The Review Of The Use Of Microorganisms In Biodegradation Of Crude Oil Spill: Challenges And Prospects

Ikhimiukor, Odion O. [1] and Nneji, Lotanna, M. [2]

[1]Department of Microbiology, University of Ibadan, Ibadan
[2]Department of Biological Sciences, University of Abuja, FCT, Abuja
Odido1@yahoo.com, lotannanneji@gmail.com

Abstract: Biodegradation is the process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition. It is a biologically catalyzed reduction in complexity of chemical compounds that involve two basic processes: growth and co-metabolism. Specific indigenous microbial communities are likely to contain microbial populations of differing taxonomic relationships which are capable of degrading crude oil spill. The most important genera of hydrocarbon utilizers in aquatic environment include *Pseudomonas*, *Achromobacter*, *Arthrobacter*, *Micrococcus*, *Nocardia*, *Vibrio*, *Acinetobacter*, *Brevibacterium*, *Corynebacterium*, *Flavobacterium*, *Candida*, *Rhodotorula*, *Sporobolomyces*, cyanobacteria and some algal species such as *Oscillatoria* spp., *Microcoleus* sp., *Anabaena* spp., *Agmenellum* sp., *Coccochloris* sp., *Nostoc* sp., *Aphanocapsa* sp., *Chlorella* spp., *Dunaliella* sp., *Chlamydomonas* sp., *Ulva* sp., *Cylindretheca* sp., *Amphora* sp., *Porphyridium* sp., and *Petalonia.* Microorganisms are capable of degrading hydrocarbons under both aerobic and anaerobic conditions. Challenges militating against the use of microorganisms in the biodegradation of crude oil spill include local environmental conditions of the polluted site, rate of uptake and mineralization of the organic compounds, concentration of the crude oil, limited bioavailability of the organic compounds etc. The use of molecular techniques in producing 'super bugs' or genetically modified organisms under strict non-proliferation rules provides a huge advantage in improving the use of microorganisms in oil biodegradation.

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1.0 INTRODUCTION

The ever burgeoning population and rapidly increasing industrialization in the world has led to high rate of dependence on crude oil especially in developing countries where efficient and adequate use of alternative sources of energy still remains farfetched. Perhaps there isn't any other raw material like petroleum oil that has impacted so much, and found wide application on human civilization.

Crude oil and its various factions are used in virtually all aspects of any society in developing countries ranging from transportation, construction, generators and cosmetics to various forms of commercial activities. All this is done without a comprehensive evaluation of impacts of these activities to the environment and to the health as in the case of oil spills. Oil spills have become a thing of great concern in developing countries. Oil spills in Nigeria have been a concerning matter for the Nigerian population, especially in the Niger-Delta region. Oil spills may occur for numerous reasons such as equipment failure, disasters, deliberate acts or human error (Anderson and LaBelle, 2000).Oil spills on the land and sea has been on the increase with explorative activities in the country. On the 28th of May, 1991, Angola witnessed a major oil spill of about 1, 907, 000 barrels of crude oil (Wang *et al*., 2011).Below is a list of major oil spills in some developing countries (Table 1).

Aquatic ecosystem has been contaminated as a result of oil spills and the public health acutely threatened. According to researchers, the oil clean-up will take up to 30 years to go back to full recovery with all of the environmental damage it has caused (Okwoche, 2011). Fifty percent (50%) of oil spills in Nigeria is due to corrosion, while twenty eight percent (28%) to sabotage and twenty one percent (21%) to oil production operations. One percent (1%) of oil spills is due to engineering drills, inability to effectively control oil wells, failure of machines, and inadequate care in loading and unloading oil vessels (Nwilo and Badejo, 2005).

When a big spill occurs there is usually a large group of volunteers, local inhabitants, who mobilize and take part in the cleanup work to minimize the impact of the spill on the natural and economic resources and recover the environment as soon as possible. These individuals constitute an exposed population whose health may be potentially affected by the noxious properties of the oil (Aguilera *et al*, 2010). Toxicity of crude oil includes liver necrosis, congestion of the liver, fat degeneration, and dissociation of hepatocytes (Sathishkumar *et al*., 2008).

Table 1: Major Oil Spill in some Developing Countries

Date	Location	Spill Type	Tons of crude oil	Barrels
1/23/1991	Iraq, Persian Gulf and Kuwait	Gulf War oil spill	270, 000 – 820, 000	2, 000, 000 – 6, 000, 000
6/3/1979–3/23/1980	Mexico, Gulf of Mexico	IxtocI	454 000 – 480 000	3 329 000- 3 520 000
7/19/1979	Trinidad and Tobago	Atlantic Empress/Aegean Captain	287 000	2 105 000
3/2/1992	Uzbekistan	Fergana Valley	285 000	2 090 000
2/4/1983	Iran, Persian Gulf	Nowruz Field Platform	260 000	1 907 000
5/28/1991	Angola	ABT Summer offshore	260 000	1 907 000
8/6/1983	South Africa, Saldanha Bay	Castillo de Bellver	252 000	1 848 000

Source: Wang *et al.* (2011)

Remediation of oil polluted environments is a difficult matter and can be very costly when using the following conventional methods of oil clean-up such as; use of dispersants, manual removal, use of chemicals, burning, cutting vegetation, passive collection sorbents, debris removal, trenching, removal of sediment, slurry and blasting and others(Michel *et al*., 2010). According to Porto *et al.* (2011), the damages from oil polluted environments are practically irreparable. The conventional methods above used to decrease the effects of oil spillage in the environment are usually a form of palliative solution and never definitive for the problems caused.

Due to the detrimental drawbacks of oil spills incidents which are becoming more rampant in developing countries scientists, researchers and environmentalists have developed new methods and forms of technology to facilitate the tasks of cleaning up oil spills (UN, 2011). One promising treatment method is to exploit the ability of microorganisms to remove these organic pollutants from contaminated sites (Finley *et al*, 2010). This is an alternative treatment strategy that is effective, minimally hazardous, economical, versatile and environment-friendly, is the process known as bioremediation (Finley *et al*, 2010). Bioremediation methods are currently receiving favorable publicity as promising environmental friendly treatment technologies for the remediation of hydrocarbons. Moreover, biological methods can have an edge over the physico-chemical treatment regimes in removing spills as they offer cost effective *in situ* biodegradation of oil fractions by the microorganisms (Sathishkumar *et al*., 2008).

1.1 Bioremediation

Lovely (2003) defines bioremediation as any process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition. Bioremediation technology using microorganisms was reportedly invented by George M. Robinson (Meagher, 2000).He was the Assistant County Petroleum Engineer for Santa Maria, California. During the 1960s, he spent his spare time experimenting with dirty jars and various mixes of microbes. The process by which microorganisms achieve oil bioremediation is by first being able to utilize these organic pollutants for their own metabolism by means of biodegradation.

According to Diaz (2004), biodegradation is the process by which organic substances are broken down into smaller compounds by the enzymes produced by living microbial organisms. The microbial organism transforms the substance through metabolic or enzymatic processes. The biogeochemical capacities of microorganisms seem almost limitless, and it is often said that microorganisms are “Earth’s greatest chemists” (Madigan *et al*., 2012). The activities of these great little chemists have been exploited in many ways.

Furthermore, Alexander (2001) sees biodegradation as the biologically catalyzed reduction in complexity of chemical compounds. It is based on two processes: growth and co-metabolism. The underlying principle behind microbial biodegradation of oil polluted environments is the ability to exploit the metabolic activities of these microorganisms in their natural environments where their nutritional and physical requirements can be influenced.

Microbial biodegradation should not be confused with microbial biodeterioration, where biodegradation is a useful activity vital for the recycling of matter, biodeterioration is an unfavourable activity which leads to the spoilage of an object or material by microorganisms that is still useful (Singleton and Sainsbury, 2006). Hence, biodegradation is a positive activity while biodeterioration is a negative activity.

According to Joanne *et al.* (2008), biodegradation can be used to describe three major changes in a molecule , it could either describe a minor change in the functional groups attached to an organic compound, such as the substitution of a hydroxyl group for a chlorine group or it could refer to an actual breaking of the organic compound into organic fragments in such a way that the original molecule could be reconstructed and finally it can describe the complete degradation of an organic compound to minerals, otherwise known as biomineralisation.

1.2 Role of Microorganisms in Biodegradation of Crude Oil Spills

Crude oil is a liquid mixture of a variety of hydrocarbon compounds derived from ancient algal and plant remains and found in reservoirs under the earth's surface (AAM, 2011). From the hundreds of individual components, several classes, based on related structures, can be recognized. Since these hydrocarbons from crude oil are naturally occurring organic compounds, it is of little or no surprise that microorganisms have evolved the ability to utilize these compounds. A diverse group of microorganisms have been implicated in petroleum hydrocarbon biodegradation in both aquatic and terrestrial habitats (Coronelli, 1996; Ijah and Antai, 2003).

When natural ecosystems are contaminated with petroleum hydrocarbons, the indigenous microbial communities are likely to contain microbial populations of differing taxonomic relationships which are capable of degrading the contaminating hydrocarbons (Atlas, 1981). According to the American Academy of Microbiology (2011), microbes that use oil as their source of energy have been around for hundreds of millions of years indeed, for as long as this energy rich substance has been available. Where oil is naturally present, for example, on the floor of the Gulf of Mexico, the community of microbes that collectively feeds on all the different compounds contained in the oil is well established and diverse (AAM 2011). Even where the background levels of oil are low, a few microbes with the capability of degrading oil always seem to be present.

The ability to metabolize oil is displayed by many different types of microbes, some more versatile than others. Research done by Ekpo and Udofia (2008) on rate of biodegradation of crude oil by microorganisms isolated from oil sludge environment showed that individual microbial species of *Bacillus subtilis, Micrococcus varians* and *Pseudomonas aeruginosa* degraded oil at different rates at 72.3%, 85.7% and 97.2% respectively with *P. aeruginosa* having the highest rate of degradation. Complex mixtures of petroleum hydrocarbons, such as crude oil and refinery sludge can be expected to alter the activity and structure of natural microbial communities (Van Hemme *et al.*, 2000). Song and Bartha (1990) found out that the proportion of hydrocarbon degrading microorganisms increases substantially upon exposure to hydrocarbons, reflecting the selectivity of carbon source by the microorganisms.

The ability to degrade petroleum hydrocarbons is not restricted to a few microbial genera; a diverse group of bacteria and fungi have been shown to have this ability (Atlas, 1981). ZoBell (1946) in his review noted that more than 100 species representing 30 microbial genera had been shown to be capable of utilizing hydrocarbons. In a previous review, Bartha and Atlas (1977) listed 22 genera of bacteria, 1 algal genus, and 14 genera of fungi which had been demonstrated to contain members which utilize petroleum hydrocarbons; all of these microorganisms had been isolated from an aquatic environment. The most important (based on frequency of isolation) genera of hydrocarbon utilizers in aquatic environments were *Pseudomonas*, *Achromobacter*, *Arthrobacter*, *Micrococcus*, *Nocardia*, *Vibrio*, *Acinetobacter*, *Brevibacterium*, *Corynebacterium*, *Flavobacterium*, *Candida*, *Rhodotorula* and *Sporobolomyces* (Bartha and Atlas, 1977).

Some cyanobacteria and algae have been tested and found to be capable of utilizing hydrocarbon from crude oil factions. Experiments carried out by Walker *et al.* (1975) described a hydrocarbon utilizing achlorophyllous strain of the alga *Prototheca*. Also, Cerniglia *et al.* (1980) tested nine cyanobacteria, five green algae, one red alga, one brown alga, and two diatoms for their ability to oxidize naphthalene. They found that *Oscillatoria* spp., *Microcoleus* sp., *Anabaena* spp., *Agmenellum* sp., *Coccochloris* sp., *Nostoc* sp., *Aphanocapsa* sp., *Chlorella* spp., *Dunaliella* sp.,*Chlamydomonas* sp., *Ulva* sp., *Cylindretheca* sp., *Amphora* sp., *Porphyridium* sp., and *Petalonia* all were capable of oxidizing naphthalene. Their results indicate that the ability to oxidize aromatic hydrocarbons is widely distributed among the cyanobacteria and algae (Atlas, 1981). Table 2 shows predominant bacterium in soil samples polluted with aliphatic and Aromatic Hydrocarbons, Polycyclic Aromatic hydrocarbon and Chlorinated compounds

Table 2: Representative Bacteria with Broad Hydrocarbon Biodegradative Abilities

Gram negative bacteria	Gram positive bacteria
Pseudomonas spp	*Norcardia* spp
Acinetobacter spp	*Mycobacterium* spp
Allcaligenes spp	*Corynebacterium* spp
Flavobacterium spp	*Arthobacter* spp
Xanthomonas spp	*Bacillus* spp

Source: Wolfgang (2007)

1.2.1 The Genus *Pseudomonas*

Pseudomonas is an aerobic gram negative rod shaped bacterium, it shows no fermentative activities and seem to have the highest degradative potential e.g., *Pseudomonas putida* and *P. fluorescens* (Wolfgang, 2007). Although, a wide phylogenetic diversity of bacteria is capable of degradation of pollutants, *Pseudomonas* spp and closely related organisms have been the most extensively studied owing to their ability to degrade so many different contaminants (Wackett, 2003). The immense potential of the *Pseudomonads* does not solely depend on the catabolic enzymes, but also on their capability of metabolic regulation (Wackett, 2003).The presence of multiple dioxygenases in *Pseudomonas* spp. and related strains dramatically expands the range of substrates capable of being catabolized (Palleroni *et al*, 2005). The dioxgenases which act on aromatic hydrocarbonns are dramatically broad in their substrate specificities (Wackett, 2001).

1.2.2 *Rhodococcus* spp

A second important group of degrading bacteria are the gram-positive rhodococci and coryneform bacteria. Many species, now classified as *Rhodococcus* spp. had originally been described as *Nocardia* spp., *Mycobacterium* spp., and *Corynebacterium* spp. Rhodococci are aerobic actinomycetes showing considerable morphological diversity. A certain group of these bacteria possess mycolic acids at the external surface of the cell. These compounds are unusual long-chain alcohols and fatty acids, esterified to the peptidoglycan of the cell wall. Probably, these lipophilic cell structures have significance for the affinity of rhodococci to lipophilic pollutants. In general, rhodococci have high and diverse metabolic activities and are able to synthesize biosurfactants (Wolfgang, 2007).

1.2.3 *Sphingomonas yanoikuyae* B1

S. yanoikuyae B1 previously known as *Beijerinckia* sp. strain B1, was originallyisolated for its ability to use biphenyl as a carbon source (Wackett, 2001). It was the first bacterium for which definitive metabolites were shown for multi-ring polycyclic compounds, such as benzo[a]pyrene and benzo[a]anthracene (Gibson *et al.,* 1975). *S. yanoikuyae* B1 is remarkable as a bacterium capable of oxidizing many aromatic compounds as its source of carbon and energy (Wackett, 2001). As such, it is representative of the genus *Sphingomonas,* a group of gram-negative bacteria generally known for their diverse catabolic activities.

2.0 PROCESS INVOLVED IN THE USE OF MICROORGANISMS IN OIL BIODEGRADATION

Polycyclic Aromatic Hydrocarbon (PAH) compounds are usually degraded under aerobic and anaerobic conditions. In both cases, a key step is the activation of the inert aromatic ring (Walter, 2001). In the presence of oxygen, this is carried out by oxygen-dependent enzymes. Under anaerobic conditions, however, the rate and extent of hydrocarbon biodegradation decreases and the variety of substrates degraded is typically narrower (Coates *et al*., 1997; Bertrand *et al*., 1989).

2.1 Aerobic Biodegradation

Wolfgang (2007) defines aerobic biodegradation as the breakdown of organic pollutants by microorganisms when oxygen is present. More specifically, it refers to a microbial catalytic process occurring or living only in the presence of oxygen; therefore, the chemistry of the system, environment or organism is characterized by oxidative conditions. Many organic contaminants are rapidly degraded under aerobic conditions.

Aerobic microorganisms have an oxygen based metabolism where aerobes through cellular respiration, use oxygen to oxidize substrates in order to obtain energy. Before cellular respiration begins, glucose molecules are broken into smaller molecules in the cytoplasm. Oxygen of the cells is used in the chemical reactions that breakdown small molecules into H_20 and CO_2 in a reaction that releases energy. Wolfgang, (2007) reported that the most important classes of organic pollutants in the environment are mineral oil constituents and halogenated products of petrochemicals, therefore , the capacities of aerobic microorganism are of particular relevance for the biodegradation of such compounds as exemplarily described with reference to the degradation of aliphatic and aromatic hydrocarbons as well as their chlorinated derivatives (Wolfgang, 2007). Examples of some microorganisms that carry out aerobic biodegradation include *Rhodococcus* sp. , *Burkholderiaxenovorans, Pseudomonas spp* (McLeod and Eltis, 2008)

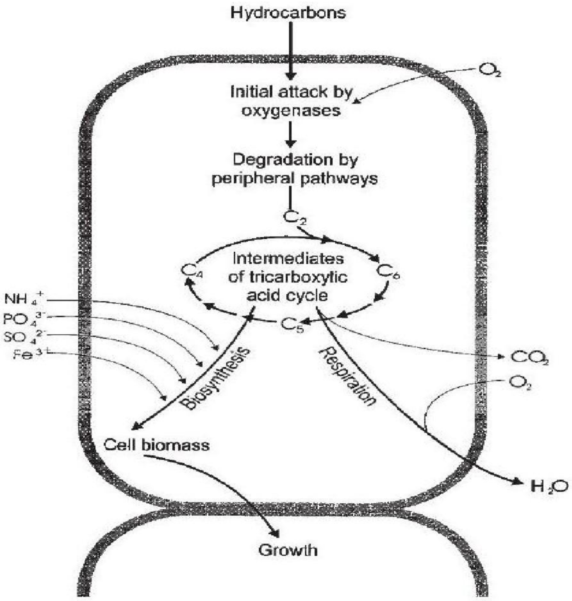

Fig 1: Main principles of aerobic degradation of Hydrocarbons (Wolfgang, 2007)

2.2 Anaerobic Biodegradation

Many polluted environments are often anoxic, e.g., aquifers, aquatic sediments and submerged soils. In such environments, biodegradation is carried out by strict anaerobes or facultative microorganisms using alternative electron acceptors, such as nitrate (denitrifying organisms), sulfate (sulfate reducers), Fe(III) (ferric-ion reducers), CO_2 (methanogens), or other acceptors such as chlorate, Manganese, Chromate, and others (Gibson and Harwood, 2002; Lovely, 2003; Widdel, 2001). Anaerobic biodegradation involve a series of processes in which microorganism's breakdown biodegradable materials in the absence of oxygen (Murphy, 2004).

The use of electron acceptors other than oxygen is based on: The electron-acceptor availability and the competition of different respiratory types of microorganisms for electron donors. For example, reduction of Fe(III) is the most frequent mechanism for oxidation of organic matter in subsurface environments. Sulfate is a major electron acceptor for the anaerobic degradation of contaminants in marine environments due to the high concentrations of sulfate in seawater (Lovely, 2003).

In terms of energy, whereas degradation of aromatics using nitrate and Fe(III) as terminal electron acceptors is almost as efficient as that using oxygen, sulfate reducers and methanogenic conditions generate comparatively much less energy (Field *et al.*, 1995). Consequently, the molar cell yields under methanogenic and sulfidogenic conditions are rather low.

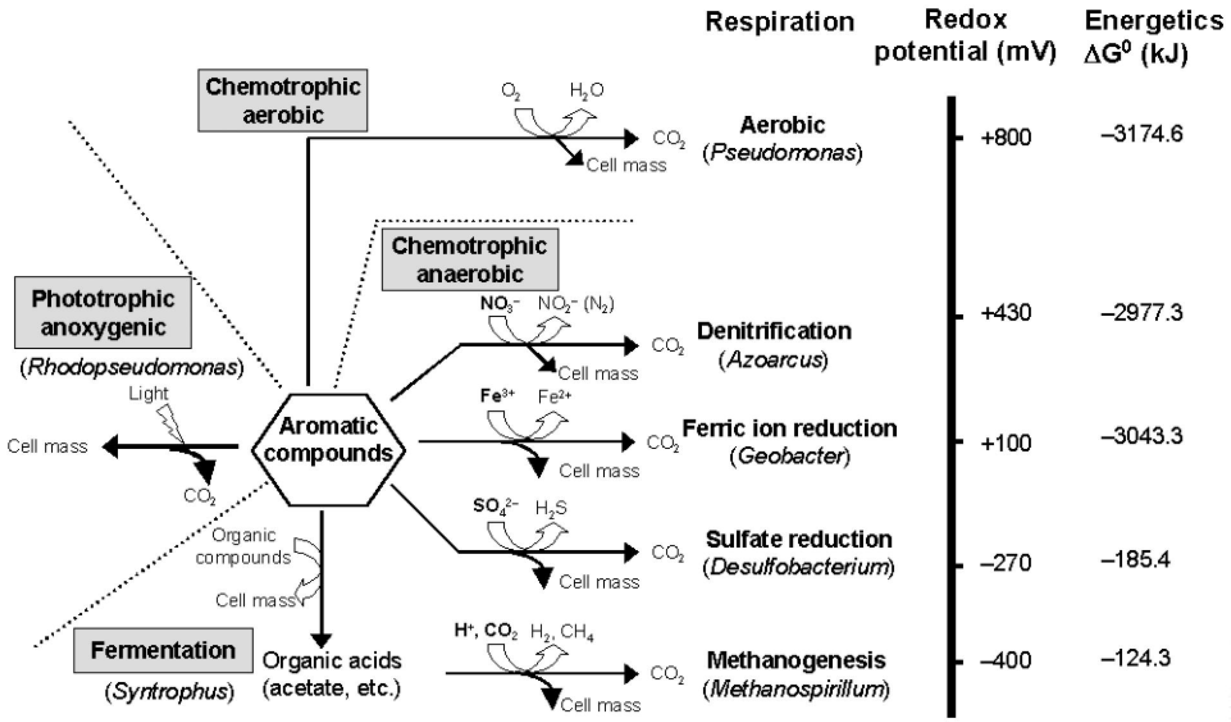

Fig 2: Microbial utilization of aromatic compounds. The different terminal electron acceptors in respiration are indicated in bold and they are aligned with the redox potential bar. The energetics (free-energy changes) of the aerobic and anaerobic degradation of a model aromatic compound, benzoate, are indicated on the right. Methanogenesis needs to be coupled to fermentation reactions. Bacterial genera representative of each type of metabolism are shown in parentheses. Source: Diaz (2004)

2.3 Aerobic versus Anaerobic Microorganisms in Biodegradation

The presence or absence of oxygen often dictates the type of biodegragative pathways and the types and number of bacteria involved in the biodegradation of a particular compound (Wackett, 2001). In practical biodegradation, the choice of fostering aerobic or anaerobic condition is often a acrucial one.Wackett (2001) reported that, more knowledge is accumulated about Aerobic and facultative Aerobic microorganism. Organisms such as *Pseudomonas* sp or *Escherichia coli* can be grown overnight with simple equipment to yield high cell densities. In contrast, anaerobic enrichment cultures may initially show very long lag before significant biodegradation occurs. Upon repeated transfers, the lag phase often shortens continually. Still many years may be required to achieve significant high rates of biodegradation and they may never approach the rates of comparable aerobic biodegradation (Wackett, 2001). Aerobic processes typically yield more energy, generate a commensurately greater number of ATP equivalents, and generate more biomass per unit of compound transformed (Dagley, 1975; Thauer *et al.,* 1977). Thus, compounds with a significant energy yield upon oxygenative metabolic to carbon dioxide and water are often rapidly biodegraded under aerobic conditions.

3.0 CHALLENGES INVOLVED IN THE USE OF MICROORGANISMS IN OIL BIODEGRADATION

There are a number of challenges to the use of microorganisms for the biodegradation of oil. The biodegradation of oil pollution or spills in the environment is a complex process where quantitative and qualitative aspects depend on the nature and amount of the pollutant present, the ambient and the seasonal environmental condition, and the constitution of the indigenous microbial community (Leahy and Colwell, 1990; Hinchee and Olfenbuttel, 1991).

Sathishkumar *et al*. (2008) carried out a research which aimed to explore the possibility of the use of selected bacterial cultures and a mixed bacterial consortium to degrade crude oil at various pH, temperatures, and oil concentrations, since extreme pH and temperature conditions are expected to have a negative influence on the ability of microbial populations to degrade the hydrocarbons (Rahman *et al*., 2008). Sathishkumar *et al*. (2008) found out that microorganisms show best biodegradative potential at optimum Temperature and pH. Hence, where these environmental conditions are not at optimum for the degrading species of microbes present at the oil polluted site, biodegradation is known to occur at a less optimum rate, since the fate of hydrocarbon degradation is largely determined by the local environmental conditions, which influence the microbial growth and enzymatic activities.

The rates of uptake and mineralization of many organic compounds by a microbial population depend on the concentration of the compound (Olivera *et al*. 1997). Inhibition of biodegradation may occur due to high concentrations of undispersed volatile hydrocarbons. Bacteria used to remediate pollutants probably undergo environmental stress due to high concentrations of toxic contaminants, toxic solvents, extreme pH, temperature, ionic strength, etc. (Timmis and Pieper 1999).

To determine the effect of concentration on microbial degradation, Sathishkumar *et al*. (2008) experimented with selected isolates and mixed bacterial consortium on various concentrations of crude oil (1, 3, 6, 9, and 12%). For all the concentrations, the experiment was conducted at 35°C and pH 7. The inoculated flasks were incubated for 25 days and bacterial growth and crude oil degradation were estimated. The effects of crude oil concentrations on the growth of individual bacterial cultures and the mixed bacterial consortium, and crude oil degradation by them were tested and the results showed that the mixed bacterial consortium had 76% degradation at 1% BH crude oil, followed by 72% at 3%, 63% at 6%, 52% at 9%, and 41% at 12%. The individual cultures also showed the good degradation potential at 1% BH crude oil and decreased degradation at higher concentrations of the crude oil.

Another major challenge for the persistence of some aromatic hydrocarbon compounds in the environment is their limited bioavailability (Diaz, 2004). Typically, petroleum hydrocarbons present are frequently attached to soil particles, making them non bioavailable to the degrading microorganisms.The hydrocarbon degrading microorganisms produce biosurfactants of diverse chemical nature and molecular size (Jagadevan and Mukherji, 2004). These surface active materials increase the surface area of hydrophobic insoluble substrates and increase their bioavailabilty, thereby enhancing the growth of bacteria and the rate of bioremediation. A phenomenon unique to surfactants is the self assembly of molecules into dynamic clusters called micelles (Jagadevan and Mukherji, 2004). Volkering *et al*. (1993) looked for an enhancement in the biodegradation rates due to the production of surface active compounds produced by bacteria. Surprisingly, there was no improvement in the biodegradation rates in their study because the solubilising effect of surfactants is attributed mainly to the formation of micelles.

Some other challenges in oil biodegradation can be seen in the Fig 3.0 below.

3.0 PROSPECTS FOR THE USE OF MICROORGANISMS IN OIL BIODEGRADATION

Despite the advantages to the use of microorganisms in oil bioremediation, due to the fact that microorganisms have acquired the ability to use polyaromatic hydrocarbons as carbon and energy sources. Their efficiency at removing such pollutants might not be optimal for cleaning up present-day pollution (Diaz, 2004). In fact, microorganisms have evolved towards ecological fitness rather than biotechnological efficiency; thus, it would take a long time for bacteria capable of cleaning up anthropogenic pollution to evolve by natural selection (Diaz, 2004).

New methodological breakthrough in sequencing, genomics, proteomics, bioinformatics and imaging are producing vast amount of information in the field of Environmental microbiology, genetic engineering and gene manipulation to open a new era providing unprecedented *in silico* views of metabolic and regulatory networks, as well as clues to the evolution of degradation pathways and to molecular adaptation strategies to changing environmental pollution conditions using microorganisms (Diaz, 2004). These novel techniques are known to effectively contribute to cost reduction, minimizing chemical use and also improving cost-benefit ratios (Peixoto *et al*., 2011). Hence, studying the physiology, biochemistry and genetics of the catabolic pathways becomes crucial to recreate and accelerate natural processes in the test tube as well as to accomplish their rational manipulation to design more efficient biocatalysts for different biotechnological applications. These include: (i) bioremediation of polluted sites, (ii) biotransformation of toxic compounds into fine chemicals and other high added-value products (green chemistry), and (iii) development of *in situ* biomonitoring devices and biosensors to monitor pollutant bioavailability (De Lorenzo, 2001; Schmid *et al*., 2001; Timmis and Pieper, 1999).

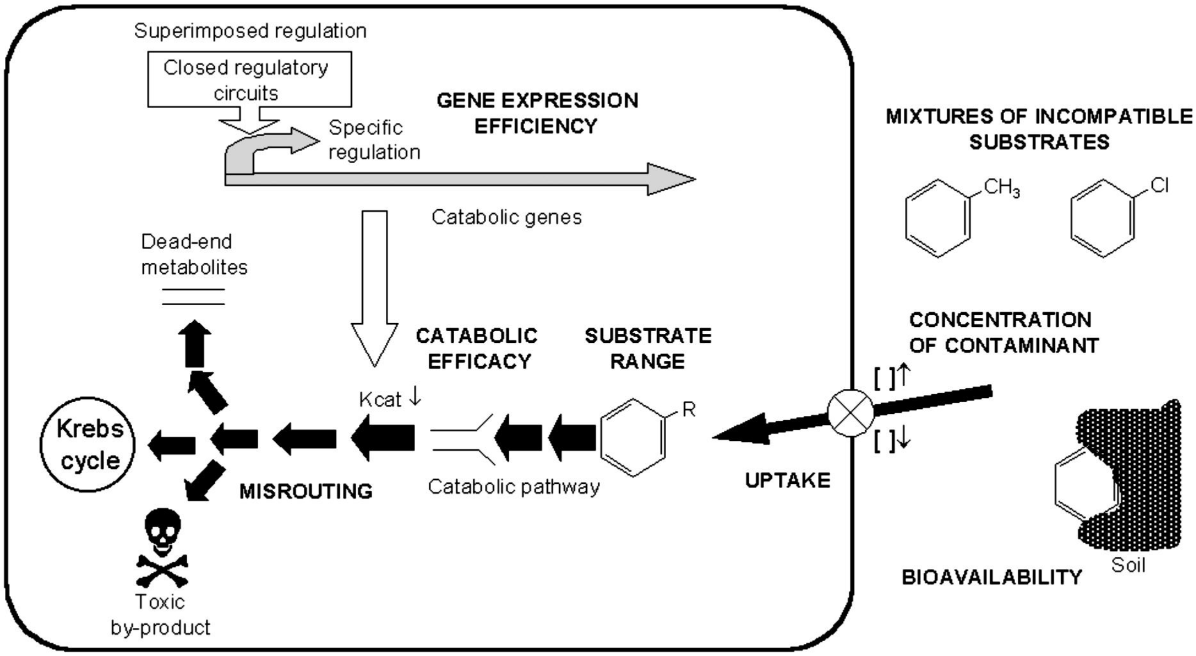

Fig. 3.0: Potential limitations of biological treatment approaches. The major problems that challenge biocatalyst performance are indicated bycapital letters. **Gray arrows**, genes; **black arrows**, proteins.
Source: Diaz, (2004).

Many PCR primers that target genes related to petroleum-degrading enzymes, both in aerobic and anaerobic conditions are now available. The utilization of these already-characterized primers may facilitate environmental screening of degrading abilities and may help to evaluate the potentials of microbial isolates (Peixoto *et al*., 2011). More primers can be described for specific pathways or to improve the comprehensiveness of known primers using available databases.

Also, the host cell can be manipulated to enhance bioavailability of the pollutant by engineering the production of biosurfactants or by promoting chemotaxis of the biodegrader to the toxic compound (Dua *et al*., 2002; Reineke, 1998). Surfactant production has been combined with the ability to selectively cleave carbon-sulfurbonds in the sulfur-containing compounds present in oil (biodesulfurization) and, hence, efficient recombinant biodesulfurizers have been obtained (Gallardo *et al*., 1997). Some anaerobes release Fe(III) chelators, which solubilize Fe(III) from Fe(III) oxides, and electron-shuttling compounds, which accept electrons from the cell surface and then reduce Fe(III)oxides. It has been shown that enhancing the availability of some electron acceptors, such as the insoluble Fe(III)oxides, by adding suitable ligands can greatly stimulate anaerobic degradation of contaminants in subsurface environments (Lovely, 2003).

The benefits provided by molecular tools can open unlimited windows of opportunity, as it is possible to detect genes from cultivable or noncultivable organisms (using metagenomics) and to express these genes in cultivable organisms, using enzymes that were not yet described (Peixoto *et al*., 2011).

Biosafety is a major issue when releasing recombinant microorganisms into any open environment. In order to address this concern, several genetic circuits have been developed to allow survival of the recombinant microorganism only when present in the polluted site and during the time required for removal of the pollutant (biological containment). To avoid dispersal of the recombinant trait from the recombinant bacteria to the native microbial population, different gene-containment circuits based on a toxin and its cognate antidote have also been developed. Such active containment systems reduce significantly the potential risks that release of recombinant bacteria might cause in the ecosystem (Ramos *et al*., 1995; Torres *et al*., 2004).

5.0 CONCLUSION

The versatility of the use of microorganism in addressing human problems cannot be overemphasized. In an economy where virtually all aspects is run by application of petroleum oil directly or indirectly without innovative sourcing and application of alternate forms of energy to relieve the dependence on crude can lead to varied adverse effect in cases of oil spills. These effects require less expensive and environmentally friendly way to tackle them and the most viable option involves the use of the great degradative abilities offered by some of these indigenous microorganisms. The abundance of microorganism, together with their high growth rates allows them to evolve quickly and to adapt to environmental changing conditions, even to extreme environments that do not allow proliferation of other living organisms. The use of molecular techniques in producing 'super bugs' or genetically modified organisms under strict non-proliferation rules provides a huge advantage in combating the menace of oil spills in these developing countries.

Correspondence to:
Names: Ikhimiukor Odion O., Nneji Lotanna, M.
Address^1: Department of Microbiology, University of Ibadan, Ibadan, Nigeria
Address^2: Department of Biological Sciences, University of Abuja, FCT
E-mail address: odido1@yahoo.com, lotannanneji@gmail.com

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