Review on the Efficiency of Microorganism in Degrading HydrocarbonsPollutants throughBioremediation process

Mohamad Ridhuan Mohd Akil, Joy Ann M. De Guzman, Geasean J. Johnson, KaterynaSukhodolska and Siu Yeon Tan

UNEP-Tongji Institute of Environment for Sustainable Development, College of Environmental Science and Engineering,Tongji University, Shanghai, China, 200092.

Abstract — The environmental degradation that currently happening is caused by increasing number of anthropogenic activities such as the production of fossil fuels, accidental releases of petroleum products, ore mining, smelting, illegal chemicals dumping, agriculture runoffs, industrial and municipal waste disposal. According to numerous studies that been done on environmental degradation issues, oil contamination is recorded to have the biggest impacts on the environment. Oil contamination can affect the environment, human health and economy of the country. Bioremediation is a technique or process of using microorganism to degrade or transform the contaminants from hazardous substances to less hazardous or non-hazardous substances. Numerous studies have shown that multiple groups of microorganisms were used as agents in degrading pollutants starting from bacteria, algae, fungi and even plants which the process also known as phytoremediation. Bioremediation is a slow process which mainly depending on the microorganisms to digest the pollutants and convert them into non-harmful substances. Some studies regards bioremediation as a process of using microorganisms in immobilizing and destroying waste and harmful substances. This review will focus in detail on the bioremediation process and how this process can be improved for better and efficient pollutant degradation in our environment.

***Keywords**: Athropogenic activities, Principle of Bioremediation, Biostimulation, Bioaugmentation, Microbial activity, Environmental Degradation Issues, Oil Contamination.*

1.0 Introduction

In this modern era, environmental degradation has been one of the major concern experienced throughout the world. The environmental degradation that currently happening is caused by increasing number of anthropogenic activities such as the production of fossil fuels, accidental releases of petroleum products, ore mining, smelting, illegal chemicals dumping, agriculture runoffs, industrial and municipal waste disposal [14], [2].Globally, human activities such as oil exploration and processes have been seen as threats to environmental health as well as to the human health. This is because such activities increase the tendency of oil spill incidents to happen which will release petroleum hydrocarbon into the environment. Historically, recorded oil spills incidents were due to numerous causes such as incidental or voluntary releases of crude oil from tankers, spills from offshore platforms, drilling rigs and wells. Besides that, the presence of hydrocarbon in the environment also come from oil spills of refined petroleum products especially gasoline and diesel as well as their by-products [28].

- *Mohamad Ridhuan Mohd Akil is currently pursuing master's degree program in Environmental Management and Sustainable Development inTongji University, Shanghai, China, E-mail: ridhuanakil@hotmail.com*

The most well-known case of oil spill accidents was Exxon Valdez accident in the year 1989. It is ranked on the 35th world oil spill accidents where 40 000 tons of crude oil contaminated the Alaska waters which give a severe impact to the marine environment and the marine animals. Other major oil spills incidents that have been recorded since 1960s includes the Torrey Canyon oil spill (25-36 million gallons) in 1967, the Sea Star oil spill (35.3 million gallons) in 1972, Odyssey oil spill (40.7 million gallons) in 1988, M/T Haven Tanker oil spill (45 million gallons) in 1991, Amoco Cadiz oil spill (69 million gallons) in 1978, Castillo de Bellver oil spill (79 million gallons) in 1983, Nowruz oil field spill (80 million gallons), Kolva River oil spill (84 million gallons) in 1983, Atlantic Empress oil spill (90 million gallons) in 1979, Ixtoc 1 oil spill (140 million gallons) in 1979,Arabian Gulf/Kuwait (380-520 million gallons in 1991 and the latest was Gulf oil spill (206 million gallons) in 2010 [39]. Besides the major events, there was a small spill occurred in the Niger Delta region in Nigeria but because of the area is secluded and far from civilization has affected the emergency responses by the authorities. Thus, proven that even small spill incident will give adverse impacts to the ecosystems.

According to numerous studies that been done on environmental degradation issues, oil contamination is recorded to have the biggest impacts on the environment. For example, the oil can penetrate into the fur of mammals and also into the structure of the plumage of birds which eventually causing reduction in their insulating ability, making them more threaten to temperature changes as well as their ability to control their buoyancy in the water. Besides that, according to Hogan [31], the strong scent emitted from oil will affect the animals that rely on scent to find their babies. The strong scent will cause the mothers to get confused and at the end abandoning and rejecting their babies which causing the babies to starve and at the end die. Other bad effects of oil to bird's population are, oil may affect the bird's ability to fly which will prevent them to go find food or to escape from predators, as this ability is disrupted, it will cause them to starve to death and also make them to be easily captured by the predators. Moreover, the oil may enter the bird's digestive system as they trying to clean their feathers using their beak, this may cause irritation to the digestive tract, altering liver function and can also cause kidney damage. In other cases, some of the birds that exposed to oil contaminant did experiencing changes in their hormonal balances as well as changes in their luteinizing protein [28]. Most of the birds that affected by oil contaminants will definitely die without any human intervention. The statement was supported by Dunnet[22], saying that most studies found out that less than one percent of oil-soaked birds survive.

As for human population, oil spill can cause major fire hazard. For example, the Kuwaiti oil spill has caused hazardous fires that produced air pollution that cause respiratory problems to the community near the area. Besides causing air pollution, other factors such as oil rig's explosion may also causing threats to human populations. The Deepwater Horizon explosion was one of the event that cause causality to its eleven workers and another 47 personnel were killed in fire caused by Lac- Megantic derailment's explosion. Oil spill also may contaminate the clean drinking water supplies in the impacted area which will limit the water resource availability for the living organisms that live in that area. Such event has happened in Miri, Malaysia in the year 2013 where the oil spills has contaminated the water supplies in the area which been publicly used by more than 300 000 people. Another incident reported in the same year where this time the oil spills has contaminated a water supplies that been supplied to an area with 80 000 people known as Coca in Ecuador.

Besides that, oil spill contamination might also have impact towards the tourism activities and to the marine resource extraction industries which will cause an economic stress to both of the sectors. According to Yang [57], the explosion of Deepwater Horizon rig has caused contamination along the beach which impacted the tourism and fishing activities along the gulf. The economic stress were added to the loss when the responsible parties were required to pay to compensate the victims. As for the terrestrial area, oil contamination has been proven to decrease the ability of soil to support the plants growing process, the oil also seeps deep into the ground and contaminated the groundwater as well as increasing the concentration of heavy metals in the soil which will cause bioaccumulation and biomagnification that can give major impacts towards the human health [13].As mentioned earlier, toxic heavy metals contamination is another major type of pollution that threatening our environment and health, this is because the pollution that caused by industrialization are continuously to increase throughout the year. Generally, heavy metal is a term use to a certain category of metals and metalloids which having atomic density more than 4000 kgm^{-3} or when compared to water to have 5 times of their density [36].Currently, in Western Europe alone, there are more than 300 000 sites that been known to be contaminated by heavy metals [42]. The heavy metals poisoning has been found to occur from drinking a contaminated water as well as by food intake and by inhaling in the heavy metal concentrated surrounding especially near the emission sources [38].

In recent years, more detailed studies has been done to focus on the diffusion process of the heavy metals through the soil layers and also the movement of the heavy metals in the ground water [35]. As the level of contaminant in the soil is increasing, it will disturb the productivity rate of the plants and also decrease the soil microbial processes that occur in the soil.This is because as the heavy metals mixed with the ground water, it will changed the soil pH value as well as created multiple reactions in the soil environment itself such as acid base reaction, oxidation, reduction, sorption, dissolution and precipitation which can affect the properties of the contaminants. The most common and dangerous types of heavy metals that can be found in the contaminated area are mercury, cadmium, lead and chromium [21]. Numerous techniques has been introduced to treat or remove the pollutants from the environment and although these techniques were proven effective but at the same time it gave more bad impacts to the environment itself. All these conventional techniques are complex, getting no consent from the public and above all it is uneconomical. So over the years, more focus and efforts been given towards improving the modern-day bioremediation technique as a replacement to the conventional methods.

2.0 Principle of bioremediation

Bioremediation is a technique or process of using microorganism to degrade or transform the contaminants from hazardous substances to less hazardous or non-hazardous substances. Numerous studies has shown that multiple groups of microorganisms were used as agents in degrading pollutants starting from bacteria, algae, fungi and even plants which the process also known as phytoremediation [43],[44]. Besides that, bioremediation is a slow process which mainly depending on the microorganisms to digest the pollutants and convert them to non-harmful substances. Some studies regards bioremediation as a process of using microorganisms in immobilizing and destroying waste and harmful substances [53]. Bioremediation also can only be considered as effective depending on their suitability to the environment as the process rely on the productivity and the growth of the microorganisms. So in order to achieve efficiency status in removing pollutants in a contaminated area, most studies found that it is important to manipulate or alter the environmental parameters in the area such as their nutrient level, temperature, pH and others.

The bioremediation process also involves in creation of energy from the redox reaction occur in the microbial cells. For example, reactions such as respiration and several other biological functions that are needed for cell reproduction and maintenance. Besides that, other factors that are compulsory to be taken into consideration is the different types of the microbial electron accepter classes such as oxygen-reducing, manganese-reducing, nitrate-reducing, iron (III)-reducing, carbon dioxide-reducing, sulfate-reducing and also each of the accepter's redox potentials. This is essential as for oil pollutants only certain microorganisms that have a proper metabolic capabilities can be considered [54].In order to find the suitable microorganisms, usually such microorganism will be collected from the impacted area itself. This is because the microorganisms populations that been exposed to the pollutants such as hydrocarbon can adapted, undergo genetic changes and also showing selective enrichment in their functions[40]. As a results, the microorganisms can act fast when exposed to hydrocarbon as well as managed to degrade it efficiently compared to microorganisms that has never been exposed to hydrocarbon contamination. Some studies also found out that since pollutants such as crude oil is composed on several mixture of compounds, it has been considered as one of the limiting factor for a single type of microorganism to degrade all of the mixtures and in which several other different group of microorganisms were needed to degrade more pollutants [12],[15].

As mentioned earlier, there are few factors that might affecting the efficiency of microorganisms in degrading pollutants. One of the factor is the contaminant concentration itself. Even though the microorganisms are able to degrade pollutants but as the concentration of the contaminants are too high, it will have a toxic effects which will affect the microbial activity of the microorganisms itself. But if the concentration of the contaminant is too low, it will inhibit the growth or multiplication of the microorganism's degradation enzymes [28]. Besides, microorganisms need enough supply of carbon sources for them to be able to generate degradation enzymes that attacking the pollutants in the molecules level. So lack of this degradation enzymes will eventually limit the ability of the microorganism's populations in completely degrading the contaminants [16].

Contaminant bioavailability also one of the factor that will affect the efficiency of bioremediation process. This is because it depends on the level to which the contaminants are absorbs into the soils or any other media. The rate of absorption of the contaminants into soil or sediment as well as in the depth of water column will make it difficult for the microorganisms to access to it let alone to be able to degrade the pollutants [33].

Parameters	**Condition required for microbial activity**	**Optimum value for pollutants degradation**
Soil moisture	25-28% of water holding capacity	30-90%
Soil pH	5.5-8.8	6.5-8.0
Oxygen content	Aerobic, minimum air filled pore space of 10%	10-40%
Nutrient content	N and P for microbial growth	C:N:P=100:10:1
Temperature (°C)	15-45	20-30
Contaminants	Not too toxic	Hydrocarbons 5-10% of dry weight of soil
Heavy metals	Total content 2000 ppm	700 ppm
Type of soil	Low clay or silt content	

Table 1: The environmental characteristics that affecting the bioremediation process [43].

Another factor is the site characteristics, it can give significant impact to the bioremediation processes. As can be seen in the Table 1, environmental characteristics that mainly affecting the pollutants degradation especially for hydrocarbon mixtures are the soil moisture, pH, oxygen content, nutrient content, temperature, contaminants

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concentration, heavymetals concentration and also the type of soil. Besides, in the table shows the optimum requirement for the microorganisms to work at the maximum level. In order to achieve that phase, the soil or the media moisture should be between 30 to 90 percent of its water retention capacity. It is also necessary for the media's pH to be in the neutral range from 6.5 to 8.0 where the condition is not too alkaline and not too acidic.Since most of the bioremediation process are working under aerobic conditions, it's only logical that the oxygen content in the media should be range from 10 to 40 percent. Besides, the optimum level of temperature should be between 20 to 30 °C, this is because the metabolism rate of the microorganisms are directly affected by the temperature changes where the metabolism rate increase when temperature increase and decrease when the temperature decrease. As for the nutrient content, it is compulsory that the nutrients ratio between Carbon (C), Nitrogen (N) and Phosphorus (P) to be 100:10:1 to achieve optimum level in pollutants degradation process. It is also important to see on the hydrocarbons toxicity level as to get the optimum bioremediation process is to have the less toxic level between 5 to 10 percent of the soil's dry weight. Lastly, it is also necessary to have a little concentration of heavy metals range from less than 700ppm in order to guarantee the optimum efficiency of bioremediation process.

Element	**Percentage**	**Element**	**Percentage**
Carbon	50	Potassium	1
Nitrogen	14	Sodium	1
Oxygen	20	Calcium	0.5
Hydrogen	8	Magnesium	0.5
Phosphorus	3	Chloride	0.5
Sulfur	1	Iron	0.2
Others	0.3		

Table 2: The nutrient composition of a microbial cell [52].

The last factor that affecting the rate of bioremediation process is the nutrient compositions that available in the contaminated site. As shown in Table 2, the necessary elements required by the microorganisms to be in optimum level. It is necessary to stimulate the growth of microorganisms in a contaminated area to increase the rate of pollutants degradation processes. As carbon is the basic nutrients that needed in the growing phase of microorganisms, study shows that microorganisms need about 50 percent carbon nutrient from the total nutrients intake.Besides that, microorganisms also need about 14 percent of nitrogen and another 3 percent of phosphorus intake because the microorganisms also requires these macronutrients to increase the efficiency of oil pollutants degradation process [16]. The table also shows that other nutrients and elements such as oxygen (20 percent), hydrogen (8 percent), sulfur (1 percent), potassium (1 percent), sodium (1 percent), calcium (0.5 percent), magnesium (0.5 percent), chloride (0.5 percent), iron (0.2 percent) and other trace elements (0.3 percent) are affecting the effectiveness of contaminants degradation process.

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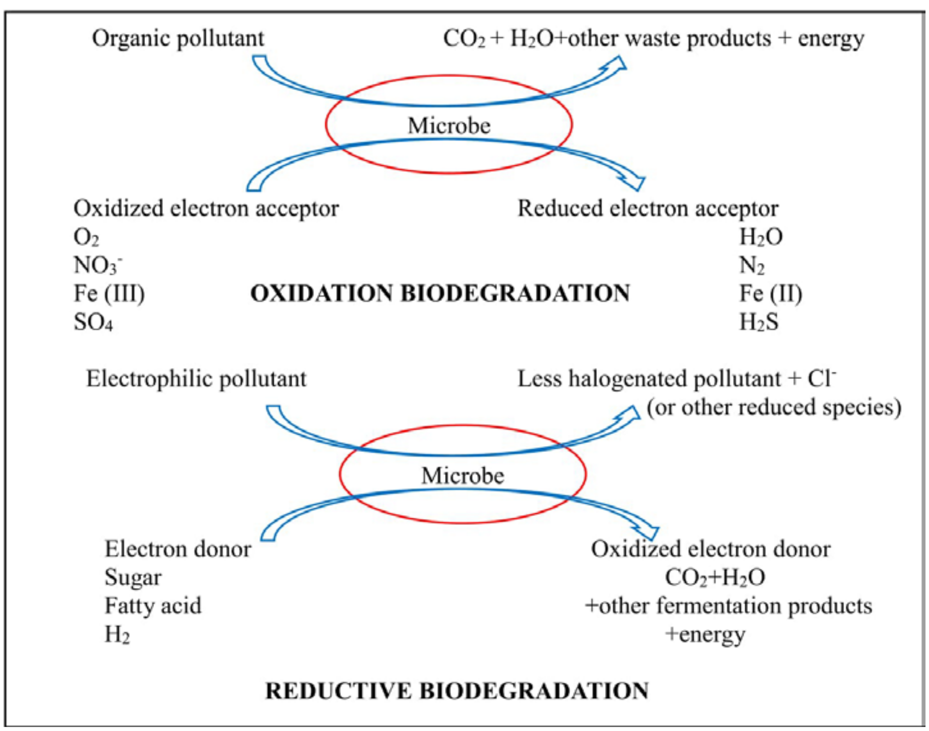

Picture 1: The general process of organic contaminant degradation [50].

3.0 Types of bioremediation

There are few types of bioremediation process that been identified throughout the years. As for contamination that happened in terrestrial area, decision that are needed to be made is whether to treat the impacted soil in the impacted area or is it necessary to transfer the impacted to the treatment facilities. Researcher define this activity as *insitu* if the impacted soil or contaminated water is treated in the same location as the impacted environment while define it as *ex situ* if it is necessary for them to treat the impacted soil and the water elsewhere [37], [48], [29]. In order to fully understands about the types of bioremediation that already exist in the modern days, more detail observations are needed to see what is the techniques or methodology that been used in the *insitu* and *exsitu* treatment methods.

The most common *insitu* technique type that been used in bioremediation process is the intrinsic bioremediation. Intrinsic bioremediation means an approach of stimulating the existing microorganisms or the indigenous population that naturally occurring in the contaminated area by adding nutrients and other necessary elements to increase their metabolic activity [37]. The other type for *in situ* technique is the engineered *in situ* bioremediation, this approach is really different compared to the previous one where engineered *in situ* bioremediation involves introducing specific microorganisms into the contaminated areas to expedite the biodegradation process by improving the physico-chemical conditions in order to increase the growth rate of the microorganisms. *In situ*bioremediation has been proven to have more advantages over disadvantages especially in term of financial and cost as this technique does not have the necessity for excavation of the contaminated media and to transfer to the treatment centres. Through this technique, it will only provide minimal site disturbances which will limit the amount of dust particles created from the treatment process. And this technique also allows the treatment for contaminated soil and groundwater to happen simultaneously. As for the disadvantages of*in situ* bioremediation, first of all this technique is been known to consume a lot time and also some of the pollutant's degradation microorganisms can have adverse changes in their microbial activity when exposed to extreme environmental changes that are out of human-control. As been said before, as the extreme changes occur in the environment, it will affect the microorganism's ability to utilize nutrients to produce energy that is crucial for the development of new cells [37].

Next is the *ex situ* bioremediation technique, this method requires excavation of the contaminated media such as soil or groundwater in purpose to increase the microbial degradation process. In this modern age, more disadvantages has been identified compared to the advantages. *Ex situ* bioremediation can be categorized into 2 categories which are known as solid phase system (land treatment and soil stockpile) and slurry phase system (solid-liquid suspensions in bioreactors). In detail, solid phase system is a treatment processes that include soil bio piles, land farming and composting. It is suitable for soil that been contaminated with various contaminants such as the agricultural wastes, domestic and industrial wastes, municipal solid wastes and sewage sludge. Land farming is the simplest technique as the contaminated soil will be excavate and distributed over a large surface area and the soil will be periodically tilled until the pollutants in the contaminated soil is completely degraded. The purpose of tilting is to expedite the aerobic degradation of the contaminants and at the same time to stimulate the native pollutant's degrader microorganisms in the contaminated soil.

As for composting, it is a technique that requires combination of non-harmful organic materials such as the agricultural wastes and animal wastes with the contaminated soil. The concept of this method is to supply enough organic substances that can supports the growth of rich microbial population and at the same time increases the temperature characteristics of the contaminated soil during the composting process. Lastly the technique that included in the solid phase system treatment is bio piles. Bio piles is a combination of both composting and land farming technique. This technique is usually use to treat the surfaces of an area that been contaminated by petroleum hydrocarbons. The main focused of this technique is to elevate the growth of pollutant's degrader microorganisms as this technique will provide an optimum environment for both native anaerobic and aerobic microorganisms.

Compared to the previous treatment system as well as other available treatment processes, slurry phase bioremediation technique is considered to be faster. In this treatment system, the contaminated media is mixed with additives and water in a large container known as bioreactor. The condition in the bioreactor will be regulated to the optimum level to make sure the growth of the native microorganisms that already existed in the contaminated media. The water in the bioreactor will be then remove from the mixture and the process will be repeated if there are still pollutant detected in the treated media.

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3.1 Biostimulation

As been discussed earlier, pollutant degradation process such as hydrocarbon in the soil or water can be limited by several factors such as the pH, nutrients level, moisture, temperature, oxygen, the media properties and the concentration of the contaminant itself [11], [10], [17]. The definition of biostimulation is the alteration of the environment to generate or to stimulate the existing microorganisms in the impacted area to be able to have an efficient bioremediation process. The stimulation usually done by adding various forms of electron acceptors and limiting nutrients such as nitrogen, phosphorus, carbon and oxygen, the substances are usually in the form of molasses in which these substances can already be found presence in the impacted environment but in a very low concentration which inhibit the microbial activity of the microorganisms [51], [49], [26].

In other study done by Bordenave*et al*., [15], described that by adding more oxygen molecules, nutrients, electron accepters and donor will elevate the number of the population of pollutant degradation microorganisms in the contaminated area. Other researcher supported the statement that biostimulation is a process of natural remediation that help to improve the degradation process by optimizing the impacted area characteristics such as through aeration, addition of nutrients, pH and temperature control [45]. It is believed that biostimulation technique is appropriate in degrading oil contamination in the soil but at the same time still requires the observation of both the intrinsic removal capacities of the autochthonous microorganisms and the environmental parameters during the *insitu* process.

The main advantage of using biostimulationin bioremediation process is as the environmental characteristics is being alter, it will promote the indigenous microorganisms in the contaminated environment to properly adapt and capable to optimally degrade the pollutants. But, biostimulation also have it downside. The challenge of biostimulation technique is during the delivery process of the additives or the elements itself. It is really difficult to make sure that the needed additives can be properly delivered and readily available for the native microorganisms to use it.

The effectiveness of the nutrients delivery is still depending on the local geology and the subsurface of the contaminated area [37]. This is because for the type of subsurface that are impermeable subsurface lithology such as fine-grained material or tight clays will deter the process of additives delivery throughout the contaminated area. This is because tight and impermeable subsurface will not have fractures or gaps for the additives to flow through, hence make it impossible for the additives to be properly distributed. Besides that, the additives used might also benefit the growth of other heterotrophic microorganisms that incapable of degrading pollutants which in the end creating a competition between the different types of microorganisms within the contaminated area [13].

3.2 Biostimulation using Organic Nutrients

Nutrient added	Type of contaminant	Initial TPH concentration	Percentage removed	Duration	Comment	Sources
Compost made from wood chips and sewage sludge	Petroleum Hydrocarbon-crude oil	38,000mg/kg	100 percent removal of 2-3 ringed PAHs within the first 3 months.	570 days	Showed 100 percent removal over a 19 months period with removal linked to the native microbial population and improved growth in the system.	[9]
Brewery used-grains, banana skin and spent mushroo m compost	Petroleum hydrocarbons -products lubricating oil	Not indicated	79 percent and 92 percent for 5 percent oil contamination linked to the presence of organic waste and low contamination while reduction was between 17	84 days	Showed significant removal of TPH using the organic nutrient sources.	[8]

			percentand 24 percent in 15 percent w/w oil contamination initially and 36 percent to 55 percent after 84 days linked to high initial concentration.			
Poultry droppings	Marine sediment that contaminated by petroleum hydrocarbon	106.4ppm-116 ppm TPH and 96.6 ppm-104ppm PAH	95.35 percent for TPH and 98.92 percent for PAH.	56 days	Significant degradation of PAH and TPH in a bioreactor using 20 g poultry litter and 1 litre seawater.	[20]
Cow dung	Hydrocarbon polluted mangrove swamp	14,103.02mg/kg	62.96 percent.	70 days	Significant reduction observed when compared with control using cow dung as Biostimulation agent.	[48]
Tea leaf, soy cake and potato skin	Petroleum hydrocarbon-diesel fuel	100000mg/kg and 200000mg/kg variation in concentration	Between 25 percent and 82 percent.	126 days	Showed significant degradation of TPH for the treatment with soy cake.	[23]
Oil palm empty fruit bunch and sugar cane bagasse	Petroleum hydrocarbon crude oil	Not indicated	100 percent for sugarcane bagasse and up to 97 percent for empty palm fruit bunch.	20 days	Showed significant biodegradation using these supplements for stimulating microbial growth.	[30]

Table 3: Studies that used organic nutrients as biostimulation agent.

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There were various studies that used organic nutrients as a biostimulation agent in improving the bioremediation process. As already mentioned before, the nutrients additives can be either naturally occur in the environment in a small quantity or by purposely introduced to the environment. Many of the nutrients additives in the world can either be natural or synthetic and also organic or inorganic. From Table 3, it shows that some studies using organic nutrients for stimulation of petroleum hydrocarbon's degrader microorganisms in the contaminated areas. As seen from the table, all of these organic nutrients does stimulate the bioremediation process in the contaminated sites. For example, study by Atagana[9] shows that by using composted mixture of sewage sludge and wood chips, it capable to stimulate the growth of the pollutant's degrader microorganisms that succeeded to remove 100 percent of the contaminant in 570 days. Even though it took about 19 months in order to completely degraded all 38 000 mg/kg of the total petroleum hydrocarbon (TPH), but in just 3 months, the microorganisms able to remove all the 2-3 ringed polycyclic aromatic hydrocarbons (PAHs) in the contaminated media. Besides, this study were mainly focused on the temperature level in the compost systems, moisture level and also the nutrient composition. While doing the study, he also observed the temperature changes in the controlled-system that ranged from 12 to 30 ^{o}C. He also recorded that in the sewage sludge compost treatment, there was an elevation in temperature which the temperature recorded rose to 58 ^{o}C during the 2 month of the study which indicated an increase of microbial activity and concentration.

Besides that, he also observed changes in the nitrogen contents in the same composting system as the concentration of nitrogen decreased rapidly compared to the controlled system. The rapid decrease in nitrogen content also can be seen as indicator to an increase of microbial activity as well as increase in the rate of pollutants degradation in the compost system. Lastly, Atagana also compared the concentration of total petroleum hydrocarbon (TPH) in the compost system and the control system after 2 month of experiment. From the observation, he found out that about 68.8 percent total petroleum hydrocarbon has been degraded in the compost system compared to only 10 percent reduction in control systems. From his experiment proven that pollutant's degrader microorganisms need supply of nutrients in order to efficiently undergone the bioremediation process.

Another study done by Abioye*et al*., [8], they used various kind of organic nutrient in order to treat soil that been contaminated with used motor oil. The set up for the experiment was an *ex situ* technique where the soil that were contaminated with 5 percent and 15 percent w/w of used lubricating oil will be tested using 15 percent of banana skin (BS), spent mushroom compost (SMC) and brewery spent grain (BSG) for 84 days. From the observation done after 84 days, Abioye*et al*. recorded that about 92 percent of the pollutants were removed from the soil that been contaminated with 5 percent w/w of used lubricating oil added with the 15 percent brewery spent grain (BSG). As for the sample that have 15 percent used lubrication oil contamination with the same substrate (BSG) recorded reduction about 55 percent throughout the study period. They concluded in the study that brewery spent grain (BSG) was considered the most effective organic nutrient to facilitate the growth of pollutant's degrader microorganisms during bioremediation process when compared to the other nutrients such as the banana skin (BS) and spent mushroom compost (SMC). This is because they identified that the moisture content in the contaminated soil also play a major role in pollutants degradation process and among all the nutrients used in the experiment, only brewery spent grain (BSG) have the highest volume of moisture content which accounted about 71.84 percent from its total volume. According to Nduka*et al*. [47], in order to improve the microbial activity while increasing the rate of pollutants degradation within the contaminated media with high pollution concentration, it is necessary to have a mixture of nutrients source as an improved "diet" for the microorganisms. By having enough nutrients supply also can help in reducing the time taken for degradation process, the more nutrients presence in the contaminated media, the faster the microorganisms can degrade the pollutants [47].

Another similar studies also been carried out by Dadrasnia and Agamuthu[23] where they used bio waste such as tea leaf, potato skin and soy cake to observed the potential of these bio waste in degrading diesel contaminated soil. The study took about 126 days to complete and they were observing the rate of degradation of the diesel in the soil. They found out that for their sample that been contaminated with 10 percent diesel have a degradation rate of 82 percent for total petroleum hydrocarbon (TPH) reduction while the test sample with 20 percent contamination only reached about 25 percent degradation rate. Both of the researchers also identify that the soil enzyme activity also known as dehydrogenase enzyme was greatly improved by the used of the bio waste. The study achieved the highest degradation rate with a fixed rate of 0.152 day^{-1} in the sample that been contaminated with 10 percent diesel when they used soy cake as the nutrients supplier. It is also stated that in the control sample, the concentration of Nitrogen and Phosphorus were really low with a concentration of 0.8 percent and 0.6 percent respectively when organic wastes were mixed in the control sample. From this we can see that by using organic wastes will help in supplying nutrients such as Nitrogen and Phosphorus.

Research done by Chikere et al. [20] have identified and isolated about fifty six bacterial strains that have potential in degrading hydrocarbon. Several of the isolated bacterial strains were *Flavobacterium sp*., *Escherichia sp*., *Acinetobacter sp*., *Proteus sp*., *Bacillus spp. Rhodococcus spp*., *Alcanivorax spp*., *Alcaligenes sp*., *Serratia spp*., *Arthrobacter spp*., *Nocardia spp*., *Staphylococcus spp*., *Pseudomonas spp*., *Citrobacter sp*., *Klebsiella sp*., *Micrococcus spp*, and *Corynebacterium spp.* All these bacterial strains were isolated and identified from contaminated soil and also from the poultry litter. In this study, bioreactor was used where the vessel was filled with 1 litre of seawater, 1 kg wet weight of sediments, 20 ml of crude oil, 20 g of poultry litter and 20 mg of anthracene. Besides, a control sample also was prepared with the same amount of sediment but without any additives added into the sediment.

There were 2 control samples where control sample 1 is the sediments without nutrients while another sediment that been autoclaved beforehand also with no nutrients was labelled as control sample 2.The focus of this study were mostly into observing the native bacteria in the mixture of contaminated sediments and sea water to see whether it susceptible to degrade petroleum hydrocarbon as well as to observe and measure whether the loss of petroleum hydrocarbon were related to the abiotic factors of the samples. As a result, it was reported that the total hydrocarbon degrading bacteria and heterotrophic bacteria were in the range of 10^5cfu/g and the larger population of bacteria growth indicates that the bacteria can withstand and degrade

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petroleum hydrocarbon. At the end of the experiment, the researchers found out that the vessel that have the mixture of the contaminated sediments and nutrients displayed a drastic decreased in the polycyclic aromatic hydrocarbon (PAH) and total petroleum hydrocarbon (TPH) concentration with a total reduction of 98.92 percent and 95.35 percent respectively. Even though the reduction rate were really high, the researchers also identified that the autochthonous microorganisms isolated gram-negative bacteria in the sediment were really specific in growing without using amino acids and carbohydrates as their growth substrates.

Other researchers such as Head *et al*. [32] and Yakmov*et al*. [56]agreed that gram negative bacteria has an ability in producing their own gluco-lipids to be use as their carbon source in the absence of carbon in the environment. Several other researchers also suggested that the gram negative bacteria are able to utilize the presence of phosphorus and nitrogen to elevate their natural degradative ability in the environment [13], [23], [8].The study done by Chikere*et al*. [20] also showed that, how the abiotic factors is essential to the bioremediation process such as the homogenization and agitation done during the entire duration of the experiment.As mentioned earlier that the concentration of the pollutants itself might affect the bioremediation process. By looking at the baseline data of the study, it shows that the concentration for both polycyclic aromatic hydrocarbon (PAH) and total petroleum hydrocarbon (TPH) were relatively low in the contaminated sediments which allow the bacterial to survive and degrade the pollutants. But the researchers were still not convinced whether the high degradation rate were because of the concentration of microorganisms alone or the contaminated sediment might have a natural capacity in fully biodegrade both of the polycyclic aromatic hydrocarbon (PAH) and total petroleum hydrocarbon (TPH).

So far, most of the studies justify that biostimulation really help in increasing the rate of bioremediation process but it is still crucial to find a way that make bioremediation process much faster as the current progress as the longer the pollutant stays in the environment, the larger ecological impacts. Another similar study also done by Orji *et al.* [48], where they used cow dung as the organic nutrient sources to elevate the rate of degradation process. The study took place for about 70 days in which 500 g of contaminated mangrove soil was collected and mixed with 50 ml of Bonny light crude oil to simulate major oil spill condition. The mixture that then later were considered as contaminated soil were mixed again with 50 g of dried cow dung. The focus of the study was also quite similar to the previous studies where the researcher wanted to observe the effectiveness of using cow dung in the contaminated soil as the only nutrient source for the microorganisms. From the study, the researchers identified that at the end of the experiment, about minimum value of 3.6 x 10^4 cfu/g and 2.4 x 10^4cfu/g of hydrocarbon degrader bacteria and fungi were recorded respectively. The study also indicate that the bacteria managed to degrade about 62.08 percent of the crude oil at the end of the 70th day compared to the control sample which only managed to degrade only 20 percent of the crude oil.When compared to other nutrients used in the prior studies, cow dung as the nutrients sources seem to be less effective in facilitate the growth of the bacteria to degrade the pollutants.

3.3 Biostimulation using Inorganic Nutrients

Globally, inorganic fertilizer are commonly been used as biostimulation agents. Some researcher also did some study to investigate the ability of inorganic fertilizer (NPK) in improving and facilitate the microbial degradation of total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) in the soil [21]. The result from the study did stated that the microorganisms that uses inorganic nutrients managed to degrade between the range of 40 to 60 percent for normal paraffin and isoperoid such as Phitane and Pristane in just 10 weeks. The study also observed that with an addition of the inorganic fertilizer facilitated further the microbial degradation activity. A similar study also done [23], where they purposely contaminated soil *ex situ* with spent engine oil to study the effectiveness of non-ionic surfactant and inorganic NPK fertilizer as biostimulation agent. From the study, they observed a reduction in total petroleum hydrocarbon (TPH) concentration with a reduction value of 67.20 percent after 42 days. By further analysed the data using numerical optimization technique, they have reported that the optimum values for NPK and non-ionic surfactant to be effectively act as biostimulation agent was 4.22 g and 10.69 ug/g respectively.

Dadrasnia and Agamuthu[23] also continued their research by using kerosene as total petroleum hydrocarbon (TPH) source with 4.30 g of inorganic NPK as source of nutrients. At the end of the experiment, they recorded a reduction of 75.06 percent which reflected that inorganic NPK fertilizer are more susceptible in degrading total petroleum hydrocarbon (TPH) in kerosene contaminated soil when compared to earlier experiment. This might due to the existence of lighter chains of hydrocarbons in the kerosene. The result was further supported by Venosa*et al*. [54]stated that the microorganisms were more prepared in degrading light end hydrocarbon compared to the heavy end hydrocarbons. Lastly, another study that focuses on the inorganic nutrients had used a modified Fenton and NPK fertilizer in their experiment [35]. The results from the

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experiment shows that in the soil that been contaminated with 20 000 mg/kg diesel,the total petroleum hydrocarbon (TPH) was degraded with 58 percent in the surface layer, 57 percent in the non-saturated layer and 32 percent in the saturated layer. The study also found out that during the experiment, right after the soil was contaminated by the diesel, a differentiation and specializationof bacterial community has taken places in the contaminated soil. This indicated that there was a stimulation improvement in the degrading biological activity and the degrading microbiota.

3.4 Bioaugmentation

Country	Biological System Used
USA	Pure or mixed cultures of *Bacillus, Clostridium, Pseudomonas,* and Gram-negative rods; mixed cultures of hydrocarbon degrading bacteria; mixed cultures of marine source bacteria; spore suspension of *Clostridium*; indigenous strata microflora; slime-forming bacteria; ultra-micro bacteria
Russia	Pure cultures of *C. tyrobutiricum;* bacteria mixed cultures; indigenous microflora of water injection and water formation; activated sludge bacteria; naturally occurring microbiota of industrial (food) wastes
China	Mixed enriched bacterial cultures of *Bacillus*, *Bacteroides, Eubacterium, Fusobacterium, Pseudomonas* ;slime-forming bacteria: *Brevibacteriumviscogenes, Corynebacterium gumiform, Xanthomonascampestris*
Australia	Ultra-micro bacteria with surface active properties
Bulgaria	Indigenous oil-oxidizing bacteria fromwater injection and water formation
Canada	Pure culture of *Leuconostocmesenteroides*
Former Czechoslovakia	Hydrocarbon oxidizing bacteria (predominant *Pseudomonas sp.*); sulfate-reducing bacteria
England	Naturally occurring anaerobic strain, high generator of acids; special starved bacteria, good producers of exopolymers.
Former East Germany	Mixed cultures of thermophilic *Bacillus* and *Clostridium* from indigenous brine microflora
Hungary	Mixed sewage-sludge bacteria cultures(predominant: *Clostridium*, *Desulfovibrio, Pseudomonas*)
Norway	Nitrate-reducing bacteria naturally occurring in North Sea water
Oman	Autochthonous spore-forming bacteria from oil wells and oil contaminated soil
Poland	Mixed bacteria cultures (*Arthrobacter*,*Clostridium*, *Mycobacterium*, *Peptococcus,Pseudomonas*)
Romania	Adapted mixed enrichment cultures(predominant: *Bacillus*, *Clostridium*,*Pseudomonas*, and other Gram-negativerods)
Saudi Arabia	Adequate bacterial inoculum accordingto requirements of each technology
The Netherlands	Slime-forming bacteria(*Betacoccusdextranicus*)
Trinidad-Tobago	Facultative anaerobic bacteria highproducers of gases
Venezuela	Adapted mixed enrichment cultures

Table 4: The bioaugmentation studies done in different countries in the world.**Source:**[41], [7], [11], [6]. [4].

Table 4 shows the bioaugmentation experiments that been done all over the world. As can be seen, the country that actively study using the bioaugmentation technique are USA, Russia, China, Australia, Bulgaria, Canada, former Czechoslovakia, England, Former East Germany, Hungary, Norway, Oman, Poland, Romania, Saudi Arabia, The Netherlands, Trinidad-Tobago and Venezuela. The table also displayed that each countries has their own specific focus and need from the studies where most of the bacteria and micro-organisms studied were for degradation purposes of total petroleum hydrocarbon (TPH) and polycyclic aromatichydrocarbon (PAH).Bioaugmentation is a process of adding a supplement or nutrients to oil-degrading microorganisms in order to facilitate the bioremediation process. It has been publicly introduced and used as the alternate strategy in removing pollutants in the contaminated environments since 1970s.

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The concept of bioaugmentation is to introduce the pollutant's degrader microorganism to facilitate the microbial activity in the contaminated area, this is because the native microbial community in a complex mixture of petroleum contaminated environment might not be able to degrade the pollutant in the wide range without any assistance [40].Besides that, bioaugmentation will also be in consideration when the rate of degradation is the primary factor, when seeding may decrease the lag period to start the degradation process as well as when the native pollutant's degrader population is low in the contaminated area. The success rate of bioaugmentation solely depend on the ability of the microorganisms to maintain genetic stability and viability during storage, able to reduce most of the petroleum substances, able to effectively compete with the other indigenous microorganisms, able to survive in hostile and foreign environments and also must be able to move through the pores of the sediment in the contaminated area [28]. There are different types of microbial species in the environment and each possess different preferences and enzymatic abilities for the reduction of pollutants. Some microorganisms prefer poly- or mononuclear aromatics while other prefer to jointly break both the aromatics and alkanes. In short, bioaugmentation is a process to introduce microorganisms that been collected from contaminated area either from specific site or genetically modified microorganisms to facilitate the bioremediation process in the selected contaminated areas.

Type of contaminants	**Initial TPH concentration**	**Microorganisms added**	**Source of microorganisms**	**Duration of research**	**TPH reduction**	**Comment**	**Sources**
TPH (Crude oil)	4200mg/kg	*Acinetobacter baumannii* T30C	Tapis crude oil contaminated soil of oil refinery plant	35 days	43 percent	TPH induction was not entirely induced by the introduction of A. baumannii T30C as the reduction observed was not significantly different from the control.	[19]
TPH (Crude oil)	>100mg/kg	*SerratiaSp* BF40	Crude oil contaminated saline soils	Unknown	>60 percent	BF40 Strain of Serratia showed high utilized potential for biodegradation of crude oil contaminated saline soils due to its high surface activity and salt tolerance.	[55]

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TPH, contaminate by parking trucks, human activities and diesel	10,000mg/kg	*Bacillus cereus, Gordonirubripertincta, Kociriarosea, Bacillus subtilis* (Strains 7A and 9A), *Aspergillus terreus, Aspergillus carneus*	Isolated from pre contaminated sites and assayed for ability to degrade TPH	90 days	52 percent	Soil TPH degradation was not significantly different from reduction observed in control, although there was a significant reduction (P<0.05) relative to initial TPH concentration.	[24]
TPH (diesel)	16,300mg/kg	*Candida tropicalis* SK 21	Petroleum contaminated soil	120 days	83 percent	Inoculation of yeast resulted in 83% TPH removal as against TPJH61%using indigenous microorganisms	[27]

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Table 5: Researches on specific bioaugmentation process.

From Table 5, it shows the researches that been specifically on the bioaugmentation process.A study done by Chang et al. [19] has focused on the Tapis crude oil contaminated soil, the study is done to investigate the effectiveness of a selected potential hydrocarbon which is *Acinetobacter baumanni* T30C in degrading the crude oil in the soil. The study took about 35 days to complete and through the observation made, there were adecrement in the amount of nutrients and also low level of degradation process were recorded. From the result, another researcher stated that it is necessary to facilitate the bacterial growth and the microbial activity in order to effectively degrade the pollutants [46].

Another study done by Wu et al. [55] focuses more on the surface activity of the *Serratia Spp.* (salt tolerant microorganisms) and the reduction of oil in the saline soil. The experiment was done by isolating the *Serratia Spp.* BF40 from the environment that been contaminated by crude oil and at the same time to study its salt tolerance , ability to degrade crude oil in saline soils and also its surface activity. At the end of the experiment, it was concluded that *Serratia Spp.* will be able to induce hydrocarbon degradation and also be able to decrease surface tension of oily soil surfaces. Besides that, study by Diaz-Ramirez *et al*. [24] also quite similar to the previous study, where focus was given to the potential hydrocarbon degrader microorganisms that are exogenous and native in the Mexican tropical soils. A laboratory experiment were set up by using artificially contaminated soil which was contaminated with Olmeca crude. The bioaugmentation strains were later mixed with the microorganisms that been collected earlier from the Mexican tropical soils and the reaction between the populations were observed. At the end of the experiment, it was recorded that both populations were able to coexist, able to stimulating a synergistic effect and no significance difference can be observed in term of the two studies on the degradation rate of the pollutant. It is also recorded that, in the first 30 days of the experiment, rapid decrease in hydrocarbons concentration were able to be observed.

Lastly the study done by Fan *et al*. in [27], the study was focused on the strain known as *Candida tropicalis* (SK 21 strain) in order to see its bioaugmentation ability. There were 2 samples prepared for the experiment, one sample as the control sample and the other one was the study sample. The sample was a mixed of 51 percent saturated hydrocarbons and 31 percent aromatic hydrocarbons. During the study period, the degradation process where observed under the optimum pH value and the result was 96 percent of the pollutants were degraded in the study sample while only 61 percent reduction can be observed in the control sample. The researcher later found out that yeast inoculation have great effect in facilitate polyphrenoloxidase and dehydrogenase activities in the soil.

3.5 Combination of biostimulation and bioaugmentation

Types of contaminant	**Initial TPH concentration**	**Microorganisms and nutrient added**	**Source of microorganisms**	**Duration of study**	**TPH reduction (percent)**	**Comment**	**Sources**
TPH from diesel oil	Not stated	A consortia isolated from previously contaminated soil and $(NH_4)_2 SO_4$ and K_2HPO_4	Previously contaminated soil	84 days	45 percent and 72.7 percent for biostimulation and bioaugmentation respectively	Bioaugmentationusing microbial load from a pre-contaminated soil enhanced the autochthonous strain and overall degradation as opposed to biostimulation as stated by the authors	[18]
TPH from motor oil	40,000 mg/kg	*Bacillus Sp.*, *Pseudomonas Sp.* and *Proteus,* NPK fertilizer	Isolated from automobile workshops	42 days	65 percent reduction	Nutrient addition improved the rate of remediation as control option amended with water only returned 42% reduction	[5]
TPH from crude artificially spiked	50,000 ppm	Halotolerant actinobacterial strains and sodium chloride. *Rhodococcus Sp., Gordoniarubripectincta, Rhodococcus Sp., G. Alkanivorans, R. equi, and Rhodococcus Sp.*	Previously contaminated soil	Unknown	Up to 36.2 percent degradation for first soil sample and 51 percent of second soil sample	This research indicated a higher degradation for n-alkanes than total petroleum hydrocarbons; 67.7 percent 77.3 percent for sample A and B as against 36.2 percent, 51 percent respectively	[3]

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TPH from diesel contaminated site	30,000 ppm	Rhodococcus Sp. EH 831 and Tween 80 surfactant	Isolated from previously contaminated soil	46 days	>50 percent reduction	The addition of surfactant seemed to enhance the availability of the diesel for microbes subsequently improving TPH degradation	[25]
TPH from spent motor oil	14,100 mg/kg	*Pseudomonas aeruginosa, Bacillus subtilis* and fertilizer; NPK 20:10:10	Stock culture obtain from a research institute	70 days	75 percent reduction	Nutrient availability was optimum for the entire duration of the research as observed in the Nitrogen, Phosphorus and Potassium content measured throughout the research	[1]

Table 6: The study on combination of biostimulation and bioaugmentation process.

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Table 6 shows the study on the combination of biostimilation and bioaugmentation process that been done all over the world. Currently, many scientists are pursuing to try in combining both mechanisms in order to achieve and improve to higher degradation rate.The combination of both mechanism are feasible as long as the nutrients are not harmful and threaten the ability of microorganisms to survive. For example, research done by Bento *et al.* in [18] where the contaminated soils were collected and compared to see the effect of nutrients and microbes on the degradation rate of diesel. The experiment took about 12 weeks to finish and the samples contained the light (C_{12}-C_{23}) components and heavy components (C_{23}-C_{40}). At the end of the experiment, there were 63-84 percent reduction observed for the light diesel component in the sample that only used microorganism and 72 percent reduction was recorded in the sample that used combination of both biostimulation and bioaugmentation. As for the heavy diesel components, a reduction of 19 percent and 31 percent were recorded for the combination mechanisms and only bioaugmentation mechanism respectively. From the result, it can be conclude that addition of nutrients in the bioaugmentation process will not give any impact on the degradation rate of the microorganisms. But the researcher believed that it is important to understands the limitation and detailed information regarding the characterization and site specification before deciding the mechanisms to use in bioremediation process.

The similar result also been observed in different study by Abdulsalam and Omale in [5] where the experiment took about 42 days to finish. The samples were mixed with spent motor oil and the result shows that biostimulation shows better degradation rate compared to the combination treatments with 69.2 percent reduction and 65.2 percent reduction respectively. The study also used specific types of microorganisms such as *Bacillus Sp., Pseudomonas Sp.,* and *Proteus Sp.* as well as NPK fertilizer but all these specifications did not facilitate or expedite the degradation rates of the pollutants. Alvarez et al. in [3] also tried to approach the possibility of increasing the degradation rate by combining both of the mechanisms. Throughout the study done by them, it was reported that the microorganisms population in the contaminated soil was mostly affected by the duration of the experiment in which they suggested that closed monitored of the process is the best way in achieving higher degradation rates.

Another experiment was done by EunHee et al. [25] by using the combination of *Rhodococcus Sp*. EH831 and a surfactant that later was used in diesel contaminated soils. Comparison was made between this mixed with another sample that only have the presence of *Rhodococcus Sp*. without surfactant. The researcher then concluded that the addition of the surfactant did not provide any significant impacts on the bioremediation process. Another fellow researcher stated that the surfactants alone were great in achieving higher degradation rate but as the focus on the study were neither on the physiological characteristic or the total petroleum hydrocarbon (TPH) removability itself became the reason of the failure (Reznik*et al*. 2010). The last study that will be included in this review is a research by Abdulsalam*et al.* in [1], the experiment involved demonstration of microorganisms and nutrients interactions in the bioremediation process. The experiment also used an aerobic fixed bed reactors to make comparison between the bioremediation that using bacteria and inorganic fertilizer with a sample that only be using bacteria. At the end of the experiment, the combination treatment managed to degrade about 66 percent of the pollutant while the bioaugmentation sample managed to degrade about 75 percent which was more than the combination treatment. Even though the results from all the combination studies were not that impressive, scientists still believe that combination technique can be used to develop a more comprehensive technology in pollutants degradation process [34].

4.0 Conclusion

As a conclusion, oil contamination has been the most major issue that been face by the human population all over the world. The contamination are caused by human activities such as production of fossil fuels, accidental releases of petroleum products, ore mining, smelting, illegal chemicals dumping, agriculture runoffs, industrial and municipal waste disposal. Bioremediation has been the easiest and the economical way in removing pollutants from the environment but bioremediation have its advantages and disadvantages. As one of the advantage is bioremediation is really economical compared to other conventional cleaning methods which can save a lot of public money in cleaning the pollutants in the environment but at the same time, bioremediation take a long time to completely remove all the pollutants which has become its biggest disadvantages. Throughout the years, more scientists focusing on how to decrease the duration for bioremediation process inremoving pollutants. Scientists also has categorized bioremediation into 2 categories which are *in situ* and *ex situ*. They also managed to find a solution to increase the biodegradation rate by applying biostimulation and bioaugmentation techniques. Even though the biostimulationand bioaugmentation techniques really help in increasing the degradation rate of bioremediation process, more improvement can still be develop and apply to the technique. More studies are needed to develop the most efficient bioremediation processes in the future.

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