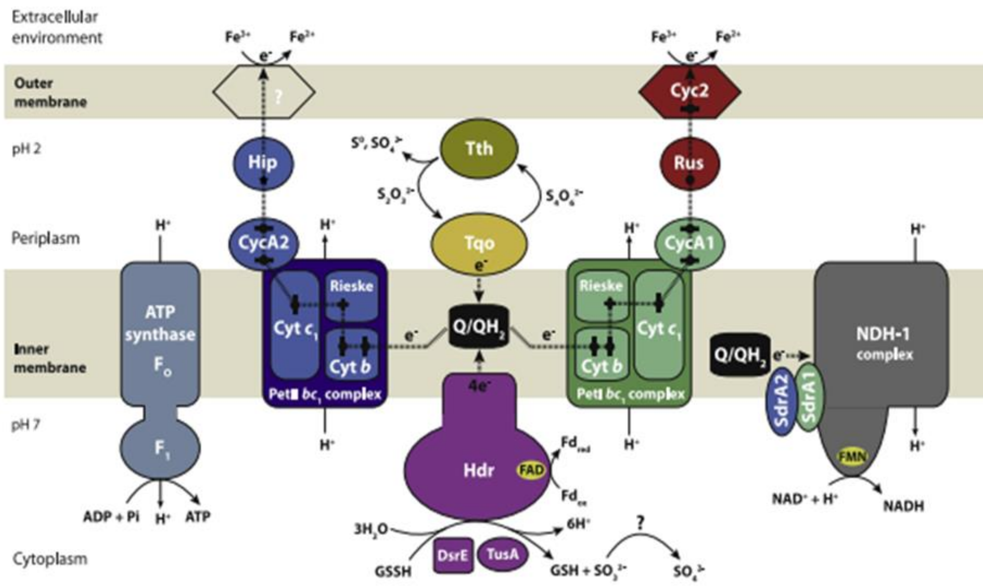

Figure 1: Model of anaerobic electron pathways within T. ferrooxidans.(Kucera et al.*, 2016)*

ORGANIC REMEDIATION CELL (ORC)

The Organic Remediation Cell (ORC) is designed similarly to the Microbial Fuel Cell (MFC) (Xu *et al.*, 2022). A typical MFC consists of two chambers: an anaerobic anode chamber and an aerobic cathode chamber, separated by a proton exchange membrane (Yen *et al.*, 2023). The BGC chamber houses the bacteria in an oxygen-deprived environment (Chen *et al.*, 2024). ORC cells combining *T. ferrooxidans* with iron substrate technology can utilize the oxidation of DMS and DMDS (Demas and Rabenhorst, 1999). Coupling *T. ferrooxidans* with ORCs could offer a synergistic approach, where the bacteria facilitate the breakdown of DMS and DMDS, and a gas recovery cycle could be employed to capture sulfur and hydrogen that can be used for other purposes. The bacteria oxidize organic matter, releasing electrons in the process. These electrons are then transferred to the RxC chamber. From the anode, the electrons flow through an external circuit to the cathode chamber, generating an electrical current. The theoretical ORC cell will use a similar process but will collect hydrogen and sulfur gas for future purposes. The ORC cell has three chambers. The bacterial growth chamber (BGC), the organic material chamber (OMC), and the reaction chamber (RxC). The OMC chamber is bottom-fed from a pulse injector inserted into the soil, with a self-flushing sediment filter. The BGC is the home of the *T. ferrooxidans* and the iron substrate as a production source. The BGC and OMC chambers are connected to the RxC chamber via independent injectors. The RxC chamber is three times the size of the OMC chamber to allow for variations in flow and reaction rates. Approximately 2/3 of the RxC is segmented off with a gaseous membrane to allow H^+ and S^0 to escape to a collection point. The bottom of the RxC has a small water outlet to allow decontaminated water to gently pass back into the water column.

Components of the ORC will be made of a special polymer that meet the following requirements. Must be stable at pH levels as low as 2.5, can exist underwater indefinitely, has inert contact reactions, and will not absorb S^0 or H^+ gases. Chemistry of the currently available polymers would suggest that either polyetheretherketone (PEEK), specifically PEEK 30GF®, or polyethylene terephthalate (PET), specifically the TECAPET/1400®, would be good candidates for cell material. Water absorption for PEEK 30 GF® is 0.11%, and TECAPET/1400®, being 0.04%, is a small criteria concern because it is not known how absorption will affect the ongoing reaction.

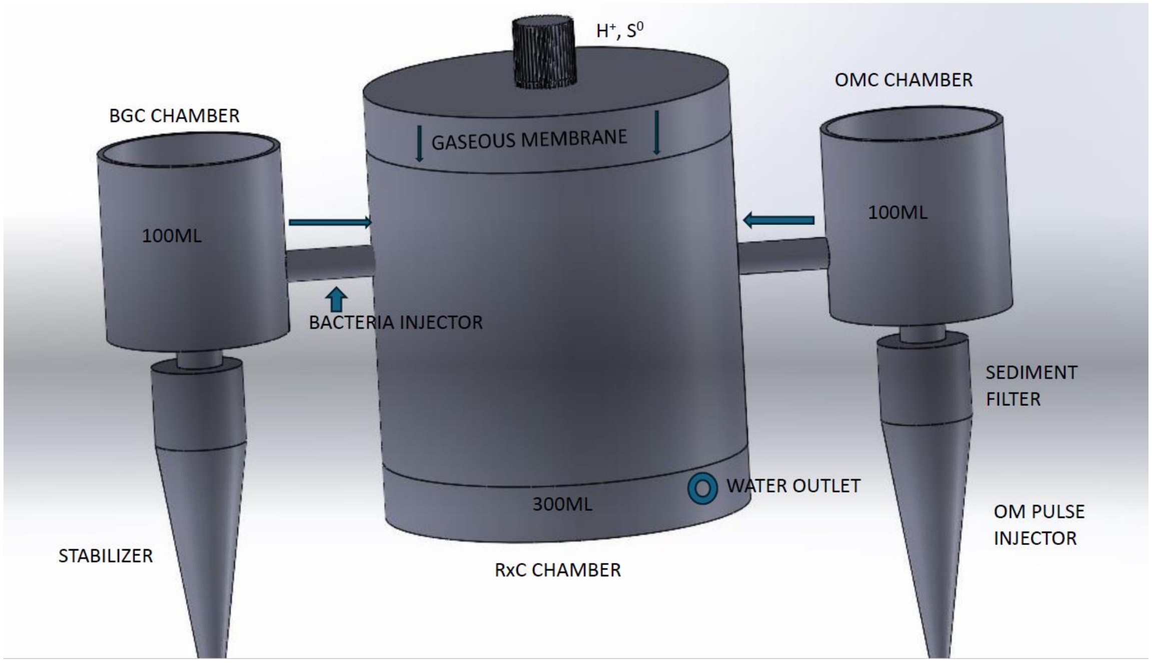

Figure 2 ORC cell design v1(personal design)

COMBINING CHEMICAL AND BIOLOGICAL COMPONENTS

This theoretical research explores an innovative approach to enhance the oxidation of ferrous sulfate ($FeSO^4$), a critical process in various environmental and biotechnological applications, by synergistically integrating biological and chemical methods. At the heart of this strategy lies *T. ferrooxidans*, a chemoautotrophic bacterium renowned for its remarkable ability to catalyze the oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) (Nemati and Webb, 1999; Zhang *et al.*, 2018). This microbial transformation is central to the processes of bioleaching, acid mine drainage treatment, and bioremediation in subaqueous soils (Sharma, 2021). My study aims to harness and optimize this inherent biocatalytic capability of *T. ferrooxidans* for improved efficiency and stability in ferrous iron oxidation and the breakdown of DMS/DMDS. A key aspect of the proposed research involves the design and implementation of an oxidation-reduction composite device that integrates biological and chemical oxidation mechanisms. *T. ferrooxidans* will be immobilized within a carbon matrix embedded with Fe^{2+}, where it can contribute to the oxidation of ferrous iron to sustain the bacterial colony and degrade organic sulfur compounds. The ORC device is hypothesized to create a synergistic environment where biological and chemical processes work in concert to accelerate DMS and DMDS breakdown. This synergistic effect, combined with the benefits of bacterial immobilization, has the potential to yield a more efficient and robust system for subaqueous organic compounds, with broad implications for various environmental and industrial applications.

METHODS

My systematic approach will begin with site selection and characterization. Site selection will study sites that are downstream of industrial manufacturing facilities, the mouths of old vein mines, and chemical spill sites (Li *et al.*, 2015). Site characterization will include a thorough analysis of contaminated subaqueous soil. Analysis will be conducted at different depths and recorded (Tripathi, Singh and Hills, 2019). All samples will be analyzed for physical and chemical properties (Han *et al.*, 2018) (pH, OM, full MS analysis, and XRF x-ray analysis). Another site characteristic that must be assessed is the identification of indigenous bacteria potential for added bioremediation and the presence of other pollutants that may inhibit remediation. When DMS/DMDS is identified, the samples will be tested in the lab specific to *T. ferrooxidans* to assess remediation capabilities. The experiment will involve setting up microcosms with contaminated soil, *T. ferrooxidans* in the experimental ORC RxC chamber, and monitored for loss of DMS and DMDS in soil. The release of gases will be collected and quantified to help determine the rate of pollutant breakdown. hydrogen and sulfur gas will be collected and measured. Control groups will be set aside to compare with a known baseline of pollutant degradation and gas release. The microbial community will be monitored for activity and abundance. After Lab experimentation, rRNA sequencing will be conducted to check for any other bacteria that may have been in the *T. ferrooxidans* composition within the BGC chamber. Data analysis using AQUASIM, an online available software (Martins *et al.*, 2022) will determine if the results are promising; then, the project will move forward to a pilot-scale test at a predetermined site.

EVALUATION AND CONCLUSION

The integration of bacteria, specifically *T. ferrooxidans*, with ORC cell technology for bioremediation offers several potential economic advantages compared to traditional methods. Traditional methods like dredging or chemical treatments often involve high energy consumption, significant labor costs, and the need for specialized equipment. Bioremediation using T. ferrooxidans and ORCs can potentially reduce these costs. *T. ferrooxidans* is a self-replicating biocatalyst that reduces the need for continuous chemical inputs (Nemati and Webb, 1999). ORCs can generate H^+ and S^0 during the bioremediation process, offering the possibility of offsetting operational costs or even generating revenue. These byproducts can be used for other industries requiring hydrogen or sulfur gas. This could potentially make this process more sustainable and economically attractive. The economic value of ecological services, such as bioremediation, is increasingly being recognized around the world. While directly monetizing these services can be complex, the cost savings associated with reduced pollution and improved ecosystem health can be substantial.

The use of bacteria and ORC technology for bioremediation also presents several environmental advantages. Traditional remediation methods can have negative impacts on the environment, such as habitat destruction, release of pollutants during dredging, and the introduction of harmful chemicals (O. Gessner *et al.*, 2004). Bioremediation using *T. ferrooxidans* offers a more environmentally friendly alternative. *T. ferrooxidans* naturally occurs in many environments and can effectively remove pollutants without introducing harmful foreign bacteria, foreign chemicals, or causing significant habitat disruption.

It is crucial to conduct a thorough environmental impact assessment before implementing this technology. Potential risks include the unintentional introduction of *T. ferrooxidans* into non-target environments, the formation of harmful byproducts during the bioremediation process, and the potential for bioaccumulation of pollutants in the food chain (Fang *et al.*, 2020).
Overall, the integration of bacteria and ORC technology holds significant promise for sustainable and cost-effective bioremediation. There are also economic challenges associated with this technology. The initial investment in ORC construction and optimization can be high. Furthermore, the long-term stability and performance of ORCs in real-world subaqueous soil environments need to be thoroughly evaluated to ensure a reliable return on investment.

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