FACULTY OF SCIENCE AND TECHNOLOGY

MASTER'S THESIS

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Author: Marit Henriksen	
Supervisor at UiS: Krista Michelle Kaster Co-supervisor: External supervisor(s):	
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Abstract

The Norwegian approach to contaminated soil management typically involves excavation and transportation of soil to a landfill or redistribution within a construction site if the soil is moderately contaminated. Bioremediation, an eco-friendly method, naturally degrades pollutants in soil. Bioremediation can be performed either by monitoring the activity of naturally occurring soil microorganisms or can be enhanced by supplementing essential elements such as electron acceptors, nutrients, and water.

This study assessed both *in situ* and *ex situ* bioremediation techniques and their potential effectiveness in the Norwegian climate, emphasizing hydrocarbon biodegradation. Eleven bioremediation techniques were assessed to determine their suitability. To determine whether bioremediation is currently employed in Norway, requests for data were sent to waste management facilities and research centers.

The findings of this study showed that several methods for bioremediation have been investigated in colder climates. Of the methods examined, several techniques have yielded effective degradation rates. *In situ* methods carry more uncertainties for consistent and rapid degradation. *Ex situ* methods can be controlled and adapted to cold climates and demand little effort to incorporate into the Norwegian model for handling contaminated soil.

Biopiles were deemed the most cost-effective and easily applicable method of the eleven techniques explored. A company employing biopiles for bioremediation was identified. However, the method was not optimized for recycling the sand silt. The inquiries in this study revealed a knowledge gap in incorporating bioremediation within the Norwegian soil management model.

Abbreviations

BAF	Bioaccumulation Factor
BCF	Bio Concentration Factor
BTEX	Benzene, Toluene, Ethylbenzene, Xylene
DDT	Dichlorodiphenyltrichloroethane
DEHP	(2- Ethylhexyl) Phthalate
EVOH	Ethylene Vinyl Alcohol
HDPE	High-Density Polyethylene
PAH	Polycyclic Aromatic Hydrocarbons
PCB	Polychlorinated Biphenyls
PFAS	Poly-And Perfluorinated Alkyl Substances
PFAA	Perfluorinated Alkyl Acids
SOM	Soil Organic Matter
SVE	Soil Vapor Extraction
TBC	Trichlorobenzenes
THC	Total Hydrocarbons
TOC	Total Organic Carbon
TPH	Total Petroleum Hydrocarbons

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1 Introduction

Arable soil, a limited and non-renewable resource formed through weathering and erosion, is crucial for sustaining life(FAO and ITPS, 2015). Yet, human activities like agriculture, mining, industry, and waste disposal have contaminated the soil, reducing soil fertility and posing a risk to living organisms(FAO and UNEP, 2021). This calls for urgent soil preservation and effective remediation strategies. Bioremediation, the degradation of pollutants by the use of soil fauna or plants, has been extensively researched for several decades(Atlas & Philp, 2005; Filler et al., 2008; Hasanuzzaman & Prasad, 2021). Several reviews from the last 20 years are available regarding methodologies, practical implications, as well as biotic and abiotic factors influencing the degradational processes(Aislabie et al., 2006; Atlas, 1981; Chaudhary & Kim, 2019; Rahmeh et al., 2021; Tomei & Daugulis, 2013; Truskewycz et al., 2019; Yap et al., 2021a). Among these, methods for bioremediation of contaminated soil in cold climates have been reviewed by Yap et al. in 2021 and Chaudhary et al. in 2019.

Our reliance on industrial activities has caused and continues to exacerbate environmental pollution (FAO and ITPS, 2015; Norwegian Environment Agency, 2022a). The "Global Assessment on Soil Pollution" report by FAO and UNEP (2021) underscores the pressing necessity to tackle soil pollution and protect the ecosystem services provided by soils. The report proposes a multi-faceted approach, focusing on prevention as the primary strategy. It outlines a plan to close knowledge gaps in soil pollution, enforce legislative and technical frameworks, enhance public awareness and communication, and cultivate international collaboration. In addition, increased use of natural and eco-friendly sustainable management techniques and pollution clean-up methods, such as bioremediation, are encouraged. Soil pollution does not only pose a threat to human health; it affects the food chain and biodiversity as well (Abbasian et al., 2016; Eggen et al., 2020; G. Wu et al., 2010). Given the scarcity of soil and the increased emphasis on sustainable waste disposal, soil restoration has become an essential field of study for environmental scientists, politicians, and communities worldwide. Several contaminated sites have been successfully remediated in Europe, including several hydrocarbon-contaminated brownfield sites, which have been treated with physical remediation like excavation and isolation, and sometimes combined with biological techniques like biodegradation by heterotrophic digestion(JRC, 2015). More than 10,000 polluted sites have been discovered in Norway, and the probability of unidentified polluted sites still exists (Norwegian Environment Agency, 2022c). Local landfills have been cleaned up or are currently being cleaned up, focusing on physical separation of wastes and physical treatment, isolation, and monitoring of runoffs (Nesse & Sundal, 2019). Nevertheless, despite considerable research in temperate and cold regions, evidence of biological treatment for site restoration in Norway is surprisingly sparse.

1.1 Disposal of Contaminated Soil in Norway

Since the inception of soil excavation practices, Norway has primarily relied on landfill disposals for handling soil pollution. This is an act undertaken in accordance with the Norwegian guideline for soil pollution "Health-based states for contaminated ground - TA 2553."(Norwegian Environment Agency, 2009). During the last ten years, there has been an increase in the number of manufacturers in Norway that treat contaminated soil and waste, including both chemical/physical treatments like washing stations and incineration plants (Envir AS, 2023; Lindum AS, 2023; Velde AS, 2023). Lindum AS has been identified as a company that uses full-scale bioremediation to deal with sludge contamination (Rosenvinge, 2019). Lindum AS uses a biopile/landfarming hybrid to biodegrade hydrocarbon-contaminated sludge. In 2022, the company dewatered 20 000 tons of sludge before piling the sludge together with fillers and conventional compost to biodegrade hydrocarbon contamination (A. H. Rosenvinge, personal communication, May 31, 2023). Regarding other major waste management companies, the practice is either physical treatment or isolating the contaminated soil in landfills. There are a few companies known who perform physical treatment, Velde AS and ENVIR AS. These companies wash and separate valuable fractions, like sand, stone, and gravel, to recycle larger proportions of the excavated soil (Envir AS, 2023; Velde AS, 2023). Despite these businesses, a significant volume of contaminated waste and soil is still disposed of in landfills instead of being separated, followed by a reduced amount of waste disposal (Statistics Norway, 2021).

Statistics Norway (2021) provides data on waste disposal to landfills in Norway from 2017 to 2021, as shown in Table 1. According to the statistics supplied, the patterns are stable. Most of the biologically treated waste consists of organic wastes from agriculture and food production, garden and home wastes, and a small quantity of sludge. Composting, biogas production, and general treatment are the reported methods in the biological treatment statistics. Bioremediation is not explicitly specified in the statistics for either biologically treated waste or landfill waste. The total amount of biologically treated waste, which includes composting or biogas production of organic wastes, was 666 thousand tons, compared to 5,600 thousand tons of landfill waste.

Table 1 Presentation of soil deposition in Norwegian waste management sites.

Waste Type per thousand tons	**2017**	**2018**	**2019**	**2020**	**2021**
Disposed and used in landfilling and covering, total	5945	5706	5384	5355	5611
Condition category 2 +3, Total	2841	2572	2748	2692	3081
Condition category 2 +3, Landfills	2256	2527	2677	2668	2975
Condition category 2 +3, Coverage bulk fill	585	44	71	25	106
Condition category 4 +5, Total	3052	3081	2572	2593	2450
Disposed hazardous waste[1]	1175	797	887	864	882
Disposed ordinary waste[2]	1876	2284	1685	1729	1568
Ordinary waste disposal, total, used in landfills[3]	52	53	63	69	80
Biologically treated waste	538	532	605	612	666

1. Contaminated concrete and similar above threshold levels for hazardous waste
2. Moderately contaminated concrete and similar below threshold levels for hazardous waste
3. Clean concrete and similar products

The prolonged disposal of contaminated soil in landfills increases greenhouse gas emissions from transportation and depletes essential resources (Norwegian Environment Agency, 2021). Methane is generated in landfills through methanogenesis, which involves the anaerobic degradation of organic waste materials under oxygen-depleted conditions (Kebreab et al., 2006). Regarding hydrocarbon-contaminated soil, Yang and colleagues (2018) investigated *in situ* methane emissions from a crude oil-contaminated area and discovered that the emissions were significantly greater than those from clean soil. Large portions of the soil's composition are regarded as inert, and landfills are governed by the acceptable concentration of total organic carbon (TOC) in the soil to be deposited. Since 2009, disposal of organic waste in landfills has been prohibited in Norway (Avfallsforskriften, 2004, § 9-4). As of this date, no known landfill in Norway can accept soil with a TOC content above 5% for ordinary waste and 1% for hazardous waste (NOHA AS, 2023).

Nevertheless, the landfills are often off-site with long transport routes, and the volumes sent to storage can be vast. Bioremediation of soil is a promising technology used to restore polluted soil by using microbes to break down and transform contaminants into less harmful forms(Chaudhary & Kim, 2019). However, the effectiveness of bioremediation depends on various factors such as soil properties, temperature, pH, the type and concentration of contaminants, and presence of suitable degrading organisms(Varjani & Upasani, 2017). In Norway, where transporting contaminated soil to disposal sites results in significant carbon dioxide emissions and other pollutants, applying bioremediation to reduce the volume of soil disposed of in landfills represents an intriguing possibility. This study will examine the efficacy of several bioremediation procedures with an emphasis on hydrocarbon degradation, such as both *in situ* and *ex situ* bioremediation methods, to eliminate or significantly reduce pollutants from soil with hope that information obtained in this study could be used in minimizing the amount of soil transported to landfills.

2 Methods

The articles for this comprehensive review were mainly collected through systematic searches in the academic database Scopus, PubMed, Google Scholar, and Web of Science. The research methodology was inspired by the PRISMA method for systematic reviews and meta-analysis, although additional searches had to be performed to cover each topic (Page et al., 2021). Bioremediation nomenclature is not universal, so additional searches for synonyms had to be performed to cover each topic in this review. Search phrases used to compile the literature for this review involved "hydrocarbons," "bioremediation," "soil," and "cold" or "temperate.". The further search included the bioremediation techniques to ensure the relevant literature was evaluated, as displayed in Table 2. Searches performed in the database Scopus were evaluated initially by screening the abstract before the paper was evaluated for relevance. Only literature written in English or Norwegian was included.

Table 2 List of searches performed in Scopus

ID	Initial search phrases in Scopus, completed May 2023	Results
1	Hydrocarbons AND bioremediation AND soil AND cold	164
2	Hydrocarbons AND bioremediation AND soil AND temperate	20
	Expanded search for technologies: ("technology" AND bioremediation AND soil AND cold)	
3	"Natural attenuation"	13
4	Biosparging	0
5	Bioventing	5
6	Bioslurping	0
7	Phytoremediation	14
8	Landfarming	11
9	Biopile/composting	20/8
10	Bioreactor	10

Bioremediation of hydrocarbons in soil has been a topic for research for many decades, resulting in tremendous amounts of published literature. To narrow the number of articles in the review, the papers were evaluated based on the research territory and the year of publication Research published prior to 2010 was excluded due to a general timeframe limitation. For some bioremediation techniques, however, no new studies were published after 2010. In these cases, the older publications were evaluated. Papers concerning field scale trials in regions with a climate similar to Norway were emphasized and evaluated based on the individual papers methodology. For example, colder regions include Antarctica, but the climate in Antarctica varies significantly from the climate in the northern hemisphere. Consequently, some articles were excluded based on location, but studies of significance for this review were included. The reference lists from the included papers were used to include relevant literature not retrieved through the searches. The records included in this review proceeded, as shown by the modified PRISMA diagram in Figure 1

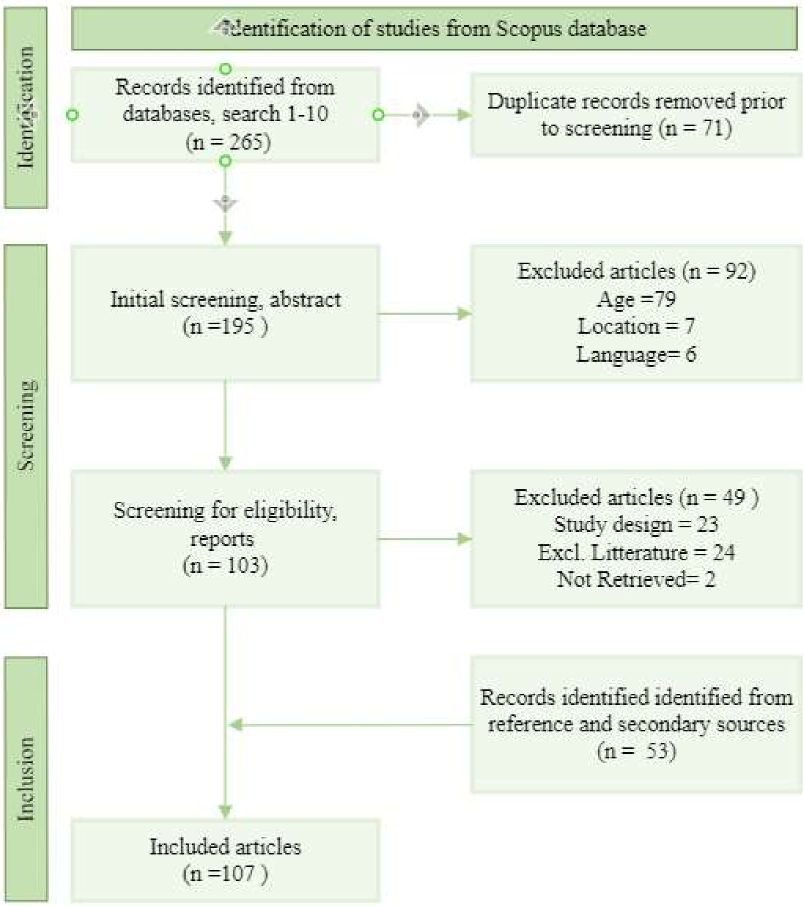

Figure 1 PRISMA flow chart for included articles.The figure presents the article selection process and search strategy employed in the bioremediation review. The boxes represent exclusion criteria, indicating the number of articles included or excluded at each stage.

3 Hydrocarbons

Hydrocarbons are organic compounds primarily made up of carbon and hydrogen atoms(Hart et al., 2007, p. 16). Hydrocarbons can exist as gases, liquids, and solids, with liquids and solids being the most prevalent forms at atmospheric pressure (Walker et al., 2012, Chapter 1). Hydrocarbons have low water solubility as they are of low polarity. The hydrocarbons are classified into two primary divisions based on the structure of their molecules; aliphatic alkanes and alkenes, and aromatics (Hart et al., 2007, Chapters 2–4). Polycyclic aromatic hydrocarbons (PAHs) are compounds with two or more aromatic rings. The hexagonal benzene ring Benzene and its methyl derivatives Toluene, Ethylbenzene, and Xylene are common examples of aromatics that are crucial for human and ecological toxicology. Among the vast array of PAHs, benzo(a)pyrene stands out as a particularly toxic member, earning its reputation as the most hazardous and studied PAH. (Breedveld & Arp, 2022d, p. 24, 2022a, p. 19). These compounds are of particular concern due to their potential toxicity and persistence in the environment(Joner et al., 2004).

Branched hydrocarbons, PAHs, and other aromatic hydrocarbons are often more resistant to biodegradation than aliphatic hydrocarbons due to their higher energy demand for bond cleavages and complex structures. Since microorganisms tend to prefer readily degradable substrates over complex structures, it is assumed that the degradation of the alkanes is preferred over complex aromatic compounds. Although this is not necessarily a "rule of thumb,"; Brzeszcz and Kaszycki (2018) thoroughly discussed the possibilities for simultaneous *n*-alkane and aromatic hydrocarbon degradation. One example is the simultaneous degradation of alkanes and naphthalene (an aromatic hydrocarbon) performed by *Rhodococcus sp.* (Andreoni et al., 2000). Contamination is often composed of a very complex mixture of hydrocarbons, and as discussed by the review authors, the majority of the studies reviewed were performed in mixtures of few substances and are not necessarily relatable to the *in situ* situations (Brzeszcz & Kaszycki, 2018).

Separating the total petroleum hydrocarbons (TPH) into sub-fractions allows for a better focus on specific compounds and variations in volatility. By fractionating hydrocarbons based on carbon chain length, it can be easier to understand the composition and complexity when dealing with soil contamination. A known nomenclature in the scientific literature is hydrocarbons of low molecular weight (LMW), medium molecular weight (MMW), and high molecular weight (HMW). However, there is inconsistency in the scientific literature regarding the specific fractions used. For instance, the Norwegian guidelines determine C_8-C_{10} as LMW, C_{10}- C_{12} as MMW, and C_{12}-C_{35} as HMW hydrocarbons (Norwegian Environment Agency, 2009). Pinedo et al. (2014) separated the hydrocarbons in C_5-C_{10} as volatile hydrocarbons and the semi-volatiles as the lighter C_{10}- C_{20} and heavier C_{20}-C_{40} fractions. Other articles refer to the total fraction of C_{10}-C_{40} as total petroleum hydrocarbons (TPH) (Mao et al., 2009). Studies from more recent years tend to refer to the Canadian CCME (2008) guidelines, were the fractions are F1=C_6-C_{10}, F2=C_{10}-C_{16}, F3=C_{16}-C_{34}, and F4=C_{34}-C_{50}. To simplify this in this review and make the findings form different studies comparable, the carbon chain lengths will be used instead of fraction numbers or abbreviations, as far as possible. Therefore, the choice of hydrocarbon fractions for toxicology studies depends on the intended purpose and requires careful consideration of the compounds that may pose greater risks to human health.

Naturally occurring hydrocarbons, contrary to those of anthropogenic origin, have long been intrinsic components of marine and terrestrial ecosystems, profoundly influencing the biochemical composition of soil and seawater over geological timescales. The hydrocarbon status of an unaltered soil system is determined by the flux of organic matter from natural biochemical and geological processes.(Gennadiev et al., 2015). For instance, the natural polycyclic aromatic hydrocarbons (PAHs) in soils predominantly originate from the lithogenic basis of the soil cover, extending to deep lithosphere horizons. Hydrocarbons are also very common in sea water, for instance, originating from oil seeps and from primary producers. The marine primary production contributes significantly, as pentadecane produced by marine cyanobacteria was estimated to be 100 to 500 times greater than oil from spills and natural seeps acids (Love et al., 2021)

4 Physical and chemical properties of soil

Soil, a crucial resource, consists of a diverse mixture of sand, silt, clay, organic compounds, living organisms, moisture, and gases, making it a heterogeneous matrix. Hence, it is important to protect and conserve it (FAO and ITPS, 2015; Montanarella et al., 2016). Soil promotes plant development, regulates water and nutrient cycling, filters and purifies water, store and releases gases, and provide habitats for a diverse community of microorganisms and animals. Consequently, soil is an indispensable element within terrestrial ecosystems, playing a vital role in sustaining life on Earth.

4.1 Pedogenesis

Pedogenesis is the process of soil development, encompassing the creation of soil profiles. The diagram presented in Figure 2 outlines the various processes involved in soil formation (Buol, 2006). Diurnal radiation governs the exchange of energy, while transpiration and precipitation facilitate water exchange (Hartemink et al., 2020). Physical and chemical weathering, erosion, and deposition collectively contribute to the alteration and movement of minerals. Intrasolum translocation refers to activities occurring within the soil column, while lateral displacement is primarily driven by groundwater flow. The formation of horizons is influenced by biocycling, which involves the uptake and utilization of nutrients through plant and animal processes. However, as water percolates beyond the rooting depth of the existing vegetation, leaching mechanisms remove soluble organic and inorganic components from the soil, thereby compromising the biocycling capacity of the vegetation (Coleman & Crossley, 2018, Chapters 1–3).

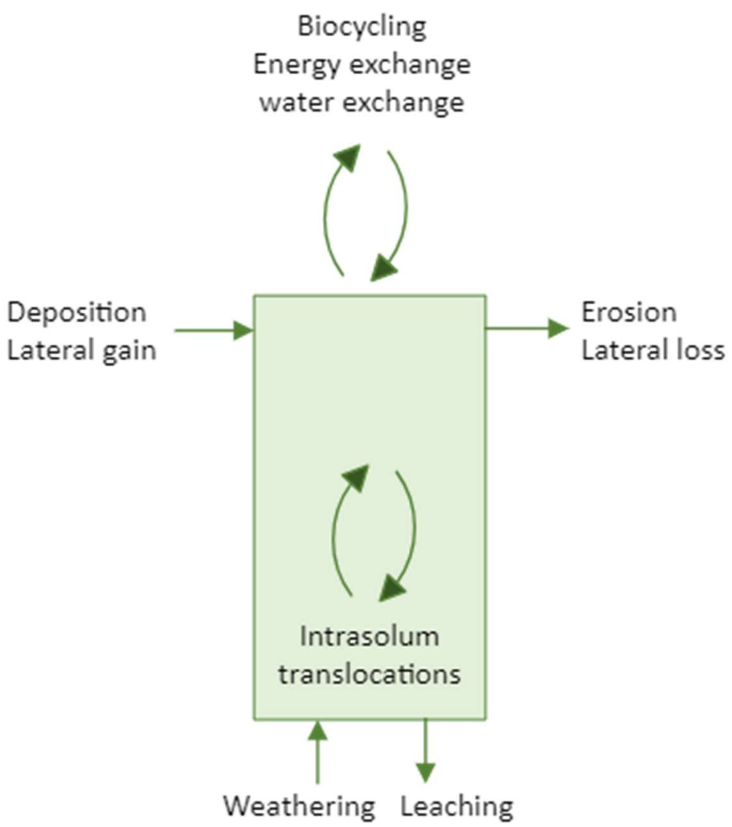

Figure 2 Soil forming processes.
The figure illustrates how forces affect the development of soil over time. The figure is modified from Buol, S. (2006).

4.2 Soil characteristics and composition

Soil formation is a complex process and is the result of time, climate, organisms present, and parent material (Arnold et al., 1990; Bockheim et al., 2014). The relief of the region contributes to soil profiles, as the varying slopes and elevations influence erosion, deposition, and the distribution of soil materials. In our modern civilization, soil fertility is depleted of nutrients faster than erosion, and weathering can replenish them (FAO and ITPS, 2015; Francaviglia et al., 2023). Therefore, this non-renewable resource should be handled with care, and efforts should be made to remediate contaminated sites.

Bioremediation is influenced by soil properties (Haghollahi et al., 2016). Soil is a heterogenous matrix of minerals, soil air, soil water, and both living and decomposing organic matter, shown in Figure 3 (Stirling et al., 2016). The mineral particles typically encompass 45% of the composition and often consist of sand, silt, and clay. Sand, silt, and clay refer to the soil particle size, where sand is the largest, silt is smaller, and clay is the smallest. These variations in particle size result in complex aggregates that make up the soil texture and further affect water retention, drainage, and nutrient availability (Basset et al., 2023). Sandy soils are more porous compared to clay-rich soils, and the texture of the soil can influence properties like water-holding capacity, nutrient availability, and soil structure (Scalenghe, 2006). Soil air contributes to gas exchange between the soil particles and the atmosphere, and the organic content, the most minor yet important fraction, typically contributes by approximately 5%.

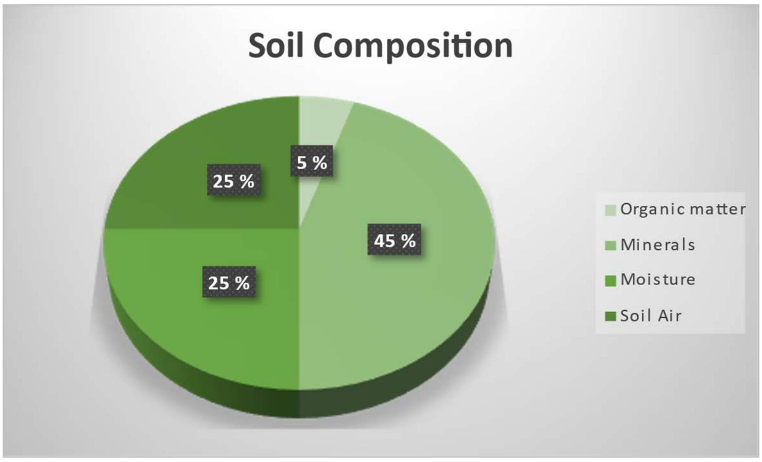

Figure 3 Physical composition of the soil, each slice representing the four soil main content. Redrawn from Stirling et al. (2016), chapter 2.

4.2.1 Soil organic matter

Soil organic matter (SOM) is a mixture of degrading organic compounds that are mixed with other soil components(Lehmann & Kleber, 2015). SOM significantly enhances soil chemical fertility by acting as a nutrient reserve and preventing nutrient leaching into groundwater. Typically, soil organic matter content remains relatively low, around 5% (Stirling et al., 2016). SOM holds a tremendous cation exchange capacity and is a source of carbon and energy for heterotrophs (Kaiser et al., 2008). SOM has a significant impact on soil physical quality by enhancing water retention and aggregating mineral particles, contributing to a favorable soil structure, and preventing soil erosion. Organic particles can retain organic pollutants due to their hydrophobic nature (Ahmed et al., 2015). Hence, where contaminants are spilled, the contaminants will be adsorbed by soil particles, and only a low percentage of the contaminants will be bioavailable (Barriuso et al., 2008)

Within SOM, humic acid represents a fraction of random amorphous polyaromatic organic macromolecules known as humic substances. These substances serve as redox compounds, increasing biodiversity and facilitating electron shuttling for humic-reducing microorganisms, thereby aiding in bioremediation(Kulikova & Perminova, 2021). Pollutants adsorb to the functional groups of humic substances, such as naphthalene and carboxylic acid, enhancing pollutant bioavailability and preventing transportation and leaching(Ahmed et al., 2015; Jednak et al., 2017)

Soil inorganics consist of weathered parent rock material and can be separated into primary and secondary minerals. Primary minerals generally are negatively charged, more resistant to weathering, and have a crystalline structure, whereas secondary minerals usually contain positive charges, are less resistant to weathering, and frequently possess an amorphous structure(Sagbo et al., 2015). Certain clay minerals, for instance, are noted for their high cation exchange capacity and net negative charge. These charges will greatly affect pollutant transport (Durães et al., 2018).

4.2.2 Soil liquid phase

The liquid phase of soil plays a pivotal role in facilitating nutrient uptake by plants and microbial activity, both crucial to ecosystem functionality. Soil-water interactions heavily influence the bioavailability of pollutants to soil microbes, as these before-mentioned interactions can affect the sorption of pollutants to soil particles, thereby limiting their catabolic activity (Barriuso et al., 2008). The dynamics of water movement in soil are greatly influenced by factors such as weather patterns, topography, soil porosity, and slope formations(Hoylman et al., 2019). Compounds and pollutants are dissolved and moved through the soil by three essential processes: infiltration through the soil surface, downward and lateral movement by percolation, and capillary action into soil pores (Durães et al., 2018).

Well-structured soils have larger pore spaces that can hold more water and allow for better infiltration. In contrast, less percolative soils have smaller pore spaces that can become easily clogged, hindering water infiltration (Basset et al., 2023). In the context of bioremediation, it is crucial to comprehend soil-water interactions. The water impacts how contaminants might emerge more readily in porous soils than in more compact clayey soils, the effect of freeze-thaw cycles on the amount of liquid water available, as well as the significant role of abiotic variables (Akbari & Ghoshal, 2014; Chang, Klemm, et al., 2011).

4.2.3 Soil atmosphere

The soil atmosphere, or soil air, is a mixture of gases and vapors and contributes significantly to bioremediation efficiency (Walworth et al., 2013). Whether biodegradation occurs aerobically or anaerobically is determined by the amount of oxygen in the soil atmosphere. Inorganic gases such as nitrogen, oxygen, and carbon dioxide are the major components, but the presence of vapors like ammonia and volatile organic components such as carbohydrates, organic acids, alcohols, oils, and pesticides (Davie-Martin et al., 2015; D.-G. Kim et al., 2012). Depending on soil parameters such as nutrients, species richness, moisture, and available carbon sources, these gases can be a result of soil metabolism, with a minor contribution from abiotic processes such as volatilization, adsorption, and dissolution. Soil air supplies oxygen vital for aerobic biomass respiration and can ventilate CO_2 from metabolism and volatilized contaminants from contaminated soils (Davie-Martin et al., 2015; Kuzyakov, 2006).

4.2.4 Soil biomass

Both micro and macrofauna in soils are of impotence for nutrient cycling, transformation of organic compounds, and soil aggregation. Larger organisms, especially invertebrates, contribute to soil cycles by creating tunnels and channels that are vital for facilitating airflow and infiltration of water, can increase bioremediation rates by decomposing macromolecules an thus bioavailability to microorganisms, and detoxify pollutants (Hickman & Reid, 2008). The microorganisms responsible for bioremediation will be discussed in detail in chapter 6.

4.3 The soil profile

Soil profiles are vertical cross-sections of soil that include information about the soil's history, including characteristics and property distribution. A soil profile can be divided into horizons, wich is stratas parallel to the soil surface whose characteristics is different from the layers above and beneath. The soil horizon plays a crucial role in the selection of appropriate *in situ* bioremediation methods for soil treatment. Understanding the distinct layers and their properties is essential for determining the most effective approach to address contaminant remediation within a specific soil profile. Many soil mechanisms are similar to the mechanism that translocate and weather pollutants (Quinton & Rickson, 1993). Most surface horizons, or epipedons in soil taxonomy, are formed by the biocycling and mixing of organic and mineral material. Most subsurface horizons are caused by mineral fluxes, accumulations, and mineral transformations of parent materials. Eluviation, the emergence of suspended and soluble particles from upper soil layers, and illuviation, the accumulation of similar substances in deeper subsoils, drive the creation of subsurface horizons (Bockheim et al., 2014; Hartemink et al., 2020). Each horizon has its own unique characteristics and properties, which will be determined by the soil texture and composition. Further, water movements through the different horizons can reallocate contaminants. For instance, precipitation tends to accumulate in top soils; hence the contaminants can be concentrated in the upper horizons (Dougill et al., 1998).

5 Biodiversity and ecosystem dynamics

The soil serves as a habitat for a vast range of microorganisms, plants, and animals, which together contribute to the notable biodiversity found in soil ecosystems (Kent & Triplett, 2002; Torsvik & Øvreås, 2002; van Bruggen & Semenov, 2000). Within this complex network of interactions, soil micro- and meso- and macrofauna cohabit with plants and play a significant role in linking plant primary production to soil fauna's secondary production. The existence of healthy soil fauna is imperative for preserving soil structure and stimulating plant growth (Bardgett & van der Putten, 2014; García-Segura et al., 2018). Environmental changes can induce transformations in soil communities, where pollutants often encourage the proliferation of pollutant-degrading organisms (Van Dorst et al., 2016). For instance, Yergau et al. (2012) used metagenomic sequencing to investigate the changes in a biopile community from Nunavut, Canada, and found that the biopile treatment increased the abundance of hydrocarbon-degrading organisms. Unfortunately, this transition also diminishes species diversity (Ferguson et al., 2020; S. Yang et al., 2016). For instance, bacterial communities in hydrocarbon-contaminated soil undergo changes when exposed to different alkanes as substrates, even with minor differences in the alkane chain-length leading to distinct community structures (Kuc et al., 2019). This was also shown at Ellesmere Island, were an oil spill in 1972 had a lasting impact, with the diversity of bacterial communities significantly altered even 40 years post-incident (Ferguson et al., 2020). The oil spill drastically reduced bacterial diversity, indicated by the Shannon-Weaver index of pristine soils around 5.2, compared to 2.6 in the oil-contaminated soil.

Biomass in soil consists of organisms in many physiological states, such as active, dormant, or dead organisms. The active biomass typically resides in the upper 30 cm of the soil. However, Min et al. (2021) showed how potentially active communities at a depth of 240 cm could be activated by the addition of substrates within hours to days after substrate addition. The driving force behind biogeochemical processes, including those involved in bioremediation, is the active fraction of biomass (Blagodatskaya & Kuzyakov, 2013). This active microbial biomass is defined by its capacity to utilize substrates, rapidly respond to substrate input, and sustain growth and reproduction. Microorganisms in soil that can increase their metabolic activity in response to substrate exposure within minutes to hours represent potentially active microorganisms. These organisms reside between active and dormant stages. Estimating the active proportion of microbial biomass in soil can provide insights into how the microbial community adapts to pollution and how effectively the biomass can aid in bioremediation. For instance, Mukherjee and colleagues(Mukherjee et al., 2014) observed that bacterial diversity decreased in a creosote-contaminated site while the total microbial activity measured by basal respiration and FDA hydrolysis rates increased in these areas. This observed increase in total microbial activity amidst a decline in bacterial diversity suggests that potentially active organisms may play a significant role in microbial responses to contamination. Understanding the behavior of these organisms under various conditions can enhance the capacity to design and implement more effective bioremediation strategies in diverse ecosystems.

5.1 Toxicologic effects of soil pollution

Pollution can be defined as the presence of any substance in quantities sufficient enough to endanger humans, animals, or the environment (Walker et al., 2012). Many substances, including both organic and inorganic compounds, can contribute to soil pollution. Toxicological evidence can manifest in both biochemical and physiological ways. Evidence of toxicity can manifest in both biochemical and physiological forms. Biochemical responses involve molecular-level changes, such as modifications in gene expression, enzyme activity, or protein synthesis. Physiological responses, on the other hand, are changes that occur at the organism level, which may include alterations in growth, reproduction, or behavior.

Toxicological responses can be used to evaluate the impact of pollutants on organisms and ecosystems. These responses provide different information and are sensitive to different exposure scenarios. Biochemical responses can detect acute exposure to high pollutant concentrations, whereas physiological responses can detect chronic exposure to lower quantities, and both forms of responses can be utilized in conjunction to understand pollution impacts truly.

The Bioconcentration Factor (BCF) serves as a predictive metric for the accumulation of metals within biological tissues. BCF is formally characterized as the ratio of a chemical concentration within an organism to that in the organism's surrounding environment. It is important to note that the BCF can vary across different organisms for a given substance. This variation is attributed to each organism's different uptake and utilization pathways, leading to distinct bioconcentration outcomes (Walker et al., 2012, Chapter 4). This can be illustrated by the BFC of benzo(a)pyrene in vegetables, having a BCF of 2,06 l/kg w.w. for stem vegetables and a BCF of 1531 l/kg w.w. for root vegetables(Breedveld & Arp, 2022b, p. 67).

Where BCF is essential to aquatic systems and plant uptake, the bioaccumulation factor (BAF) is critical for terrestrial systems (Walker et al., 2012, p. 83). BAF is a measure of the extent to which a substance accumulates in an organism relative to its concentration in ingested food, where a high BAF indicates a greater tendency for an organism to accumulate a substance.

5.1.1 Inorganic pollutants

A major group of inorganic pollutants are metals(Walker et al., 2012). Metals are inorganic, persistent toxicants and can promote oxidative stress, neurotoxicity, carcinogenicity, and fertility loss (Briffa et al., 2020; Paithankar et al., 2021). Additionally, heavy metal pollution can cause ecological imbalances, leading to the loss of biodiversity and ecosystem services. As a protective response to metal toxicity, metals can accumulate in biological tissue (Briffa et al., 2020). The mining, excavation, and quarrying of rocks can release metal contaminants into the soil, increasing the risk of soil pollution. In the presence of the right geominerals, these activities expose large surfaces to weathering and particle migration, potentially leaching vast quantities of heavy metals. The Norwegian Geotechnical Institute has performed surveys to investigate the heavy metal content in Norwegian agricultural soil and revealed the uptake and presence of heavy metals in plants (Jeng & Bergseth, 1992; Mellum et al., 1998). Recently, new sites with a possibility for acidic bedrock in Norway have been discovered and are suspected of leaching heavy metals into the surroundings (Rogaland County Governor, 2023a, 2023b). The weathering of alum slate and other acidic bedrocks may be potentially harmful to human health when crops are grown in soil rich in acidic minerals. These health effects can include a wide array of known diseases, ranging from reduced energy levels and organ damage to longterm-exposure diseases like Alzheimer’s disease, Parkinson’s disease, and muscular dystrophy (Jaishankar et al., 2014). As a result, oral exposure is now proposed to be included as an assessment criterion in revising the current acceptance criteria for contaminated soil (Breedveld & Arp, 2022c; Norwegian Environment Agency, 2009).

Anions are inorganic ions that can have serious environmental consequences, such as when nitrogen and phosphorus induce eutrophication of water bodies. Excessive phosphates and nitrogen lead to a rapid increase in the population of autotrophs, followed by oxygen depletion of the water bodies (X. Wu et al., 2021). Anions can damage ecosystems due to the high concentration of nitrogen compounds in the environment, even though the substance itself is harmless. Excess nitrates can transform into hazardous nitrite, which binds to hemoglobin and decreases its oxygen-binding capacity, resulting in methemoglobinemia (Ludlow et al., 2022). Algal blooms proliferate under excess phosphorus and can produce toxins, microcystins, that enter the food chain and can cause neurological and gastrointestinal damage (Hallegraeff et al., 1995). It is recognized that eutrophic lakes may contain microcystin-producing cyanobacteria, which can be lethal to both microbes and mammals (de Figueiredo et al., 2004). Microcystins specifically target liver cells, producing cytoskeleton damage and subsequent hepatic hemorrhage (internal bleeding) (Dawson, 1998). These cases demonstrate how a seemingly harmless substance can lead to detrimental outcomes.

5.1.2 Organic pollutants

There are many organic contaminants: Hydrocarbons, halogenated organic chemicals, insecticides, organometallic compounds, solvents, flame retardants, personal care products, and radioactive isotopes are some of the most important classes. A xenobiotic is a chemical foreign to an organism's normal physiology and metabolism (Katayama et al., 2010). Depending on the chemical properties, dose, exposure time, and organism susceptibility, the effect of an individual xenobiotic on a living organism can range from harmless to toxic. Some xenobiotics can be metabolized or eliminated by the organism's detoxification systems, while others can accumulate in tissues and cause damage over time. Hydrophobic compounds like hydrocarbons can penetrate the hydrophobic region of the cell membrane, disrupting its structure and function (Sikkema et al., 1995). This can lead to increased membrane permeability, loss of membrane potential, and altered ion transport. Furthermore, hydrocarbons can also interfere with and inhibit membrane-bound proteins. These effects can cause damage to cellular processes such as energy production, signal transduction, and nutrient uptake, causing cell lysis and apoptosis.

5.1.3 The fate of pollutants in the environment

Petroleum spills in the environment can lead to a variety of consequences. These can involve petroleum volatilizing into the atmosphere, dissolving into water, undergoing photo-oxidation or auto-oxidation, or becoming adsorbed to soil particles (Truskewycz et al., 2019). in terrestrial systems, the interaction between the hydrocarbon and soil particles is the most significant factor determining bioremediation efficiency (Lăcătușu et al., 2021). In this context, it is critical to consider specific hydrocarbons, where targeted substances like TPH, PAH, and BTEX can be of interest. These compounds have different physical and chemical properties, influencing how they interact with soil and move through the environment. Lăcătușu et al. (2021) investigated how TPH, PAH, and BTEX from crude oil percolated in lysimeters containing three soil types of different permeability, organic content, and conductivity: a sandy-textured permeable soil, a moderately permeable loamy-textured soil, and a loamy-clayey highly permeable soil. Lysimeters are containers with core extractions of soil, which are frequently used in soil studies as they provide valuable insights into soil profiles. The study by Lăcătușu et al. (2021) showed that TPH and PAH accumulated in the upper 20 cm of the clayey soil, while the BTEX accumulated in the upper 30 cm with a tendency to emerge unevenly through the soil profile. The loamy soil had an even distribution of TPH and PAH across the complete profile. In the sandy soil, TPH showed an even distribution down to 70 cm depth and PAH to 30 cm. for both the loamy and sandy soil, BTEX was unevenly distributed throughout the complete soil profile.

5.2 Hydrocarbon-degrading organisms in cold, terrestrial ecosystems

Hydrocarbon-degrading organisms represent a diverse array of prokaryotes and eukaryotes, capable of utilizing hydrocarbons as their primary carbon source (Prince, 2010; Prince et al., 2010). Under aerobic conditions, heterotrophic prokaryotes can degrade large amounts of hydrocarbons in a relatively short time frame (Brzeszcz & Kaszycki, 2018). A few eukaryotes are also known to grow on hydrocarbons as a sole source of carbon (Zhang et al., 2019). The adaptability of these organisms to diverse environments is critical to their function in bioremediation. Microorganisms have evolved to inhabit an extensive range of climates, ranging from warm to cold and even extreme conditions. Psychrotolerant organisms are of interest when regarding bioremediation. They can grow in near-freezing conditions, yet their optimal growth temperature is above 20°C. These organisms are more frequently found and isolated from permanently cold environments (<5°C), compared to their psychrophilic counterparts, which can only grow at temperatures below 20°C (Giudice et al., 2010). Psychrotolerant organisms have been shown to degrade Naphthalene at a rate three times higher in Arctic seawater compared to temperate seawater (Bagi, 2013). Chaudhary & Kim (2019) stated in their review how pathways for hydrocarbon degradation are similar in mesophilic and psychrophilic organisms. They further emphasized how the enzymatic reactions are slow, indicating slower bioremediation rates. The findings from Bagi's (2013) research clearly showed how this may not be correct. Noteworthy, the study was performed on seawater, not on soil, and studies comparing bioremediation rates of mesophilic and psychrophilic or psychrotolerant soil organisms were not identified during this thesis, thus could be an area for further studies.

Since the discovery of the first hydrocarbon-degrading organisms in the early 1900s, countless genera of hydrocarbon-degrading microorganisms have been identified (Bushnell & Haas, 1941). Since then, the technological world has evolved significantly. Now, highly advanced tools that enable DNA amplification and sequencing for accurate taxonomic classification aid in rapid investigation. These technologies allow researchers to identify multiple strains simultaneously. In a study by Dziurzynski et al. (2023), the active layer of permafrost at Spitsbergen Island has been studied for fungal diversity, revealing 14 psychrotolerant multi-metal resistant strains by PCR amplification. DNA sequencing by the bacterial 16S rRNA gene was used to monitor the microbial community change in an oil-polluted soil and identified the relative abundance of five phyla; *Actinobacteria, Proteobacteria, Chloroflexi, Acidobacteria,* and *Gemmatimonadetes* (Yan et al., 2018). The technology of rapid taxonomy identification has revealed that *Pseudomonas, Rhodococcus, Acinetobacter,* and *Sphingomonas* are well-known hydrocarbon degraders (Aislabie et al., 2006). Table 3 provides additionally identified genera, complemented by an annotation for how the organism was tested for biodegradation of a target compound. The table also includes data for organisms that have been optimized by the use of models. However, the table is far from comprehensive.

During the research for this review, an excess of articles regarding species identification in Antarctica was observed. With its harsh and dry climate, Antarctica must be the most challenging area where bioremediation can be performed. In addition to being cold, dry, and often very dark, the Protocol on Environmental Protection to the Antarctic Treaty Consultative Parties (1991) prohibits the introduction of alien organisms. Both factors necessitate the isolation and characterization of native microorganisms for use in bioremediation strategies. Despite the promise of these organisms for hydrocarbon degradation, it is crucial to acknowledge the risks and challenges involved in developing cultures for bioaugmentation (VKM, 2016). The successful establishment of inoculated microorganisms in soil matrixes can be complex, and the stresses of transitioning from laboratory to field conditions, competition with natural microbiota, and predation can all become limiting factors.

Table 3 Hydrocarbon degrading organisms and removal efficiency for degraded contaminants.

Organism	**Degraded pollutant**	**Origin**	**Method and temperature**	**Removal efficiency**	**Reference**
Sphingobium sp.	Carbazole	Antarctica	Lab, 15 °C, 15d	25%	(Sato et al., 2023)
"consortium BS24"	Diesel	Antarctica	Model, 12,5 °C	94.77%	(Roslee et al., 2021)
Arthrobacter sp, *Rhodococcus sp.* *Pseudomonas sp.* *Stenotrophomonas sp.* *Sphingobacterium*	TPH	Alpine meadow snow	Lab, 10 °C, 30d	53%	(Teng et al., 2021)
Arthrobacter sp. Strain AQ5-05	Diesel	Antarctica	Lab, 10 °C, 7d	56%	(Abdulrasheed et al., 2020)
Lysinibacillus fusiformis str 15-4	"petroleum hydrocarbons"	Qinghai-Tibet	Lab, 20 °C, 96h	56%,	(Li et al., 2020)
Exophiala macquariensis sp.	Toluene	Antarctica	Nm.	Nd.	(Zhang et al., 2019)
Dietzia maris *Arthrobacter rhombi*	Arctic Diesel Hexadecane	Canada	Soil slurry, 10 °C 5% NaCl 0% NaCl	21% 37%	(Chang et al., 2018)
Chryseobacterium *Bacillus* *Pseudomonas*	polybrominated diphenyl ethers (PBDEs)	China	Soil, 10 °C 150d	61-78%	(L. Wang et al., 2016)
Arthrobacter sp. strain AQ5-05 *Arthrobacter sp. strain* AQ5-06 *Rhodococcus sp. strain* AQ5-07	Phenol	Antarctica	Lab, (aq), 10-20 °C	Nr.	(Lee et al., 2018)
Pseudomonas caribbica	nC_{11}-nC_{14} Diesel	Antarctica	Lab, 15 °C	Nr.	(Martorell et al., 2017)
Pseudomonas Citronellis *Arthrobacter Aurescens*	Atrazine	Finland	Lab, 10 °C, 115d	21%,	(Nousiainen et al., 2015)

Organism	Degraded pollutant	Origin	Method and temperature	Removal efficiency	Reference
Pseudomonas sp. *Stenotrophomonas sp.* *Pedobacter sp*	Octane Dodecane Hexane Toluene Naphthalene Phenanthrene Pyrene Diesel oil JP1 Crude oil	Antarctica	Lab, 15 °C 15 d	nr	(Vázquez et al., 2013)
Acinetobacter sp., *Pseudomonas sp.,* *Ralstonia sp.* *Microbacterium sp*	Phenanthrene Anthracene Pyrene Perylene Total PAH	Spain (pristine Atlantic forest)	Lab, 5-15 °C, 137 d	94% 58% 54% 69% 67%	(Raquel et al., 2013)
Sphingomonas sp. *pseudomonas sp.* *Variovorax sp.*	Phenantrene	Greenland	Soil, 0 °C, 150 d	22-30%	(Sørensen et al., 2010)
Pseudomonas sp. ADL15 *Rhodococcus sp.* ADL36	n-Dodecane	Antarctica	Model	33,77% 95,67%	(Habib et al., 2018)
Rhodococcus sp Strain AQ5-07	Diesel	Antarctica	Model 23,5 °C	90,39%	(Roslee et al., 2020)
"consortium BS24"	Diesel	Antarctica	Model, 12,5 °C	94.77%	(Roslee et al., 2021)
Conventionally produced consortium	TPH	Canada	Model,	90,7%	(Gomez & Sartaj, 2014)

5.3 Hydrocarbon biodegradation

Microorganisms inhabit remarkable abilities to metabolize and utilize hydrocarbons, which is essential for biodegradation. Scientists have been aware of the hydrocarbon-degrading ability of microorganisms since the early 20th century (Bushnell & Haas, 1941). Metabolism is an intricate and vital process that underlies the functioning of every living organism (Madigan & Brock, 2015). Log_{Kow}, the octanol-water partition coefficient, quantifies the tendency of hydrocarbons to partition into hydrophobic phases. Hydrocarbons generally have high Log_{Kow} values, indicating a strong affinity for hydrophobic environments. This will limit hydrocarbon bioavailability to microorganisms.

5.3.1 Bioavailability and uptake of hydrocarbons

Multiple mechanisms exist for hydrocarbon uptake. The hydrocarbon can adhere to the surface of microorganisms and undergo transmembrane passive diffusion across concentration gradients or through energy-demanding active transport (Miyata et al., 2004; Z. Wang et al., 2022). Despite being hydrophilic, bacteria absorb and destroy hydrophobic substances by interfacial absorption (Bouchez et al., 1997; Westgate et al., 1995). This process involves the contact of microorganisms with hydrocarbon substrates at the liquid-liquid interface for adsorption and uptake. Many microorganisms produce surfactant compounds to emulsify hydrocarbon molecules into micelles, a process discussed in greater detail in section 7.5.4 (Trudgeon et al., 2020). Surfactants enhance the bioavailability and degradation of PAHs in soil since they aid in desorbing or detaching the PAHs from soil particles, enhancing their transfer into the aqueous phase where they become more bioavailable for microbial degradation (L. Wu et al., 2020).

5.3.2 Degradational Pathways for Hydrocarbons

Even though hydrocarbon structures are heterogenous, they are generally degraded by the common intermediate pathways used for assimilation and respiration (Abbasian et al., 2015; Ladino-Orjuela et al., 2016). Hydrocarbons are initially degraded through peripheral and central pathways to break down highly complex structures into smaller intermediates. The smaller molecules are suitable for the intermediary pathways such as the tricarboxylic acid cycle (TCA cyle), β-oxidation and the glycolytic pathway (Abbasian et al., 2015; Ladino-

Orjuela et al., 2016). Figure 4 illustrates how hydrocarbons are often aerobically degraded by oxygenases, which catalyze the incorporation of oxygen into the hydrocarbon molecule, producing alcohols from aliphates or catechols from aromatics (Yap et al., 2021b). The aliphatic hydrocarbons will be further converted by central metabolic pathways into fatty acids, which undergo β-oxidation to produce acetyl coenzyme A, an intermediate in the TCA cycle. The catechols from the aromatic compounds will undergo ring-cleavage, more precisely called dearomatization, and eventually produce intermediates that enter the TCA cycle. In the absence of oxygen, the terminal electron acceptors that can be used include nitrate, manganese, iron, sulfate, and carbon dioxide (Ladino-Orjuela et al., 2016).

Anaerobically, the initial activating enzymes are synthases, dehydrogenases, and carboxylases, and the methods for activation are diverse. There are five different activation mechanisms for anaerobic degradation, including phosphorylation, methylation, carboxylation, oxygen-independent hydroxylation, and fumarate insertion (Abbasian et al., 2015). In the absence of molecular oxygen, anaerobic organisms rely on alternative sources such as nitrate (NO_3^-), sulfate (SO_4^{2-}), carbon dioxide (CO_2), and iron (Fe^{3+}) as electron acceptors for hydrocarbon degradation. After activation, the intermediates will enter a central pathway suitable for the central intermediate, and eventually, the enzymatic transformations lead to β-oxidation and the TCA cycle.

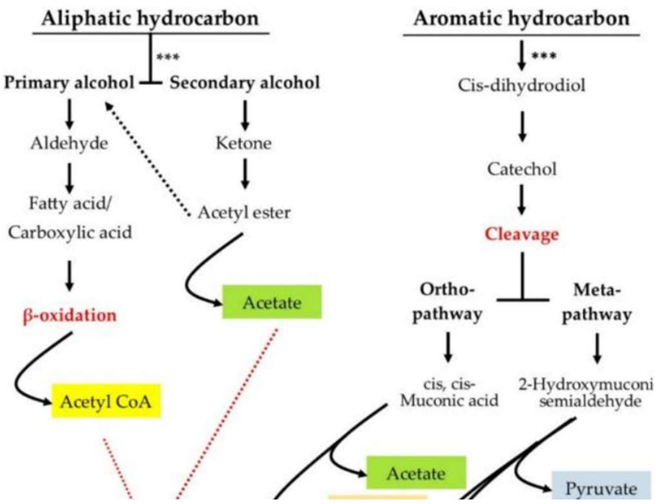

Figure 4 Aerobic pathways hydrocarbon metabolism. The figure illutstrate the pathway for aerobic hydrocarbon activation by dioxygenases and introduction of oxygen to the contaminant. The figure is collected from Yap et al. (2021). CC BY 4.0

5.3.3 Enzymes and psychrozymes

Microorganisms have evolved specialized enzymes to enable microorganisms to efficiently break down numerous contaminants (Abbasian et al., 2015; Cabral et al., 2022; Ladino-Orjuela et al., 2016). The enzyme classes known for participation in hydrocarbon metabolism are oxygenases, synthases, hydrolases, carboxylases, reductases, co-enzymes, hydratases, and dehydrogenases (Cabral et al., 2022). Monooxygenases and dioxygenases are responsible for the initial activation of hydrocarbons and require one or two oxygen atoms to oxidize hydrocarbons, respectively (Widdel & Musat, 2010). Enzymes exist in all microbial communities in petroleum-contaminated sites, and the enzymatic activity of some enzymes tends to decrease with temperature (Kang et al., 2009). In cold environments, cold-active enzymes, known as psychrozymes, are required for hydrocarbon breakdown (Miri et al., 2019, 2021, 2022). Miri et al. (2021) isolated the psychrophilic Pseudomonas S2TR-14, which produces cold-active toluene p-xylene monooxygenase and Catechol 1,2-dioxygenase. These enzymes were upconcentrated in laboratory at 10°C and immobilized onto a biochar-chitosan matrix. The covalent attachment of these enzymes on micro and nano biochar-chitosan matrices resulted in high enzyme stability and the ability to break down more than 80% of BTEX molecules at 10°C. Furthermore, a soil column test performed by Miri and colleagues (2022) used an enzyme cocktail of 10 U/ml p-xylene monooxygenase and 20 U/ml catechol 2,3-dioxygenases to eliminate 92-94% p-xylene. However, the p-xylene clearance rate produced by a lower enzyme-concentration solution was less than 30% and close to the untreated control column (22.2% removal). Despite the promising results from these laboratory studies, a pilot-scale study showed that the biodegradation rate decreased as the experimental scale increased (Miri et al., 2023). Despite these limitations, the potential for utilizing large quantities of cold-adapted enzymes for biostimulation in colder regions remains promising, paving the way for effective hydrocarbon degradation in such environments

5.3.4 Toxicity of intermediates

Toxic intermediates produced during degradation can significantly impact degradability if the hydrocarbon-degraders become terminated or inhibited due to toxicological caused by the intermediates (Imam et al., 2022). Although some active intermediates are rapidly further metabolized and do not cause significant harm, some intermediate metabolites have been identified as toxic or carcinogenic, as demonstrated by Cámara et al. (2004)and Sikkema et al.

(Sikkema et al., 1995). For instance, PAHs can be hydroxylated or carboxylates, resulting in genotoxicity and developmental toxicity (Chibwe et al., 2015). In response, some organisms have developed transmembrane efflux pumps, such as those discussed by Bugg et al. (2000). The efflux pumps play a crucial role in eliminating harmful substances like polycyclic aromatic hydrocarbons (PAHs) from cells. However, they might not be effective in removing epoxide intermediates due to their lower polarity (Abdel-Shafy & Mansour, 2016).

6 Contaminated Soil Regulations in Norway

The regulated classification of soil contamination for Norway is given in the guidelines TA2553/2009 (Norwegian Environment Agency, 2009). The regulation is divided into five different condition categories and assigned color codes, see Table 4.

Table 4 Classification of soil contamination level

Condition category	1	2	3	4	5
Category description	**Very Good**	**Good**	**Moderate**	**Poor**	**Very Poor**
Upper limit regulated by	Normative levels	health-based acceptance criteria	health-based acceptance criteria	health-based acceptance criteria	Levels regarded hazardous waste

The Norwegian guideline TA 2553 includes threshold values for controlled chemicals such as aliphatic hydrocarbons, polyaromatic hydrocarbons (PAHS), benzene, toluene, ethylbenzene, and xylene (the BTEX group), heavy metals, polychlorinated biphenyls (PCBs), and DDT. Also regulated are di (2- ethylhexyl) phthalate (DEHP), dioxins and furans, phenol, benzene, and trichloroethene. The threshold values are listed in Table 5. However, the parameter total petroleum hydrocarbons (TPH, known as total hydrocarbon (THC) in Norwegian) are missing from the current guidelines. Between November 2022 and February 2023, a draft consultation to regulate the threshold values was published to classify contaminated soil (Breedveld & Arp, 2022d). The new draft consultation introduces several major proposals that urge the reconsideration of current guidelines. It argues that the current guidelines were formulated on outdated information and did not consider the soil ecosystem. The current guidelines also fail to account for individual risks associated with multiple-source exposure to substances that pose threats to health and the environment. These guidelines were initially constructed on the premise that the tolerable weekly intake (TWI) of contaminants could originate solely from polluted grounds. However, TWI, which denotes the maximum exposure to a contaminant that will not harm health over a lifetime, may come from other sources, like food intake. Additionally, the draft consultation proposes threshold values for THC, as opposed to the current regulations that only consider individual aliphatic fractions of C_8-C_{10}, C_{10}-C_{12}, and C_{12}-C_{35}.

Table 5 Condition categories for contaminated soil in Norway

Condition category and description *Conc. in mg/kg*	**1** **Very Good**	**2** **Good**	**3** **Moderate**	**4** **Poor**	**5** **Very Poor**
Arsenic	< 8	8-20	20-50	50-600	600-1000
Lead	< 60	60 -100	100-300	300-700	700-2500
Cadmium	<1,5	1,5-10	10-15	15-30	30-1000
Mercury	<1	1-2	2-4	4-10	10-1000
Cupper	< 100	100-200	200-1000	1000-8500	8500-25000
Zink	<200	200-500	500-1000	1000-5000	5000-25000
Chromium (III)	<50	50-200	200-500	500-2800	2800-25000
Chromium (IV)	<2	2-5	5-20	20-80	80-1000
Nickel	< 60	60- 135	135-200	200-1200	1200-2500
PCB_7	< 0,01	0,01-0,5	0,5-1	1-5	5-50
DDT	<0,04	0,04-4	4-12	12-30	30-50
PAH_{16}	<2	2-8	8-50	50-150	150-2500
Benzo(a)pyrene	< 0,1	0,1-0,5	0,5- 5	5 -15	15-100
Aliphates C_8-C_{10} 1)	< 10	≤10	10-40	40-50	50-20000
Aliphates > C_{10}-C_{12} 1)	< 50	50- 60	60-130	130-300	300-20000
Aliphates > C_{12}-C_{35}	< 100	100-300	300-600	600-2000	2000-20000
DEHP	<2,8	2,8-25	25-40	40-60	60-5000
Dioksines/furanes	<0.00001	0,00001-0,00002	0,00002-0,0001	0,0001-0,00036	0,00036-0,015
Phenol	<0,1	0,1-4	4-40	40-400	400-25000
Benzene 1)	<0,01	0,01-0,015	0,015-0,04	0,04-0,05	0,05-1000
Trichloroethylen	<0,1	0,1-0,2	0,2-0,6	0,6-0,8	0,8-1000[1]

[1] 1) For volatile substances, gas as an exposure route will give low limit values for human health. If gas in buildings is not a relevant route of exposure, a site-specific risk assessment should be carried out to calculate site-specific acceptance criteria.

7 Overview of bioremediation

Bioremediation is the utilization of the metabolic capabilities of living microorganisms, including bacteria, fungi, and plants, to enzymatically transform or degrade contaminants present in the environment (Atlas & Philp, 2005). Bioremediation can be performed either *in situ* at the contamination site or by excavation and transport from the contaminated site to an *ex situ* treatment facility. Bioremediation is regarded as an environmentally preferable method for decontaminating contaminated environments and has been utilized and studied thoroughly (Abbasian et al., 2015; Dehnavi & Ebrahimipour, 2022; Margesin & Schinner, 1999; Miri et al., 2019; G. Wu et al., 2010; Yap et al., 2021a). This review has investigated both *in situ* and *ex situ* approaches to bioremediation, illustrated in Figure 5.

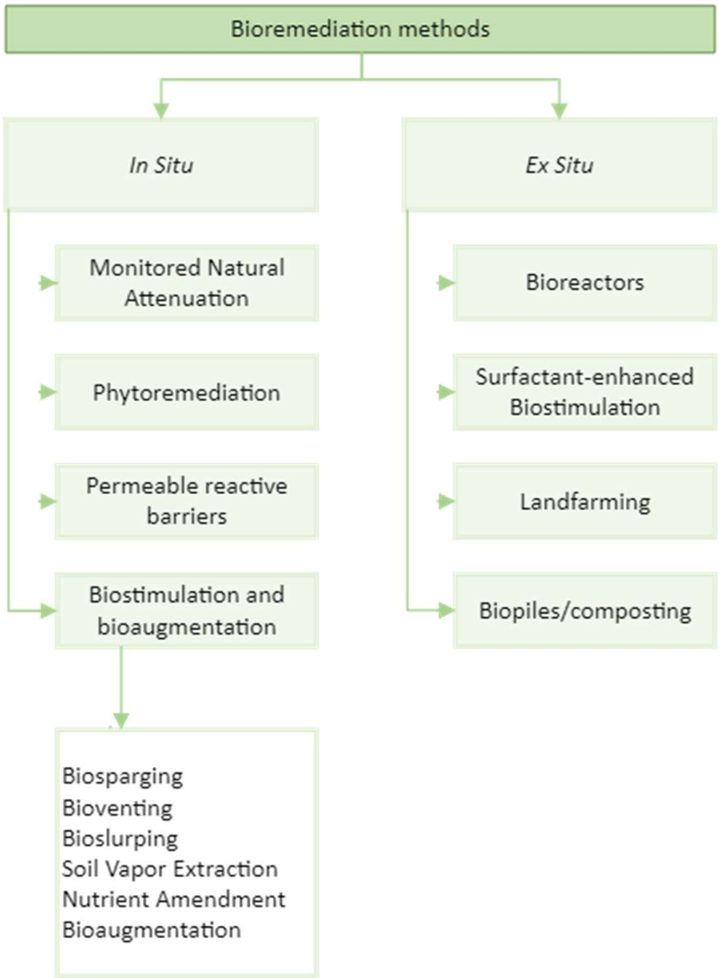

Figure 5 Bioremediation method flow chart. The flow chart illustrate which bioremediation processes are regarded as in situ or ex situ methods.Please note that the methods can be used interchangeably, and therefore, the distinction is not fixed

Appropriate environmental parameters, such as temperature, pH, moisture, and the presence of degrading organisms, are essential for bioremediation. The process is regarded to be relatively slow in colder climates and requires adequate time and contact between organisms and pollutants to be successful(McWatters, Wilkins, et al., 2016; Song et al., 2023). Engineering solutions such as air injection wells, nutrition supplementation, and water supply could be implemented to increase the pollutant removal rate. In the absence of indigenous species capable of degrading the target pollutant at the contaminated site, bioremediation can be aided by an inoculum, although the effectiveness of bioaugmentation is uncertain (Bento et al., 2005; Kauppi et al., 2011).

Details for bioremediation methods included in this review are presented in Table 6. Each method is associated with its target pollutant and the temperature conditions applied during trials. This table aims to facilitate a straightforward comparison and comprehensive understanding of the different bioremediation strategies. Subsequent sections will provide a detailed discussion of these methods, elucidating their operational mechanisms, benefits, and potential drawbacks.

Table 6 Bioremediation methods and contaminant removal rates, duration of study, and temperature.

Method	Location	Temp, °C	Contaminant	Removal efficiency	Duration	Reference
Monitored natural attenuation	Antarctica	Annual	C_{10}-C_{40} TCB	90% To n.d.**	2 years	(Song et al., 2023)
Monitored natural attenuation	Antarctica	Annual	TPH	80%	10 years	(Ferguson et al., 2020)
Bioventing	Norway	8°C	TPH	87%	1 year	(Sparrevik & Breedveld, 1997)
Bioslurping	Korea	n.r*	TPH BTEX	90% 93%	2 years	(S. Kim et al., 2014)
Biopile with meat and bone meal + cyclodextrin	Finland	Lab, 21°C	Diesel	96%	12 weeks	(Cavazzoli et al., 2022)
Biopile with fish meal	Antarctica	annual	TPH	71%	7 weeks	(Dias et al., 2015)
Biopile	Finland,	Lab, 10°C	Atrazine	52%	16 weeks	(Nousiainen et al., 2015)
Biopile, augmented	Finland	Lab, 10°C	Atrazine	76%	16 weeks	(Nousiainen et al., 2015)
Biopiles, woodchips	Finland	8 - 10 °C	Diesel	75%	11 months	(Kauppi et al., 2011)
Phytoremediation *salix alaxensis*	Canada	Annual	C_6-C_{50} $>C_{50}$	65% 75%	5 years	(Robichaud et al., 2019)
Phytoremediation *Populus sp.*	Finland	Annual	TPH	78%	3 years	(Lopez-Echartea et al., 2020)
Landfarming Diurnal	Canada	1 – 10°C	TPH C_{10}-C_{16} C_{16}-C_{34} Sum UCM	55% 63% 53% 47%	2 months	(Chang, Whyte, et al., 2011)
Landfarming Diurnal	Canada	6 °C	TPH C_{10}-C_{16} C_{16}-C_{34} Sum UCM	19% 36% 21% 19%	2 months	(Chang, Whyte, et al., 2011)
Landfarming augmented	Korea	6 °C	TPH	73%	30 days	(Jeong et al., 2015)

Method	Location	Temp, °C	Contaminant	Removal efficiency	Duration	Reference
Pseudomonas sp						
Biopiles, geomembrane	Antarctica	6,5 °C	TPH	75%	40 days	(Martínez Álvarez et al., 2017).
Biopiles, geomembrane repeated	Antarctica	5,4 °C	TPH	55%	40 days	(Martínez Álvarez et al., 2020).
Biopile, freeze-thaw	Canada	Frozen	C_{10}-C_{16} C_{16}-C_{34}	13% 33%	9 months	(J. Kim et al., 2018)
		Thaw, acc.	C_{10}-C_{16} C_{16}-C_{34}	47%, 39%		
		Final, acc	C_{10}-C_{16} C_{16}-C_{34}	57% 58%		
Biopile, Clay	Canada	15 °C	TPH C_{16}-C_{34}	43% 38%	110 days	(Akbari & Ghoshal, 2014)
Biopile, Clayey soil	Canada	5 - 15 °C	C_{10}-C_{16} C_{16}-C_{34}	48%	70 days	(Akbari & Ghoshal, 2015)
Biopiles, sandy soil, compost + inoculum	Canada	0 - 10 °C	TPH	82%	14 weeks	(Gomez & Sartaj, 2013)
Biopile/landfarm hybrid - summer	Norway	Annual	C_8-C_{35}	71%	50 days	(Rosenvinge, 2019)
Biopile/landfarm hybrid - winter	Norway	Annual	C_8-C_{35}	25% 55%	50 days 90 days	(Rosenvinge, 2019)
Bioreactor, enzymatic biostimulation	Canada	24 °C	xylene	88-90%	2 months	(Miri et al., 2023)

* n.r. not reported
** n.d. not detected

7.1 Bioremediation and the Norwegian Climate

The Norwegian climate demonstrates considerable geographical variation(The World Bank Group, 2023). The terrestrial region predominantly exhibits a temperate climate in the lowlands, transitioning to a polar climate in the NorthDespite its high latitude, Norway has a mild climate due to the warming influence of ocean currents and wind systems, which is exacerbated by the geographic distribution of marine and terrestrial ecosystems, as well as mountain range design (Balling et al., 1987). Wind patterns and precipitation distribution are complex, interlinked with temperature variations and topographical characteristics. The nation experiences significant precipitation, with an average annual rainfall approximating 1100 millimeters (The World Bank Group, 2023). The west coast is characterized by milder winters, while the interior regions exhibit a cold-temperate climate with persistent snow cover.

Mean annual temperatures in Norway present noticeable regional differences. The summer mean annual temperature is approximately 15°C, although the temperature can reach 30°C during summer. The winter mean annual temperature differs more across the country, ranging from -1°C in the South to - 10°C in the North. The temperatures can differ significantly in winter, with mild winters in the South and temperatures reaching -20-30°C in the North. Further, insolation differs significantly between winter and summer. In the southern regions, there are approximately eighteen hours of sunlight during summer and about six hours of daylight during winter. Conversely, daylight persists 24 hours in the northern parts during the summer, while it can be absent during winter. The multifaceted nature of the Norwegian climate poses a challenge to designing bioremediation systems.

7.2 Economics of bioremediation

Bioremediation is not a universal solution for all instances of hydrocarbon pollution; it necessitates detailed site-specific evaluation and technique tailoring. The financial implications of five different bioremediation methods for prolonged hydrocarbon-polluted soils differed substantially regarding initial investment and ongoing costs (Orellana et al., 2022a). The expense of addressing formerly hydrocarbon-contaminated soils varied considerably. Relatively simple biostimulation with 10% compost had a cost of USD 50.7/m^3 soil. A combined bioaugmented and aerated biopile with a soil-to-compost ratio of 3:2 to

USD 310.4/m^3 due to the complexity of the equipment required. Simultaneously, Orellana and colleagues (2022b) compared their costs with summarized prices from 130 bioremediation studies, with the majority located in the United States of America (Figure 6). They found that the increase in costs was correlated with the complexity of required equipment, the efficiency of pollutant removal, and the use of materials for bioaugmentation. However, it is essential to note that although the costs are higher for advanced methods, this does not necessarily imply that the more expensive methods lack value or are unworthy of consideration. Depending on the level of contamination, the type of pollutants present, and the desired outcome, these more expensive methods could still be the preferred or only viable solution. The cost analysis should be part of a broader decision-making process that also considers environmental and health impacts, legal requirements, and other considerations.

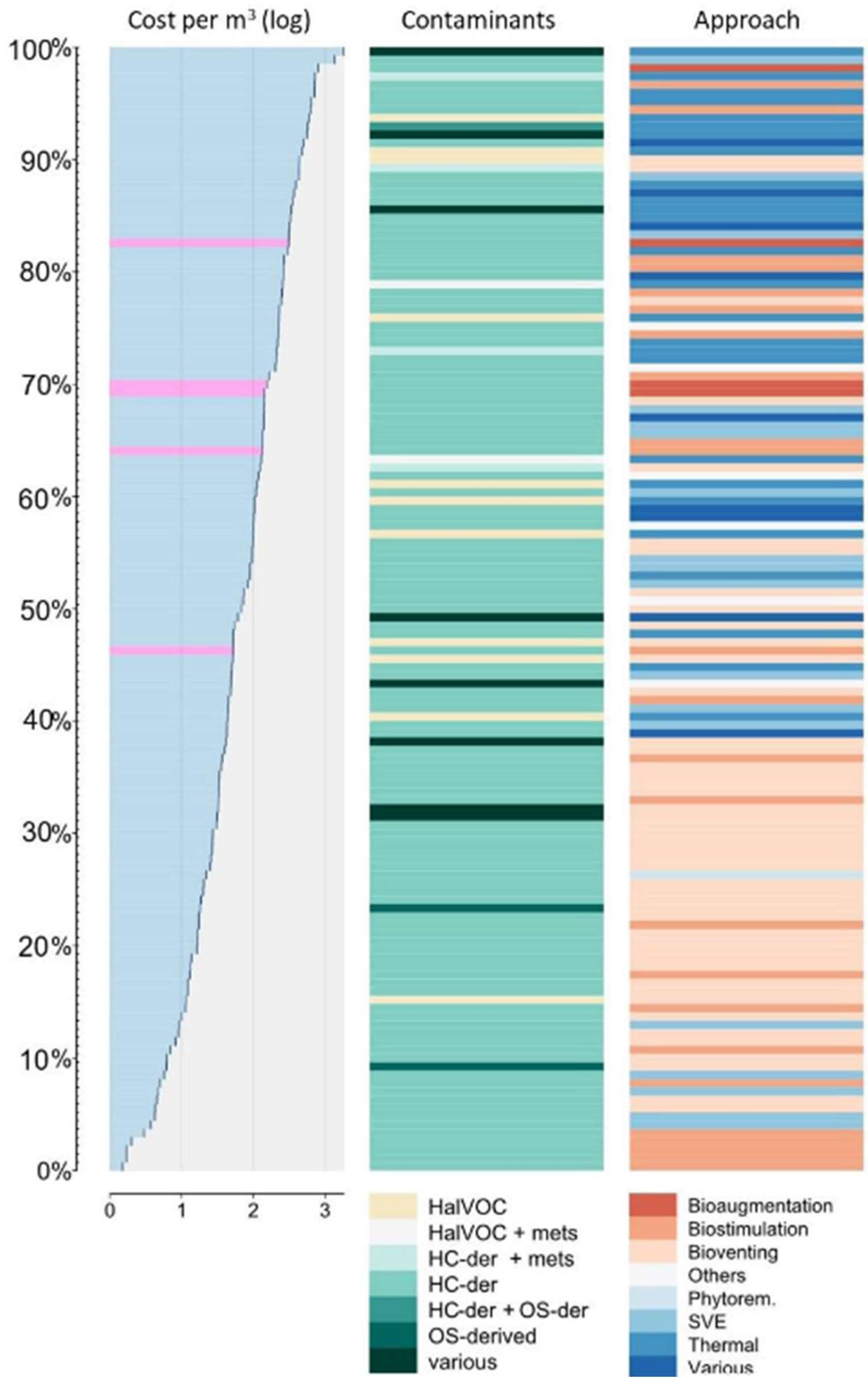

Figure 6 Comparison of costs and bioremediation methods. The bar chart on the left represents the costs of bioremediation for a set of projects involving contaminated soils. The costs are sorted from the cheapest (0%) to the most expensive (100%). The cost of bioremediation is expressed in USD per cubic meter (m_3) of contaminated soil on a logarithmic scale. Retrieved from Orellana et al., 2022. CC BY 4.0

7.3 Abiotic factors affecting bioremediation

Several factors are known for affecting bioremediation of hydrocarbons. These factors differ depending on the environment. Temperature influences degradation in both marine and terrestrial environments. However, the behavior of pollutants in the soil is also subject to adsorption to soil particles, undergoes freeze-thaw cycles, and is affected by age (Bagi, 2013; Varjani & Upasani, 2017; Xiao & Zytner, 2019).

7.3.1 Temperature and moisture

Temperature and moisture are perhaps the most important abiotic factors affecting bioremediation (Varjani & Upasani, 2017). Natural attenuation in Antarctica showed no significant degradation for 12 years (McWatters, Wilkins, et al., 2016), compared to a restoration period of approximately three years in a temperate region in China with an average temperature of 8°C (Song et al., 2023). These sites differed in temperature and annual precipitation regimes, thus showing their importance. In addition, in colder and temperate climates, the soil will be subject to freeze-thaw cycles; hence, as the temperature rises, the degradation rate will increase (Okonkwo et al., 2022). Okonkwo and colleagues showed that the activity of lipase and dehydrogenase decreased during freezing events, while the thawing process compensated for the loss. The study also found a switch in removing different fractions of hydrocarbons during freeze-thaw events. Active removal of C_{10}-C_{23} was observed during the freezing phase, but during the thawing phase, the C_{23}-C_{34} was degraded. Notably, this study was performed on freshly contaminated soil. For the long-term polluted soil, the C_{23}-C_{34} was degraded most rapidly.

7.3.2 Freeze-thaw cycles

The availability of terminal electron acceptors will determine if biodegradation occurs under aerobic or anaerobic conditions. Aerobic biodegradation is energetically most favorable for organisms, but as the air circulation in soils can be low and affected by soil porosity, oxygen depletion occurs relatively often. When the freezing phase proceeds over a couple of weeks, like in natural seasonal cycles, solutes tend to be excluded from the solid ice phase and thus alter the chemical composition of the liquid phase (Konrad & McCammon, 1990). During the cold season, the presence of nutrients can thus extend the period of residual unfrozen water in the soil due to osmotic pressure. This phenomenon can depress the freezing point of the soil and stimulate microbial activity, thereby sustaining the biodegradation process for an extended period (J. Kim et al., 2021). In addition, Kim et al. observed that the biodegradation of long-term polluted soils under subzero temperatures differed slightly from that of above-freezing temperatures. C_{16}–C_{34} was degraded more effectively than C_{10}–C_{16}. It was postulated that lighter hydrocarbons are bound to frozen water, rendering them inaccessible to microorganisms and that the heavier hydrocarbons might interact with salt-rich unfrozen water films, making them more susceptible to deterioration.

7.3.3 Aging and adsorption

The age of the contaminant to be treated is important in biostimulation treatments; long-term exposure can affect the acclimatization of the indigenous microbial community, and reduce bioavailability by sorption to soil particles, prohibiting contaminant extractability (Mosco & Zytner, 2017; Xiao & Zytner, 2019). Sparrevik and Breedveld (1997) observed slow degradation rates in a Norwegian field scale trial of bioventing and nutrient stimulation of aged diesel-polluted soil, where aged pollution degraded at one-third of the rate compared to a previous study looking at freshly polluted soil. Mosco and Zytner (2017) observed similar findings in a laboratory trial using formerly clean soils amended with diesel, followed by aging for four months prior to a bioventing experiment. The degradation rate was reduced by a factor of 2 using the aged, polluted soil, compared to the immediately polluted soil before the degradation test. Further, Freshly polluted soil showed no significant reduction in TPH for 11 months, but acclimatized soil treated with nutrients at lower temperatures was degraded by 82% in five months (King et al., 2014). King and colleagues demonstrated significant TPH and short-chain aliphatic degradation at 10°C and 20°C, although the test executed at 10°C required a higher air flow rate at 275 cm^3/min to degrade TPH by 82,5%, compared to the low air flow rate at 140 cm^3/min and removal rate at 92,5%. Degradation of long-chained aliphatic and aromatic components was also detected.

Xiao and Zytner (2019) observed the acclimation of indigenous organisms where soil was polluted for 300 days via a process called wet aging, in which clean soil is polluted and kept moist during the aging period. During the time of wet aging, biodegradation rates moderately increased, indicating the adaptation of the indigenous species. The same study evaluated the extractability of naphthalene, isooctane, toluene, xylene, and mesitylene after 270 days of aging. The compounds were moderately extractable from sandy soil and nearly unextractable from clayey soils. These studies highlight the significant role that the age of pollution and the nature of soil play in biostimulation treatments, with aged pollutants exhibiting quicker response to biostimulation, and how the bioavailability of contaminants is being affected by soil type. Understanding these variables is paramount for the effective implementation of bioremediation strategies when choosing technology.

7.4 *In situ*

In situ bioremediation refers to cleaning up pollution at the site of contamination by using or supporting the microbial life already present there. The methodologies for *in situ* bioremediation include natural attenuation, aeration and stimulation technologies for field application, and phytoremediation. Bioaugmentation, the use of specific microorganisms selected or engineered to degrade the target contaminants effectively and can be added to a contaminated site, can be a helpful tool for *in situ* bioremediation. The success of *in situ* bioremediation heavily depends on the type and concentration of the contaminants, the presence of suitable microorganisms, and the environmental conditions at the site (Azubuike et al., 2016). The *in situ* methods are often referred to in the literature as potentially more cost-effective (Chaudhary & Kim, 2019; Yap et al., 2021a). However, this relies on the contaminant's position relative to the treatment facility, the intended use of the contaminated land, and the potential biostimulation equipment required. Processes like natural attenuation or phytoremediation may be cost-effective due to the absence of required measures, particularly if the monitoring site is adjacent to civilization, yet these are the most time-consuming remediation strategies(Ferguson et al., 2020; McWatters, Wilkins, et al., 2016). An offsite contaminated location requiring biostimulation, such as air sparging or bioventing, would require infrastructure to power aeration pumps, which may be expensive. Thus, *in situ* methods should be examined separately for each scenario.

7.4.1 Natural attenuation

Continuous monitoring of polluted locations where native bacteria reduce the target contaminant without human intervention is referred to as monitored natural attenuation

(Ferguson et al., 2020). The contaminated areas can be monitored using methods such as soil- and water sampling, the use of monitoring wells for groundwater monitoring, and monitoring of soil vapor (Martí et al., 2014; Song et al., 2023). When using natural attenuation, an environmental risk assessment should be conducted to prevent the spreading of contaminants to groundwater and nearby surface waterways and to prevent agricultural activities in the contaminated area (Breedveld et al., 2021). In addition to the human risk factors, microbial communities are at risk for more permanent alterations, as discussed in section 5 (Ferguson et al., 2020).

When hydrocarbons are spilled in the environment, the spilled contaminants start interacting with soil particles and will be adsorbed. The adherence will become more pronounced in weeks to months, affecting the bioavailability of the compounds. As a result, branched or large molecules can become more challenging to utilize, thus extending the remediation timespan (Zytner et al., 2019). Natural attenuation becomes monitorable when a microbial community has acclimatized to a spill and started to degrade or cometabolize the contaminant. Without assistance, this process can be exceedingly slow. Song and colleagues (2023) tracked the remediation of a site contaminated with heavy metals, hydrocarbons in the range C_{10}-C_{40}, and tetrachlorobiphenyl (TCB). The initial concentration of 13.50–782.33 mg/kg C_{10}-C_{40} hydrocarbons decreased to 12.2–72.0 mg/kg, whit an average half-life of 693 days. The TCB concentration decreased to undetectable levels over three years. The contamination levels reported by Song et al. were not alarmingly high and could correspond to a condition category class 4 "poor," as shown in Section 6, Table 4. Ferguson and colleagues (Ferguson et al., 2020) showed how the natural attenuation in Antarctica could degrade <1200 µg TPH per decade; nevertheless, this was a removal rate of 80%.

7.4.2 *In situ* Biostimulation and bioaugmentation

Biostimulation refers to the process of enhancing the activity of indigenous microorganisms in the environment by providing them with nutrients, electron donors or acceptors, or other amendments that they need to thrive(Margesin, 2000). Biostimulation mechanisms like biosparging, bioslurping, and bioventing involve the use of air or oxygen injection or extraction and potentially the addition of other nutrients to promote the growth of aerobic hydrocarbon degraders. The technological solutions are separated by what mechanism air and nutrients are supplied to or extracted from the contaminated site or soil zone, illustrated in Figure 7 (Cadotte et al., 2007). Aeration techniques are occasionally combined with nutrition to support adequate degradation (Shewfelt et al., 2005). These methods require the application of technologies and are thus more costly compared to natural attenuation. However, they may increase the rates of biodegradation (Orellana et al., 2022a; Simpanen et al., 2016).

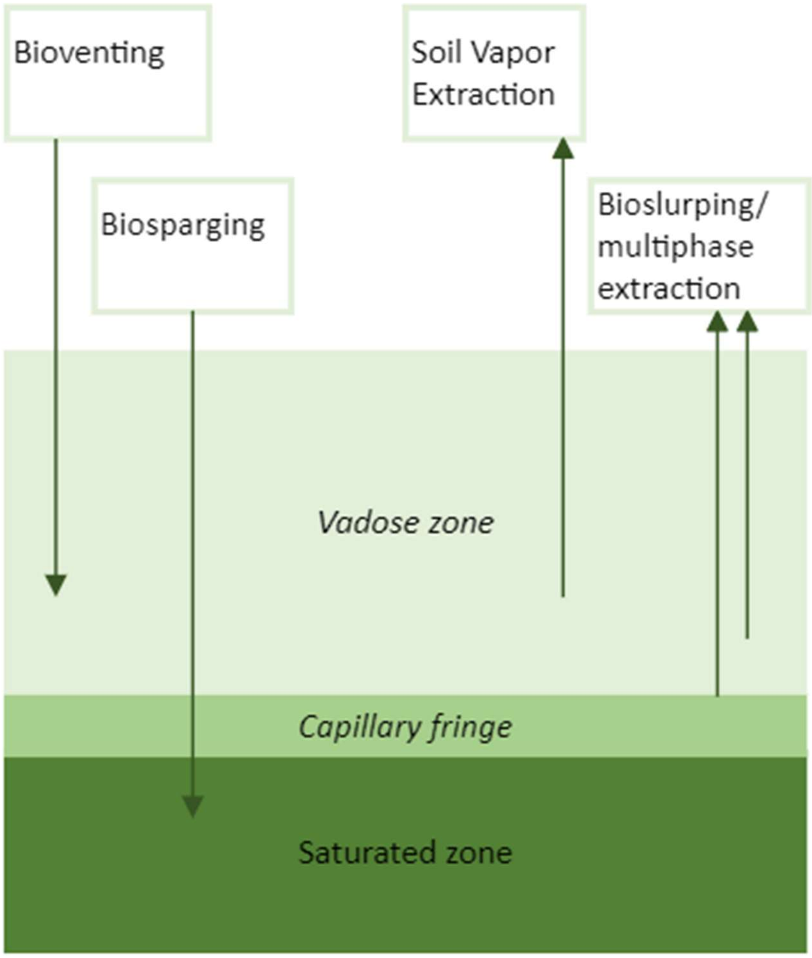

Figure 7 Illustration of air injection and extraction zone. The arrows represent either air injection or air extraction. The arrow start or end point illustrates what soil zone the air is injected or extracted from.

When operating an aeration system, it is imperative to carefully regulate airflow rates, as excessive airflow may inadvertently favor volatilization over the desired biodegradation (Azubuike et al., 2016; Amin et al., 2014). The impact of temperature and airflow rates was assessed by Sanscartier et al. (2011), who evaluated both volatilization and biodegradation rates under two airflow and temperature regimes. They found that the airflow stimulated biodegradation at 7°C and 22°C, and hydrocarbon-degradation was observed at low and high airflow. The exception was the high airflow of 45 ml/s at 22°C, where 51% of the TPH was lost to volatilization. Interestingly, the aeration rate of both 13 and 45 ml/s resulted in 99% and 98% removal rates for hydrocarbons >nC_{15} at 7°C, respectively.

7.4.2.1 Bioventing

Bioventing enhances the biodegradation of contaminants in soil and groundwater by supplying oxygen to the vadose zone (Leeson et al., 1993; Sparrevik & Breedveld, 1997). The air is directly injected into the soil by airflow pumps, creating more movement of the soil air, and can be supported by monitoring wells for controlling the remediation efficiency. Sparrevik & Breedveld (1997) carried out a pilot scale study in the Norwegian climate, investigating glaciofluvial soil that is generally nutrient-poor and challenging for remediation due to its geological properties. Four soil cores were collected for the trial, each containing diesel-contaminated soil up to 30 years old, with a concentration range of 2000 to 5000 mg/kg soil. During the trial, the cores were placed in a rock cavern with the ambient temperature of 8°C, simulating a typical Scandinavian soil temperature. The trial evaluated bioventing only, bioventing in combination with ammonium and orthophosphate, and bioventing in combination with nitrate and meta-phosphate. The last core sample functioned as an untreated control. By measuring CO_2 in the off gas from the columns, Sparrevik and Breedveld where able to determine that the decrease in TPH was a result from bacterial activity, rather than volatilization. After one year of biostimulation, all three columns showed reduced TPH-content compared to the control, where the column where nitrate and meta-phosphate where added showed the highest removal rate of 87% TPH over a year.

Sparrevik and Breedveld's study on bioventing of hydrocarbons in soil was one of the most relevant articles for this thesis about soil bioventing, as it was the sole field-scale trial conducted in Norway. Sadly, the study lacked relevant information, such as the initial TPH concentration for each test column. Further, the removal efficiency was only calculated for the column gaining the highest removal rate. Some results could imply that TPHs had not been degraded but percolated through the soil and accumulated in the column bottom layers. The authors noted how hydrocarbons had degraded in the upper and middle layers of the soil, although not in the lower layer. Without access to more of the results in the study, it will be challenging to utilize the findings for large-scale bioremediation

7.4.2.2 biosparging

Air or gas injection below the groundwater table in the saturated zone is termed biosparging. In temperate regions, biosparging is an efficient method for biostimulation that can promote the degradation of both soil and groundwater contamination (Heaston et al., 2010; Kao et al., 2008). On the contrary, for colder regions, there are limited studies available on biosparging, an opinion that is supported by several reviews discussing the limitations of biosparging in colder regions (Azubuike et al., 2016; Chaudhary & Kim, 2019). An intriguing paper concerning a laboratory study on Poly-and perfluorinated alkyl substances (PFASs) was found relevant to this thesis (Nickerson et al., 2021). PFASs are suspected to be converted by biosparging to highly recalcitrant perfluorinated alkyl acids (PFAAs) (McGuire et al., 2014). The study suggests that biological activity promoted by biosparging, along with high salt concentrations in the artificial groundwater, likely enhances the release of PFASs (Nickerson et al., 2021). The researchers ruled out non-biological transformations, as sterilized soil exhibited similar PFAS release patterns to non-sterilized soil. Thus, biosparging can make PFAS easier to reallocate through the soil subsurface of contaminated sites and extracted for disposal. The application of biosparging on sites simultaneously contaminated with PFAS and hydrocarbons may result in the uncontrolled mobilization or transformation of these substances. Without meticulous management, this process could inadvertently exacerbate the contamination within the soil subsurface, thereby adding complexity to remediation efforts and potentially posing a more significant threat to both environmental health.

7.4.2.3 Vacuum-enhanced recovery (bioslurping and soil vapor extraction)

Soil Vapor extraction (SVE) uses vacuum pumps to create pressure differences in wells to extract air containing volatile contaminants. SVE is often regarded as a physical methodology, although the increased airflow in the vadose zone will promote similar effects to bioventing(Magalhães et al., 2009). In the bioslurping technique, a vacuum-aided pumping system enables the ascension of NAPLs from the water table and extracts them from the capillary fringe (S. Kim et al., 2014). Combining air injection with soil vapor extraction free-phase products can be removed from the capillary fringe and simultaneously support aerobic degradation in the vadose zone in bioslurping (Khan et al., 2004). Compared to bioventing and biosparging, the variable transition capacity of bioslurping is a distinguishing feature. When free-phase pollutants are recovered, the bioslurping configuration can be changed seamlessly to a conventional bioventing system to complete the remediation process. Since 2010, very few field-scale trials for bioslurping/multiphase extraction have been published, and they have all been conducted in warmer climates. Kim et al. (2014) performed bioslurping on a site contaminated with TPH and BTEX in the Republic of Korea. The removal efficiencies of TPH from groundwater by depth were 89.4% (at 2.5–3.5 m) and 91.9% (at 3.5–4.5 m), respectively. The removal rate for BTEX was 93%.

7.4.2.4 Biostimulation with nutrient additives

The conventional approach to providing nutrients in biostimulation has been to add phosphorus and nitrogen (J. Kim et al., 2018; Nousiainen et al., 2015; Sparrevik & Breedveld, 1997). Boreal soils, which cover extensive areas in Norway, host microbial communities adapted to the colder climates of subarctic and cold temperate regions. These regions experience harsh winters characterized by seasonal freeze-thaw episodes and snow cover(Männistö et al., 2018). Given that the biomass in boreal forests is limited by carbon availability rather than nitrogen or phosphorous, nutrient supplementations may not be necessary regarding the bioremediation of simpler alkanes (Ekblad & Nordgren, 2002). Providing extensive nitrogen can further inhibit bacterial growth and thus inhibit bioremediation processes (J. Kim et al., 2018). For instance, a comparison of four lysimeters proved how biostimulation with nitrogen and phosphorous was more effective in degrading BTEX, C_5-C_{10}, and C_{10}-C_{40} compared to natural attenuation and chemical oxidation (Simpanen et al., 2016). The study did not report individual biodegradation rates. For more complex hydrocarbons, exemplified by the pesticide atrazine, biodegradation may not be possible without biostimulation (Nousiainen et al., 2015). Sandy boreal soils contaminated by atrazine showed that biostimulation of the indigenous, psychrophilic bacteria removed 52% of atrazine at 10°C. The removal rate could increase to 76%, but that required biostimulation and bioaugmentation by *Pseudomonas Citronellolis* and *Arthrobacter Aurescens.*

Researchers have been studying natural soil amendments and actively manipulating soil structure and porosity recently. Cyclodextrin and meat and bone meal have been shown to greatly increase bacterial diversity and facilitate bioremediation in contaminated soils, with cyclodextrin producing a major alteration in the bacterial community over time (Cavazzoli et al., 2023). In a room-temperature laboratory trial, the combination of meat and bone meal and cyclodextrin degraded diesel by 96% over 12 weeks (Cavazzoli et al., 2022). The substitution of fish meal as an alternative nutrient source demonstrated a noteworthy enhancement in the bioremediation of hydrocarbons within biopile systems with a removal rate of 71% (Dias et al., 2015). In a study conducted by (Kauppi et al., 2011), the effectiveness of woodchips and nutrients in degrading diesel fuel in boreal clayey soils was examined. The addition of woodchips provided structure and reduced the required airflow.

Högfors-Rönnholm and colleagues (2020) investigated biodegraded peat to retain metals and restore the biodiversity of Acid sulfate soil (ASS). The substitution with biodegraded peat resulted in higher microbial diversities and retention of metals, although acid-tolerant and acidophilic microbes still dominated as species. Bedrock in Norway is rich in sulfates, which in contact with air and water, can produce ASS (Norwegian Environment Agency, 2022b). ASS can contribute to natural, long-term heavy metal contamination in soils and runoff as the sulfates react with soil minerals and leach heavy metals. This is a pollution scenario that could exacerbate during construction activity. The acidic environment will favor acidophiles, an organism thriving in conditions with a pH <4.0 and with a capability to further increase the heavy metal content of soils by catalyzation of dissolution of heavy metals (Högfors-Rönnholm et al., 2020). Both acidophilic bacteria and fungi can degrade PAHs and several n-alkenes (Gemmell & Knowles, 2000; Qi et al., 2002). Given this understanding, we can better inform the selection of bioremediation strategies for oil spills and, in fact, metal retention in ASS. Notably, these soils may already harbor organisms capable of digesting contaminants due to their inherent metabolic pathways. Thus, leveraging these native organisms could provide a natural, efficient solution to mitigate oil pollution in such environments.

7.4.3 Phytoremediation

Phytoremediation is a process that utilizes plants to gather, convert, or stabilize contaminants, offering various methods by which plants contribute to bioremediation. The routes for decontamination encompass extraction of contaminants into plant roots, stem, or leaf, immobilization of the contaminants in soil, a transformation of the contaminants by rhizosphere organism or by plant metabolism, volatilization primarily through stomata and phytofiltration of water sources (Iyyappan et al., 2023; Susarla et al., 2002). Among all the bioremediation methods, phytoremediation should be suspected to be the most applicable, where more than 450 plant species suitable for phytoremediation have been identified(Ghori et al., 2016). The high level of applicability for phytoremediation is assessed mainly by how readily it can be implemented, as opposed to how effectively it eliminates contaminants. To increase the use of phytoremediation, a database was created to promote the wide variety of phytoremediation benefits better and make the scientific knowledge available to the layman (Famulari & Witz, 2015). In recent years, the heavy metal hyper-accumulating properties of many plant species have been of increasing interest to scientists and developers (Iyyappan et al., 2023). The biomass produced during phytoremediation measures can be reintegrated into the circular value chain by producing biofuels and biochar, and this area of study is attracting increasing attention.

In colder climates, phytoremediation can be affected by shorter growing seasons and reduced rates of degradation as a result of temperature. An important aspect is the use of native plants because of the plant adaptation to the current climates and to avoid spreading invasive species (Robichaud et al., 2019). A field trial in Canada used the site native *salix alaxensis* in combination with compost, wood chips, and a fungal spawn consisting of the white-rot fungus *Trametes versicolor* to create a micro-ecosystem on-site. The initial TPH concentration in the soil was a stunning 200 000 mg/kg and was effectively degraded: C_6-C_{50} by 65% and >C_{50} by 75%. It should be highlighted that during the first year of the test, none of the *salixes* survived due to the toxic levels of contaminants and had to be reintroduced in the second year. The researchers then established life pockets of clean soil before installing the *salix* cuttings, securing their establishment. Heavy metal was translocated during the trial from soil to plant leaves. Supplementary, contaminants like PAH and TCE were degraded to below detection limits. In this trial, the fungus did not colonize, and its participation in the ecosystem was uncertain.

In the aftermath of an oil tanker truck accident, a phytoremediation effort was launched at a facility in Finland to mitigate total petroleum hydrocarbon (TPH) pollution in boreal soil (Lopez-Echartea et al., 2020). This study used poplar trees to facilitate the process in a boreal climate, demonstrating substantial efficacy. The initial concentration of contaminants, at 7300 mg/kg, was reduced by 78% over three years and decreased to undetectable levels after six years of continued growth. Bacterial dynamics within the soil were closely monitored throughout the trial using 16S rRNA gene sequencing, revealing an overall increase in bacterial diversity associated with the rhizosphere. This suggests a positive correlation between TPH degradation and bacterial diversity, wherein a reduction in TPH leads to enhanced microbial diversity. This enhanced diversity may contribute to an increasingly robust and resilient soil ecosystem capable of further enhancing bioremediation processes.

Phytoremediation can have a significant impact when applied to ecosystems, both for plant successional trajectory and the structure of the soil bacterial community (Leewis et al., 2013). Therefore, great care should be taken when considering species for phytoremediation. Leewis and colleagues investigated a formerly phytoremediated field from 1996, performed in 1995 and 1996 (Reynolds et al., 1999). Fifteen years later, the planted annual non-native grass was overgrown by native species. The initial trial restored the elevated hydrocarbon content to lower levels, but the grasses needed repeated seeding and effort to maintain.

7.4.4 Permeable reactive barriers

Permeable reactive barriers (PRB) are effective for removing contaminants in groundwater. Their performance relies on carefully placing reactive substances that adsorb or degrade migrating pollutant plumes (Snape et al., 2001). This intervention allows the transformation of the contaminant into a less harmful substance or to be immobilized. Although permeable reactive barriers can be considered a physical treatment, some barriers use microbes to metabolize contaminants by providing oxygen or nutrients to encourage biodegradation (Gholami et al., 2019). Mumford et al. (2013, 2015) have investigated the use of PBRs in Antarctica. Barriers could be designed from numerous materials, like zero-valent ions, compost, activated carbon, pumice, and others (Gillham & O'Hannesin, 1994; Henry et al., 2004; Moraci & Calabrò, 2010). The barriers researched were designed as a fully abiotic system, although using granulated activated carbon inside the barriers adsorbs microorganisms and pollutants.

Given their operation principle, PBRs hold significant relevance for groundwater flow, acting as a filtration system to prevent the spread of contaminants (Mumford et al., 2015). However, it is essential to note that there appears to be a lack of current research regarding field-scale trials for biotic PBRs, particularly in studies focused on colder climates. While physical treatment studies conducted by Mumford et al. (2013, 2015) have provided valuable insights into using PRBs in Antarctica, these studies only encompass the physical removal of contaminants. Nevertheless, one functional implementation of such barriers could be combining PRB with a biosparging approach for PFAS mobilization. Combining the finding from the research done by Nickerson et al. (2021) with a PFAS-adsorbing PBR could provide a helpful tool to aid in tackling the persistent and pervasive problem of PFAS contamination.

7.5 *Ex situ*

Ex situ bioremediation involves excavation and transport for off-site treatment of contaminated soil, commonly employed when *in situ* treatment is impractical or when removal is necessary for health concerns. The excavated soil can sometimes undergo physical pretreatment prior to bioremediation to extract stone fractions or support the soil structure. Landfarming, biopiling, and bioreactors are known methods.

7.5.1 landfarming

Landfarming involves spreading a thin layer of soil which is amended with strategies such as nutrient addition, pH buffering, bulking agents, and periodic tilling (US EPA, 1994). The treatment area resembles a conventional field and is managed using conventional farming machinery. The operational depths of the tillers, which frequently reach a maximum of 0,5 meters, determine the thickness of the field. Landfarming promotes the volatilization and biodegradation of hydrocarbon contaminants, utilizing microbial processes to break them down into less harmful substances. Landfarming can be tailored to site-specific characteristics to optimize microbial activity and facilitate the natural degradation of contaminants in the soil. Additionally, measures such as lining to prevent leakage to groundwater, irrigation systems, and collective systems for gathering runoffs can be applied.

A review by Camenzuli and Freidman (2015) confirmed the widespread success of landfarming in temperate and tropical environments. However, they noted a scarcity of information and research on landfarming trials in colder regions, which was also found to be correct in this study. In a pilot-scale study, Chang et al. (2011) examined the impact of variable and constant temperatures on the biodegradation of petroleum hydrocarbons. The results highlighted the significant influence of temperature fluctuation. Variations in temperature between 1 – 10°C resulted in a biodegradation efficiency for TPH of 55%, C_{10}-C_{16} of 63%, and C_{16}-C_{34} of 53%, respectively. These results were markedly higher than those under continuous temperature at 6°C, of 19%, 36%, and 21%, respectively. It was discussed if the higher removal rate was due to the exponential growth during thawing periods. The study also found that temperature variations of 1 – 10°C were more effective at breaking down complex structures of hydrocarbons, the unresolved complex mixture (UCM), with a 47% reduction, compared to only a 19% reduction at 6°C. Chang and colleagues highlight how the natural conditions in colder environments can be exploited to optimize landfarming and increase the removal of hydrocarbons that are considered resistant and complicated.

Since Camenzuli and Friedmans Review in 2015, one single study on landfarming in colder climates was identified; a laboratory trial performed by Jeong et al. (2015) to investigate the use of bioaugmentation and surfactants to increase biodegradation of TPH. The sprayed foam fulfills three roles: restricting wind-borne particles and volatilized contaminants, regulating soil temperature, and supplying additives and microbes for bioaugmentation. Foam shares some similarities with geomembranes used to cover biopiles in how it can reduce wind-borne pollutants and increase temperature (McWatters, Rutter, et al., 2016). A highly stable foam engineered from a surfactant and the bacteria *Pseudomonas sp*. G2–2 was sprayed over contaminated soil and kept at a constant temperature of 6°C (Jeong et al., 2015). The augmented foam had a TPH degradation efficiency of 73,7% compared to traditional, bioaugmented landfarming yielding a 46,2% removal rate. Given this, foam spraying seems to present an innovative approach to tackling soil remediation in colder climates, although it would require further studies to show replicability, and surveying to cover the engineering requirements and environmental impacts.

7.5.2 Biopiles and composting

Biopiles consist of stacked contaminated soil, preferably onto an impermeable base, and can be covered with an impermeable liner. The biopile design commonly incorporates distribution systems for oxygen and nutrient supplies and should also implement an irrigation and drainage water system (Dias et al., 2015). Liners can consist of geosynthetic clay liners, high-density polyethylene (HDPE) geomembranes, geotextiles, and a co-extruded HDPE with ethylene vinyl alcohol (EVOH) geomembrane, their primary function being the protection of the surrounding environment from the piled pollutions (McWatters et al., 2014). Compared to the previously discussed landfarming technique, extensive studies have been performed on biopiles in colder climates to assess their feasibility of bioremediation under harsh conditions. Biopiles in Antarctica have succeeded in remediating a hydrocarbon content of 2180 mg/kg with a removal rate of 75% in 40 days to an end concentration of 527 mg/kg (Martínez Álvarez et al., 2017). A follow-up trial to investigate the robustness and reproducibility of the 2017 paper, conducted at equal design and location, had significantly lower removal rates of 55% for hydrocarbons (Martínez Álvarez et al., 2020). Biopiles averaged 6,5°C and 5.4°C during experiments, and both trials supplemented nutrients and tilled the piles for aeration. Geomembranes protected the biopiles from rain, snow, and wind and raised their temperature. There were differences in initial concentrations of hydrocarbons in the studies, as the 2020 trial used a start concentration of 6098 mg/kg with a final concentration of>3000 mg/kg. Thus, the removal efficiency decreased, but the quantified removal yield was higher in the 2020 trial. Further, insolation, the measure of solar energy over a specific time range, was higher during the 2017 trial than in the 2020 trial and was considered significant for the remediation efficiency.

One interesting observation is how heavier hydrocarbons tend to be degraded more rapidly during a freezing event compared to thawing events. A comparison of one biostimulated and one unstimulated biopile during a freezing and thawing period was performed in field by Kim et al. (2018) in Saskatchewan, Canada. During the frozen season, the removal rate of C_{16}-C_{34} was 33% compared to C_{10}-C_{16} at 13%. The removal rates accumulated to 39% and 47% during the thawing phase, respectively.

Akbari and Ghosal (2014, 2015) have performed two studies on bioremediation in temperatures above zero degrees, comparable to the Norwegian conditions during summer. In their laboratory trial on the effect of diurnal temperature changes, they found an increased TPH and C_{16}-C_{34} removal at diurnal temperatures of 5 to 15 °C, compared to moderate removal rates at constant temperatures of 5 °C (Akbari & Ghoshal, 2015). In the pilot scale trial, biopiles of clayey soil showed reductions in TPH of 35–43% and C_{16}-C_{34} of 24–38% over 110 days in three different set-ups (Akbari & Ghoshal, 2014). A very high nitrate dosage of 1340 mg N/kg inhibited degradation. In comparison, Gomez and Sartaj (2013) reported a removal rate for TPH in sandy soils of 82% at temperatures of 0-10°C. The clayey-soil removal rates are lower than previously discussed removal rates and can be attributed to the high surface area and the numerous micropores of clay aggregates, which decrease the accessibility and availability of hydrocarbons to microorganisms.

Biopiles are the only known bioremediation method currently in full-scale operation by the waste treatment company Lindum AS (2023). Sludge from oil separators and sand traps is delivered to the facility and dewatered before being mixed with structural materials, nitrogen, and phosphorous (A. H. Rosenvinge, personal communication, May 31, 2023). The mixture is then piled up in large piles and tilled during degradation. The piles are stacked for six to twelve months before the concentration is below the safe limit to dispose on a landfill. The treatment can be considered a hybrid bioremediation method composed of landfarming and biopiles. In 2018, Lindum conducted a field experiment to investigate whether the start-up process differed between summer and winter (Rosenvinge, 2019). The biodegradation rates were faster during summer, with a removal of 71% during the first 50 days, and slower during winter by 25% in the first 50 days, and proceeded to 55% after 90 days. Post initial degradation, the removal rate was not reported, although it showed a reduction from 5000 mg/kg to >3000 mg/kg at the end of the test.

The data provided by Lindums field study, presented in Figure 8 with permission from the author, can be further illuminated by the insights gained from this review (Rosenvinge, 2019). Despite slower initial degradation, a detailed examination of the results from the November batch revealed a lower final concentration of total aliphatic than the summer sample. Kim et al. (2018) and Chang et al., (2011) found that during a relatively cold period, where the biopiles or landfarms were exposed to diurnal variations, the heavier fractions of the hydrocarbons were typically preferred by microorganisms. However, it is essential to acknowledge that this field trial was not published in a scientific paper, and the poster presentation where the study was described did not undergo peer review. Nevertheless, the findings presented in the poster conveyed valuable information for the purpose of this review, and thus, it was deemed necessary to include the review. In general, removal rates appear to be higher when hydrocarbons are subjected to freeze-thaw cycles and changing temperatures (J. Kim et al., 2018). The observed patterns in Figure 9 suggest that the bioremediation process may prefer colder, more variable temperature conditions, contrary to what might be expected. It is also possible that the contaminants in the summer batch volatilized more rapidly during the warm winter temperature. This insight could lead to a deeper understanding of how biopile processes function under fluctuating conditions and how they can be optimized.

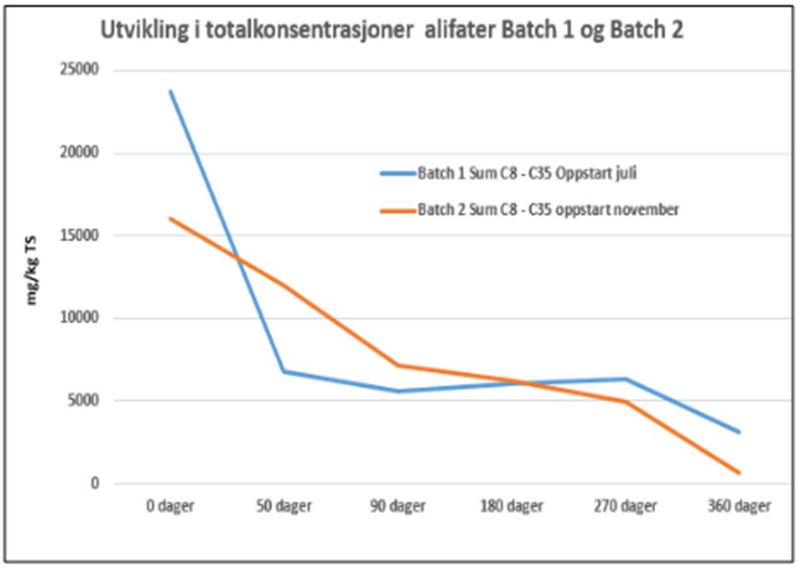

Figure 8 Development of total concentration of Aliphatic hydrocarbon from Lindum field study, 2019. The illustration show the trend for hydrocarbon concentration in the summer and winter batch. Reused with permission by Rosenvinge (2019)

7.5.3 Bioreactors

Owing to their remarkable flexibility and control, bioreactors are paramount technology in fostering the ideal environment for bioremediation. Bioreactor systems are engineered tanks in which temperature, nutrient and moisture supply, and availability of terminal electron acceptors can be controlled flawlessly. Slurry bioreactors are among the most sophisticated soil bioremediation treatment technologies (Robles-González et al., 2008). Numerous operating modes exist, including batch reactors, fed-batch, sequencing batch, continuous flow reactors, and multistage reactors. Uncountable designs of bioreactors exist, as such designs are continuously under development for optimizing treatment processes. The typical features of a slurry bioreactor are a feeding system for the slurry phase, the reactor container, and an effluent separation system for the slurry phase. The reactor container usually possesses inlets for biostimulation factors and treatment for effluent gases from the bioremediation process Figure 9. The effluent slurry can be treated to separate soil from the water phase, which can be treated and recycled in a continuous loop. Such designs can be tailored to fit various budgets, ranging from manual to fully automatic sensor-regulated processes.

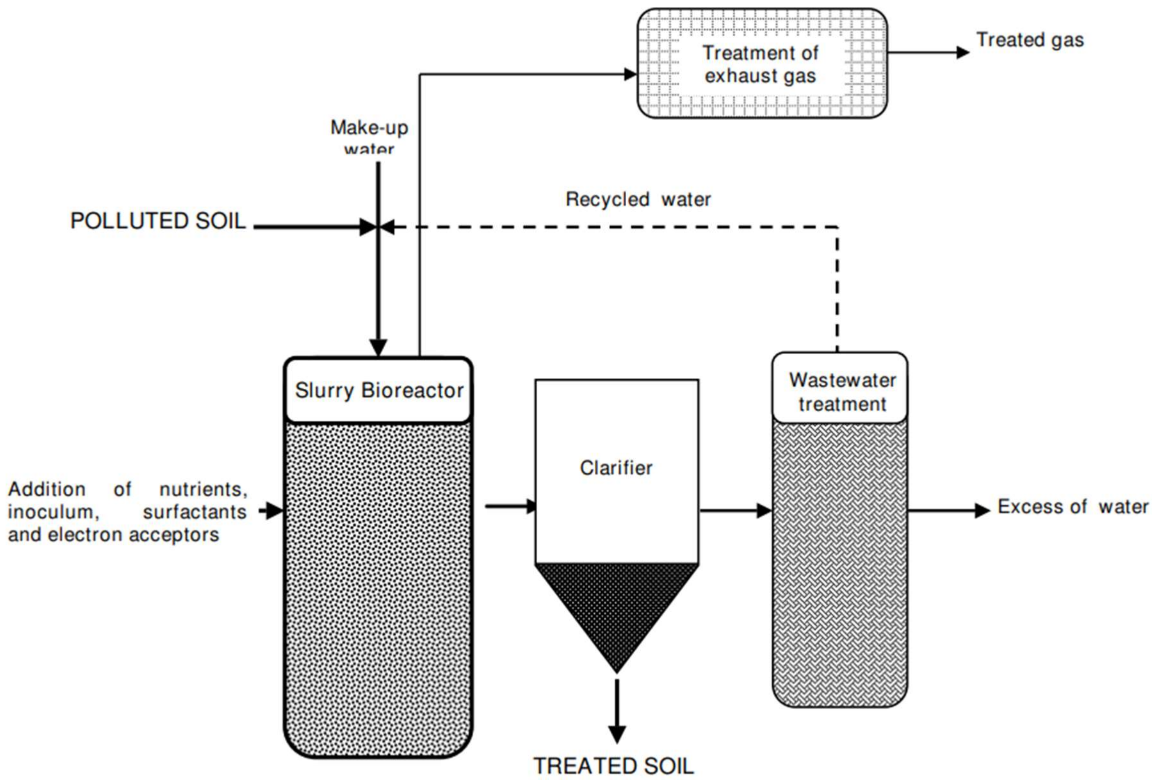

Figure 9 Conventional bioreactor flow scheme. The contaminated soil slurry is fed into the bioreactor and the amendment suited for the process is added. Post biodegradation, the effluent is separated in a clarifier, and wastewater can undergo treatment for recycling into reactor. The figure is collected from Robles-González et al., 2008. CC BY 4.0

Bioreactors can, for instance, be a valuable tool for producing cold-active enzymes or specified consortia (Davoodi et al., 2023; Miri et al., 2021). In a pilot scale trial, the enzymes were produced in batch and feed batch reactor from *Arthrobacter sp*, and injection with the enzyme solution into a large-scale pilot tank contaminated with p-xylene successfully reduced the xylene concentration by approximately 88-90% in two months (Miri et al., 2023). It is worth mentioning that, although xylene monooxygenase and catechol 2,3 dioxygenases are cold-active enzymes, and were collected from a cold site in Montreal, Canada. The pilot scale trial described above was performed indoors at temperatures of 24 °C. Despite the test conditions, cold-adapted enzymes generally have higher reaction rates at lower temperatures than their mesophilic counterparts, which implicates the usefulness of up-concentrating the enzymes for biostimulation *in situ* (Davoodi et al., 2023; Santiago et al., 2016).

7.5.4 Surfactant-enhanced bioremediation

The use of surfactants to aid in the bioremediation of hydrophobic compounds is gaining attention. Biosurfactants are biodegradable and non-toxic surface-active chemicals generated by microorganisms (Perfumo et al., 2018). Surfactants reduce the surface tension and increase the solubility of hydrophobic substances. Critical micelle concentration (CMC), the concentration of added surfactant where aggregates tend to form, is vital in determining degradation efficiency (Chrzanowski et al., 2012). Reaching the CMC indicates that an adequate amount of surfactant is present to reduce surface tension to its maximum extent. At this point, contaminants like NAPLs get effectively trapped inside the micelles and can be assimilated. Therefore, a lower CMC for a specific surfactant implies that less surfactant is needed to form micelles to emulsify contaminants.

Biosurfactants have numerous applications and can be utilized *in situ* and *ex situ* (Chrzanowski et al., 2012; Parus et al., 2023). Microorganisms in cold environments inhabit several survival mechanisms like metabolic adaptation and cold shock proteins, where the production of biosurfactants is believed to be one of the crucial adaptations (Tribelli & López, 2018). Several genera have been identified that produce biosurfactants, including Pseudomonas, Rhodococcus, Bacillus, Burkholderia, and Sphingomonas (Perfumo et al., 2018). Perfumo and colleagues (2018) suggested that the original function of biosurfactants was to aid in utilizing decomposed plant materials like lignin and cellulose, components that also encompass hydrocarbons and PAHs. However, while surfactants enhance the absorption of hydrocarbons, they do not directly facilitate degradation, as biodegradation depends on the inherent genes that code for the enzymes and metabolic pathways necessary for degradation (Chrzanowski et al., 2012).

Rhamnolipids are extensively researched biosurfactants, and the specie *Rhodococcus fascians*, a rhamnolipid-producing bacteria, have been identified and successfully isolated from soil in Antarctica (Gesheva et al., 2010). While biosurfactants have been extensively studied, there is a notable scarcity of field trials conducted in colder regions that utilize biosurfactants as additives for bioremediation of hydrocarbons. In soil bioremediation, rhamnolipids can be purposeful in both the saturated zone for increasing the bioavailability of NAPLs and in the vadose zone to desorb contaminants to increase their bioavailability. For instance, rhamnolipids can desorb phenanthrene from soil, but high concentrations of rhamnolipids reduce the desorption of phenanthrene under freeze-thaw cycles (Yao et al., 2017). Further, addition of surfactants, as with any other additive to an ecosystem, should be carefully evaluated and risk assessed. Even though biosurfactants can desorb particles, they can become adsorbed before CMC, so higher amounts of surfactant are required to emulsify contaminants in soil compared to biosurfactants in pure water solutions (Parus et al., 2023). Parus and colleagues (2023) further reviewed that increased surfactant concentrations could be phytotoxic at elevated concentrations and, therefore, could interfere with bioremediation.

8 Discussion

This literature review aims to investigate the present research concerning the bioremediation of hydrocarbons in colder climates. In this review, eight different bioremediation techniques were evaluated. These approaches encompassed both *in situ* and *ex situ* cleanup techniques, and the evaluation included both field-scale trials, pilot studies, and laboratory-scale tests. Several literature reviews recently assessed bioremediation in colder climates; Azubuike et al. (2016) assessed *in situ* bioremediation, focusing on practical site applications. New insights into these methods have been reviewed by Chaudhary and Kim (2019). Very recently, Holmberg and Jørgensen (2023) provided valuable contributions to understanding how prokaryotes adapt, their abundance, and prokaryotic activity in Arctic and Antarctic habitats, which are colder climates compared to Norway. What sets this review apart from the mentioned is the unique focus on how bioremediation methods can be applied to the present practices of managing contaminated soils in Norway. Considering recent field-scale trials, pilot studies, and laboratory tests, evaluating the implementations, effectiveness, and environmental implications of both in situ and ex situ bioremediation approaches is necessary.

8.1 Bioremediation methods

When looking into the different bioremediation approaches, it becomes evident that the study of bioremediation methods is somewhat unbalanced regarding geographical focus. While a considerable amount of research is undertaken in warmer climates, research pertinent to colder regions is more scarce. For warmer climates, for instance, all bioremediation strategies are thoroughly researched. In cold regions, some bioremediation strategies have been extensively studied, for example, biopiles and landforming. Field-scale trials were lacking for some bioremediation strategies, such as biosparging in cold climates. This discrepancy between the number of studies conducted on the different bioremediation strategies is highlighted in Table 6, where thirteen of the twenty-three listed studies were undertaken in biopiles.

8.1.1 *In situ* bioremediation

The *in situ* remediation methods examined in this study hold significant potential for effective environmental restoration and management implementation. *In situ* treatments looked at in this study—including monitored natural attenuation, various aeration techniques in biostimulation, biostimulation with nutrient additives, and phytoremediation—all demonstrate distinct potential and constraints. While *in situ* remediation strategies may necessitate a substantial initial expenditure on equipment, they can prove to be cost-efficient compared to the expenses associated with excavation, transport, and the advantage of allowing simultaneous land use.

Monitored natural attenuation provides the least expensive approach to site rehabilitation, utilizing the intrinsic biodegradative capabilities of existing microbial communities. While the appeal of this method lies in the low intervention and cost-effectiveness, the time scales necessary for significant remediation can be extensive. Two case studies observing natural attenuation in Antarctica reported how contaminants from oil spills still were present decades after the incidents (Ferguson et al., 2020; McWatters, Wilkins, et al., 2016). However, in a temperate climate with significantly elevated annual precipitation, even TBC experienced natural biodegradation within a span of three years (Song et al., 2023). Furthermore, the effectiveness of natural attenuation is highly contingent upon site-specific factors and the adsorption of contaminants to soil particles. In some cases, natural attenuation may be the suitable method, whereas other methods may be preferred when excavation is unavoidable.

Biostimulation methods using aeration and nutrient amendments share some similarities in their general approach, although they have distinct differences in terms of their intended use and specific mechanisms (Cadotte et al., 2007). The distinctions are the technological solutions, like air injection or vacuum extraction, to apply the electron receptors (oxygen), at what depth or zone the air is injected, and the possibility of pollutant extraction at different soil depths. Biostimulation proves its usefulness by amplifying the biodegradation rate, mainly via optimized aeration techniques and nutrient enrichment. The addition of air increased bioremediation rates in all studies evaluated in this research; however, their effectiveness and practicality hinge on numerous site-specific conditions and the nature of the pollutants.

Sanscartier et al. (2011) demonstrated how fluctuations in temperature and airflow influenced the degradation rates of chain lengths $<nC_{11}$, nC_{11}-C_{15}, and $>nC_{15}$. Specifically, higher air rates at 45 ml/s resulted in more extensive degradation of alkanes $>nC_{15}$ compared to airflow rates of 13 ml/s. A field trial conducted in Norway used bioventing in combination with nitrogen and phosphorous to evaluate natural attenuation compared to bioventing (Sparrevik & Breedveld, 1997). In this study, TPH was successfully biodegraded by a removal rate of 87 % in one year, indicating this technology is suitable for the Norwegian climate. These are only a few of the numerous biostimulation options available, highlighting the need for careful engineering design. Moreover, the most effective remediation may require the integration of multiple types of biostimulation. Therefore, initial tests and site evaluations are crucial to identify the most appropriate airflow applications and nutrient choices before deciding which method or combination of methods are best suited and how bioremediation can be implemented at each individual polluted site.

Hydrocarbon degradation often demands a high oxygen-to-hydrocarbon ratio, necessitating approximately three moles of oxygen for each mole of hydrocarbon (US EPA, 2004, p. XII–22). This high oxygen demand necessitates a large air or gas volume and sufficient delivery time to the polluted site. Aeration equipment is expensive, and prolonged treatment can add up (Orellana et al., 2022a). While it is often suggested *in situ* treatments may be more cost-effective than *ex situ* conterparts; this might not hold true in remote areas where adequate infrastructure to support such equipment may be lacking. In such cases, the equipment may need to rely on fossil energy for operation, further adding to operational costs. Furthermore, physical limitations of the soil, such as density or the presence of buried rocks, can restrict airflow to smaller areas than initially intended and cause zones of inadequate aeration. This soil heterogeneity may lead to uneven distribution of remediation, complicating the process further, and needs to be considered before implementing this technology.

Bioslurping has the advantage of removing NAPLs (Non-Aqueous Phase Liquids), which are typically challenging to degrade (Khan et al., 2004). This technique should be viewed primarily as a physical treatment concerning NAPLs, as it centers on extracting the contaminant from the soil for further management. However, the effects of bioslurping extend beyond simple extraction; the process directly affects the water table elevation and may create a smear zone where pollutants are dispersed.

Phytoremediation, owing to the wide variety of plant species that contribute to bioremediation, is a promising and environmentally friendly approach with extensive application potential. In colder climates, this method encounters challenges that include shorter growing seasons and reduced degradation rates (Bramley-Alves et al., 2014). While the capability to accumulate heavy metals has traditionally garnered significant interest, the potential to degrade hydrocarbons in colder climates is an area of increasing focus (Lopez-Echartea et al., 2020). The focus on using native plants in colder climates addresses two primary concerns: adaptation to the local climate and prevention of invasive species introduction (Robichaud et al., 2019). As previously noted, a field trial using a non-native annual grass required elevated levels of maintenance, and after 15 years, the seeded species were eliminated in favor of native trees (Leewis et al., 2013). Conversely, selecting an inappropriate species can potentially lead to harmful consequences. An invasive species is a non-native organism that proliferates and takes root swiftly in a new ecosystem (Norwegian Environment Agency, 2023b). It can inflict severe environmental damage by out-competing native species and disturbing the ecological balance. Notably, several of the invasive species, like the highly aggressive *Solidago Canadensis* and members of the *Reynoutria sp.* (formerly *Fallopia sp.*), are extraordinary metal accumulators (Berchová-Bímová et al., 2014; Bielecka & Królak, 2019; Królak, 2021). Their rapid growth and adaptability to arid environments render these species an attractive choice for heavy metal extraction. However, these same traits also make them a threat in ecosystems where they are not native species, and as such, their use should be avoided in favours phytoremediation efforts.

8.1.2 *Ex situ* treatments

The bioremediation strategies discussed here included landfarming, biopiles, composting, bioreactors, and surfactant-enhanced bioremediation. These methods have substantial potential for treating contaminated soil, particularly in colder regions. Nevertheless, as highlighted in this review, each technology presents unique challenges that require meticulous attention to ensure the best possible results.

The study by Chang et al. (2011) on landfarming showed the pronounced effect of diurnal temperature fluctuation, suggesting that natural diurnal temperature variations may not impede biodegradation. These temperature fluctuations may act as a booster for bioremediation, particularly in southern Norway, which experiences similar temperature fluctuations. An innovative suggestion from Jeong et al. (2015) highlighted that bioaugmentation using foam and surfactants in the landfarming process could significantly enhance the degradation efficiency of TPH to 73.7% compared to traditional landfarming at 46.2%. The foam application might also safeguard against the wind spreading the contaminants with dust. Foam and surfactants may potentially be used in the future to enhance bioremediation. However, additional studies are needed to validate the effectiveness of biosurfactants and environmental compatibility.

Biopiles have demonstrated remarkable success even in harsh conditions, such as Antarctica, as evidenced by the removal rates of 75% and 55% achieved within 40 days, as reported by Martínez Álvarez (2017, 2020). Among all the reviewed remediation methods, biopiles emerge as the most user-friendly, easily manipulable with additives, and highly efficient biotechnology. Biopiles require less space compared to landfarming, while aeration equipment is more cost-efficient compared to *in situ* bioventing. In addition, the soil can be homogenized. Moreover, *ex situ* bioaugmentation can be more straightforward to implement, with protective measures such as impermeable liners to prevent contaminants from spreading into the environment. Additionally, using liners will prevent the introduced organisms from escaping and merging with the surrounding environment. As presented in Table 6, section 7, biopiles generally showed efficient removal rates between 50 – 96%, where no trial extended beyond one year of operation. These results demonstrate a significant dependence on carefully selecting specific nutrients, soil composition, temperature variations, and the target contaminant.

Biopiles are the only known bioremediation technique presently being implemented at full scale in Norway by the waste treatment company Lindum (Lindum AS, 2023). As mentioned in section 7.5.2, Lindum AS operates a hybrid bioremediation method that combines landfarming and biopiles (Rosenvinge, 2019). The operational efficiency of the plant has not been optimized. Their removal rates are 25-55% for C_8-C_{35}, implying that significant gains in performance could be made with dedicated efforts. Lindum performs bioremediation to ensure that dewatered oil-containing sludge is below the legislative threshold levels for disposal in a landfill. Given the simplicity of operation and cost-effectiveness of Lindum AS approach, many waste recipients could adopt it. This could reduce the transportation of heavily contaminated soil, thereby reducing CO_2 emissions.

Bioreactors, with their ability to tightly regulate treatment conditions, hold significant potential for the enhancement of bioremediation practices. In a study by Miri et al. (2023), it was found that enzymes adapted to colder environments, and produced within bioreactors, could effectively break down pollutants such as xylene by up to 90%. However, this was achieved in laboratory and small pilot studies, and a comprehensive evaluation of these enzymes with *in situ* conditions is yet to be fully explored. Utilizing bioreactors for the remediation of soil pollution on a large scale would necessitate considerable construction akin to that of resource recovery facilities or smaller wastewater treatment systems. Since soil recovery bioreactors are currently not in operation in Norway, a move toward their implementation could potentially contribute to land restoration efforts. Typically, bioreactors are permanent constructions, necessitating land reclamation and potential excavation with lengthy transit routes. In contrast, within existing facilities in Norway, bioreactor treatment systems could be combined with soil washing systems and applied at landfills to promote resource recovery. On the contrary, the designs of bioreactors typically involve complex processes, necessitating extensive tools and engineering costs, which may discourage their desirability.

8.2 Emerging trends and innovations in bioremediation

Advancements in research have shed light on emerging trends in bioremediation, where the utilization of biosurfactants and enzymatic biostimulation has gained prominence, contrasting the more traditional approach of bioaugmentation with single strains or a consortium of microorganisms. Models for optimization of the conditions for bioremediations evolve rapidly. The use of cold-adaptive enzymes as additives instead of bioaugmentation with inoculums is gaining interest. Both biosurfactants and cold-adaptive enzymes require bioreactors for upscaled production, making them costly (Miri et al., 2023). Ex situ biostimulation in slurry-phase bioreactors can prove beneficial for generating cold-active enzymes and biosurfactants for in situ bioremediation (Davoodi et al., 2023; Miri et al., 2023). The higher reaction rates of cold-adapted enzymes at lower temperatures present promising opportunities for biostimulation in cold environments.-Research on biosurfactants focuses on their ability to desorb contaminants from soil particles, enhancing contaminant bioavailability (Yao et al., 2017). Surfactants are being explored for their feasibility in improving the ability of an organism to assimilate contaminants (Gesheva et al., 2010; Perfumo et al., 2018; Trudgeon et al., 2020). With their environmentally friendly properties and capacity to enhance the degradation of hydrophobic compounds, biosurfactants are gaining attention as sustainable alternatives. However, caution must be exercised when implementing biosurfactants in bioremediation strategies, as high concentrations could potentially have phytotoxic effects and disrupt the bioremediation process (Parus et al., 2023). Future research and development efforts should continue to focus on these avenues, driving forward the evolution of time-effective and sustainable bioremediation strategies

Predictive models forecast bioremediation processes and optimize efficiency (Habib et al., 2018; Roslee et al., 2020, 2021). Modeling microorganisms is an upcoming technology that optimizes bioremediation efficiency (Gomez & Sartaj, 2014). A model is a simplified representation or simulation of a complex system or phenomenon that helps us understand, predict, or analyze behavior and outcomes. Integrating models in bioremediation research has shown promising results for predicted removal rates of 90-95% at temperatures below 15 °C (Gomez & Sartaj, 2014; Habib et al., 2018; 2020, 2021). Predictive models in bioremediation research enhance effectiveness. Integration of modeling microorganisms minimizes resource-intensive experimentation, reduces environmental impact, and identifies efficient approaches, all contributing to overall efficiency and sustainability. These advancements reflect a growing emphasis on optimizing efficiency and considering long-term environmental impact in bioremediation practices.

8.3 The current situation on bioremediation practice in Norway

While developing this thesis, a detailed evaluation of the current status of bioremediation in Norway was conducted. One company, Lindum AS, was discovered by using a hybrid bioremediation technique, a combination of landfarming and biopiling. Sparrevik and Breedveld (1997) conducted a field-scale trial for bioventing and biostimulation in Norway, achieving an 87% removal of TPH in one year through simultaneous aeration and nutrient addition with nitrate and meta-phosphate. This apparent scarcity of research and practice in bioremediation was echoed in the views expressed by three companies consulted during this thesis (Rosenvinge, Blaalid, J.Joner, personal correspondence, 2023). Bioremediation as an effective treatment for denser and more complex hydrocarbons was viewed with skepticism, and it was acknowledged that the current legislative framework may be inflexible. This consensus suggests a significant knowledge gap exists in Norway concerning enhancing bioremediation to suit its unique environment. It is worth exploring whether this gap arises from the challenges the Norwegian climate poses or a lack of knowledge about the latest technological advancements in bioremediation.

The current strategies for handling contaminated soil in Norway are limited to contamination burial onsite for moderately contaminated soil and *ex situ* soil isolation for condition categories 4 and 5 (Norwegian Environment Agency, 2009). The strategy choice largely depends on the planned land use - be it residential, industrial, or agricultural. Sites contaminated with compounds exceeding threshold levels in condition categories 4 and 5 usually require deposition in a certified treatment facility or contamination deposit. Such certified deposits are legally regulated and often necessitate the long-distance transport of the contamination, consequently impacting the carbon footprints of construction sites. One question that arises from these practices is how bioremediation could be better integrated into the Norwegian contaminated soil management cycle. The regulation governing contaminated soil processes, TA 2553, primarily concerns safe soil redisposition within construction areas. However, if contamination poses a threat to human health, the material must be removed from these sites (Norwegian Environment Agency, 2009). Notably, the decision on managing the received waste lies entirely with the treatment facilities, as long as its within Norwegian legislation.

Modern technological advancements have revolutionized how data is gathered, analyzed, and distributed; an additional practice in Norway is to register terrestrial pollution by coordinates in an official database/website named «Grunnforurensing» (translation;Ground Pollution) (Norwegian Environment Agency, 2023a). The website provides a map of all registered contaminated sites and results from investigations for contaminated soil and sediments in Norway since 1989 (Riksrevisjonen, 2003). Grunnforurensing offers an established and effective system known to professionals, which is well-designed to monitor natural attenuation. The interactive functionality of the web-based mapping system facilitates local authorities in visualizing and comprehending the progress and efficacy of pollution control initiatives for specific locations within an accurate geographical framework. This is exemplified by the continuous surveillance of decommissioned landfills, as illustrated in Figure 10. The location, previously employed for waste disposal, possesses a "monitoring" status, and information regarding the regulatory permissions associated with the closed landfill is attached. The system is designed to accommodate the uploading of extensive data linked to geotagged locations within the database. This feature enables local authorities to maintain rigorous supervision of site progress. Furthermore, the web-based application offers detailed information on remedial measures executed at contaminated sites, such as the extraction of pollutants. The tracking of ongoing natural attenuation, phytoremediation, and other *in situ* treatments could be pursued by utilizing this web-based mapping system. Future enhancements to the system may incorporate bioremediation data to increase utility and provide an improved understanding of the Norwegian environmental restoration efforts.

Figure 10 Map section from the web tool Grunnforurensing (ground pollution)

https://grunnforurensning.miljodirektoratet.no. The figure shows triangle markings where ground pollution has been detected, and information on the contamination level and process status can be found. The Norwegian text "process status: overvåking" indicates the ongoing monitoring of the closed landfill.

8.4 Costs and circularity

The application of bioremediation in environmental recovery strategies poses a complex challenge, given the interplay of economic feasibility and circularity aspirations. Initial costs, long-term sustainability, and resource recovery must be carefully assessed. However, bioremediation can reduce waste, recover resources, and restore the ecosystem, promoting economic circularity. For instance, in the case of soil masses, they can be separated and cleansed to reclaim valuable resources like sand, gravel, and crushed stone. Occasionally, authorized facilities with the capacity to separate and clean uncontaminated stone fractions are also permitted by regional authorities to handle contaminated soil. This process requires a specialized wastewater treatment facility to manage the wastewater generated during separation. A noteworthy example is the facility operated by Velde AS, where both clean soil masses and contaminated soil are subjected to washing. The resulting wastewater undergoes detoxification through flocculation and filtration, with the treated water subsequently reintroduced into the washing facility. As contaminants become trapped in the sludge, it is dewatered and transported to a local landfill. However, subjecting the sludge to bioremediation, it can be further processed to recycle additional waste, consequently enhancing soil recovery.

According to Orellana et al. (2022), bioremediation methods vary in cost. The financial burden ranges from a modest USD 50.7/m3 of soil for biostimulation employing compost only to a more significant USD 310.4/m3 for an intricate bioaugmented, aerated biopile system. It is pertinent to note that an increase in the complexity of bioremediation apparatus and methodology corresponds with a rise in the cost. An economic lens might initially deem this less attractive; however, broadening the perspective to incorporate environmental benefits is essential. More intricate and financially demanding methods might be the only viable solution for certain contamination types, offering substantial environmental value. In a circular economy, it is essential to recognize the environmental implications of transportation. As previously discussed, contaminated soil often necessitates long-distance transportation to certified deposits, thereby contributing to the carbon footprint of construction sites. Due to workforce operating hours and fuel consumption, this transportation embodies a 'hidden' environmental and economic cost. Integrating bioremediation methods into local waste management practices could mitigate these transportation costs and associated environmental impacts. The direct costs of various bioremediation techniques present a broad spectrum. However, analyzing costs within the expansive context of circular economy principles and resource recovery is paramount. Strategic investments in complex and higher-cost bioremediation techniques can yield significant profit by enhancing resource recovery and diminishing environmental impacts. Moreover, leveraging technological advances and improved data management can augment the efficiency and circularity of bioremediation practices. A deeper exploration into this domain, coupled with robust public-private collaboration, can catalyze the adoption of these principles within the Norwegian contamination management protocols.

8.5 Feasibility of bioremediation to the Norwegian soil handling system

Norway, characterized by a vast latitudinal span, manifests an exceedingly diverse climate. This, combined with its varied bedrock and unique natural and topographic diversity, results in a wide array of soil types across the country. The climatic diversity extends from temperate regions in the south to sub-arctic zones in the north, reaching arctic conditions if one includes Svalbard. In this context, a uniform approach to bioremediation is not possible. For instance, Martínez Álvarez et al. (2017, 2020) illustrated how immutable factors such as insolation, which is daily sunlight duration, can alter hydrocarbon removal rates by up to 20% within the exact location at two separate years.

With its protracted, dark winters, extensive snow cover, and permafrost, Northern Norway markedly contrasts the South, which features temperate, marine climates and eastern boreal zones. Consequently, bioremediation outcomes during winter could exhibit significant discrepancies between the North and the South. This review underscores the aptitude of bioremediation for the Norwegian climate, particularly emphasizing biopiles as a viable approach, albeit with necessary adaptations to accommodate the specific environment in which it is applied. Of the 23 studies examined (Table 6, section 7), 13 field studies were performed on biopiles. Notably, these biopile studies were already conducted at a pilot scale, implying that scaling up to more extensive applications should be relatively straightforward for this method. Conversely, other studies were conducted in highly controlled laboratory settings or modeled, which could present challenges when scaling up these methods.

Observations indicate that even in colder climates, hydrocarbons, including complex ones, can be degraded relatively quickly. Key to this is carefully adapting the process to the specific compound and process at hand. Furthermore, contamination is usually not homogeneous but rather heterogeneous and complex, which calls for a detailed, layered approach. Due to their persistent and non-biodegradable nature, heavy metals may pose the greatest challenge. As such, future studies should prioritize addressing multi-component removals and filling the existing knowledge gaps. In addition to the knowledge gap, the current legislation for dealing with polluted soil might be enhanced to include bioremediation options. As waste from condition categories 4 and 5 is frequently transported to landfills, biopiles could break down contaminants to acceptable levels in situ. As condition categories 2 and 3 can be reused onsite at different soil depths, the degraded soil could be reallocated *in situ* at a safe soil depth, according to TA 2552.

More significant volumes of field trials are necessary at this juncture to enable the industrial application of these methods. Given our advanced technological and research capabilities, we should aspire to evaluate multiple combinations of various remediation technologies. This will facilitate the integration of biodegradation and hydrocarbon extraction with the treatment of highly recalcitrant compounds such as PFAS and heavy metals.

9 Conclusion

This research has critically examined both *in situ* and *ex situ* bioremediation techniques, tailored explicitly towards cold climates, focusing on the adaptability to conditions prevalent in Norway. Of the methods examined, several techniques have yielded effective degradation rates. *In situ* methods carry more uncertainties for consistent and rapid degradation, while *Ex situ* methods can be more controlled and adapted to cold climates.

The research into the methods demonstrates the feasibility of implementing bioremediation in cold environments. Biopiles, in particular, distinguished themselves as a subject of meticulous scrutiny, demonstrating highly favorable outcomes. The efficacy, operational simplicity, and relative cost-efficiency of biopiles substantiate this preference. Moreover, the study reveals that the supplementation derived from seafood waste and meat- and bonemeal can significantly enhance the removal of hydrocarbons. This approach was shown to boost bioremediation efficiency and can pave the way for converting more waste into resources for environmental enrichment.

However, applying these methodologies necessitates an expansion of knowledge, specifically about the bioremediation of heavy hydrocarbons and the optimal stimulation conditions required for their breakdown. The Norwegian practice of handling soil pollution already poses an opportunity to implement *ex situ* bioremediation methods in operative landfills. The existing legislative framework in Norway could be strategically refined to permit or even promote bioremediation to reduce pollution to less hazardous levels. This would provide an avenue for the bioremediation of heavily contaminated soil and the soil mass potential redistribution within specific areas of intervention, thus reducing transport and CO_2 emissions and preventing excessive rock excavation.

10 Future perspective

Despite significant advancements in bioremediation, several areas warrant further exploration and development. The following future perspectives highlight potential avenues for research and innovation.

1. Integration of Multiple Bioremediation Approaches: Considering the complexity of hydrocarbon contamination in colder climates, integrating multiple bioremediation approaches may yield better results. Combining *in situ* and *ex situ* techniques can enhance the overall effectiveness of remediation strategies. Further investigations are needed to identify synergistic effects and determine the optimal combination of approaches for specific contaminated sites.
2. Communicating Science: To bridge the knowledge gap in bioremediation optimization and foster collaboration among scientists and experts across disciplines through conferences, workshops, and online platforms. Simultaneously, raise public awareness about the benefits of bioremediation through outreach programs, campaigns, and education to garner support for these efforts.
3. Advancements in Bioaugmentation and Biostimulation: Bioaugmentation and biostimulation techniques have shown promise in enhancing bioremediation efficiency. However, further research is needed to explore the potential of using cold-adaptive enzymes and biosurfactants as additives instead of relying solely on traditional bioaugmentation with specific microbial strains or consortia. Optimizing the production of cold-adaptive enzymes and biosurfactants and assessing their long-term effectiveness and environmental compatibility should be pursued.
4. Integration of Modeling Approaches: Using modeling tools and predictive models can aid in designing and optimizing bioremediation strategies. These models can simulate and predict the behavior of contaminants, microbial populations, and environmental factors over time. Future research should focus on developing accurate and reliable models that can assist in decision-making processes, optimize remediation designs, and predict the long-term efficacy of bioremediation approaches in colder climates.
5. Sustainable and Cost-Effective Solutions: As bioremediation techniques continue to evolve, emphasis should be placed on developing sustainable and cost-effective solutions. This includes exploring renewable energy sources to power aeration

equipment and bioreactors, minimizing the environmental footprint of remediation operations, and maximizing resource recovery from contaminated soils. Collaborative efforts between researchers, industry stakeholders, and regulatory bodies can foster the development of innovative and economically viable approaches.

By focusing on these future perspectives, the field of bioremediation in Norway can continue to advance and provide sustainable solutions for the remediation of hydrocarbon-contaminated soils in colder climates. Through interdisciplinary collaborations and ongoing research, the development of innovative techniques, improved understanding of microbial processes, and implementation of environmentally friendly practices can be achieved.

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