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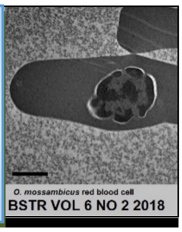

Biodegradation of Petroleum Hydrocarbons by *Bacillus* spp.: A Review

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ABSTRACT

This mini review aims to provide an overview of petroleum hydrocarbons degradation by *Bacillus* species. The first half of the scientific assessment is focusing on the impact of usage of petroleum hydrocarbons such as diesel fuels towards organisms and the surrounding environments. The other section of the literature collection discusses on the microbial remediation of this recalcitrant compounds by microbial species with special highlight on the genus *Bacillus*. This short evaluation will improve our present comprehension of bacterial degradation of petroleum hydrocarbons and their respective derivatives while providing an insight on the role of *Bacillus* species in microbial remediation communities.

INTRODUCTION

Due to the increase of demands for hydrocarbon fuels such as diesel to support the anthropogenic activities, the occurrences of hydrocarbon pollution in many parts of the world are not shocking. The persistency of these hazardous complexes causes many toxic effects towards human health and the environment. Several toxic effects towards human are chronic cough and phlegm [1], persistent respiratory symptoms [2] and potential of skin and lung cancer [3].

For this reason, various attempts to remove hydrocarbon pollutants from the contaminated environment via physical, chemical and biological methods have been established. Although the primary response to a source of contamination such as fuel spills is more biased to the physicochemical method, the fate of the spilled fuels on the site is unpredictable due to the disturbances of the natural ecosystems [4]. As demands for an environmentally-friendly technique are on the rise, an alternate petroleum hydrocarbons removal method such as microbial remediation is highly anticipated. Bioremediation defines as the process used to treat contamination by exploiting the enzymatic mechanism of microbes and plant [5,6].

Current decades have shown reports and review that addresses the distinctive application of *Bacillus* species in xenobiotics degradation and remediation. The present review focuses on the degradation of petroleum hydrocarbons, especially from the diesel fuels by the genus *Bacillus*. We hope that this review will increase our understanding of the impact of hydrocarbon pollution and the subsequent microbial remediation by this specific genus.

Petroleum hydrocarbon (diesel)

Petroleum, derived from a Greek word πέτρα (Latin *petra*) - rock + ἔλαιον (Latin *oleum*) - oil) or crude oil is an oily, heavy or unrefined liquid form that occurs naturally in deposits, usually beneath the surface of the earth. Meanwhile, petroleum hydrocarbons are compounds that carry predominantly carbon and hydrogen atoms. Petroleum hydrocarbons consist of a complex mixture of non-aqueous and hydrophobic components of hydrocarbon grouped as aliphatics (straight, branched, saturated and unsaturated), alicyclic, cycloalkanes, aromatics, polycyclic aromatics, asphaltenes, and resins [7]. A wide sort of petroleum hydrocarbon is the result of variations in branching, chain length and ring condensations.

The other constituents present in oil are sulfur, oxygen, nitrogen and metal atoms although in very small amounts [8]. Petroleum or crude oil may appear black or brown since it varies depending on the composition and also level of flammability [9]. Most petroleum is lighter than water or rock thus floats above a layer of water and is held under pressure beneath a natural gas layer. It will move upward through adjacent rock layers until it is trapped beneath impermeable rocks, within porous rocks called reservoirs [10]. The formation of crude petroleum is formed from the preserved remains of zooplankton and algae that populated the sea or lake bottom in large quantities under anoxic conditions. Series of transformation processes is carried out on the organic matters before it becomes petroleum. According to geologists, petroleum is believed to be the product of repeated compression and heating of prehistoric organic materials over time.

Several refined products of petroleum such as engine fuels, diesel oil, lubricating oil, jet fuel, liquefied petroleum gas and gasoline is obtained through the fractional distillation [11]. These groups are called as the petroleum hydrocarbon fractions [12]. Among the petroleum-refined products, diesel oil proved to be the most utilized hydrocarbons due to their immense production for fuel industry. Diesel is a complex mixture of liquid and solid hydrocarbon, whose composition also varies with the source. Most of hydrocarbons in diesel oil consist of straight aliphatic molecules and smaller portions of cyclic or branched hydrocarbons [13].

The aliphatic alkanes (paraffins), *n*-, *iso*- and cycloalkanes (naphthenes) are saturated hydrocarbons and composed approximately 80-90% of the diesel oils. Aromatics hydrocarbons (naphthalenes and alkyl benzenes) and olefins (styrene and indene) compose of 10-20% and l% of the fuel oils, respectively. This oil mixture includes both the volatile and semi-volatile hydrocarbons [13]. Diesel is typically heavier, more viscous, immiscible with water and less volatile than gasoline [14]. It also represents an excellent substrate in the study of hydrocarbon biodegradation due to its composition. According to Richard and Vogel [15], a typical diesel fuel comprises commonly *n*-hexadecane, alkanes with 16 carbons ($C_{16}H_{34}$).

Uses of diesel fuels

Diesel fuels have been extensively used as the principal source of energy to power a diesel engine for transportation such as ships, boats, motorcycles. Diesel-based fuels also have been used as locomotives, lubricants and generation of electricity (power) [16]. In terms of utilization of fuel, diesel is more advantageous than gasoline as it is less flammable and commonly used as armoured fighting vehicles like trucks and tanks for military [17].

Compared to the liquefied petroleum and petroleum ether gas engines, the ignition of spark plugs when using diesel as fuels is also more valuable as low voltage is being used [18]. Besides, engines running on diesel-based fuels are less likely to stall and provide more rotating force as they are controlled by a mechanical or electronic governor. Moreover, consumption of diesel fuel in motorized vehicles is more environmentally friendly as it produces fewer amount of greenhouse gas emission and more economical in comparison to an equivalent gasoline engines [19].

Hydrocarbon fuels pollution

Hydrocarbon fuels are important energy resources used by industry and in our everyday life. For the past few decades, the growth of modern industrialization has caused a rise in demand of petroleum and associated products. This action is to fulfill the energy requirement for current population [20]. Despite the fluctuations in fuel prices, hydrocarbon fuels will remain as the principal source of energy within the next several decades due to the lack of any reliable alternative energy that are more dominant than petroleum fuels.

According to Plohl et al. [21], petroleum hydrocarbons posed as a globally environmental pollutant among the most common anthropogenic contaminants despite its important usage. The most common sources of fuel contaminations id from the leakage from storage tanks and pipelines and the release during accidental spills [22]. The contaminations of petroleum hydrocarbons into the natural environment provide serious problems for many countries [23] including Malaysia. Gallego et al. [22] also reported that diesel oil is the most frequently reported contaminants to pollute soil, groundwater and marine environment due to accidents involving motorized vehicles (ships, cars, trucks), leaking of underground oil storage tanks, spillage during transferring oil from production facilities to refinery sites and rupture of pipelines.

The other contributing source of oil pollution results from a variety of human activities including bilge waste and ballast water discharges from ships (fishing, merchant, and tanker ships), abandoned gasoline manufacturing sites and illegal of industrial and urban discharges [24]. Several causes of leakage of storage tanks includes the corrosion of old bare steel tanks and the connecting pipes or lines, improper connection and incompatibility between pipes and their fittings, shifting of the piping after installation and pump failures [25].

Petroleum released to the soil may move through the soil to the groundwater [3]. Individual compounds may then separate from the original mixture, depending on the chemical properties of the compound. Some of these compounds will evaporate into the air and others will dissolve into the groundwater and move away from the release area. Other compounds will attach to particles in the soil and may stay in the soil for a long period of time, while others will be broken down by organisms found in the soil [26].

By 2025, with world oil demand expected to increase to 50%, the oil pollution is likely to remain as significant hazard to organism, ecosystem and industry for many more year even after the event took place [27]. This is because hydrocarbons can be persisting for ages in one environment and completely biodegraded in a short time, under different condition [28].

Effect of hydrocarbons pollution

The hydrocarbons that contaminate the soil can also lead to the pollution of underground water and coastal marine water [29]. Soil contamination with hydrocarbons causes' extensive damage towards local ecosystems since the accumulation of pollutants in animals and plants tissues may cause progeny's death or mutation [30]. Acute and chronic oil effects on ecosystems and marine organisms such as fish and shellfish may kill eggs of marine fauna's egg, fish and invertebrate [31,32] as well as bioaccumulation of polyaromatic fractions that are harmful to the natural environment. In addition, several congeners of polyaromatic hydrocarbons are known for their carcinogenic and teratogenic properties.

The aromatics in oils also have numerous adverse effects on the environment particularly to the local microbial flora. Benzo(a)anthracene, chrysene, and benzo(a)pyrene are some residues in the diesel that may harm human life [33]. The impact of oil pollution is particularly complex in a forest and woodland environmental system. It may lead to the decline of vegetation, which is directly reflected on the environmental and site value of a forest [34,35].

Additional impairment inflicted during the process of restoring an accident site may cause the soil to be compacted or the roots and damaged the above ground plant parts [35]. Mud ditches have a special status in an oil field. They were formerly used as disposal sites for drilling waste. Although such sites have been restored, it still represent the points of threat in an oil field [16]. From economical aspect, the revenues from sources of fisheries, recreational, marine activity and tourism will be affected by the pollution [36].

Petroleum compounds are considered to be recalcitrant to microbial degradation and persist in ecosystems because of their hydrophobic nature as well as expensive disposal methods [37]. Uncontrolled releases of these compounds into soil and ground water are frequent as a result of accidents, improper storage and handling of fuels, and the indirect booming of tourism that initiates many anthropogenic activities [38–40].

Toxicity of petroleum hydrocarbons

Hydrocarbon compounds such as petroleum are essential elements of life. Since they do not naturally occur in the forms that are more convenient to humans, they can be hazardous due to the composition of highly concentrated toxic materials and can become stable and recalcitrant in subsurface [41]. This makes their biodegradation efficiency considerably challenging thus can negatively influence to human, animals, soil microbes and plants, as well as contaminate groundwater, which may be used for drinking or agriculture [7]. The toxicity of petroleum and its derivatives is mostly attributed to its soluble part, which contains low weight molecular polar compounds, such as monoaromatic hydrocarbons (benzene, toluene and xylene) and small polyaromatic hydrocarbons (PAHs) [11].

Methodical and accidental discharge of dangerous hydrocarbon compounds into the aquatic environment has the impending disturbance to the structure and functioning of natural ecosystem [42]. The constituents of these contaminants such as diesel oil are carcinogenic, mutagenic and are potent immunotoxicants, thus posing a serious threat to human and animal health [43,44]. It has been observed that diesel oil presented a higher toxicity than crude oil in all mesocosms [45]. Petroleum and its refined products are found all over the planet, which justifies the recent global concern with pollution originated from oil spills that can result in the deposition of large quantities of aromatic hydrocarbons in the aquatic ecosystem [11] . In regards to toxicity of hydrocarbon composition, the aromatic constituents are usually more toxic than aliphatics with the same number of carbon atoms and they are typically found at higher concentrations in water because their solubility is three to five times greater [14].

Petroleum hydrocarbons and heavy metals can impact soil ecosystems sufficiently to result in significant losses in soil quality [46–48]. Their negative impact results from their toxicity to biological processes catalyzed by soil microorganisms. Field studies of contaminated soils have demonstrated that elevated loadings of these contaminations can result in diminished microbial biomass, reduced viable bacterial population densities, inhibition of organic matter mineralization as well as decreased leaf litter decomposition [49–51], and mycorrhizal infection of clover (*Trifolium* sp.) roots [52].

Saturated alkanes with C12 to C18 chains or longer are readily attacked by a large variety of bacteria. The degradation of water-soluble short chain alkanes such as pentane, hexane, heptane and octane, which are toxic for the environment, is less frequent [41]. In general, different compounds in petroleum fractions affect the human body in different ways. The smaller compounds such as benzene, toluene and xylene can affect the human central nervous system (CNS) and can cause death when exposed in elevated concentration [53,54]. Inhalation of concentrated toluene around 100 ppm in a short time period can cause fatigue, headache, nausea, and drowsiness [55]. Although these symptoms do not prolong, continuous exposure may damage the central nervous system permanently. Hexane, an alkane also can affect the central nervous system but in a different mode. It will cause numbness in the feet and legs and, in severe cases, paralysis. It also known as “peripheral neuropathy” [56].

Gasoline, kerosene or diesel ingestion can cause irritation of the throat and stomach, central nervous system depression, breathing difficulties, and pneumonia from breathing liquid into the lungs. The compounds in the petroleum portions can also affect the blood, immune system, liver, spleen, kidneys, lungs, and fetus development. Certain hydrocarbon compounds can be irritating to the skin and eyes. Other hydrocarbon compounds, such as some mineral oils, are not very toxic and are used in foods [7].

Besides that, toxicity of crude oil includes liver necrosis, congestion of the liver, fat degeneration, and dissociation of hepatocytes [7]. Birds and animals in oil-contaminated area are found to have black emulsion in the digestive tract with a petroleum odour. This leads to a decrease in the absorption of nutrients and finally leads to death of these birds and animals due to rupture of capillaries and hemorrhage, hepatocellular dissociation, hemosiderosis, renal tubular necrosis, and anemia [57].

Crabs, shellfish and mussels can be tainted from small oil spill in shallow shore areas [29]. Fish eggs and first larvae stage will be destroyed and cannot reach the next larval stage even with the low concentration (0.1 mg/L) diesel. Exposure to crude oil and derivatives can induce a variety of toxic symptoms in experimental animals. Petroleum hydrocarbons can act as a mediator in free radical generation in fish [58]. Studies with the goldfish *Carassius auratus* has shown an increase in antioxidant defenses in animals after exposure to different concentrations of the water-soluble fraction of diesel oil (WSD) for various experimental times [59,60]. Other studies have also indicated that the exposure of fish to a water-soluble fraction of petroleum derivatives causes different effects in cortisol plasma concentrations [61–63], suggesting that these contaminants might interfere in the fish stress response.

Hydrocarbon removal from contaminated environment

Hydrocarbon pollution such as oil spills cause short-term as well as long-term damage to the environment especially in soil, water, animals and aquatic flora and fauna. Remediation of the affected sites helps to reduce the environmental damage and aid in its recovery. Several physical and chemical techniques for decontamination have been developed and used such as incineration, combustion, solvent extraction, washing with detergent, mechanical collection, sorbent materials utilization, sinking, dispersion and diffusion. Although these techniques are well developed and established, these methods have limited efficacy. The synthetic detergents used in petroleum remediation often led to more destruction of the environment, production of more toxic compounds thus can cause recontamination by secondary waste products, costly and demand the custom usage of elaborate equipment and large amounts of energy [64–67].

For the past few decades, researchers have established the use of microorganism to decompose petroleum products and this approach shows to be a promising technological alternative pollution treatment [14]. One documented example is the contamination cleanup of the *Exxon Valdez* oil spill occurred in Prince William Sound, Alaska on March 1989, which resulted in the release of large quantities of crude oil. It was reported that the oil affected about 1300 miles of shoreline and considered as one of the most devastating man-made environmental tragedies to ever to occur at sea. Various methods from high-pressured dispersant to hot water beach treatment were used to remove oil from the beaches.

Mechanical methods such as backhoes and other heavy equipment were also employed to dig the beaches in order to expose the oil underneath so that it could be washed out. Concurrent to these methods, research focusing on remediation of contaminated area has increased. It is an important tool in attempts to lessen the environmental contamination. In a bioremediation effort, beaches were fertilized to promote growth of bacteria that utilize hydrocarbons. This was successful on several beaches where the oil was not too thick. Some solvents and chemical agents were also used, although none extensively [68,69].

Bioremediation of petroleum hydrocarbons has been proposed as more economical compared to current conventional technologies, can be carried out on-site with minimal site disruption, have low energy requirements and most importantly, capable of a complete destruction of the contaminants in a shorter time span and perceived as environmental friendly technology [70,71]. In general, bioremediation is defined as the process that exploits plants and microorganisms to revert back the contaminated environments to their original state [72,73]. Specifically, it is an organized or spontaneous process that exploits the catalytic capabilities of living organisms to degrade, transform and/or detoxify organic contaminants to less or non-toxic products in order to reduce the environmental pollution [74] to a safer environmental levels.

Bioremediation are the one of the most effective methods and give more advantages over the conventional methods to cleaning up hydrocarbons environmental contamination. The advantages of bioremediation include small capital expenditures, less energy requirement, fairly inexpensive and also greater removal of contaminant with less disturbance to the environment [75]. By this process, it will lowered the public's health and safety compared to the conventional systems [76].

Conversely, bioremediation also have some disadvantages such as slow process and may not removes all quantities of contaminants [77]. Furthermore, there will be limited areas for bioremediation application. Last but not least, bioremediation also need a stronger scientific basis for rational designing of process and success while requiring an extensive monitoring [78].

Currently, bioremediation technologies are well established for the clean-up process in industrial sector and also being applied commercially in large scale [79]. There are three types of bioremediation which are natural attenuation, biostimulation, and bioaugmentation. Biodegradation by natural populations of microorganisms, or in situ attenuation is a primary mechanism by which petroleum hydrocarbons could be eliminated from contaminated sites such as soil or water [80,81]. For natural attenuation to be viable approach, the site must have a high natural supply of nutrients and oxygen, and the source of contamination must be small. Significant evaluation up front and follow-up monitoring are necessary to ensure removal of contaminants of concern at reasonable rates [67].

Biostimulation is a process in which the indigenous microorganisms are stimulated with oxygen supply, optimized temperature and pH conditions [81,82] and appropriate nutrient supplements to ensure that the microbial growth is sustained so that they can effectively degrade contaminants. Other than that, the ratios of carbon to other nutrients such as nitrogen and phosphorus are limited only by carbon [83], hence speeding up the bioremediation process. However, different strategies are used to enhance the rate of degradation process in certain cases such as low or absence of natural communities of degrading bacteria therefore unable to meet the required demand.

The inoculation of an enriched mixed microbial consortium into the contaminated site is called as bioaugmentation [81,82]. As mentioned above, enrichment of degrading microbial communities and inoculation those into the contaminated site can be useful for removing oil pollutant from the environment thus the best bioaugmentation performance can be approached [75].

Hydrocarbon-degrading *Bacillus* spp.

Microorganisms survive in contaminated habitat because they are metabolically capable of utilizing its resources and can occupy in a suitable niche. Many microorganisms have been reported to degrade fuel and diesel oils and distributed in soil and marine environments [84,85]. This is possible because microorganisms have enzyme systems to degrade and utilize diesel oil as a source of carbon and energy [86]. Previous study have reported on several genus that have been able to utilize hydrocarbon such as *Pseudomonas, Bacillus, Proteus, Aeruginosa, Klebsiella, Aeromonas, Micrococcus, Serratia, Acinetobacter,* and *Flavobacterium* [87,88]. Such bacterial genera of *Acinetobacter, Arthrobacter, Bacillus, Corynebacterium, Flavobacterium, Vibrio* and *Pseudomonas* contain species that together can degrade most constituents of crude oil, including the aliphatic, alicyclic, aromatic, and polycyclic [89].

The normal, branched, cyclic alkenes and aromatics compound in diesel were shown to be readily biodegradable by bacteria [90]. A large number of bacterial genera have been identified as being involved in crude oil degradation. Among the bacteria, *Bacillus* sp. have been reported have diesel-degradation ability [91–93]. Diesel-oil degrading *Bacillus* strains have been isolated from a number of oil polluted sites [94–96].

The genus *Bacillus* is a Gram positive, rod-shaped bacterium which sporulate in aerobic environment. This genus belongs to the class of *Bacilli* and family of *Bacillaceae*. They can be obligate aerobes or facultative anaerobes, motile, and have positive production of cytochrome C oxidase and catalase [97]. According to Kumar and Lèon [98], *Bacillus* species are able to grow in minimal salt, commonly found as mesophilic with temperature optima between 30 and 45°C with small percentage of thermophiles with optimal temperature as high as 65°C. The organism capable to form a distinctive type of resting cell called an endospore. During stressful environmental conditions, *Bacillus* cells produce oval endospores that can remain dormant for extended periods. These characteristics originally defined the genus, but not all such species are closely related. Each bacterium creates only one spore, which is resistant to heat, cold, radiation, desiccation, and disinfectants [71].

There are several characteristics of the genus *Bacillus* that are more beneficial over other microorganisms in hydrocarbon biodegradation. *Bacillus* species display an array of physiological abilities that permit them to live in a wide range of habitats, including many extreme habitats such as desert sands, hot springs, and Arctic soils. Species in the genus *Bacillus* can be thermophilic, psychrophilic, acidophilic, alkaliphilic, halotolerant, or halophilic and are capable at growing at pH values, temperatures, and salt concentrations where few other organisms can survive. This bacterium is highly resistant to extreme environmental conditions such as low or no nutrient availability, desiccation, irradiation, H_2O_2 and chemical disinfections [99].

Besides, *Bacillus* species are commonly available in nature [100]. Most bacilli are saprophytes and they are the most widely represented organisms found in soil, marine [101], and fresh water sediments. They are often found in petroleum-contaminated environments [102–107]. A key and pivotal factor in the rate of biodegradation of hydrocarbons either in soil or in liquid phase is based on the survival of microorganisms in diesel medium after their inoculation. *Bacillus* can use hydrocarbon as their carbon source and energy in order to grow in diesel media. Hence, gram-positive bacteria particularly *Bacillus* species has been attracting interest in both environmental bioremediation strategies and biotechnological applications. **Table 1** shows the list of bacterial species from the genus *Bacillus* that able to utilize hydrocarbons as their carbon source.

Table 1. Hydrocarbon-degrading strains from the genus *Bacillus*.

Strain	Comments	Reference(s)
Bacillus badius D1	Alkaliphilic anthracene degrader. Able to degrade 50 mg/100 ml of anthracene at pH 9 within 60 hours.	[108]
Bacillus cereus	Potent degraders of crude oil isolated from petroleum-refined area in Kaduna, Nigeria	[109]
Bacillus cereus CPOU13	Strain was able to degrade PAHs up to 250 ppm and optimal pH for degradation was 6-8. The strain degraded 73.46% of phenanthrene, anthracene (85.76%), and pyrene (47.88%) within 14 days incubation period.	[110]
Bacillus cereus DRDU1	Isolated from automobile engine and widely used in hydrocarbon degradation studies	[111,112]
Bacillus cereus JMG-01	Anthracene-degrading strain with 98% of substrate (500 ppm) degradation within 21 incubation days	[113]
Bacillus lichenformis	The thermophilic bacteria was evaluated for the degradation of Maya crude oil, a type of Mexican heavy oil using bubble column reactor	[114]
Bacillus lichenformis DHT	A halotolerant and thermotelerant strain capable to degrade wide range of hydrocarbons and yield biosurfactant as the side product	[98]
Bacillus cibi and Bacillus megaterium	Used in bacterial consortium study which demonstrated an outstanding sludge degradation capacity in liquid medium (90.7% reduction of aliphatic fraction, 51.8% of aromatic fraction)	[115]
Bacillus pumilus 28-11	Naphthalene degrading strain isolated from oils sludge and proficient in producing biosurfactant	[94]
Bacillus pumilus JL_b	Used to test the bioaugmentation efficiency of diesel degradation in contaminated soils with/without the supplementation of nutrients via fertlizers	[116]
Bacillus pumilus KS2	Strain isolated from oil fields of Sivasagar (district of Assam), India was able to degrade 80.44% of TPH by 4 weeks of aerobic incubation	[117]
Bacillus sp.	One of the isolated bacterial species from soil and bottom sediments of hypersaline environments that able to degrade PAHs	[118]
Bacillus sp. Ege B.6.2i	Showed 60% and 33% of chrysene and naphthalene degradation with more than 30% of emulsification activity	[119]
Bacillus sp. Ege B.1.4ka	Showed 36% and 55% of chrysene and naphthalene degradation	[119]
Bacillus stearothermophilus	Capable to grow obligately on crude oil at high temperature (optimal at 60°C) and grew best on middle-chain alkanes	[120]
Bacillus subtilis	Able to utilize petrol fuel as sole carbon and energy sources	[121]
Bacillus subtilis 22BN	Strain was isolated based on their proficiency to utilize *n*-hexadecane and naphthalene simultaneously while producing surface-active compound (rhamnolipid) at 1.5-2.0 g/L	[122]
Bacillus subtilis A1	Biosurfactant-producing and alkane-degrading strain. Biodegradation efficiency of the strain was about 87% within short period of time (7 days)	[123]
Bacillus subtilis BMT4i (MTCC 9447)	First report on the specific species that was able to degrade benzo-[a]-pyrene as sole carbon source	[124]
Bacillus subtilis C9	Produce lipopeptide-type biosurfactant, and showed fast degradation of alkanes up to C19	[125]
Bacillus subtilis DM-04	Crude oil degrading bacteria used in consortium shake flask study	[91]

CONCLUSION

Petroleum hydrocarbons and its derivatives such as diesel and gasoline are one of the most reported pollutants that can be found around the globe. Extensive usage of these xenobiotic compounds for transportation and power generation caused the substantial spread of this recalcitrant complex. Due to this, the demand for cleanup of this compound is very critical. Microorganisms are the minute scavengers that could be found enormously in the environment. Their natural competency to utilize and manipulate petroleum hydrocarbon components into less harmful constituents makes the exploitation of microbial flora and fauna more valuable than the mechanical physical and chemical remediation practice. One of the main vehicles for microbial remediation is *Bacillus* spp. Regarded as one of the potential bioremediator agent, this genus is more beneficial than other genera in respect to their outstanding defense mechanism. The genus' high rate of survivality also strengthen their ability to survive in many polluted environments. Although scientific reports have proved the remarkable proficiency of this bacterial genus in degrading wide-range of aliphatic and aromatic hydrocarbons, a profound understanding on the total diversity of biodegradation pathways in this genus remains understudied. Hence, by understanding all the inferior vital feature in the genus catabolic pathway that often overlooked in most study may enhance the genus overall capability to mineralize these recalcitrant compounds.

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