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Review

Approaches to bioremediation of fossil fuel contaminated soil: An overview

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A reliance on fossil fuels as a source of energy has resulted in the generation of pollutants which have entered the environment. Health of humans, animals, plants and microorganisms has been compromised due to activities linked to fossil fuel extraction, processing and use. Coal conversion to value added products has been investigated in an effort to reduce the cumulative effects of waste generated during mining. Clean coal technology, developed to convert coal into value added products with reduced pollution, has been a major source of liquid petroleum in South Africa. Although the conversion process, neither generates waste nor pollutes the soil environment, the final products either through accidental or deliberate spillage can have a severe and protracted impact. Biological methods for combating pollutants generated within the fossil fuels sector are preferred to mechanical or physicochemical practices. This is due to the production of non- or less toxic by-products, cost effectiveness and safety. In this manuscript, an overview of the approaches adopted and factors influencing microbial metabolism of fossil fuel contaminants in soil and water bodies is presented. In particular, emphasis is placed on bacteria as biocatalysts of choice and their ability to degrade waste coal and liquid petroleum hydrocarbons.

Key words: Fossil fuels, coal, petroleum hydrocarbons, biodegradation, pollutants.

INTRODUCTION

Fossil fuels are natural substances formed from the remains of ancient plants and animals. Over time, heat and pressure converted these remains into fuels which release energy when burned. The term fossil fuel also includes hydrocarbon-containing natural resources that are not derived from animal or plant sources. These are sometimes called mineral fuels. For the purpose of this review, the hydrocarbons derived from decayed plants and animals will be referred to as fossil fuels. The age of these ancient plant and animal fossil fuels is typically millions of years, and in some cases, in excess of 650 million years (Mann et al., 2003). Different types of fossil fuels are formed depending on the combination of animal and plant debris present. However, the length of time for which the material was buried and the temperature and pressure during decomposition also contributed to the

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type of fossil fuel formed. Fossil fuel has been broadly divided into three categories based on the mode of its formation. These are solid, liquid and gaseous fossil fuels and each is characterized by a high carbon and hydrogen content. Within these categories are volatile materials with low carbon: hydrogen ratios such as methane, liquid petroleum and the non-volatile materials composed almost of pure carbon, like anthracite coal. Fossil fuels have played an important role in providing energy for transportation, power generation, industrial growth, agricultural production and other basic human needs (Basha et al., 2009). Irrespective of the major roles that fossil fuels have played in sustaining the global economy, combustion of these fuels is a major source of anthropogenic CO_2 emissions (Muradov, 2001). For the purpose of this review, only the solid and liquid fossil fuels will be discussed.

Fossil fuel resources are generally a major source of revenue for the main oil and gas producing countries in Africa (Zalik and Watts, 2006). South Africa, which is one of the world's largest producers (5^{th}) and consumers (7^{th}) of fossil fuels (BP, 2012), has experienced a boost in her economy due to the production, consumption and exportation of coal (UNECA, 2011). Increased production over the last 30 years and an over reliance on coal as a source of energy has stimulated revenue accrual (ERC, 2004). BP statistics in 2011 showed that Africa has enormous potential in the fossil fuels sector with proven reserves accounting for about 9.5, 8 and 4% of the crude oil, natural gas and coal in the world, respectively (BP, 2011). The generation of electricity from fossil fuels cannot be neglected as more than 80% of electricity generated across the continent of Africa is from fossil fuels (IEA, 2011). The generation and supply of energy from fossil fuels has also been documented. IEA, in their 2011 annual report, stated that fossil fuels account for about 50% of the total energy supply and one-third of the energy consumed (IEA, 2011).

Huge problems have emerged due to an over reliance on fossil fuels and when viewed from an environmental and social perspective, it affects societies locally, regionally and globally (UNECA, 2011). Some of these problems include ozone depletion, global warming, acidification, and depletion of non-renewable resources. According to Höök and Tang (2013), energy production is the principal contributor to release of greenhouse gases, in particular CO_2, with fossil fuel combustion the major source. Of the three categories of fossil fuels, liquids (petroleum) and solids (coal) are the major contaminants in the environment. Any unwanted substance introduced into the environment is referred to as a 'contaminant' and the deleterious effects of these contaminants leads to 'pollution', a process in which a resource (natural or man-made) is rendered unfit for use, more often than not, by humans (Megharaj et al., 2011).

The drastic increase in the demand for coal has led to an increase in mining of this natural resource in countries like South Africa with subsequent generation of wastes and an increase in the level of pollution. One of the basic reasons behind the increase in demand for coal is due to the various products derived from coal during its conversion processes. For instance, in South Africa where liquid and gaseous fossil fuels are not readily available, coal liquefaction is one option available for obtaining these products. However, the utilization of coal and coal derived products is associated with serious environmental problems from the mining stage through to its final utilization by consumers (Geo-4, 2007). To reduce environmental damage by this energy source, new conversion technologies are urgently needed. One of the strategies adopted in reducing environmental damage is clean coal technology. Clean coal technologies which make use of biological processes to effect pollutant biodegradation have received considerable attention in recent years (Klein et al., 2008; Sekhohola et al., 2013). Similarly, the use of biocatalysts to remediate liquid petroleum hydrocarbon and diesel contaminated sites has been the subject of much recent attention (Sander et al., 2010; Diya'uddeen et al., 2011; Vaidehi and Kulkarni, 2012; Elazhari-Ali et al., 2013; Kang, 2014). In this paper, we present an overview of some of the approaches used in the biodegradation of coal, coal related contaminants, and liquid hydrocarbon pollutants in an effort to stimulate the search for and emergence of successful bioremediation strategies.

BIODEGRADATION OF COAL AND COAL-RELATED PRODUCTS

Studies on the breakdown of coal by bacteria and fungi started as far back as 1920 (Olson and Brinckman, 1986). Although, it was accepted that microorganisms are capable of degrading coal, significant research effort occurred only after demonstration of the successful breakdown of coal by bacteria (Fakoussa, 1981). One year later, Cohen and Gabriele (1982) demonstrated the breakdown of low rank leonardite using wood rot fungi. Following these breakthroughs, intensive study by various research groups was carried out with the aim of establishing a better understanding of the mechanisms involved in the biological transformation of coal and in combination with the production of value-added products (Polman et al., 1994; Fakoussa and Frost, 1999; Fakoussa and Hofrichter, 1999; Gotz and Fakoussa, 1999; Ralph and Catcheside, 1999; Machnikowska et al., 2002; Igbinigie et al., 2008; Jiang et al., 2013).

The complexity and recalcitrance of coal suggested initially that microorganisms might not be able to modify the physicochemical structure of this substrate. Thus, according to Klein et al. (2008), the colonization and breakdown of coal by microorganisms was not possible

unless certain necessary conditions such as moisture content, mineral salt availability, additional nitrogen sources and a stable pH were met. To date, a number of microorganisms have been identified as being able to modify the structure of coal (Yuan et al., 2006; Kang, 2014). Different mechanisms as suggested by various authors appear to be used to achieve modification of the coal structure and these include enzymatic changes (Cohen et al., 1987; Pyne et al., 1987; Fakoussa and Hofrichter, 1999), alkaline solubilisation (Strandberg and Lewis, 1987; Quigley et al., 1989a), metal ion chelation, and the action of surfactants (Fakoussa, 1988; Quigley et al., 1989b; Fredrickson et al., 1990).

Enzymatic modification of coal structure

A large number of biological molecules responsible for many chemical interconversions have been linked to the structural modification of coal otherwise called depolymerization (Hofrichter and Fakoussa, 2001). The depolymerization of brown coal occurs at low pH values (pH 3-6) resulting in the cleavage of bonds inside the coal molecular structure which leads to the formation of yellowish, fulvic-like substances with low molecular mass (Hofrichter and Fakoussa, 2001). Although a wide range of enzymes with coal degrading ability have been identified the majority appear to be from fungi. For instance, Sekhohola et al. (2013) provided a detailed list of the purported catalysts used in coal biodegradation which shows that nearly all of the enzymes that have been linked to coal biodegradation are of fungal origin. Even so, contradictory reports have been published with regard to fungal activity and breakdown of coal (Torzilli and Isbister, 1994). For instance, studies by Cohen et al. (1987) initially suggested that the ability of fungi to degrade coal was the result of enzymatic activity. However, in a subsequent report, these authors identified the coal solubilizing agent from *T. versicolor* by infrared spectroscopy and x-ray studies as ammonium oxalate monohydrate (Cohen et al., 1990) while Fredrickson et al. (1990) argued that the coal solubilizing activity of *T. versicolor* was not ammonium oxalate monohydrate but a siderophore-like compound. In addition to fungi, several gram positive and negative bacteria have been implicated in the biodegradation of coal.

Studies by Crawford and Gupta (1991) demonstrated that extracellular bacterial enzymes were capable of depolymerizing a soluble coal polymer although the enzymes involved were neither specified nor identified. Nevertheless, the depoly-merisation process appears to be non-oxidative which may indicate that non-oxidative, enzymatic depolymerization of coal is possible. Reports on the utilization of low rank coal as a source of carbon by several bacteria including *Pseudomonas oleovorans, Rhodococcus ruber* and *Bacillus* sp. Y7 have also been published (Fuchtenbusch and Steinbuchel, 1999; Jiang et al., 2013). The ability of *Bacillus* sp. Y7 to degrade lignite was attributed to the production of extracellular substances (Jiang et al., 2013) while oxidized lignin solubilisation was ~90% in the presence of *Pseudomonas putida* (Machnikowska et al., 2002). For the latter example however, it was stated that pre-treatment of lignite with nitric acid was essentially responsible for the enhanced rate of biodegradation. In an experiment carried out by Tripathi at al. (2010) on the fungal biosolubilisation of lignite and the subsequent production of humic acid, these authors concluded that the likely mechanism of lignin breakdown by fungi was somehow linked to action of oxidative (peroxidases and laccases) and hydrolytic enzymes (esterases) initially secreted by bacteria confirming an earlier observation based on a comparative study of coal solubilisation by both bacteria and fungi (Torzilli and Isbister, 1994). Some of the enzymes secreted by fungi which are believed to play a major role in the biodegradation of coal include lignin peroxidase (Hofrichter and Fritsche, 1997b; Laborda et al., 1999), laccase (Fakoussa and Frost, 1999), esterase (Laborda et al., 1999) and phenol oxidase (Laborda et al., 1999) and although their precise role in coal biodegradation remains unclear, a model for the phyto-biodegradation of low rank coal by mutualistic interaction between ligninolytic microorganisms and higher plants has recently been proposed (Sekhohola et al., 2013).

Alkaline substance modification of coal structure

A different mechanism of coal biodegradation has been suggested based upon results which indicate microbial secretion of alkaline substances that facilitate the breakdown of coal (Quigley et al., 1988). During this non-enzymatic process, often the formation of black liquids is observed coincident with higher pH (pH 7-10). The increase in pH has been attributed to the release of alkaline substances by bacteria which aid in coal solubilisation (Hofrichter and Fakoussa, 2001). The actual mechanism of coal biodegradation by bacteria due to alkaline substances is not well defined and as a consequence, not fully understood. Thus, Machnikowska et al. (2002), in an experiment on the microbial degradation of low rank coals, reported an increase in pH of medium containing sub-bituminous coal and suggested that the pH change arose as a result of the production of alkaline substances. Details of the alkaline substances involved however, in this and other studies and the effect of these on coal biodegradation remain obscure. As highlighted by Sekhohola et al. (2013) many different bacteria appear capable of secreting alkaline substances when inoculated into coal media including; *Pseudomonas putida, Arthrobacter* sp., *Streptomyces viridosporous, Streptomyces setonii, Bacillus pumilus,* and *Bacillus cereus.*

Metal ion chelation and the action of surfactants on the modification of coal

Experiments on coal bio-solubilisation carried out by Yin et al. (2011) pointed to the importance of surfactants in the synthesis of enzymes responsible for coal breakdown. These authors went further and showed that in the absence of surfactants; limited enzymes were adsorbed onto the coal surface while the reverse was the case in the presence of surfactants. Thus, interaction between enzyme and coal is possibly due to the presence of surfactants which modify the charge and the hydrophilic properties of the coal surface (Yin et al., 2011). Nonetheless, studies on the biological breakdown of coal have concentrated on fungi as the biocatalysts of choice and very few reports have examined the contribution by bacteria. A summary of the historical progress made so far in the field of coal biodegradation is presented in Table 1.

BIODEGRADATION OF PETROLEUM HYDROCARBONS

The biosolubilisation of coal and the serial production of liquid fuels has been investigated (Ackerson et al., 1990). In this report, bio-extracts from solubilized coal were converted to liquid alcohols, one of the earliest clean coal technologies for petroleum production. In South Africa many petroleum products are derived from coal using Fischer-Tropsch synthesis including fuels, plastics, oils, synthetic rubbers etc. Globally, there is high demand for petroleum products (Hasan et al., 2010) and during transportation of these from point of production to point of consumption spillage is inevitable (Das and Chandran, 2011). It has been estimated that natural crude oil seepage exceeds 600000 metric tons per year with a range of uncertainty of 200000 metric tons per year (Kvenvolden and Cooper, 2003). Accidental or deliberate release of crude oil into the environment has also led to serious pollution which affects both water and soil resources (Atlas, 1981; Okoh, 2006). Just like coal, different strategies including mechanical, chemical and biological have been developed and used to remediate sites contaminated with these petroleum hydrocarbons (Lohi et al., 2008).

A common mechanical means of remediating petroleum contaminated waters includes floating booms, skimmers, and oil-water separators (Ventikos et al., 2004; Yang et al., 2000). Unfortunately, removal of spilled oils from contaminated sites by these means is usually incomplete leading to progressive accumulation of residual hydrocarbons (Yang et al., 2000). Chemical remediation of oil contaminated sites on the other hand has been associated with increased dissolution of oil in seawater, which affects both water bodies and benthic biota (Doerffer, 1992). The reason why this technology is associated with increased dissolution of oil in water is because it makes use of chemical dispersants such as surfactants (Lohi et al., 2008). In contrast to the above, biological remediation technologies which have been intensively studied both in controlled conditions and field experiments (Okoh, 2006), appear to be the most environmentally friendly methods for removal of hydrocarbon pollutants (Barathi and Vasudevan, 2001; Balba et al., 2002; Urum et al., 2003; Liu et al., 2008; Das and Chandran, 2011). Bioremediation, which is one example of a biological remediation process, has been defined as the use of microorganisms to detoxify or remove pollutants from contaminated water and soil bodies (Medina-Bellver et al., 2005; Mukherjee and Bordoloi, 2012) and a comparison of treatment costs for South Africa reveals that it is by far the most economical technology (Table 2).

Different microorganisms including bacteria and fungi have been used to remediate hydrocarbon contaminated sites. Addition of nutrients to an oil spilled site to stimulate the growth of resident microorganisms in degrading contaminants is known as biostimulation while isolation, growth and introduction of microorganisms (that can degrade contaminants) from a different environment into oil spilled sites to remediate those sites is known as bioaugmentation. It has been argued that biostimulation is a superior technique to bioaugmentation (Alexander, 1999; Van Hamme et al., 2003; Philp and Atlas, 2005; Lohi et al., 2008) based on the outcome of field experiments (Abdulsalam et al., 2011). Studies by Devinny et al. (2000) and Bento et al. (2005) seem to support the above conjecture and show that augmented microorganisms easily lose their intrinsic degradation ability during the time it takes for acclimatisation to the new environment. Different amendments have been used to stimulate resident microorganism populations in oil spilled environments and a summary of these is presented in Table 3.

According to D'Annibale et al. (2006) and Yi et al. (2011), fungi are the organisms of choice with regards to bioaugmentation as these synthesize relatively unspecific enzymes involved in cellulose and lignin decay. Fungal enzymes degrade high molecular weight, complex and more recalcitrant toxic compounds, including aromatic structures (Grinhut et al., 2007; Mancera-Lopez et al., 2008). However, Sutherland (1992) explained how fungi degrade hydrocarbons indirectly by co-metabolism and stated that fungi generally do not utilize petroleum hydrocarbons (PHC) as their sole carbon and energy source but transform these compounds co-metabolically to detoxified metabolites. Different fungal species have been implicated in bio-augmentation studies involving both low and high molecular weight polyaromatic hydrocarbons (PAHs) in soils. For instance, Mancera-Lopez et al. (2008) carried out studies on petroleum hydrocarbon contaminated soils using *Rhizopus* sp.,

Table 1. Historical overview of advances in coal bioconversion.

Year	Progress	Reference
1981	Effects on hard coals by *Pseudomonas* strains, simultaneous biotenside-excretion	Fakoussa (1981)
1982	Solubilization of lignite to droplets on agar plates by fungal action	Cohen and Gabriele (1982)
1986	Acceleration of solubilisation by pre-treatment of coal	Scott (1986), Grethlein (1990)
1986	Solubilisation of coal by an extracellular component produced by *Streptomyces setonii* 75Vi2 in submerged culture	Strandberg and Lewis (1987)
1987	First solubilisation mechanism elucidated: production of alkaline substances (fungi + bacteria)	Quigley et al. (1988), Quigley et al. (1989a), Quigley et al. (1991)
1988	Second mechanism elucidated: production of chelating agents	Quigley et al. (1988), Quigley et al. (1989), Cohen et al. (1990), Quigley et al. (1991)
1989	First product on market: Solubilized lignite as fertilizer	Arctech Inc. (2007)
1991	Evidence that chelators alone are not responsible for all effects	Fakoussa (1994)
1994	Decolourisation and reduction of molecular weight of soluble lignite-derived humic acids proves catalytic enzymatic attack	Ralph and Catcheside (1994), Hofrichter and Fritsche (1997a and b)
1994	Analysis of low-molecular mass products from bio-solubilised coal	Toth-Allen et al. (1994)
1997	In vitro systems shown to degrade humic acids and attack matrix and coal particles	Hofrichter and Fritsche (1997a and b)
1997	First fine chemical produced successfully from heterogeneous humic acid mixtures to polyhydroxyalkanoates (PHA, “Bioplastic") by pure cultures	Fuchtenbusch and Steinbuchel (1999)
1999	Involvement of laccase in depolymerization of coal implied by conversion of coal humic acid to fulvic acids *in vivo* by *Trametes versicolor* (basidiomycetous fungi)	Fakoussa and Frost (1999)
2001	Microbial solubilisation of lignites. Preliminary gasification tests with solubilized coal yielding 21% energy recovery from methane	Gokcay et al. (2001)
2006	Mechanisms of coal solubilisation in *Penicillium decumbens* P6 combination of production of alkaline materials, peroxidase and esterase. First report on involvement of biosurfactant in coal solubilisation by fungi	Yuan et al. (2006)
2007	Degradation of LRC by *Trichoderma atrovide* (ES 11)	Silva-Stenico et al. (2007)
2007	Phytoremediation of coal mine spoil dump through integrated biotechnological approach	Juwarkar and Jambhulkar (2008)
2008	The effect of the particulate phase on coal biosolubilisation mediated by *Trichoderma atrovide* in a slurry bioreactor	Oboirien et al. (2008)
2008	Fungal biodegradation of hard coal by a newly reported isolate, *Neosartorya fischeri*	Igbinigie et al. (2008)

Table 1. Contd

2013	Formation of biosolubilised humic acid from lignite using *Bacillus* sp. Y7	Jiang et al. (2013)
2013	Production of methane from coal by a fungal isolate *Penicillium chrysogenum* MW1	Haider et al. (2013)

Table 2. A comparison of soil remediation treatment technology costs in South Africa.

Method of treatment	Approximate cost (ZAR/tonne soil)
Biological	70 - 2 395
Chemical	169 - 8 455
Physical	282 - 2 395
Solidification/stabilization	239 - 2 409
Thermal	423 - 10 569

Table 3. Examples of various biostimulation methods used to treat hydrocarbon contaminated sites.

Amendment type	Reference
Chelating agents	Da Silva et al. (2005)
Activated sludge from wastewater treatment	Juteau, et al. (2003), Maki et al. (1999)
Bio-solids and maize	Sarkar et al. (2005), Rivera-Espinoza and Dendooven (2004)
Immobilized-cell systems	Chen et al. (2009)
Nitrogen and phosphorous	Jiménez et al. (2006), Bento et al. (2005), Evans et al. (2004)
Surfactants or bio-surfactants	Rahman et al. (2002)
Bulking agents e.g. wheat straw, hay and sawdust	Namkoong et al. (2002), Rahman et al. (2002), Rhykerd et al. (1999)
Biocompatible hydrophobic solvents	Zawierucha et al. (2011)

Penicillium funiculosum and *Aspergillus sydowii* isolated from two aged soils contaminated with petroleum hydrocarbons and showed that each fungus was able to degrade PAHs effectively when compared to biostimulated soils. Bacteria on the other hand, though able to degrade aromatic hydrocarbons, only degrade low molecular weight PAHs. Many pure cultures of bacteria, including various strains of *Pseudomonas putida*, have been evaluated for their benzene, toluene and xylene (BTX) biodegradation potential (Jean et al., 2002, 2008). The highest PAHs that bacteria have been recorded to degrade are the PAHs containing four benzene rings such as pyrene and chrysene (Boonchan et al., 2000).

Mukherjee and Bordoloi (2011) reported that remediation of oil spilled sites usually requires the cooperation of more than a single species of microorganism because individual microorganisms can metabolize only a limited range of hydrocarbon substrates. Therefore, assemblages of mixed populations with overall broad enzymatic capabilities are required to energize the rate and extent of petroleum hydrocarbon degradation. Thus, various researchers have shown that consortia comprising bacteria and fungi are better bioaugmentation agents than individual bacterial and fungal isolates (Boonchan et al., 2000; Jacques et al., 2008). Table 4 presents a brief summary of single isolates of bacteria and fungi that are known to degrade aromatic hydrocarbons using bioaugmnetation as a strategy and various consortia of bacteria and fungi that successfully carry out this process.

Aliphatic hydrocarbons on the other hand which are basically made up of straight, branched and cyclic structures are more readily degraded by microorganisms than aromatic hydrocarbons (Das and Chandran, 2011). For instance, Colombo et al. (1996) investigated the biodegradation of aliphatic and aromatic hydrocarbons by natural soil microflora and pure cultures of imperfect and ligninolytic fungi. In their experiments, they discovered that the natural microbial soil assemblage isolated from an urban forest area was unable to significantly degrade

Table 4. A summary of microorganisms involved in the degradation of aromatic hydrocarbons using bioaugmentation as a strategy.

Microorganism	Contaminants treated	Reference
Single strains		
Mycobacterium sp.	Pyrene (PAH)	Heitkamp et al. (1988)
Pseudomonas paucimobilis	Fluoranthene (PAH)	Weissenfels et al. (1990)
Pseudomonas cepacia	HMW PAHs	Juhasz et al. (1996)
Sphingomonas paucimobilis	PAHs	Ye at al. (1996)
Burkholderia cepacia	fluoranthene, pyrene, benz[a]anthracene and dibenz[a,h]anthracene	Boonchan et al. (1998)
Comamonas testosteroni BR60	Crude oil, PAHs	Gentry et al. (2001)
Arthrobacter chlorophenolicus A6L	4-Chlorophenol	Jernberg and Jansso (2002)
Absidia cylindrospora	Fluorene	Garon et al. (2004)
Pseudomonas sp. ST41	Marine gas oil	Stallwood et al. (2005)
Pseudomonas aeruginosa WatG	Diesel oil	Ueno et al. (2006)
Sphingobium chlorophenolicum ATCC 39723	Pentachlorophenol	Dams et al. (2007)
Burkholderia sp. FDS-1	Fenitrothion	Hong et al. (2008)
Aspergillus sp. LEBM2	Phenol	Santos et al. (2008)
Gordonia sp. BS29	Aliphatic/aromatic hydrocarbons	Franzetti et al. (2009)
Pseudomonas putida ZWL73	4-Chloronitrobenzene	Niu et al. (2009)
Aspergillus sp.	LMW-PAHs (2–3 rings)	Silva et al. (2009a)
Trichocladium canadense, Fusarium oxysporum, Aspergillus sp.*, Verticillium* sp., *Achremonium* sp.	HMW-PAHs (4-7 rings)	Silva et al. (2009a)
Neosartorya sp. BL4	Total petroleum hydrocarbons	Yi et al. (2011)
Consortia		
Rhodococcus sp.*, Acinetobacter* sp., *Pseudomonas* sp.	PAHs (fluorene, phenanthrene, pyrene)	Yu et al. (2005)
Bacillus subtilis DM-04, *Pseudomonas aeruginosa* M and NM	Crude petroleum-oil hydrocarbons	Das and Mukherjee (2007)
Mycobacterium fortuitum, *Bacillus cereus*, *Microbacterium* sp., *Gordonia polyisoprenivorans, Microbacteriaceae bacterium*, *Fusarium oxysporum*	PAHs (anthracene, phenanthrene, pyrene)	Jacques et al. (2008)
Rhizopus sp., *Penicillium funiculosum*, *Aspergillus sydowii*	Petroleum hydrocarbons	Mancera-Lopez et al. (2008)
Bacillus strains B1F, B5A and B3G, *Chromobacterium* sp. 4015, *Enterobacter aglomerans* sp. B1A, *Achremonium* sp., *Aspergillus* sp., *Verticillium* sp.	Mixture of PAHs (naphthalene, phenanthrene, anthracene, pyrene, dibenzo[a]anthracene, benzo[a]pyrene)	Silva et al. (2009b)

crude oil, whereas pure fungi cultures effectively reduced the residues by 26-35% in 90 days. They also reported that normal alkanes were almost completely degraded in the first 15 days, whereas degradation of aromatic compounds (for example, phenanthrene and methylphenanthrene) exhibited slower kinetics. Another experiment conducted on the kinetics of the degradation of aliphatic hydrocarbons by the bacteria *Rhodococcus ruber* and *Rhodococcus erythropolis,* showed that the growth of these bacterial isolates on *n*-alkanes was intense when compared to growth in diesel medium (Zhukov et al., 2007). A comparative study on the degradation of both

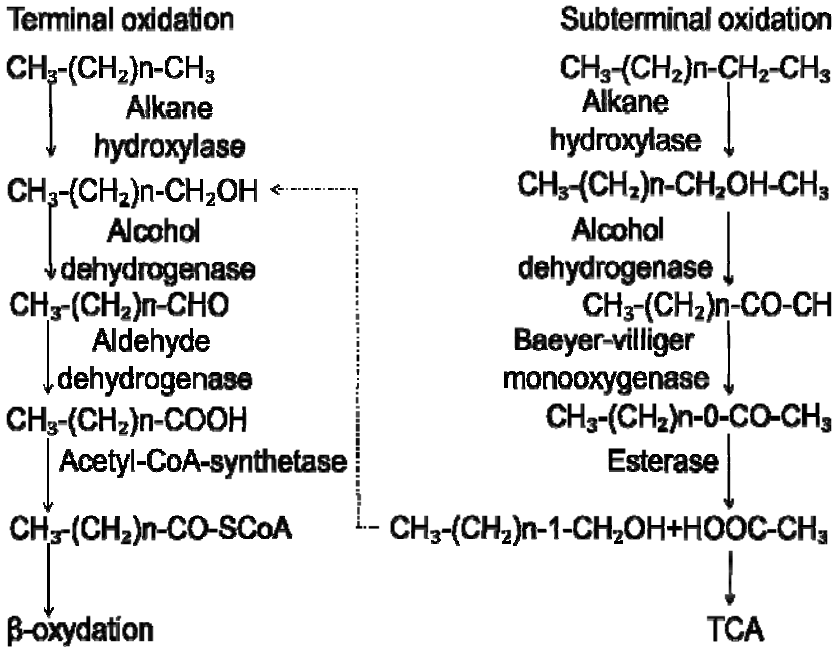

Figure 1. Simplified pathways for the degradation of *n*-alkanes by terminal and sub-terminal oxidation.

aliphatic and aromatic hydrocarbons by *Nocardia* sp. H17-1 was conducted and the results obtained showed a 99.0±0.1% and 23.8±0.8% reduction of both classes of hydrocarbons (Baek et al., 2006).

Mechanisms involved in petroleum hydrocarbon degradation

Various mechanisms of biodegradation of pollutants in the environment have been proposed by different researchers. For effective biodegradation of pollutants in environment, the chemicals must be accessible to the biological catalyst (Fritsche and Hofrichter, 2000). The first mechanism for degradation of petroleum hydrocarbons involves enzymes and was proposed by Fritsche and Hofrichter (2000). These authors stated that for complete degradation of the majority of organic pollutants to be accomplished, aerobic conditions are a requirement. Thus, introduction of oxygen into the environment is vital as a co-substrate in reactions catalysed by oxygenases and peroxidases (Kariga and Rao, 2011) which are the main enzymes responsible for the aerobic degradation of most pollutants. The conversion of organic pollutants step by step through peripheral pathways such as the tricarboxylic acid cycle into intermediates of central intermediary metabolism is one of the results achieved during the microbial degradation process (Fritsche and Hofrichter, 2000; Das and Chandran, 2011). Biosynthesis of cell biomass occurs from the central precursor metabolites acetyl-CoA, succinate, and pyruvate derived from sugars via gluconeogenesis. Different pathways for the aerobic degradation of the various components of petroleum hydrocarbons have been proposed. For instance *n*-alkanes, a major group in crude oil contamination have several pathways through which it is biodegraded.

Pathways for degradation of *n*-alkanes

Aerobic degradation of *n*-alkanes begins with the oxidation of a terminal methyl group which renders a primary alcohol to be oxidized to the corresponding aldehyde, and finally conversion into a fatty acid (van Hamme et al., 2003; Wentzel et al., 2007). The fatty acids which are formed are subsequently transformed to acyl-CoA by aldehyde dehydrogenase and acyl-CoA synthetase respectively (Wentzel et al., 2007). Figure 1 shows the general degradation pathways for *n*-alkanes by two types of oxidation systems. Different enzymes are involved in the initial terminal hydroxylation of *n*-alkanes by bacteria (van Beilen et al., 2003; van Beilen and Funhoff, 2007). Methane monooxygenases are the major group of enzymes that carry out the hydroxylation of short chain-length alkanes (C_2-C_4) (Hamamura et al., 1999) while the non-heme iron monooxygenases and soluble cytochrome P450 (CYP153) are known to degrade

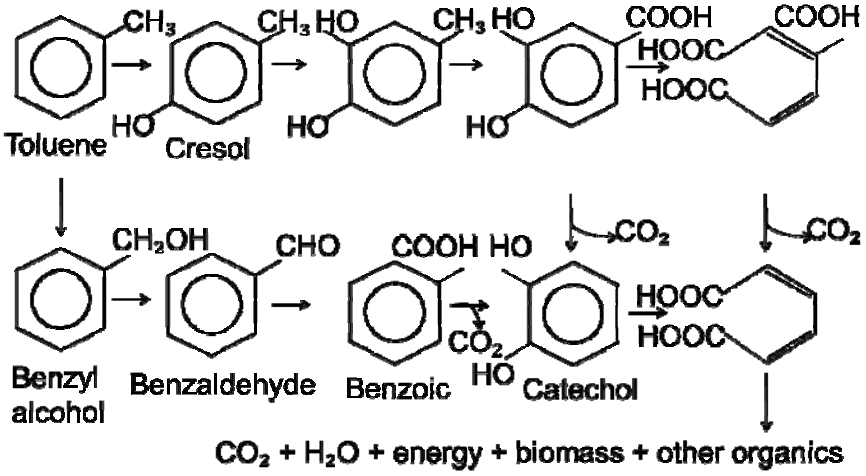

Figure 2. Mechanisms of toluene metabolism.

medium chain alkanes (C_5-C_{11}) (Maier et al., 2001; van Beilen et al., 2005). The long chain alkanes (C_{10}-C_{30}) are easily degraded by alkane hydroxylases such as LadA, the thermophilic flavin-dependent monooxygenase (Wentzel et al., 2007).

Anaerobic biodegradation of petroleum hydrocarbons has been achieved using different bacterial strains (Widdel et al., 2006; Foght, 2008; Salehi et al., 2008) and reports show that these bacteria activate hydrocarbons by unprecedented biochemical mechanisms that differ completely from those employed in aerobic hydrocarbon metabolism. These unprecedented biochemical mechanisms may be initiated by bacteria through metabolic pathways by oxygen-independent hydrocarbon-activating reactions (Heider and Schuhle, 2013).

Pathways for degradation of aromatic hydrocarbons

The biodegradation of aromatic compounds has been extensively studied due to its importance in the biogeochemical carbon cycle. Since many aromatic compounds such as benzene, toluene, ethylbenzene and xylene (BTEX) are major environmental pollutants; their detection and removal from contaminated sites are of great biotechnological interest (Dıaz et al., 2013). Different catabolic pathways for the degradation of aromatic compounds have been described. According to Harayama et al. (1999), toluene is degraded by bacteria along five different pathways and for the purposes of this review, only two of these pathways are highlighted.

Different enzymes are involved in the degradation of toluene and they include toluene monooxygenase, benzyl alcohol dehydrogenase, benzaldehyde dehydrogenase and catechol-2, 3-dioxygenase. These enzymes are organized into two different pathways with the upper pathway, coding for enzymes that convert aromatic alcohols to acids, while the lower pathway is involved in aromatic acid metabolism via an *ortho* and *meta* pathway (Hamzah et al., 2011). Ring hydroxylation, which is involved in the second pathway, yields methyl catechol as the metabolic intermediate with toluene dioxygenase as the key enzyme. A second mechanism involved in petroleum hydrocarbon degradation involves attachment of microbes to the substrates while a third mechanism involves the production of bio-surfactants (Figure 2).

Factors influencing rate of petroleum hydrocarbon biodegradation

For a successful bioremediation technology to be achieved, a full knowledge of the characteristics of the contaminated site and the parameters that affect the biodegradation of the pollutants must be accounted for. Different abiotic factors have been highlighted in various studies which influence the rate of hydrocarbon degradation in any environment. These factors include temperature, pH, nutrient availability, moisture content, and chemical composition of the contaminant, salinity of the environment, concentration and physical state of the contaminant (Leahy and Colwell, 1990; Salleh et al., 2003; Okoh, 2006).

The effect of temperature on the degradation of pollutants is very important as it affects solubility of the contaminants in the environment (Foght et al., 1996). Degradation of hydrocarbons occurs over a very wide range of temperatures.

However, the biodegradability of a contaminant decreases with a decrease in temperature (Das and Chandran, 2011). Researchers have isolated a number of hydrocarbon utilizing bacteria which include psychrotrophic, mesophilic and thermophilic bacteria. Psychrotrophic bacteria such as *Rhodococcus* sp. were reported

by Whyte et al. (1998 and 1999) to have successfully degraded short chain alkanes at 0°C. However in a report by Atlas (1981), a direct correlation between increased microbial degradation with an increase in temperature was recorded. This means that when microorganisms that are isolated from a cold region are introduced into an environment that has an elevated temperature, their metabolic activities tend to be faster (Atlas, 1981). According to Okoh (2006), highest degradation rates generally occur in the range 30-40°C in soil environments, 20-30°C in some fresh water environments, and 15-20°C in marine environments.

Biodegradation rates have also been measured in relation to pH (Strandberg and Lewis, 1988). Outcomes from various experiments conducted show that biodegradation is effectively carried out at an optimum of pH 7.0 (Zaidi and Imam, 1999). In a contaminated environment such as soil that is acidic in nature, the dominant microbial species that are capable of metabolising the contaminants in a short space of time appear to be fungi (Jones et al., 1970). The isolation of bacteria from an alkaline medium that were able to degrade phenol at pH 7.0-10.6 has also been reported (Kanekar at al., 1999). The importance of nutrients in the degradation of hydrocarbons has also been stressed (Cooney, 1984). During biodegradation of hydrocarbons, lack of nutrients such as nitrogen, phosphorus, potassium, and iron may either hinder the breakdown process or result in an incomplete breakdown of contaminants. In fresh water environments, nutrients are particularly deficient. The supply of carbon significantly increases during major oil spills in marine and fresh waters with nitrogen and phosphorus serving as limiting factors (Atlas, 1985). A deficiency in these nutrients in fresh water is due to demand by plants, and photosynthetic and non-photosynthetic microorganisms. Enhancement of biodegradation in different experiments has been achieved through the addition of nutrient supplements (Breedveld and Sparrevik, 2000; Li et al., 2006; Xia et al., 2006; Vyas and Dave, 2010). It should be noted however, that excessive nutrient concentration can impact the microbial degradation of hydrocarbons negatively (Oudot et a., 1998; Chaîneau et al., 2005).

The stability of water activity in aquatic environments has caused researchers to focus more attention on soils. For instance, Bossert and Bartha (1984) stated that the water activity of an aquatic environment is 0.98 while that of soil has a range between 0.0 and 0.99. The wide range of water activity in soils has made biodegradation of petroleum hydrocarbons very difficult. For effective biodegradation in soils, water activity must be kept constant and at an optimum level.

The chemical composition of contaminants in any environment is another factor that influences microbial degradation of such contaminants. Petroleum hydrocarbons which is made up of four classes; saturates, aromatics, asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and resins, differ in their susceptibility to microbial attack. Biodegradation of hydrocarbons in decreasing order of susceptibility is ranked in the following order: *n*-alkanes > branched alkanes > low-molecular-weight aromatics > cyclic alkanes (Leahy and Colwell, 1990). According to Okoh (2006) the biodegradation of heavier crude oils is generally much more difficult than lighter ones. However, a report contrary to that of Okoh (2006) published by Cooney et al. (1985) stated that the degradation of more complex compounds such as naphthalene was faster than that of hexadecane in water-sediment mixtures from a freshwater lake. This observed result could be as a result of the action of co-metabolism by the organisms acting on the substrates.

Metabolic rate of microorganisms in mineralising contaminants in different environments tends to decline with increasing salinity (Ward and Brock, 1978; Minai-Tehrani et al., 2006). The ability of different microorganisms to degrade hydrocarbons in contaminated environments in the presence of elevated concentration of salts has been tested. Results showed that almost 100% of initial phenanthrene and dibenzothiophene were degraded at a salt concentration of 35 g/L (Díaz et al., 2002) while Abed et al. (2006) reported that at salinities ranging from 60 to 140 g/L, alkane biodegradation rates were 50 to 60% with a lesser degradation rate of less than 30% at 180 g/L. Contrary to these reports, Bertrand et al. (1990) isolated an Achaeon from a water-sediment interface with salinity of 310 g/L which was able to degrade eicosane more efficiently at a rate of 64% in a medium that contained sodium chloride at a concentration of 146 g/L.

Due to the dispersion of oil in water during spillage, a slick typically forms which gives rise to emulsions (mousse) (Leahy and Colwell, 1990). The formation of an emulsion in water increases the surface tension of the oil thereby making it available for microorganisms to degrade (Salleh et al., 2003). Emulsion formation through microbial production and release of biosurfactants has been documented (Kosaric, 2001; Kumar et al., 2008; Aparna et al., 2011; Mnif et al., 2011). Kumar et al. (2008) reported that a hydrocarbon degrading and biosurfactant producing strain of *Pseudomonas*, DHT2, which was isolated from oil contaminated soil was able to degrade crude oil, fuels, alkanes and PAHs. These authors also established that the biosurfactants which were produced by the organism lowered the surface tension of the medium from 54.9 to 30.2 dN/cm and formed a stable emulsion.

CONCLUSION

Bacterial degradation of fossil fuels (solids and liquids) is an important and emerging aspect of biotechnology which

is neither fully described nor understood and as a consequence, technologies for implementation as commercial remediation strategies are few. While fungal biodegradation/biosolubilisation of coal and coal related products has been widely reported, it appears that work with bacteria has lagged and in some cases it has been completely ignored. In contrast, the use of bacteria and bacterial consortia for the remediation of petroleum hydrocarbon contamination is well established (Pinedo-Rivilla et al., 2009; Basha et al., 2010; Zhang et al., 2013; Ma et al., 2013; Martin et al., 2013) and as a consequence, commercial remediation protocols and the associated biocatalysts are widely available. Even so, there is a growing realisation that a mutualistic relationship between microorganisms and higher plants is necessary for complete remediation of contaminated sites (Ndimele, 2010; Sekhohola et al., 2013). Thus, further study is needed to enhance our understanding of the processes involved in the bacterial bioconversion of coal and petroleum hydrocarbon contaminants in order to facilitate both a reduction in pollutant levels and to explore the potential for generating products of value. While the use of single strains to degrade coal and liquid hydrocarbon contaminants has been widely reported, consortia of bacteria or bacteria together with fungi appear to be the biocatalysts of choice as biodegradation agents.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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