Microbiological remediation - oil contaminated sites.

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**Abstract:**

Waste engine oil (WEO) and repeatedly heated cooking oil (RHCO) pose an insidious threat to environment due to improper disposal of oil spills. While existing techniques focus on converting waste oil into value-added products, residual spills and improper disposal remain a crucial issue. Various bioremediation techniques like Augmentation, using specialized bacteria, biostimulation-boosting existing microbial activity, phytoremediation using plants, and Nano remediation are compared and contrasted.

This chapter highlights the effects caused by accidental oil spills and human negligence, leading to soil contamination and harm to lifeforms in terrestrial and marine environments. It delves deeper, exploring bioremediation as a promising, eco-friendly solution. Further it focuses on the advantages of bioremediation, emphasizing its use of local bacteria with oil degradation potential. This localized approach offers sustainable and less disruptive solution compared to traditional methods. Furthermore, it acknowledges the challenges associated with each bioremediation technique, including difficulty in selecting suitable microorganisms, scaling up for large-scale implementation, and ensuring complete degradation or neutralization of harmful oil components. Despite these challenges, bioremediation presents a strong potential for mitigating the environmental impact of WEO and RHCO spills. Benefits of bioremediation are summarized highlighting its promising future as an eco-friendly solution for a cleaner and healthier planet.

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**I .Introduction:**

 Oil spillage is a significant contributor to environmental contamination, posing a threat to ecosystems and human well-being. Large-scale oil spills in marine waters have been the cause of major ecological disasters worldwide. These incidents highlight the urgent need for effective and sustainable remediation strategies to mitigate the adverse impacts of oil pollution. The estimated annual amount of natural crude oil seepage is around 600,000 metric tons, with an associated uncertainty range of 200,000 metric tons. The market for waste cooking oil is anticipated to increase to $10.08 billion in 2028. (1)

 Approximately 47% of the crude oil entering the marine environment is attributed to natural seeps, while the remaining 53% is the result of human negligence, including spillage or leaks during various phases of oil exploration, storage, and transportation [2]. The average cost of cleanup often is as high as about $200 per liter of spilled oil [3]. Spent engine oil and waste cooking oil are two significant pollutants.

 Waste Engine Oil Contamination (WEO): Improper disposal of waste engine oil, generated from industrial machinery, automotive engines, and power generators, contributes to soil and water pollution. Petroleum industry as a major source of hydrocarbon pollutants. Specifically, it highlights the release of polycyclic aromatic hydrocarbons (PAHs) into the environment through petroleum exploration activities. The indiscriminate accumulation of these hydrocarbon pollutants poses hazards to both human life and aquatic biota due to their toxic nature [4]. Inadequate storage, leakage, or illegal dumping of waste engine oil poses a significant ecological threat, potentially harming soil, water, and air quality, and disrupting ecosystems.

 Repeatedly heated Cooking Oil (RHCO): The widespread practice of reusing repeatedly fried cooking oil, often without awareness of its health hazards, poses a significant risk to all forms of life. While preventing this practice among hawkers is challenging, we can focus on discouraging its use at the household level where we have more control and influence [5]. In the food industry, cooking oil undergoes degradation and chemical transformations during repeated frying, resulting in the accumulation of harmful byproducts such as polar compounds, free fatty acids, and trans fats. Improper disposal of used cooking oil further contributes to environmental pollution. The lack of awareness regarding proper disposal methods is prevalent, with approximately 80% of individuals lacking knowledge in this regard [6]. Reusing used oil carries serious health risks, including the potential development of fatal diseases like cancer. It is crucial to refrain from selling or using non-edible oil for cooking purposes to safeguard both human health and the environment [7, 8]. The increased awareness of renewable energy sources and these oil wastes can turn out to be raw materials in production of various products such as Biodiesel, Lubricants solvents etc

1. **Effect of oil spills on the environment:**

Oil spills cause significant harm to ecosystems and the organisms that inhabit them. These spills can result in the following environmental consequences:

1. Marine Life Contamination: Oil spills contaminate water bodies, coating the surface and harming marine life in various ways. The oil can suffocate marine animals, impair their ability to move and feed, and damage their reproductive systems. Birds and mammals that come into contact with the oil may experience reduced insulation and be unable to fly or swim properly. The extensive damage caused by soil contamination with hydrocarbons is attributed to the accumulation of pollutants in animals and plant tissue, leading to severe consequences such as death or mutations [9]. Oil contamination can taint fish and shellfish, making them unappealing to consumers and harming fisheries. It can also disrupt fish metabolism and harm organisms at the base of the food chain, like plankton, even at low levels. For instance, detectable oil traces along shipping routes affect the feeding and burrowing behavior of sand crabs (*Emerita holthuisi*) [10].
2. Habitat destruction: **Oil spills devastate marine habitats like marshes and coral reefs, crucial breeding and feeding grounds for countless species. The oil smothers plants and animals, disrupting ecosystems and reducing biodiversity. From microscopic larvae to adult fish, marine life suffers as oil coats their bodies, often fatally. These impacts extend beyond the immediate spill, with PAHs (toxic oil components) harming organisms throughout their life cycle [9].**
3. Water Quality Degradation: Oil spills, from both natural seeps (50%) and human activities (50%), contaminate water with toxins. This disrupts oxygen exchange, harming aquatic life (hypoxia) and hindering plant growth (reduced light penetration). Land runoff (38%), lubricating oil (wastewater), and maritime transport (13%) are major human contributors [11].
4. Long-Term Effects: Even after the visible oil is cleaned up, the long-term effects of spills can persist. Oil can sink to the ocean floor, where it can remain for years, affecting benthic communities and disrupting the food chain. Contaminated sediments can release oil slowly over time, further impacting the ecosystem [12].
5. Impact on Coastal Areas: Oil spills devastate coastal ecosystems like beaches, mangroves, and estuaries, harming biodiversity and tourism. Even small, undetected spills can cause chronic damage, while large ones have immediate and lasting effects. Understanding this overall ecological impact is crucial for protecting our coasts [13].
6. Ripple Effects: Oil spills wreak havoc on food webs, causing species decline and disrupting predator-prey relationships in marine ecosystems. While dispersants are used to minimize shoreline damage in the US (3 million gallons spilled annually), their effectiveness and long-term impacts require careful consideration [14].
7. Alteration of Soil Characteristics :Waste oil adversely affects the physical and chemical properties of soil resulting in increase in bulk density ,organic carbon ,moisture content and reduction in pH ,porosity, water holding capacity ,phosphorus and potassium content (15). It even makes the soil less fertile and result in the inactivity of beneficial microorganisms.
8. **Methods of treating oil spills**:

Treatment of UCO and RHCO depends on the size, location, resources, and desired outcome. A combination of methods may be used for effective cleanup and minimal environmental impact.

1. Sorbent Materials: Sorbents like activated carbon or natural materials like kenaf fibers have the potential to soak up oil spills. Kenaf fibers, tested in various oil-water mixtures, absorb oil most effectively within 2 minutes and saturated sorbents are then disposed [16].
2. Skimming and Mechanical Recovery: **For large spills, skimmers are used to remove surface oil. However, this method is limited by oil behavior, weather, equipment, and recovers 10-30% of oil and their effectiveness in large offshore spills is minimal** [17].
3. Chemical Dispersants: Dispersants consist of surfactants which are effective agents that reduce the interfacial tension between oil and water and break oil slicks into small droplets, solvents, and other compounds. It is efficient in removing floating oils [18].
4. Thermal Treatment: It involves the application of heat to contaminated soils with the intention of volatilizing hydrocarbons, which are then carried away by vacuum and eventually destroyed through incineration or carbon adsorption. (19) Recovering from oil spills takes time, and ecosystems rarely fully return to their original state [20]
5. Chemical Oxidation: **It involves using compounds like hydrogen peroxide,** as its hydroxyl radicals react rapidly and nonselective with nearly all organic compounds. It **breaks down oil but is best for small spills due to potential environmental impacts.** [21].
6. Bioremediation: **This process harnesses microbes (bacteria, fungi) to break down oil into harmless substances. This eco-friendly method can be done on-site (in-situ) or in a controlled setting (ex-situ). Adding nutrients and oxygen boosts the microbes' activity. Bioremediation works in various environments and is most effective when using a mix of microbes (consortia) rather than a single type [**22]. Microbes naturally break down pollutants using special enzymes. These enzymes, like laccases and hydrolases, make microbes effective tools for cleaning up contaminated sites [23].
7. **Bioremediation and its various strategies:**

Microbial remediation is a promising field with new applications for environmental cleanup. Some of them are:

1. Genetically Engineered Microorganisms (GEMs): **Bioremediation gets a boost from genetically engineered microbes. Scientists can equip native bacteria with genes for faster oil degradation, allowing them to target specific pollutants. These supercharged microbes break down contaminants more efficiently by producing enzymes and following different metabolic pathways** [24) **Prof. Chakrabarthy pioneered this approach by genetically engineering *Pseudomonas putida* bacteria with enhanced oil-degrading abilities in an eco-friendly way [**25, 26].
2. Bioaugmentation: It involves injecting specialized microbes (indigenous or nonindigenous) into polluted areas to speed up contaminant breakdown. This strengthens the existing microbial community's ability to tackle specific pollutants [27]. Microbial species belonging to *Pseudomonas*, *Acinetobacter*, *Rhodococcus*, *Burkholderia* etc., have been successfully used for bioaugmentation of PAHs.(28)
3. Biostimulation: This process acts like fertilizer for natural cleanup. By adding nutrients, it strengthens existing microbes at a spill site, speeding up their breakdown of pollutants like oil or plastics. This eco-friendly approach works in various environments, from open seas to chronically polluted areas [29]
4. Nano remediation: Nanotechnology is transforming bioremediation by making contaminant removal significantly more efficient. **Tiny particles like** nano zero-valent iron (nZVI), [carbon nanotubes](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/carbon-nanotube), sponges, [aerogels](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/aerogel) and [nanocomposites](https://www.sciencedirect.com/topics/engineering/nanocomposite), metal and non-metal nanostructurized oxides, [nitrides](https://www.sciencedirect.com/topics/engineering/nitride), salts, and zeolites are used **to help microbes break down pollutants faster.[30]** **These nanoparticles can also trap contaminants and deliver nutrients, boosting the effectiveness of bioremediation. In oil spills, they act like magnets or emulsifiers, aiding marine microbes in degrading the oil.** Unlike traditional methods like dispersants and burning, this eco-friendly approach offers a long-lasting solution, minimizing harm to marine ecosystems [31].   Some of the nanoparticles can be prepared by “greener” methods at lower costs and without damage to the environment.
5. Phytoremediation: Cleaning up oil spills can be done using plants and this can be enhanced by Addition of organic matter, organic chelates, soil amendments, adoption of suitable cropping systems, intercrops and fertilizer selection of Indian mustard. [32].
6. In-situ Bioremediation: In-situ bioremediation techniques like bioventing and biosparging treat contaminated soil and groundwater without excavation. Studies show that adding specific microbial inoculants significantly enhances oil degradation in soil, suggesting a promising strategy for cleaning up oil sludge contamination [33].
7. **Physical and chemical factors influencing bioremediation:** Bioremediation success depends on careful site analysis and monitoring of physical and chemical factors.

 Table 1: Factors influencing Bioremediation [34]

|  |  |
| --- | --- |
| **Factors** | **Optimal Range** |
| Temperature | 30-40°C |
| pH | 5-8 |
| Moisture Availability | 30-90% |
| Carbon/Nitrogen/Phosphorus Ratio | 100:20:1 |
| Oxygen Levels | 10-40% (for aerobic degradation) |

1. **Microbiological remediation** :

Bioremediation is considered one of the preferred methods to treat WEO and RHCO spills due to following reasons:

1. Environment Friendly: Bioremediation, a natural method using microbes to break down oil spills, was tested in a lab. The most effective approach (combining stimulation and adding microbes) achieved nearly 90% oil removal, suggesting that boosting microbial activity is key for successful bioremediation [35].
2. Targeted Degradation: Scientists enhanced *E. coli* with genes to break down specific oil components. These genes were then transferred to native bacteria in polluted sediments, significantly reducing the oil content. This targeted approach leverages natural microbial abilities for a more effective and complete oil spill cleanup compared to traditional methods [36].
3. Cost-Effective: Bioremediation remains a cost-effective option, especially for large oil spills. This is because it utilizes natural microbes, eliminating the need for expensive equipment or chemicals compared to alternative methods [37].
4. Minimizes Disruption to Ecosystems: Bioremediation leverages existing microbes and their natural breakdown processes, minimizing disruption to surrounding ecosystems. This method harnesses nature's cleanup crew – fungi, bacteria, and plants – to effectively eliminate organic contaminants [38].
5. Versatility: Bioremediation tackles oil spills in diverse environments (soil, water, sediment) through in- situ (on-site) or ex-situ (off-site) methods. It offers three main techniques: land treatment for soil/groundwater, biofiltration for air, and bioreactors for water. This versatility makes bioremediation a powerful tool for environmental cleanup [39].
6. Long-Term Effects: Bioremediation uses microbes to break down pollutants like PAHs (harmful chemicals) in soil. A genetically engineered microorganism (*Pseudomonas fluorescens* HK44) effectively removed most PAHs within two years. [40].
7. Reduced Secondary Pollution: Bioremediation offers a significant advantage over methods like dispersants or thermal treatment by offering a minimal risk of secondary pollution. [41].
8. **Challenges associated with Bioremediation:**

Bioremediation also comes with certain challenges that need to be addressed for successful implementation (42). Some of the key challenges include:

1. Microbial Selection: Selecting the appropriate microbial species or consortia for bioremediation is crucial. Different contaminants require specific microbial capabilities for degradation. Identifying and isolating the most effective and efficient microbial strains for a particular contaminant can be challenging [43].
2. Scalability: Scaling up bioremediation processes from laboratory-scale to field-scale can be complex. Maintaining optimal conditions, such as temperature, pH, and nutrient availability, becomes more challenging in large-scale applications. Ensuring consistent microbial activity and contaminant degradation across a larger area requires careful planning and monitoring [44].
3. Residual Contaminant Management: Bioremediation may not completely eliminate contaminants but may transform them into less harmful forms. Managing the residual contaminants and byproducts is essential to prevent their reentry into the environment or their accumulation in the treated area. Proper disposal or further treatment of residual contaminants is necessary [45].
4. Site Heterogeneity: Environmental sites are often heterogeneous, with variations in soil types, moisture content, and contaminant distribution. The effectiveness of bioremediation can be influenced by these site-specific factors. Understanding the site heterogeneity and developing strategies to address variations in microbial activity and contaminant availability is crucial [46].
5. Time Requirements: Bioremediation is generally a slower process compared to some other remediation methods. It may take weeks, months, or even years to achieve significant contaminant reduction or complete remediation. Patience and long-term monitoring are necessary to ensure the success of bioremediation projects [47].
6. Regulatory Approvals: Compliance with regulatory requirements and obtaining necessary permits for bioremediation projects can be a challenge. Meeting the standards and regulations set by environmental agencies is essential to ensure the safety and effectiveness of the bioremediation process [48]. Successful bioremediation strategies require interdisciplinary integration [49].
7. **Regulatory frameworks and guidelines**:

Guidelines play a pivotal role in governing the bioremediation of oil wastes to ensure safe and effective environmental cleanup. The specific regulations and guidelines may vary among countries or regions, but some common aspects include:

1. Environmental Protection Laws: Countries have environmental protection laws that govern the management and cleanup of hazardous substances, including oil wastes. These laws set the legal framework for bioremediation activities and provide guidelines for compliance.
2. Remediation Standards: Regulatory bodies establish specific standards or guidelines for acceptable levels of contaminants in soil, water, or air. These standards serve as benchmarks to assess the effectiveness of bioremediation and determine when a site has been successfully remediated.
3. Permitting and Reporting Requirements: Bioremediation projects require permits or approvals from environmental agencies or regulatory bodies. These permits outline the conditions, monitoring requirements, and reporting obligations for the bioremediation process. Compliance with these requirements ensures that the project meets regulatory expectations.
4. Health and Safety Regulations: Bioremediation activities involving hazardous substances must comply with health and safety regulations to protect workers and the surrounding community. These regulations include guidelines for personal protective equipment, handling and storage of hazardous materials, and emergency response protocols.
5. Monitoring and Reporting: Regulatory frameworks require regular monitoring and reporting of bioremediation activities. This includes collecting and analyzing data on contaminant levels, microbial activity, and the progress of cleanup efforts. Reporting obligations ensure transparency and accountability throughout the bioremediation process.
6. Risk Assessment: Risk assessment is an important component of regulatory frameworks for bioremediation. It involves evaluating the potential risks associated with the remediation process, considering factors such as contaminant toxicity, exposure pathways, and potential impacts on human health and the environment. Risk assessment helps decision-making and the implementation of appropriate mitigation measures.

 Compliance with relevant regulatory frameworks and guidelines is crucial for successful bioremediation projects. It is important to review key federal statutes such as toxic Substances Control Act (TSCA), the Resource Conservation and Recovery Act (RCRA), the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), and the Federal Plant Pest Act (FPPA). Working closely with regulatory agencies, following best practices, and maintaining open communication ensures compliance and safeguards the environment and human health [50, 51, 52, and 53].

1. **Conclusion:**

Bioremediation is an ecofriendly as it is known to use no toxic chemicals. It involves the usage of biological agents (Indigenous / GEM’s) to decontaminate the environment and making it a better place to live.

1. **Challenges and Future Perspectives**:

Selection of suitable microbe, type of pollutant, site conditions andfactors like excavation costs, equipment installation, are the prime challenges faced in controlling the polluted environment. The selection of the appropriate bioremediation technique is vital for successfully reducing pollutants. Bio stimulation and bio augmentation are common approaches used to enhance bioremediation, with environmental factors playing a crucial role in their effectiveness.

 By exploring the latest research and advancements in the field of Nano-remediation can contribute to the success of bioremediation. Understanding of the potential and limitations of bioremediation as a sustainable solution for mitigating the environmental impacts of these oil wastes.

 **Declaration of competing interest:**

 The authors state that they have no competing financial interests or personal relationships that could have influenced the findings reported in this paper.

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