

FMH606 Master's Thesis 2024

Master of Science – Energy and Environmental Technology

Microbial Induced Corrosion (MIC) Effects on Wastewater Treatment Plant Infrastructures



Zahra Sanidanesh

Faculty of Technology, Natural sciences and Maritime Sciences
Campus Porsgrunn

Course: FMH606 Master's Thesis, 2024

Title: Microbial Induced Corrosion (MIC) - Effects on Wastewater Treatment Plant Infrastructures

Number of pages: 85

Keywords: Microbial Induced Corrosion, Hydrogen Sulfide, Wastewater Treatment, Sewer Sediments, Sulfate-Reducing Bacteria, Emission Modeling, H₂S Removal Techniques

Student: Zahra Sanidanesh

Supervisor: Eshetu Janka and Vibeke Bredvold Karlsen

External partner: NORPART Project South Partner Universities

Summary:

This thesis presents an in-depth analysis of Microbial Induced Corrosion (MIC) and the impact of hydrogen sulfide (H₂S) on municipal wastewater treatment plants. The study aims to explore the mechanisms of H₂S production, its effects on infrastructure, and the various techniques for its removal. Emphasis is placed on both small- and large-scale treatment facilities, including centralized and decentralized systems. A comprehensive literature review forms the basis of the study, utilizing a wide range of scholarly articles sourced through Google Scholar. The review covers the biological processes leading to sulfide production, the role of sulfate-reducing bacteria, and the factors influencing MIC. Environmental and health risks associated with H₂S production and mitigation were also evaluated. The findings highlight the significant role of sewer sediments in H₂S production, underscoring the importance of accurate modeling and simulation for emission predictions. Furthermore, the economic implications of MIC, as well as the costs and benefits of different H₂S removal techniques, are examined. Various H₂S removal methods, including chemical, biological, and physical techniques, are assessed for their effectiveness and practicality in different treatment plant settings.

The University of South-Eastern Norway takes no responsibility for the results and conclusions in this student report.

Preface

This study contributes to a better understanding of MIC and H₂S impacts on wastewater infrastructure, providing insights that can help develop more sustainable and corrosion-resistant systems. The conclusions drawn emphasize the need for integrated approaches to manage MIC and mitigate H₂S effects, ensuring the longevity and efficiency of wastewater treatment plants.

I would like to express my deepest thanks to my supervisors, Eshetu and Vibeke, for their invaluable guidance and dedication. Their helpful comments and feedback greatly improved my understanding of the thesis, and they were always available to assist me whenever I needed help. Thank you for your constant guidance and dedication throughout this research journey.

Additionally, I would like to extend my gratitude to my friends and family for their companionship and encouragement.

Porsgrunn, May 29, 2024

Zahra Sanidanesh

Contents

1	Executive Summary	7
1.1	Objectives of the Research.....	8
1.2	Scope of the Study	8
2	Methodology	9
3	Literature review	10
3.1	Introduction to MIC in wastewater treatment.....	10
3.1.1	<i>MIC Effect on Metallic Materials (e.g., Steel, Iron)</i>	11
3.1.2	<i>MIC Of Concrete and Cement and Other Advanced Materials</i>	18
3.2	Economic Implications.....	23
4	Biological Processes involved in Sulfide Production and MIC	26
4.1	Sulfide Production	29
4.2	Effect Of Process Parameters on Sulfide Production And SRB Activity	29
5	Quantification of Sulfide Production in Sewer System	41
6	Human exposure to H₂S in different treatment plants (i.e., centralized or decentralized), seasons (temperature) and geographical location	44
6.1	Seasonal and geographical aspects of H ₂ S production	47
7	Removal process of Hydrogen Sulfide	49
8	Discussion	52
9	Conclusion	53
	References	54
	Appendices	82

Abbreviations

AMP	Adenosine Monophosphate
APS	Adenosine-5'-Phosphosulfate
As ⁵⁺	Arsenate Ion
ASOB	Acidophilic Sulfur-Oxidizing Bacteria
ASR	Assimilated Sulfate Reduction
ASTM	American Society for Testing and Materials
ATP	Adenosine Triphosphate
CH ₃ COO ⁻	Acetate Ion
CMIC	Chemical Microbiologically Influenced Corrosion
CCP	Current Cathodic Protection
COD	Chemical Oxygen Demand
CO	Carbon Monoxide
CO ₂	Carbon Dioxide
CP	Cathodic Protection
DET	Direct Electron Transfer
DFB	Down-Flow Fluidized Bed
DO	Dissolved Oxygen
DSR	Dissimilatory Sulfate Reduction
DWDS	Drinking Water Distribution System
E ⁰	Standard Reduction Potential
EET	Extracellular Electron Transfer
EMIC	Electrical Microbiologically Influenced Corrosion
EPS	Extracellular Polymeric Substances
FAD	Flavin Adenine Dinucleotide
FA	Fatty Acid
FISH	Fluorescence In Situ Hybridization
G	Gibbs Free Energy Change
GDP	Gross Domestic Product
H ₂ S	Hydrogen Sulfide
H ₂ SO ₄	Sulfuric Acid
HP ₂ O ₇ ³⁻	Pyrophosphate
HCO ₃ ⁻	Bicarbonate Ion
HCO ₃ ⁻	Bisulfite
HOS	Heterotrophic Oxidizing Sulfate
HRT	Hydraulic Retention Time
ipy	Inches per year
LPS	Lipopolysaccharide
MA	Methanogenic Archaea
MET	Mediated Electron Transfer
MFCs	Microbial Fuel Cells
MIC	Microbiologically Influenced Corrosion
MICC	Microbiologically Induced Concrete Corrosion
mm/y	Millimetres per year

Abbreviations

MoO_4^{2-}	Molybdate Ion
mpy	Miles per year
NADH	Nicotinamide Adenine Dinucleotide (Reduced Form)
NACE	National Association of Corrosion Engineers
NRB	Nitrate-Reducing Bacteria
NSOB	Neutrophilic Sulfur-Oxidizing Bacteria
ORP	Oxidation-Reduction Potential
Pd0	Palladium
Pd^{2+}	Palladium Ion
RH	Relative Humidity
SEM	Scanning Electron Microscopy
SeO_3^{2-}	Selenite Ion
SeO_4^{2-}	Selenate Ion
SOB	Sulfur Oxidizing Bacteria
SO ₄	Sulfate
SOMs	Sulfur Oxidizing Microbes
SRB	Sulfate-Reducing Bacteria
UASB	Upflow Anaerobic Sludge Blanket
XPS	X-ray Photoelectron Spectroscopy
ZVI	Zero-Valent Iron

1 Executive Summary

Microorganisms have been on Earth for billions of years before plants and animals, aiding in the rise of higher life forms. They can also harm, causing disease and infrastructure damage under certain conditions. Industrial systems create new environments for microorganisms, leading to unwanted microbial activities, like microbiologically influenced corrosion (MIC). MIC involves bacteria, archaea, microalgae, and fungi corroding metallic structures and equipment. Microbiologically influenced corrosion (MIC) contributes significantly to global corrosion costs, accounting for around 20% of total expenses, especially affecting underground pipelines. MIC can result in rapid corrosion rates over 10 mm annually, leading to premature equipment failure and environmental damage (Machuca Suarez, 2019). Prior to the mid-1980s, the origins of the destructive effects caused by sulfate-reducing bacteria (SRB) was linked to CMIC (Chemical Microbiologically Influenced Corrosion) parameters, which emphasized the role of chemical species in the corrosion mechanism. Following this, a novel viewpoint emerged with the advent of EMIC (Electrical Microbiologically Influenced Corrosion) particularly concerning SRB, offering an alternative rationale for their corrosive conduct alongside preexisting hypotheses. EMIC assumes that bacteria adhere directly to the metal surface and acquire electrons from the metal via three mechanisms mentioned in (3.1.1.1) are collectively referred to as Extracellular Electron Transfer (EET). This process designates the bacteria as the cathode, while the metal functions as the anode. An established method for evaluating the extent and advancement of corrosion involves quantifying the corrosion rate, typically denoted in units such as mpy (miles per year), ipy (inches per year), or mm/y (millimetres per year). Whereas CMIC can elucidate documented corrosion rates of 16 mpy, EMIC provides a mechanistic foundation for scenarios in which the corrosion rate escalates to 36 mpy. EMIC has been detected not only in sulfate-reducing bacteria (SRB) but also in other bacterial types including nitrate-reducing bacteria and methanogens (Javaherdashti, 2023).

Microbial corrosion, a form of corrosion, is recognized by various interchangeable terms. It is referred to as microbial corrosion, bacterial corrosion, bio-corrosion, microbiologically influenced corrosion, or microbial induced corrosion (MIC). This form of corrosion arises from biological entities or microorganisms. Depending on the metal base and the specific setting, it may appear as localized corrosion like pitting or as general corrosion. It has the potential to occur in all aqueous surroundings. MIC is prevalent due to the extensive presence of microbes, sufficient nutrients, and corrosive substances in both liquid systems and industrial operations and is relevant to both metallic and non-metallic substances. The disintegration of materials, primarily metals, due to the influence of microorganisms, characterizes microbiologically influenced corrosion. The phrase microbiologically influenced corrosion is also utilized to describe corrosion stemming from the existence and actions of microorganisms in biofilms on metal surfaces. MIC impacts not only metals but also non-metallic surfaces like concrete, leading to significant losses in various industries. This form of MIC stands out and has attracted substantial research attention from numerous researchers and engineers in recent years, especially with the advancement of surface assessment and electrochemical methodologies capable of measuring the microbial impact on electrochemical occurrences and elucidating corrosion mechanisms (Loto, 2017).

1.1 Objectives of the Research

The primary aim of this thesis is to conduct a rigorous and critical review focusing on the overall impact of Microbial Induced Corrosion (MIC), with a specific emphasis on the effects induced by hydrogen sulfide. The investigation will centre on both small- and large-scale municipal wastewater treatment plants, encompassing variations in configurations such as centralized and decentralized systems. Through this examination, the thesis aims to provide a comprehensive understanding of the challenges posed by MIC in the context of wastewater treatment, contributing valuable insights to the industry's knowledge base and addressing the demand for effective strategies in both small and large-scale treatment plant infrastructures.

1.2 Scope of the Study

The thesis will thoroughly investigate the removal process of hydrogen sulfide (H₂S) within the specific context of wastewater treatment plant infrastructures. It will focus exclusively on elucidating methodologies aimed at mitigating the effects of H₂S, particularly in relation to microbially induced corrosion (MIC). Both small- and large-scale municipal wastewater treatment plants will be encompassed in this exploration, aiming to comprehensively understand various removal techniques and their applicability.

This study will critically examine the effectiveness of different H₂S removal processes, evaluating their efficiency in diverse treatment plant configurations. Emphasis will be placed on elucidating the underlying mechanisms and principles guiding each removal method, ranging from chemical treatments to biological and physical processes.

Furthermore, the research will assess the economic implications associated with H₂S removal, considering the costs, benefits and limitations of different approaches. This analysis will encompass not only the initial investment required for implementing H₂S removal technologies but also the long-term operational and maintenance expenses.

Moreover, the thesis will explore the environmental and human health impacts of H₂S production and removal processes, considering factors such as emissions, by-products, and potential risks to workers and surrounding communities. Through a comprehensive examination of these aspects, the research aims to provide valuable insights for enhancing the sustainability and resilience of wastewater treatment plants in the face of H₂S-induced corrosion challenges.

2 Methodology

1. Literature Review

The methodology for this research involved a comprehensive review of literature pertinent to the topic of Microbial induced corrosion. The initial exploration of available literature highlighted a limitation in accessing comprehensive articles solely through platforms like ScienceDirect and Scopus. Therefore, to ensure a thorough examination of relevant research findings, Google Scholar was chosen as the primary platform for literature search and retrieval.

2. Platform Selection

Google Scholar was selected due to its extensive database, which encompasses various scholarly sources including journals, theses, and books. Unlike other platforms, Google Scholar offers a broader range of publications, potentially overcoming the limitations observed in accessing comprehensive articles solely through platforms like ScienceDirect and Scopus.

3. Utilization of Filters

To improve the effectiveness of the literature search and to identify articles relevant to the research subject within the specified time span (2014-2024), specific filters were implemented in Google Scholar. These filters encompassed the activation of Library Links to reach articles accessible through our institution's subscriptions, thereby guaranteeing the availability of a broad array of scholarly articles not directly obtainable via Google Scholar but accessible through our institutional subscriptions. Furthermore, Open-Access Links were chosen to pinpoint freely accessible articles, thus broadening the range of accessible literature beyond subscription-based outlets. The incorporation of open-access links enriched the comprehensiveness of the literature survey by integrating articles openly accessible to researchers globally.

4. Search Strategy

The search strategy involved the use of relevant keywords and phrases related to the research topic such as "Microbially induced corrosion", "Biocorrosion", "Mechanisms of microbially induced corrosion". These keywords were carefully chosen to capture the breadth and depth of the subject matter, ensuring that no relevant literature was overlooked during the search process. The selected articles were then thoroughly analysed to extract key findings, methodologies, and insights relevant to the research objectives.

5. Ethical Considerations

Throughout the literature review process, ethical considerations were paramount. Proper citation and attribution were ensured to acknowledge the contributions of previous researchers.

3 Literature review

In the literature review, details, and information about microbial-induced corrosion (MIC) will be thoroughly assessed.

3.1 Introduction to MIC in wastewater treatment

MIC has been defined by NACE and ASTM as 'corrosion influenced by the presence or activity, or both, of microorganisms'(Knisz et al., 2023). Various expressions are utilized to describe this occurrence such as microbial influenced corrosion (Microbially Influenced Corrosion, Microbiologically Influenced Corrosion), MIC, biodeterioration, and biocorrosion. Nevertheless, due to the presence or activity of microorganisms can also reduce the speeds of corrosion. Therefore, utilizing the term "induced" to exclusively signify that microorganisms always boost corrosion may not precisely capture the several ways in which microorganisms can interact with metal surfaces. Consequently, expressions like "influenced" or "affected by" are frequently favoured over "induced" when discussing MIC to encompass both situations where microorganisms can either accelerate or decelerate erosion procedures (Knisz et al., 2023). Microbiologically influenced corrosion (MIC) is the degradation process of materials due to the direct or indirect influence of microscopic microorganisms. MIC is primarily responsible for localized corrosion types such as pitting corrosion, crevice corrosion, and stress corrosion cracking (M. Khan et al., 2023).

Microbial corrosion results from the interaction of metal, media, and microorganisms. The process of colonizing a surface by bacteria is called bacterial adhesion, which is the start of biofilm growth. Microbial biofilms are found ubiquitously as the predominant life forms for microorganisms in various systems. Consequently, they offer numerous potential applications across different industries. These biofilms have a significant impact on both human and animal health, present challenges to food safety, disrupt oil and gas production, and contaminate drinking water sources. However, they can also be advantageous in areas such as wastewater treatment processes, enhancing nutrient availability in soil, and addressing oil spill remediation. Biofilm process involves molecular and physical interactions. Bacterial biofilm formation is a complex process that can be broken down into five main phases. Firstly, in the reversible attachment phase, bacteria initially attach non-specifically to surfaces. This is followed by the irreversible attachment phase, where bacterial cells interact with the surface using adhesins such as fimbriae and lipopolysaccharide (LPS). Subsequently, in the third phase, resident bacterial cells produce extracellular polymeric substances (EPS). The biofilm maturation phase comes next, during which bacterial cells synthesize and release signalling molecules to sense each other's presence, leading to the formation of microcolonies and the maturation of biofilms. Finally, in the dispersal/detachment phase, bacterial cells detach from the biofilms and return to an independent planktonic lifestyle (Cámara et al., 2022; Muhammad et al., 2020; Pal & Lavanya, 2022).

Biofilms are complex communities of microorganisms that adhere to surfaces, composed of diverse species which offer enhanced resistance compared to single-strain biofilms. Within these structures, some microbes are sessile (attached to surfaces) while others are planktonic (suspended in the surrounding fluid), with sessile cells capable of detaching and colonizing new areas. Extracellular polymeric substances (EPS), consisting of proteins, polysaccharides,

and nucleic acids, are secreted by microorganisms to support the embedding of sessile cells within the biofilm matrix. Biofilms are recognized as major contributors to Microbiologically Influenced Corrosion (MIC), leading to material degradation across various environments including industrial, medical, marine, and aerospace sectors. MIC can exacerbate other forms of corrosion such as crevice corrosion, under-deposit corrosion, and stress corrosion cracking, causing extensive damage to materials like aluminium alloy, magnesium, zinc, and even concrete (Jia et al., 2019).

Wastewater treatment facilities play a crucial role in maintaining the environmental safety of the hydrosphere within cities. However, the operation of these facilities involves unique challenges due to the biologically active nature of the environment, leading to the formation of biofilms on metal and concrete surfaces. Microbial corrosion within these biofilms, driven by metabolic and corrosive activities, can lead to significant degradation of metal and concrete surfaces (Abdulina et al., 2019). Sulfate reducing bacteria (SRBs) cause corrosion due to sulfate presence in anoxic and anaerobic environments. Archaea are significant in extreme environments with high temperatures. Fungi are key in warm, humid climates like Southeast Asia for MIC studies. Bacteria and fungi produce organic acids leading to corrosion of various metals. Organic acids cause corrosion on metals like magnesium alloy and zinc (Jia et al., 2019).

3.1.1 MIC Effect on Metallic Materials (e.g., Steel, Iron)

MIC contributes to 20% of corrosion losses with SRB being the main agents, utilizing organic carbons for energy. The electrons harvested are used to reduce sulfate, producing hydrogen sulfide gas that is toxic and corrodes infrastructure. SRB reside in biofilm communities providing protection against environmental conditions. They adapt their electron donor sources, transitioning from organic carbon to iron, initiating pitting corrosion. Apart from SRB, other electrogenic microbes like nitrate reducing bacteria (NRB) and methanogens are involved in MIC. Gu and Xu (2013) classify these microbes as X-reducing bugs, with 'X' representing various electron acceptors including sulfate, nitrate/nitrite, CO₂, and others (Gu et al., 2015).

Xu et al. discuss the corrosion of metal under aerobic conditions, involving Fe²⁺-oxidizing and Mn²⁺-oxidizing bacteria as early biofilm colonizers. These bacteria consume O₂, produce oxide coatings on the metal, and create a low O₂ environment near the biofilm-metal interface. Heterotrophic microorganisms help remove O₂ within corrosion biofilms by utilizing organics present in various environments where corrosion occurs. As O₂ levels decrease in biofilms, microorganisms with fermentative metabolism and anaerobic respiration establish themselves near the metal-biofilm interface. In anaerobic conditions, microorganisms are vital in promoting corrosion of ferrous metals like FeO. Nitrate, sulfate, and CO₂ do not react with FeO spontaneously unlike oxygen; microbial catalysis is required for oxidation. Anaerobic microorganisms aid FeO oxidation through various mechanisms such as metabolite production and electron transfer mediation. These mechanisms involve diverse microorganisms and have been studied rigorously in some cases, while in others they are inferred from indirect evidence. Studying corrosion dynamics in complex environments is challenging but focusing on FeO as the electron donor in studies, along with genetic or molecular analysis, can provide valuable insights (D. Xu et al., 2023).

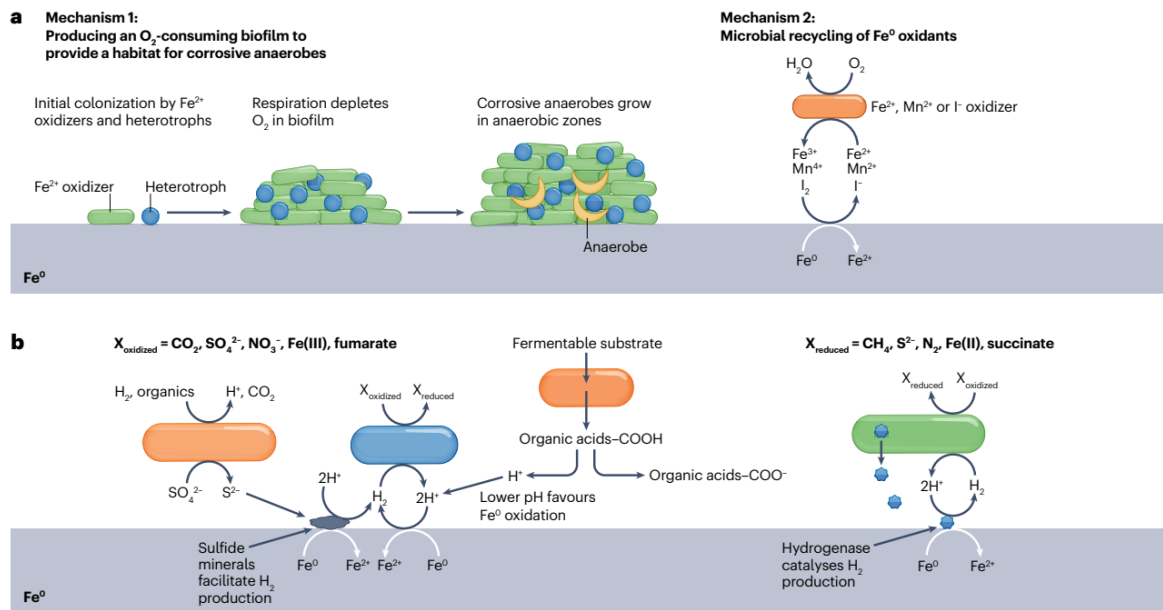


Figure 1. Aerobic (a) and Anaerobic (b) mechanisms for microbial corrosion of ferrous metal (Xu et al., 2023).

In (Figure1), the processes described the initial stages under aerobic and anaerobic conditions. Under aerobic conditions, iron-oxidizing microorganisms colonize metal surfaces and form biofilms, creating zones of low oxygen. This facilitates the growth of anaerobic sulfate reducers. Additionally, certain microorganisms oxidize iron, manganese, and iodine, generating oxidants for the abiotic oxidation of metallic iron (Fe^0). Under anaerobic conditions, sulfide minerals formed through microbial sulfate reduction, along with protons released from organic acid fermentation, promote the oxidation of Fe^0 coupled with the reduction of protons (H^+) to hydrogen (H_2). Extracellular hydrogenases released from microorganisms also contribute to this process (D. Xu et al., 2023).

3.1.1.1 Mechanisms and Theories

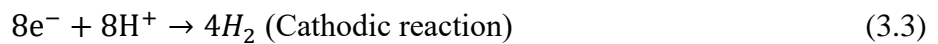
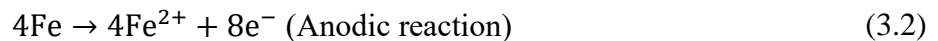
MIC has been categorized into three groups for the purpose of distinguishing the various mechanisms. Nevertheless, there are two distinct types of anaerobic metabolisms: respiration and fermentation (Gu et al., 2015; D. Xu et al., 2016). Type I MIC is initiated by electrogenic bacteria, which have the capability to actively generate pili for electron transfer and energy dissemination during respiration metabolism. These bacteria deliberately utilize carbon steel or other non-noble metals as electron donors due to the sufficiently negative reduction potentials of the ions present in these metallic substances, resulting in thermodynamically favourable redox reactions when combined with the reduction of an oxidant like sulfate and nitrate. The bacteria harness electrons released from the oxidation of elemental metal to internally reduce the oxidant (Pillai, 2015). Type II-MIC is caused by metabolites like volatile fatty acids secreted by microbes. The Microbially Influenced Corrosion (MIC) induced by acid-producing bacteria (APB) is classified within this group as indicated by Gu and Xu (2013). Similarly, the Copper MIC resulting from Sulfate-Reducing Bacteria (SRB) is also categorized as Type II-MIC. Additionally, Type III-MIC is attributed to microbial organisms that release enzymes or other corrosive substances, leading to the deterioration of

non-metallic materials comprising organic carbon among their constituents (Pillai, 2015). MIC impacts various metals by engaging microorganisms that adhere to the metal surface, produce extracellular polymeric substances (EPS), and create biofilms. These biofilms can trigger localized corrosion processes like pitting corrosion, crevice corrosion, and stress corrosion cracking, compromising the structural strength of the metals. The presence of microorganisms and their corrosive actions can expedite the degradation of steel alloys, magnesium alloys, aluminium alloys, and titanium alloys, rendering them vulnerable to damage caused by MIC (M. Khan et al., 2023).

Sulfate-reducing bacteria (SRBs) play a significant role in inducing corrosion through a combination of biological, chemical, and physical processes. Although the precise mechanisms are not fully understood, several hypotheses have been proposed (Y. G. & Mulky, 2023). Two primary hypotheses for microbially influenced corrosion (MIC) by sulfate-reducing bacteria (SRB) are widely acknowledged: the cathodic depolarization theory (CDT) and the biocatalytic cathodic sulfate reduction (BCSR) theory. The former suggests that SRB induce corrosion by forming a hydrogen film on corroded metal surfaces, which they depolarize by removing hydrogen via the enzyme hydrogenase. The latter, more broadly, involves SRB and other microorganisms deriving energy through extracellular electron transfer (EET) mechanisms. EET is seen as a critical aspect of MIC, but it is also noted as a bottleneck in EET-MIC processes (Anguita et al., 2022).

- Cathodic Depolarization Theory (CDT)

CDT, focuses on the role of SRB in accelerating corrosion by consuming hydrogen ions at the metal surface, leading to increased corrosion rates. The theory examines hydrogen produced by iron dissolution or water reactions consumed by SRB for sulfate reduction pathway (Y. G. & Mulky, 2023).

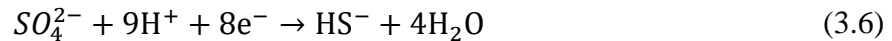


CDT offers understanding of electrochemical processes in MIC. Yet, it lacks clarity on MIC cases caused by hydrogenase-negative SRB and other non-sulfate-reducing bacteria (Pillai, 2015).

- Biocatalytic Cathodic Sulfate Reduction (BCSR)

Based on principles of bioenergetics, Gu et al. devised a theoretical framework known as biocatalytic cathodic sulfate reduction (BCSR) theory. This framework aims to explain the

thermodynamic processes involved in Type I microbially influenced corrosion (MIC) by sulfate-reducing bacteria (SRB)(D. Xu et al., 2016).BCSR proposes that Sulfate Reducing Bacteria (SRB) harness electrons from iron oxidation through sulfate reduction within their cellular processes. This mechanism entails the reduction of sulfate (SO_4^{2-}) as the final electron acceptor, resulting in the generation of hydrogen sulfide (H_2S) and the initiation of corrosion on steel surfaces. The fundamental reactions in BCSR include:(Y. G. & Mulky, 2023).



By employing these techniques, different SRB biofilms can be simulated, with some relying on Direct Electron Transfer (DET) while others utilize Mediated Electron Transfer (MET). In this scenario, there is no physical cathodic surface where the reduction reaction occurs. Instead, the reaction takes place within the cytoplasm of SRB cells, which are located in proximity to the metal surface to facilitate electron transfer across cell walls. Electrons produced from iron oxidation are conveyed into the cytoplasm of SRB cells through Extracellular Electron Transfer (EET). The term "cathodic" in the Biofilm-Corrosion-Sulfate-Reducing Bacteria (BCSR) model is employed for consistency in depicting an electrochemical corrosion process, which typically encompasses both anodic and cathodic reactions, akin to classical CO_2 corrosion modeling(D. Xu et al., 2016).

- Extracellular Electron Transfer Microbiologically Influenced Corrosion (EET-MIC)

Biofilms have the capability to generate corrosive metabolites in high concentrations within their vicinity, resulting in metabolite-MIC (M-MIC). Conversely, biofilms are also able to acquire electrons from energetic metals, thereby facilitating extracellular electron transfer (EET)-MIC. When an electron donor enters a microbe's cytoplasm, there is no need for EET as oxidation and reduction reactions happen inside the cell. EET is necessary for electron donor releasing electrons outside the cell like in Fe (0) corrosion. This process is referred to as inward EET where a biofilm on metal surface acts as a biocathode. If oxidation happens inside cells, the biofilm functions as a bioanode, where electrons generated by microbial metabolism are transferred out of the cells to an external electron acceptor (Gu et al., 2021).

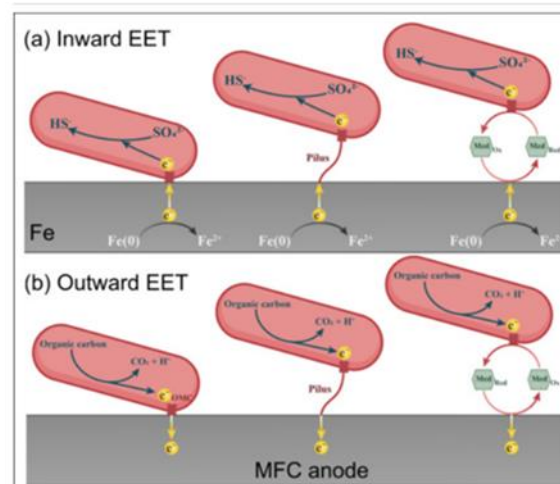


Figure 2. Bidirectional EET. a) Inward EET in a biocathode of microbial fuel cell. b) outward EET in a bioanode of a microbial fuel cell (Gu et al., 2021)

The concept that both outward and inward extracellular electron transfer (EET) processes can involve Direct Electron Transfer (DET) mechanisms explained in (Figure 2). (Gu et al., 2021)

In the absence of a biofilm, the utilization of electrons by planktonic cells in the liquid is hindered as electrons lack the ability to move freely in the liquid medium, unlike ions. Electron Extracellular Transfer (EET) encompasses three primary mechanisms: direct contact, conductive pilus, and electron mediator. The initial two strategies are referred to as direct electron transfer (DET), while the third approach is known as mediated electron transfer (MET) (Y. Li et al., 2018). MET involves the use of electron transfer mediators like potassium hexacyanoferrate ($\text{K}_3[\text{Fe}(\text{CN})_6]$), phenazine methosulfate, osmium complexes, phenothiazin, and naphthoquinone. These small and mobile mediators transport electrons from FAD, derived from an enzyme-catalytic process, to the electron acceptor (Ishida et al., 2018). In other words, mediated electron transfer (MET) entails the use of electron carriers like hydrogen or electron transfer mediators such as riboflavin and flavin adenine dinucleotide (FAD) (Lv MeiYing & Du Min, 2018).

- The impact of sulfate-reducing bacteria on different type of steel.

Carbon steel is an extensively researched steel type in the context of corrosion induced by Sulfate Reducing Bacteria (SRB). Its high iron content and limited resistance to environmental conditions such as pH and temperature make carbon steel highly vulnerable to SRB-induced corrosion. SRB can expedite the corrosion process in carbon steel through multiple mechanisms, including the generation of corrosive byproducts like hydrogen sulfide (H_2S). Besides carbon steel, various other types of steel, such as stainless steel, low-alloy steel, and galvanized steel, are also prone to corrosion caused by sulfate-reducing bacteria (SRB). The impact of SRB on these diverse steel types can be attributed to a combination of

direct and indirect processes. Direct processes may encompass the generation of corrosive and the hindrance of protective coatings and inhibitors. In addition, among carbon steel, X70 steel, and X80 steel, studies have shown that X80 steel exhibits better resistance to SRB-induced corrosion compared to carbon steel and X70 steel. Based on microstructure analysis, they proposed that the issue is attributable to the less uniform structure of X70 relative to other types of steel (Y. G. & Mulky, 2023).

The study on microbiologically influenced corrosion of X80 pipeline steel by sulfate-reducing bacteria (SRB) involved immersing steel coupons in sterile and SRB-inoculated culture media. Carbon source starvation tests were conducted with varying organic carbon content (0%, 10%, and 100%). The exposure duration was 14 days, and analysis techniques such as SEM and XPS were used for characterization. The experiments were carried out in a controlled anaerobic environment with pH regulation. Sample preparation included machining, grinding, polishing, cleaning, and sterilization of steel coupons. The findings revealed that Sulfate-reducing bacteria (SRB) spurred the creation of FeS corrosion products, leading to crust formation on steel surfaces. Despite this, the crust failed to mitigate corrosion in carbon-deficient environments where electron transfer prevailed. Corrosion rates peaked in a 10% carbon source medium, indicating heightened SRB activity accelerating the corrosion process. Depletion of carbon sources resulted in a porous corrosion layer, exacerbating corrosion by facilitating direct contact between the solution and the steel substrate. Overall, SRB activity, carbon sources, and corrosion product characteristics significantly influenced the corrosion behaviour of the steel (*Laboratory Investigation of Microbiologically Influenced Corrosion of X80 Pipeline Steel by Sulfate-Reducing Bacteria | Journal of Materials Engineering and Performance*, n.d.).

- Microbial corrosion affecting metal water supply pipelines

Distribution networks comprise various components like pipes, plumbing, valves, pumps, meters, and storage tanks. Microbially-induced corrosion affects these components, releasing harmful substances into the water. This corrosion can lead to the growth of metal-oxides scale, causing pipeline blockages and breakages. Microbial attachment to the rough surfaces of metal-based pipelines initiates the corrosion process, followed by the formation of mineral deposits that create a protective barrier resistant to disinfectants. This sets the stage for microbially induced corrosion (MIC) within the pipeline's microenvironment, driven by microbial metabolic processes and extracellular polymer generation. As oligotrophic microbes multiply, they dissolve metal pipe materials, causing damage to the pipe walls. Interaction among corrosive microorganisms leads to various forms of corrosion, reflected in

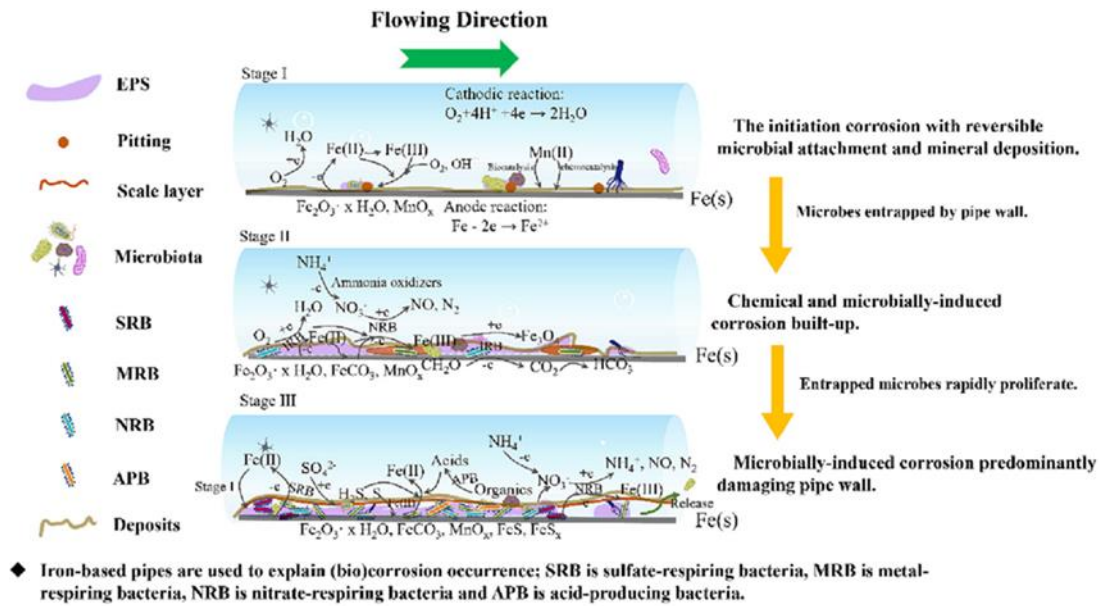


Figure 3. Potential MIC paths in DWDS (Song et al., 2023).

The development of scale layers on pipe surfaces that indicate microbial diversity. Microbial metabolic activities, including acid production, contribute to the formation of porous scale layers, enhancing microbial community stability and accelerating MIC (X. Song et al., 2023).

The MIC process in DWDSs comprises three stages is shown in (Figure3.) In the initiation stage, HOS microbes attach to metal pipelines, initiating corrosion. Progression involves mineral deposit formation induced by attached microbes, leading to metal dissolution and pipe damage. Completion sees corrosive microorganisms interact with pipes, causing various corrosion forms. Understanding these stages is vital for developing strategies to control MIC, ensuring system integrity, water quality, and minimizing economic and environmental impacts (X. Song et al., 2023).

Cast iron pipes utilized in urban water distribution systems are prone to corrosion upon exposure to water, leading to the development of "corrosion tumours" which change the colour and turbidity. Studies suggest that during the initial phases of biofilm development within cast iron pipes, corrosion may be accelerated, whereas in later stages, mature biofilms can offer protection against surface degradation. *Thiobacillus ferrooxidans*, a specific bacterium, is recognized as a significant corrosive agent on cast iron pipes under specific temperature conditions, highlighting the crucial role of microbial activity in corrosion mechanisms. The collective bacterial community plays a substantial role in the corrosion of cast iron pipes in real-world scenarios, contributing significantly to the total weight loss attributed to microbial corrosion. To combat microbial corrosion in cast iron pipes, protective measures such as lining inner walls with materials like cement mortar, epoxy resin, and other inert substances are commonly employed as effective mitigation strategies (Yang et al., 2022).

3.1.2 MIC Of Concrete and Cement and Other Advanced Materials

Microbiologically induced corrosion (MIC) represents a highly distinct form of deterioration mechanism observed in concrete structures that are subjected to sewer environments. This phenomenon has the potential to significantly decrease the durability of concrete structures potentially reducing the expected lifespan from 100 years to a range of 30-50 years, and in severe cases, as low as 10 years or less (J. Wu et al., 2019).

3.1.2.1 MIC In Concrete Exposed to Sewer Environments

MIC in concrete exposed to sewer environments initiates with anaerobic sulfate reducing bacteria converting sulfates to hydrogen sulfide (H_2S), which then accumulates and reacts with moisture to form sulfuric acid. This acid, along with other biogenic acids, deteriorates the concrete matrix, causing progressive disintegration (M. Wu, Wang, Wu, et al., 2020).

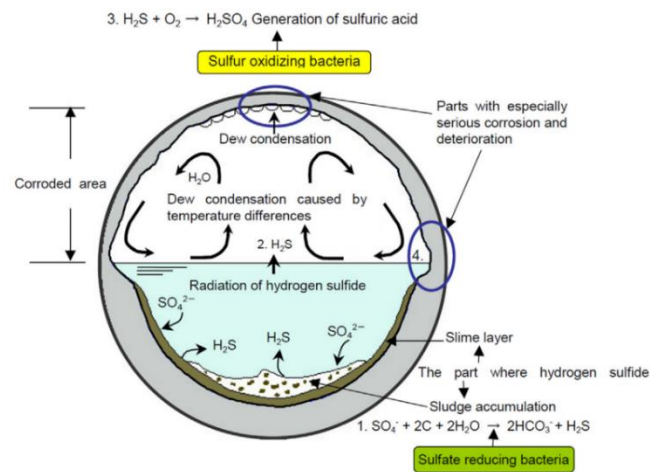


Figure 4. The main events tied to microbiologically induced corrosion of concrete in sewer environments (Wu, Wang, Wu, et al., 2020).

The key phases in microbiologically induced corrosion (MIC) of concrete in sewer environments comprise 4 main stages. According to (Figure 4.) the formation of hydrogen sulfide in the solution, the release and buildup of hydrogen sulfide gas, the generation of sulfuric acid, and the deterioration of the concrete materials. These processes entail a blend of biological, chemical, and physical mechanisms that contribute to the corrosion of concrete in sewer systems (M. Wu, Wang, Wu, et al., 2020).

Consequently, progression and the degradation of concrete structures by microbial concrete corrosion (MICC), involve distinct phases including abiotic acid-base reactions, the dominance of sulfur-oxidizing bacteria, and the intensification of corrosion rates. The initial stage is marked by abiotic acid-base reactions, resulting in carbonation and the formation of weak acids like thiosulfuric and polythionic acid. Following this, the biotic dominated part ensues, where neutrophilic sulfur-oxidizing bacteria (NSOB) become prevalent in the

concrete's pore spaces. These bacteria thrive in humid conditions and oxidize reduced sulfur species, generating sulfuric acid that contributes to the corrosion of the concrete structure. As the corrosion progresses, the last stage emerges, characterized by the dominance of acidophilic sulfur-oxidizing bacteria (ASOB) with low pH levels and high corrosion rates. Notably, *Acidithiobacillus trioxidans* is a common bacterium found in this stage. The corrosion intensifies in this final stage, leading to substantial material loss, with reported corrosion rates exceeding 10 mm/year. This sequential progression from abiotic reactions to biotic interactions underscores the complex nature of MICC and the detrimental impact it can have on concrete infrastructure (Grenng et al., 2018).

Concrete corrosion in sewers begins with abiotic processes such as carbonation and H₂S acidification, leading to a decrease in surface pH. Subsequently, biological sulfide oxidation produces sulfuric acid, further lowering pH levels. The corrosion process is influenced by the water content of concrete, which is affected by sewer relative humidity (RH) and occasional flooding. Higher RH levels accelerate corrosion initiation and progression. Moisture condensation on sewer crown regions fosters microbial colonization and sulfide oxidation, contributing to corrosion (X. Li et al., 2017). In concrete sewer pipes, corrosion is often concentrated at the crown and waterline levels. Crown corrosion is intensified by temperature variations, enhancing H₂S(g) transfer to sewer walls. Acid-containing condensates around the waterline trigger rapid corrosion rates, leading to the formation of deteriorated products that are intermittently washed away by the waste stream, exposing fresh surfaces for further corrosion (M. Wu, Wang, & Wang, 2020).

The mechanisms underlying Microbially Induced Concrete Corrosion (MICC) in sewer wastewater were confirmed through 14-month laboratory experiments. The gradual decrease in pH levels from 6.87 to 3.0 facilitated the growth of sulfur-oxidizing bacteria, leading to corrosion product formation and concrete weight loss. This extended monitoring period allowed for the observation of morphological changes and the identification of specific corrosion by-products, highlighting the cumulative impact of biogenic sulfuric acid on concrete degradation. *Acidithiobacillus trioxidans* and similar bacteria were found to play a critical role in sulfuric acid production, which led to the dissolution of calcium compounds and the formation of corrosion by-products such as gypsum and ettringite. These findings emphasize the complex interplay between microbial activity, pH levels, and sulfate concentrations in sewer environments (D & R.H, 2021). Additionally, Biocorrosion, fuelled by microbial activity, can trigger significant changes in both the outward appearance and internal structure of concrete. These effects encompass alterations in geometry, surface crack formation, material removal from the surface, and colour variation. Biocorrosion-induced degradation can also lead to the formation of microcracks, permeable gaps, and changes in the chemical composition of the concrete material (Chaudhari et al., 2022). The investigation of the concrete structure within a sewage treatment facility in Curitiba, Brazil, which has been subjected to an aggressive environment for close to 20 years, leads to observations of variations in the concrete structure. These include the formation of surface cracks, removal of material from the surface, and shifts in chemical composition due to the impact of biogenic sulfur attack. Additionally, the study identifies the presence of microcracks and structural modifications within the concrete as a direct result of biocorrosion-induced degradation (Pereira Godinho & De Medeiros, 2021).

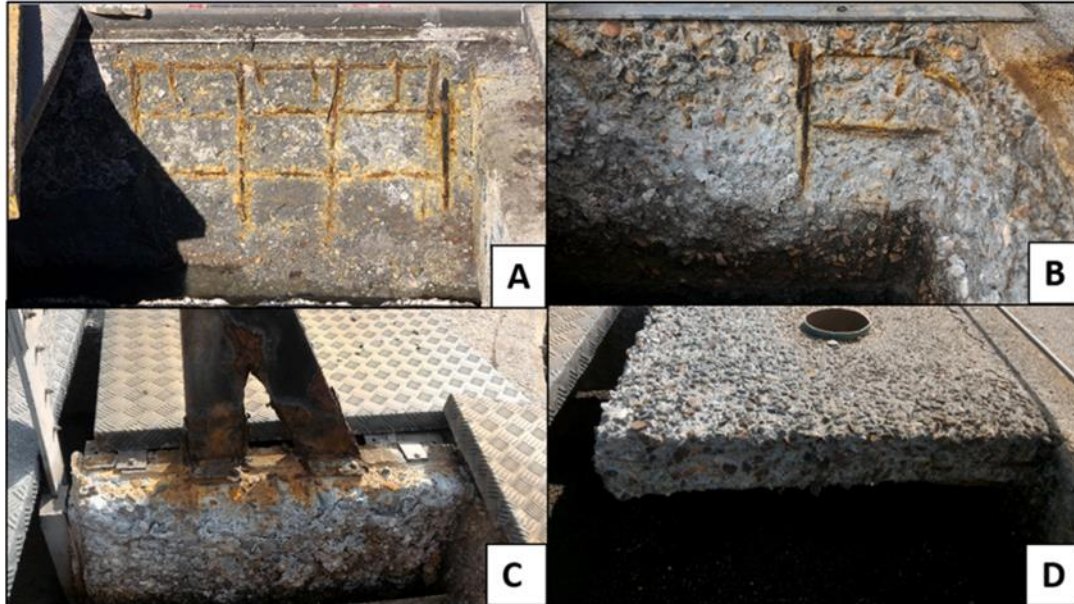


Figure 5. Degradation of concrete. A and B - Walls of the drainage channels in the sanitary sewer show exposed reinforcement. C - Supplementary metallic structures of the Upflow Anaerobic Sludge Blanket (UASB) reactor exhibit intense corrosion. D - The slab above the flow channel displays exposed aggregates (Pereira Godinho & De Medeiros, 2021).

During the recent assessment, it was observed that the sewage treatment plant is operational, with concrete showing surface disintegration in the form of flaking that can be manually removed. This disintegration has caused a depletion in the reinforcement cover in certain tank areas, leading to reinforcement corrosion and exposing the underlying carbon steel, as shown in (Figure 5)(Pereira Godinho & De Medeiros, 2021).

- **Addressing Corrosion Issues in Different Components of Wastewater Treatment Plants**

Various components in wastewater treatment plants are prone to corrosion due to exposure to corrosive atmospheres and immersion in corrosive fluids. Critical components such as sludge scrapers, gratings, ladders, electrical junction boxes, steel lamp posts, valve handles, and immersed steel components are particularly susceptible to deterioration in high humidity, corrosive atmospheres containing hydrogen sulfide (H_2S), and corrosive aqueous environments, leading to operational inefficiencies. Corrosion in wastewater treatment plants is affected by various factors, including microbiologically influenced corrosion (MIC), anaerobic conditions, Thiobacillus bacteria acting on sulfur compounds to produce hydrogen sulfide (H_2S), sulfur oxidizing bacteria (SOB) creating dilute sulfuric acid (H_2SO_4), and environmental elements such as elevated temperatures, sunlight exposure, and humidity. These combined factors play a significant role in the corrosion of both metallic and non-metallic components within the wastewater treatment plant setting. To address this corrosion challenge, advanced materials and coatings like coal tar epoxy, petrolatum tape coatings,

corrosion-resistant steels, polymers, and fiberglass-reinforced plastics are employed to safeguard these components. Furthermore, the implementation of corrosion prevention measures such as cathodic protection (CP) systems for selected steel components and ceramic anode ICCP systems in clarifiers plays a crucial role in effectively managing corrosion issues and prolonging the lifespan of wastewater treatment plant infrastructure (Stephenson & Kumar, 2009).



Figure 6. The valve handle on the clarifier displays significant corrosion. (Stephenson & Kumar, 2009).

(Figure 6) illustrates an instance of dissimilar metal corrosion cells observed at the wastewater treatment plant, where a bronze water valve experienced severe corrosion due to a galvanized steel pipe fitting screwed into it. This example underscores the importance of using compatible materials to prevent corrosion issues in such environments.



Figure 7. The a) initial and b) final states of the valve-operator stands at a wastewater treatment plant. (Stephenson & Kumar, 2009).

The (Figure 7), shows the before-and-after states of the valve-operator stands, illustrating the extent of corrosion damage, the abrasive cleaning process to achieve a particular finish, and the application of protective coatings to prevent future corrosion. This visual representation underscores the maintenance efforts undertaken to address.

3.1.2.2 MIC in Cementitious Materials and Mortar

Kashaija et al. (2024) studied cement mortars as a replacement for concrete in wastewater treatment facilities. Degradation of cement-based materials in pumping stations and sand-trap structures was examined. Exposure to wastewater vapours caused more deterioration than liquid exposure, forming gypsum and ettringite due to biogenic sulfuric acid attack. An in-situ experiment was conducted for 1 to 7 months on cement samples in wastewater treatment structures. specimens exposed to wastewater environments within pumping stations and sand-trap structures (Kashaija et al., 2024).

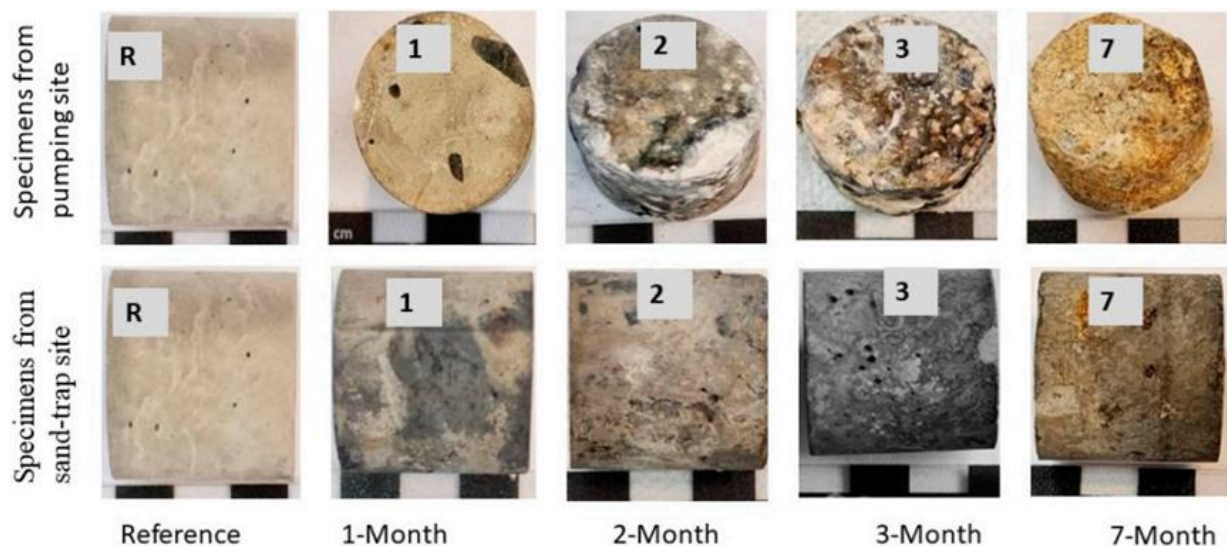


Figure 8. physical changes in cement paste specimens over time. Pumping station specimens at 2-, 3-, and 7-months display surface deterioration, while sand-trap counterparts do not (Kashaija et al., 2024).

According to (Figure 8), the specimens located within the pumping station underwent a noticeable transformation in coloration following a period of two months, contrasting starkly with the specimens housed within the sand-trap structure which retained their original grey hue. As the timeline extended to seven months, it was observed that the paste specimens within the pumping station exhibited the development of white, tender, and pulpy by-products, presenting a distinct contrast to the sand trap specimens which did not showcase similar changes over the same duration.

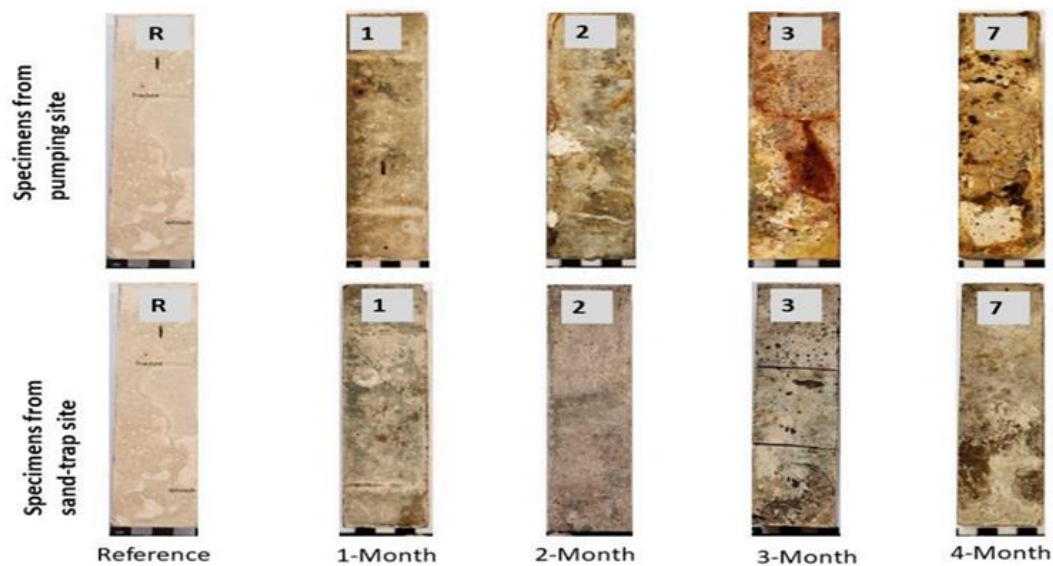


Figure 9. The physical changes in cement mortar specimens over different exposure periods. Pumping station specimens at 2, 3, and 7 months display more significant surface changes compared to those from sand-trap structures (Kashaija et al., 2024).

(Figure 9), shows more physical damage on pumping station specimens' edges compared to sand trap specimens (Kashaija et al., 2024). The laboratory simulated microbially induced corrosion test involved the evaluation of four types of mortars: low calcium fly ash based geopolymer mortar (FA-GPm), alkali-activated slag-based mortar (AASm), calcium aluminate cement-based mortar (CACm), and sulfate resistant Portland cement mortar (SRPCm). These mortars were exposed to a nutrient solution containing sulfur oxidizing microbes to assess their performance and resistance to biocorrosion over a six-month period. The results showed varying degrees of deterioration and resistance to biocorrosion among the different mortar types. Different mortars have distinct chemical compositions, porosities, and microstructures, which can affect their susceptibility to biocorrosion. For example, mortars with higher porosity may provide more favourable conditions for microbial growth and acid formation compared to denser mortars. Additionally, the presence of certain minerals or compounds in the mortars may influence the biodegradation process differently. Specifically, the neutralization of CACm after exposure to the biotic reactor was lower compared to other mortars, indicating its resistance to biocorrosion. Formation of sulfuric acid (H_2SO_4) and growth of sulfur oxidizing microbes (SOMs) were dependent on the type of mortar, with different mortars exhibiting varying levels of degradation (H. A. Khan et al., 2022).

3.2 Economic Implications

MIC is recognized as the most hazardous form of corrosion in industries like power generation, wastewater treatment plant, and oil and gas production, among others, and it is

classified as one of the eight distinct types of corrosion, known as biocorrosion (Lavanya, 2021).

Hydrogen sulfide emissions in sewer systems pose numerous challenges, including biogenic concrete degradation, unpleasant odours in urban areas, and potential health risks to sewer workers due to sulfide gas. Instances of minor concrete degradation have been observed when the total sulfide concentration in wastewater ranges from 0.1 to 0.5 mg S L⁻¹, while significant concrete corrosion tends to occur at sulfide levels exceeding 2.0 mg S L⁻¹. In Los Angeles County, approximately 10% of sewer pipes suffer from substantial sulfide-related corrosion, leading to restoration costs estimated at around \$400 million. In Flanders, Belgium, annual costs associated with biogenic sulfuric acid corrosion in sewers are estimated to be about €5 million, representing roughly 10% of the total expenditure for wastewater collection and treatment. To address the issue of biogenic corrosion, various chemical and biological technologies have been proposed to prevent or reduce hydrogen sulfide emissions in sewer systems, as will be detailed in chapter 10. However, the primary drawback of current hydrogen sulfide emission control technologies is their cost (h1.9–7.2 kg⁻¹ S removal). There is a need for a cost-effective and efficient method to manage hydrogen sulfide emissions in sewer systems, such as the use of formaldehyde, MgO₂/CaO₂, microbial fuel cells (MFCs), and phages. Formaldehyde is a cost-effective chemical (h1.3–3.6 kg⁻¹ S removal) that inhibits sulfide generation in sewers. Solid-phase oxygen is appealing because it can provide long-term inhibition of sulfate-reducing bacteria (SRB) activity. Sewer MFCs is advantageous as they can generate electricity while preventing sulfide formation. Additionally, phages that efficiently lyse SRB could be cost-effective due to their potential to reproduce in sewers. (L. Zhang et al., 2008a).

A study conducted by Koch et al. (2002) evaluated the direct costs of corrosion across 26 industrial sectors where corrosion is commonly found. By extrapolating their findings nationwide, they determined the total direct cost of corrosion to be \$276 billion annually, equivalent to 3.1 percent of the U.S. gross domestic product (GDP). Additionally, they conservatively estimated the indirect costs to society to be equal to the direct costs. This implies that the overall societal cost of corrosion could potentially amount to as much as 6 percent of the GDP. Typically, only the direct costs are accounted for, while the indirect costs, despite being borne by society, are often overlooked as they are paid solely by the owner/operator (Koch et al., 2002). In 2002, the total annual direct cost of corrosion for the nation's drinking water and sewer systems was estimated to be \$36.0 billion. This comprehensive figure included expenses related to infrastructure replacement, water loss through leaks, corrosion inhibitors, internal mortar linings, and protective coatings. The drinking water and sewer systems sector was highlighted for its substantial contribution to the overall corrosion cost, ranking among the industries with the highest direct corrosion impacts. Moreover, this sector's corrosion cost was part of the total direct corrosion cost, which was estimated at \$276 billion per year, equivalent to 3.1 percent of the U.S. gross domestic product (GDP). Conservatively estimated, the indirect costs of corrosion in the sewer and water sector were deemed equivalent to the direct costs, suggesting an overall societal cost that could potentially reach 6 percent of the GDP.

In 2015, the overall global cost of corrosion reached \$2.5 trillion, and of this amount, microbial-induced corrosion (MIC) accounted for approximately 20%, although the specific

impact of MIC on corrosion losses in wastewater treatment was not clearly indicated (Lavanya, 2021) .

According to (Grengg et al., 2018), microbial-induced concrete corrosion (MICC) poses substantial economic burdens on wastewater networks globally. In Germany, annual rehabilitation expenses for MICC-related damages exceed €450 million, while in the UK, they surpass £85 million. Additionally, the United States is expected to allocate approximately \$390 billion over the next two decades to sustain the functionality of its existing wastewater infrastructure.

The study (Zhong et al., 2023) examined the noteworthy economic consequences of corrosion, which typically represents 3-4% of a nation's gross domestic product (GDP) and results in substantial losses across diverse industries. To illustrate, in 2014, corrosion in water distribution networks resulted in expenses of around 10 billion RMB in China, while public drinking water systems suffered annual losses of \$22 billion and sewage systems incurred losses of \$14 billion in the USA. Urban water systems are particularly susceptible to corrosion, leading to system failures and economic setbacks, particularly in relation to pipeline infrastructure and the quality of drinking water. The cost of corrosion varies depending on the region, with China and the USA demonstrating significant research interest and potential costs, while Belgium holds considerable influence in the field based on citation rates. Ongoing research is focused on comprehending the effects of corrosion inhibitors and biocides on wastewater treatment processes and elucidating the mechanisms of biocorrosion in reclaimed water distribution systems.

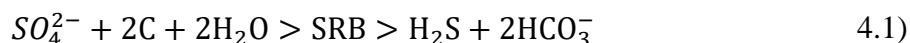
the cost implications of microbial corrosion on concrete structures are indeed mentioned by (D. Wang et al., 2023) which draws attention to the considerable financial burdens that arise from the need to repair and rebuild sewer systems affected by microbially influenced concrete corrosion (MICC). For instance, it is noted that the replacement of 11% of a concrete pipe in Los Angeles, California, due to microbial corrosion incurred a cost of USD 400 million. Moreover, estimations for the restoration of sewage systems in the United States indicate that the total costs could reach up to USD 1.6 trillion. These figures serve to highlight the substantial economic impact that MICC has on the maintenance and repair of infrastructure.

4 Biological Processes involved in Sulfide Production and MIC

A group of anaerobes, collectively called sulfate-reducing bacteria (SRB), generate different sulfide species (H_2S , HS^- and S^{2-}) that coexist in equilibrium in wastewater and the distribution of each species is a function of pH (Mara & Horan, 2003). Over the past decade, extensive research has been conducted on the reduction of sulfate to sulfide by sulfate-reducing bacteria (SRB) under anaerobic conditions (Z. Zhang et al., 2022). Several factors contribute to its notable performance:

- I. Sulfide formation is enhanced through sulfate reduction, which consumes more substrates with limited electron donors, consequently reducing sulfate levels (J. Wu et al., 2019). By reducing sulfide formation, this process mitigates the inhibitory effects of sulfide on microorganisms, prevents the corrosion of metal pipelines or equipment by sulfide, and enhances the purity of biogas (Oliveira et al., 2020; Shi et al., 2020).
- I. By reducing sulfide formation, this process mitigates the inhibitory effects of sulfide on microorganisms, prevents the corrosion of metal pipelines or equipment by sulfide, and enhances the purity of biogas (Oliveira et al., 2020; Shi et al., 2020).
- II. Metal sulfide precipitation occurs as sulfate is reduced to sulfide, leading to the formation of metal sulfide, which can then be precipitated by adjusting pH for removal or recovery (Y.-N. Xu & Chen, 2020).

In sulfate bioconversion, SRB play indispensable roles. They reduce sulfate through the oxidation of low molecular weight substrates (such as hydrogen, acetic acid, lactic acid, ethanol, etc.) to acetic acid salts as incomplete oxidation products, with CO_2 produced as complete oxidation products (Piña-Salazar et al., 2011). SRB are obligate anaerobes that derive energy for growth through the oxidation of organic substrates, utilizing sulfate as the terminal electron acceptor as (O. J. Hao, 1996).



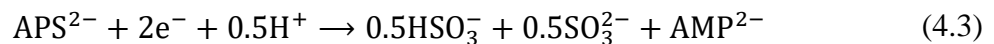
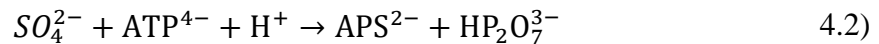
The preferred carbon sources for sulfate-reducing bacteria (SRB) include low molecular weight compounds such as organic acids (e.g., lactate, pyruvate, formate, and malate), fatty acids (e.g., acetate), and alcohols (e.g., ethanol, propanol, methanol, and butanol). These compounds are largely fermentation products resulting from the anaerobic degradation of carbohydrates, proteins, and lipids. Consequently, SRBs act as terminal degraders, analogous to methanogenic bacteria, which produce methane and CO_2 as end products. The rate of sulfide production in sewers has been observed to correlate with dissolved carbohydrate and volatile fatty acids concentrations.

The pathways involved in sulfate reduction encompass assimilated sulfate reduction (ASR) and dissimilatory sulfate reduction (DSR) (see Fig. 1). ASR is a vital biochemical synthesis process that yields reduced sulfur, holding significant importance in industrial biotechnology. Methionine production stands out as a crucial energy requirement within ASR, garnering

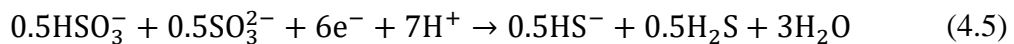
considerable attention in biotechnological applications (Rückert, 2016). However, during sulfate reduction, only a small portion

of sulfate is converted into sulfur-containing compounds through the ASR pathway, while a considerable amount is reduced to sulfide via the DSR pathway (J. Li et al., 2019). As a result, the primary focus is on sulfate-reducing bacteria (SRB) involved in the dissimilatory sulfate reduction (DSR) process.

In chemical reactions, E^0 (pronounced "E zero prime") refers to the standard reduction potential. It represents the tendency of a chemical species to gain electrons and undergo reduction, compared to a standard reference electrode, usually the hydrogen electrode. The E^0 value is a measure of the "strength" of a redox couple, indicating how easily a species can accept electrons. More positive E^0 values indicate a stronger tendency to accept electrons (i.e., a better oxidizing agent), while more negative values indicate a weaker tendency to accept electrons (i.e., a better reducing agent). The standard reduction potential is often expressed in volts (V) or millivolts (mV). The dissimilatory reduction of sulfate can be broadly divided into a two-step process. Sulfate poses challenges as an electron acceptor for microorganisms due to its unfavorable redox potential (E^0 of the sulfate-sulfite pair is -516 mV), which cannot be efficiently reduced by intracellular electron mediators like ferrite protein or NADH (having E^0 values of -398 mV and -314 mV, respectively). Thus, in the initial step, sulfate is activated by sulfate adenylyltransferase (sat), forming adenosine-5'-phosphosulfate (APS) at the expense of two ATP equivalents. APS reductase then utilizes two electrons to reduce APS to bisulfite (HSO_3^-) as represented in Eqs 4.2) and (4.3). The hydrolysis of the released pyrophosphate ($\text{HP}_2\text{O}_7^{3-}$) drives Eq.(4.4) activation reaction. The redox potential (E^0) of the APS-sulfite pair plus adenosine monophosphate (AMP) is approximately -60 mV, facilitating the reduction of APS by reduced ferredoxin or NADH (Z. Zhang et al., 2022).



In the subsequent step, bisulfite reductase reduces HSO_3^- with six electrons to form sulfide (HS^-) (Eq. (4.5), with a potential E^0 of approximately -116 mV for the redox sulfite-sulfide pair. It's still under debate whether sulfite reduction to sulfide follows a route through trithionate and thiosulfate, allowing for reduction in three two-electron steps (Muyzer & Stams, 2008). The overall result of sulfate reduction involves the consumption of 8 moles of electrons and 8.5 moles of protons (Keller & Wall, 2011).



4 Biological Processes involved in Sulfide Production and MIC

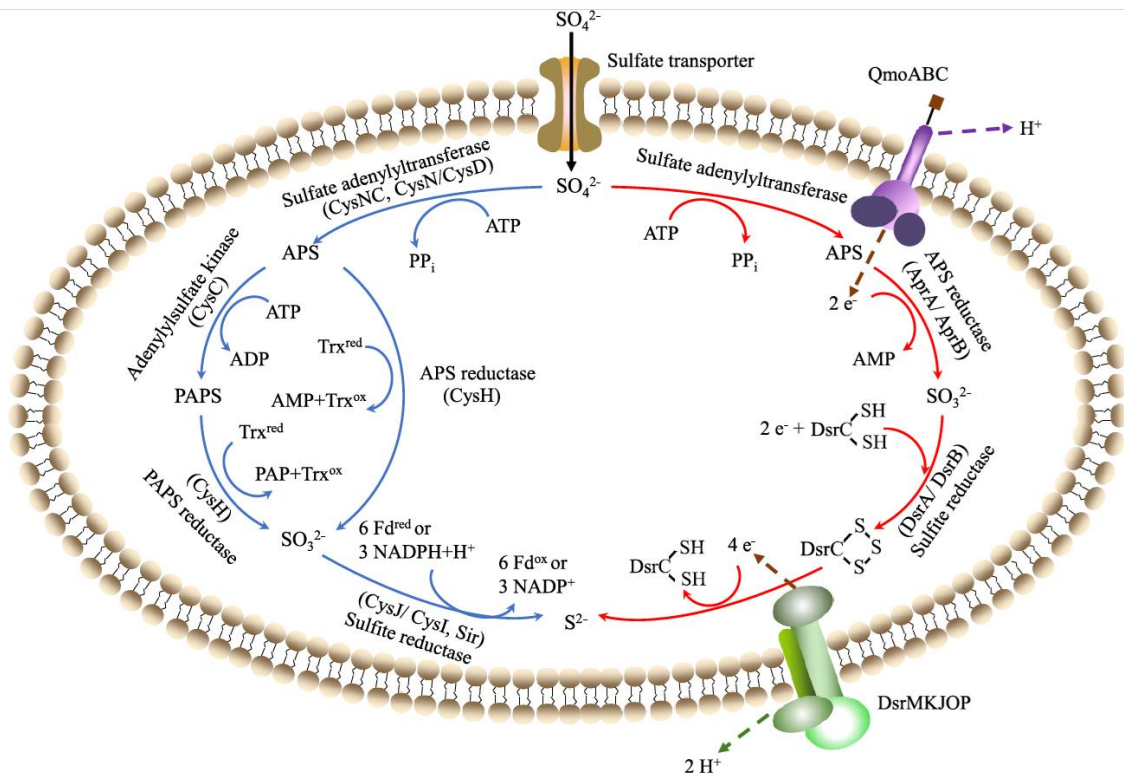


Figure 10. . Assimilation and dissimilatory sulfate reduction pathways. (blue line: assimilated sulfate reduction (ASR); red line: dissimilatory sulfate reduction (DSR)) (Z. Zhang et al., 2022)

For three bacterial phyla and one archaeal phylum, over 120 species and 40 genera of SRB have been identified by (Barton & Hamilton, 2007), Zhang et al., 2022 lists the SRB for 40 species, the majority of which have morphologies that are rod, vibrio, or curved. Further details are provided in (Appendix B)(Z. Zhang et al., 2022). SRB can be classified into three groups based on their inability to further oxidise substrates to carbon dioxide in the absence of acetyl-CoA: those that fully degrade organic compounds to CO₂, those that partially degrade organic compounds to acetate, and those that exhibit both complete and incomplete degradation(Colleran et al., 1995). Some particular SRBs can also employ nitrate, nitrite, iron, and other chemicals as electron acceptors in addition to sulfate, sulfite, thiosulfate, and elemental sulfur(Muyzer & Stams, 2008). The species within the genus *Desulfotomaculum*, such as *Desulfotomaculum solfataricum*, *Desulfotomaculum kuznetsovii*, *Desulfotomaculum australicum*, and *Desulfotomaculum thermoacetoxidans*, are capable of using acetate as an electron donor. However, *Desulfotomaculum luciae*, *Desulfotomaculum thermocisternum*, *Desulfotomaculum geothermicum*, and *Desulfotomaculum thermobenzoicum* subsp. *Thermobenzoicum* lacks the ability to metabolize acetate. Both *Desulfotomaculum* and *Desulfomonile* genera contain species that exhibit both complete and incomplete oxidation, which is attributed to different substrates(T. Hao et al., 2014).

Most SRB reactors use incomplete oxidation as their primary metabolic route since it typically performs better than complete oxidants in terms of substrate utilisation during anaerobic degradation (Muyzer & Stams, 2008). One of the causes for the SRB reactor's inferior effluent is residual acetate from incomplete oxidative metabolism, which can be

found in the bioreactor's effluent. Reports on SRB of full oxidants are scarce, nevertheless. Therefore, in order to improve sulfate reduction technology in the future, it is required to research the SRB of full oxidation(Z. Zhang et al., 2022).

4.1 Sulfide Production

Oxidized sulfur compounds like sulfate, sulfite, and thiosulfate are commonly found in industrial wastewaters and to a lesser extent in municipal wastewaters. These compounds can act as electron acceptors for sulfate-reducing bacteria, which utilize organic compounds in anaerobic reactors and generate hydrogen sulfide H_2S . The heightened toxicity of un-ionized hydrogen sulfide (H_2S) in comparison to its ionized counterpart emphasizes the crucial role of solution pH in determining the overall toxicity of H_2S . Moreover, the toxicity levels are intricately influenced by factors such as the nature of anaerobic biomass (whether granular or dispersed), the specific composition of methanogenic population, and the ratio of Chemical Oxygen Demand (COD) to sulfate (SO_4) in the feed. Elevated levels of COD lead to heightened methane generation, resulting in the dilution of H_2S and its subsequent transfer into the gaseous phase. In aqueous mediums, hydrogen sulfide may manifest as H_2S gas, the bisulfide ion (HS^-), or the sulfide ion (S^{2-}), depending on the prevailing pH conditions, as illustrated in the equilibrium reaction provided (Tchobanoglous et al., 2014).

4.2 Effect Of Process Parameters on Sulfide Production and SRB Activity

The effectiveness of biological treatments and its successful integration into water treatment are determined by SRB metabolism. SRB is able to operate better in complex anaerobic microbial communities when certain environmental factors and operational conditions are fulfilled, including pH, temperature, COD/SO_4^{2-} ratio, electron donor, ORP, HRT, concurrent ions, and syntrophic/competitive bacterial groups(Z. Zhang et al., 2022).

pH

First, the breakdown of cell homeostasis resulting from a pH shift is caused by passive transfer of free acid or alkali across the cell membrane and subsequent destruction within the cell. Furthermore, pH is strongly correlated with the concentration of free acid or base in relation to the equivalent ionic molecule(Sharma et al., 2014). More short-chain fatty acids can be produced by hydrolyzed acidifying bacteria in an alkaline environment, and SRB can use these fatty acids efficiently(J. L. Chen et al., 2014). More protons diffuse across the cell membrane at lower pH values than at neutral pH, which puts diffusion pressure on the membrane. Most of the energy from the redox process is needed to maintain pH homeostasis if the pH gradient between the external and intracellular environments is too great. Various ways for maintaining pH homeostasis comprise active processes like amino acid decarboxylase and proton pump as well as passive ones such changes in lipid composition and positively charged surface protein expression. Due to this procedure, very little redox energy is used for cell development, which is highly harmful to the metabolism of SRB growth(Meier et al., 2012).

Second, different types of chemicals have varied impacts on SBR, and the pH will influence each substance's presence in the solution (Eqs. (4.7) and (4.8)) because pH can alter the chemical equilibrium among the sulfide species, the sulfide (H_2S , HS^- and S^{2-}) species in solutions rely on the pH of the solution (H. Wang et al., 2013).

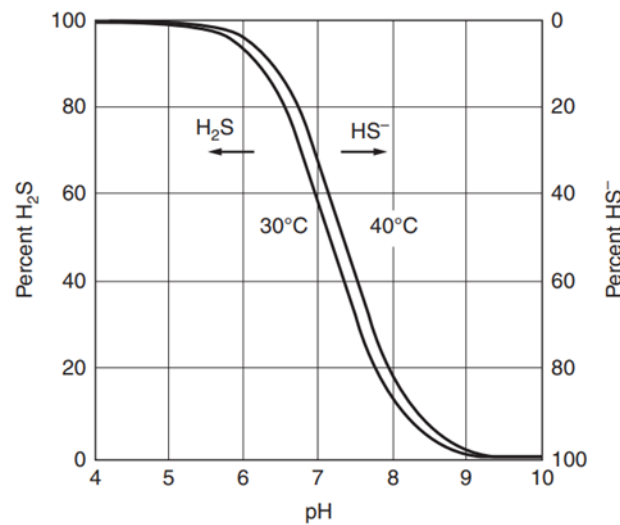
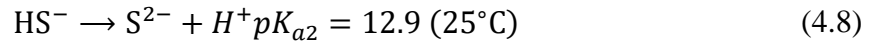
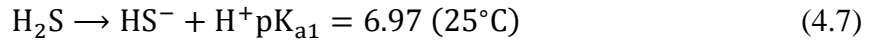


Figure 11. Fraction of hydrogen sulfide in H_2S form is dependent on the pH level (Tchobanoglous et al., 2014).

According to the (Figure 11), The distribution of hydrogen sulfide (H_2S) and bisulfide ions (HS^-) in a solution varies with pH and temperature. In acidic conditions, H_2S predominates, with its percentage higher as the pH decreases. Conversely, as pH becomes more neutral or basic, the proportion of HS^- increases due to its greater stability under less acidic conditions. At pH 7, the intersection point, both H_2S and HS^- are equal in percentage. Temperature influences this equilibrium, with higher temperatures favouring the formation of HS^- over H_2S . For instance, at 30°C , at pH 6, H_2S might be around 80%, decreasing to 20% at pH 8, while HS^- increases proportionately. At 40°C , these percentages would shift slightly towards HS^- at lower pH levels compared to 30°C . While these values are hypothetical, the exact percentages depend on specific conditions and concentrations, typically determined through chemical equilibrium models or direct experimental measurements.

At lower pH levels, approximately 99% of sulfide species exist as H_2S , which poses risks of corrosion and toxicity, particularly as a major component of biogas. Conversely, under neutral to slightly alkaline conditions (pH 7-8), the bisulfide ion (HS^-) becomes predominant, while at highly alkaline conditions (pH > 9), the sulfide ion (S^{2-}) prevails, crucial in industrial processes for forming stable metal sulfides.

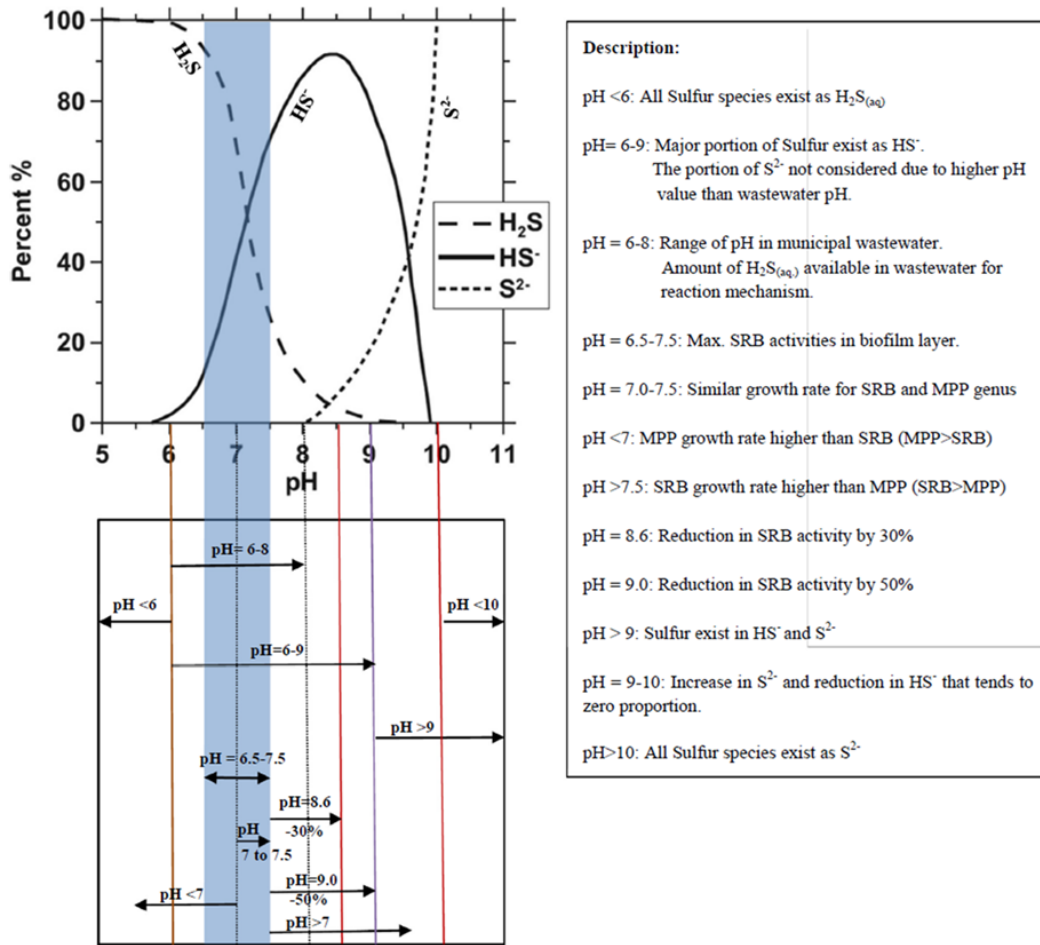


Figure 12. The relationship between pH, temperature, and the emission of sulfide species in wastewater system (Anwar et al., 2022).

A lower pH levels (4.5-5), based on (Figure12), hydrogen sulfide (H_2S), can diffuse through cell membranes, causing denaturation of proteins and cellular disruption in microorganisms, including sulfate-reducing bacteria (SRB). The optimal pH for sulfate-reducing bacteria (SRB) activity, key contributors to biogenic corrosion, is 6.5-7.5, where they produce sulfide by reducing sulfate. Changes in pH levels can lead to shifts in the dominance of SRB and methane-producing prokaryote (MPP) strains in the biofilm layer(Tran et al., 2021).

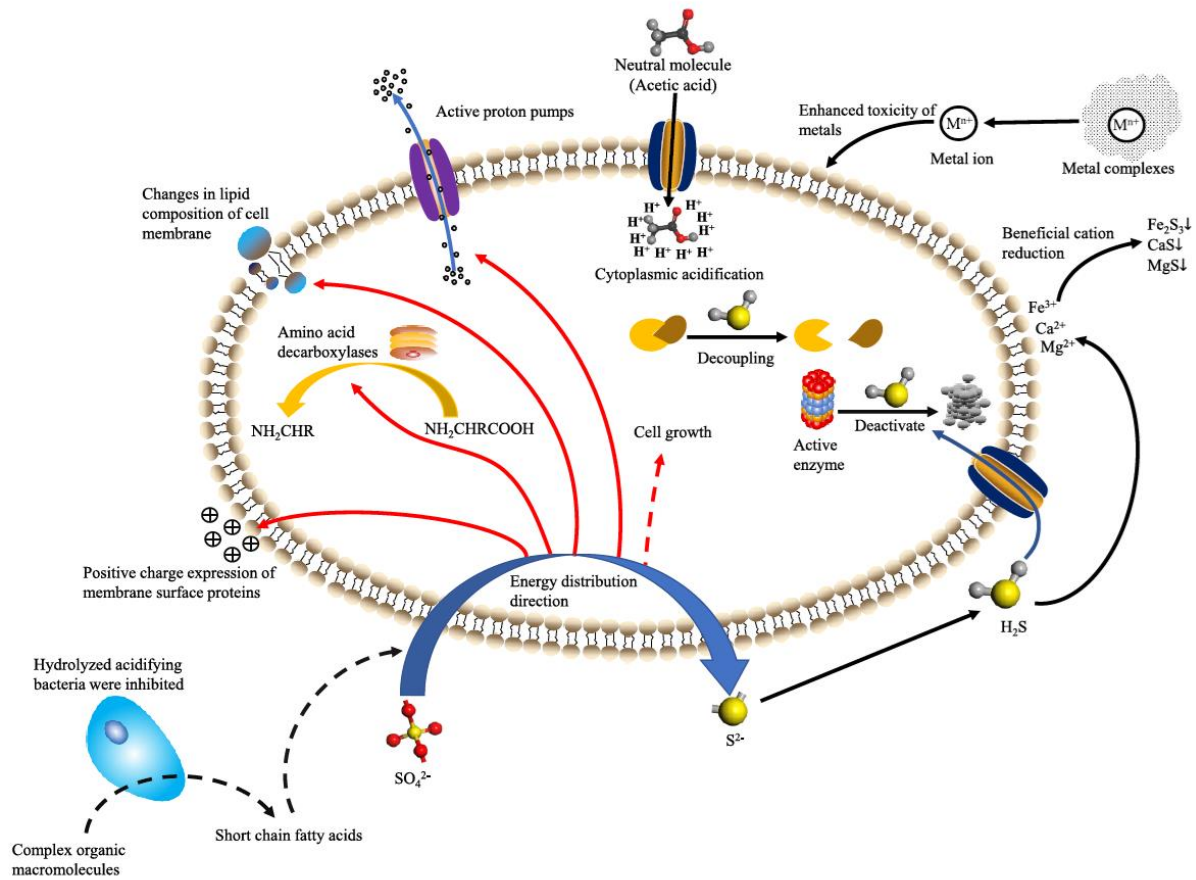


Figure 13. Inhibitory mechanism of low pH on SRB (full line: increasing trend; dashed line: decreasing trend)

Eventually, the inhibitory impact of low pH on sulfate-reducing bacteria (SRB) is multifaceted and intricate (see Fig.13). The influence of pH on SRB can be elucidated from several perspectives. Firstly, pH directly impacts the metabolism of SRB, disrupting cellular homeostasis, disrupting the pH balance inside and outside the cell, ultimately leading to energy loss. Secondly, pH indirectly affects SRB by altering the chemical forms of certain substances in the environment, such as sulfides, heavy metals, and organic acids. Thirdly, pH influences the metabolism of microorganisms that interact or compete with SRB, such as acidogenic bacteria and methanogenic archaea (Z. Zhang et al., 2022).

Temperature

SRBs are capable of thriving across a broad range of temperatures, although the majority prefer moderate temperatures. Psychrophilic SRB exhibit optimal growth between 7-18°C (Knoblauch & Jørgensen, 1999). Moderately temperature-resistant SRB thrive best between 40-60°C (Aüllo et al., 2013). Thermophilic SRB, on the other hand, have an optimal growth temperature ranging from 65-70°C, with those able to grow above 80°C being exclusive to marine hydrothermal environments (Sánchez-Andrea et al., 2013). The ideal temperature range for most sulfate-reducing bacteria to grow is between 28-30°C. Furthermore, temperature affects the solubility of H₂S in wastewater, with higher temperatures resulting in decreased solubility of H₂S. This, in turn, reduces the inhibitory

effect of H₂S on sulfate-reducing bacteria SRB (Kaksonen & Puhakka, 2007a). While moderate temperature increases benefit both SRB and methanogenic archaea (MA), their competitive dynamics vary depending on temperature conditions. Research indicates that SRB outperform MA in wastewater treatment at high temperatures particularly when temperature rises to 55-65° (Omil, Bakker, et al., 1997). Sulfate reduction rates are significantly influenced by temperature, typically increasing by 2 to 3.9-fold with a temperature rise of 10°C. The optimal temperature for most pure cultures of SRB falls within the range of 28 to 32°C. Among SRB, some *Desulfobacterium* strains and a curved *Desulfobacter* strain exhibit lower optima at 24-28°C, while the highest observed temperature is around 70°C for *Thermodesulfobacterium commune*. However, most SRB species experience rapid death at temperatures above 45°C (O. J. Hao et al., 1996a).

Temperature Mechanism

Temperature changes affect the average structural composition of membrane lipids, such as acyl and alkyl. Microorganisms located in remote cold climate regions are particularly interested in removing sulfate and metals using SRB at low temperatures. Virpiranta et al. successfully enriched SRB with high metabolic activity (with reduction rates ranging from 13- 42 mg/L/d) at 6°C using sediment samples from the Arctic in northern Finland (Virpiranta et al., 2019). The primary mechanism through which low temperatures affect SRB metabolism is the stiffening of cell membrane lipids and the subsequent reduction in the activity of membrane proteins. This phenomenon limits the transport capacity of cell membranes for both electron donors and acceptors (Nevatalo et al., 2010a). Temperature influences membrane fluidity by altering lipid order, protein lateral and rotational diffusion, and membrane resistance to shear forces. Although chains, they do not alter the ratio of different lipids (Vinçon-Laugier et al., 2017). Microorganisms adapt to temperature changes by adjusting the properties of fatty acid (FA) composition and phospholipid head groups to maintain sufficient membrane transport capacity (Ernst et al., 2016). One-way microorganisms adapt to temperature variations is by adjusting the carbon chain length of phospholipid fatty acids. Longer carbon chain lengths result in higher melting points and reduced membrane fluidity at higher temperatures, and vice versa (Mykytczuk et al., 2007). Maintaining the fluid state of the cell membrane and the activity of transporters are crucial for ensuring the metabolic activity of microorganisms at low temperatures.

COD/SO₄²⁻

The ratio of chemical oxygen demand to sulfate (COD/SO₄²⁻) directly impacts the proportion of SRB in the microbial community, as SRB can utilize some carbon sources shared by other microorganisms. When the COD/SO₄²⁻ ratio is below 0.67, organic matter can be fully degraded through sulfate reduction, with all electrons flowing to SO₄²⁻. However, when the ratio exceeds 0.67, competition between SRB and other microorganisms for common electron donors intensifies (Dar et al., 2008). Predicting the outcome of this competition becomes more feasible at higher COD/SO₄²⁻ levels. When the ratio falls between 1.7 and 2.7, there is intense competition between SRB and MA. It is generally accepted that excessive sulfate favors the dominance of SRB over methanogens. Under high COD/SO₄²⁻ ratios, it is challenging to prevent SRB from utilizing hydrogen, and acetate methanogens play a significant role in this competition with SRB (Paulo et al., 2015).

The higher the ratio COD/SO₄²⁻, the higher the rate of sulfate removal. The consumption rate of substrate by sulfate-reducing bacteria depends on the concentration of the electron donor

and sulfate, thus affecting the competition between SRB and MA. Mizuno et al. found that when the COD/SO₄²⁻ ratio was 2.0 or higher, MA utilized over 80% of the total electron flow, while SRB accounted for more than 50% of the total electron flow at a COD/SO₄²⁻ ratio of 0.5 (Mizuno et al., 1994). However, O'Reilly and Colleran noted that SRB species could not outcompete MA species for acetate when influent COD/SO₄²⁻ ratios ranged from 2 to 16 (O'Reilly & Colleran, 2006a). These discrepancies may be due to variations in carbon source composition, sulfate concentration, and other environmental factors such as PH, temperature, and oxidation-reduction potential (ORP). Hu et al. demonstrated that at both higher and lower COD/SO₄²⁻ ratios, all SRBs exhibit incomplete oxidation (Hu et al., 2015). Li et al. conducted upflow anaerobic sludge blanket (USAB) treatment of sulfate-containing pharmaceutical wastewater and found that a COD/SO₄²⁻ ratio of 8 resulted in nearly 70% COD reduction with biogas containing 63% methane, while a COD/SO₄²⁻ ratio of 1.5 inhibited methanogenesis (W. Li et al., 2015).

Electron donors

An electron donor, in the context of sulfate-reducing bacteria (SRB) and environmental processes, refers to a substance or compound that can donate electrons to support biological reactions, particularly sulfate reduction. The consumption of electron donors is a significant constraint in the application of sulfate-reducing bacteria (SRB), particularly in scenarios like mine wastewater, where the dissolved organic carbon is below 10 mg/L (Ayangbenro et al., 2018). Hence, it is essential to introduce supplementary electron donors (carbon sources) and maintain the COD/SO₄²⁻ ratio above 0.67 or higher to ensure complete sulfate reduction. The following aspects need to be considered when selecting additional electron donors (Ayangbenro et al., 2018; Cao et al., 2012; Papirio et al., 2013).

1. The ability of sulfate-reducing bacteria (SRB) to utilize extra carbon sources.
2. The residual organic load resulting from incompletely degraded carbon sources.
3. The expense of carbon sources utilized in reducing sulfate to sulfide.
4. The appropriateness of external carbon sources for specific applications.

Autotrophic SRB are capable of growth using carbon monoxide, carbon dioxide, or hydrogen as sole electron donors (Muyzer & Stams, 2008). Houten et al. utilized H₂ and CO₂ as substrates and observed that medium-temperature bioreactors achieved high sulfate reduction rates within a span of 10 days (R. T. Van Houten et al., 1997). Research has demonstrated that using syngas (a mixture of H₂, CO₂, and CO, with small amounts of other components such as methane and nitrogen) as electron donors, a sulfate conversion rate of 15 kg/m³/d was attained after 7 weeks, with an average removal rate of 88% of sulfate (B. H. Van Houten et al., 2006). Syngas proves to be economical with no organic residues in the effluent, and SRB surpasses MA in hydrogen production, making it an ideal electron donor for large-scale applications. However, there are challenges associated with syngas utilization, such as limitations in reaction rate due to H₂ transfer, competition with other organisms, reduced efficiency of hydrogen utilization caused by methane formation, and safety requirements related to hydrogen. Moreover, CO is toxic to SRB within concentrations ranging from 2% to 70% (volume fraction) (Parshina et al., 2010), highlighting the need to investigate SRB's resistance to CO toxicity.

Oxidation Reduction Potential

The oxidation-reduction potential (ORP) reflects the redox properties of the solution and affects SRB metabolism. The sulfate reduction reaction occurs only under suitable ORP conditions. The reduced potential indicates that sulfates' capacity to accept electrons is much lower than that of O₂ and nitric acid (NO³⁻). A negative reduction potential in the system maximizes the reduction effect of sulfate (Liamleam & Annachatre, 2007). Research has shown that SRB can maintain metabolism with ORP below -100 mV, whereas metabolism is inhibited when ORP exceeds -100 mV (M. Y. Wang et al., 2005). In anaerobic treatment, ORP is influenced by dissolved oxygen in the system and some redox substances, such as nitrite (NO₂⁻), nitrate (NO₃⁻), and zero-valent iron (ZVI). ORP accurately reflects trace amounts of dissolved oxygen (DO) in the reactor, with ORP increasing as DO levels rise (Z. Zhang et al., 2021). Although SRB are strictly anaerobic microorganisms, they remain active when exposed to trace amounts of oxygen, mainly because the anaerobic zone inside aggregate particles provides a suitable environment (Van Den Brand et al., 2015). Additionally, increasing DO appropriately benefits sulfide oxidation, thereby reducing sulfide's toxic effects on microorganisms. Khanal's research demonstrated that dissolved and gaseous sulfides were completely eliminated when ORP was increased by 50 and 100 mV (Khanal & Huang, 2003).

Hydraulic Retention Time

The hydraulic retention time (HRT) impacts reactor efficiency by altering microbial activity and reactant composition. The balance between organic matter degradation and sulfate reduction is crucial for stable SRB bioreactor operation, partly determined by HRT, microbial metabolism, and community structure (Cunha et al., 2019; Vasquez et al., 2016). A short HRT may result in incomplete reactions and reduced biomass. Moreover, a short HRT leads to rapid inflow of DO into the anaerobic environment, elevating ORP and disrupting the favorable anaerobic conditions for SRB. In acid mine drainage (AMD), a shorter HRT increases acid and heavy metal concentrations in the reactor, inhibiting SRB due to excess acidity and inadequate metal precipitation. Conversely, a longer HRT may deplete the substrate, leading to excessive microbial growth and mineral precipitation, ultimately reducing hydraulic conductivity and causing system short-circuiting (Aoyagi et al., 2017). Longer HRTs inhibit SRB, increase residual sulfide, and diminish effluent quality. The optimal HRT must be determined based on reactor type and influent characteristics. In packed bed bioreactors, the optimal HRT is 6 hours under neutral PH conditions, increasing to 20 hours under acidic conditions, attributed to microbial adaptation and the production of abundant HCO₃⁻ to neutralize acidity. In UASB and down-flow fluidized bed (DFB) reactors, a low HRT enhances sulfate reduction rates (Piña-Salazar et al., 2011). Furthermore, HRT affects the interaction between SRB and other microorganisms. Longer HRTs increase H₂ production, intensifying competition between SRB and MA. For instance, shorter HRTs reduce the efficiency of hydrolytic acidifying bacteria in degrading macromolecular organics, resulting in fewer degradation products utilized by SRB.

Concomitant Ions (Metal Cations and Oxyanions)

The primary challenge for SRB in practical engineering applications is the inhibition caused by high concentrations of metal cations and oxyanions. It is widely acknowledged that heavy metals have toxic effects on microorganisms in anaerobic systems, including SRB. Since heavy metals are resistant to biodegradation, they tend to exert inhibitory effects (Nguyen et al., 2020; Zhu et al., 2022). Heavy metals bind to thiols and other groups or replace naturally occurring metal cofactors in enzyme prosthetic groups, disrupting enzyme function and structure (J. L. Chen et al., 2014). The toxicity of metals depends on factors such as temperature, PH, carbon source, metal type, concentration, and complexes (Y. Chen et al., 2020; Qian et al., 2015). Additionally, the negative effects of metals on SRB are diverse, including (a) inhibiting SRB growth, (b) reducing SRB metabolism, (c) delaying sulfide production, and (d) causing microbial decay (Papirio et al., 2013). Previous studies have shown that pure cultures of SRB could tolerate 0.3-0.8 mM Cu and 1.5 mM Zn. Furthermore, the half-inhibitory concentration (EC_{50}) for pure culture of SRB for Cu/Zn/Cd/Pb was 0.17/0.25/0.35/0.4 mM, respectively (Shahsavari et al., 2019).

Various oxidation states of elements, such as As(V), As (III), Se (VI), and Se(IV), can cause various toxic effects. Inorganic cations may affect heavy metal toxicity by competing with heavy metals for anionic sites on the cell surface (Kaksonen et al., 2004a). The combined toxic effect of several heavy metals may be higher than the sum of the individual components, attributed to a synergy effect. Many metal oxyanions could inhibit SRB. For example, selenate (SeO_4^{2-}), molybdate (MoO_4^{2-}), and monofluorophosphate are competitive inhibitors. Molybdate ions (MoO_4^{2-}) inhibit sulfate reduction by depleting ATP reserves, while selenate (SeO_4^{2-}), and monofluorophosphate do not affect the reduction of sulfite or thiosulfite. High concentrations of Na^+ and Ca^{2+} inhibit SRB activity, although moderate levels of Na^+ and Ca^{2+} can be beneficial. Studies have demonstrated that in up-flow anaerobic sludge bed (UASB) reactors fed with ethanol and propionate, when the NaCl concentration exceeded 50 g/L, the sulfate reduction rate decreased to 3.7 g/L d (Vallero et al., 2004). Ca^{2+} can indirectly cause toxicity by leading to the precipitation of $CaCO_3$ and $Ca_3(PO_4)_2$, which cover biomass and hinder assimilation. Furthermore, the precipitation of $Ca_3(PO_4)_2$ can result in phosphorus deficiency in cells (Kaksonen & Puhakka, 2007a).

On the other hand, the sensitivity of SRB to heavy metals varies among genera. Some genera, such as *Desulfovibrio desulfuricans* or *Desulfovibrio vulgaris*, can alleviate toxicity by reducing MoO_4^{2-} , SeO_4^{2-}/SeO_3^{2-} , As^{5+} , and Pd^{2+} to MoO_2 , Se^0/Se^{2-} , As^{3+} , and Pd^0 , respectively, thereby reducing metal toxicity (Paulo et al., 2015). Sulfides produced by SRB are beneficial for heavy metal detoxification because they react with heavy metals to form metal sulfides (MeS), which precipitate rapidly, reducing sulfide content and alleviating metal toxicity (Y.-N. Xu & Chen, 2020).

Syntrophic/Competitive Groups of Bacteria

In a syntrophic relationship in a chemical reaction, two or more organisms work together cooperatively to degrade complex organic compounds into simpler substances. This relationship is characterized by the mutual dependence of the organisms involved, where the metabolic activities of one organism depend on the byproducts produced by another organism. In other words, one organism carries out a metabolic step that produces compounds needed by another organism for its own metabolism, and vice versa. This interdependence allows the organisms to achieve reactions that they cannot accomplish

individually, making syntrophic relationships crucial in various environmental processes, such as anaerobic degradation of organic matter.

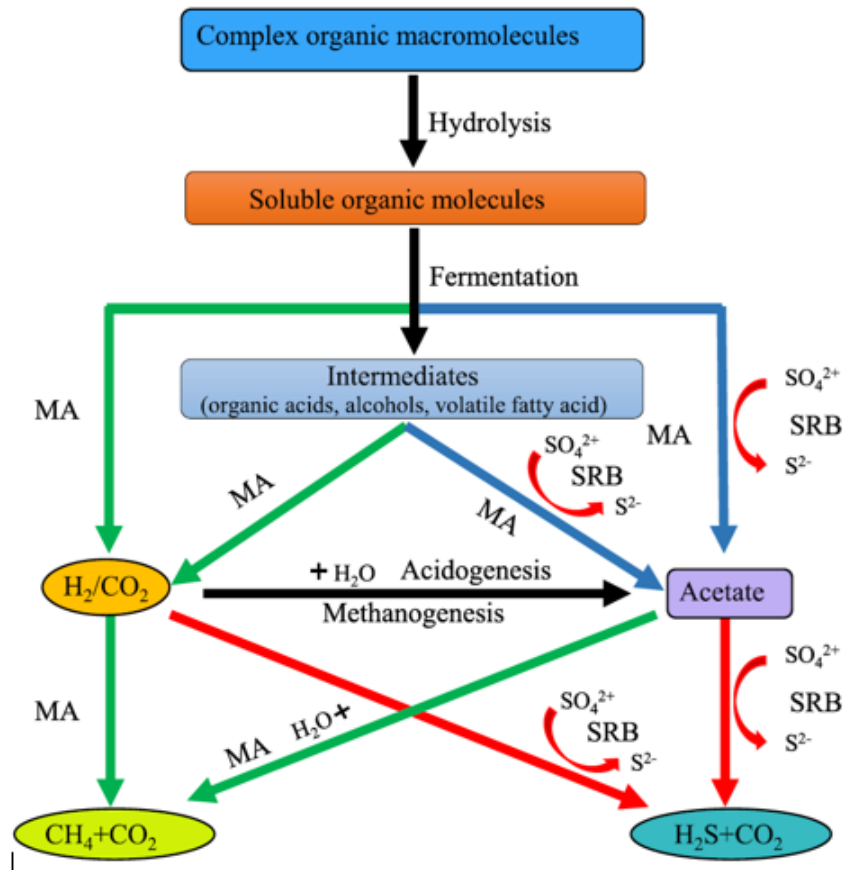
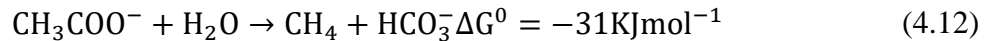


Figure 14. Conceptual diagram of sulfate reduction and other biological anaerobic processes. (Red line: SRB; Green line: MA; Blue line: SRB and MA) (Z. Zhang et al., 2022)

In intricate sulfate reduction systems, the primary biological transformations include sulfate reduction, acetogenesis, methanogenesis, hydrolysis, and fermentation. A schematic representation of sulfate reduction and other anaerobic processes is depicted in (Fig. 14). These diverse microbial communities possess complementary roles in their niches, enhancing the reactor's stability (T. Hao et al., 2014). However, concerning SRB metabolism, the impact of these various bacterial groups varies, broadly classified as either syntrophic or competitive relationships.

In anaerobic environments, the competition between SRB and methanogenic archaea (MA) is significant (Hidalgo-Ulloa et al., 2020). When sulfate enters the methanogenic zone, there are notable changes in the anaerobic microbial community. In low redox potential environments, SRB competes with other anaerobic bacteria, including MA, for common substrates. SRB has an advantage in competing with MA for electron acceptors like hydrogen, formate, and acetate, as well as for electron donors such as propionate and butyrate. For instance,

comparing the Gibbs free energy (See Appendix C) changes for the conversion of H₂ and acetate, sulfide formation from acetate has a much higher Gibbs free energy value than methane formation as it showed in Eqs 8 to 11.



Therefore, the H₂ and acetate conversion to sulfate reduction has a thermodynamic advantage over methane generation. In addition to their superior thermodynamics, SRB have a higher affinity for H₂ than MA, making them more competitive in environments with excessive sulfate (Paulo et al., 2015). Consequently, in anaerobic systems, organic carbon sources are more efficiently utilized by SRB, without being disrupted by external conditions. Interestingly, SRB can also thrive without sulfate by forming syntrophic associations with methanogens or other hydrogen-scavenging organisms (Plugge et al., 2011).

When dealing with complex macromolecular compounds as substrates, SRB depend on hydrolytic and fermentative bacteria to break down these complex organics into simpler electron donors, as SRB lack the ability to directly degrade complex organics (Mulopo et al., 2011). The degradability of cellulose plays a crucial role in its performance as a substrate for reactors. The main genera of bacteria that utilize cellulose as a substrate include Bacteroides, Clostridium, Acetivibrio, Spirochaeta, Ruminococcus, and Cellulomonas. Acetogenic bacteria convert CO₂ into acetate via the acetyl-CoA pathway, providing acetate as an electron donor for sulfate reduction (Sánchez-Andrea et al., 2014). Acetogens found in reactors treating acid mine drainage (AMD) include Acetobacterium and Clostridium, as well as other acetogenic bacteria like Ruminococcus or Treponema (Hiibel et al., 2011; Sánchez-Andrea et al., 2012). Additionally, Oxobacter and other acetogens have been detected in acid sulfide enrichment (Sánchez-Andrea et al., 2013). Acetogens and heterotrophic SRB exhibit synergistic behavior, but whether they compete with autotrophic SRB for CO₂ remains unreported and requires further investigation.

The interaction between SRB and other microorganisms during sulfate reduction is highly intricate and influenced by microenvironmental factors, process parameters, and products like H₂S (Lu et al., 2016). Enhancing the performance of bioreactors for sulfate reduction necessitates considering the entire microbial community, not just the SRB.

Table 1. Common factors affecting the efficiency and performance of sulfate reducing bioprocesses in reactors summarized in (T. Hao et al., 2014)

Table 1 - Common factors affecting the performance of sulfate reducing reactors.			
Factor	Effects	Conditions Preferred	reference
Sludge type (flocs/ biofilm/ aggregate)	Biomass concentration; Reaction rates, local pH, temperature, toxicity resistance etc.	SRB granular sludge/biofilm	(T. Hao, Lu, et al., 2013)
SRB species	Organics oxidations complete to CO ₂ or acetate (effluent COD concentration); Growth rate; aggregation ability	Organics complete oxidizers SRB species	(Omil, Oude Elferink, et al., 1997) (Z. Zhang et al., 2022)
Syntrophic structure	Enhance sulfate reduction rate	SRB collaborates with other organisms to acquire electron or energy source	(T. Hao, Wei, et al., 2013) (Mulopo et al., 2011)
Competition model	Compete with methanogens, acidogenic/hydrogenotrophic bacteria and acetoclastics for the available substrates	SRB predominate the microbial community with syntrophic bacteria	(Muthumbi et al., 2001) (Koschorreck et al., 2004) (O'Reilly & Colleran, 2006b)
Influent components Sulfate concentration	Affects SRB growth and activity; May be out-competed at low concentration; High concentrations inhibit SRB activity	Typical COD/SO ₄ ²⁻ values range between 0.7 and 1.5 depending on the carbon source	(O. J. Hao et al., 1996b) (Rzeczycka et al., 2010)
Trace element	Fe, Cu, Zn, Co, Mo, Ni are needed in electron transport, redox-active metalloenzymes and composition of some protein and enzymes: High Mo level inhibits SRB metabolism	High levels of Fe in culture media to compensate for that precipitated by sulfide. Mo above 2 mM completely inhibits SRB	(Bridge et al., 1999) (Biswas et al., 2009) (Postgate, 1984)
Metal concentration Not mostly present in domestic sewage	Elevated heavy metal concentration can reduce or terminate SRB activity	Desired concentration and the order of decreasing toxicity. (mg/L) Cu < 4, Cd < 11, Ni < 13, Zn < 16.5, Cr < 35, Pb < 80	(Kaksonen & Puhakka, 2007b) (Utgar et al., 2001) (Naz et al., 2005)
Nitrate concentration Nitrite is a strong inhibitor in growth and activity of SRB	Nitrite is a strong inhibitor in growth and activity of SRB	The impact level: 70 mM NO ₃ ⁻ inhibits growth significantly; Long term 0.25_0.33 mM injection inhibits the number and activity	(L. Zhang et al., 2008b) (He et al., 2010) (Bødtker et al., 2008)
pH	Effect the growth and activity; Influence the SRB species diversity and out-compete with methanogens. Effects dissolved sulfide quantity	pH range for SRB:5.5-10	(Aerts, 2009) (Gormly, 2005)
Salinity	Influence the species of SRB present; Generally, sulfate reducing rate is inversely correlated with salinity	Optimum salinity range 6-12%.	(Kerkar & Loka Bharathi, 2007) (Srensen et al., 2004)

4 Biological Processes involved in Sulfide Production and MIC

Operation Conditions Substrate/Sulfate	Effect growth and activity and microbial diversity; Proper C/S ration favors SRB out-compete with other organisms	Optimal COD/SO ₄ ²⁻ ratio for COD removal is 0.6-1.2; for sulfate removal is 2.4-4.8	(Rzeczycka et al., 2010)
Oxidation reduction potential (ORP)	Effect the competition between SRB and other organisms i.e. mathanogens. Effect the performance of SRB	Suitable ORP for SRB is -50 to -300 mV; Optimal ORP readings of -270 mV using standard hydrogen probe.	(Kamlage, 1996) (Khanal & Huang, 2006)
Temperature	Control the activity and growth; Initial cultivation temperature. effects SRB diversity; Lower H ₂ S solubility at high temperature	SRB tolerate temperatures between -5 and 75 °C. Optimum temperature for most SRB ranges 28-32 °C	(Nevatalo et al., 2010b) (Mara & Horan, 2003)
Sludge retention time (SRT)	Effect the reactor's performance and sludge production; Effect the competition between SRB and methanogens/homoacetogenic bacteria	Elevated SRTs delay the outcompetition of methanogens, and methanogens could be rapidly removed by applying a low SRT	(Esposito et al., 2003) (Weijma et al., 2002)
Hydraulic retention time (HRT)	Influence SRB activity; Biomass concentration; Competition with other organisms	Overall optimum HRT of 20-30 h for SRB activity	(Sipma et al., 2007) (Polo et al., 2006)
H ₂ S concentration	High H ₂ S direct and reversible toxicity effect on SRB, and inhibit the activity	Nitrogen purging: Decrease the activity when H ₂ S is higher. than 60-70 mg/l	(Reis et al., 1992) (Kaksonen et al., 2004b) (Jin, 2010)
Mixing condition	Mixing frequency significantly impacts SRB activity; Effect the SRB distribution and detachment and hydraulic loss of biomass		(Gantzer & Stefan, 2003)

5 Quantification of Sulfide Production in Sewer System

The quantification of sulfide production is a critical aspect of understanding and managing wastewater treatment processes, particularly in systems prone to sulfide build-up. Sulfide accumulation can lead to severe odour issues, toxic environments, and infrastructure corrosion. Accurate measurement and modelling of sulfide production are essential for developing effective control strategies.

The study by L. Sutherland-Stacey et al. describes the development and validation of a continuous monitoring method for dissolved sulfide in sewer systems using an in-situ UV-VIS spectrometer and a pH probe, similar to the work of Guenther et al. (2001) on bisulfide ion detection. Hydrogen sulfide, generated under anaerobic conditions in wastewater, causes infrastructure corrosion, health risks, and foul odors. The spectrometer identifies bisulfide ion peaks around 230 nm, and calibration involves curve fitting and Partial Least Squares algorithms. Field validation at sites in Gold Coast, Australia, showed accuracy with a prediction error under ± 1.2 mg/L. In addition to UV-VIS spectroscopy, the method uses ion chromatography (Keller-Lehmann et al., 2006) for calibration, where sodium sulfide standards are prepared and analyzed to validate the spectroscopic measurements. This continuous monitoring technique provides real-time data and can be integrated into existing wastewater systems, offering improvements in speed, safety, and cost-effectiveness over traditional sampling methods (Sutherland-Stacey et al., 2008).

In the study "Sulfide and methane production in sewer sediments," sulfide production was quantified using batch tests, microsensor measurements, and pore water analysis (Liu, Ni, et al., 2015). In batch tests, the reactor was filled with fresh sewage, and liquid samples were taken at intervals to measure dissolved sulfide concentrations using ion chromatography, with production rates calculated via linear regression. Microsensors provided high-resolution profiles by recording H₂S concentrations at fine depth intervals within the sediment. Additionally, pore water samples were extracted from various depths, preserved to prevent oxidation, and analyzed for sulfide species. These methods together enabled detailed quantification of H₂S production rates and their spatial distribution within the sediment layers (Y. Liu, Ni, et al., 2015).

In parallel study, Liu et al. 2015 used an advanced online measuring device for monitoring of dissolved methane and total dissolved sulfide concentrations in sewers (Y. Liu, Sharma, et al., 2015). This device uses a gas-phase sensor to measure methane concentrations under equilibrium conditions after being stripped from the sewage. For sulfide measurements, the device employs a similar principle, capturing sulfide in the liquid phase and converting it into a detectable form. The device is equipped with sensors capable of continuously measuring dissolved sulfide levels directly in the sewer environment. These measurements are taken by the online device, which maintains equilibrium conditions to ensure accuracy. The sulfide is stripped from the sewage, and its concentration is determined using specialized sensors that provide real-time data. This continuous monitoring approach offers several advantages over traditional methods. It allows for the immediate detection of fluctuations in sulfide concentrations, which is critical for managing odour and corrosion in sewer systems.

Additionally, the online system reduces the need for frequent manual sampling and laboratory analysis, saving time and resources. The data collected from the online measurements help in identifying trends and variations in sulfide levels, enabling prompt and effective responses to potential problems in the sewer network. Overall, this method provides a reliable and efficient solution for monitoring dissolved methane and sulfide concentrations in sewers, enhancing the ability to manage and mitigate associated risks (Y. Liu, Sharma, et al., 2015).

The study (Y. Liu, Ni, et al., 2015) focuses on quantifying sulfide production in sewer sediments using discrete sampling methods and laboratory analysis, while the study by (Y. Liu, Sharma, et al., 2015), describes the use of an advanced online device for continuous monitoring of sulfide and methane in sewers. The continuous monitoring device provides real-time data, which is advantageous for managing immediate fluctuations in sulfide concentrations.

In Table 2, the sulfide production rates from different studies have been summarized in to provide a clear and detailed overview of the results from various conditions and research methodologies.

Table 2. Different quantification methods of sulfide production

Quantification Method	Scale of Test	Condition	Sulfide Production Rate	Units	Types of sulfide form	Reference
Batch Tests	Laboratory Reactor	Every 2-3 weeks after 300 days of reactor operation	9.20 ± 0.39	g S/m ² ·d	Total Dissolved Sulfide	(Y. Liu, Ni, et al., 2015)
Online Monitoring	Field Scale	Rising main sewer network during summer and winter	0.75 to 1.4 mg/L	mg/L	Total Dissolved Sulfide	(Y. Liu, Sharma, et al., 2015)
Empirical Modelling	Model Simulation	Various hydraulic conditions	0.3 to 70 mgS/m ² ·h	mgS/m ² ·h	Total Dissolved Sulfide	(Carrera et al., 2016)
Field Measurements	Full Scale	Two stretches of an intercepting sewer system with intermittent flow conditions in a coastal village, Portugal	Dissolved sulfide concentrations around 12 mg S/L; H ₂ S gas concentrations above 250 ppm upstream, below 40 ppm downstream	mg S/L (dissolved), ppm (gas)	Total Dissolved Sulfide, Gaseous Hydrogen Sulfide	(Matias et al., 2017)
Empirical Modelling	Full Scale	Different seasons and operating conditions in a sludge rising main	2.30 to 3.24 g S ² /m ² ·d	g S/m ² ·d	Total Dissolved Sulfide	(Matos et al., 2018)
Batch Test	Lab Scale	Anaerobic, Marine Sediments	5-50 mg/L (varies with experimental setup)	mg/L	Total Dissolved Sulfide	(Moura et al., 2019)
Batch Fermentation	Lab Scale	Cider fermentation with varying YAN	UCD522 strain: Low (123.75)	µg/100 ml	Gaseous Hydrogen	(Y. Song et al., 2020)

5 Quantification of Sulfide Production in Sewer System

		levels (Low: 86 mg/L, Intermediate: 208 mg/L, High: 433 mg/L)	$\mu\text{g}/100\text{ ml}$, Intermediate (288.25 $\mu\text{g}/100\text{ ml}$), High (44.125 $\mu\text{g}/100\text{ ml}$)		Sulfide	
Dynamic Quantification	Field Scale	Spatially heterogeneous sediments in gravity sewers	0 to 4.5 g S/m ² ·d	g S/m ² ·d	Total Dissolved Sulfide	(Zuo et al., 2021)
Empirical Modelling	Model Simulation	Various hydraulic conditions and sewer factors	0.3 to 70 mgS/m ² ·h	mgS/m ² ·h	Total Dissolved Sulfide	(Zuo et al., 2021)
Physical Twin	Pilot Scale	Controlled introduction of ferrous iron to reduce sulfide concentration	0.8 mg/L throughout the system	mg/L	Total Dissolved Sulfide	(Kim et al., 2024)

6 Human Exposure to H₂S In Different Treatment Plants (I.E., Centralized Or Decentralized), Seasons (Temperature) and Geographical Location.

Hydrogen sulfide (H₂S) is crucial in cellular physiology and pathology, acting as a key gaseous mediator in various biological processes. Recent studies emphasize its roles in multiple body systems. Understanding H₂S chemistry and properties is vital. Challenges include selecting appropriate chemicals for generating H₂S in vitro and in vivo and accurately measuring sulfide levels in biological fluids. The term "sulfide" covers total sulfide, while H₂S, SH⁻, and S²⁻ are used as needed. It's essential to recognize the limitations and potential errors in these processes (L. Li & Moore, 2008; Pryor et al., 2006; Wallace, 2007).

Hydrogen sulfide (H₂S) is extremely toxic because it binds to cytochrome c oxidase in mitochondria, disrupting cellular respiration. It also affects the olfactory nerves, eyes, and brain. Being more toxic than hydrogen cyanide, H₂S requires careful handling. When preparing and using H₂S solutions, it is essential to work in well-ventilated fume hoods to prevent accumulation, especially since H₂S is denser than air and can collect in low, unventilated areas. Exposure to H₂S at around 50 ppm can cause a loss of the ability to smell it, and concentrations over 100 ppm can lead to a loss of smell within minutes. Exposure to 500 ppm for 30 minutes can be lethal. It is unsafe to rely on the sense of smell to detect H₂S. In laboratory settings, H₂S must be trapped and neutralized with appropriate chemicals, and air quality must be continuously monitored to avoid toxic exposure (Hughes et al., 2009).

Emphasizing the effects of hydrogen sulfide (H₂S), a potent gas with a distinct rotten egg odour, the study in Curitiba, Brazil, monitored hydrogen sulfide (H₂S) levels at two wastewater treatment plants (WWTPs) - WWTP "A" and WWTP "B" - using passive samplers over eight campaigns from August 2013 to March 2014. H₂S, the primary pollutant causing nuisance odours from degradation processes, was measured at specific points (A1-A13 for WWTP "A" and B1-B5 for WWTP "B") and analysed using a spectrophotometer at 665 nm after desorption with ultrapure water and a ferric chloride-amine solution. Mean H₂S concentrations ranged from 0.14 to 32 µg m⁻³. The study also involved three cohorts: residents living and working near the WWTPs, residents living near but working/studying away, and individuals working near but living far from the WWTPs. Five campaigns were designed to capture temporal and seasonal variations in H₂S concentrations at the sampling points (Godoi et al., 2018).

6 Human Exposure to H₂S In Different Treatment Plants (I.E., Centralized Or Decentralized), Seasons (Temperature) and Geographical Location.

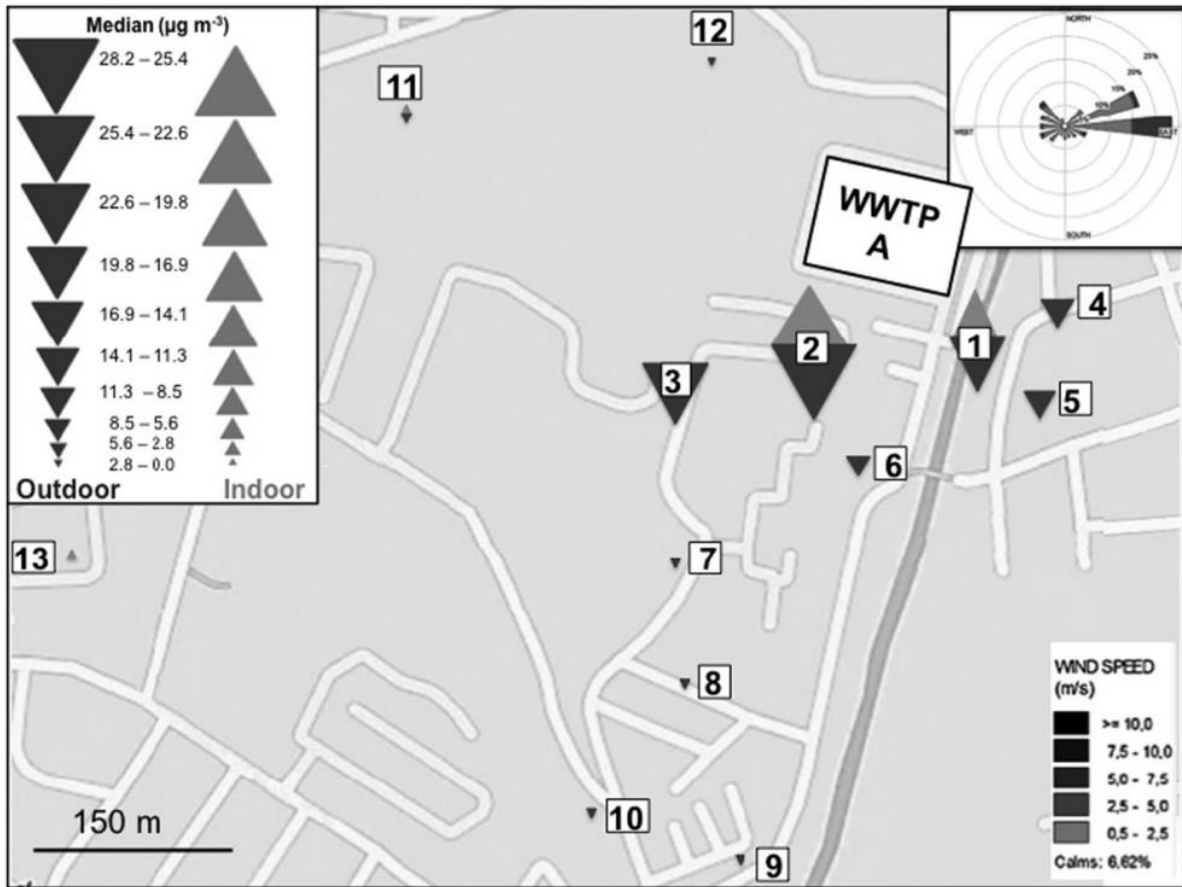


Figure 15. A total of 13 sampling points near WWTP "A" for the assessment of hydrogen sulfide (H₂S) concentrations (Godoi et al., 2018).

In WWTP "A," the study assessed hydrogen sulfide (H₂S) concentrations both indoors and outdoors to understand the pollution distribution around the wastewater treatment plant (Figure 15). Indoor measurements focused on H₂S levels inside buildings or structures near WWTP "A," evaluating air quality for residents and workers. Outdoor monitoring involved tracking H₂S concentrations in the ambient air around the plant, analysing the extent of H₂S emissions and their potential environmental and community impact.

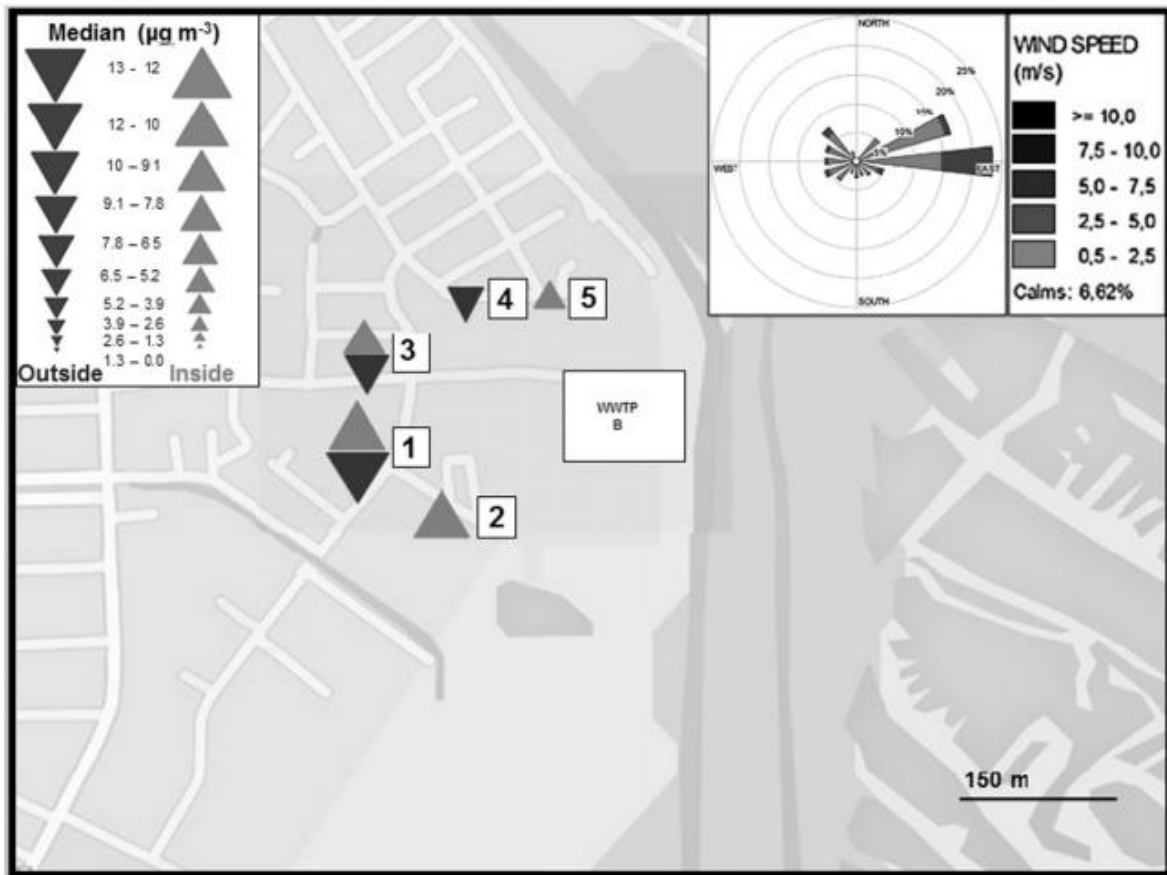


Figure 16. Assessment of Indoor and Outdoor Hydrogen Sulfide (H₂S) Concentrations near WWTP 'B': Understanding Pollution Levels and Health Risks in the Surrounding Community (Godoi et al., 2018).

The assessment of hydrogen sulfide (H₂S) levels near WWTP "B" (Figure 16) was conducted to analyse the dispersion of pollution, assess potential health hazards, and investigate the environmental consequences linked to H₂S exposure in the proximity of the wastewater treatment facility.

The study on hydrogen sulfide (H₂S) concentrations at two wastewater treatment plants (WWTPs) in Curitiba, Brazil, found significant variations based on location, season, campaign, and cohort. At WWTP "A," H₂S levels were highest near the plant (A1-A3), intermediate at mid-range sites (A4, A5), and lowest at distant sites (A7-A13). For more detailed information, please refer to (Appendix D), which contains graphs and data related to seasonal variations and location of specific H₂S concentrations. At WWTP "B," similar distance-related patterns were observed, with higher risks for cohorts 1 and 2, particularly downwind at B1 and B₂ for cohort 3. Seasonal analysis revealed H₂S concentrations were 3.7 times higher in summer than in winter, though some winter points had higher levels. Campaign analysis showed unexpected decreases in H₂S levels in campaigns 7 and 8 at WWTP "B," suggesting microenvironmental influences. Health effects from H₂S exposure included acute symptoms like eye irritation, olfactory paralysis, respiratory irritation, pulmonary edema, convulsions, and death, as well as chronic issues like nausea, headaches,

and respiratory problems. Wind direction significantly affected H₂S distribution, with downwind areas showing the highest concentrations, highlighting the need for effective monitoring and control to protect public health(Godoi et al., 2018).

Researchers reviewed human studies on Hydrogen Sulfide from 2006 to 2020, examining expert reports, referenced studies, and conducting searches in PubMed and Embase. They focused on health outcomes associated with Hydrogen Sulfide exposure in various settings from 404 citations. Additionally, studies on occupational exposures were analysed, including one on wastewater treatment plant workers in Iowa. This study compared health effects of wastewater workers to unexposed water treatment plant workers. Tasks like sludge handling and plant inspection were associated with health symptoms in the study which encompasses respiratory, ocular, and dermal irritation, neurological complications, as well as gastrointestinal symptoms. While the paper does not specifically address exposure limits for Hydrogen Sulfide (H₂S) in wastewater treatment plants (WWTPs), it discusses general occupational exposure limits for H₂S as recommended by various expert groups. The European SCOEL report cited in the paper recommends exposure limits of 10 ppm for a short-term 15-minute average and 5 ppm for an 8-hour time-weighted average. These limits are intended to protect workers in various industries, including WWTPs, where H₂S exposure may occur. It is crucial for workers and employers in WWTP facilities to follow these recommended limits and implement appropriate safety measures to mitigate health risks associated with H₂S exposure(Elwood, 2021).

The research paper by Muzaini et al. examines the impact of exposure to PM (Particulate Matter) 2.5 and H₂S on work-related respiratory symptoms among sewage workers in Malaysia. The study found that chronic cough was the most common symptom, followed by chronic phlegm, shortness of breath, and chest tightness. Significant determinants of respiratory symptoms included shift work, working at a sludge treatment facility, longer work duration, and individual cumulative exposure to PM 2.5 and H₂S. Sewage workers at treatment facilities had higher symptom prevalence and exposure levels compared to office workers. The developed model explained 47.8% of symptom variance and emphasized the need to address occupational respiratory hazards to safeguard sewage workers' respiratory health(Muzaini et al., 2022).

6.1 Seasonal And Geographical Aspects of H₂S Production

The study discusses the impact of temperature on the production and release of hydrogen sulfide (H₂S) in urban sewage treatment plants. The sewage temperature is positively correlated with the H₂S discharge concentration in the inlet pump house, grille, and aeration grit tank. This indicates that higher temperatures can lead to increased H₂S emissions in these units. Furthermore, the change in H₂S emissions during different seasons, with lower concentrations in winter and higher concentrations in summer, is influenced by temperature variations(Z. Liu, 2021).

Geographical factors are crucial in odour dispersion modeling at sewage treatment works. The article "Hydrogen sulphide dispersion modelling – urban and rural case studies" by S.A. Parsons et al. highlights the importance of defining surrounding land use accurately for

predicting odour dispersion. Distinguishing between urban and rural land use types is essential for selecting suitable dispersion coefficients. Auer's meteorological land use typing scheme is referenced as a valuable method for classifying land use within a specified radius. The article also discusses a population density procedure to determine which dispersion coefficients to use. Population density threshold guides the selection of dispersion coefficients based on geographical features. The study shows that urban or rural land use significantly affects predicted odorous compound concentrations. Considering geographical factors like land use, meteorological conditions, and population density is crucial in odour dispersion modeling. Evaluation of these aspects is vital for accurate predictions and effective odour control strategies in wastewater treatment facilities(Parsons et al., 2000).

In the study "Monitoring risks in association with exposure levels among wastewater treatment plant workers" Austigard et al., 2018, the authors investigated how geographical factors impact hydrogen sulphide (H₂S) exposure levels among wastewater treatment workers. The research focused on variations in exposure due to facility location, job tasks, seasonal changes, and flushing practices. Analyzing data from urban and rural areas, the study developed an exposure index considering peak height, duration, and number of peaks. Results showed significant geographical variations: urban areas had different H₂S exposure levels than rural areas due to industrial activities and population density. Job tasks like sludge handling and tank maintenance had higher exposure, especially during warmer months due to increased microbial activity. Extensive flushing reduced H₂S levels, while inadequate flushing led to higher concentrations. Specifically, City 1 workers had a moderate H₂S index of 180 in summer, City 2 workers had a high index of 230 in winter with extensive flushing, Rural Area 1 showed a low index of 90, and Rural Area 2 had a higher index of 150 due to less frequent flushing. The study underscores the need to consider geographical factors to mitigate H₂S exposure risks effectively(Austigard et al., 2018).

7 Removal Process of Hydrogen Sulfide

Hydrogen sulfide (H₂S) is a hazardous and corrosive gas commonly detected in biogas, typically ranging from 100 to 10,000 parts per million (ppm). The generation of H₂S in biogas predominantly stems from the actions of reducing microorganisms thriving in anaerobic conditions, such as those within biodigesters. These microorganisms play a key role in reducing sulfur-containing proteins, resulting in the production of H₂S. Furthermore, the transformation of inorganic sulfur compounds, like sulfates, can also contribute significantly to the H₂S levels in biogas. External factors such as dietary protein intake can impact the discharge of sulfur-containing compounds, potentially elevating the H₂S production levels in anaerobic settings. Due to the severe toxicity and corrosiveness of H₂S, it is imperative to deploy efficient removal techniques to mitigate the associated risks in biogas and uphold the safety and effectiveness of biogas utilization processes (Fonseca-Bermúdez et al., 2023). Based on the review by Pardon Nyamukamba et al., the detailed description of each process presented as following (Nyamukamba et al., 2022).

Adsorption

Involves the use of materials such as zeolites modified with metals or metal oxides to physically or chemically bind H₂S molecules, providing a high sulfur loading capacity and good regenerability. The adsorbents must possess a stable structure to ensure effective H₂S removal in biogas purification processes (Ozekmekci et al., 2015).

Absorption

Entails passing biogas through alkaline solutions like sodium hydroxide or calcium oxide for H₂S removal. Chemical absorption methods, such as employing iron-chelated solutions catalyzed by Fe/EDTA, have demonstrated high efficiency in H₂S removal and selective elimination of H₂S. This process converts H₂S to elemental sulfur, offering benefits such as low chemical usage and regenerable pseudo-catalyst properties (Horikawa et al., 2004).

Biological Treatment

Various biological methods are used for removing H₂S from biogas in wastewater treatment plants. Biofilters use microorganisms on a fixed bed to degrade H₂S as the gas passes through the biofilm formed on materials like peat, compost, and wood bark. Biotrickling filters involve gas flowing through a fixed-bed irrigated with a nutrient-rich aqueous solution, where microorganisms grow as a biofilm and degrade H₂S. Bioscrubbers consist of an absorption tower and a bioreactor; H₂S is absorbed into a recirculated liquid and then biodegraded by microorganisms. In activated sludge systems, microorganisms oxidize H₂S under aerobic conditions in aerated biological reactors, offering high removal efficiency (Barbusiński & Kalemba, 2016).

Chemical Scrubbing

Chemical scrubbers are widely used for the removal of hydrogen sulfide (H₂S) from biogas due to their high efficiency and reliability. The process involves the absorption of H₂S into a scrubbing liquid, typically an oxidizing agent like sodium hypochlorite (NaOCl), hydrogen

peroxide (H₂O₂), or potassium permanganate (KMnO₄). The performance of the scrubber depends on several factors, including the gas flow rate, the empty bed residence time (EBRT), and the scrubbing liquid flow rate. Increasing the air flow rate can decrease the H₂S removal efficiency, particularly with oxidants like H₂O₂. Optimal conditions for H₂S removal typically achieve efficiencies above 95%, with NaOCl and KMnO₄ showing the best results. However, chemical scrubbers are associated with high operational costs due to the consumption of chemicals and energy, making them a costlier option compared to some biological methods (Alinezhad et al., 2019).

Hollow Fiber Membrane Contactor (HFMC)

A study explored the use of hollow fiber membrane contactors (HFMC) with hydrophobic polypropylene fibers to effectively remove over 98% of dissolved hydrogen sulfide (H₂S) from synthetic wastewater. The experimental setup involved a lab-scale system using aqueous monoethanolamine (MEA) as the extractant, achieving high removal efficiency across various H₂S concentrations and flow rates. A mathematical model was developed to elucidate the mass transfer mechanism, incorporating both pore diffusion and adsorption/desorption on the membrane walls. The model's predictions closely matched the experimental results, demonstrating HFMC's potential as an efficient and environmentally friendly solution for H₂S removal from wastewater (Agrahari et al., 2013).

However, another study summarises different types of process with advantages and their disadvantages in a (table 3) (Ghimire et al., 2021) .

Table 3. Advantages and disadvantages of techniques for removal of H₂S ((Angelidaki et al., 2018; Awe et al., 2017; Muñoz et al., 2015; Ryckebosch et al., 2011; Sun et al., 2015)

H ₂ S removal process		Advantages and features	Disadvantages
Biological	In situ microaeration	No separate reactors needed for H ₂ S removal, treatment cost of 0.0037 (pure oxygen) and h0.0026 m ³ (air) of biogas treated (Khoshnevisan et al., 2017)	May lead to deposition of elemental sulfur on the reactor system, control of air/O ₂ dose or O ₂ /H ₂ S is challenging, needs additional upgrading steps, requires careful designing of reactor. headspace
	Biological air filtration (BF, BS, BTF)	Operation at ambient temperature and pressure, low operation and maintenance cost, high removal possible (.97% CH ₄), possible to bring H ₂ S to ,50 ppm, cost associated with BTF at h0.013 to 0.016 m ³ of biogas treated (Khoshnevisan et al., 2017)	Controlling O ₂ /N ₂ in biogas implies difficult and additional upgrading steps in the case of aerobic technologies, elemental sulfur deposition on the media requires periodic cleaning, and also strict control of process parameters like pH, moisture, and nutrient conditions, concentration in the treated biogas could be still high. (100300 cm ³ /m ³)

7 Removal Process of Hydrogen Sulfide

	Microalgal technologies	H ₂ S and CO ₂ could be simultaneously removed, operation at ambient temperature and pressure, use of algae biomass for energy generation	No/limited full-scale experiences, O ₂ /N ₂ in biogas needs additional upgrading steps
Absorption	Water scrubbing	Proven and simple technology, economical if water is available, CO ₂ can be removed along with H ₂ S, energy requirements of 0.30.9 kWh/Nm ³ clean biogas	Requires high pressure and low temperature, expensive, lower flexibility toward variation of input gas, clogging due to bacterial growth
	Organic solvents (amine)	High efficiency (.99% CH ₄), low CH ₄ losses (.0.1%)	High (CAPEX and O&M) costs, heat requirements for regeneration, foaming problems
	Absorption with NaOH and FeCl ₃	Low electricity requirements, low CH ₄ losses, operating cost around h0.03/m ³ of biogas	High (CAPEX and O&M) costs, not regenerative (standalone process)
Adsorption	Activated carbon	High efficiency (H ₂ S,3 cm ³ /m ³), high purification rate, low operation temperature, high loading capacity, cost of treatment around h3.85/kg H ₂ S	Regeneration requires high temperature, (450C) and residues are present up to 850C, requires pretreatment for O ₂ and H ₂ O, CH ₄ losses
	Metal oxides (iron-impregnated wood chips, iron oxide pellets)	Simple technology, low investment cost, high removal efficiency (.99% CH ₄) along with removal of mercaptans	Sensitive for water (pretreatment required), risk of ignition of the impregnated wood chips (regeneration is exothermic)
	PSA	Compact technique, tolerant to impurities, efficient technology (96-98% CH ₄), energy requirements of 0.251 kWh/Nm ³ clean biogas, flexibility for small applications	High (CAPEX and O&M) costs, CH ₄ losses (.4%), extensive process control
Membrane	Gasgas; gasliquid	Compact process, removal of more CO ₂ and H ₂ , energy requirements of 0.140.26 kWh/Nm ³ clean biogas, less than 0.6% CH ₄ loss, 9698% CH ₄ recovery	High investment cost, expensive operation and maintenance, biogas requires pretreatment, less operational experience of gas liquid membranes
BF, biofilter; BS, bioscrubber; BTF, biotrickling filters; O&M, operation, and maintenance; PSA, pressure swing adsorption.			

8 Discussion

This study focuses on the impact of hydrogen sulfide (H_2S) on microbial-induced corrosion (MIC) in wastewater treatment plants (WWTPs). It evaluates the effectiveness of various H_2S removal processes and explores the economic, environmental, and health implications associated with H_2S management. The study highlights the severe corrosive effects of H_2S on different materials used in WWTPs, including metals and concrete. H_2S , produced primarily by sulfate-reducing bacteria (SRB), leads to the deterioration of structural components, resulting in increased maintenance costs and potential failures in wastewater infrastructure. The corrosive nature of H_2S necessitates robust monitoring and mitigation strategies to ensure the longevity and safety of treatment facilities. The biological production of H_2S is intricately linked to the activity of SRB. These bacteria thrive in anaerobic conditions typically found in sewer systems and WWTPs, where they reduce sulfate to sulfide. This study underscores the variability of SRB activity based on environmental conditions such as temperature, pH, and the availability of organic matter. Seasonal variations significantly affect sulfide production, with higher temperatures generally accelerating microbial activity and consequently increasing H_2S levels.

Accurate quantification of sulfide production is crucial for effective management. This research employed various methodologies, including empirical models and laboratory-scale experiments, to define sulfide levels through the table. The findings suggest that existing predictive models for sewer biofilms may underestimate the sulfide production potential in sewer sediments. The empirical data gathered can inform the development of more accurate predictive tools, enhancing the capability to forecast and mitigate H_2S -related issues. Additionally, the study reviews several H_2S removal technologies, including chemical, biological, and physical methods. Chemical treatments, such as the addition of iron salts, effectively precipitate sulfide, but they are often costly and generate secondary waste. Biological treatments leverage the activity of sulfide-oxidizing bacteria (SOB) to convert H_2S to less harmful substances. Physical methods, like aeration, can also reduce H_2S levels but may not be feasible for large-scale applications. The economic analysis indicates that while initial investments in advanced removal technologies can be substantial, the long-term benefits outweigh the costs. Effective H_2S management reduces maintenance expenses, extends the lifespan of infrastructure, and minimizes health risks for workers and surrounding communities. The study advocates for a balanced approach that considers both the upfront costs and the long-term savings associated with efficient H_2S removal systems.

H_2S poses significant environmental and health risks. Its characteristic rotten egg smell is not only a nuisance but also a health hazard at high concentrations, potentially leading to respiratory issues and other health problems. The study emphasizes the need for stringent monitoring of H_2S levels and the implementation of safety protocols to protect workers and residents in the vicinity of WWTPs.

Future research should focus on developing integrated management strategies that combine multiple H_2S removal methods to enhance efficiency and cost-effectiveness. Additionally, more comprehensive studies are needed to understand the long-term effects of various H_2S mitigation techniques on both infrastructure and the environment. Collaborative efforts between academia, industry, and regulatory bodies can drive innovations in H_2S management and contribute to more sustainable wastewater treatment practices.

9 Conclusion

This thesis has reviewed the existing literature on Microbial Induced Corrosion (MIC) and the impact of hydrogen sulfide (H_2S) on wastewater treatment plant infrastructures. By synthesizing research across multiple aspects, the study concludes with a comprehensive understanding of MIC as follows:

1. The MIC in wastewater treatment has substantial effects on treatment plants infrastructure, due to the biological processes involved in sulfide production, particularly the sulfate-reducing bacteria (SRB).
2. The quantification of sulfide production in sewer systems were examined, along with the effects of human exposure to H_2S in different treatment plant configurations and environments.
3. Various H_2S removal processes, including chemical, biological, and physical methods, are assessed for their efficacy and practical application by comparing the pros and cons of each method.
4. The literature highlights the economic and environmental implications of MIC, emphasizing the need for accurate emission modeling and effective mitigation strategies. The review underscores the importance of integrated approaches to manage MIC and mitigate the effects of H_2S , ensuring the sustainability and efficiency of wastewater treatment systems.

In summary, this thesis provides a thorough understanding of MIC corrosion by assessing it from multiple perspectives, including biological processes, economic implications, and removal techniques. Furthermore, including a figure that illustrates the relationship between pH and temperature for various forms of sulfide, as recommended by the supervisors, provides a clear understanding of how environmental conditions affect corrosion processes. Following this, discussing the pros and cons of different H_2S removal methods offers a balanced evaluation of each technique's effectiveness and limitations. This combined approach aids in identifying the most suitable strategies for specific operational conditions, ultimately enhancing the efficiency of H_2S mitigation efforts, and protecting the integrity of wastewater treatment facilities. Continued research and development of advanced materials and monitoring practices are crucial for enhancing the resilience of wastewater infrastructures against MIC.

References

- Abdulina, D. R., Kurmakova, I. N., Bondar, E. S., Demchenko, N. R., & Bondarenko, A. I. (2019). Seasonal Dynamics of Bacteria in Corrosive Biofilms Formed on the Surface of Wastewater Treatment Plants. *Journal of Water Chemistry and Technology*, *41*(1), 44–51. <https://doi.org/10.3103/S1063455X19010077>
- Aerts, S. (2009). Effect of geochemical conditions on bacterial activity. *SCK• CEN Report (SCK• CEN-ER-75)*.
- Agrahari, G. K., Rawat, A., Verma, N., & Bhattacharya, P. K. (2013). Removal of dissolved H₂S from wastewater using hollow fiber membrane contactor: Experimental and mathematical analysis. *Desalination*, *314*, 34–42. <https://doi.org/10.1016/j.desal.2012.12.027>
- Alinezhad, E., Haghghi, M., Rahmani, F., Keshizadeh, H., Abdi, M., & Naddafi, K. (2019). Technical and economic investigation of chemical scrubber and bio-filtration in removal of H₂S and NH₃ from wastewater treatment plant. *Journal of Environmental Management*, *241*, 32–43. <https://doi.org/10.1016/j.jenvman.2019.04.003>
- Angelidaki, I., Treu, L., Tsapekos, P., Luo, G., Campanaro, S., Wenzel, H., & Kougias, P. G. (2018). Biogas upgrading and utilization: Current status and perspectives. *Biotechnology Advances*, *36*(2), 452–466. <https://doi.org/10.1016/j.biotechadv.2018.01.011>
- Anguita, J., Pizarro, G., & Vargas, I. T. (2022). Mathematical modelling of microbial corrosion in carbon steel due to early-biofilm formation of sulfate-reducing

- bacteria via extracellular electron transfer. *Bioelectrochemistry*, 145, 108058.
<https://doi.org/10.1016/j.bioelechem.2022.108058>
- Aoyagi, T., Hamai, T., Hori, T., Sato, Y., Kobayashi, M., Sato, Y., Inaba, T., Ogata, A., Habe, H., & Sakata, T. (2017). Hydraulic retention time and pH affect the performance and microbial communities of passive bioreactors for treatment of acid mine drainage. *AMB Express*, 7(1), 142.
<https://doi.org/10.1186/s13568-017-0440-z>
- Aüllo, T., Ranchou-Peyruse, A., Ollivier, B., & Magot, M. (2013). Desulfotomaculum spp. And related gram-positive sulfate-reducing bacteria in deep subsurface environments. *Frontiers in Microbiology*, 4, 362.
<https://www.frontiersin.org/articles/10.3389/fmicb.2013.00362/full>
- Austigard, Å. D., Svendsen, K., & Haldal, K. K. (2018). Hydrogen sulphide exposure in waste water treatment. *Journal of Occupational Medicine and Toxicology*, 13(1), 10. <https://doi.org/10.1186/s12995-018-0191-z>
- Awe, O. W., Zhao, Y., Nzihou, A., Minh, D. P., & Lyczko, N. (2017). A Review of Biogas Utilisation, Purification and Upgrading Technologies. *Waste and Biomass Valorization*, 8(2), 267–283. <https://doi.org/10.1007/s12649-016-9826-4>
- Ayangbenro, A. S., Olanrewaju, O. S., & Babalola, O. O. (2018). Sulfate-reducing bacteria as an effective tool for sustainable acid mine bioremediation. *Frontiers in Microbiology*, 9, 371482.
<https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2018.01986/full>
- Barbusiński, K., & Kalemba, K. (2016). Use of Biological Methods For Removal of H₂S From Biogas In Wastewater Treatment Plants – A Review. *Architecture*,

- Civil Engineering, Environment*, 9(1), 103–112.
<https://doi.org/10.21307/acee-2016-011>
- Biswas, K. C., Woodards, N. A., Xu, H., & Barton, L. L. (2009). Reduction of molybdate by sulfate-reducing bacteria. *BioMetals*, 22(1), 131–139.
<https://doi.org/10.1007/s10534-008-9198-8>
- Bødtker, G., Thorstenson, T., Lillebø, B.-L. P., Thorbjørnsen, B. E., Ulvøen, R. H., Sunde, E., & Torsvik, T. (2008). The effect of long-term nitrate treatment on SRB activity, corrosion rate and bacterial community composition in offshore water injection systems. *Journal of Industrial Microbiology and Biotechnology*, 35(12), 1625–1636.
- Bridge, T. A. M., White, C., & Gadd, G. M. (1999). Extracellular metal-binding activity of the sulphate-reducing bacterium *Desulfococcus multivorans*. *Microbiology*, 145(10), 2987–2995. <https://doi.org/10.1099/00221287-145-10-2987>
- Cámara, M., Green, W., MacPhee, C. E., Rakowska, P. D., Raval, R., Richardson, M. C., Slater-Jefferies, J., Steventon, K., & Webb, J. S. (2022). Economic significance of biofilms: A multidisciplinary and cross-sectoral challenge. *Npj Biofilms and Microbiomes*, 8(1), 1–8. <https://doi.org/10.1038/s41522-022-00306-y>
- Cao, J., Zhang, G., Mao, Z.-S., Li, Y., Fang, Z., & Yang, C. (2012). Influence of electron donors on the growth and activity of sulfate-reducing bacteria. *International Journal of Mineral Processing*, 106, 58–64.
<https://www.sciencedirect.com/science/article/pii/S0301751612000269>
- Carrera, L., Springer, F., Lipeme-Kouyi, G., & Buffiere, P. (2016). A review of sulfide emissions in sewer networks: Overall approach and systemic modelling.

- Water Science and Technology*, 73(6), 1231–1242.
<https://doi.org/10.2166/wst.2015.622>
- Chaudhari, B., Panda, B., Šavija, B., & Chandra Paul, S. (2022). Microbiologically Induced Concrete Corrosion: A Concise Review of Assessment Methods, Effects, and Corrosion-Resistant Coating Materials. *Materials*, 15(12), 4279.
<https://doi.org/10.3390/ma15124279>
- Chen, J. L., Ortiz, R., Steele, T. W., & Stuckey, D. C. (2014). Toxicants inhibiting anaerobic digestion: A review. *Biotechnology Advances*, 32(8), 1523–1534.
<https://www.sciencedirect.com/science/article/pii/S0734975014001542>
- Chen, Y., Ge, J., Huang, T., Shen, L., Chu, Z., & Xie, Z. (2020). Restriction of sulfate reduction on the bioavailability and toxicity of trace metals in Antarctic lake sediments. *Marine Pollution Bulletin*, 151, 110807.
<https://www.sciencedirect.com/science/article/pii/S0025326X19309634>
- Colleran, E., Finnegan, S., & Lens, P. (1995). Anaerobic treatment of sulphate-containing waste streams. *Antonie van Leeuwenhoek*, 67(1), 29–46.
<https://doi.org/10.1007/BF00872194>
- Cunha, M. P., Ferraz, R. M., Sancinetti, G. P., & Rodriguez, R. P. (2019). Long-term performance of a UASB reactor treating acid mine drainage: Effects of sulfate loading rate, hydraulic retention time, and COD/SO₄²⁻ ratio. *Biodegradation*, 30(1), 47–58. <https://doi.org/10.1007/s10532-018-9863-8>
- D, W.-P., & R.H, G. (2021). Study on Microbiologically Induced Corrosion of Concrete in Sewer Waste Water. *International Journal of Advances in Scientific Research and Engineering*, 07(09), 09–17.
<https://doi.org/10.31695/IJASRE.2021.34075>

- Dar, S. A., Kleerebezem, R., Stams, A. J. M., Kuenen, J. G., & Muyzer, G. (2008). Competition and coexistence of sulfate-reducing bacteria, acetogens and methanogens in a lab-scale anaerobic bioreactor as affected by changing substrate to sulfate ratio. *Applied Microbiology and Biotechnology*, 78(6), 1045–1055. <https://doi.org/10.1007/s00253-008-1391-8>
- Elwood, M. (2021). The Scientific Basis for Occupational Exposure Limits for Hydrogen Sulphide—A Critical Commentary. *International Journal of Environmental Research and Public Health*, 18(6), 2866. <https://doi.org/10.3390/ijerph18062866>
- Ernst, R., Ejsing, C. S., & Antonny, B. (2016). Homeoviscous adaptation and the regulation of membrane lipids. *Journal of Molecular Biology*, 428(24), 4776–4791. <https://www.sciencedirect.com/science/article/pii/S0022283616303084>
- Esposito, G., Weijma, J., Pirozzi, F., & Lens, P. N. L. (2003). Effect of the sludge retention time on H₂ utilization in a sulphate reducing gas-lift reactor. *Process Biochemistry*, 39(4), 491–498.
- Fonseca-Bermúdez, Ó. J., Giraldo, L., Sierra-Ramírez, R., & Moreno-Piraján, J. C. (2023). Removal of hydrogen sulfide from biogas by adsorption and photocatalysis: A review. *Environmental Chemistry Letters*, 21(2), 1059–1073. <https://doi.org/10.1007/s10311-022-01549-z>
- Gantzer, C. J., & Stefan, H. G. (2003). A model of microbial activity in lake sediments in response to periodic water-column mixing. *Water Research*, 37(12), 2833–2846.
- Ghimire, A., Gyawali, R., Lens, P. N. L., & Lohani, S. P. (2021). Technologies for removal of hydrogen sulfide (H₂S) from biogas. In N. Aryal, L. D. Mørck

- Ottosen, M. V. Wegener Kofoed, & D. Pant (Eds.), *Emerging Technologies and Biological Systems for Biogas Upgrading* (pp. 295–320). Academic Press. <https://doi.org/10.1016/B978-0-12-822808-1.00011-8>
- Godoi, A. F. L., Grasel, A. M., Polezer, G., Brown, A., Potgieter-Vermaak, S., Scremin, D. C., Yamamoto, C. I., & Godoi, R. H. M. (2018). Human exposure to hydrogen sulphide concentrations near wastewater treatment plants. *Science of The Total Environment*, 610–611, 583–590. <https://doi.org/10.1016/j.scitotenv.2017.07.209>
- Gormly, S. (2005). *Development of a psychrophilic, halophilic, SRB biomass production digester as a tool for European astrobiology research*. University of Nevada, Reno. <https://search.proquest.com/openview/48d5ddd9aaa8e76006ea044b358442c2/1?pq-origsite=gscholar&cbl=18750&diss=y>
- Grengg, C., Mittermayr, F., Ukrainczyk, N., Koraimann, G., Kienesberger, S., & Dietzel, M. (2018). Advances in concrete materials for sewer systems affected by microbial induced concrete corrosion: A review. *Water Research*, 134, 341–352. <https://doi.org/10.1016/j.watres.2018.01.043>
- Gu, T., Wang, D., Lekbach, Y., & Xu, D. (2021). Extracellular electron transfer in microbial biocorrosion. *Current Opinion in Electrochemistry*, 29, 100763. <https://doi.org/10.1016/j.coelec.2021.100763>
- Gu, T., Xu, D., Zhang, P., & Amy L. Lindenberger, Y. L. and. (2015). Microbiologically Influenced Corrosion and Its Impact on Metals and Other Materials. In *Microbiology for Minerals, Metals, Materials and the Environment*. CRC Press.

- Guo, G., Ekama, G. A., Wang, Y., Dai, J., Biswal, B. K., Chen, G., & Wu, D. (2019). Advances in sulfur conversion-associated enhanced biological phosphorus removal in sulfate-rich wastewater treatment: A review. *Bioresource Technology*, *285*, 121303. <https://doi.org/10.1016/j.biortech.2019.03.142>
- Hao, O. J. (1996). *Handbook of Water and Wastewater Microbiology*.
- Hao, O. J., Chen, J. M., Huang, L., & Buglass, R. L. (1996a). Sulfate-reducing bacteria. *Critical Reviews in Environmental Science and Technology*. <https://doi.org/10.1080/10643389609388489>
- Hao, O. J., Chen, J. M., Huang, L., & Buglass, R. L. (1996b). Sulfate-reducing bacteria. *Critical Reviews in Environmental Science and Technology*. <https://doi.org/10.1080/10643389609388489>
- Hao, T., Lu, H., Chui, H. K., Van Loosdrecht, M. C. M., & Chen, G. H. (2013). Granulation of anaerobic sludge in the sulfate-reducing up-flow sludge bed (SRUSB) of SANI® process. *Water Science and Technology*, *68*(3), 560–566. <https://doi.org/10.2166/wst.2013.182>
- Hao, T., Wei, L., Lu, H., Chui, H., Mackey, H. R., Van Loosdrecht, M. C. M., & Chen, G. (2013). Characterization of sulfate-reducing granular sludge in the SANI® process. *Water Research*, *47*(19), 7042–7052. <https://doi.org/10.1016/j.watres.2013.07.052>
- Hao, T., Xiang, P., Mackey, H. R., Chi, K., Lu, H., Chui, H., van Loosdrecht, M. C. M., & Chen, G.-H. (2014). A review of biological sulfate conversions in wastewater treatment. *Water Research*, *65*, 1–21. <https://doi.org/10.1016/j.watres.2014.06.043>
- He, Q., He, Z., Joyner, D. C., Joachimiak, M., Price, M. N., Yang, Z. K., Yen, H.-C. B., Hemme, C. L., Chen, W., Fields, M. M., Stahl, D. A., Keasling, J. D., Keller, M.,

References

- Arkin, A. P., Hazen, T. C., Wall, J. D., & Zhou, J. (2010). Impact of elevated nitrate on sulfate-reducing bacteria: A comparative Study of *Desulfovibrio vulgaris*. *The ISME Journal*, *4*(11), 1386–1397.
<https://doi.org/10.1038/ismej.2010.59>
- Hidalgo-Ulloa, A., Sánchez-Andrea, I., Buisman, C., & Weijma, J. (2020). Sulfur Reduction at Hyperthermoacidophilic Conditions with Mesophilic Anaerobic Sludge as the Inoculum. *Environmental Science & Technology*, *54*(22), 14656–14663. <https://doi.org/10.1021/acs.est.0c02557>
- Hiibel, S. R., Pereyra, L. P., Breazeal, M. V. R., Reisman, D. J., Reardon, K. F., & Pruden, A. (2011). Effect of Organic Substrate on the Microbial Community Structure in Pilot-Scale Sulfate-Reducing Biochemical Reactors Treating Mine Drainage. *Environmental Engineering Science*, *28*(8), 563–572.
<https://doi.org/10.1089/ees.2010.0237>
- Horikawa, M. S., Rossi, F., Gimenes, M. L., Costa, C. M. M., & Da Silva, M. G. C. (2004). Chemical absorption of H₂S for biogas purification. *Brazilian Journal of Chemical Engineering*, *21*, 415–422.
<https://www.scielo.br/j/bjce/a/qSGS4pnwV5SP8GjcWnBRdPH/?lang=en&format=html>
- Hu, Y., Jing, Z., Sudo, Y., Niu, Q., Du, J., Wu, J., & Li, Y.-Y. (2015). Effect of influent COD/SO₄²⁻ ratios on UASB treatment of a synthetic sulfate-containing wastewater. *Chemosphere*, *130*, 24–33.
<https://www.sciencedirect.com/science/article/pii/S0045653515001216>
- Hughes, M. N., Centelles, M. N., & Moore, K. P. (2009). Making and working with hydrogen sulfide: The chemistry and generation of hydrogen sulfide in vitro

- and its measurement in vivo: A review. *Free Radical Biology and Medicine*, 47(10), 1346–1353. <https://doi.org/10.1016/j.freeradbiomed.2009.09.018>
- Ishida, K., Orihara, K., Muguruma, H., Iwasa, H., Hiratsuka, A., Tsuji, K., & Kishimoto, T. (2018). Comparison of Direct and Mediated Electron Transfer in Electrodes with Novel Fungal Flavin Adenine Dinucleotide Glucose Dehydrogenase. *Analytical Sciences*, 34(7), 783–787. <https://doi.org/10.2116/analsci.17P613>
- Javaherdashti, R. (2023). Editorial: Microbiologically influenced corrosion (MIC): its mechanisms, technological, economic, and environmental impacts. *Frontiers in Microbiology*, 14, 1249565. <https://doi.org/10.3389/fmicb.2023.1249565>
- Jia, R., Unsal, T., Xu, D., Lekbach, Y., & Gu, T. (2019). Microbiologically influenced corrosion and current mitigation strategies: A state of the art review. *International Biodeterioration & Biodegradation*, 137, 42–58. <https://doi.org/10.1016/j.ibiod.2018.11.007>
- Jin, Y. (2010). *Effects of sulfide and pH on microbial sulfate reducing efficiency* [PhD Thesis]. <https://scholarworks.unr.edu/handle/11714/4239>
- Kaksonen, A. H., Franzmann, P. D., & Puhakka, J. A. (2004a). Effects of hydraulic retention time and sulfide toxicity on ethanol and acetate oxidation in sulfate-reducing metal-precipitating fluidized-bed reactor. *Biotechnology and Bioengineering*, 86(3), 332–343. <https://doi.org/10.1002/bit.20061>
- Kaksonen, A. H., Franzmann, P. D., & Puhakka, J. A. (2004b). Effects of hydraulic retention time and sulfide toxicity on ethanol and acetate oxidation in sulfate-reducing metal-precipitating fluidized-bed reactor. *Biotechnology and Bioengineering*, 86(3), 332–343. <https://doi.org/10.1002/bit.20061>

- Kaksonen, A. H., & Puhakka, J. A. (2007a). Sulfate Reduction Based Bioprocesses for the Treatment of Acid Mine Drainage and the Recovery of Metals. *Engineering in Life Sciences*, 7(6), 541–564.
<https://doi.org/10.1002/elsc.200720216>
- Kaksonen, A. H., & Puhakka, J. A. (2007b). Sulfate Reduction Based Bioprocesses for the Treatment of Acid Mine Drainage and the Recovery of Metals. *Engineering in Life Sciences*, 7(6), 541–564.
<https://doi.org/10.1002/elsc.200720216>
- Kamlage, B. (1996). *Methods for General and Molecular Bacteriology*. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood and N. R. Krieg. 791 pages, numerous figures and tables. American Society for Microbiology, Washington, D.C., 1994. Price: 55.00 £ (Vol. 40).
<https://onlinelibrary.wiley.com/doi/abs/10.1002/food.19960400226>
- Kashaija, N. T., Gável, V., Gergely, K., Akos, K., Kürthy, M., Szabó, C., Tóth, E., & Szabó-Krausz, Z. (2024). Deterioration of Cementitious Materials in Wastewater Treatment Plants' Pumping Stations and Sand-Trap Structures. *Journal of Composites Science*, 8(2), Article 2.
<https://doi.org/10.3390/jcs8020060>
- Keller, K. L., & Wall, J. D. (2011). Genetics and Molecular Biology of the Electron Flow for Sulfate Respiration in *Desulfovibrio*. *Sec. Microbial Physiology and Metabolism*, 29(2). <https://doi.org/10.3389/fmicb.2011.00135>.
- Keller-Lehmann, B., Corrie, S., Ravn, R., Yuan, Z., & Keller, J. (2006). Preservation and simultaneous analysis of relevant soluble sulfur species in sewage samples. *Proceedings of the Second International IWA Conference on Sewer Operation and Maintenance*, 26, 28.

- <https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=cf892716508c0571de885ae864a0b2ac323d9032>
- Kerkar, S., & Loka Bharathi, P. A. (2007). Stimulation of Sulfate-Reducing Activity at Salt-Saturation in the Salterns of Ribandar, Goa, India. *Geomicrobiology Journal*, 24(2), 101–110. <https://doi.org/10.1080/01490450701266597>
- Khan, H. A., Yasir, M., & Castel, A. (2022). Performance of cementitious and alkali-activated mortars exposed to laboratory simulated microbially induced corrosion test. *Cement and Concrete Composites*, 128, 104445. <https://doi.org/10.1016/j.cemconcomp.2022.104445>
- Khan, M., Yang, K., Liu, Z., Zhou, L., Liu, W., Lin, S., Wang, X., & Shang, C. (2023). Microorganisms Involved in the Biodegradation and Microbiological Corrosion of Structural Materials. *Coatings*, 13(10), 1683. <https://doi.org/10.3390/coatings13101683>
- Khanal, S. K., & Huang, J. (2006). Online Oxygen Control for Sulfide Oxidation in Anaerobic Treatment of High-Sulfate Wastewater. *Water Environment Research*, 78(4), 397–408. <https://doi.org/10.2175/106143006X98804>
- Khanal, S. K., & Huang, J.-C. (2003). ORP-based oxygenation for sulfide control in anaerobic treatment of high-sulfate wastewater. *Water Research*, 37(9), 2053–2062. <https://www.sciencedirect.com/science/article/pii/S0043135402006188>
- Khoshnevisan, B., Tsapekos, P., Alfaro, N., Díaz, I., Fdz-Polanco, M., Rafiee, S., & Angelidaki, I. (2017). A review on prospects and challenges of biological H₂S removal from biogas with focus on biotrickling filtration and microaerobic desulfurization. *Biofuel Research Journal*, 4(4), 741–750. <https://doi.org/10.18331/BRJ2017.4.4.6>

References

- Kim, M., Zaman, M., Jang, E., Nakhla, G., Ward, M., Gutierrez, O., Willis, J., Walton, J., & Santoro, D. (2024). Experimental investigation on hydrogen sulfide production, wastewater characteristics and microbial ecology profiles in anaerobic sewer lines using a sewer physical twin. *Journal of Environmental Chemical Engineering*, *12*(2), 111965.
<https://doi.org/10.1016/j.jece.2024.111965>
- Knisz, J., Eckert, R., Gieg, L. M., Koerd, A., Lee, J. S., Silva, E. R., Skovhus, T. L., An Stepec, B. A., & Wade, S. A. (2023). Microbiologically influenced corrosion—More than just microorganisms. *FEMS Microbiology Reviews*, *47*(5), fuado41. <https://doi.org/10.1093/femsre/fuado41>
- Knoblauch, C., & Jørgensen, B. B. (1999). Effect of temperature on sulphate reduction, growth rate and growth yield in five psychrophilic sulphate-reducing bacteria from Arctic sediments. *Environmental Microbiology*, *1*(5), 457–467. <https://doi.org/10.1046/j.1462-2920.1999.00061.x>
- Koch, G. H., Brongers, M. P. H., Thompson, N. G., Virmani, Y. P., Payer, J. H., CC Technologies, Inc., & NACE International. (2002). *Corrosion Cost and Preventive Strategies in the United States [Final report]* (FHWA-RD-01-156, R315-01). <https://rosap.nhtl.bts.gov/view/dot/40697>
- Koschorreck, M., Kunze, T., Luther, G., Bozau, E., & Wendt-Potthoff, K. (2004). *Accumulation and inhibitory effects of acetate in a sulphate reducing in situ reactor for the treatment of an acidic pit lake.*
Laboratory Investigation of Microbiologically Influenced Corrosion of X80 Pipeline Steel by Sulfate-Reducing Bacteria | Journal of Materials Engineering and

- Performance*. (n.d.). Retrieved April 20, 2024, from <https://link.springer.com/article/10.1007/s11665-021-05974-z>
- Lavanya, M. (2021). A Brief Insight into Microbial Corrosion and its Mitigation with Eco-friendly Inhibitors. *Journal of Bio- and Tribo-Corrosion*, 7(3), 125. <https://doi.org/10.1007/s40735-021-00563-y>
- Li, J., Cai, M.-H., Miao, Y., Luo, G., Li, W.-T., Li, Y., & Li, A.-M. (2019). Bacterial community structure and predicted function in an acidogenic sulfate-reducing reactor: Effect of organic carbon to sulfate ratios. *Bioresource Technology*, 293, 122020. <https://doi.org/10.1016/j.biortech.2019.122020>
- Li, L., & Moore, P. K. (2008). Putative biological roles of hydrogen sulfide in health and disease: A breath of not so fresh air? *Trends in Pharmacological Sciences*, 29(2), 84–90. [https://www.cell.com/trends/pharmacological-sciences/fulltext/S0165-6147\(07\)00284-2](https://www.cell.com/trends/pharmacological-sciences/fulltext/S0165-6147(07)00284-2)
- Li, W., Niu, Q., Zhang, H., Tian, Z., Zhang, Y., Gao, Y., Li, Y.-Y., Nishimura, O., & Yang, M. (2015). UASB treatment of chemical synthesis-based pharmaceutical wastewater containing rich organic sulfur compounds and sulfate and associated microbial characteristics. *Chemical Engineering Journal*, 260, 55–63. <https://www.sciencedirect.com/science/article/pii/S1385894714011498>
- Li, X., Kappler, U., Jiang, G., & Bond, P. L. (2017). The Ecology of Acidophilic Microorganisms in the Corroding Concrete Sewer Environment. *Frontiers in Microbiology*, 8, 683. <https://doi.org/10.3389/fmicb.2017.00683>
- Li, Y., Xu, D., Chen, C., Li, X., Jia, R., Zhang, D., Sand, W., Wang, F., & Gu, T. (2018). Anaerobic microbiologically influenced corrosion mechanisms interpreted using bioenergetics and bioelectrochemistry: A review. *Journal of Materials*

- Science & Technology*, 34(10), 1713–1718.
<https://doi.org/10.1016/j.jmst.2018.02.023>
- Liamleam, W., & Annachhatre, A. P. (2007). Electron donors for biological sulfate reduction. *Biotechnology Advances*, 25(5), 452–463.
<https://www.sciencedirect.com/science/article/pii/S0734975007000535>
- Liu, Y., Ni, B.-J., Ganigué, R., Werner, U., Sharma, K. R., & Yuan, Z. (2015). Sulfide and methane production in sewer sediments. *Water Research*, 70, 350–359.
<https://doi.org/10.1016/j.watres.2014.12.019>
- Liu, Y., Sharma, K. R., Fluggen, M., O'Halloran, K., Murthy, S., & Yuan, Z. (2015). Online dissolved methane and total dissolved sulfide measurement in sewers. *Water Research*, 68, 109–118. <https://doi.org/10.1016/j.watres.2014.09.047>
- Liu, Z. (2021). Urban sewage treatment odor gas release characteristics and regional differences. *Environmental Technology & Innovation*, 21, 101190.
<https://doi.org/10.1016/j.eti.2020.101190>
- Loto, C. A. (2017). Microbiological corrosion: Mechanism, control and impact—a review. *The International Journal of Advanced Manufacturing Technology*, 92(9–12), 4241–4252. <https://doi.org/10.1007/s00170-017-0494-8>
- Lu, X., Zhen, G., Ni, J., Hojo, T., Kubota, K., & Li, Y.-Y. (2016). Effect of influent COD/SO₄²⁻ ratios on biodegradation behaviors of starch wastewater in an upflow anaerobic sludge blanket (UASB) reactor. *Bioresource Technology*, 214, 175–183.
<https://www.sciencedirect.com/science/article/pii/S096085241630596X>
- Lv MeiYing, L. M., & Du Min, D. M. (2018). *A review: Microbiologically influenced corrosion and the effect of cathodic polarization on typical bacteria*.
<https://www.cabidigitallibrary.org/doi/full/10.5555/20183306636>

- Machuca Suarez, L. (2019). Understanding and addressing microbiologically influenced corrosion (MIC). *Corrosion & Materials*, 44(1), 88–96.
<https://core.ac.uk/download/pdf/195698106.pdf>
- Mara, D., & Horan, N. J. (2003). *Handbook of Water and Wastewater Microbiology*. Elsevier.
- Matias, N., Matos, R. V., Ferreira, F., Vollertsen, J., & Matos, J. S. (2017). Release of hydrogen sulfide in a sewer system under intermittent flow conditions: The Ericeira case study, in Portugal. *Water Science and Technology*, 75(7), 1702–1711. <https://doi.org/10.2166/wst.2017.040>
- Matos, R. V., Matias, N., Ferreira, F., Santos Silva, J., & Matos, J. S. (2018). Assessment of sulfide production in a full scale wastewater sludge rising main. *Journal of Environmental Management*, 209, 505–514.
<https://doi.org/10.1016/j.jenvman.2017.12.073>
- Meier, J., Piva, A., & Fortin, D. (2012). Enrichment of sulfate-reducing bacteria and resulting mineral formation in media mimicking pore water metal ion concentrations and pH conditions of acidic pit lakes. *FEMS Microbiology Ecology*, 79(1), 69–84. <https://academic.oup.com/femsec/article-abstract/79/1/69/672273>
- Mizuno, O., Li, Y. Y., & Noike, T. (1994). Effects of sulfate concentration and sludge retention time on the interaction between methane production and sulfate reduction for butyrate. *Water Science and Technology*, 30(8), 45.
<https://search.proquest.com/openview/b1e09806fe1340116e96f1d4dbod4929/1?pq-origsite=gscholar&cbl=2044520>
- Moura, A. G. L., Centurion, V. B., Okada, D. Y., Motteran, F., Delforno, T. P., Oliveira, V. M., & Varesche, M. B. A. (2019). Laundry wastewater and

- domestic sewage pilot-scale anaerobic treatment: Microbial community resilience regarding sulfide production. *Journal of Environmental Management*, 251, 109495. <https://doi.org/10.1016/j.jenvman.2019.109495>
- Muhammad, M. H., Idris, A. L., Fan, X., Guo, Y., Yu, Y., Jin, X., Qiu, J., Guan, X., & Huang, T. (2020). Beyond risk: Bacterial biofilms and their regulating approaches. *Frontiers in Microbiology*, 11, 928. <https://www.frontiersin.org/articles/10.3389/fmicb.2020.00928/full>
- Mulopo, J., Greben, H., Sigama, J., Radebe, V., Mashego, M., & Burke, L. (2011). The Relationships Between Sulphate Reduction and COD/VFA Utilisation Using Grass Cellulose as Carbon and Energy Sources. *Applied Biochemistry and Biotechnology*, 163(3), 393–403. <https://doi.org/10.1007/s12010-010-9047-4>
- Muñoz, R., Meier, L., Diaz, I., & Jeison, D. (2015). A review on the state-of-the-art of physical/chemical and biological technologies for biogas upgrading. *Reviews in Environmental Science and Bio/Technology*, 14(4), 727–759. <https://doi.org/10.1007/s11157-015-9379-1>
- Muthumbi, W., Boon, N., Boterdaele, R., De Vreese, I., Top, E., & Verstraete, W. (2001). Microbial sulfate reduction with acetate: Process performance and composition of the bacterial communities in the reactor at different salinity levels. *Applied Microbiology and Biotechnology*, 55(6), 787–793. <https://doi.org/10.1007/s002530100623>
- Muyzer, G., & Stams, A. J. (2008). The ecology and biotechnology of sulphate-reducing bacteria. *Nature Reviews Microbiology* 6, 441–454. <https://doi.org/10.1038/nrmicro1892>
- Muzaini, K., Yasin, S. M., Ismail, Z., & Ishak, A. R. (2022). Characteristics and Determinants of the Presence of Respiratory Symptoms among Sewage

- Workers in Malaysia. *Journal of Environmental and Public Health*, 2022, 1–12. <https://doi.org/10.1155/2022/8567594>
- Mykytczuk, N. C. S., Trevors, J. T., Leduc, L. G., & Ferroni, G. D. (2007). Fluorescence polarization in studies of bacterial cytoplasmic membrane fluidity under environmental stress. *Progress in Biophysics and Molecular Biology*, 95(1–3), 60–82. <https://www.sciencedirect.com/science/article/pii/S0079610707000430>
- Naz, N., Young, H. K., Ahmed, N., & Gadd, G. M. (2005). Cadmium Accumulation and DNA Homology with Metal Resistance Genes in Sulfate-Reducing Bacteria. *Applied and Environmental Microbiology*, 71(8), 4610–4618. <https://doi.org/10.1128/AEM.71.8.4610-4618.2005>
- Nevatalo, L. M., Bijmans, M. F., Lens, P. N., Kaksonen, A. H., & Puhakka, J. A. (2010a). The effect of sub-optimal temperature on specific sulfidogenic activity of mesophilic SRB in an H₂-fed membrane bioreactor. *Process Biochemistry*, 45(3), 363–368. <https://www.sciencedirect.com/science/article/pii/S1359511309003183>
- Nevatalo, L. M., Bijmans, M. F., Lens, P. N., Kaksonen, A. H., & Puhakka, J. A. (2010b). The effect of sub-optimal temperature on specific sulfidogenic activity of mesophilic SRB in an H₂-fed membrane bioreactor. *Process Biochemistry*, 45(3), 363–368.
- Nguyen, H. T., Nguyen, H. L., Nguyen, M. H., Nguyen, T. K. N., & Dinh, H. T. (2020). Sulfate Reduction for Bioremediation of AMD Facilitated by an Indigenous Acid-and Metal-Tolerant Sulfate-Reducer. *Journal of Microbiology and Biotechnology*, 30(7), 1005. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9728233/>

- Nyamukamba, P., Mukumba, P., Chikukwa, E. S., & Makaka, G. (2022). Hydrogen Sulphide removal from biogas: A review of the upgrading techniques and mechanisms involved. *International Journal of Renewable Energy Research (IJRER)*, *12*(1), 557–568.
<https://www.ijrer.com/index.php/ijrer/article/view/12668>
- Oliveira, F. R., Surendra, K. C., Jaisi, D. P., Lu, H., Unal-Tosun, G., Sung, S., & Khanal, S. K. (2020). Alleviating sulfide toxicity using biochar during anaerobic treatment of sulfate-laden wastewater. *Bioresource Technology*, *301*, 122711. <https://doi.org/10.1016/j.biortech.2019.122711>
- Omil, F., Bakker, C. D., Pol, L. W. H., & Lettinga, G. (1997). Effect of pH and Low Temperature Shocks on the Competition between Sulphate Reducing Bacteria and Methane Producing Bacteria in UASB Reactors. *Environmental Technology*, *18*(3), 255–264. <https://doi.org/10.1080/09593331808616535>
- Omil, F., Oude Elferink, S. J. W. H., Lens, P., Hulshoff Pol, L. W., & Lettinga, G. (1997). Effect of the inoculation with *Desulforhabdus amnigenus* and pH or O₂ shocks on the competition between sulphate reducing and methanogenic bacteria in an acetate fed UASB reactor. *Bioresource Technology*, *60*(2), 113–122. [https://doi.org/10.1016/S0960-8524\(97\)00014-X](https://doi.org/10.1016/S0960-8524(97)00014-X)
- O'Reilly, C., & Colleran, E. (2006a). Effect of influent COD/SO₄²⁻ ratios on mesophilic anaerobic reactor biomass populations: Physico-chemical and microbiological properties. *FEMS Microbiology Ecology*, *56*(1), 141–153. <https://doi.org/10.1111/j.1574-6941.2006.00066.x>
- O'Reilly, C., & Colleran, E. (2006b). Effect of influent COD/SO₄²⁻ ratios on mesophilic anaerobic reactor biomass populations: Physico-chemical and

- microbiological properties. *FEMS Microbiology Ecology*, 56(1), 141–153.
<https://doi.org/10.1111/j.1574-6941.2006.00066.x>
- Ozekmekci, M., Salkic, G., & Fellah, M. F. (2015). Use of zeolites for the removal of H₂S: A mini-review. *Fuel Processing Technology*, 139, 49–60.
<https://www.sciencedirect.com/science/article/pii/S0378382015301272>
- Pal, M. K., & Lavanya, M. (2022). Microbial Influenced Corrosion: Understanding Bioadhesion and Biofilm Formation. *Journal of Bio- and Tribo-Corrosion*, 8(3), 76. <https://doi.org/10.1007/s40735-022-00677-x>
- Papirio, S., Villa-Gomez, D. K., Esposito, G., Pirozzi, F., & Lens, P. N. L. (2013). Acid Mine Drainage Treatment in Fluidized-Bed Bioreactors by Sulfate-Reducing Bacteria: A Critical Review. *Critical Reviews in Environmental Science and Technology*, 43(23), 2545–2580.
<https://doi.org/10.1080/10643389.2012.694328>
- Parshina, S. N., Sipma, J., Henstra, A. M., & Stams, A. J. (2010). Carbon monoxide as an electron donor for the biological reduction of sulphate. *International Journal of Microbiology*, 2010.
<https://www.hindawi.com/journals/ijmicro/2010/319527/abs/>
- Parsons, S. A., Smith, N., Gostelow, P., & Wishart, J. (2000). Hydrogen sulphide dispersion modelling—Urban and rural case studies. *Water Science and Technology*, 41(6), 117–126. <https://doi.org/10.2166/wst.2000.0100>
- Paulo, L. M., Stams, A. J. M., & Sousa, D. Z. (2015). Methanogens, sulphate and heavy metals: A complex system. *Reviews in Environmental Science and Bio/Technology*, 14(4), 537–553. <https://doi.org/10.1007/s11157-015-9387-1>
- Pereira Godinho, J., & De Medeiros, M. H. F. (2021). Biogenic sulfur attack in a reinforced concrete sewage treatment plant. Re-visited mechanism and

- rehabilitation proposal. *Engineering Failure Analysis*, 124, 105354.
<https://doi.org/10.1016/j.engfailanal.2021.105354>
- Pillai, A. (Ed.). (2015). *Microbiology for minerals, metals, materials and the environment*. CRC Press.
- Piña-Salazar, E. Z., Cervantes, F. J., Meraz, M., & Celis, L. B. (2011). Biofilm development during the start-up of a sulfate-reducing down-flow fluidized bed reactor at different COD/SO₄²⁻ ratios and HRT. *Water Science and Technology*, 64(4), 910–916. <https://iwaponline.com/wst/article-abstract/64/4/910/16944>
- Plugge, C. M., Zhang, W., Scholten, J. C., & Stams, A. J. (2011). Metabolic flexibility of sulfate-reducing bacteria. *Frontiers in Microbiology*, 2, 81.
<https://www.frontiersin.org/articles/10.3389/fmicb.2011.00081/full>
- Polo, B. C., Bewtra, J. K., & Biswas, N. (2006). Effect of hydraulic retention time and attachment media on sulfide production by sulfate reducing bacteria. *Journal of Environmental Engineering and Science*, 5(1), 47–57.
<https://doi.org/10.1139/s05-013>
- Postgate, J. R. (1984). *The Sulphate-Reducing Bacteria*. Cambridge University Press.
https://books.google.dk/books?hl=en&lr=&id=odU9AAAAIAAJ&oi=fnd&pg=PA8&ots=yzm8UJWibE&sig=sKxfOfvRxYRwJYpWby7vHJyRK1o&redir_esc=y#v=onepage&q&f=false
- Pryor, W. A., Houk, K. N., Foote, C. S., Fukuto, J. M., & Ignarro, L. J. (2006). *Free radical biology and medicine: It's a gas, man! Am J Physiol Regul Integr Comp Physiol*.
- Qian, J., Zhu, X., Tao, Y., Zhou, Y., He, X., & Li, D. (2015). Promotion of Ni²⁺ removal by masking toxicity to sulfate-reducing bacteria: Addition of citrate.

- International Journal of Molecular Sciences*, 16(4), 7932–7943.
<https://www.mdpi.com/1422-0067/16/4/7932>
- Reis, M. A. M., Almeida, J. S., Lemos, P. C., & Carrondo, M. J. T. (1992). Effect of hydrogen sulfide on growth of sulfate reducing bacteria. *Biotechnology and Bioengineering*, 40(5), 593–600. <https://doi.org/10.1002/bit.260400506>
- Ryckebosch, E., Drouillon, M., & Vervaeren, H. (2011). Techniques for transformation of biogas to biomethane. *Biomass and Bioenergy*, 35(5), 1633–1645. <https://doi.org/10.1016/j.biombioe.2011.02.033>
- Rzeczycka, M., Miernik, A., & Markiewicz, Z. (2010). Simultaneous Degradation of Waste Phosphogypsum and Liquid Manure from Industrial Pig Farm by a Mixed Community of Sulfate-Reducing Bacteria. *Polish Journal of Microbiology*, 59(4), 241–247. <https://doi.org/10.33073/pjm-2010-037>
- Sánchez-Andrea, I., Sanz, J. L., Bijmans, M. F., & Stams, A. J. (2014). Sulfate reduction at low pH to remediate acid mine drainage. *Journal of Hazardous Materials*, 269, 98–109.
<https://www.sciencedirect.com/science/article/pii/S030438941300962X>
- Sánchez-Andrea, I., Stams, A. J. M., Amils, R., & Sanz, J. L. (2013). Enrichment and isolation of acidophilic sulfate-reducing bacteria from T into R iver sediments. *Environmental Microbiology Reports*, 5(5), 672–678.
<https://doi.org/10.1111/1758-2229.12066>
- Sánchez-Andrea, I., Triana, D., & Sanz, J. L. (2012). Bioremediation of acid mine drainage coupled with domestic wastewater treatment. *Water Science and Technology*, 66(11), 2425–2431. <https://iwaponline.com/wst/article-abstract/66/11/2425/16442>

- Shahsavari, S., Seth, R., Chaganti, S. R., & Biswas, N. (2019). Inhibition of anaerobic biological sulfate reduction process by copper precipitates. *Chemosphere*, *236*, 124246.
<https://www.sciencedirect.com/science/article/pii/S0045653519314572>
- Sharma, K., Derlon, N., Hu, S., & Yuan, Z. (2014). Modeling the pH effect on sulfidogenesis in anaerobic sewer biofilm. *Water Research*, *49*, 175–185.
<https://www.sciencedirect.com/science/article/pii/S0043135413009305>
- Shi, X., Gao, G., Tian, J., Wang, X. C., Jin, X., & Jin, P. (2020). Symbiosis of sulfate-reducing bacteria and methanogenic archaea in sewer systems. *Environment International*, *143*, 105923.
<https://www.sciencedirect.com/science/article/pii/S016041202031878X>
- Sipma, J., Osuna, M. B., Lettinga, G., Stams, A. J., & Lens, P. N. (2007). Effect of hydraulic retention time on sulfate reduction in a carbon monoxide fed thermophilic gas lift reactor. *Water Research*, *41*(9), 1995–2003.
- Song, X., Zhang, G., Zhou, Y., & Li, W. (2023). Behaviors and mechanisms of microbially-induced corrosion in metal-based water supply pipelines: A review. *Science of The Total Environment*, *895*, 165034.
<https://doi.org/10.1016/j.scitotenv.2023.165034>
- Song, Y., Gibney, P., Cheng, L., Liu, S., & Peck, G. (2020). Yeast Assimilable Nitrogen Concentrations Influence Yeast Gene Expression and Hydrogen Sulfide Production During Cider Fermentation. *Frontiers in Microbiology*, *11*.
<https://doi.org/10.3389/fmicb.2020.01264>
- Srensen, K. B., Caneld, D. E., & Oren, A. (2004). Salt responses of benthic microbial communities in a solar saltern (Eilat, Israel). *Applied and Environmental Micro-Biology*, *70*, 16081616.

- Stephenson, L. D., & Kumar, A. (2009). Corrosion control technologies for wastewater treatment plants. *Mater Perf*, *48*, 42–47.
<https://www.materialsperformance.com/uploads/documents/Magazine/2009/JAN%202009%20Stephenson.pdf>
- Sun, Q., Li, H., Yan, J., Liu, L., Yu, Z., & Yu, X. (2015). Selection of appropriate biogas upgrading technology-a review of biogas cleaning, upgrading and utilisation. *Renewable and Sustainable Energy Reviews*, *51*, 521–532.
<https://doi.org/10.1016/j.rser.2015.06.029>
- Sutherland-Stacey, L., Corrie, S., Neethling, A., Johnson, I., Gutierrez, O., Dexter, R., Yuan, Z., Keller, J., & Hamilton, G. (2008). Continuous measurement of dissolved sulfide in sewer systems. *Water Science and Technology*, *57*(3), 375–381. <https://iwaponline.com/wst/article-abstract/57/3/375/12593>
- Tran, T. T. T., Kannoorpatti, K., Padovan, A., & Thennadil, S. (2021). Sulphate-Reducing Bacteria's Response to Extreme pH Environments and the Effect of Their Activities on Microbial Corrosion. *Applied Sciences*, *11*(5), 2201.
<https://doi.org/10.3390/app11052201>
- Tchobanoglous, G., Stensel, D. H., Tsuchihashi, R., Burton, F., Abu-Orf, M., Bowden, G., Pfrang, W., Metcalf & Eddy, Inc, & AECOM (Eds.). (2014). *Wastewater engineering: Treatment and resource recovery* (Fifth edition). McGraw-Hill Education.
- Utgikar, V. P., Chen, B., Chaudhary, N., Tabak, H. H., Haines, J. R., & Govind, R. (2001). Acute toxicity of heavy metals to acetate-utilizing mixed cultures of sulfate-reducing bacteria: EC100 and EC50. *Environmental Toxicology and Chemistry*, *20*(12), 2662–2669. <https://doi.org/10.1002/etc.5620201202>

- Vallero, M. V. G., Sipma, J., Lettinga, G., & Lens, P. N. L. (2004). High-rate sulfate reduction at high salinity (up to 90 mS.cm⁻¹) in mesophilic UASB reactors. *Biotechnology and Bioengineering*, *86*(2), 226–235.
<https://doi.org/10.1002/bit.20040>
- Van Den Brand, T. P. H., Roest, K., Chen, G. H., Brdjanovic, D., & Van Loosdrecht, M. C. M. (2015). Occurrence and activity of sulphate reducing bacteria in aerobic activated sludge systems. *World Journal of Microbiology and Biotechnology*, *31*(3), 507–516. <https://doi.org/10.1007/s11274-015-1807-4>
- Van Houten, B. H., Roest, K., Tzeneva, V. A., Dijkman, H., Smidt, H., & Stams, A. J. (2006). Occurrence of methanogenesis during start-up of a full-scale synthesis gas-fed reactor treating sulfate and metal-rich wastewater. *Water Research*, *40*(3), 553–560.
<https://www.sciencedirect.com/science/article/pii/S0043135405007062>
- Van Houten, R. T., Yun, S. Y., & Lettinga, G. (1997). Thermophilic sulphate and sulphite reduction in lab-scale gas-lift reactors using H₂ and CO₂ as energy and carbon source. *Biotechnology and Bioengineering*, *55*(5), 807–814.
[https://doi.org/10.1002/\(SICI\)1097-0290\(19970905\)55:5<807::AID-BIT11>3.0.CO;2-8](https://doi.org/10.1002/(SICI)1097-0290(19970905)55:5<807::AID-BIT11>3.0.CO;2-8)
- Vasquez, Y., Escobar, M. C., Neculita, C. M., Arbeli, Z., & Roldan, F. (2016). Biochemical passive reactors for treatment of acid mine drainage: Effect of hydraulic retention time on changes in efficiency, composition of reactive mixture, and microbial activity. *Chemosphere*, *153*, 244–253.
<https://www.sciencedirect.com/science/article/pii/S0045653516303678>
- Vinçon-Laugier, A., Cravo-Laureau, C., Mitteau, I., & Grossi, V. (2017). Temperature-dependent alkyl glycerol ether lipid composition of mesophilic and

- thermophilic sulfate-reducing bacteria. *Frontiers in Microbiology*, 8, 273580.
<https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2017.01532/full>
- Virpiranta, H., Taskila, S., Leiviskä, T., Rämö, J., & Tanskanen, J. (2019).
Development of a process for microbial sulfate reduction in cold mining
waters—Cold acclimation of bacterial consortia from an Arctic mining district.
Environmental Pollution, 252, 281–288.
<https://www.sciencedirect.com/science/article/pii/S0269749119314988>
- Wallace, J. L. (2007). Hydrogen sulfide-releasing anti-inflammatory drugs. *Trends
in Pharmacological Sciences*, 28(10), 501–505.
[https://www.cell.com/trends/pharmacological-sciences/fulltext/S0165-6147\(07\)00211-8](https://www.cell.com/trends/pharmacological-sciences/fulltext/S0165-6147(07)00211-8)
- Wang, D., Guan, F., Feng, C., Mathivanan, K., Zhang, R., & Sand, W. (2023). Review
on Microbially Influenced Concrete Corrosion. *Microorganisms*, 11(8), 2076.
<https://doi.org/10.3390/microorganisms11082076>
- Wang, H., Chen, F., Mu, S., Zhang, D., Pan, X., Lee, D.-J., & Chang, J.-S. (2013).
Removal of antimony (Sb (V)) from Sb mine drainage: Biological sulfate
reduction and sulfide oxidation—precipitation. *Bioresource Technology*, 146,
799–802.
<https://www.sciencedirect.com/science/article/pii/S0960852413012315>
- Wang, M. Y., Liang, X. B., Zheng, Y. P., Zhao, Y. Z., & Wei, Z. Q. (2005). Advanced in
Identification of Sulfate-Reducing Bacteria and Its Detection Method. *J.
Microbiol*, 25 (6), 81–84. <https://doi.org/10.3969/j.issn.1005-7021.2005.06.022>

- Weijma, J., Gubbels, F., Hulshoff Pol, L. W., Stams, A. J. M., Lens, P., & Lettinga, G. (2002). Competition for H₂ between sulfate reducers, methanogens and homoacetogens in a gas-lift reactor. *Water Science and Technology*, *45*(10), 75–80.
- Wu, J., Liu, Q., Feng, B., Kong, Z., Jiang, B., & Li, Y.-Y. (2019). Temperature effects on the methanogenesis enhancement and sulfidogenesis suppression in the UASB treatment of sulfate-rich methanol wastewater. *International Biodeterioration & Biodegradation*, *142*, 182–190.
<https://doi.org/10.1016/j.ibiod.2019.05.013>
- Wu, M., Wang, T., & Wang, S. (2020). Corrosion and sulfur-related bacteria. *Environmental Technologies to Treat Sulfur Pollution: Principles and Engineering*; Lens, PNL, Ed. https://www.researchgate.net/profile/Min-Wu-50/publication/344804869_Chapter_5_Corrosion_and_sulfur-related_bacteria/links/5f913127a6fdccfd7b746aee/Chapter-5-Corrosion-and-sulfur-related-bacteria.pdf
- Wu, M., Wang, T., Wu, K., & Kan, L. (2020). Microbiologically induced corrosion of concrete in sewer structures: A review of the mechanisms and phenomena. *Construction and Building Materials*, *239*, 117813.
<https://doi.org/10.1016/j.conbuildmat.2019.117813>
- Xu, D., Gu, T., & Lovley, D. R. (2023). Microbially mediated metal corrosion. *Nature Reviews Microbiology*, *21*(11), 705–718. <https://doi.org/10.1038/s41579-023-00920-3>
- Xu, D., Li, Y., & Gu, T. (2016). Mechanistic modeling of biocorrosion caused by biofilms of sulfate reducing bacteria and acid producing bacteria.

- Bioelectrochemistry*, 110, 52–58.
<https://doi.org/10.1016/j.bioelechem.2016.03.003>
- Xu, Y.-N., & Chen, Y. (2020). Advances in heavy metal removal by sulfate-reducing bacteria. *Water Science and Technology*, 81(9), 1797–1827.
<https://doi.org/10.2166/wst.2020.227>
- Y. G., A., & Mulky, L. (2023). Biofilms and beyond: A comprehensive review of the impact of Sulphate Reducing Bacteria on steel corrosion. *Biofouling*, 39(9–10), 897–915. <https://doi.org/10.1080/08927014.2023.2284316>
- Yang, G., Gong, M., Zheng, X., Lin, L., Fan, J., Liu, F., & Meng, J. (2022). A review of microbial corrosion in reclaimed water pipelines: Challenges and mitigation strategies. *Water Practice and Technology*, 17(3), 731–748.
<https://doi.org/10.2166/wpt.2022.007>
- Zhang, L., De Schryver, P., De Gusseme, B., De Muynck, W., Boon, N., & Verstraete, W. (2008b). Chemical and biological technologies for hydrogen sulfide emission control in sewer systems: A review. *Water Research*, 42(1), 1–12.
<https://doi.org/10.1016/j.watres.2007.07.013>
- Zhang, Z., Yu, Y., Xi, H., & Zhou, Y. (2021). Review of micro-aeration hydrolysis acidification for the pretreatment of toxic and refractory organic wastewater. *Journal of Cleaner Production*, 317, 128343.
<https://doi.org/10.1016/j.jclepro.2021.128343>
- Zhang, Z., Zhang, C., Yang, Y., Zhang, Z., Tang, Y., Su, P., & Lin, Z. (2022). A review of sulfate-reducing bacteria: Metabolism, influencing factors and application in wastewater treatment. *Journal of Cleaner Production*, 376, 134109.
<https://doi.org/10.1016/j.jclepro.2022.134109>

References

- Zhong, H., Tang, Y., Yan, H., Zhang, Y., Dong, L., & Wang, B. (2023). Corrosion of pipelines in urban water systems: Current research status and future trends based on bibliometric analysis. *Journal of Water Process Engineering*, *56*, 104288. <https://doi.org/10.1016/j.jwpe.2023.104288>
- Zhu, S., Chen, Y., Khan, M. A., Xu, H., Wang, F., & Xia, M. (2022). In-Depth Study of Heavy Metal Removal by an Etidronic Acid-Functionalized Layered Double Hydroxide. *ACS Applied Materials & Interfaces*, *14*(5), 7450–7463. <https://doi.org/10.1021/acsami.1c22035>
- Zuo, Z., Ren, D., Qiao, L., Li, H., Huang, X., & Liu, Y. (2021). Rapid dynamic quantification of sulfide generation flux in spatially heterogeneous sediments of gravity sewers. *Water Research*, *203*, 117494. <https://doi.org/10.1016/j.watres.2021.117494>

Appendices

Appendix A. Master Thesis Contract



Faculty of Technology, Natural Sciences and Maritime Sciences, Campus Porsgrunn

FMH606 Master's Thesis

Title: Microbial induced corrosion (MIC) effects on wastewater treatment plant infrastructures

USN supervisor: Eshetu Janka and Vibeke Bredvold Karlsen

External partner: NORPART project South partner universities.

Task background:

Microbial induced corrosion (MIC) of wastewater treatment plant infrastructures are the main problems that incur additional maintenance cost every year on treatment plants worldwide. In wastewater treatment system where anaerobic environment is present, sulphate-reducing bacteria (SRB) reduce sulphate to sulphide which in turn can combine with hydrogen to form hydrogen sulphide (H_2S). Hydrogen sulphide gas, which could accumulate to high levels in the treatment system infrastructure, particularly in net-work pipes and joints with a long transport time. The accumulated H_2S gas can be oxidized biological to sulfuric acid which is corrosive to concrete sewer pipes, pumps, gate valves and other infrastructures. This corrosive effect is known as 'Crown rot' can seriously threaten the treatment plant infrastructure integrity in the long term and shorten the lifespan of the treatment plant. Hence, nowadays there is growing demand across the wastewater treatment industry for sustainable construction materials that can be used under such corrosive environments. The aim of this thesis is to conduct a critical review on the overall effect of MIC especially due to hydrogen sulphide on small- and large-scale (i.e., centralized or decentralized) municipal wastewater treatment plants.

Task description:

- Conduct a critical review of MIC effects on wastewater treatment plant infrastructures. E.g., cost.
- Identify biological processes involved in sulphide production and microbially induced corrosion in sewers.
- Describe the mechanisms underlying H_2S production and how to quantify sulphide production in sewer systems.
- To review the effect of human exposure to H_2S in different of treatment plants (i.e., centralized or decentralized), seasons (temperature) and geographical place.

Student category: EET or PT students

Is the task suitable for online students (not present at the campus)? Yes. The task is suitable for online students.

Practical arrangements: Literature review, secondary data analysis and theoretical mathematics.

Supervision: As a general rule, the student is entitled to 15-20 hours of supervision. This includes necessary time for the supervisor to prepare for supervision meetings (reading material to be discussed, etc).

Signatures:

Supervisor (date and signature):

Student (write clearly in all capitalized letters): Zahra Sanidanesh

Student (date and signature):

Eshetu Janka 15.03.24


Appendix B. SRB Components

Table 1 – SRB components.(Z. Zhang et al., 2022)

Explanation of components in SRB	
ADP	Adenosine diphosphate, a nucleotide formed when ATP loses a phosphate group, releasing energy
AMP	Adenosine monophosphate, a nucleotide involved in energy transfer
APS	Adenosine 5'-phosphosulfate, an intermediate in the sulfate reduction pathway
ATP	Adenosine triphosphate, the primary energy carrier in cells
CysD	A protein involved in the biosynthesis of cysteine
CysI	A protein involved in sulfur metabolism and the biosynthesis of cysteine
CysJ	A protein involved in the biosynthesis of cysteine
CysH	A protein involved in sulfur metabolism and the synthesis of cysteine
CysNC	A protein complex involved in cysteine biosynthesis
DsrABC	A set of proteins involved in dissimilatory sulfite reduction
Fd ^{ox}	Oxidized ferredoxin, the oxidized form of ferredoxin in electron transfer reactions
Fd ^{red}	Reduced ferredoxin, an electron carrier involved in various metabolic pathways
DsrMKJOP	A set of proteins involved in the dissimilatory sulfate reduction pathway in SRB. Specifically:
DsrJ	A cytoplasmic protein with a role in sulfur trafficking
DsrK	A cytoplasmic protein involved in the reduction of sulfate to sulfite
DsrM	A membrane-bound protein involved in electron transfer
DsrO	A cytoplasmic protein involved in the final step of sulfate reduction
DsrP	A cytoplasmic protein involved in sulfur transfer in the Dsr pathway
E0'	Standard Reduction Potential volts (V) or millivolts (mV)
nDHA	Nicotinamide adenine dinucleotide, a coenzyme involved in cellular respiration and energy production
PAP	3'-Phosphoadenosine 5'-phosphate, a product of the reaction between APS and PAPS
PAPS	3'-Phosphoadenosine 5'-phosphosulfate, a sulfate donor in the sulfate reduction pathway
QmoABC	A set of proteins involved in quinone-dependent sulfite reduction
SIR	Sulfite reductase, an enzyme involved in dissimilatory sulfate reduction
Trx ^{ox}	Oxidized form of thioredoxin, involved in redox reactions
Trx ^{red}	Reduced form of thioredoxin, involved in redox reactions

Appendix C. The Gibbs free energy involved in biological sulfate reduction processes

Table 2 - Chemical equations and Gibbs free energy of biological sulfate reduction reactions with various electron donors. calculated from.(Guo et al., 2019)

Electron donors	Reaction	ΔG °(kJ/reaction)
Hydrogen	$\text{SO}_4^{2-} + 4\text{H}_2 + \text{H}^+ \rightarrow \text{HS}^- + 4\text{H}_2\text{O}$	-151.9
Methane	$\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$	-16.6
Formate	$4\text{HCOO}^- + \text{H}^+ + \text{SO}_4^{2-} \rightarrow 4\text{HCO}_3^- + \text{HS}^-$	-146.7
Acetate	$\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} \rightarrow 2\text{HCO}_3^- + \text{HS}^-$	-47.6
Propionate	$\text{C}_2\text{H}_5\text{COO}^- + 2\text{SO}_4^{2-} + \text{H}_2 \rightarrow 3\text{HCO}_3^- + 2\text{HS}^- + \text{H}_2\text{O}$	-122.7
Propionate	$\text{C}_2\text{H}_5\text{COO}^- + \text{SO}_4^{2-} + \text{H}_2 \rightarrow \text{CH}_3\text{COO}^- + \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$	-75.8
Butyrate	$\text{C}_3\text{H}_7\text{COO}^- + 3\text{SO}_4^{2-} + 2\text{H}_2 \rightarrow 4\text{HCO}_3^- + 3\text{HS}^- + 2\text{H}_2\text{O}$	-198.4
Butyrate	$\text{C}_3\text{H}_7\text{COO}^- + \text{SO}_4^{2-} + 2\text{H}_2 \rightarrow 2\text{CH}_3\text{COO}^- + \text{HS}^- + 2\text{H}_2\text{O}$	-103.8
Pyruvate	$4\text{CH}_3\text{COCOO}^- + \text{SO}_4^{2-} + 2\text{H}_2 + 6\text{H}_2\text{O} \rightarrow 4\text{CH}_3\text{COO}^- + 4\text{CO}_2 + \text{S}^{2-}$	-331.1
Lactate	$2\text{CH}_3\text{CHOHCOO}^- + \text{SO}_4^{2-} \rightarrow 2\text{CH}_3\text{COO}^- + \text{HS}^- + 2\text{HCO}_3^- + \text{H}^+$	-159.6
Methanol	$4\text{CH}_3\text{OH} + 3\text{SO}_4^{2-} \rightarrow 4\text{HCO}_3^- + 3\text{HS}^- + 4\text{H}_2\text{O} + \text{H}^+$	-361.7
Methanol	$2\text{CH}_3\text{OH} + \text{SO}_4^{2-} \rightarrow 2\text{HCOO}^- + \text{HS}^- + 2\text{H}_2\text{O} + \text{H}^+$	-108.3
Ethanol	$2\text{C}_2\text{H}_5\text{OH} + \text{SO}_4^{2-} \rightarrow 2\text{CH}_3\text{COO}^- + \text{HS}^- + 2\text{H}_2\text{O} + \text{H}^+$	-132.7
Glucose	$\text{C}_6\text{H}_{12}\text{O}_6 + \text{SO}_4^{2-} \rightarrow 2\text{CH}_3\text{COO}^- + \text{HS}^- + 2\text{HCO}_3^- + 3\text{H}^+$	-358.2
Glucose	$\text{C}_6\text{H}_{12}\text{O}_6 + 3\text{SO}_4^{2-} \rightarrow 3\text{HS}^- + 6\text{HCO}_3^- + 3\text{H}^+$	-452.5

Remark: If new appendix → New page (i.e. don't put several appendices on the same page)

Appendix D. Seasonal and Location-Specific H₂S Concentration Graphs

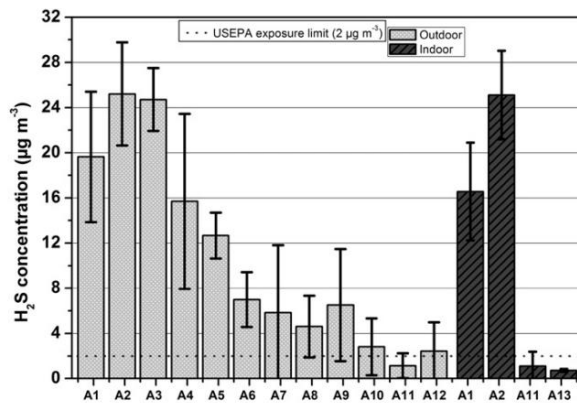


Figure C1: H₂S concentration levels at different locations (A1-A13) near WWTP "A".(Godoi et al., 2018)

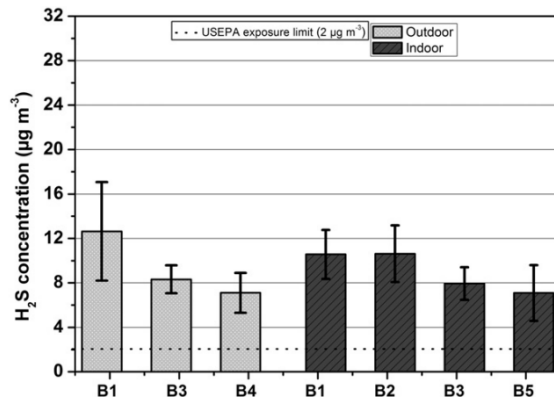


Figure C3: Comparison of H₂S concentration levels at different distances from WWTP "B" for various cohorts (Godoi et al., 2018)

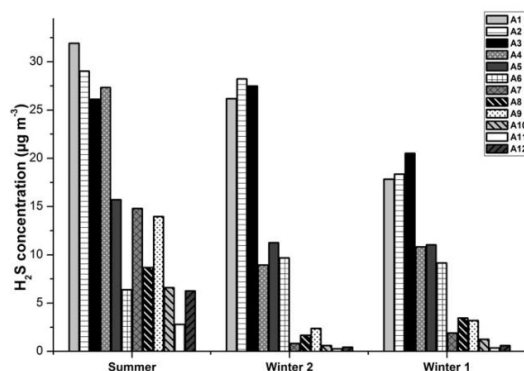


Figure C3: Seasonal variation of H₂S concentrations at WWTP "A" and WWTP "B" during the summer and winter campaigns (Godoi et al., 2018)