EVALUATING THE EFFICACY OF A FUNGAL CONSORTIUM IN HYDROCARBON BIOREM EDIATION

BY

UYONWU, PEACE OJOCHIDE
GOU/ U22/ MCB/519

MICROBIOLOGY
DEPARTMENT OF BIOLOGICAL SCIENCES
FACULTY OF NATURAL SCIENCE AND ENVIRONMENTAL STUDIES
GODFREY OKOYE UNIVERSITY, UGWUOMU NIKE, ENUGU. ENUGU STATE

JULY, 2026

EVALUATING THE EFFICACY OF A FUNGAL CONSORTIUM IN HYDROCARBON BIOREM EDIATION

BY

UYONWU, PEACE OJOCHIDE
GOU/ U22/ MCB/519

A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCE S, FACULTY OF NATURAL SCIENCE AND ENVIRONMENTAL STUDIES, GODFREY OKOY E UNIVERSITY, UGWUOMU NIKE, ENUGU. ENUGU STATE.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE BACHEL OR OF SCIENCE (B.Sc.), DEGREE IN MICROBIOLOGY

SUPERVISOR: DR LINDA OBI

JULY, 2026

APPROVAL

This research titled "Evaluating the Efficacy of Fungal Consortia for Hydrocarbon Bioremediation" has been assessed and approved by the Committee of Department of Biological Sciences and the Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University Enugu, Nigeria.

Dr Linda Obi ________________ ________________
Supervisor Signature Date

__________________ ________________ ___________
Ass.Prof. Vivian Nonyelum Anakwanze
External Examiner Signature Date

Dr. F. N. Olisaka ________________ ________________
Head of Department Signature Date

Prof. Chidi C. Uhuegbu ________________ ________________
Dean of FNASE Signature Date

CERTIFICATION

This is to certify that this research titled "Evaluating the Efficacy of Fungal Consortia for Hydrocarbon Bioremediation" was carried out by UYONWU OJOCHIDE PEACE with registration number GOU/U22/MCB/519 under supervision in the Department of Biological Sciences, Godfrey Okoye University Enugu, Nigeria.

Uyonwu Peace Ojochide ________________ ________________
Student Signature Date

Dr. F. N. Olisaka ________________ ________________
Head of Department Signature Date

DEDICATION

"In every step of this journey, I acknowledge and give thanks to God, for 'I can do all things through Christ who strengthens me' (Philippians 4:13). To Him, I dedicate this project. I'm grateful for his unwavering grace and guidance that sustained me throughout this academic pursuit.

ACKNOWLEDGEMENTS

First and foremost, I return all glory, honour, and adoration to God Almighty for His mercy, grace, strength, and faithfulness throughout my academic journey and during the completion of this research work. Without Him, this achievement would not have been possible.

My sincere appreciation goes to the management and staff of Godfrey Okoye University for providing a suitable environment for learning and research.

With deep respect and gratitude, I sincerely appreciate my supervisor, Dr. Linda Obi , for the patience, guidance, corrections, encouragement, and support given to me throughout this project. Your valuable advice and dedication played a major role in the success of this work.

I also extend my heartfelt gratitude to my co-supervisor, Mrs Cynthia Ada , for the encouragement, assistance, and academic support rendered during this research.

My profound appreciation goes to the Head of Department, Dr. Frances N. Olisaka, and my lecturers, Prof. C. Oyeka, Prof. J.I . Okafor, Mr. Solomon, and all the laboratory Technologist staffs for their assistance in one way or the other which led to the successful completion of my stay in school.

A very special and emotional appreciation goes to my beloved late father, Mr. Paul Uyonwu. Thank you for believing in my dreams and supporting my education while you were alive. Even though death took you away before the completion of this journey, your sacrifices, labour, love, and encouragement will never be forgotten. You started this journey with me, and though you could not stay to witness the end, this achievement is also yours. Your memories continue to inspire me every day. May your gentle soul continue to rest in perfect peace.

To my loving and prayerful mother, Mrs. Felicia Uyonwu, words are not enough to express my gratitude. Thank you for your endless prayers, sacrifices, sleepless nights, encouragement, and unwavering love. In moments when things became difficult, your prayers and strength kept me going. You stood by me through every challenge, and I am deeply grateful for everything you have done for me.

I also appreciate my siblings, Chiamaka Uyonwu, onyeka Uyonwu, chibuzor Uyonwu and my wonderful friends especially Detso Doochivir Joy, Otio Allwell Asuquo, Christian Ekegbo, Frances Ihegwuagu, Gift Uzor, Tito, Daisy ogwulu, Nwuba Noela, Obi Vivian for their support, encouragement, advice, and motivation throughout this journey. Your kindness and belief in me meant a lot.

Finally, to everyone who contributed directly or indirectly to the success of this work, I say a heartfelt thank you. May God bless and reward you all abundantly.

ABSTRACT

Petroleum hydrocarbons (PHCs) are important energy sources widely used in industries and daily activities. However, accidental spills from petroleum industries and other human activities have resulted in severe soil contamination, making PHC pollution a major environmental concern worldwide. The accumulation of these pollutants in soil threatens environmental safety and public health, thereby increasing the need for effective remediation strategies. Among the available methods, bioremediation has emerged as an environmentally friendly and sustainable approach for restoring PHC-contaminated soils. This study aimed to evaluate the efficiency of fungal isolates in the bioremediation of PHC-contaminated soils. Fungi were isolated from PHC-contaminated soils using Potato Dextrose agar. The PHC-degrading efficiency of single fungal isolates was compared with fungal consortium by screening for their hydrocarbon degrading ability using lacto phenol blue and Microscope for identification method. Findings revealed that several microbial species have been successfully applied in the bioremediation of PHCs, highlighting the importance of diverse microbial communities in hydrocarbon degradation. Among the fungal isolates studied, *Aspergillus spp.*, *Penicillium spp.*, and *Trichoderma spp*. Were identified as the most effective organisms for degrading PHC-contaminated soils.The study is significant because it provides a comprehensive understanding of the mechanisms involved in PHC degradation and highlights the potential of fungal bioremediation as a sustainable and cost-effective solution to environmental pollution. It also emphasizes the need for further research, complete degradation of contaminants, and flexible regulations guiding the safe use of genetically engineered microbes to improve the future application of bioremediation technologies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

Appendix 2: Fungal isolate B (*Penicillium chrysogenum*)

37

Appendix 3: Fungal isolate C (*Trichoderma viride*)

38

CHAPTER ONE

INTRODUCTION

1 .1 Background of the Study

Hydrocarbon pollution is now one of the biggest environmental problems being faced, especially in areas with a lot of industries, oil exploration, and fast-growing cities. Most of this pollution comes from things like crude oil spills, factory waste, vehicle exhaust, and the careless dumping of petroleum products (Tao *et al.,* 2024). Because these substances do not break down easily, they stay in the environment for a long time and can damage soil quality, water sources, ecosystems, and even human health.

One group that is especially dangerous is polycyclic aromatic hydrocarbons, or PAHs. They are toxic, hard to break down, and can cause cancer (Minwei *et al.*, 2023). With more oil drilling and other human activities, more of these chemicals keep getting released into the environment. When they enter soil and water, they change the physical and chemical nature of the soil, lower its fertility, and interfere with natural biological processes.

Traditional cleanup methods like digging up contaminated soil or using chemicals are usually expensive, harmful to the environment, and still do not remove all the pollutants. This is why bioremediation has become popular. It uses living microorganisms to break down or change hydrocarbon pollutants into safer compounds. It’s a cleaner and more sustainable way to handle pollution (Arif *et al.,* 2024).

Fungi are now getting a lot of attention in bioremediation research. This is because they can survive in harsh, toxic conditions and they have the enzymes needed to break down complex hydrocarbons (Tasiu *et al.,* 2022). Through their normal activities, some fungi c an even turn petroleum hydrocarbons into carbon dioxide, water, and simpler organic substances.

The problem is that not all hydrocarbons are easy for one type of microbe to degrade. Long-chain alkanes and PAHs, for example, often stay in the soil for a long time, especially in areas affected by drilling or oil spills (Grmasha *et al.*, 2023). Because of this, researchers are now looking more into using a mix of different fungi, called fungal consortia, to improve hydrocarbon breakdown.

Recent studies suggest that fungal consortia, which are made up of different fungal species that work together, can make bioremediation more effective in polluted soil and water (Bala *et al.*, 2022). The teamwork between these fungi increases the range of enzymes they produce, and this helps them degrade pollutants better than when a single fungal isolate is used alone.

Even with these improvements, there is still limited research on how to optimize fungal consortia, how the species interact with each other, and how they compare to single isolates in real performance. Most studies focus only on one fungal species or on mixed microbial systems, without directly testing if fungal consortia are more effective than single fungi. As a result, there is not enough strong evidence to confirm whether fungal consortia are truly better or practical for advanced hydrocarbon cleanup.

1.2 Statement of Problem

Hydrocarbon pollution is still a major environmental problem because petroleum contaminants can remain in soil and water for a long time. Although using fungi for bioremediation has shown good results, single fungal isolates often struggle when faced with compl ex mixtures of hydrocarbons. Also, while fungal consortia are being suggested as a better option, there are few detailed studies that compare their degradation ability with that of single fungal species. This gap in knowledge makes it difficult to develop reliable and well-optimized fungal-based bioremediation method.

1.3Aim and Objectives

Aim

Evaluating the efficacy of fungal consortia in hydrocarbon bioremediation

Objectives

1. Isolate and identify fungal strains from hydrocarbon-contaminated soil.
2. Characterize the hydrocarbon-degrading abilities of the isolated fungal strains.
3. Compare the bioremediation potential of fungal consortia with that of single fungal isolates in hydrocarbon degradation.

CHAPTER TWO

LITERATURE REVIEW

Petroleum hydrocarbons (PHs) are long lasting, toxic environmental pollutants. They damage both land and water organisms (Truskewycz *et al.*, 2022). Oil pollution extends far beyond extraction sites due to widespread human activity. Ocean transit spills, vehicular oil leaks and compromised underground fuel tanks constantly release these toxins, creating an expensive environmental threat that requires urgent mitigation (Lmman *et al*, 2022). Terrestrial oil spills happens when petroleum hydrocarbons accidentally coat the ground. Pipeline ruptures, failing storage tanks and transport crashes drive these events, making infrastructure failures the primary cause of onshore contamination.

Infrastructure failures cause most land-based oil spills. Extensive research confirms that these complex petroleum hydrocarbon mixtures heavily drive global soil degradation. Spilled ground oil seeps through soil into aquifers endangering ecosystems and human health. As the oil migrates, physical and chemical interactions alter soil structures and reduce permeability. These persistent toxins remain in the ground for decades causing prolonged damage to plants, microbes and water supplies.

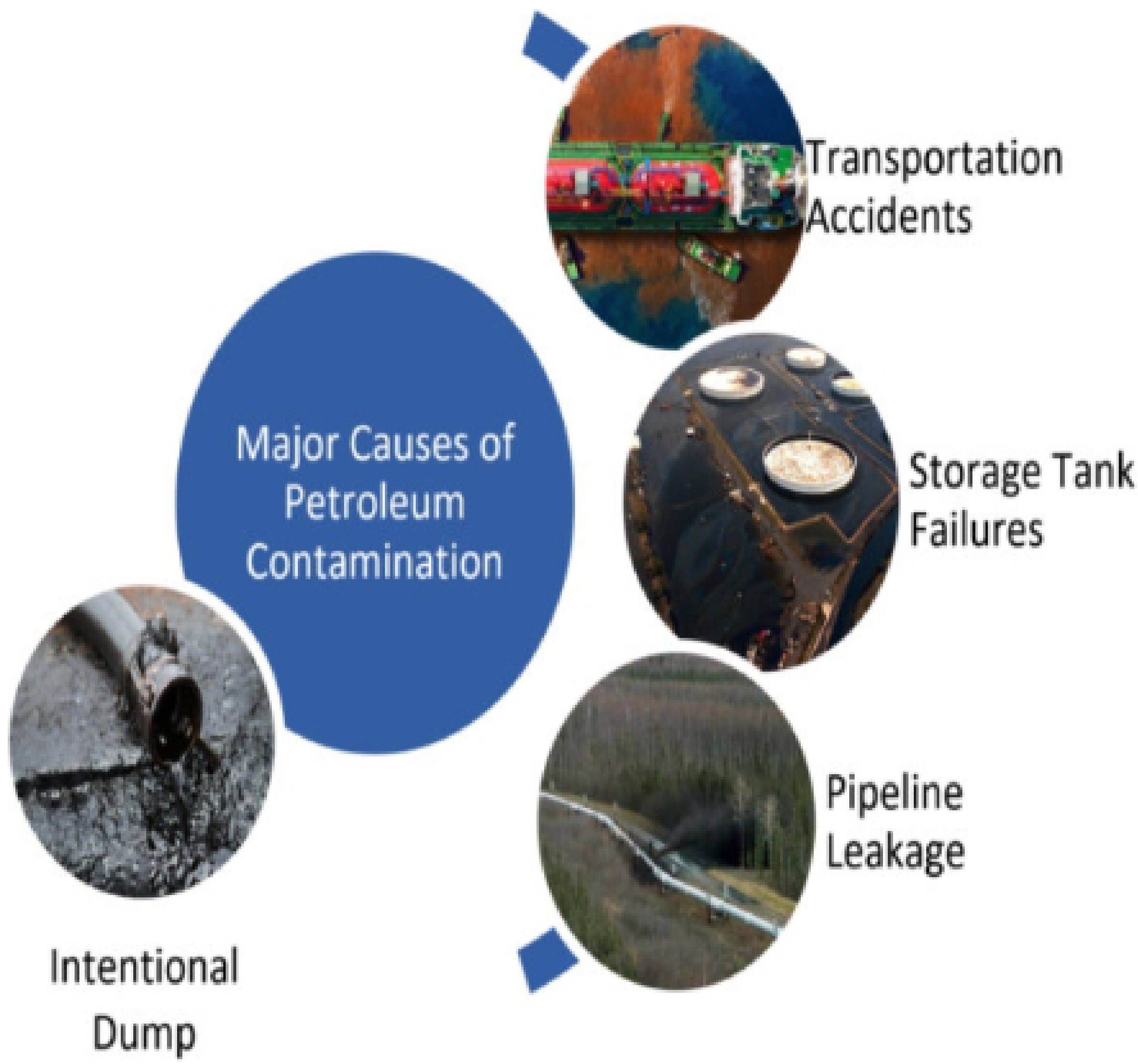

Figure 1. Main Reasons behind Major Petroleum Hydrocarbon (PH) Contamination in En vironmental Incidents
Source: (Guzik *et al*,. 2023)

2.1 The problem: Hydrocarbon pollution

2.1.1 Sources and environmental fate of petroleum hydrocarbon

Industrial operations joined to the petroleum sector, including exploration, drilling, storag e, movement, processing, and refining of petroleum are major ways petroleum hydrocarb ons are released into the environment. These activities continuously cause terrestrial oil contamination and often involve water-repellent (Nonpolar compounds that do not mix well with water), (Chettri *et al.*, 2021). The introduction of petroleum hydrocarbons (PHC s) into natural environments causes serious ecological threats and may result in a very s erious environmental and human health consequences (Guarino *et al.*, 2023). Current est imates indicate that approximately 0.6 million tons of crude oil are discharged into the e nvironment each year (Kvenvoldrler *et al.*, 2023).. Furthermore, high concentrations of re adily degradable organic matter in surface soils disrupt dissolved oxygen, which reducin g oxygen availability and slowing its movement into deeper soil layers (Dindar *et al.*, 202 1). As a result, the remediation of PHC-polluted soils has become a major environmental challenge, requesting the urgent need for the development of sustainable, eco-friendly, a nd efficient remediation strategies to maintain healthy soil ecosystems (Cui *et al.*, 2022). The process of restoring soil that has been destroyed by polycyclic aromatic hydrocarbo ns (PAHs) is a global ecological concern due to its damages for public health. The rehabi litation of petroleum-polluted soils is essential for removing hazardous compounds from the environment and can be achieved through multiple remediation approaches that involve contaminant extraction, containment, or transformation. Basically, a variety of soil restoration techniques are available, including thermal, chemical, physical, and biological cleanup approaches can be used either directly at the disruptions site (in situ) or after the material has been excavated and treated somewhere (ex situ) (Table 1)

Table 2.1: Strategies for the remediation of petroleum-affected soils Bioremediation

Strategies for the remediation of petroleum- affected soil Bioremediation	**References**
Plant-based remediation	Lu *et al.*,(2022)
Root-associated remediation	Rostami *et al.*,(2023)
Nutrient -enhanced bioremediation	Wei *et al.*,(2021)
Microbial inoculation	Patel *et al.*,(2023)
Bio electrochemical remediation systems	Hao *et al* .,(2022)
Microbial electrochemical remediation	
Plasma -based oxidation processes	Lu *et al.,* (2023)
Physicochemical separation	Yen *et al* ., (2022)
Physical treatment techniques	
Ultrasonic treatment	Copik *et al.,* (2023)
Electrokinetic remediation	Pourfadakari *et al* ., (2022)
Soil vapor extraction	Cao *et al.,* (2021)
Desorption	Wei *et al.*,(2022)

In-situ remediation is carried out directly at the polluted location and presents several benefits. This approach is generally associated with reduced operational risks, lower financial costs, and minimal human intervention. In addition, natural environmental conditions at the site can actively support the remediation process by facilitating the transformation or degradation of contaminants. In contrast, Ex-situ remediation involves digging up contaminated materials and transporting them to another site for treatment. Although this method is more expensive, it is performed under highly controlled conditions, requires greater human participation, and often results in increased direct exposure to pollutants. Ex-situ remediation is also subject to strict regulatory limitations. Common techniques within this category include solvent extraction, ultraviolet (UV) oxidation, photochemical or photocatalytic degradation, as well as biological approaches such as bioremediation and phytoremediation. The choice of an appropriate remediation strategy for petroleum-contaminated soils is influenced by several factors, which involves the nature and chemical compositions of the pollutant and the intended future use of the affected soil, soil characteristics, and available financial resources.

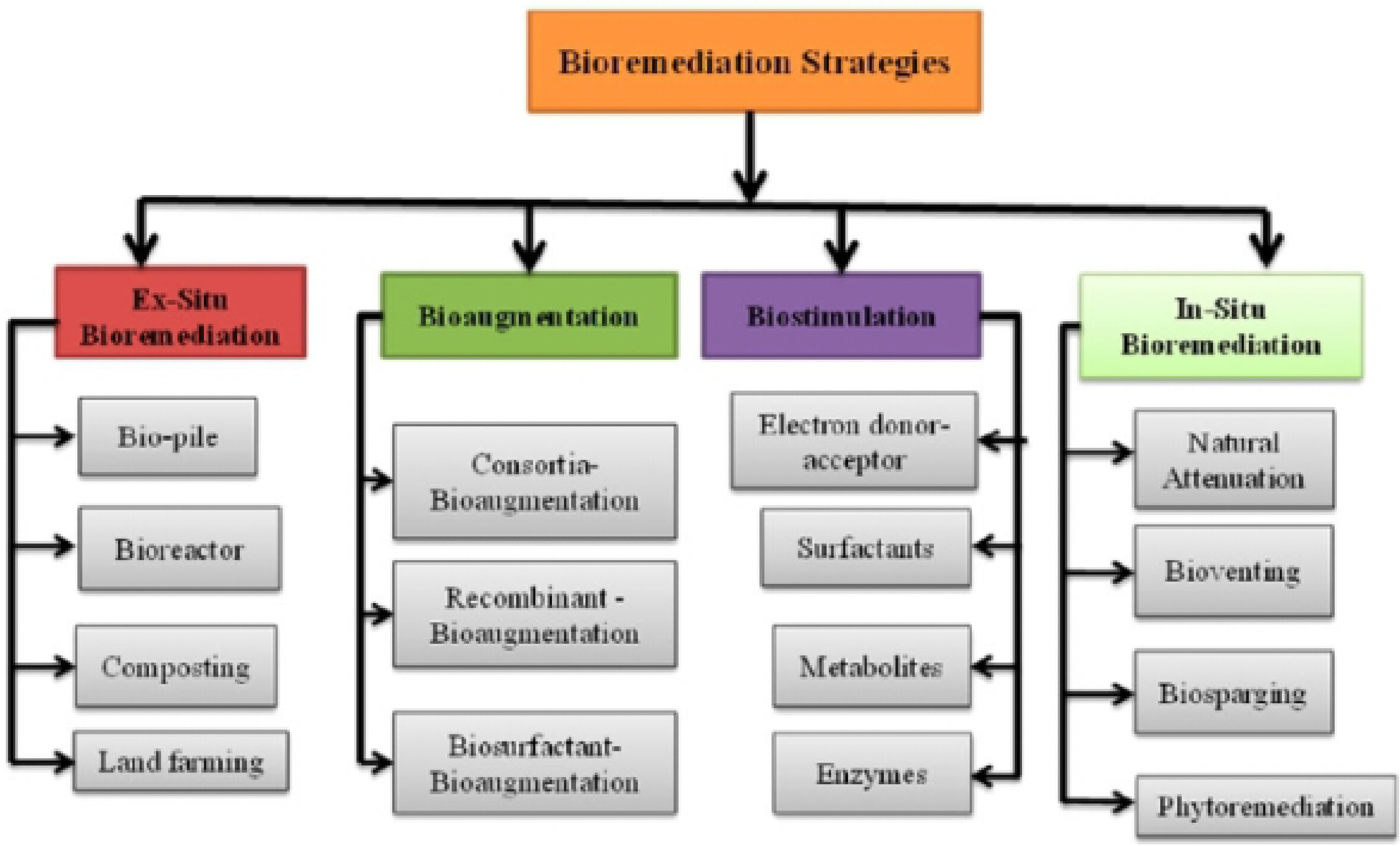

Figure 2. Strategies employed in the bioremediation of petroleum hydrocarbon–contami nated soils, adapted and refined from earlier research.
Source: (Chaudhary *et al*,. 2022)

2.2.2 Ecotoxicology impacts

Aquatic ecosystems are among the most susceptible to environmental damage, particularly those situated close to busy maritime corridors, oil terminals, and offshore drilling facilities. Oil discharges from vessels or drilling operations can spread across extensive water surfaces, forming a thick layer that restricts the aquatic environment and the exchange of gases between the atmosphere. Over time, this disruption can suffocate marine life by blocking sunlight for phytoplankton photosynthesis and reducing dissolved oxygen levels. Freshwater bodies such as wetlands, ponds, and lakes located near refineries, storage tanks, or pipelines are also at high risk of pollution. Because the water is mostly still, hydrocarbons can remain and accumulate on the surface. This leads to a decline in water quality, which upsets the balance of freshwater food webs. When oil blocks the exchange of air and water, it causes ecological stress, reduces the number of species, and damages aquatic habitats. In addition, the chance of oil getting into drinking water sources increases, which poses a direct threat to human health. Identifying where oil spills come from and understanding their environmental effects is important for planning better cleanup and control measures, since people can be exposed to these hydrocarbons in different ways. Often, these medical conditions develop silently over time rather than showing up right away which can lead to lasting organ damage or death. As a result, Figure 3 demonstrates the pathways through which hydrocarbons enter and are absorbed by the human body, while Figure 4 illustrates the associated short- and long-term health impacts.

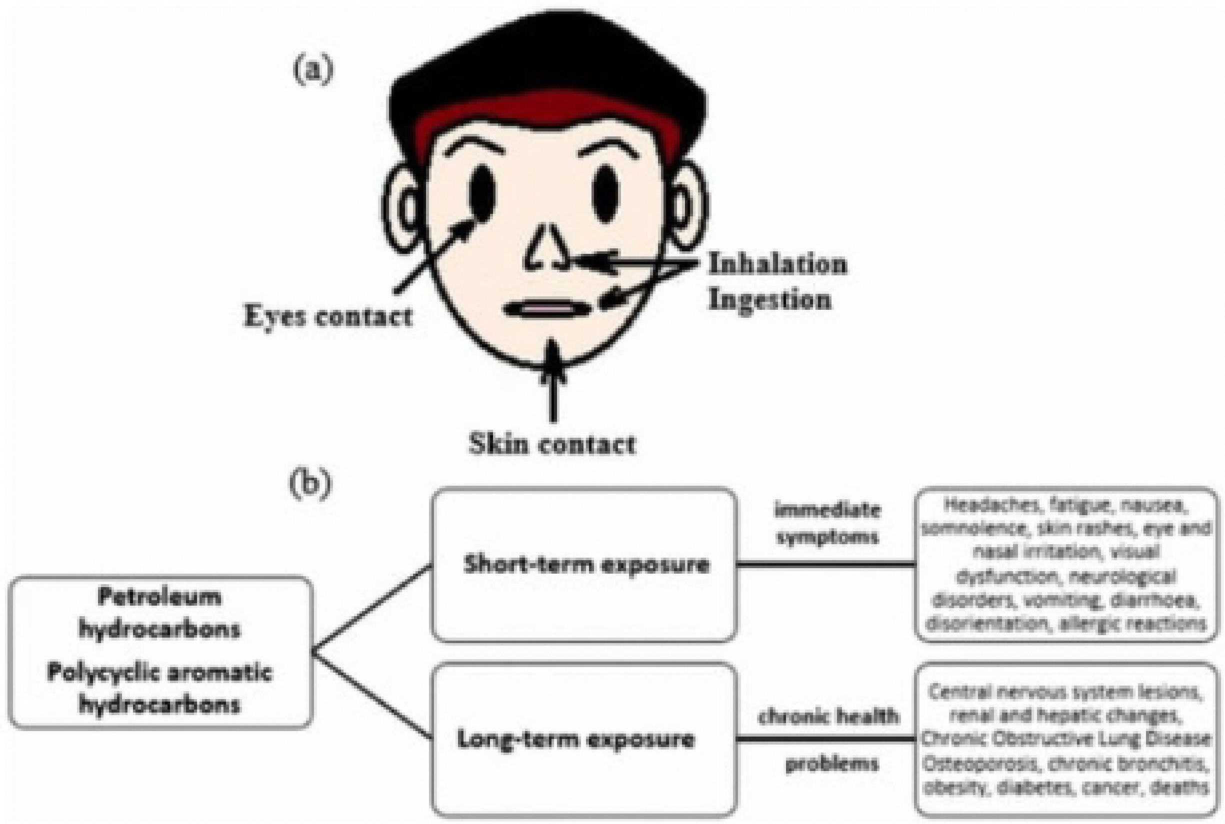

Figure 3: Illustration showing the different routes through which petroleum hydrocarbons enter the human body and associated health effects they may cause.
Source: (Mastrangelo, *et al*,. 2022)

2.2.3 Regulatory and economic drivers for remediation

The efficacy of fungal bioremediation is reflected in its ability to decompose persistent Organic pollutants, such as hydrocarbons derived from petroleum, polychlorinated chemical compounds (PCBs) and multi-ringed hydrocarbon pollutants (PAHs). Moreover, fungi can stabilize as well as alter heavy metals through processes such as biosorption and bioaccumulation. The application of microbial consortia for converting a wide range of contaminates into harmless or less hazardous substances is considered as more effective strategy (Azubuike *et al.*, 2022). Currently, bioremediation utilizing microbial consortia has become increasingly significant in practical applications and has already been implemented in various real-world settings. As illustrated in Figure 4, these microbial communities are capable of breaking down complex substances such as plastics, petroleum-derived materials, antibiotics, azo dyes, and a wide array of pollutants commonly present in wastewater. Moreover, these mixed microbial populations is capable of being employed in consolidated bioprocessing (CBP), a promising approach for addressing shortages of energy. Petroleum based compounds and plastic waste might likewise serve as feedstock's for generating valuable byproducts. Owing to their superior capacity to degrade complex compounds, microbial consortia are often preferred over individual microbial strains for environmental cleanup. They effectively decompose persistent pollutants in contaminat ed soils and wastewater systems. Additionally, soils restored through such remediation can support the growth of shrubs and trees, while treated wastewater can be safely reused for irrigating commercially grown crops unsuitable for eating, (Biswas *et al.*, 2021).

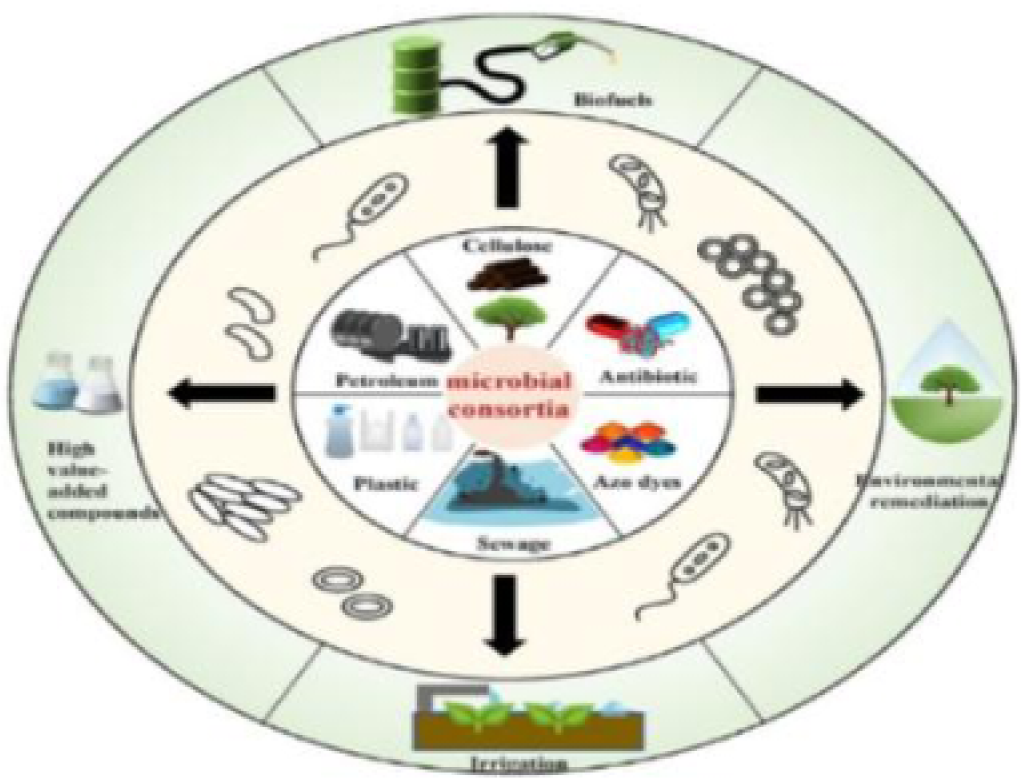

Figure 4: Biological cleanup that harnesses microbial consortia to reutilize complex compounds

Source: (Cui *et al.,* 2022)

2.3 Bioremediation as a remediation strategy

2.3.1 Definition and principles

Fungal bioremediation, often referred to as mycoremediation, is the process of using fungi to break down organic waste materials and harmful chemical pollutants in the environment. These fungi secrets multiple enzymes, which includes amylases, lipases, nucleases, and proteases, which aid in breaking down cell walls, nucleic acids, and other structural elements. The purpose of this technique is to rehabilitate contaminated environments by converting organic waste into less toxic or chemically stable end products. In addition, fungi can process metalloids by releasing enzymes that alter their chemical forms, thereby affecting the mobility, reactivity, and bioavailability of related contaminants. In addition, fungi are highly resilient and can thrive under diverse environmental conditions, processing a broad spectrum of compounds within their surroundings and utilizing the resulting by-products as nutrients. Through the action of extracellular oxidoreductases, they mitigate pollution and remove contaminants by decomposing lignocellulosic materials.

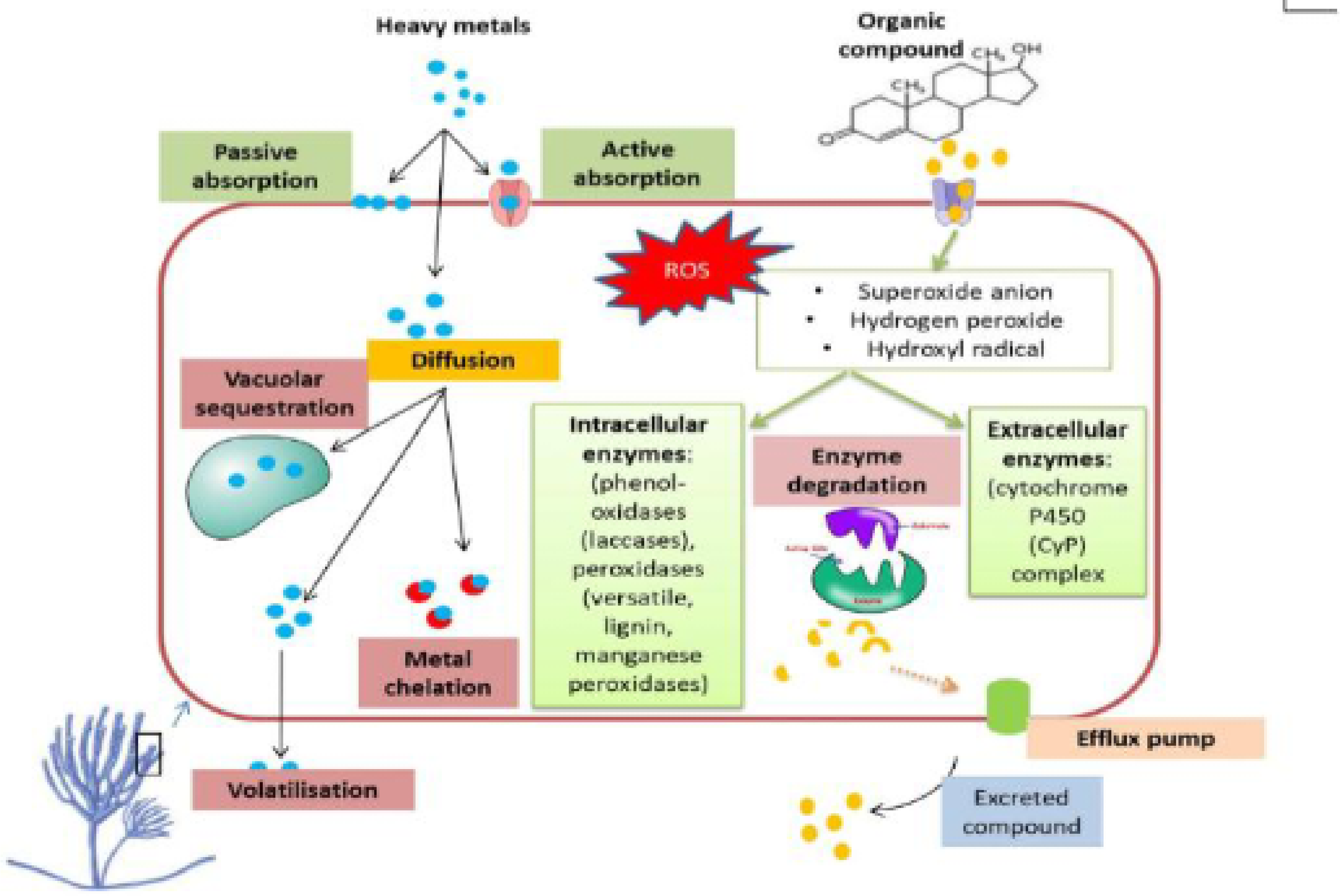

Figure 5: This figure shows the different ways fungi help clean up pollutants, such as br eaking down contaminants through enzyme-driven processes, binding toxic substances t o their cell walls, and absorbing and storing pollutants inside their cell
Source: (Kurade *et al*,. 2022)

Fungi play a crucial role in cleaning up a wide range of toxic pollutants, including coal by-products, synthetic chemicals from the persistent organic pollutants (POPs), textile dyes, paper industry, tannery wastes, pharmaceutical residues and other emerging contaminants (PPCPs), pesticides, and polycyclic aromatic hydrocarbons (PAHs).A growing body of research shows that fungal species such as *Penicillium*, alkalophilic white-rot fungi, and *Aspergillus*, particularly *Phanerochaete chrysosporium* are highly effective in bioremediation processes. These fungi have been successfully applied in the removal and decolorization of textile dyes, synthetic compounds generated during kraft pulp production, leather tannery effluents, and sugar-industry wastes. Short-term incubation of contaminated soils with *Aspergillus niger* and *Phanerochaete chrysosporium* in the presence of petroleum hydrocarbons has resulted in marked reductions in total organic carbon (TOC), as well as significant degradation of diesel and gasoline contaminants. White-rot fungi are distinguished by their ability to secrete one or several extracellular enzymes, These processes differ depending on the type of fungus involved and the environmental conditions in which they operate. Comparative research shows that the production of ligninolytic enzymes, For example, enzymes like lignin-degrading peroxidase (LiP), oxidative enzymes called laccases, and manganese dependent peroxidase (MnP)., can vary considerably depending on factors like pH, the availability of carbon and nitrogen sources, and the concentration of metal ions. As a result, effective bioremediation relies on the ability of fungi and their enzymes to degrade complex xenobiotic substances, including synthetic dyes, through specific and well-regulated biochemical pathways.

2.3 Bacteria: Traditional workhorse

Bacteria are the dominant microbial agents involved in the degradation of petroleum-derived contaminants. These organisms multiply quickly, are simple to grow and breakdown and oil more efficiently than fungi or algae (Chunyan *et al.*, 2023). Two main biochemical pathways allow bacteria to destroy Petroleum hydrocarbons (PHs). First, bacteria trigger and modify organic pollutants through processes like oxygenation, hydration and carboxylation to create essential metabolic intermediates. The cells then use these intermediate as vital carbon sources within their normal metabolic pathways. Second, bacteria produce biosurfactants that boost the solubility of hydrophobic materials, making the sticky hydrocarbons easier to absorb and digest (Victor *et al.*, 2020) (fig.6). The actual activation steps vary deeply between aerobic and anaerobic microbes because bacteria utilize diverse electron acceptors to process Petroleum (fig6). Aerobic breakdown happens much faster than anaerobic tracks since oxygen functions as an excellent terminal electron acceptor. Therefore, aerobic digestion serves as the primary pathways for the biodegradation of petroleum hydrocarbons (Victor *et al*., 2020).

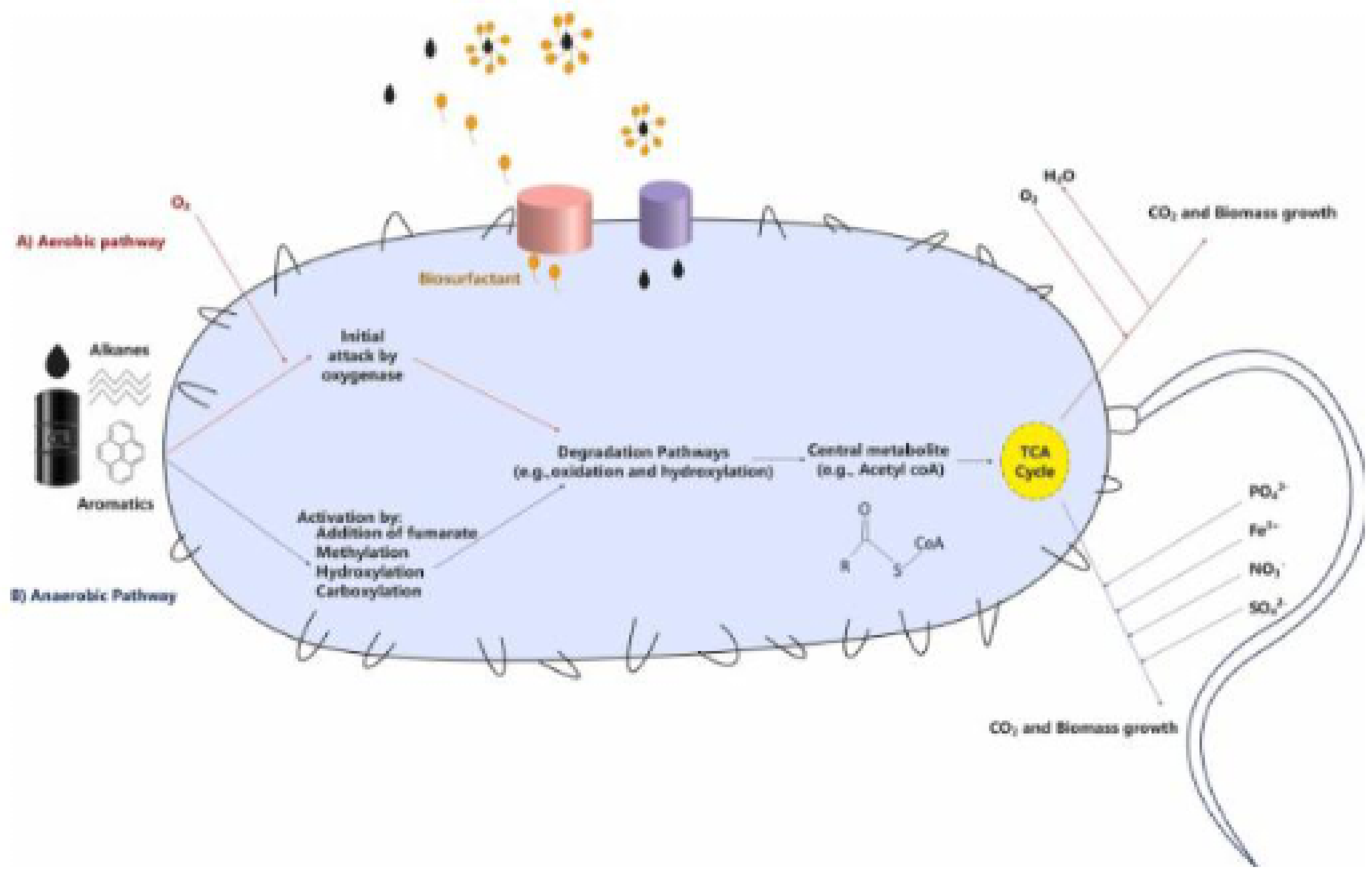

Figure 6: Bacterial Pathways for Petroleum Hydrocarbon Degradation. The processes res ponsible for hydrocarbon activation differ markedly between aerobic (A) and anaerobic (B) microorganisms, with bacteria utilizing a variety of electron acceptors during the brea kdown of petroleum hydrocarbons. In addition, bacteria synthesize biosurfactant that inc rease the solubility of hydrophobic pollutants and improve the bioavailability and cellular uptake of hydrocarbon contaminants for effective degradation.

Source: (Khan *et al*,. 2021)