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Research Paper

Bioremediation Techniques for contaminated Land: Focusing on Land Contaminated by Petroleum Hydrocarbon Pollutants

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Abstract
Oil spills on land is one of major environmental issues. The spills have been known to affect not only environment but also human health. Certain bioremediation techniques, biostimulation, bioaugmentation, and land farming, have been widely applied to solve the problem. Approaches using these methods, over the years, have given varying result and rate of success. Generally, biostimulation and bioaugmentation are preferred since they are more practical and cost effectives compared to land farming. However, geographical structure of subsurface and competition among indigenous microorganism are of major concerns when applying biostimulation. For bioaugmentation, the ability of the new engineered microorganisms to survive the new environment and to find its way to the pollutant are also big challenges. Therefore, finding the best bioremediation methods that effectively can degrade oil spill must be done carefully. In this paper, advantages, disadvantages, and limitation of the methods when applied will be discussed.

Keywords
Biostimulation, Bioaugmentation, Land Farming, Oil Spill

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1. INTRODUCTION

Land contaminated by petroleum hydrocarbon is one of environmental problems that have been long causing serious impacts to both environment and human. Numerous cases of oil spills containing petroleum hydrocarbons on land have been reported consistently from many countries in the world. Newer cases usually occur in oil producing countries, such as in Mexico where 1284 Ha of soil is contaminated [1]. In Nigeria, over 13 million tonnes of oil has been spilled in Niger Delta and contaminating food chain in the area (Sam and Zabbey, 2018). Similar cases are also reported in Libya Alzien et al. (2019), Kuwait Mostagab et al. (2018), Iraq (Arbili and Karpuzcu, 2018). Various methods, like chemical and mechanical treatment methods have been developed over the years to find the best way to deal with the problem (Okoh et al., 2019). However, the outcome of those methods applications is often environmentally unsustainable and not cost effective. As a result, alternative methods which are more economically and environmentally sustainable are preferred. Bioremediation is one of them (Okoh et al., 2019). This method utilizes microbes, plants, and earthworms ability to degrade various types of contaminants Sinha et al. (2009). The method is economically profitable since the treatment cost is generally lower compared to other treatment methods Megharaj et al. (2011). Another advantage of the method is it does solve the contamination problem permanently, unlike the conventional method which usually moves the problem to other places Gordon et al. (2018). This method also does not harm environment for there is no involvement of hazardous chemical (Perelo, 2010a). Simple maintenance and "applicable over large areas" are other factors that make bioremediation more attractive to be implemented (Perelo, 2010b). In this review, focus of the discussion will be on bioremediation techniques that utilize microorganisms that already live in the soil. The techniques are biostimulation, bioaugmentation, and land farming. The techniques will be discussed using land contaminated by petroleum hydrocarbon pollutant cases. This review will be divided into three parts of discussion. The first part will discuss both environmental and health impacts of petroleum pollutants on soil. It will be followed by discussion of bioremediation methods that could work to resolve the contamination of petroleum hydrocarbon on land. Advantages and disadvantages of each proposed treatment will be discussed in this part. Lastly, a conclusion of this review is presented.

2. RESULTS AND DISCUSSION

2.1 Environmental and health effects of petroleum hydrocarbon pollutants

2.1.1 Environmental effects

Impacts that petroleum hydrocarbon spills caused to the environment is profound. The impacts may vary depending on the amount and types of petroleum hydrocarbon contaminating the land (Baker, 1970). External factors, such as time of spills as well as weathering degree, also determine level of damage petroleum hydrocarbon left on soil (Baker, 1970). Once petroleum hydrocarbon contaminates soil, it can damage soil properties, chemically and physically (Wang et al., 2013). According to Sutton et al. (2012), the presence of petroleum hydrocarbon stimulates formation of anaerobic environment in soil (Sutton et al., 2012). This can happen because petroleum hydrocarbons are able to smother soil particles by preventing soil pores from getting air diffusion (Sutton et al., 2012). Furthermore, the hydrocarbons film can also be formed when oil is spilled on the soil (Gordon et al., 2018). In addition, the presence of petroleum hydrocarbon also increases oxygen demand needed by microorganisms to decompose the oil (de Jong, 1980). This could lead to the decrease number of microbial community that presents in the soil (Sutton et al., 2012). Petroleum hydrocarbon spills also causes soil pH rise from acidic to alkaline (Yu et al., 2020). All of these factors result in the decrease of concentration level of important nutrients for plants, such as phosphorus and nitrogen, while there is possibility of concentration level of toxic compound such as Mn increases de Jong (1980). Eventually, this could deteriorate soil physical properties and fertility, adversely harming plants living on the soil. Real example of this case is when crude oil spilled in Arctic region (Raisbeck and Mohtadi, 1974). Growing tissue in this area was entirely dead and various plants and trees, like sedge and mosses, could not recover as well (Raisbeck and Mohtadi, 1974). De Jong also finds that cereal yield decreases significantly when oil contaminated wheat field in Moose Jaw, Saskatchewan, Canada (de Jong, 1980). For the cereal case, the decrease of yield specifically happened because other than factors mentioned previously; oil also prevents water from being taken up by wheat (de Jong, 1980).

2.1.2 Health effects

Studies over the years have found that petroleum hydrocarbon spills indeed cause negative impacts to human health. For example, spilled petroleum hydrocarbon on land, after contaminating top soil (Gay et al., 2010), is highly possible to find its way to enter groundwater which often is source of drinking water (Duffy et al., 1980). On top of that, the spills could contaminate air and live stock as well (Gay et al., 2010). All these possibilities lead to the greater chance for human's health to be affected. A number of serious illnesses have been recorded in some areas which are exposed to spilled petroleum hydrocarbon. Palinkas finds that the impacts of the spills on the psychosocial environment are just as bad as the impacts on the environment (Palinkas et al., 1993). People, who were exposed to the spills, were found to have higher possibility, up to 3.6 and 2.9 times to have generalized anxiety disorder and post-traumatic stress disorder (PTSD) respectively (Palinkas et al., 1993). Studies by Lyons et al. reports that people live in the area contaminated by crude oil spills are associated with "higher depression and anxiety, self reported headache, and worse mental health" (Lyons et al., 1999). Other studies by Campbell et al., Morita et al., and Janjua et al. also presents similar findings in which oil spills do worsens psychological condition of people who are exposed to the spills [Campbell et al. (1994), Morita et al. (1999), Janjua et al. (2006)].

In addition to psychological condition, physical illnesses also are a threat in the oil contaminated places. Studies done by Zock et al. (2007). and Rodriguez-Trigo et al. show that people exposed to petroleum hydrocarbon may have higher chance to experience lower respiratory tract symptoms [Zock et al. (2007), Rodríguez-Trigo et al. (2010)]. People aged between 40 and 60 have higher chance to suffer from stomach and skin cancer (Gay et al., 2010). Children under 10 years old are found to have higher chance to suffer hematopoietic cancers and leukemia as well (Gay et al., 2010). This could be because petroleum hydrocarbon exposure to human body could induce the increase of genotoxic effects, a damaging effect on DNA/RNA (Pérez-Cadahía et al., 2008). Miscarriage rates are also found more significant in the area that is close to the contamination area Gay et al. (2010). Sam and Zabbey (2018) also notes other numerous health illnesses human could suffer from oil contamination, such as genetic mutation and reproductive defects, birth defects, and many other diseases (Sam and Zabbey, 2018).

2.2 Bioremediation technique

Basically bioremediation is an effort to enhance hydrocarbon-degrading microorganisms' activities in soil so the rates of natural degradation could increase to significant higher rates (Atlas and Cerniglia, 1995). The method is believed to be environmentally friendly, for in the right circumtances, eventually, majority petroleum hydrocarbon spilled on the soil will be degraded by the microorganism. The degradation process provides energy they need to grow and to reproduce (Xu et al., 2018). Some examples of native microorganism that have been identified in oil contaminated soil and are potential to be used for degradation process are Acetobacteria, Plantomycetes, Bacteriodetes, Actinobacteria, Chloroflexi, Pseudomonas, *Collimonas sp*, *Rhodococcus coprophilus*, *Nocardioides albus*, and *Rhodococcus erythropolis* [Peng et al. (2015), Saadoun et al. (2008), Hamamura et al. (2006)].

However, generally, the number of these indigenous microorganisms that naturally exist in soil is less than 1% of total microbial communities (Atlas and Cerniglia, 1995). As a result the rate of natural degradation process by these microorganisms is very low, especially when the amount of petroleum that needs to be degraded is high. There is possibility that the number of the indigenous microorganisms to increase, up to 10%, with the presence of bigger oil amount (Atlas and Cerniglia, 1995). Nonetheless, the percentage increase does not always directly translate into higher degradation rate by the microorganism for the ratio of the oil and the microorganism will be still too low. In addition, bioremediation process always depends on various factors supporting microorganism functions, including site characteristics, moisture content, temperature, nutrients, redox potential, oxygen content, contaminant bioavailability and contaminant concentrations (Adams et al., 2015). Therefore, various techniques have been developed to increase the natural degradation rates of the hydrocarbon-degrading microorganisms. Below, a more detailed discussion of three bioremediation methodologies, biostimulation, bioaugmentation, and land farming are provided.

2.2.1 Biostimulation

Biostimulation is one of *in situ* bioremediation processes. This method tries to increase the indigenous microorganism activities by adding nutrient Benyahia and Embaby (2016), water, acceptors or donors of electron (Megharaj et al., 2011), and oxygen (Malina and Zawierucha, 2007). Generally, nutrient is the one that most often added. The addition of nutrient is important, especially N since it works as limiting nutrient (Agamuthu et al., 2013) during bioremediation. Nutrition addition is also needed since with the increase number of carbon from petroleum contaminant, nutrient concentration in the soil will also deplete faster (Margesin and Schinner, 2001). This means less source of food for the microorganism. Less source of food means less enzyme production that is needed to degrade the contaminants (Vidali, 2001). According to Atlas, this method successfully increases rate of biodegradation up to three to five times compared to without the addition of nutrients (Atlas and Cerniglia, 1995). In addition, Tyagi et al. (2010). report a better result in which degradation rate increases up to 96% when nutrient, biosolid and commercial fertilizer, are added Tyagi et al. (2010). Tangahu et al. also report that the use of another commercial fertilizer, NPK, manged to reduce total petroleum hydrocarbon value from 8,37% TPH to 4.23% (Tangahu et al., 2017). However, unwanted side effects, such as euthropication, could occur. Nonetheless, this method has been proven successful to enhance microorganism activities to break down hydrocarbon pollutants faster (Megharaj et al., 2011), such as for Alaskan Oil Spill in Alaska in 1989 (Atlas and Cerniglia, 1995).

The main advantage of having this method implemented is because bioremediation process utilizes the indigenous microorganisms that are already compatible with their environment and evenly distributed “within the subsurface” (Adams et al., 2015). Moreover, nutrients added in this method could be from both organic and inorganic substances (Tyagi et al., 2010), meaning more options available to produce the nutrients. On the other hand, the main challenge when implementing this method is it relies heavily on geological structure of subsurface where the indigenous microorganisms live (Jayaprakash et al., 2019). This factor should not be overlooked as the geology structure will affect whether the additives could reach the microorganisms or not. For example, impermeable and tight subsurface, like clays, will be a constraint to distribute the additives evenly in the contaminated area (Adams et al., 2015). Another challenge is the additives might not only promote indigenous microorganisms’ growth that can degrade petroleum hydrocarbon pollutants, but also other microorganisms which have nothing to do with the biodegradation process (Adams et al., 2014). As a consequence, a competition between these two types of microorganisms often could not be avoided.

2.2.2 Bioaugmentation

This is a bioremediation process that utilizes non-indigenous microorganisms that is known to have the ability to break down the contaminants (Megharaj et al., 2011). The microorganisms come from either genetically modified microorganisms or isolated microorganisms from the contaminated areas (Tyagi et al., 2010). The addition of these microorganisms also aims to increase contaminant biodegradation rate (Abdulsalam and Omale, 2009). The reason is because there is possibility that the indigenous microorganism species that are available in the contaminated area cannot degrade the complex mixtures of petroleum hydrocarbon pollutant since metabolic capacities of the microorganisms is different one from another (Tao et al., 2017). Another reason is because the indigenous species might not work optimally due to stress triggered by the petroleum hydrocarbon contaminants (Adams et al., 2015). The low number of the indigenous microorganisms could be the factor as well (Adams et al., 2015). Therefore, the introductions of the non-indigenous species could be a support to the indigenous species which could lead to the increase of biodegradation rate (Perelo, 2010a). In order for this method to be successful, the non-indigenous species must able to degrade the petroleum hydrocarbon contaminant and make this process faster than natural decontamination rate (Perelo, 2010b). The non-indigenous species should also able to survive the new harsh environment and compete with the indigenous species, as well as able to find its way to the contaminants (Adams et al., 2015).

This method works well to degrade petroleum contaminant having complex mixtures. With the addition of more microorganisms groups or consortia, the wider the range of hydrocarbon substrates that could be degraded. Usually one group of microorganism can only degrade limited type of hydrocarbon substrates (Adams et al., 2015). For instance, *Alcanivorax degrade* linear alkanes Harayama et al. (2004) and *Mycobacterium* degrade alfalfa (Shi et al., 2020). In addition, it is possible to choose exactly the right microorganisms that can work optimally to break down appropriate petroleum pollutants since nowadays the microorganisms are studied well first before they are applied (Shi et al., 2020). Tao et al. (2017). report that a consortium consisting mainly "Burkholderials order (98.1%) for degrading crude oil" [2017]. Another study cited in Tyagi et al. (2010) give similar result in which the use of a microbial consortium degrades diesel oil completely. However, this method also has limitations. Vidali notes that usually non-indigenous microorganism fails to compete with the indigenous microorganism, leading to the failure of developing sufficient populations that can degrade the contaminant (Vidali, 2001). Vidali also highlights the fact that soil that has been long exposed to biodegradable organic waste, including petroleum, usually has indigenous microorganisms that can effectively degrade the waste (Vidali, 2001). This means, for such a case, the addition of non-indigenous microorganisms will be futile. Another challenge of using non-indigenous and engineered microorganism is there are many aspects that have yet to be fully understood when the microorganisms are released to the environment (Benjamin et al., 2019).

2.2.3 Land farming

This method is a simple bioremediation technique which requires the excavation of contaminated soil (Vidali, 2001). After the contaminated soil is excavated the soil is spread on a thin surface (Dzionek et al., 2016). This soil then tilled or plowed time to time to prompt the biodegradation process aerobically (Pavel and Gavrilescu, 2008). By doing this, it is expected that indigenous microorganism that already exist in the contaminated soil could work on degrading the contaminants (Vidali, 2001). To stimulate activities of the indigenous microorganism, nutrients and minerals are added as well (Wang et al., 2016). The addition of additives in this method making this method is not that different from biostimulation method, except this method is conducted not in the original contaminated sites.

In practice, this method usually is only to treat superficial soil (Dzionek et al., 2016). Soil must be graded and cleared first before treatment using this method is run, according to The US Environmental Protection Agency (EPA). This is done by building treatment system, placing leachate collection and installing vapor treatment facilities (Pavel and Gavrilescu, 2008). Land farming could be an effective solution for contaminated land located in the remote areas which are far away from residential areas, for soil having "low concentration of volatile compound", and for area that can manage all potential emission to water, air, and land (EPA, 2014). To implement this method properly there are a lot of factors that must be considered, including concentration, volume and characteristic of the contaminants, area and duration for treatment, and criteria of remediation to be achieved (EPA, 2014). As a result, this method would be more costly when it is done properly relative to other in situ bioremediation techniques (Vidali, 2001). However, nowadays, this method is quite popular as it works well for contaminants with low concentration (Dzionek et al., 2016) and cost for maintenance, monitoring and clean-up liabilities is not as expensive as it was in the past (Vidali, 2001).

2.3 Overall evaluation

Generally, *in situ* bioremediation process is more preferred than *ex situ* process. The reason is usually because cost needed for in situ treatment is lower than that of *ex situ* (Vidali, 2001). There will be no excavation fee for *in situ* methodologies as the treatment is conducted on site. Moreover, on site treatment could prevent the spread of pollutants to other areas because there is no excavation as well as transportation process. Considering these factors, among the three methods discussed, biostimulation and bioaugmentation are the more desirable option compared to land farming. Biostimulation is also well preferred since US EPA has recognised it as an effective method to be implemented in the field (Malina and Zawierucha, 2007). *Ex situ* method such as land farming is difficult to apply when the area contaminated by petroleum is wide and deep and located in non-remote area. Thus, it can be said that *in situ* treatments are more beneficial in term of practicality and cost effectiveness.

For biostimulation and bioaugmentation cases, both methods are effective in their own way. There are many factors that can affect the final result of these methods. Numerous studies have been done over the years using these methods which give various results from time to time. For example, a study carried out by Bento et al. which aims to compare bioremediation techniques, biostimulation, bioaugmentation, and natural attenuation, to remediate soil contaminated by diesel oil, shows that the three treatments give different result after 12 weeks of incubation (Perelo, 2010b). Soils used in this study come from California and Hong Kong. Bioaugmentation works the best for soil from Long Beach, California, with the degradation rate is up to 72.7% in the light (C_{12}–C_{23}). Biostimulation, however, does not give better result or even an equivalent result like bioaugmentation according to (Perelo, 2010b). Other studies using bioaugmentation to degrade petroleum contaminant gives varying result of total petroleum hydrocarbon (TPH) removal from 32% to 83% (Adams et al., 2015). This indicates that other factors play a significant role in determining the final result of TPH removal.

On the other hand, a study by Xu and Lu indicates that biostimulation is a better choice for bioremediation of soil contaminated by crude oil (Xu and Lu, 2010). They find that biostimulation treatment gives up to 61% of TPH removal. Wu et al. also reports that biostimulation promotes 60% TPH removal compared to 34% of bioaugmentation (Wu et al., 2016). Similar findings is also revealed by Haleyur et al., Wu et al., and Ortega et al. with removal efficiency of 99%, 28%, and 49-62% respectively using biostimulation technique [Haleyur et al. (2019), Wu et al. (2019), Ortega et al. (2018)]. According to Adams et al., other biostimulation studies that use TPH removal rate to measure the success of bioremediation process also show a promising removal rate with TPH removal from 25% up to 100% (Adams et al., 2015).

Because biostimulation and bioaugmentation techniques give varying result, an approach to combine biostimulation and bioaugmentation to achieve better result is proposed by (Sun et al., 2012). Sun et al. (2012). finds that dry soil containing 375 mg of total PAH in each kg could be cleaned more effectively using combination of these two techniques (Sun et al., 2012). It was found that 43.9% of total PAH and 55% of 4-6 ring-PAH can be degraded using the combination techniques. This is far higher than degradation rate when using biostimulation and bioaugmentation separately (Sun et al., 2012). Safdari et al. (2018). also report combination of biostimulation and bioaugmentation gives highest TPH degradation rate (89,7±0.3%) compared to individual treatment (Safdari et al., 2018). However, other study carried out by Abdulsalam and Omale give opposite result. In their study, they measure TPH removal in soil which is contaminated by motor oil (Abdulsalam and Omale, 2009). Concentration of the oil is 40.000 ppm. They found that combination of biostimulation and bioaugmentation gives a little bit lower TPH removal rate (65.2±0.25%) compared with biostimulation TPH removal rate (69.2±0.05%).

Based on the various result given by bioaugmentation and biostimulation above, it is clear that there are various factors that affect the final result of these two techniques. Concentration of contaminants is one of them. Contaminants concentration in each study is different. Adams et al. (2015). points out that when contaminant concentration still can be tolerated by microorganisms, biodegradation process will be more optimum and faster (Adams et al., 2015). But when the concentration of contaminants is beyond "microbial tolerable limit" the rates of biodegradation will be slower and will take longer times to finish (Adams et al., 2015). Other factors, such as soil characteristic, scale of studies, length of studies as well as various different controls applied during studies will definitely affect how well biodegradation process proceed (Perelo, 2010b). Thus, comparing result of bioaugmentation and biostimulation directly cannot be done without the same condition of study. Comparing one study result with others also should be done carefully by observing factors and parameters affecting the studies.

2.4 Limitations

Due to many benefits bioremediation offers, this methodology seems like a promising way to resolve land contamination because of petroleum hydrocarbon. However, this method still has its own limitations. One of them is there is no clear bench-marking value that can be used as a standard to determine whether a bioremediation process is a success or not (Megharaj et al., 2011). Different studies gives different TPH removal rate. Some gives impressive number, up to 90% but there are other studies that give less than 50% of TPH removal rate. Furthermore, every study uses different contaminant concentration and length of study. Therefore, it is hard to compare the success of one study to others. If there was a definite number to measure the efficacy of bioremediation technique, for example a success is when TPH removal rate is X% for every Y ppm of contaminants in Z days, it would be easier to determine goals and process that need to be done to achieve such results. Another limitation is lack of understanding regarding contaminants bioavailability (Megharaj et al., 2011). This is an important factor that still needs better understanding. Once pollutants enter soil, there will be sorption process, precipitation and complexation processes that determine how the pollutants will interact with soil. Sorption process by soil will determine pollutants's "susceptibility to microbial degradation", therefore affecting how effective the process of bioremediation will run (Megharaj et al., 2011). The next limitation is lack of knowledge of whether there will be impacts in the future from the addition of nutrients and genetically-engineered microorganisms to the soil (Benjamin et al., 2019). The knowledge of how the addition of nutrients and genetically-engineered microorganisms will affect ecology in the future is yet fully understood. Having this kind of knowledge fulfilled will be a great advantage in improving bioremediation techniques in the future.

3. CONCLUSIONS

In conclusion, bioremediation technique is one of promising the solutions that can be applied to remedy land contaminated by petroleum hydrocarbon. There are two types of bioremediation, *in situ* and *ex situ*. Both of these methods have been proven to be more environmentally friendly and more cost effective to solve the problem compared to technical and chemical methods. However, when *in situ* and *ex situ* methods are compared, currently *in situ* methods are thought as the better methodology. That is why methodology such as biostimulation and bioaugmentation are widely used in the studies that aim to degrade petroleum hydrocarbon pollutants. Using these two methodologies will save more money for operational cost as well as will prevent possibilities of pollutants to spread. However, there are many factors that need to be considered in order to achieve best result of TPH removal when applying biostimulation and bioaugmentation techniques. These factors include contaminant concentration and characteristics, length of study, soil condition, and many other factors. These factors also make contribution to the fact that result achieved using biostimulation should not be compared directly to result achieved using bioaugmentation. In addition taking into account these factors, to get the best rates of TPH removal, limitations of bioremediation methodologies, such as the absence of bench-mark value to measure the success of TPH removal, the lack of understanding of contaminant bioavailability and fate of additives and genetically-engineered microorganisms added to the soil should be recognized as well.

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