Bioremediation of Petroleum Sludge by Chemotrophic Bacteria

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Introduction

Hydrocarbon compounds or petroleum hydrocarbon specifically are essential elements of life, which are regularly processed, stored, transported and used by a large population. But disposal of hydrocarbon lead to contamination the quality of environment globally (Das and Chandran 2011). Fuel and lubricating hydrocarbon spillage are the major hazardous to the environment till date (Srivastava et al. 2019). Several studies and researches have already given out the fate of hydrocarbons in various aspects of ecosystem. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution (Peter 2011). Taking in consideration, oil spills can affect the life of sea and seashore organisms. Such pollution problems usually create huge disturbances to both biotic and abiotic components of the ecosystems. Some components of hydrocarbon are known to belong to family of carcinogenic and neurotoxic organ pollutants. We cannot ignore such spillage and contamination occurring during transportation and processing (Saadoun 2015).

It is observed and figured out that between 1.7 and 8.8 million tons of oil are disposed in water per year, 90% of which are in direct contact of human activities and 30% enter in freshwater systems (Ferronato and Torretta 2019). In order to reduce the hydrocarbon contamination, researchers are being motivated towards many aspects of environment biotechnology for a sustainable development (Ezeonu et al. 2012). The biological treatments are more cost friendly and efficient in comparison of physical and chemical ones. Biological treatments such as bioremediation is the breakdown of hydrocarbons from highly toxic to very less toxic by biological means, by use of microorganism or a consortium of microorganisms (Singh et al. 2016, 2018). Bioremediation functions basically on biodegradation, which may refer to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds by biological agents like microorganisms (Kumar et al. 2015b, 2016). Hydrocarbon degrading bacteria are widely present in our environment distributed in marine, freshwater and soil habitat (Brooijmans et al. 2009). Bioremediation is comparatively low technology technique, cheap and provides public acceptance and can be carried out on site too. It is not so new concept instead it has been studied by many in controlled conditions (Mishra et al. 2016; Kumar et al. 2017).

The current book chapter intends to highlight the scenario about hydrocarbons, importance of microbial degradation, and significance of Bioremediation. Additionally, it will also discuss about the different strategies of bioremediation.

Brief Information about Hydrocarbons

Hydrocarbons of petroleum are continuously used as a main energy source at global level. On sea if due to any mishappening, oil spill is there then these petroleum hydrocarbons act as a hazardous pollutant (Carpenter 2019). It mainly consists of aromatic carbons which are difficult to degrade like toluene, benzene, xylene etc. As due to its spillage on the surface of sea there is complete cut off of oxygen availability to the microorganisms residing inside sea water (Nicholson and Fathepure 2004). Not only from these oil spills, had an enormous amount of petroleum sludge come from oil refineries, bottom waste of oil storage tanks and water-oil separation plants. These are expensive methods thus create a lot of problems (Johnson and Affam 2019). In spite of research for over several decades it is still challenging for us to successfully carry out bioremediation with great efficiencies. As we know that petroleum sludge is a mixture of many hydro carbonic compounds like benzene, toluene, paraffin, asphaltenes, anthracene and other alkane and aromatics so these are very difficult for micro-organisms to degrade (Kumar et al. 2013a, 2014b; Wani et al. 2017). Members of the genera like *Haloarcula*, *Marinobactor*, *Halomonas*, *Haloferax*, and *Halobacterium*, *Alcanivorax* mostly are there in published articles (Fathepure 2014).

Importance of Microbial Degradation

Here in this project mainly our target is to isolate chemotrophic bacteria and for that we mainly use Mineral Salt Media, Bushnell Haas Media and Centrimide Agar. As M.S.M media and Bushnell Haas mainly lack carbon so that bacteria could use oil as a carbon source, Centrimide Agar is used to determine whether Pseudomonas is there or not. Moreover, bacterial consortium will also be used to increase the efficiency of degrading the hydrocarbon components of sludge (Jayanthi and Hemashenpagam 2015).

Bacterial consortium can help us in different ways. We can use it as a group of micro-organisms in which bacteria degrade the sludge components with different efficiencies or the other option could be that some of bacteria actually degrade the sludge components and other just help them in degrading the bacteria by boosting up the condition for degradation (Kumar et al. 2013b, 2014a, 2015a). Like by producing various types of bio-surfactants. These are basically surfactants that help in reducing the surface tension of liquid substances or between solid and liquid substances. Bio-surfactants are the compounds having good affinity towards water molecules (Singh et al. 2017; Datta et al. 2018). These get deposited at the interface between oil and water and then increase the surface area of oil resulting in free movement of oil molecules present in that of sludge. Thus, it increases the bioavailability of sludge particles to bacteria that in turns help in biodegradation (Karlapudi et al. 2018). As these are eco-friendly, easily digestible by bacteria and are non-harmful compounds. There are many chemosynthetic bacterial species that produce biosurfactants Ex: *Bacillus megaterium*, *Klebsiella ozaenae* etc (Randhawa and Rahman 2014). So, bioremediation by using bacterial consortium is an effective and natural process for reclaiming the hydrocarbons of petroleum sludge (Kumar et al. 2018b; Kaur et al. 2018; Sidhu et al. 2019). Also, another way to enhance the biodegradation process is by providing our bacteria or bacterial consortium with enhancing nutrients such as Potassium, Nitrogen and Phosphorus (PNK) that eventually boost up the digestibility of sludge by bacteria. Many of the compounds in bilge water and petroleum sludge oil are environmentally persistent, but significant traces are mutagenic (Tahri et al. 2013). These mutagenic substances are the ones that should be destroyed. Bio-remediation is a new revolution in technology that is promising in converting the toxic and mutagenic compounds to non-hazardous products without affecting our environment furthermore (Azubuike et al. 2016).

Whole of the process regarding bioremediation has certain mechanism that is followed by bacteria. Firstly, there is the breakdown of sludge particles in the starting step then a hydroxyl group is added at the end terminal of chain of alkane and to an unsaturated ring of aromatic component of sludge that lead to the formation of alcoholic compound (Nzila et al. 2016; Sharma et al. 2018). After that there is an uninterruptable process of conversion to aldehyde by oxidation reaction that is followed by carboxylic acid compound formation. Eventually after that there is reduction of these carboxylic acid components to carbon dioxide and water. Also, certain amount of biomass is also released along with it. Similarly ring fission reaction in case of aromatic compounds takes place that lead to its conversion to carbon dioxide and water. This process is called as mineralization (Jain et al. 2011). Also, there is another reason for using bacterial consortium. As in case of degradation of partially degraded components of sludge their oxidation could be difficult at large depths so we have to take care somewhat about the mechanics of water with its thermochemical and thermodynamic properties (Dhall et al. 2012).

All the contaminates of our concern in bilge water and petroleum sludge are readily bio-degradable under certain conditions so the rate of its succession solely dependent on the skills and ability to create that type of optimum conditions in the surroundings and environment of those micro-organisms (Sharma and Singh 2015; Kumar et al. 2018a; Singh et al. 2019b). Foremost the microbe should have that metabolic activity or capability that will suffice the purpose i.e. Effective digestibility of sludge components. If we could able to develop or induce such conditions by providing sufficient amount of nutrients, their ability to grow in wider range then easily we can maximize their biodegradation power. As mentioned earlier if we will use these microbes for biodegradation on sea water then the physical and chemical properties of water will also play an important role in bio-digestibility of sludge components (Sharma and Singh 2015; Mukherjee et al. 2018; V.Kumar and S. Singh 2018; Singh et al. 2019c, d; Bhati et al. 2019). Petroleum sludge with bulky hydrocarbon components such as xylene, asphaltene etc. are comparable less vulnerable to biodegradation then that of light chain hydrocarbons such as alkanes. These easily degradable components mostly have aliphatic compounds majorly. So that is the main reason for using a bacterial consortium so that the surface area between sludge and water could be altered to enhance bio-digestibility of sludge components (Roy et al. 2018).There are some factors that are easy to alter or to manipulate than of others. Like we can't do anything regarding chemical composition of sludge components or the components of bilge water as that kind of technology is not present right now but the environmental conditions as mentioned earlier can be altered (Bhatia et al. 2014; Girdhar et al. 2014; Singh et al. 2015).

Significance of Bioremediation

Bioremediation of crude oil by these indigenous microbes is one of the primary methods by which petroleum and other waste hydrocarbon can be eliminated out from the environment. The degradation of hydrocarbons in soil is a multi-step process which comprises of many chemical reactions converting big toxic hydrocarbons to simpler and less toxic products including water and carbon dioxide, by catalytic proteins which are also consider as effective indicators for checking various impact on soil (Singh et al. 2019a, e, 2020b, a). Bioaugmentation(BA), Biostimulation (BS) and natural attenuation(NA) are the three processes which are used to degrade the hydrocarbon contaminated soil and helping local flora and fauna to protect from toxic hydrocarbons .Other group researchers figured out that no single microbe can degrade more than two classes of compounds of crude oil due to its enzymatic metabolic activities, so basically there is a need of consortium depending on their survival and efficiency, which too depends upon the environmental factors varying from region to region (Dhanjal et al. 2018; Karnwal et al. 2019; Kumar et al. 2019; Singh et al. 2020c). The victory over biodegradation of oil spillage by oil tanker Exxon Valdez of 1989 and the Gulf of Alaska, both of these successes created worldwide interest among researchers to work upon biodegradation and bioremediation (Atlas and Hazen 2011).

That type of bacterial consortium should be there that has minimum requirement of additive nutrients as otherwise it could lose its advantage of being economical. As most of the sludge degrading bacteria majorly grows by the utilization of 1 (rarely on 3-4) components and are ubiquitous to the site of sludge contamination so there is no use of using a consortium that only degrade 1-2 components only (Puyol et al. 2017). The capacity of our bacterial consortium to bear those harsh conditions regarding the wave dispersion in sea, predation by other bacteria i.e. Indigenous bacteria already present there etc. are also an important factor in the success of bacterial consortium. Here for the sludge clean-up at the shoreline could be difficult due to tidal waves by using consortium. An effective approach for biodegradation by bacterial consortium requires bacteria and nutrient to be in the contact of water or oil interfaces to have maximum growth rate of sludge degrading bacteria throughout whole biodegradation process (Saxena and Bharagava 2020).

According to National Academy Sciences report (2012) about 5.2 – 12.2 metric tons of crude oil, petroleum hydrocarbons and sludge of various hydrocarbons are released or drained out or spillage in various water bodies every year. Most of this contamination of hydrocarbons is due to various activities of humans and only a very small part is from natural means. Example of oil spillage is that of by "Exxon Valdez" in Prince Williams Sound, Alaska in 1989. This spill was estimated to release about 11 million gallon of crude oil that still is responsible for various catastrophic results even by this year of 2015. Other release of oil includes bilge water from ships and runoff of oil by urban areas mainly (Xu et al. 2018). There has been a less usage of bioremediation technique in Bilge water treatment. However only a little information regarding use of bioremediation is there in this bilge water treatment as mostly physical and chemical methods are mainly applied to treat waste water. It is estimated that about 18% oil comes through bilge water in marine environment (Kapahi and Sachdeva 2019). In current scenario the International Regulation doesn't allow ship companies to release bilge water that contains about 15mg/l of oil. For that purpose, i.e. to reduce amount of oil per litre of the bilge water ships has their own installations such as fuel separators at air vents, avoid overfilling of fuel, proper maintenance of engine and proper check of engines for fuel leaks. But bioremediation of this bilge water is the effective and efficient method for treating bilge water (Okoh 2006).

Different strategies of Bioremediation

Bio-remediation is mainly applied to treat petroleum hydrocarbon contamination in environment mainly marine environment under a wide range of various environmental factors. We can have bioremediation in in-situ environment as well as in ex-situ environment i.e. native and non-native environment. Generally, it is used as a secondary approach due to time taken by it. But still it can be used as a primary approach like in marine oil spills where it is difficult to use physical mechanical or chemical treatment exclusively (Pande et al. 2020). Moreover, Bio-augmentation also has a little effectiveness in open environment treatment according to some case studies but when it is applied along with bio-stimulation its efficiency goes on increasing rapidly in bio-degradation. Also, that it would be better if we use bio surfactants instead of chemical surfactants in bio remediation techniques as is more beneficial for us (Gkorezis et al. 2016). Types of different approaches in bio remediation are as follows:

Bio-augmentation: In which an alien species of bacteria is added with natural occurring biodegrading bacteria and that what is the main approach that will be follow. Bio augmentation may be elaborated as a technical way of improvement of the capacity of degradation of contaminated sites by introducing specific competent strains of microbes. Its efficiency is determined by various biotic and abiotic factors. Like Structure of pollutant its concentration and ease of availability of pollutants moreover various physical and chemical properties of soil. Here from the biotic factors to select particular microorganisms that can mainly degrade contaminants and also successfully compete with indigenous microbes present is more important (Nzila et al. 2016).

Bio-stimulation: In which extra amounts of nutrients are added for the enhanced growth of indigenous specie. In this approach we add some extra amount of nutrients like N, P, K, i.e. nitrogen, phosphorus and potassium for the already occurring microbial population in a contaminated site so that they could be boosted up to feed on the pollutants more effectively and efficiently. Most of these bio-simulative agents are generally used for stimulating sub surface microbes more effectively but due to impermeable restrictions of geographical locations i.e. lithology of some areas like more clay containing or fine-grained material containing soil make it a bit difficult to work effectively with this technique (Sarkar et al. 2016).

Anaerobic Degradation: In most of the bio-remediation methods enhancement of oxygen supply is there as it is assumed generally that decomposition of hydrocarbons need mostly aerobic conditions. But in some methods, we add ammonia-based fertilizers that can potentially exert an oxygen demand due to biological oxidation of ammonia. But on some sites, it is difficult to have aerobic conditions readily so in that case anaerobic degradation may have fine relevancy. Land Farming: Land farming is a technique in which we spread contaminated soil over a bed which is prepared with some fertilizer and occasionally rotated. This led to enhancement of microbial activity and stimulates oil degradation. For determining proper site location certain criteria are followed i.e. minimum distance from ground surface should be 3 feet and slope shouldn't be exceeding from 8% (Ghattas et al. 2017).

Composting: Composting is a technique of making a heap or pile of soil that is contaminated with organic contaminants or agricultural waste or household or industrial waste. Some organic nutrients are added to stimulate the development of rich microbial population and to elevate the temperature of heap or pile. This stimulation by organic nutrients results in enhanced bio-degradation in a short period of time (Kästner and Miltner 2016).

Conclusion

Wide range of hydrocarbon has contaminated the environment and became the pollutant in the environment. Although, the microbes have aided in removing the petrochemical pollutant from the environment and has gained the significant attention. This attention has prompted the exploration of biodegradation mechanism of the petrochemical degrading microbes so that they can be used to transform the pollutants to less toxic form. Even, certain efforts are being made to genetically modified the microbes to enhance their ability to remediate the petrochemicals from the contaminated site. Hence, the better understanding of the pathways, metabolism and factor affecting the growth of microbes will allow us to improve and manipulate the microbes according to our need for petro-hydrocarbon remediation.

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