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MICROBES AS TOOLS FOR ECO-SUSTAINABLE BIOREMEDIATION: A REVIEW

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ABSTRACT

Environmental pollution has been on the rise in the past few decades owing to increased human activities on energy reservoirs, unsafe agricultural practices and rapid industrialization. Among the pollutants that are of environmental and public health concern due to their toxicities are petroleum hydrocarbons, polycyclic aromatic hydrocarbons, heavy metals, polyfuoroalkyl substances and pesticides. Remediation of polluted sites using microorganisms has proven effective and reliable due to its eco-friendly features. We reviewed two enhanced bioremediation approach for the cleanup of polluted sites. Biostimulation is the addition of nutrients and supplements such as vegetable oil, carbon and nitrogen to support microbial metabolic activities and growth while Bioaugmentation is the addition of exogenous microbial living cells with degradative capabilities. These strategies can be influenced by biological, biotic and environmental factors such as temperature, moisture content, and availability of nutrients and supplements. This review paper focuses on these two technologies and efforts are directed towards the process of bioremediation for the decontamination of environmental pollutants.

KEYWORDS

Environmental pollutants, microorganisms, bioremediation, biostimulation, bioaugmentation, petroleum hydrocarbons

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INTRODUCTION

Petroleum hydrocarbons are a major source of energy, providing over 50% of the energy used worldwide (Barr *et al.*, 2018). It is the principal source of lubricants, solvents and a variety of large volumes of chemical feedstock for the synthesis of plastics, fibres, detergents, pharmaceuticals and cosmetics products. In 2020, it was estimated that approximately 1,128 billion barrels of crude oil were produced globally, and global production has increased by an average of 1.5% per year (Panagiotakis *et al.,* 2020). Traditionally, most of the oil extracted from the earth comes from onshore sources or shallow offshore reservoirs. Today, it is estimated that 40% of global oil extraction is conducted offshore. This makes offshore and onshore crude oil extraction and production locations more susceptible to minor and major oil spills from extraction, production, and transportation operations (Besaltatpour *et al.,* 2018).

Large amounts of petroleum hydrocarbon contaminants are released into the environment as a result of both natural and anthropogenic activities (Delille *et al.,* 2008). While releases such as industrial emissions can be easily controlled and carefully regulated, catastrophic releases such as major oil spillage from tankers, pipelines and storage tanks pose severe immediate and long-term environmental and ecological repercussions, since many petroleum hydrocarbons are toxic and considered as persistent organic pollutants (POPs) in terrestrial and aquatic environments (Adams *et al.,* 2015).

Several physical and chemical methods are available for the reclamation of polluted environmental compartments but are usually expensive, labour intensive and often risk the spread of pollution to other environment (Bouwer *et al.,* 2017). A clean and healthy environment is extremely important to protect not only ecological health but also the general public health. As human beings, we all significantly depend on clean air to breathe as well as clean water to drink and for use in agriculture and industry.

Unfortunately, during the past few decades, the magnitude of environmental pollution has been continuously rising at an alarming pace, primarily due to population explosion, rapid urbanization and industrialization, man-made chemicals are increasing by the day and many of them are recalcitrant with most being xenobiotic. As per estimates every year 10 million tons of toxic chemicals are released into the environment around the globe. Due to the addition of dangerous toxic chemicals such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polyfluoroalkyl substances (PFAs) and heavy metals in soil and water systems. These pollutants are carcinogenic and are persistent, causing great harm to ecosystems harming the health of the environment and causing damage to all life forms (Dias *et al.,* 2015).

Bioremediation is a process that uses microorganisms to degrade environmental contaminants in soil and groundwater. Bioremediation is considered an economical, sustainable, versatile, efficient, and eco-friendly way of decontaminating environmental pollutants compared to physical and chemical methods (Pandey et al., 2009). Bioremediation can also be enhanced to achieve rapid degradation rate with two best approaches such as biostimulation and bioaugementation (Obayori et al., 2017). This paper reviews these two bioremediation technologies and efforts are directed towards the decontamination and cleanup of environmental pollutants in polluted sites.

Biostimulation

Petroleum hydrocarbons in the environment can be limited by many biotic and abiotic factors including nutrients and supplements hence, biostimulation is the modification of the microcosm with nutrients and supplements in their right proportions to stimulate indigenous microorganisms with biodegradative potentials. This is mostly done by the addition of limiting nutrients such as phosphorous, nitrogen, oxygen, carbon, vegetable oil, rhamnolipids, animal dungs, sewage waste, and diary droppings (Perfumo *et al.*, 2007). The addition of nutrients, oxygen, electron donor and acceptor increases the microbial biomass for bioremediation in the polluted site.

Bioaugmentation

This is the introduction of exogenous microbes with known degradative potentials and capabilities to facilitate, support the native microbial population in the polluted environment. The rationale for this approach is that indigenous or native microorganism may not be capable of degrading the persistent organic pollutant. The exogenous microorganisms help to maintain genetic stability, viability, metabolic activity and survival in hostile environment (Iwamoto *et al.*, 2015). The exogenous microbes with degradative potentials includes *Pseudomonas* sp, *Bacillus subtilis, Rhodococcus, Alcaligens, Burkholderia xenovarans* and *Bacillus cereus.*

Advantages of Biostimulation and Bioaugmentation

i. Biostimulation enhances the metabolic activities of the indigenous microorganisms in the polluted community through nutrients amendments such as nitrogen, carbon, phosphorous, yeast extracts, meat extract, peptones, rhamnolipids, vegetable oil (John *et al.*, 2011).
ii. Biostimulation practices is tolerant to various environmental pollutants such as heavy metals, pesticides, petroleum hydrocarbons and the microorganisms can utilize it as a sole source of carbon for their growth and metabolic activity (Ali *et al.*, 2023).

iii. Biostimulation accepts a standard ratio of carbon, nitrogen, phosphorous and other supplements in quantity of 100/10/1/1 as a formular (Leys *et al.,* 2005).
iv. Bioaugmentation of exogenous microorganisms into contaminated soil with low indigenous population of petroleum hydrocarbon degraders facilitate the degradation kinetics of the pollutants (Wu *et al.,* 2012).
v. Degradation process can start immediately when specific microbial degraders are inoculated in the microcosm (Bao *et al.,* 2017).
vi. They share genetic materials such as plasmids and sessile bodies which help protect against the toxic effect of the pollutants on the native microorganisms (Liu *et al.,* 2013).

Disadvantages of Biostimulation and Bioaugmentataion

i. Biostimulation largely depends on environmental factors that controls its degradation potentiality (Sunggyu, 2018).
ii. Biostimulation is extremely site specific and requires immense scientific observation (Viesser *et al.,* 2020).
iii. Environmental pollutants engrossed to the soil particles are most times not biodegradable
iv. The success of bioaugmentation process depends mainly on the adaptation of the microbial consortia to the site that needs to be decontaminated (Godleads *et al.,* 2015).

Factors Affecting Biostimulation and Bioaugementation

Enhanced Bioremediation (biostimulation and biougementation) can be best achieved in degrading, removing, altering, immobilizing, or detoxifying various environmental pollutants by understanding the biotic and abiotic parameters needed for their metabolic activity and increase in biomass (Obiakalaije *et al.,* 2015). Microorganisms act against pollutants only when they have access to a variety of material compounds to help them generate energy and nutrients to build more cells (Adeyemo *et al.,* 2018).

Biological Factors

Biotic factors affect the degradation of environmental pollutants through competition between microorganisms for limited carbon sources, antagonistic interactions between microorganisms or the predation of microorganisms by protozoa and bacteriophages (Salam *et al.,* 2014). The rate of contaminant degradation is often dependent on the concentration of the contaminant and amount of catalyst present. In this context, the amount of "catalyst" represents the number of organisms able to metabolize the contaminant as well as the amount of enzymes produced by each cell. The expression of specific enzymes by the cells can increase or decrease the rate of contaminant degradation (Aislable *et al.,* 2016). The major biological factors include mutation, horizontal gene transfer, enzyme activity, interaction such as competition, succession, and predation (Smith *et al.,* 2018).

Environmental Factors

The metabolic characteristics of the microorganisms and physicochemical properties of the targeted environmental contaminants determine possible interactions during the process and degradation rate. (Muneer *et al.,* 2013). The actual successful interaction between the two however, depends on the environmental conditions of the site of the interaction. Biodegradation rate, and metabolic activity are affected by temperature, moisture, nutrients, supplements and exogenous microbial inoculum (Marzan *et al.,* 2017).

Availability of Nutrients and Supplements

The addition of nutrients and supplement amendments increases the metabolic activity of the native microorganisms and impacts the biodegradation rate and effectiveness (Baric *et al.,* 2014). Nutrient balancing especially the supply of essential nutrients such as nitrogen, carbon and phosphorous can improve biodegradation efficiency by optimizing the bacteria. To survive and continue their microbial activities microorganisms need a number of supplements such as yeast extract, meat extract, blood, peptone, lipids, and vegetable oil to stimulate microbial biomass and growth (Kumar *et al.*, 2016).

Temperature

Temperature is the most important factor in determining the survival of indigenous microorganisms and the composition of petroleum hydrocarbons. In cold environments such as the Arctic, petroleum hydrocarbon degradation via natural processes is very slow and puts the native microbes under more pressure to clean up the oil spilt sites (Chikere *et al.,* 2016). The subzero temperature of water in this region causes the transport channels within the microbial cells to shut down or may even freeze the entire cytoplasm of the microbial cell thus, rendering most oleophilic microbes metabolically inactive (Zavareh *et al.,* 2016).

Temperature also speeds up the biodegradation rate because it highly influences microbial physiological properties (Alam *et al.,* 2014).). The rate of microbial activities increases with temperature, and reaches its maximum level at an optimum temperature. Temperature affects the rate of degradation by stimulating evaporation and weathering of lighter components which may be toxic to exposed microbial strains (Nakamura *et al.,* 2007). Degradation rate for biostimulation and bioaugmentation is faster at the mesophilic temperature of 25^{O}C.

Moisture content

Indigenous microorganisms for enhanced bioremediation needs adequate water for metabolic activity, production of surfactants which help in the degradation of environmental pollutant (Obayori *et al.,* 2017).

Exogenous Microbial Inoculum

Inoculum with high specific degrading capabilities can be used to enhance bioremediation of environmental pollutants. Microorganisms that can be used to augment indigenous flora within the polluted sites are *Pseudomonas* sp, *Bacillus subtilis*, *Alcaligens*, *Rhodococcuss* sp (Obayori *et al.*, 2017).

Table 1: Biostimulation and bioaugmentation carried out in different countries

Country	Biological system used
United State	Pure or mixed cultures of *Bacillus* and *Pseudomonas* sp
Russia	Pure cultures of *Rhodococcus* sp of water injection and formation
China	Mixed enriched bacterial cultures
Australia	Indigenous oxidizing bacteria from water injection and formation
England	Naturally occurring anaerobic strains
Hungary	Mixed sewage sludge bacteria cultures
Saudi Arabia	Adequate bacteria inoculum from animal dungs

Source: Villaverde *et al.* (2019).

Table 2: Bioaugmentation and the environmental pollutant degraded

Microorganisms used	Environmental pollutants degraded	References
Pseudomonas putida PDH5	Petroleum hydrocarbons	Wu *et al.*,(2012)
Burkholderia xenovarans	Pesticides	Colla *et al.*, (2014)
Pseudomonas aeuroginosa	Heavy metals and engine oil	Chang (2011)
Pseudomonas fluorenscens	Polycyclic aromatic hydrocarbons	Fan *et al.*, (2013)
Bacillus subtilis	Phenanthrene	Kuran *et al.*, (2014)

Table 3: Biostimulation and the environmental pollutants degraded

Nutrients	Environmental pollutants degraded	References
Phosphorous and Nitrate	Heavy Metals	Atagana (2008)
Animal manure and sewage sludge	Poly cyclic aromatic Hydrocarbons	Abioye *et al.*, (2012)
Vegetable oil and Rhamnolipids	Pyrene and Phenanthrene	Ali *et al.*, (2023)
Dairy Manure	Petroleum Hydrocarbons	Chikere *et al.*,(2016)
Carbon and peptone	Engine oil	Orji et al., (2012)
Yeast and meat extract	Polyfluoroalkyl substances	Hamzah *et al.*, (2014)

CONCLUSION

Bioremediation is a very productive and attractive option for the detoxification and cleaning of contaminated environments through microbial activity. Enhanced Bioremediation can only be effective where environmental conditions allow microbial growth and metabolic activity of microorganisms. Biostimulation and Bioaugmentataion strategies in decontaminating polluted sites is achievable. There seem to be a general agreement that indigenous microorganisms is needed to be promoted and nourished for better biodegradation performance.

Furthermore, a successful implementation of a remediation strategy requires a consideration of the indigenous biota, nutrient availability as well as other environmental parameters necessary to achieve optimum results. Finally, a combination of technologies regulated within stringent conditions and allowed enough time will prove tremendously important in returning contaminated soils to fit-for-purpose states.

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