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Extraction, Analysis and the Role of Co-contaminants on Sulfolane Biodegradation

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Extraction, Analysis and the Role of Co-contaminants on Sulfolane Biodegradation

by

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A THESIS

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Abstract

Sulfolane's extensive use in various oil and gas industries has led to its increased environmental contamination throughout Alberta. The impact of co-contaminants and complex matrices in sulfolane polluted areas can produce challenges in analytical and remediation efforts and generate vastly different results from those obtained in a laboratory setting. Particularly, the low regulatory guidelines of 0.18 mg/kg in soils and 0.09 mg/L in groundwater has created challenges for commercial testing laboratories as these levels are often too close to their instrument detection limits. Potential interferences and false positives have become a cause for concern especially in complex matrices with high organic content. Similarly, groundwater bioremediation efforts can be positively or negatively impacted depending on the type of co-contaminant present alongside sulfolane.

A spiking study was conducted to investigate sulfolane analytical challenges faced by various testing labs when analysing sulfolane in peat and clay soils, as well as groundwater samples. It was observed that soil spiked with high sulfolane concentrations (>0.5mg/kg) resulted in more reliable data compared to low concentrations (<0.5mg/kg), with mineral soils providing more reproducible data than the highly organic peat soil. Similarly, groundwater analysis also provided less variable results in higher concentrations (0.5 mg/L) than lower levels (0.1 mg/L). Soil water extraction efficiency of sulfolane improved with an increase in soil to water ratio, however, clay soils will require additional aliquots to achieve maximum recovery. GC-MS analysis demonstrated that organic soils can produce false positives. Therefore, at low concentrations, interferences, loss of sulfolane due to biodegradation and sample heterogeneity will significantly impact results.

Impact of co-pollutants (As (III), fulvic acid, and diisopropanolamine) on sulfolane biodegradation in groundwater was also investigated in this research. Three concentrations of As

(III) (low, medium and high) were observed to have negligible impact on sulfolane degradation. Similarly, at environmentally relevant concentrations, fulvic acid did not significantly effect sulfolane removal. Diisopropanolamine, however, was observed to positively and negatively impact sulfolane removal depending on the biological, chemical and physical characteristics of the water matrix used.

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To: Mom, Dad, Maryam, Mina, & Saeid

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List of Symbols, Abbreviations, and Nomenclature

Symbol	Definition
C	Concentration of substrate, mg/L
C_0	Initial concentration of substrate, mg/L
nm	Nanometer
μm	Micrometer

Abbreviation	Definition
BTEX	Benzene, Toluene, Ethylbenzene, and Xylene
DIPA	Diisopropanolamine
USEPA	United States Environmental Protection Agency
CCME	Canadian Council of Ministers of the Environment
GC	Gas Chromatography
FID	Flame Ionization Detector
MS	Mass Spectrometry
EI	Electron Ionization
UV	Ultraviolet
UVC	Ultraviolet, sub-type C
HPLC	High Performance Liquid Chromatography
DCM	Dichloromethane
I.D.	Internal Diameter
AOP	Advanced Oxidation Processes
N	Nitrogen
P	Phosphorus
C	Carbon
S	Sulfur
TOC	Total Organic Carbon
FA	Fulvic Acid
HA	Humic Acid
HS	Humic Substances
NOM	Natural Organic Matter
AT1	Alberta Tier 1
GW	Groundwater

Chapter one: INTRODUCTION

1.1 Background

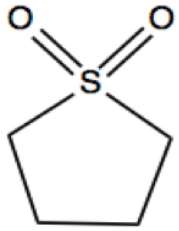


Figure 1.1: Sulfolane

Sulfolane is an organosulfur compound commonly used in the pre-treatment process of sour natural gas. It was first developed by Shell® and has since demonstrated its versatility in various organic material applications due to its thermal stability (until 220 °C) and inertness. Besides natural gas processing, sulfolane has practical uses in high performance dye-sensitized solar cells, advancement of lithium ion batteries, pharmaceutical manufacturing, and BTEX extraction (Zhang et al., (2018), Mahmoudi & Lotfollahi (2008)). However, it's extensive use in oil and gas refining processes has led to its release into the environment and is now an emergent problem.

Environmental contamination of sulfolane, particularly in soils and groundwater, has prompted research for a better understanding of this anthropogenic pollutant. Sulfolane often enters the environment via spills, unlined process water storage ponds and landfill leachate (Greene & Fedorak, 1998). Its miscibility in water inevitably results in groundwater pollution while its low adsorption to aquifer sediments (Luther et al., 1998) makes sulfolane a mobile recalcitrant contaminant.

Sulfolane can readily absorb into the human body via oral and inhalation routes, but not through the skin (Stewart and Minnear, 2010). Due to its low volatility, however, inhalation at ambient temperatures is not a cause for concern. Therefore, the main route of exposure is through drinking of contaminated water. While potential health effects of sulfolane on humans have not

been studied, its adverse effects on aquatic animals such as zebrafish larvae have been investigated. Shah (2018) observed sulfolane concentrations greater than 0.010 mg/L to disrupt gene development and above 0.8 mg/L to significantly decrease the survival rate of zebrafish. With high concentrations being prevalent in the environment where up to 800 mg/L sulfolane was recorded in shallow till aquifer (Gieg et a. 1998), effective remediation methods and regulatory measurements are warranted. The Alberta Tier 1 (AEP, 2019) remediation guidelines for groundwater and soils is 0.09 mg/L and 0.18 mg/kg, respectively.

Commercial testing laboratories have repeatedly shown to struggle with detecting sulfolane in the environment as the low regulatory guidelines are often too close to their instrument detection limits. Since sulfolane is regularly extracted with a polar organic solvent (CCME 2016) co-extracts can potentially lead to interferences during analysis (Headley et al., 2002). Depending on the analytical methods used, such as a GC-FID where a non-selective detector is used, false positives can be a cause for concern particularly for complex matrices with high organic carbon. Since the recommended extraction and analytical methods suggested by US EPA and CCME are not specific to sulfolane, it is difficult to know if such techniques result in optimum extraction and analytical results and if they are best suited for the compound. Therefore, spiked soil and groundwater samples submitted to commercial testing labs are studied for potential analytical challenges and means to possibly mitigate those issues.

Besides analytical difficulties, complex matrices and co-contaminants can also pose a challenge during remediation efforts. Several methods have shown to successfully degrade sulfolane such as advanced oxidation processes and bioremediation. Izadifard et al., (2018) fully mineralized sulfolane using ozone/CaO_2 and ozone/CaO while Yu et al., (2016) effectively treated the compound using various combinations of UVC and H_2O_2 and ozone. Both studies

demonstrated that when using real groundwater compared to pure spiked water, components such as the humic material and inorganic ions or other unknown components can impact results by quenching the hydroxyl radicals produced.

Similarly, co-contaminants found in groundwater can have a tremendous impact on sulfolane bioremediation efforts and is an area that has received very little attention. Naturally occurring co-contaminants such as inorganic minerals can have adverse effects on sulfolane degrading bacteria due to their toxicity. On the other hand, humic substances can enhance biodegradation as it has shown to do with hydrophobic compounds. Anthropogenic organic co-contaminants, conversely, can have a positive, negative or no impact at all depending on their structure, intermediates, or end-products. Kasanke and Leigh (2017) observed a 30% reduction in 0.750 mg/L sulfolane degradation rate when around 400 mg/L kerosene was present.

1.2 Research Objectives and Scope

This research has two main objectives. These are:

- Investigate the analytical challenges faced by commercial testing laboratories when analysing sulfolane in different types of matrices such as organic and mineral soils as well as groundwater.
- Examine the impact of co-contaminants on sulfolane biodegradation in groundwater from both anthropogenic and natural sources.

To address the first objective a soil and groundwater spiking study was conducted to investigate the following research questions:

1. Examine if sulfolane analytical results from soil and groundwater are comparable to the expected concentration in the spiked samples.
2. If not, inspect if the differences are related to soil type (highly organic soil versus mineral soil) or spiked sulfolane concentration (high, medium or low).
3. Investigate variance in sulfolane analytical results for soil and groundwater samples and determine if they are similar/different and under what conditions (concentration or matrix).
4. Investigate sulfolane recovery from soil using water and optimize sulfolane recovery with changing extraction parameters.
5. Examine if soil organic matter can be a source of interference during analysis.

The second objective is to understand the impact of co-contaminants on the aerobic biodegradation of sulfolane in groundwater. The co-contaminants chosen are often found alongside sulfolane, as a result of sour natural gas treatment processes or are naturally prevalent in groundwaters. Impact of DIPA (diisopropanolamine), As (III) and fulvic acids on sulfolane biodegradation is the main focus of this research question. The work plan included:

1. Investigate if DIPA has a positive, negative or negligible impact on sulfolane biodegradation. If there is a significant impact, determine if it's observed at a specific DIPA concentration.
2. Investigate the impact of As (III) at three concentrations on sulfolane degradation and determine the fate of arsenic in samples.
3. Investigate the impact of fulvic acid at two different concentrations on sulfolane removal.

4. Investigate the mixture of DIPA and As (III) on sulfolane degradation at environmentally relevant concentrations.
5. Examine the matrix effect on sulfolane removal with and without the addition of co-contaminants.

1.3 Thesis Overview

This thesis is divided into five chapters and a brief description of each is outlined below.

Chapter 1. describes background information regarding sulfolane. The chapter also briefly highlights the goals and scope of this research.

Chapter 2. presents a literature review on the properties of sulfolane, its extraction/analytical methods, remediation methods, principles of bioremediation, and possible impacts of co-contaminants.

Chapter 3. describes the spiking study carried out between various testing labs for two types of soils (organic and mineral) and groundwater. The chapter further delves into sulfolane water extraction efficiency and soil organic matter interference identification by GC-MS.

Chapter 4. investigates the impact of co-contaminants on sulfolane biodegradation in groundwater. The three co-contaminants investigated are DIPA, As (III) and fulvic acid.

Chapter 5. summarizes major conclusions drawn from this investigation as well as future directions of research.

Chapter two: LITERATURE REVIEW

2.1 Sulfolane Characteristics

Sulfolane is synthesized by reacting sulfur dioxide and butadiene in a cheletropic (Diels-Alder) reaction to give sulfolene. Sulfolene is then hydrogenated using Raney nickel catalyst to produce sulfolane (Figure 2.1). This is a highly inert compound and can withstand highly acidic and basic conditions, demonstrating its versatility in various reaction conditions. Sulfolane is also a thermally stable solvent with a vapour pressure of 0.0091 kPa at 30°C, but at elevated temperatures (> 200 °C) the compound starts to breakdown.

Even though sulfolane contains a 4-carbon alkane as part of its ring (Figure 1), saturated hydrocarbons are insoluble in the solvent due to sulfolane-alkane dispersion force interactions not being strong enough to overcome the sulfolane-sulfolane interactions (Langford, 1960). This is a dipolar aprotic solvent with a large dipole moment of 4.69D (Tilstam, 2012). Its dipoles can induce a sizeable dipole-induced dipole interaction in unsaturated and aromatic hydrocarbons by polarizing the pi electrons to promote solvation (Langford, 1960). As a result, sulfolane becomes an excellent solvent in aromatic BTEX extractions from aliphatics (CCME 2006). Various characteristics of sulfolane are listed in Table 2.1.

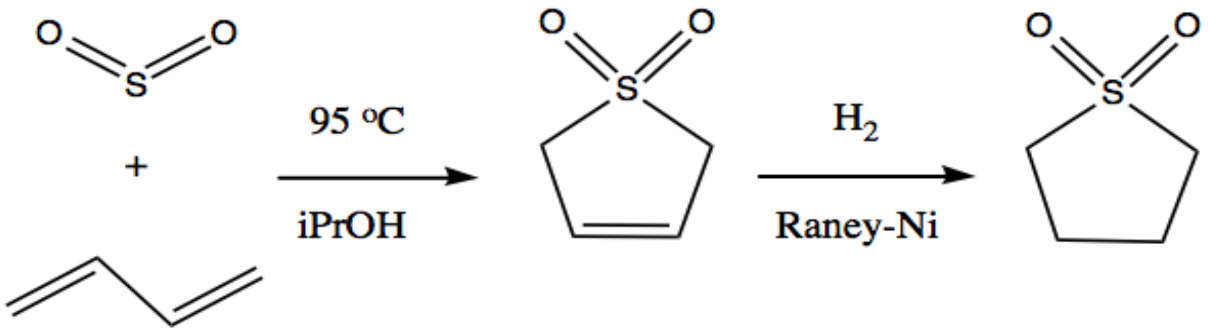


Figure 2.1: Synthesis of sulfolane (Adapted from Tilstam, 2012).

Table 2.1: Sulfolane characteristics (CCME, 2006)

Property	Value
Molecular formula	$C_4H_8SO_2$
Molecular weight	120.17 $g.mol^{-1}$
Melting point	28.5 °C
Boiling point	287.3 °C
Flashpoint	165-178 °C
Vapour pressure	
20 °C	0.01 mm Hg
118 °C	5 mm Hg
150 °C	14.53 mm Hg
160 °C	21.55 mm Hg
200 °C	85.23 mm Hg
210 °C	115.1 mm Hg
260 °C	421.4 mm Hg
Henry's law constant	$8.9x10^{-10}$ $Atm.m^{-3}.mol^{-1}$
Solubility in water	
20 °C	1,266 $g.L^{-1}$
25 °C	379, miscible $g.L^{-1}$
30 °C	Miscible $g.L^{-1}$
Pka	12.9
Dielectric constant	43.3
Dermal permeability constant (kp)	0.0002 $cm.hour^{-1}$

2.2 Sulfinol Process

Natural gas reservoirs often contain sour gas impurities such as CO_2, H_2S, COS, thiols and other organosulfur compounds and must be treated before use to be considered "pipeline quality" (Isaacs et al., 1977). The Shell Sulfinol® process utilizes sulfolane to purify natural gas and is consequently one of the main causes of environmental sulfolane contamination.

Sulfolane, however, is not the only compound used in this treatment process. Alkanolamines are also utilized as they can chemically react with sour gas impurities. Such chemical solvents can reduce the sour gas contaminants to low concentrations at low partial pressures with fast reaction rates (Bak et al., 2018). However, the outcome is extremely energy intensive due to the highly exothermic nature of the reaction and the large quantity of heat that is required for solvent regeneration. Chemical solvents are also limited by the 1:1 stoichiometric reaction ratio, indicating if the concentration of contaminants increases, the reaction rate will be limited (Bak et al., 2018). The alkanolamine commonly used is either a tertiary amine (methyl diethanol amine – MDEA) or a secondary amine (diisopropanolamine – DIPA), with the former being capable of forming stronger bonds with impurities.

Physical solvents (like sulfolane) on the other hand depend on the acid gas solubility instead of a chemical reaction. They are a highly attractive alternative due to their high absorption capacity out of stoichiometry, reduced energy for regeneration, and the ability to readily remove mercaptans and other sulfur compounds in comparison to amines (Angaji et al., 2013). The downside to physical solvents, however, is their inability to economically recover higher molecular weight hydrocarbons (C_{3+}) from the natural gas stream as they get removed alongside acidic gases.

In the Shell Sulfinol® process a combination of physical (sulfolane) and chemical solvents is utilized to obtain the benefit of both, maximizing the treatment performance (Ghanbarabadi & Khoshandam, 2015). Consequently, it is common to detect the presence of alkanolamines alongside sulfolane in contaminated environments.

2.3 Environmental Contamination

Environmental contamination of sulfolane is mostly a result of spills, landfills and unlined surface storage ponds from oil and gas processing plants (Headley et al, 2002). Due to its low vapour pressure (0.0091 kPa at 30°C), sulfolane is considered non-volatile. Its complete miscibility in water results in the high mobility of the compound with a potential to become an off-site contaminant.

Sulfolane attenuation was examined by investigating the sorption behaviour of sulfolane in various aquifer sediments, soils, and reference clays (montmorillonite and kaolinite) (Luther et al., 1998, Saint-Fort, 2006). It was observed the sulfolane partition coefficient in reference clays to be much higher (0.18 – 0.94 L/kg) than in humus-rich soils (0.099 L/kg) with montmorillonite resulting in the highest partition coefficient (0.94 L/kg). This is indicative that sorption of sulfolane is governed by clay content when organic soil content is low (< 1%). Sulfolane sorption was found to be reversible (Saint-Fort, 2006) by up to 90%. It was determined that in aquifer sediments, sulfolane will migrate at the same speed as groundwater in saturated zones as the compound has a low retardation factor. Therefore, sulfolane will likely become an off-site contaminant as the result of its low sorption affinity (Luther et al., (1998)).

2.4 Extraction and Analysis

With sulfolane contamination recorded in groundwater, surface water, soils and plants especially near gas treatment sites, Alberta Tier 1 guidelines have been set to 0.18 mg/kg for soils and at 0.09 mg/L for groundwater (AER, 2019). Such low regulatory guidelines are bound to produce analytical challenges for commercial testing laboratories as regulations are often close to detection limits of analytical instruments and could potentially lead to false positives due to matrix interference.

The Canadian Council of Ministers of the Environment (CCME-2016) has published a set of procedures following US EPA methods for soil, sediment and water sample preparation as well as analysis, applicable for *all* organics. It is recommended for water samples to be analysed using direct aqueous injection in GC-FID (or GC-MS if increased sensitivity is needed) and for soils to be subject to a leaching test and the leachate analysed. Other approaches, such as preparation method 3540C (USEPA) which outlines a Soxhlet extraction for general nonvolatile organics from matrices such as soils, sludge, and solid wastes have also been recommended for sulfolane extraction. Soxhlet requires 5-10 g of soil to be mixed with anhydrous sodium sulfate, placed into an extraction thimble and extracted using an organic solvent in semi-continuous manner.

The advantage of Soxhlet is that the sample is repeatedly extracted with fresh portions of the solvent, resulting in high extraction efficiency. However, this process takes a long time to complete (16 to 24 hrs) and also uses large volumes of solvent. The extensive use of solvents in highly organic matrices can also produce co-extracts that can potentially interfere with sulfolane analysis (Headley et al., 2002).

Preparation procedure for organic extraction from aqueous samples such as groundwater is listed in Method 3510C (USEPA), which involves solvent extraction of the analyte using a

separatory funnel. The advantage to using this method is the low time requirement (three 2-minute extractions followed by filtration), however, utilization of solvents can potentially lead to inaccuracies due to their high volatility and extraction inefficiencies.

Various studies have successfully demonstrated sulfolane extraction from soils, sediments and water without the use of extensive methods. Greene & Fedorak (2001) studied sulfolane extraction by extracting 1 g soil samples with 2 successive 5 mL aliquots of water, with extraction efficiency of 92-102%. Headley et al., (1999) followed a similar method extracting sulfolane from wetland vegetation, but after performing the aqueous extraction a back-extraction with water-saturated toluene was carried out with an efficiency of 80 ± 12%. Utilization of DCM for sulfolane extraction from aqueous matrices is also common throughout literature. Izadifard et al., (2018) obtained a sulfolane extraction efficiency of 80% using DCM with 1:2 ratio of water sample:DCM in a cold-shake extraction.

While the recommended preparation methods for various matrices differ, their analysis is often the same. As stated by CCME (2016), Method 8015D, which incorporates the use of a GC-FID instrument, is the recommended method of analysis. The techniques used for introducing sulfolane samples to the GC are: 1) direct aqueous injection of samples or 2) solvent extraction. The downside to using an FID instrument is that it employs a non-selective detector, resulting in risk of interference by non-target compounds as well as poor resolution for samples containing interfering organic compounds. Greene et al., (1998) employed a GC-FID direct aqueous injection analysis using a 2 m x 0.3 mm stainless steel column packed with 5% polyphenyl ether 6-ring coated Tenax-GC, 60/80 mesh, however, the method was applicable to concentrations above 0.5mg/L.

Several studies (Kasanke and Leigh (2017), Headley et al., (1999)) have employed a GC with a MS detector to obtain lower detection limits and to mitigate potential sources of interference. Headley et al., (1999) employed a GC system fitted with a 25 m x 0.25 mm I.D. DB5-MS fused-silica open tubular column. Selective ion mode (SIM) was used for ions at m/z 120, 56, & 41. Ion m/z 41 was used for quantification of sulfolane due to its high intesity. Ions m/z 120 and 56 were also monitored for confirmation of sulfolane's presence, obtaining a detection limit of 90 ng/g for wetland vegetation.

With sulfolane's miscibility in water and negligible interaction with soil components (Luther et al., 1998) water extraction and direct aqueous injection are favoured, which can possibly reduce backgroud noise associated with solvent extraction. One analytical technique missing from the recommended list of methods in CCME is the utilization of HPLC-MS for sulfolane. Limited literature is available on the analysis of sulfolane using this instrument. Headley & Peru (2002) analysed a sulfolane metabolite (3-hydroxysulfolane) using HPLC-MS, however, the technique was considered to be rugged and further study is required to obtain optimum conditions.

In this study, a spiking experiment was carried out between various testing labs with different methods of sulfolane analysis. The variation of their results was compared based on concentration and matrix (soil and water). Interference in soil samples and groundwater have been flagged as a concern when analysing sulfolane from oil and gas processing plants. The purpose of this study is to further understand any potential issues that arise when analysing sulfolane in different matrices.

2.5 Remediation Methods

Sulfolane's miscibility in water, thermal stability, low vapour pressure, and low affinity for sorption to soils makes the compound persistent in the environment. Therefore, a suitable remediation method is warranted particularly for groundwater contamination. Advanced oxidation processes and bioremediation are the few treatment techniques used to successfully remove sulfolane.

2.5.1 Advanced Oxidation Processes

Advanced oxidation processes are powerful methods for degrading and mineralizing persistent compounds in the environment. These methods can be light induced (UV light) or non-light induced (H_2O_2 and O_3). Often times a combination of different methods are incorporated to achieve maximum degradation particularly for compounds like sulfolane that do not absorb UV light above 200 nm (Yu et al., 2016). UV irradiation and ozone have been used for water disinfection for many years. The reaction chemistries of AOPs often involve the production of free hydroxyl radicals produced with oxidants like ozone and hydrogen peroxide. There are several pathways to produce hydroxyl radicals which include: UV light dissociating H_2O_2 and forming two $^{\cdot}OH$ radicals via bond cleavage; ozone reacting with hydrogen peroxide; and UV initiating the reaction between ozone and water to form hydrogen peroxide, which subsequently reacts with ozone and UV to form hydroxyl radicals (Staehelin and Hoigne, (1982), Chang et al., (2015)).

Yu et al., (2016) successfully degraded sulfolane by utilizing UVC irradiation in conjunction with O_3 and H_2O_2 and their various combinations in synthetic waters. With UVC alone, a 27% reduction of sulfolane was observed within 3 hours and in conjunction with H_2O_2 and ozone a higher degradation rate was observed. Mehrabani-Zeinabad et al., (2016) confirmed that the

combination of UVC/ozone/H_2O_2 had a greater degradation rate compared to UVC/O_3 and UVC/H_2O_2 alone. Izadifard et al., (2018) demonstrated that sulfolane can be degraded under non-light induced oxidative processes, for example, with CaO/O_3 and CaO_2/O_3. The studies mentioned observed positive or negative impacts of sulfolane degradation when the experiments were carried out in real groundwater versus in synthetic lab waters. This indicates that the presence of humic substances, inorganic minerals and other unknown factors can potentially influence the reaction chemistry.

While AOPs are extremely effective, utilization of such strong oxidants may at times lead to the formation of toxic by-products particularly in the complex matrix of groundwater. Formaldehyde, acetaldehyde, glyoxal, methyl glyoxal are a few organic by-products found in groundwater as a result of incomplete mineralization of natural organic matter under ozonation. Bromates have also been known to form during advanced oxidation. The high energy and cost associated with ex-situ treatment required for such methods also make this technique unfavourable.

2.5.2 Bioremediation

Biodegradation is the utilization of microorganisms (bacteria) to destroy or transform contaminants in soils, waters and sediments. Microbes can either completely convert organic compounds into inorganic products (known as mineralisation), produce new organic products from the parent contaminant, or have negligible impact on the chemical in question. Microorganisms possess enzymes that enable them to use organic pollutants as carbon sources and electron donors to grow and reproduce new cells. By breaking the chemical bonds of the contaminant (electron donor) and transferring the electrons to an electron acceptor (such as oxygen), the microorganisms are able to oxidize part of carbon in the contaminant to CO_2 and use the remaining to produce new cell mass (National Research Council, 1993).

In aerobic biodegradation, microorganisms (aerobes) use oxygen as an electron acceptor. However, in anoxic environments devoid of O_2, anaerobes can use sulfate (SO_4^{2}), nitrate (NO_3^{-}), iron (Fe^{3+}), manganese (Mn^{4+}) or even CO_2 as the electron acceptor in anaerobic degradation (National Research Council, 1993). Similar to aerobic conditions, the end-product of anaerobic biodegradation is water, carbon dioxide and biomass, but additional by-products such as reduced metals, hydrogen sulfide or methane may also be produced which does not occur in aerobic respiration.

Several environmental aspects can impact the behaviour of indigenous microorganisms and consequently effect the process of biodegradation. For example, decreased microbial activity correlates with low temperatures as microbial enzymatic activity declines (Zou and Crawford, 1994) and extreme pH levels can cause great stress on the bacteria past their tolerance levels, and retard their growth (Alexander, 1995). However, often times the most significant factor hindering bioremediation of organic compounds is the availability of electron acceptors.

The dissolved oxygen content controls the fate of contaminants in the aquifers as it limits the type and the number of microorganisms capable of degrading the compound (Rose and Long, (1988)). Oxygen is frequently depleted in aquifers contaminated with hydrocarbons, particularly if the contaminant concentration (electron donor) is significantly higher than the electron acceptor concentration. Fortunately, remediation technologies such as bio-sparging (Johnston et al., 1998), ozone injection (Hu and Xia, 2018) and even use of oxygen releasing compounds (Kunukcu, 2007) can be used to increase the oxygen concentration for remediation efforts (USEPA, 2003).

One other factor impacting bioremediation is the low levels of inorganic nutrients such as N and P, particularly if there is a large influx of carbon compounds. Nutrient supplementation is not typically required for biodegradation to occur as naturally occurring N & P levels have been deemed sufficient to sustain bacterial growth. However, their addition has shown to increase degradation rate (Alexander, 1995).

2.6 Aerobic Bioremediation of Sulfolane

Several studies have investigated biodegradation as a mean to treat sulfolane and have often focused on bio-stimulation rather than bio-augmentation. Bio-stimulation is a cost-effective approach which stimulates indigenous microorganisms of the contaminated site to treat pollutants (Tyagi & Fonseca, 2011). Bio-augmentation, on the other hand, is the introduction of specific competent bacterial strain to the polluted area to enhance the genetic diversity of the native microbial population incapable of degrading a contaminant.

Sulfinol® polluted soils, sediments, and groundwaters have repeatedly demonstrated to contain viable microorganisms capable of degrading sulfolane when stimulated with appropriate levels of oxygen and inorganic nutrients. Shake-flask slurries are a common method to study these parameters in a laboratory setting as the vigorous mixing provides high oxygen levels. Fedorak and Coy, (1996) used aerobic shake flask cultures and solid phase soil bioreactors (representing bioventing) to investigate sulfolane biodegradation. The native microorganisms in soils, groundwater and sandstone used from contaminated aquifers demonstrated their ability to fully degrade sulfolane. An increase in sulfolane removal rate was observed (up to 5 times) when samples were supplemented with inorganic nutrients (K_2HPO_4, KNO_3, NH_4Cl) in comparison to non-supplemented samples.

Greene and Fedorak (2001) evaluated nutrient stimulation of sulfolane biodegradation in shake-flask cultures. Since shake-flask experiments don't fully represent field conditions due to the lack of oxygen present in real aquifers, an air-sparged microcosm was also developed to represent bio-pile remediation in fields. Both contaminated and uncontaminated soils (with and without previous exposure to sulfolane) were used to determine if they contain a diverse microbial community to degrade sulfolane. Sulfolane was indeed removed in both soils and the addition of

N & P decreased the lag times. The air-sparged microcosms, however, demonstrated a longer removal rate as oxygen was a rate limiting factor in this scenario.

In another study by Greene et al., (1999), a gently aerated 2.5 L microcosm was used to demonstrate sulfolane biodegradation in an attempt to replicate *in situ* conditions since intense mixing does not occur in fields as it does in bench-scale experiments. Under slow agitation, sulfolane removal rate was much slower in comparison to shake-flask slurries. The dissolved oxygen concentration of the 2.5 L microcosm (7.5-10mg/L) was deemed sufficient to fully remove sulfolane. Therefore, as long as contaminated site materials are supplemented with oxygen and sufficient nutrients (Khan et al., 2019), sulfolane degradation will occur. The degradation rate, however, will vary depending on the concentrations of said nutrients. It can be concluded that soils, sediments and groundwater contaminated previously exposed to sulfolane contain viable bacterial communities capable of degrading sulfolane.

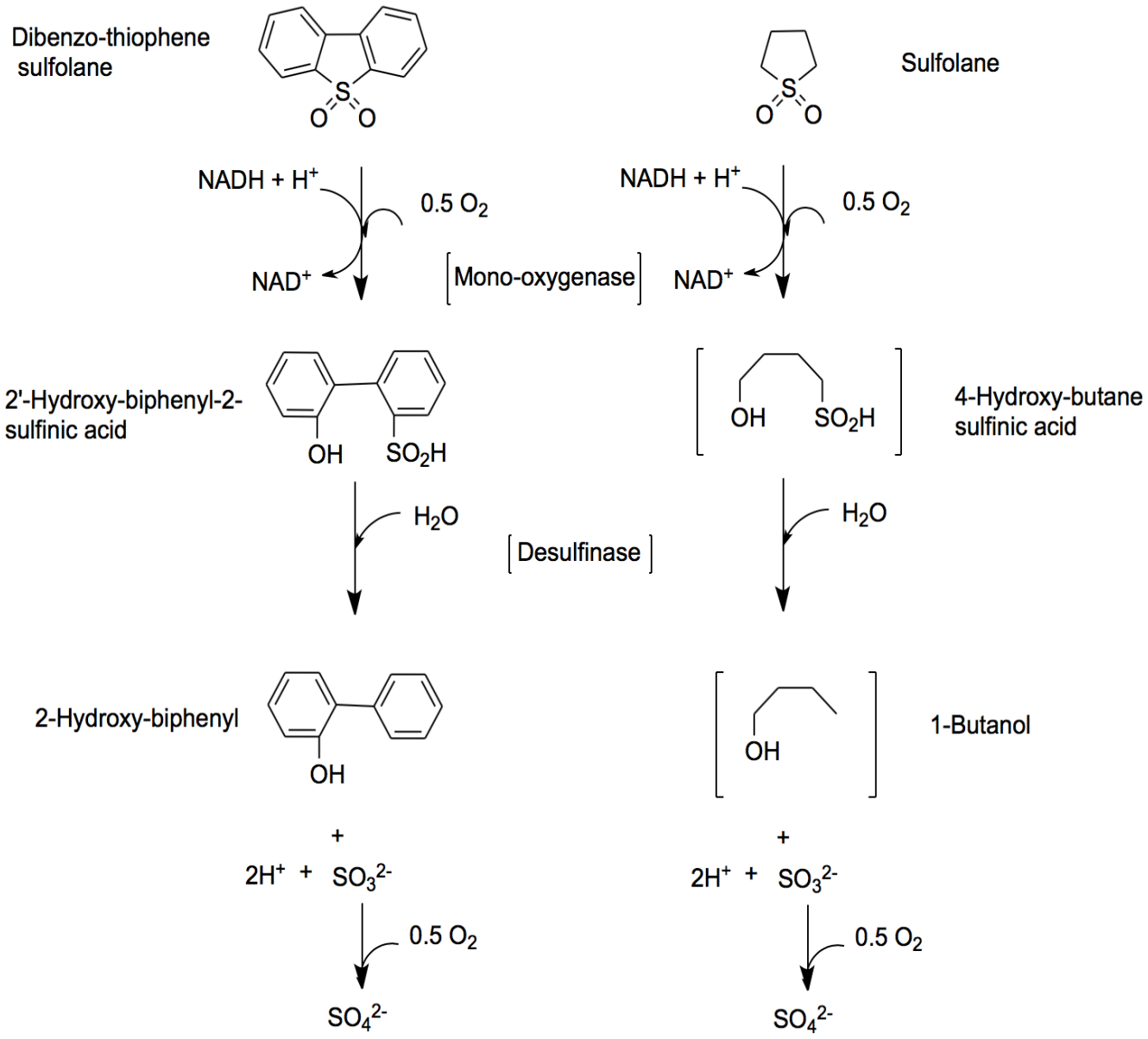
2.7 Bio-Mechanism

Greene et al., (2000) demonstrated sulfolane biodegradation using mixed cultures in comparison to the bacterial isolate similar to *Variovorax paradoxus* grown on sulfolane as the sole C, S and energy source. Mixed cultures showed a greater capability (60 – 80 %) in degrading sulfolane versus the bacterial isolate (40 - 40 %), indicating that a diverse range of bacteria is more competent than a single strain. The study also hypothesized the biotic degradation pathway of sulfolane under aerobic conditions (Figure 3) based on the established degradation mechanism of dibenzothiophene sulfone stated in the literature.

An end product of both dibenzothiophene sulfone and sulfolane is sulfate (confirmed by Chou and Swatloski (1983)) and by using the same analogy, sulfolane's bio-mechanism is hypothesized (Figure 2.2). The study predicts that sulfolane ring cleavage starts by C-S bond breakage as they are typically weaker than C-C bonds, forming 4-hydroxybutane sulfinic acid first, then consequently forming 1-butanol after removal of the sulfone group (called desulfinase). The end products are then CO_2, H_2O and SO_4^{2-} (equation 1). A total organic carbon analysis (TOC) done by Khan et al., (2019), indicates that at 50% sulfolane removal 35% TOC is removed during sulfolane biological treatment with TOC linearly decreasing with sulfolane removal. Therefore, it is unlikely any intermediates formed during sulfolane degradation will persist in the environment, however, further study is required to confirm this hypothesis.

$$C_4H_8O_2S + 6.5O_2 \rightarrow 4CO_2 + 3H_2O + 2H^+ + SO_4^{2-} \quad (1)$$

Figure 2.2: Biodegradation pathway of sulfolane and dibenzothiophene sulfone (Greene et al., (2000))



2.8 Anaerobic Bioremediation of Sulfolane

While aerobic biodegradation of sulfolane has shown to be successful, anaerobic conditions have yet to demonstrate the same results. It is critical to understand the fate of sulfolane in conditions devoid of oxygen as in real aquifers the dissolved oxygen content will be depleted as a result of bacterial metabolism (Rose and Austin, 1988).

Greene et al., (1998) studied anaerobic degradation of sulfolane under nitrate, Mn(IV), Fe(III), sulfate and CO_2-reducing conditions. Only 4 out of the 60 anaerobic microcosms supplemented with sulfolane demonstrated degradation which were under Mn(IV) and nitrate reducing conditions. Kim et al., (1999), on the other hand, observed a 64% sulfolane removal under anaerobic conditions, however, they do not specify which electron acceptor is utilized for the study.

Similar to Greene et al., (1998), Kasanke and Leigh (2017) also did not observe any anaerobic sulfolane degradation under nitrate, sulfate or iron-reducing conditions. All were incubated for 1021 days for nitrate and sulfate reducing conditions, and 391 days for iron-reducing conditions, yet no sulfolane removal was observed. Therefore, anaerobic degradation of sulfolane has not been a reliable remediation method as a treatment technique.

2.9 Sulfolane Co-contaminants in Alberta's Wells

While biodegradation has shown to be a promising technique for treating sulfolane, rarely has the impact of co-contaminants on its degradation been a subject of a study. In real environmental conditions, organic compounds are not found individually but rather alongside other pollutants that can be used by one or more of the indigenous microbiota. These substrates can be synthetic, naturally formed minerals, and even dissolved organic matter. As a result, biodegradation of a single substrate will differ when multiple substrates are present.

Simultaneous metabolism of two substrates has been reported throughout the literature. The degradation rate of glucose in activated sludge has shown to be unaffected by the simultaneous degradation of acetate. Linear alkanes ($C_{16\text{-}30}$) have also demonstrated coinciding degradation alongside oil-contaminated sediments and waters (Alexander, 1995). There have even been instances where one substrate improves the degradation rate of another. Toluene has shown to stimulate the degradation of benzene and p-xylene by pseudomonas (Alvarez & Vogel, 1991).

However, it is entirely possible for the presence of one substrate to inhibit the degradation rate of another. Organisms metabolizing one compound could be competing for oxygen and nutrients with organisms metabolizing another substrate (Steffensen and Alexander, 1995). As a result, the degradation of one is inhibited. Suppression of degradation rate by the presence of toxic co-contaminants can also play a major role. Even if the pollutants present are not considered toxic to the microorganisms, they might eventually degrade to products harmful to the bacterial population degrading a second contaminant (Lindley & Heydeman, 1986).

The focus of this study is to examine the impact of co-contaminants on sulfolane biodegradation in groundwater to investigate a more representative scenario of real environmental conditions. Results published by Kasanke and Leigh (2017) is one of the few studies investigating

the impact of co-pollutants on sulfolane biodegradation. They observed a ~30% reduction in sulfolane's degradation rate with the addition of kerosene using Alaska's subarctic aquifer groundwater. However, in this study the co-contaminants of interest are specific to an Alberta site and include DIPA, As (III) and fulvic acid. It is critical to investigate the impact of such co-contaminants of sulfolane bioremediation since different compounds can impact sulfolane degradation differently and can hinder/enhance remediation efforts.

2.9.1 DIPA

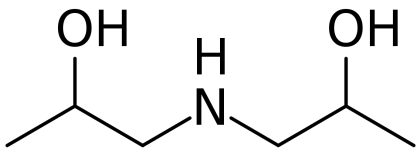


Figure 2.3: DIPA

DIPA is a chemical solvent used in the Sulfinol® process where spills containing 45% sulfolane and 40% DIPA have been previously reported. Similar to sulfolane, DIPA is miscible in water and is non-volatile with a vapour pressure of 0.0027 kPa. Conversely, DIPA has a higher distribution coefficient (3.5-43 L/Kg) compared to sulfolane (0.18-0.94 L/Kg), indicative of its high attenuation in soils, particularly clays. As a result, DIPA moves 3 to 8 times slower than sulfolane in ground waters (Luther et al., 1998). Nonetheless, concentrations of 6 ppm and 590 ppm have been documented in sand aquifers and shallow till aquifers, respectively (Greene et al., 1999).

Sulfinol® contaminated aquifers have shown to contain viable microbial populations capable of degrading DIPA both aerobically and anaerobically when appropriate levels of N and P are present (Greene et al., 1999, Gieg et al., 1998). Methylglyoxal, a compound with antimicrobial

properties, was found to be one of DIPA's biodegradation intermediates (Gieg et al., (1999)). However, ammonia was reported as the end product of aerobic biodegradation of DIPA which can serve as potential nitrogen nutrient for sulfolane-degrading bacteria (CCME, 2005). The biochemical reaction for biodegradation of DIPA is shown in equation (1). Gieg et al., (1999) found that DIPA's biodegradation would contribute to the total available nitrogen and can potentially provide a source of nutrient to sulfolane degrading bacteria. As a result, it is critical to investigate the impact of DIPA on sulfolane biodegradation as both compounds often co-exist in groundwater wells.

$$C_6H_{15}O_2N + 8O_2 \rightarrow 6CO_2 + 6H_2O + NH_3 \qquad (1)$$

2.9.2 As (III)

Arsenic is a naturally occurring metalloid that can be found alongside sulfolane in contaminated groundwaters. In Alberta, arsenic-containing bedrock formations are often associated with naturally elevated levels in of this metalloid (Silver & Phung, 2005) which contain marine shale deposits known to be enriched with arsenic (Lemay, 2002). For example, in Alberta's Southern Oil Sand Regions (SOSR), shallow groundwater often contains dissolved arsenic concentrations well above the 0.010 mg/L guideline levels (Moncur et al., 2015).

The predominant oxidation states of arsenic are As (III) and As (V), with As (III) being the more toxic of the two (Lloyd & Oremland, 2006). Since As (III) is poorly sorbed onto mineral phases in comparison to As (V), it is found in higher concentrations and is more mobile. This is attributed to reducing conditions caused by dissolution of Fe-oxyhydroxides from pyrite

weathering, which is a common cause of As (III) transport in groundwater throughout the world (Yadav et al., 2015). Consequently, this research focuses on the impact of As (III) specifically, as it is less bound to surfaces.

While As (III) is extremely toxic to many microorganisms (Silver & Phung, 2005), some bacteria have evolved to detoxify this metalloid. Under aerobic conditions, As (V) prevails and in anaerobic conditions As (III) predominates. Over 30 strains of bacteria have been identified to oxidize As (III) to As (V) (Lloyd & Oremland, 2006) with strains including Actinobacteria, Microbacterium, Pseudomonas and Rhizobium, commonly found in As-rich groundwater samples of West Bengal. Such bacteria are categorized as heterotrophic As (III)-oxidizing bacteria where they simply detoxify the metalloid while respiring oxygen and using organic matter to grow. Chemolithoautotrophic bacteria, on the other hand, have demonstrated to gain energy through As (III) oxidation, but with the fixation of inorganic carbon (CO_2) for cell material (Omeland & Stolz, 2005).

With varying possible impacts of As (III) on microorganisms, it is critical to investigate the impact of a common groundwater metalloid such as arsenic on sulfolane biodegradation and as to the best of my knowledge, no other study has delved into this topic previously.

2.9.3 Fulvic Acid

Natural organic matter (NOM) is a naturally found constituent of groundwaters and is the outcome of decomposed plant and animal tissues. They vary in size, molecular weight, elemental composition, structure and position of functional groups depending on its origin and age. As a result, they are a mixture of organic compounds, oligomers, polymers, and small molecules (Kordel et al., 1997). They are found occurring in soils, water (surface and groundwater) and

sediments throughout all environments (Gaffney et al., 1996). These organic matters can interact with various contaminants through adsorption, ion exchange and complexation and impact the bioavailability of chemicals in the environment.

In aquatic systems, NOM portions smaller than 0.45 μm in diameter are termed dissolved organic carbon (DOC), which constitute about 90% of the total organic carbon of NOM. Traditionally, soil derived aquatic DOC have been separated into two categories and further subdivided based on their solubilities (Leenheer and Crouè, 2003). These categories include humic substances (HS) and non-humic substances (NHS), with the former making up to 70% of the dissolved organic carbon. HS consist of humic acids (HA), fulvic acids (FA) and humin and are fractioned based on their solubilities under acidic or basic conditions. Fulvic acids are the fraction soluble in water at all pH levels, while humic acids are insoluble at pH levels below 2. Humins are the insoluble organic material regardless of pH.

In general, aquatic FA have a lower molecular weight (500-2000 atomic mass units) compared to HA (1000-10000), with soil derived material being larger than aquatic material (Gaffney et al., 1996; Thurman and Malcolm, 1981). HS consists of alkyl/aromatic units cross-liked mainly by oxygen and nitrogen groups. The main functional groups present in these organic materials are carboxylic acids, phenolic and alcoholic hydroxyls, ketones and quinone groups. Compared to HA, the structure of FA is less aromatic and is richer in carboxylic acid and ketonic functional groups (Thurman and Malcolm, 1981; Matilainen et al., 2011). As a result, FA have more polar groups per unit mass than HA and are consequently more soluble in water. FA contributes as much as 95% of the humic substance fraction in groundwater, with concentrations as high as 10 mg/L. This can be attributed to the higher solubility of FA and limited solubility of HA since the latter has a stronger affinity for sorption on surfaces (Gaffney et al., 1996).

Colloidal HS (> 10 μm) found in groundwaters have the ability to bind with both water-soluble and water-insoluble species as they contain polar and nonpolar functional groups (McCarthy and Zachara, 1989) and can negatively or positively impact a compounds bioavailability for degradation. On the other hand, microorganisms could preferentially use NOM as a carbon source instead of the pollutant in question, decreasing the degradation rate of the compound as a result. Presence of HS was observed to have inhibitory effects on the biodegradation of m-cresol, m-aminophenol and p-chlorophenol (Shimp and Pfaender, 1985). The bacterial colonies in question, however, had previous exposure to HS and were adapted to the organic matter. Conversely, with short-term previous exposure to HS, no suppressive effect was observed on degradation of the phenols. Lu and Speitel Jr (1991) investigated the effect of NOM on pentachlorophenol (PCP) biodegradation. They observed a negative impact on PCP removal in the presence of NOM, and the degradation was enhanced once the organic matter was treated with oxidants. Therefore, it would be crucial to investigate the impact of organic materials such as FA on sulfolane biodegradation as remediation efforts could be significantly impacted in the presence of NOM.

Chapter three: EXTRACTION AND ANALYSIS

3.1 Introduction

Sulfolane is a highly polar, water miscible compound. It is very mobile in the aqueous phase and quickly becomes a groundwater contaminant once spilled onto soils (Luther et al., 1998). Environmental contamination due to extensive use of sulfolane in oil and gas processing plants in Alberta has driven the need to define methods of analysis in soil and water, and to establish representative procedures for testing of this compound. Nonetheless, there are limited publications available documenting optimum analytical and extraction techniques in complex matrices. Sulfolane guideline for soils and groundwater are set at 0.18 mg/kg and 0.09 mg/L, respectively (AER, 2019). Commercial testing labs often face challenges with such concentrations as the guideline levels can be too close to their instrument detection limits.

The Canadian Council of Ministers of the Environment (CCME 2016) has published a set of procedures following USEPA methods for soil, sediment and water sample preparation as well as analysis, applicable for general organics. However, the recommended methods suggested by the environmental regulatory bodies are not specific to sulfolane, but for general organic contaminants.

Method 3540C (CCME 2016) recommends a Soxhlet extraction to recover sulfolane from soils, sludges, and solid wastes (USEPA 3540C). While Soxhlet can provide effective extractions, this method is not specific for sulfolane and is a common technique recommended for general nonvolatile organics. Soxhlet also takes a long time to complete (16 to 24 hrs), uses large volumes of solvent and is mainly recommended for water-insoluble and slightly water-soluble organics. Similarly, method 3510C (US EPA) which involves solvent extraction using a separatory funnel

has been suggested for sulfolane extraction from aqueous mediums. These methods are labor-intensive, require large volumes of solvent, can potentially extract non-target compounds (Headley et al., 2002) and risk biasing the results high due to solvent volatility.

There are several papers available that demonstrate simpler extraction methods of sulfolane from soils and aqueous media. Izadifard et al., (2018) extracted sulfolane from 1 mL of water into 2 mL of DCM and obtained an extraction efficiency of about 80%. Greene and Fedorak (2001) took advantage of sulfolane's miscibility in water and low sorption to soils and extracted the compound from 1g of soil using 2x5mL aliquots of water, resulting in a recovery of 90-102%. Headley et al., (1999) followed a similar method for extracting sulfolane from plants, but after performing the aqueous extraction a back-extraction with water-saturated toluene was carried out, obtaining a recovery of 80 ± 12%.

A recommended analytical method for sulfolane (CCME 2016) suggests the utilization of GC-FID either through a direct aqueous injection or solvent extraction. There is a cause for concern, however, that the non-selective detector of an FID results in risk of interference by non-target compounds when dealing with complex environmental matrices. Besides GC-FID, other analytical methods have been successfully utilized for sulfolane analysis. Headley et al., (1999) used a GC-MS with a DB5-MS column (25 m x 0.25 mm id, 0.25 μm thickness) with a detection limit of 9 $x10^{-6}$ mg/L using Selective Ion Mode and Headley and Peru (2002) analysed a sulfolane metabolite (3-hydroxysulfolane) using HPLC-MS with a C18 column. Thus, there are other analytical and extraction techniques of sulfolane available not listed by regulatory bodies which can be used for this compound.

The current study was inspired by erratic environmental monitoring results suggesting potential interferences affecting sulfolane results in soils (particularly highly organic) and groundwater samples from an industrial site in Alberta. Spiked soils and groundwater were submitted to several commercial testing laboratories and the variance in their results was quantified. The aim of this research is to investigate potential challenges that arise with analyzing environmental samples from oil and gas processing plants in an effort to possibly mitigate these issues.

3.2 Methods

3.2.1 Soil Spiking

Organic and mineral soils were dried and ground by testing lab A to ensure homogenization before spiking. Soils were autoclaved for 2x30 minutes at 121 ^{o}C to minimize microbial activity. Each type of soil was then spiked to 100 mg/kg, 10 mg/kg and 0.5 mg/kg sulfolane using a spray bottle and mixed thoroughly to fully coat soils with solution. A brine solution of 1500 mg/kg NaCl was used to help with homogenization of mineral soils. Once dried, soils were again homogenized and a sample splitter was used to increase randomness of each sample. Soil and groundwater samples were prepared in laboratory-supplied containers and stored on ice in coolers until submission to each testing laboratory. The specific analytical and extraction method for each laboratory used is listed in Table 3.1. University of Calgary is denoted as Lab E. Soil characteristics are listed in Table 3.2. A Soxhlet extraction using DCM was carried out according to US EPA method 3540C for both soils in Lab E using 5-10 g of soil and sodium sulfate to remove sample moisture.

Table 3.1: Chosen laboratories and their corresponding instrumentation, extraction method and detection limit for sulfolane analysis.

Lab	Sample Matrix	Instrumentation	Extraction Method	Detection limits
A	- Soil	- GC/FID	- Water mechanical extraction	- 0.15 mg/kg
	- Water	- GC/FID	- Direct injection	- 0.2 mg/L
	- Water	- GC/MS	- DCM	- 0.001 mg/L (Low level)
B	- Soil	- HPLC/MS	- Acidified water mechanical extraction	- 0.10 mg/kg
	- Water	- HPLC/MS	- diluted with glacial acetic acid	- 0.05 mg/L
C	- Soil	- HPLC/MS	- Organic free water mechanical extraction	- 0.05 mg/kg
	- Water	- HPLC/MS	- No extraction, direct analysis	- 0.003 mg/L
D	- Soil	- GC/MS	- DCM soxhlet extraction	- 0.05 mg/kg
	- Water	- GC/MS	- DCM:ethyl ether	- 0.003 mg/L
E	- Soil	- GC/MS	- DCM soxhlet extraction	- 0.010 mg/kg
	- Water	- GC/MS	- DCM	- 0.005 mg/L

Table 3.2: Characteristics of organic and till soils used in this study (obtained from Lab A)

Soil Characteristic	Organic	Mineral
pH	4.94	7.51
Conductivity (dS/m)	0.36	1.1
Sodium Adsorption Ratio	0.55	0.57
Total Organic Carbon (mg/kg)	485000	9900
Texture	Peat	Clay (45%), Sand (30%), Silt (25%)
Cation Exchange Capacity (cmol+/kg)	160	20

3.2.2 Groundwater Spiking

A 3L stock solution of groundwater was prepared with 0.5 mg/L and 0.1 mg/L sulfolane. Sulfolane (99%) was obtained from Sigma Aldrich. The groundwater characteristics are listed in Table 3. Triplicates of each concentration were given to each laboratory listed in Table 1 and analysed using their specific instrumentation and extraction method. Groundwater characteristics are listed in Table 3.3. A 30 minute DCM-cold shake extraction (water:DCM 5mL:3mL) was used by Lab E to extract sulfolane from groundwater. An 80% extraction efficiency was obtained.

Table 3.3: Characteristics of the groundwater used in this study (obtained from Lab A)

Characteristics	**Water**
pH	7.9
Ca	95 mg/L
Mg	18 mg/L
Na	11 mg/L
K	0.61 mg/L
Cl	16 mg/L
SO_4	18 mg/L
Bicarbonate	300 mg/L
Total Dissolved Solids	310 mg/L
Total Organic Carbon	3.1 mg/L

3.2.3 Extraction Efficiency Test

A separate batch of organic and mineral soil (different from section 2.1) was spiked to 100 mg/kg sulfolane using 100 g of each soil. Soil samples of 2 g were extracted with 10, 20, and 40 mLs of water and further extracted into DCM (5:3 water:DCM) for GC-MS analysis. The water extraction method was later investigated at extractions times of 30, 60 and 90 minutes.

3.3 GC-MS Method

An Shimadzu QP2010SE Gas Chromatograph equipped with a mass spectrometer and electron ionization (EI) was utilised for sulfolane analysis. An RTX-5MS (15 ft x 0.25 μm thickness x 0.25 mm ID) column was used for chromatographic separation with a flow of 1.53 mL/min and pressure of 85.0 kilopascals (kPa). The starting oven temperature was 40 °C which was ramped to 200 °C at 10 °C/min where it was held for 2 min, then ramped at 20 °C/min to 280 °C where it was held for 5 minutes with a total run time of 18 minutes. Split/split-less inlet mode with 1 μL injections with an EST Flextest autosampler was used for all samples. All samples were analysed in selective ion mode (SIM mode) with He as the carrier gas. Organic soil leachate was collected with water, which was further extracted into DCM and analysed with GC-MS in Scan Mode to identify potential sources of interference.

3.4 Z_L-score

A Z_L-score was used to measure acceptable deviation from expected soil and groundwater results as shown in equation (1) (AMCTB-74). The acceptable uncertainties chosen for soils include extraction uncertainty (u_{ex}) of ± 20% with analytical uncertainty (u_{an}) of ±10%. Variables $\boldsymbol{x_i}$ and $\boldsymbol{x_{ex}}$ represent the value obtained and value expected, respectively. Based on calculations, a z-score of ± 2 is deemed acceptable, while between ± 2-3 is questionable, with z >3 is unacceptable. For groundwater, an analytical uncertainty of 10% was chosen to calculate the Z_L-score. For non-detectable samples, 50% of the reported instrument detection limit was used for graphs and statistical evaluations.

$$Z_L score = \frac{(x_i - x_{ex})}{\sqrt{(u_{ex})^2 + (u_{an})^2}} \quad (1)$$

3.5 Results and Discussion

3.5.1 Soils

Sulfolane recovery from organic and mineral soils (spiked at 0.5, 10, and 100 mg/kg) from 5 testing laboratories are shown in Figures 3.1 and 3.2, respectively and are listed in Table 3.4. At higher concentrations (>1 mg/kg), both soils demonstrated a bias low in sulfolane recovery. The variability in data also significantly increased with a decrease in the spiked concentration, and more so in organic than mineral soil. Samples spiked at 0.5 mg/kg were more likely to be undetectable in organic soil. Testing labs B (water extraction - HPLC-MS analysis) and D (Soxhlet extraction - GC-MS analysis) were forced to raise their instrument detection limits due to increased background noise caused by organic samples. Yet, sulfolane detection was still not successful after such manipulations.

According to Lab B, the organic soil samples appeared to be ash-like and thus were not behaving like typical field soils when extracted, with a potential to affect accuracy of results. Lab B also indicated that elevated sodium levels in the mineral soil had the potential to affect the laboratory's HPLC-MS detector and consequently impact the accuracy of the reported results. Consequently, there was potential that modifications might be required in their routine procedures. Due to such challenges, samples were delivered to Lab C from Lab B for analysis. However, after discussions, Lab B was willing to report their initial results using their routine procedures although in some cases the detection limit needed to be raised. Besides those mentioned, no significant challenges were reported by the other testing laboratories.

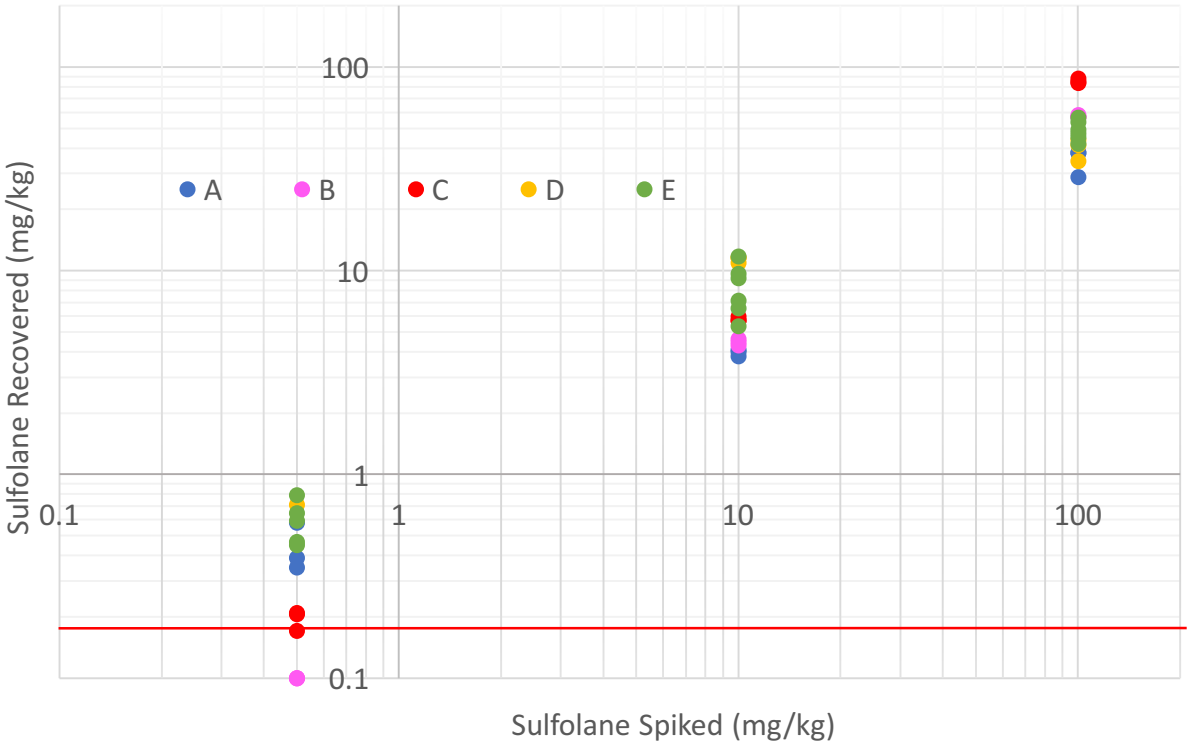


Figure 3.1: Sulfolane recovered from organic soil obtained from testing labs A, B, C, D and E. *Red line indicates AT1 guidelines.*

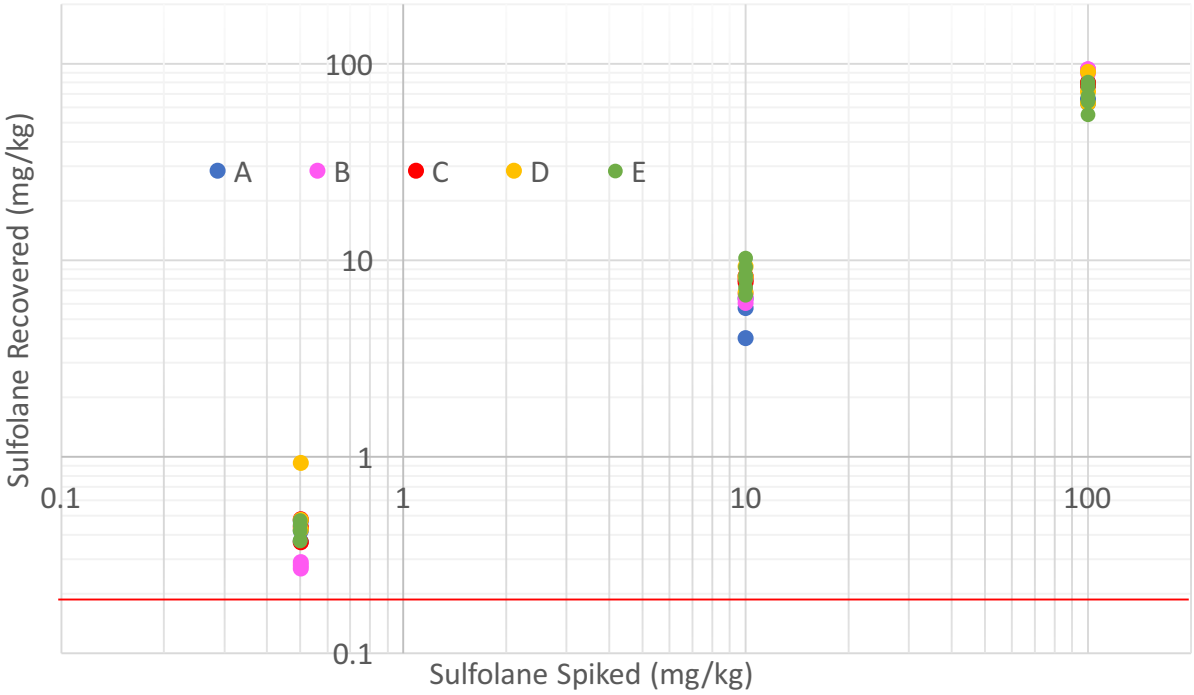


Figure 3.2: Sulfolane recovered in mineral soil from testing labs A, B, C, D and E. *Red line indicates AT1 guidelines.*

Table 3.4: Average sulfolane recovered from soil samples corresponding to each lab

Lab	Organic 100 mg/kg	Mineral 100 mg/kg	Organic 10 mg/kg	Mineral 10 mg/kg	Organic 0.5 mg/kg	Mineral 0.5 mg/kg
A	35.0	64.7	4.0	5.4	0.44	0.42
B	57.6	92.1	4.5	6.4	<DL	0.28
C	85.6	79.0	5.8	8.0	0.196	0.43
D	40.1	75.3	11.4	8.1	0.71*<DL	0.61
E	49.0	70.1	8.3	8.2	0.59	0.47

*<DL=Value listed is for 1 soil sample only as the other 2 were below detection limit
<DL= All three samples were below detection limit

Figure 3.3 showcases the deviation of sulfolane recovered from sulfolane spiked between the two soils (obtained following the method in section 4). Soil data obtained from all testing labs are compiled in the figure. Z-score scattering of 100, 10, and 0.5 mg/kg spiked samples increases from ~0 to -3, ~1 to -3 and ~4 to -3, respectively. The figure also reveals sulfolane recovery from organic soil to fall outside of the acceptable range of ±2 z-score more frequently than mineral soil. Therefore, we can conclude mineral soil extractions to produce much more reliable data than organic, and at higher concentrations than low ones. While extraction inefficiency and sulfolane loss by biodegradation can significantly increase variation in sulfolane recovery (especially at low concentrations), soil organic matter and sample variation can also lead to deviations from spiked concentrations.

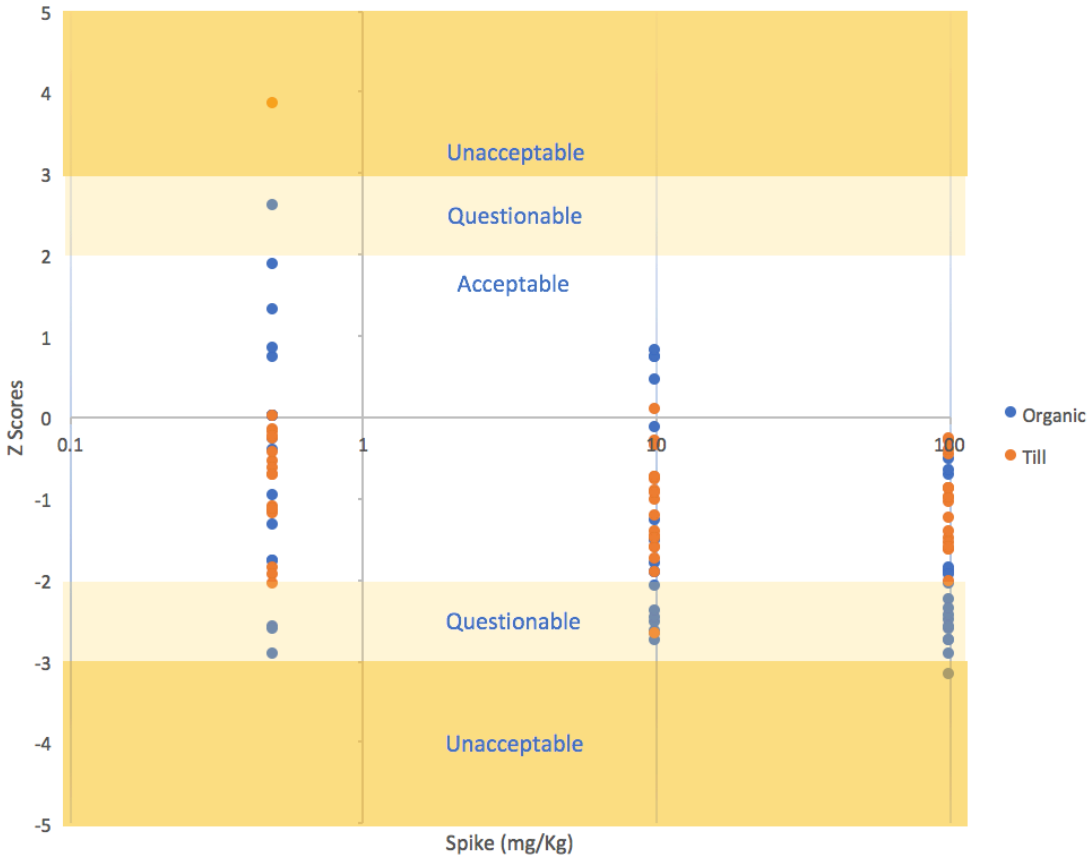


Figure 3.3: $Z_L - score$ distribution of all sulfolane recovery values obtained from organic and mineral soil at 0.5 mg/kg, 10 mg/kg, and 100 mg/kg.

The heterogeneous nature of organic soils will greatly influence how sulfolane is distributed throughout such a complex matrix. Organic soils can have highly irregular and interconnected large pores, smaller open pores, dead-end pores and those that are closed to partially closed. Peats, for example, are characterized as a dual-porosity medium with water-mobile and water-immobile regions (Rezanezhad et al., 2016). Therefore, heterogeneous pore distribution of this media will strongly affect water movement, and consequently sulfolane scattering in this soil due to the compound's miscibility in water. As a result, sulfolane is likely unevenly distributed in organic soil in comparison to mineral soil and one small subsample from a large impacted area can vary significantly from another.

3.5.2 Soil Extraction Using Water

Sulfolane's low organic carbon-water partition coefficient (K_{ow} log=-0.77) is indicative of its low potential to sorb readily onto organic matter and partition in aquifer sediments (Luther et al., (1998)). In conjunction with its miscibility in water, sulfolane should readily extract into water from soils. Therefore, one possible way to mitigate low sulfolane recovery is to extract soils with water.

Sulfolane water extraction from organic and mineral soils at 100 mg/kg is summarized in Table 3.5. For 100 mg/kg in organic soil, an increase in sulfolane recovery was observed with a decrease in soil:water ratio from 1:5 to 1:10 to 1:20 resulting in 87.8 mg/kg (3.0%), 92.3 mg/kg (3.7%) and 103.0 mg/kg (2.7%), respectively. Longer extraction times of 30, 60 and 90 minutes did not result in a significant increase in sulfolane recovery, giving: 92.3 mg/kg (3.7%), 96.3 mg/kg (0.4%), 93.3 mg/kg (7.1%), respectively. Walczak et al., (2002) demonstrated that an increase in soil organic matter content leads to an increase in the total porosity and as a result increases a soil's water retention capacity. Due to sulfolane's miscibility in water, it is hypothesized that porous components of organic soils can retain sulfolane-containing water (Greene and Fedorak (2001)) and lower sulfolane recovery. Consequently, higher volumes of water are required to extract sulfolane from porous matrices such as organic soils. Therefore, decreasing the soil:water ratio will result in higher sulfolane recovery.

In mineral soil, decreasing the soil:water ratio from 1:5 to 1:10 increased sulfolane recovery from 58.6 mg/kg (3.0%) to 66.5 mg/kg (7.7%), however, an insignificant difference was observed from 1:10 to 1:20 ratio (66.5 mg/kg (7.7%) versus 66.0 mg/kg (0.4%)). Increasing the time of extraction also had negligible impact on sulfolane recovery. Greene and Fedorak (2001) observed similar recoveries from mineral soils with a single water extraction. Their study revealed that an

increase in the number of aliquots used to extract such soils resulted in higher sulfolane recovery. The lower sulfolane recovery observed from mineral soils can be attributed to the clay bound water capable of retaining the compound (Saarenketo, 1998).

Table 3.5: Sulfolane recovered from organic and mineral soils spiked at 100 mg/kg

Soil to water ratio	Extraction Time (mins)	organic 100 mg/kg (RSD%)	mineral 100 mg/kg (RSD%)
1:5	30	87.8 (3.0%)	58.6 (3.0%)
1:10	30	92.3 (3.7%)	66.5 (7.7%)
1:20	30	103.0 (2.7%)	66.0 (0.4%)
1:10	60	96.3 (0.4%)	71.2 (2.0%)
1:10	90	93.3 (7.1%)	66.4(3.7%)

3.5.3 Groundwater

Figure 3.4 exhibits sulfolane recovered from groundwater spiked at 0.1 mg/L and 0.5 mg/L, the values are summarized in Table 3.6. Variation in sulfolane recovery was less pronounced in groundwater compared to soil extracts. Generally, results were biased low, however, the outcome is much more reliable compared to soils. Figure 3.5 displays the variance in sulfolane recovery of 0.1 and 0.5 mg/L spiked groundwater. Samples spiked at 0.5 mg/L are within ±2 z-scores, but, scattering of data increases for 0.1 mg/L spiked concentrations and falls outside the acceptable range. In particular for Lab B, sulfolane recovery results are at z-scores -3 and -4. Similar to soils, extraction inefficiencies and sulfolane loss by biodegradation can play a major role in scattering of results. Several studies (Kasanke and Leigh (2017), Greene et al. (1999)) have effectively degraded sulfolane under aerobic conditions. Simple aeration can successfully degrade this compound as groundwaters often contain sufficient nutrients capable of promoting bacterial growth (Table 3.3). The groundwater in use was obtained from a processing plant with a history

of sulfolane contamination. Therefore, the bacterial community have likely had previous exposure to sulfolane which increases their ability to degrade the compound. Consequently, to mitigate sulfolane loss in groundwater sample preservation and cold storage of samples prior to analysis will play a key role in obtaining results true to field conditions.

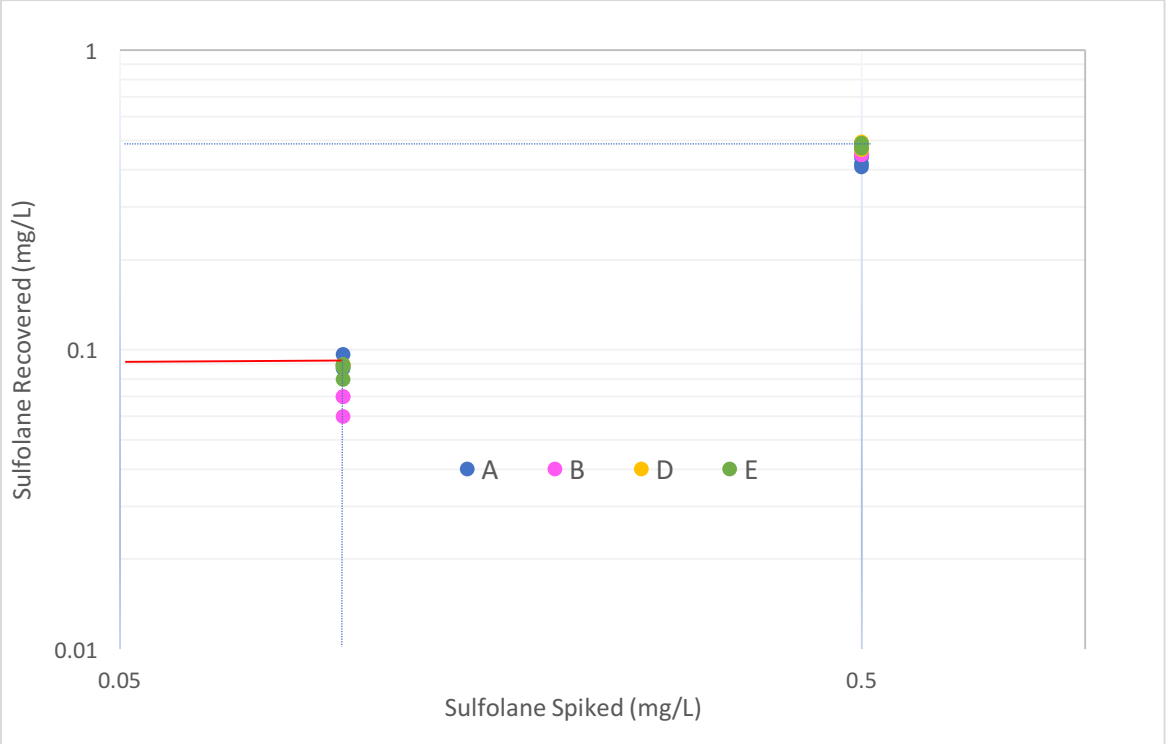


Figure 3.4: Groundwater recovery results of 0.1 and 0.5 mg/L from labs A, B, D, and E. *Red line indicates AT1 guidelines.*

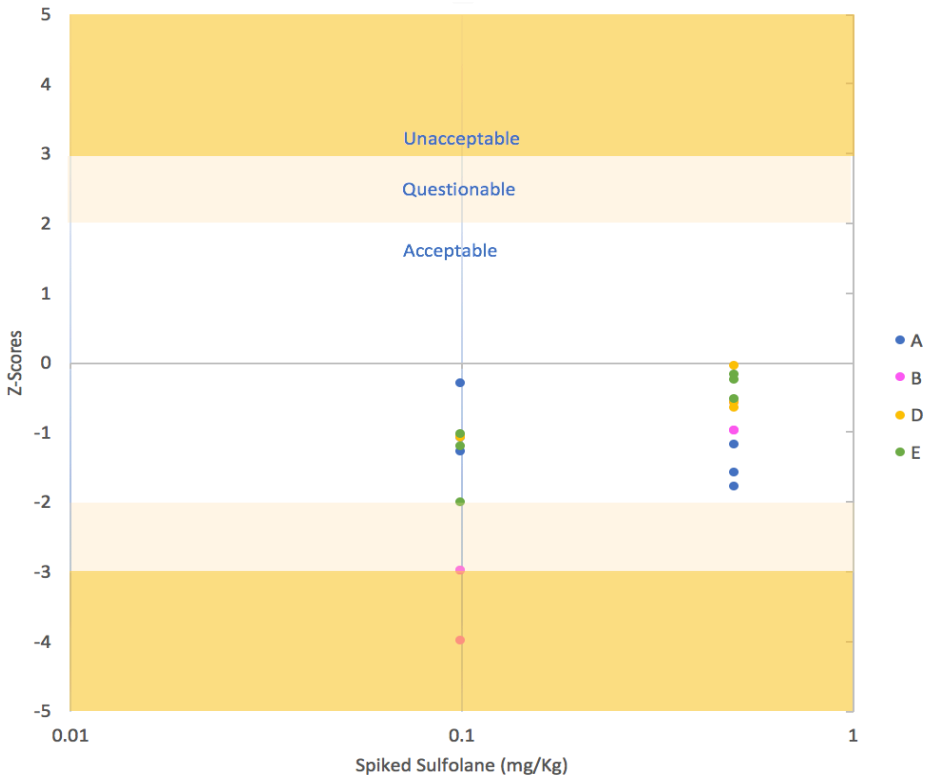


Figure 3.5: $Z_L - score$ distribution of sulfolane recovered from values obtained from 0.1 and 0.5 mg/L spiked groundwater from labs A, B, D, and E.

Table 3.6: Recovered sulfolane concentration from groundwater

Spiked Sulfolane (mg/L)	Sulfolane Recovered (mg/L)			
	A	B	D	E
0.5	0.44	0.47	0.47	0.491
	0.42	0.45	0.467	0.473
	0.41	0.45	0.497	0.487
0.1	0.087	0.07	0.0893	0.0896
	0.089	0.07	No data	0.0879
	0.097	0.06	0.0891	0.0798

3.5.4 Potential Interferences

An RTX-5MS column was used for the purposes of this study in Lab E. This is a low-polarity column consisting of 5% diphenyl 95% dimethyl polysiloxane and is a common stationary phase used throughout literature for sulfolane analysis (Fedorak and Coy, (1996), Headley et al.,

1999). Due to sulfolane's polarity, a slightly polar stationary phase is required for the sulfolane-column interaction to occur. Analysis of sulfolane in GC-MS and electron ionization was carried out in Selective Ion Mode (SIM) to reduce interference. Four ion peaks were used to finger print the chemical, ensuring the compound identified is in fact sulfolane. The parent ion of sulfolane (m/z 120) and 3 daughter ions with m/z 41, 55, and 56 were used to distinguish the compound (Fig 3.6). Ion peaks m/z 41, 55, 56 and 120 have a relative intensity of 100%, 71.40%, 67.80% and 29.50%, respectively. The relative intensity of these 4 peaks in relation to one another was used to further characterize the compound, resulting in greater than 95% similarity to sulfolane and better eliminating the concern for interferences.

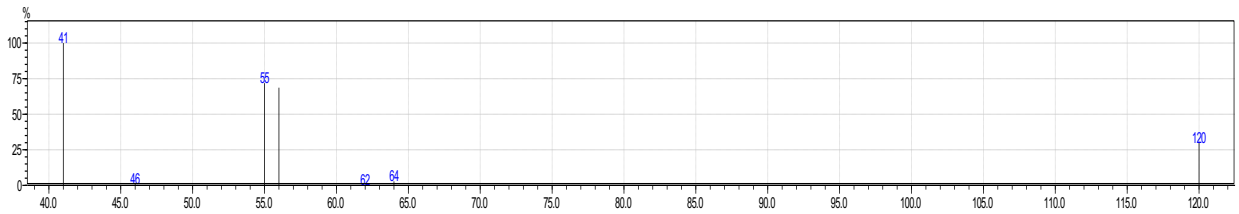


Figure 3.6: Four ion peaks used to fingerprint sulfolane in SIM mode (Intensity vs m/z).

Analysis of potential sulfolane interferences in organic soil leachate was done using Scan Mode with a library search and compared to SIM mode as shown in Fig 3.7. A significant peak is observed at the same retention time of sulfolane in Scan mode chromatogram in comparison to SIM. The organic soil used in this study consists of 48.5% organic carbon (Table 3.2) which is a contributing factor to analytical difficulties observed for some testing labs. Leachate analysis of the organic soil revealed up to 24 different compounds shown to match sulfolane with up to 80% similarity via mass spectral libraries. Some of these fragments include: stearic hydrazide, 6-methyloctadecane, 2-propyl-1-pentanol, 2-butenyl hydrazine, butyric acid hydrazide and allyl methallyl ether just to name a few.

It is assumed that the origin of these compounds is from the organic soil fragments as they are from the same families characterized by Saiz-Jimenez (1986). The study found more than 100 structural segments can be evolved from heating of humic acids alone, in which none of those dominate. Therefore, it is difficult to exclude any of the compounds, as the organic matter alone can have many molecules which can fragment similar to sulfolane. There is no guarantee that any of these potential interferences are high in concentration, however, their impact might be amplified at low concentrations of sulfolane, depending on the analytical method. As a result, higher sulfolane concentrations tend to be more reliable in comparison, and more so in mineral soils than those with high organic content.

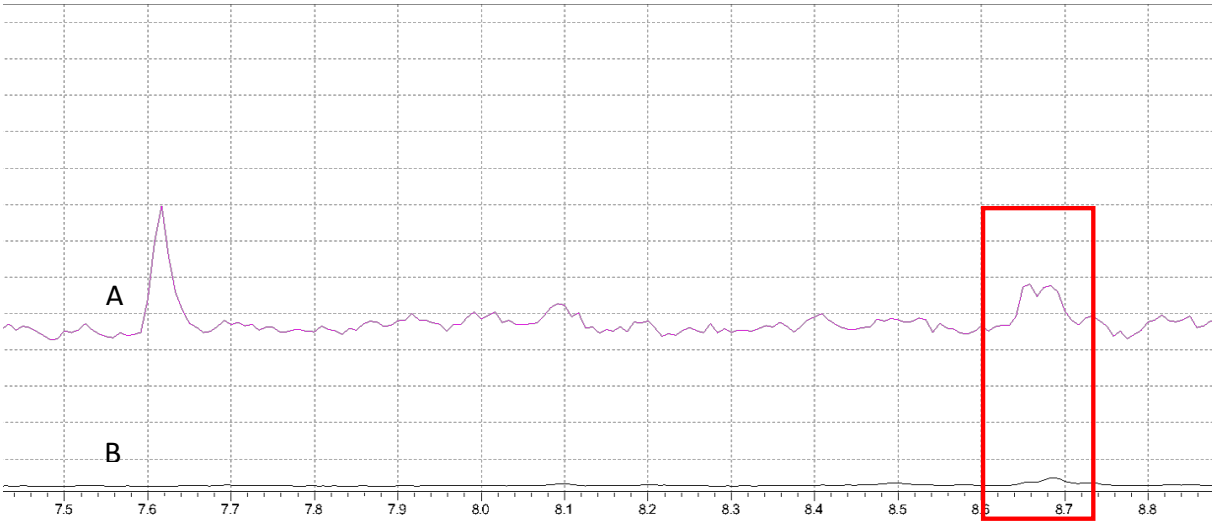


Figure 3.7: Chromatogram of organic soil in SCAN (A) and SIM (B) mode. Retention time (min) of sulfolane is highlighted in the red box.

3.6 Conclusion

Testing laboratories have varying analytical and extraction methods for sulfolane in soils and groundwater. The results obtained demonstrated sulfolane recovery was biased low in all three matrices used. However, higher concentrations of sulfolane in soils showed to have more consistent results. Organic soil had a greater variability in sulfolane recovery particularly at low

concentrations, in comparison to mineral soils. Groundwater results were more reliable, however, similar to soils the scattering increased at low sulfolane concentrations. Therefore, sulfolane analysis at higher concentrations is more reliable. Sulfolane recovery using single aliquot water extraction were successful in extracting sulfolane, particularly in organic soil. Decreasing soil:water ratio resulted in higher sulfolane recovery. However, mineral soils will require multiple aliquots to obtain maximum recovery. GC-MS analysis demonstrated that there is potential for interference from organic component of soils and testing labs should be cautious of this aspect when developing suitable methods for sulfolane, particularly at low concentrations of sulfolane.

Chapter four: IMPACT OF CO-CONTAMINANTS ON SULFOLANE BIODEGRADATION IN GROUNDWATER

4.1 Introduction

Over the past couple of decades sulfolane (tetrahydrothiophene 1,1-dioxide) contamination has become a concern in places where oil and gas exploration and processing activities take place. The Sulfinol® gas sweetening process developed by Shell in 1960s utilises sulfolane as a solvent to remove acidic components from natural gas (Isaacs et al., 1977). The miscibility of sulfolane in water and its consequent mobility in groundwater and/or surface water has led to wide spread contamination in areas around processing facilities where it is used. (Luther et al., 1998).

A number of treatment methods ranging from advanced oxidation processes (AOPs) to bioremediation of sulfolane in water are being investigated (Mehrabani-Zeinabad et al., 2016; Yu et al., 2016: Izadifard et al., 2018). Amongst others, bioremediation has been shown to be a simple and cost-effective approach in degrading sulfolane (Khan et al., 2019). Chou and Swatloski (1983) successfully degraded sulfolane using activated sludge under aerobic conditions. Fedorak and Coy (1996) used aerobic shake-flask slurry cultures and determined that supplementing the water with nutrients such as nitrogen and phosphorus accelerates sulfolane degradation. However, degradation of sulfolane under anaerobic conditions have not provided consistent results **(**Kasanke and Leigh, 2017, Greene et al., 1998, Kim et al., 1999).

In most cases, sulfolane contaminated groundwater plumes contain more than one pollutant. These can be products from the same industrial activity or naturally occurring compounds in the groundwater. The role of co-pollutants on the degradation of a primary contaminant is quite complex and varies not only with the co-contaminant's characteristics but also with concentration and form (Alexander, 1995). They might actively inhibit the bioactivity of the bacteria or compete

with primary contaminant as viable carbon sources. Toxic co-contaminants can destroy primary contaminant-degrading bacteria and impede its degradation. In cases where the co-contaminants are not toxic, competition for oxygen, nitrogen and phosphorus (O_2, N and P) between primary contaminant degrading bacteria and co-contaminant metabolizing bacteria (assuming they are different) can also hinder degradation of the primary contaminant (Alexander, 1995). McNally et al. (1999) studied the aerobic biodegradation of polycyclic aromatic hydrocarbons (PAHs) such as phenanthrene, naphthalene and pyrene in varying combinations. They determined that the presence of naphthalene stimulated phenanthrene and pyrene degradation while phenanthrene inhibited pyrene metabolism. Steffensen and Alexander, (1995) examined the competition of two substrates in the presence of low phosphorus. They found that if benzylamine and caprolactam were present along with two bacterial strains designated for metabolizing each, then the degradation of one or the other compounds got suppressed. So far, the impact of co-contaminants on sulfolane biodegradation has rarely been investigated. Kasanke and Leigh, (2017) examined the impact of kerosene on sulfolane degradation kinetics and demonstrated that a high concentration of kerosene (around 400 mg/L) inhibited 0.750 mg/L sulfolane degradation by 30%.

In this study, the focus was on groundwater co-contaminants found at a sulfolane impacted site, which included diisopropanolamine (DIPA), trivalent arsenic and fulvic acid. The impacts of these co-contaminants on sulfolane biodegradation were studied considering the type of co-contaminants, their concentrations and the mixtures of co-contaminants and variations in characteristics of the groundwater matrices. A short description of each co-contaminant used in the study is provided in the subsequent sections below.

Diisopropanolamine (DIPA) is a solvent utilised in the Sulfinol® process in conjunction with sulfolane and is often detected in sulfolane-contaminated aquifers (Isaacs et al., 1977). Gieg et al.

(1999) found that methylglyoxal, a compound with antimicrobial properties, was one of the DIPA biodegradation intermediates that could inhibit microbial activity. On the other hand, ammonia is the final product of DIPA degradation (equation below), which can be a source of nitrogen for microbes and accelerate remediation if nutrient limitation is an issue (Greene et al., 1999).

$$C_6H_{15}O_2N + 8O_2 \rightarrow 6CO_2 + 6H_2O + NH_3 \quad (1)$$

Arsenic is commonly found in Alberta groundwaters as a result of natural weathering of glaciofluvial, sandstones and shale sediments alongside deep oilfield brines (Moncur et al., 2015). Up to 0.780 mg/L arsenic has been recorded throughout the province's groundwater wells with over 50% exceeding the drinking water guideline levels of 0.01 mg/L (Alberta Environment and Parks [AEP], 2019) in the southern oil sand regions alone (Moncur et al., 2015). Two common groundwater oxidation states of arsenic are As(III) and As(V), with the former being up to 60 times more toxic than the latter (Lloyd & Oremland, 2006). Under aerobic conditions As (V) prevails while in anaerobic environment As(III) persists.

Fulvic acid (FA), a component of natural organic matter (NOM) was also studied in this research. Natural organic matter concentrations from deep groundwater range from 0.1 to 10 mg/L with FA contributing as much as 95% due to its high solubility (Gaffney et al., 1996). Adding FA to the study was necessary as binding of organic molecules to NOM can have an impact on its bioavailability (Gaffney et al., 1996).

4.2 Materials and Procedure

4.2.1 Materials

Sulfolane (99% purity), DIPA (≥98% purity) and sodium (meta) arsenite (≥90% purity) were obtained from Sigma Aldrich. Fulvic acid (2S101F) was purchased from International Humic Substances Society (IHSS). Sulfolane contaminated samples were obtained from two different groundwater-bearing zones via groundwater wells labeled as GW1 and GW2 at an inactive sour gas processing plant in Alberta. GW1 is installed in the uppermost groundwater-bearing zone while GW2 is deeper and in the secondary groundwater-bearing zone. The groundwater characteristics from each well are summarized in Table 4.1. Tap water was used as the third matrix for the experiments.

Table 4.1: Water Characteristics

	Concentration (mg/L)		
Parameter	GW1*	GW2*	Tap Water**
DIPA	3.0	5.7	N/A
Benzene	0.32	0.0032	N/A
Toluene	0.0011	< 0.00040	N/A
Ethylbenzene	0.053	< 0.00040	N/A
Bicarbonate	610	320	144
Chloride	48	22	11
Sulphate	2.7	7.8	74
Iron	4.2	0.57	< 0.010
Manganese	5.5	2.2	< 0.0005
Magnesium	22	17	17
Sodium	17	11	9.0
Calcium	170	84	54
Total dissolved solids	570	300	265
Nitrate + Nitrite (as Nitrogen)	< 0.014	< 0.014	< 0.08
Hardness	520	280	214

*Groundwater data based on analytical results obtained from a testing laboratory

**Obtained from City of Calgary Glenmore Water Treatment Plant

4.3 Procedures

4.3.1 Incubation of sulfolane degrading bacteria in Bioreactor

An aerobic bio-stimulating reactor was created in a 1 L column to incubate sulfolane degrading microbes. The column was supplied with 700 millilitres (mL) of tap water, aquifer sediments containing sulfolane-degrading microbes, 100 mg/L of sulfolane and various nutrients. Tap water was used to provide micronutrients for the growth of microbes; the sediments were obtained from the sulfolane contaminated groundwater; ammonium chloride was used as a N source and monopotassium phosphate/dipotassium phosphate were used as sources of P; N/P nutrients were added into the column according to C:N:P=100:5:1. The column was constantly aerated using an atmospheric air diffuser to provide saturated oxygen conditions and ensure sediment suspension in solution. Every 48 hours, the air diffuser was shut down for 30 minutes and the solid/biomass in the column settled at the bottom. A volume of 500 mL of the water on the top layer of the column was siphoned out and another 500 mL of tap water containing 100 mg/L sulfolane and nutrients as stated above was added into the column. After 14 days, the column showed the capacity to degrade 100 mg/L sulfolane to below detection limits of 1 mg/L within 24 hours, ensuring the biomass was fully acclimated to sulfolane. A schematic setup of the bioreactor is shown in Fig 4.1.

4.3.2 Jar test

All jar tests were conducted in 500 mL sterile high-density polyethylene (HDPE) bottles. The total volume in each jar was set at 300 mL. Each bottle was filled with 10 mL of acclimated

sediments from the bioreactor. Nutrients (C:N:P) were added to each jar in a 100:5:1 ratio similar to the reactor with 100 mg/L sulfolane. The remaining volume was filled with the same matrix (GW1, GW2 or tap water) and spiked with co-contaminants as necessary. For control experiments, similar jar conditions were set up but without any co-contaminant being added (Table 4.2). All jars were exposed to the atmosphere to ensure aerobic conditions and each jar was placed on a table shaker continuously rotating at 220 revolutions per minute (rpm). The weight of each jar was recorded and adjusted based on the amount of water evaporated between samples. Aliquots of 10 mL were drawn at specific times and filtered using 0.45 μm syringe filters. A schematic setup of the jar tests is shown in the Fig 4.1.

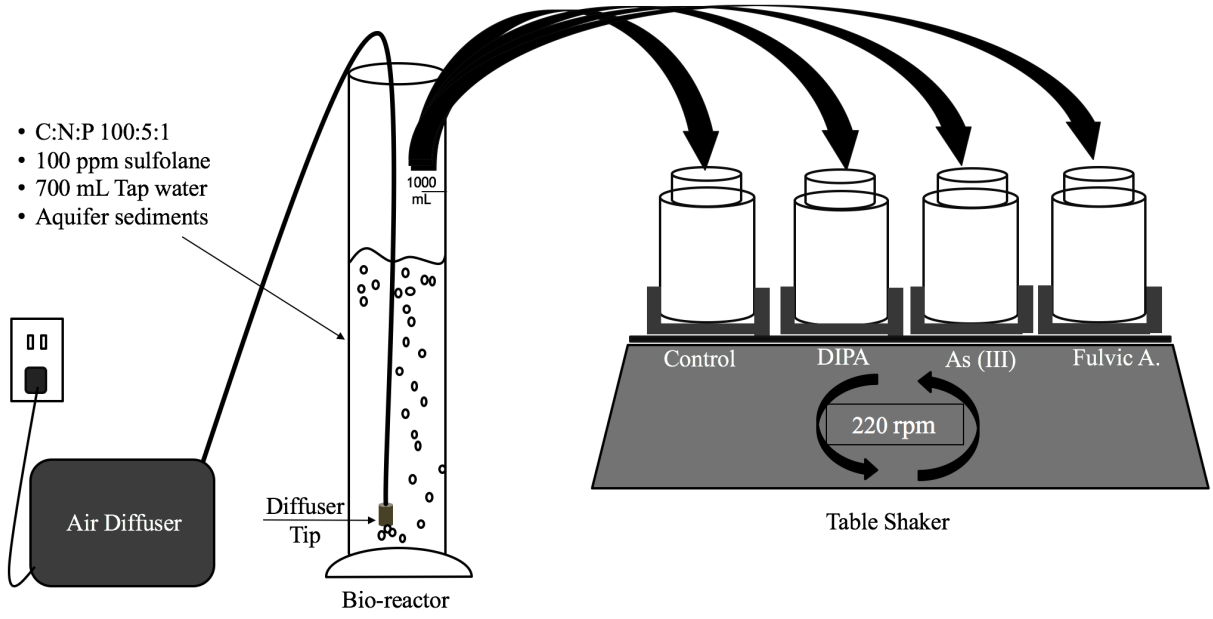


Figure 4.1: Schematic setup of the bioreactor and the jar tests.

Table 4.2: Jar experimental conditions

	Co-contaminant concentration (mg/L)		
Jar ID*	DIPA	As(III)	FA
GW1-Control	-	-	-
GW1-A	250	-	-
GW1-B	10	-	-
GW1-C	-	25	-
GW1-D	-	5	-
GW1-E	-	0.5	-
GW1-F	10	0.5	-
GW1-G	-	-	50
GW1-H	-	-	250
GW2-Control	-	-	-
GW2-A	250	-	-
GW2-B	-	25	-
Tap-Control	-	-	-
Tap-A	250	-	-
Tap-B	-	25	-

*Note: All jars contain C:N:P (100:5:1) where C comprised of 100 mg/L added sulfolane.

4.3.3 Analysis of Sulfolane

An Agilent Gas Chromatograph equipped with a Flame Ionization Detector (GC-FID Model 6890 with auto-sampler) was utilised for sulfolane analysis. A Phenomenex Zebron (ZB-5MSi 30m x 0.25 mm x 0.25 μm) column was used for chromatographic separation with a flow of 0.9 mL/min and pressure of 85.9 kilopascals (kPa). The starting oven temperature was 80°C which was ramped up to 160 degrees C (°C) at 10 °C/min where it was held for 2 min, then increased 20°C/min to 280°C where it was held for 5 min with a total run time of 21 min. Split/split-less inlet mode with 2 μL injections were used for all samples. Sulfolane detection limit of this instrument was 1 mg/L.

All experimental samples were extracted using dichloromethane (DCM). Sulfolane calibration standards were prepared in DI water and extracted using DCM in order to account for the extraction efficiency. A 5:3 ratio of solution to DCM was used for all samples and standards, resulting in approximately an 80% extraction efficiency of sulfolane.

4.3.4 Analysis of DIPA

A Shimadzu GC-FID (Model GC2010Plus AF with auto-sampler) was utilised for all DIPA analysis. An RTX-5 (30m x 0.25 mm x 0.25 μm) amine column was used for chromatographic separation with a flow of 1.2 mL/min. The starting oven temperature was 60°C which was ramped up 10°C/min where it was held for 2 minutes, then increased to 280°C with a total run time of 22 minutes. Split/split-less inlet mode with 1 μL injections were used for all samples. All solutions were diluted with 50% methanol used as solvent for the analysis.

4.3.5 Analysis of As (III)

Samples containing As (III) were diluted 50 times and passed through an aluminosilicate adsorbent arsenic speciation cartridge (obtained from MetalSoft) to remove As (V) from solution. Arsenic analysis was done using an Agilent 8900 Inductively Coupled Plasma Triple Quadrupole Mass Spectrometer (ICPQQQMS). The radio frequency (RF) generator was set at 1550W. The dilution gas, carrier gas and cell gas (O_2) flow rates were 0 L/min, 1 L/min and 0.15 mL/min, respectively. Extract 1 and 2 were -15.6V and -225V with Omega bias, Omega lens and Octopole bias set at -130 V, 8.0 V and -3 V, respectively.

4.3.6 QGA Cellular ATP (cATP)

Cellular ATP measurements (cATP) are indicative of the total living biomass in water. The Qunech-Gone™ Aqueous Test Kit from LuminUltra (Canada) was purchased for this analysis. A

10-mL sample of each water (GW1, GW2 & tap) was pushed through a 0.45 μm filter using a 12-mL syringe. 1 mL of UltraLyse 7 was then added to the syringe and filtered through the same filter once more, depositing into a 9 mL UltraLute (dilution) tube. This process allowed for the release of ATP from the microorganisms in the sample through lysis. A 100 μL sample from the tube was then combined with 100 μL of Luminase in an assay tube and analysed in Lumitester C-110 for RLU_{cATP} (Relative Light Units). Cellular ATP was then calculated using equation (2) where RLU_{ATP1} is the ATP standard calibration and V_{sample} is the sample volume.

$$cATP\ \left(pg\frac{ATP}{mL}\right) = \frac{\mathrm{RLU_{cATP}}}{\mathrm{RLU_{ATP1}}}\ x\ \frac{10{,}000\ (pg\ ATP)}{V_{Sample}} \qquad (2)$$

cATP was then converted to Microbial Equivalents (ME/mL) using equation (3) based on the assumption that 1E. coli-sized bacteria (ME) is 0.001 pg (1 fg) ATP per cell. Table B summarizes the ME of all 3 water matrices used prior to any experimental manipulation.

$$cATP\ \left(\frac{ME}{mL}\right) = cATP\ \left(pg\frac{ATP}{mL}\right) x\ \frac{1\ ME}{0.001\ pg\ ATP} \qquad (3)$$

4.4. Results and Discussions

4.4.1 DIPA

Experiments containing DIPA (10 or 250 mg/L) using GW1 degraded sulfolane to a greater extent in a shorter amount of time compared to the control sample (see Table 4.3). No sulfolane was detected after 97 hours with 10 or 250 mg/L DIPA supplementation while the sulfolane breakdown in the control experiment plateaued after 97 hours and no further degradation was observed until the experiment was ceased after 247 hours. It was also observed that for jar GW1-B (250 mg/L DIPA), DIPA concentration decreased by 50% within 48 hours. Since DIPA has a low vapour pressure, one can reasonably argue that abiotic processes such as volatilisation were

not impacting the results (CCME, 2005). Ammonia has been reported as a by-product of aerobic biodegradation of DIPA (Greene et al., 1998), which can serve as potential nutrient for sulfolane-degrading bacteria (CCME, 2005). The biochemical reaction for degradation of DIPA is shown in equation (1). Gieg et al. (1999) reported that DIPAs biodegradation would contribute to the total available nitrogen. Previous studies (Greene & Fedorak, 2001, Fedorak & Coy, 1996, and Kasanke & Leigh, 2017; Khan et al., 2019) have shown that sulfolane biodegradation can be enhanced by adding more nutrients. In addition to its potential to providing nitrogen, DIPA, a weak base, can also help maintain the pH of the solution. Sulfuric acid is expected to be produced during the aerobic biodegradation of sulfolane, inhibiting microbial activity (Chou and Swatloski, 1983). DIPA can react with sulfuric acid and prevent the drop of pH in the solution. Available nutrients for biodegradation are usually limited in aquifers. Presence of DIPA can provide additional nitrogen and therefore enhance the rate of biodegradation of sulfolane.

Table 4.3: Sulfolane removal in presence or absence of DIPA supplement

		Sulfolane Removal (%)			
Samples	DIPA added (mg/L)	After 48 hours	After 97 hours	After 150 hours	After 247 hours
GW1-Control	-	75.7% ± 0.3%	92.5% ± 0.1%	94.3% ± 0.1%	93.4% ± 0.3%
GW1-A	250	78.4% ± 1.2%	>99%	>99%	>99%
GW1-B	10	84.2% ± 0.6%	>99%	>99%	>99%

4.4.2 Impact of As (III)

Addition of As (III) at various concentrations (0.5, 5, 25 mg/L) had negligible impact on aerobic sulfolane degradation in GW1. As can be observed from Figure 4.2, the concentration of

sulfolane plateaued in all four jars after a period of 97 hours (hrs). No inhibitory impact on sulfolane degradation was observed as the degradation kinetics were comparable to the control. As(III) concentration was monitored during the sulfolane biodegradation yielded that over 60% of As(III) (25 mg/L initial concentration) oxidized to As(V) within 48 hrs as shown in Fig 4.3. Alberta's natural groundwater arsenic levels have been found to average around 0.5 mg/L. Across Canada, however, instances of 5 mg/L arsenic have been reported where about 50% of the population relies on groundwater as a drinking water source (McGuigan et al., 2010).

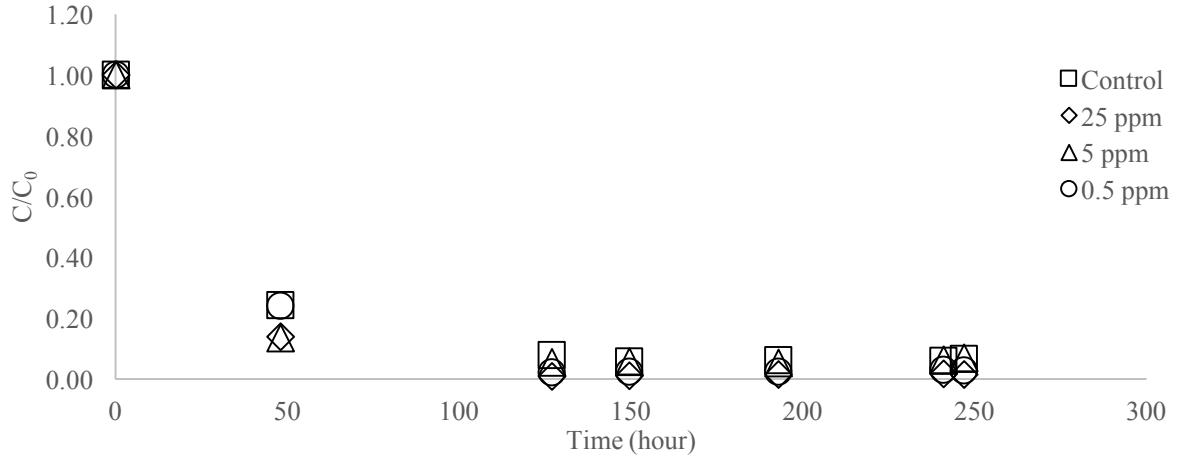


Figure 4.2: Impact of As (III) on sulfolane degradation at initial concentration C_0 = 110 mg/L sulfolane.

The electron pair from As (III) reduces molecular oxygen and forms water. Even though oxygen has the ability to oxidize As (III), the conversion is very slow (Wang et al., 2012). Therefore, one can rule out abiotic reactions as the cause of this conversion. Aerobic bacterial oxidation of As(III) to As(V) has been previously found in chemolithotrophic and heterotrophic microorganisms (Lloyd & Oremland, 2006). Heterotrophs require an organic source of carbon for their growth and will simply detoxify As (III) to the lesser toxic form of As(V). Chemolithotrophs, on the other hand, need an inorganic carbon source (eg. CO_2) and will benefit energetically from the oxidation process (Lloyd & Oremland, 2006).

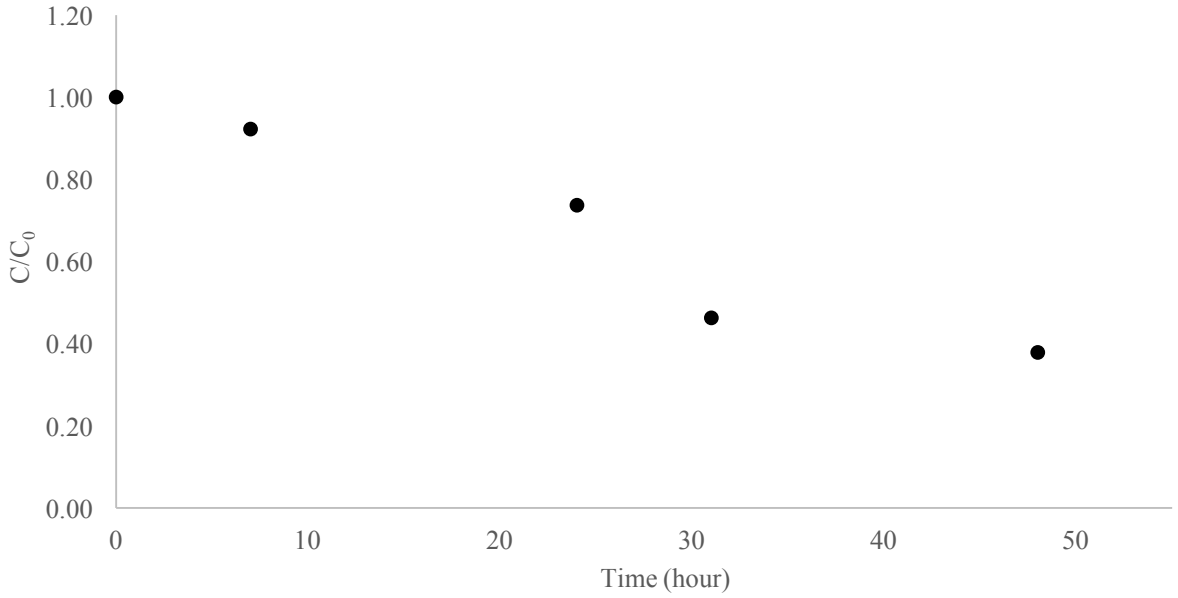


Figure 4.3: As (III) concentration during sulfolane biodegradation (C_0=25 mg/L As (III))

Dong et al. (2016) examined the impact of As (III) on bacterial community structure and diversity in soil. They found that under high As (III) concentrations As(III)-resistant bacteria grew 37-times while the microbial diversity was at its lowest. Tang et al. (2013) studied the bioremediation of phenanthrene (PHE) in conjunction with As (III). Using a bacterial consortium, the study identified *pseudomonas* and *alcaligenes* as the dominant groups in oxidizing As (III), while *pseudomonas* and *achromobacter* were mainly responsible for degrading PHE. Therefore, it is possible that sulfolane degrading bacteria and As (III) oxidizing bacteria are the same strain in contaminated aquifers even at high As (III) toxicity levels.

Simultaneous sulfolane and As (III) oxidation using indigenous aquifer microbes is a much more environmentally friendly and cost-effective approach in remediating both contaminants. While As (III) oxidation does not remove the toxic metalloids from aquifers, it can certainly be a first step in the remediation process as the As(V) produced is less mobile compared to its trivalent

counterpart due to its higher ability to adsorb to iron oxide, aluminum oxide and clay materials (Lloyd & Oremland, 2006).

4.4.3 Fulvic Acid

As shown in Figure 4.4, 50 mg/L FA had a negligible impact (<5%) on sulfolane biodegradation rate in GW1. However, at a high FA concentration (250 mg/L) the rate decreased by 20%. The system pH dropped from 7.8 to 5.5 under high FA loads. The slower degradation rate is attributed to the low pH since the microorganisms were naturally acclimated to a slightly alkaline environment. Even though the groundwater had elevated bicarbonate content (610 mg/L), the high FA load exceeded the water's natural buffering capacity. In general, biodegradation tends to be faster at moderate pH levels. However, it is likely that GW1 contains a diverse group of microorganisms capable of degrading sulfolane within a broad range of pH levels. Groundwater FA levels typically do not exceed 10 mg/L, thus their impact can be deemed negligible on sulfolane degradation (Gaffney et al., 1996). Natural organic matter can increase the mobility and solubility of compounds (Gaffney et al., 1996) and enhance their bioavailability to microorganisms. A study conducted by Ortega-Calvo et al., (1997) showed that soil organic matter enhances the biodegradation of phenanthrene. However, this phenomenon was not observed in this study. Sulfolane is highly water soluble and its solubility is not a limiting factor for its biodegradation.

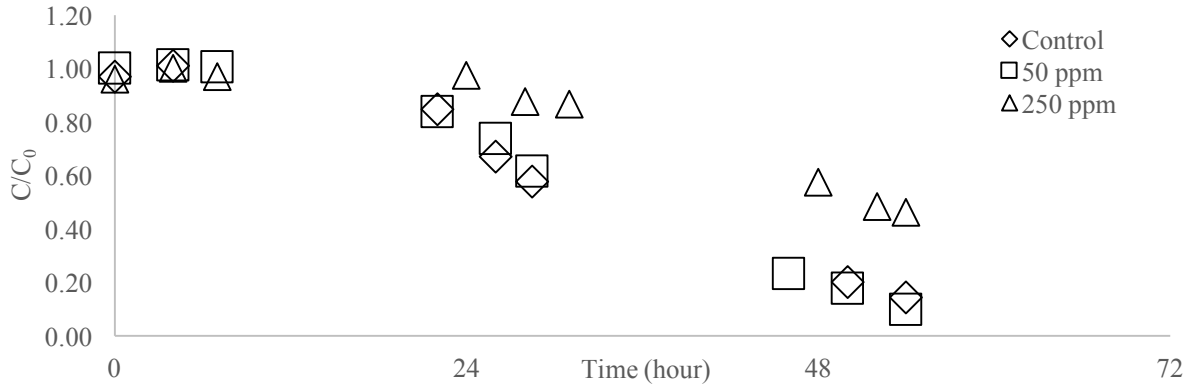


Figure 4.4: Impact of 50 & 250 mg/L fulvic acid on sulfolane biodegradation in GW1 at initial sulfolane concentration C_0 = 110mg/L.

4.4.4 Mixture

Combination of GW1-B (10 mg/L DIPA) and GW1-E (0.5 mg/L As(III)) on sulfolane degradation was examined and the results are presented in Fig 4.5. The combination chosen in this study is intended to represent typical sulfolane, DIPA and As (III) concentrations at the at the investigated site (i.e. inactive sour gas processing plant). Within 48 hrs, 75.7% (± 0.3%) of sulfolane degraded in the control sample and 75.9% (± 0.9%) sulfolane degraded in GW1-E. GW1-B had 84.2% (± 0.6%) sulfolane degradation and in combination with 0.5 mg/L As (III) (GW1-F), 81.5% (± 0.5%) degradation was observed within 48 hrs. Both GW1-B and GW1-F degraded sulfolane to below detection limits (what are the detection limits) in less than 100 hrs while the sulfolane in control and GW1-E plateaued until the end of the experiment. These results indicate that not only does As(III) not impact sulfolane degrading bacteria, but also does not hinder DIPA degrading bacteria as it neither impeded nor accelerated the rates. As previously discussed in Section 3.1, DIPA has a positive influence on sulfolane degradation due to its N-containing

mineralised products, helping to degrade sulfolane to below detection limits under nutrients scarce environments.

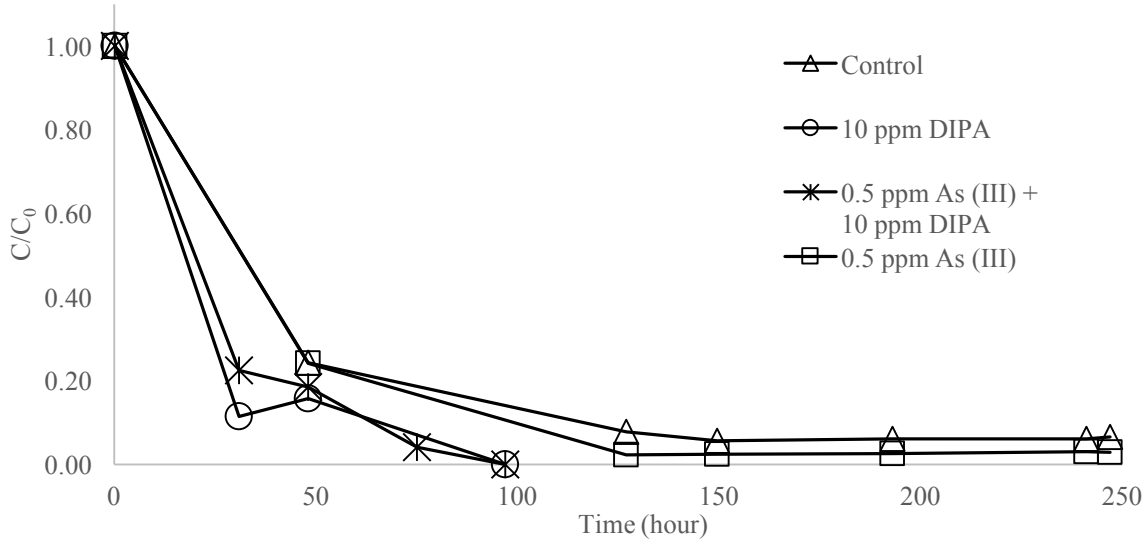


Figure 4.5: Impact of co-contaminant mixture on sulfolane biodegradation in GW1, initial sulfolane concentration C_0 = 110 mg/L.

4.4.5 Matrix effect

Sulfolane degradation without addition of co-contaminants, in different water matrices (tap water, GW1 and GW 2) is shown in Fig 4.6. In 48 hrs, about 40% of sulfolane in tap water degraded, while approximately 60% and approximately 75% degradation was noted for sulfolane in GW2 and GW1, respectively. The difference in the results from the control experiment can be explained using the different characteristics of the water matrices. The three waters have different microbial populations as demonstrated through ATP measurements. ATP results indicated GW1 to have a higher Microbial Equivalent count (8.4×10^5) than GW2 (3.8×10^5) and tap water (3.0×10^5). Higher microbial population leads to faster aerobic degradation of sulfolane. It is also possible that the chemistry of the groundwater makes it more amenable to biodegradation of sulfolane.

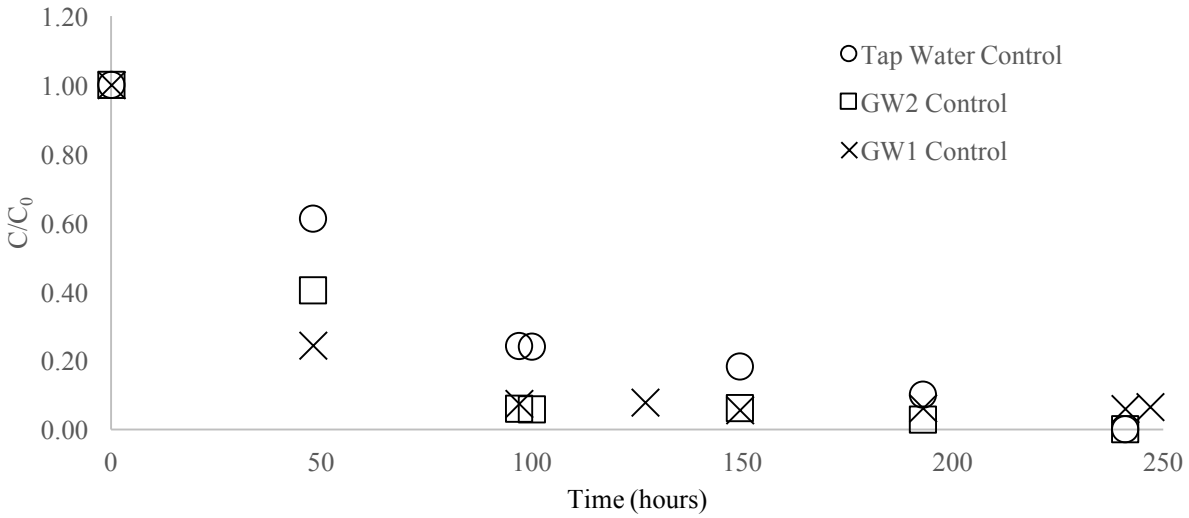


Figure 4.6: Water matrix effect on sulfolane degradation in control experiments. (C_0 = 110 mg/L sulfolane for tap water and GW1 for GW2 C_0 = 140 mg/L sulfolane).

It is also probable that the sulfolane-degrading bacteria have adapted to the groundwater environment which provides better growth conditions when compared to tap water. In addition, other constituents in the groundwater also make them favourable for sulfolane treatment such as high levels of bicarbonate, calcium and the presence of necessary micronutrients. This agrees with Kasanke and Leigh, (2017) who observed that the presence of mineral nutrients stimulated biodegradation of elevated sulfolane levels.

Supplementing with bicarbonate is necessary in bioremediation as it buffers the acidic end products of biological reactions and has been used to maintain alkalinity in treatments of various chemicals such as chlorinated ethenes (Schaefer et al., 2010). There were significant differences in the bicarbonate contents of the three waters, with GW1 having a higher level (610 mg/L) compared to GW2 (320 mg/L) and tap water (144 mg/L). Low bicarbonate levels mean the water will be pH sensitive. A stoichiometric conversion of the sulfur in sulfolane results in sulfate and sulfuric acid formation (Chou & Swatloski, 1983). Even though a strong acid is produced,

biodegradation is still possible but slower removal rates should be expected if the buffering capacity is exceeded (Greene et al., 1999).

$$C_4H_8O_2S + 6.5O_2 \rightarrow 4CO_2 + 3H_2O + 2H^+ + SO_4^{2-} \quad (4)$$

In addition to macronutrients such as other carbon, micronutrients like calcium also play a significant role in bacterial growth. GW1 contained elevated calcium levels (170 mg/L) compared to GW2 (84 mg/L) and tap water (54 mg/L) (see Table 1). Onoda et al., (2000) demonstrated that *E. coli* L –form requires calcium for growth and cells will eventually lyse if calcium is insufficient.

This study further investigated the impact of water matrix on co-contaminant influence of sulfolane biodegradation and the results are summarized in Table 4.4. The presence of DIPA in GW1 enhanced sulfolane biodegradation while in GW2 sulfolane degradation was inhibited. In tap water, however, negligible impact of DIPA was observed on sulfolane removal (Fig 4.7). DIPA provided a greater sulfolane removal in GW1 compared to control. However, in GW2, DIPA significantly inhibited sulfolane degradation (Fig 4.8). When DIPA was added to GW2, less than 70% sulfolane degraded after 100 hours. The same experiment showed more than 90% of sulfolane degraded when DIPA was not added.

Table 4.4: Summary of impact of co-contaminants on sulfolane biodegradation in comparison to the extent of sulfolane removal in their respective control experiment.

Co-contaminant	Water Matrix		
	GW1	GW2	Tap
DIPA	Positive	Negative	negligible
As (III)	Negligible	Negligible	negligible
As (III) + DIPA	Positive	-	-
FA 50 mg/L	Negligible	-	-
FA 250 mg/L	Negative	-	-

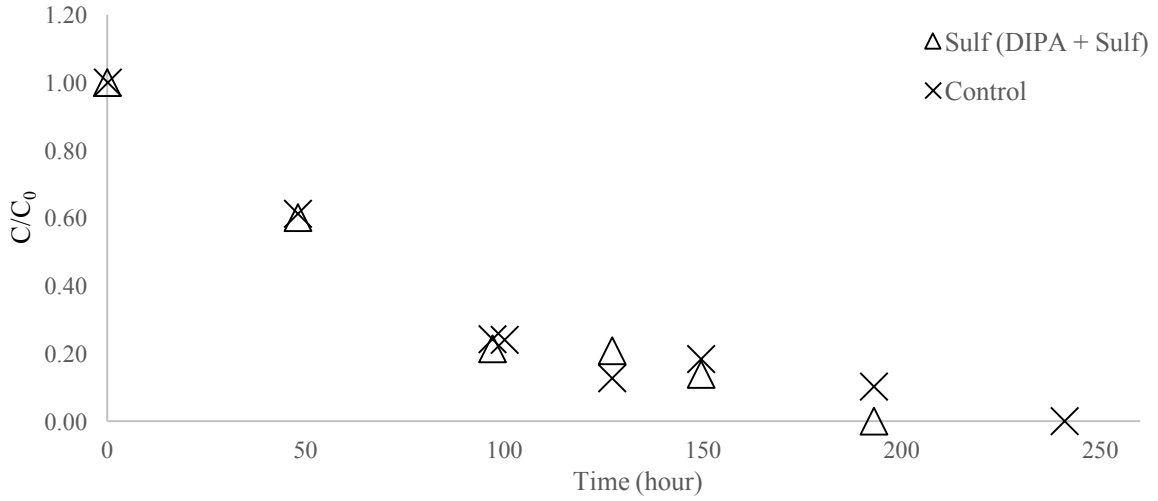


Figure 4.7: Degradation of sulfolane in control sample and with 250 mg/L DIPA using tap water. (C_0 = 110 mg/L sulfolane).

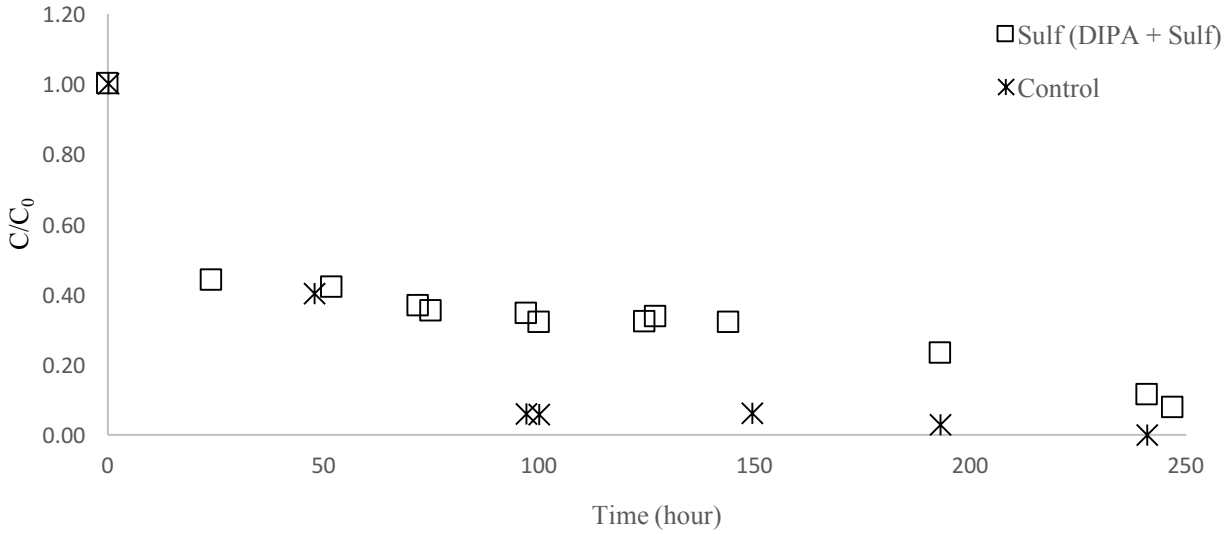


Figure 4.8: Degradation of sulfolane in control sample and with 250 mg/L DIPA using GW2 with initial sulfolane concentration C_0 = 140 mg/L.

As stated earlier, GW1 contained higher Microbial Equivalent, indicating that it possibly had higher capacity to degrade pollutants. GW1 also contained high levels of organic pollutants

compared to tap water and GW2 (Table 1). It is probable that the bacteria in GW1 have been acclimated to high food stress and those diverse microbes can effectively degrade any organic compound even sulfolane and DIPA. However, in GW2, the microorganisms have been exposed to a low level of organic contaminants, therefore, it is possible that they are sensitive to external organic sources. When organic pollutants were introduced to GW2, the competition between the biodegradation of DIPA and sulfolane became significant. On the other hand, As(III) had negligible impact on the sulfolane degradation rate in all three matrices.

4.5 Conclusions

Presence of co-contaminants in sulfolane-contaminated groundwater can impact its degradation differently depending on the type of co-pollutant, pH, and water matrix. Under aerobic conditions, DIPA enhanced sulfolane degradation in one well water, but inhibited its degradation in another. Therefore, DIPA's impact will vary depending on biological, chemical and physical characteristics of each matrix. The metalloid As (III) showed no significant impact on sulfolane-degrading bacteria regardless of concentration and was converted to As(V) under aerobic conditions. Therefore, during sulfolane bioremediation efforts, both pollutants can be oxidized (aerobically) without As (III) significantly impacting the treatment process of sulfolane. Lastly, at environmentally relevant concentrations, natural organic matter (such as fulvic acid) will have a negligible impact on sulfolane degradation in groundwater.

Chapter 5: CONCLUSION AND FUTURE WORK

5.1 Extraction and Analytical challenges of Sulfolane

An aim of this research was to investigate the analytical challenges produced by various environmental matrices such as soil (organic and mineral) and groundwater, particularly at low sulfolane concentrations and to determine the reliability of sulfolane. Various testing laboratories were involved in analysing spiked sulfolane samples and their data were compared. Sulfolane recovery generally demonstrated a bias low. Greater variability of data was observed in sulfolane recovery from organic soils in comparison to mineral soils. Data scattering especially increased at low spiked concentrations (>0.5 mg/kg), more so in organic than mineral soil. The background noise from organic soil forced some testing labs to increase their instrument detection limits and some were unsuccessful in quantifying sulfolane in samples at such low concentrations.

A z_L-score was calculated incorporating analytical and extraction errors. Sulfolane recovery from organic soils often fell outside the acceptable range of ±2, primarily at low concentrations. This is indicative that mineral soil extractions produce much more reliable data than organic, with high concentrations creating more consistent results than low ones. Similarly, sulfolane groundwater recovery had less variability in higher concentrations than lower ones. GC-MS analysis demonstrated soil organic matter to potentially cause false positives during sulfolane analysis. Therefore, at low concentrations of sulfolane in organic soil, sample heterogeneity, biodegradation, interference and extraction inefficiency will significantly impact results.

5.2 Impact of Co-contaminants on Sulfolane Biodegradation in Groundwater

The impact of co-contaminants such as DIPA, As (III) and fulvic acid were examined on sulfolane biodegradation in groundwater. Three different levels of arsenic (0.5, 5 and 25 mg/L)

had negligible impact on sulfolane removal. In GW1, the sulfolane concentration plateaued after 97 hours regardless of the presence of arsenic, even with varying As (III) concentrations. Over 60% of 25 ppm As (III) was converted to As (V) with simultaneous degradation of sulfolane. Fulvic acid had insignificant impact on sulfolane degradation at environmentally relevant concentrations. DIPA, on the other hand, had varying impact on sulfolane removal depending on the biological, chemical and physical characteristics of the groundwater matrix used. Groundwater (GW1) previously contaminated with various organic pollutants and a high microbial count resulted in greater sulfolane degradation in presence of DIPA. No sulfolane was detected after 97 hours with the addition of DIPA while in the control experiment the sulfolane concentration plateaued. GW2 with lower microbial count and less previous exposure to various pollutants inhibited sulfolane removal alongside DIPA. Less than 70% sulfolane degraded after 100 hours while more than 90% of sulfolane was removed when DIPA was not added. As a result, DIPA's impact on sulfolane biodegradation will vary depending on the characteristics of the matrix used.

5.3 Future Research

Future research can consider:

- Analysing the variance in contaminated field samples to better understand the analytical challenges observed from real polluted environments
- Examine the impact of fulvic acid, DIPA and As (III) as a mixture on sulfolane biodegradation
- Study field application of sulfolane-contaminated groundwater of such co-pollutants
- Isolate bacterial strains capable of oxidizing both sulfolane and As (III)
- Observe impact of co-contaminants on sulfolane removal under low oxygen levels

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