SRYAHWA PUBLICATIONS

Annals of Ecology and Environmental Science
ISSN: 2637-5338 | Volume 6, Issue 1, 2024
https://doi.org/10.22259/2637-5338.0601004

REVIEW ARTICLE

Effect of Biostimulation on Biodegradation Rate of Hydrocarbon-Polluted Soil: A Review

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Received: 18 March 2024 Accepted: 15 April 2024 Published: 22 April 2024

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Abstract

Hydrocarbon pollution is a global concern in view of its devastating effect on the natural ecosystem and human health. The severity of the impact has necessitated concerned efforts and plans for control or remediation. One of such strategy for remediation of polluted environment is biostimulation. Biostimulation is a cost-effective and environmentally friendly approach for bioremediation of severely contaminated environment. Hydrocarbons spill reduces soil pH and increases the total hydrocarbon (THC) content. The pH of organic biostimulants are in the alkaline range (7.00-11.2), which make them good acidic soil neutralizer. Addition of biostimulant increases pH (reduce acidity), enabling biodegradation and hydrocarbon reduction by hydrocarbonistic microorganisms and also making the hydrocarbon pollutant more bioavailable. This paper presents a review on the strategy of biostimulation and its effect on pH, biodegradation rate and percent hydrocarbon reduction in oil polluted soil. It equally highlights the advantages and pitfalls of biostimulation, and noted that it may lead to bioaccumulation in plant tissues with serious health implications. Omics technology is considered as an upscaling approach to effective biostimulation and bioremediation.

Keywords: *Biostimulation, pH, Petroleum Hydrocarbon, Biodegradation, Omics.*

1. Introduction

Petroleum hydrocarbon pollution occurs due to oil and gas activities as well as processing and utilization of petrochemicals. Majority of hydrocarbon component are stable, toxic, carcinogenic and even mutagenic (Das & Chandran, 2010).While the low molecular weight hydrocarbons are readily degraded, the higher fractions are recalcitrant (difficult to be degraded) and thus persist in the environment (Chandran et al, 2020). Several physical and chemical methods have been adopted for removing or reducing hazardous toxic pollutants from the environment. Some of the methods such as incineration and landfills are costly and also pollute the environment the more and hence, cause more damages to ecosystem and human wellbeing (Johnson & Affam, 2019; Goswami et al; 2018). In view of their deleterious effect, there is need to remove them or at least reduce their levels. Ability of organisms to metabolize contaminants is known as biodegradation, and often lead to mineralization of hydrocarbon to carbon dioxide and water (Alotiabi et al, 2018; Hamound et al, 2018; Chikere et al; 2011) or detoxification.

Petroleum hydrocarbons differ in their susceptibility to microbial attack. The most degradable being n-alkanes while the least biodegradable is polycyclic aromatic hydrocarbon (PAH). The technique of using microorganisms for degrading hydrocarbon and eventually remove particularly the non-volatile fractions from the environment is referred to as bioremediation (BR). BR is achieved naturally by microorganisms already present in the contaminated site (natural attenuation), introducing new microorganisms (bioaugmentation) or could be

Citation: Dirisu, Chimezie Gabriel. Effect of Biostimulation on Biodegradation Rate of Hydrocarbon-Polluted Soil: A Review. Annals of Ecology and Environmental Science. 2024;6(1): 32-38.

facilitated by adding essential nutritional supplements that enhance microbial growth and biodegradation of the hydrocarbon (biostimulation). This paper presents a review of biostimulants applied to enhance biodegradation of hydrocarbon and hence remediate the contaminated environment. pH, percentage hydrocarbon reduction and biostimulation efficiency (rate of biodegradation) as well as challenges and prospect of using biostimulants are presented as well as the potential of omics approach to biostimulation in bioremediation.

2. Biostimulation (BS)

Biostimulation is the addition of electron donors, oxygen or rate-limiting nutrients like nitrogen (N) and phosphorus (P) to polluted sites in order to stimulate the existing microorganisms to degrade the hazardous and toxic contaminants (Ali et al; 2020; Kapahi & Sachdeva, 2019; Varjani & Upasani, 2017). Biostimulants are of organic and inorganic origin. Inorganic biostimulants include NPK fertlizer, Urea, ammonium and sulphate fertilizers. Others include chemically synthesized surfactants such as Tween 80 or microbiologically synthesized biosurfactants. Organic biostimulants are usually derived from plant and animal waste or plant /animal processing wastes/ effluent as listed in Figure 1.

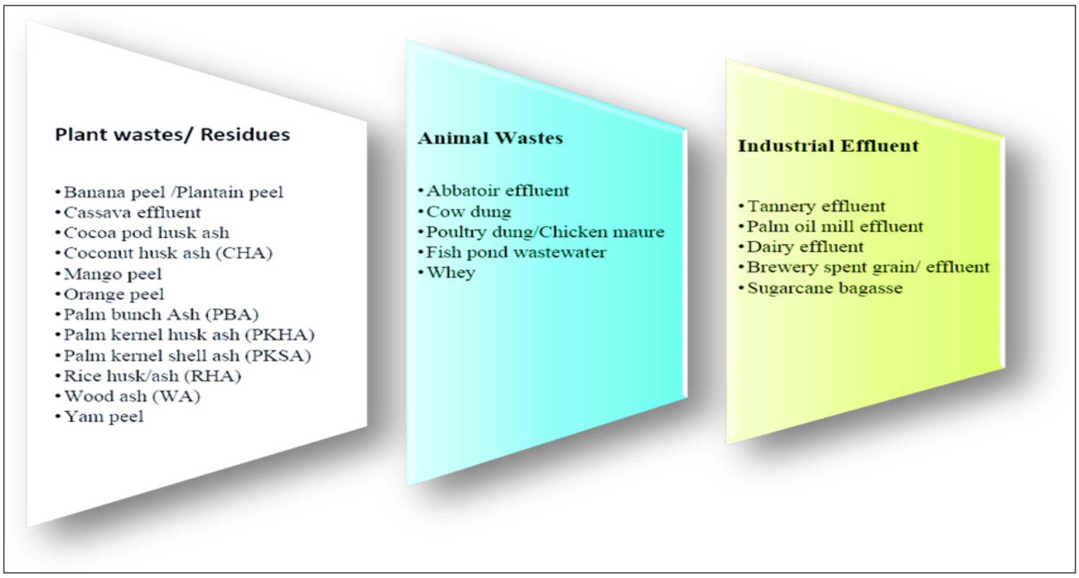

Figure 1. *Variety of biostimulants for bioremediation of hydrocarbon-contaminated environment*

The success of a bioremediation project using biostimulant can be quantified by calculating Biostimulation efficiency (B.E) and percent degradation.

B.E. represents the percentage removal of crude oil in the biostimulated soil and, is the percentage removal of crude oil in the non biostimulated soil. B.E is determined using the equation:

Percent degradation is the difference between the final and initial TPH expressed in percentage

2.1 Advantages and Challenges of Biostimulation

Biostimulation (BS) is an eco-friendly and cost -effective bioremediation strategy which can be performed anywhere (Canak et al, 2019; Goswami et al; 2018). Organic residues /effluents are regarded as wastes, which should be discarded to avoid accumulation. Using them for this purpose helps in pollution reduction. Also, BS helps in the degradation of contaminants internally, which prevents any kind of disturbances to the environment.

In spite of the advantages of BS, its adoption has some challenges:

BS is applied in certain sites and not others and requires careful observation to monitor parameters such as pH, time, concentration applied, degradation etc. Furthermore, BS can be hampered by non-availability of contaminant to the hydrocarbon degrading microorganisms, especially when contaminants are firmly adsorbed to soil particles such as clay or the contaminant is toxic or recalcitrant. Finally, environmental factors can limit the progress or efficiency of the method adopted. The factors affecting bioremediation have been discussed elsewhere and include moisture content, temperature, oxygen, pH as well as concentration of pollutant and organic matter load (Goswami et al, 2018; Yuniati, 2017).

2.2 Biostimulation Experimental Trials

The process of biostimulation involves making nutrient available to microorganisms in a contaminated environment. The biostimulants thus provided, maintain the favourable conditions for growth of soil microorganisms. The mechanisms involved in using biostimulants have been elucidated by Goswami et al (2018). This has been supported by the results of experimental trials with different biosurfactants as highlighted below:

2.2.1 Rate Limiting Nutrient Enrichment

Nutrients, especially Nitrogen (N) and phosphorus (P) is considered as the limiting factors for bioremediation of contaminated soils. Once provided by the biostimulants, they will greatly enhance the growth of hydrocarbon degrading bacteria. Several studies of biostimulants' effect on nutrient status of contaminated soil indicate that nutrients such as N, P, K, Ca, K and Mg are all influenced by their addition. Factors that affect populations and activities of microorganisms in soil may significantly affect soil characteristics and environmental quality. For example, Oludele et al (2019) reported that N increased from 0.04 to 0.17 mg/kg and P also increased from 1.72 to 6.73 mg/kg after applying biostimulant for 42 days. Haque et al (2021) supported the view that addition of organic biostimulant significantly influence soil chemical properties. They observed that addition of palm bunch ash (PBA) produced the highest available P and exchangeable K followed by rice husk bunch. Similarly, Adjei-Nsiah & Obong (2018) compared the efficacy of PBA and NPK fertilizer and noted that addition of PBA significantly increased soil pH, soil P and exchangeable cations, and hence can be used for soil liming and fertilizer supplementation. Osu et al (2021) equally applied PBA and poultry manure to crude oil contaminated environment and observed that total organic carbon increased but later reduced while total N and average P equally increased. In another instance, Gbosidom & Teme (2015) studied the effect of PBA (10g-60g/soil) on soil chemical property and reported that soil organic carbon and organic matter increased with levels of amendment material over time. Soil available phosphorus increased with amendment levels and was subsequently depleted over time. PBA had positive effect on soil total nitrogen as well as significant reduction of total hydrocarbon when 30 - 40g were applied.

2.2.2 Increasing Bioavailability of Pollutants

Biostimulants have the ability to release biosurfactants that increase the bioavailability of poorly soluble hydrocarbon (Johnson & Afam, 2019; van Hommes et al; 2003). Thus, biostimulants act by emulsifying hydrocarbon fractions and hence make them available to hydrocarbon degraders Etok et al (2015) studied the rate of crude oil remediation in soils by applying surfactants (wood ash, PBA, and tween 80) to contaminated soil. The highest reduction of 94.54% was observed in bioaugmented soil treated with oil palm fruit bunch ash (OPFBA). while the control sample with indigenous population and no surfactant treatment had 36.32% reduction. They therefore concluded that biosurfactant aided the utilization of the crude oil by hydrocarbonoclastic bacteria in the soil and enhanced bioremediation. This is particularly important for hydrocarbon that adsorb to soil particles. Kumar et al (2016) explored the use of orange peel as a source of biosurfactant to enhance degradation of crude oil in a polluted soil sample. They reported that orange peels yielded 1.796 g/L biosurfactant and emulsification activity of 75.17% against diesel.

2.2.3 Reduction of Acidity

Biostimulants are alkaline in nature (basic pH). The pH of some agriculture/agro-wastes reported is shown in table 1.

Thus compared with crude oil contaminated soil, organic waste are either near neutral or alkaline in pH. This alkalinity makes biostimulants suitable as a

Table 1. *pH of some biostimulants*

Biostimulant	pH	Reference
PKHA	6.1	Osu et al; 2021
PBA	8.98	
PBA	10.64	Ofoegbu et al, 2015
PBA	7.04	Gbosidom & Teme2015
PBA	9.60	Eremrena & Mensah, 2017
PBA	10.8	Etok et al, 2015
PBA	10.9	Udoetok, 2012
PBA	11.04	Israel & Akpan, 2016

liming agent to reduce acidic soil, which is typical of crude oil or heavy metal contaminated soil and soil impacted by acid mine drainage. pH values ranging from 7.7–7.8 is known to be optimal for hydrocarbon degradation. Supporting this, Boopathy et al (2021), Olubodun et al (2020) and Evbuomwan et al (2019) concluded from evidences in their respective studies that while crude oil introduction reduced the pH of the affected environment (making it acidic), addition of soil amendments raised the pH to neutral/alkaline for active microbial catabolic capacity. Hamoudi-Belarbi et al (2018) and van Hommes (2003) however observed that lower pH values may result in partial inhibition of degradation.

2.2.4 Biostimulants, Tph Reduction and Biodegradation Rate

Effect of remediation time on reduction of total petroleum hydrocarbon (TPH) has been elucidated and or predicted using rate kinetic models. Percent reduction or removal for various organic biostimulant have been reported: Hamoudi-Belarbi et al (2018) reported that TPH concentration in carrot peel waste amended oil polluted soil reduced from 38 - 1.4% after 35 days to 31- 0.67% after 15 days and 29.5- 0.70% at the end of day 30. In unamended (control) soil, TPH reduced gradually at the end of the 15th day (37.6- 1.20%) and the 30th day (36- 0.52%) respectively. By the end of 45 days, the final TPH % reduction were 27.0- 1.90% and 36.0- 1.27%, for polluted and control soil respectively. In a related experiment, Boopathy et al (2021) treated polluted soil with 125 g of poultry droppings and 125 g of sunflower seed husk for 20 - 60 days and observed that TPH concentration reduced drastically. In the same vein, Oludele et al (2019) reported that hydrocarbon-polluted soil amendment with poultry dropping at the end of 42 days reduced from an initial value of 4550.08 mg/kg in control soil to 3410.61, 2664.90 and 1598.95 mg/kg, respectively. Using rice husk for soil amendment, Amaechi et al (2017) observed a 97.85% biostimulation efficiency compared to the 53.15% reported for remediation by enhanced natural attenuation RENA over a two- month period. Apart for plant residue ash, plant peels have been applied with varying biostimulation efficiency. In another experiments, banana peel produced as low as 8.74%TPH reduction (Thani et al, (2017), but a much higher reduction rate ranging from 87.82- 97.45% (Aliyu et al, 2015). Osu et al (2021) evaluated the effectiveness of OPBA and Dried Poultry manure (DPM) as organic supplement. This correlated with decrease in soil TPH from 156.45 mg/kg to 146.73 mg/Kg. Thus, oil toxicity decreased and soil status improved. Albert & Anyanwu (2016) studied the synergistic effect of combined amendment of 6.7% crude oil contaminated soil with OPBA and sawdust (SD) each and in combination with no amendment as control. Results showed that TPH reduced by 65% and 52% with OPBA and SD-treated soil respectively. They however observed that combination of OPBA and SD did not synergistically reduce soil TPH content.

Similarly, Ofoegbu et al (2015) applied cow dung (CD), palm kernel husk ash (PKHA) and NPK fertilizer, individually and in 50:50 combinations (CD+NPK), (CD + PKHA) and observed that combination of CD and NPK yielded the highest percent degradation (84.62%). From the estimated B.E and biodegradation rate constant (k), the orders of remediation from the most treated are: A combination (NPK + CD)> inorganic fertilizer used singly> cow dung used singly> combination (CD+PKHA)> and finally PKHA used singly at 2, 4% crude oil contamination, and NPK preceding before a combination (NPK+CD) at 6% crude oil contamination. B.E. of various biostimulants reveals that the combination of NPK fertilizer + Cow dung (CD), NPK, CD, PKHA + CD, PKHA were able to remediate the soil by 62.1, 58.2, 51.9, 50.2 and 46.0% respectively. In summary, the degree of biodegradation decreased with an increase in levels of crude oil contamination.

From the foregoing comparative experimental trials of biostimulants applied singly or in combination, it is obvious that the results seem to follow the same trend although with slight variations. The significant effects included acidity reduction (increased pH), increase in nutrients, reduction of TPH, increase in hydrocarbon degrading bacteria and fungi as well as improvement in morphometric parameters and plant yield.

2.3 Biostimulation Outlook

Microbial succession naturally occurs in contaminated environment. The chemical compounds in organic biostimulants are initially processed by indigenous microorganisms into desirable intermediate, which in turn are used as final electron acceptors by other new groups of microorganisms. Omics technology enables biostimulation (and bioaugmentation) by characterizing hydrocarbon degrading microorganisms with novel genes coding for degradative enzymes for biodegradation of hydrocarbon as well as heavy metals and biosurfactant production. Nutrient uptake and hydrocarbon degradation following biostimulation can only be fully understood when one researchers have insight to the important microbial taxa and biodegradation pathways. To this effect, genomics may contribute to this knowledge at both levels of single species and microbial communities (Chandran et al; 2020; Abbasian et al; 2015). Since the use of native microorganisms for in situ removal of these contaminants has shown promise in several studies, investigating the microbial diversity in contaminated areas and of the effects of environmental conditions on the degradation ability of these organisms can improve bioremediation rate in these sites. The genes involved in degradation of hydrocarbons can be detected. Also, since the levels of N, P, S, and Fe of the hydrocarbon -contaminated sites are very low (Yuniati, 2017), investigations of the genes involved in the acquiring these nutrients can also be useful in developing optimized conditions that may improve biodegradation rate and efficiency. More so, the contribution of genomics may help for a thorough understanding of interactions of microbes with pollutants. Devarapalli & Kumavath (2015) adopted genomic data from 16s RNA sequencing to assess the effect of biostimulation on uranium and nitrate decontamination from ground water. They confirmed that there was distinct changes in microbial comminutes with different physiologies. The principle behind biostimulation as a strategy for bioremediation relies on establishing favorable environmental conditions through nutrients addition (Qin et al; 2013). Several authors have investigated the impacts of in situ biostimulation treatment on bacterial diversity to understand the relationships between dominance, physiology and functions of specific microbial genera with catabolic genes able to degrade contaminants of concern. Their observations therefore suggest that identifying the hydrocarbon degraders that drive community structure is important in order to understand, model, monitor and control bioremediation (Canak et al, 2019; Lee et al, 2018; Ezekonye et al; 2018). In view of the above, omics technology will be of benefit in monitoring changes in microbial diversity during hydrocarbon bioremediation.

3. Summary

Biostimulation is a strategy used for bioremediation and reduction of total petroleum hydrocarbon in polluted environment. It is compatible to the environment and uses readily available organic wastes. Biostimulation achieves its success by increasing soil pH by virtue of its alkaline nature. Control of environmental factors is critical for achieving maximum hydrocarbon reduction. Bioaccumulation of heavy metals in plant tissues with potential public health concerns and competition with other biotechnological applications are some of the limitations. Organic loading of soil can result in slow biodegradation and hence low rate of bioremediation. It is therefore important to ensure the optimal C: N ratio are attained for bioremediation projects success. The uptake of heavy metals by microbial degraders must be balanced with the desired hydrocarbon reduction, thus, challenge of heavy metal toxicity must be put into consideration. In spite of this, biostimulation is a bioremediation strategy which when properly managed will significantly enhance decontamination of a polluted environment.

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